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Aspergillus and aflatoxin in groundnut (*Arachis hypogaea* L.) and groundnut cake in Eastern Ethiopia

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ABSTRACT

This study was conducted to assess major *Aspergillus* species and aflatoxins associated with groundnut seeds and cake in Eastern Ethiopia and evaluate growers' management practices. A total of 160 groundnut seed samples from farmers' stores and 50 groundnut cake samples from cafe and restaurants were collected. Fungal isolation was done from groundnut seed samples. *Aspergillus flavus* was the dominant species followed by *Aspergillus parasiticus*. Aflatoxin analyses of groundnut seed samples were performed using ultra performance liquid chromatography; 22.5% and 41.3% of samples were positive, with total aflatoxin concentrations of 786 and 3135 ng g^{-1} from 2013/2014 and 2014/2015 samples, respectively. The level of specific aflatoxin concentration varied between 0.1 and 2526 ng g^{-1} for B_2 and B_1, respectively. Among contaminated samples of groundnut cake, 68% exhibited aflatoxin concentration below 20 ng g^{-1}, while as high as 158 ng g^{-1} aflatoxin B_1 was recorded. The study confirms high contamination of groundnut products in East Ethiopia.

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Introduction

Groundnut (*Arachis hypogaea* L.) is one of the most important food and oil crops cultivated and utilised in most parts of the world as annual legume (Gibbons et al. 2002). In Ethiopia, groundnut is one of the five widely cultivated oilseed crops (Wijnands et al. 2009), and the total production area and yield of groundnut are estimated at 49,603 ha and 71,606.8 t, respectively (CSA 2011). Eastern and Western Hararghe zones of Oromiya region, especially low land areas have the highest groundnut production.

Pre- and postharvest infection by storage moulds and subsequent mycotoxin accumulation in groundnut are serious problems in the tropical hot and humid areas of the world (Savage & Keenan 1994). The mycotoxins produced by these moulds are toxigenic contaminants of food and feeds that are frequently responsible for health and economic concerns in many countries (Bhatnagar et al. 2003). Aflatoxins are a group of naturally occurring highly carcinogenic compounds which are mainly produced by *Aspergillus flavus, Aspergillus parasiticus* and *Aspergillus nomius* (Strosnider et al. 2006). The most abundant and important are aflatoxin B_1, B_2, G_1 and G_2. Among those, Aflatoxin B_1 (AFB_1) is the most toxic of the aflatoxins known in nature (IARC 1993). Consumption of aflatoxin-contaminated food affects human health causing aflatoxicosis, cancer, stunted growth in children and immune suppression among others (Shephard 2008). The worst aflatoxicosis outbreak recorded in the world occurred in Kenya in 2004, where 317 cases of aflatoxin poisoning and 125 deaths were reported (CDC 2004). Other documented fatal aflatoxicosis outbreaks reported in Kenya are 20 cases in 1981 (Ngindu et al. 1982); 80 cases and 30 deaths in 2005 (Lewis et al. 2005) and 9 deaths in 2006 (Wagacha & Muthomi 2008). Even though there are no documented cases of aflatoxicosis in Ethiopia, this does not mean that there is no disease or mortality, as clinical diagnostic and post-mortem examination are not common practices.

One of the pioneering efforts in the survey of aflatoxin content of agricultural and food products in Ethiopia reported mean levels of aflatoxin (AFB_1) of 34.7 and 105 ng g^{-1} from samples of groundnut and peanut butter, respectively (Besrat & Gebre 1981). Later Ayalew et al. (1995) revealed that 85% of *A. flavus* isolated from groundnuts in East Ethiopia were able to produce aflatox-

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ins in a range of 1–>300 ng g^{-1} in liquid medium. Recently, Chala et al. (2013) reported values near 12,000 ng g^{-1} total aflatoxins in stored groundnut seeds in Babile district, which are extremely high compared to the 4 ng g^{-1} limit set for the European Union (OJEU 2010). No information about aflatoxin concentration in the groundnut food product "*Halawa*" (local cake) from Ethiopia has been documented thus far. Groundnuts that are used as raw materials for groundnut cakes or food products are prone to colonisation by *Aspergillus* species along the value chain and this leads to subsequent aflatoxin accumulation and exposure of consumers to health risks (Okun et al. 2015). In eastern and lowland parts of Ethiopia, where the bulk of groundnut is produced (Kebede & Tana 2014), there is limited research work on specific aflatoxin contamination of groundnut and food products. In the current study, assessment of major *Aspergillus* species and the levels of aflatoxin B_1, B_2, G_1 and G_2 from groundnut seeds and groundnut cake "*Halawa*" from different agro-ecological areas of Eastern Ethiopia were investigated. Also, growers were interviewed about cultural and storage practices that may be promoting aflatoxin contaminations in groundnut seeds. Establishing such data is of paramount importance for all of the stakeholders, from growers to consumers.

Materials and methods

Survey and sampling of groundnut seed and food product "Halawa" (local cake)

A survey was undertaken in four important groundnut production districts of Eastern Ethiopia, three from East Hararghe, namely Babile, Fedis and Gursum, and one, Darolabu, from West Hararghe during the 2013/2014 and 2014/2015 cropping seasons. The survey covered a total of 16 farmers associations (FAs) and 160 farmers. During each season survey, five different groundnut samples (500 g each) were collected from each of the four selected FAs of each district that gave a total of 20 samples ($4 \times 5 = 20$) per district per season, then totally 80 ($20 \times 4 = 80$) samples per cropping season. This made the total number of groundnut samples collected 160 ($2 \times 80 = 160$) over the 2 years period. The collected samples were labelled and stored at 4°C until analysis. Each sample was uniformly blended and representative of 100 g was removed from a total of 500 g, for mycological and aflatoxin analysis (50 g for each experiment). Additionally, the physical condition of each sample was observed and moisture content was tested. Background information of the areas is provided in Table 1. During survey and sample collections, questionnaires were prepared to assess management practices with emphasis to postharvest conditions of the growers.

Table 1. Sample collection sites.

District	Latitude (*N*)	Longitude (E)	Altitude (m.a.s.l)	Rainfall (mL)	Temperature (°C)
Babile	09°11′933″	042°18′049″	1642	450–600	24–28
Darolabu	08°26′078″	040°20′388″	1750	636–963	14–26
Fedis	09°8′22″	042°2′53″	1664	604–926.5	8.8–27.8
Gursum	09°37′268″	042°43′951″	1694	650–850	14–24

m.a.s.l.: Metres above sea level.
Source: Agricultural offices of the districts and Mersha Belete (2014).

Table 2. Moisture content (%) of groundnut samples collected from survey areas.

	2013/2014		2014/2015	
District	Mean	Range	Mean	Range
Babile	8.6	7.1–10.7	8.3	6.5–10.2
Darolabu	7.8	6.3–9.8	7.1	5.3–8.3
Fedis	4.3	3.1–5.6	4.6	3.4–6.3
Gursum	6.8	4.1–7.4	6.5	4.6–8.4

Additionally, a total of 50 (20 g each) samples of groundnut food product "*Halawa*" (local cake) were collected from eastern parts of Ethiopia, Babile, Dire Dawa, Haramaya, Harar and Jijiga city. Both groundnut seed samples and "*Halawa*" (local cakes) were mailed to USDA-ARS-NPRL, Dawson, GA, USA for further laboratory analysis. Moisture content of each sample was measured using BURROWS DMC 500 instrument (Miami, FL, USA) by adjusting the operation to the specific crop (groundnut) according to the manufacturer's guidelines. Then 50 g groundnut seeds per sample were tested in triplicate and the mean values were calculated (Table 2).

Isolation of Aspergillus spp. from groundnut seeds

Groundnut seed samples were diagnosed for fungal contamination with emphasis on the prevalence of *Aspergillus* species. Medium of modified dichloran rose Bengal (MDRB) with a composition of 10 g of glucose (dextrose), 2.5 g of peptone, 1.0 g of KH_2PO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of yeast extract, 0.5 ml of Rose Bengal stock solution (5 g Rose Bengal and 100 mL water), 20 g of Agar and a final volume of 1.0 L distilled water by adjusting pH to 5 using hydrochloric acid (HCl) and autoclaved for 20 min at 121°C, was prepared according to Horn and Dorner (1998). The pH of the medium was adjusted to 5 using HCL. Antibiotics, Streptomycin, Tetracycline, and dichloran (Botran) were added to a final concentration of 30, 0.15 and 0.8 mg L^{-1}, respectively, after cooling the medium to 50°C. Plates were poured using a WHEATON/Omnispense® (ELITE, Millville, NJ, USA) 26 mL per plate on 90-mm petri-dishes. Twenty grammes of groundnut seeds were weighed from each ground sample and suspended to 50 mL Falcon™ tubes

round-bottom with 25 mL of sterile distilled water. The suspension was mixed in a KLECO pulveriser (Visalia, CA, USA) for 1 min. From each sample, 50 and 100 µL suspension were spread plated on MDRB medium in a laminar flow hood, and then incubated at 37°C for 72 h. After incubation, spores from individual colonies of *Aspergillus* section Flavi were transferred to fresh MDRB plates using a sterile needle, and then streak isolated using a sterile loop to obtain separate individual colonies. Plates were incubated at 30°C for 72 h, and then small pieces of agar containing hyphal tips were transferred to Czapek medium slant prepared according to Horn et al. (1996).

Identification of Aspergillus *species*

Aspergillus species were identified after growing the pure cultures for 10–14 days on Czapek Dox Agar (OXOID Ltd, Hampshire, England) slants at 30°C. Morphological characteristics, microscopic and macroscopic examinations were performed according to Diba et al. (2007). Further, all isolated *Aspergillus* fungal species were identified to the species level using taxonomic systems of *Aspergillus* by Klich (2002) and confirmation was done by comparison with reference cultures of Dr Bruce Horn's collection (USDA National Peanut Research Laboratory, Dawson, GA, USA). Pure cultures of isolates representing each species as preserved in silica gel at the USDA National Peanut Research Laboratory, Dawson, GA, USA.

Aflatoxin analysis

Clean-up column preparation

Polypropylene columns measuring 1.5 mL with two matching polyethylene porous (20 µm) frits (Alltech, Deerfield, IL, USA) were packed with 200-mg basic aluminium oxide (40 µm flash; Scientific Adsorbent, Inc, Atlanta, GA, USA) according to Sobolev and Dorner (2002).

Aflatoxin extractions and detection

Fifty grammes of groundnut seed and 100 mL of methanol/distilled water (80/20, v/v, respectively) were blended at high speed for 1 min in a glass blender jar (Waring Products Div., Torrington, CT, USA) according to the procedure developed by Sobolev and Dorner (2002). A prepleated filter paper Whatman No. 4 was inserted in the mixture, 500 µL of filtrate was transferred to a disposable glass test tube followed by addition of 500 µL of acetonitrile to the same tubes and mixed thoroughly. Then, 500 µL of the mixture were pipetted into the 1.5-mL columns prepared for cleaning. The eluate containing aflatoxins was collected into 500 µL-ultra performance liquid chromatography (UPLC) glass vials and immediately closed with caps with septa (Arias et al. 2015). The limit of detection for aflatoxins was 1.0 ng g^{-1} for B_1 and G_1, and 0.05 ng g^{-1} for aflatoxin B_2 and G_2. Aflatoxin standards of B_1, B_2, G_1 and G_2 were obtained from (Sigma Chemical Co., St. Louis, MO, USA). Stock and spike solutions of each aflatoxin B_1, B_2, G_1 and G_2 were prepared according to the protocol developed by Sobolev and Dorner (2002).

Aflatoxin detection was done by UPLC Acquity, using column ACQUITY UPLC® BEH C18 1.7 µm 2.1 × 50 mm (Waters, Milford, MA, USA), at a temperature of 40°C, and with fluorescent detection. Mobile phase was methanol/water/acetonitrile (20/70/10%, v/v/v, respectively) and flow rate of 0.25 mL/min with injection volume of 1 µL. All instrument control, analysis and data processing were performed via Waters® Empower 3® Chromatography Data Software. Concentrations of aflatoxins were calculated by reference to calibration curves obtained by injecting different amounts of corresponding commercial standards of aflatoxins B_1, B_2, G_1 and G_2 as suggested by the UPLC manufacturer and determined by the Empower software. Aflatoxin concentration was calculated as ng g^{-1} which is equivalent to (1 in 10^9) ppb. Aflatoxin extraction from groundnut cake "*Halawa*" was done by blending 20 g of sample in 40 mL of methanol/distilled water (80/20, v/v) for 1 min. Cleaning up by using column and detection by UPLC was done following same procedures as described above for groundnut seeds.

Data analysis

Frequency of *Aspergillus* species and contaminated samples per district and FAs were recorded to determine the proportions of infected and non-infected samples. *Aspergillus* species distributions across the geographical area were computed and statistical analysis by general linear models for mean comparison was done by SAS (2002) for Windows 9 (SAS Institute Inc., Cary, NC, USA). Concentration of aflatoxin B_1, B_2, G_1 and G_2 were recorded and computed as ng g^{-1}.

Results and discussion

Moisture content of groundnut seeds

FAO (2002) indicated that 7.5% moisture content is the best storage condition for groundnuts seeds. Though the average moisture content of farmer stock groundnut is usually 8–9% when graded, under typical weather conditions it equilibrates to 5–7% moisture content (APSA 2013). In the current study, the average recorded moisture content from samples obtained from Babile was

8.5%, while the rest of samples were around 7.5% or lower (Table 2). It is known that in a 3-day period a 10-fold increase in aflatoxin contamination by *Aspergillus* can occur when seeds are stored with high moisture contents (Hell et al. 2008).

Aspergillus *species associated with groundnut seeds*

Aspergillus species were present across the surveyed districts although the level of contamination varied with location (Table 3). During the 2013/2014 cropping season, the proportion of infested samples ranged from 10% (Fedis) to 100% (Babile), whereas in 2014/2015 *Aspergillus* presence decreased in this order: Darolabu, Babile, Fedis and Gursum district. Groundnut seed moisture was twice as high in samples collected from Babile (8.5%) than those from Fedis (4.5%). Mohammed and Chala (2014) reported higher level of contamination of groundnut samples from Babile and Gursum.

The distribution of *Aspergillus* species contaminating groundnut seeds was significantly different among districts in 2013/2014 than 2014/2015, with the highest presence of *A. flavus* found in Babile ($p \leq 0.05$) (Table 4). It might be because of several factors like background of the areas and management practices performed by the growers or genetic variations among crops of different districts. Result of moisture contents shows variation between samples of different areas. Awuah and Ellis (2002) reported groundnuts dried to 6.6% moisture levels are free of fungi for 6 months regardless of the storage protection used, and it supports our finding. These safe moisture levels are applicable to both unshelled and shelled groundnuts. This study supports our results obtained from Fedis district, where the average moisture content was 4.5% from and relatively less infected (47.5%) while the maximum was 95% from Babile and Darolabu districts. Babile district had low altitude coupled with minimum rainfall (525 mL per year) and high moisture content of samples compared to those three districts. Subsequently, maximum fungal contamination of samples and aflatoxin concentrations were recorded from the same district (Tables 4 and 5).

Table 3. Incidences of *Aspergillus* contamination of groundnut samples.

	2013/2014		2014/2015	
District	Infected	Non-infected	Infected	Non-infected
Babile	20 (100.0)	0 (0.0)	18 (90.0)	2 (10.0)
Darolabu	18 (90.0)	2 (10.0)	20 (100.0)	0 (0.0)
Fedis	2 (10.0)	18 (90.0)	17 (85.0)	3 (15.0)
Gursum	14 (70.0)	6 (30.0)	14 (70.0)	6 (30.0)
Total	54 (67.5)	26 (32.5)	69 (86.3)	11 (13.8)

Numbers in brackets show percentage of infected samples.

The recorded *Aspergillus* species were *A. flavus, A. parasiticus, A. flavus* S strain, *A. flavus* L strains, *Aspergillus caelatus, Aspergillus niger, Aspergillus tamarii* and *Aspergillus ochraceus*. One species obtained from Fedis district was unknown; its spores are beige. *A. flavus* ranked first and was followed by *A. parasiticus*, while *A. ochraceus* and *A. caelatus* were each observed only in one season. Some of these species, including L and S strains of *A. flavus*, have been reported in Kenya by Wagacha et al. (2013); in this survey, *A. flavus* L and S strain morpho-types, and *A. tamarii* were found in Ethiopian groundnut.

Aflatoxin in groundnut seeds

In the current study among collected samples, 22.5% (18) and 41.3% (33) of 2013/2014 and 2014/2015, respectively, were found to be contaminated by aflatoxin. Aflatoxin G types were not detected in samples from Babile district. Therefore, this study indicates that

Table 4. Average *Aspergillus* spp. cultures found in groundnut seeds across four districts of Eastern Ethiopia on crop seasons 2013/2014 and 2014/2015 (n = 20 samples per district per crop season). Mean comparison by Duncan test.

District	*A.flavus* (*N.s)	*A.flavus* L-strain	*A.flavus* S-strains	*A.parasiticus*	*A.caelatus*	*A.tamarii*	*A.ochraceus*	*A.niger*	CV (%)
2013/2014									
Babile	7.8^{a}	0.5^{a}	0.5^{ab}	2.75^{a}	0.25^{b}	0.0^{a}	0.0^{a}	0.0^{a}	51.6
Darolabu	2.5^{bc}	0.75^{a}	2.0^{a}	2.5^{a}	1.5^{a}	1.25^{a}	0.0^{a}	1.0^{a}	19.9
Fedis	0.8^{c}	0.0^{a}	0.0^{a}	0.5^{b}	0.0^{b}	0.0^{a}	0.0^{a}	0.0^{a}	12.3
Gursum	3.5^{b}	1.0^{a}	1.3^{ab}	1.3^{ab}	0.0^{b}	0.0^{a}	0.0^{a}	0.25^{a}	8.9
LSD (0.05)	2.6	1.6	1.6	1.6	1.1	1.5	0	1.7	
2014/2015									
Babile	4.3^{a}	0.0^{a}	2.5^{a}	2.0^{a}	0.0^{a}	0.0^{b}	0.0^{a}	2.0^{a}	51.6
Darolabu	4.8^{a}	0.0^{a}	2.3^{a}	4.5^{a}	0.0^{a}	0.0^{b}	0.3^{a}	2.5^{a}	20
Fedis	2.3^{a}	0.8^{a}	1.0^{a}	3.3^{a}	0.0^{a}	0.5^{a}	0.0^{a}	1.5^{a}	12.3
Gursum	3.0^{a}	0.3^{a}	2.8^{a}	3.8^{a}	0.0^{a}	0.0^{b}	0.0^{a}	2.3^{a}	8.9
LSD (0.05)	2.6	0.9	2.2	4.9	0	0.5	0.4	2.4	

Means in a column followed by the same letter are not significantly different according to LSD at $p \leq 0.05$.
*N.s = Non-sclerotia.

Table 5. Proportion of positive samples for specific aflatoxins and concentration range in both crop seasons (N = 20 samples per district per season).

		2013/2014		2014/2015	
Districts	Aflatoxin	% Positive samples	Levels (ng g^{-1})	% positive samples	Levels (ng g^{-1})
Babile	B_1	45	1.8–723	30	7.1–2526
	B_2	50	0.1–63.5	30	0.3–113
	G_1	<LOD	<LOD	<LOD	<LOD
	G_2	<LOD	<LOD	<LOD	<LOD
Darolabu	B_1	10	4.5–80.2	60	1.6–1358
	B_2	10	0.5–18.8	60	0.2–195
	G_1	7.5	5.99–61.9	20	199–736
	G_2	8.8	0.4–8.2	20	15.7–171
Fedis	B_1	<LOD	<LOD	45	1.7–2368
	B_2	<LOD	<LOD	45	0.3–237
	G_1	<LOD	<LOD	10	238–477
	G_2	<LOD	<LOD	10	53.0–96.9
Gursum	B_1	<LOD	<LOD	25	4.5–682
	B_2	<LOD	<LOD	25	0.1–35.5
	G_1	<LOD	<LOD	5	187.1
	G_2	<LOD	<LOD	5	14.9

LOD: Limit of detection.

Aspergillus species isolated from samples from Babile district were most probably contaminated by aflatoxigenic strains of *A. flavus* or non-toxigenic strains of *A. parasiticus*. Whereas from the rest of three districts, Darolabu, Fedis and Gursum, both G and B types of aflatoxins were detected from groundnut seed (Tables 5 and 6). Such results confirm infection of groundnut seeds with toxigenic *A. flavus* and *A. parasiticus* strains in these areas.

In the current study, the extremely high levels of AFB_1 that greatly exceed the maximum limits set by European Union, 2 ng g^{-1} (OJEU 2010) was recorded in samples from Babile district (Table 6). This shows that the aflatoxin contamination of groundnut seed in Eastern Ethiopia is at critical levels to cause health and economic threat to growers and consumers. Those samples were collected from farmers' stores intended for home consumption or local markets. Previously, Chala et al. (2013) had detected a total aflatoxin concentration up to 12,000 ng g^{-1} from the same district. The researchers reported that the level of aflatoxin concentration associated with groundnut seeds in Babile district was higher than the tolerable levels for human consumption in other countries, which range from 2 to 20 ng g^{-1}. Ayalew et al. (1995) reported 85% infection by *A. flavus* that was able to produce aflatoxins in a range of 1–>300 ng g^{-1} in liquid medium isolated from groundnuts in East Ethiopia. In this study, in both years among all samples, of AFB_1 was predominant; the research was consistent with the finding of Kamika et al. (2014) which reports AFB_1 was the analogue with the highest concentration among samples from both Kinshasa and Pretoria.

The maximum limit set by the European Union for total aflatoxin and for AFB_1 in groundnut is 4 and 2 ng g^{-1} (CEC 2006), while the corresponding limits (total aflatoxin and for AFB_1) by the Kenya Bureau of Standards are 10 and 5 ng g^{-1}, respectively (KEBS 2007). However, there is no such standard in Ethiopia. This suggested the urgent need to set standards based on scientific evidence and risk assessment. In addition, regulatory measures should be put in place to avoid/lessen health risks to the consumers as a result of selling heavily contaminated crop.

Table 6. Proportions of samples with different levels of aflatoxin contamination.

		2013/2014				2014/2015			
Sample sources	AF (ng g^{-1})	<4	4–20	20–100	>100	<4	4–20	20–100	>100
Groundnut seed samples									
Babile (20)	B_1	5	15	10	15	0	5	0	25
	B_2	35	5	10	0	5	15	5	5
Darolabu (20)	B_1	0	15	15	0	10	10	10	30
	B_2	20	10	0	0	25	20	10	5
	G_1	0	15	5	0	0	0	0	20
	G_2	20	5	0	0	0	10	0	10
Fedis (20)	B_1	0	0	0	0	5	10	20	10
	B_2	0	0	0	0	20	10	5	10
	G_1	0	0	0	0	0	0	0	10
	G_2	0	0	0	0	0	0	10	0
Gursum (20)	B_1	0	0	0	0	0	15	0	10
	B_2	0	0	0	0	15	0	10	0
	G_1	0	0	0	0	0	0	0	5
	G_2	0	0	0	0	0	5	0	0
Groundnut cake "*Halawa*" (2014/2015)									
		<4	4–20					20–100	>100
Babile Town (10)	B_1	20	20					30	0
	B_2	60	10					0	0
Dire Dawa city (10)	B_1	20	10					30	20
	B_2	60	20					0	0
Jijiga City (10)	B_1	10	90					0	0
	B_2	100	0					0	0

Aflatoxin in groundnut cake "Halawa"

Since the raw groundnut seeds used for food products are vulnerable to contamination by *Aspergillus* species in all of the production and processing stages (Kaaya et al. 2006), it requires measuring the levels of aflatoxin in "*Halawa*", locally made cakes from groundnut seeds. In the current study, 50 groundnut cake "*Halawa*" samples were collected from Eastern Ethiopia. Among those samples, 50% (25) of them were contaminated by AFB_1 and B_2 with levels ranging from undetected to 158.1 ng g^{-1}. Some cakes were also found to be free of aflatoxin contamination (Table 7). The levels and ranges of concentration from groundnut cake samples from each area were mentioned below (Table 6). Only aflatoxin B types were detected from groundnut cake and neither of G type was detected. This indicates that aflatoxin B type has capability to resist the temperature of cake making, and aflatoxin G type could have been eliminated by food processing temperature.

In Ethiopia, there is limited research on the contamination of groundnut-based products by aflatoxins. The first report by Besrat and Gebre (1981) revealed contamination of groundnut and groundnut butter by AFB_1 at levels of 34.7 and 105 ng g^{-1}, respectively. Another report by Younis and Kamal (2003) from Sudan showed that the levels of aflatoxin concentration in groundnut butter and groundnut cakes ranged from 32 to 54 ng g^{-1} and from 7 to 10 ng g^{-1}, respectively. In the current study, the maximum AFB_1 level found was 158.1 ng g^{-1} (Table 7) in groundnut cake "*Halawa*" collected from Eastern Ethiopia, which was 15 times the value of the Sudan samples. However, Kayode et al. (2013) reported maximum aflatoxin concentration of AFB_1 and B_2 1041 ng g^{-1} from groundnut snack in Nigeria, which is much higher than the results obtained from Ethiopia in this study.

The factors of aflatoxin contamination in food products include seed contamination. So, a fundamental distinction must be made between aflatoxin formation in crops before or after harvest and that of aflatoxin occurrence in stored commodities or foods. Therefore, prevention of the formation of aflatoxins mainly relies on avoidance of contamination after harvest, using rapid drying and good storage practise, followed by improvement on buying of healthy seeds and sorting out shrivelled and discoloured seeds from the healthy groundnut seeds.

Table 7. Aflatoxin in samples from groundnut food product "*Halawa*" (local cake) from Eastern Ethiopia (N = 10 samples per city or town).

Sample sources	Aflatoxin	% positive samples	Levels (ng g^{-1})
Babile town	B_1	70	0.7–39.1
	B_2	70	0.2–4.6
Dire Dawa city	B_1	80	1.5–158.1
	B_2	80	0.2–15.3
Haramaya town	B_1	<LOD	<LOD
	B_2	<LOD	<LOD
Harar city	B_1	<LOD	<LOD
	B_2	<LOD	<LOD
Jijiga city	B_1	100	2.8–9.4
	B_2	100	0.2–0.8

LOD: Limit of detection.

Growers' management practices and Aspergillus *fungi contaminating groundnut seed*

During sample collections, all the interviewed farmers (100%) grew local varieties, mainly Sartu and Oldhale. The common local groundnut varies in Eastern Ethiopia are "Oldhale", "Sartu" and "Jawsi" (Tefera & Tana 2002; Mohammed & Chala 2014). Eighty five per cent of the farmers have awareness of mould fungi associated with groundnuts; despite they did not properly manage the crops. The common storage materials around the survey areas are polyethylene bags or sacks, 58% of growers store their products in old sacks which could have contained contaminated seeds.

About 78% of interviewed farmers did not properly dry their harvested groundnut and lacked awareness about increasing fungal invasion in relation to improper drying and storage methods. Though 100% of respondents claim harvesting is done at optimum maturity of the crop, this depends on labour availability. Up to 37% of growers use pieces of wood to strike the pods during shelling; this causes mechanical damage of seeds which increases mould invasion. Chala et al. (2014) reported about 30% of the groundnut growers in Babile district threshed groundnuts with sticks. Mechanical damage during threshing groundnut makes them much more vulnerable to the invasion by moulds and subsequent aflatoxin contamination (Heathcote & Hibbert 1978). About 55% of growers did not remove the uncoloured, shrivelled, immature or broken seeds and stored entire pods in the same sack. Among interviewed farmers', 77% added water to make pod soft during shelling, on top of that 94% of farmers leaking water at the time of selling to make it heavy as the commodity is sold by weight. A total of 54% samples had mould, insect scales and visible infections observed during sample collection. Scientist revealed that insect damage of groundnut pods creates a portal for the fungus invasions and aflatoxin accumulation in groundnut (Waliyar et al. 2008).

Apart from biological and physical factors, farmers' practices that lead to contamination with *Aspergillus* fungi and aflatoxin accumulation include use of damaged and shrivelled kernels as seed for planting 94%. Several researchers have shown correlation between the improper post-harvest storage/handling and mould development and aflatoxin accumulation (Graham 1982; Hill

et al. 1984). ICAR (2002) revealed the lack of awareness and actual skills on groundnut post-harvest technologies have caused significant losses starting from harvesting to curing, drying and storage. Therefore, aflatoxin management should start in farmers' fields, though creating awareness both for growers and extension workers, with proper crop management and handling, post-harvest storage, followed by marketing and processing conditions need priority in Eastern Ethiopia.

Summary and conclusion

Eastern part of Ethiopia is the leading groundnut producing area in the country and consumption is also high in the region. However, several fungal species do constrain the production and productivity of the crop in all of the crop value chain from fields to forks and from threshing floor to consumers. In the current study, *Aspergillus* species like *A. flavus* that included L and S morphotypes, *A. parasiticus, A. caelatus, A. niger, A. tamarii* and *A. ochraceus* were isolated from groundnut seed samples collected from Eastern Ethiopia. In both cropping seasons (i.e. 2013/2014 and 2014/2015), occurrence of *A. flavus* ranked first and followed by *A. parasiticus*.

The level of specific aflatoxin concentration was measured from groundnut seeds based on farmers' stores and the groundnut food product "*Halawa*" (local cake). The obtained results were extreme values in both seeds and cakes compared to the international acceptable levels. Therefore, these aflatoxin findings imply an urgent call for management of aflatoxin associated to groundnut crops in Eastern Ethiopia and the Ministry of Health has to give due attention for the expected problems and health risks due to *Aspergillus* species and aflatoxin contamination. In case of aflatoxin contamination to groundnut cake "*Halawa*", the producing factories should be bearing the primary responsibility to buy only sound seeds and segregation of discoloured or shrivelled seeds. Subsequently, to improve the marketing potential of their products and create safety for consumers; simultaneously, there is a requirement for action towards quality control organisation among the food factories.

Many countries have set maximum levels or limits for potent mycotoxins in foods and feed stuffs, especially AFB_1 and/or total aflatoxin in food products. Otherwise, infection of plant products by toxigenic fungi and associated mycotoxins would become a major impediment to the global exchange/trade of plants and plant products. In Ethiopia, however, there is lack of adaptation to such technologies for domestic food grains; even there is a report of aflatoxin contamination from various food commodities. So, such a study serves as a springboard and can be considered as a benchmark to generate data on specific aflatoxin concentrations to be used by growers, traders, food factories and policy makers.

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