ORIGINAL ARTICLE

Survey of Aflatoxin Contamination in Ethiopia

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ABSTRACT

Background: ***Aflatoxins are highly toxic, hepatocarcinogenic, secondary metabolites of Aspergillus species produced in most agricultural commodities stored at inappropriate temperatures and water activities. The aim of the present paper was to analyse the levels and frequency of aflatoxin contamination in samples of most commonly consumed agricultural commodities collected from various regions of the country.***

Methods: ***A total of 595 food samples collected from Southern Peoples Nations and Nationalities, Oromia and Harari Regional States were collected and screened for aflatoxin contamination. Commodities sampled included barley, wheat, maize, millet, sorghum, tef, pepper, peanut, broad beans and dry peas. Aflatoxins B_1 and G_1 were the only mycotoxins detected in the food samples.***

Results: ***Aflatoxin B_1 was the predominant form, the incidence of samples containing it was 30% and then accompanied by aflatoxinG_1, 6%. The highest levels of aflatoxin B_1 was observed in peanut and sorghum samples (738 and 692μgkg^{-1}, respectively). The highest level of aflatoxin G_1 found was 201μ gkg^{-1}. Groundnut, sorghum and millet samples have been identified as high-risk commodities based on the incidence rate of aflatoxin contamination. Levels of total aflatoxin greater than 20 μgkg^{-1}, were most frequently encountered in all aflatoxin positive samples of corn, sorghum, wheat, red pepper and peanut followed by barley (17%) and teff (13%).***

Conclusion: ***The presence of aflatoxins in commonly consumed foods emphasize a public health concern and the need to develop mycotoxin prevention and control strategies in Ethiopia.***

Keywords: Aflatoxin, fungi, mycotoxin, Aspergillus species

INTRODUCTION

Aflatoxins are secondary metabolites of the storage fungi *Aspergillus flavus* and *Aspergillus parasiticus* produced in most agricultural commodities stored at inappropriate temperatures and water activities. *A. parasiticus* and *A. flavus* are common and widely distributed in tropical and sub-tropical parts of the world.

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Commonly, the molds produce aflatoxins B_1, B_2, G_1 and G_2 (1).

Aflatoxins, basically difuranocoumarin compounds, are highly toxic and hepatocarcinogenic. The most commonly occurring aflatoxin, aflatoxin B_1, is the most toxic, a potent hepatocarcinogen as well as immunosuppressive. It is increasingly implicated in human and animal pathology (2). Primary liver cancer (PLC) is one of the leading causes of cancer mortality in Asia and Africa. Studies carried out in Kenya, Swaziland, Mozambique and Uganda, provide evidence for involvement of aflatoxin consumption in the causation of PLC (3).

It is also believed that there are synergistic effects between aflatoxins and hepatitis B virus infection causing primary liver cancer (4).

In many parts of Africa, human food staples exist which contain 10 to 30 times the recommended maximum and evidences from various parts of Africa have been produced suggesting that the level of constituents of local foods is directly related to the incidence of liver cancer in the various communities (5).

Fungal spoilage of stored commodities and aflatoxin production highly depends on several important factors including moisture content, relative humidity in the air, and temperature of the environment (6). Although the ideal temperature for mycotoxin production by many molds is in the range of 25 to 28°C, *A. flavus* is known to grow at temperatures as low as 10-15°C (7). However, constant temperature (25°C) is generally accepted as the temperature near the optimum for aflatoxin production (8). The activities of molds are also governed by the relative humidity of surrounding air and moisture content of stored products. There is fairly defined relationship between water content in the grain and relative humidity of the surrounding atmosphere. The food samples surveyed in the present study contained moisture equal to or slightly higher than the critical moisture content for safe storage of cereals and legumes (6). Storage fungi mostly species of *Aspergillus*, grow when agricultural commodities are stored with moisture contents above 13 or 14% (8).

Ethiopia, with its various agro-climatic regions, produces a variety of crops. Most of the produces are stored under poor and unsatisfactory storage conditions for considerable periods. Traditional storage structures in Ethiopia are usually made up of mud, bamboo strips and underground pits. In addition to these structures grains are stored in polyethylene and gunny bags. Extended storage under unsatisfactory storage conditions predisposes the produce to growth of storage fungi and productions of mycotoxins (6).

Several studies have reported increased rate of liver diseases and a significant degree of morbidity and mortality attributed to the diseases in Ethiopia. Primary hepatocellular carcinoma, the commonest malignancy seen in medical wards, is one of the forms of occurrences of liver diseases in Ethiopia (9). Although the aetiological agents were not elucidated, Coady (1976) however, indicated the possibility of causal association between primary hepatocellular carcinoma and consumption of aflatoxin contaminated diets in Ethiopia (10).

The problem of aflatoxin contamination of agricultural commodities in Ethiopia is much more serious than commonly visualized. The growth and proliferation of aflatoxigenic fungi depends on several factors including high temperature and humidity under which natural substrates are stored (6). In addition, particular social conditions and behavior including methods of preservation of food products and traditional feeding may play a significant role. In all these aspects, Ethiopia is considered to provide a favorable situation

for aflatoxigenic mold proliferation of agricultural commodities. Previous reports have been made of aflatoxin contamination in cereals and cereal products and spices taken from silos, warehouses, shops and market places in Addis Ababa (11-13).
The present paper however, reports the results of analysis on levels and frequency of aflatoxin contamination in samples of most commonly consumed agricultural commodities collected from various regions of the country.

MATERIALS AND METHODS

A total of 595 samples of cereal grains, legumes, oil seed and a spice were used for analysis. The food samples were collected randomly from warehouses, silos, bins, stores and foods offered for sale by grain retailers in the markets. Twenty-six locations in the Southern Peoples Nations and Nationalities, Oromiya and Harari Regional States were visited and samples of 10 varieties of the most commonly consumed agricultural commodities were collected. Commodities sampled included barley (26), wheat (30), maize (105), millet (42), sorghum (64), tef (71), pepper (96), peanut (74), broad beans (44) and dry peas (43). Observations were also made regarding the temperature of the environment and relative humidity in the air. All samples were drawn applying the sampling method of Bacha *et al.* (14), the number of samples collected depending on the size of the lot. Temperature and relative humidity of sample collection sites were measured using a portable Okaton Hygrothermograph (Cole-Parmer, Vernon Hills, IL., USA). Before taking the analytical sample, the samples were mixed thoroughly to achieve effective distribution of contaminated portions. One-two kg of the bulk samples was well ground and 100g of the flour served as the analytical sample for the analysis. Samples were analyzed to detect aflatoxins B_1, G_1, B_2, andG_2. Aflatoxin standards (aflatoxins B_1, B_2,G_1, and G_2) were obtained from Sigma Chemical Co. (St. Louis, Mo., USA) and thin layer chromatography plates ((20x20 cm, 0.25mm thickness, Silica Gel 60) were obtained from Merck (Darmstadt, Germany). Aflatoxin standards were prepared following the AOAC (Association of Official Analytical Chemists) Official Method 970.44 (15). ACS (American Chemical Society) grade chemicals or reagents were obtained from Sigma or Merck.

Aflatoxin analysis: Extraction, purification and separation techniques qualitative and quantitative determination of individual aflatoxins were carried following the AOAC Romer Minicolumn method (15). In brief, aflatoxins were extracted from the commodities by acetone: water (85:15,v/v), and interfering compounds were removed by adding cupric carbonate and ferric chloride gel. The aflatoxins were subsequently extracted from the aqueous phase with chloroform and the chloroform extract is then applied to the top of a minicolumn containing successive layers of neutral alumina (top), silica gel, and florosil (bottom), with calcium sulfate as drier at both ends. The column was developed with chloroform: acetone (9:1), and the aflatoxins are trapped as a tight band at the top of the florosil layer. The fluorescence was measured directly by inserting the minicolumn in the fuorotoxinmeter. Chemical confirmation of aflatoxins was achieved by spraying sulfuric acid: water solution (1:1, v/v) on TLC plates (16). For recovery studies, wheat samples just as with corn and sorghum samples were spiked with standard aflatoxins and processed as already described for food samples. All results were determined on a dry weight

basis and reported as the average value of assays of four samples.

RESULTS

Mean recoveries were 97% for aflatoxin B_1, 103% for aflatoxin G_1 and 96% for the other aflatoxins. The limits of detection of the method were 12μg kg^{-1} for aflatoxins B_1 and B_2 and 15μg kg^{-1} for aflatoxins G_1 andG_2

Table 1 shows geographical distribution of relative humidity in air, environmental temperature and moisture content of the food samples collected. There was less variation in the moisture content of samples with the exception of those samples collected from Jimma, Arbaminch and Bako where the highest temperature was recorded. There was also a positive correlation between relative humidity in air and moisture content of the collected food samples.

Table 1. Environmental temperature, relative humidity and moisture content of the grains. EHNRI, 1998

Sample Type	Locality	Temp °C	RH %	MC %	Sample type	Locality	Temp °C	RH %	MC %
Barley	Dabasso	21	65	13.5	Sorghum	Harar	22	54	13.0
	Assela	18	64	14.0		Alemaya	28	69	14.5
	Shirka	22	55	13.0		Asbe Teferi	26	56	13.0
Wheat	Assela	18	64	13.5		Nazareth	31	60	13.5
	Shirka	22	55	13.0	Peanut	Alemaya	28	69	15.0
Pepper	Shirka	22	55	13.0		Harar	22	54	13.0
	Mareko	18	66	14.0		Jimma	31	70	15.0
	Arbaminch	35	68	14.5		Babile	29	66	14.0
	Alaba	24	62	13.5		Dabasso	21	65	13.0
	Jimma	31	70	14.8	Millet	Bedele	24	67	14.0
	Bako	30	69	15.0		Woliso	25	68	14.0
Broad beans	Dedo	27	56	13.0		Gedo	24	66	14.0
						Ambo	25	69	14.5
	Gedo	24	66	14.0	Dry peas	Gedo	24	66	14.0
	Assendabo	23	68	14.5		Ambo	25	69	14.5
	Bedele	24	67	14.0		Bedele	24	67	14.0
	Dodolla	22	64	13.5		Assela	18	64	13.5
Maize	Shashamene	25	62	13.3	Teff	Ambo	25	69	14.5
	Yirgachaffe	26	60	13.1		Gedo	22	54	13.0
	Walaita					Jimma	30	70	15.0
	Sodo	26	62	13.3		Asendabo	23	68	14.5
	Dilla	27	64	13.9		Bedele	24	67	14.0
	Jimma	31	70	15.0		Nazareth	31	60	13.5.
	Bedele	24	67	14.0		AsbeTeferi	26	56	13.0
	Dedo	27	56	12.8					
	Aleta Wondo	22	57	13.0					

MC= moisture content food samples. RH= relative humidity of the bulk. Number food samples in bracket

Table 2. Occurrence of aflatoxins in cereal samples. EHNRI. 1998.

Sample type	Location	No. of samples	Aflatoxin type	Percent samples	Average μgkg^{-1}	Max μgkg^{-1}
Barley	Dabasso (10)	2	B_1	20	23	48
	Assela (8)	1	B_1	13	17	17
	Shirka (8)	3	B_1	38	32	61
Wheat	Assela (11)	4	B_1	36	42	75
	Shirka (9)	3	B_1	33	21	42
	Itaya (10)	2	B_1	20	19	23
Maize	Shashamene(15)	3	B_1	20	41	90
	Dila (16)	2	B_1	13	32	45
	Dedo(12)	3	B_1	25	48	55
	Jimma (10)	5	B_1	50	83	285
	Bedele (15)	4	B_1	27	58	123
	Aleta Wondo (10)	2	B_1	20	42	51
	Wolaita Sodo (12)	1	B_1	8	24	24
	Yirgachafe (15)	5	B_1	33	87	213
Millet	Bedele (7)	2	B_1	28.6	21	32
		2	G_1	28.6	12	18
	Woliso (10)	1	B_1	10	36	36
	Jimma (8)	4	B_1	50	47	203
		4	G_1	50	31	57
	Gedo (5)	1	B_1	20	31	31
	Bako (12)	2	B_1	16.7	45	53
Sorghum	Harar (20)	4	B_1	20	62	238
	Alemaya (15)	6	B_1	40	72	692
		6	G_1	40	32	76
	Asbe Teferi (18)	5	B_1	27.8	28	97
	Nazareth (11)	4	B_1	36.4	104	363
Teff	Ambo (8)	ND	ND	ND	ND	ND
	Gedo (7)	ND	ND	ND	ND	ND
	Jimma (12)	5	B_1	41.7	57	283
	Asandabo (14)	4	B_1	28.6	32	193
	Bedele (8)	2	B_1	25	17	28
	Nazareth (10)	3	B_1	30	63	114
	Asbe Teferi (12)	2	B_1	16.7	13	18

ND= Not detected. Number food samples in bracket

Table 2 indicates cereal grains according to their aflatoxin load at the time of collection. The aflatoxins found in cereals were identified as aflatoxin B_1 and aflatoxin G_1. The incidence of contaminated cereal samples were 25% for aflatoxin B_1. G_1 aflatoxin was detected in 12 of the 338 cereal samples analyzed. 2 and 4 from Jimma millet samples and 6 sorghum samples from Alemaya.

Mean aflatoxin B_1 concentrations in corn varied from 24 to 87µg kg^{-1} and from 17 to 32 µg kg^{-1} in the barley samples. As for the incidence of aflatoxin contamination in sorghum, 19 samples (29.7%) were contaminated with aflatoxin B_1. The maximal levels found in Alemaya sorghum were 692 µg kg^{-1} for aflatoxin B_1. On the other hand, of 20 Harar and 18 Asbe Teferi sorghum samples, 4 (20%) and 5 (28%) were contaminated with aflatoxin B_1 at a mean concentration of 62 and 28 µg kg^{-1}, respectively. Although the maximal level of aflatoxin B_1 was 363µg kg^{-1}, the mean aflatoxin value in the Nazareth sorghum was 104 µg kg^{-1}. The mean concentrations of aflatoxin B_1 in positive samples varied from 19 to 42 µg kg^{-1} for wheat and from 13 to 57 µg kg^{-1} for the teff samples. This toxin was however, absent in

Table 3. Occurrence of aflatoxins in legumes and pepper. EHNRI, 1998.

Sample type	Locality	No. of samples	Aflatoxin type	Percent Samples	Average µgkg^{-1}	Maximum
Pepper	Mareko (25)	4	B_1	16	26	133
		4	G_1	16	120	183
	Shirka (20)	3	B_1	15	68	95
	Arbaminch(25)	4	B_1	16	75	79
		4	G_1	16	32	85
	Alaba (10)	1	B_1	10	55	55
	Jimma (8)	3	B_1	37.5	73	220
	Bako (8)	2	B_1	25	45	61
Peanut	Alemaya (18)	7	B_1	38.9	205	738
	Jimma (8)	3	B_1	37.5	53	139
	Harar (11)	3	B_1	27.3	37	94
	Jimma (13)	6	B_1	46.2	183	493
		7	G_1	53.8	68	201
	Babile (16)	9	B_1	56.3	295	400
		8	G_1	50	116	133
	Dabaso (8)	2	B_1	25	115	145
		3	G_1	37.5	105	105
Broad beans	Dedo (10)	1	B_1	10	12	12
	Gedo (8)	ND	ND	ND	ND	ND
	Bedele (7)	2	B_1	29	27	30
	Asendabo (10)	3	B_1	30	36	88
	Dodolla (9)	1	B_1	11	29	29
Dry peas	Gedo (10)	ND	ND	ND	ND	ND
	Ambo (12)	1	B_1	8	37	37
	Bedele (8)	2	B_1	25	41	51
	Assela (13)	ND	ND	ND	ND	ND

ND= Not detected. Number food samples in bracket

the teff samples collected from Ambo and Gedo.

The natural occurrence of aflatoxins in red pepper and legume samples is summarized in Table 3. Aflatoxins were detected in 48 of the 74 peanut samples. Highest concentrations of aflatoxin B_1 in positive samples were detected in peanut samples from Alemaya, Jimma and Babile were 738, 493 and 400µg kg^{-1}, respectively. Aflatoxin G_1 however, was detected in peanut samples from Jimma, Babile and Dabasso with mean concentrations ranging from 68 to 116 µg kg^{-1}. Other mycotoxins were not detected in the peanut samples. Among the 96 pepper samples, aflatoxins B_1 and G_1 was found at the same incidence in both Mareko and Arbaminch samples. The mean concentrations of aflatoxin B_1 in positive sample ranges from 26 in Mareko to 75µg kg^{-1} in Arbaminch. The highest level (183µg kg^{-1}) of aflatoxin G_1 was recorded from Mareko pepper samples. Aflatoxin B_1 was detected at relatively low concentrations, mean values ranging from 12 to 41 µg kg^{-1} in only 10 of 87 legume samples. It was not found at all in Gedo and Assela legume samples.

DISCUSSION

In the present study, all food samples come from regions with temperatures ranging from 18°C to 31°C which supports the growth of *Aspergillus* species. Under tropical conditions, stored products are more susceptible to *Aspergillus* species than other fungi, as many *Aspergillus* species are favored by the combination of low water activity (a_w) and relatively high storage temperature (17). Water activity is numerically equal to the equilibrium relative humidity (ERH) expressed as a decimal. Studies elsewhere have also shown that at moisture contents of 8- 9% in oil seeds, 8-10% for cereal grains and 10-12% for wheat, fungi may grow and elaborate mycotoxins at normal tropical temperature (18). This is true for the storage fungi *A. flavus*, a xerophilic fungi, which are capable of growth at water activity (a_w) of a food or commodity 0.8 or below (17, 19).

Stored commodities always have reduced a_w, and the a_w should be less than 0.6 for them to be microbiologically stable. However, stored commodities are often moister than this, or there are damp pockets due to moisture migration, and xerophilic fungi are able to grow. The detection of aflatoxin B_1 in sorghum, peanut, and red pepper samples at relative humidity in the air of as low as 54% may be attributed to these factors or development of insects in the crops during storage. Even in dry grains, insect activity creates a moist microclimate within the infested kernels or cotyledon and facilitates growth of storage fungi and production of mycotoxins.

Although mycotoxins were found to be contaminating foods in Ethiopia, there were no specific regulations or detailed proposals on the control of mycotoxins in agricultural commodities. All the food samples in the present study come from parts of the country with a high humidity level and temperatures ranging from 18-31°C. This climatic factor potentiates hazards associated with aflatoxin production which leads to the view that the incidence of contamination in the humid regions would be greater. Thus, aflatoxins B_1 and G_1 were predominantly detected in food samples collected from places where temperature and humidity was high.

The detection of aflatoxin G_1 in conjunction with aflatoxin B_1 is not unusual. A biochemical distinction between isolates of the two species is that *A. parasiticus* produces aflatoxins B_1, B_2, G_1,G_2 and M_1 whereas *A. flavus* usually produces only B_1 and B_2 (20). Aflatoxin B_1 was detected in conjunction with aflatoxin

G_1 in the same positive samples of millet, sorghum, red pepper and peanut samples similar to other studies (9,11). The absence of G aflatoxins in other food samples indicated the presence of *A. flavus* in agreement with previous reports (20). On the other hand, in the case of teff samples collected from Gedo and Ambo, no mycotoxin contamination occurred. It was found only in 16 of the 56 teff samples collected from other locations, and essentially in 9 of the 30 wheat and 6 of the 26 barley samples. These cereals constitute the principal component of the traditional diet sourdough bread *Injera*. Earlier studies have shown that aflatoxins persist throughout the traditional fermentation processes of *Injera* preparation as practiced in Ethiopia (10-11).

Aflatoxin is a natural contaminant on legumes and retains remarkably high viability on whole seeds and in flour stored for up to 20 months (21). The highest mean total aflatoxin level which was 36μg kg^{-1} was found in broad beans from Asandabo whereas, in dry peas from Bedele it was 41μg kg^{-1}, respectively. Legumes including dry peas and broad beans are usually used to prepare the traditional vegetable sauce, *shiro wot* (9). While levels of aflatoxin produced on dry peas and broad beans may not be as great as those on peanuts or corn, the potential for human health hazards associated with improperly stored and processed legumes must be considered in overall handling systems followed in the home. It appears from this study that legumes are good substrates for aflatoxin production in agreement with previous studies (21).

Of greatest concern is the aflatoxin incidence at levels equal to or exceeding the maximum tolerated concentrations decided by countries like USA (20μg kg^{-1}) and UK (10μg kg^{-1}) in foods for human use (22). Total aflatoxin was found to occur mainly (90%) above the 20μg kg^{-1} tolerance level in cereal and legume samples. All aflatoxin positive peanut and red pepper samples however, exhibited concentrations greater than the tolerance limits. This high level of contamination was consistent with the findings previously reported elsewhere (10,11,18).

Although no direct evidence has implicated aflatoxins as the causal agents for primary hepatocellular carcinoma in Ethiopia, the above finding emphasize that the presence of aflatoxins in commonly eaten foods as a public health concern and aflatoxin contamination as a problem in the country. Since no agricultural commodity is absolutely immune to aflatoxin contamination, results of the present study will help identify sources of contamination and areas where control measures should be improved. Implementation of national prevention and control strategies like, proper post-harvest handling and good storage practices for food ingredients are required to reduce or eliminate contamination by the *Aspergillus* storage fungi.

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