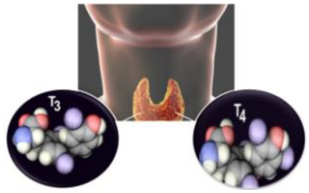

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Impact of Aflatoxins Exposure on Human Health and its Management Strategies

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ABSTRACT: *Aflatoxins are common contaminants in human food supplies that affects the world's developing economies. These are cosmopolitan in distribution and found everywhere in nature and can grow under drought, warm, and humid conditions. The aflatoxins are secondary metabolites and mostly found in feed and food products. These are oncogenic, mutagenic, and immunosuppressive in nature. On the basis of the aflatoxin producing fungi, the aflatoxins are roughly split into two distinct groups: those that penetrate in pre-harvest circumstances and those that are generated in post-harvest conditions. Aflatoxins exposure has great public health impact in economically developing nations and lot of research is taking place to reduce its harmful impacts; as a result, we need to establish preventive strategies that are feasible for these high-risk populations. This review provides in-depth information regarding the presence of aflatoxins, their analysis, and potentially harmful consequences on human health, as well as various detoxification approaches.*

Keyword: *Aflatoxin, toxic, ELISA, detection, prevention, detoxification*

INTRODUCTION

Mycotoxins are secondary metabolites produced by organisms of kingdom *Fungi* and are present in food and feed. Mycotoxins have the ability to cause different diseases and even death in animals and humans. Due to their high toxicity, they are one of the main causes of public health issues. Mycotoxins have been the subject of research around the globe to determine their severity and prevalence in various dietary entities (Berthiller et al., 2018). Mycotoxins influence and jeopardize the economy, global trade, and also cause irreversible health impacts in living things. Contamination by mycotoxins is unavoidable and occasionally unpredictable, making it a unique problem for food safety (Stadler et al., 2018).

Poor hygienic conditions during transportation and storage, moisture, torrential rain, and high temperatures all contribute to the production of mycotoxins including aflatoxins, ochratoxins, patulin, deoxynivalenol, citrinin and zearalenone (Elkenany et al., 2021). Aflatoxin growth is mainly found in cereals, ground nuts, oil seeds, grains and different spices such as wheat, maize, red chilli, turmeric, black pepper, almond, cotton, peanut etc. Aflatoxin contamination can also be found in vegetables and fruits, as well as meat, animal tissues, and animal products. Aflatoxins in feed and food have different permissible limits in different countries (Makau et al., 2020).

Poor storage practices and lack of technical capability have resulted in 50–60 % losses in cereal grains around the world (Akhtar et al., 2018). The loss of stored grains varies from region to region. In temperate climates, the loss is nearly 10 % while in humid tropical climates the loss is about 50 % (Zampieri et al., 2020). Every year, Pakistan loses 76 to

90 million dollars due to the presence of aflatoxins and about $80 million to $1.68 billion per year loss to crops in US. It is anticipated that Indonesia, and Thailand collectively lost $1 billion USD each year due to the contamination of food (Tabak et al., 2019). According to the estimation of Food and Agriculture Organization, aflatoxins affect 25 percent of world food crops each year (FAOSTAT, 2019). It is unprofitable to increase output without lowering postharvest losses because one-third of total output is lost at this stage (Bradford et al., 2020).

Aflatoxins

Aflatoxins belong to the family of harmful metabolites produced by fungi and are found in nature under drought, warm, and humid conditions. The ideal temperature for the production of aflatoxins is 33ºC. The species that produce aflatoxins are mainly *Aspergillus flavus* and Aspergillus parasiticus (Mwakinyali et al., 2019). Aflatoxin-producing fungi are particularly prevalent in warm and humid climates (Agriopoulou et al., 2020). Temperature, soil, humidity, and storage conditions all affect the rate and degree of aflatoxins accumulation (Roila et al., 2021). They were first discovered in the United Kingdom in 1960, when more than one million poultry birds in Turkey died of eating meal contaminated with *A. flavus* (Tahir et al., 2018).

Aflatoxins have catastrophic effects on humans, as well as are potential cause of liver and other organ cancer. Food ingestion, as well as other methods such as dermal contact and inhalation, is common sources to aflatoxin exposure (Garduño-García et al., 2017). There are main types of aflatoxins i.e Aflatoxin B1, Aflatoxin B2, Aflatoxin G1 and Aflatoxin G2, whereas AFM1 is a form of AFB1, mainly found in

milk and other dairy products (Moral et al., 2018). The alphabets “B” and “G” denote the blue and green fluorescence characteristics of aflatoxin when we visualize it under ultra-violet light respectively, while the number 1 and 2 denote the aflatoxins' relative position on thin layer chromatography. The toxicity potential of aflatoxins is B1 > B2 > G1 > G2.

The structures of aflatoxins G1 and B1 were proposed in 1963 and structures of aflatoxins G2 and B2 were proposed shortly thereafter (Fig. 1).

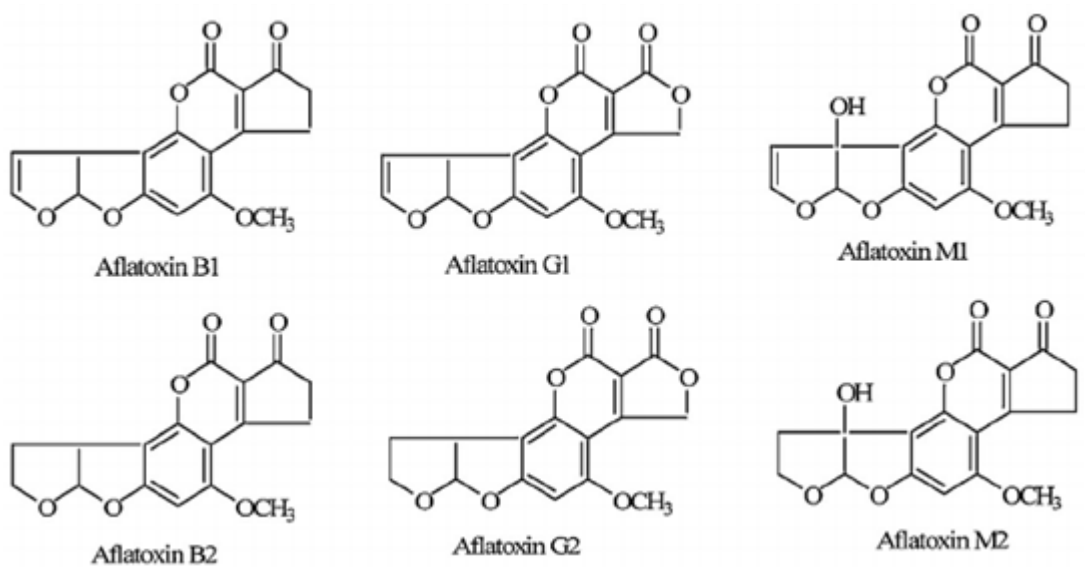

Fig. 1. Chemical Structures of Aflatoxins (https://www.researchgate.net/publication/262878341_Military_potential_of_biological_toxins)

Physicochemical Properties

Aflatoxin B1 undergoes complete catalytic hydrogenation, which culminates in the intake of 3 moles of hydrogen with the creation of the tetrahydrodeoxy derivative (Peng et al., 2020). Aflatoxin B2 is produced in a quantifiable yield when the hydrogenation process is interrupted after taking 1 mole of hydrogen. Additionally, it has been noted that, when a powerful acid acts as a catalyst, aflatoxin B1 reacts additively with a hydroxyl group (Javanmardi et al., 2020).

Although there have been few comprehensive investigations on the stability of aflatoxins, general experience would seem to suggest that some degradation occurs under a variety of circumstances. For instance, the compounds seem to partially disintegrate when left standing in methanolic solution. Beyond work related to structure elucidation, there has been comparably little systematic investigation of the chemical interaction and conduct of the aflatoxins (Yunus et al., 2019). The physical features of aflatoxins are given in Table 1.

Table 1. A **s**summarized data on physical features of aflatoxins

Aflatoxins	**Structural Formula**	**Molecular Mass**	**Liquefaction Point (˚C)**
G_1	$C_{17}H_{12}O_7$	328	244-246 ˚C
B_1	$C_{17}H_{12}O_6$	312	268-270 ˚C
B_2	$C_{17}H_{14}O_6$	314	286-289 ˚C
G_2	$C_{17}H_{14}O_7$	330	237-240 ˚C

Hazardous Health Effects of Aflatoxins

A large exposure to aflatoxin in the case of acute aflatoxicosis can result in about 25% of deaths. Aflatoxin exposure is common in countries that are still developing, resulting in major disorders and death (Puzanov et al., 2017). The incidence of acute aflatoxicosis, on the other hand, is relatively low because human beings are mostly immune to these toxins, and people generally keep away from taking food that is tainted with aflatoxins. However, this problem is frequent in penurious areas where people have no other option but to eat the poisonous food (Tahir et al., 2018, de Sousa et al., 2022).

Dullness, hydrops, liver necrosis, liver cirrhosis, jaundice, enlarged liver are all symptoms of severe aflatoxicosis. The immune system of children is less strong as compared to adults, so aflatoxins contamination is more vulnerable to children (Kumar and Kalita, 2017). Chronic aflatoxins exposure has a significant impact on an animal's nutritional status. In such cases, aflatoxins bind to DNA covalently shortly after exposure and protein synthesis start decreasing as a result (Asghar et al., 2018). There are several allowable limits for aflatoxins in food and animal feed components that have been adopted by different authorities.

The permitted levels of aflatoxin in multiple food items in different nations are listed in Table 2.

Table 2. Acceptable levels for aflatoxins (Yadav et al., 2021)

Aflatoxins	Maximum Permitted Value µg/kg	Products	Country
Aflatoxins (B2, G2, B1, G1)	10	Nuts	Australia, Taiwan, Indonesia, Malaysia
(AFB1, AFB2, AFG1, AFG2)	10	Groundnuts	Japan, Thailand, Egypt, Turkey
Aflatoxins (G1, B1,G2,B2)	4	Groundnuts	European Union
Total aflatoxins	10	Food entities	Vietnam
Total aflatoxins	20	All foods except milk	USA
Aflatoxins (B1, B2, G1,G2)	5	Groundnuts	Singapore
Total aflatoxins	30	Food stuffs	India
Total aflatoxins	20	Nuts	Philippines
Total aflatoxins	15	Nuts and their derivatives	Canada
Total aflatoxins	20	Groundnuts	Kenya

Techniques for Detection and Quantification of Aflatoxins

Various analytical techniques are present for aflatoxins detection and quantification. Some of the techniques are given as follow:

1. Thin Layer Chromatography

One of the most common separation techniques used for the detection of aflatoxins is thin-layer chromatography (TLC). It comprises a stationary phase that is made up of silica or alumina that is immobilized on an inert material made up of plastic or glass known as matrix (Folarin-Ottun 2018). The mobile phase includes water, methanol and acetonitrile. The sample is carried through the solid stationary phase by the mobile phase. The difference in solvability of the analytes in the two phases determines the distribution of aflatoxins between the mobile and

stationary phases in TLC (Jallow et al., 2021). The TLC method can be used to detect several types of mycotoxins in a single run. Although, TLC has excellent sensitivity, it also needs an expert to run it, sample pre- treatment, and this equipment is expensive. The development of High-Pressure Thin Layer Chromatography (HPTLC) has overcome the problems related to TLC (Sipos et al., 2021).

2. High Pressure Liquid Chromatography

The most widely used chromatographic technique for separating and determining organic compounds is High Pressure Liquid Chromatography (HPLC). HPLC is used to determine about 80% of all organic compounds on the planet. HPLC technique is also comprised of stationary and mobile phases. The sample which is to be analyzed is coated on top of the column, it flows through both the mobile and stationary phases and distributes evenly. HPLC also necessitates time-consuming pre- and post-column derivatization processes to refine aflatoxins detection limits. A change in the HPLC method, in which the HPLC is incorporated with mass spectroscopy, was developed to tackle the challenges associated to aflatoxins analysis (Malviya et al., 2010). High Pressure Liquid Chromatography-Mass Spectrometry (HPLC-MS), on the other hand, is a large and expensive piece of equipment that can only be handled by an expert and professional personnel (Kumar et al., 2021).

3. Gas Chromatography

In GC, the mobile phase is a carrier of gas and stationary phase is a liquid that is placed on inert solid particles. The sample which is to be analyzed is evaporated into gas phase and is detected using either Lame Ionization Detector (FID), Electron Capture

Detector (ECD) or Mass Spectrometry (MS). Gas chromatography is not commercially used as it is an expensive technique (Kaminiaris et al., 2020).

4. Enzyme Linked Immuno-Sorbent Assay

ELISA is a safe and suitable technique for the determination of aflatoxins. In ELISA, the antigens or antibodies are not labelled with isotopes but enzymes. ELISA is sensitive and simple to use. It also gives accurate measurement of anti-body or antigen concentration (Pereira et al., 2020).

Agricultural products are being detected for aflatoxins using ELISA method and different ELISA kits are available in market these days. The ELISA test can be performed on a 96-well assay platform, allowing for the simultaneous analysis of a large number of samples. ELISA kits are inexpensive, easy to use and there are no such health risks involved. However, ELISA method involves washing process multiple times which is laborious and time consuming (Omar et al., 2020). The strengths and weaknesses of aflatoxins detection tools are given in Table 3.

Table 3. Strengths and weaknesses of aflatoxin detection tools

Approach	Strengths	Weaknesses	References
HPLC	• Precise and efficient quantitative approach	• Quite pricey to test a significant number of samples • Samples destruction during preparation	(Abbas et al., 2004)
ELISA	• Fast, precise and quiet easy to use • Simultaneous examination of several samples • Partial use of organic solvents	• Chances of false negative/ positive results • Confined detection limit	(Do and Choi, 2007)
GC/MS	• Concurrent analysis of aflatoxins • Sensitive • Provides confirmation (MS detector)	• High variability in regard to repetition and replication	(Kasoju et al., 2020)
TLC	• Involves no complex equipment • Easy and reliable semi-quantitative way	• Outdated method • Samples destruction during preparation • Need HPLC analysis for quantification of results	(Zahra et al., 2019)
Strategies for Preventing and Reducing Aflatoxins	Due to frequent presence of aflatoxins in food and feed, a variety of strategies		

have been developed to remove contamination and to restore food quality and edibility. Pre-harvest and post-harvest are the two types of control strategies. Pre-harvest strategies include use of genetically modified crops that show resistance against Aspergillus infection, use of pesticides and planting time of crops (Rushing and Selim, 2019). Post-harvest strategies include physical, chemical and biological methods. Physical methods include heat, washing, proper drying, storage techniques, use of pesticides etc. Chemical methods include treatment with HCl, citric acid, lactic acid etc. Biological methods include treatment with plants extracts such as Allium sativum, Nigella sativa.

Detoxification of Aflatoxins

The reduction of mycotoxin in food requires optimal storage conditions. Mycotoxin growth may be influenced by elements such as temperature, water activity, gas composition and microbiological interactions of the preserved items (Kumar et al., 2022). These factors could be effectively controlled if they were all under an integrated control. For the removal of mycotoxins, numerous methods are present. Maintaining food's savoriness and nutritional value is important. Many strategies and techniques have been examined to reduce mycotoxin infection rate in both food and feed products (Tiwari et al., 2022). These techniques fall into three categories: chemical, biological and physical.

Physical Methods

Physical techniques like color-based sorting, mechanical separation, fines removal and density segregation can lower the amount of mycotoxin contamination that is present in food. Sodium carbonate or distilled water is used to wash the grains of different food and feed to lower the levels of aflatoxins (Shen et al., 2021).

Chemical Methods

Because aflatoxin infestation is unpredictable and unavoidable, it poses a unique threat to the safety of both feed and foods because it can cause both animals and people discomfort. Despite the availability of other chemicals, it has been discovered

that 0.5% HCL is the most effective one for removing aflatoxin B1 from fish feedstuffs and fowl samples (Awan et al., 2022). Ammoniation is indeed an effective technique that has been around for a while and is mostly used to reduce the amount of aflatoxins in feed, but it has an impact on food quality. Similar to this, aflatoxin levels in food items may be decreased by alkalization, heat treatment and acidification. Deamination also reduces the toxicity of AFB1 (Yang et al., 2022).

Biological Methods

Mycotoxins can be reduced through biotransformation by adsorption, binding, or detoxification. In order to diminish mycotoxins as biocontrol agent, probiotic microorganisms (Saccharomyces cerevisiae and Lactobacillus delbrueckii) were studied. This research revealed the use of probiotics as an alternate option to stop the synthesis of aflatoxin in food items (Nazir et al., 2021). Plant extracts of Nigella sativa and Allium sativum are very effective for the detoxification of aflatoxins (Zahra et al., 2019).

CONCLUSION

Aflatoxicosis may result in serious, fatal diseases. The presence of various aflatoxins in feed and food products could be harmful to both animal and human health and can also badly affect the nation's economy. To prevent severe and difficult situations, there must be strict regulation of food standards in both developed and developing countries alike. Aflatoxin exposure can sometimes be unavoidable and result in significant financial damage. Regarding the quality of food stuffs, precautionary measures are important. To ensure food safety, there must be strict enforcement of laws and regulations in every state that allows the presence of these dangerous aflatoxins within permissible ranges. Adequate aflatoxin control can be achieved by using approaches like HACCP (Hazard analysis and critical control points).

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