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Review Article

Aflatoxin B1 as a Global Food Safety Threat: Toxicity Mechanisms, Detection Technologies and Control Strategies

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Abstract:

Aflatoxin B1 (AFB1) is one of the most toxic and prevalent mycotoxins that can be found in food and feed worldwide. AFB1 is a by-product of Aspergillus flavus and A. parasiticus that poses threat to food security, human health and agriculture's economy. In this review paper, the status, structure, metabolism, toxicity, health effects, detection and prevention and detoxification strategies of AFB1 are discussed. Cereal, other crops (nuts, oilseeds), milk and agricultural products are typically vulnerable to contamination at harvest, at warm and humid temperature during transport and storage. The AFB1 is metabolized in the liver to form reactive epoxide metabolites that can react with DNA and proteins to cause oxidative stress, mutagenesis and hepatocellular carcinoma. Acute exposure can cause aflatoxicosis; chronic exposure can cause liver cancer, suppression of immune system, developmental abnormalities and growth retardation in children. There are several analytical techniques for detection of aflatoxin that are currently available, including TLC, HPLC, LC-MS, ELISA and biosensors. Other AFB1 reducing processes for food and feed have been developed and are termed prevention and detoxification processes such as adequate food storage, irradiation, acid and ozone processing, ammoniation and biological methods. Climate change, lack of advanced technologies and monitoring systems still pose difficulties to global aflatoxin control. Therefore, continued research, improved laws and sustainable mitigation strategies are essential to minimize the negative health and food security impacts of AFB1.

Keywords: Aflatoxin B1; Mycotoxins; *Aspergillus flavus*; food contamination; hepatocellular carcinoma, detection methods, detoxification, food safety

Introduction

Mycotoxins are found all over the world. These chemically varied chemicals are particularly significant agricultural pollutants since both acute and chronic food exposures can cause a range of negative health impacts in both humans and animals. They usually coexist in agricultural crops, and exposure to mycotoxin combinations is frequent due to varied diet (Eskola 2020).

Many significant phytopathogenic and food spoilage fungus, such as Aspergillus, Penicillium, Fusarium, and Alternaria species, create mycotoxins, which are fungal secondary metabolites that have been linked to extremely harmful effects on vertebrates. Aflatoxin contamination of food and animal feed is a global issue (Kabak 2006).

A class of naturally occurring and structurally similar poisonous, mutagenic, and carcinogenic secondary metabolites generated by specific Aspergillus species, aflatoxins (AFs) were originally identified in 1960 and 1961(Winter 2019; Diedhiou 2011). The most prevalent and significant of the more than 20 identified AFs are AFB1, AFB2, AFG1, and AFG2 (Enyiukwu 2014; Falade 2022). Milk, milk products, and meat include AFM1 and AFM2, which are the hydroxylated derivatives of AFB1 and AFB2, respectively (Giray 2007; Kuilman 2000). AFB1, AFB2, AFG1, AFG2, and AFM1 are more significant than the other AFs due to their frequency in food (Benkerroum 2020).

AflatoxinB1 circulate in human bloodstream with concentration 10-30 ng /liter of blood, also transferred to woman milk (AFM1) with concentration ranged from 16-1990 ng / liter of milk (Dharumadurai 2011). AFB1 is the most common and widespread food and feed contamination worldwide (Hussain 2008). Because of its effects on the immune systems of both

humans and animals, this toxin is especially concerning, as well as its ability to induce cancer (Diedhiou 2011).

Aflatoxins, which cause liver cancer and have also been linked to acute toxicoses and growth impairment in children, fumonisins, which have been linked to esophageal cancer (EC) and neural tube defects (NTDs), deoxynivalenol (DON) and other trichothecenes, which are immunotoxic and cause gastroenteritis, and ochratoxin A (OTA), which has been linked to renal diseases (Felicia Wu 2014).

Sources and occurrence

A. flavus is most prevalent in corn, cottonseed, and tree nuts, while A. parasiticus is dominant in peanuts. A. flavus contains structures including mycelium, conidia, or sclerotia and can grow at temperatures 12-48◦C (Hedayati et al., 2007).

Aflatoxins occur in range of products such as cereals, oilseeds, spices, and nuts (Lancaster et al., 1961; Weidenborner, 2001; Reddy, 2010; Iqbal et al., 2014). These Aspergillus colonize among themselves and produce aflatoxins, which contaminate grains and cereals at various steps during harvesting or storage. Fungal infestation may occur in the field, or during harvest, transport and storage (Kader and Hussein, 2009). Inadequate storage conditions cause Aflatoxin contamination in Wheat and Barley (Jacobsen, 2008).

About 230 samples of rice Aflatoxin B1 was detected in samples collected from several parts of Brazil during the 2007–2009 beriberi pandemic. The results showed that the concentration of aflatoxin B1 in those samples was as high as 180.74μg/kg (Almeida et al., 2012). Despite the fact that the average levels of contamination in rice are frequently lower than those seen in maize or groundnuts, the sheer amount of rice ingested daily can account for a significant amount of total exposure (Koshiol 2026).

Chemical structure and Properties

Aflatoxin B1 ($C_{17}H_{12}O_6$, MW_{312}) is a crystalline chemical that dissolves in water, methanol, chloroform, and dimethylsulfoxide at concentrations of 10–20 mg/litre. When AFB1 is exposed to UV light, it fluoresces.

In the absence of light, particularly UV radiation, the crystalline form of AFB1 is stable at temperatures of 100°C. AFB1's chemical composition indicates that it is a desirable alternative coumarin structure with a fused dihydrofurofuran moiety (Kensler 2011).

The **properties** of aflatoxin are, soluble in polar organic solvents but water (MdQuadri 2013). They are thermostable, so techniques like pasteurization are non-efficient (Jard 2011). They become unstable at extreme pH values (3 or >10) and in UV radiation when oxygen is present.

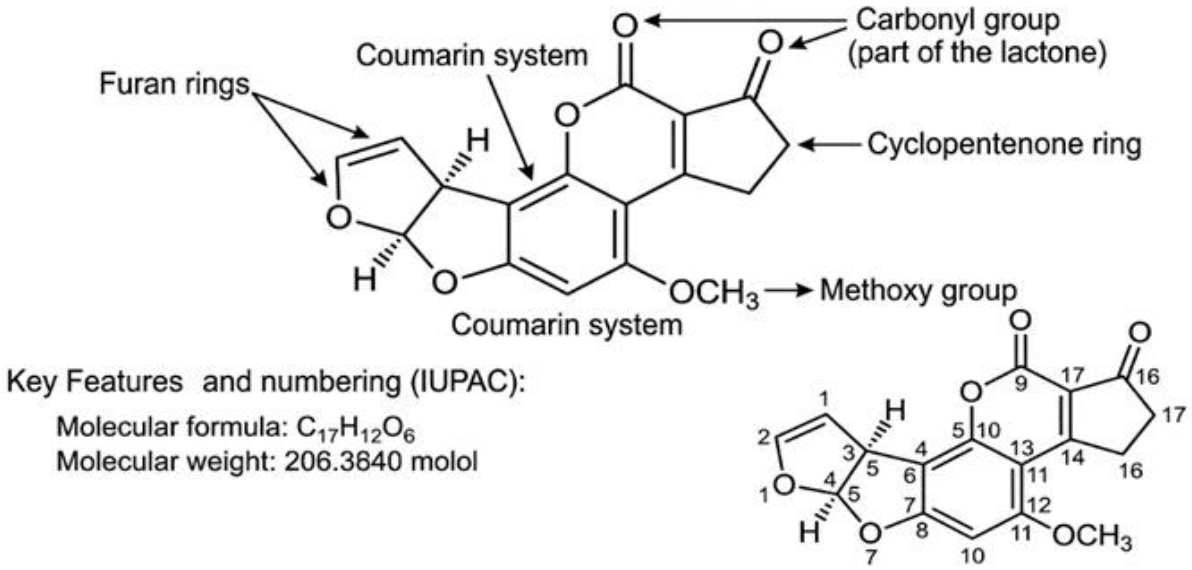

Figure 1: Chemical Structure of Aflatoxin B1.

Aflatoxin is destroyed when the lactone ring opens in an alkaline environment, but this process is reversible in an acidic environment. Aflatoxins undergo irreversible decarboxylation when ammoniation opens the lactone ring at high temperatures (Kumar 2018).

Metabolism of Aflatoxin B1

Aflatoxins are absorbed by cell membranes as they enter the body and eventually make their way into the bloodstream. The primary organ for processing xenobiotics is the liver, which transports them to various tissues via the blood. The liver mostly breaks down aflatoxins into a reactive epoxide intermediate, or the less harmful aflatoxin M1. The cytochrome P450 (CYP450) microsomal enzymes in humans and susceptible animal species produce aflatoxin-8, 9 epoxide, a reactive form of aflatoxin that binds to DNA and albumin in blood serum to produce DNA-damaging adducts. Several CYP450 enzyme isoforms in the liver convert aflatoxin into aflatoxin-8, 9-epoxide. This reactive oxygen species can bind to DNA to cause liver cancer or to proteins to cause acute toxicity that is aflatoxicosis (Wu and Khlangwiset 2010). While prostaglandin H synthase and lipoxygenases seem to play a significant role in the biotransformation of human AFB1 in the lung, the main CYP enzymes engaged in its metabolism in the liver are CYP3A4, CYP3A5, and CYP1A2 (Wild 2002; Dohnal 2014).

CYP3A4, a unique isoform of P450, breaks down AFB1 into AFB1-endo-epoxide, a less dangerous compound that may be removed in a number of

methods and doesn't attach to nucleic acids. Both exo- and endo-epoxides can hydrolyze rapidly in the absence of enzymes to create AFB1-8, 9 dihydrodiol by interacting with the lysine amino group in serum albumin (Wild 2010).

AFB1 also binds to DNA, changing its structural characteristics and causing changes in genes, telomere length, and cell cycle regulatory points. Tumors form as a result of the liver cells' AFB1 attaching to DNA at the guanine base, corrupting the genetic blueprint that regulates cell proliferation (Vermeulen 2003).

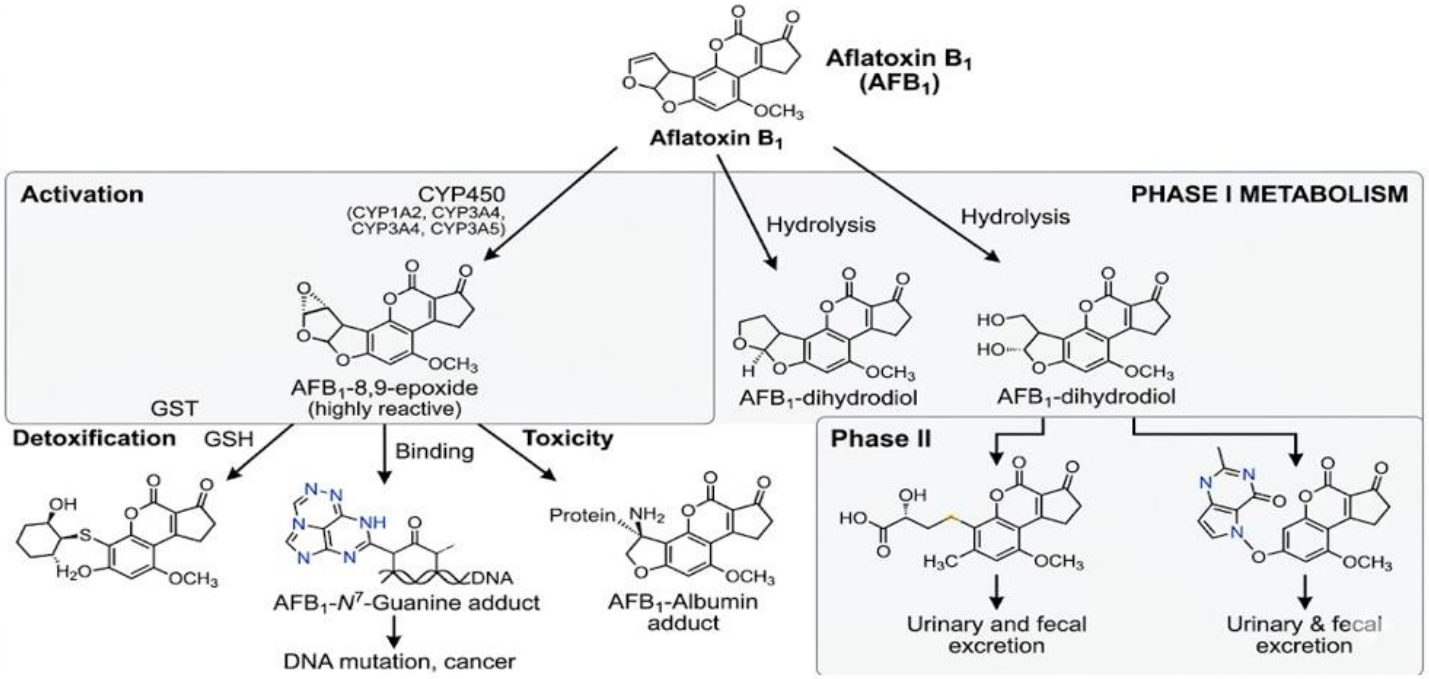

Figure 2: Metabolism of Aflatoxin B1 with the metabolites involved in the Hepatocytes.

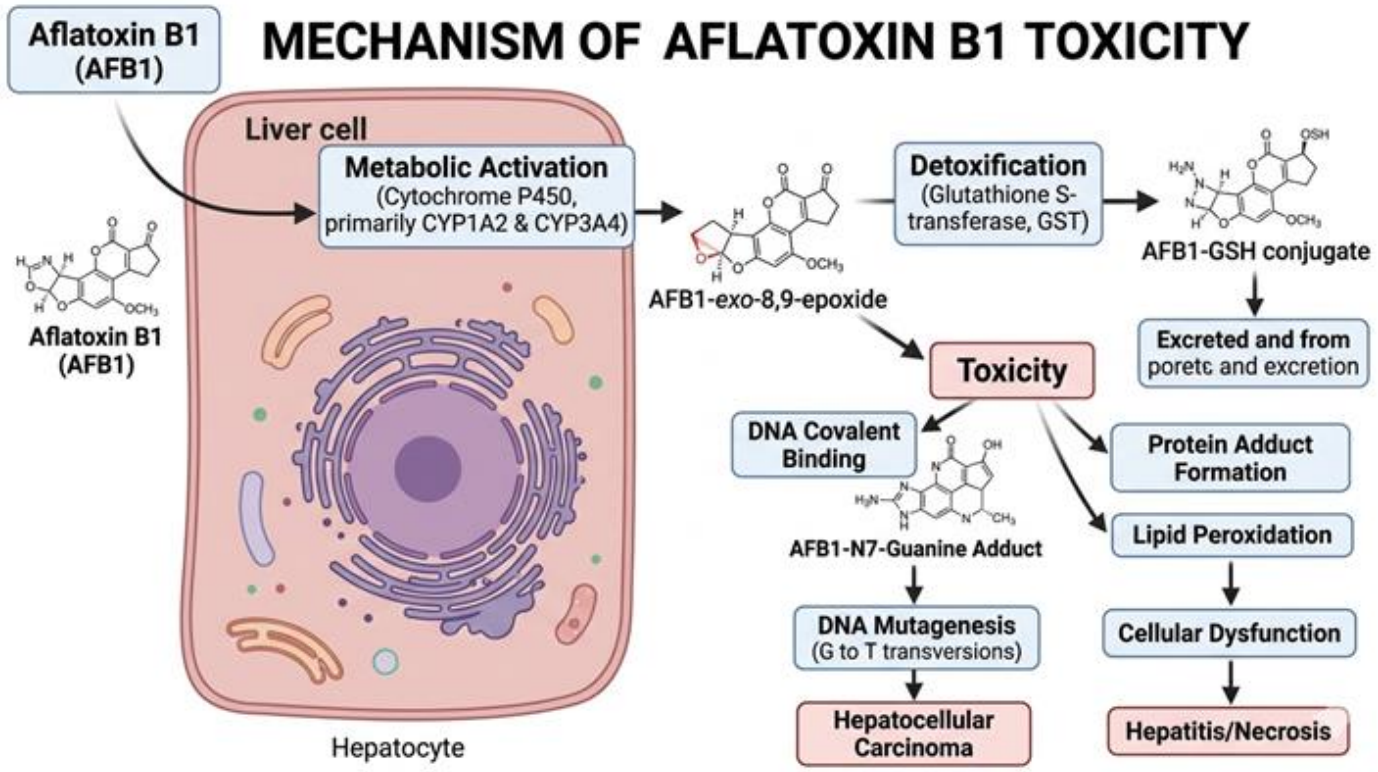

Figure 3: Metabolic activation of aflatoxin B1 (AFB1) to AFB1-8,9-epoxide and subsequent formation of the primary AFB1 DNA adduct 8,9-dihydro-8-(N7-guanyl)-9-hydroxyaflatoxin B1 (AFB1-N7-Gua).

Health Impact of Aflatoxin B1

Aflatoxins are poisonous substances made by certain molds that often end up in our food and negatively affect human health, food security, and economic trade in much of SubSaharan Africa (Demissie, 2018). It has been reported that aflatoxin affects multiple organs and systems in the body. There is no specific antidote for aflatoxins. It is estimated that more than 5 billion individuals in developing nations are chronically exposed to aflatoxins, as these toxins are present in their food supply (Alloysius and Ositadinma, 2016). The health consequences of AF contamination are especially dire in the areas with warm and humid climates. The long-term exposure to AFs, and in particular AFB1, the most toxic variant, is related to a variety of health issues, such as HCC, immune suppression, growth retardation in children, and reproductive complications (Balan et al. 2024; Urugo et al. 2023; Jallow et al. 2021).

Acute aflatoxicosis

Apart from the loss of life, acute aflatoxicosis can cause exacerbated malnutrition, which has devastated impact on affected populations. When dairy animals were fed aflatoxin-contaminated feed, they excreted aflatoxin metabolites in their milk. Consequently, dairy consumers may develop aflatoxicosis. Because of those government regulations indicates that it is essential that milk should be free from contamination by aflatoxin B1. Regardless nothing is done until the aflatoxin level in market milk exceeds 0.5ppb, below which the public is not at risk (Abebe, Abriham, and Yobsan, 2018).

Chronic impacts on child development and growth

Aflatoxins have the potential to harm the development of the human brain by damaging brain tissue and can cross the blood-brain barrier. Even though dietary aflatoxin exposure is common in children, there is a limited data on aflatoxin exposure and child developmental outcomes. Aflatoxin can affect a child in utero through breast milk and from complementary feeding after weaning (Khlangwiset et al., 2011). Delayed growth in children may be caused by aflatoxin as it damages enterocytes, which can lead to poor absorption of vital nutrients. Systemic immune activation is also influenced by aflatoxin and decreases insulin-like growth factors (IGF) through liver toxicity (Smith, Stoltzfus, and Prendergast, 2012). Child growth failure characterized by stunting (height-for-age Z-score (HAZ) < -2 standard deviations (SDs), wasting (weight-to-height Z-score (WHZ) < -2 SDs, underweight (weight for age Z-score (WAZ) < -2 SDs has still remained a serious problem in many low- and middle-income countries (Smith, Stoltzfus, and Prendergast, 2012). That's why, early childhood development is considered a difficult stage to diminish aflatoxin exposure and prevent its long-lasting outcomes.

Detection Methods for AFB1

For the detection of aflatoxins various Conventional techniques are currently being used including Thin Layer Chromatography (TLC), Gas Chromatography (GC)-Mass Spectroscopy (MS), High Performance Liquid Chromatography (HPLC), ultraviolet absorption, fluorescence technique, and Enzyme Linked Immunoabsobent Assay (ELISA) (Mahato et al., 2019). We can detect and identify aflatoxins based on their absorption and emission spectra, with peak absorbance occurring at 360 nm. B toxins demonstrate blue glow at 425, on the other hand G toxin display green glow at 540 nm under UV irradiation. This florescence is the best widely used phenomenon for the detection of aflatoxins (Fallah et al., 2011). Various analytical methods have been developed, having different levels of sensitivity and accuracy, which can be employed for different objectives (Rahmani, Jinap, and Soleimany, 2009). Although these techniques are the best choice for a qualitative study of food content because of the accurate detection findings, to use these entrenched techniques we call for well-equipped laboratories, and trained personnel, are very expensive, harmful chemicals for experimental procedures (Bhardwaj, Rajesh, and Sumana, 2021). According to the approximation of AfB1-lysine (metabolite of AFB1 toxin) concentration in the blood, metabolite of AFB1 toxin can be identify by using ELISA. Precisely, from this test can detect as low as 5 pg/mg albumin of AFB1 in blood which make it a cost effective method for routine monitoring that can also be utilized for the detection of hepatitis B virus (Costa- Fernandez and Sanz-Medel, 2000).

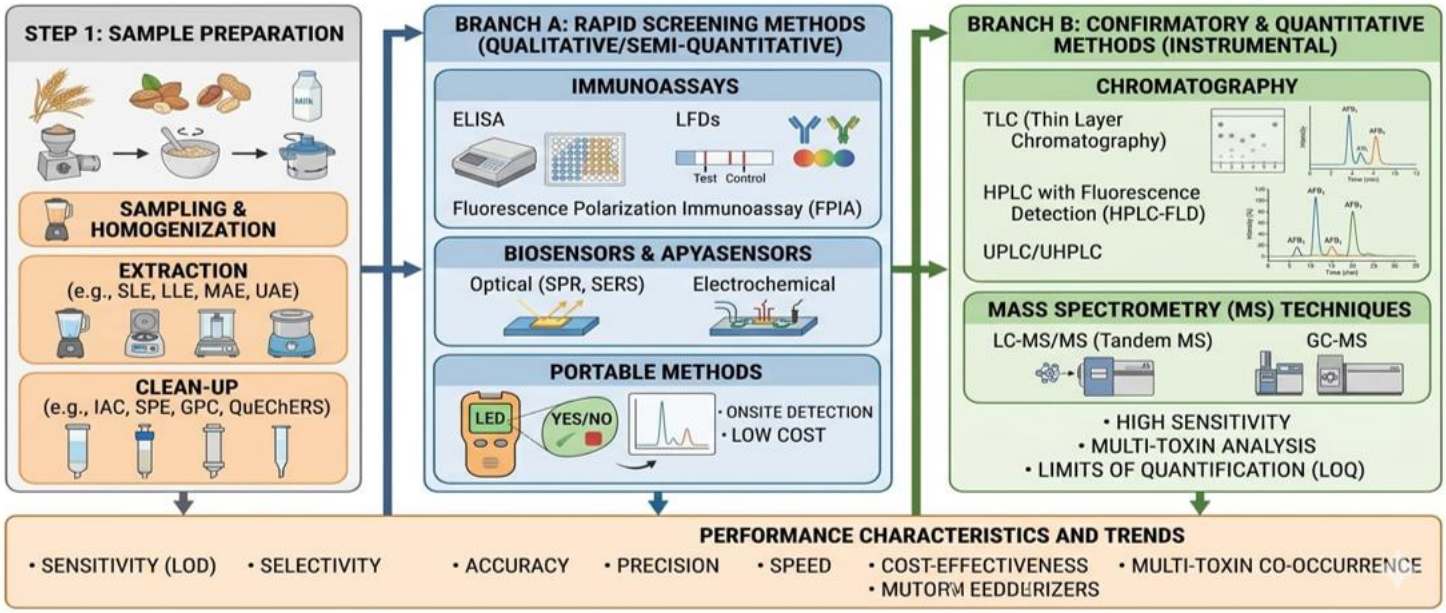

Figure 4: Overview of detective analytical methods for Aflatoxin B1 in food samples.

Prevention and control Strategies

China has come up with a number of ways to deal with aflatoxins (and keep them under control), and these fall into a few different types. The majority of ways of stopping or reducing the amount of aflatoxin that forms are breeding crops to be resistant to it, using genetic engineering, using natural biological controls, managing the conditions in the environment, good practices in the field, storing things correctly, using chemicals to prevent issues, adsorbing (or soaking up) the toxins, detoxifying, and altering what is eaten (Hamid et al., 2013; Liaoet al., 2009). Moreover, the application of post-harvestin gtechniques for aflatoxin reduction, such as sorting, cleaning, fast and proper drying, post-harvest insect control, sprayingand smoking of synthetic pesticides or botanicals were used as storage protectants (Guan et al., 2011b; Hell et al., 2010; Liao et al., 2009). Another approach to decrease aflatoxin contamination is Adsorption. Research has suggested that Negative impact of aflatoxin on animals can be reduced by adding various types of nutrition adsorbent to the animal feed. We can reduced the adsorption capacity of toxins in the intestinal trac of chickens by the combination of some bacteria, as a result of that, their biological activity reduces in vivo and prevent liver cancer (Gratz et al., 2005; Wang etal., 2018).

Detoxification Methods

Aflatoxin B1 is challenging food security and safety as it attacks plenty of commodities therefore many efficient methods to prevent or eliminate AFB1 in polluted. Some control has been made, commodities have been developed. techniques are used before harvesting the crop and others are applied after the harvesting (pre-harvesting and postharvesting). Strategies such as in pre-harvesting include strategies such as: applying pesticides on crop in field ,crop rotation, timing of planting, and use of genetically engineered seeds resistant to Aspergillus infection and environmental stressors. Post-harvest strategies that can be employed are proper drying, packaging, storage and preservatives/pesticide. In this review we are highlighting strategies used to detoxify the contaminated crops using some different techniques including Physical treatment, Chemical treatment.

Physical strategies

Physical methods of detoxification have a wide variety of methods such as Heating treatment, Irradiation, Electrolyzed water (EOW), Pulsed light technology to remove AFB1.

- **Heating treatment**

In modern industries heating is mostly used technique in many processing units and is also employed to decontaminate food/feed containing aflatoxin B1 but this is not an efficient strategy. Since aflatoxins have high resistance to heat, in order to eliminate fascinating amount of AFB1 rugged heating is required. Temperatures between 150–200°C can remove huge amounts of AFB1when the humidity is high (Lee et al., 2015). Heating treatment have some advantages such as is cheap, quick and easily performed. However, it also has its negative sides such as the temperatures applied can interfere with other nutrients that are delivered in food. Further the extent of AFB1 loss is based on the beginning level of mycotoxin concen-tration, the scope of binding of mycotoxin and food or feed products, heat penetra-tion, moisture content and processing conditions.

- **Irradiation treatment**

γ-Radiation is also a method of detoxification of AFB1 contaminated food, this is a usual method and is mostly applied in groundnuts, grains, palm juice, soybean and animal feed. Current studies have demonstrated that during these techniques a γ-ray source is compulsory and it used to irradiate food products until an ionizing radiation of 6-60 kGy is obtained. Moreover studies with this method demonstrated a reduction efficacy of 65% at high irradiation. Gamma irradiation is efficacy to feedstuffs which have moderate damage by fungi. Current study in Sudanese peanut oil using titanium dioxide (TiO2) in which photocatalysis ≥99.4% of AFB1 were removed in 4 minutes of irradiation (Magzoub et al., 2019).

- **Electrolyzed water (EOW) treatment**

The newly developed technique that employs electrolyzed water to decontaminate the AFB1 contaminated food or feed are being used currently. The EOW contain many OH groups which play a significant role in killing fungis like A. flavus by this way AFB1 is reduced when EOW is used. A study conducted using peanuts samples in order to investigate the effectiveness of electrolyzed oxidizing water for removal of aflatoxin B1 showed that after 15 minutes of treatment AFB1 was mostly eliminated (Xiong et al., 2012). Two types of water are produced and can be used in this treatment: neutral electrolyzed water (NEW) and acidic electrolyzed water (AEW). The antimicrobial mechanism of both water rely on three properties: pH, oxidation reduction potential concentration (ACC).

Chemical strategies

Chemical detoxification comprises the employ of acids, bases, oxidizing agents, reducing agents, chlorinating agents, and other reagents to inert or removes mycotoxins from contaminated foods and feeds.

- **Acid Treatment**

During HCl treatment, hydrolysis reaction occurred and it turned out to be the most powerful reaction for the degradation of AFB1. A survey was executed to analyze degradation of AFB1. From the results, it proved that HCL concentration, temperature, and time are the vital components affecting the efficiency of HCL. Initially, the conditions were 1 mol L−1 HCl at 110°C for 4 h degradation was 27.7% resulted in degradation of AFB1 by 27.6% (33.07 μg kg−1) after 4 h when the conditions changed the results improved to 42.5% after 8 h using 3 mol L−1 finally full elimination was observed when 5 mol L−1 HCl after 4 h at 110°C were employed (Aly and Hathout, 2011). Recent studies showed that lactic acid displayed highest efficiency using other organic acid such as acetic and citric acid in degrading AFB1. The out comings showed that 85% of AFB1 were removed after 2 hours of heating (Aiko et al., 2015).

- **Ozone Treatment**

According to United States Food and Drug Administration (FDA), Implementation of ozone does not have any consequences so it can be imployed for decontamination of food contain AFB1, as it is utilized in food processing as oxidizing agent and is well known (GRAS). Additionally, a study executed using 60 mg/L of Ozone, 8 hours of exposure time and 1 kg maize samplewhich showed a substantial reduction of AFB1 upto 57% (Porto et al., 2019).

- **Ammonia Treatment**

Ammoniation was completed in a closed ammoniation tank. The ammoniating test equipment was independently designed and developed by the research group of Northeast Agricultural University. The equipment consisted of an ammonia gas tank with a capacity of 170 kg, an ammonia vapor generator (2 L), and an electronic digital controlling box that shows pressure and time; the safe working pressure of the equipment is 0.3 MPa. The ammoniation conditions were 0.1, 0.2, and 0.3 MPa and 1, 2, and 3 h, respectively. The concentrations of AFB1 in all samples were analyzed by the HPLC method described by the China Feed Industry Standardization Technical Committee (Zhang et al. 2022).

Prevalence and Levels of AFs in the Asians

In Asia, AF contamination is an important and recurrent issue, which is affected by climatic conditions, agricultural activities, and supply chain management. In China, a large-scale study conducted on 16,604 food samples found out that 34.93% of the food was tested positive of AFs. In particular, contamination was most severe in peanut oil (49.14%) and corn (29.10%), with corns in Guangxi province recording mean contamination of 148.57umg/kg, almost 10 times the Codex Alimentarius limit (Umar et al. 2023; Chen, Liu, et al. 2022; Chen, Fang, et al. 2022).

Policy and Regional Initiatives

The strong governance framework and the transboundary cooperation are also necessary in controlling AF. The Strategic Framework of the Holistic AF Control by the African Union is an illustration of the efforts to coordinate the regulation, build awareness, and improve the institutional capacity of the member states (African Union (AU), 2023). The recent addition of 12 other countries (including Ethiopia, Ghana, and Kenya) to the framework is an indication of an increasing continental commitment to fight AFs. In addition, the regional organizations, such as Comite Sahelloen des Pesticides (CSP), under the Permanent Interstate Committee for Drought Control in the Sahel (CILSS) facilitate joint registration of biocontrol agents, such as Aflasafe, allowing them to be deployed across borders cost-effectively (Bonkoungou et al. 2024; Ortega-Beltran and Bandyopadhyay, 2023).

WHO Regulatory standards

Governments and the intergovernmental Codex Alimentarius Commission (the food standards-setting body) use the risk assessment on mycotoxins in food by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) to establish maximum levels in food or to give other mycotoxins risk management recommendations. The Codex standards are the international reference to the national food supply as well as for trade in food, where people from all over the world can be reassured that the food they purchase complies with the agreed Codex safety and quality standards regardless of where it is produced.

Mycotoxins are highly toxic and the maximum limits for mycotoxins in food are extremely low. For example, the maximum levels for aflatoxins set by the Codex in various nuts, grains, dried figs and milk are in the range of 0.5 to 15µg/kg (a µg is one billionth of a kilogram). Patulin's Codex limit in apple juice is 50µg/L.

FDA Policy

Aflatoxins are toxins, things that can make food harmful to your health. The Food and Drug Administration (FDA) will likely see food with more than 20 micrograms per kilogram (or 20 parts per billion) of total aflatoxins as “contaminated” according to section 402(a)(1) of the main law about food, drugs and cosmetics (21 U.S.C. 342(a)(1).

Future Challenges

Despite its terrible health, food and economic impacts on the global agri-food systems, AF contamination, mostly caused by Aspergillus species, still remains a serious threat to the global agri-food systems (Bhardwaj et al. 2023; Jallow et al. 2021). As a key factor that increases the prevalence of AF, recent studies have highlighted the role of climate change in this context. Increased global temperatures, changing patterns of precipitation and high humidity levels provide favourable conditions in the growth of fungi in staple crops like maize, groundnuts and other cereals. These fungi are not only able to survive in changing climatic conditions but also generate AFs that are highly resilient and able to withstand conventional methods of food processing (Bunny et al. 2024; Umar et al. 2023; Urugo et al. 2023; Nji et al. 2022).

The monitoring and detection of AFs are also essential issues. The conventional techniques, including the HPLC and enzyme-linked immunosorbent assay (ELISA) are precise but can be very expensive, time-consuming and require well-equipped laboratories, which makes it undoable in practice in the low-resource setting (Kumar et al. 2022). To address this, there has been an increasing interest in sustainable and innovative solutions which include the development of resistant crop varieties, biotechnology applications; advanced food processing methods, nanotechnology, and machine learning-based predictive models. These tools are potentially useful as real-time, on-site detection and specific treatment. However, as (Nazareth et al. 2024) highlight, the innovations need thorough field testing in different agro-ecological regions and capacity building of stakeholders to achieve successful adoption. The lack of correspondency between technological innovation and ground implementation is a key factor towards ineffective AF risk reduction (Jallow et al. 2021).

Conclusion

Aflatoxin B1 (AFB1) is considered to be one of the most poisonous and carcinogenic mycotoxins, and can be dangerous to humans, food safety and the agricultural industry. AFB1 is produced primarily by Aspergillus flavus and Aspergillus parasiticus and is usually found in cereals, nuts, animal’s feed and dairy products when stored and grown under poor conditions. Acute exposure may lead to aflatoxicosis and intense liver damage, while chronic exposure may lead to hepatocellular carcinoma, immune deficiency and growth retardation.

The monitoring and detection of AFB1 contamination have been improved by various analytical techniques including TLC, HPLC, LC-MS, biosensors and rapid detection kits. Of course, other methods of detoxification, such as ammoniation, biological degradation, or improved storage techniques, have proven to be effective to lower levels of toxins. But aflatoxin contamination is a serious problem, particularly in developing countries where warm and humid conditions promote the growth of fungi.

Consequently, it is critical to have strict food safety standards, food storage procedures, new detection methods, and ongoing food safety awareness to reduce the presence of AFB1 and safeguard public health. The use of environmentally friendly, cost-effective and sustainable

approaches to control and detoxify aflatoxins should be a target for future research.

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