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Incidence of *Aspergillus* contamination of groundnut *(Arachis hypogaea* L.) in Eastern Ethiopia

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The production of groundnut is constrained by several factors, among which is *Aspergillus* spp. In addition to causing quantitative losses, *Aspergillus* spp. produce highly toxic and carcinogenic chemical substances known as aflatoxins. This study was conducted with the objectives to (i) identify *Aspergillus* species associated with groundnuts, (ii) determine the frequency of seed contamination, and (iii) survey agro-ecological conditions related to groundnut contamination by *Aspergillus* spp. About 270 groundnut samples were collected from farmers' storage, fields and local markets of three districts that is, Babile, Darolabu and Gursum of Eastern Ethiopia for mycological analysis in the year 2010. Results of the mycological analysis suggested heavy infestation of groundnut samples by various molds including *Aspergillus niger, Aspergillus flavus, Aspergillus ochraceus, Aspergillus parasiticus* and *Pencillium* species. At the district level, the incidence of infected groundnut kernels ranged from 50 to 80%. Within the district kernel infection varied between 36.3 and 100%. The common *Aspergillus* symptoms (yellowing or chlorotic leaves, wilting, drying and brown or black mass covered by yellow or greenish spores) were also observed in groundnut fields. The current results were consistent with our earlier report of heavy aflatoxin contamination of groundnut from the same places, suggesting the urgent need to apply control measures against toxigenic fungi and associated mycotoxins.

Key words: *Aspergillus* spp., groundnut, *Penicillium* spp., Ethiopia.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an important food and feed crop, which also serve as component of crop rotation in many countries (Pande et al., 2003; Upadhyaya et al., 2006). Groundnuts are also significant source of cash in developing countries that contribute significantly to food security and alleviate poverty (Smart et al., 1990). Developing countries account for 97% of the world's groundnut area and 94% of the total production (FAOSTAT, 2010). However, groundnut yield in this part of the world and particularly in Africa is lower than the world average due to prevailing abiotic, biotic and socio-economic factors (Pande et al., 2003; Upadhyaya et al., 2006; Caliskan et al., 2008).

In warm climates grains are easily infected with toxigenic microorganisms like *Aspergillus* species. *Aspergillus* spp. are facultative parasites. They 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 harvest equipment (Pettit and Taber, 1968). They are distributed worldwide, mainly in countries with tropical climates that have extreme ranges of rainfall, temperature and humidity (Pettit and Taber, 1968). Many strains of this fungus are capable of producing aflatoxins that render the seed unacceptable due to high toxicity for human or animal consumption (Reddy et al., 1996). Aflatoxins are highly toxic metabolites associated with Reye's syndrome, Kwashiorkor and acute hepatitis

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(Wild and Hall, 1999; Wild and Turner, 2002).

The Eastern lowland areas of Ethiopia have considerable potential for increased oil crop production including groundnut. Particularly areas such as Babile, Darolabu and Gursum are the major producers of groundnuts for local and commercial consumption (Getnet and Nugussie, 1991; Chala et al., 2012). Nevertheless, the area may also be very conducive for toxigenic fungi like *Aspergillus* spp. owing to its warm and dry climate. Moreover, farmers' practices of production and handling of groundnut at pre- and pos-harvest stages may provide favorable conditions for outbreaks of fungi and their mycotoxins. As Chala et al. (2012) reported, groundnut from East Ethiopia is heavily contaminated by aflatoxins at levels much more than international standards, and this might be associated with infection of the crop with *Apsergillus* spp., mainly *A. falvus* and *A. parasiticus* that are known producers of aflatoxins. However, up to date information on the prevalence of fungi, and studies on environmental factors and farmers' practices that promote fungal contamination, which could be basis for the reduction of mycotoxins are limited under Ethiopian conditions. On the other hand, such studies are of paramount importance to give valid recommendations for safe consumption and marketing of groundnut. The objectives of this study were to: i) identify *Aspergillus* species associated with groundnut in East Ethiopia, ii) determine the frequency of seed contamination, and iii) survey agro-ecological conditions related to contamination of groundnut by *Aspergillus* spp.

MATERIALS AND METHODS

Surveys of groundnut and sample collection

Groundnut samples were collected from three districts (Babile, Darolabu and Gursum) of Eastern Ethiopia in the year 2010. The samples were collected in three groups that is, from farmers' stores, fields and from local markets of selected locations of the three districts. Geographic description of survey locations are given in Table 1. In each district, five locations were chosen based on the groundnut productions potential. Six samples were collected from each location and hence 30 samples were collected per district making the total number of samples collected from all over the three districts 270 (3 districts × 5 locations per district × 6 samples per location × 3 sample groups per district). Data were also gathered on planting and harvesting dates of the crop, the variety, soil type, previous crop, and types of cultural practices. The common methods of storage structures, drying materials and grain moistures were observed.

Fungal isolation, species identification

Fungal isolation

Fifty groundnut seeds per sample were surface sterilized with 10% Chlorox solution for 1 min, followed by immersion in sterile distilled water for 1 min. Surface sterilized seeds were then placed on freshly prepared potato dextrose agar (PDA) plates (five seeds per plate) and incubated for three days at 25°C. Pure cultures of different out growing fungi were obtained by transferring fungal colonies to new PDA plates 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.

Species identification

Isolates were identified to a species level based on morphological (phenotypic) features as described by Cotty (1994), Egel et al. (1994), Kurtzman et al. (1997), and Okuda et al. (2000). For this purpose: Isolates representing each pure culture were grown on Czapek Dox Agar (CZDA) and PDA at 25°C for 5-7 days. Fungal colonies that grew rapidly and produced colors of white, yellow, yellow-brown, brown to black or shades of green, mostly consisting of a dense felt of erect conidiophores were broadly classified as *Aspergillus* spp. while those that produce blue spores were considered as *Pencillium* spp. (Okuda et al., 2000). Isolates with dark green colonies and rough conidia were considered *A. parasiticus* (Klich, 2002). 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 (Raper and Fennell, 1965). Those that showed brown colony with orange and cream reverse sides were considered *A. sojae* and *A. oryzae*, respectively (Cotty, 1994). Those, which produce conidia with smooth surface on CZDA and colonies typical of *A. flavus* were recorded as *A. flavus.*

Data analysis

Data on frequencies of kernel infection by *Aspergillus* and *Penicillium* species for samples collected from different locations of the districts were subjected to analysis of variance (ANOVA) using the SAS computer package, version 9.11 (SAS, 2003). LSD test at the 0.05 probability level was used for mean comparison.

RESULTS AND DISCUSSION

Identification of *Aspergillus* species associated with groundnut

Four different *Aspergillus* spp. were found to be associated with groundnut samples collected from Eastern Ethiopia (Figure 1). The first species isolated from the collected samples was *A. 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 (Raper and Fennell, 1965). The current study also confirmed the production of black or brown-black or black conidia by this species (Figure 1).

A. flavus was the second species identified in this study. Colonies of this fungus 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 (Gourama and Bullerman, 1995). In general, *A. flavus* was known as a velvety, yellow to green or the old colony was brown mould with a goldish to red-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 Gourama and Bullerman (1995), who reported *A. flavus* colonies as being initially

Table 1. Geographic description of locations included in the survey.

District	Location	Latitude	Longitudes	Altitude (m)
	Ausharif	09^{0}11'933"	042^{0}13'080"	1733
	Shekabdi	09^{0}12"890"	042^{0}13'65"	1437
Babile	Shekusman	09^{0}12'89"	042^{0}13'105"	1784
	Ifa	09^{0}17'762"	042^{0}17'161"	1500
	Bishan Babile	09^{0}15'872"	042^{0}18'049"	1500
	Sakina	08^{0}35'023"	040^{0}20'388"	2100
	Gadulo	08^{0}26'078"	040^{0}15'732"	1400
Darolabu	Odalaku	08^{0}35'067"	040^{0}19'842"	1760
	Sororo	08^{0}35'036"	040^{0}19844"	1608
	Haroadi	08^{0}34'954"	040^{0}20'280"	1788
	Audal	09^{0}37'297"	042^{0}43'951"	1792
	Ilalam	09^{0}37'268"	042^{0}43'758"	1838
Gursum	Kassa oromiya	09^{0}37'282"	042^{0}43'610"	1650
	Oda oromiya	09^{0}37'273"	042^{0}43'633"	1732
	Harobate	09^{0}37'298"	042^{0}43'832"	1550

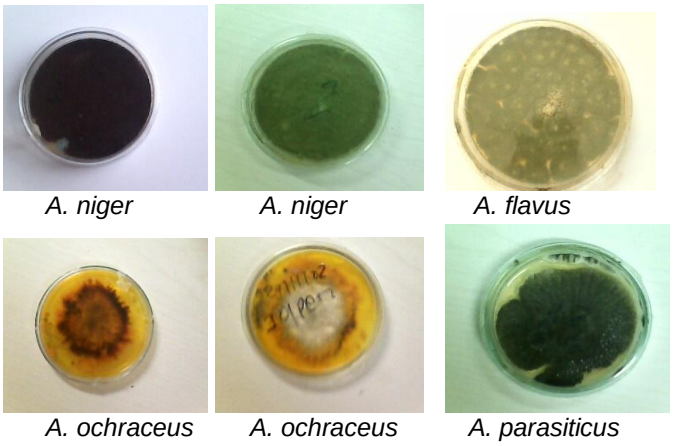

Figure 1. *Aspergillus* spp. isolated from groundnut samples.

Figure 2. Symptoms of *Aspergillus* infection on groundnut.

yellow, turning to yellow-green or olive green with age and appearing dark green with smooth shape and some having radial wrinkles.

The third species identified in the current work, *A. ochraceoroseus,* produced yellow-gold conidia (Bartoli and Maggi, 1978). 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 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.

A. parasiticus was the fourth species isolated from groundnut samples tested in the current study. Colonies representing this species produced dark green and rough conidia on CZDA at 25 and 37°C after 5-7 days of incubation. Similar study by Peterson et al. (2001) distinguished *A. parasiticus* from *A. bombycis* by its typically dark green color on CZDA.

Groundnut plants infected by *Aspergillus* spp. showed typical symptoms of infection as shown in (Figure 2). These include yellowing of leaves or chlorosis and premature death and dropping of leaves, wilting, drying single plant and patching or drying of localized areas within the fields. Underground grains were rotten and distorted, and the plants were pulled out of the ground easily. On some dried plants, brown or black mass covered by yellow or greenish spores were observed in infected fields. A study by Subrahmanyam and Ravindranath (1988) stated the presence of shriveled and dried grains covered by yellow or green spores, when groundnut plants are infected by *A. flavus* (Aflaroot or yellow mold). The same study reported highly stunted seedlings; reduced leaf size with pale to light green; crown rot or collar rot, with germinating seeds covered with masses of black conidia; and rapid drying of plants due to infection of groundnut by *Aspergillus* spp.

Frequency of kernel contamination

Proportion of kernel contamination by *Aspergillus* spp. varied from 50% at Babile market to about 80% at farmers'

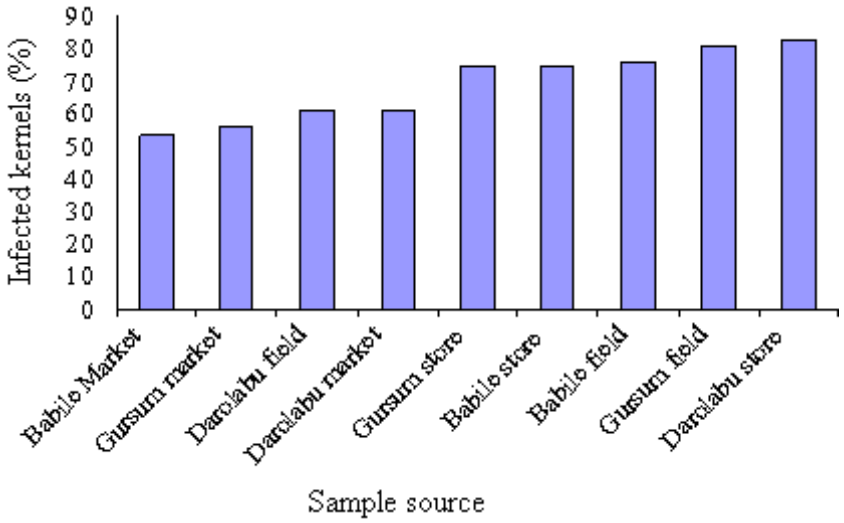

Figure 3. Proportion of groundnut kernels infected with *Aspergillus* spp. in East Ethiopia (N= 150 seeds/samples).

fields in Gursum district and storage houses in the district of Darolabu (Figure 3). Groundnut samples collected from storage houses in the districts of Gursum and Babile, and farmers' fields at Babile had the second highest kernel contamination (70%).

When samples were analyzed at the location level (within district), kernel contamination varied significantly ($p<0.05$) from 36% at Bishan Babile market (Babile district) to 100% in farmers' fields of Harobate (Gursum district) (Table 2). Samples from stores in Bishan Babile and Shekabdi kebeles (Babile district), stores in Sororo location of Darolabu district, farmers' field in Kasa Oromiya of Gursum district were infected at a proportion of 93%.

In addition, groundnut samples collected from farmers' fields in Ausharif and Shekabdi location of Babile district, stores of Sakina and Odalaku (Darolabu district), and stores of Audal and Harobate in Gursum district were found to be contaminated at a rate of 90%. On the other hand, samples from markets at Shekusman location (Babile district), Sororo of Darolabu district and Oda Oromiya (Gursum district) had only 43% kernel infection. Generally, the current results suggest heavy contamination of groundnut kernels with *Aspergillus* spp. Groundnut samples collected from markets had the lowest level of *Aspergillus* infection compared to samples from farmers' fields and stores across the survey districts and locations. This may suggest possible sorting of kernels by farmers to avoid those with visible symptoms of infection (discoloration) before bringing the samples to the local markets. The results were in agreement with the study by Pitt and Hocking (2009) in which *Aspergillus* spp. were more prevalent in the field and stored foods than in the markets.

The frequency of *Aspergillus* isolated from the groundnut samples analyzed in the current study is presented in Table 3. Of the several species isolated from the groundnut samples, *A. niger* and *A. flavus* were the most prevalent mycotoxigenic fungi across the storage, field and market samples in East Ethiopia. These two species were isolated at a rate of 21-48% (*A. flavus*) and 35-66% (*A. niger*). On the other hand, *A. ochraceus* accounted for 0-14%, while *A. parasiticus* was associated with 2-21% of kernel infection. *Pencillium* spp. were also isolated from 6-13% of samples analyzed (data not shown). In another experiment, Eshetu (2010) reported the most frequent occurrence of *Aspergillus* spp. (*A. flavus, A. niger* and other *Aspergilli*) in wet shelled one year stored peanut sample from Gursum district of Hararghe region in East Ethiopia.

In this study; *A. flavus* and *A. niger* were isolated at higher frequencies from samples collected from farmers' fields and stores than markets while *A. parasiticus* was consistently isolated at higher frequencies from market samples. In contrast to these, isolation frequency of *A. ochraceus* was not consistently high or low in any of the sample collection sites (fields, markets and stores) across the survey districts. The current results were consistent with Chala et al. (2012), who detected 5-11,900 µg/kg total aflatoxin from same samples suggesting heavy groundnut contamination by *Aspergillus* spp. and associated aflatoxins in the region. *Aspergillus flavus* and aflatoxins contamination was also prevalent in stored groundnuts in Ghana (Awuah and Kpodo, 1996). Abdela (2009) also reported contamination of groundnut samples from Sudan by *A. niger* and *A. flavus*, which were isolated at frequencies of 29-60% for *A. niger* and 4-52% for *A. flavus*. *Fusarium oxysporum, A. niger, R. bataticola* and *S. rolfisii* 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 (Tefera and Tana, 2002).

Table 2. Proportion of groundnut seeds infected by *Aspergillus* spp. in different districts of East Ethiopia.

District	Location	Percent kernel infection		
		Store	Field	Market
Babile	Ausharif	50[c]	90[a]	53.3[bc]
	Shekusman	70[b]	63[b]	43.3[cd]
	Shekabdi	93.3[a]	90[a]	70[a]
	Ifa	66.7[b]	63[b]	56[b]
	Bishan babile	93.3[a]	70[b]	36.3[cd]
	LSD (0.05)	12	11	21.8
	CV (%)	14	13	20
Darolabu	Sakina	90[a]	70[b]	56.7[ab]
	Gadulo	73.3[b]	53.3[b]	66.7[a]
	Odalaku	90[a]	56.7[b]	66.7[a]
	Sororo	93[a]	53.3[b]	43[c]
	Haroadi	66.7[b]	76[a]	46.7[bc]
	LSD (0.05)	12	11	11
	CV (%)	12	15	17
Gursum	Audal	90a	73.3b	56.7c
	Ilalam	56.7c	70b	66ab
	Kasa Oromiya	63.3b	93.3a	65bc
	Oda Oromiya	73.3b	66.7b	43.3cd
	Harobate	90a	100a	46.7cd
	LSD (0.05)	13.4	11	7
	CV (%)	15	11	12

Means in a column followed by the same letter are not significantly different according to LCD at $p<0.05$.

Table 3. Frequency of *Aspergillus* species isolated from groundnut seeds collected from three districts in East Ethiopia.

District	Fungal specie	Percent kernel contamination		
		Store	Field	Market
Babile	*A. flavus*	20.5	29	26.25
	A. niger	66	47.8	38.75
	A. parasiticus	3.6	14	21.25
	A. ochraceus	9.8	9.7	13.75
Darolabu	*A. flavus*	31	32.6	33.7
	A. niger	56	53.6	41
	A. parasiticus	4	2.1	10.8
	A. ochraceus	9	10.5	8.7
Gursum	*A. flavus*	48.2	41	34.5
	A. niger	34.82	40.5	39
	A. parasiticus	4.46	10.74	14.3
	A. ochraceus	9.82	7.43	0

Description of local groundnut varieties around selected areas

Description of groundnut varieties in around Babile and Gursum districts are different from that of Darolabu districts. Their naming was based on the morphology standing from the ground and leaf shape, size, color and seed size. Around Babile and Gursum districts, the common

local groundnut varieties were “Oldhale”, “Sartu”, and “Jawsi” (Data from Agricultural and Rural Develop-ment Bureaus of the districtcs). Another similar study re-ported that predominant groundnut local variety (Oldhale) was commonly produced by farmers around Babile district (Tefera and Tana, 2002). The shape and leaf size of these three varieties were different from each other. Two groundnut varieties that is, “Basuqa”and “Qacine” are found to grow commonly around Darolabu district. They are identified based on the plant morphology and seed size. The “Basuqa” variety has larger seeds and leaves than “Qacine”. It gives higer yield than “Qacine”. But it is more susceptible to diseases and drought as revealed by the Agricultural and Rural Development Bureau of the district.

Factors associated with *Aspergillus* spp. contamination of groundnuts in the study areas

Cultural practices

Across the study areas, the groundnut shell is removed and left in farms, on the roads or water furrows then irrigation water takes it to the farms and gardens. Such a scenario generally favors over seasoning of plant patho-gens including *Aspergillus* for subsequent contamination of the next crops. Besides, groundnut in the survey dis-tricts is cultivated either as a sole crop or intercropped with different crops like sorghum (*Sorghum bicolor*), maize (*Zea mays*), haricot bean (*Phaseolus vulgaris*), and under the shade of chat (*Khata edulis*), coffee (*Coffea arabica*) and mango (*Mangifera indica*) trees. Although intercrop-ping is generally known to decrease disease pressure in agricultural fields, the companion crops in groundnut fields may not be very effective against *Aspergillus* spp.

Mechanical damages caused during pulling and digging out groundnuts may also have predisposed kernels to infection by *Aspergillus* and associated aflatoxins. Seeds in split pods are frequently invaded by *A. flavus* and subsequently become contaminated with aflatoxin as suggested by (Graham, 1982). Shriveled kernels contain higher amounts of aflatoxin as a result of higher *A. flavus* infection (Hill et al., 1984). Other factors, which may aid the contamination of groundnut with *Aspergillus* and then aflatoxins might be exchanging of equipment or plowing materials and groundnut seeds between households.

Time of planting and harvesting of groundnuts

Rain fall is usually unpredictable at the time of planting and harvesting around the study areas. The planting time of groundnut around the study areas usually lasts from the beginning of April to the end of May, and the har-vesting time is from the end of September to the first half of November according to the field conditions and availa-bility of labor. However, pods may overstay in the field after optimum maturations due to lack of labor, which further increases *Aspergillus* infection. Drought stress during late stages of pod development favors inva-sion of groundnut by *A. flavus* and subsequent aflatoxin conta-mination. End of season drought was very critical as it affects grain filling and pod formation in groundnut (Hill et al., 1984).

Drying method of groundnut around the study areas

Groundnut crops harvested in the survey districts are usually sun dried on materials such as matting. Curing of groundnut was done for few days in the farms before drying and removing of the seed from the hulms, to re-move some moisture content from the kernels. In cases, where the moisture content of threshed unshelled and shelled pods was too high, the pods were sometimes bagged and every day the bags were brought out from the stores and left in the open. Although such practices may help reduce *Aspergillus* invasion, it would have been made more effective had it been coupled with fumigation with burning cow dung fumes and sun drying for one day as suggested by Gehewande et al. (1986).

Traditional groundnut storage system

Storage structures commonly found in the survey districts are made from mud and animal dung. In mud house there was no improved aerations in some stores and in some rain could percolate from the top and from the sides. Groundnuts in such houses are usually stored in sacks from the previous years, and this may also increase the contamination of groundnuts by *Aspergillus* and aflatoxins. Dickens et al. (1973) associated moisture condensation on roofs, improper application of insecticide sprays or leaking hoses and application equipment, conveyance of water from flooded elevator dump pits into warehouse, and storage of peanuts on concrete floors that are damp or have no vapor barriers with increased *A. flavus* growth. According to Bankole and Adebanjo (2003), traditional storage structures used for on farm storage include containers made of plant materials (woods, bamboo, and thratch).

Conclusion

Results of the current survey revealed heavy contamina-tion of groundnuts by *Aspegillus* spp. Infection of kernels were higher in farmers’ store and farmers’ fields than markets with incidence of kernel infection at district level ranging from 36% at Babile market to 100% at Gursum field. *A. niger* and *A. flavus* were the most dominant spe-cies infecting groundnuts in East Ethiopia.

In combination with our earlier report on aflatoxin contamination of groundnut from East Ethiopia, the cur-rent results should serve as a wakeup call to create aware-

ness on toxicogenic fungi and associated mycotoixns in the country. As aflatoxins are associated with health risks, reducing their level in food stuff to a level accepted by international standard is paramount importance to ensure the safety of these food stuff to consumers thereby facilitate trade both within and between countries.

Five local groundnut varieties are found to grow in the survey districts. These are “Sartu”, “Oldhale”, “Jawsi”, “Basuka” and “Qacine”. Although these varieties vary in terms of morphological features and disease resistance, their resistance to *Aspergillus* and aflatoxins under differing environmental conditions is not established beyond ambiguity. Thus future work should also focus in testing varieties for Aspergillus resistance under different environmental conditions and management practices.

This study has identified some important factors that may contribute for aflatoxin contamination of groundnuts both pre- and post-harvest. These include: weather conditions, seed and equipment sharing, planting time; harvesting time and methods; curing, drying and storage practices. The roles of additional factors that contribute to aflatoxin contamination down in the value chain need further investigation. In addition, the role of none chemical seeds treatments especially essential oils and that of biological control agents in reducing groundnut contamination should be studied to come up with a more effective and sustainable management strategy. Farmers’ association and extension agents should also be encouraged in creating awareness about aflatoxins and management techniques.

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