Chapter 3

Mycotoxins Dangers and Prevention Methods

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Aflatoxins are mycotoxins produced by some fungi on crops such as peanuts, tree nuts, and corn. The major molds that produce aflatoxins include *Aspergillus* species commonly found in humid and warm regions of the world. Aflatoxin-producing molds can infect the production in the field, during harvest, and in storage facilities. There are various methods for the detection of aflatoxins in food and feed. Aflatoxin control is required during both pre-harvest and post-harvest treatments. Major lasting, sustainable solution to control preharvest infection of aflatoxin is via improving the capability of crops to prevent fungal infections or preventing aflatoxins productions by fungal invasion.

3.1 Introduction

The name Mycotoxin comes from the Greek language (*mykes* meaning fungus, and *toxikos* meaning poisonous). They are by-products of metabolism from toxin-producing fungi that can cause death and disease in humans and animals, and more than 400 types of mycotoxins have been discovered in different places around the world. The presence of toxins in foods has long-term effects on health, such as carcinogenic effects and immunodeficiency. Among the hundreds of mycotoxins discovered up to the present time, twelve poisons have received great attention due to their dangerous effects on human health and they are present in foods [1].

Mycotoxins are metabolic by-products produced by filamentous fungi at appropriate temperatures and humidity. Poisoning with it can occur through ingestion, inhalation, or contact through the skin, and it can be consumed directly by humans through food contaminated with toxins or through eating foods such as meat, milk, or eggs of animals that have previously fed contaminated feed [2, 3]. Many studies have shown that the organs of the body that

receive more blood are more susceptible to poisoning since the amount of blood that they receive plays a role in increasing the possibility of these organs being exposed to poisoning. Liver and kidneys are among the organs most exposed to poisoning because they are among the organs that receive large amounts of blood [2].

3.2 Mycotoxins

3.2.1 Chemical Structure

Most of the mycotoxins are aromatic hydrocarbon compounds and very rarely are open chains. Their molecular weight is lower than 1000 Dalton, and because of their low molecular weight, they are resistant to harsh environmental conditions, as well as the inability of the immune system of humans and animals to produce antibodies to them, and that is where danger lies immunologically [4, 5].

Many mycotoxins have structures from single rings with a molecular weight of at least 50 Dalton, to groups of six or eight rings with a molecular weight of 500 Dalton, and the smallness of these molecules does not cause any stimulation of the human immune system. There are neurotoxins that, in small amount, may cause continuous shivering in the animal, but in slightly higher doses, it may cause brain damage or lead to death [6]. Aflatoxins belong to the group of Difuranocoumarins and are soluble in different polar solvents such as chloroform, acetone, and methanol [7, 8]. The chemical structure of some aflatoxins are shown in Figure 3.1.

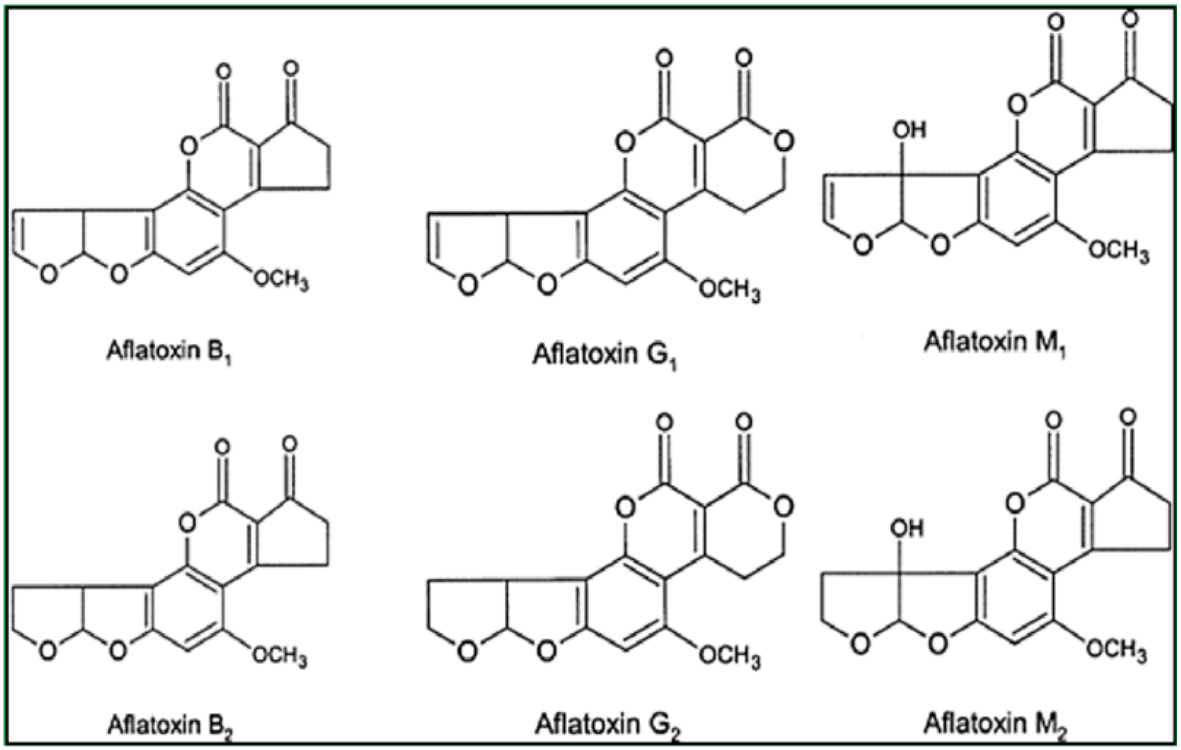

Figure 3.1: The chemical structure of aflatoxin B1, B2, G1, G2, M1, M2.

3.2.2 Nature and Properties

Mycotoxins are toxic chemical compounds that have several properties and are tasteless, odorless, and invisible [9]. Mycotoxins are resistant to high temperatures, storage, and cooking. Most of them are aromatic hydrocarbons or open-chained Aliphatic hydrocarbons, which resist decomposition within the digestive system of humans and animals [10]. It is chemically stable and does not stimulate the immune system due to its low molecular weight [11].

3.3 Aflatoxin

3.3.1 History

Mycotoxins were known throughout history through the cases of poisoning that humans were exposed to during the consumption of toxic elements, resulting in deaths. A case of a poisoned horse after ingesting feed contaminated with the fungus *Stachybotrys spp.* was reported in Ukraine in 1930 [12]. In Russia, during World War II, symptoms of poisoning and a decrease in white blood cells appeared as a result of exposure to one of the triglycerides, T2-Toxin, as well as St. Anthonys, which is caused by eating grains contaminated with stone bodies of the fungus *Claviceps purpurea* [13]. The beginning of the establishment of Mycotoxicology (officially and globally) happened after the incident of turkey excretion poisoning in England in 1960, which led to the death of 100,000 turkeys in one of the breeding farms [14, 3, 15, 16, 17].

3.3.2 Toxicity for Animals Organisms

Aflatoxins are metabolically produced by certain types of fungi and are found naturally throughout the world. In countries of the world, there are several types of aflatoxins, but B1, B2, G1, and G2 are very dangerous, as they are found in the vast majority of the main foods included in the human diet [18]. Aflatoxins are one of the most dangerous and toxic toxins and are produced by some types of *Aspergillus* such as *A. flavus* and *A. niger*, which are found in soil and plant residues, grains, etc., and are widespread. It has also been proven that aflatoxins cause mutations in the genetic system, which causes damage to nucleic acids [19, 20, 21]. Approximately 4.5 billion people in developing countries are exposed to aflatoxins through foods that are not subject to health and safety control conditions, which has led many countries to put in place regulations on the levels allowed for their presence in food and feed, and these limits vary in different countries. Concentrations in many countries ranged between 1-20 micrograms and an average of 5 micrograms per kilogram for aflatoxin B1, as for total aflatoxin 0-35 micrograms and an average of 10 micrograms per kilogram, while the United States was set at 20 micrograms per kilogram grams of total aflatoxin in all foodstuffs [22].

AFB1 is the most common and frequently present and has been classified by the International Agency for Research on Cancer in sequence I group A as a liver and bile duct carcinogen (IARC 2019). AFB1 is metabolized to an intermediate complex, AFB1-8,9 epoxide, and then converted to other compounds by changing the DNA and changing the positions

of the nitrogenous bases from T to G at codon 294 in p53, which is responsible for carcinogenic effects in the liver and kidneys [23]. Cancer resulting from exposure to aflatoxins B1 and G1 occurs due to the metabolic activation of the oxidative double bond 8-9 epoxide reaction, and the different animal species in the metabolism have a role in the different degrees of toxicity [24]. Aflatoxin B1 causes a wide range of harmful effects on humans and various animals, especially poultry, such as liver damage, poor reproductive efficiency and productivity, lack of eggs, and others [25]. When aflatoxins B1 and B2 reach lactating young cattle by eating contaminated feed, approximately 1.5% of it is added to a hydroxyl group and is excreted in milk in the form of M1 and M2, and they are compounds with toxicity that are less than B1 and B2, so many countries have set limits for aflatoxin in Feed [26]. AFB1 and AFM1 are carcinogens and hepatotoxins, AFM1 are the only toxins for which the maximum residue limits (MRL) in milk and milk products [27] are set by the World Health Organization (WHO). During pasteurization of milk or its derivatives [28], it can cause cancer of the liver, lungs, and colon [29]. Reports indicate that 43.9% of cases of liver cancer in Bangladesh are related to eating food contaminated with AF toxins [30].

Toxic problems are very many and widespread, especially in low-income countries as well as developing countries. Their presence may be very common [31], resulting in contamination of breast milk [32] and infant milk [33], therefore studies and research on mycotoxins have become very necessary [34] to maintain and protect human health [35]. [36] mentioned that B1 toxin causes the death of half the number of mice (LD50) at a dose of 9 mg/kg, while Ochratoxin A caused the same effect, but at a dose of 22 mg/kg, and this confirms that the aflatoxin poison is more toxic than OCH. Aflatoxins are ring structures that have unique and heterogeneous high-oxygen shapes [37]. The two main types were named B and G, as a result of their fluorescence in green and blue under ultraviolet rays. This fluorescence represents the basis of the work of aflatoxin detection techniques. The chemical structure of aflatoxins is not very different, but there is a difference in the degrees of their toxicity. Its chemical structure consists of a nucleus of coumarin and two furan rings. In group B there is a five-sided ring, while group G contains a lactone ring, as one of the most important properties of aflatoxin is its ability to fluorescence. Which is important in detecting and determining its low concentrations, especially when UV rays are shed on it. The toxic substance was separated by Thin Layer Chromatography (TLC) method, and it was found that it is composed of two spots, each with a different glowing color and a rate of flow, the first being a blue glow. Violet and Rf (0.6) which is aflatoxin B and the second with a green glow and Rf (0.4) which is aflatoxin G [38].

The physicochemical properties of aflatoxins are presented in Table 3.1

Table 3.1: Physicochemical properties of aflatoxins.

Toxin	Molecular structure	Molecular weight (KD)	UV	Rf
B_1	$C_{17}H_{12}O_6$	312	Blue	0.56
B_2	$C_{17}H_{14}O_6$	314	Blue	0.53
G_1	$C_{17}H_{12}O_7$	328	Green	0.48
G_2	$C_{17}H_{14}O_7$	330	Green	0.46
M_1	$C_{17}H_{12}O_7$	328	Blue	-
M_2	$C_{17}H_{14}O_7$	330	Blue	-

Aflatoxin is the cause of liver, gastric, and colon cancer, with a median lethal dose of aflatoxin B1 of 0.36 mg/kg body weight [39]. [40] mentioned that consumption of aflatoxins may be toxic in several ways (Figure 3.2). Aflatoxins can be metabolized in the liver and converted to AF-8,9-epoxied, which can bind to liver proteins to render them ineffective or bind to DNA that induces hepatocellular carcinoma and can also be associated with the hepatitis B virus. Viral and synergistic effects can lead to a significant increase in liver risk. In addition, it can modulate the expression of cytokines that can compromise the immune system or gut integrity, resulting in stunted growth in children. Acute toxicity is characterized by acute liver injury, hemorrhage, high fever, vomiting, dark urine, jaundice, and rapid progression to ascites, edema, and death in humans [41]

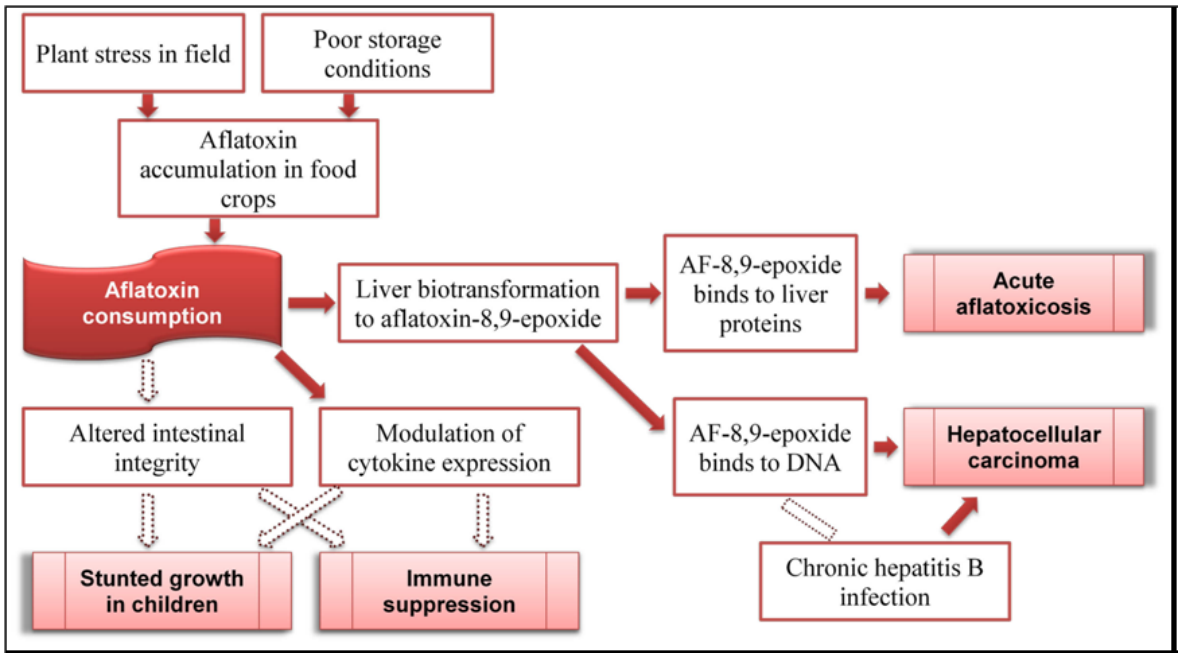

Figure 3.2: Aflatoxin and Disease Pathway in Humans.

3.4 Dangers of the Presence of Mycotoxins in Food

The economic losses resulting from a decrease in productivity due to mycotoxins are estimated at nearly USD 1.5 billion annually, due to only three mycotoxins, namely aflatoxin, fumonisin, and triglycerides [42]. It is possible to contaminate human food with mycotoxins at certain and different stages in the food chain [43]. Approximately 25% of grain production annually is contaminated with mycotoxins, while more than 800 million people face the problem of poverty and hunger around the world, according to reports of the World Food Program [44]. Researchers have identified many mycotoxins, and the most common, harmful, and worrying to the health of living organisms are aflatoxin, patulin, ochratoxin, piomenzin, zearalenone, and methanol. These appear due to the contamination of crops with the fungi that make them up before harvest, during germination, or after harvesting the crops as they are transmitted through the food chain [45].

Consuming food contaminated with mycotoxins results in acute and chronic diseases in humans and animals in general. Aflatoxins have been found in the livers of African children with Kwashiorkor disease (malnutrition and protein deficiency), as well as in Indonesia, where nearly 20,000 deaths from liver cancers are recorded annually due to eating food contaminated with aflatoxin [46, 47]. Fungi change both the chemical and physical properties of the rotting materials, changing the taste (rancidity, bitterness), appearance (black mold colors, etc.), composition and nutritional value, such as vitamin A deficiency, and the decomposition and oxidation of fats [48, 49]. The synergistic effect of the combined toxins causes a greater effect than if they act alone, as many cases of synergism have been studied, for example, fumonisin with aflatoxin B1 and DON with fumonisin, and aflatoxin B1 with aflatoxin B2 [50, 51].

Mycotoxins are of great concern in human foods, as the high levels of mycotoxins recorded in foods, blood samples, and urine of people in developing countries confirm that these toxins are an important cause of death in Africa and Southeast Asia [52]. There are many factors that control the distribution and occurrence of fungal poisoning, mainly climate, type of crops, biological factors, conditions and methods of harvesting, storage, and treatment, as well as moisture content and the damage that can be caused by insects before and after the harvest [53, 54]. The natural ecosystems of plants are affected by climate change, which affects the distribution of mycotoxin-excreting fungi in their places of growth and presence. Heat waves, ultraviolet radiation, and drought also affect the biological system and may cause the emergence of new mutagenic strains of mycotoxin-producing fungi due to selective adaptation, which may be more tolerant to new environmental conditions and more toxin production [55, 56]. Environmental conditions have direct effects on the ability of pathogenic organisms to survive and live outside the host, such as heat, humidity, and sunlight, as heat-tolerant species are adapted to live in a warmer climate [57].

3.5 Prevention of Mycotoxins

Prevention of infection in the fields is of paramount importance to control the infection of toxin-producing fungi and their contamination by maintaining appropriate conditions for the growth of fungi by adopting methods through which pollution can be reduced in light of the use of biological resistance methods, and setting appropriate harvest and post-harvest dates, reducing seed damage by insects or others, and controlling storage conditions to reduce damage [58].

The main reason for the occurrence of fungal poisoning in crops during storage is due to the availability of suitable conditions for the growth of fungi that produce toxins in the stored crop. Incorrect and bad storage conditions such as high humidity and varying temperatures are among the most important factors that lead to fungal contamination [59, 60, 61].

The conditions of drying, packaging, storage, and transportation are conditions that cause the transfer and growth of fungi and increase the risk of mycotoxin production [62]. 500 types of mycotoxins have been identified and there are nearly 1000 species that have not been detected, which pose a danger because there are no studies and methods to identify them [63]. To reduce exposure to mycotoxins, it is recommended to follow the following steps [64]:

- Diversity in the diet;
- Not keeping food and grains for long periods;
- Store raw foodstuffs in good ways to protect them from insect infestation, drought, moisture, and heat;
- Purchasing fresh and untreated foodstuffs with preservatives;
- Examination of foodstuffs, such as cereals, dried figs, pistachios, peanuts, and coconuts, which are usually contaminated with aflatoxin, as well as detecting the presence of molds contaminating them;

3.5.1 Identification Methods

Several methods were used to identify and differentiate species related to the genus *Aspergillus*. The classical method considered several taxonomic keys and guides that should be used by expert mycologists under standard laboratory conditions to ensure accuracy. The keys depended on morphological traits like colony diameter, color, the existence of sclerotia and/or cleistothecia, production of pigments and other exudates in addition to the microscopical traits which include style of seriation, the morphology of cleistothecia and ascospores shape and size of conidia, vesicles and stips [65]. Selective media were also used to support morphological identification methods including Czapek Dox Agar (CZA), which can differentiate *A. flavus*, which appears in a yellow-green color colony, from *A. parasiticus*, which is dark green. The Coconut Cream Agar (CCA) is used to identify isolated aflatoxin-producing by exposing the colony to UV light which reflects a blue fluorescent color when aflatoxin has been existing. The medium of *Aspergillus flavus* and *parasiticus* Agar (AFPA) can detect the *A. flavus* group selectively by the orange color developed on the reverse side of the plate. The modified Rose Bengal (M-RB) was found to be very selective media used for the isolation of *A. flavus* from soil samples and showed a high ability to prevent competition with other genera, especially members of Mucorales [66].

Molecular identification methods were found to be the most robust, fast, and sensitive method applied to a wide spectrum of microorganisms. This potent tool included the amplification of specific regions of DNA, then sequencing those pieces followed by sequence analysis. Genetically, *Aspergillus* was one of the best-studied fungi, and the complete genome sequence of *A. flavus*, in addition to several strains of *A. flavus* group strains, are available now at the National Center for Biotechnology Information (NCBI) [67]. Internal transcript spacer (ITS1 to ITS4) is a specific region located in rDNA, broadly used to differentiate *Aspergillus* species in addition to the 28S rRNA region (D1-D2) [68]. Furthermore, other studies utilized sequencing of other target genes in the molecular identification of *A. flavus*, including β-tubulin, topoisomerase II, and calmodulin genes for distantly related species because of low variability in those regions, in addition to the mitochondrial cytochrome b and aflR genes which are used to differentiate between closely related species like *A. flavus* and *A. oryzae*, and *A. parasiticus* and *A. sojae*. However, 18S rRNA is stills the most variable and reliable target region for molecular identification of *A. flavus* [69]. Random Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP) analysis

is also used significantly to distinguish some *Aspergillus*-related species. According to the high similarities between closely converged *Aspergilli*, some of the studies employed PCR product of the amplified specific regions in several molecular techniques like Single Strand Confirmation Polymorphism (SSCP), Restriction Fragment Length Polymorphism (RFLP), and Heteroduplex Mobility Assay (HMA) that showed multiple degrees of success [70].

3.5.2 Detection of Aflatoxins in Humans

Two main methods (techniques) are commonly used to detect aflatoxin levels in humans. The first one involves measuring AFB1-guanine (aflatoxin B1-guanine) adducts in the urine of patients. The presence of aflatoxin B1 guanine adducts (breakdown products) indicates exposure to aflatoxin B1 within the past 24 hours, i.e., this method only measures the current load. Measured aflatoxin B1 guanine levels may vary with diet due to the half-life of the metabolites, making long-term exposure an ineffective assessment. The second technique is the measurement of AFB1-albumin (aflatoxin B1-albumin) adducts in serum. This method provides additional cohesive measures of exposure to aflatoxins over weeks or even months. Identifying aflatoxin poisoning in humans and animals is also challenging due to differences in clinical symptoms presence of some conditions including immune system suppression resulting from infectious diseases. Of these two techniques commonly used to detect aflatoxin levels in humans, one measures a specific breakdown product in the urine (which is only present for a day after exposure, however), while the other measures an AFB1–albumin level in the blood serum, giving information on exposures over weeks or months. Measurement of these biomarkers is critical for studying outbreaks of suspected aflatoxin infection. Various methods for detecting the presence of aflatoxins in food/feed have been developed for different requirements. Aflatoxins and their analysis and detection techniques are very important and have been extensively studied to develop products that are very specific, practical, and useful. Many approaches have been developed for different needs, ranging from methods (and techniques) for Regulatory control in official laboratories (e.g. HPLC-MS) to rapid test kits such as ELISA (enzyme-linked immunosorbent assay) used in factories and granaries. Many potential new systems for aflatoxins detection based on new technologies include hyperspectral imaging, dipstick kits, molecularly imprinted polymers, electronic noses, and aptamer-based biosensors (small organic molecules that can bind to specific target molecules) [71, 72, 73, 74].

3.6 Conclusion

Average dietary exposures to aflatoxins are typically below 1 ng/kg body weight per day in developed countries, while estimates in many sub-Saharan African countries exceed 100 ng/kg body weight per day. Country estimates of dietary exposure to AFM1 rarely exceed 1 ng/kg body weight per day. No animal species have shown immunity to the acute toxicity of aflatoxins.

The major target organ in mammals is the liver, and aflatoxin poisoning is primarily a liver disease. A regular diet of celery-like vegetables like parsnips, carrots, parsley, and celery can reduce the carcinogenic effects of aflatoxins. There are various methods for the

detection of aflatoxins in food and feed. Aflatoxin control is required during both pre-harvest and post-harvest treatments. A key permanent, sustainable solution to aflatoxin infection control before harvest is to improve The ability of a crop to prevent fungal infection or fungal invasion from forming aflatoxins.

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