On Farm Varietal Diversity and Prevalence of *Aspergillus* Contamination of Groundnut (*Arachis Hypogaea* L.) in Major Production Areas of Oromia Region, Ethiopia

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Abstract

Background: Groundnut is one of the most valuable cash crops and food sources in low lands of Ethiopia. In spite of its food and economic importance, very few studies have been conducted yet on the factors that influence groundnut production including aflatoxigenic fungi in different socio-ecological conditions. Therefore, this study aimed at i) assessing groundnut varietal diversity ii) to determine the existing use values and management practices iii) to isolate and determine the prevalence of *Aspergillus* species associated with groundnut seed.

Methods: Field data were collected using structured and semi-structured interviews from 144 general informants and 24 key-informants. 88 groundnut seed samples were collected from farmers' fields for *Aspergillus* species isolation. Diversity of groundnut was analyzed using the Shannon diversity index. Frequency of *Aspergillus* contamination was determined using plate counting methods.

Results: In the study areas, six varieties were grown with varying distribution. Shannon diversity indices ranged from 0.20 to 1.27 in warm sub-humid lowland and warm moist lowland agro-ecological zones, respectively. Of the four varieties observed in East Hararghe, oldhale was the most commonly grown variety (66% of groundnut growers), whereas farmers interviewed in East Wellega zone grow bure variety (87% of growers), due to greater drought tolerance and higher yields relative to other varieties. Results of the fungal species analysis showed infestation of groundnut seeds by *A.niger*, *A. flavus*, *A. prasaticus* and *A. ochraceous*. Of the four *Aspergillus* species, *A. niger* and *A. flavus* were the most prevalent, with frequency ranging from 17.8 to 37.5% and 14.4 to 32.5%, respectively. However, when we compare infection rates among varieties, we did not find statistically significant differences in the infection rates of different varieties collected from similar agroecology of the study area.

Conclusion: Different varieties of groundnut were grown with varying distribution based on the farmers' preference criteria in different socio-ecological zones of the study areas. Although these varieties vary in terms of morphological features and disease resistance, there is not sufficient evidence to conclude they are differentially resistant to *Aspergillus* infection. Therefore, further breeding for more varieties using farmer selection criteria in mind and research identify field management measures against *Aspergillus* will further increase groundnut production and hence income for resource poor farmers

Background

Groundnut (*Arachis hypogea* L.) is one of the world's most important oil seed crops, cultivated in tropical and subtropical countries [1]. Groundnut is primarily cultivated in the USA, Nigeria, Senegal, Sudan, China, West Africa, Indonesia and India. It is ranked as the four most important oilseed crop and the 13th most important food crop of the world [2]. It is the principal source of digestible protein, cooking oil and vitamins and is the chief crop rotation component in many sub-Saharan countries [2, 3]. With growing interest in bio-energy, possibilities are being tested for using groundnut as a bio-diesel crop, because groundnut produces more oil per hectare than any other food crop [4]. Groundnut is also one of the leguminous plants that plays an important role in improving soil fertility through symbioses with Rhizobia, which fix nitrogen and thereby

Groundnut was introduced to Ethiopia by Italian explorers in the 1920s [6]. It is one of the five widely cultivated oilseed crops in Ethiopia [7]. The production is concentrated in the south-eastern and north-western part of the country. It is produced in the five of nine regions found in Ethiopia with Oromia and Benishangul Gumuz regions account for nearly 90% of the groundnut grown in the country. Within these regions, Eastern Hararghe zone in Oromia region and Metekel zone in Benishangul-Gumuz region are the main centers for groundnut production [6, 8, 9].

In spite of its food, economic and industrial importance, groundnut production in Ethiopia is constrained by erratic and low rainfall, poor water management practices, poor soil fertility, crop diseases and insect pests, poor crop management practices, lack of higher-yielding improved varieties and lack of production technologies [8, 10]. The main biotic constraints in Ethiopia are termites, leaf spots and aflatoxin-producing fungal species [10–13]. The *Aspergillus* contamination and its associated aflatoxin are one of the most critical constraints of groundnut production [6, 8]. Aflatoxins are mutagenic, immunosuppressive and carcinogenic chemicals endangers health of humans and animals [14].

Genetic diversity is one of basic requirements for development of improved varieties through plant breeding [15]. Although groundnut is self-pollinating and possesses limited variability, significant genetic diversity has been observed among the studied groundnut varieties in Ethiopia [16, 17]. Knowledge of groundnut diversity remains a prerequisite for effective implementation of on-farm conservation strategies and improvement of varieties against *Aspergillus* infections. However, there is limited data on traditional groundnut landraces, their ability to resist *Aspergillus* contamination, and farmers' preference criteria in different socio-ecological settings of Ethiopia.

Although a survey-based study cannot detect variation at the genotypic level, it provides the way of identifying high-performing landraces and a feasible approach to estimate infraspecific diversity as well as the relative abundance of landraces [18]. Furthermore, identification of high-performing landraces based on farmer's preference criteria and isolation of *Aspergillus* species associated with each landrace will provide a better understanding of existing varietal diversity and important attributes breeders should use to develop new varieties to overcome production constraints. Therefore, the present study aims 1) to document farmers' groundnut landraces and the characteristics they use to differentiate them, 2) to analyze patterns of groundnut diversity in relation to agro-ecological and socio-cultural factors, 3) to determine the existing values of groundnut landraces to farming communities in different agro-ecological and socio-cultural contexts, 4) to describe existing management practices, including gender roles in groundnut production, use and conservation, and 5) to identify the *Aspergillus* species associated with groundnut landraces and estimate their prevalence in the study areas.

Methods

Study area description

The survey was undertaken in major groundnut growing areas identified based on reconnaissance surveys and production data obtained from Central Statistical Agency [19]. Field data and groundnut seed samples

were collected from twelve *kebeles* (village) (Table 1) spread over four *woreda* (districts) in two administrative zones, namely East Wellega and East Hararghe, both within Oromia Regional State (Fig. 1). Across all study areas, mixed farming of annual crops, tree and fruit crops and livestock are the dominant activity. The main crops produced in the area include maize, sorghum, groundnut, chat (*Catha edulis* (Vahl) Endl.) and sweet potatoes (*Ipomoea batatas* (L.) Lam.). Maize, sorghum and sweet potatoes are grown for home consumption while groundnut and chat are cash crop in the area. Although the majority of farmers in both East Wellega and East Hararghe identify as Oromo and speak Afaan Oromo, there are some cultural differences between the two sites on agronomic practice in farming system that may influence the production and use of crops, including groundnut.

Study design and informant selection

In order to maximize varietal diversity, the area was divided into four smaller areas based on a combination of agro-ecological and cultural factors. Each stratum had a unique combination of agro-ecological zone (as defined by Ethiopia's Ministry of Agriculture and Rural Development, [add citation]) with the different cultural identities found in East Wellega and East Hararghe. Three agro-ecological zones (Table 1) were selected based on the requirements of groundnut growth in Ethiopia [20]. One of these agroecological zones (SM2, or warm sub-moist lowlands) is found only in East Hararghe, and another (SH2, or warm sub-humid lowlands) is found only in East Wellega. The third agroecological zone (M2, warm moist lowlands) is found in both administrative zones and therefore provides a basis for comparison of cultural differences. Three kebeles were selected randomly from each agro-ecological and socio-cultural stratum. Groundnut growers were identified by the help of developmental agents in each kebele, who also assisted in stratifying them into two groups: those with medium-to-high income and those with relatively low income. From each randomly selected kebele, six households in each income groups (for a total of twelve households) were randomly selected for interviews. Within each income category, three women and three men were recruited, based on alternating the gender of interviewees as the research team moved from one household to the next.

A total of 144 groundnut producers (72 males and 72 females) were interviewed following a structured interview guide, using Open Data Kit (ODK) Collect on a mobile phone. Additionally, two key informants were interviewed in each kebele (Table 1), for a total of 24. Key informants were purposively selected based on recommendations from local farmers as well as local guides and agricultural extension experts in the district offices. Key informants were interviewed with a semi-structured interview guide. Free and informed consent was obtained prior to all interviews.

Table 11
Detail of the study sites and respondents

Major Agro-ecology	Zone	Districts	Kebeles (sub-districts)	No. of informants	
				General Informants	Key informants
Warm moist low land (M2)	East Hararghe	Babile	Tofik Ifaden Erer Guda	36	6
	East Wellega	Diga	Meda Jalela Bikila Lalisa Dimtu	36	6
Warm sub-moist lowland (SM2)	East Hararghe	Babile and Fedis	BishanBabile Berkele Umar Kule	36	6
Warm sub-humid lowland (SH2)	East Wellega	Gida Ayana	Tulu Lenca Warabo Gutin	36	6
Total	2	4	12	144	24

Sources and method of data collection

Structured and semi-structured interviews, field visits and group discussions [cite Martin and Cotton] were conducted with local farming households in October through December 2016. Interviews were conducted in Oromiffa and Amharic; answers were translated and documented in English. Topics included the landraces grown by the interviewee and others in their locality, including vernacular names and characteristics used to differentiate them; seed sources; use values; parts of the plant used, such as stem, pods and seed; preference for different landraces based on specific criteria; and gender roles in farming and management of groundnut.

Seed sample collection

Samples of unshelled groundnut seeds belonging to the four most common landraces were collected from the areas where they were mentioned. Two landraces (oldhale and sartu) were collected from the moist (M2) and sub-moist (SM2) lowlands of East Hararghe zone. The other two landraces (bure and dima) were collected in the moist (M2) and sub-humid (SH2) lowlands of East Wellega zone. For each landrace, composite seed samples of approximately 1 kg were collected, including four sub-samples per kebele, taken from different locations (Table 2). In each field, plants were randomly selected from three to five locations (based on the size of the field). Pods (unshelled groundnut seeds) were collected, pooled and placed in cloth

bags to allow air circulation, thereby reducing condensation and limiting fungal growth until analyses [10, 13].

Collected sample were labeled with relevant information on the date of sampling, GPS coordinates, altitude, local name of the landrace, soil type and cropping practice. A total of 88 samples were collected (one composite sample per kebele except dima which is collected from one kebeles in SH2), and then brought to Haramaya University Micro Biology laboratory. The collected samples were dried for one week and then the pods were hand shelled and maintained at about 4 C° until laboratory analyses.

Table 2
Description of groundnut seed sampling site and total sample

AEZ	Zone	Kebele	Latitude	Longitude	Altitude (m)	Cropping system	Varieties	Total sample
SM2	East Hararghe	Bishan Babile	9°11'33"	42°18'17"	1515	LCR	oldhale, sartu	8
	East Hararghe	Berkele	9°12'7"	42°18'22"	1530	IC	oldhale, sartu	8
	East Hararghe	Umar kule	9°10'19"	42°4'15"	1800	IC	oldhale, sartu	8
M2	East Hararghe	Tofik	9°15'42"	42°14'12"	1170	LCR	oldhale, sartu	8
	East Hararghe	Ifadin	9°13'20"	42°13'6"	1382	LCR	oldhale, sartu	8
	East Hararghe	Ererguda	9°13'55"	42°14'50"	1310	LCR	oldhale, sartu	8
	East Wellega	Bikila	9°4'17"	36°19'4"	1361	LCR	bure, dima	8
	East Wellega	Lalisadimtu	9°3'47"	36°14'8"	1251	LCR	bure, dima	8
	East Wellega	Madajalala	9°2'7"	36°13'30"	1282	LCR	bure, dima	8
SH2	East Wellega	Gutin	9°33'55"	36°37'51"	1375	IS and LCR	bure	4
	East Wellega	Warabo	9°32'13"	36°37'20"	1355	LCR	bure, dima	8
	East Wellega	Tulu-lenca	9°35'54"	36°38'19"	1367	LCR	bure	4

LCR = Legume-cereal rotation, IC = Intercropping with cereals, IS = Intercropping with sesame AEZ = agro-ecological zone, SM2 = Warm sub-moist lowlands, M2 = Warm moist lowlands, SH2 = Warm sub-humid lowlands, * = GPS signal is not available

A total of 880 (ten groundnut seeds per sample) were selected randomly and surface sterilized with 10% Clorox solution for one minute, followed by immersion in sterile distilled water for one minute. Surface sterilized seeds were placed on potato dextrose agar (PDA) plates and incubated for three days at 25°C. After few days of growth, pure cultures of different fungi were obtained by transferring each colony to new PDA plates using sterile toothpicks, and incubated for five to seven days at 25°C. Pure cultures of each isolate were then maintained at 4°C on PDA plates for identification to species level. Isolates representing each pure culture were grown on Czapek Dox Agar (CZDA) and PDA at 25°C for seven days. Fungal species were identified to species level based on morphological (phenotypic) features such as color and texture of mycelia, type of pigmentation, and conidial structure. Microscopic characteristics of spores, such as shape and color, were also used [21–24].

Prevalence of *Aspergillus* Contamination

After the initial isolation, data were recorded on the number of infected and non-infected seed. The frequency of *Aspergillus* species in groundnut seed samples were measured as the proportion of kernels contaminated by each fungal species to the total number of plated seed [13, 24]

Data analysis

Frequency distributions were computed to analyze quantitative data from interviews, including percentage of farmers growing each variety/landraces of groundnut and the sources of each seed (saved at home, local market, neighbors and research center)

The Use Value Index (UV) for groundnut within each stratum was calculated using the following formula:

$$UV = \sum \frac{U}{n}$$

Where U = number of use citations per landrace/variety (home consumption, income generation, forage and fuels) and n = number of informants [cite source of formula].

Landrace diversity within each stratum was analyzed using the Shannon-Wiener Diversity Index (H') according to Krebs (1989), using the following formula:

Where, P_i = the proportion of households reporting the i^{th} landrace. In other words, $P_i = n_i / N$, where, n_i is the number of households reporting the use of i^{th} landrace and N is the total number of households visited.

Data on frequencies of seed infection by *Aspergillus* species for samples collected from four landraces in different locations were subjected to analysis of variance (ANOVA) using R (version 3.3.2). Tukey's test of honest significant differences (HSD) was conducted *post hoc* to identify statistically significant differences among the samples, with a significance level alpha of 0.05.

Results

Groundnut varieties and their distinguishing characteristics

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Six types of groundnut, including five landraces and one released variety, were found to be grown in the kebeles visited in East Hararghe and East Wellega zones. According to farmers, the five landraces and one released variety differed in their agronomic, culinary and economic traits (Table 3). About 98% of the farmers interviewed cultivate one or more of the five landraces. The one released variety, which can be traced back to breeding programs at Melkawerer, Malkasa and Haramaya University Agricultural Research Center, was grown by few farmers (2% of respondents), and only in East Hararghe zone.

Table 3: Groundnut landraces and released varieties cultivated in East Hararghe (EH) and East Wellega (EW) zones, their distinguishing features according to local producers interviewed in 2016.

Image of seed	Local name (in Oromiffa)	Meaning of the name	Habit and agronomic features	Culinary and economic features
oldhale or ejja	Prefix ol means 'up' and dhale means giving birth; oldhale means pods on the upper surface of the soil	Relatively high yield under optimal and drought conditions because pod does not remain in the soil and is confined to the base of the plant.	Less tasty, not preferred for food. Low nutrient content. Less weight resulting in low market value.
sartu or sara	sartu and sara both refer to having more vegetative branches.	Traditional, spreading landrace collected from EH zone. Difficult to harvest since it produces pods along all branches and requires more digging. Relatively low yields; some pods lost in soil at harvest, especially under drought condition. Does not tolerate excessive rain (flooding).	Tasty and preferred for food. High nutrient content. High market value. Highly vegetative and preferred for fodder.
roba	Named by Haramaya University Research Center.	Early maturing and erect released variety collected from EH Zone. Tolerates drought and is easy to harvest, but sometimes germinates before harvesting, resulting in yield losses.	Less tasty, not preferred for food. Low market demand and low prices.
jambo or yambo	jambo refers to the relatively large size of the pod and seed	Traditional, neglected erect landrace collected from EH zone. Low yield, producing a large number of vegetative branches and few pods. Does not tolerate drought but is easy to harvest.	Less tasty, not preferred for food. No market demand and low prices.
bure	bure means having multiple of colors, referring to the variegated color of the seed.	Traditional, erect landrace collected from EW zone. Easy to harvest. Sharing many features with oldhale landrace (see above).	Less tasty than dima but used as food. Low market demand and low prices.
Dima	'Dima' means a state of having red colors So groundnut variety with	Traditional, spreading landrace collected from EW zone. Does not tolerate drought. Late (long) maturing.	Tasty and preferred for food. High market demand and high prices.

Farmers usually distinguish groundnut landraces by morphological characteristics. In East Hararge zone, growth habit and site of pod formation were the most common characteristics used by respondents to differentiate landraces, although they also used pod and seed size for some landraces. In East Wellega zone, seed color is the main distinguishing feature.

Farmers naming conventions for groundnut landraces varies within the study area. Names are based on the unique characteristics of each landrace. In East Hararge oldhale (which literally means 'giving birth up') refers to an erect type that forms pods on the surface of the soil at the base of the plant, whereas sartu refers to a spreading type that forms pods along all running stems. Color of the seed is also important for naming groundnut landraces in East Wellega zone; dima (meaning 'red') and bure (meaning 'variegated') both refer to the color of the seed (Table 3).

Farmers' seed sources

Farmers in East Hararghe zone tend to obtain their groundnut seeds from different sources than those in East Wellega (Fig. 2). The majority of farmers in East Hararghe (82.8%) used the seeds they have saved at home and few of them (2.8%) obtained seed from Haramaya University Research Center found nearby in Babile district or from the local market (5.6%). The second most common source of seed source was neighbors (17.2%). By contrast, most farmers in East Wellega reported that they had obtained their seeds from the local market (80.6%), with a much smaller percentage saving them at home (19.4%) and there is no research center distributing groundnut seed in that area (Fig. 2)

Diversity and distribution of groundnut varieties/landraces

A total of six groundnut varieties (two landraces from East Wellega and three from East Hararghe, as well as one released variety from East Hararghe) were observed, with varying distribution among farming households (Fig. 3). Among the four varieties observed in East Hararghe zone, oldhale was the most commonly grown, with a relative frequency of 68.0%, followed by sartu (24.7%), jambo (4.1%) and the released variety roba (3.1%). Farmers in this area preferred oldhale due to its high yield under normal conditions and when there is terminal (end of season) drought. Furthermore, since it produces pods on the surface of the soil, that reduces the number of pods that remain in the soil during harvesting. The majority of farmers interviewed in East Wellega zone grow the similar variety bure (relative frequency 86.7%), which is easier to harvest and has higher availability of seed, but is less tasty and has lower market demand than the one other variety observed, dima (13.3%).

Calculation of diversity indices for each stratum revealed variation in varietal diversity among agro-ecological and administrative zones (Table 4). The total number of varieties was higher in East Hararghe than in East Wellega, resulting in higher Shannon Weiner Diversity indices (H'). There were slight difference between the two agroecological zones in East Hararghe, due to the fact that oldhale is slightly more dominant (as a higher relative frequency) in the moist (M2) than the sub-moist (SM2) conditions. There are greater differences in East Wellega, where the same two varieties are found in both agroecologies, but they are each planted by a more similar number of farmers in moist (M2) than sub-humid (SH2) communities. The variety bure is more dominant in the wetter, sub-humid areas, resulting in lower evenness.

Table 4

Varietal diversity of groundnut landraces and released varieties among three agro-ecological and two administrative zones

Administrative Zone	Agro-ecology	Total number of varieties	Shannon-Wiener Diversity Index (H')
East Hararghe	SM2	4	0.79
East Hararghe	M2	4	0.77
East Wellega	M2	2	0.51
East Wellega	SH2	2	0.20

Use values of groundnut and their selection criteria

Respondents reported a variety of uses of groundnut within different zones, including income generation, food, fodder and fuel (Table 5). The seed was the highly valued part of the crop and contributes to smallholders' income (100% responses). All respondents also reported the use of seed for human consumption in different forms, such as boiled seed, which is an ingredient in dishes known as danfa, shorba, mullu or shumo. Danfa, for example, is a local stew prepared from split seeds of roasted groundnut seeds with some spices. This stew is a well-known food in East Hararghe, whereas shorba (soup), prepared from roasted split groundnut seeds and barley is commonly consumed in East Wellega. Seeds can also be eaten fresh during the harvest. Stems and leaves were also reported as having fodder value (80.6%) and the remaining shell after threshing (locally known as kola) is used as a fuel (30.6%). A few farmers in Babile district of East Hararghe and Gida Ayana district of East Wellega reported that bees forage for nectar from groundnut during its flowering. Therefore, Use Value (UV) index values ranged from 3.60 at Babile in warm sub-moist lowland agro-ecology to 2.97 at Gida Ayana in warm sub-humid lowland. The second highest use value index (2.67) were observed at warm moist lowland (Table 5).

Table 5

Use values of groundnut in four districts of East Hararghe (EH) and East Wellega (EW) reported by farmers during the 2016 cropping season.

Lists of uses	Parts used	District				Total count of farmers	% of Responses
		Babile (EH)	Fedis (EH)	Diga (EW)	Gida Ayana (EW)		
Food	Seeds	60	12	36	36	144	100
Fodder	Stems & leaves	56	12	34	14	116	80.6
Income	Seed	60	12	36	36	144	100
Fuel	Husks	24	1	15	4	44	30.6
Bee forage	Flowers	16	0	1	6	23	16.0
Sum		216	37	122	96	471	
Number of informants		60	12	36	36	144	
Use Value Index		3.60	3.08	3.39	2.67	3.27	

Gender roles in production, management, and use of groundnut

Although the activities of men and women vary between farmers in East Hararghe and East Wellega, including in the production and use of groundnut, some gender roles are consistent across the study area (Fig. 4). For example, farmers practiced some form of selection process to save seed for planting the next season. Most of the farmers reported selecting seed at harvest time, just before threshing, but some of them also used combined practices of selection on the field and after-harvest seed sorting for their preferred varieties. When selecting plants or seeds to renew seed lots, farmers most often selected for well-filled grains, heavy pod load (large number of pods per plant), absence of any damage, seed size (mostly larger size preferred), clean pods (field resistance against insect pests) and growth habit of the plants. In both zones, seed selection is mostly undertaken by males (94.1% in East Wellega and 90.2% in East Hararghe), with very low participation by females.

Land preparation (clearing crop residues and preparing for plowing) starts in March in East Hararghe and April in East Wellega, before the rains have started. These activities were done by all family members except for young children less than 10 years old. However, activities such as plowing and hoeing, which both require a lot of energy, were reported to be carried out by males (100% of responses). In East Hararghe zone, planting commences towards the first half of April, right after the first substantial rains, through to the first half of May. In East Wellega, planting starts in May and continues through the first half of June. Planting groundnut is done by both males and females, including children. When it comes to fertilizer, a considerable numbers of farmers (90.3%) in East Hararghe zone use urea and DAP in addition to crop residue and animal

dang. By contrast, few farmers (19.4%) in East Wellega zone use fertilizer on groundnut, and these are limited to crop residue. Fertilizer application is dominated by males in East Hararghe as well as East Wellega. Weeding is done by manual hand-weeding and hoeing and is performed by both males and females in both zones, although females more often participate in East Wellega than in East Hararghe. By the end of October in East Hararghe and November in East Wellega, farmers are harvesting groundnut. The harvest requires the participation of the entire household, including males and females. Post-harvest activities such as storing, threshing and marketing were performed by both males and females with little variation between East Hararghe and East Wellega, especially on storage in which females in East Wellega more participate than females in East Hararghe. In addition food preparation, one of the Post-harvest activities is dominated by females in both East Hararghe and East Wellega (Fig. 4).

Groundnut is mainly grown as a rain fed crop and is typically rotated with sorghum (85.5% of responses) in both zones. In addition to sorghum, maize (60.5%) and sesame (16.7% of responses) are also rotated with groundnut. Groundnut-sesame rotation is more commonly practiced by the farmers in East Wellega.

Farmers reported that groundnut is usually grown as a sole crop (55.6% of responses) but is often intercropped with cereals and other species (44.4% of responses) (Fig. 5). The most common intercrops are sorghum (25.7%), sesame with groundnut (9%, mostly in East Wellega) and maize (also 9%) with groundnut (Fig. 5). In East Hararghe, groundnut is sometimes grown between rows of khat (*Catha edulis*, 2.1% of responses).

Characterization and occurrence of *Aspergillus* species

Four different *Aspergillus* species (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus ochraceus*) were recorded on groundnut seed samples collected from East Hararghe and East Wellega. Figure 6 presents an illustration of different types of fungi growing from the groundnut seed samples after three days of incubation and pure culture of identified species after seven days of incubation. Frequency of seed infected by *A. niger* and *A. flavus* in the current study ranged from 17.8 to 37.5% and 14.4 to 32.5%, respectively (Table 6). Of the four species isolated from the groundnut seed sample, *Aspergillus niger* and *Aspergillus flavus* were the most prevalent species (Table 6). The highest frequencies of both species were recorded in warm moist lowland (M2) of East Hararghe.

Groundnut seed samples collected from the different agro-ecological zones of East Hararghe and East Wellega zone were also moderately infected by *A. parasiticus*. Frequency of this species ranged from 5.0 to 24.4%. *A. ochraceus* was the least prevalent *Aspergillus* species and was not detected on any sample collected from East Wellega zone

When varieties were compared, the highest infection rate (93.3%) was recorded on oldhale landrace collected from warm moist lowlands of East Hararghe and the least (37.8%) of infection was recorded on dima landrace in the same agro-ecology in East Wellega. However at the $\alpha = 0.05$ level of significance, there was no sufficient evidence to draw any conclusion about differences in the percentage infection of different varieties collected from similar agro-ecology, with the sole exception of bure having a significantly higher

rate of infection (70.0%) than dima (50.0%) on collections from warm sub-humid lowlands (SH2) in East Wellega

Aspergillus niger *Aspergillus flavus*

Aspergillus parasiticus *Aspergillus ochraceus*

Percent seed infection by <i>Aspergillus</i> spp.						
AEZ	Variety	Total infected seed	<i>A. niger</i>	<i>A. flavus</i>	<i>A. parasiticus</i>	<i>A. ochraceus</i>
EHM2	oldhale	93.33 ^a	37.50 ^a	32.50 ^a	15.00 ^a	8.33 ^a
	sartu	80.83 ^a	31.66 ^a	23.33 ^a	13.33 ^a	11.66 ^a
	LSD	NS	NS	NS	NS	NS
SM2	oldhale	80.00 ^a	30.00 ^a	25.83 ^a	14.16 ^a	10.00 ^a
	sartu	84.44 ^a	31.66 ^a	20.27 ^a	24.44 ^a	9.16 ^a
	LSD	NS	NS	NS	NS	NS
EWM2	bure	59.16 ^a	23.33 ^a	24.16 ^a	11.66 ^a	0.00 ^a
	dima	37.77 ^a	17.77 ^a	14.44 ^a	5.55 ^a	0.00 ^a
	LSD	NS	NS	NS	NS	NS
SH2	bure	70.00 ^a	32.50 ^a	31.66 ^a	5.00 ^a	0.00 ^a
	dima	50.00 ^b	25.00 ^a	17.50 ^a	7.50 ^a	0.00 ^a
	LSD	13.3	NS	NS	NS	NS
Means in a column followed by the same letter are not significantly different according to LSD at $p < 0.05$. Note EHM2 = Warm moist lowland in East Hararghe zone, SM2 = Warm sub-moist lowland in East Hararghe zone, EWM2 = Warm moist lowland in East Wellega, SH2 = Warm sub-humid lowland in East Wellega, LSD = Least Significant Difference, NS = Not significantly different						

Discussion

Farmers in the surveyed areas use local names for groundnut varieties based on morphological characteristics such as growth habit, site of pod formation, seed size and seed color. Use of morphological, as well as physiological and agronomic traits by farmers to distinguish their varieties has been reported in various studies [10, 25]. Of course, the naming system adopted by groundnut farmers depends on the cultural group and specific community. It is possible that the same name is applied to genetically distinct

Loading [MathJax]/jax/output/CommonHTML/fonts/TeX/fontdata.js different names in two communities may refer to

the same genotype [25–27]. To elucidate the situation, agro-morphological characterization followed by genetic analyses with molecular tools are recommended [28]. On the other hand, several studies have demonstrated consistency between farmers' varietal classification and molecular markers [29], suggesting that assessing varietal diversity based on farmers' taxonomy is to some extent reliable.

Farmers use specific criteria in selecting groundnut varieties for planting. Although selection criteria depend on the uses and roles of a crop within the farming system [25, 30], we found that groundnut growers in our study areas prefer varieties that are high yielding, have drought tolerance, and require less labor during harvest [10]. In both the moist and sub-moist lowlands of East Hararghe, farmers prefer the traditional bunch type landrace called oldhale, because it is a traditional variety that has been grown by them for a long time, the seed is widely available, it is relatively high yielding with heavy nuts, is resistant to drought and easy to harvest [10]. Oldhale and other bunch type landraces develop pods at the base of the plant; therefore, when the plant is pulled up during harvest, most all pods are recovered. By

Due to its spreading habit, farmers report that harvesting sartu requires intensive digging and it has low yields under dry conditions, especially during terminal drought [31]. In East Wellega, key informants reported that the landraces known as bure and dima have both been grown for a long time. Some farmers had abandoned groundnut production and switched to sesame because of higher market demand and lower labor requirements, but in the five years before our survey (2011 to 2016), demand for groundnut increased and farmers started to grow their traditional varieties of groundnut again. Bure is the most commonly grown landrace because it is higher yielding and easier to harvest, similar to oldhale in East Hararghe. Although dima has a higher market value and better taste, few farmers grow it because it is more difficult to harvest.

Multiple uses of groundnut were recorded across most of the study areas. Groundnut is a multi-purpose crop that generates cash income, food, fodder and fuel for households across both study areas [4, 32, 33]. Some other uses of groundnut, such as cooking the fresh leaves to be consumed as a vegetable have been reported from West Africa [32] but were not recorded in this study. As in different parts of Ethiopia and other regions, groundnut cultivation fits into a wide variety of farming systems [5, 30]. In crop rotation, it can follow cereals (maize, sorghum, or finger millet) or root crops (cassava and sweet potatoes). In the present study, groundnut was rotated with sorghum, maize or sesame. Although groundnut is usually grown as a sole crop, it is often intercropped with sorghum or maize or sesame. Previous studies [4] confirm that groundnut is usually grown either as a sole crop or mixed with cereal crops including sorghum and millets.

Although land preparation occurs around the same time (March and April), planting of groundnut commences earlier in East Hararge than East Wellega. Farmers in East Hararghe combine inorganic fertilizers (urea and DAP) with crop residue and animal manure, farmers in East Wellega use crop residue as their primary fertilizer. Most of the farmers select the seed at harvest just before threshing, and some of them also used combined practices of field plant selection and after-harvest seed sorting from their preferred varieties. When select seeds or plants to renew seed lots, farmers most often selected for well-filled grains, heavy pod load (large number of pods per plant), absence of any damage, seed size (mostly larger size preferred), clean pods (field resistance against insect pests) and growth habit of the plants

Within our study areas, groundnut was frequently intercropped with sorghum, sesame, maize, khat (*Catha edulis*) and mango. Although inter-cropping is generally known to decrease disease pressure in agricultural fields, these crops may not have a very good ground cover as they grow taller than groundnut and hence may not be able to protect groundnut plants from soil borne inoculums. Thus, selecting the appropriate time of planting in accordance with the weather conditions and avoidance of cropping under the shade might be of importance in reducing groundnut contamination by *Aspergillus* species

Gendered division of labor in rural Ethiopia varies in terms of farming systems, cultural settings, and relative wealth categories [34]. Many farming activities are carried out by both men and women, while others are dominated by one gender [35]. Previous studies of groundnut production found that women play important roles in both pre- and post-harvest activities [36]. Some studies report that men prepare land for planting, apply fertilizers, and handle chemicals, while women are primarily responsible for weeding, harvesting, storage and processing groundnut [37]. A similar pattern is observed in the present study; while men are solely responsible for plowing and hoeing, females participate in weeding, harvesting, threshing, storing, and marketing groundnut. Women are primarily responsible for food preparation. Interestingly, gender roles do not appear to vary between East Wellega and East Hararghe. Furthermore, gender roles shape farmers' preference for groundnut varieties. For example, women were more concerned with the taste of groundnut varieties and their contributions to household food supply, which is likely associated, their responsibility to prepare food [38]. Therefore, women in East Hararghe often preferred sartu to oldhale because it is an important source of food.

Four *Aspergillus* species were found associated with groundnut seed samples collected from the study area. The first species isolated from the samples was *Aspergillus niger*, which is distinguished from other species by black or dark brown spores [24, 39]. This species comprised compact white colonies covered by a dense layer of dark brown to black conidial heads with smooth-walled, biseriate and globose to subglobose spores. Colonies of the second species, *Aspergillus flavus*, were characterized by yellow to dark, yellowish-green pigments with smooth conidial surface [11, 22, 23]. The conidiophores were variable in length, smooth to finely roughened or moderately roughened, pitted and spiny [12]. *Aspergillus parasiticus*, the third species isolated, was characterized by production of dark green spores with rough conidia [10, 21]. The fourth species, *Aspergillus ochraceus*, was recognized by its production of yellowish-gold conidia [21]. Other studies have distinguished *A. ochraceus* from other species by its pale yellowish conidia [12, 13] or by orange-red conidiophores with coarsely roughened walls and light colored sclerotia [12]. The current study confirmed the production of yellowish pale brown color on PDA and orange-red conidiophores with coarsely rough surface on CZDA.

The proportion of groundnut seed contamination by *Aspergillus* ranged from 48.5% in warm moist lowlands of East Wellega to 87.1% in the same agroecological zone in East Hararghe. This finding aligns with those of Mohamed and Chala [13] and Ephrem *et al.* [12] who reported 80 and 85% contamination of groundnut seed samples collected from a farmer's field in East Hararghe. Higher levels of seed contamination in East Hararge may be ascribed to continuous cultivation of groundnut on the same land, as the zone has long been a major groundnut production area. Ortiz *et al.* [40] suggest that continued cultivation of groundnut on

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When infection rates for different varieties were compared, the only significant difference was detected between bure and dima in the sub-humid lowlands of East Wellega. The highest infection was recorded for oldhale landrace collected from warm moist lowlands of East Hararghe zone and the least of infection was recorded on dima landrace in a similar agro-ecology in East Wellega. However at the $\alpha = 0.05$ level of significance, there was no sufficient evidence to draw any conclusion about differences in the percentage infection of different varieties collected from similar agro-ecology of the study area. This might be due to the existence of resistant gene in BURE variety which needs further varietal screening against observed disease.

Of the four *Aspergillus* species identified in the groundnut seed samples, *A. niger* and *A. flavus* were the most prevalent. The highest frequencies of both species were recorded in warm moist lowlands of East Hararghe. These findings are consistent with Ephrem *et al* [12] who reported that *A. niger* and *A. flavus* were the most prevalent *Aspergillus* species contaminating groundnut seed, and found similar frequencies ranging from 23.83 to 40.25% and 22.05 to 27.65%, respectively. Similarly, a study conducted in Yemen by Almod [41] reported 37 and 34.5% of groundnut seed infection by *A. niger* and *A. flavus* respectively.

Groundnut seed samples were also moderately infected by *A. parasiticus*, with frequencies ranging from 5.00 to 24.44%. These findings are consistent with those of Mohamed and Chala [12] who reported that groundnut kernel infection by *A. parasiticus* from 2.1 to 14.0% on samples from farmers' fields. A study by Tadesse and Kiros of groundnut varieties in northern Ethiopia [42] reported a similar infection rate of 12.3% by *A. parasiticus*. Finally, *A. ochraceus* was the least prevalent *Aspergillus* species, and was not recorded on any sample collected from East Wellega. This may be attributed to higher humidity due to more precipitation in western Ethiopia and *A. ochraceus* is known to thrive at low humidity [43], and may not compete with other species under wetter conditions.

Conclusion

Farmers in the administrative and agro-ecological zones included in this study consider a multitude of factors as they differentiate and select groundnut varieties for planting. Drought tolerance, seed yield and ease of harvest were frequently cited as important criteria. Therefore, these are important attributes for breeders to consider when developing new varieties. Ultimately, responding to farmers' preferences will save resources that go to waste when a variety is developed and subsequently rejected by end users. Future breeding efforts should continue to incorporate these traits identified by the farmers who are experts regarding the specific environmental conditions on their farmlands.

The central finding of this study is that multiple landraces of groundnut are grown with varying frequency across the different agro-ecological zones of Ethiopia's major production areas. Although these varieties vary in terms of morphological features, there is not sufficient evidence to conclude that there are significant differences in their resistance to *Aspergillus* infection. Therefore, further breeding for disease-resistant varieties with farmers' other selection criteria in mind will be necessary to increase groundnut production and enhance food and livelihood security of groundnut growers in Ethiopia.

Declarations

Acknowledgement

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Ethics approval and consent to participate

Prior to data collection participant gave oral consent to participate in the study. No other ethical approval was needed.

Consent for publication

The respondents were informed that their opinions were to be shared with others and gave approval

Availability of data and materials

All data set and material supporting the conclusion of this article are included within the article

Competing interest

No competing interest

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Figures

Figure 1

Map of the study areas

Figure 2

Sources of groundnut seed reported by farmers (n = 144) in East Hararghe and East Wellega zones.

Figure 3

Relative frequency of groundnut varieties reported grown in East Hararghe (EH) and East Wellega (EW) during the 2015-2016 growing season (n = 144).

Figure 4

Gender role in groundnut production use and conservation (n = 144)

Figure 5

Groundnut cropping system in study area (n = 144)

Aspergillus niger

Aspergillus flavus

Aspergillus parasiticus

Aspergillus ochraceus

Figure 6

Aspergillus species identified from groundnut seed samples in current study

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