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Aflatoxin and its Public Health Significance: A Review

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Abstract

Aflatoxins are a group of mycotoxins produced by certain fungi, especially Aspergillus flavus and Aspergillus parasiticus. Aflatoxicosis is a condition caused by aflatoxin in both animals and humans. The contamination of food and feed with aflatoxin has serious health consequence in animal and humans as well as economic impacts especially in developing countries. Aflatoxin M1 is stable in raw milk and processed milk products and is unaffected by pasteurization or processing into cheese and yogurt. The ability of this toxin to induce cancer in experimental animals and the relatively large consumption of milk by children has made in food contaminant of worldwide concern. Aflatoxin exposure is mainly problem in poor and developing countries with poor regulation authority in food processing, storage and weather condition as well as with high level malnutrition all species of animal are susceptible to aflatoxicosis. Although we can control and eradicate the incidence of aflatoxin by using different strategy. A primary strategy should aim to eliminate aflatoxins by reducing mould proliferation during Cultivation and storage and also different methods are used to decontaminate food and feed before ingestion. Different methods for detection and quantification of aflatoxins have been employed in this document for agricultural food crops and feeds have been explored. While chromatographic methods such as TLC, HPLC and ELISA are thus the most widely used techniques in aflatoxins analysis.

Keywords: Aflatoxicosis; Economic; Milk Product; Public Health; Tumor

Abbreviations: Ab: Antibody; AFB1: Aflatoxin B1; AFBO: Aflatoxins B1-8, 9-epoxide; AFS: Aflatoxins; CYP450: cytochrome P450 enzyme; DO: Deoxynivalene; ELISA: Enzyme Linked Immune Sorverbent Assay; GH: Gas Gromatography; GIT: Gastrointestinal tract; HPLC: High performance Liquid chromatography; TLC: Thin layer chromatography; ZEA: Zearalenone

Introduction

Aflatoxins are a group of mycotoxins produced by certain fungi, especially *Aspergillus flavus* (*A. flavus*) and *Aspergillus parasiticus* (*A. parasiticus*) [1]. A. flavus and A. parasiticus are economically important molds that produce exclusively aflatoxin B1, B2, G1 and G2, and all the other aflatoxins are derivatives of these four aflatoxins [2]. According to Ruiqian [3], A. flavus and the closely related species, A. parasiticus have a worldwide distribution and normally occur as saprophytes in soil and many kinds of decaying organic matter. It colonizes a wide variety of food commodities including maize, oil seeds, spices, groundnuts, tree nuts, milk, and dried fruit [4]. Aflatoxigenic fungi produce four major aflatoxins: B1, B2, G1 and, G2 plus two additional metabolic products, M1 and M2, that are of significance as direct contaminants of foods and feeds [5].

Aflatoxin B1 (AFB1) has a great concern because of its detrimental effects on the health of animals and, humans including carcinogenic, mutagenic, teratogenic, and immune suppressive effects [6]. Upon ingestion by ruminants, AFB1 is partially destroyed in the rumen whereas the absorbed AFB1 rapidly undergoes metabolic processes in the liver to various secondary metabolites [7]. Consequently, over 5 billion people in developing countries worldwide are at risk of chronic exposure to aflatoxins through contaminated foods (Williams et al., 2004). Risk of human exposure to AFM1 contamination of milk is a major concern in Ethiopia where dairy farmers commonly use different mixed concentrate feeds containing traditional brewery by-product ("atela"), wheat bran, noug (Guizotia abyssinica) cake, maize grains, and silage to increase production. However, these feeds are susceptible to contamination with AFB1 [8].

Milk shed is selected for studies because it is a rapidly intensifying system where aflatoxins are likely to be an increasing problem. Fresh milk which is often consumed in developing countries without treatment poses a high risk to consumers [9]. Aflatoxin poisoning has been associated with eating home grown maize and storing it under damp conditions [10]. The application of these strategies in developing countries is difficult, given differences in technology, agriculture, and trade practices, as well as other issues contributing to occurrence of aflatoxins and incidence of exposure. Studies have indicated aflatoxin contamination in Ethiopia in staple cereals [11]. Aflatoxins must be removed from food and feed sources through various methods such as detoxification on larger scale so that to prevent great

economic loss and illnesses in human beings and animals [12]. Agricultural interventions are methods or technologies that can be applied either in the field ("pre-harvest") or in drying, storage, and transportation ("post-harvest") to reduce aflatoxin levels in food [13]. Aflatoxins are regulated in more than 80 countries; their legislation is not yet completely harmonized at the international level [14].

Therefore, the objectives of this review paper are:

- To highlight on the aflatoxin.
- To review on public health and economic significance of Aflatoxin.

Aflatoxicosis and its Predisposing Factors

Aflatoxins are secondary metabolites produced by the common molds of A. flavus, A. parasiticus and A. nominus. These fungi are ubiquitous in the environment and produce aflatoxin in warm (30-35 °C) and high-humidity conditions. The occurrence of aflatoxins in agricultural commodities depends on region, season and the conditions under which a particular crop is grown, harvested or stored. Crops grown under warm and moist weather in tropical or subtropical countries are more prone to aflatoxin contamination than those in temperate zones. Aflatoxin production is also stimulated by high zinc concentration in feed [15].

Aflatoxin contamination occurs at every stage of the supply chain, from preproduction to post-harvesting, marketing and distribution. Aflatoxin accumulation during post-harvesting is a particular challenge for Africa. Food and feed contamination with AFS like soyabeans, maize, oilseeds, spices, peanuts, tree nuts (almonds, pistachios, hazelnuts, pecans, Brazil nuts, and walnuts), milk (in the form of aflatoxin B1‟s metabolite aflatoxin M1), milk product and dried fruit [16]. These are found in many foods and considered as major public health problem especially in developing countries where long term food storage is often inadequate for high heat and humidity, which encourage the growth of mold. Their production can be influenced by several factors, including temperature, water activity, pH, available nutrients, and competitive growth of other microorganisms [17].

The most important factors that help predict the occurrence of aflatoxins in food include weather conditions, agronomical practices (crop rotation and soil cultivation) and internal factors of the food chain (drying and storage conditions). A comprehensive approach is needed to identify and control risks related to good production system that could present a potential hazard to human health, being necessary to identify emerging risks which may include "newly" identified risks, not previously observed risks in human or animal food chain as well as known risks. The emerging risks need to be identified as early as possible in order to take appropriate preventive measures. Thus, the specific risk can be prevented from becoming a danger [18]. Subsistence farmers in developing countries are perhaps the most at risk. They often lack the capacity to protect crops against aflatoxin contamination. In addition, food insecurity due to drought and other causes of crop failure seem to contribute to behaviors that increase risk of exposure. Finally, 60-85% of the population in the developing world is subsistence farmers and is not protected by commercial food safety regulation [19] (Figure 1).

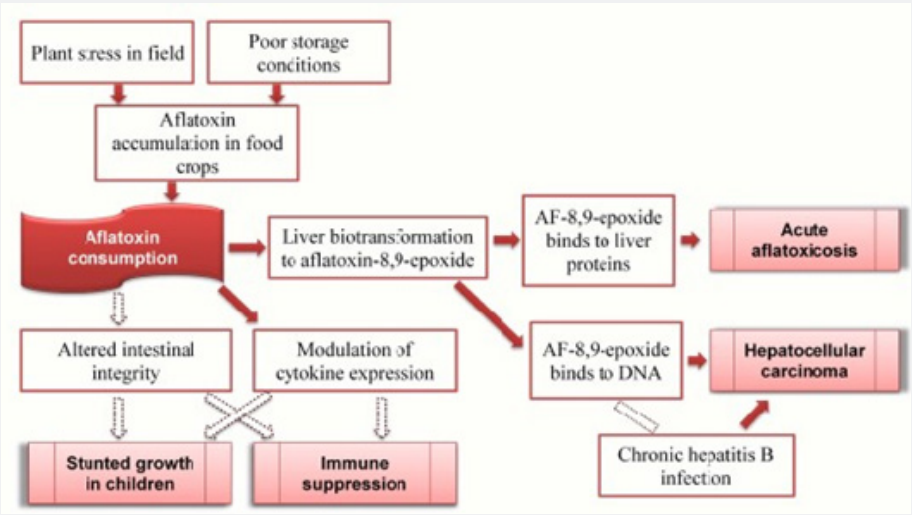

Figure 1: Aflatoxin disease pathways in humans [13,50].

Toxicity and Detoxification of Aflatoxin

Toxicity of Aflatoxin: Aflatoxins are highly lipid soluble compounds and are readily absorbed from the site of exposure usually through the gastrointestinal tract (GIT) and respiratory tract into blood stream [20]. Aflatoxin B1 itself is not a potent toxin, and phase I bioactivation is needed to exert toxic effects. Phase I reactions are mainly oxidation of AFB1 to hydroxylated metabolites such as aflatoxin M1 [21]. Bioactivation is required for AFB1to be toxic and this processing predominantly occurs in hepatocytes [22]. Aflatoxins have been recently considered as an important sanitary problem because it has been demonstrated that human exposure to mycotoxins may result from consumption

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of plant derived foods that are contaminated with toxins and their metabolites (which are present in animal products such as milk, meat, visceral organs and eggs) or exposure to air and dust containing toxins [23].

Aflatoxin can cause both acute and chronic aflatoxicosis. Aflatoxins, once ingested (because of their low molecular weight), are rapidly adsorbed in the gastro-intestinal tract through passive mechanism, and then quickly appear as metabolites in blood after just 15 minutes and in milk as soon as 12 hours post-feeding [24]. Early toxicological studies focused on the acute toxic effects of aflatoxins on animals, and demonstrated that ducklings, hamsters, rabbits, trout, rats, and a number of other vertebrates were all susceptible. Soon it was discovered that aflatoxins administered in lower doses over longer periods of time could induce tumors, particularly in the liver. Rats and trout were highly susceptible to the carcinogenic effect of aflatoxin B1 [25].

Aflatoxicosis is defined as the poisoning that results from ingesting aflatoxins. There are two forms that have been identified, the first is acute severe intoxication, which results in direct liver damage and subsequent illness or death, and the second is chronic sub symptomatic exposure. The effects are usually subclinical and difficult to recognize. Some of the common symptoms are impaired food conversion and slower rates of growth with or without the production of overt aflatoxin syndrome [26]. The effects of aflatoxins on humans, as with animals, are dependent upon dosage and duration of exposure [27]. The toxicity and carcinogenicity of AFB1 is thought to be directly linked to its bioactivation, resulting in a highly reactive AFB1 8, 9-epoxide (AFBO). This bioactivation of AFB1 occurs primarily by a microsomal cytochrome P450 (CYP450) dependent epoxidation of the terminal furan ring of AFB1 and is responsible for binding to cellular macromolecules such as DNA, RNA and other protein constituents [28]. The formation of AFB -DNA adduct is highly correlated to the carcinogenic effect of AFB in both animal and human cancer cases [29]. The International Cancer Research Institute identifies aflatoxin B1 as a Class 1 carcinogen, resulting in the regulation of this mycotoxin at very low concentrations in traded commodities (20 ppb in grain and 0.5 ppb in milk in the United States; 4 ppb in foods in some European countries) [30].

Detoxification of Aflatoxin: The increasing number of reports on the presence of aflatoxins in food and feedstuffs dictates the need for decontamination procedures; such procedures should not only reduce the mycotoxin content to "safe" levels below regulatory limits but should also have the following characteristics: easy to use, inexpensive and free of the potential for forming compounds that are still toxic or compromising the nutrinal value of the treated commodity [31]. Although numerous detoxification methods have been tested, only some of them seem to be able to fulfill the efficacy, safety, safeguarding measures of nutritional elements and costs requisites of a detoxification process. These methods can be divided into three subcategories, which are physical, chemical and biological techniques [32]. Physically, aflatoxin contaminated seeds can be removed by hand picking or photoelectric detecting machines, but this is labor intense and expensive. Heating and cooking under pressure can destroy nearly 70% aflatoxin. Dry roasting can reduce about 50-70% of aflatoxin and sunlight drying of aflatoxin contaminated feed could reduce the toxin level by more than 70% [33].

Biologically, which are based on the action of microorganisms on mycotoxins and their mechanism of action is based on competition by nutrients and space, interactions, and antibiosis, among others [34]. Biological control of mycotoxin is a promising approach for reducing both pre harvest and postharvest mycotoxin contamination in food crops [35]. Different organisms, including bacteria specially, probiotics and dairy strains of lactic acid bacteria, yeasts strains of Saccharomyces cerevisiae and nontoxigenic Aspergillus fungi, have been tested for their ability in the control of AFs contamination [36]. Chemically, there is no reliable method for feed decontamination from aflatoxin, various workers have screened a large number of chemicals viz. benzoic acid, propionic acid, copper sulphate, synthetic zeolites, citric acid etc. these chemicals have shown the reduction of aflatoxin in vitro [37].

Detection Methods of Aflatoxin

Various analytical methods employed in analysis of aflatoxins in agricultural food crops and feeds have been explored. While chromatographic methods such as TLC, HPLC and ELISA are considered the gold standard and are thus the most widely used techniques in aflatoxins analysis, they remain largely cumbersome, requiring extensive sample preparations, let alone very expensive equipment. This makes their routine use in analysis confined to laboratories. It is on the account of such limitations that it was necessary to develop more sensitive and better techniques for aflatoxins analyses [38]. Mainly used methods for analysis of aflatoxins in food and feed are the thin layer chromatography (TLC), liquid chromatography (LC), and immunochemical methods. TLC is one of the most widely used separation techniques in aflatoxin analysis. Since 1990, it has been considered the AOAC official method and the method of choice to identify and quantitate aflatoxins at levels as low as 1ng/g. Similar in many respects with TLC is LC. Usually TLC is used as a preliminary work for optimization of LC separation conditions [39].

Chromatography

Chromatography is one of the most popular methods to analyze mycotoxins such as Aflatoxins. Gas chromatography (GC), liquid chromatography (LC), High performance liquid chromatography (HPLC) and thin layer chromatography (TLC) are the most common techniques of chromatography. Out of these methods, LC and HPLC are the most used. In many cases, they are followed by fluorescence detections stage [40]. LC, TLC and HPLC are the most used quantitative methods in research and routine analysis of aflatoxins [41]. These techniques offer excellent sensitivities, but they frequently require skilled operators, extensive sample pretreatment and expensive equipment [42].

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High performance liquid chromatography: High Performance Liquid Chromatography system of aflatoxin estimation has high precision, high sensitivity, and high automation. This method retains two phase systems: normal phase (liquid/solid, polar stationary phase) and reverse phase (liquid/liquid, polar mobile phase) in conjunction with UV absorption and fluorescence detection. Reverse-phase HPLC is widely used for aflatoxin analysis [43]. HPLC is the most popular method for the analysis of mycotoxins in foods and feeds. Actually, it is a quantitative technique that is suited for online cleanup of sample extract and could be combined with different detectors [44].

The mycotoxins extracted from field samples undergo clean-up using commercial immune affinity columns before their analysis by HPLC. The columns are available for all the important mycotoxins: AFB1, AFB2, AFG1, AFG2, AFM2, ochratoxin A, T2 toxin, deoxynivalenol (vomitoxin), citrinin, fumonisins FB1, FB2, FB3, zearalenone, patulin and moniliformin. Multiplex columns are available for AFs, ochratoxin A and zearalenone. The rationale beyond the multiplex columns and for multiplex detection methods is the frequent production of more than one mycotoxin by a single fungus, and the frequent contamination of crops or silage with several species of fungi [45].

Thin layer chromatography: Thin layer chromatography (TLC), also known as flatbed chromatography or planar chromatography is one of the most widely used separation techniques in aflatoxin B1 analysis. Since 1990, it has been considered the AOAC official method. The TLC method is also used to verify findings by newer, more rapid techniques. The technique is widely used in laboratories throughout the world for food analysis and quality control. Applications of TLC have been reported in areas of food composition, intentional additives, adulterants, contaminants, etc. TLC has been used to analyze agricultural products and plants. It has advantages as, simplicity of operation; availability of many sensitive and selective reagents for detection and confirmation without interference of the mobile phase; ability to repeat detection and quantification; and cost effectiveness analysis, because many samples can be analyzed on a single plate with low solvent usage, and the time that TLC employs to analyze the sample is less that LC method [46]. Presumptive aflatoxin detection can be performed with thin-layer chromatography (TLC) as this method is a simple, robust technique, which is relatively is an inexpensive compared to high performance liquid chromatography methods [47].

Enzyme Linked Immune Sorbent Assay Detection Method

Aflatoxin detection has largely relied on competitive ELISA formats that are semi quantitative and depend on the use of conjugated toxin molecules. However, the limitation of conventional immunoassay formats is largely due to the requirement that at least two epitopes of an antigen to be recognized and occupied. It is more challenging to develop a sandwich immunoassay assay for low molecular weight hapten molecules such as aflatoxins since the antibodies are much larger in size (150 K Da) than the aflatoxin molecules (~0.312 K Da) themselves preventing combinatorial association of antibodies due to steric hindrance and limiting access of the antigen to secondary antibodies. It is also reported that the sandwich immunoassay for molecules of 1000 Daltons MW is less amenable for such applications.

Economic and Health Impacts of Aflatoxin

Aflatoxins have economic and health importance because of their ability to contaminate human food and animal feeds, in particular cereals, nuts and oilseeds. The toxins have adverse effects on plants, animals and humans. They are responsible for damaging up to 25% of the world's food crops, resulting in large economic losses in developed countries and human and animal disease in under-developed countries [48]. The toll of the effects on human health includes the cost of mortality, the cost of productive capacity lost when people die prematurely, the cost of morbidity, losses resulting from hospitalization and the cost of health care services, both public and private. There is intangible cost of pain, suffering, anxiety and reduction of the quality of life [49].

Economic Impacts

The magnitude of the economic impacts of the health consequences associated with consumption of aflatoxin-contaminated food in developing countries is not known due to a lack of good data. According to them, the quantification of economic losses and estimation of the effects of aflatoxin on health will encourage Health Ministries to enforce standards and provide crucial advocacy to benefit the rural poor, such as improving their level of education about aflatoxin exposure [50]. The economic impact of aflatoxins derives directly from crop and livestock losses as well as indirectly from the cost of regulatory programs designed to reduce risks to animal and human health [51]. The chronic and acute exposures of cattle to aflatoxin cause significant economic loss. In addition to financial losses and economic damage to agricultural and animal husbandry, losses due to aflatoxin contamination of foods include major pharmaceutical and health costs to treat food poisoning. Consumption of aflatoxin contaminated feed reduces productivity of livestock [52].

Aflatoxin cause huge economic loss to Ethiopia and many developing countries. Commodities can be contaminated with aflatoxigenic fungi and aflatoxin at any time, before harvest and after harvest. The prevention of aflatoxin once occurs and treatment of aflatoxicosis is difficult. However, there are some mitigation mechanisms pre- and post-harvest. Moreover, awareness creation on aflatoxin contamination, its effect and management are essential.

Public Health Significance

Humans are exposed to aflatoxins by consuming foods contaminated with products of fungal growth. Evidence of acute aflatoxicosis in humans has been reported from many parts of the world, namely the Third World Countries. Conditions increasing the likelihood of acute aflatoxicosis in humans include limited availability of food, environmental conditions that favor fungal development in crops, and lack of regulatory systems for aflatoxin

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monitoring and control. The expression of aflatoxin related diseases in humans may be influenced by factors such as age, sex, nutritional status, and/or concurrent exposure to other causative agents such as viral hepatitis (HBV) or parasite infestation [53]. Over 5 billion people in developing countries worldwide are estimated to be at risk of chronic exposure to aflatoxins through contaminated foods. Aflatoxins are naturally occurring contaminants of food according to Guo [54].

Animals and humans are exposed to aflatoxins through consumption of contaminated products such as dairy products (e.g. milk, cheese, and yogurt) [55] Aflatoxin is both a food safety and public health issue because of its toxicity. When it is consumed, it can exert toxicity by altering intestinal integrity or modulate the expression of cytokines which can result to stunted growth in children and/or immune suppression. In the liver, aflatoxin may be transformed by certain p450 enzyme to its DNA reactive formAflatoxin-8-9-epoxide which binds to liver proteins and lead to their failure, resulting in acute aflatoxicosis or it may bind to DNA, contributing to aflatoxin induced hepatocellular carcinoma (liver cancer) [56].

Control and Prevention of Aflatoxin

Ultimately an early warning system should rely on multiple sources of information and triggers that would set in motion various responses for preventing or reducing an outbreak of aflatoxicosis. Triggers for action could also be based upon other factors which indicate or influence aflatoxin contamination, such as reporting of death among livestock or domestic animals which are often given lower quality grain. Modeling of aflatoxin contamination based on weather conditions from planting to post-harvest could also serve as a trigger [57]. To minimize risks associated with unavoidable exposure to AFs, regulation and monitoring measures must be supported by in field (pre-harvest) and storage (postharvest) interventions which may be applied to minimize AF contamination. AFM1 is excreted in milk of dairy animals following metabolism of AFB1 ingested with feed. Contamination of milk may, thus, be reduced either directly, decreasing AFM1 content of contaminated milk, or indirectly, decreasing AFB1 contamination in feed of dairy animals. Many methodologies developed to reduce AFM1contamination with both direct and indirect approaches have been extensively reviewed [58].

The presence and growth of Aspergillus on pre-harvested crops is dependent on the environment. Agricultural practices including proper irrigation and pest management can reduce aflatoxin contamination. Pre-harvest interventions include choosing crops with resistance to drought, disease, and pests and choosing strains of that crop which are genetically more resistant to the growth of the fungus and the production of aflatoxins. Feeds have to be kept hygienically and prevent molds formation by using available methods that is accessible for them in their environment Aware Extension workers and owners of livestock on impact of aflatoxin in feeds: implications to livestock and human health. Systemic vaccination of dairy cows and heifers has recently proved to be effective in reducing AFB1 carry-over as AFM1 in milk [59]. Reduction through food processing procedures: Sorting can remove a major part of aflatoxin contaminated units, but levels in contaminated commodities may also be reduced through food processing procedures that may involve processes such as washing, wet and dry milling, grain cleaning, dehulling, roasting, baking, frying, nixtamalization and extrusion cooking. These methods and their impact on aflatoxin reduction have been reviewed [60-72].

Conclusion and Recommendations

Generally, Aflatoxins are a group of mycotoxins produced by certain fungi, the occurrence of aflatoxins is influenced by certain environmental factors; hence the extent of contamination will vary with geographic location, agricultural and agronomic practices, and the susceptibility of commodities to fungal invasion during pre-harvest, storage, and or processing periods. Aflatoxins and the associated health disorders in humans and animals(which includes immune suppression, carcinogenic, teratogenic etc.) have been recognized as a major health and economical problem which dictates measures to minimize the exposure by applying proper agricultural practice, storage of products and control of the products intended for human or animal consumption. The high toxicity and carcinogenicity of the aflatoxin contaminated compounds and their ability to cause various pathological conditions has led to widespread screening of foods and feeds potentially contaminated with them.

Therefore, based on the above conclusion the following recommendations are forwarded;

a. Feeds should be kept hygienically and stored dry condition to prevent molds formation

b. The farmers should select crops strains resistant to drought, disease, and pests

c. Awareness should be created on public about aflatoxicosis and its economic impact.

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