## Mold contamination of agricultural products

A major problem associated with the utilization of agricultural crops is contamination by toxigenic molds. Molds are ubiquitous organisms that grow almost everywhere. Given suitable temperature and humidity conditions, molds can grow and produce mycotoxins, toxigenic secondary metabolites, on agricultural crops before harvesting, during storage or transport. Mold damage during grain storage ranks second after damage caused by insect pests.

Fungal toxins, one of the most dangerous contaminants of food and feed have been detected in many food commodities world over. Mycotoxins affect cereals such as maize, wheat, oats, barley, rice; seeds, nuts, peanuts, beans, cassava, fodder and feed intended for animal consumption as well as milk and dairy products. However, not all molds are toxigenic and not all secondary metabolites from molds are toxic. Similarly, although all mycotoxins are metabolites of fungi, not all toxic substances synthesized by fungi is termed mycotoxin. Molds belonging to *Aspergillus*, *Penicillium* and *Fusarium* also known as field fungi, are the producers of majority of mycotoxins of significant effect on health of human and animals. Mold contamination in foods does not always correlate with mycotoxin production as molds may be present in commodities without producing toxins.

Mold contamination and subsequent mycotoxin occurrence may become inevitable under certain environmental conditions. For example, countries in Asia and Africa experience high temperature (26‒39°C) and high relative humidity (67‒98%) which support the growth of mycotoxigenic fungi on agricultural crops and consequently the production of mycotoxins on these crops. Fungal growth and the risk of mycotoxin contamination in agricultural products is further encouraged by poor harvesting practices, improper drying, handling, packaging, storage, and transport conditions. Contamination may be directly when the food or feed become infected by a toxigenic fungus and formation of mycotoxin occurs, or indirectly when a previously contaminated food or product has been processed to eliminate the fungus but the mycotoxin remains in the final product.

Survival and growth of spoilage, pathogenic microorganisms on foods and food ingredients are affected by storage temperature and relative humidity. However, the requirements for toxin synthesis and optimal growth differ among fungi, even within the same genus. Fungi grow between 10 and 40°C, over a pH range of 4‒8, and at a water activity level (aw) above 0.70.

**Mycotoxins in food and feed**

Mycotoxin is derived from two words, a Greek word *mukes* meaning fungi and a Latin word *toxicum* referring to poison. They are large molecules, heat stable and not significantly volatile; and are carried over into processed foods due to their stability. Their structures vary from simple heterocyclic rings to groups with molecular weights ranging from 50 to 500 Da.

Contamination by mycotoxin-producing fungi result in wastage of massive amount of foods annually. The Food and Agricultural Organization of the United Nations has estimated that 25% of the world’s food crops are contaminated by molds and become affected by mycotoxins, translating to loss of billions of dollars annually. Mycotoxin contamination results in loss to crop producers and sellers who will be required to give market discounts for the contaminated products or disposal of such products in cases of severe contamination. It can also lead to loss of business, product recall and thereby adversely affecting international trade . About 4.5 billion persons in developing countries are exposed to unchecked amounts of mycotoxins yearly. The ingestion in high quantities or over a long period of time of mold-contaminated foods may become harmful.

Mycotoxins can be inhaled, ingested or absorbed through the skin. Mycotoxicosis is a disease condition resulting from ingestion of food contaminated with mycotoxins and it has been linked to many human diseases. It may have chronic effects leading to cancer, immune suppression, and other ‘slow’ pathological conditions. Acute mycotoxicosis results in death. Aflatoxins produce clinical toxicosis, reduce resistance to diseases and interfere with vaccine induced immunity in poultry birds. Feeding livestock and poultry with aflatoxin contaminated feed can result in death, immune suppression and growth reduction. The type and dose of mycotoxin, health condition, extent of exposure, gender and age of the individual all determine the severity of mycotoxicosis. Figure 1 shows the chemical structures of mycotoxins that are common in foods.



Figure 1 **Commonly occurring mycotoxins in food. AF (Aflatoxin) B1, B2, M1, G1, G2; OT (Ochratoxin) A, B; DON Deoxynivalenol; NIV Nivalenol; T2 T-2 toxin (Insariotoxin); FB1 Fumonisin B1; ZON Zearalenone; PAT Patulin.**

Mycotoxin production is influenced by fungal species potential, relative humidity, type of substrate, water activity, use of fungicides, duration of fungal growth, aeration, temperature, and storage conditions. Toxin production is further enhanced by stress factors which include shortage of water, insect infestation and attacks by other pests.

## Mycotoxins of significance to human and animal health

Several previous studies reported on the existence of mycotoxins in food systems. Researchers agree that there are about 400 confirmed existing mycotoxins. Research underway also confirm that there are other various emerging toxins whose toxicokinetics are still largely unexplored.

Few of the mycotoxins discovered are a public health concern due to high prevalence, teratogenic, carcinogenic, mutagenic, and immune suppression effects. Mycotoxins of concern are aflatoxins (AFs), ochratoxin A (OTA), trichothecenes (deoxynivalenol (DON) and T-2 toxin), zearalenone (ZEN), fumonisins (FMN) and patulin (PAT). This is due to their studied economic impact, frequency of occurrence in food systems and severity on humans and animals. These mycotoxins as well as ergot alkaloids pose threat to food safety; and are produced by fungi belonging to *Aspergillus*, *Penicillium*, *Fusarium* and *Claviceps*.

Since mycotoxins are heat stable and chemically stable, various regulatory bodies have set maximum tolerable limits for their presence in foods and feed. Table 1 presents an overview of the worldwide legislation on mycotoxins.

**Table 1 Overview of the worldwide legislation on mycotoxins**

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| --- | --- | --- | --- |
| Mycotoxin  | Commodity | Country | Maximum tolerable levels\* (µg/kg**)**  |
| AFs  | Oil seeds, nuts, dried fruits, cereals, spices  | EUAustralia, Canada, Nigeria, New Zeland, South Africa, USA, Brazil India  | 4–15\* (2–12\* for AFB1)(15 for AFB1)2030 |
| AFM1 | Milk and infant formula  | EU,Turkey,SouthAfrica, Argentina, China, India, Kenya, Mexico, Uruguay, USA Brazil | 0.25–0.05\*0.50.5–5\* |
| DON  | Cereals, bakery products  | EUBrazilRussia Canada, China, India, Japan, USAa  | 500–1750\*750–3000\*700–10001000 |
| FMs | Maize | EU, Turkey, NorwaySwitzerland USA Brazil | 800–4000\*2000–4000\*2000–5000\* |
| OTA | Cereals, dried fruits, coffee, cocoa, wine, beer, grape juice, spices, liquor, rice, blood products  | EU, Egypt China, Kenya, Nigeria, Russia India Brazil Uruguay  | 2–10\*5202–30\*50  |
| Patulin  | Fruit juice, apple products  | Brazil, China, EU, India, Japan, Kenya, Nigeria, Russia, South Africa, USA  | 50 |
| T-2 and HT-2  | Cereals  | EU Russia  | Not permitted 50-100\* |
| ZON | Cereals, bakery products, maize oil  | EU Brazil China, Russia, Chile  | 75–400\*200–1000\*200,000 |

\*Depends on the commodity (lowest-highest MTL)
a Advisory level

### Aflatoxin

Toxigenic strains of *Aspergillus* species produce cancerous secondary metabolites in agricultural foodstuffs known as aflatoxins. They are very stable and can withstand conventional food processing. Aflatoxins have been isolated in various commodities including barley, wheat, sorghum pear millet, oilseeds, tree nuts, milk, and butter. Aflatoxins have been linked with carcinogenicity, mutagenicity, teratogenicity, and immune suppression in animals and humans. Previous research show that they are the most hazardous of all mycotoxins, causing damage to the liver. Aflatoxins have also been linked with aggravation of kwashiorkor and impaired childhood growth. They can depress cell-mediated immunity and cause stunting in children. Evidence from studies indicates that aflatoxins increase the rate of progression from HIV infection to AIDS. Consumption of food heavily contaminated with aflatoxin (> 20 ppb) has been linked to death.

Eighteen different aflatoxins have been identified, of which six are of concern in agricultural foodstuff and they include AFB1, AFB2, AFG1, AFG2, AM1and AM2. Aflatoxin B1 (AFB1), AFG1, AFB2, and AFG2, in order of decreasing toxicity, occur naturally. The International Agency for Research on Cancer (IARC) has classified these aflatoxins (AFB1, AFG1, AFB2, AFG2) as Group 1 carcinogens. Aflatoxins M1 and M2 are hydroxylated products of the B1 and B2 aflatoxins respectively and they are found in milk, dairy products or meat.

Aﬂatoxin B1 is the most potent naturally formed carcinogen and it is regarded as the strongest hepatocarcinogenic agent known. It is also an immune suppressor, inflammation promoter and growth suppressor in animals and humans. About 75% contamination in food and feed can be traced to aflatoxin B1 contamination. It is themost common and wide-spread of the identified aflatoxins. AFB1 is so far the only mycotoxin regulated by EU with a limit of 5 µg/kg for finish feed for dairy animals to 20 µg/kg for raw materials and other finish feed. The toxicological effects are dependent on dose; at high doses, they are lethal and cause liver, myocardial and kidney tissue damage. At sub-lethal doses chronic toxicity such as liver cirrhosis occurs. The maximum acceptable levels of AFB1 in cereals, peanuts, and dried fruits, either for direct human consumption or as an ingredient in foods is 4 ppb for total aflatoxins (AFB1, AFG1, AFB2, AFG2) and 2 ppb for AFB1 alone.

When livestock consume mycotoxin-contaminated feed, there is a carry-over effect of mycotoxin residues into the animal (cow) milk, meat, or eggs and can be consumed by humans. Although rumen flora act as defence against mycotoxin by metabolizing some of these toxins to less toxic compounds, the rumen barrier can be altered by diseases in the animal, a change in the diet or a very high concentration of mycotoxin in the feed. This is of great concern as contaminated milk can be harmful to humans and especially to children who are more susceptible to action of toxic compounds.

#### Fungal species associated with aflatoxin production

Recent reports have suggested the production of aflatoxins by thirteen species belonging to three sections of the genus *Aspergillus* section *Flavi* (*A. arachidicola*, *A. bombycis*, *A. flavus*, *A. parvisclerotigenus*, *A. pseudotamari*, *A. minisclerotigenes*, *A. nomius* and *A. parasiticus*), *Nidulantes* (*Emericella astellata*, *E. venezuelensis* and *E. olivicola*) and *Ochraceorosei* (*A*. *ochraceoroseus* and *A. rambelli*). In dry conditions and elevated temperature, aflatoxins remain stable but can be metabolized into toxic derivatives such as epoxide, aflatoxin M1 or M2 in human and animals, and by microorganisms to less toxic derivatives such as B2a.

*Aspergillus flavus* is found in plants, animals, and insects. Optimum temperatures for growth of *A. flavus* and aflatoxin production are 33°C and 16‒31°C respectively; and optimum water activity for growth and aflatoxin production are 0.98 aw and 0.95‒0.99 aw respectively. *A. flavus* normally produce aflatoxin B derivatives while *A. parasiticus* produces both aflatoxins B and G derivatives. Both B and G aflatoxin derivatives are produced by other species that include *A. toxicarius*, *A. nomius, A.* *bombycis, A. parvisclerotigenus, A. minisclerotigenes,* and *A. arachidicola*.

#### Aflatoxin contamination in foods

. Aflatoxin discovery dates to the 1960s where more than 100, 000 turkeys and other farm animals died from the consumption of Brazilian peanut contaminated with *Aspergillus flavus* in the UK known as the Turkey ‘X’ disease outbreak. In Gujurat and Rajasthan, India in 1974, 106 deaths arising from 397 cases of hepatitis caused by consumption of maize heavily contaminated with *A. flavus* and aflatoxin concentration between 6.25 and 15.6 µg/g was reported. In Eastern Kenya, between 2004 and 2011, 40% deaths resulted from 477 cases of acute aflatoxicosis reported . Among the 317 people who became ill from consumption of maize contaminated with aflatoxin, 125 deaths occurred. In 2010, an outbreak of aflatoxicosis also due to contaminated maize killed at least one child. A study demonstrated a striking relationship between exposure of children to aflatoxin, with both stunting of growth and malnutrition. Blood samples of 480 children (aged between nine months and five years) from Benin and Togo were analysed to determine dietary exposure to aflatoxin. Aflatoxin-albumin adducts were detected in 99% of the samples. Children with stunted growth or low body weight had 30–40% higher mean aflatoxin-albumin concentrations.

### Ochratoxin A

Ochratoxins are a group of mycotoxins produced as secondary metabolites by *A. niger*, *A. ochraceus*, *A. carbonarius*, *Penicillium verrucosum*, *Neopetromyces spp*., and *Petromyces spp*. Significance in this group of mycotoxins are OTA, OTB, OTC, methyl ester of OTA and ethyl ester of OTB. Ochratoxin A (OTA) is the most common pollutant among these and commonly found in feed and feed ingredients; it is resistant to heat and affects protein, DNA and RNA synthesis in the body. Ochratoxin A, a potent nephrotoxin, is produced at optimum temperature and water activity of 0.98 *a*w and 25 to 30°C. It contaminates various commodities such as corn, coffee, wine, dried fruits, cocoa, beans, spices, cereal grains and rice. It has been found in oats, barley, wheat and other products consumed by human and animals.

Ochratoxin A is also teratogenic and immunotoxic, and has been classified as a probable human carcinogen. European Union limits for OTA differ according to food type. Limits of 5 µg/kg in unprocessed cereals and 3 µg/kg in cereal-based processed foods; 10 µg/kg in coffee, and raisins and maximum of 15 µg/kg in spices.

### Fumonisin

Fumonisins are recently isolated mycotoxins that are nephrotoxic and hepatotoxic, known to possess high cancer-inducing properties. Fumonisins cause neural tube defects in experimental animal species and consumption of fumonisin-contaminated corn grains by humans has been reportedly linked with oesophageal cancer in Transkei region of southern Africa, in China and in north-eastern Italy. This toxin has also been reported to be immunosuppressive and has been classified under group 2B (possibly carcinogenic to humans) according to the International Agency for Research on Cancer. Fumonisins have been found to contaminate beer, rice, corn, figs amongst others. *Fusarium* species are plant pathogens that produce fumonisins. *F. proliferatum*, *F. verticillioides* (previously classified as *F. moniliforme*)are the primary producers of fumonisins; as well as *F. nygamai*. Other producers are *F. polyphialidicum*, *F. anthophilum*, *F. dlamini*, *F. napiforme*, *F. subglutinans* and *F. oxysporum*. *A. niger* also produce fumonisins, especially FB2.

**Zearalenone**

*Fusarium* species, especially *F. graminearum* and *F. culmorum* produce zearalenone (ZEN) as a secondary metabolite. Zearalenone has worldwide contaminationrecord for various cereals and animal feed. It is hepatotoxic, hematotoxic, genotoxic and immunotoxic. Also referred to as mycoestrogen, it has ability to bind to estrogen receptors and significantly affect the reproductive system and may cause ovarian dysfunction, increase in abnormal spermatozoa, infertility, and other severe reproductive disorders. It is linked to early onset of puberty in young children and is a potential stimulator of human breast tumorigenesis. In swine, zearalenone may lead to vaginal and/or rectal prolapse, vulvovaginitis, swelling of vulva and mammary glands, disrupted conception, abortion and infertility.

###  Deoxynivalenol

Deoxynivalenol (DON) is one of the most frequently detected tricothecenes contaminants in grains, more common in seeds of safflower, rye, barley and wheat. It is produced majorly by *F. graminearum* and inhibits the synthesis of RNA, DNA, and protein. In experimental animals, it causes genotoxicity, cytotoxicity, teratogenicity, and induction of foetal skeletal deformities. It is also associated with diarrhoea, nausea, headache, dizziness, gastroenteritis, and ataxia in animals and humans. Vomiting arise from consumption of DON-contaminated food but may become lethal at exceedingly higher concentrations.

Ingestion of DON-contaminated foods by animals at higher doses causes vomiting, diarrhoea and feed refusal leading to severe weight loss, damage to the hematopoietic system and immune dysregulation. At small doses, in pigs and other animals, its ingestion cause nausea, gastrointestinal tract lesions, decreased nutritional efficiency and weight loss, and the refusal to eat. Hence it is known as vomitoxin or feed refusal factor. Deoxynivalenol is very heat stable, although its concentration can be reduced by boiling in water.

### Patulin

Patulin, otherwise known as clavacin, claviformin, expansin, micoine C and penicidin, was first isolated as an antimicrobial compound from *P. patulum* now known as *P. griseofulvum*. It is also produced by *A. clavatus*, *A. giganteus* and *A. terreus*. Initially used as a nose and throat spray to treat common cold and ointment to treat skin infections, its toxicity to animals and plants was discovered during the 1960s which led to its re-classification as a true mycotoxin. *P. expansum*, a natural and most efficient producer of patulin is the causative agent of ‘blue mold’ in apple, pear, cherry, and other fruits. Although commonly found in non-fermented apple juice, it is efficiently metabolized by yeasts during fermentation.

## Management and control of mycotoxins in food commodities

In the control or prevention of mycotoxins in food commodities, the fungal strain and food commodity determine the effectiveness of any method. Preventive measures at pre-harvest include 1. the use of mold-resistant varieties such as transgenic plants, which possess antifungal or anti-mycotoxin compounds.

2. Proper agronomic practices can also prevent mycotoxin contamination. These include crop rotation, avoiding overcrowding of plants, monitoring of soil pH and mineral deficiency, avoiding planting crops at periods of high temperature and water stress, and use of harvesting techniques that will minimize mechanical damage to grains.

3. Timely management guided by predictive models which can predict growth of toxigenic molds and mycotoxin contamination, forecast optimum harvest and antifungal application time will contribute to preventing mycotoxin contamination.

4. Postharvest strategies aimed at mycotoxin control focuses on drying and cleaning. These include drying of commodities to water levels too low to favour growth of fungi.

5. Dry, clean, insect-free containers should be used in transporting commodities.

6. Freshly harvested crops with high moisture contents should not be piled for longer periods before drying or processing.

7. During storage, cooling and aeration systems are necessary to regulate temperature, humidity and gas atmosphere .

8. Storage facilities should protect commodities from rain water and ground water drainage.

9. The use of appropriate anti-fungal agents and pesticides to protect against rodents, insects and fungal growth is recommended.

10. Essential oils are naturally-occurring plant based volatile compounds with antimicrobial and antioxidant activities. Being volatile and biodegradable in nature, they may be used as fumigants for stored food commodities.

11. Regular check for any rise in temperature and moisture levels that could support mold growth should be implemented.

### Chemical methods

The use of acids, bases, oxidizing agents, reducing agents, chlorinating agents in the degradation of mycotoxins have not been entirely effective. Some of them have detrimental effects on the nutritional values of the foods in which they are used. The use of ammonia and hydrochloric acid, although achieved efficient detoxification, however reduced the nutritional values of the treated commodities. Some molds have developed resistance to chemicals and some preservatives. For example, some *Penicilli* can grow in the presence of potassium sorbate while others are able to degrade sorbate. Antifungal activity against aflatoxin-producing fungi and inhibition of aflatoxin formation have been reported with extracts and powders of various spices, herbs, and essential oil.

###  Processing operations

Pre-processing operations such as sorting, trimming and cleaning may reduce mycotoxin contamination of food commodities but may not achieve complete elimination of the contamination. During milling, mycotoxins undergo re-distribution and concentration in certain mill fractions, particularly in the germ and bran fractions during dry milling. In wet milling, for example wet milling of corn, mycotoxins are not destroyed but may be dissolved in the steep water or distributed among the process by-products. Roasting as a processing method may also achieve considerable reduction of mycotoxin in the food commodities. Partial roasting and blanching reduced aflatoxin production in peanut by above 72% during storage. Roasting at 150°C for 30 min also degraded AFB1 and AFB2 by 66% and 63% respectively in naturally contaminated pistachio kernel.

### Biological control

Various physical methods such as microwave heating, electronic eye sorting, UV irradiation, solar irradiation, solvent extraction, adsorption, ozone gas, gamma rays and floatation have been used to control mycotoxins in foods. These methods have had limited efficiency based on safety issues, losses in nutritional values and palatability of feeds, cost implications and limited efficacy with different mycotoxins. Thus, there is need to seek for an effective, specific, feasible and environmentally sound decontamination technique.

Bio-preservation is a method of preserving food products that is based on the principle of employing the use of one organism to control another. In recent years, it has received much attention for control of spoilage in foods. Biological control can be achieved using non-toxigenic strains of *A. flavus* and *A. parasiticus* in the soil of growing crops, by inoculating grains with conidia suspension or applied directly to the seedling or soil surface before planting. The naturally occurring toxigenic strains are then competitively eliminated by the non-toxigenic strain. Lactic acid bacteria (*Lactobacillus spp*.,) and yeast *Saccharomyces spp*. can also contribute to inhibition of molds development and consequently, production of mycotoxins.

Table 2 Mycotoxin detection methods

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| Methods  | Advantages | Disadvantages |
| TLC | Simple, inexpensive, and rapidCan be used for screeningSimultaneous analysis of multiplemycotoxinsSensitive for aflatoxins & ochratoxin A | Poor sensitivity (for some mycotoxins)Poor precisionAdequate separation may require two-dimensional analysis Quantitative only when used with a densitometer |
| GC | Simultaneous analysis of multiplemycotoxinsGood sensitivityMay be automated (autosampler)Provides confirmation (MS detector) | Expensive equipmentSpecialist expertise requiredDerivatization requiredMatrix interference problemsCarry-over effects from previous sampleVariation in reproducibility & repeatability |
| HPLC | Good sensitivityGood selectivityGood repeatabilityMay be automated (autosampler)Short analysis timesOfficial methods available | Expensive equipmentSpecialist expertise requiredMay require derivatization |
| LC/MS | Simultaneous analysis of multiplemycotoxinsGood sensitivity (LC/MS/MS)Provides confirmationNo derivatization required | Very expensiveSpecialist expertise requestedSensitivity relies on ionization technique |
|  |  |  |
| ELISA | Inexpensive equipmentHigh sensitivitySimultaneous analysis of multiplesamplesSuitable for screeningLimited use of organic solventsVisual assessment | Cross-reactivity with related mycotoxinsMatrix interference problemsPossible false positive/negative resultsConfirmatory LC analysis required |
| Rapid Tests (membrane-based card test; antibody-coated tube; immunodot cup test) | Simple and fast (5-10 min)No expensive equipment requiredLimited use of organic solventsSuitable for screening purposesCan be used *in situ* | Possible false positive/negative resultsCross-reactivity with related mycotoxinsMatrix interference problems |

 Surface Plasmon Resonance (SPR); Fiber Optic Immunosensors (FOI); Quartz Crystal Microbalance (QCM); Screen-Printed Carbon Electrodes (SPCE); Molecularly Imprinted Polymer (MIP); Thin Layer Chromatography (TLC); Gas Chromatography (TLC); High Performance Liquid Chromatography (HPLC); Liquid Chromatography/Mass Spectrometry (LC/MS); Enzyme-Linked Immunosorbent Assay (ELISA).

Reference: Pascale and Visconti (2008)