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Review

Occurrence, detection and detoxification of mycotoxins

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Mycotoxins have been identified as important toxins affecting animal species and humans ever since the discovery of aflatoxin B_1 in 1960. Mycotoxigenic fungi are ubiquitous in nature and are held responsible for economic loss as they decrease crop yield and quality of food. The presence of fungi and their mycotoxins are reported not only in food grains but also in medicinal herbs and processed foods. Since prevention is not always possible, detoxification of mycotoxins have been attempted using several means; however, only few have been accepted for practical use, e.g. ammonia in the corn industry. Organizations such as the World Health Organization, US Food and Drug Administration and European Union have set regulations and safety limits of important mycotoxins, viz. aflatoxins, fusarium toxins, ochratoxin, patulin zearalenone, etc., to ensure the safety of the consumers. This review article is a brief and up-to-date account of the occurrence, detection and detoxification of mycotoxins for those interested in and considering research in this area.

[Aiko V and Mehta A 2015 Occurrence, detection and detoxification of mycotoxins. *J. Biosci.* **40** 943–954] **DOI** 10.1007/s12038-015-9569-6

1. Introduction

The word mycotoxin is derived from the Greek word ‘mykes’ meaning ‘fungus’ and the Latin word ‘toxicum’ meaning ‘poison’. They are low molecular weight molecules produced as secondary metabolites by saprophytic fungi, especially Aspergillus, Penicillium and Fusarium. Mycotoxins have been known to mankind since the 1800s as ‘St. Anthony’s Fire’ caused by ergot alkaloids, and as ‘Alimentary Toxic Aleukia’ caused by T2 toxins during World War II in Russia (Richard 2003). It was not until the 1960s, with the outbreak of ‘Turkey X’ disease in England, that mycotoxins were identified as important toxins. Turkey X disease refers to the death of 100,000 Turkey poults due to consumption of peanut meal contaminated with fungi (Blount 1961). The responsible fungus was identified as *Aspergillus flavus* and the toxin as aflatoxin. Aflatoxins are acutely and chronically toxic to humans and animals. They cause liver and kidney damage, and induce mutagenic, carcinogenic and immunosuppressive effects. Among the mycotoxins, aflatoxin B_1 is considered the most toxic and is classified as a group I carcinogen by the International Agency for Research on Cancer (IARC 2002). Some of the important mycotoxins of significant health hazards are listed in table 1.

There have been several outbreaks of mycotoxicosis in the human population. In 1974, an outbreak of hepatitis in India resulted in the death of 100 people due to consumption of contaminated maize (Krishnamachari *et al*. 1975). In another case in India, an outbreak of gastrointestinal disorder associated with consumption of bread made of contaminated wheat was reported in 1987. The contaminating moulds consisted of Fusarium sp. and Aspergillus sp., and the toxins were identified as deoxynivalenol, nivalenol, acetyl-deoxynivalenol and T2 toxin (Bhat *et al*. 1989). The Kenyan outbreak in 2004 was one of the largest outbreaks, where 125 people died due to liver failure caused by acute aflatoxicosis after consumption of contaminated maize (Muture and Oqana 2005).

Keywords. Aflatoxin; contamination; degradation; toxic effect

Published online: 28 November 2015

Table 1. Important mycotoxins affecting humans (CAST 2003)

Mycotoxins	Producer fungi	Commodities	Toxic effect
Aflatoxins (B_1, B_2, G_1, G_2, M_1, M_2)	*A. flavus, A.parasiticus, A.nominus, A. tamari*	Peanuts, corn, wheat, rice, milk, cheese, figs, herbs	Mutagenic, carcinogenic, hepatotoxic, immunosuppressive
Citrinin	*P. citrinum, P. viridicatum*	Wheat, barley, corn, rice	Nephrotoxic, carcinogenic.
Deoxynivalenol (Trichothecenes)	*F. graminearum, F. culmorum*	Corn, wheat, barley, oats	Vomiting, diarrhea, immunosuppressive
Fumonisin	*F. verticillioides, F.proliferatum*	Corn, wheat	Tumors of the kidney and liver
Ochratoxin A	*A. ochraceus, A. flavus, P. viridicatum*	Cereals, beans, peanuts, cheese, coffee, dried fruits, grapes, wine	Nephrotoxic, hepatotoxic, teratogenic, carcinogenic,
Patulin	*P. patulum, P. expansum*	Apples, apple juice	Subcutaneous sarcomas, hemorrhage, carcinogenic
T-2 toxin (Trichothecenes)	*F. sporotrichoides, F. poae, F. roseum*	Corn, wheat, barley, oats	Emetic, cytotoxic, teratogenic
Zearalenone	*F. graminearum, F. tricinctum, F. Culmorum*	Corn, hay	Hyper-estrogenic, abortion

2. Occurrence of mycotoxins

The ubiquitous nature of fungi makes food crops vulnerable to fungal contamination during pre-harvest and post-harvest conditions. In the field, the contaminating fungi are airborne or transmitted by insects, and damaged kernels often become infected. Stress conditions like drought, floods, insect infestation and delayed harvest increase the level of contamination. Post-harvest conditions such as inadequate drying, warm humid environment during storage lead to mould formation. The extent of contamination by fungi depends on various factors like geographic location, processing and storing periods of the crops. Environmental factors like temperature, water activity or pH, damage of crop by insects, crop densities, etc., influence the growth of fungi and mycotoxin production (Magan and Olsen 2004).

2.1 *Mycotoxins in food and feed*

A survey of mycotoxin contamination in animal feeds in European and Mediterranean markets and Asia-Pacific regions was carried out by Binder *et al*. (2007). Their result showed the occurrence of deoxynivalenol, zearalenone and T2 toxin as the major contaminants in the European samples, while aflatoxins, fumonisins, deoxynivalenol and zearalenone were mainly found in the samples from Asia and Pacific regions. Herzallah (2009) has reported the presence of aflatoxin M_1 and M_2 in milk samples and aflatoxins B_1, B_2, G_1 and G_2 in meat samples collected from the local markets in Jordan. Rice is the staple food of India, consumed almost daily in every household, and if contaminated with aflatoxins, will have high impact on human health. A survey by Reddy *et al*. (2009) revealed the presence of several species of Aspergillus and aflatoxin B_1 in rice samples consisting of paddy and milled rice. Occurrence of Aspergillus and aflatoxins in rice grains were also reported from other countries like China, Nigeria and United Arab Emirates (Hussaini *et al*. 2007; Osman *et al*. 1999; Zuoxin *et al.* 2006). Maize samples in Vietnam intended for human and animal consumption were reported to be contaminated with high fungal load and aflatoxin B_1 and fumonisin B_1 (Trung *et al.* 2008). Alborch *et al*. (2012) isolated several species of Aspergillus, Fusarium, Penicillium and Mucorales from maize flour and popcorn kernels in Spain, and reported the natural contamination of aflatoxin B_1 and ochratoxin A in the samples. Mycotoxins such as aflatoxins, ochratoxin and fumonisins have been detected in processed foods which were sold in the market (Sugita-Konishi *et al*. 2006; Romagnoli *et al*. 2007; Mushtaq *et al*. 2012). Aflatoxins and fumonisins were also detected in maize-based products intended for human consumption in Glasgow, UK (Candlish *et al*. 2000).

2.2 *Mycotoxins in medicinal herbs*

Studies in India showed the natural occurrence of aflatoxin B_1 and citrinin in medicinal plants and herbal drugs (Roy and Kumari 1991). Aflatoxin B_1, citrinin, ochratoxin A and zearalenone were detected in several medicinal plants such as *Asparagus racemosus, Carum ajmoda, Cinnamomum zeylanicum, Cuminum cyminum, Elettaria cardamomum, Emblica officinalis, Piper longum, P. nigrum, Saraca indica* and *Zingiber officinale* (Chourasia 1995). Thirumala-Devi *et al.* (2001) reported the contamination of *Coriandrum sativum, Piper nigrum, Zingiber officinale* and *Curcuma longa* with ochratoxin A in India. The incidence of toxigenic fungi producing aflatoxins, ochratoxin A and fumonisin on medicinal herbs was reported from Argentina (Rizzo *et al.* 2004). The medicinal herb ginseng was reported to be contaminated with aflatoxin B_1, ochratoxin A and zearalenone (Gray *et al.* 2004; Trucksess *et al.* 2006). An investigation from South Africa showed the presence of fumonisin B_1 in dietary (*Rumex lanceolatus, Zantedeschia aethiopica, Raphanus raphinastrum, Solanum nigrum*) and medicinal (*Catha edulis, Dalbergia obovata, Brunsvigia* sp.*, Datura stramonium*) wild plants (Sewram *et al.* 2006). Aflatoxins-, ochratoxin-A- and citrinin-producing Aspergillus and Penicillium were isolated from medicinal herbs in Brazil (Bugno *et al.* 2006). The presence of aflatoxin B_1 in *Pimpinella anisum*, *Piper nigrum*, *Mentha piperita* and *Origanum majorana* was reported by Bokhari (2007) in Saudi Arabia. In Korea, a survey was conducted on spices and processed spice products for aflatoxin contamination, where aflatoxin B_1 was detected in 13.6% of the spices (Cho *et al.* 2008). Multi-contamination of mycotoxins with T2 toxin, zearalenone, aflatoxins, ochratoxin A, deoxynivalenol, citrinin and fumonisin were detected in 84 medicinal herbs surveyed in Spain (Santos *et al.* 2009).

3. Mechanism of toxicity

3.1 *Aflatoxin*

Aflatoxin B_1 is a well-known human carcinogen. The World Health Organization has reported that hepatocellular carcinoma (HCC) or liver cancer is the third leading cause of cancer death worldwide and chronic aflatoxicosis leading to the development of HCC has been implicated (Wild and Gong 2010). Aflatoxin B_1 is activated by cytochrome P450 to form aflatoxin B_1-8,9-epoxide, which is responsible for the mutagenic activity of aflatoxin B_1 (McLean and Dutton 1995). Aflatoxin B_1-8,9-epoxide specifically binds to the N^7 position of guanine of DNA and RNA to form aflatoxin B_1-N^7-guanine adduct (Croy *et al.* 1978). Aflatoxin B_1 inhibits DNA, RNA and protein synthesis, resulting in immuno-suppressive, hormonal and teratogenic effects (McLean and Dutton 1995).

3.2 *Ochratoxins*

Ochratoxin primarily affects the kidney of all animal species and at high concentration can affect the liver. The toxic effect has been attributed to inhibition of phenylalanine tRNA synthetase. It is also an immune suppressor, teratogen and a carcinogen (Kuiper-Goodman and Scott 1989) and its role in the Balkan endemic nephropathy has been implicated (Hult *et al.* 1982). Studies have shown the formation of ochratoxin A-DNA adducts in the kidney and bladder tissues of Bulgarian patients undergoing surgery for cancer (Pfohl-Leszkowicz *et al.* 1993).

3.3 *Citrinin*

Citrinin is hepatotoxic and nephrotoxic to a number of animal species, and the possible role of citrinin and ochratoxin A in the Balkan endemic nephropathy was implicated (Vrabcheva *et al.* 2000). Citrinin has been shown to inhibit RNA, DNA and protein synthesis in porcine kidney at 0.01 mM concentration (Braunberg *et al.* 1992).

3.4 *Fumonisins*

Fumonisin B_1 and B_2 are known to cause leukoencephalomalacia in horse (Marasas *et al.* 1988), and pulmonary edema and hydrothorax in swine (Colvin and Harrison 1992). This toxin has been reported to interfere with sphingolipid metabolism by inhibiting ceramide kinase. The high incidence of esophageal cancer in South Africa, China and Italy has been correlated with fumonisin B_1 (Peraica *et al.* 1999). In a recent study, Wang *et al.* (2014) reported that fumonisin B_1 stimulated the proliferation of normal human esophageal epithelial cells increasing the protein expression of cyclin D1 and decreasing cyclin E, p21 and p27.

3.5 *Trichothecenes*

Deoxynivalenol, diacetoxyscirpenol and T2 are the most common trichothecenes. They are known to cause nausea, vomiting, diarrhea and suppress the immune system in animals. Trichothecenes are reported to be potent inhibitors of protein synthesis by direct inhibition of peptidyl transferase in the large ribosome subunit (Feinberg and McLaughlin 1989).

3.6 *Zearalenone*

Zearalenone, also known as a mycoestrogen or phytoestrogen, mainly affects swine causing hyperestrogenism (Kurtz and Mirocha 1978). The exact mechanism of toxicity is not known however it has been reported that zearalenone is metabolized into α- and β-zearalenol which conjugates with glucoronic acid (Olsen 1989).

4. Detection of mycotoxins

Mycotoxins occur naturally and frequently in food and feeds, as mentioned earlier. The toxic nature of mycotoxins makes their detection an absolute necessity. Several detection methods have been developed, and among them chromatographic techniques are widely used. The procedure for detecting mycotoxins involves extraction from sample material, purification, and qualitative and quantitative analysis. The most common methods currently used are described here.

4.1 *Thin layer chromatography (TLC)*

TLC is one of the traditional methods of detecting mycotoxins. This technique enables screening of large number of samples, easy identification and is cost-effective. A silica gel layer is most commonly used; however, phenyl non-polar bonded, silanized and polyamide are also been used (Lin *et al*. 1998). Mycotoxins are visualized on the TLC plate by observing under UV light or by spraying chemicals which react with mycotoxins and enhance the fluorescence or produce colour products (Betina 1985). Aflatoxins, citrinin and ochatoxin are naturally fluorescent compounds; hence, they are identified based on their fluorescent properties. For example, the B and G aflatoxins are differentiated by blue and green fluorescence, respectively, while citrinin is identified by yellow fluorescence (Betina 1985). Aflatoxins have been identified by chemical confirmation by spraying trifluoroacetic acid and sulphuric acid on the TLC plate (Stack and Pohland 1975). Serralheiro and Quinta (1985) have reported that spraying sulphuric acid improves the limit of detection of aflatoxin M_1 from 0.5 μg/kg to 0.3 μg/kg. Semi-quantitative analysis has been carried out for mycotoxins by TLC; however, the method has low sensitivity.

The TLC method has been improved in high-performance thin layer chromatography (HPTLC) to enhance the resolution and accuracy. HPTLC has been used to determine aflatoxins in peanut products and was shown to be equivalent to liquid chromatography in precision, accuracy and sensitivity (Tosch *et al*. 1984).

4.2 *High-performance liquid chromatography (HPLC)*

HPLC provides higher accuracy and precision of mycotoxin determination. Normal and reversed-phase HPLC are used with a variety of detection systems. UV and fluorescence detectors are most commonly used. Pons and Franz (1978) reported accurate and sensitive detection of all aflatoxins at levels of 0.3–1 μg/kg, whereas aflatoxins B_1 and B_2 were detected by a UV detector at 360–365 nm, and G_1 and G_2 by fluorescence. Akiyama *et al*. (1998) detected non-fluorescent mycotoxins, fumonisins, by using *o*-pthalaldehyde post-column derivatization and then detection by fluorescence detector. The detection limit of fumonisin by this method was reported to be 10 μg/kg of corn. Ochratoxin A in wine has been accurately detected by HPLC following immuno-affinity clean-up with a detection limit of 0.01 ng/mL (Visconti *et al*. 1999). HPLC with fluorescence detector has been used for detecting aflatoxin B_1, citrinin and ochratoxin in rice, and a detection limit of 0.07, 0.11 and 0.08 μg/kg, respectively, for these mycotoxins was reported by Nguyen *et al*. (2007).

4.3 *Liquid chromatography-mass spectrometry (LC-MS)*

Liquid chromatography coupled with mass spectrometry eliminates the need for sample derivatization for fluorescent activity. LC-MS is a very selective and sensitive method for identification and quantification of mycotoxins. Spanjer *et al.* (2008) had developed an LC-MS/MS method to detect 33 mycotoxins simultaneously in various food materials. The mycotoxins include aflatoxin B_1, B_2, G_1 and G_2, and ochratoxin A with a limit of quantification of 1 μg/kg and 50 μg/kg for deoxynivalenol. An ultra-high-performance liquid chromatography combined with electrospray ionization triple quadrupole tandem mass spectrometry (UHPLC-ESI-MS/MS) has been developed to determine aflatoxin M_1, ochratoxin A, zearalenone and α-zearalenol in milk. The limits of quantification of these toxins were reported to be in the range 0.003 to 0.015 μg/kg (Huang *et al*. 2014). LC-MS/MS was used for detection and quantification of mycotoxins in blood and urine samples. In a recent study, 23 mycotoxins and their metabolites were monitored in human population of Bangladesh and Germany (Gerding *et al.* 2015).

4.4 *Gas chromatography (GC)*

This method is also used for detecting mycotoxins especially trichothecenes in food samples. Most mycotoxins are non-volatile and hence are derivatized for detection (Scott 1995). Electron capture detection (ECD), mass spectrometry (MS) and flame ionization are the common detectors used with

GC. Croteau *et al.* (1994) analysed trichothecenes in corn using GC. The trichothecene mycotoxins were derivatized using heptafluorobutyric anhydride and detected by ECD. The limit of quantification was reported in the range 50–200 µg/kg of corn. In another report, trichothecenes were determined in corn by MS detector with a detection limit of 10–40 µg/kg and limit of quantification of 70–200 µg/kg (Milanez and Valente-Soares 2006). The GC method has also been used for analysing multi-mycotoxins such as patulin, zearalenone and trichothecenes in wheat (Rodriquez-Carraso *et al.* 2012). There are a few disadvantages with this method such as the need for derivatization and thermal stability of mycotoxins, where heating degrades the samples.

4.5 *Fluorescence spectrometry*

Fluorescence property of several mycotoxins is an important characteristic for their detection. A novel biosensor based on surface plasmon-enhanced fluorescence spectroscopy has been developed for detecting aflatoxin M_1 in milk with a detection as low as 0.6 pg/mL (Wang *et al*. 2009).

4.6 *Fourier transform infrared spectroscopy*

The use of infrared spectroscopy has proved to be a promising technique for the fast and non-destructive detection of mycotoxins in food grains. Deoxynivalenol was detected in wheat kernel samples using near-infrared spectroscopy at concentrations above 400 µg/kg (Pettersson and Aberg 2003). In a similar study, Kos *et al*. (2003) used mid-infrared to detect deoxynivalenol at concentrations as low as 310 µg/kg in corn samples. Near-infrared spectroscopy technique has also been used to detect aflatoxin B_1 and ochratoxin A in red paprika in Spain (Hernandez-Hierro *et al*. 2008).

4.7 *Enzyme-linked immunosorbent assay (ELISA)*

ELISA technique has been used for determining aflatoxin in a large number of foods. This method is based on direct and indirect competitive assay. ELISA has been used for detecting deoxynivalenol and zearalenone in maize (Cavaliere *et al*. 2005). Reddy *et al*. (2009) have used indirect competitive ELISA for detecting aflatoxin B_1 in rice with a detection limit of 0.02 ng/kg. Other mycotoxins like fumonisin have also been detected using this method with a detection limit of 3 ng/mL of beer (Torres *et al*. 1998). This method has the advantage of screening bulk samples and is highly specific. The performance of various detection methods differs for different food materials. Dell *et al*. (1990) has reported the determination of aflatoxin in peanut butter using high-performance TLC which gave precise and consistent data than HPLC and ELISA.

4.8 *Radioimmunoassay (RIA)*

The other immunological methods for mycotoxin detection include radioimmunoassay. Sun and Chu (1977) had developed a solid-phase RIA for detecting aflatoxin B_1 in corn and wheat. Commercial kit for the application of RIA has been validated for determining ochratoxin A in food and feed stuffs with a detectable limit of 1 µg/kg (Fukal 1990). RIA was also used for detecting nivalenol in barley and the result was reported to be consistent with gas chromatographic analysis (Teshima *et al*. 1990).

4.9 *Lateral flow devices*

Lateral flow or dipstick immunoassay, developed using the principal of ELISA, are been successfully used for detecting mycotoxins. This technique was used for screening aflatoxin B_1 and ochratoxin A simultaneously in chili samples, with limit of quantitation of 2 and 10 µg/kg, respectively (Saha *et al*. 2007). Deoxynivalenol and zearalenone were detected in wheat samples using colloidal gold-based lateral flow immunoassay (Kolosova *et al*. 2007). They have reported cut-off levels of 1500 and 100 µg/kg for deoxynivalenol and zearalenone, respectively. Other studies include aflatoxin B_1 detection in pig feed (Delmulle *et al*. 2005), T2 toxin in wheat and oat (Molinelli *et al.* 2008) and aflatoxin M_1 in milk (Zhang *et al.* 2012). This method is very simple and rapid where the result is obtained within 10 min and offers a convenient on-site screening tool.

4.10 *Signal amplification method*

With the advancement of technology, very low concentration of mycotoxin can be detected using the signal amplification technique. Pal and Dhar (2004) had developed an analytical device for immunoassay using an improved catalysed reporter deposition method of signal amplification. Groundnut, corn, wheat, cheese and chili were analysed for aflatoxin B_1 using this method, and a detection limit of 0.1 ng/mL was reported. In another study, a gold nano-particle-enhanced surface plasmon resonance imaging chip was designed to detect multiple mycotoxins using a competitive immunoassay (Hu *et al*. 2014). They have reported high specific and sensitive detection of aflatoxin B_1, ochratoxin A and zearalenone with low detection limits of 8, 30 and 15 pg/mL, respectively.

5. Detoxification of mycotoxins

Removal or detoxification of mycotoxins has been studied using physical, chemical or biological methods. Efficient degradation of mycotoxins is a challenge since most mycotoxins are heat-stable and form toxic degradation products. Although several detoxification methods have been developed, only a few have been accepted for practical use. Some of the common methods are described here.

5.1 *Physical treatment*

Physical treatment includes cooking, boiling, roasting, microwave heating, extrusion, irradiation, etc. Food undergo heat treatment during the processing stage, and hence thermal inactivation of mycotoxins is practical. Mycotoxins are relatively heat-stable and so are not easily destroyed (Bullerman and Bianchini 2007). The level of mycotoxin degradation by thermal process depends on factors like temperature, moisture content and time period. In heat treatment, temperature and time period are important in determining the level of degradation. Higher level of aflatoxin degradation was achieved when heated at 200°C for longer exposure time (Levi 1980). The moisture content of a product also plays an important part in degrading aflatoxins. At high moisture content, degradation was found more efficient (Mann *et al*. 1967). Under dry conditions, citrinin was decomposed at 170°C, whereas under moist condition it was detoxified at 140°C (Kitabatake *et al*. 1991). Heating ochratoxin A in the presence of sodium hydroxide (NaOH) resulted in the detoxification of the toxin (Trivedi *et al*. 1992). Roasting fumonisin B_1 contaminated cornmeal at 218°C for 15 min resulted in almost complete degradation of the toxin (Castelo *et al*. 1998). The presence of ammonia during extrusion of aflatoxin B_1 led to higher amount of degradation (Hameed 1993).

Aflatoxins are photosensitive in nature; hence, various radiations such as sunlight, UV light and gamma rays have been employed for degradation studies. Sunlight was efficiently used for degrading aflatoxin B_1 in olive oil, groundnut oil, etc. (Shantha and Sreenivasa Murthy 1977; Mahjoub and Bullerman 1988). Aflatoxin B_1 was found to be more susceptible to irradiation when present in liquid medium than in solid media. The cytotoxicity and mutagenecity of aflatoxin B_1 has been shown to reduce after treatment with UV in aqueous medium (Liu *et al*. 2011). On the other hand, it was reported that irradiated fungal inocula may produce increased levels of mycotoxins especially aflatoxins (Applegate and Chipley 1974) and ochratoxin (Applegate and Chipley 1976; Paster *et al*. 1985).

5.2 *Chemical treatment*

Treatment with chemicals efficiently degrades aflatoxin B_1; however, formation of degradation products was observed. Acids convert aflatoxin B_1 into several products such as aflatoxins B_2, B_{2a}, D_1, etc., rather than complete degradation. Shukla *et al*. (2002) reported the conversion of aflatoxin B_1 into aflatoxin B_2 and aflatoxin G_1 into aflatoxin G_2 by lactic acid. In a recent study, lactic acid has been shown to degrade aflatoxin B_1 into aflatoxin B_2 and B_{2a} efficiently, with aflatoxin B_{2a} as the major degradation product under heat treatment (Aiko *et al.* 2015). Citric acid causes the hydration of aflatoxin B_1 at the 8,9-olefinic bond of the terminal furan ring to form aflatoxin B_{2a} (Ciegler and Peterson 1968). Treatment of aflatoxin B_1 with hydrochloric acid at elevated temperatures completely destroyed the toxin without the formation of toxic residues (Williams and Dutton 1988; Wattanapat *et al*. 1995). Other acids like salicylic, sulphamic, sulposalicylic, anthranilic, benzoic, boric, oxalic and propionic acids were efficiently used for degrading aflatoxin B_1 by more than 90% in sorghum (Hasan 1996). Alkalis cause the hydrolysis of the lactone ring in aflatoxin B_1; however, it can revert back under acidic conditions (Price and Jorgensen 1985; Camou-Arriola and Price 1989). Boiling aflatoxin B_1 contaminated corn with NaOH decreased the level of aflatoxin B_1 by 93%, with 18% reversion level after treatment with acid (Camou-Arriola and Price 1989). Nixtamalization (alkaline cooking of grains) was reported to be an efficient method in degrading fumonisin. Fumonisin-contaminated kernel corn on nixtamalization resulted in reduced toxicity of fumonisin (Voss *et al*. 2012). Sodium bisulphate and hydrogen peroxide were also used in degrading aflatoxin B_1 efficiently (Altug *et al*. 1990).

Among the many chemicals used for detoxification of mycotoxins, ammonia is the most efficient and it has been accepted for use by the corn production industry. Ammonia degrades aflatoxin B_1 into aflatoxin D_1 which has reduced toxicity and mutagenic potential (Lee and Cucullu 1978). Ozone has been used to degrade aflatoxin B_1 by more than 90% in animal feed (Prudente and King 2002). Maeba *et al.* (1988) showed that ozone-treated aflatoxins were not toxic and mutagenic. Aflatoxin B_2 and G_2, fumonisin, ochratoxin, patulin and zearalenone were also efficiently degraded by ozone.

5.3 *Biological treatment*

5.3.1 *Microorganism:* Few strains of lactic acid bacteria have been reported to remove aflatoxins B_1 and M_1 by binding non-covalently (El-Nezami *et al*. 1998). Heat-treated and acid-treated *Lactobacillus rhomosus* GG and *L. rhamosus* were able to remove zearalenone, indicating

that binding and not metabolism is the mechanism by which the toxins are removed (El-Nezami *et al*. 2002). Another bacteria *Flavobacterium aurantiacum* B-184 was found to remove aflatoxin B_1 irreversibly (Lillehoj *et al*. 1967). Line *et al*. (1994) studied the mechanism and suggested that the degradation of aflatoxin B_1 by *F. aurantiacum* is probably a mineralization phenomenon. A number of fungal species, especially *Phoma* sp., were reported to prevent the synthesis of aflatoxin B_1 and degrade the toxin as well (Shantha 1999). Degradation of mycotoxins occurs during fermentation of various foods such as milk (Megalla and Mohran 1984), dough fermentation in making bread (El-Banna and Scott 1983) or during beer brewing (Chu *et al*. 1975). Some toxigenic fungi (*Aspergillus parasiticus, A. flavus*) have been reported to degrade their own toxins (Doyle and Marth 1978; Hamid and Smith 1987). Degradation of aflatoxin B_1 depends on the type of substrate and the fungal strains used.

5.3.2 *Enzymes:* Several fungal enzymes have been reported to degrade aflatoxin B_1. *Armellaria tabescens* produced a multienzyme system which detoxified aflatoxin B_1 by opening the difuran ring (Liu *et al*. 1998). The enzyme peroxidase from *A. flavus* and *A. parasiticus* has been shown to degrade aflatoxins B_1 and G_1 (Singh 1998; Doyle and Marth 1979). A horseradish peroxidase enzyme from the plant *Raphinus sativa* has also been reported to degrade aflatoxin B_1 (Das and Mishra 2000).

5.3.3 *Plant extracts:* Use of botanicals as anti-fungal and anti-mycotoxin agent is considered safe to humans and environmental friendly. Various extracts from plants such as piperine from black and long peppers (Singh *et al*. 1994); lutein and xanthrophylls from Aztec marigold (Meija *et al*. 1997); carotenoids from fruits and vegetables (Rauscher *et al*. 1998) were reported to suppress the toxicity and mutagenicity of aflatoxin B_1. The essential oils of several plants have been documented to possess strong anti-microbial property. The oil of *Illicium verum*, *Cymbopogon martini, Eucalyptus globulus, Cinnamon zylenium*, etc., are reported to be anti-fungals (Bansod and Rai 2008; Huang *et al*. 2010). The powder and essential oil of *Cymbopogon citratus* have been successfully used for inhibiting aflatoxin B_1 contamination and preserving the quality of melon seed under storage (Bankole and Joda 2004). The essential oils of *Cinnamomum jensenianum* (Tian *et al.* 2011), *Ocimum sanctum* (Kumar *et al*. 2010) and *Zataria multiflora* (Gandomi *et al.* 2009) were used efficiently against toxigenic fungi and aflatoxin B_1 and their safe use as natural preservative of food has been implicated. Among the number of plants, *Syzygium aromaticum* (clove) has been extensively studied for its anti-microbial property (Pinto *et al*. 2009). The oil of clove and its main component, eugenol, has been reported to inhibit Aspergillus growth and aflatoxin B_1 production by various investigators (Bullerman *et al*. 1977; Jayashree and Subramanyam 1999). Whole clove has also been shown to inhibit the growth of *A. flavus* and *P. citrinum* and their toxins in culture media and rice grains (Aiko and Mehta 2013a, b). Removal of toxigenic fungi and mycotoxins by botanicals are usually preferred over chemical treatments.

Table 2. The regulation of mycotoxins in human food (μg/kg)

Mycotoxins	European Union	US FDA
Aflatoxin B_1	2–8	20
Aflatoxin M_1	0.05	0.5
Deoxynivalenol	200–700	1000
Fumonisins (FB_1, FB_2, FB_3)	200–1000	2000–4000
Ochratoxin	3–10	-
Patulin	10–50	-
Zearalenone	20–200	-

6. Management and regulation of mycotoxins

The Food and Agricultural Organization has reported that 25% of the world's food crops are contaminated with mycotoxins. This not only causes economic loss but also reduces the world's food supply. The contaminating fungi and mycotoxins are found in food crops as well as in a number of processed foods intended for human consumption. Mycotoxins pose higher risk of causing cancer than contaminants in food such as anthropogenic contaminants, pesticides, phycotoxins and food additives (Kuiper-Goodman 1998).

Several national and international organizations and agencies have set regulations and safety limits of various

Table 3. Permissible limits of Aflatoxin B_1 in food set by various countries (Moss 2002)

Country	Aflatoxin B_1 (μg/kg)	Products
Argentina	0	Groundnuts, maize and products
Brazil	15	All foodstuffs
China	10	Rice and edible oils
Czech Republic	5	All foods
Hungary	5	All foods
India	30	All foods
Japan	10	All foods
Nigeria	20	All foods
Poland	0	All foods
South Africa	5	All foods
Zimbabwe	5	Foods

mycotoxins. The maximum levels for mycotoxins in foods and feeds have been set to ensure the safety of the consumers. The US Food and Drug Administration (FDA) and European Union (EU) have set the maximum limit of the major mycotoxins in human food as given in table 2. With the recognition of aflatoxin B_1 as a human carcinogen, several countries have set the regulation of aflatoxin B_1 in foods (table 3).

7. Conclusion

Food safety is a major concern around the world. A number of researches are focused on the prevention and removal or detoxification of mycotoxins from food and feed. Control of mycotoxins largely depends on taking proper care during pre-harvest and post-harvest conditions. Use of fertilizers, pest control and fungal-resistant crops, and maintaining low moisture content and temperature during storage conditions can prevent fungal and mycotoxin contamination. However, prevention of mycotoxin contamination is not always possible; hence, many reduction or detoxification methods have been developed as mentioned above. These methods either degrade mycotoxins completely or reduce the toxin concentration to a safe level. Ammonia is currently used for degrading aflatoxin B_1 in feedstuffs; however, it also forms a degradation product aflatoxin D_1 which is not completely non-toxic (Lee and Cucullu 1978). It is of utmost importance to develop a safe and suitable detoxification technique without compromising the nutritional value of food. There is scope for developing an efficient and safer technique for mycotoxin detoxification and the intense research in the field can be very useful.

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MS received 08 April 2015; accepted 12 August 2015

Corresponding editor: Luis M Corrochano