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Implication of Aflatoxin Contamination in Agricultural Products

Ephrem Guchi*

Department of Applied Biology, Samara University, School of Natural and Computational Sciences, Samara, Ethiopia
*Corresponding author: ephremg21@gmail.com

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Abstract Aflatoxins are secondary fungal metabolites that contaminate agricultural commodities and can cause sickness or death in humans and animals. Risk of aflatoxin contamination of food and feed in Africa is increased due to environmental, agronomic and socio-economic factors. Temperature, food substrate, strain of the mould and other environmental factors are some parameters that effect mycotoxin production. Preventing mycotoxin production at farm level is the best way to control mycotoxin contamination. Advances in molecular techniques and other decontamination methods such as gamma-irradiation and microwave heating could help to deal with these issues. Mycotoxins could be used as an energy source for a group of aerobic microorganisms, which are suitable to mycotoxin biodegradation. Several protocols have been provided to biodegrade mycotoxins in food and feed using potential bacteria such as *Lactobacillus* and *Bifidobacterium.* However, there are varieties of responses between different microorganisms against mycotoxins. For example, *Bacillus brevis* were not affected by high concentrations of trichothecene. Application of microorganisms needs to be evaluated from a safety point of view. Application of microorganisms on mycotoxin degradation, food and feed materials also need to be investigated. Further studies need to be conducted to address the seasonal variation of aflatoxin contamination in food and feed. Understanding the seasonal variation could help demonstrate and develop more effective decontamination methods. For example, it is postulated that mycotoxin issues due to monsoons in Hungary could possibly be concluded to technical difficulties in pre- and post-harvest operations. Application of advanced methods such as DNA biosensors and infrared spectroscopy for rapid and accurate detection of mycotoxin and related fungi is increasing dramatically. Application of new and advanced detection techniques could enable the agricultural industry to deal more effectively with the occurrence of aflatoxin contamination.

Keywords: *aflatoxin contamination, agricultural products*

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1. Introduction

Aflatoxins are a group of mycotoxins produced by *Aspergillus species*, including *A. flavus*, *A. parasiticus*, and *A. nomius*. A quarter of the world's food crops are estimated to be affected by mycotoxins; creating a large economical loss in the developed and developing countries (Kumar and Rajendran, 2008). Other reports indicate even higher contamination rate of aflatoxin (Njobeh *et al*., 2009). Exposure to higher levels of aflatoxin contamination increases cancer incidence, including risk of hepato-cellular carcinoma especially in 6- to 9-year-old girls and neural tube defects (Umoh *et al*., 2011).

One of the reasons which make aflatoxins one of the most challenging mycotoxin is the fact that it could be produced by the responsible fungi not only at pre-harvest time but also at post harvest stages including storage. However, later on, lack of regulations or poor enforcement, which makes the use of such contaminated commodities inevitable, could lead to severe human and animal diseases too. Aflatoxin B1, B2, G1 and G2 are the most important members of the aflatoxin group, which chemically are coumarin derivatives with a fused dihydrofurofuran moiety. Presence of aflatoxin B1, B2, G1 and G2 may naturally occur in different ratios depending on different matrices. However, it was concluded that when aflatoxins are limited only to AFB1 and AFB2, such ratio is 1.0 to 0.1, while when all four aflatoxins occur (AFB1, AFB2, AFG1 and AFG2), they may be found in a ratio of 1.0:0.1:0.3:0.03 (Abbas *et al*., 2010). Cereals notably corn, nuts such as peanuts, pistachio and Brazil nuts, oil seeds such as cottonseed, as well as copra, the dried meat of coconut, are some of the commodities with greater risk of aflatoxin contamination (Cornea *et al.*, 2011; Idris *et al.*, 2010). Because peanuts, cottonseed and copra constitute the most important source of edible oils, they are of particular interest (Idris *et al.*, 2010). Commodities which are resistant or only moderately susceptible to aflatoxin contamination in the field include wheat, oats, millet, barley, rice, cassava, soybeans, beans, pulses and sorghum. However, when any of these commodities are stored under high moisture and temperature conditions, aflatoxin contamination may occur (Smith and Moss, 1985). Other commodities such as

cocoa beans, linseeds, melon seeds and sunflower seeds have been infrequently contaminated with mycotoxins with lower importance rate compared to other commodities (Bankole *et al*., 2010). Aflatoxin is the single most important contaminant on The Rapid Alert System for Food and Feed (RASFF) of the European Union in a way that in 2008, aflatoxins alone were responsible for almost 30% of all the notifications to the RASFF system (902 notifications) (Energy, 2009). With increasing knowledge and awareness of aflatoxins as a potent source of health hazard to both human and animals, a great deal of effort has been made to completely eliminate the toxin or reduce its content in foods and feedstuffs to significantly lower levels. Although prevention is the most effective intervention, chemical, biological and physical methods have been investigated to inactivate aflatoxins or reduce their content in foodstuffs (Rustom, 1997).

2. Natural Occurrence of Aflatoxin Contamination in Raw Agricultural Products

Natural occurrence of aflatoxins in raw agricultural products poses severe health and economic risks worldwide. The Food and Agriculture Organization (FAO) estimates that many basic foods could be contaminated with mycotoxin producing fungi, contributing to huge global losses of foodstuffs, about 1000 million metric tons each year (Bhat and Karim, 2010). Contamination of feed materials with mycotoxins is an important issue for farmers due to both acute and chronic intoxication in animals. The economic impact of feed contamination with mycotoxins includes productivity reduction and organ damage (Upadhaya *et al.*, 2010). Aflatoxins, zearalenone, trichothecenes, fumonisins and ochratoxin A are the most frequently investigated toxins, although there are more than 300 recognized mycotoxins in animal feed (Rustemeyer *et al*., 2010). Mycotoxin contamination reports in animal feed indicate a variety of contamination levels (Monbaliu *et al*., 2010). Fungi which produce mycotoxin belong to *Aspergillus, Penicillium* and *Fusarium species* (Rustemeyer *et al*., 2010). Aspergillus and penicillium constitute a major part of the fermented feed microbiota (Roige *et al*., 2009). Intrinsic and extrinsic factors during storage and at field condition may interact with mycotoxin contamination. Animal feeds, such as hay and straw, might be contaminated during pre-harvest or drying stages (Bhat and Karim, 2010). Mycotoxin contaminated animal feed cause's serious effect on monogastric animals. However, the ruminants may be more resistant to mycotoxins due to biotransformation ability of the rumen microbiota. Other factors such as age, aflatoxin concentration and duration of exposure might also have some effect (Rustemeyer *et al*., 2010; Upadhaya *et al.*, 2010). The affected commodities by aflatoxins are corn, peanuts, cottonseed, millet, sorghum and other feed grains. In ruminants, a part of aflatoxin B1 is degraded into aflatoxicol and the remaining is hydroxylated in the liver into aflatoxin M1 (Upadhaya *et al.*, 2010). Aflatoxin B1 is considered as a group I carcinogen for humans by International Agency for Research on Cancer (IARC) (Seo *et al.*, 2011). Aflatoxicosis may cause death in ruminants (Pierezan *et al*., 2010). Despite extensive research done during the last few decades, which helped authorities around the world to establish control measures, still aflatoxin contamination in food and agricultural commodities remains as one of the most challenging and serious food safety problem.

Close study of the annual reports in the last decade (2003-2009) of the Rapid Alert System for Food and Feed (RASFF) showed four aforementioned groups contributed to the most aflatoxin contamination. Although, one should be careful in jumping to a bigger conclusion as these data also depends on the policy of EU countries, on products that go on a 100% check and those checked randomly. The detailed results are included in Table 1. According to a study by Vinod *et al.* (2008) the incidence of mycotoxins in some commercially important agricultural commodities concluded that high-risk commodities for mycotoxin contamination were corn and groundnut.

Table 1. Comparison of number of aflatoxin allert notifications according to product category reports in the RASFF system in years 2003–2009

Year	Total		Nuts, nut products & seeds		Fruit & vegetables		Cereal products		Herbs & spices		Feed for food producing animals		Pet food	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%
2003	763	95	695	91	33	4	6	1	5	1	-	NA	-	NA
2004	844	95	699	83	42	5	12	1	7	1	-	NA	-	NA
2005	943	95	827	88	81	9	9	1	57	6	2	0	-	NA
2006	800	91	684	86	69	9	5	1	37	5	4	1	-	NA
2007	705	93	568	81	70	10	21	3	35	5	6	1	4	1
2008	902	97	710	79	103	11	46	5	26	3	11	1	3	0
2009	638	95	517	81	64	10	13	2	23	4	9	1	11	2

Source: (RASFF, 2011).

2.1. Nuts, Nut Products and Seeds

As it is clear from Table 1 based on RASFF reports; nuts, nut products and seeds were the most rejected lots, and thus the most contaminated products in general too. These serve as very good substrates due to their high fat content. Also, aflatoxin producing fungi can cause toxin production in all steps including pre-harvest, drying process as well as storage. Environmental conditions such as prolonged drought stress play a major role in increasing the risk of aflatoxin contamination (Kumar and Rajendran, 2008). Similar conclusion was also drawn by Wagacha & Muthomi (2008) from the African perspective too, in which aflatoxins widely occured in groundnuts. A close study of all mycotoxins rejected lots (1000 reports of 5979 at the time) based on online available information of RASFF from 16/12/2009 till 02/05/2011 revealed that highest aflatoxin levels were found again in this group (Table 2).

A Korean survey of different nuts and their products marketed in South Korea showed that 9 out of 85 samples including peanuts, peanut butter, and pistachios were contaminated with aflatoxins (10.6% of incidence). The most contaminated nut was peanut (roasted) with values ranging from 2.00–28.24 µg/kg and a mean of 10.67 ± 12.30 µg/kg for total aflatoxins (7.97 ± 7.75 µg/kg for aflatoxin B1). Similar data at slightly lower levels was found in one assorted nuts and 2 peanut butter samples (Chun *et al.*, 2007). A Turkish study conducted from September 2008 to February 2009, detected aflatoxin B1 contamination in almost 49.2% (59/120) of un packed and packed pistachio nut samples at levels lower than action limits of 5 µg/kg (Set and Erkmen, 2010). Abdulkadar *et al.* (2004) found aflatoxin B1 contamination in different nuts in the range of not detected (ND)–81.64 µg/kg. In a study by Ismail *et al.* (2010), from about 196 nuts and their products in Malaysia, 16.3% of the products showed contamination between 17.2 to 350 µg/kg. Forty-eight samples out of 95 were contaminated within a range of 0.007 to 7.72 µg/kg in pistachio nuts (Set and Erkmen, 2010).

Table 2. Some of the highest values of aflatoxin contamination in the rejected lots of Nuts, nut products and seeds, based on The Rapid Alert System for Food and Feed (RASFF)

Raw	Origin	Commodity	Maximum Contamination Levels µg/kg)		Date of case
			B1	Total	
1	United States	Almonds	43800	47800	13/04/2010
2	Italy, with raw material from Afghanistan	Shelled roasted Pistachios	973	--	27/07/2010
3	Georgia	Hazelnut kernels	638	713	24/03/2011
4	Ghana	Groundnut paste	622	810	25/10/2010
5	Egypt	Groundnuts in shell	614.0	646.4	07/12/2010
6	Turkey	Pistachios in shell	594	665	25/11/2010
7	Iran	Pistachios	562	597.7	30/04/2010
8	Syria	Pistachios kernel	333	369	29/03/2011
9	Algeria	Dried Apricot kernels	333	342.5	16/12/2009
10	Iran	Pistachio nuts	210	230	21/03/2011
11	China	Peanuts in shell	192	214	24/03/2010
12	Italy	Dried sweet chestnut flakes	184	-	26/11/2010
13	Nigeria	Ground melon Seeds	136.3	154.1	03/02/2011
14	United states	Raw pistachios	120	134	18/03/2010
15	India	Groundnut Kernels	118.0	281.0	15/04/2011
16	United states	Salted almonds	95.1	127.3	04/02/2010
17	United states	Almonds	61.5	69.2	12/11/2010
18	Ukraine	Hulled walnuts	38	-	08/02/2011

Source: (RASFF, 2011).

2.2. Fruits and Vegetables

Close study of all mycotoxin rejected lots (277 reports of 672 at the time) from 01/01/2008 till 19/04/2011, based on online information from RASFF, revealed that highest aflatoxin levels were found in dried figs from Turkey followed by dried figs from Greece (Table 3). Natural occurrence of aflatoxin in fruits came to light more in the recent years.

Table 3. Some of the highest values of aflatoxin contamination in the rejected lots of fruit and vegetables, based on The Rapid Alert System for Food and Feed (RASFF)

Raw	Origin	Commodity	Maximum Contamination Levels (µg/kg)		Date of case
1	Turkey	Dried figs	91.1	133.4	4/7/2008
2	Turkey	Dried figs	76	84	18/01/2008
3	Greece	Dried figs	70.6	76.4	22/12/2010
4	Turkey	Dried figs	63.5	117.5	3/1/2008
5	HUNGARY raw material from Turkey	Dried figs	62.2	104.2	22/12/2008
6	Turkey	Dried figs	55	113	26/11/2010
7	Turkey	Dried figs	54.2	58.3	20/10/2010
8	Greece	Sun dried figs	47.9	86.7	3/12/2009
9	Turkey	Dried figs	41.80	51.23	19/11/2009
10	Turkey	Dried figs	25.0	36.25	17/01/2008

Source: (RASFF, 2011).

Reports indicated that figs, dates and citrus fruits grown in susceptible regions (the high temperature conditions) could get contaminated with aflatoxins (Rivka, 2008), of which fig is most vulnerable to aflatoxin contamination. The reason for such high susceptibilities apart from their chemical properties is based on the fact that *A. flavus* is able to enter and colonize in the internal cavity of the fruit (Rivka, B., 2008). Although some surveys found only

trace amount of aflatoxins in fig (Blesa *et al.*, 2004), others found quite high levels and the contamination levels might go as high as 77,200 ng/g. Aflatoxins were also reported, but in lesser extent, in other fruits such as dates, citrus fruits, raisins and olives (Ferracane *et al*., 2007). In case of citrus fruits, at least there is sound evidence of potential contamination risk (Bamba and Sumbali, 2005).

2.3. Cereal Products

A study was conducted in Iran on Aflatoxin contamination of foodstuffs. Fifty-one maize samples, intended for animal feed and human consumption, were collected from the four main maize production provinces in Iran and analyzed by high-performance liquid chromatography for contamination by four naturally occurring aflatoxin analogues (AFB1, AFB2, AFG1, and AFG2). AFB1 was detected in 58.3% and 80% of the maize samples obtained from Kermanshah and Mazandaran provinces, respectively (Ghiasian *et al.*, 2011).

High levels of aflatoxin B1 contamination in rain-affected maize and rice at a level of 15600 and 1130 µg/kg respectively, was reported. Also, high levels of aflatoxins were found in parboiled rice (max 130 µg/kg). However, relatively lower values were reported in normal crops (Vasanthi, 1998).

The crops with higher risk of aflatoxin contamination were groundnuts, maize and chilies. In one study, 21% and 26% of groundnuts and maize samples respectively, exceeded their national maximum limit of 30 µg/kg of aflatoxin contamination (Vasanthi, 1998).

Vargas *et al*. (2001) reported that 38.3% of maize samples were contaminated with aflatoxin B1 with a mean of 9.4 µg/kg and a maximum of 129 µg/kg. The investigators have reported that only 3.7% showed levels above 20 µg/kg. They found the co-occurrence of aflatoxin B1 and fumonisin B1 in all of the 82 aflatoxin-contaminated samples. Co-occurrence of these 2 mycotoxins with zearalenone was observed only in 18 samples.

Maize and groundnuts were reported to be a major source of aflatoxin contamination around the globe particularly in India, South America and the Far East in the late 1990's. Other commodities which raised concerns with regard to high susceptibility to aflatoxin contamination were tropical and subtropical cereals, oilseeds, and tree nuts as well as cotton-seed meal.

The largest and the most severe documented aflatoxin poisoning has been reported at a level as high as 8,000 µg/kg in Kenya in 2004, causing 125 deaths out of 317 case-patients (Wagacha and Muthomi, 2008).

According to a study conducted by Sugita-Konishi *et al*. (2006) about the contamination in various Japanese retail foods with aflatoxin B1, B2, G1, and G2, and other mycotoxins, between 2004 and 2005, aflatoxins were detected only in almost half of the peanut butter samples with the highest concentration of aflatoxin B1 at about 2.59 µg/kg. While in other products such as corn products, corn, peanuts, buckwheat flour, dried buckwheat noodles, rice, or sesame oil, aflatoxin contamination was not detected.

Aflatoxin was also detected in the majority of dried yam chips samples surveyed in Benin with levels as high as 220 µg/kg, although the average was much lower (14 µg/kg). More than 54% of dried yam chips in Nigeria were found positive for aflatoxin contamination, while high levels of aflatoxins ranging from 10–120 µg/kg was detected in slightly more than one third of the tiger nut (*Cyperus esculentus*) samples in the same country (Bankole and Mabekoje, 2004).

High aflatoxin levels in maize, in some other African countries, notably Benin and Togo have been reported and one third of the household grain, contained aflatoxins in the range of five-fold the safe limit (Wagacha and Muthomi, 2008).

Maize (*Zea mays L.*) grain was shown to be a good substrate for mould infection including *A. flavus, A. parasiticus* and production of aflatoxins. Indian scientists have reported several cases of aflatoxin epidemic in humans over the last decade mainly due to the consumption of heavily contaminated maize that nominates maize as a high risk crop. Rice is another member of the cereal family which shows high level of aflatoxin contamination, as high as 2830 µ g/kg, which according to some reports was even higher than levels compared to wheat and maize. Aflatoxin contamination in rice occurs in the preharvest stage. Delayed drying as well as high moisture content and crop storage can cause postharvest contamination. Although both white rice and parboiled rice could be contaminated with aflatoxin, parboiled rice (boiled rice in the husk), despite improvement in its nutritional profile especially its vitamin-B content (Beri-beri disease is common among the white rice-eating people), is more suitable for the storage fungi to enter if later drying is not adequate (Kumar and Rajendran, 2008). Nguyen *et al*. (2007) investigated the possible coexistence of aflatoxin B1, citrinin and ochratoxin in Vietnam. From 100 rice samples collected countrywide, 35 samples showed values higher than the limit of quantification (LOQ) of 0.22 µg/kg, with a mean of 3.31 µg/kg and a highest value of 29.8 µg/kg, for aflatoxin B1. The results also indicated a high percentage in co-occurrence of aflatoxin B1 and ochratoxin A in rice. Their findings showed significant effect of monsoons that increased the average of quantifiable samples of AFB1 and the ratio of detectable samples in rice, compared to those in the dry season. In some provinces, these were 5 times higher (mean of 10.08 µg/kg compared to 1.77 µg/kg) or even more (mean of 4.5 µg/kg compared to less than LOQ of 0.22 µg/kg). Given the average daily intake of rice by aVietnamese adult to be 500 g, there is a cause for concern (Nguyen *et al.*, 2007). Reports raised concern over the presence of citrinin in red yeast rice (Monascus fermented rice); a traditional natural food colorant in Asia, while no reports on aflatoxin was obtained (Lin *et al.*, 2008). A study on Turkish wheat samples published in 2008 revealed 60% contamination level in a very low range indeed (maximum of 0.644 µ g/kg) (Giray *et al.*, 2007).

No aflatoxin was found in the 60 samples of corn meal and flour obtained from Sao Paulo Market in 2000 (Bittencourt *et al.*, 2005). A market research of various food products (cereal and cereal products, nuts and nut products, spices, dry fruits and beverages) in Qatar in 2002, revealed no detected levels of aflatoxin contamination in rice and wheat (Abdulkadar *et al.*, 2004).

The highest aflatoxin levels were found in stone ground corn meal from India followed by mixed snacks from India, and rice from Thailand. Aflatoxin contamination in raw and processed food can be monitored using

chromatography or antibody platforms (Seo *et al.*, 2011). Aflatoxin B1 was detected at the following levels in all samples of Nigerian grains: 17.01-20.53 µ g/kg in wheat, 34.00-40.30 µ g/kg in millet, 27.22-36.13 µ g/kg in guinea corn, and 40.06-48.59 µ g/kg in bread fruit (Odoemelam and Osu, 2009).

Close study of all mycotoxins rejected lots (249 reports of 249 at the time) from 14/02/2000 till 28/04/2011, based on online information available from RASFF, revealed that the third highest aflatoxin levels were found in this group (Table 4).

2.4. Herbs and Spices

Medicinal plants are various plants with medicinal properties, which were the core of traditional therapy for the most of human history. Although the toxic effect of some were known for centuries, only in the recent modern time, the safety of these plants from the contamination point of view come to light.

Table 4. Some of the highest values of aflatoxin contamination in the rejected lots of cereals and cereals products, based on The Rapid Alert System for Food and Feed (RASFF)

Raw	Origin	Commodity	Maximum Contamination Levels (µg/kg)		Date of case
			B1	Total	
1	India	Stone ground corn meal	410	430	08/08/2008
2	Ghana	Dried roasted corn	336	383.6	15/10/2007
3	India	Mixed snacks	184.07	188	12/12/2007
4	United Kingdom withraw material from Ghana	Kenkey (maize based product)	134	153	28/04/2011
5	Ghana	Fermented banku Flour	57	127	03/09/2010
6	Ghana	Maize flour	56	67	04/07/2008
7	Thailand	Black rice	52.2	72.2	01/07/2004
8	India	Corn meal in retail packs	47	51	06/02/2009
9	India	Unpolishedbasmati rice	46.2	50.7	07/12/2007
10	Hong kong	Egg cake	45	54	01/12/2007
11	-	Rice – red	35	43.6	14/08/2001
12	Malaysia	Glutinous rice balls with peanut butter	35*	-	19/03/2008
13	Canada	Roasted red rice flour	32	37	18/09/2009
14	Pakistan	Broken rice	28	32.3	15/05/2006
15	Pakistan	Brown basmati rice	27	-	01/03/2007
16	Pakistan	Brown basmati rice	22.1	23.7	13/03/2008
17	Pakistan	Long grain white rice	18.9	25.6	03/03/2008
18	Poland	Long grain white rice	16.7	18.4	23/03/2007
19	Bangladesh	Rice flakes	12.7	16.8	23/12/2008
20	Pakistan	Basmati rice	12	14	27/11/2009
21	Pakistan	Broken rice	11.5	13	25/02/2009

Source: (RASFF, 2011).

One of the safety concerns in herbal medicine now a days is the presence of mycotoxins, notably aflatoxins, as their use have been increasing in the recent years after a decline in their use for almost a century. It has been reported that spices and herbs that was used for the improvement of some forms of liver disorder might be contaminated with high concentrations of aflatoxins, with aflatoxin B1 at an alarming level of 2230 µg/kg (Moss, 1998). Abdulkadar *et al.* (2004) found aflatoxin B1 contamination in mixed spices powder in the range of 0.16–5.12 µg/kg, while chilli powder showed a higher range of 5.60–69.28 µg/kg.

A Turkish study conducted from September 2008 to February 2009, detected aflatoxin B1 contamination in 80% (48/60) of unpacked and packed ground red pepper samples within the range of 5-55.9 µg/kg (Set and Erkmen, 2010). Zinedine *et al.* (2006) reported relatively low contamination levels in spice samples including paprika; ginger, cumin, and pepper. The highest level of aflatoxin was found in red paprika (9.68 µg/kg) (Zinedine *et al.*, 2006). Close study of all mycotoxin rejected lots (211 reports of 432 at the time) from 06/12/2007 till 19/04/2011, based on online information available from RASFF, revealed that the highest aflatoxin levels were found in curry powder from Nigeria, whole nutmeg from Indonesia, dried paprika from Peru and suya pepper from Ghana, followed by paprika powder from UK (Table 5). Contrary to the long history and the wide use of herbal medicines, there are only a few publications in regard to their mycotoxin contamination compared to the large number of publications on the contamination of cereals and oil seeds (Trucksess & Scott, 2008). The European Pharmacopeia has set limits for aflatoxin B1 and total aflatoxins at 2 and 4 µg/kg respectively, for some medicinal herbs (Pharmacopeia, 2007). Although in one study in South Africa, no aflatoxin contamination was found in some medicinal plants (Sewram *et al.*, 2006), while others reported levels ranging from 2.90–32.18 µg/kg (Yang *et al.*, 2005). Roy *et al.* (1988) reported both high incidence (>93%) and high levels ranging from 90–1200 µg/kg in some common drug plants. *Piper nigrum* with a concentration of 1200 µg/kg was the highest contamination level reported. The second highest reported value was in the seeds of *Mucuna prurita* at a level of 1160 µg/kg. The third highest value was 1130 µg/kg, which found in the roots of *Plumbago zeylanica* (Roy *et*

al., 1988). Aflatoxins were only found in 1 out of 5 Aerra lanata medicinal plant samples from Sri Lanka at 500 µg/kg (Abeywic *et al.*,1991). In another survey in India, 60% samples of medicinal plant seeds were contaminated with AFB1, ranging from 20 to 1180 µg/kg (Trucksess and Scott, 2008). In Thailand, five out of 28 herbal medicinal products were found to be contaminated with aflatoxins at 1.7–14.3 µg/kg using an immunoaffinity column (IAC) and high performance liquid chromatography (HPLC) method (Tassaneeyakul *et al*., 2004). None of the samples contained aflatoxins at levels above 20 ng/g (Tassaneeyakul *et al.*, 2004). In Malaysia and Indonesia, 16 of the 23 commercial traditional herbal medicines, jamu and makjun, analyzed using IAC/LC method contained a low level of total aflatoxins (0.36 µg/kg) (Ali *et al*., 2005). Romagnoli *et al.* (2007) analyzed aflatoxins in 27 aromatic herbs, 48 herbal infusions and medicinal plants using LC with post-column derivatization and fluorescence detection. They found no contamination with aflatoxins (Romagnoli *et al.*, 2007). In a study by Hitokoto *et al*. (1978) aflatoxins were not detected in the 49 powdered herbal drugs, Ten percent of the tablets of *Cascara sagrada* dried bark were contaminated with aflatoxins in Argentina.

In a study on garlic samples, no aflatoxins were found at levels >0.1 µg/kg. However, aflatoxin levels between 4.2-13.5 µg/kg were detected in ginger (Patel *et al.*, 1996).

A detailed UK study of aflatoxin contamination in some herbs and spices including curry powder, pepper, cayenne pepper, chilli, paprika, ginger, cinnamon and coriander showed 95% contamination below 10 µg/kg of total aflatoxins, while only 9 out of the 157 retail samples had higher levels (Macdonald & Castle, 1996). Study of ginseng root samples, both simulated wild and cultivated ones by D'Ov idio *et al*. (2006), showed approximately 15 µg/kg of total aflatoxins in only 2 of the simulated wild roots while none of the cultivated roots were contaminated with aflatoxins. Similar results (16 µg/kg) were found in just one mouldy ginseng root purchased from a grocery store (D'Ovidio *et al*., 2006). Trucksess and Scott (2008) found that 30% of the ginseng products purchased in USA was contaminated with AFB1 at levels of about 0.1 µg/kg. In an aflatoxin survey done in Turkey, 17.1% and 23.1% of unpacked and packed ground red peppers respectively, were contaminated with total aflatoxins and aflatoxin B1, with one out of the 82 samples over the legal limit (Set and Erkmen, 2010).

Table 5. Some of the highest values of aflatoxin contamination in the rejected lots of herbs & spices, based on The Rapid Alert System for Food and Feed (RASFF)

Raw	Origin	Commodity	Maximum Contamination Levels (µg/kg)		Date of case
1	India	Ground turmeric and whole nutmeg	700	1200	18/10/2010
2	Nigeria	Curry powder	570	1100	03/09/2008
3	Indonesia	Whole nutmeg	384.5	455.3	20/12/2007
4	India	Ground and broken nutmeg	230	249	03/09/2008
5	Peru	Dried paprika	216	221	23/12/2009
6	Ghana	Suya pepper	169	215.9	04/01/2008
7	Spain	Paprika powder	145.3	160.8	10/08/2010
8	United Kingdom with raw material from Spain	Paprika powder	120.3	135	11/2010
9	Indonesia	Nutmeg	120	140	03/12/2009
10	Spain	Nutmeg	98	105	04/04/2011
11	India	Nutmeg powder	79+/-24	97+/-29	21/01/2010
12	Indonesia	Nutmeg shrivels	57	-	26/10/2010
13	Indonesia	Ground nutmeg	56	70.5	19/08/2010
14	India	Ground nutmeg	50	58.2	27/04/2010
15	India	Turmeric powder	48	53	24/12/2010
16	India	Turmeric powder	48	52	29/04/2009
17	India	Chili powder	47.2	48.7	05/11/2010
18	India	Organic ground nutmeg	41.1	-	28/05/2010
19	India	Crushed chillies	38	40	27/08/2010
20	Pakistan	Chilli powder	30.3	32.1	16/08/2010
21	India	Clove powder	-	29	17/02/2009
22	India	Curry powder	26.4	27.4	14/07/2010
23	India	Chilli powder	24	25	31/08/2010
24	India	Dried red chilli	23	25	17/12/2010
25	China	Red pepper powder	22	26	09/07/2010
26	India	Dry whole chillies	20	21	24/11/2010
27	India	Ginger	13.2	24	19/04/2011

Source: (RASFF, 2011).

3. Aflatoxigenic Fungi

Aflatoxins are produced by four *Aspergillus* species. These include *Aspergillus flavus* Link ex Fr, *Aspergillus nomius* Kurtzman, Horn and Hesseltine, *Aspergillus parasiticus* Speare, and *Aspergillus tamarii* (Goto *et al*., 2013). The agronomically and economically most important aflatoxin producers are the closely related *A. flavus*, hence the name afla-toxin, and *A. parasiticus*. Both

species are soil-borne fungi that grow on living and decaying plant matter. These fungi produce aflatoxins on various commodities, but they are a concern on corn, groundnut and cottonseed. *A. flavus* can be distinguished from *A. parasiticus* by its smooth spores and yellow-green colonies on potato dextrose agar (PDA) medium. *A. parasiticus* produces dark yellow-green conidia with nearly spherical vesicles that produce roughened conidia. It can be readily distinguished from *A. flavus* by its rough-walled conidia (Goto *et al*., 2013).

Dominant aflatoxins produced by *A. flavus* are B_1 and B_2, whereas *A. parasiticus* produces two additional aflatoxins G_1 and G_2 (Goto *et al*., 2013). *A. flavus* of the section Flavi is the most common species involved in pre-harvest aflatoxin contamination of crops and causes aflaroot or yellow mould. *A. flavus* is the most common mycotoxin-producing fungus found in groundnuts; this is true across various climates and geographic regions.

Aflatoxigenic fungi are soil-borne imperfect filamentous fungi, which are saprophytic during most of their life cycle, and grow on wide variety of substrates, including decaying plant and animal debris. Two major factors that influence soil populations of these fungi are soil moisture and soil temperature. These storage fungi can grow at temperatures ranging from 12 to 48 ^{o}C, with optimum of 25 to 42 ºC, and at water potentials as low as -35 MPa. Under high soil temperatures and low moisture, which are associated with drought stress, these fungi become highly competitive and dominant, produce abundant inocula, and outcompete other microflora on corn, cotton, and groundnut (Goto *et al*., 2013).

Neither *A. flavus* nor *A. parasiticus* has a known sexual stage; they reproduce only by asexual means but undergo genetic recombination through a parasexual cycle. Morphology of the conidiophore, which bears asexual spores, is the most important taxonomic character in the identification of *Aspergillus*. Other important morphological structures used in identification are cleistothecia, hulle cells, and sclerotia (Bennett, 2013). These fungi can survive either as mycelium or as resistant structures known as sclerotia. *A. flavus* type fungi are genetically and phenotypically diverse. There are of two types, L isolates producing abundant conidiophores, large sclerotia, and variable amounts of aflatoxin, while the S isolates produce abundant, small sclerotia, fewer conidiophores, and high levels of aflatoxins.

Aflatoxigenic fungi are ubiquitous in nature and have important roles in natural ecosystems and human economy. *Aspergillus* species are capable of recycling starches, hemicelluloses, celluloses, pectins and other sugar polymers. Some species of *Aspergillus* degrade more refractory compounds, such as fats, oils, chitin and keratin. Maximum decomposition occurs in the presence of sufficient nitrogen, phosphorus and other essential inorganic nutrients. Foods utilized by humans and domestic animals are also good nutritional sources for *Aspergillus* species (Bennett, 2013).

3.1. Life Cycle of *Aspergillus* Species

Aspergillus flavus is a saprophytic fungus that survives on dead plant tissue and sometimes behaves as a weak and opportunistic pathogen (Yu *et al*., 2012). The sources of inocula for *A. flavus* and *A. parasiticus* are sporogenic sclerotia, conidia and mycelia that over-winter in plant debris (Scheidegger and Payne, 2012). In fields repeatedly cropped to groundnut or rotated between groundnut, maize and cotton, conidia from sporogenic sclerotia are the primary source of *A. flavus* inocula. Conidia adjacent to the developing groundnut pods germinate in the soil following the release of carbon and nitrogen substrates by injured groundnut pegs and result in colonization of the pods. Hot humid conditions favor the release of spores on plant residues, and these spores are dispersed by wind through the field (Scheidegger and Payne, 2012). Conidia that adhere to insect bodies are physically moved to plant parts and flowers in groundnut. Smaller, generally immature kernels are more easily infected in a shorter period of time than kernels in more mature pods. Infections of groundnut kernels at other maturity stages are relative to the survival of the fungus and not necessarily to a new infection at a later stage of maturity. *Aspergillus flavus* does not always establish a successful systemic infection in groundnut plants.

4. Methods of Detecting Aflatoxins

The above stated agricultural products were analyzed for aflatoxin contamination using ELISA test. A total of 300g from each sample was grind to a 1mm particle size using laboratory mill (Thomas- WILEY, LABORATORY MILL, Model 4. ARTHUR H. THOMAS Company PHILADELPHIA, PA., U.S.A.). About 100g of each sample was taken to Haramaya University Plant Sciences laboratory, Haramaya, Ethiopia, for aflatoxin analysis. Then 5g of groundnut flour from each sample was blended to 25 ml of 70% methanol. The extractions was done by mixing the solution with magnetic stirrer or soft shaking over a 10min period by flask shaker until the powder was thoroughly pulverized. The obtained solution was filtered through a Whatman No. 1 (Whatman International Ltd. Maidstone, UK) filter paper and 15 ml of distilled water and 0.25 ml of Tween20were added to 5ml of the filtered solution. The resulting suspension was then stirred for 2 minby vortex mixer. Immuno affinity column (RIDA® Aflatoxin column) (Art No: R5001, ArtNo: R5002, RBiopharm AG, Darmstadt, Germany) was used for sample clean up prior to analysis of aflatoxin. The columns were rinsed with 2ml of distilled water and filled with 1ml of the extracted samples solutions. The suitable adopter was attached on the top of column and syringe was used as sample reservoir. The samples passed slowly and continuously through the columns (approximately 1 drop/sec) and the syringes were filled with residual samples solutions. The passed solutions were discarded and the columns were rinsed by 10 ml of distilled water. This was repeated again and some air was pressed through the column and absorbed with light - 32 -vacuum (approximately 10 sec) to make sure that all the residual fluids were removed from the columns. Then the syringe was removed and placed on a clean and closable vial directly below the column and eluted with 0.5 ml of 100% methanol (methanol had to passed slowly through the column at flow rate of approximately 1 drop/sec) to ensure complete elution of the aflatoxins. All eluted residues were collected by pressing air thoroughly through the column to vials.

4.1. Aflatoxin Analysis by ELISA

Aflatoxin analysis was carried out using the ELISA (RIDASCREEN® Aflatoxin total Enzyme Immunoassay for the quantitative analysis of Aflatoxin R-Biopharm AG, Darmstadt, Germany) according to the manufacture's recommendation. Fifty µl of the standard solutions and prepared samples were transferred to each well of the micro-titer plate in duplicate. Then 50µl of enzyme conjugate and 50 µl of the antibody solution were added to each well, and the contents of the wells were mixed gently by shaking the platemanually. After incubation for 30 min at room temperature (20-25°C) in the dark, the wells were emptied by inverting the microwell holder upside down and tapping it vigorously against absorbent paper. This was followed by washing the wells with 250 µl washing buffer two times, 100µl of substrate (Chromogen) was added to each well, mixed gently by shaking the plate manually, and the reaction was stopped by adding 100µl of stop solution into each well aft er 15min incubation at room temperature in the dark. Finally, absorbance of each well was measured by ELISA reader (Multiscan Ex microplate photometer; Thermo Electron Corporation, Vantac, Finland) at 450 nm within 30 min after addition of stop solution. Toxin concentration was read directly from the standard curve.

5. Summary

Temperature, food substrate, strain of the mould and other environmental factors are some parameters that effect mycotoxin production. Preventing mycotoxin production at farm level is the best way to control mycotoxin contamination. Advances in molecular techniques and other decontamination methods such as gamma-irradiation and microwave heating could help to deal with these issues. Mycotoxins could be used as an energy source for a group of aerobic microorganisms, which are suitable to mycotoxin biodegradation. Several protocols have been provided to biodegrade mycotoxins in food and feed using potential bacteria such as *Lactobacillus* and *Bifidobacterium.*

However, there are varieties of responses between different microorganisms against mycotoxins. For example, *Bacillus brevis* were not affected by high concentrations of trichothecene. Application of microorganisms needs to be evaluated from a safety point of view. Application of microorganisms on mycotoxin degradation, food and feed materials also need to be investigated. Further studies need to be conducted to address the seasonal variation of aflatoxin contamination in food and feed. Understanding the seasonal variation could help demonstrate and develop more effective decontamination methods. For example, it is postulated that mycotoxin issues due to monsoons in Hungary could possibly be concluded to technical difficulties in pre- and post-harvest operations. Application of advanced methods such as DNA biosensors and infrared spectroscopy for rapid and accurate detection of mycotoxin and related fungi is increasing dramatically. Application of new and advanced detection techniques could enable the agricultural industry to deal more effectively with the occurrence of aflatoxin contamination.

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