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Occurrence of *Aspergillus* Species in Groundnut (*Arachis hypogaea* L.) along the Value Chain in Different Agro-Ecological Zones of Eastern Ethiopia

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Abstract Groundnut is an important cash crop for domestic markets as well as for foreign trade in several developing and developed countries. It is also one of the most valuable cash crops in eastern Ethiopia. However, its production is constrained by *Aspergillus* species, which cause quantitative losses and produce highly toxic and carcinogenic chemical substances known as aflatoxins. A reconnaissance survey was conducted in 2014 cropping season with a research objective to determine the levels of infection of groundnut seed by *Aspergillus* species along the groundnut value chain in different agro-ecological zones of eastern Ethiopia, including three major groundnut growing areas, namely Babile, Gursum and Fedis Districts of East Hararghe Zone, Oromia Regional State. In this study 210 groundnut seed samples were collected from farmers' fields, farmers' stores, market retailers and vendors at Babile, Fedis and Gursum districts in 2014 cropping season, and were tested for seed moisture content using electronic moisture meter. The frequency of *Aspergillus* species contamination on groundnut seeds was determined using plate counting methods. The seed moisture data showed that the moisture contents of seed samples ranged between 3 and 15%; the lowest was obtained from groundnut seed samples collected from vendors at Babile district and the highest was from farmers' fields at harvest in Fedis district The proportion of seed contamination by *Aspergillus* species varied from 30% in seed collected from vendors to 85% in seed samples from farmers' fields in Babile, Fedis and Gursum districts. Groundnut seed samples collected from farmers' fields was the first (85%), where as from farmers' stores had the second highest seed contamination with 80% infection, and groundnut samples collected from that of market retailers had 60% contamination. Five different *Aspergillus* species were associated with 210 groundnut seed samples along the groundnut value chain actors. Of the several *Aspergillus* species isolated from the groundnut seed samples, *A. flavus* and *A. niger* were the most prevalent mycotoxigenic fungi across the farmers' fields, farmers' stores, market retailers and vendors at Babile, Fedis and Gursum districts in the five agro-ecological zones of eastern Ethiopia such as low-land dry (LLD), low-land moist (LLM), mid-land dry (MLD), mid-land moist (MLM), and high-land humid (HLH). The occurrence of these two species ranged from 22.05-49.05% (*A. flavus*) and 23.83-48.11% (*A. niger*). Their relative dominance in number of isolates from the total associated fungi was 23 and 22%, respectively. On the other hand, *A. parasiticus*, *A. ochraceus*, and *A. tamarii* occurred rarely. The highest frequencies of occurrence of *A. flavus* and *A. niger* were on groundnut seed samples from farmers' fields in Fedis district in mid-land moist agro-ecological zone and the least was from roasted groundnut seed samples from vendors in Babile, Fedis and Gursum districts in all the five agro-ecological zones. From the present findings, it could be concluded that there was high occurrence of aflatoxigenic fungi along the groundnut value chain actors. Also, the current results imply the urgent need for application of management measures against aflatoxigenic fungi to maintain the quality of groundnut in the various value chain processes and to avert human and animal health risks.

Keywords: *Aflatoxin, Arachis hypogaea, Aspergillus species, Ethiopia, groundnut, occurrence, value chain actors*

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1. Introduction

Groundnut (*Arachis hypogaea* L.), which is also known as peanut, earthnut, monkeynut and goobers, is an annual legume crop. It is one of the world's most important

oilseed crops [1], ranking as the 13th most important food crop and 4th most important oilseed crop of the world [2], and is cultivated in more than 100 countries in six continents [3]. Groundnut kernels contain 40-50% fat, 20-50% protein and 10-20% carbohydrate and are rich in vitamins and minerals [4].

Cultivated groundnut originated from South America [5]. Its cultivation is mostly confined to the tropical, subtropical, and warm temperate (zones) countries between 40°N and 40°S latitude. It is currently grown on 25.2 million hectares worldwide with a total production of 35.9 million metric tons and productivity of 1.425 tons per hectare, with developing countries in Asia (66%) and Africa (25%) as the major producers [6]. In 2009, China, India and the United States were the three largest producers of groundnut [7].

Groundnut kernels are consumed directly as raw, roasted or boiled kernels or oil extracted from the kernel is used for culinary purpose. Oil pressings, seeds, and the haulms of groundnut are used as animal feed, while the oilcake is used as an industrial raw material and fertilizer [8]. These multiple uses of groundnut plant make it an excellent cash crop for domestic markets as well as for foreign trade in several developing and developed countries.

Groundnut is relatively new to Ethiopia. It was introduced from Eritrea to Hararghe in the early 1920s by Italian explorers [9]. Major groundnut producing areas in Ethiopia are Babile, Gursum, Beles, Didessa, Gambella and Pawe. Gamu Gofa, Illubabor, Gojam, Wello and Wellega are identified as potential production areas [9]. During the 2014, it was cultivated on 79943.03 ha of land and 112088.7 tons of groundnuts were produced, with average yield of 1.402 tons per ha [10].

Groundnut is affected by several diseases, such as late leaf spot (*Phaeoisariopsis personata* Berk and Curt), early leaf spot (*Cercospora arachidicola* Hori), collar rot (*Aspergillus niger*), rust (*Puccinia arachidis* Speg), and bud necrosis (bud necrosis virus (BNV). Apart from these, infection of groundnut seed by molds, mainly *Aspergillus flavus* Link ex Fries and *Aspergillus parasiticus* Speare, can result in the contamination of the seed with aflatoxins, which are toxic fungal metabolites (mycotoxins). Aflatoxins are a group of structurally related toxic polyketide-derived secondary metabolites produced by certain strains of *Aspergillus flavus* and *Aspergillus parasiticus* [11].

In warm climates, grains are easily infected with toxigenic microorganisms like *Aspergillus* species. *Aspergillus* species are facultative parasites and can invade host plant tissues directly or attack tissues that have been predisposed by environmental stresses, such as dry weather or damages caused by insects, nematodes, natural cracking, and harvestequipment [12]. They are distributed worldwide, mainly in countries with tropical climates that have extreme ranges of rainfall, temperature and humidity. Many strains of this fungus are capable of producing aflatoxins that render the seed unacceptable due to high toxicity for human or animal consumption [13].

Aflatoxins are acutely toxic, immunosuppressive, mutagenic, teratogenic and carcinogenic compounds targeting mainly the liver for toxicity and carcinogenicity [14]. Aflatoxin contamination of agricultural commodities poses considerable risk to human and livestock health and has significant economic implication for the agricultural industry worldwide [15]. In the USA, it was reported that income losses due to aflatoxin contamination cost an average of more than US$100 million per year to US producers [16]. According to Cardwell [17], aflatoxin contamination of agricultural crops, such as groundnut and cereals, causes annual losses of more than US$750 million in Africa.

Aflatoxins are the major mycotoxins that are most commonly associated with groundnuts [18]. Aflatoxin contamination of groundnut prevents groundnut producers from accessing bigger western markets, increases dependency on foreign food aid, stifles economic opportunities, and adversely affects consumer health. According to FAO [19], 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 aflatoxin contamination. For instance, a food processing company in Ethiopia imported groundnuts from India, while groundnut producers in Gursum and Bablie could not find market to sell their crop (Amare Ayalew, personal communication).

Aflatoxin contamination of groundnut could occur before harvest while the crop is maturing in the field, particularly favored by drought stress and high soil temperature, in storage and during marketing [18,20,21,22,23]. Aflatoxin contamination and associated fungi in groundnut continue to attract worldwide attention and have been reported from various countries. Aflatoxin contamination can occur on pods and seed in the soil near harvest, during harvest, and post-harvest in storage. Pre-harvest infection by *Aspergillus* spp. and the environmental factors that lead to colonization, infection of the seeds, plants, and aflatoxin accumulation have been reviewed in detail [24]. Outbreaks of acute aflatoxicosis from contaminated groundnut in humans have been documented in Kenya, India, Malaysia and Thailand [25]. One of the first major documented reports of aflatoxins in humans occurred in 150 villages of western India in 1974 where 397 persons were affected and 108 persons died [26].

The lowland areas of eastern Ethiopia have considerable potential for increased oil crop production, including groundnut. Particularly areas such as Babile, Fedis and Gursum are the major producers of groundnuts for local and commercial consumption [27,28]. Nevertheless, the area may also be very conducive for aflatoxigenic fungi, like *Aspergillus* species owing to its warm and dry climate. Moreover, farmers' practices of production and handling of groundnut at pre- and post-harvest stages may provide favorable conditions for outbreaks of fungi and their mycotoxins.

In Ethiopia, an earlier report showed mean levels of aflatoxin B1 of 34.7 and 105 µg/kg in samples of groundnut and peanut butter, respectively [29]. Amare [30] reported aflatoxin levels of 5-250 µg/kg in groundnut seed from eastern Ethiopia. Recently, Alemayehu [28] reported that total aflatoxin levels in *Aspergillus flavus* positive samples of groundnut seed varied between 15 and 11865 µg/kg. These results indicated heavy aflatoxin contamination of groundnut samples from Ethiopia, at levels much higher than any international acceptable standards, e.g. FAO and WHO acceptable limit being 15 µg/kg. On the other hand, these reports have been based

on limited single-season survey of market samples and there is a need to extend the database both spatially and temporarily to get conclusive evidence on the level of contamination.

Information on aflatoxigenic fungi in Ethiopia is likewise limited and further studies are warranted. Such studies would be more meaningful if they 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.

Although groundnut has a huge potential as a cash crop to improve livelihoods of farmers and traders in various parts of Ethiopia, its market is declining and export of the crop has come to a standstill. This is due to aflatoxin contamination of the crop and the difficulty of meeting tolerance limits by importers and food processors, leading to rejection of the crop and reduction in market demand. Aflatoxin contamination is both a pre-harvest and postharvest problem. It can occur during all stages along the groundnut value chain unless the fungus is inactivated before chances of multiplication and re-invasion. Information on aflatoxin contamination of groundnut and the associated fungi in Ethiopia is scanty, confined to limited market samples, and does not particularly address the situation at harvest. Moreover, despite the importance of the problem, there are no recommended research findings for aflatoxigenic fungi management in Ethiopia. Therefore, the present study was conducted with the specific objective to determine the levels of infection of groundnut seed by *Aspergillus* species along the groundnut value chain in different agro-ecological zones of eastern Ethiopia.

2. Materials and Methods

2.1. Description of the Study Area

The study dealt with field work (field survey, groundnut sampling, and groundnut varietal resistance evaluation) 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 in 2014 crop season. The areas were selected purposively as they represent the bulk of groundnut production in Ethiopia [31]. These areas have high potentials for rain-fed groundnut production nationally. The study areas are described in Ephrem Guchi *et al*. [32].

2.2. Description of Groundnut Value Chain in Eastern Ethiopia

The groundnut value chain in Eastern Ethiopia comprised farmers, and traders (wholesalers and retailers); rural, urban and semi-urban markets, and consumers. Descriptions of groundnut value chain in eastern Ethiopia are described in Ephrem Guchi *et al*. [32].

Groundnut sampling along each segment of the groundnut value chain is described in the following subsections.

2.3. Sampling

Samples were collected along the groundnut value chain in the three districts (Babile, Fedis and Gursum). Accordingly, samples were drawn from farmers' fields at groundnut harvest, from farmers' stores, from traders (both wholesalers and retailers as well as from rural and urban markets). Hence, a total of 210 samples were collected from the various groundnut seed sources.

Composite seed samples consisted of 1 kg each comprising five sub-samples drawn from different parts of the groundnut lot. The samples were placed in cloth bags to allow air circulation that reduced condensation and limited fungal growth after sampling until analyses. Samples were properly labeled and relevant information on the locality, GPS coordinates altitude, cultivar, date of sampling, date of harvest, type and duration of storage were recorded. Seed moisture content was measured at the time of sampling using electronic seed moisture meter. Samples were transported on the same day to Plant Pathology Laboratory, Haramaya University, and were maintained at about 4 °C until laboratory analyses.

2.3.1. Sampling from farmers' fields and storage

Table 1. Description of Groundnut Seed Sampling Site in Five Agro-ecologies of Eastern Ethiopia

District	Name of site	Altitude (m)	TM (°C)	Rain fall (mm)	Cropping system	Agro-ecology
Babile	Shek Hussien	1401	18-27	<900	LCR	LLD
Babile	ShekAbdi	1419	18-27	<900	LLR	LLD
Babile	Kito	1420	18-21	<900	LLR	LLD
Babile	Iffa	1431	18-27	<900	LCR	LLM
Babile	Ausharif	1431	18-27	<900	LCR	LLM
Babile	Shekusman	1431	18-27	<900	LCR	LLM
Fedis	Umer Kulle-1	1483	18-24	650-900	IC	MLD
Fedis	Umer Kulle-2	1497	18-24	650-900	IC	MLD
Fedis	Hussien	1581	18-21	650-900	IC	MLD
Fedis	Tuka Kanesa	1710	18-21	900-1000	IC	MLM
Fedis	Ido Basso-1	1841	18-20	900-1000	IC	MLM
Fedis	Ido Basso-2	1899	18-20	900-1000	IC	MLM
Gursum	Audal	2201	18-20	>1000	LLR	HLH
Gursum	Oda Oromia	2509	14-18	>1000	LLR	HLH
Gursum	Kassa Oromia	2525	14-18	>1000	LLR	HLH

*LCR=Legume-cereal rotation, LLR=Legume-Legume rotation, IC=Intercropping with cereals, FLR=Fallow-legume rotation
*LLD=Lowland dry moist, LLM=Lowland moist, MLD=Midland dry moist, MLM=Midland moist, HLH=Highland humid

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 [8,33,34]. Accordingly, low-land dry (LLD)

(Shek Hussien, Shek Abdi and Kito, from Babile), low-land moist (LLM) (Iffa, Ausherif, and Shekusman, from Babile), mid-land 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 (Table 1).

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 150 farmers' groundnut samples were collected, i.e., 75 groundnut samples (5 AEZs x 3 sites x 5 samples) were collected from farmer's fields at harvest and 75 samples were collected from farmers' stores.

2.3.2. Sampling from Traders and Marketing Cooperatives

A total of 30 groundnut samples were collected from retailers', i.e. 10 samples from each district. These samples were collected 4 – 6 months after harvest parallel to farmers' storage samples.

2.3.3. Open-air Vendors

A total of 30 samples were collected from rural, and urban and semi-urban market places. The samples consisted of ten roasted kernel samples from markets in each of the three districts.

2.4. Isolation and Identification of *Aspergillus* Species

Hundred groundnut seeds per sample were surface-sterilized with chlorox solution (10% NAOCl) for 1 min, followed by immersion in sterile distilled water for 1 min, then placed on dichloran rose bengal chloramphenicol agar (DRBC) supplemented with 3% NaCl plates (ten kernels per plate) and incubated at 25°C for three days. Pure cultures of different outgrowing fungi were obtained by transferring fungal colonies to new DRBC plates by using sterile toothpicks, and incubating the plates for 5-7 days at 25°C. Pure cultures of each isolate were then stored at 4°C in vials containing 2.5 ml of sterile distilled water for further use.

Isolates were identified to a species level based on morphological features and descriptions used by other scholars [35,36,37,38]. For this purpose, isolates representing each pure culture were grown on Czapek Dox Agar at 25°C for 5-7 days. Fungal colonies that grew rapidly and produced white, yellow, yellow-brown, brown to black or shades of green, mostly consisting of adense felt of erect conidiophores were broadly classified as *Aspergillus* species, while those that produced blue spores were considered as *Pencillium* species [38]. Isolates with elongate sclerotia and relatively smooth conidia were considered *A. nomius* [35,36]. On the other hand, isolates with dark green colonies and roughconidia were considered as *A. parasiticus*. Those that produced rough-brown conidia and a brown colony on reverse side were considered *A. tamarii* [39]. The major distinction currently separating *A. niger* from the other species of *Aspergillus* is the production of carbon black or very dark brown spores from biseriate phialides [40]. Those that showed brown colony with orange and cream reverse sides were considered *A. sojae* and *A. oryzae*, respectively [41]. Those, which produced conidia with smooth surface on Czapek Dox Agar and colonies typical of *A. flavus* on A. flavus and A. parasiticus Agar, were considered as *A. flavus*.

2.5. Frequency of Groundnut Seed Contamination

After the initial isolation, data were recorded on the number of infected and non-infected kernels. The frequency of *Aspergillus* species in groundnut samples was determined as proportion of kernels contaminated by each fungal species to the total number of kernels plated.

2.6. Data Analyses

The data were analyzed using Statistical Package for Social Studies (SPSS) Version 16 for Windows. *Aspergillus* species counts were normalized by log transformation before subjecting to analysis. Frequency (out of the total samples) of occurrence of each species and relative density of each pecies was determined. Poisson regression was used in the analysis of frequency of *Aspergillus* species.

3. Results and Discussion

3.1. Moisture Content of Samples

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 [42].

Comparison between the moisture content of groundnut seeds along actors of the value chain showed that the highest moisture contents were recorded from farmers' field samples, which was 15%, and sixty-two samples from the total seed samples were also above the maximum allowable Codex standard of moisture content (10%). Moreover, six samples from farmers' stores were above the maximum allowable Codex standard of moisture content. On the contrary, all samples from market retailers and vendors were below the maximum allowable Codex standard of moisture content. This is because samples from vendors were roasted groundnut seeds and were used directly for consumption. Overall, the moisture contents of groundnut seeds from vendors were lower than that from market retailers, farmers' stores and farmers' fields, respectively. This might imply that during storage, groundnut seeds continued to dry as long as they were stored in well-aerated and protected structures.

The present finding is consistent with the investigation by Eshetu [43] who reported that the moisture content of groundnut seed samples ranged between 7 and 15%; the

lowest moisture content was obtained from Babile area where the groundnut was stored in sack for one year and the highest was from Garamuleta area where the wet groundnut was shelled from newly harvest product. Also, the current finding is comparable to the findings of Sseruwu [44] who sampled groundnut seeds, from five districts in Uganda, which were stored for three to eight months, and reported moisture content of 10.5 to 14.6%. Comparison between the results of moisture contents with other reported data by Amare [30] shows higher difference in the percentage of seed moisture content, which ranged between 3.0 to 6.8%. The difference could accrue from the methods employed to determine the seed moisture content, i.e. oven drying method.

3.2. Identification and Characterization of *Aspergillus* Species from Groundnut Seeds

To isolate the various fungal species from the infected groundnut samples, simple growth analyses made on agar plates were carried out. Several fungal species became obvious from the growth media seven days after incubation. From seven days onwards, infected samples displayed fungal species, which were obvious enough for isolation, identification and characterization using identification keys.

Five different *Aspergillus* species (Figure 1) were associated with groundnut seed samples collected from the groundnut value chain actors in different agro-ecologies of eastern Ethiopia. One of the *Aspergillus* species identified in this study was *Aspergillus flavus.* Colonies of this species grew rapidly often at 28 to 37°C. These colonies were characterized by yellow to dark, yellowish-green pigments, consisting of a dense felt of conidiophores or mature vesicles bearing phialides over their entire surface [45]. The colony of *A. flavus* was velvety, yellow to green or the old colony turned to brown mould with golden to reddish-brown on the reverse. The conidiophores were variable in length; walls of *A. flavus* conidia were smooth to finely roughened or moderately roughened, pitted and spiny. These observations were consistent with the findings of Abdi and Alemayehu [46] who reported *A. flavus* colonies as being initially yellow, turning to yellow-green or olive-green with age and appearing dark-green with smooth shape and some having radial wrinkles.

Another species isolated from the collected groundnut samples was *Aspergillus niger*. The major distinction currently separating *A. niger* from the other species of *Aspergillus* is the production of carbon black or dark brown spores of biseriate phialides [40]. The current study also confirmed the production of black or brown-black conidia by this species. On Czapek dox agar, colonies consist of a compact white or yellow basal felt covered by a dense layer of dark-brown to black conidial heads. Conidial heads are large (up to 3 mm x 15-20 um in diameter), globose, dark brown, becoming radiate and tending to split into several loose columns with age. Conidiophores are smooth-walled, hyaline or turning dark towards the vesicle. Conidial heads are biseriate with the phialides borne on brown, often septate metulae. Conidia are globose to subglobose (3.5-5.0 um in diameter), dark brown to black and rough-walled.

Aspergillus parasiticus was the third *Aspergillus* species isolated from groundnut samples collected from groundnut value chain actors. Colonies representing this species also grew fast on CZDA at 25 and 37°C producing dark-green and rough conidia 5-7 days after incubation. A similar study by Peterson [47] distinguished *A. parasiticus* from *A. flavus* by its typically dark-green color on CZDA. *A. parasiticus* had dark-green colonies with a diameter of between 24–36 mm, was predominantly uniseriate or uniseriate with 20% biseriate and had rough conidia.

The fourth *Aspergillus* species identified in the current work, *A. ochraceus*, produced yellow-gold conidia [48]. Historically, the *A. ochraceus* group has embraced Aspergilli with biseriate sterigmata and heads of yellow or ochraceus conidia that are small, thin-walled, and smooth or nearly so according to Raper and Fennell [40]. The *A. ochraceus* was characterized particularly by its pale yellow conidial heads, orange-red conidiophores with coarsely roughened walls, light colored sclerotia, and salmon-pink mycelial turf on the reverse side of Czapek's Dox Agar (CZDA). Colonies of this species also produced near white to light yellow pigment and were dull yellow to dark yellow or sometimes brown on the reverse. They also had wrinkled mycelial growth [49]. The other species identified in the current work, *Aspergillus tamari*, had dark-brown colonies with a diameter of 2–10 mm, was uniseriate with spiky globose conidia. The reverse of *A. tamarii* was bright orange in early stages and turned to dark-brown four days after incubation. Isolates with brown to yellow-brown colonies on agar were classified as belonging to *A. tamarii*.

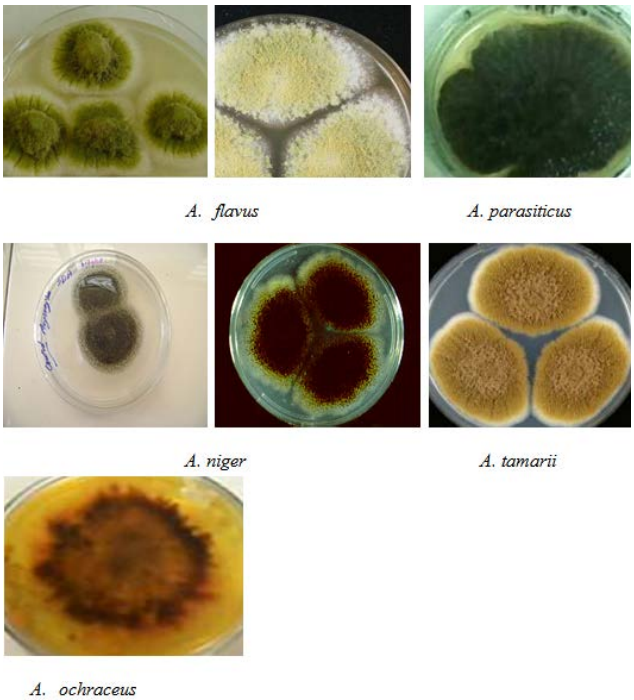

Figure 1. *Aspergillus* species identified from groundnut seed samples along the value chain actors

3.3. Frequency of Groundnut Seed Contamination

In this study, 210 samples were collected from the groundnut value chain actors, i.e. from farmers' fields, farmers' stores, market retailers and vendors within the three districts in different agro-ecologies of eastern Ethiopia and analyzed in the laboratory to identify

Aspergillus species associated with the seed samples and to determine their relative frequencies.

Proportion of groundnut seed contamination by *Aspergillus* species varied from 0.5% from vendors of roasted groundnut seed samples to 85% at farmers' fields in Babile, Fedis and Gursum districts in eastern Ethiopia. Groundnut samples collected from farmers' storehouses had the second highest (80%) seed contamination, whereas groundnut samples collected from that of market retailers had 60% contamination. The finding of the present study is in agreement with the findings of Abdi and Alemayehu [46] who reported that groundnut samples collected from farmers' fields and farmers' store houses in Babile and Gursum districts had 80 and 70% kernel contamination, respectively, by *Aspergillus* species.

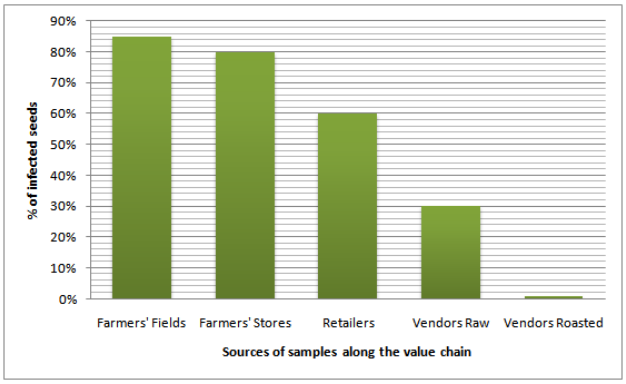

Figure 2. Proportion of groundnut seeds infected with *Aspergillus* species along the value chain in eastern Ethiopia in 2014 (N= 210)

Table 2. Frequency of *Aspergillus* species isolated from groundnut seeds along the value chain actors in different agro-ecologies of eastern Ethiopia in 2014

Distri-ct	Agro-ecology	Fungal species	Percent seed infection				
			Farmers' Fields	Farmers' Stores	Retailers	Vendors	
						Raw	Roasted
Babile	LLD	*A. flavus*	22.05	30.02	27.05	20.25	0.00
		A. niger	40.25	36.75	30.25	23.05	0.00
		A. parasiticus	15.05	8.05	6.05	5.27	0.00
		A. ochraceus	10.07	10.07	9.25	3.16	0.00
		A. tamari	2.05	0.00	0.00	0.00	0.00
	LLM	*A. flavus*	27.65	20.77	16.01	17.75	0.00
		A. niger	23.83	20.25	14.75	12.03	0.00
		A. parasiticus	20.22	19.74	12.22	11.05	0.00
		A. ochraceus	22.11	21.25	18.23	9.75	0.00
		A. tamari	4.99	3.95	4.18	2.01	0.00
Fedis	MLD	*A. flavus*	32.55	33.04	30.15	20.84	0.00
		A. niger	48.11	44.13	42.16	25.01	0.00
		A. parasiticus	11.75	9.25	8.25	2.07	0.00
		A. ochraceus	10.32	12.45	8.75	3.12	0.00
		A. tamari	6.00	2.00	0.00	0.00	0.00
	MLM	*A. flavus*	49.05	42.14	35.21	30.27	0.00
		A. niger	35.80	41.25	40.00	27.32	0.00
		A. parasiticus	11.09	8.31	7.05	3.00	0.00
		A. ochraceus	10.75	8.50	7.09	5.03	0.00
		A. tamari	12.00	11.00	9.06	4.00	0.00
Gursum	HLH	*A. flavus*	45.27	39.13	32.25	20.19	0.00
		A. niger	30.45	32.34	30.14	19.27	0.00
		A. parasiticus	23.46	11.75	3.09	6.17	0.00
		A. ochraceus	1.65	6.45	2.05	0.00	0.00
		A. tamari	0.00	0.00	0.00	0.00	0.00

The frequency of *Aspergillus* species isolated from the groundnut seed samples along the groundnut value chain actors has been analyzed and presented for different agro-ecologies in the current study (Table 2). Five different *Aspergillus* species were identified from 210 groundnut seed samples along the groundnut value chain actors. Of the several *Aspergillus* species identified from the groundnut seed samples, *A. flavus* and *A. niger* were the most prevalent mycotoxigenic fungi across the farmers' fields, farmers' stores, market retailers and vendors in Babile, Fedis and Gursum districts in the five agro-ecological zones of eastern Ethiopia. However, *A. parasiticus*, *A. ochraceus*, and *A. tamarii* occurred rarely in association with the groundnut seed samples.

The frequency of occurrence of *A. flavus* and *A. niger* was the highest from farmers' fields in Fedis districts in mid-land 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 such as low-land dry (LLD), low-land moist (LLM), mid-land dry (MLD), mid-land moist (MLM), and high-land humid (HLH). The percentage frequency of the five identified *Aspergillus* species in this study was almost nil from roasted groundnut seed samples of vendors in the five agro-ecological zones. This was because roasting kills aflatoxin producing-fungi and hence groundnut seed roasting processes would reduce the risk of aflatoxigenic fungi contamination. The present finding is in agreement with previous studies by Govrama and Bullar [45] who reported that groundnut seed roasting processes reduced the risk of aflatoxigenic fungi contamination. The current study revealed that there was higher risk of exposure to aflatoxigenic fungi through raw than roasted groundnuts. Roasting is one of the effective physical methods to remove or reduce aflatoxin content in foodstuff, therefore, this will reduce possible health risks associated with aflatoxin to the consumers. In another experiment, Eshetu [43] reported the most frequent occurrence of *Aspergillus* species (*A. flavus*, *A. niger* and other Aspergilli) in wet shelled one year stored peanut sample from Gursum district of Hararghe region in East Ethiopia.

From among the *Aspergillus* species isolated in the current study, *A. flavus* and *A. niger* were more prevalent species infecting groundnut samples collected from

farmers' fields than from farmers' stores, market retailers and vendors in the five agro-ecologies of eastern Ethiopia. The current results are in agreement with the study by Pitt and Hocking [50] who indicated that *Aspergillus* species were more prevalent in the field and stored foods than in the markets. These two species were isolated at a rate of 22.05-49.05% (*A. flavus*) and 23.83-48.11% (*A. niger*). Their relative dominance in number of *A. flavus* and *A. niger* isolates from the total fungi was 23 and 22%, respectively.

Groundnut seed samples along the value chain actors were also moderately infected by *A. parasiticus* (0.00-15.05%), *A. ochraceus* (0-22.11%) and *A. tamarii* (0-12.00%). The isolation frequency of *A. parasiticus, A. ochraceus* and *A. tamarii* was not consistently high or low in any of the seed samples from the various collection sites (farmers' fields, farmers' stores, market retailers and vendors). When agro-ecological zones were compared, the frequency of occurrence of *A. flavus* and *A. niger* identified in the current study was highest in mid-land moist zone and lowest in the low-land dry zone. The high temperature and periodic drought prevalent in mid-land moist zone could be ascribed to the higher levels of *A. flavus* and *A. niger* in that climate. In addition, unfavorable drying and storage practices might aggravate the problem. Moreover, the environmental conditions, especially temperature, relative humidity and/or moisture prevailing in the mid-land moist zone, might be responsible for this established trend. Moreover, the reason that *A. flavus* and *A. niger* was high in farmers' fields was that the sources of inocula are sporogenic sclerotia, conidia and mycelia that over-winter in plant debris and the farmers' fields was repeatedly cropped to groundnut since it is the main groundnut production areas nationally in Eastern Ethiopia, so that conidia from sporogenic sclerotia are the primary source of *A. flavus* and *A. niger* inocula.

Aspergillus species were commonly found in stored grain or food and could produce aflatoxins [51]. Abdela [52] showed that *Aspergillus niger* and *Aspergillus flavus* were isolated from all samples of investigated peanut in the Sudan; *A. niger* occurred in 29–60% and *A. flavus* in 4–52% of the kernels. *Fusarium oxysporum*, *A. niger*, *R. bataticola* and *S. rolfsii* were the predominant species of fungi associated with diseased plants indicating the involvement of these fungi in pre- and post-emergence death of groundnut plants in Babile district [53,54].

4. Conclusion

Groundnut is an important cash crop for domestic markets as well as for foreign trade in several developing and developed countries. It is one of the most important cash crops in eastern Ethiopia too and its production is constrained by *Aspergillus* species, which cause quantitative losses and produce highly toxic and carcinogenic chemical substances known as aflatoxins.

In this current study, 210 groundnut seed samples were collected from farmers' fields, farmers' stores, market retailers and vendors in Babile, Fedis and Gursum districts, and were tested for their moisture contents and *Aspergillus* species contamination. The seed moisture data showed that the moisture content of samples ranged between 3 and 15%; and the lowest moisture content was obtained from groundnut seed samples collected from vendors and the highest was recorded in seed samples collected from farmers' fields at harvest. According to Codex Alimentarius Commission, the maximum allowable moisture content in groundnut is 10% and above this maximum range can support mould growth during storage and aflatoxins contamination.

In the current study, five different *Aspergillus* species were associated with groundnut seed samples collected from the groundnut value chain actors in different agro-ecologies of eastern Ethiopia and were identified and characterized. The proportion of seed contamination by *Aspergillus* species varied from 0.5% in roasted groundnut seeds from vendors to 85% in seed samples collected from farmers' fields in Babile, Fedis and Gursum districts in eastern Ethiopia. Groundnut samples collected from farmers' store houses had the second highest (80%) seed contamination, whereas groundnut samples collected from that of market retailers had 60% contamination.

In the current study, five different *Aspergillus* species were identified from 210 groundnut seed samples along the groundnut value chain actors. Of the several *Aspergillus* species identified from the groundnut seed samples, *A. flavus* and *A. niger* were the most prevalent mycotoxigenic fungi across the farmers' fields, farmers' stores, market retailers and vendors in the five agro-ecological zones such as low-land dry (LLD), low-land moist (LLM), mid-land dry (MLD), mid-land moist (MLM), and high-land humid (HLH), of Babile, Fedis and Gursum districts in eastern Ethiopia. On the other hand, *A. parasiticus, A. ochraceus* and *A. tamarii* occurred rarely. The frequency of occurrence of *A. flavus* and *A. niger* was high in seed samples from farmers' fields in mid-land moist agro-ecological zones of Fedis district and the least was in roasted groundnut seed samples obtained from vendors in all the five agro-ecological zones of Babile, Fedis and Gursum districts.

In conclusion, *A. flavus* and *A. niger* were the most dominant species infecting groundnuts in eastern Ethiopia. These two species were isolated at a rate of 22.05-49.05% (*A. flavus*) and 23.83-48.11% (*A. niger*). Their relative dominance in number of isolates from the total fungal population was 23 and 22%, respectively.

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