

AFLATOXINS IN PAKISTANI FOODS: A SERIOUS THREAT TO FOOD SAFETY

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Abstract

Finished products have always been the main focus of food safety. Aflatoxins are the fungal toxins mainly produced by *Aspergillus flavus* and *Aspergillus parasiticus* species. The contamination level depends upon pre and post-harvest practices, processing as well as storage conditions of the foods. The warm and humid environment of Pakistan is very favorable for the growth of aflatoxigenic fungi. Moreover, the lack of awareness among the masses, improper pre and post-harvest practices, usage of contaminated equipment and poor processing and storage conditions are the major contributing factors towards the contamination of aflatoxins in a variety of foods in Pakistan. In this study, occurrence and toxicity of aflatoxins in Pakistani foods i.e. cereal grains, chilies, dry fruits and milk etc., are briefly described.

This base line data about the prevalence of aflatoxin contamination will certainly help to devise the effective strategies to tackle this significant problem. Moreover, different approaches for the exposure assessment of aflatoxins to the consumers are also discussed and analyzed. Advances in research have developed some techniques to minimize the risk of aflatoxins contamination. These include the application of bio-control agents (amycotoxigenic and antagonistic activities), bio-degrading agents (microbes and/or their enzymes) and some other physical or chemical treatments during processing of foods.

Continuous monitoring and surveillance, as well as improved testing and analytical facilities at grass root level will facilitate to tackle this potential food safety threat in Pakistan.

Key words: Aflatoxins, Pakistan, Exposure assessment, Remediation.

1. Introduction

Pakistan is categorized as an agrarian society, as more than 65% of its population lives in rural areas. Agriculture

sector imparts 23% of Pakistan's total GDP and employs about 41% of its work force. A variety of food crops i.e. rice, wheat, chilies, citrus and mangoes etc are produced in surplus and exported to other parts of the world from Pakistan. However, the problem of mycotoxins contamination in its agricultural produce is affecting the agricultural exports from Pakistan, and also posing serious food safety threats to its local population, consuming these contaminated foods. Mycotoxins are metabolites of fungi and about 400 types of mycotoxins have been reported. Mycotoxins are comprised of diverse organic structures with variety of functional groups forming: fumonisins, aflatoxins, ochratoxins, deoxynivalenol (DON), and others as trichothecenes, zearalenone and patulin etc. Among all these, aflatoxins are of major concern being unavoidable contaminants present in a variety of foods. High temperature coupled with periods of drought intensifies the production of aflatoxins in fields. So, the majority of world's population (approximately 4.5 billion people) living in hot zone of the Earth are chronically exposed to the aflatoxins. These are type of mycotoxins naturally produced by fungus mainly by *Aspergillus* species in a large variety of foods i.e., rice grains, maize, milk, peanut, chilies ([1], [2], and [3]).

Aflatoxin B1, B2, G1 and G2 are most important members of this group, and chemically are coumarin derivatives with a fused dihydrofurofuran moiety. These compounds are reported as carcinogenic and mutagenic in humans and various animals, and cause a disorder known as aflatoxicoses [4]. Aflatoxins are also reported as immune suppressors, inflammation promoters and growth suppressors, both in animals and humans. Another reason making aflatoxins most challenging mycotoxins, is the ability of the contaminating fungus to produce these toxins not only at pre-harvest stages, but also during post-harvest stages, including storage of the agricultural produce. Climate of maximum Pakistani cultivate able area is warm and humid, so Pakistani foods are more susceptible to aflatoxins production, and the condition is increasing due to

inappropriate agronomic practices of farmers, processors and in adequate facilities of storage ([1], and [5]). Due to high level contamination of aflatoxins, export of chilies and rice from Pakistan is badly affected as Pakistan is one of the biggest exporters of these two commodities in the world. So it is an alarming situation for Pakistan's economy [6].

Worst effects of aflatoxins in human body include: liver cancer, abdominal pain, eyes inflammation, joint disorders, gastrointestinal inflammation, bowel syndromes and different neurological disorders [3]. Humans can consume aflatoxins directly i.e., by consumption of contaminated foods or indirectly i.e., by consumption of milk, in which aflatoxin M1 and M2 are present, that are formed by biotransformation of aflatoxin B1 and B2 from contaminated feed consumed by animals [7]. According to a survey conducted in Karachi, the biggest city of Pakistan, prevalence of high level of liver cancer is associated with aflatoxins contamination in different foods ([8], [9]). About 4.6 billion populations of developing countries is being exposed to aflatoxins continuously due to improper handling, processing and storage conditions, that is a remarkable factor in reducing immunity and in alteration of nutritional composition of various food items [10].

With increased awareness of aflatoxins as potential health hazards in foods and feeds, efforts are being made for complete elimination or maximum reduction of aflatoxins level in food commodities. These efforts include: Good Agricultural Practices (GAPs), use of hygienic farm equipment, improved pre and post-harvest practices, and Good Storage Practices (GSPs). Though, prevention of fungal contamination in foodstuff is most effective intervention, however, various strategies i.e. physical, chemical or biological are being investigated to inactivate, reduce or eliminate these potential contaminants. This study is an overview of occurrence and recent approaches on the fate, toxicity and decontamination of aflatoxins in Pakistani foods.

2. Prevalence in Pakistani foods

The Food and Agricultural Organization (FAO) has reported that many of the basic foods can get contaminated by mycotoxigenic fungi, contributing towards a huge global loss i.e. 1000 million metric tons per year, of agricultural produce. Poor post-harvest techniques, improper handling, lack of novel food processing and preservation technologies, tropical conditions, temperature, water activity, moisture contents, initial contamination load, and poor agricultural practices are main reason for prevalence of aflatoxins in various types of Pakistani foods ([1], [6], [11], and [12]). Different surveys and studies conducted in Pakistan, showed aflatoxins contamination in different food groups are summarized as following:

Cereals and Animal Feed

Aflatoxin is found in a variety of cereals i.e., wheat, maize, rice, and barley reported from different parts of Punjab, Pakistan as a serious problem to local consumers [6]. Wheat is staple food in Pakistan with annual production of 24 million ton on average that is sufficient for domestic consumption. Improper conditions of storage environment in wheat godown i.e., high humidity and improper ventilation systems are major causes of *Aspergillus* growth, ultimately causing the production of aflatoxins. Average mean value of aflatoxins in wheat has been reported as 5.2 µg/Kg [12]. Maize is also consumed widely in Pakistan, and approximately 4.2 million tons of maize was produced in the year 2012-13 with average production of 4 tons per hectare. Unfortunately high level of aflatoxins in maize grains is also reported with average mean value of 30.34 µg/Kg. The minimum mean value was reported as 10.4 µg/Kg, and the maximum mean value was 49.35 µg/Kg ([12], and [13]).

Rice is 2nd main income crop of Pakistan and it is consumed widely, as main meal course and parts of different dishes. Rice is also major export crop of Pakistan as it contributes about 6.11% of total value added in agriculture while about 1.4% of total Gross Domestic Product (GDP) is contributed by rice export [14]. Hygroscopic nature of rice grains make it more susceptible to aflatoxins production. Aflatoxin B1, B2, G1 and G2 were reported in rice taken from different parts of Pakistan, by using thin layer chromatography [15]. Broken and damaged rice grains are more susceptible to aflatoxins production than whole rice kernels ([16], [17]). Aflatoxin contamination was observed in 70% of rice samples with mean concentration of 4.9 µg/Kg, which is higher than the specifications [17].

Aflatoxins contamination in animal feed is an important issue for farmers due to both chronic and acute intoxication in animals, which ultimately lead towards the economic loss due to reduced productivity and organ damage in the animals. The contamination may occur in cottonseed, millet, sorghum and other feed grains. Hay and straw may also be contaminated with aflatoxins. Direct correlation between aflatoxin B1 concentration in fodder and aflatoxin M1 concentration in milk of the animal, feeding on contaminated fodder was analyzed in various animal species from Punjab, Pakistan [16].

Milk

Pakistan produces 29 million tons of milk annually, and holds 5th position regarding worlds total milk production. About 70% of total milk production in Pakistan is from buffaloes, while 98% of the total milk production is sold in raw form. A very minute percentage i.e. 2% of the total milk production goes under UHT processing

in Pakistan. Milk producing animals in Pakistan are usually fed on fodder, cotton seed cake, threshed wheat straw, wheat bran, soybean and paddy straw, rather than on prepared feed. Aflatoxins have also been reported in milk, as milk animals feeding on contaminated feed ingest aflatoxins i.e. B1, B2, G1 and G2, which are bio-transformed into another type of aflatoxins i.e. M1 and M2 in digestive tract of animals, becoming part of milk and ultimately reaching into the humans, consuming such milk. Aflatoxin M1 contamination in milk and milk products requires continuous surveillance as 3% of the total analyzed sample of milk were found contaminated higher than the US specifications e.g. concentration more than 0.5 µg/L. Similarly, many other reports showed the high concentration of aflatoxin M1 in milk i.e. 34.5%, 37.5%, 20% and 16.7% in the samples of buffalo, cows, goats and sheep etc. Even the milk products like yoghurt, and butter samples indicated the high level contamination of aflatoxins ([18], [19], [20], and [21]).

Nuts and Dry fruits

A variety of nuts i.e. peanut, pistachios, almonds etc., and dried fruits i.e. dried apricot, figs, dates and mulberries etc., are being produced in Pakistan. Studies showed very high level of aflatoxins contamination in all these products. For example the percentage of the total samples detected: dried apricot (20%), dates (10%), dried figs (50%), dried mulberries (26%), and raisins (20%), while in apricot kernels (26%), almonds without shell (30%), walnuts with shell (40%), walnuts without shell (70%), peanut with shell (40%), peanuts without shell (50%), pistachios with shell (20%), pistachios without shell (50%), and pine nuts with shell (20%) were contaminated with aflatoxin and showed higher concentration comparing with the suggested limit of 4 µg/kg, set by EU regulations [11]. High aflatoxin levels have been investigated in dried figs, found mainly in the fruit cavity [22].

Chilies

Pakistan is a large exporter of chili peppers by contributing 1.5 % of total gross domestic products. Pakistan ranks 6th position worldwide in export of chilies. Aflatoxin has been reported in various varieties of chilies in Pakistan especially in red chilies. Contamination level is found alarming with respect to food safety, as chilies are used as main food ingredient in number of recipes for typical taste and flavor. Chilies production and export in Pakistan may be highly affected by aflatoxin contamination, [23]. A number of studies have reported the high level contamination of aflatoxins in chilies. On average 30% of the chilies samples were found contaminated with average mean value of 30 µg/kg, which is six fold higher than the EU permissible limits ([12], [24]).

The situation becomes more drastic when we see the reports on aflatoxins contamination in different water bodies, tap water that is not properly filtered or at those places in which water is stored for a long period of time without drained. Concentrations are found high enough to pose potential health risks to the consumers [17]. Pakistan is suffering from Dengue epidemic since last three year. The situation may relate to the continuous exposure of consumers to aflatoxins in different food, causing the immune deficiencies, which may leads towards the Dengue fever etc. [11].

2.1 Toxicology and exposure

Aflatoxins have been shown highly mutagenic and carcinogenic in different animals, in many organs, primarily targeting the liver cells. There are sufficient evidences reported by different studies for carcinogenic effects of major aflatoxins, such as aflatoxin B1, B2, G1 and G2 etc. ([25], [26]). Among these, aflatoxin B1 is most prevalent and toxic compound. The contamination of aflatoxin B1 becomes more serious threat, if it is present in animal feed, because after getting ingestion in the animal stomach, it is bio transformed into another type of mycotoxins named aflatoxin M1, which is secreted into animal milk, and ultimately finds its way into human food chain. The International agency for Research on Cancer has classified the aflatoxin B1 and M1 as Group 1 human carcinogen even though the aflatoxin M1 is ten times less carcinogen than aflatoxin B1 ([21], and [22]).

Toxicity of aflatoxins can be categorized into two major types as; acute toxicity and chronic toxicity. Disorder occurred due to toxicity of aflatoxins is known as aflatoxicosis, symptomized as food poisoning leading to liver cancer. Acute poisoning is less common in animals and humans; it occurs only due to the consumption of very highly contaminated food items frequently ([27], [28]). Symptoms of acute human poisons are vomiting, fatty liver and accumulation of necrotizing enterocoliticus (necrosis), mental stress and neurological disorders. Chronic poisoning is caused by consumption of less contaminated food items for long period of time. Toxicological effects of chronic consumption depend on many factors mainly including age, gender, time and length of exposure. Symptoms of chronic poisoning are not specific. Normally symptoms may include weight loss, reduction in normal weight gain, reduced immunity etc. In humans, consumption of aflatoxin contaminated food for long duration may cause many dangerous diseases including hepatitis B and C, loss in reproductive effects in males, degeneration of testis, inflammation, reduction in immunity, risk of stroke and pulmonary disorders ([29], [30], [31], [32], [33], and [34]).

Aflatoxin consumption has direct interference in protein and energy metabolism in human body. After consumption of aflatoxins contaminated food, these

are readily absorbed in human digestive tract and transported to circulatory system of human body, ultimately accumulated in liver. They bind DNA bases and bind protein resulting in severe liver aches leading towards liver cancer ([31], [32]). Aflatoxin B1 is found to work as pro-carcinogen, and inhibiting the activity of many proteinase enzymes in body. Aflatoxins also poses some teratogenic effects, involve in disturbing the tissues development in children suffering from Protein energy malnutrition, and also key factor for Kwashiorkor ([25], [26], and [29]).

Regarding the exposure assessment, a very common approach to estimate the exposure is the combination of contamination data with consumption data. Contamination data is provided by the researchers, while consumption data is collected from the National dietary surveys etc. It is interesting to mention that despite of the several reports on prevalence of aflatoxins in various foods, a very little information about exposure assessment data are available. One of the major limitations is the management of left-censored data from occurrence studies, which ultimately make the exposure estimation inaccurate and unreliable. Though, there are many scientific reports proposing the best methodology for the purpose of exposure assessment, to date the harmonization is very far being achieved [35].

2.2 Control and management strategies

Control strategies for aflatoxins include detoxification by different methods including pre-harvest and post-harvest management at farm level, chemical treatments, physical methods including processing techniques, biological control i.e., bio-degradation and bio-control etc. Another approach is to reduce the bio-availability of aflatoxins by the use of enterosorptions, which is done by using the mycotoxins sequestrants, which inhibit the absorption of these toxins into gastrointestinal tract. However, this method is not widely practiced due to the safety issues of such sequestrants in human foods. There is no specific rapid method for aflatoxin detection and decontamination in food items; however aflatoxins can be controlled and minimized by using good agricultural, storage and processing practices [36]. A brief summary of different decontamination strategies is as following:

2.2.1 Physical Treatments

Currently different physical treatments are present to reduce aflatoxins in different food items including mechanical sorting, thermal treatment or heat inactivation, irradiation, density segregation etc. However, the level of reduction depends upon initial level of contamination. Aflatoxins are normally resistant to heat but detoxification by heat has been reported, especially in nuts and dry fruits i.e. the roasting of almonds at about

200 °C can significantly reduce the level of aflatoxins ([37], [38], and [39]). Cooking, washing, steaming, broiling and boiling showed considerable extent of aflatoxin reduction in different food items [38]. Irradiation (UV light treatment) and ionization are successful methods to reduce aflatoxins level, inhibition of sprouting, rupturing the cell wall and destruction of food borne pathogens ultimately resulting in the extended shelf life [39]. Detoxification effect of irradiation on aflatoxins is remarkable, and about 97% detoxification has been documented by combined treatment of irradiation with an enzyme [40]. On average, 60% positive results of aflatoxins decontamination have been reported in fruits and vegetables by using gamma rays treatment ([41], [42]).

Adsorbent treatment technologies are novel techniques for decontamination and detoxification of aflatoxins. Big advantage in using adsorbents is that, food items are prevented from production of secondary contaminants and metabolites that can cause alteration in composition of foods. Activated carbons, sorbents and clays showed more ability for decontamination of aflatoxins B and G, especially in perishable foods ([43], [44]). Animal feed treated with phosphosilicates have been documented effective in detoxification and reduction in production of aflatoxins in milk ([45], and [46]). Large range of inorganic compounds and their synthesized products including hydrated sodium calcium aluminosilicates and phyllosilicates, bentonite, zeolite and silicates that are special clays are used successfully for decontaminating aflatoxins. These are porous harboring ringed structures and tetrahedrons that trap aflatoxins by electrical elementary charges [47].

2.2.2 Chemical Treatments

Chemical decontamination/ detoxification of aflatoxins is documented by treating with ammonia, sodium bisulfite, propionic acid and different antioxidants treatments [43]. More than 100 chemical compounds have effects of inhibition or reduction of mold growth ultimately reducing aflatoxins. Chloroform and hexane extracts from a variety of fruits showed remarkable reduction in aflatoxins level. Fungitoxic effects of many antioxidants like butylated hydroxyanisole, butylated hydroxytoluene and propyl paraben on *Aspergillus flavus* have also been reported ([47], [48], and [49]).

However, both physical and chemical methods have their own limitations in terms of high cost or nutritional losses to the food products. Inefficiency of physical of chemical treatments is another consequence.

2.2.3 Biological Treatments

Biodegradation or biological control of aflatoxins is an innovative technique, successfully affected against a wide variety of mycotoxins. This concept is rising in world because of its cost effectiveness, and

environment friendly behavior. Biological methods may involve the use of microbes i.e. bacteria, fungi, yeast, algae etc, or their products i.e. enzymes etc. Mechanism of action based upon the competition for nutrients and space or interaction and/or antibiosis among each other etc. Amycotoxigenic fungal species may also be used as effective bio-control agents in the field. Biodegradation technology has offered an attractive alternative to control or eliminate the aflatoxins, while preserving the quality and safety of the food and feed. Moreover the use of biological agents gives more "natural" appeal against the ever growing resistance of customers against the use of chemical and synthetic compounds.

Biological control also includes the concept of probiotics; as probiotic mixture of *Propionibacterium* and *Lactobacillus* have shown ability to reduce aflatoxins. *Enterococcus faecium* have been documented as reducing probiotic for highly perishable foods and also effective in removal of patulin. *Streptococcus lactis* and *Bacillus subtilis* isolated from groundnut has been effective against the propagation of *Aspergillus flavus*. Similarly, plant bioactive compounds i.e. essential oils etc., are being used for many years in food processing and preservation for the prevention of mold growth. O-methoxycynamaldehyde and thyme are effective against mold growth and reduction of aflatoxins contamination ([50], [51], and [52]).

3. Conclusions

- Aflatoxins contamination in our local foods is posing a serious threat to Pakistani population resulting in increase of several chronic and acute diseases. The situation is alarming for policy makers and masses too. - In Pakistan's local environmental conditions, it is very difficult to prevent the contamination of aflatoxigenic fungi. However, creating awareness among different stake holders like farmers, processors, exporters and consumers as well, may be helpful to combat the situation.

- Several physical, chemical and biological methods for the control of aflatoxins in different foods have been reported internationally.

- Adoption of good agricultural practices and proper pre-harvest and post-harvest technologies can play an imperative role. Food hygiene trainings, awareness of food safety, and implementation of strict rules and regulations with changing food safety perspectives might be helpful to curtail the contamination of aflatoxins in Pakistani foods.

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