Mycotoxins

Toxins are highly specific poisonous substances produced by some micro-organisms. The toxins produced by Fungi are called mycotoxins. Mycotoxicosis is the term applied to any disease that results from consumption of food contaminated by fungal toxin.

Mycotoxins can be produced in food as a result of fungal growth and eaten in low concentration by vertebrate animals, will produce toxic effects. The particular toxic effects vary widely, ranging from food refusal and vomiting in farm animals to cancer in human. Mycotoxins can be found in a wide variety of food derived from plants including cereals, nuts, fruits and vegetables, because of their high moisture content and nutrient level.

More than 100 species of filamentous fungi are known to produce mycotoxins and to cause toxic effects under naturally occurring conditions. One of the most important of them is the aflatoxin.

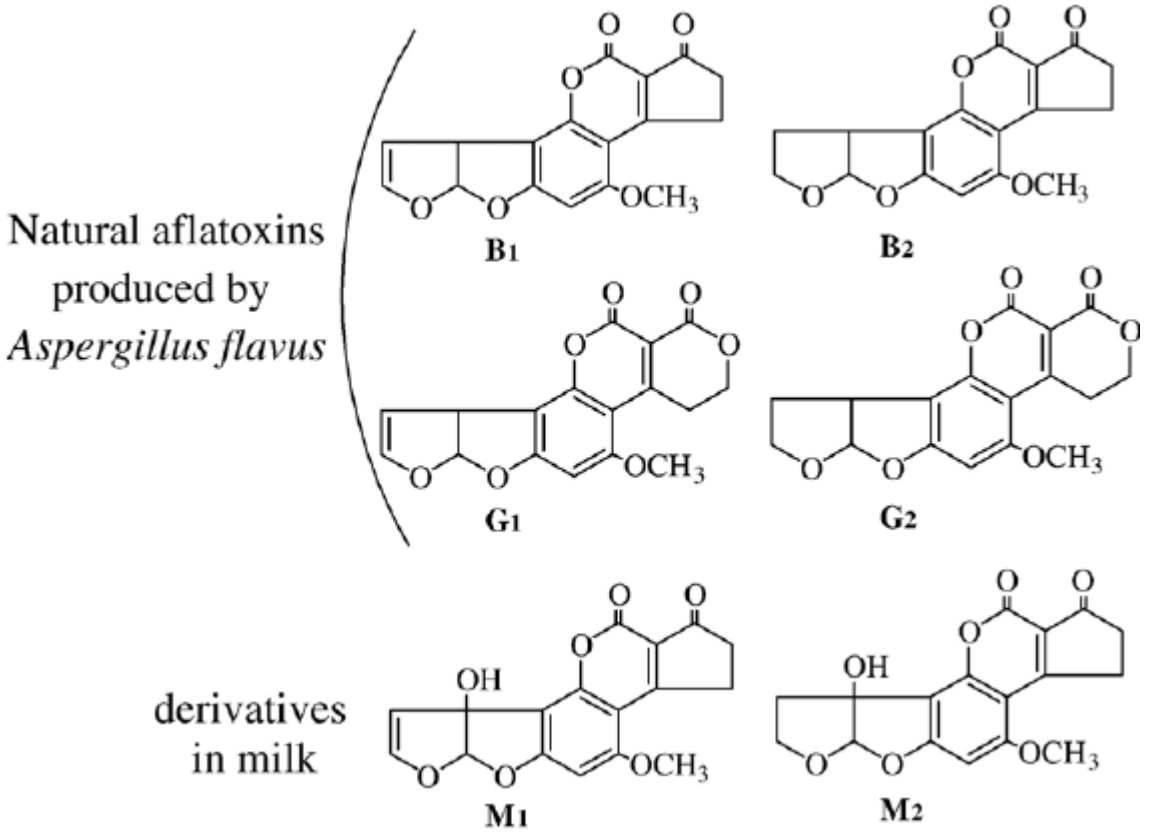
Aflatoxin

Aflatoxins are potent toxic, carcinogenic, mutagenic, immunosuppressive agent produced as secondary metabolised by the fungi on variety of food products. The aflatoxin problem was first recognised in 1960, when there was a severe outbreak of a disease referred as Turkey-X disease in UK in which over 100,000 Turkey birds died. They were found to consume groundnut meal infested with *Aspergillus flavus, A. parasiticus, A. fumigatus* etc.

Aflatoxin normally refers to the group of difuranocoumarins and are classified in two broad groups according to their chemical structure- the difuranocoumarocyclopentano series and the difuranocoumaroacetone series. They intensely fluoresce under UV rays (360-365 nm). Aflatoxin B1 and B2 produce the blue fluorescence while G1 & G2 produce a green fluorescence. Among the 18 types of Aflatoxins identified B1, B2, G1 & G2 are majorly found with M1 & M2, which are of less significance. Crystals of Aflatoxin are colorless to pale yellow and freely soluble in methanol, chloroform, acetone, and dimethyl sulphoxide; insoluble in non-polar solvents and partly soluble in water. Physical and chemical properties of different Aflatoxins are given below:

Table-2. Physical and chemical important properties of the Aflatoxin.

Aflatoxin	**Molecular formula**	**Molecular weight**	**Melting point**	**UV absorption max UV(ε (L $mol^{-1}cm^{-1}$)), metanol**	
				265 nm	360-362 nm
B_1	$C_{17}H_{12}O_6$	312	268-269	12400	21800
B_2	$C_{17}H_{14}O_6$	314	286-289	12100	24000
G_1	$C_{17}H_{12}O_7$	328	244-246	9600	17700
G_2	$C_{17}H_{14}O_7$	330	237-240	8200	17100



The actual growth of *A. flavus* or *A. parasiticus* on the food does not always mean that aflatoxins are present. Moisture, temperature and insect or other injury are particularly important factors in determining whether or not an *Aspergillus* isolate actually produces aflatoxin while growing on seeds or grains.Both species of *Aspergillus* commonly occur as soil saprophytes or on grains and other food in storage. Under particular conditions these fungi can also live parasitically on growing plants.*A. flavus* can infect the seed or grain of peanuts, cottonseeds, corns etc. while the plant is maturing in the field. Mycelial growth and aflatoxin production can continue after harvest so long the moisture level remain high within the seeds or grains. Aflatoxin production is favoured by very moist conditions. Aflatoxins are produced only between temperatures of 12 to 42^0c. Further, aflatoxin production is particularly encouraged in the absence of competing fungi.

Toxic effects of Afltoxin:

Aflatoxins can produce a variety of toxic effects in vertebrates. These are genomic mutations, chromosomal aberrations, developmental abnormalities after prenatal or postnatal exposure of embryos, a suspension of the immune system and cancer. The most important effect of aflatoxin B_1 consumption is the development of liver cancer in humans and domestic animals. For most animals eating a diet containing a low level of aflatoxin (about 15 parts per billion) in the food over a time period will eventually cause liver tumour. The clinical symptoms associated with AFB_I consumption include jaundice, rapidly developing ascites, portal hypertension etc. In case of human beings, it is sometimes also associated with lung, kidney and colon tumours.

The toxic effect of aflatoxin have been frequently observed in farm animals such as poultry, cattle or pigs that have been accidentally poisoned by contaminated feed. Young animals are more susceptible to aflatoxins to older ones. Again, animals fed a protein deficient diet are more sensitive to aflatoxin

than are animals fed a well-balanced diet. Symptoms of chronic poisoning are at first loss of appetite and reduction of growth. The liver becomes congested, parts of the liver may show haemorrhage or become necrotic or tumours often develop.

Various changes occur at the molecular and cellular levels to induce mutations and cancer after consumption of food contaminated with aflatoxins. It binds with DNA and prevents its transcription. As a result, protein synthesis is inhibited. Degranulation of endoplasmic reticulum is also seen in the electron photomicrographs of aflatoxin treated cells.

Aflatoxin B_I has been specifically shown to be genotoxic, that is, mutagenic carcinogen. The carcinogenic properties of AFB_I is expressed when it is activated by cytochrome P_{450} and other oxidative enzymes. The AFB_I is transformed into several products most of which are excreted through urine. But AFB_I-exo8, 9-epoxide is responsible for inciting the carcinogenesis in liver. It is shunted to liver cells by glutathione-s-transferase. The epoxide molecules intercalate between DNA base pairs and produce N^7- guanyl adduct. Similar adducts have also been detected in kidney or lung. Some of these adducts are resistant to DNA repair system and thus, bring out mutation and carcinogenesis. AFB_I induced liver tumours show p53 tumour suppressor gene mutation, which, thus, leads to carcinogenesis. Activated AFB_I can also suppress the synthesis of all types of RNA.

Control of aflatoxin production in foods:

1. Breeding of crops can be done to produce resistant varieties that can prevent the growth of the fungus or can inhibit toxin production.
2. Insecticides should be sprayed as insects carry the spores of *Aspergillus.*
3. Because aflatoxin production is favoured by moist conditions, rapid drying of food right after harvest and maintaining low levels of moisture during storage are particularly important.
4. It is important to determine the aflatoxin content before the food is processed into another form or consumed by farm animals or humans. The presence and quantity of aflatoxin can be determined in samples of food by chemical extraction and identification by chromatographic method, mass spectrometry, immunological processes, fluorescence of samples or by bioassays.
5. Molded food commodities are discarded, and if feasible, damaged grains or nuts are eliminated.
6. Treatment of foods with anhydrous ammonia actually destroys aflatoxins, making these foods safe. Ammonisation is widely practiced for corn, cottonseed and peanut meal that is intended for use of animal food.
7. Other chemical techniques that can reduce aflatoxin contamination in food include treatment with chlorine gas, gamma rays or ozone.
8. Moisture level above 15% in grains are favourable for fungal contamination and thus the grains like maize, which is often harvested at 30% moisture level should be well dried rapidly to bring it down to 15%.