Food and feed mycotoxins: A review

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Mycotoxins are chemicals produced by fungi that are harmful to humans and domestic animals. These chemicals may contaminate staple foods and feeds worldwide, posing a number of significant food safety concerns. Mycotoxins may be fatal or cause severe illness at very small concentrations, often measured in parts per million (ppm) or parts per billion (ppb). There may be thousands of mycotoxins on the planet earth, but only a small fraction of these toxic chemicals have the potential to cause plant and animal diseases. The study of mycotoxins, known as mycotoxicology, began in 1960 on a farm in England over 100,000 young turkeys died from ‘Turkey-X disease’ after eating a peanut meal that was contaminated with aflatoxins—A then new group of mycotoxins produced by the fungus Aspergillus flavus. In the years since this massive fatality, other important mycotoxins including ergot alkaloids, fumonisins, ochratoxins, trichothecenes, and zearalenone have been discovered and described, many the result of other devastating intoxications.

Ergotism is the oldest identified mycotoxicosis in human. This mycotoxin represents a group of alkaloids that grow on the heads of grasses, such as wheat and rye. Ergot was responsible for a disease of the middle ages known as “St. Anthony’s Fire,” so named for the burning sensation caused in victim’s limbs. The Spartans apparently suffered an ergot epidemic in 430 B.C. and European epidemics date back as far as 857 A.D. (Bove, 1970). Ergotism has also been associated with the Salem witch trials in the 1600s in Massachusetts (Caporael, 1976). More recent outbreaks, associated with economic upheaval and war, have occurred in Russia (1924 and 1944), Ireland (1929), France (1953) and Ethiopia (1978). Although ergot poisoning continues to pose a challenge for the livestock industry, the toxin is less of a challenge for the food industry because current food quality control procedures screen out ergot-infected grains.

Major loss of fresh harvest that renders it to be an impediment for safe consumption can be attributed mainly to 3 factors: biological (storage pests), microbial (bacteria, fungi), and chemical (insecticide, fungicide residues). These 3 factors, singly or in combination, can readily react with the substrate or the raw material leading to the production of off-flavors, discoloration of the product, and reduction in nutritional value. Today, in most of the cases, chemical fumigants or chemical-based protective agents are used for the safe preservation of fresh produce. However, increasing concern and demand by consumers for safe and high-
Quality foods have made it mandatory to look for better alternatives to chemicals. In this regard, it has been a major challenge for the scientific community around the world, as some of the chemical fumigants (like ethylene dioxide, methyl bromide), which are routinely used for postharvest preservation purposes, have been reported to be highly toxic. Some of these chemicals are either banned in developed countries or are likely to be banned in developing countries (by 2015) (Anonymous, 1995; FAO, 2005). Worldwide, it is generally claimed that natural products are safe. However, contamination of human or animal food (or feed) via natural biotoxins produced by microbes might result in outbreaks of several diseases. Among the microbes, fungal toxins assume more importance due to their worldwide distribution. The colonizing fungi are capable of producing toxins, and can cause deleterious health effects in humans or in livestock consuming the contaminated products. Such cases of fungal poisoning may cause death in animals, but are rarely fatal to humans (Pfohl- Leszkowicz, 2000). As there is an increasing concern among consumers regarding food safety, as well as demand for high-quality foods with minimal “bio” or “chemical” contaminants, frequent occurrence of these toxins will definitely have a negative impact on the economy of the affected region/country.

This review provides an overview of economically important mycotoxins that may contaminate livestock feed or human staple foods and threatens the health of humans and domestic animals [Table 1].

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Contaminated products</th>
<th>Animals affected</th>
<th>Clinical effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxins</td>
<td>Corn, peanuts, cottonseed, tree nuts, dairy products</td>
<td>Swine, dogs, cats, cattle, sheep, young birds, humans</td>
<td>Liver damage, intestinal bleeding, cancer</td>
</tr>
<tr>
<td>Ergot alkaloids</td>
<td>Rye, sorghum, pasture grasses</td>
<td>Cattle, sheep, humans</td>
<td>Hallucinations, gangrene, loss of limbs, hastening of birth</td>
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<tr>
<td>Fumonisins</td>
<td>Corn, silage</td>
<td>Horses, swine, humans</td>
<td>Pulmonary edema, leukoencephalomalacia, esophageal cancer, neural tube defects, liver damage, reduced growth</td>
</tr>
<tr>
<td>Ochratoxins</td>
<td>Cereal grains, coffee, grapes</td>
<td>Swine, humans</td>
<td>Kidney and liver damage, cancer</td>
</tr>
<tr>
<td>Trichothecenes</td>
<td>Wheat, barley, oats, corn</td>
<td>Swine, dairy cattle, poultry, horses, humans</td>
<td>Feed refusal, diarrhea, vomiting, skin disorders, reduced growth</td>
</tr>
</tbody>
</table>
Zearalenone | Corn, hay | Swine, dairy cattle | Enlargement of uterus, abortion, malformation of testicles and ovaries
---|---|---|---
Patulin | Fruits, vegetables and silage | Cattle, humans | Affect the functions of gastrointestinal tissue, kidney, liver, and the overall immune system
Citrinin | Fruits, barley, maize, cheese, dietary supplements | Cattle, humans, rabbits, poultry, dogs, and rats and mice | Mycotoxic nephropathy in swine and Balkan endemic nephropathy in humans

**Aflatoxins**

Aflatoxins are highly toxic, mutagenic, teratogenic, and carcinogenic compounds that are produced as secondary metabolites by fungi belonging to several *Aspergillus* species, mainly *A. flavus* and *A. parasiticus* (Romagnoli et al., 2007; O’Riordan and Wilkinson, 2008). Aflatoxins have a high presence in tropical and subtropical regions where humidity and temperature conditions are optimal for toxin production. The name aflatoxin has been derived from the combination of “a” for the *Aspergillus* genus and “fla” for the species *flavus*, and toxin meaning poison (Ellis et al., 1991). Discovery of aflatoxins dates back to the 1960s following the severe outbreak of turkey “X” disease (in the other farm animals. The cause was attributed to feed (Brazilian peanuts) contaminated with *A. flavus*. Aflatoxins are encountered in a wide range of important agricultural commodities, including cereals (maize, sorghum, pearl millet, rice, wheat), spices (chillies, black pepper, coriander, turmeric, ginger), oilseeds (groundnut, soybean, sunflower, cottonseed), tree nuts (almond, pistachio, walnut, coconut), milk (human and animal), and butter. Until now, nearly 18 different types of aflatoxins have been identified wherein the major ones include aflatoxin B1, B2, G1, G2, and M1. Fungal species belonging to *Aspergillus flavus* typically produce AFB1 and AFB2, whereas *A. parasiticus* produces AFG1 and AFG2 as well as AFB1 and AFB2. The 4 major aflatoxins (aflatoxin B1, B2, G1, and G2) are based on their fluorescence under blue or green light and their relative mobility during separation by thin-layer chromatography (TLC) (Stroka and Anklam, 2002; Bennett and Klich, 2003). Four other types of aflatoxins, M1, M2, B2A, G2A, that are produced in minor amounts, have been isolated from cultures of *A. flavus* and *A. parasiticus*. A number of closely related compounds, aflatoxin GM1, parasiticol, and aflatoxicol are produced by *A. flavus*. Aflatoxin-producing fungi show wide variations in their growth requirements. For example, the minimum temperature range for growth of *A. parasiticus* is 6 to 8 °C and maximum is 44 to 66 °C, optimum being 25 to 35 °C (Diener et al., 1982), while *A. flavus* can produce toxin between 12 and 42 °C and its optimum is 28 to 30 °C (Brackett, 1989). Presently, it is estimated that human consumption of aflatoxins ranges between 0 and 30,000 ng/kg/d with an average intake
of 10 to 200 ng/kg/d (Revankar, 2003). The maximum acceptable levels of AFB1 in cereals, peanuts, and dried fruits, either for direct human consumption or as an ingredient in foods, has been set by the European Committee Regulations (ECR) as 4 ppb for total aflatoxins (AFB1, AFG1, AFB2, and AFG2) and 2 ppb for AFB1 alone. Out of the nearly 18 different types of aflatoxins identified to date, the Intl. Agency for Research on Cancer (IARC) has classified 4 aflatoxins (AFB1, AFG1, AFB2, and AFG2) as Group 1 carcinogens (Chiavaro et al., 2001).

Factors favoring aflatoxin production

Fungal growth and aflatoxin contamination are the consequence of interactions among the fungus, the host and the environment. The appropriate combination of these factors determines the infestation and colonization of the substrate, and the type and amount of aflatoxin produced. However, a suitable substrate is required for fungal growth and subsequent toxin production, although the precise factor(s) that initiates toxin formation is not well understood. Water stress, high-temperature stress, and insect damage of the host plant are major determining factors in mold infestation and toxin production. Similarly, specific crop growth stages, poor fertility, high crop densities, and weed competition have been associated with increased mold growth and toxin production. Aflatoxin formation is also affected by associated growth of other molds or microbes. For example, preharvest aflatoxin contamination of peanuts and corn is favored by high temperatures, prolonged drought conditions, and high insect activity; while postharvest production of aflatoxins on corn and peanuts is favored by warm temperatures and high humidity.

Health risks associated with aflatoxin consumption

Aflatoxin poisoning (aflatoxicosis). Consumption of foods/feeds contaminated with high levels of aflatoxins may lead to acute aflatoxicosis and regular intake, even at low levels (ppb), is reported to be responsible for stunting and loss of weight among children, and in some cases has led to the development of hepatocellular cancer (Bhat and Vasanthi, 2003; Hall and Wild, 2003). Aflatoxins have also been linked with kwashiorkor, a protein-energy malnutrition disease (Adhikari et al., 1994). Reports are available wherein AFB1 and aflatoxicol (a metabolic product of AFB1) were detected more frequently in the serum, liver, urine, and stools of children suffering from kwashiorkor (De Vries et al., 1990).

The role of aflatoxins in the development of Reye’s syndrome (encephalopathy with severe lesions in kidney and liver following influenza or varicella) has never been proved, regardless of the frequent detection of aflatoxins in the liver of children who have died of this disease. Egal et al., (2005) have reported that 90% of children in West Africa (Benin and Togo) are exposed to aflatoxins due to consumption of contaminated maize and groundnuts, which leads to a measurable impairment of child growth. Severe liver lesions in malnourished adults during the 1970s, with fatal outcome have been reported after severe cases of acute aflatoxicosis in parts of Asia and Africa (Krishnamachari et al., 1975; Bhat and Krishnamachari, 1977). Aflatoxins are perceived to be co-factors in the higher
incidence of liver cancer (hepatocellular carcinoma) along with hepatitis-B virus in tropical Africa (FAO, 1997). Hepatitis-B virus (HBV) interferes with the ability of hepatocytes to metabolize aflatoxins, and an aflatoxin M1-DNA conjugate exists for a longer time in the liver, increasing the probability of damage to tumor suppressor genes. This effect is synergistic with the resulting damage far greater than just the sum of aflatoxins or HBV individually (Williams et al., 2004).

The FDA tolerance level for aflatoxin in human food is 20 μg/kg; for breeding livestock feed 100 μg/kg; breeding cattle feed 20 μg/kg; and poultry feed 300 μg/kg (Park and Liang, 1993). According to the FAO/WHO expert committee recommendations (1990) the tolerance limit for AFB1 is 5 μg/kg food products, for AFM1 it is 0.05 μg/kg milk products, and for B1+G1+B2+G2 it is 15 μg/kg, as for example in raw peanuts.

**Ergot alkaloids**

Ergot and ergotism are most closely associated with rye (Matossian 1989) compiled records of Neolithic, Bronze Age, and Iron Age sites from which C. purpurea sclerotia were recovered in excavations in Poland, Scotland, Germany, Sweden, and Denmark. The fungus was found in association with rye, wheat, barley, and wild grasses

**Associated health risks of ergot toxins:**
The Claviceps purpurea toxin is of not much significance today and human ergotism is extremely rare, which might be attributed to 2 reasons: primarily, due to the recent improvements in the cleaning and milling processes that are able to remove most of the ergots leaving very low levels of the alkaloids in the flour, and, second, these alkaloids might be relatively unstable and can be destroyed easily by conventional processing (baking, cooking, milling). However, it is necessary to cover a few aspects on C. purpurea toxins. Earlier reports are available on ergot poisoning of domestic animals by ingestion of feeds containing Claviceps purpurea sclerotia (Groger, 1972). Ergot alkaloids have been reported in sleepy grass (Stipa robusta) which is common in the South Western parts of the U.S.

The most common intoxications associated with ergot alkaloids is “fescue toxicosis” wherein the “tall fescue” (Festuca arundinacea) pasture grass common to the U.S. was infected by Claviceps spp. that produced ergovaline (an alkaloid), which proved to be toxic to animals (Botha et al., 2004). These ergot alkaloids have also been reported in pasture grasses of Northern Europe (Fink- Gremmels, 2005).

Toxicity symptoms of Claviceps toxins include delirium, prostration, violent head pain, abscesses, and gangrene of the extremities. The toxin most likely acts as a vasoconstrictor. Some of the secondary metabolites of fungi that were used as antibiotics in earlier years are now considered toxins (Peraica et al., 1999). However, with regard to ergot alkaloids, they are still being used in the treatment of Parkinson’s disease, as prolactin inhibitors, in cerebrovascular insufficiency, and in migraine treatments. Ergotamine, a major alkaloid involved, possesses greater biological activity than the other components of ergot and is used in human
medicine (mainly as a vasoconstrictor and an oxytoxic).

**Fumonisins**

Fumonisins are toxins produced by *Fusarium* species that grow on several agricultural commodities, mainly corn, in the field or during storage. The disease, *Fusarium* kernel rot of corn, is caused by *Fusarium verticillioides* and *F. proliferatum*, common producers of fumonisin. More than ten chemical forms of fumonisins have been isolated, of which FB$_1$ is the most prevalent in contaminated corn and is believed to be the most toxic.

**Crops and Weather Conditions**

Levels of fumonisins in corn are influenced by environmental factors such as temperature, humidity and rainfall during pre-harvest and harvest periods. High levels of fumonisins are associated with hot and dry weather, followed by periods of high humidity, and may also occur in corn that has been damaged by insects and birds. Improper storage conditions, such as moisture above 18%, will lead to increase fumonisin levels.

Reports are available on the presence of fumonisins in several agricultural products like corn, cornflour, dried milled maize fractions, dried figs, herbal tea, medicinal plants, bovine milk, and others (Omurtag and Yazicioglu, 2004; Gazzotti et al., 2009; Karbancioglu-Guler and Heperkan, 2009; Pietri et al., 2009; Seo et al., 2009), indicating high risks to public health. It has been estimated that consumption of fumonisin B1 (FB1) by humans in the U.S. is about 80 ng/kg/d (WHO, 2002). Occurrence of fusarial toxins in ensiled grass or hay, originating mainly from pre-harvest contamination, has been reported by Baath *et al.*, (1990). Associated health risks of fumonisins. Consumption of fumonisin-contaminated foods by humans has been correlated with increased incidence of esophageal cancer in various parts of South Africa, Central America, Asia, and among the black population in Charleston, South Carolina (Sydenham *et al.*, 1994). Similar observations have been reported from China (Abnet *et al.*, 2001), Italy (Franceschi *et al.*, 1990), and Brazil (Van der Westhuizen *et al.*, 2003). This toxin has also been reported to be immunosuppressive (WHO, 2002). The IARC (International Agency for Research on Cancer 1993c) has classified fumonisins under group 2B (possibly carcinogenic to humans). Among the various types, FB1 is known as a cancer promoter and plays an important role in carcinogenesis in humans (Chu and Li, 1994). Fumonisin consumption has also been related to neural tube defects in human babies as they (especially FB1) reduce the uptake of folate in different cell lines (Marasas *et al.*, 2004).

**Ochratoxin**

Ochratoxin is present in a large variety of foods because it is produced by several fungal strains of the *Penicillium* and *Aspergillus* species that have varied physiologies and ecologies. The presence of chlorine in its structure makes it unique. Ochratoxin is considered to be nephrotoxic, teratogenic, and immunotoxic, and has been classified by the IARC as a Class 2B carcinogen, probable human carcinogen. Ochratoxin A, the main toxin in this group, is found in wheat, corn, and oats having fungal
infection and in cheese and meat products of animals consuming ochratoxin-contaminated grains (Aish et al., 2004). *A. ochraceus* is found on dry foods such as dried and smoked fish, soybeans, garbanzo beans, nuts, and dried fruit. *A. carbonarius* is the major pathogen in grapes and grape product including raisins, wines, and wine vinegars. Although reported to occur in foods around the world, the main regions of concern are Europe and, for some foods, Africa. The Joint Expert Committee on Food Additives of the Food and Agriculture Organization of the United Nations and the World Health Organization (JECFA, 2000) presented data indicating that cereals, wine, grape juice, coffee, and pork are the major sources of human ochratoxin exposure, at levels of 58%, 21%, 7%, 5%, and 3% of total ochratoxin intake, respectively. Levels reported range from 100 to 700 ng/kg in cereals, 30 to 9000 ng/L in European wines, 170 to 1300 ng/kg in coffee, and 150 to 2900 ng/kg in pork (Sage et al., 2004). Ochratoxin presence in European wines is a relatively recent concern, with red wines typically containing higher ochratoxin levels than rose or white wines. Because of the large variety of food matrices in which ochratoxin has been found, there is no universally suitable method of analysis. Differences in extraction conditions and clean-up are as varied as the foods. Analysis is typically accomplished by high performance liquid chromatography, although liquid chromatography-mass spectrometry-is an important secondary confirmation technique.

The impact of processing on ochratoxin A-contaminated foods is not very well understood. Polishing and milling of wheat (to remove outer layers for white flour production) lowered ochratoxin levels; however, no effect was seen for whole-wheat flours (Osborne et al., 1996). Wet milling of corn resulted in reductions of ochratoxin levels in germ and grits of 96% and 49%, respectively.

**Trichothecenes**

Trichothecenes are produced by *Fusarium* species. Trichothecenes are also known to be produced by other fungal genera like *Trichoderma*, *Trichotecium*, *Myrothecium* and *Stachybotrys* (IPCS, 1990). Trichothecenes are sesquiterpenoid mycotoxins that accumulate in kernels of infected spikelets rendering the grain unsuitable for human or animal consumption. Trichothecenes are usually found to be contaminants of cereals and their derivatives (Foroud and Eudes, 2009). Nearly 160 trichothecenes have been identified and are classified into 4 groups depending on their chemical structure. The major ones are T-2 and HT-2 toxins (group A) and nivalenol (NIV) (group B).

**Associated health risks of trichothecenes:**

Trichothecene mycotoxicosis (scabby grain toxicosis) has been reported to occur within hours after ingestion of contaminated foods (wheat, corn, rice). The main symptoms of trichothecene mycotoxicosis are abdominal pain, nausea, vomiting, diarrhea, dizziness, and headache.

Trichothecenes have strong impacts on the health of animals and humans due to their immunosuppressive effects. Group- A trichothecenes are of major concern as they are more toxic than the type B trichothecenes. In animals, these mycotoxins
are held responsible for reduced feed uptake, vomiting, and immuno-suppression. In instances of chronic poisoning, Group-A trichothecenes produce significant changes in the blood cell count and in immune function.

Among the group A, T-2 toxin is the most important one. It is readily metabolized by the gut microflora of mammals into a number of other metabolites. HT-2 toxin is a primary metabolite in the gut and is absorbed into the blood after ingestion of T-2 toxin. Metabolism continues in the liver along with biliary excretion, resulting in a substantial combined first-pass effect in the gut and liver (WHO, 2002). The principal effects of perturbed protein synthesis from T-2 toxin are usually observed in the immune system and include changes in leukocyte counts, delayed hypersensitivity, depletion of selective blood cell progenitors, and depressed antibody formation (WHO, 2002). Compounds of the other group of trichothecenes (Group B) generally cause a reduction in dietary consumption, especially in pigs.

**Zearalenone**

Amycoestrogen, zearalenone has attracted recent attention due to concerns that environmental estrogens have the potential to disrupt sex steroid hormone functions. Occasional outbreaks of zearalenone mycotoxicosis in livestock are known to cause infertility. Alternatively, derivatives of zearalenone are used in some livestock feeds for growth promotion (for example, Ralgro in beef cattle), as alternatives to the more potent and controversial synthetic estrogen, diethylstilbestrol. This toxin is found almost entirely in grains and in highly variable amounts ranging from a few nanograms per gram to thousands of nanograms per gram. The appearance of mold on grain plants cannot be relied upon to warn of toxin production because *Fusarium* infected grain does not necessarily appear visibly moldy in the presence of high concentrations of mycotoxins (Murphy et al., 1996). The average human intake of zearalenone was estimated to be approximately 0.02 μg/kg bw/d on the basis of limited data obtained in Canada, the United States, and Scandinavian countries, but it is likely that intakes are greater in countries from the regions of the world having less well-controlled grain storage systems. Genotoxicity is a reported concern with respect to zearalenone. Although this estrogenic compound showed no mutagenicity in Ames tests (1 to 500 μg zearalenone/agar plate), the substance induced chromosomal anomalies in some lymphocyte, oocyte, and kidney cell cultures when present within a range of 0.1 to 20 μM (Stopper et al., 2005). This dose range is difficult to extrapolate to likely human exposures because no human bioavailability estimates are available. With estimated human intake of approximately 1 to 2 μg per person, however, occurrence of blood or tissue concentrations remotely close to 0.1M (approximately 30 μg/L) seems extremely unlikely.

**Patulin**

Generally, fruits and vegetables are easily contaminated by toxigenic molds leading to quality deterioration (Moss, 2008). Agronomic practices employed during fruit cultivation and juice making have been reported to significantly influence the occurrence and production of patulin and
Citrinin. Patulin (molecular weight: 145.1) is a mycotoxin that forms the smallest group of toxic metabolites referred to as polyketides, and is reported to be produced by fungi belonging to *Aspergillus* spp., *Penicillium expansum*, and *Paecilomyces* spp. (*Byssochlamys nivea*, *B. fulva*) (Moss 2008; Chun *et al.*, 2009). Patulin is being considered as a “possible toxin” in Europe and New Zealand and is regarded as the most dangerous mycotoxin in fruits, particularly apples, pears, and their products (Murillo-Arbizu *et al.*, 2009). Patulin is mainly associated with surface-injured fruits, which renders them vulnerable to fungal infection, mainly by *Penicillium* spp. (Sewram *et al.*, 2000).

**Associated health risks of Patulin:** Patulin toxin is reported to affect the functions of gastrointestinal tissue, kidney, liver, and the overall immune system (Wichmann *et al.*, 2002).

This toxin is regarded to be genotoxic, carcinogenic, can induce oxidative stress response in mammalian cells, generate reactive oxygen species (ROS), and induce apoptosis in human leukemia cells (HL-60) (Wu *et al.*, 2008). However, the IARC has classified patulin as category 3; not classifiable as to its carcinogenicity in humans (IARC, 1993b). The permissible limit for patulin in apples and their products in the U.S. and EU has been set at 50 ppb. A permissible limit of patulin content in apple juice, and as juice ingredients in other beverages, has been set at 50 μg/kg, in solid apple products at 25 μg/kg, and in baby food of 10 μg/kg (Mycotoxin Certification Standard 2008, www.mycotoxin-certification.eu).

**Citrinin**

Citrinin (molecular weight: 250.25) is the secondary metabolite produced by *Penicillium expansum* and some of the *Aspergillus* and *Monascus* spp. (Kim *et al.*, 2007; Abramson *et al.*, 2009). Citrinin often occurs as a common contaminant of food and feed (fruits, barley, maize, cheese, dietary supplements) (Meister, 2004). Barley, as well as other cereals employed for producing beer, has been reported to be a good substrate for the growth of many toxigenic fungi capable of producing citrinin (Galvano *et al.*, 2005).

**Associated health risks of citrinin:** In humans, reported health risks due to citrinin are scarce. Some reports do indicate citrinin’s association with mycotoxic nephropathy in swine and Balkan endemic nephropathy in humans. However, details available on the toxic effects of citrinin in animals show its nephrotoxic nature as well as teratogenic effects in rabbits, poultry, dogs, and rats and mice along with induction of apoptosis (Chan, 2007; Kumar *et al.*, 2007; Singh *et al.*, 2008).

**Alternaria toxins**

Mycotoxins produced by fungi belonging to *Alternaria* species are referred to as *Alternaria* toxins. *Alternaria* species commonly occur during the pre- and postharvest stages of fruits and vegetables. These fungi are capable of producing a range of mycotoxins and other less toxic metabolites. The most important toxin producing species is *Alternaria alternate*, which usually contaminates cereals, sunflower seeds, rapeseed, olives, and fruits. Among the various *Alternaria* toxins,
alternariol (AOH) and alternariol monomethyl ether (AME) are reported to be the most toxic. The toxins AOH and AME have been detected in sorghum, sunflower seeds (Chulze et al., 1995), barley, wheat, oats (Azcarate et al., 2008), olives, tomatoes, mandarin oranges, peppers, and melons (Logrieco et al., 1988). Also, apart from AOH and AME, other naturally occurring Alternaria toxins include tenuazonic acid, altenuene, and altertoxin.

The significance of tenuazonic acid in fresh tomatoes used for the production of tomato sauce has been detailed by Mislivic et al., (1987). The other fungal species producing these toxins include A. alternata, A. dauci, A. cucumerina, A. solani, and A. tenuissima.

**Associated health risks of Alternaria toxins:** Alternaria toxins have been implicated in humans and animal health disorders. AME is reported to be cytotoxic and along with AOH has been shown to possess synergistic effects. AOH is lethal to unborn mice at levels of 100 mg/kg body weight (Pero et al., 1973). Presently, no limits are set for Alternaria mycotoxins as various Surveys conducted have shown their natural occurrence in foods to be very low and the prospects for direct human exposure are limited.

**Preventive Practices**

Prevention is the best method to control mold growth and possible toxin formation. The following practices can help minimize mold growth and subsequent toxin production in storage.

**Pre harvest**

Clean inside and outside of grain bins and dryers. Prior to storage, check the condition of the bin for possible water leaks, and clean it properly by removing dust, dirt, leftover grain and other foreign material. Crop rotation in many regions or tillage can reduce the risk of Gibberella ear rot in corn and Fusarium head blight of wheat. These practices have little effect on other corn ear rots. Some corn hybrids are more resistant to ear rots than others, but overall, resistance to ear rots is not widely available. Some BT hybrids, those that produce BT in the kernels, have less ear rot due to insect control resulting in less toxin problems. Control of second generation European corn borers and other insect pests of corn ears can greatly reduce infection by Fusarium and Aspergillus.

Few wheat varieties have high levels of resistance to Fusarium head blight (scab). Plant moderately resistant varieties whenever it is available. Planting several varieties that differ in maturity will reduce the risk of disease to the whole crop. As with any crop pest, early detection through scouting and early harvest can reduce serious losses and avoid crises. Decisions on handling moldy grain should be made before it is harvested. After harvest, spoilage can occur quickly if delays result from indecision.

If extensive ear rot development is observed (10% or more of the ears with more than 10-20% mold), the field should be harvested as soon as moisture content reaches a level that can be harvested. Even if some drying costs are incurred, this will be
less expensive than loss of crop value due to mycotoxins and resulting feeding problems.

**Postharvest**

The crops should be allowed to mature in the field to the following moisture contents: shelled corn, 23-25%; ear corn, 20-25%; small grain, 12-17%; and soybeans, 11-15%.

Harvesting equipment should be adjusted to minimize damage to seeds or kernels and allow for maximum cleaning. Cracked or broken seeds or kernels are more susceptible to mold invasion.

Upon storage, dry the grain to 13-14%, if possible, within 48 hours. Long-term storage can be achieved at uniform moisture of 18% for ear corn; 13% for sorghum, wheat and shelled corn; and 11% for soybeans. After drying, store under cool temperatures (36-44° F). Every few weeks check the condition of the grain for temperature, wet spots and insects.

**Grain treatments**

Antifungal treatments can be applied to grain to reduce mold growth in storage. These products, such as propionic acid, do not kill the mold already present nor do they reduce toxins already present in the grain. Do not use antifungal agents on stored grain unless you are certain the grain can be marketed after treatment.

Hydrated sodium calcium aluminosilicate (HSCAS) can reduce the effects of aflatoxins when fed to swine, cattle, or poultry. HSCAS at 10 lb. /ton provides substantial protection against dietary aflatoxins.

**Detection of mycotoxins**

For qualitative, quantitative, and accurate determinations of mycotoxins in foods and feeds, several analytical methods have been developed and refined since the 1960s. Accurate detection of mycotoxins depends on various factors, as their distribution is not uniform in a substrate.

According to Whittaker *et al.*, (1991), a statistically valid sample must be drawn from a single lot; if not, a sampling error of up to 90% may occur. Also, mycotoxin analysis should always be performed in replicates ($n = 3$ to $5$) for confirmation of the actual concentration in the samples. However, if the method is validated in a proper way, and the validation results are satisfactory, there is no need to run every sample for 3 to 5 times. Care should be taken to finely grind a sample and further divide it into subsamples for analysis.

**Analytical methods to detect mycotoxins:**

Thin-layer chromatography (TLC), liquid chromatography, high-performance liquid chromatography (HPLC) with fluorescence or diode array detector, gas chromatography coupled to mass spectrometry (GC–MS) or electron capture detection (GC–ECD), enzyme-linked immunosorbent assays (ELISAs), and a combination of immuno-affinity column techniques (WHO, 2002). In recent years, liquid chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry (LC-MS/MS) technique has
also been often applied for multiplemycoxin detection, and this approach looks to be the most promising one at the moment. These analytical methods are exclusively employed for the detection of various types of mycotoxins and have been proven successful, especially with aflatoxins, ochratoxin A, fumonisins, and deoxynivalenol (DON) in different commodities, like cereals and legumes (Scudamore et al., 2003; Yumbe-Guevara et al., 2003), coffee (Sibanda et al., 2002; Vatinno et al., 2008), black pepper (Gatt et al., 2003), wine and beer (Leitner et al., 2002; Stefanaki et al., 2003), and cheese (Zambonin et al., 2002; Manetta et al., 2009).

CONCLUSION

Mycotoxins are chemicals that may contaminate staple foods and feed worldwide. Apart from practicing good sanitary measures, awareness has to be created to indicate the toxic effects associated with mycotoxin poisoning in humans and livestock. Mycotoxins are a food safety risk globally. International risk assessments have been performed by JECFA for aflatoxin B1, aflatoxin M1, DON, fumonisins, ochratoxin A, T-2 toxin and HT-2 toxin. Research needs to be focused on the generation of data dealing with epidemiological and toxicity effects, especially in humans. Implementation of strict quarantine rules with regard to mycotoxin contamination has to be made mandatory worldwide. Emphasis should be laid towards development of newer low-cost mycotoxin detection instruments, which are portable, reliable, and easy to handle at field levels. Development of new genetically modified plants by the application of genetic engineering that might be resistant to fungal invasion might also prove to be a good option. Further, developing new protocols and strategies to compare the costs and benefits of various remedial measures against fungal pathogens and mycotoxin production also helps in alleviating the problem.

REFERENCES


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