

ORIGINAL ARTICLE**Survey of Aflatoxin Contamination in Ethiopia****Habtamu Fuffa, MSc¹ and Kelbessa Urga, MSc*, FUNU¹****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 B₁ and G₁ were the only mycotoxins detected in the food samples.*

Results: *Aflatoxin B₁ was the predominant form, the incidence of samples containing it was 30% and then accompanied by aflatoxin G₁, 6%. The highest levels of aflatoxin B₁ was observed in peanut and sorghum samples (738 and 692 µgkg⁻¹, respectively). The highest level of aflatoxin G₁ found was 201 µgkg⁻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 B₁, B₂, G₁ and G₂ (1).

Aflatoxins, basically difuranocoumarin compounds, are highly toxic and hepatocarcinogenic. The most commonly occurring aflatoxin, aflatoxin B₁, 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 B₁, G₁, B₂, and G₂. Aflatoxin standards (aflatoxins B₁, B₂, G₁, and G₂) 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 florisil (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 florisil layer. The fluorescence was measured directly by inserting the minicolumn in the fluorometer. 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 B₁, 103% for aflatoxin G₁ and 96% for the other aflatoxins. The limits of detection of the method were 12µg kg⁻¹ for aflatoxins B₁ and B₂ and 15µg kg⁻¹ for aflatoxins G₁ and G₂.

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	Peanut	Nazareth	31	60	13.5	
	Shirka	22	55	13.0		Alemaya	28	69	15.0	
Pepper	Shirka	22	55	13.0	Millet	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		Bedele	24	67	14.0	
Broad beans	Bako	30	69	15.0	Dry peas	Woliso	25	68	14.0	
	Dedo	27	56	13.0		Gedo	24	66	14.0	
	Gedo	24	66	14.0		Ambo	25	69	14.5	
	Assendabo	23	68	14.5		Gedo	24	66	14.0	
	Bedele	24	67	14.0		Ambo	25	69	14.5	
	Dodolla	22	64	13.5		Bedele	24	67	14.0	
	Maize	Shashamene	25	62		13.3	Teff	Assela	18	64
Yirgachaffe		26	60	13.1	Ambo	25		69	14.5	
Walaïta					Gedo	22		54	13.0	
Sodo		26	62	13.3	Jimma	30		70	15.0	
Dilla		27	64	13.9	Asendabo	23		68	14.5	
Jimma		31	70	15.0	Bedele	24		67	14.0	
Bedele		24	67	14.0	Nazareth	31		60	13.5	
Dedo		27	56	12.8	AsbeTeferi	26		56	13.0	
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
Millet	Yirgachafe (15)	5	B ₁	33	87	213
	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 B₁ and aflatoxin G₁. The incidence of contaminated cereal samples were 25% for aflatoxin B₁. G₁ 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 B₁ concentrations in corn varied from 24 to 87 µg 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 B₁. The maximal levels found in Alemaya sorghum were 692 µg kg⁻¹ for aflatoxin B₁. On the other hand, of 20 Harar and 18 Asbe Teferi sorghum samples, 4 (20%) and 5 (28%) were contaminated with aflatoxin B₁ at a mean concentration of 62 and 28 µg kg⁻¹, respectively. Although the maximal level of aflatoxin B₁ was 363 µg kg⁻¹, the mean aflatoxin value in the Nazareth sorghum was 104 µg kg⁻¹. The mean concentrations of aflatoxin B₁ 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
			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 B₁ in positive samples were detected in peanut samples from Alemaya, Jimma and Babile were 738, 493 and 400 µg kg⁻¹, respectively. Aflatoxin G₁ 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 B₁ and G₁ was found at the same incidence in both Mareko and Arbaminch samples. The mean concentrations of aflatoxin B₁ in positive sample ranges from 26 in Mareko to 75 µg kg⁻¹ in Arbaminch. The highest level (183 µg kg⁻¹) of aflatoxin G₁ was recorded from Mareko pepper samples. Aflatoxin B₁ 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 B₁ 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 B₁ and G₁ were predominantly detected in food samples collected from places where temperature and humidity was high.

The detection of aflatoxin G₁ in conjunction with aflatoxin B₁ is not unusual. A biochemical distinction between isolates of the two species is that *A. parasiticus* produces aflatoxins B₁, B₂, G₁, G₂ and M₁ whereas *A. flavus* usually produces only B₁ and B₂ (20). Aflatoxin B₁ was detected in conjunction with aflatoxin

G₁ 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 $36\mu\text{g kg}^{-1}$ was found in broad beans from Asandabo whereas, in dry peas from Bedele it was $41\mu\text{g kg}^{-1}$, 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 ($20\mu\text{g kg}^{-1}$) and UK ($10\mu\text{g kg}^{-1}$) in foods for human use (22). Total aflatoxin was found to occur mainly (90%) above the $20\mu\text{g kg}^{-1}$

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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