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Mycotoxin problem in maize

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Mycotoxins are secondary fungal metabolites produced by certain toxigenic fungi on various food and feed substrates and are capable of causing ill-effects on the body of the consumers. Aflatoxins, ochratoxins, sterigmatocystin, citrinin, citreo-viridin, patulin, zearalenone, trichothecenes, fumonisins, moniliformin and *Alternaria* toxins are some of the common and important mycotoxins which are produced by the species of *Aspergillus, Penicillium, Fusarium* and *Alternaria*. These mycotoxins are having specific target organs for their toxicity and exhibit acute and/or chronic disease syndrome in affected hosts. Mycotoxins and particularly aflatoxins have been analysed as natural contaminants in almost all the agricultural crops and other consumable items. Problem of aflatoxin contamination, however, varies with the nature of substrates, availability of toxigenic fungal strains and prevailing climatic conditions. Maize (*Zea mays* L.) is one of the important cereal crops of the country and also one of the most susceptible commodities for aflatoxin elaboration. Natural incidence of mycotoxins has been reported in standing crops as well as in the grains of stored maize from different parts of India. Out of various preventive measures suggested for the control of mycotoxin problem, selection of resistant crop varieties has been found to be the best.

Key words: Mycotoxin, maize, problem

At the outset I must thank the Executive Council of the Indian Mycological Society and particularly Prof. N. Samajpati and Dr. A. K. Manna, who have given me a chance to be here today before this august gathering to deliver a talk in the name of the great Indian Mycologist, Prof. S. N. Banerjee, who had been one of the Founder Members of this Society and had served it in various capacities.

I also feel honoured because the First Memorial Lecture in this Series was delivered by my teacher, supervisor and also one of the great Mycologists, Late Prof. K. S. Bilgrami. I pay my regards and homage to both those Scientists especially at this historic moment.

I have selected this topic for my talk because I am working in this field since last 38 years. In fact the concern over exposure to moulds and human diseases dates back to biblical times. In recent centuries, the majority of mass poisonings attributed to moulds have been due to the ingestion of mouldy foods that contain secondary metabolites of moulds, also known as mycotoxins.

Mycotoxins are fungal poisons. The word '*mycotoxin*' is derived from the Greek word "*mykes*" meaning fungus and Latin word "*toxicum*" meaning poison. These are toxic metabolites produced by certain fungi especially by saprophytic moulds

growing on food and feed. These are hazardous for man and domestic animals but their effects have largely been overlooked. During sixties it was established scientifically that some fungal metabolites were responsible for various animal diseases and death. Some mycotoxins have multiple effects and may cause phytotoxic and antimicrobial syndromes in addition to animal toxicity. On the basis of the origin and target of toxicity in the living organisms, there have been different definitions of mycotoxins proposed by different scientists.

Uraguchi and Yamazaki (1978): "Mycotoxins are secondary fungal metabolites capable of causing pathological changes or physiological abnormalities in man and warm blooded animals".This definition is not correct because some cold-blooded animals are also influenced by such toxic effects.

Moreau (1979): "Mycotoxins are extra-cellular zootoxic metabolites produced by moulds in food consumed by man and animals." His view is also not clear about the substrate, where moulds produce toxins, and the nature of toxicity. Some of these toxins are also known to affect the growth and metabolism of plants.

International Society for Human and Animal Mycology (ISHAM) in 1982 defined mycotoxins as "A metabolite of micro-fungus which when ingested by a natural route in sufficient concentration will cause ill-effects in animals (including birds and men)". This definition does not include the toxins produced by mushroom and other higher fungi and toxic effects of these compounds on plants, microorganisms and insects.

Pitt (1996): "Mycotoxins are fungal metabolites which when ingested, inhaled or absorbed through the skin can cause lowered performance, sickness or death in man or animals, including birds."

Bilgrami and Choudhary (1998): "Mycotoxins are fungal secondary metabolites which occur naturally as contaminants of agricultural and other consumable products and which show toxicity in animals via a natural route of administration."

Mycotoxins may now be defined as "Chemically diverse group of secondary metabolites of certain toxigenic strains of fungi, which are produced on food and feed substrates and are lethal to living organisms causing abnormalities at cellular and physiological levels especially in animals, human beings and plants".

Mycotoxicoses in Historical Perspective

The disease symptoms created by mycotoxins are called mycotoxicoses. There are several kinds of mycotoxicoses, which are specific to the respective group of mycotoxins. It is now well established that mycotoxicoses have been associated with major epidemics in man and animals in the past due to ingestion of highly contaminated food and feed stuffs. The most important examples are of ergotism, ATA, stachybotryotoxicosis, aflatoxicosis etc. which were known to be caused by the growth of specific moulds producing one or more potent toxins in one specific kind of food commodity or feed.

Ergotism

Also known as St. Antony's fire, ergotism is thought to be the earliest known mycotoxicoses which occurred thousand years back in Central Europe. The disease has been caused by different species of *Claviceps* mainly *Claviceps purpurea* and *C. paspali*. These fungi grow in the ovaries of grains especially rye and other cereals and produce dark brownish fruiting bodies *viz.*, sclerotia or ergots. But these ergots are difficult to be separated from normal grains at milling and become dispersed in flour made from grains. These sclerotia (ergots) contain alkaloids representing acidamide derivatives of D-lysergic acid, i.e. ergotamine and alkaloids of ergotoxin group(ergocorine, ergocryptin and ergocysten).

Ergotic epidemics have been reported from France, India and Ethiopia (Krishnamachari and Bhat, 1976). Ergotism causes constriction in blood vessels especially those of limbs. In acute cases cell death (necrosis), gangrene and hallucinations may occur.

Alimentary Toxic Aleukia (ATA)

ATA is a notorious disease of human beings causing fever, bleeding from skin, nose, throat and gums, necrosis and suppression of immune system. It caused death of thousand of population in USSR, between 1942 and 1948 (World War II) after consumption of over-wintered millet and buckwheat contaminated by *Fusarium poe* and *F. sporotrichioides* (*F. tricintum*). During 1970 it became clear that the toxin responsible for ATA was trichothecenes, also called T-2 toxins. These were

responsible for a variety of diseases both in men and domestic animals. Most of the epidemics occurred in Europe, Russia, Japan and United States. Marasas *et al.* (1979) have suggested that trichothecenes may be involved in the high incidence of oesophageal cancer in the Republic of Transkei (South Africa).

Stachybotryotoxicoses

Stachybotryotoxicoses are primarily an equine disease reported from horses consuming hay infected with *Stachybotrys alterans,* however, it also affects human population particularly in the regions where this disease is prevalent. Some of the common symptoms of the disease include severe dermatitis, buccal inflammation and pain with burning sensation in mouth. The disease results from a series of trichothecenes designated as satratoxins C,D,F,G and H.

There are some other mycotoxicoses which seriously affected the humans, include acute Cardiac Beriberi in Japan caused by citreo-viridin, a mycotoxin produced by *Penicillium citreo-viride*, Red Mould Disease caused by *Fusarium* spp. which appeared in Japan during 1940's and 1950's, Endemic (Balkan) Nephropathy, a fatal renal disease reported from Bulgaria, Romania and Yugoslavia, Onyalai caused by fungus *Phoma sorghina* on millet in Africa .

The year 1960 is the landmark in the history of Mycotoxicology when over one lakh young turkeys in England died within short span after consuming contaminated peanut meal. The disease was later called "Turkey-X-Disease" (Blount, 1961). Coincidently, at the same time high incidences of liver disease also occurred in ducklings in Kenya. Thorough examinations of the feed sources (Brazilian peanut meal) established the cause of disease to be the toxins produced by the mould, *Aspergillus flavus* and *A. parasiticus* (Blount, 1961). The active ingredient (toxin) was given the name 'Aflatoxin' on the basis of the name of producing organism, where *A* represents *Aspergillus* and *'fla'* represents *flavus* (Sargeant *et al.*, 1963).

Mycotoxins and Mycotoxin Producing Fungi

Mycotoxins have been reported to be produced by the toxigenic strains of a wide range of fungi. Toxigenicity of fungus by and large depends on its genetic makeup, food/host range as well as ecological factors that influence toxin production. However, the important mycotoxin producing fungi belong to four genera viz., *Aspergillus, Penicillium, Fusarium* and *Alternaria*. Nearly four hundred mycotoxins have been discovered to date and are generally categorized into groups based on structural similarities. Some of the most common types of mycotoxins that can cause health problems in animals and humans are aflatoxins, ochratoxins, sterigmatocystin, citrinin, citreo-viridin, patulin, trichothecenes, zearalenone, moniliformin and fumonisins. Major mycotoxins, their main producer fungi as well as their principal toxic effects on animal and human beings have been given in Table 1. All mycotoxins have their specific target organs for their toxicity (Table 2) and on that basis these have been classified (Table 3).

Aflatoxins are produced by the closely related species of the genus *Aspergillus*: *Aspergillus flavus* Link ex Fries, *A. parasiticus* Speare and *A. nomius* (Payne and Brown, 1998).

Besides aflatoxins, other major mycotoxins produced by *Aspergillus* species are sterigmatocystin and ochratoxin which are produced by *A. versicolor* and *A. ochraceus*, respectively. *Penicillium* also produces a good number of mycotoxins viz., citrinin, patulin, citreo-viridin etc. which are produced by the species like *P. citrinum*, *P. patulum*, *P. citreo-viride*, respectively. *Fusarium,* another major mycotoxin producing genus, elaborates a variety of mycotoxins. These include trichothecenes, produced mainly by *F. tricinctum,F. solani* and *F. equiseti*, zearalenone by *F. graminearum* and moniliformin and fumonisins by *F. moniliforme* etc.

Since aflatoxins are the most common mycotoxins produced by the species of *Aspergillus*, emphasis has been given here on these mycotoxins.

Physico-Chemical Properties of Aflatoxins

Hartley *et al.* (1963) first isolated and characterised the four mother compounds of aflatoxins from the culture extracts of *A. flavus* grown on crushed groundnuts at the Tropical Product Institute, London. The major compounds were designated as B_1 and G_1 while the minor compounds were B_2 and G_2 on the basis of colour of their fluorescences (viz., B-blue, G-green) and their respective *Rf* values. Aflatoxins in crystalline state look colourless to pale yellow. They fluoresce strongly under long wave UV light. Isolation of the toxins was facilitated

by the discovery that characteristic blue fluorescence paralleled the toxicity observed in duckling test. Later on the extract could be separated chromatographically into four distinct zones or compounds. Aflatoxins are readily soluble in moderately polar solvents e.g. chloroform, methanol and dimethyl sulfoxide (DMSO), insoluble in non-polar solvents and sparingly soluble in water. Aflatoxins, in pure state, are very stable at high temperatures when heated in air, but relatively unstable, when exposed to light and particularly to UV radiation and air on a TLC plate and especially when chloroform and benzene solutions are stored for years in dark and cold. Physico-chemical properties of some important aflatoxins and their derivatives are represented in Table 4.

Compounds of aflatoxin group are highly oxygenated heterocyclic coumarin derivatives. They consist of coumarin nucleus fused with bifuran. In aflatoxin B_1 there is a pentanone structure which is replaced by a six member lactone in aflatoxin G_1. More than two dozen aflatoxins and their derivatives are known but the major members of this group are aflatoxin B_1, B_2, G_1 and G_2. Of the four aflatoxins, B_1 is found in highest concentration followed by G_1, B_2 and G_2. Some of the derivatives of these aflatoxins include aflatoxin M_1 and M_2, B_{2A} and G_{2A} which have been shown to be produced in minor amounts by the toxigenic strains of *A. flavus* and *A. parasiticus*. Aflatoxin B_2 is dihydroderivative of aflatoxin B_1. Aflatoxin G_1 exhibits a green fluorescence under high wavelength ultraviolet light and contains coumarin nucleus linked to bifuran and a six member lactone. Aflatoxin G_2 is hydroxylated derivative of aflatoxin G_1. Allcroft (1969) showed that lactating animals on ingestion of aflatoxin B_1 secreted aflatoxin in their milk which was later designated as M_1. Aflatoxin M_1 is 4-hydroxylated derivative of aflatoxin B_1. Another milk toxin M_2 was also isolated from crude extract of fungal culture. It is produced from catalytic hydrogenation of aflatoxin M_1.

Aflatoxin B_1 and G_1 are the most potent toxins among all other toxins of this group. Other aflatoxins seem to be less important as their effects are less. The structural formulae of some major aflatoxins are illustrated in Fig. 1.

Biosynthesis of Aflatoxins

Aflatoxin biosynthetic pathway consists of at least eighteen-step multi-enzyme conversion initiated by polypeptide synthesis from acetate. The general accepted pathway for aflatoxin B_1 formation is as follows:

Acetate→Polyketide→Norsolorinic acid (NOR)→ Averantin (AVN)→Hydroxyaverantin (HAVN)→ Averufanin (AVNN)→Averufin (AVF) →Hydroxyversicolorene (HVN)→ Versiconalhemiacetal acetate (VH)→VersicolorinA (VERA →Dimethylsterigmatocytin (DMST)→Sterigmatocytin (ST)→O-methylsterigmatocystin (OMST)→Aflatoxin B_1.

Table 1 : Major mycotoxins, their producer fungi and principal biological effects

Mycotoxins	Main Producer Fungi	Principal Biological Effects
Aflatoxins	*Aspergillus flavus, A. parasiticus*	Hepatotoxic, Carcinogenic, Teratogenic, Mutagenic, Immuno-suppressive
Sterigmatocystin	*A.versicolor*	Carcinogenic
Ochratoxins	*A.ochraceus*	Nephrotoxic, Immuno-suppressive
Citrinin	*Penicillium citrinum*	Nephrotoxic
Patulin	*P. patulum*	Death of cattle, Haemorrhage in brain and lungs
Citreo- viridian	*P. citreo- viride*	Nephrotoxic
Trichothecenes	*Fusarium tricinctum, F. solani, F. equiseti*	Dermal necrosis, Haemorrhage
Zearalenone	*F. graminearum*	Vulvovaginitis, Abortion
Moniliformin	*F. moniliforme*	Cell death
Fumonisins	*F. moniliforme*	Carcinogenic
Alternaria toxins	*Alternaria* sp.	Teratogenic

In this pathway norsolorinic acid (NOR) is the first stable state intermediate. The conversion of sterigmatocystin (ST) to OMST and OMST to aflatoxin, which represent the final steps of the pathway are unique to the aflatoxin producing fungi *A. flavus* and *A. parasiticus*. Some of the enzymes involved in aflatoxin biosynthesis have been characterized and their respective genes have been cloned. These include *pksA, pksL1, fas1A, nor1, norA, avf1, vbs, ver1, stcP, omtA, ord1, avnA* and *aflR* genes which code for a regulatory factor (AFLR) that activates the transcription of these pathway genes. Studies reveal that all the identified genes related to aflatoxin biosynthesis are lo-

cated within 75-kb DNA region in both *A. parasiticus* and *A. flavus* and their relative position in the cluster of both fungal species are similar (Yu *et al.*, 1995).

Natural occurrence of Aflatoxins in Food and Feed

The problem of aflatoxin contamination of food and feed is unavoidable. No part of the world can be considered to be aflatoxin free zone due to the movement of various foodstuffs from one part of the globe to the other. The risk is, however, most acute in developing countries where it is not possible to discard mouldy foods and feet from the food chain particularly among the poor section.

Almost all the food and feed commodities are prone to invasion by aflatoxin producing fungi and subsequent aflatoxin production. The level of contamination, however, varies with the nature of crop, prevailing agronomic practices and the susceptibility of the plants to fungal invasion during different phases of growth, storage and/or processing. The commodities, which have been shown to be contaminated naturally with aflatoxins, include groundnut and its products, maize, wheat, rice, sorghum, barley, oats, soybean, sunflower seeds, cotton seed and their products, pulses, cheese etc. (Jelinek, 1987). Maize, groundnut, rice, cotton seed and some millets are categorized as high risk crops on the basis of the periods of natural occurrence and level of aflatoxins from different parts of India (Bilgrami, 1996). A number of reviews on this aspects have been published by different parts of the world (Bilgrami and Sinha, 1984; Pohland and Wood, 1987; Frisvad, 1995).

In India also there are number of reports on the natural occurrence of aflatoxin contamination from different parts of the country. A wide range of food commodities including cereals, pulses, oil seeds, dry fruits, spice, fruits, vegetables, milk and milk products, poultry and cattle feed etc. are known to be adversely affected.

Maize or corn (*Zea mays* L.) is one of the major cereal crops of worldwide importance and traded as food, feed in several countries including India. In this country maize grown in 6.59 million hectares (ha) of land (1.4% of the world) and its production is more than 13.30 million tons. Particularly in Bihar state maize is cultivated under 0.60 M ha of land and the total production is less than 2.00 million tons. After UP, Bihar is the 2nd largest maize cultivating state. Maize is grown in this country in a wide range of soil texture ranging from plains to the hilly regions, and environment extending from extreme semi-arid to sub-humid/humid conditions. It is also grown in all the seasons of the year because of its wide adaptability under diverse rainfall, temperature and all types of soil from alluvial of the Indo-Gangetic plains to heavy clay. Organic or chemical fertilizers are added to the needs of the soil. Several varieties of maize, like Kanchan, Sweta, Popcorn, Hemant, Devki, Suwan, Ganga, Shaktiman, Surya, Laxmi and Local etc. are cultivated in Bihar state both in rainy and winter seasons on commercial scale. The crop is harvested when the sheath turns brownish and grains become fairly dry and hard. After removing the grains from the cob, precaution is taken by the farmers to ensure perfect dryness of the grains under regular exposure of sunlight.

Maize yields during the winter season are higher than yields during the rainy season. On an average, the difference is up to 1.0 t/ha for hybrids and 0.5 t/ha for local varieties. Bihar, where about 32% of total maize growing fields are occupied by hybrids, achieve considerably higher yields ranging between 5.5 and 6.5 t/ha in Munger district, 6.0 to 7.0 t/ha in Siwan district, and 6.0 to 9.0 t/ha in Begusarai district, during the winter season where maize crop enjoys a favourable environment of cooler temperatures and higher solar radiation and is less affected by insect pests. In Bihar, winter maize is usually cultivated on diara lands, which are river flood plains and considered most fertile. These lands are used for cultivation of winter crop, after the river water is receded.

India being a tropical country has great diversity in agro-climatic conditions which adversely affect the standing crops as well as stored food materials by favouring the growth of moulds and production of various types of mycotoxins. Natural contamination of mycotoxins in agricultural commodities is a worldwide problem but it is greater in tropics than temperate zone (Bhat *et al.,* 1978; Sinha, 1990). About 25% of the food crops are being affected by different mycotoxins of which aflatoxins have attracted great attention throughout the world. The variable agro-climatic conditions i.e., unseasonal rains, severity of floods or droughts, warm and humid weather, traditional farming and storage

Table 2 : Target organs of mycotoxins

System/Organs	Mycotoxins
Digestive organs: Liver, Bile duct, Gall bladder, Oesophagus, Stomach, Intestine	Aflatoxins, Sterigmatocystin, Ochratoxins, Patulin, Yellowed rice toxins
Urinary organs: Kidney, Uterus	Aflatoxins, Sterigmatocystin, Ochratoxin A, Citrinin, Trichothecenes
Hematopoietic/Circulatory organs: Heart, Bone marrow, Spleen, Nymph nodes (Immune suppression)	Aflatoxin B_1, Ochratoxin A, Trichothecenes
Skin	Aflatoxins, Patulin, Trichothecenes
Reproductive organs: *Female* (Ovary, Uterus, Vulva & Mammary glands)	Zearalenone
Male (Testes), Embryo toxic	Aflatoxins
Nervous system	Aflatoxin B_1, Patulin, Yellowed rice toxins
Respiratory organs Lungs	Patulin, Trichothecenes

systems of Bihar state make maize seeds more vulnerable to *Aspergillus flavus* contamination and aflatoxin elaboration. *A. flavus* may parasitically colonize silks and invade maturing corn kernels in the field producing aflatoxins (Payne *et al.*, 1988).

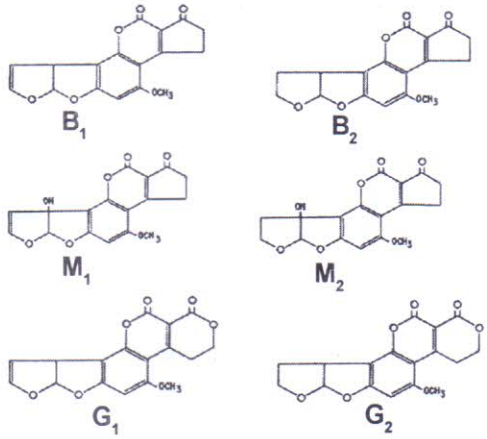

Fig. 1 : Structural formulae of some major aflatoxins

Thus, aflatoxigenic fungi may infect the crop prior to harvest and remain present even during storage and which finally appears in corn/corn products. In Bihar maize is cultivated and consumed on large scale by the weaker section of the society but only fragmentary reports are available on aflatoxin elaboration in maize varieties and their consequential detrimental effect on consumers (Bilgrami *et al.*, 1980, 1982).

Aflatoxin contamination has been reported in this country in maize (Krishnamachari *et al.*, 1975; Bilgrami *et al.*,1983; Sinha, 1980, 1983, 1987, 1990; Sinha and Ranjan, 1990; Mukherjee and Nandi, 2001), wheat (Prasad *et al.*, 1987; Jeswal, 1986; Sinha and Sinha, 1990), paddy and rice (Sreenivasamurthy, 1975; Prasad *et al.*, 1986; Sinha and Ranjan, 1990), oil-seeds like groundnut, mustard, cottonseed (Kumar and Singh, 1991; Kumar, 1993; Ahmad and Sinha, 2002), dry fruits and spices (Singh, 1983; Bilgrami, 1985;), vegetables (Sinha and Singh, 1982), milk and milk products (Sinha and Ranjan, 1990), cattle/poultry feed (Sinha *et al.*, 1999; Thirumala Devi *et al.*, 2002) and drug plants (Roy *et al.*, 1988).

Table 3 : Important groups of mycotoxins based on target organs

A) Hepatotoxins	Aflatoxins Sporidesmin Luteoskyrin Cyclochlorotine Rubratoxins Sterigmatocystin
B) Nephrotoxins	Ochratoxins Citrinin
C) Neurotoxins	Penitrem Patulin Citreoviridin Ergots
D) Cytotoxins (Alimentary Tract Toxins)	Trichothecenes T-2 toxins Diacetoxyscripenol Neosolaniol Nivalenol Deoxynivalenol(DON, vomitoxin) HT – 2 toxin
E) Estrogenic mycotoxins	F – 2 toxin (Zearalenone)

Toxic Effects of Aflatoxins

Aflatoxins have received extensive attention as the possible causative agents due to their established potential to cause adverse health effects when ingested with contaminated food stuffs. Diseases caused by the consumption of aflatoxin contaminated food and feed is known as Aflatoxicoses. A massive literature is available on the toxic effects of aflatoxins in animal systems. Aflatoxin poisoning is reported from all parts of the world in almost all the domestic and non-domestic animals like cattle, horses, rabbits and other primates. Aflatoxicosis is also reported in humans in many parts of the world.

Table 4 : Aflatoxins and their physico-chemical properties

Aflatoxin	Mole. Formula	Mole. Weight	Melting Point(°C)	UV Absorp. (362 nm)	Fluoresc. Emission
B_1	$C_{17}H_{12}O_6$	312	268-269	21,800	425
B_2	$C_{17}H_{14}O_6$	314	286-289	23,400	425
G_1	$C_{17}H_{12}O_7$	328	244-246	16,000	450
G_2	$C_{17}H_{14}O_7$	330	237-240	21,000	450
M_1	$C_{17}H_{12}O_7$	328	299	19,000 (357nm)	425
M_2	$C_{17}H_{14}O_7$	330	293	--	--
B_{2A}	$C_{17}H_{14}O_7$	330	240	20,400	--
G_{2A}	$C_{17}H_{14}O_8$	346	190	18,000	--

After wide experimentation on animal species, aflatoxins especially aflatoxin B_1 has been confirmed as a potential carcinogen (IARC, 1993). Metabolism plays a major role in deciding the degree of toxicity (Eaton *et al.*, 1994). After ingestion, aflatoxin is metabolized by cytochrome p450 group of enzymes in liver, where it is converted to many

Table 5 : The acute LD_{50} of aflatoxin B_1(mg/Kg body weight) for domestic and experimental animals (FAO web library)

Rabbit	0.30
Duckling(11 day old)	0.43
Cat	0.55
Pig	0.60
Dog	0.50-1.0
Sheep	1.00-2.00
Guinea pig	1.40-2.00
Baboon	2.00
Chicken	6.30
Rat(female)	17.9
Rat(male)	16.00
Macaque	7.8
Mouse	9.00
Hamster	10.20

metabolic products like aflatoxicol, aflatoxin Q_1, aflatoxin P_1 and aflatoxin M_1, depending on the genetic predisposition of the species. Alongwith the above chemicals another metabolite, aflatoxin 8, 9 epoxide is also formed. The amount of this metabolite decides species susceptibility as it can induce mutations by intercalating into DNA, by forming an adduct with guanine moiety in the DNA (Smela and Curier, 2001). This intercalation of epoxide causes GAT transversion at codon in p53 gene in liver, which may lead to hepatic carcinoma. This was observed in most of the experimental models and it is presumed that this is the major reason for aflatoxin carcinogenicity (Katherine *et al.*, 1997). Moreover, species susceptibility to aflatoxin mainly depends on its liver detoxification systems, genetic makeup, age and other nutritional factors (Ramdell and Eaton, 1990).

Sensitivity toward aflatoxin varies with species, age and sex of animals as well as the composition of diet and route of ingestion. The effect of aflatoxin exposure may be acute or chronic depending primarily on the test system, dosage and frequency of exposure (Coker *et al.*, 1984).

Exposures to aflatoxin B_1 manifestation include mutagenicity, hepatotoxicity, immune-suppression, lung injury and birth defect (teratogenic) to domestic animals, monkey and laboratory animals. Aflatoxin induces a variety of symptoms in affected animals. Acute toxicity is expressed as a death of the animal within a limited time in which liver becomes generally pale and discoloured and increases in size (Moreau, 1979).There is a wide range of acute toxicity level of aflatoxin B_1 expressed as single lethal dose LD_{50}, ranging from 0.3 mg/Kg for rabbits to17.9 mg/Kg for mature female rat (Table 5). Aflatoxin B_1 is a potent liver carcinogen in a variety of experimental animals. It causes liver tumours in mice, rats, fish, hamster, monkeys etc. following administration by various routes (IARC, 1976).

Aflatoxins and Human Health

Scientific literature is replete with studies addressing indoor mould and aflatoxicosis in human beings. Evidence of acute and chronic aflatoxicosis in humans has been reported in many parts of the world especially Third World countries, like Taiwan, Uganda, India and many others. In African and Asian countries the threat to human health from aflatoxin is very high as environmental conditions in these countries favour aflatoxin contamination. The epidemic in Kenya has resulted in 125 deaths out of over 500 recognized cases in 1974. World Health Organization and Kenya Ministry of Health (KMOH) discovered in their investigation an outbreak of jaundice with a high fatality rate in the districts of Makueni and Kitui in the Eastern Province of Kenya. In the same year India too witnessed a major outbreak of severe aflatoxicosis in western part where the acute hepatitis among tribal of Rajasthan and Gujrat revealed the consumption of aflatoxin contaminated corn by the patients (Kishnamachari *et al.*, 1975). It resulted in 397 reported cases and 106 deaths. The syndrome was often characterized by jaundice, rapidly developing ascites, pulmonary oedema, portal hypertension and high mortality rate. Death usually occurred from excessive gastrointestinal haemorrhage. The heavily contaminated maize grain contained afla-

toxin at concentration of 6.25-15.6ppm and the average daily intake was 1-6 ìg of aflatoxin (Aflatoxins, National Library of Medicines, 2002).

The report revealed that children exposed to aflatoxin through breast milk might fell into a condition known as Indian Childhood Cirrhosis (ICC). Antibodies of aflatoxin B_1 have been reported in humans, however, antibodies are considered indicative of exposure and may or may not be related to disease.

Effect of Aflatoxins on Plant System

Phytotoxic effects of aflatoxin have been shown on various plant groups, including higher plants, ferns, algae, fungi and bacteria (Reiss, 1978; Chohan, 1983; Sinha, 1996).

Aflatoxins induced inhibition of germination and seedling growth has been reported in many crop plants. Schoental and White (1965) showed considerable inhibition of elongation of the hypocotyls and roots in *Lepidium sativum* after the treatment with crude aflatoxin. *L. sativum* was the most susceptible plant studied and exhibited the maximal inhibitory response above at concentrations of 8 ìg aflatoxin/ml. Aflatoxin was also found to inhibit germination and seedling growth in many cultivars of lettuce (*Lactuca sativa*), cowpea, sorghum , maize, wheat (Sinha, 1991), mung (Sinha and Kumari, 1989), gram (Kumar, 1993) and mustard (Sinha *et al.*, 1992; Ahmad and Sinha, 2002). Chohan and Gupta (1968) reported a new disease of groundnut seedling (Aflaroot disease) induced by the toxigenic strain of *Aspergillus flavus*.

Besides seed germination and seedling growth aflatoxins also inhibit chlorophyll synthesis of newly emerged leaves of the affected plants (Kang, 1970). Aflatoxins inhibit chlorophyll synthesis and produce virescence or albinism in affected parts. Slowatizky *et al.* (1969) demonstrated inhibition of grana formation in chloroplast while Jackquet *et al.* (1971) observed disintegration of chloroplasts in *Nasturitium officinale*. Chohan (1983) suggested that aflatoxin blocks the syntheses of chlorophyll by inhibiting growth hormone induced synthesis of protein in the leaf.

Aflatoxin B_1 influences respiratory rates of maize seeds during germination and inhibits the gibberellic acid stimulated synthesis of á-amylase and lipase in cotton seed .Aflatoxin B_1 suppressed the syntheses of RNA, DNA and protein in germinating seeds of maize (Sinha and Kumari, 1989; Prasad *et al.*, 1997; Prasad, 1998), mung (Sinha and Kumari, 1989a), gram and mustard (Kumar, 1993; Sinha, 1996) as well as wheat (Sinha and Sinha, 1993). Respiratory Quotient (R.Q.) values of germinating seeds of maize (Prasad, 1993), gram and mustard (Kumar, 1993) have also been influenced by aflatoxin B_1.

Aflatoxin B_1 has also been found to induce cytological abnormalities in treated cells. Reiss (1995) found chromosome bridges, c-mitosis and reduction of mitotic index due to aflatoxin poisoning.

Control of Aflatoxins

The massive economic implications of the aflatoxin problems and its potential health threat to humans have clearly created a need to eliminate or at least control aflatoxin contamination of food and feed. Many of the strategies used for other commodities can generally also be applied to maize. The three basic approaches, which can be adopted to alleviate this problem, include :

- Prevention of the initial growth of mould and subsequent contamination by aflatoxins.
- Removal of contaminated portion, and
- Inactivation or detoxification of aflatoxin by chemical, physical and/or biological methods.

Prevention

Prevention is supposed to be the best approach to alleviate aflatoxin problem. Pre-harvest prevention of aflatoxin contamination is the best and most widely explored strategy, since *A. flavus* infects all the affected crops prior to harvest.

The step of prevention should initially be carried out before the fungal infestation and aflatoxin contamination in pre-and post-harvest conditions and inhibition of fungal growth can be achieved by physical, chemical and biological treatments (FAO, 1979).

Prevention or reduction in the incidence of pre-and post-harvest infection is the critical factor in reducing aflatoxin accumulation since concentration of aflatoxin has shown to be greater symptomatic that non-symptomatic in maize ears or kernels. Environmental factors that favour *A. flavus* infection include high soil or air temperatures, drought stress, nitrogen stress, crowding of plants and con-

dition that aid dispersal of conidia . The growth of *A. flavus* and *A. parasiticus* and subsequent aflatoxin production in storage are favoured by high humidity (>85%), high temperature (>25°C) and insect or rodent activity (CAST, 1989). Tillage practices, crop rotation, weed control, late seasonal rainfall, wind and post vectors all affect the amount and sources of fungal inoculum that maintain the disease in maize.

Any action that interrupts the cycle reduces the probability of silk and kernel infection. So the effective strategy for pre-harvest control of aflatoxin may include:

- Reduction in plant stress through irrigation, mineral nutrition protection from insect damage.
- Avoidance of environmental conditions that favour infection in the field.
- Minimization of inoculum in the field.

One of the potential effective pre-harvest strategies is breeding of maize cultivars for resistant to fungal infection and use of antifungal chemicals for crop protection. Breeding of resistance against toxin production has met with limited success. Fungal resistance is genotypic. Resistance to invasion of *A. flavus* has been attributed to several biochemical, environmental and physical factors. Maize cultivars that are resistant to aflatoxin production have been reported (Windham and Williams, 1998). Varietal resistance has also been studied in Indian maize (Nagrajan and Bhat, 1972; Priyadarshini and Tulpule, 1978; Bilgrami *et al.*, 1982; Chourasia, 2001), groundnut (Nagrajan *et al.*, 1973), sunflower (Nagrajan *et al.*, 1974), paddy (Prasad and Jeswal, 1987) and wheat (Sinha, 1991) etc. Some reports are also available to show the relationship with the sources of resistance in maize seed towards aflatoxin production. Kumar *et al.* (2001) have established relationship between phenolic content of maize kernel with that of resistance to *A. flavus*.

The use of chemicals, fungicides and antifungal chemicals is a very attractive strategy to prevent aflatoxin production. Some chemical treatments prevent mould growth and potentially reduce aflatoxin production in the field and in storage. Use of organic acids such as acetic acid, propionic acid and butyric acid , benzoic acid, lactic acid as well as citric acid and their sodium salts has been proved to reduce mycotoxins. Besides that, sodium chloride, benzoic acid derivatives like *O*-nitrobenzoate, *O*-aminobenzoate, paminobenzoate have also shown significant responses towards mycotoxin reduction in crops. Fumigants like ammonia and phosphine have been used as grain preservatives against aflatoxin production. But before suggesting any of the compounds, the residual effects on the consumer population are required to be evaluated (Bilgrami, 1996). However, with regard to maize and mycotoxins, the economic and ecological hurdles seem to be quite high because few of these approaches are being marketed widely.

In the recent years, considerable interest has been shown towards the study of inhibitory effects of plant extracts or natural plant products against the growth of aflatoxin producing fungi and aflatoxin production. Aqueous extracts of several plants *viz.*, *Adiantum* sp., *Azadiracta indica, Euphorbia hirta, Riccinus communis, Thysonolaena maxima, Lawsonia alba, Artemisia indica, Streblus asper, Xanthium pungens* etc. have been reported to inhibit aflatoxin production by *A. flavus* and *A. parasiticus* (Bilgrami *et al.*, 1979, 1992; Sinha, 1985, 1990; Singh and Sinha, 1985, 1986). Allicin and related substances from garlic and onion extracts, cinnamon extracts like trans-cinnamic acid, trans-cinnamaldehyde and ferulic acid as well as clove oil have also been reported to inhibit growth of *A. flavus*. Some essential oils were also found to inhibit *A. flavus* growth .

Decontamination

Contamination of aflatoxins in foods and feeds can be removed, inactivated or detoxified by physical, chemical and biological means depending on the conditions. However, the treatment has its own limitations, since the treated products should be health safe from the chemicals used and should retain their essential nutritive value. Hand picking or photoelectric detecting machines can remove physically fungal infested seeds. The principle is based on the identification of damaged kernels in the seed lots because of variation in size, shape, colour and more often mould growth on the affected kernels. It is because aflatoxin contaminated kernels are usually damaged, shrivelled or discoloured. Therefore, combination of sieving and electronic sorting can be helpful in eliminating most of the undesired kernels and leave the remaining nuts virtually free

from aflatoxins (Ciegler, 1976). Belt separation, shelling colour sorting and blanching can be other effective means of removal of contaminated seeds. Mechanical sorting on the basis of the energy reflected from the particles illuminated by UV light has also been found to be effective. Aflatoxin in crude peanut oil can be separated by filtration. Basappa and Sreenivasmurthy (1979) developed a special filter pad system which can easily be adopted in oil mills to remove aflatoxins from crude oil. Milling has also been found successful in reducing the levels of aflatoxin and other mycotoxins (Basappa and Sreenivasmurthy, 1974).

Aflatoxins can also be removed chemically by extraction of contaminated commodities with suitable solvents (Sinha, 1998). Organic solvents (chloroform, acetone, hexane and methanol) have been used to extract aflatoxins from agricultural products. However, application of these methods may have several limitations in the form of high cost of additional equipment and material, formation of undesirable derivatives in the extracted meal and loss of nutrients from the raw materials.

Inactivation of Toxins or Detoxification

Detoxification strategies are primarily dependent on physical, chemical and biological means depending on the conditions that detoxify by destroying, modifying or absorbing the mycotoxins, so as the reduce or eliminate the toxic effects.
Physical methods include thermal inactivation, light irradiation etc. (Sinha, 1998). Heating and cooking under pressure can destroy nearly 70% of aflatoxin in rice. Conventional processing of food like cooking, roasting, frying, spray drying, baking etc. has also been shown to destroy aflatoxins present in the food. Detoxification of aflatoxins from contaminated food has been reviewed by Basappa (1983), Basappa and Shantha (1996) and Sinha (1998).

Chemical treatment has been used as the most effective means for the removal of mycotoxins from contaminated commodities. The method should ensure that the detoxification system is capable of converting the toxin to a non-toxic derivatives without deleterious changes in the raw product. Several chemicals have been tested for their effectiveness in detoxification of aflatoxins. These include acetic acid, ammonia gas or ammonium salts, calcium hydroxide, gases like chlorine, sulphur dioxide, ozone, hydrogen peroxide, sodium bisulfite, methylamine etc. (Sinha, 1996).

Although some of the above mentioned chemicals are known to destroy aflatoxins, these can also decrease the nutritive value of the processed material or produce toxic products or products having undesirable side effects. Therefore, use of these chemicals should be done very cautiously. Detoxification of aflatoxin can also be achieved by biological methods (CAST, 1989). Ciegler (1976) and Bhatnagar *et al.* (1991) screened several microorganisms against aflatoxin production and found *Flavobacterium aurantiacum* of being capable of removing aflatoxin from the liquid medium.

The biological control method for aflatoxin can also increase crop safety by decreasing toxin content and that is based on the displacement of toxigenic isolates using atoxigenic isolates of the same species. Bio-control of aflatoxin producing strains with atoxigenic strains of *A. flavus* has been developed for corn, cottonseed, peanuts, rice kernel and wheat seeds (Cotty, 1994).

Filamentous fungus *Trichoderma* sp. has been accepted as the most potent biological control agent for fungal disease. *T. viride* was also found to inhibit production of aflatoxin B_1 (73.5%) and aflatoxin G_1 (100%) when cultured with *A. flavus* together (Choudhary, 1992).

Besides fungal antagonism, bacteria like *Azospirillum* sp. have also been reported to degrade aflatoxin B_1 production by *A. flavus* by 95%. Bacteria like *Bacillus subtilis* and *Streptococcus lactis* have the ability to inhibit aflatoxin when grown along with *A .flavus* in the medium.

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