REVIEW ARTICLE

The AJIEC
THE ASIAN JOURNAL OF EXPERIMENTAL CHEMISTRY
Volume 8 | Issue 1&2 | June & December, 2013 | 24-29

Mycotoxins : toxicology, health risks and integrated management

■ K.K. CHAHAL, S. KAUSHAL AND P. SAHOTA

Author for Correspondence -
K.K. CHAHAL
Department of Chemistry, Punjab Agricultural University, LUDHIANA (PUNJAB) INDIA

See end of the article for authors affiliation

Abstract - Mycotoxins are secondary metabolites produced by certain filamentous fungi which are toxic to crop plants, man and animals; the disease caused by them is called Mycotoxicosis. Based on intermediates from which these secondary metabolites are derived, there are three board categories of mycotoxins, they are polyketide derived mycotoxins, formation of which requires acetyl coenzyme (Patulin, Aflatoxin and Citonin), terpene mycotoxins having mevalonic acid as intermediate (Trichothecin) and cyclic polypeptide derived mycotoxins (Giotoxin and Sparldesnium). The effect of mycotoxins may be categorized into three forms acute primary mycotoxicosis, chronic primary mycotoxicosis and secondary mycotoxin diseases. There are geographic and climatic variations in the production and occurrence of mycotoxins, exposure of these substances occur all over the world and much of the world's food supply is contaminated to some extent. FDA has estimated direct economic loses of nearby a billion dollars a year due to crop loss and another half a billion dollars in mitigating costs. Monitoring of mycotoxins is needed to avoid adverse effects on health. Risk analysis approach dealing with the problem of mycotoxins involves risk assessment and risk Management. Risk management is to ensure safe food supply will range from prevention of mould growth and setting of regulatory limits to diversion into alternate uses, control through good agricultural practices, control through processing and consumer / producer education.

Key words - Mycotoxins, Mycotoxicosis, Aflatoxins, Fungi, Secondary metabolites

How to cite this paper - Chahal, K.K., Kaushal, S. and Sahota, P. (2013). Mycotoxins : toxicology, health risks and integrated management. *Asian J. Exp. Chem.,* **8** (1&2) : 24-29.

Paper history - Received : *10.05.2013;* **Sent for revision :** *10.11.2013;* **Accepted :** *23.11.2013*

Among the filamentous fungi or moulds associated with crop plants there are many which produce secondary metabolites which are toxic to man and other animals. These toxic metabolites are called Mycotoxins.These are present in mycelium which colonizes senescing plant organs such as leaves, fruits and seeds. They are also present in spores and may be excreted into the surrounding plant tissues. The disease caused by them is referred to as mycotoxicosis.It is caused by the ingestion of mycotoxin contaminated food. Greek Physician, Galen was first to record mycotoxicosis when during famine many people were forced to eat stored barley or wheat. Mycotoxicosis caused by consumption of cereals and bread containing the sclerotia of *Claviceps purpurea* was wide spread in Europe. From the middle ages there were numerous episodes pertaining to gangrenous and convulsive ergotism. In the first half of the present century well documented evidence of mycotoxicosis caused by *Fusarium* species in cereals emerged from Russia. However research on mycotoxins did not really burgeon until after 1960, when one lakh turkey poults in England died from turkey X disease in which there is acute necrosis of liver and hyperplasia of the bile duct. It was discovered that this disease developed due to the consumption of peanut meal. It was found that

toxin named aflatoxin was responsible for producing disease. Aflatoxin was produced by fungus, *Aspergillus flavans* and found to be carcinogenic in nature. Little is known about the natural occurrence of most of the mycotoxins and consequently their true significance. This article is therefore devoted to classification, effects, production, economic impact and regulatory limits of mycotoxins.

Classification of mycotoxins:

There are three broad categories of Mycotoxins.These are based on intermediates from which these secondary metabolites are derived as the growth related processes of primary metabolism are restricted.

In the first category these are polyketide derived mycotoxins, formation of which requires acetyl coenzyme A that is normally involved in fatty acid synthesis during growth. These include the toxin Patulin which is derived from tetraketide, Citrinin from pentaketide, Zearalenone from nonaketide and Aflatoxins from decaketide.The second category consists of terpene mycotoxins, which have mevalonic acid as a key intermediate. It includes around 80 sesquiterpene trichothecenes including Trichothecin.The third category consists of cyclic polypeptides and their derivatives. It includes Gliotoxin and Sporidesmin.

Effect of mycotoxins:

A number of cereals and other crops are susceptible to fungal attack, either in the field or during storage. The fungi involved may produce mycotoxins as secondary metabolites. The level of mycotoxins in food can fluctuate widely and vary significantly from year to year. These fluctuations depend on many factors, including adverse conditions that favour fungal invasion and growth either in the field or during storage.Mycotoxins are diverse group of chemical substances. When present at high levels in the diet, may cause acute and /or chronic adverse health effects in animals and humans. Mycotoxin can affect many target organs and systems, notably the liver, kidneys, nervous, endocrine and immune systems. There is a much concern about secondary effects brought about by low levels of exposure. The effect of mycotoxins has been broadly categorized into three forms:

Acute primary mycotoxicosis:

At acute level every system of an animal's body can be effected by one or a combination of mycotoxin *viz.*

– Vascular system-increased vascular fragility, haemorrhage into body tissues e.g. Aflatoxins.

– Digestive system-diarrhoea, intestinal haemorrhage and hepatotoxic effects causing liver necrosis, bile duct proliferation and fibrosis e.g. Aflatoxins.

– Respiratory system- adenomatosis e.g. 4-Ipomenol.

– Nervous system-tremors, incordination, mania, coma e.g. ergotamine and related alkaloids.

– Cutaneous system-photosensitization e.g. Sporidesmin.

– Urinary system-Nephrosis, uremia e.g.Ochratoxin.

– Reproduction system-infertility, prolonged oestrus e.g.T-2 toxin.

Chronic primary mycotoxicosis:

In these types of mycotoxicosis, there is a relative lack of macroscopically visible changes in the infected animals and this prevents an easy diagonisis based on symptoms.

It appears as reduced productivity in the form of slower growth rates, reduced reproductive efficiency and inferior market quality. Reduced milk yields in dairy cows and reduced egg production and increased cracked eggs in poultry have been noted in mature animals exposed to Aflatoxins and Ochratoxin A.

Secondary mycotoxin diseases:

Consumption of low levels of certain mycotoxins can lead to impairment of the native and acquired resistance to infectious diseases causing health related economic losses. In particular, low concentration Aflatoxin induced immunologic deficiency is believed to be associated with the specific failure of the cell mediated immune system while at slightly higher levels; antibody production may also be impaired. Several mycotoxins have been classified by International agency for research in cancer (IARC) as human carcinogens or potential human carcinogens. The sources and toxic effects of some important moulds are given in Table 2.

Production of mycotoxins:

The usual conception of secondary metabolites is of molecules which are produced in submerged liquid culture when some nutrient such as nitrogen and phosphorus sources become limiting. Exponential growth of the mould can no longer be sustained and as a stationary phase is entered the secondary metabolites are synthesized. In laboratory

Table 1: Formation of mycotoxins from intermediates

Primary metabolites	Intermediate	Secondary metabolites	Mycotoxins formed
Fatty acids	Acetyl Coenzyme A	Polyketide	Patulin,Citrinin,Zearalenone,Aflatoxins
Sterols	Mevalonic Acid	Terpenes	Trichothecin
Proteins	Amino Acids	Cyclic peptides	Gliotoxin,Sporidesmin

fermentation conditions can be manipulated so as to maximize production and the metabolites are recovered both from mycelium and culture broth. In laboratory it is possible to study the interaction of various variables such as temperature and water availability on growth and toxin production. The situation is highly complicated in nature, since so many variables are involved. Other organisms present on the substratum also exert an influence on toxin production. Simultaneous inoculation of maize with *A.flavans* and *A.niger* may lead to significant reduction in the amount of aflatoxin which would otherwise be produced by *A.flavans* alone. The application of pesticides and fungicides may also affect mycotoxin production either by directly affecting the toxic fungi or by affecting competing microorganisms.For example the fungicide triademenol which inhibit sterol biosynthesis has been shown to stimulate *invitro* biosynthesis of Aflatoxins. It appears that *A.flavans is* adapted to aerial and foliar environment and predominates over *A.parasiticus* on maize and cottonseed, while the latter seems better adapted to the soil environment and is therefore prominent in peanuts. It has been found that colonization by the mould and the aflatoxins contamination is strongly influenced by the temperature around the maize cob and of the soil around the geocarp of the peanut. Drought results in elevation of soil temperature, which promotes fungal growth and Aflatoxin production in peanuts.

Aflatoxins as major mycotoxins:

Aflatoxins are produced by *Aspergillus flavans* and *A.parasiticus*. They have world wide distribution and are mainly prevalent in tropical and sub-tropical regions, where they may colonize plant organs both in field and under poor storage conditions after harvest. In temperate parts of the world they tend to be regarded primarily as storage fungi, since they are seldom found under field conditions. A wide range of plant products are known to be contaminated with aflatoxins but the toxins are most often associated with peanuts, maize, rice and cottonseed.The Aflatoxin molecule contains a coumarin nucleus linked to a bifuran and either a pentanone as in AFB_1 and the dihydro derivative in AFB2 or a six membered lactone as in AFG_2. *A.parasiticus* produces all four of these Aflatoxins but *A. flavans* produces only AFB_1 and AFB_2. Two 4-hydroxylated derivatives of these last toxins have been found in maize and peanuts. These derivatives are AFM_1 and AFM_2 were first isolated from the milk of cows fed on Aflatoxin contaminated rations but they have been recovered from meat, liver, kidneys, urine of various animals and eggs. One of the metabolites of AFB_1 produced in the animal body is its 8, 9 epoxide and is considered to be the active form of AFB_1. Electrophilic attack at the N-7 position of guanyl residues in DNA by the epoxide is likely to be the major cause of lesions which appear to account for the mutagenic and carcinogenic nature of AFB_1. The epoxide may also be hydrated to the dihydrodiol, which will form Schiff bases with the amino groups of bases. The hydrodiol binds to DNA *in vitro* and acts directly as a mutagen. In addition to attacking DNA, AFB_1 and its derivatives also bind to histones, ribosomal RNA and proteins. A primary biochemical effect of aflatoxins in the target organ, the liver, is the inhibition of DNA synthesis, which occurs at concentration below *i.e.* those which affect RNA and protein synthesis. Interference with RNA synthesis may not block the subsequent synthesis of inducible enzymes but also synthesis of various components of immune system. Dietary AFB_1 has been shown to significantly suppress the cell mediated immune response in chickens and has a residual effect on the delayed type hypersensitivity and phagocytic activity of reticuloendothelial cells. Such damage to the defense mechanisms of the body would contribute to the observed reduction in resistance of animals to viral, bacterial and fungal infections.Aflatoxins show both acute and chronic toxicity towards animals.The symptoms of acute aflatoxicosis are pathological changes in liver including enlargement, fat deposition, necrosis and hyperplasia of the bile duct. Other effects observed are less specific e.g. the loss of appetite, lethargy, weakness and gastrointestinal

Table 2 : Food sources and toxic effects of some important moulds

Mycotoxin	Fungus	Foods involved	Toxicity
Aflatoxins	*Aspergillus* sp.	Peanuts,maize,wheat,milk and cottonseed	Mutagenic and carcinogenic
Ochratoxins	*Aspergillus* sp.	Maize,wheat,barley, peanut, hen's egg, coffee	Carcinogenic and liver damage
	Penicillum sp.		
Fumonisins	*Aspergillus* sp.	Maize,peanut,cottonseed,	Nausea, vomiting and giddiness
	Fusarium sp.	soyabean	
Trichothecenes	*Aspergillus* sp.	Wheat, maize , rye	Gastrointestinal disorders
	Fusarium sp.		
Patulin	*Penicillum* sp.	Apple products	Human susceptibility not known
Zearalenone	*Aspergillus* sp.	Wheat, maize	Human susceptibility not known
	Fusarium sp		

hemorrhage.

Economic impact of mycotoxins:

Although there are geographic and climatic variations in the production and occurrence of mycotoxins, exposures of these substances occur all over the world and much of the world's food supply is contaminated to some extent. The regulations against sale of contaminant commodities have put a very severe economic burden. FDA has estimated direct economic losses of nearby a billion dollars a year due to crop loss, and another half a billon dollar in mitigating costs. These losses are only from three toxins namely Aflatoxins, Fumonisins and Trichothecenes.Monitoring of mycotoxins is needed to avoid adverse effects on health. Although these toxicants can never be completely removed from food supply, risk analysis based on sound scientific knowledge makes it possible to define the levels in foods (tolerances, guideline levels and maximum residue levels) that are unlikely to be detrimental to health. In terms of exposure and the severity of the chronic lesions (especially cancer) they cause, mycotoxins appear at present to pose a higher risk than anthropogenic contaminants, pesticides and food additives. The Joint FAO/WHO Expert Committee on food additives (JECFA) has been meeting regularly since 1956 to evaluate food contaminants, naturally occurring toxicants etc in foods. To date more than 20 contaminants including mycotoxins have been evaluated by the committee. Risk analysis approach dealing with problems associated with mycotoxins is proposed to be made up of two parts:

- Risk assessment
- Risk management

This approach considers scientific principles related to human and animal health, as well as socioeconomic factors. The aim is to achieve guidelines for preventing the problems. Scientific evaluations have become the basis for recommendations regarding international regulations of mycotoxin levels (Aflatoxins, Ochratoxins, Patulin, Zearalenone) made by Codex Committee on food additives and contaminants.

Risk assessment:

Risk is likelihood of an adverse health effect weighed for its severity in humans as a result of exposure to a biological, chemical or physical agent in food. Hazard is intrinsic property of a biological, chemical or physical agent to cause adverse health effects under specific conditions. Risk assessment involves toxicological, epidemiological and exposure assessments along with a risk characterization.

Hazard identification:

Many toxins have carcinogenic properties and may cause developmental effects including birth defects and affect the immune system; some also exhibit hormonal activity or are neurotoxic.In addition disturbances in gastro intestinal system, skin irritation and haemotological effects have been observed. Identification studies usually determine the 'no-observe-effect –level' (NOAEL) which can be regarded as 'threshold'. Several carcinogenic mycotoxins appear to have both initiating and promoting properties and also contribute to tumor progression. It is presumed that there is no threshold for this process. Other mycotoxins predominantly exert tumor promoting activity for which it is presumed that a threshold level does exist. These two types of carcinogens are treated differently and have an impact on determination of their safe levels. However, the differences in two groups may not always be clear. Such diversity of biological effects demands a case-by-case evaluation and may require a variety of extrapolation techniques.

Hazard characterization:

Hazard characterization is extrapolation phase of risk assessment. Its aim is to make a predictive characterization of the hazard to humans, based on animal studies (species extrapolation) under low exposure conditions (extrapolation from high to low dose). The end point of hazard characterization is estimation of safe dose (provisional tolerable daily intake, TDI). To derive a TDI for humans, it has been a common practice to divide NOAEL by a safety factor of 100 when extrapolating from animals to humans. For insufficient data additional uncertainty factors may be added. For carcinogens with no threshold level, TDI is generally not determined.

Exposure assessment:

Reliable analytical methods are available for only a few mycotoxins and non-homogenous distribution of mycotoxins in food commodities cause problems. Exposures to mycotoxins depend on their level in different foods and intake of these foods. There can be large regional variations, therefore exposure assessments are area specific. Monitoring data for food commodities collected over years will provide input data for mycotoxin levels and estimates of mycotoxin intake calculated from actual consumption of such foods. There may be different age and target groups depending upon investigation (*i.e.* acute intake verses chronic intake) as young children are being exposed at a much higher rate in terms of body weight for commodities such as milk (upto sevenfold). Exposure assessments can also be based on measurements of biomarkers in humans (aflatoxins, ochratoxin) and their intake. Potential hazards to human health arising from mycotoxins in animal food products need to be assessed. At high levels in feed, mycotoxins may affect health of animals or even kill them whereas at low levels, their residues and related substances may move up the food

chain. Indirect intake of mycotoxins from such foods may pose a health hazard to humans, though this risk is somewhat lower than direct exposure from cereal and other food crops.

Risk characterization:

Risk characterization is the qualitative and quantitative estimation of potential adverse health effects on an exposed population. Risk characterization can also be established of levels of daily exposure at which risk is insignificant over a life time (*i.e.* below TDI).Where TDI cannot be determined, safety margin between human exposure and adverse effects seen in animal species may serve an indication of ill effects in humans. This has been done in evaluation of Fumonisins.Vulnerable groups such as children (because of lower body weight) and elderly (due to differences in bioavailability, metabolism and genetic disposition) need to be considered separately for risk characterization. Detailed risk assessments have been performed only for Aflatoxins, Deoxynivalenol, Ochratoxin A, Zearalenone and Fumonisins.

Risk management:

Risk management to ensure a safe food supply will range from prevention of mould growth and setting of regulatory limits to diversion into alternate uses. One impediment to harmonization in trade relates to setting of residue limits (MRLs) for mycotoxins.To date Joint FAO/ WHO Expert Committee on food additives has evaluated three mycotoxins:

- Aflatoxins B,G and M
- Patulin
- Fumonisins

Regulatory limits for mycotoxins in food and feed:

The U.S. Food and Drug Administration (FDA) have issued Regulatory limits for aflatoxins and other mycotoxins.

Reductions in intake of aflatoxins can be achieved through avoidance measures such as improved farming and proper storage practices and / or enforcing standards for levels of contamination in food / feed which are costly. However, risks are significantly reduced when the most highly contaminated samples are eliminated. When two alternative standards for aflatoxin contamination in foods are considered, the higher standard will carry the same risk as the lower standard if the samples of fraction excluded under the two standards are similar. When a substantial fraction of current food supply is heavily contaminated with aflatoxins, reducing the level of contamination is beneficial. Conversely when a small fraction of current food supply is heavily contaminated, reducing the standard by apparently the substantial amount may have little appreciable affect on health. Some of the methods used for detoxification of mycotoxins are:

Table 3: Regulatory limits for mycotoxins in food and feed

Sr. No.	Mycotoxins	Limits ($\mu g\ kg^{-1}$)
1.	Aflatoxins	
	Human foods (except milk)	20
	Milk	0.5
	Animal feeds except listed below	20
	Cotton seed meal(as a feed ingredient)	300
	Corn and peanut products for poultry)	100
	Corn for immature animals and for dairy cattle	20
2.	Deoxynivalenol	
	For finished wheat products for human use	1
	For animal feed	5-10
3.	Fumonisins	
	For human foods	2-4
	For animal feeds	5-100
4.	Patulin	
	In apple juice	50

· *Physical removal:* hand picking or electronic colour sorting

· *Heat inactivation*: dry heating like roasting, frying, microwave roasting at high temperature; pressure cooking may destroy 49-82 % of Aflatoxins.

· *Irradiation*

· *Chemical methods:* chlorine, hydrogen peroxide, alkali, bisulfite etc are of practical importance.

The different phases of food safety management programme for naturally occurring toxins should include:

Setting of regulatory limits:

– Commodity surveys to determine contamination levels.

– Dietary intake surveys to determine consumption levels.

– Evaluation of toxicological data.

– Establishment of analytical capabilities.

– Availability of food/feed supply based on different regulatory limits.

Establishment of monitoring programme:

– Establishment of sampling plan.

– Sample collection.

– Preparation of test portion.

– Analysis of test portion.

– Permitted uses of mycotoxin contaminated products.

Control through good agricultural practices:

– Post harvest decontamination of commodities

– Development of biomarkers

- Preharvest control of mycotoxins.
- Enhancement of host resistance.
- Biological control of toxigenic fungi.

Control through processing:

- Good manufacturing practices.
- Quality control.

Decontamination through specific treatments:

- Evaluation of the final product.
- Designation of use of treated product.
- Consumer/ producer education.

Thus, mycotoxins are a concern of everyone working to protect health, achieve adequate and safe food supplies and raise living standards. Reducing the risk of mycotoxin contamination of food and animal feed require the combined efforts of agricultural research centers, nutrition and health institutions, training and extension programmes and regulatory agencies.

Conclusion:

Any realistic assessment of the present day risk to human health posed by mycotoxins is hampered by the difficulty of quantifying exposure to mycotoxins in the diet. Many mycotoxins are stable and are either not or only partially destroyed by cooking. It has been found that mycotoxin uptake from the alimentary canal into the tissues can be greatly reduced by the addition of fibre or clay minerals in food stuffs. It is possible to remove toxins from commodities by solvent treatment.Ammoniation for example is effective in detoxifying aflatoxin contaminated oilseeds and cereals. However careful attention to husbandary, drying and storage will do much to prevent mycotoxin development and reduce health hazards.

Authors Affiliation :

S. KAUSHAL, Department of Chemistry, Punjab Agricultural University, LUDHIANA (PUNJAB) INDIA

P. SAHOTA, Department of Microbiology, Punjab Agricultural University, LUDHIANA (PUNJAB) INDIA

REFERENCES

Barug, D., Van, E.H.V., Garcia, R.L., Osenbruggen, T.V. and Visconti, A. (2004). Wageningen Academic Publishers, The Netherlands. pp. 17-40.

Goodman, T.K. (1999). *Food Nutr. Agric*., **23**: 10-15.

Herrman, J.L. and Walker, R. (1999). *Food Nutr. Agric*., **23**:17-21.

Lopez-Garcia, R., Park, D.L. and Phillips, T.D. (1999). *Food Nutr. Agric*., **23**:38-47.

Piya, G., Galvano, F., Pietri, A. and Piva, A.(1995). *Nutr. Res*., **15**: 689-715.

Price, R.L. and Jorgensen, K.V. (1985). *J. Food Sci*., **50** : 347.

Pussa, T. (2008). CRC Press, New York. pp 195-209.

Smith, J.E. and Moss, M.O. (1985). John Wiley and Sons, Great Britain. pp. 50-58.

Turner, N.W.,,Subrahmanyam, S. and Piletsky, S.A.(2009). *Anal. Chem. Acta*, **632** (2) : 168-180.

Booner, J., Malysheva, S., Taevemier, L., Diana, D., Mavungu, J., De, S.S. and De, S.B. (2012). *Toxicology*, **301** (1-3) : 21-32.

Shedhard, G.S.(2008).*Chem. Soc. Rev*., **57**(11): 2468-2477.

Berthiller, F., Crew, C., Dall, A.C., Sager, S.D., Haesart, G., Karlovsky, P., Oswald, I.P., Seefelder, W., Speiger, G. and Stroka, J.(2013). *Mol. Nutr. Food Res*., **57**(1):165-186.

8th Year
★★★★★ of Excellence ★★★★★