National Journal of Biological Sciences, (2020), Volume 1, Issue II

An Overview of Aflatoxins in the Developing World Regarding Food Security and Safety

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Received: 9th September, 2020 / Revised: 30th September, 2020 / Accepted: 1st October, 2020/ Published: 16th December, 2020

Abstract

Food security and food safety are alarming issues in the developing world. Aflatoxins are among the naturally occurring poisons that are fatal for all animals due to their carcinogenic potential. Recent studies have shown the correlation of these toxins with food chains. This correlation has been found to be more significant in developing countries as compared to developed countries. Since the discovery of aflatoxins, great efforts have been exerted to investigate their biochemical nature, biosynthesis, mode of pathogenesis and health-associated effects. However, no sound remedy has yet been designed to completely control their development. The main purpose of this review is to consolidate the findings of published studies on the status of aflatoxins in developing countries, including recent perspectives in this field. The areas that need to be elucidated further are also highlighted.

Keywords: *aflatoxins, developing world, natural poisons, food safety, food chains*

Introduction

Fungi can inhabit a wide range of environmental conditions due to their diverse morphological and functional characteristics (Garcia *et al.,* 2009). They play an important role as decomposers in food chains. Association of fungi with a myriad of diseases in plants, animals, and humans has led to several economic setbacks (Placinta *et al.,* 1999; Schnürer and Magnusson, 2005; Binder, 2007; Magan and Aldred, 2007; Zinedine *et al.,* 2007; Zinedine and Mañes, 2009; Duarte *et al.,* 2011; Kalač, 2011; Bryden, 2012). Their pathogenicity is due to the *production* of certain enzymes and toxins. Out of these, mycotoxins have captured much attention for centuries, as they affect all the major groups of living organisms (Bennett, 1987; Miller, 1995; Sweeney and Dobson, 1998; Richard, 2007; Bokhari, 2010).

Fungi have long been investigated for their ability to produce toxic substances including antibiotics against bacteria, phytotoxins against plants, and mycotoxins against other fungi (Reddy *et al.,* 2010a). Fungi produce diverse compounds, out of

which only few low-molecular-weight compounds are regarded as mycotoxins (Bennett, 1987). Mycotoxins are secondary metabolites of filamentous molds, so far never reported to be produced by yeasts (Moss, 1996; Reddy *et al.*, 2010b). Being secondary metabolites, these moieties do not have a significant role in the growth and development of the fungal strains but they provide them with a competitive edge over non mycotoxigenic fungi by developing hostile conditions against other species (Bushell, 1989; Payne and Brown, 1998; Abdin *et al.*, 2010). Nevertheless, secondary fungal metabolites exhibit beneficial as well as harmful effects. Cholesterol-reducing, lovostatin, immunosuppressive, cyclosporine; hemorrhage and migraine controlling, ergot alkaloids, and various other antibiotics are miraculous fungal secondary metabolites (Payne and Brown, 1998; Etzel, 2002; Medeiros *et al.*, 2012).

Fungal toxicity, due to mycotoxins, is a major concern of the contemporary world. About 300-400 mycotoxins have been discovered so far. These are mainly produced by *Alternaria*, *Penicillium*, *Aspergillus* and *Fusarium* species (Sweeney and Dobson, 1998; Hussein and Brasel, 2001; Zinedine and Mañes, 2009; Tsitsigiannis *et al.*, 2012), while other genera are considered nontoxigenic. Mycotoxins including aflatoxins (AF), deoxynivalenol, ergot alkaloids, fumonisins (F), ochratoxins (OT), patulin, tremorgenic toxins, trichothecenes, and zearalenone (ZEN) are known to pose drastic harm to living beings (Moss, 1996; Zain, 2011; García-Cela *et al.*, 2012). The heat-resistant nature of mycotoxins makes them even worse and their removal becomes difficult by routine feed or food-processing procedures at household and industrial levels (Bakirci, 2001; Bullerman and Bianchini, 2007; Sherif *et al.*, 2009).

Aflatoxins, produced by *Aspergillus* spp., have been ranked first in terms of their virulence as these are carcinogenic, genotoxic, nephrotoxic, and teratogenic (Placinta *et al.*, 1999; Binder, 2007; Wagacha and Muthomi, 2008; Turner *et al.*, 2009; Bokhari, 2010). Aflatoxins are of 4 major types (B_1, B_2, G_1, G_2), and are named so due to their fluorescent characteristics under UV light, blue and green, respectively, as well as due to their relative mobility on silica gel during thin-layer chromatography (Yu, 2012). These mycotoxins usually accumulate in animal feed because of the common association of producer molds with cereals, grains, and other plants (Sweeney and Dobson, 1998; Magan and Aldred, 2007; Kalač, 2011; Bryden, 2012). In addition, M_1 and M_2 are hydroxylated derivatives of B_1 and B_2, respectively, and are called so by dint of their presence in milk and its products. Lactating mammals are found to be responsible for the conversion of B_1 and B_2 into M_1 and M_2, respectively, in the liver and subsequent secretion into milk (Bakirci, 2001; Hussein and Brasel, 2001; Torkar and Vengušt, 2008; Heshmati and Milani, 2010; Zain, 2011; Iha *et al.*, 2013). Contamination of food and animal feed with these mycotoxins has seen an alarming extent worldwide. The problem that needs special attention is that most of the farm animals are made to feed on mycotoxin-contaminated feed. These toxins affect their health and their products such as meat and milk. High incidence of aflatoxins B_1, B_2 in silage feed and M_1, M_2 in milk and its products (sweets, yogurt, cheese, and so on) has been cited in various research reports from many developing countries

(Anjum and Naseem, 2000; Williams *et al.,* 2004; Azziz-Baumgartner *et al.,* 2005; Hanif *et al.,* 2005; Hussain *et al.,* 2008). If contaminated with these toxins, milk being a staple food, can be very risky for immunocompromised individuals as well as infants and the elderly (Prandini *et al.,* 2009; Sherif *et al.,* 2009; Zinedine and Mañes, 2009). Out of numerous physical, chemical, and biological methods for detoxification of mycotoxins, biological control is of utmost significance (Sweeney and Dobson, 1998; Bata and Lásztity, 1999; Young *et al.,* 2007; He *et al.,* 2010; Yuttavanichakul *et al.,* 2012). Utilization of GRAS (Generally Regarded as Safe) microorganisms to combat these toxins would not only lessen the risk of toxicity but would also aid in preventing antimicrobial resistance being caused by excessive use of commercially available drugs (Varga *et al*., 2005).

Aflatoxins

Although 50 different *Aspergillus* species are well recognized for producing more than dozens of mycotoxins such as gliotoxin, helvolic acid, fumitremorgin A, fumagillin, asphemolysin, aflatrem, cyclopiazonic acid, aspergillic acid, beta nitropropionic acid; scientists unanimously rank aflatoxins as the most toxic natural compounds (Cole, 1986; CAST, 1989; GASGA, 1997; Kamei and Watanabe, 2005; Heperkan, 2006; Yu, 2012).

A great deal of information has been obtained on aflatoxins, their biosynthesis, mode of action, and impacts on human and animal health. Aflatoxins are known to be produced by various species of *Aspergillus*, including *A. flavus*, *A. parasiticus*, *A. bombycis*, *A. nomius*, *A. tamari*, and *A. pseudotamarii* (Klich *et al.,* 2000; Frisvad *et al.,* 2005). Nevertheless, most attention has been given to 2 agro-economically important *Aspergillus* species, *A. flavus* and *A. parasiticus* (Lanyasunya *et al.,* 2005). Continuous research on aflatoxins is being undertaken worldwide due to their immense importance in food safety and human health.

The renowned Turkey-X-disease was the first major incident that provided scientists with a new direction in the field of mycotoxicology. In England, thousands of turkeys died in 1960 due to consuming aflatoxins-infested peanut meal that had been imported from Brazil. The association of that disease with secondary metabolites of *A. flavus* led to the coinage of the well-known term ‗a-fla-toxin' for the causative agent (Wogan, 1966; Bennett and Klich, 2003).

Utilizing 3 major routes of administration, inhalation, absorption, and ingestion, these toxins are now known to be pronounced teratogens, mutagens, immunosuppressants, and, most importantly, carcinogens (Piva *et al.,* 1995; Pitt, 1996; Zain, 2011). The serious health effects of these aflatoxins in plants, animals, and humans caused the International Agency for Research on Cancer (IARC) to categorize them as Group-I carcinogens in 1987 and to confirm their belonging to this group in 1992 (Speijers and Egmond, 1993; Kumar *et al.,* 2008; Hussain *et al.,* 2011). *Aspergillus flavus* is the most prevalent soil-borne saprophytic fungus, abundantly found in dead plants and animal bodies. It also resides on aerial plant bodies. Its ability to survive in diverse temperature ranges equips it with the ability to inhabit a wide range of environmental niches (Dvorockova, 1990; Yu, 2012). After their absorption in the GI tract, aflatoxins are rapidly transferred to the liver through the circulatory system (Rogers, 1993; Fung and Clark, 2004). In the liver,

aflatoxins are reported to be activated by cytochrome P450 enzymes, which transform them into epoxides for instance B1-8,9-epoxide. These epoxides have the ability to bind with the guanine base (G) of DNA in hepatocytes (Mulunda *et al*., 2013). This DNA binding and adduct formation ability characterize their role as mutagen, genotoxin, and carcinogen. Aflatoxin B_1 was first identified in 1977 as DNA-adduct (Kensler *et al*., 2011).

Although aflatoxins have affected all human populations indiscriminately, they have had more drastic impact on developing countries as compared to developed ones. Developed countries have overcome the problem to some extent due to continuous efforts of careful monitoring and control systems. In contrast, developing countries are still facing a high rate of aflatoxin contaminations of staple food and the consequent outbreaks leading to large-scale economic and health issues (40% disease burden). Various research studies present aflatoxins as a common source of morbidity and mortality in Asia, Latin America, and Africa. Similarly, WHO ranked aflatoxicosis as the sixth major disease among the top 10 health risks in developing countries (Azziz-Baumgartner *et al*., 2005; Shukla *et al*., 2012). Despite such knowledge, 99% of children and 98% of adults in Africa are still found to have aflatoxin as a biomarker in their blood and serum, respectively. Exposure to this high dose of aflatoxins is making every individual prone to aflatoxicosis and predisposes every affected child to hepatomegaly (Bankole *et al*., 2006). Some examples of aflatoxin-associated deaths include the loss of 125- 200 lives in Kenya in different outbreaks, the death of 220 dogs in Gauteng, as well as 500 acute infections in Africa (Yard *et al*., 2013; Adjovi *et al*., 2014; Iheanacho *et al*., 2014). In the world, 5-30% of liver cancers are caused by aflatoxins and the highest incidence of this aflatoxin-induced live cancer (40%) is reported in Africa. Moreover, 25,200-155,000 individuals in developing countries are annually affected by aflatoxins-induced hepatic cancer. The same hepatomas were found to be responsible for 10% of adult deaths of in China and 10% of deaths in the male population in Gambia (Williams *et al.*, 2004; Liu and Wu, 2010).

Biosynthesis of Aflatoxins

In addition to aflatoxin production, *Aspergillus* species are also well known to synthesize economically important secondary metabolites such as antibiotics for example helvolic acid; pharmaceutical products such as gluconic acid; antitumor drugs such as fumagillin; and enzymes of industrial significance such as pectinases, amylases as well as cutinases (Prandini *et al.*, 2009; Yu, 2012). The need to increase the yield of important metabolites and suppress the production of toxic ones acted as an incentive to study the biosynthetic pathways of these secondary metabolites (Payne and Brown, 1998). Since 1965, great efforts have been made to study the complete biosynthetic pathways of aflatoxins. It is the second most studied biosynthetic pathway after the regulatory metabolic pathway of penicillin (Payne and Brown, 1998). Complete biosynthetic pathways have been studied extensively for aflatoxin B, however, genes involved in production of aflatoxin G have not been completely deciphered so far (Yu, 2012).

The complex ring-like structure of aflatoxins requires more than one gene for its synthesis, contrary to bacterial toxins that can be formulated employing transcription of a single gene (Levin, 2012). There are about

12,197 genes in *Aspergillus flavus* that are arranged on eight chromosomes and account for a genome size of 36.8 Mb. Scientists have further revealed that there are about 20-30 aflatoxin synthesizing genes organized in the form of a gene cluster of about 70-75 kb (Woloshuk and Shim, 2013). In both *A. flavus* and *A. parasiticus*, the aflatoxin biosynthesis gene cluster is present on chromosome number 3. About 25 enzymes are responsible for the final conversion of acetyl Co-A into B_1, B_2, G_1, and G_2 (Ruiqian *et al.,* 2004; Wen *et al.,* 2005). The gene cluster of aflatoxin is reported to be conserved in most of the *Aspergillus* species belonging to section Flavi; although it is documented to be functional only in a few aflatoxigenic species such as *A. flavus* and *A. parasiticus*. Certain other *Aspergillus* species such as *A. oryzae* and *A. sojae* possess the same gene cluster, but it remains non-functional throughout their lifecycle. The non-functionality of aflatoxins-producing genes in *A. sojae* and *A. oryzae* makes them safe for their usage in various industrial processes and thus they are commonly used in the dairy industry as koji molds (Tominaga *et al*., 2006; Chang *et al*., 2007).The reason behind their non-aflatoxigenicity is either partial or complete deletion of the aflatoxigenic gene cluster. For instance, *aflR* has been found to be a regulator of the aflatoxin biosynthetic pathway; however, this gene is defective in *A. sojae* (Criseo *et al.,* 2008).

Aflatoxins are produced during idiophase or morphological differentiation after repression of the rapid primary growth phase (Price *et al.,* 2005; Uppala, 2011). The nutritional and environmental factors influencing aflatoxin production include availability of N, C, and lipids, pH, temperature, water activity, ATP level, oxidative stress, metal ions (Mg, Mn. Mo, Cu, Zn, Fe), and salt concentration (van Egmond, 1983; Coulumbe, 1993; Luchese and Harrigan, 1993; Ruiqian *et al.,* 2004; Uppala, 2011; Yu, 2012). A correlation has also been found to be present between aflatoxin biosynthesis and fungal development. The absence of sexual reproduction, in *A. flavus* and *A. parasiticus*, leads to 97-98% nucleotide sequence similarity and about 95% sequence conservation. Recombination in these fungal strains is thus probably found due to the parasexual life cycle (Payne and Brown, 1998; Obrian *et al.*, 2007).

Types of Aflatoxins

About 20 different types of aflatoxins have been discovered so far. Out of them, B_1, B_2, G_1, and G_2 are recognized as the most important types (Sherif *et al.,* 2009). B_2 and G_2 are reported to be dihydro-derivatives of B_1 and G_1, respectively. These 4 types are independently produced (Yabe *et al.,* 1988). Moreover, despite the fact that all of the 4 types can be found in any of the aflatoxigenic mold extracts, B_1 remains dominant in relative concentration; however, B_2 and G_2 are found in comparatively low amounts (Wogan, 1966; Ruiqian *et al.,* 2004). Aflatoxins B and G are designated so due to their ability to fluoresce blue and yellowish green under UV light, respectively, and also due to their relative running (mobility) positions on TLC plates (Ellis *et al.,* 1991; D'Mello and Macdonald, 1997). Other less studied types of aflatoxins include aflatoxicol, AFB_{2a}, P, G_{29},and Q, et al., and most of them represent the endogenously produced metabolites of aflatoxins in animals (Crespi *et al.,* 1991). B_1 is known to be produced by almost all aflatoxigenic strains of *Aspergillus*, (*A. flavus*, *A. ochraceoroseus*, *A. pseudotamarii*, *A. nomius*, *A. parasiticus*, and *A. bombycis*). However, there are a few

species that have acquired the ability to produce both B and G types of aflatoxins, such as *A. parasiticus, A. nomius,* and *A. bombycis* (Yu *et al.,* 2002). *A. parasiticus* displays a narrow host range and habitat range as compared to *A. flavus.* It mostly prefers soil habitat; furthermore, it is also reported that almost all of the isolated strains are aflatoxigenic, in contrast to *A. flavus* in which almost half of the isolated species are non-aflatoxigenic (Frisvad *et al.,* 2005).

The most important derivatives of aflatoxins B_1 and B_2 are M_1 and M_2 respectively. Although these are less toxic than the parent molecule, they still possess the potential of carcinogenicity (Carnaghan *et al.,* 1963). B_2 and G_2 are comparatively less toxic in contrast to B_1 and G_1. As far as pathogenicity of these types of aflatoxins is concerned, the order of virulence is as follows: B_1> M_1> G_1> B_2> G_2 (Gourama and Bullerman, 1995; Torkar and Vengušt, 2008; Kensler *et al.,* 2011). The diverse nature of different types of aflatoxins and their toxic potency reveal the marvellous ability of the aflatoxigenic *Aspergillus* species to ruin affected plants, animals, and humans.

AFM_1

M_1 and M_2 are oxidative hydroxyl-derivatives of B_1 and B_2, respectively. The attempt of liver to detoxify AFB_1 leads to its transformation into the less toxic but still carcinogenic metabolite regarded as AFM_1 and it is named so due to its presence in milk and other dairy products (Neal *et al.,* 1998; Polovinski-Horvatović *et al.,* 2009; Al-Zuhair and Omar, 2012; El Marnissi *et al.,* 2012). It is found in animals‘ body fluids, including milk, blood from the umbilical cord, serum as well as urine (Yu *et al.,* 2002 ; Lanyasunya *et al.,* 2005). The presence of AFM_1 in such body fluids acts as a biomarker that indicates the contamination of the ingested food and feed with AFB_1 (Groopman *et al.,* 1985; Kensler *et al.,* 2011). Various studies have resulted in establishing the fact that the amount of AFM_1 in milk is directly proportional to the concentration of AFB_1 in the feed of the consumer (Kamkar, 2008). Therefore, when the amount of aflatoxin reaches 20 ppb or more in animal feed, the lactating animals produce milk contaminated with AFM_1 (Maqbool *et al.,* 2009; Mulunda *et al.,* 2013). Approximately 1-3% of the ingested AFB_1 gets converted into AFM_1 in lactating cattle and about 95% of the ingested B_1 is secreted into human breast milk (Gong *et al.,* 2004; Mulunda *et al.,* 2013). According to a study, when lactating mammals eat aflatoxin free feed, their milk production increases up to 25% (Panahi *et al.,* 2011).

Just like its parent molecule ‗B_1‘, M_1 has the ability to synthesize adducts with DNA and thereby acts as mutagen, genotoxin as well as carcinogen. It causes gene mutation, chromosomal aberrations and cell damage (Prandini *et al.,* 2009; Elzupir and Elhussein, 2010; Heshmati and Milani, 2010). The adduct formation ability of M_1 enables it to accumulate in tissues of animals and humans. This milk-toxin appears violet-blue under UV light and is found to be transitory because it disappears from milk after 4-5 days soon after the removal of aflatoxin B_1 from cattle‘s feed (Wogan, 1966). Although initially AFM_1 was categorized as a Group-IIB carcinogen, probable human carcinogen, due to inadequate studies and data, later on, it was classified as Group-I, a known human carcinogen by IARC in 2002 (Mulunda *et al.,* 2013). Due to its association with milk proteins, AFM_1 is reported to be found in curd in higher amounts rather than being evenly distributed in whey and curd. Because of this affiliation, the concentration of AFM_1

increases to several folds in milk products such as cheese, as compared to the milk used for product formation (Anfossi *et al.,* 2012).

Biological and Chemical Nature of Aflatoxins

Biologically aflatoxins are secondary metabolites that are produced in the secondary growth phase of aflatoxigenic molds. Chemically these are polyketide coumarin-derived difuranocoumarins, displaying the characteristics of poly aromatic compounds (Wogan, 1999; Yu *et al.,* 2002 ; Halász *et al.,* 2009; Reddy *et al.,* 2010b). The oxygenated penta-heterocyclic conformation is the reason underlying the fluorescent characteristics of aflatoxigenic fungi under UV-light (Fente *et al.,* 2001; Uppala, 2011). Aflatoxigenic *Aspergillus* species also have an excellent ability to manufacture a wide range of enzymes such as amylases, cellulases, hemicellulases and pectinases (Gadgile and Chavan, 2010; Uppala, 2011). This enzyme producing ability enables them to adapt to a diverse range of habitats; and this feature adds another factor for their successful colonization and disease-causing ability.

Stability of Aflatoxins

A host of literature studies reveal that mycotoxins resist the heat of cooking and many other processing strategies; thus, mycotoxins are heat stable moieties that cannot be removed during conventional food preparatory procedures (Rao *et al.,* 1982; Sherif *et al.,* 2009; Zhang *et al.,* 2011). Hanif *et al.* (2005) stated that it is nearly impossible to get rid of aflatoxins, once these are produced, because of their highly stable nature (Hanif *et al.,* 2005).

Oluwafemi and Ikeowa (2005) carried out a study to find out the effect of fermentation and refrigeration (4^oC) on the persistence of aflatoxins in a local fermented maize product (ogi). They concluded that fermentation for 24 and 48 hrs as well as refrigeration did not affect aflatoxin concentration in maize. However, fermentation of the product for 72 hrs decreased the concentration of aflatoxins up to 50%, possibly due to increase in the population of lactic acid bacteria in fermented maize (Oluwafemi and Ikeowa, 2005). Bullerman and Bianchini (2007) stated that there are various food processing techniques such as thermal processing, extrusion processing and corn flake processing which are known to reduce the level of aflatoxins up to 50% in food items at temperature as high as above 150^oC. Nevertheless, they indicate that such processing practices are unable to completely eliminate aflatoxins due to their high level of stability (Bullerman and Bianchini, 2007).

Since most of the characteristics of aflatoxin M_1 are similar to its parent molecule AFB_1, so is the case with its resistance to a wide range of high temperatures. It endures pasteurization and fermentation and is not degraded under such elevated temperature conditions (Heperkan, 2006; Maqbool *et al.,* 2009; Atasever *et al.,* 2010). In addition, AFM_1 is also resistant to ultra-high temperature, sterilization as well as various milk processing techniques (Nachtmann *et al.,* 2007; Kamkar, 2008; Al-Zuhair and Omar, 2012). A study was conducted in 1975 by Stoloff and his colleagues to determine the effect of pasteurization and refrigeration on the stability of AFM_1 in milk. They found that AFM_1 withstood pasteurization at 63^o C for half an hour and at 77^o C for 16 minutes. However, storage at -18^o C caused a 45% reduction in M_1 level of milk after 120 days of refrigeration (Stoloff *et al.,* 1975).

Likewise, a study by Prandini and co-workers (2009) indicates that low temperature treatment and concentrating milk through water evaporation do not affect AFM_1 concentration in milk and other fermented milk products. These studies provide evidence to establish the fact that aflatoxin M_1 is hard to eliminate through conventional cooking and heating practices and necessitate new safety techniques in order to escape its harmful effects (Prandini *et al.,* 2009).

Techniques to Analyze Aflatoxins and Aflatoxigenic Fungi

The diverse nature of mycotoxins makes the availability of a single monitoring technique for all the known mycotoxins nearly impossible. For this reason, several analytical methods are practiced examining mycotoxins in food matrices (Smith *et al.,* 1995). Several studies have been carried out to deal with the issues of devising strategies to monitor aflatoxins in feed and food as well as discriminating aflatoxigenic *Aspergillus* species from that of non-aflatoxigenic. A parasitic wasp, *Microplitis croceipes*, due to its olfactory sense, can differentiate between aflatoxin producing and non-aflaotoxin producing strains of *A. flavus*. It can detect the toxin even without presence of the toxigenic mold strain at the site of contamination (Tertuliano *et al.,* 2005).

Due to the unavailability of sufficient sophisticated equipment as well as the inability to bear the expenses of such techniques, developing countries mostly rely upon cost-effective culture-based methods to investigate aflatoxin contamination. These cultural methods include detection of colony color change employing vapour induction of ammonium hydroxide, blue fluorescence under UV, enhancement of fluorescence using β-cyclodextrin, yellow pigmentation and combination of such aflatoxigenicity detection tests (Hara *et al.,* 1974; Davis *et al.,* 1987; Fente *et al.,* 2001; Abbas *et al.,* 2004 ; Shier *et al.,* 2005 ; Cigić and Prosen, 2009). Contrary to developing countries, developed countries utilize modern, rapid and sophisticated practices for aflatoxin monitoring such as high-performance liquid chromatography (HPLC), immunoaffinity columns (IAC), thin layer chromatography (TLC), liquid chromatography-mass spectroscopy (LC-MS), enzyme-linked immunosorbent assay (ELISA), gas chromatography (GC), pressurized fluid extraction (PFE), capillary electrophoresis (CE), electrochemical biosensors, polarized fluorescence method and immuno assays and so on (Abbas *et al.,* 2004; Shier *et al.,* 2005; Zheng *et al.,* 2006; Cigić and Prosen, 2009; Sheibani and Ghaziaskar, 2009; Turner *et al.,* 2009; Paniel *et al.,* 2010). Currently, modern molecular techniques such as PCR in combination with single stranded conformational polymorphisms (SSCP) and restriction fragment length polymorphism (RFLM), DNA fingerprinting and microarray-based processes, are dominating the field of analysis in the aforementioned context (Somashekar *et al.,* 2004a; Abdin *et al.,* 2010; Stark, 2010).

PCR is one of the molecular methods, which researchers have been utilizing as a rapid and reliable mean of detecting aflatoxigenic *A. flavus*. The aflatoxin biosynthetic genes are arranged in the form of a cluster and identifying these genes through polymerase chain reaction allows differentiation of aflatoxigenic strains from non-aflatoxigenic ones at the genomic level (Geisen, 1996; Somashekar *et al.,* 2004b;

Manonmani *et al.,* 2005; Russell and Paterson, 2006; Erami *et al.,* 2007; Rahimi *et al.,* 2008; Rashid *et al.,* 2008; Abdel-Hadi *et al.,* 2011; Gallo *et al.,* 2012). Reverse Transcription- Polymerase Chain Reaction (RT-PCR) is another advanced molecular technique, which in addition to fulfilling the above-mentioned purpose of differentiation, is also used to get insight into expression of aflatoxin producing genes (Scherm *et al.,* 2005; Abdel-Hadi *et al.,* 2011). According to Sardiñas *et al.* (2011) and Rodríguez *et al.* (2012), qPCR is an efficient technique to distinguish strains of *A. flavus* from that of *A. parasiticus* on molecular basis, since both these species are difficult to differentiate because of morphological and about 97% genomic similarity. In addition, this process enables quantification of spores of the two *Aspergillus* species in food or feed samples (Sardiñas *et al.,* 2011; Rodríguez *et al.,* 2012).

Fourier Transform Infrared Spectroscopy-Attenuated Total Reflectance cell (FTIR-ATR) is a rapid method of detection as well as quantification of aflatoxin content in contaminated food items. Its efficiency in sample analysis with reduced time (about 1 minute) provides an ideal method for aflatoxin analysis (Abbott *et al.,* 1988; Kaya-Celiker *et al.,* 2011). FTIR can also be employed to isolate *A. flavus* from other *Aspergillus* species and to discriminate between toxigenic and atoxigenic strains (Gordon *et al.,* 1997; Garon *et al.,* 2010). HPLC is another highly practised technique for scrutinizing aflatoxins in contaminated samples. It can also be used for simultaneous determination of all the four major forms of aflatoxins, B_1, G_1, B_2, and G_2 (Ghali *et al.,* 2009). A comparison was made between HPLC and Fluorometery to define the better method for AFM_1 detection in fermented milk products. It was found that lower cost and lesser time of analysis taken by fluorometer makes it a preferable practice as compared to HPLC for examining aflatoxin level in cheese (Pena, 2010). Berthiller *et al.* (2007) attempted to explain multi-toxin analysis techniques such as LC/MS for simultaneous detection of multiple mycotoxins in cereals and other food articles. He claimed that such practices can help to identify up to 90 mycotoxins, if present in a single food item (Berthiller *et al.,* 2007). Correspondingly, Sørensen and Elbæk (2005) successfully utilized LC/MS for detecting numerous mycotoxins and metabolites in milk from bovine (Sørensen and Elbæk, 2005).

Health Impact of Aflatoxins

Aflatoxins are regarded as natural pollutants (Maqbool *et al.,* 2009). Their toxicity nullifies the conventionally held concept that only artificially synthesized products can be toxic and natural products are beyond the phenomena of toxicity. The toxicity of aflatoxins can either be acute, when a high dose is ingested, inhaled or absorbed at once, and leads to death; or chronic, when a low dose of aflatoxins enters the body continuously over a prolonged period of time. Acute aflatoxicosis leads to sudden death; however, chronic aflatoxicosis impairs health (Fink, 1999; Oluwafemi and Ikeowa, 2005; Qazi and Fayyaz, 2006; Panahi *et al.,* 2011). Depending upon the concentration of dose intake, aflatoxins induce three types of effects; (1) lethal, when administered acutely in high amounts (2) histological abnormalities, when intake is sub-acutely in smaller amounts and (3) tumor formation, when administration is chronic (Wogan, 1966; Hesseltine, 1985; Qazi and Fayyaz, 2006).

Impact on Humans

In 1987, aflatoxins were regarded as carcinogens since they had been reported to induce liver, kidney and colon cancers. About 28% of cancers are found to be associated with aflatoxin contamination (Kensler *et al.,* 2011). Likewise, inhalation of aflatoxigenic mold spores predisposes lung problems (Wogan, 1999). When aflatoxins enter the body, they formulate oxidative products in the form of epoxides with the help of cytochrome P450 enzymes. Being unstable in nature, epoxides, immediately after their formation, form adducts with macromolecules such as proteins, DNA and RNA. For instance, with DNA they synthesize aflatoxin-N7-guanine adduct and with proteins such as albumin they formulate aflatoxin-B1 lysine adduct in serum (Skipper and Tannenbaum, 1990). These protein and DNA adducts lead to destruction of hepatocytes which in turn result in toxicity and liver cancer (Tandon *et al.,* 1978).

Presence of aflatoxins and their derivatives in milk, blood and urine can serve the purpose of biomarker of aflatoxin infestation (Groopman *et al.,* 1985; Kensler *et al.,* 2011; Bryden, 2012). The lipophilic characteristic of aflatoxins enhances the accumulative ability of AFB_1 and AFM_1 in plants and the adipose tissue of mammals (Hussein and Brasel, 2001; Muhammad *et al.,* 2010; Hathout *et al.,* 2011). Nevertheless, a study by Stoloff and Rodricks (1977) suggests that the lower accumulative ability of aflatoxins as compared to other mycotoxins is responsible for its rapid excretion in body fluids (Stoloff and Rodricks, 1977).

In addition to being carcinogens and toxins, aflatoxins are well known as mutagens and immunosuppressants (Yu *et al.,* 2002). Teratogenic effects include anomalies in fetus development. Haemorrhaging, attack on CNS, jaundice, weight loss, skin disorders, cirrhosis, reduction in lactation, stunting in infants, protein synthesis inhibition, loss of appetite, reproductive abnormalities, hormonal defects and hemorrhagic diarrhoea are various symptoms of aflatoxicosis (Payne and Brown, 1998; Wangikar *et al.,* 2005; Uppala, 2011). Exposure to AFB_1 in hepatitis B patients increases the risk of hepatocellular carcinoma up to 30% and enhances the severity of the disease as they lose the ability to detoxify aflatoxins (Qian *et al.,* 1994; Yu *et al.,* 2012). Aflatoxins have also been found to cross the placenta, and thereby affect fetus development, leading to still births and neonatal mortality (Wild *et al.,* 1991; Maxwell, 1998). A correlation has also been found between aflatoxin exposure and certain childhood diseases such as Reye‘s syndrome, Kwashiorkor and Neonatal Jaundice (Blunden *et al.,* 1991; Daele and Eggermont, 1994; Verma, 2004; Sherif *et al.,* 2009). Thus, it can be presumed that aflatoxins increase infants‘ susceptibility to infections and reduce their immunity (Hendrickse, 1997).

It is estimated that about 4-5 billion people in developing countries are chronically exposed to aflatoxins, causing the suppression of the efficiency of nervous, endocrine and immune systems (Uppala, 2011). Wogan (1966) found that even small concentrations of AFB_1 halt mitotic divisions in lung cells of the human embryo. Moreover, about 2-6 mg/day ingestion of aflatoxins can lead to death by causing acute hepatitis (Lanyasunya *et al.,* 2005). A large number of outbreaks, associated with aflatoxins contamination, have been documented in literature. In 1970 and 1974,

outbreaks were reported in India that caused several deaths due to consumption of moldy maize. Likewise, outbreaks of in Malaysia in 1988, Italy in 2003 and Kenya in 1981, 2004, 2005 and 2007 occurred due to utilization of aflatoxin contaminated maize (Probst *et al.,* 2007; Kensler *et al*., 2011; Gallo *et al.,* 2012). In various case studies related to aflatoxicosis outbreaks in Kenya, it was found that there was a direct association between aflatoxin concentration in contaminated maize and concentration of aflatoxins-albumin adducts in the serum of suffering individuals (Azziz-Baumgartner *et al.,* 2005). Similarly, an outbreak was caused in Serbia due to consumption of AFM_1 contaminated milk in 2013 (Škrbić *et al.,* 2014). It has also been found that there is no threshold level for aflatoxins to cause cancer, and any concentration above zero can be carcinogenic (WHO, 1999; Bokhari, 2010). The studies of Oluwafemi (2000), and Shuaib *et al*. (2010), threw light on the noteworthy effects of aflatoxins on reproductive health. A direct correlation was demonstrated between aflatoxin infestation of semen and infertility in 40% of the tested male subjects. Similarly, a direct relationship was found between the presence of aflatoxins in serum, breast milk as well as cord blood of the mother with still birth, reduced body weight and neonatal jaundice in infants. Moreover, the results were more pronounced for developing countries as compared to developed ones.

Impact on Animals

In animals, chronic aflatoxicosis is more likely to occur than acute intoxication. Rabbits, chickens, monkeys, ducks, pigs, turkeys, rodents, ruminants, fish and rats develop disease symptoms upon exposure to aflatoxins. The liver has been found to be a major target unit in all these animals; for example, 1 ppb aflatoxin ingestion results in liver cancer in trouts (Miller, 1995; Abdel-Wahhab *et al.,* 2007; Hussain, 2009; García-Cela *et al.,* 2012). Decreased growth rate, nephrosis, low productivity, immunosuppression, lesser feed intake, hemorrhage, hepatitis, decreased fertility, reproductive complications and reduced performance are among the symptoms and consequences of aflatoxicosis in animals (Wyatt and Hamilton, 1975; Kensler *et al.,* 2011). Monkeys have been used in several studies as model animals to study the effects of aflatoxicosis. Rhesus monkeys, when fed with aflatoxin contaminated meal for 18 days, first developed liver lesions as well as anorexia and then died after 2-4 weeks (Wogan, 1966).

In a study conducted by Bokhari (2010) in Saudi Arabia, it was found that about the entire test feed samples were contaminated with mycotoxigenic fungal species. In the contaminated feed samples, the CFU/g for *Aspergillus* species was counted in the range of $223x10^3$ and out of them 6-30% of strains were detected to be aflatoxigenic. Moreover, the concentration of aflatoxins in feed samples was up to 25 ppb. The study showed the association of aflatoxin contamination of feed with camel‘s health issues and their large-scale death in Saudi Arabia (Bokhari, 2010). Another study conducted in Brazil, found the contamination of aflatoxins in 92% of feed samples utilized by laying hens and 88.2% of feed samples for broiler hens in a concentration of 19.75-20.83 ng/g and 8.41-10.48 ng/g, respectively. Aflatoxins, after their consumption by poultry, become a constant part of its tissues, liver and eggs which in turn become potential health hazards for human consumers (Rossi *et al.,* 2012).

Impact on Plants

Aflatoxigenic *A. flavus* and *A. parasiticus* are opportunistic plant pathogens. They are notorious for unavoidable devastation to various crops, both in pre- and postharvest states. Aflatoxin infestation inhibits seed germination and biosynthesis of chlorophyll (Wogan, 1966).

Strategies to Combat Aflatoxins (Prevention and Control)

Designing strategies to combat harmful and life-threatening substances has been associated with the history of mankind. Innumerable efforts have been made to eliminate the dangers of mycotoxins for centuries. Here, a few of the studies dealing with the prevention and control from aflatoxins are briefly discussed.

Physical Control

Analysing aflatoxin contaminated grainy food after fluorescence detection and manually separating these grains or kernels from healthy ones is one of the physical methods to control aflatoxin contamination. Blending is another method in which a small number of tainted grains are thoroughly mixed with non-contaminated crop in order to reduce aflatoxin levels up to the permissible regulatory limit. This method helps diluting the concentration of aflatoxins in cattle feed (Yiannikouris and Jouany, 2002; Turner *et al.,* 2009; Upadhaya *et al.,* 2010). Traditional practices of sorting, dry and wet milling, washing, ash mixing, heat treatment, roasting, irradiation with UV and gamma rays were considered good for reducing fungal load from the contaminated crop. Similarly, ultra-filtration and pasteurization were found better options to render milk free of AFM_1 contamination (Zain, 2011; Iqbal *et al.,* 2013).

Higuera-Ciapara *et al.* (1995) performed experiments in order to study the effects of diafiltration and ultrafiltration on the level of AFM_1 in raw, acidified and homogenized milk. It was found that ultrafiltration and diafiltration reduced M_1 concentration in raw milk up to 43.8% and 25%, respectively, due to the small size (328 D) of AFM_1. On the other hand, acidification and homogenization did not affect the level of M_1 in milk (Higuera-Ciapara *et al.,* 1995). Nevertheless, these physical control practices are not only expensive and time consuming but also unable to completely eliminate aflatoxins from food and feed.

Chemical Control

For centuries, the use of chemical fungicides has been considered as a first line of defence against fungal attack to crops (Ni and Streett, 2005; Medeiros *et al.,* 2012). López-Malo *et al.* (2005) studied the effect of natural antimicrobial compounds and chemical preservative agents on the growth of *A. flavus* and found useful results in reducing germination of spores and mycelia development (Lopez-Malo *et al.,* 2005). Mckenzie and colleagues found the use of 2% ozone as an effective way to degrade aflatoxins B_1 and G_1 in aflatoxin infested grains; whereas, degradation of B_2 and G_2 required 20% ozone treatment (Mckenzie *et al.,* 1997).

Many studies have described the use of chemicals such as ethanol, sodium hydroxide, calcium hydroxide, ammonium hydroxide, sodium bisulphite, hydrogen peroxide, propionic acid, Schiff base, ammonia gas, sulphur dioxide gas and formaldehyde in minimizing the toxic

effects of aflatoxins by altering the reactive chemical structure of these toxic compounds. Likewise, diverse chemical substrates, for instance, silicas, aluminas, phyllosilicate, aluminosilicate and bentonite clay have been reported to be well known for reducing the bioavailability of aflatoxins by physically binding to them (Scheideler, 1993; Piva *et al.,* 1995; Méndez-Albores *et al.,* 2007; Dao and Dantigny, 2011; Duraković *et al.,* 2012). Besides, the partial effectiveness of such chemical control methods, there are certain associated drawbacks, such as loss of food nutritious value, natural taste as well as less confidence of consumers on such treated food items due to health and environment protection issues, which lessen the demand of chemical control strategies.

Biological Control

Use of physical and chemical methods not only led to compromise on food nutritional value, and organoleptic features, as well as huge expenses of such treatments but also increased the resistance of pathogens to chemical preservatives. The consumer demand for food without such physical and chemical processing and the need to control multiple resistant harmful microbial strains have motivated scientists to change their direction towards devising bio-preservative and bio-control strategies to combat food contamination and spoilage issues (Piva *et al.,* 1995; Basappa and Shantha, 1996; Teniola *et al.,* 2005; Medeiros *et al.,* 2012).

Ruiqian *et al*. (2004) reviewed the biocontrol and degradation of *A. flavus* and aflatoxins, respectively. They demonstrated the significant role of atoxigenic strains of *A. flavus*, *B. subtilis* and *Trichoderma* species in inhibiting the growth and aflatoxin producing ability of *A. flavus* (Ruiqian *et al.,* 2004). Similar results were obtained by El-Shiekh *et al.* (2007) for the degradation of aflatoxins by other non-toxin producing fungal strains (El-Shiekh *et al.,* 2007). In a study carried out by Dowd *et al.* (1998), sap beetles called *Carpophilus lugubris* were used to disperse *B. subtilis* on maize kernels in order to examine the bio-control effect of *B. subtilis* on *A. flavus* and its aflatoxins. Results indicated the positive role of the bacterium in reducing the growth and toxicity of *A. flavus* (Dowd *et al.,* 1998). Likewise, Farzaneh *et al*. (2012) isolated a new strain of *B. subtilis* from pistachio nuts that displayed a remarkable ability to inhibit the growth of *A. flavus* and degrade aflatoxins. This bacterial strain was detected to degrade $AFB_{1,}$ 95% and 85.66% in pistachio and culture of nutrient broth, respectively. About 78.39% aflatoxin degradation ability was found in cell-free culture supernatant, which deciphered the extracellular and enzymatic nature of degradative compounds produced by *B. subtilis* (Farzaneh *et al.,* 2012).

Lactic acid bacteria have long been used as biopreservative agents in different food matrices (Schnürer and Magnusson, 2005). The ability of Lactic acid bacteria to inhibit fungal growth and detoxify as well as degrade mycotoxins, particularly aflatoxins, led many researchers to carry out studies in order to determine the compounds that enable these bacteria to display such antimicrobial characteristics. Consequently, a wide range of such compounds have been discovered so far such as phenyllactic acid, fatty acids, hydroxy phenyllactic acid and cyclical dipeptides (Lavermicocca *et al.,* 2003; Schnürer and Magnusson, 2005; Ström, 2005; Coloretti *et al.,* 2007; Fuchs *et al.,* 2008). Hathout *et al*. (2011), recently studied the antioxidant effects of two LAB species, *Lactobacillus reuteri* and

Lactobacillus casei against aflatoxins. They studied various biochemical and histological parameters of the liver and kidney of rats in the presence of aflatoxins. They further proposed that use of LAB can reduce the damaging effects of aflatoxins on vital body organs probably because of their binding ability to these toxins in the GI tract (Hathout *et al.,* 2011).

Several other research studies have identified the role of plant extracts such as extracts of garlic *Trachyspermum ammi* (medicinal plant) and *Hibiscus sabdariffa,* and certain oils such as that of *Nigella sativa* and *Callistemon lanceolatus* against aflatoxins producing *Aspergillus* species (Saleem *et al.,* 2017). Similarly, microorganisms for instance *Pleurotus ostreatus* (edible mushroom), *Flavobacterium aurantiacum*, nonaflatoxigenic strains of *A. flavus*, microbes of cattle rumen, *Rhodococcus erythropolis* and *Mycobacterium fluoranthenivorans*, are also well known in restraining *A. flavus* growth and detoxifying its toxic metabolites (Hamid and Smith, 1987; Sutabhaha *et al.,* 1992; Bata and Lásztity, 1999; Smiley and Draughon, 2000; Motomura *et al.,* 2003; Teniola *et al.,* 2005; Alberts *et al.,* 2006; Halász *et al.,* 2009; Wu *et al.,* 2009; Reddy *et al.,* 2010a; Upadhaya *et al.,* 2010; Velazhahan *et al.,* 2010; Degola *et al.,* 2011; El-Nagerabi *et al.,* 2012; Juodeikiene *et al.,* 2012; Shukla *et al.,* 2012; Zuo *et al.,* 2012).

Affordable Solutions to the Aflatoxin Menace

Whitehead (1995) offered valuable ideas to develop an error-proof and immaculate food safety system at the national level. In light of that system, there is a considerable need for the formulation of a policy by the government to control levels of mycotoxins particularly aflatoxins in different food articles (Whitehead, 1995). Knudsen (2010) proposed a framework for safe foods according to which risk managing officials, stakeholders and scientific experts of the relevant field must collaborate on a single platform provided by food sector governance in order to discuss risk issues of food and suggest sound solutions to them (Knudsen, 2010). This proposal can be followed to design some sort of food-safety strategy to combat hazards of aflatoxins to food. Cleveland and colleagues have suggested using non-toxigenic strains of *A. flavus* to outcompete their aflatoxigenic relatives, as well as employing bioengineered resistant crops to prevent aflatoxin infestation (Cleveland *et al.,* 2003). In addition, they have also provided certain useful antagonistic biocontrol approaches as a solution to this predicament of aflatoxins. Antagonistic and biodegradation strategies using harmless and useful microorganisms, isolated from different food matrices, and their enzymes can provide the best way to purge aflatoxins and aflatoxigenic *A. flavus* (Guan, 2011; Velmourougane *et al.,* 2011).

Presence of Aflatoxins in Food Commodities of Developing Countries

Council for Agricultural Science and Technology (CAST) has found aflatoxins' association with a diverse range of food commodities (CAST, 2003). Aflatoxins are found to be the contaminants of common staple food items such as cereals (corn, rice, sorghum maize and barley), peanut figs, hazel nuts, tiger-nuts, red pepper, soybean and so on (Krishnamurty and Raveesha, 1996; Park, 2002; Bennett and Klich, 2003; Heperkan, 2006; Kumar *et al.,* 2008; Shuaib *et al.,* 2010; Rubert *et al.,* 2012). Due to the

devastating effects of aflatoxins on health, many countries have set regulatory limits for its concentration in human food, which ranges between 4-50 µg/kg for different countries. Characterization of melon seeds for the occurrence of molds and aflatoxins was carried out in Nigeria, as a result of which the analyzed samples were found highly contaminated with *A. flavus* strains. Additionally, 26.5% samples were tainted with AFB_1 with an average concentration of 13.05 µg/kg (Bankole *et al.*, 2006). Aydin *et al.* (2007) in Turkey found 100% analysed red chilli powder samples being contaminated with aflatoxins AFB_1, where 18% of the samples contained its concentration above the maximum permitted levels, that is 5 µg/kg, 50% of the samples contained 0.025- 5 µg/kg and 32% of the samples had AFB_1 below 0.025 µg/kg (Aydin *et al.*, 2007). Set and Erkmen (2010) carried out a study to determine the total aflatoxin and AFB_1 concentration in pistachio and ground red pepper, both in packed and unpacked states. About 50.5% of the unpacked pistachio nut samples were contaminated with aflatoxins in a concentration of 0.007-7.72 ppb, however, total aflatoxin and AFB_1 concentration exceeded the regulatory limits (10 ppb and 5 ppb for AFT and AFB_1, respectively) in about 17% and 23% ground red pepper samples, respectively (Set and Erkmen, 2010). 11% of the Chinese tested spices samples were contaminated with aflatoxins in a concentration between 0.258-27.52 µg/kg. In addition, it was also found that spices were responsible for 10% exposure of the average Chinese citizen to aflatoxins (Zhao *et al.*, 2013). Golge *et al.* (2013) analyzed chilli samples for the incidence of aflatoxins in Turkey. They found AFB_1, AFB_2 and AFG_1 contamination in 82%, 46% and 17.5% of tested chilli samples, respectively. 27.5% and 19.2% of the samples exceeded the EU limits (5-10 µg/kg) of AFB_1 and total aflatoxins in examined samples, respectively (Golge *et al.*, 2013). Observation of milled and dried spices samples in Togo and Benin, countries of West Africa, found that the most dominant fungal contaminants were *Aspergillus* spp. All the *A. flavus* isolates displayed the existence of the aflatoxigenic gene cluster; moreover, 1 garlic and 4 ginger samples contained aflatoxins AFB_1 in a concentration of 390 µg/kg and 1045 µg/kg, respectively (Gnonlonfin *et al.*, 2013). Almost similar results were obtained when Cassava samples were tested in Sub-Saharan Africa (Adjovi *et al.*, 2014).

Considerable studies have regarded aflatoxin contamination being unavoidable and unpreventable. Nevertheless, many such studies demonstrated drought stress and poor storage conditions as the possible reasons underlying food contamination with these toxins (Miller, 1995; Azziz-Baumgartner *et al.*, 2005; Magan and Aldred, 2007). Wicklow and Wilson (1986) discovered that the sclerotia of *A. flavus* and aflatoxin contaminated grains, being left-over in the field after harvesting, could act as inoculums to the new crop of the field since mulching like practices did not significantly destroy them (Wicklow and Wilson, 1986). Similarly, Georgiadou *et al.* (2012) found a positive correlation between aflatoxin contamination of pistachio nuts with insect infestation of the orchards. They also found the onset of aflatoxin contamination at the early maturity step, which demands incisive measures to prevent contamination at such stages (Georgiadou *et al.*, 2012).

As a matter of fact, the toxicity of these metabolites does not necessarily require the presence of aflatoxigenic fungi at the site of contamination. Furthermore, most of the

time mycotoxin ingestion does not reveal obvious disease symptoms. Therefore, these ‗silent killers' or ‗insidious toxins' (Kumar *et al.,* 2008; Polovinski-Horvatović *et al.,* 2009; Sultana and Hanif, 2009) need tremendous attention for their prevention and control.

Status of Aflatoxin M_1 in the Developing World

The most important food commodities that also get contaminated with aflatoxins are milk and its products. Milk is often tainted by the hydroxylated metabolite of aflatoxin B_1, after its ingestion by the milk producing mammal. In addition to cattle milk, human breast milk is also reported to be contaminated with AFM_1 and thereby puts the health of both infants and elders in danger. Children are more vulnerable to the carcinogenic effects of AFM_1 than adults (Hussain and Anwar, 2008; Kumar *et al.,* 2008; Kalač, 2011).

According to the FDA, 60 countries have set permissible limits for AFM_1 to regulate its concentration in dairy products, and in 34 countries its maximum tolerable limit is defined to be 0.05 µg/kg in milk (Iha *et al.,* 2011; Picinin *et al.,* 2013). As a consequence of such efforts, a growing body of knowledge has been gathered about aflatoxin M_1 contamination in various countries of the world (Table: 1). Bakirci (2001) reported that in Van province (Turkey), 87.77% of raw milk samples were contaminated with M_1 and about 44.30% of samples contained higher than permissible level of AFM_1 in milk. Moreover, he reported that M_1 concentration increased in cheese when made from M_1 contaminated milk and pasteurization did not reduce its concentration (Bakirci, 2001). Çelik *et al.* (2005) demonstrated that 88.23% of pasteurized milk samples in Turkey contained AFM_1 and 48% of samples exceeded permitted levels of M_1 in milk by European Codex (EC), that is, 0.05 µg/L (Çelik *et al.,* 2005). Nachtmann and co-workers (2007) in Italy reported that only 0.6% milk samples contained M_1 concentration above EU allowed limits and about 1.6% samples had this concentration reaching EU limits. This study revealed that developed countries like Italy have overcome the problem of M_1 contamination by serious and continuous efforts of food-safety (Nachtmann *et al.,* 2007).

Kamkar (2008) utilized the ELISA technique to detect AFM_1 and its concentration in 52 ultra- high temperature (UHT) processed milk samples. All of the tested samples (100%) had AFM_1 contamination, with about 79.92% of samples above than the maximum permissible levels of M_1 in milk by European Union (EU) (Kamkar, 2008). Torkar and Vengušt (2008) established that about 10% of examined cheese samples in Solvenia contained AFM_1 concentration of 50 ng/kg, where special control measures are needed to be taken. They also found that out of 63.3% moldy raw milk samples, contamination of 33.8% samples was due to *Aspergillus* species (Torkar and Vengušt, 2008). Yet another study carried out in Turkey revealed that 82.6% of tested cheese and 67% of UHT milk samples contained AFM_1 in a range of about 10-690 ng/kg. Furthermore, 27.3% of cheese and 31% of UHT milk samples contained higher AFM_1 concentration than maximum allowable EU limits (Tekinşen and Eken, 2008).

Table 1-Status of Aflatoxins M_1 in the Developing World

Country	Food items (sample size)	Percentage of AFM_1 in contaminated samples	Contamination level	Regulatory limit	Reference
Brazil	Dairy products (n=123)	89.5%	10-529 ng/kg	500 ng/L	Iha *et al.* (2011)
Brazil (Minas Gerais)	UHT milk (n=75)	30.7%	1000-4100 ng/L	500 ng/L	Oliveira *et al.* (2013)
Brazil	Milk samples (n=83)	83%	<3->500 ng/L	500 ng/L	Iha *et al.* (2013)
China	Milk samples (n=135)	72%	0.16-0.50 ng/mL	500 ng/kg	Pei *et al.* (2009)
China	Pasteurized (n=26) and UHT milk (n=153)	96.2 and 54.9%	0.006-0.160 and 0.023-0.154 µg/L	0.5 µg/L	Zheng *et al.* (2013)
China	Tank milk samples (n=72)	59.7%	10-420 ng/L	0.5 µg/L	Xiong *et al.* (2013)
Croatia	UHT (n=706) and raw milk (n=3716)	100%	3.98-183.5 and 3.25-1135 ng/L	No regulatory limit	Bilandžić et al. (2014)
India (Karnataka &Tamilnadu)	UHT milk (n=45)	100%	0.05-0.5 µg/L	0.5 µg/L	Siddappa *et al.* (2012)
Indonesia (Yogyakarta)	Unpasteurized fresh milk (n=113)	100%	<5ng-25 ng/L	No legal limit	Nuryono *et al.* (2009)
Iran (Tehran)	UHT milk (n=52)	100%	19.40-93.60 ng/kg	500 ng/L	Kamkar (2008)
Iran (Ahvaz)	Raw milk (n=311)	42.1%	5-50 ng/L	500 ng/L	Rahimi *et al.* (2010)
Iran (Khorasan)	Pasteurized milk (n=196)	100%	19-126 ng/L	500 ng/L or 0.05 µg/L	Mohamadi *et al.* (2010)
Iran (Tehran)	UHT milk (n=210)	55.2%	0.008-0.249 µg/L	0.05 µg/L	Heshmati and Milani (2010)
Iran	Dairy products (298)	68%	0.013-1.200 µg/L	0.05 µg/L	Fallah (2010)
Iran (Ardebil)	Raw milk (n=122)	100%	4 -112.4 ng/L	500 ng/L	Kamkar *et al.* (2011)
Iran (Urmia)	Raw milk (n=100)	100%	61-90 ng/L	500 ng/L	Panahi *et al.* (2011)

Iran	Dairy products (n=682)	46.3%	0.013-0.394 µg/L	0.05 µg/L	Fallah *et al*. (2011)
Morocco	Raw milk (n=48)	27%	10-100 ng/L	50 ng/kg	El Marnissi *et al*. (2012)
Palestine	Raw milk (n=40)	85%	3 - 80 ppt	No regulatory limit	Al-Zuhair and Omar (2012)
Serbia (Vojvodina)	Pasteurized and UHT milk (n=65)	27.69%	0.01-0.03 mg/L	0.5 µg/L	Polovinski-Horvatović *et al.*(2009)
Serbia	Milk samples (n=50)	100%	<LOD-1.44 µg/kg	0.5 µg/kg or µg/L	Škrbić *et al*. (2014)
Serbia	Different milk samples (n=176)	74.6%	0.005-0.5 µg/kg	0.5 µg/kg	Kos *et al*. (2014)
Slovenia	Raw milk (n=60) and cheese (n=40)	15%	25-223 ng/kg	No regulatory limit	Torkar and Vengušt (2008)
Sudan (Khartoum)	Bulk milk (n=44)	95.45%	0.22-6.90 µg/L	No regulatory limit	Elzupir and Elhussein (2010)
Turkey (Van province)	Raw milk (n=90)	87.77%	0.0302-0.0636 ppb	250 ng/kg	Bakirci (2001)
Turkey	Pasteurized milk (n=85)	88.23%	5.2-127.6 ng/L	50 ng/kg	Çelik *et al*. (2005)
Turkey	UHT milk (n=132) and Cheese (n=100)	67% and 82.6%	10-690 ng/kg	50-250 ng/kg	Tekinşen and Eken (2008)
Turkey (Erzurum)	UHT milk (n=150)	59%	5-185 ng/kg	250 ng/kg	Atasever *et al*. (2010)

One of the initial studies carried out in Yogyakarta, Indonesia on AFM_1 detection through ELISA, revealed that although 100% (n=113) of fresh milk samples were contaminated with AFM_1, none of the samples exceeded the European Union regulatory limit for AFM_1 concentration in milk (25 and 50 ng AFM_1/L). Nonetheless, 30.1% milk samples were contaminated with AFM_1 above 10 ng/L Similarly, 27.4% and 42.5% of samples were found to contain AFM_1 in the range of 5-10 ng/L and below 5 ng/L, respectively (Nuryono *et al.*, 2009). In a study conducted in China, monoclonal antibodies were used to run ELISA for the detection of AFM_1 in milk. 72% of investigated milk samples contained AFM_1 in a concentration of 0.16-0.50 ng/mL (Pei *et al.*, 2009). Coffey *et al.* (2009) found that AFM_1 contamination of milk was a serious threat in Ireland because there was a probability for AFM_1 to go over

EU permissible limits in liquid milk (Coffey *et al*., 2009). In Serbia, Vojvodina, the study of Polovinski- Horvatović *et al.* (2009) revealed that about 18 out of 65 tested milk samples were found contaminated with AFM_1. Despite the fact that contaminated milk samples did not go beyond maximum allowed levels, the need for an efficient monitoring system was being clearly stated in this study (Polovinski-Horvatović *et al.*, 2009).

In Iran, Heshmati and Milani carried out a study in 2010 to monitor AFM_1 levels in UHT milk. 55% of milk samples showed M_1 contamination and 33% samples exceeded EU limits. They also determined seasonal variation in AFM_1 level and found it to be higher in February than in August (Heshmati and Milani 2010). In another study conducted in Iran, 42.1% of milk samples were reported to contain AFM_1 in a concentration range of <5 to >50 ng/L. The order of AFM_1 incidence (concentration) in different milk samples was raw cow milk> water buffalo milk> camel milk> sheep milk> goat milk. None of the milk samples exceeded the maximum tolerable limit of AFM_1 according to Iranian standard (500 ng/L); whereas about 13% of milk samples surpassed the EU standard (50 ng/L) (Rahimi et al., 2010). Sani and his colleagues (2010) carried out a study in Iran to detect the presence of aflatoxins and antibiotic residues. Hundred percent of milk samples were detected to be positive for AFM_1 presence in a range of 19-126 ng/L, out of which 80.6% of contaminated samples transcended the EU limits (Mohamadi Sani *et al.*, 2010). Elzupir and Elhussein (2010) carried out a study in Khartoum, a state of Sudan, to detect AFM_1 levels in dairy cattle milk. It was established that 95.45% of milk samples were contaminated with AFM_1 exhibiting an average of 2.07 μg/L, which is a level higher than that allowed by EU for AFM_1 concentration in milk (Elzupir and Elhussein 2010). About 59% of UHT milk samples were found contaminated with AFM_1 in Erzurum, a city of Turkey (Atasever *et al.*, 2010). Fallah (2010) used TLC to determine the amount of AFM_1 in pasteurized milk and other dairy products collected from different regions of Iran. AFM_1 was detected in 72.5%, 66.1%, 81.9%, 25.8% and 69.4% of pasteurized milk, yogurt, cheese, butter and ice cream samples, respectively. In addition, about 36.2% of pasteurized milk, 20.6% of yogurt, 30.5% of cheese, 9.6% of butter and 27.7% of ice cream samples surpassed the maximum allowed limits (Fallah 2010).

ELISA of contaminated milk samples displayed that 11% of samples had a higher concentration of AFM_1 than the limit allowed by EU. All of the 122 raw milk samples examined for AFM_1 concentration through ELISA in Adebil, Iran, contained M_1 levels (Kamkar *et al.*, 2011) with a mean level of 40 ng/L. About 15% of M_1 containing milk samples exceeded maximum allowable levels of AFM_1 in Iranian regulations and EC. In 2011, Panahi *et a*l. (2011) conducted a study to examine M_1 levels in raw milk in Iran. All the tested raw milk samples (100%) were contaminated with M_1 and moreover, all of them surpassed the maximum limits permitted by EU for M_1 concentration in milk (Panahi *et al.*, 2011). In Brazil, a study conducted by Iha *et al*. (2011) revealed that 84% of cheese and 95% of dairy drink and yogurt samples were contaminated with AFM_1. It showed that around 67% of cheese and 72% of dairy drink and yogurt samples contained AFM_1 levels in a range of 10-304 ng/kg and 10-529 ng/kg, respectively (Iha *et al.*, 2011). The study of Fallah *et al.* (2011)

reported that AFM_1 concentration was higher in contaminated raw cow milk and its industrial products than raw sheep and goat milk and their traditional products. Furthermore, they also suggested an increased level of AFM_1 in milk and its products collected in winter as compared to those collected in other seasons (Fallah *et al*., 2011). Similar results were obtained by Anfossi *et al*. (2012), when they carried out the investigation of AFM_1 in Italian cheese samples (Anfossi *et al*., 2012).

The study of Signorini *et al*. (2012), suggested that in Argentina, contamination of corn-silage and feed with AFB_1 and other mycotoxins led to the appearance of AFM_1, ZEA and DON in cow's milk samples. Although the concentration of the mycotoxins did not go beyond regulatory limits of EU, their presence in milk could not be neglected due to their serious health effects on children and elderly (Signorini *et al*., 2012). El Marnissi *et al*. (2012) in Morocco, displayed that about 27% of the 48 raw milk samples were contaminated with AFM_1 in a concentration range between 10-100 ng/L and about 8% of samples had its concentration above European regulatory limits. This study also demonstrated a link between AFM_1 concentration and seasonal variation and found it to be higher in autumn and winter (El Marnissi *et al*., 2012). Al-Zuhair and Omar (2012) in Palestine, demonstrated that 34 out of 40 tested milk samples (85%) were contaminated with AFM_1 showing an average value of 29.6 ppb. About 20% of the M_1 contaminated milk samples had M_1 concentration about 0.05 µg/L (Al-Zuhair and Omar 2012). Siddappa *et al*. (2012), reported that in India 100% of examined UHT milk samples were found contaminated with AFM_1, out of which 38% samples displayed aflatoxin concentration above the Indian regulatory limits for AFM_1 in liquid milk, that is, 0.5 µg/kg. In addition, 62.5% of flavoured UHT and 61.6% of raw milk samples also contained AFM_1, where 12.5% of flavoured UHT and 17.3% of raw milk samples exceeded the maximum permissible limits (Siddappa *et al*., 2012).

Brazilian research of Iha *et al*. (2013) revealed that fermentation and pasteurization pose negligible effects on M_1 reduction in milk and its products. They reported 83% of powdered and liquid milk samples being contaminated with AFM_1 and reduction of its concentration during its formation into cheese and yogurt by 3.2% and 6%, respectively (Iha *et al*., 2013). In another study, about 30.7% of Brazilian tested milk samples were found to contain AFM_1 in a concentration range of 1000-4100 ng/L. All of the AFM_1 contaminated samples displayed its concentration higher than the maximum permissible limits, that is, 500 ng/L (Oliveira *et al*., 2013). According to a study carried out in China for the detection of AFM_1 through ELISA, both pasteurized (96.2%) and UHT (54.9%) milk samples were found positive for its presence with a concentration ranging between 0.006-0.160 µg/L. Out of the contaminated samples, 65.4% of pasteurized and 20.3% of UHT milk samples exceeded EU regulatory limits (Zheng *et al*., 2013). In another study from China, Xiong *et al*. (2013) detected AFM_1 contamination in 59.7% of raw milk samples with a concentration of 10-420 ng/L detected through LC-MS/MS. Moreover, the AFM_1 concentration was found higher in winter (123 ng/L) than in the other three seasons (Xiong *et al*., 2013).

In Croatia, seasonal variation was reported in the concentration of AFM_1 in aflatoxin tainted milk. 9.64% of UHT and 27.8% of raw milk samples were found to

surpass the EU limits of AFM_1 in liquid milk. This trend of exceeding EU limits was higher in February as compared to the other months of the year, as 36.2% of UHT and 45.9% of raw milk samples had AFM_1 levels above 50 ng/kg in winter (Bilandžić *et al*., 2014). Detection of AFM_1 was carried out in Serbia and its occurrence in 100% of investigated milk samples was demonstrated in 76% of the samples its concentration was found beyond the maximum admissible limits of EU. Moreover, the AFM_1 exposure of the average citizen in Serbia (bw/day) was also scrutinized and found to be 1.420 ng/kg in February, 0.769 ng/kg in April and 0.503 ng/kg in May (Škrbić *et al*., 2014). Yet another study conducted in Serbia, exhibited that 98.7% of cow, 80% of goat and 60% of donkey and breast milk samples analysed were contaminated with AFM_1 in a concentration range of 0.005-0.5 μg/kg. Furthermore, it was indicated that individuals of all age groups and especially infants were exposed to a higher risk concentration of AFM_1 (ng/kg) per bw/day (Kos *et al*., 2014).

Status of Aflatoxins Particularly AFM_1 in Pakistan

Pakistan, being in a tropical region, is characterized by hot and humid climatic conditions. Since this climate favors aflatoxin production and its dissemination; contamination of food, feed and milk with aflatoxins is a common problem in Pakistan (Siddique *et al*., 1987; Majeed *et al*., 2013). A few studies have been carried out on the detection of aflatoxins in feed and incidence of AFM_1 in milk in Pakistan (Table: 2). Co-occurrence of several mycotoxins in feed has also been observed and reported (Anjum and Naseem, 2000). Study of Nizami and Zuberi (1977) revealed an association between consumption of aflatoxin contaminated food over a long period of time and incidence of liver cancer in Karachi, Pakistan (Nizami and Zuberi 1977). Hanif *et al*. (2005) demonstrated that 84% of poultry feed samples were contaminated with aflatoxins. In 2007, about 493 animals died from consuming aflatoxins and T-2 toxin contaminated feed in Landhi, Karachi (Ilyas 2007).

Study of Saleemullah *et al*. (2006) revealed that nuts such as almond, peanut and walnut as well as cereals such as maize, rice and wheat were contaminated with aflatoxins in a range of 5-17 μg/kg and 14-45 μg/kg, respectively. Although none of the sample exceeded the maximum permissible limits of aflatoxins in food items, that is, 50 μg/kg, the present concentration may act as a serious health risk for consumers (Saleemullah *et al*., 2006). Pakistani chillies both in ground and pod form were analyzed by Paterson (2007) in order to determine aflatoxin levels in these samples. Hundred percent samples were infested with aflatoxins with its concentration ranging from 0.1 to 96.2 μg/kg and mean value of 32.11 μg/kg for B_1 and 1.31 μg/kg for B_2. It was further recognized that aflatoxins contaminated samples of chillies were not necessarily being plagued by *A. flavus* (Paterson 2007). In a study conducted in Swat, maize kernels were analyzed for mold and mycotoxin incidence; *A. flavus* was isolated in the highest number among all the predominant mycotoxigenic fungal genera. In addition, maize samples were also found to contain aflatoxins in a high concentration of 0-30.92 μg/kg (Shah *et al*., 2010). Iqbal *et al*. (2011) reported 60% of chilli samples to be contaminated with aflatoxins in a range of 1.0- 34.6 μg/kg (Iqbal *et al*., 2011a).

Table 2: Status of Aflatoxins in Pakistan

Province	Food items (sample size)	Percentage of aflatoxins contamination	Contamination level	Reference
Different areas	Poultry finished feed (n=726)	84%	1-120 ppb	Hanif *et al.* (2005)
Punjab	Raw milk (n=168)	100%	0.01-0.70 µg/L	Hussain and Anwar (2008)
Punjab	Milk samples (n=480)	47.5%	0.027-0.044 µg/L	Hussain *et al.* (2008)
Punjab (Faisalabad)	Milk (n=169)	26%	0.002-0.014 µg/L	Hussain *et al.* (2010)
Punjab (Lahore)	Raw milk (n=84)	81%	0.69 to 100.04 µg/L	Muhammad *et al.* (2010)
Different areas	Rice (n=40)	70%	3.7 ng/g	Hussain *et al.* (2011)
Punjab	Sweets (n=138) and Milk (n=232)	97% and 76.3%	0.48 µg/kg - 0.252 µg/L	Sadia *et al.* (2012)
Punjab	Milk products (n=546)	47%	0.015-0.18 µg/L	Iqbal and Asi (2012)
Punjab and KPK	Maize (n=65)	46.15%	5-850 µg/kg	Khatoon *et al.* (2012)
Punjab (Faisalabad and Lahore)	Processed food (n=125)	38%	0.02–0.37 µg/kg	Mushtaq *et al.* (2012)
Punjab	Milk containing halva (n=431)	59%	0.1-0.4 µg/L	Iqbal *et al.* (2013b)

Luttfullah and Hussain (2011) carried out a study on nuts and dried fruits in Pakistan to detect their aflatoxin levels. Their study found that out of 75 dried fruit samples and 105 samples of nuts, 17 (22.6%) of dried fruits and 36 (34.2%) of nuts were contaminated with aflatoxins, with a mean value of 2.1- 6.4 µg/kg. Furthermore, they also suggested that in 16 of the 180 analyzed samples (8.8%) the aflatoxin level was found above the EC regulatory limits, that is, 4 µg/kg (Luttfullah and Hussain, 2011). In another study carried out by Luttfullah and Hussain (2012), the aflatoxin level was determined in beans and cereals in Pakistan. They demonstrated that 12 (18.4%) samples of beans and 23 (24.2%) samples of cereals were contaminated with aflatoxins out of 65 beans and 95 cereals samples, respectively. The average value of aflatoxins was found between 2.2-10.4 µg/kg, with 15 (9.3%) out of total 160 samples exceeding the limits of EC (4 µg/kg) for aflatoxins in food items (Lutfullah and Hussain, 2012). Iqbal *et al.* (2013) reported 56.7% of halva ingredients (comprised of almond, cardamom, halva puri, pistachio, raisins, semolina and wheat powder) being contaminated with AFB_1, out of which 26.7% of samples contained its concentration higher than EU allowable

limits, that is, 2 μg/kg (Iqbal *et al*., 2013b). The occurrence of aflatoxins in corn, corn products and rice was found to be 35%, 42% and 55.8%, respectively in a concentration of 5-19 μg/kg. Furthermore, 14% of corn, 20% of corn products and 28% of rice samples were found to have aflatoxin levels above the permissible limits of EU in food, that is, 5-10 μg/kg (Majeed *et al*., 2013). In another study, being conducted in Punjab, occurrence of aflatoxins was detected in 57% of raw peanut and 48% of peanut product samples and toxin concentration was found in a range of 2-12 μg/kg (Iqbal *et al*., 2013c).

Pakistan is one of the major milk producing countries in the world. Despite that, no tolerance limit for AFM_1 in dairy products has been introduced yet. Hussain *et al*. (2008) revealed that 43% of the buffalo milk samples and 53% of the cow milk samples were found to be contaminated with AFM_1. In another study, Hussain and Anwar (2008) pointed out that almost all of the tested milk samples (100%) contained M_1 contamination and about 99.4% of contaminated milk samples exceeded EC tolerance limits for M_1 in liquid milk (Hussain and Anwar, 2008).

Studies of Muhammad *et al*. (2010) showed that 81% samples of raw milk, collected from Lahore, contained AFM_1 concentration above American and EC permitted limits (Muhammad *et al*., 2010). In addition to milk, other food crops such as rice were also contaminated with aflatoxins. According to Hussain *et al*. (2011), 8% of rice samples in Pakistan exceeded maximum tolerable limit of aflatoxins and about 70% samples were contaminated with aflatoxins B_1, B_2, G_1 and G_2 (Hussain *et al*., 2011).

Asi *et al*. (2012) revealed seasonal variation of AFM_1 in milk of different mammals. According to the research, 55% of buffalo, 56% of cow, 32% of goat, 58% of sheep and 27% of camel milk samples were found to have AFM1 level above EC acceptable limits. However, in summer, the percentage of contaminated milk samples was 38%, 33%, 21%, 36% and 14%, respectively. In winter and morning times, concentration of M_1 was higher than in summer and evening times (Asi *et al*., 2012). Iqbal and Asi (2012) stated that about 71% of milk, 61% of yogurt, 78% of white cheese, 59% of cheese cream and 45% of butter samples were reported to be contaminated with AFM_1. Out of them, about 58% of milk, 47% of yogurt, 15% of white cheese, 11% of cheese cream and 52% of butter samples exceeded EC tolerable limits (Iqbal and Asi, 2012). Study of Khatoon *et al*. (2012) revealed about 28% prevalence of aflatoxins in maize samples (Khatoon *et al*., 2012). According to a report, about 38% of examined processed food contained aflatoxins and out of them around 21% exceeded EC limits (Mushtaq *et al*., 2012).

Iqbal *et al*. (2013) described that 59% of analyzed halva (a sweet dish made from milk) samples were contaminated with AFM_1. Out of them, 9% samples surpassed the EU recommended limits of AFM_1 in milk (Iqbal *et al*., 2013b).

Legislation Regarding Aflatoxins

The deleterious effects of aflatoxins on agricultural crops, animals as well as humans led food-security agencies to set regulations for controlling maximum tolerable limit of aflatoxins in various food commodities. The Food and Drug Administration of the United States and the European Union are the organizations that control aflatoxin levels in food and feed as well as milk (Kensler *et al*., 2011). About 77-100 countries have set proper regulations

for monitoring mycotoxin levels in food and feed (van Egmond 1983; FAO 2004; Zain 2011). According to Haumann (1995), 50 countries have established regulatory limits for aflatoxins in food and feed items (Haumann 1995). FDA sanctions 20 µg/kg aflatoxins in feed and food articles and 0.05 µg/L AFM_1 in liquid milk (Cleveland *et al*., 2003). The European community permits 50 ng of aflatoxin concentration per kg, however, the US allows 500 ng/kg and Australia has set even more strict limits, that is, 10 ng/kg (Kamkar 2008). Still, there are certain developing countries that lack such regulations (CAST 2003; Nuryono *et al*., 2009). Pakistan is one such country that has completely ignored this important step to be taken (Hussain and Anwar 2008; Iqbal *et al*., 2011b; Iqbal and Asi 2012). There are many examples of developed countries including Spain, Italy and so on that are worth mentioning and deserve applause in the context of eradicating aflatoxin contamination from their crops and food items (Santamakina *et al*., 1986). This could be achieved only due to the stringent and serious control efforts developed and implemented by these countries.

Once produced, it becomes quite hard to completely eliminate aflatoxins from food commodities (Scott *et al*., 1992; Wu *et al*., 2009; Panahi *et al*., 2011). There is a great need to develop regulations to monitor, control and eliminate aflatoxin contamination. Such regulations will not only lessen the economic burden but will also help attract foreign traders to enhance business terms in various food sectors (Zain 2011).

Economic Impact of Aflatoxins

According to a careful estimation by the Food and Agriculture Organization (FAO), about 25% of the world‘s crops are significantly affected by the peril effects of mycotoxins every year (WHO 1999; Spadaro and Gullino, 2004). The Council for Agriculture Science and Technology (CAST) has also estimated huge scale economic losses due to aflatoxin infested crops and food commodities. The FDA reports loss of millions of tons of food items due to mycotoxin infestation each year (FDA 1997). In addition to this direct effect on crops, these aflatoxigenic molds indirectly affect the consumers, both humans and animals, indiscriminately (Abdin *et al*., 2010). Price *et al*. (2005) reveal that *A. flavus* and *A. parasiticus* are responsible for agricultural destruction and cause 200-270 million dollars loss annually in the US (Yu *et al*., 2002; Richard and Payne 2003). Similarly, rejection of aflatoxin contaminated food consignments brings about massive economic losses mostly for the developing world (Hussein and Brasel 2001; Kensler *et al*., 2011). The ban on the import of chillies from Pakistan by Japan and the European Union due to high aflatoxin contamination is one of such examples (Iqbal *et al*., 2011a). A number of studies report the monetary losses owing to aflatoxin contamination.

Contaminated crops are destroyed in developed countries (Cleveland *et al*., 2003), but in the case of their entry into the food chain, they impart serious health effects on animals and contaminate their products such as meat and milk; consequently, these toxins provide health threats to humans also. AFM_1 contaminated milk, just like that of AFB_1 contaminated crops, is also discarded in developed countries, when the concentration of M_1 exceeds 0.05 ppb in milk (Pennington, 2005). These issues are even worse in developing countries like Pakistan, where no regulatory system exists for aflatoxin

monitoring. For this reason, untested food-items become one of the basic causes of foodborne outbreaks in these countries. For instance, outbreaks due to aflatoxin contamination, such as those which occurred in 1977, 1980 and 1988 in USA, India and Kenya, respectively, are all examples of such food- related calamities (Probst *et al*., 2007; Kensler *et al*., 2011). Therefore, it can be concluded that millions of dollars are lost annually as a consequence of the chaos that aflatoxins cause, because of their severe impacts on agriculture, animals and humans (Upadhaya *et al*., 2010).

Conclusions

Although commendable efforts have been made to get details about aflatoxins as well as to develop biocontrol strategies to eliminate these toxins, no strategy has yet been designed to completely eliminate aflatoxins from food commodities. From previous reports, it is also inferred that the developing world is facing severe challenges with respect to aflatoxin contaminated food. In Pakistan, studies have been carried out only to detect the presence of aflatoxins in certain food commodities. However, there is still a need for studies relating preventive measures and aflatoxin degradation strategies. There are also certain research areas which have been less investigated up till now; for instance, using biotechnological approaches to utilize the products of the genes against aflatoxin degradation and use of harmless microbial communities to lessen the concentration of aflatoxins in food items up to permissible limits. The most important step that should be taken by each country on a government level is to have a check and balance on aflatoxin contamination level in food items.

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