Review Article

Occurrence and health hazard status of aflatoxin in human food and animal feed of wheat from Pakistan: A review paper

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Abstract

Aflatoxins (Afs) are the most important and naturally occurring health hazard mycotoxin which is carcinogenic and mutagenic found mainly in cereal grain food and feeds. Disease caused by ingestion of aflatoxin through food and feed are called aflatoxicosis. They cause hepatocellular carcinoma (HCC), or liver cancer mainly in humans and some animals. Considering its significant impact on human, animals and poultry health, the main aim of the present review is to provide a comprehensive overview on Afs affecting human food and animal feed of wheat from Pakistan. Afs are principal toxic secondary metabolites produced by toxigenic fungal plants (viz., *Aspergillus flavus* and *A. parasiticus*) on host crops either before harvest or post-harvest mainly during grain storage period. Research focuses that there are two main external factors e.g. humidity and temperature which affect the incidence of Afs producing *Aspergillus* species. Warm and humid climatic condition prevails in most of the agricultural land of Pakistan. Humidity encourages *Aspergillus* to attack the wheat grains. Favorable condition for its growth is high temperature and high moisture content (>7%). The optimum temperature for Afs production is recorded as 30°C. In Pakistan very little information regarding Afs status of cereal grains is available on toxicological data in general and wheat grains in particular. But few researchers highlighted the presence of Afs in wheat food and feeds. Based on their limited published work I found a mixed trend regarding the status of Afs in wheat food and feeds. Some analyzed samples were found above, and some were below the health hazard limit or permissible limit as proposed by European Union (EU) and FDA (US Food Drug Administration). Therefore, more research is needed to be carried out on the potential threats to human beings, animals and poultry after consumption of Afs contaminated wheat food and feeds.

Key words: Aflatoxin (Af); wheat; food; feed; mycotoxin; aflatoxicosis

Introduction

Wheat (*Triticum aestivum* L.) is an important food crop grown throughout the

globe. It is the third most produced cereal grain after maize and rice crops. It is a staple food of billions of people. China, India,

USA, France, Russia, Australia, Canada, Pakistan, Germany and Turkey are the top ten leading countries producing about 125.6; 94.9; 61.8; 40.3; 37.7; 29.9; 27.0; 23.5; 22.4 and 20.1 million metric tones, respectively [1]. In Pakistan, wheat is the topmost cereal crop cultivated on 8649.8 thousand hectares by producing 25979.4 thousand tones during growing year 2013-14 [2].

Many agricultural commodities are vulnerable to attack by a group of fungi that are able to produce toxic secondary metabolites known as mycotoxins. There are more than six common mycotoxins viz. Aflatoxin, Ochratoxin, Fumonisin, Zearalenone, Trichothecenes and Patulin [3]. Aflatoxins (Af) are one of the most important and naturally occurring toxins were first discovered in Europe in animal feed. Af are found as contaminants in various agricultural commodities such as oats [4]; nuts [5]; maize [6]; oil seed crops [7]; chilli [8]; wheat [3]; rice [9]; sorghum [10], and spices [11]. Among various mycotoxins, Af were assumed significant due to their deleterious effects on human beings, poultry and livestock. Approximately, 5-10% of agricultural commodities world wide are spoiled by fungi, to the extent that crops can not be consumed by human, animal and birds (poultry). This situation is more serious particularly in developing countries like Pakistan, where there are poor agricultural, harvesting, storage, transportation and marketing conditions, which ultimately lead to fungal growth and increase the risk of mycotoxin in general and aflatoxin in particular [12]. According to reliable estimate, mycotoxin affect 1/4th of the world's food crop which includes many basic food and feed stuffs of human and animals [13]. Studies also showed that food contamination by Af may develop as a result of respective fungal attack before or during wheat harvest, or during storage [14].

Fungus Producing Aflatoxins (Afs)

Afs are mostly produced by filamentous fungi known as *Aspergillus*. This genus is distributed world wide and contains over 180 species. It is one of the most cosmopolitan and abundant of all groups of fungi, and also one of the most studied fungal groups [15, 16, 17]. They can colonize and contaminate grain before harvest or during storage. This is also a type of phytopathogenic organisms that infect crops and fruits [18]. *Aspergillus flavus*; *Aspergillus parasiticus*; *Aspergillus nomius* and *Aspergillus niger* are the common fungal plants which can contaminate cereals (including wheat); food, vegetable, fruits, and cattle feed [19]. The Af quantity also depends on *Aspergillus* strain [20, 21].

Types of Aflatoxins

Aflatoxins (Afs) are the most potent carcinogens both in human and animal populations. In fact, the toxin-producing fungus was identified as *Aspergillus flavus* (1961) and the toxin was given the name Aflatoxin by virtue of its origin (*A. flavus* -----> Afla). There are more than 20 different types of aflatoxins (Af), but the most important and naturally occurring major members of Af in food and food stuffs are AfB₁, AfB₂, AfG₁, AfG₂, AfM₁, AfM₂. The last two are produced in milk and milk products. While among all these, AfB₁ is the most toxic, abundantly occurring and well studied one is produced by both *Aspergillus flavus* and *Aspergillus parasiticus* [22, 23]. Letters 'B' and 'G' refer to its blue and green fluorescence colors produced by these compounds when exposed under UV light.

History of Aflatoxins (Afs)

The Afs represent a group of secondary fungal metabolites which were first discovered in Europe as contaminants of certain lots of animal feeds in year 1960. These toxic contaminants have a high order of acute toxicity both to human and many other animal species viz., chickens, swine,

cattles, horses etc and also have shown to possess potent carcinogenic properties in several animals (including human). Their discovery in agriculture based animal feed and the subsequent demonstrations of various biological effects resulting from ingestion of contaminated diets by domesticated animals and birds have emphasized the potential public hazard which might arise from contamination of the food supply by mycotoxin in general and Af in particular [24]. The first report of the toxicity syndrome in term of mortality of domestic animals were recorded in young turkeys [25], followed by the same sort of incidents in ducklings, and chickens [26], as well as in swines and calves [27, 28].

Chemistry of Aflatoxins (Afs)

They are secondary metabolites produced by the moulds on food and feed stuffs. Though there are more than 20 naturally occurring Afs, but the most important one are AfB₁, AfB₂, AfG₁ and AfG₂. Af B₂ and AfG₂ are the dihydro derivatives of the parent

compounds (Fig. 1). They all exist as colorless to pale-yellow crystals at room temperature [29]. They are oxygenated hydrocarbons and are slightly soluble in water, soluble in organic solvents like methanol, acetone, and chloroform, and insoluble in non-polar solvents. Dichloromethane has good solubility for aflatoxins and has been used in analytical procedures to extract and purify aflatoxin for their assay. Aflatoxins are relatively unstable in light and air, particularly in polar solvents or when exposed to oxidizing agents, ultraviolet light, or solutions with a pH below 3 or above 10. There are various isoforms of Af viz. B₁, B₂, G₁, G₂. Afs decompose at their melting points, which are between 268-289°C (B₁ & B₂) and 237-246°C (G₁ & G₂), but are not destroyed under normal cooking conditions (Table 1). They can be completely destroyed by autoclaving in the presence of ammonia or by treatment with bleach [30].

Fig 1. Common aflatoxins (Afs) found in wheat food and feeds.

Table 1. Physiocochemical characteristics of aflatoxins (Afs) found in wheat food and feeds.

Aflatoxins (Afs)	Molecular weight	Molecular Formula	Melting Point (°C)	Storage Temperature (°C)	Optical rotation [α] _D **	Fluorescence/ UV absorbance	UV absorption max (e), nm, methanol	
							265	360-362
B ₁	312.27	C ₁₇ H ₁₂ O ₆	268-269*	-20	- 559	Bright blue UV 360 nm	12,400	21,800
B ₂	314.29	C ₁₇ H ₁₄ O ₆	286-289*	-20	- 492	Bright blue UV 360 nm	12,100	24,000
G ₁	328.27	C ₁₇ H ₁₂ O ₇	244-246*	-20	- 533	Blue-green UV 360 nm	9,600	17,700
G ₂	330.29	C ₁₇ H ₁₄ O ₇	237-240*	-20	- 473	Blue-green UV 360 nm	8,200	17,100

*Decomposes and **Chang et al. (1963) [55]

Determination of Aflatoxins (Afs)

Many methods have been used for the determination of aflatoxins in cereal grains (including wheat) and other food materials in the past [31, 32]. There are so many latest established methods for the determination of Af, but the most common and accurate methods for the determination of Af in food and feed science are: (1) Chromatography e.g., Thin layer chromatography (TLC) [33], Highperformance Liquid Chromatography (HPLC) [34], and Gas Chromatography [35]. (2) Spectroscopy e.g., Fluorescence Spectroscopy (FS) [36] and Frontier Infrared Spectroscopy [37], and (3) Immunochemical methods e.g., Radioimmunoassay (RIA) [38], Enzyme Linked Immunosorbent Assay (ELISA) [39], Lateral Flow Devices (Immunodipsticks) [40] and Immunosensors [41].

Each of the above mentioned analytical procedure for the determination of Af having both merits and demerits. However, chromatographic methods such as TLC and HPLC are considered the gold standard, and are thus the most widely used techniques both in food crops and feeds for aflatoxins analysis.

Diseases Caused by Aflatoxins (Afs)

Illness caused by injection of aflatoxin through food and feed are called aflatoxicosis. Considering the pathogenic effects and aflatoxins of *Aspergillus*, recorded at different post harvest stages which could be either carcinogenic, cytotoxic, immunosuppressant or estrogenic causing severe disorders both in human and animals. The adverse effects of Afs both in human and animals have been categorized into two main categories:

a) Acute aflatoxicosis: It is produced when there is moderate to high levels of Afs are injected through contaminated food and feed. Acute aflatoxicosis is characterized by symptoms of hemorrhage, acute liver damage which manifests as severe hepatotoxicity as a result of which approximately 25% fatality rate occurred, edema, issues with digestion and absorption and nutrients metabolism problem also occurred [42, 43]. Other symptoms of acute aflatoxicosis include hemorrhage (bleeding), edema (swelling of somatic tissue), and changes in metabolism and nutrient absorption, which can ultimately lead to malnutrition. The published outbreaks of acute toxicosis in India (1974), Malaysia (1988), and Kenya (1982 & 2004-05) reflects a mortality rate as high as 60%. While it was recorded as 40% in Kenya (2004-05) through consumption of contaminated maize. But no any proper published record in Pakistan is available regarding mortality through aflatoxicosis.

b) Chronic aflatoxicosis: It is produced when there is a low to moderate level of Afs are ingested through contaminated food and feed. The effects are usually subclinical and difficult to recognize. Few of the common symptoms are impaired food conversion and slow rates of growth with or without having an overt Af syndrome [44]. In addition to carcinogenic effects, it also causes an immune system problem both in human and animals.

Status of Aflatoxin in Food and Feed of Wheat

There are about five billion peoples in developing countries of world which are at the risk of chronic exposure to aflatoxins through contaminated food. As compare to develop nations, there have been very few scientific enquiries for Af in wheat food and feed in Pakistan though wheat is being consumed as the topmost staple food by the general public of the country [45, 46]. A

very little attention has been paid to study the mycotoxin in general and Af in particular in wheat food and feed of Pakistan. However, based on limited published literature, the quantity of Af in 56 analyzed wheat samples from Sindh province were not detected within the detectable limits [3]. The studies of [47] indicated an average mean value of $1.42 \mu\text{g kg}^{-1}$ (Table 2). In another study about 20% of wheat samples were contaminated by total Afs and the highest level was found in one sample. Among all the contaminated samples, two wheat samples were above the suggested limit ($4 \mu\text{g kg}^{-1}$), and their average mean value of $6.6 \mu\text{g kg}^{-1}$ was also above the safe limit set by EU regulations (Lutfullah & Hussain, 2012) [21]. Similarly wheat bran and wheat bread were found to be higher than safe limit recommended by FDA [48]. [49] also stated that out of 19 wheat bran samples, 6 samples were found positive by producing average mean values of AfB_1 i.e. $19.0 \mu\text{g kg}^{-1}$. However, a maximum level of $39 \mu\text{g kg}^{-1}$ was also recorded by them. More than eight species of *Aspergillus* is recorded from stored wheat grains of Pakistan. None of the stored wheat grain samples of three provinces (viz. Punjab, Sindh & KPK) was found contaminated by Af when analyzed by ELIZA technique [50]. Same results were also obtained by [51] when they analyzed rice for Af concentration.

Factors Affecting Concentration of Aflatosins (Afs)

There are so many internal and external factors which can affect the rate Afs produced by fungus, particularly by *Aspergillus*. Generally Afs are affected by pre-harvest and post-harvest conditions, non-scientific methods of grain storage, storage duration, storage season and transportation means. But research studies revealed that there are two main external factors e.g. humidity and temperature [52].

Warm and humid climatic condition prevails in most of the agricultural land of Pakistan. Humidity encourages *Aspergillus* to attack the wheat grains or crop. Favorable condition for its growth is high temperature and high moisture content (>7%). The optimum temperature for Afs production is

recorded as 30°C, while no toxin is produced at 10°C [53]. The positive control in all the batches of *Aspergillus flavus* and *Aspergillus parasiticus* isolates produced maximum Afs in grains at 16% moisture, and 25°C temperature in a laboratory experiment [54].

Table 2. Average mean values of aflatoxins (Afs) in wheat food and feed samples reported from Pakistan.

Wheat Samples	Aflatoxin mean values (µg kg or ppb)	References and Citation Number	Safe limits of aflatoxins (Afs) by EU & FDA
Wheat	5.2 µg kg	Shah (1985) [56]	EU (4 µg kg)
Wheat	18.88 ppb	Bhatti <i>et al.</i> , (2001) [48]	FDA (20 ppb)
Wheat	1.42 µg kg	Qamar <i>et al.</i> , (2008) [47]	EU (4 µg kg) and FDA, USA (20 µg kg)
Wheat	6.6 µg kg	Lutfullah and Hussain (2012) [21]	EU (4 µg kg)
Wheat bran	24.00 µg kg	Khan <i>et al.</i> , (2011) [49]	FDA (20 ppb)
Wheat bran	20.29 ppb	Bhatti <i>et al.</i> , (2001) [48]	FDA (20 ppb)
Wheat bread	18.88 ppb	Bhatti <i>et al.</i> , (2001) [48]	FDA (20 ppb)

EU = European Union; FDA = US Food Drug Administration

Conclusions

Keeping in view the above mentioned pivotal role of fungi in Afs production in wheat food and feed, it is strongly recommended that seed lots before and after harvest should regularly be monitored through certain modern technologies in order to determine the health hazard status of seeds prior to be locally consumed or exported. The wheat crop seeds contaminated by *Aspergillus* spores should be treated at pre harvest level as a control measure of spread of disease caused by *Aspergillus*. Present review also suggests that *Aspergillus* growth needs to be controlled both in fields prior to harvest and post harvest at storage stage before to be consumed by human and animals. The chances of Afs contamination in wheat grains increase on account of high moisture content, and inadequate storage temperature. Therefore, wheat grains must be maintained at proper storage conditions to stop the

Aspergillus proliferation. Post-harvest agricultural practices also need to be properly improved by using the modern technologies in threshing, drying, storage, and transportation of wheat grains. Detoxification and decontamination and cleaning are few other remedies which could be adopted in order to minimize or reduce the rate of Afs contamination. To ensure the health safety of national and international consumers, the concerned regulatory authorities of Pakistan are suggested to take into consideration this serious issue of wheat food and feed contamination caused by fungal growth, and controlling strategies should be practiced and quality control system of food should also be adopted and needs to be improved.

Authors' contributions

Review paper designed: AKK Achakzai & Samiullah. Literature surveyed: M Rahman. Overlooked: ZA Bazai. Wrote the paper: AKK Achakzai

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