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Prevalence of Aflatoxin Contamination along the Groundnut Value Chain Actors in Different Agro-Ecology and Evaluation of Groundnut Varieties for Resistant to Aspergillus flavus and Aflatoxin Contamination in Eastern Ethiopia

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Abstract

Aflatoxins are common contaminants in groundnut and pose considerable risk to human health and have significant economic implication. Although, aflatoxin contamination of groundnut could occur in the field, in storage and during marketing, the level of contamination may vary along the value chain. The objective of this study was to determine level of aflatoxin concentration in groundnut along the value chain actors, and to evaluate groundnut varieties for resistant to *A. flavus* infection and aflatoxin contamination in Eastern Ethiopia. A total of 120 groundnut samples, which is 45 from farmers' fields, 45 from farmers' stores, and 30 from open air vendors, were collected and analysed for aflatoxin contamination in an ELISA test. Moreover, sixteen groundnut varieties were evaluated in this study. Field seed infection and colonization by *A. flavus* was determined using plate counting methods. The result revealed that, the level of aflatoxin contamination significantly varies along the value chain. Out of the total 120 samples, aflatoxin was detected on 91 samples, ranging from 1 ppb to 1012 ppb. Aflatoxin concentration were above 15 ppb in 85% of the positive samples collected from farmers' stores at Fedis district. Moisture contents and aflatoxin level of groundnut samples were positively correlated (r = 0.956) and significant ($p \leq 0.05$). There was also a significant and positive correlation (r = 0.959) between *A. flavus* infection and total aflatoxin levels. Moreover, the result revealed that all tested varieties were significantly different ($p \leq 0.05$) in response to *A.flavus* infection and aflatoxin levels. Among the varieties evaluated, *Baha Gudo* (13.70%), *Sartu* (14.00%) and *Sedi* (14.23%) were resistant to *A.flavus* infection. Inaddition, *Baha Gudo* (1.93 ppb), *Sartu* (3.70 ppb) and *Sedi* (6.40 ppb) were resistant to aflatoxin contamination. We suggest pre-harvest and post-harvest management of *A. flavus* infection so as to reduce the level of aflatoxin contamination at farmers' fields and farmers' stores and to maintein the quality of groundnut along the value chain. Also the varieties that showed resistance could form part of an integrated management of aflatoxin contamination in Eastern Ethiopia.

Keywords: Aflatoxin, Aspergillus flavus, Groundnut Varieties, Reaction, Value chain

1.0 INTRODUCTION

Groundnut (*Arachis hypogaea* L.), is a multipurpose cash crop for domestic markets as well as for foreign trade in several developing and developed countries. In Ethiopia, groundnut has a huge potential as

a cash crop to improve livelihoods of farmers and traders. Developing countries account for approximately 95% of world groundnut production, but are unable to sell large quantities of groundnut on the international market because of mycotoxin contamination (FAO, 2002). Infection of groundnut seed by certain races of *Aspergillus flavus* Link ex Fries and *Aspergillus parasiticus* Speare can result in contamination of the seed and groundnut by-products with aflatoxins, which are toxic fungal secondary metabolites (Waliyar *et al*., 2006).

Aflatoxin contamination of agricultural commodities has significant economic implication for the agricultural industry worldwide (Richard and Payne, 2003). For instance, aflatoxin contamination cost more than US$100 million per year to US producers (Coulibaly *et al*., 2008) and more than $750 million to Africa producers (Cardwell *et al*. 2004). Moreover, aflatoxin contamination of groundnut prevents groundnut producers in Africa from accessing international markets, increases dependency on foreign food aid, stifles economic opportunities, and adversely affects consumer health. In Ethiopia, groundnut market is declining and export of the crop has come to a standstill due to aflatoxin contamination and difficulty of meeting tolerance limits by importers and food processors. A food processing company imported groundnuts from India while groundnut producers in the country could not find market to sell their product (Amare Ayalew, personal communication).

In addition to the economic implication, aflatoxins pose considerable risk to human and livestock health. Aflatoxins are acutely toxic, immunosuppressive, mutagenic, teratogenic and carcinogenic compounds particularly to liver (Peraica *et al*., 1999). Outbreaks of acute aflatoxicosis from contaminated groundnut in humans have been documented in Kenya, India, Malaysia and Thailand (CAST, 2003). One of the first major documented reports of aflatoxins in humans occurred in 150 villages of western India in 1974 where 397 persons affected and 108 persons died (Krishnamachari *et al.*, 1975).

Aflatoxin contamination is both a pre-harvest and postharvest problem. It could occur during all stages along the groundnut value chain (Dohlman, 2003). In Ethiopia, Information on aflatoxin contamination of groundnut is scanty, and confined to limited market samples. Earlier studies reported that the level of aflatoxin in groundnut seed is 34.7µg/kg (Abrham and Petros, 1981), between 5 - 250 µg/kg (Amare *et al.*, 1995), and 15 - 11865 µg/kg (Alemayehu *et al.*, 2013). The aforementioned reports were based on market samples and did not address the entire groundnut value chain in particularly the situation at harvest. The present study was initiated to address the entire groundnut value chain covering major nodes from production through storage to consumption (marketing), since they could support decisions on targeting major points of aflatoxin contamination. Use of genotypes resistant to *Aspergillus flavus* and with diminished accumulation of aflatoxin is the best option for the resource constrained farmers to manage the problems of aflatoxin contamination (Kwemoi, 2011). Planting varieties that are resistant to fungal infection or that does not support high aflatoxin synthesis offers a sustainable, low cost approach for aflatoxin management that is suited for adoption by small scale groundnut producers. Amare et al. (1995) reported variations in terms of reaction to *A. flavus* infection among groundnut varieties widely cultivated in Ethiopia. A number of groundnut varieties have been released since then and evaluation of their reaction to *A. flavus* infection and aflatoxin contamination could identify varieties that could be promoted for widespread use by groundnut producers. Despite the importance of the problem, there are no recommended research results for aflatoxin management in Ethiopia. In the present study, the objective of the study was to determine prevalence of aflatoxin contamination of groundnut and its correlation with moisture contents and *A.flavus* infection along the value chain actors in different agro-ecological zones of Eastern Ethiopia, and to evaluates groundnut varieties for reaction to *Aspergillus flavus* infection and pre-harvest aflatoxin contamination in Eastern Ethiopia.

2.0 MATERIALS AND METHODS

2.1 Description of the Study Areas

The study dealt with field work (field survey and groundnut sampling) and laboratory characterization. The field work was conducted in major groundnut growing areas (Babile, Gursum and Fedis Districts) of East Hararghe Zone, Oromia Regional State, eastern Ethiopia (Fig. 1) in 2014 crop season. The areas were selected purposively as they represent the bulk of groundnut production in Ethiopia (Getinet and Nigussie,

1991). The altitudes of the study areas Babile range from 1401 to 1483 m.a.s.l, that of Fedis range from 1501 to 1899 m.a.s.l. and that of Gursum range from 1200 to 2950 m.a.s.l., and the geographical position of the study area is located between 09°02'52"N and 09°19'11"N latitude and between 42°06'03" E and 42°27'02" E longitude (Source: Agriculture Office of East Hararghe Zone, 2011).

Based on the three years meteorological data of Babile District, the area has mean annual rainfall range between 350-675 mm with much variation among years and with mean annual maximum and minimum daily temperatures of 28.27 and 20.18 °C, respectively. The average rainfall of Fedis area ranges from 650-1000 mm per year and with mean annual maximum and minimum daily temperatures of 26 and 15 °C, respectively. The average rainfall of Gursum area ranges from 650-1050 mm per year and with mean annual maximum and minimum daily temperatures of 20 and 13 °C, respectively (Source: Agriculture Office of East Hararghe Zone, 2011).

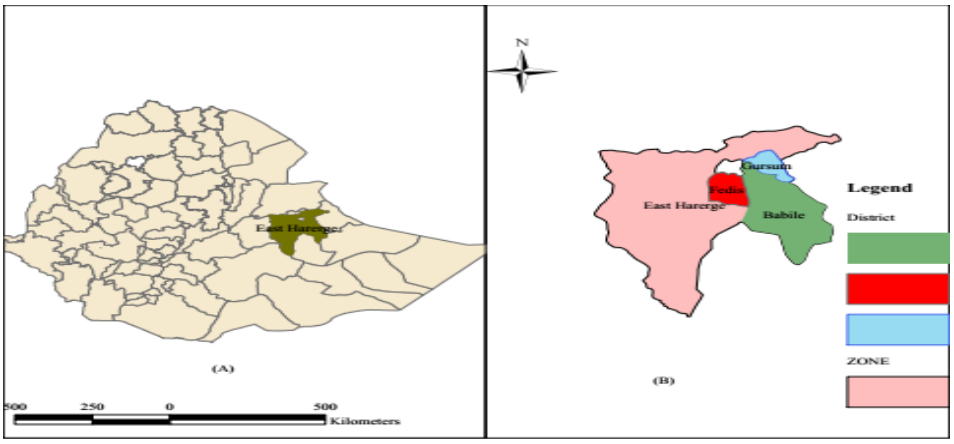

Figure 1. Map of (A) Ethiopia with study zone and (B) location map of study zone and district

2.2 Description of Groundnut Value Chain in Eastern Ethiopia

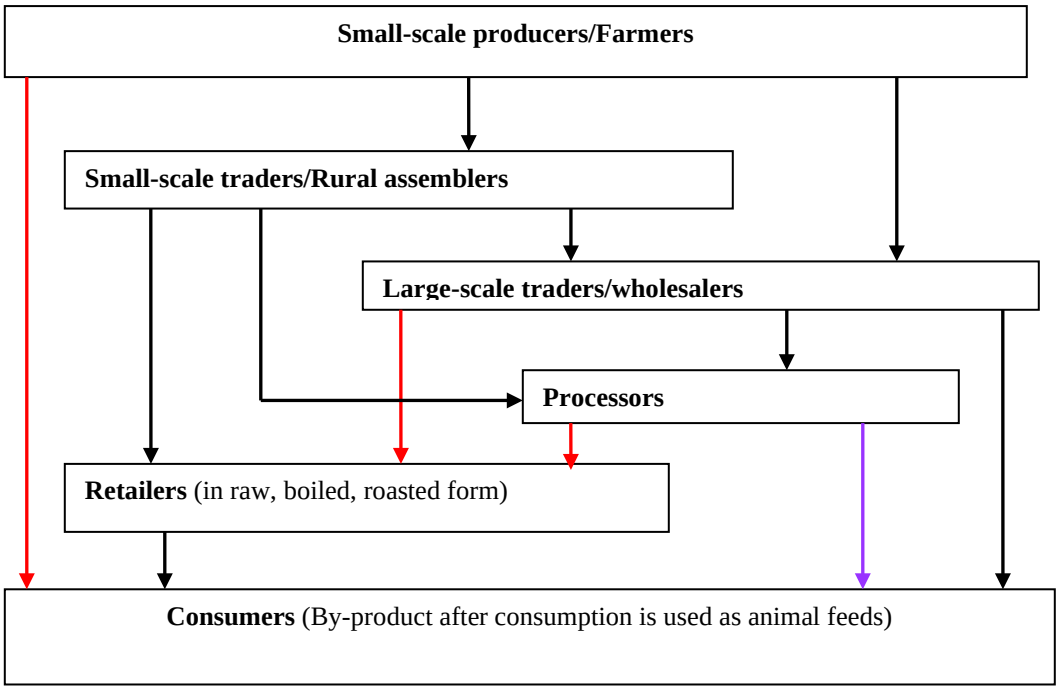

Figure 2. Flowchart of groundnut value chain and marketing channels process

The groundnut value chain in Eastern Ethiopia comprises farmers, and traders (wholesalers and retailers); rural, urban and semi-urban markets, and consumers (Fig. 2). The chain starts with the farmers who either consumed their produce or sold it locally at markets to rural retailers or local "assemblers-middlemen", who collected and transported groundnuts to larger wholesalers. Consumers were not included in the sampling since groundnuts were usually purchased in small quantities and consumed immediately and the situation was expected to be similar to that of the markets.

2.3 Sampling

Samples were collected along the groundnut value chain in the three districts. Accordingly samples were randomly drawn from farmers' fields at harvest, from farmers' storage, and from traders as follows.

2.3.1 Sampling from Farmers' Fields and Storage

Groundnut samples from farmers were collected from three representative locations of five agro-ecological zones (AEZs) that had been selected from three districts, namely Babile, Fedis and Gursum districts in eastern Ethiopia (Table 1). The AEZs were determined based on altitude, mean annual rainfall, and temperature as well as the probability of successfully growing the main crops of the zone (Ngugi *et al*., 2002; Alemayehu and Reynolds, 2006; Ayele, 2010). Accordingly, low-land dry (LLD) (Shek Hussien, Shek Abdi and Kito, from Babile), lowland moist (LLM) (Iffa, Ausharif and Shekusman, from Babile), midland dry (MLD) (Umer Kulle-1, Umer Kulle-2 and Hussien, from Fedis), mid-land moist (MLM) (Tuka Kenisa, Ido Basso-1 and Ido Basso-2, from Fedis), and high-land humid (HLH) (Audal, Oda Oromia and Kassa Oromia, from Gursum) were selected.

In each site groundnut samples were collected from three farmers' fields at harvest and the same number of samples were collected 4-6 months later from farmers' storage. Farmers' fields 5-10 km apart from each other, depending on the availability of groundnut, were sampled within each locality. So far as possible, the storage samples were taken from the same groundnut lots as those used for sampling at harvest. A total of 90 farmers' groundnut samples were collected, i.e., 45 groundnut samples (5 AEZs x 3 sites x 3 samples) were collected from farmer's fields at harvest and 45 samples were collected from farmers' stores.

2.3.2 Sampling from an Open-Air Vendor

A total of 30 samples were collected from rural, urban and semi-urban market places. The samples were consisting of roasted kernel samples from markets in each of the three districts. Samples were transported on the same day to Haramaya University and maintained at about 4 °C until laboratory analyses.

2.4 Aflatoxin Analysis using ELISA Kit

A total of 120 groundnut samples obtained from farmers' fields, farmers' stores and vendors of the three districts (Babile, Fedis and Gursum) were used for determination of total aflatoxin concentration in groundnuts. Collected groundnut samples were further air dried and brought to uniform moisture content (7%) immediately after collection and serologically assayed for total aflatoxins (AFT) contamination within four weeks of collection using the indirect Enzyme Linked Immunosorbent Assay (ELISA) following the procedure of Waliyar *et al*. (2009).

2.5 Description of Field Experimental Procedure

Twelve groundnut varieties released by Werer Agricultural Research Center (WARC) (*Shulamith, NC-343, Roba, Sedi, Lote, Bulki, Werer-961, Werer-962, Werer-963, Tole-2, Fayo* and *Fetene*) and two (*Baha Gudo* and *Baha Jido*) by Haramaya University (HU), and two Farmers' varieties (*Sartu* and *Oldhele*), a total of 16 groundnut varieties were grown in a hotspot field trial at Babile experimental field of Haramaya University in 2015 crop season. The experiment was laid out in a randomized complete block design (RCBD) with three replications. Each genotype was grown in four ridges or rows i.e. plots were 3 m long by 1.2 m wide with 30 cm spacing between ridges or rows and seeds were sown singly at 10 cm spacing

along the ridges or rows. Recommended agronomic practices (seed bed preparation, planting, weeding, digging and inverting, threshing, pre-cleaning and drying or curing) for groundnut production were applied.

2.6 Preparation of Inocula of *Aspergillus flavus*

An S-strain of *A. flavus* isolated from groundnut was used for artificial inoculation of experimental plots. Inoculum was prepared by the organic-matrix (cracked corn) method employed by (Will et al., 1994). Conidia of *A. flavus* from an 8-10 day old-culture, were suspended in sterile distilled water (900 ml/10000 g of corn) and used to inoculate sterile moisture-equilibrated (25% moisture) cracked corn. The corn was incubated at 25 to 30 °C for 3 days. Fungi did not sporulate during the three day incubation to reduce worker's exposure to airborne conidia.

2.7 Inoculation of Groundnut Plots

The inoculum was introduced into test plots to ensure the presence of sufficient aflatoxin-producing fungi in the groundnut pod zone. Artificial inoculation helps to ensure uniform testing conditions, which reduces the number of escapes and reduces variation in the data that could mask genetic differences. Each ridge/row within a plot at Babile were treated with 200 g corn infested with *Aspergillus flavus* to soil around developing pods at mid-blooming and about 4 weeks (20 to 30 days) before harvest.

2.8 Testing for Field Seed Colonization by *Aspergillus flavus*

Levels of infection of groundnut seed by *A. flavus* were determined for each plot. Undamaged, mature pods were hand shelled and 100 seeds from each plot were surface-sterilized in 0.1% aqueous mercuric chloride solution for three minute, rinsed in sterile distilled water and transferred (10 seeds per Petridish) to 14.5 cm diameter petridishes containing Potato Dextros Agar (PDA) and incubated at 30 °C for two to three days. Then, fungi growing from the seeds were recorded and the material was examined for green conidial heads of *Aspergillus* species of the *Aspergillus flavus* group and the percent infection of seed was determined according to Mehan & McDonald (1983).

$$\text{Seed infection (\%)} = \frac{\text{B}}{\text{A}} \text{ x } 100$$

Where, A = total number of seeds and B = Number of seeds with sporulating growth of *A. flavus* on their surfaces.

According to Mehan & McDonald (1983), the level of resistance to invasion and colonization by *Aspergillus flavus,* the following criteria were used:

Resistant = Sporulating growth on less than 15% of the seeds, with growth and sporulation sparse.
Moderately resistant = Sporulating growth on 16-30% of seeds, sporulation moderate to dense.
Susceptible = Sporulating growth on 31-50% of seeds, sporulation dense.
Highly susceptible = Sporulating growth on over 50% of seeds with dense growth and sporulation.

2.9 Data Analyses

The total aflatoxin concentrations determined by the ELISA test were summarized using Microsoft Excel and calculated as ppb for each sample. Regression and correlation analysis of *A. flavus* infection and moisture contents with aflatoxin levels were done using Minitab version 17 for windows. Analyses of variance (ANOVA) were done using Minitab version 17 for windows; means were compared by Fisher's protected least significant difference (LSD). Percentage of seed infection by *A. flavus* was determined using Microsoft Excel.

3.0 RESULTS AND DISCUSSION

A total of 120 groundnut seed samples were collected from farmers' fields, farmers' stores, and open-air vendors along the value chain of the three districts (Babile, Fedis and Gursum) for total aflatoxin

concentration analysis. From the total 120 samples, of which 91 samples were positive for aflatoxin. Aflatoxin concentration in the positive samples ranged from 1 ppb to 1012 ppb indicating heavy contamination of groundnut by aflatoxin beyond the maximum tolerable level by the World Health organization (WHO) (15 ppb), CODEX Alimentarius Commission (15 ppb for raw and 4 ppb for roasted seeds) and the European Union (4 ppb). Moreover, a total of 16 groundnut varieties, twelve improved groundnut varieties released in Ethiopia by Werer Agricultural Research Center, 2 improved groundnut varieties released in Ethiopia by Haramaya University and 2 local groundnut varieties were evaluated on hotspot area at Babile experimental field of Haramaya University in 2015 crop season.

3.1 Prevalence of Total Aflatoxin at Farmers' Fields

A total of 45 groundnut seed samples were collected from farmers' fields of the three districts namely Babile, Fedis and Gursum. That means 15 samples were collected from farmers' fields at Babile district, 15 samples were from farmers' fields at Fedis district and 15 samples were from farmers' fields at Gursum district. Percent of groundnut seed samples from farmers' fields with aflatoxin levels above and below 15 ppb in the three districts was shown in Figure 1. Fifteen samples were tested for total aflatoxin concentration from Babile district and only 5 samples were negative while the remaining 10 samples tested positive for aflatoxins. Percent prevalence of total aflatoxin concentration from the positive samples 55% were above 15 ppb and 45% were less than 15 ppb at farmers' fields at Babile district. From Fedis district, a total of 15 groundnut samples were tested for total aflatoxin concentration and only 3 samples were tested negative while the rest 12 samples were positive. Percent prevalence of total aflatoxin concentration from the positive samples 70% were above 15 ppb and 30% were less than 15 ppb at farmers' fields at Fedis district. As compared to Babile and Fedis districts, there was less groundnut seeds contamination by aflatoxin in Gursum district, where out of 15 samples, 8 were tested positive for aflatoxin and the remaining 7 samples were negative. Percent prevalence of total aflatoxin concentration from the positive samples 25% were above 15 ppb and 75% were less than 15 ppb at farmers' fields at Gursum district. My observations showed that these increases were due to poor harvesting, aflatoxigenic fungi infestation, pest damage, inappropriate cultural practices, and lack of knowledge of proper drying methods.

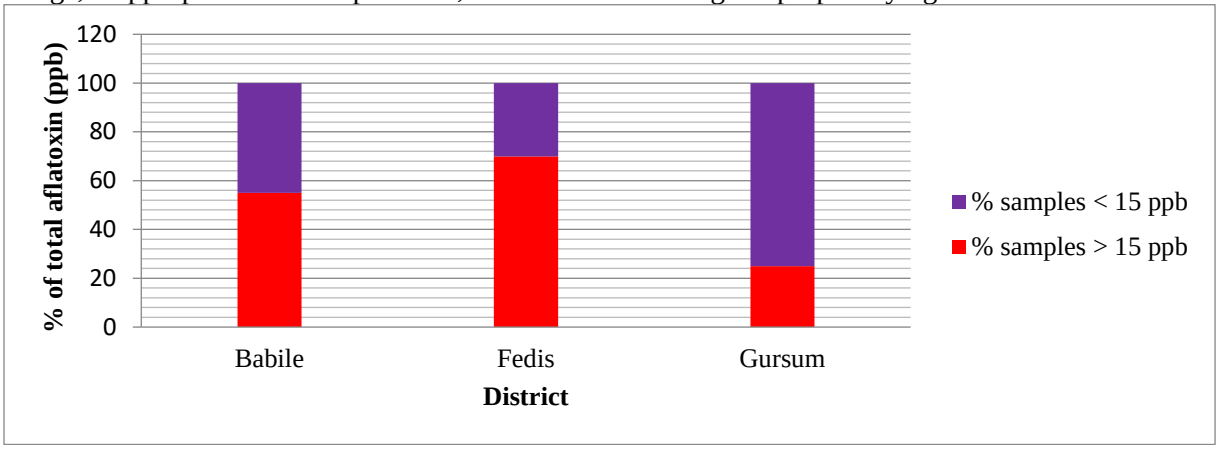

Figure 1. Percent of groundnut seed samples from farmers' fields with aflatoxin levels above and below 15 ppb in the three districts

As it was also investigated through regression analysis, aflatoxin levels with moisture contents were positively correlated (r = 0.860) and significant ($p \leq 0.05$) in groundnut seeds at farmers' fields. Aflatoxin levels were correlated to moisture contents of groundnut seeds following the equation of Total Aflatoxin at Farmers' Fields = -10991 + 1130 Moisture Ccontent at Farmers' Fields, with $R^2 = 0.74$ means that 74% of the variablity of AFT at Farmers' Fields around the mean was explained by moisture contents of groundnut seeds (Figure 2). This is because as moisture contents of the seeds increase, it promote aflatoxigenic fungi development, thereby aflatoxin contamination. Since moisture content of the seeds affect both the metabolism and physiological function of aflatoxigenic fungi.

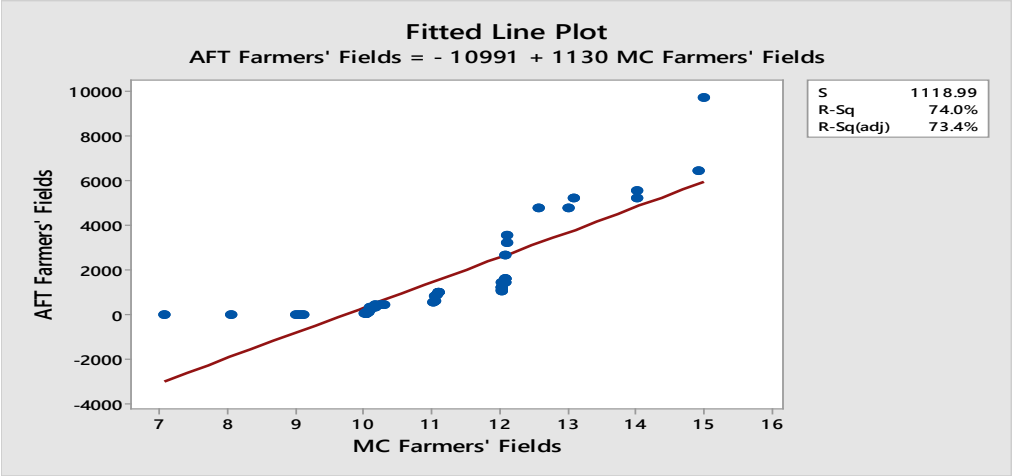

Figure 2. Regression analysis of moisture contents of groundnut seeds with total aflatoxin levels at farmers' fields

3.2 Prevalence of Total Aflatoxin at Farmers' Stores

A total of 45 groundnut seed samples were collected from farmers' stores of the three districts namely Babile, Fedis and Gursum. That means 15 samples were collected from farmers' stores at Babile district, 15 samples were from farmers' stores at Fedis district and 15 samples were from farmers' stores at Gursum district. Percent of groundnut seed samples from farmers' stores with aflatoxin levels above and below 15 ppb in the three districts was shown in figure 3. Fifteen samples were tested for total aflatoxin concentration from Babile district and only 4 samples were negative while the remaining 11 samples tested positive for aflatoxins. Percent prevalence of total aflatoxin concentration from the positive samples 76% were above 15 ppb and 24% were less than 15 ppb at farmers' stores at Babile district. From Fedis district, a total of 15 groundnut samples were tested for total aflatoxin concentration and only 2 samples were tested negative while the rest 13 samples were positive. Percent prevalence of total aflatoxin concentration from the positive samples 85% were above 15 ppb and 15% were less than 15 ppb at farmers' stores at Fedis district. As compared to Babile and Fedis districts, there was less groundnut seeds contamination by aflatoxin in Gursum district, where out of 15 samples, 14 were tested positive for aflatoxin and the remaining 1 samples were negative. Percent prevalence of total aflatoxin concentration from the positive samples 45% were above 15 ppb and 55% were less than 15 ppb at farmers' stores at Gursum district. These results were obtained because of the agro-ecologies of Fedis district was mid-land moist zones which was favorable to aflatoxigenic fungi development and thereby aflatoxin contamination as compared to low-land dry zone of Babile and highland humid zones of Gursum districts.

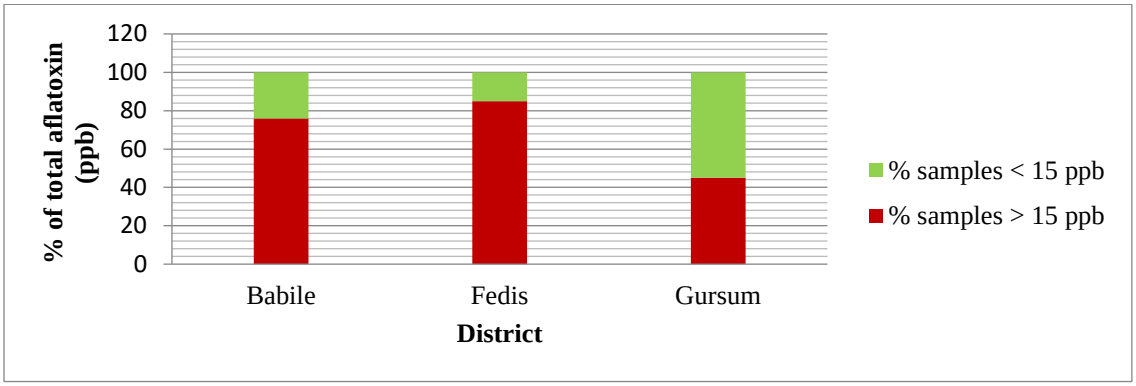

Figure 3. Percent of groundnut seed samples from farmers' stores with aflatoxin levels above and below 15 ppb in the three districts

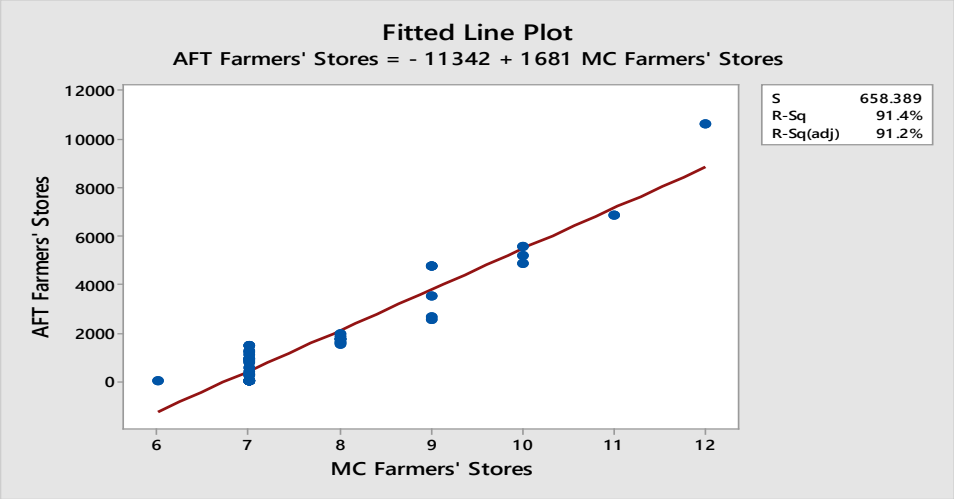

Figure 4. Regression analysis of moisture contents of groundnut seeds with total aflatoxin levels at farmers' stores

Figure 4 above showed that regression analysis of aflatoxin levels with moisture contents. The result revealed that aflatoxin levels with moisture contents were positively correlated (r = 0.956) and significant ($p \leq 0.05$) in groundnut seeds at farmers' stores. Aflatoxin levels were correlated to moisture contents of groundnut seeds following the equation of Total Aflatoxin at Farmers' Stores = -11342 + 1681 Moisture content at Farmers' Stores, with $R^2 = 0.91$ means that 91% of aflatoxin levels were due to moisture contents of groundnut seeds from farmers' stores (Figure 4). This is because as moisture contents of the seeds increase, it promote aflatoxigenic fungi development, thereby aflatoxin contamination. Since moisture content of the seeds affect both the metabolism and physiological function of aflatoxigenic fungi.

3.3. Prevalence of Total Aflatoxin at an Open-Air Vendors

A total of 30 groundnut seed samples were collected from open-air vendors of the three districts namely Babile, Fedis and Gursum. That means 10 samples were collected from open-air vendors at Babile district, 10 samples were from open-air vendors at Fedis district and 10 samples were from open-air vendors at Gursum district. Percent of groundnut seed samples from open-air vendors with aflatoxin levels above and below 4 ppb (CODEX limits for roasted seeds) in the three districts was shown in Figure 7.

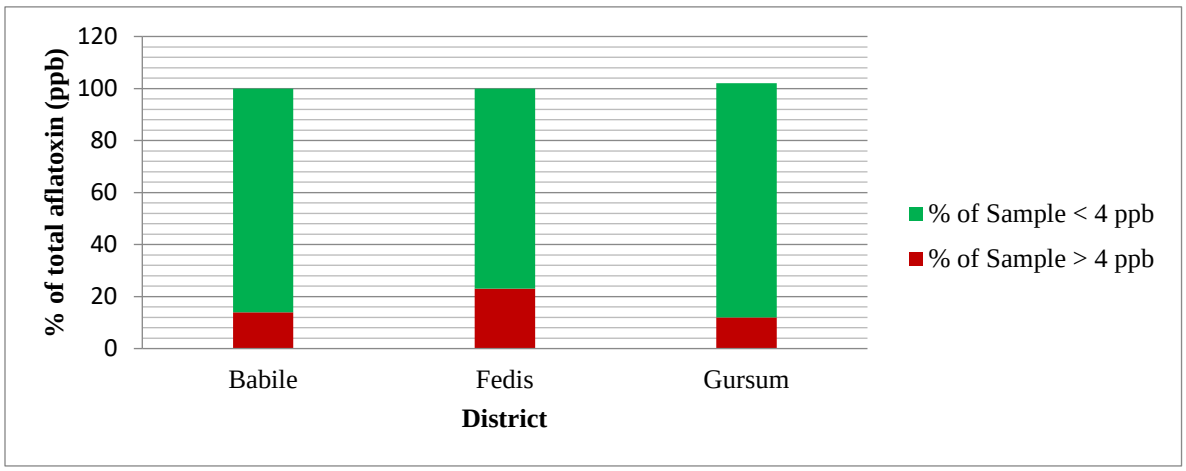

Figure 7. Percent of groundnut seed samples from open-air vendors with aflatoxin levels above and below 4 ppb in the three districts

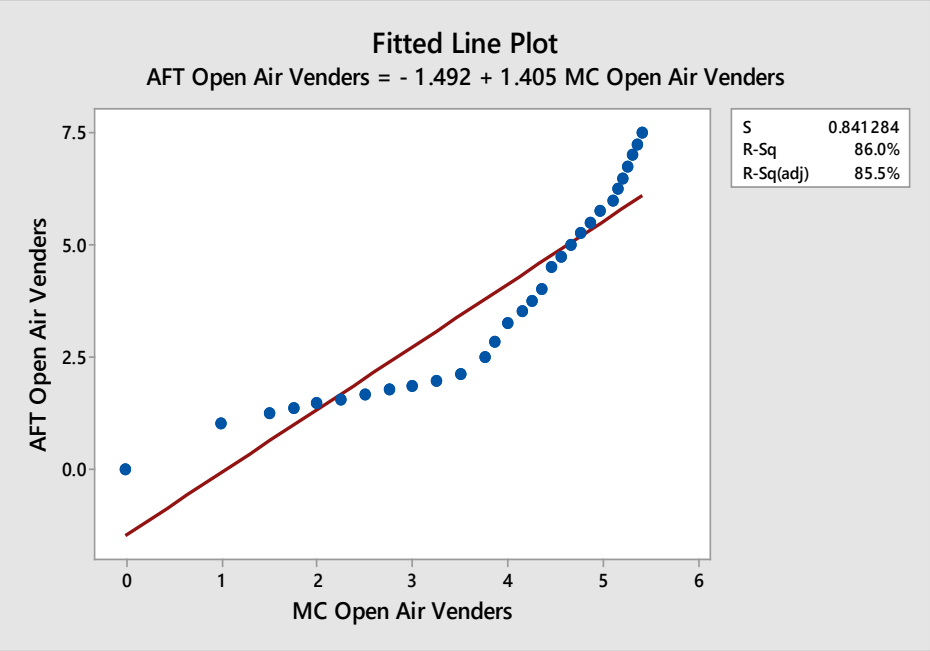

Figure 8. Regression analysis of moisture contents of groundnut seeds with total aflatoxin levels at venders

Ten samples were tested for total aflatoxin concentration from Babile district and seven samples were negative while the remaining 3 samples tested positive for aflatoxins. Percent prevalence of total aflatoxin concentration from the positive samples 14% was above 4 ppb and 86% were less than 4 ppb at open-air vendors at Babile district. From Fedis district, a total of 10 groundnut samples were tested for total aflatoxin concentration and 3 samples were tested negative while the rest 7 samples were positive. Percent prevalence of total aflatoxin concentration from the positive samples 23% were above 4 ppb and 77% were less than 4 ppb at open-air vendors at Fedis district. As compared to Babile and Fedis districts, there was less groundnut seeds contamination by aflatoxin in Gursum district, where out of 10 samples, 2 were tested positive for aflatoxin and the remaining 8 samples were negative. Percent prevalence of total aflatoxin concentration from the positive samples 12% were above 4 ppb and 88% were less than 4 ppb at open-air vendors at Gursum district. This is because roasting kills aflatoxin producing fungi and hence groundnut seed roasting processes reduce the risk of aflatoxin contamination.

Regression analysis showed that aflatoxin levels with moisture contents were positively correlated (r = 0.927) and significant ($p \leq 0.05$) in groundnut seeds at an open-air venders. Aflatoxin levels were correlated to moisture contents of groundnut seeds following the equation of Total Aflatoxin at an Open air venders = -1492 + 1405 Moisture content at an Open air venders , with $R^2 = 0.86$ means that 86% of total aflatoxin levels were due to moisture contents of groundnut seeds from open-air venders (Figure 8). This is because as moisture contents of the seeds increase, it promote aflatoxigenic fungi development, thereby aflatoxin contamination. Since moisture content of the seeds affect both the metabolism and physiological function of the aflatoxigenic fungi.

3.4. Field Seed Colonization by *Aspergillus flavus* Detected in 16 Groundnut Varities

According to Mehan and McDonald (1983) classification on the level of resistance to invasion and colonization by *Aspergillus flavus*, the improved varieties *Baha Gudo* and *Sedi*, and local variety *Sartu* were resistant in which sporulating growth on less than 15% of the seeds number. The local variety *oldhele*, and improved varieties *Bulki*, *Fayo* and *Fetene* were moderately resistant in which sporulating growth was in the range of 16-30% of the seeds number and others were susceptible (Table 2).

Table 2. Field seed colonization by *A. flavus* of the 16 groundnut varieties evaluated at Babile

No.	Varieties	Mean % of *A.flavus* Infection	Level of Resistance
1	NC-343	48.21[a]	Susceptible
2	Tole-2	45.01[b]	Susceptible
3	Baha Jido	41.10[c]	Susceptible
4	Werer 961	39.09[d]	Susceptible
5	Werer 963	35.16[e]	Susceptible
6	Lote	33.00[f]	Susceptible
7	Roba	32.50[g]	Susceptible
8	Shulamiz	32.20[g]	Susceptible
9	Werer 962	30.17[h]	Susceptible
10	Oldhele	27.63[i]	Susceptible
11	Bulki	27.00[j]	Moderately Resistant
12	Fayo	20.18[k]	Moderately Resistant
13	Fetene	19.00[l]	Moderately Resistant
14	Sedi	14.23[m]	Resistant
15	Sartu	14.00[m]	Resistant
16	Baha Gudo	13.70[m]	Resistant
Mean		29.86	

S = 3.70
LSD at p = 5%, 0.00
CV(%) = 12.39
*Significant at $p \leq 0.05$

Means that do not share same letter are significantly different

3.5. Pre-harvest Aflatoxin Contamination in 16 Groundnut Varieties

Table 3 above showed that pre-harvest aflatoxin levels (ppb) in seed samples of the 16 groundnut varieties were significantly different ($p \leq 0.05$). Results of Table 8 showed that the improved varieties *Baha Gudo* (1.93 ppb), *Sedi* (3.70 ppb), and local variety *Sartu* (6.40 ppb) were resistant to aflatoxin contamination in which their aflatoxin levels are below 15 ppb, while aflatoxin levels of the rest evaluated varieties are above 15 ppb according to CODEX Standard Limits (CODEX, 2004). This was because the resistant varieties even though they were infected, they failed to produce aflatoxins beyond a certain threshold.

Results from across the three survey districts suggest higher aflatoxin contamination of groundnut samples from the farmers' stores than from farmers' fields, and from open-air vendors, respectively. In general, when actors along the value chain were compared, the highest prevalence of total aflatoxin contamination was recorded at farmers' stores at Fedis district and the least was recorded at open-air vendors at Gursum district. When agro-ecologies were compared prevalence of aflatoxin contamination was highest from farmers' stores in Fedis districts in midland moist agro-ecological zones and the least was from roasted groundnut seed samples of vendors in Babile, Fedis and Gursum districts in five agro-ecological zones. This was because roasting kills aflatoxin producing-fungi and hence groundnut seed roasting processes would reduce the risk of aflatoxin contamination. The high temperature and periodic drought prevalent in mid-land moist zone could explain the higher levels of aflatoxin contamination in that climate.

Table 3. Aflatoxin levels (ppb) of the 16 groundnut varieties evaluated at Babile in 2015

No.	Varieties	Mean of AFT in ppb	Level of Resistance
1	NC-343	841.90[a]	Susceptible
2	Tole-2	839.10[b]	Susceptible
3	Baha Jido	838.10[c]	Susceptible
4	Werer 961	808.10[d]	Susceptible
5	Werer 963	792.10[e]	Susceptible
6	Lote	724.10[f]	Susceptible
7	Roba	721.10[g]	Susceptible
8	Shulamiz	630.30[h]	Susceptible
9	Werer 962	584.20[i]	Susceptible
10	Oldhele	300.70[j]	Susceptible
11	Bulki	273.20[k]	Susceptible
12	Fayo	273.90[l]	Susceptible
13	Fetene	93.20[m]	Susceptible
14	Sedi	6.40[n]	Resistant
15	Sartu	3.70[o]	Resistant
16	Baha Gudo	1.93[p]	Resistant
Mean		481.20	
S = 55.58 LSD at p = 5%, 0.00 CV(%) = 11.55 *Significant at $p \leq 0.05$			

Means that do not share same letter are significantly different

In addition, unfavorable drying and storage practices may aggravate the problem. Moreover, the environmental conditions especially temperature and relative humidity and/or moisture prevailing in the mid-land moist zone may be responsible for this established trend. Dereje *et al*. (2012) analyzed a total of 168 groundnut kernel samples, collected from farmers and research center fields of northern Ethiopia for Aflatoxin B1 type and were detected in all of the samples, ranging from 0.1 to 397.8 ppb (mean: 28.7 and median 5.2 ppb). The highest level of Aflatoxin was detected in groundnut samples from Tigray abergele area (55.3 ppb). Another study by Eshetu (2010) reported that aflatoxin concentration of 447 ppb and 405ppb in samples stored for three months in Babile, east Ethiopia, and for a year in Awi in North Ethiopia, respectively. The aflatoxin concentration detected in the current study was generally much higher than these last two previous reports from Ethiopia. However, the aflatoxin concentration quantified in the current work is not uniquely high in Africa.

Regression analysis showed that aflatoxin levels with moisture contents were positively correlated and significant in groundnut seeds along the value chain actors. The research results on seed moisture showed that the moisture content of samples ranged between 3 and 15%; the lowest was obtained from groundnut seed samples collected from vendors and the highest was from farmers' fields at harvest. According to Codex Alimentarius Commission, the maximum allowable moisture content in groundnut is 10% and it is known that above this maximum range can support mould growth during storage and can lead to aflatoxin contamination (CODEX, 2004). Rahmianna *et al*. (2015) reported that kernel moisture content is crucial in the incidence of aflatoxin contamination where the range of 18 to 28 % moisture content is critical level suitable for aflatoxin production.

In Ethiopia some of food materials like preparation of red pepper powder and its paste showed some aflatoxin contaminations (mean 32 µg/Kg for powder, 1 paste sample had 102.2 µg/Kg aflatoxin B1 respectively) while samples of groundnuts and peanuts butter had aflatoxin B1 at mean values of 34.7 and 105 µgKg-1, respectively (Abrham and Petros, 1981). In the European Union, regulations limit the amount of total aflatoxins to 4 ppb where as guidelines in a few developing countries and the US limit total aflatoxins to no more than 20 ppb in food stuffs intended for human consumption (FAO, 2011). International standards based on the levels of aflatoxin, the groundnut samples analyzed were grouped into three categories: samples containing 0-4 ppb, samples with 4-20 ppb, and samples with > 20 ppb. Compared to this most of the groundnut samples from east Ethiopia had aflatoxin at a level much higher than any of these three classes. Results from across the value chain actors of the three survey districts suggest higher aflatoxin contamination of groundnut samples from the farmers' stores, farmers' fields and market retailers than those from the vendors. These results were obtained due to higher kernel infection by *Aspergillus* species of groundnuts in farmers' stores, farmers' fields and market retailors than those from the vendors. The reason that lower contamination of samples from vendors was that the samples were roasted groundnut seeds which did not support *Aspergillus* species infection and there by aflatoxin contamination. The aflatoxin concentration detected in the current study is generally higher from Ethiopia.

Seeds of certain groundnut (*Arachis hypogaea* L.) genotypes were reported to be resistant to colonization by *A. flavus* based on inoculations of hand- harvested, hand-shelled seed (Kushalappa et al., 1979). Resistance was reduced or eliminated by practices that damaged the testa of the seed. Machine harvest (stripping the pods from the plants) and machine shelling decreased resistance, and abrading the testa with Carborundum or pricking it with pins eliminated resistance and seeds without testae had no resistance (Kushalappa et al., 1979). Resistant seeds seemed to have greater surface wax accumulations, more compact cells and a greater number of fibers in the testa along with smaller hila, and a greater concentration of tannins (Laprade et al., 1973).

All these studies on seed shelled by hand and established that resistance to *A. flavus* and aflatoxin contamination in groundnut seed was a function of the testa, which acts as a barrier to movement of the fungus into the seed. This was because of the intact testa and pods acting both as physical (pod) and chemical barriers (testa) to ward off fungal infection with pathogens and aflatoxins (Awuah and Ellis, 2001). Wounded seed and pod surfaces when left unprotected could also act as easy points of entry for fungi. This may account for why some of the samples had higher aflatoxin levels, which is in agreement with the present study.

4.0 CONCLUSSION

Groundnut is one of the most important cash crops in eastern Ethiopia, and aflatoxins are common contaminants in groundnut growing across the study areas. Results of the current study suggest heavy contamination of groundnuts by aflatoxin in east Ethiopia at a level much higher than any international standard, ranging from 1 ppb to 1012 ppb. Percent prevalence of (AFT) total aflatoxins concentration were higher at farmers' stores at Fedis district, indicating heavy contamination of groundnut by aflatoxin beyond the maximum tolerable level by the CODEX (15 ppb). Also moisture contents were positively correlated ($r = 0.956$) and significant ($p \leq 0.05$) with AFT levels. Moreover, *A. flavus* infection with AFT levels were positively correlated ($r = 0.959$) and significant ($p \leq 0.05$). This study revealed that there was higher risk of exposure to aflatoxin through raw than roasted groundnut seeds. Findings from the field evaluation suggest that groundnut varieties in which sporulating growth on less than 15% of the seeds number (improved varieties-*Baha Gudo* (13.70%) and *Sedi* (14.23%), and local variety-*Sartu* (14%)) were resistant to *A. flavus* infection and should be promoted as an aflatoxin management strategy. Moreover, the improved varieties *Baha Gudo* (1.93 ppb) and *Sedi* (3.70 ppb), and local variety *Sartu* (6.40 ppb) were resistant to pre-harvest aflatoxin contamination in which their aflatoxin levels are below 15 ppb. Groundnut seed, when carefully taken care of during growth, harvesting and storage, though may be subject to fungal infection, could limit pathogen infection and aflatoxin production.

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