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Investigating Groundnut Varieties for Agronomic Performance, Resistance to *Aspergillus flavus* Infection and Aflatoxin Contamination in Eastern Ethiopia

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Abstract
Aflatoxin is secondary metabolite produced by *Aspergillus flavus* and *Aspergillus paraciticus*, and posing a threat to human and animal health. The availability of resistant genotype to *A. flavus* infection and/or aflatoxin contamination urgently needed. The objective of this study was to evaluate groundnut varieties for reaction to field seed colonization by *A. flavus* and pre-harvest aflatoxin contamination in Eastern Ethiopia. Sixteen groundnut varieties were evaluated in this study. Field seed infection and colonization by *A. flavus* was determined using plate counting methods. Moreover, groundnut seeds were subjected to aflatoxin analysis using ELISA test. The result revealed that all tested varieties were significantly different ($p \leq 0.05$) in response to days to flowering and maturity, number of pods and seeds per plant, hundred seed weight, pod yield (qt/ha), *A.flavus* infection and aflatoxin levels. Among the varieties evaluated, *Baha Gudo* (13.70%), *Sartu* (14.00%) and *Sedi* (14.23%) were resistant less than 15% of seeds number infected by *A. flavus*. Moreover, *Baha Gudo* (1.93 ppb), *Sartu* (3.70 ppb) and *Sedi* (6.40 ppb) were resistant to pre-harvest aflatoxin contamination, < 15 ppb. In conclusion the varieties, *Baha Gudo* and *Sedi* (improved) and *Sartu* (local) are resistant to *A. flavus* infection and pre-harvest aflatoxin contamination. Therefore, the varieties that showed resistance could form part of an integrated management of aflatoxin contamination.
Keywords: Aflatoxin, Agronomic, *Aspergillus flavus*, Ethiopia, Groundnut, Resistance

Introduction

Groundnut (*Arachis hypogaea* L.), which is also known as peanut, earthnut, monkeynut and goobers, is an annual legume. It is cultivated worldwide in tropical, sub-tropical and warm temperate areas (Okello *et al*., 2010; Okello *et al*., 2013). It is currently grown on 25.2 million hectares worldwide with a total production of 35.9 million metric tons, with developing countries in Asia (66%) and Africa (25%) as the major producers (FAO, 2016). In Ethiopia, during 2015 it was cultivated on 67,062.40 ha of land and 103,939.53 tons of groundnuts were produced, with average yield of 1.550 tons per ha (CSA, 2019).

Infection of groundnut seed by fungi mainly *Aspergillus flavus* Link ex Fries and *Aspergillus parasiticus* Speare can result in the contamination of the seed with aflatoxins, which are toxic fungal secondery metabolites (mycotoxins). Aflatoxins are a group of structurally related toxic polyketide-derived secondary metabolites produced by certain strains of *Aspergillus flavus* and *Aspergillus parasiticus* (Waliyar *et al*., 2006). *Aspergillus flavus* is commonly found in Africa, while *Aspergillus parasiticus* is dominant in America (Xue, 2020). These fungal species produce aflatoxin that pose serious health hazard to humans and animals that consume the aflatoxin-contaminated groundnut. Aflatoxin is known to suppress the immune system of body leading to various diseases such as liver cirrhosis and cancer in humans (Xue, 2020).

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 (2002), 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 Babile could not find market to sell their crop.

Use of genotypes resistant to *Aspergillus flavus* and with diminished accumulation of aflatoxin is the best option for the resource constrained farmers to manage the problems of aflatoxin contamination (Kwemoi, 2021). Planting varieties that are resistant to fungal infection or that does not support high aflatoxin synthesis offers a sustainable, low cost approach for aflatoxin management that is suited for adoption by small scale groundnut producers. Amare *et al.* (1995) reported variations in terms of reaction to *A. flavus* infection among groundnut varieties widely cultivated in Ethiopia. A number of groundnut varieties have been released since then and evaluation of their reaction to *A. flavus* infection and aflatoxin contamination could identify varieties that could be promoted for widespread use by groundnut producers. Despite the importance of the problem, there are no recommended research results for aflatoxin management in Ethiopia. In the present study, therefore the researcher investigate groundnut varieties for agronomic performance, resistance to *A. flavus* infection and aflatoxin contamination in Eastern Ethiopia.

Materials and Methods

Description of Experimental Site and Procedures:

Twelve groundnut varieties released by Werer Agricultural Research Center (WARC) (*Shulamith, NC-343, Roba, Sedi, Lote, Bulki, Werer-961, Werer-962, Werer-963, Tole-2, Fayo* and *Fetene*) and two (*Baha Gudo* and *Baha Jido*) by Haramaya University (HU), and two Farmers' varieties (*Sartu* and *Oldhele*), a total of 16 groundnut varieties were grown in a hotspot field trial at Babile experimental field of Haramaya University.

Babile is located in eastern Oromya at about 555 km east of Addis Ababa and 50 km east of Haramaya University. The altitudes of Babile range from 1501 to 1899 m.a.s.l and the average annual rainfall ranges from 500 to 975 mm with much variation among different years. It is characterized by semi-arid conditions having sandy loam soil and with mean annual minimum and maximum daily temperatures of 14.18 °C and 28.27 °C , respectively.

The experiment was laid out in a randomized complete block design (RCBD) with three replications. Each genotype was grown in four ridges or rows i.e. plots were 3 m long by 1.2 m wide with 30 cm spacing between ridges or rows and seeds were sown singly at 10 cm spacing along the ridges or rows. Recommended agronomic practices (seed bed preparation, planting, weeding, digging and inverting, threshing, pre-cleaning and drying or curing) for groundnut production were applied.

Preparation of Inocula of *Aspergillus flavus:*

An S-strain of *A. flavus* isolated from groundnut was used for artificial inoculation of experimental plots. Inoculum was prepared by the organic-matrix (cracked corn) method employed by (Will *et al.,* 2021). Conidia of *A. flavus* from an 8-10 day old-culture, were suspended in sterile distilled water (900 ml/10000 g of corn) and used to inoculate sterile moisture-equilibrated (25% moisture) cracked corn. The corn was incubated at 25 to 30 °C for 3 days. Fungi did not sporulate during the three day incubation to reduce worker's exposure to airborne conidia.

Inoculation of Groundnut Plots:

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

Data Collection on Agronomic Parameters:

Agronomic data including days to 50% flowering, days to 90% maturity, number of pods per plant, number of seeds per plant, 100-seed weight (g), and pod yield (qt/ha) were recorded.

Days to Flowering:
A visual assessment which involved the number of days it took for 50% of the plants from the experimental plots to reach anthesis was undertaken. Or the number of days from emergence to the date on which 50% of the plants in each plot has at least the first flower.

Days to Maturity:
The days to maturity was assessed when 90% or more of the pods were mature. In each plot, 2 plants were uprooted and the number of the mature pods examined by checking the internal blackening of the kernels i.e. the number of days from emergence to the date at which 90% of the plants in a plot have reached physiological maturity; pods were considered matured when the kernels are fully developed, testa assuming the varietal color, and the inside wall of pods darken to brown.

Number of Pods per Plant:
This parameter was estimated at harvest by counting the number of pods on the five tagged plants from the two middle rows.

Number of Seeds per Plant:
Number of seeds per plant was determined from five randomly selected plants from middle rows of each plot.

One Hundred Seed Weight:
Samples were taken at random from the dried seed lots of the harvest and weighed. The weight was taken for 100 seeds for each variety.

Pod Yield:
Plants from the two middle rows were harvested together, pods collected and sundried to the safe moisture content of about 10 percent. The pods were then weighed and then the outcome extrapolated to obtain the pod yield in kg/ha using the formula. Then the outcome were converted and expressed by qt/ha.

$$\textbf{Pod yield (kg/ha)} = \frac{\textbf{Pod yield (kg)}}{\textbf{Harvested area (m2)}} \textbf{ x 10000 m2}$$

Testing for Field Seed Colonization by *Aspergillus flavus:*
Levels of infection of groundnut seed by *A. flavus* were determined for each plot. Undamaged, mature pods were hand shelled and 100 seeds from each plot were surface-sterilized in 0.1% aqueous mercuric chloride solution for three minute, rinsed in sterile distilled water and transferred (10 seeds per Petridish) to 14.5 cm diameter petridishes containing Potato Dextros Agar (PDA) and incubated at 30 °C for two to three days. Then, fungi growing from the seeds were recorded and the material was examined for green conidial heads of *Aspergillus* species of the *Aspergillus flavus* group and the percent infection of seed was determined according to Mehan & McDonald (2020).

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

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

According to Mehan & McDonald (2020), the level of resistance to invasion and colonization by *Aspergillus flavus*, the following criteria were used:
Resistant = Sporulating growth on less than 15% of the seeds, with growth and sporulation sparse.
Moderately resistant = Sporulating growth on 16-30% of seeds, sporulation moderate to dense.
Susceptible = Sporulating growth on 31-50% of seeds, sporulation dense.
Highly susceptible = Sporulating growth on over 50% of seeds with dense growth and sporulation.

Aflatoxin Analysis using ELISA Kit:
Collected groundnut samples were further air dried and brought to uniform moisture content (7%) immediately after collection and serologically assayed for total aflatoxins (AFT) contamination within four weeks of collection using the indirect Enzyme Linked Immunosorbent Assay (ELISA). From each 1 kg sample, 100 g of shelled seeds were weighed and blended. Then 100 ml of 70% methanol (v/v) containing 0.5% KCl, was added to 20 g of the blended groundnut sample and blended further. The mixture was then

transferred to a 250 ml conical flask and shaken at 300 rpm for 30 min (Gallenkamp Orbital Shaker). The mixture was then filtered through Whatman No. 41 filter paper and diluted 1:10 in phosphate buffer saline with Tween 80 (1 ml filtrate in 9 ml buffer). Microtiter plates sensitized with aflatoxin B_1 BSA conjugate were incubated at 37 °C for 2 h followed by wash with PBS Tween 80. In all the steps, 150 ul/well of appropriate wash solution was used. Then the plates were washed with PBS Tween followed by addition of blocking solution (0.2% Bovine serum albumin) before 30-45 min incubation at 37 °C and washing. The extracts of the samples, and/or AFB1 standard solution of 100 ul/well, were incubated with 50 ul/ well of polyclonal antibody solution in the plate for 60 min. Polyclonal antibodies were cross-absorbed with 0.2% BSA for 30 min at 37 °C prior to addition to the plates. Then diluted anti-rabbit Igls labeled with Alkaline Phosphotase were added to each well and the plates incubated at 37 °C for 60 min. After washing, p-Nitro phenyl phosphate prepared in 10% di-ethenolamine was added and the plates were read at 405 nm in the Multiskan Plus (Labsystem) ELISA reader. The principle of ELISA lies in immobilizing the antigen onto solid surface capturing antigen by specific antibodies and probing with specific immunoglobulin carrying an enzyme label. The enzyme retained in case of positive reaction is detected by adding suitable substrate. The enzyme converts substrate to a product, which can easily be recognized by its color. The results was compared against the standards (Waliyar *et al*., 2009).

Data Analyses:

Data were subjected to analyses of variance (ANOVA) using Minitab version 17 for windows; means were compared by Fisher's protected least significant difference (LSD). Percentage of seed infection by *A. flavus* was determined using Microsoft Excel and total aflatoxin concentrations were calculated as ppb for each sample.

Results

A total of 16 groundnut varieties, twelve improved groundnut varieties released in Ethiopia by Werer Agricultural Research Center, 2 improved groundnut varieties released in Ethiopia by Haramaya University and 2 local groundnut varieties were evaluated on hotspot area at Babile experimental field of Haramaya University in 2019 crop season.

Flowering Days of the Varieties Evaluated:

The number of days to flowering by improved variety, *Roba*, and local variety, *Sartu*, were significantly longer ($p \leq 0.05$) with 36 and 35 days respectively to flowering than the other varieties, while *Sedi* were the shortest number of days which was about 27 days to flowering (Table 1).

Table 1. Flowering days of the16 groundnut varieties evaluated

No.	Varieties	Mean no. of Days to Flowering
1	Roba	36.00[a]
2	Sartu	35.33[a]
3	Baha Jido	33.33[ab]
4	Tole-2	33.33[ab]
5	Oldhele	33.00[ab]
6	Lote	32.67[ab]
7	Shulamiz	32.33[ab]
8	Baha Gudo	32.00[ab]
9	Fayo	32.00[ab]
10	NC-343	31.65[ab]
11	Bulki	31.00[ab]
12	Werer 962	30.31[ab]
13	Werer 961	30.00[ab]
14	Werer 963	29.64[ab]
15	Fetene	27.37[b]
16	Sedi	27.32[b]
Mean		31.85

S = 3.97
LSD at p = 5%, 0.034
CV(%) = 12.47
*Significant at $p \leq 0.05$

Means that do not share same letter are significantly different.

Maturity Days of the Varieties Evaluated:

Significant differences were observed in mean days to maturity between the tested varieties. Improved varieties, *Tole-2* and *NC-343* and local varieties, *Oldhele* and *Sartu* were significantly different ($p \leq 0.05$), exceeded 145 days to reach maturity, while *Sedi* and *Fetene* had the lowest days to maturity of 98 and 103 respectively (Table 2).

Table 2. Maturity date of the16 groundnut varieties evaluated

No.	Varieties	Mean no.of Days to Maturity
1	Tole-2	150.67[a]
2	Oldhele	147.32[a]
3	Sartu	147.32[a]
4	NC-343	146.68[a]
5	Faayo	138.31[b]
6	Baha Gudo	137.33[b]
7	Baha Jido	132.34[bc]
8	Bulki	132.34[bc]
9	Roba	127.32[c]
10	Werer 961	127.00[c]
11	Werer 962	127.00[c]
12	Were 963	126.33[c]
13	Lote	126.33[c]
14	Shulamiz	117.68[d]
15	Fetene	103.56[e]
16	Sedi	98.31[e]
Mean		130.38

S = 14.92
LSD at p = 5%, 0.00
CV(%)= 11.44
*Significant at $p \leq 0.05$

Means that do not share same letter are significantly different

Average Number of Pods per Plant of the Varieties:

Among the varieties, *Fayo* and *Bulki* had the highest number of pods per plant, 43.67 and 43.33respectively and were significantly different ($p \leq 0.05$) from the other tested varieties, while *Baha Jido* and *Werer-961* had the lowest number of pods per plant 25.33 of each (Table 3).

Table 3. Number of pods per plant of the16 groundnut varieties evaluated

No.	Varieties	Mean no. of Pods/plant
1	Fayo	43.68[a]
2	Bulki	43.36[a]
3	Fetene	37.31[ab]
4	NC-343	37.31[ab]
5	Sartu	36.00[abc]
6	Tole-2	36.00[abc]
7	Shulamiz	35.00[abcd]
8	Sedi	33.34[abcd]
9	Lote	32.33[bcd]
10	Werer 962	31.31 [bcd]

11	Roba	30.33^{bcd}
12	Werer 963	28.66^{bcd}
13	Oldhele	28.00^{bcd}
14	Baha Gudo	25.69^{cd}
15	Baha Jido	25.35^{d}
16	Werer 961	25.35^{d}
Mean		33.06

S = 5.96
LSD at p = 5%, 0.014
CV(%) = 18.02
*Significant at $p \leq 0.05$

Means that do not share same letter are significantly different

Average Number of Seeds per Plant of the Varieties:

Among the varieties, *Bulki* had the highest number of seeds per plant (89.33) and were significantly different ($p \leq 0.05$) from the other tested varieties, while *Werer-963* was the least number of seeds per plant (29.67) (Table 4).

Table 4. Number of seeds per plant of the16 groundnut varieties evaluated

No.	Varieties	Mean no. of Seeds/plant
1	Bulki	89.33^{a}
2	Fayo	79.00^{b}
3	Fetene	69.33^{c}
4	Sartu	66.33^{d}
5	NC-343	65.67^{d}
6	Tole-2	62.00^{e}
7	Shulamiz	61.67^{e}
8	Werer 962	61.67^{e}
9	Lote	60.65^{ef}
10	Sedi	59.66^{f}
11	Werer 961	53.66^{g}
12	Oldhele	50.67^{h}
13	Baha Jido	44.67^{i}
14	Roba	44.67^{i}
15	Baha Gudo	43.67^{i}
16	Werer 963	29.67^{j}
Mean		58.90

S = 10.39
LSD at p = 5%, 0.014
CV(%) = 17.64
*Significant at $p \leq 0.05$

Means that do not share same letter are significantly different

One Hundred Seed Weight as Affected by Variety:

Table 5 showed the means of the different varieties in terms of 100 seed weight. The improved variety, *Bulki* gave the highest 100 seed weights of 97g and were significantly different ($p \leq 0.05$) compared with all other tested varieties, while local variety, *Sartu* had the least 100 seed weight of 28g (Table 5).

Table 5. Hundred seed weight of the16 groundnut varieties evaluated

No.	Varieties	Mean of 100 SW(g)
1	Bulki	97.30[a]
2	NC-343	91.60[b]
3	Roba	91.20[bc]
4	Werer 962	90.30[c]
5	Werer 961	81.60[d]
6	Tole-2	75.30[e]
7	Lote	66.70[f]
8	Sedi	63.17[g]
9	Shulamiz	59.17[h]
10	Fayo	54.00[i]
11	Fetene	51.60[j]
12	Werer 963	37.10[k]
13	Baha Gudo	36.57[k]
14	Baha Jido	36.50[k]
15	Oldhele	30.90[l]
16	Sartu	28.50[m]
Mean	61.97	
S = 10.36		
LSD at p = 5%, 0.00		
CV(%) = 16.72		
*Significant at $p \leq 0.05$		

Means that do not share same letter are significantly different

Influence of Variety on Pod Yield of Groundnut Varities:

The analysis of variance results for average groundnut pod yield revealed the presence of significant differences among varieties. The improved variety, *Bulki* performed the highest average pod yield (38.74 qt/ha) and were significantly different ($p \leq 0.05$) compared to other tested varieties, while the varieties, *NC-343, Sartu, Tole-2, Shulamiz* and *Werer-963* gave the least average pod yield (≤ 20.46 qt/ha) of each (Table 6).

Table 6. Pod yield (qt/ha) of the16 groundnut varieties evaluated

No.	Varieties	Mean Pod Yield
1	Bulki	38.74[a]
2	Fayo	37.40[ab]
3	Fetene	35.73[b]
4	Baha Gudo	32.03[c]
5	Lote	29.72[cd]
6	Oldhele	29.65[cd]
7	Roba	27.40[de]
8	Werer 962	27.36[de]
9	Baha Jido	25.09[ef]
10	Sedi	22.75[fg]
11	Werer 961	22.67[fg]
12	NC-343	20.46[g]
13	Sartu	20.38[g]
14	Tole-2	20.35[g]
15	Shulamiz	20.31[g]
16	Werer 963	20.27[g]
Mean	26.94	
S = 4.15		
LSD at p = 5%, 0.00		
CV(%) = 15.39		
*Significant at $p \leq 0.05$		

Means that do not share same letter are significantly different

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

According to Mehan and McDonald (2020) classification on the level of resistance to invasion and colonization by *Aspergillus flavus*, the improved varieties *Baha Gudo* and *Sedi*, and local variety *Sartu* were resistant in which sporulating growth on less than 15% of the seeds number. The local variety *oldhele*, and improved varieties *Bulki, Fayo* and *Fetene* were moderately resistant in which sporulating growth was in the range of 16-30% of the seeds number. The improved varieties *NC-343, Roba, Lote, Werer-961, Werer-962, Werer-963, Shulamiz, Tole-2* and *Baha Jido* were susceptible in which sporulating growth on 31-50% of the seeds number (Table 7).

Table 7. Field seed colonization by *A. flavus* of the 16 groundnut varieties evaluated

No.	Varieties	Mean % of *A.flavus* Infection	Level of Resistance
1	NC-343	48.21[a]	Susceptible
2	Tole-2	45.01[b]	Susceptible
3	Baha Jido	41.10[c]	Susceptible
4	Werer 961	39.09[d]	Susceptible
5	Werer 963	35.16[e]	Susceptible
6	Lote	33.00[f]	Susceptible
7	Roba	32.50[g]	Susceptible
8	Shulamiz	32.20[g]	Susceptible
9	Werer 962	30.17[h]	Susceptible
10	Oldhele	27.63[i]	Susceptible
11	Bulki	27.00[j]	Moderately Resistant
12	Fayo	20.18[k]	Moderately Resistant
13	Fetene	19.00[l]	Moderately Resistant
14	Sedi	14.23[m]	Resistant
15	Sartu	14.00[m]	Resistant
16	Baha Gudo	13.70[m]	Resistant
Mean		29.86	
S = 3.70 LSD at p = 5%, 0.00 CV(%) = 12.39 *Significant at $p \leq 0.05$			

Means that do not share same letter are significantly different

Aflatoxin Contamination in 16 Groundnut Varieties:

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

Table 8. Aflatoxin levels (ppb) of the 16 groundnut varieties evaluated

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

S = 55.58
LSD at p = 5%, 0.00
CV(%) = 11.55
*Significant at $p \leq 0.05$

Means that do not share same letter are significantly different

Discussion

The 16 groundnut varieties were significantly different ($p \leq 0.05$) in response to days to flowering, days to maturity, number of pods per plant, number of seeds per plant, hundred seed weight and pod yield (qt/ha). The improved variety, *Sedi* (27 days) and *Fetene* (27 days) flowered earliest and also matured earlier (98 days) and (103 days) respectively than all the other varieties in this study, while improved variety *Roba* (36 days) and local variety *Sartu* (35 days) took longer days to flower whereas improved varities *Tole-2* (150 days) and *NC-343* (146 days) and local varieties *Sartu* (147 daya) and *Oldhele* (147 days) took longer days to mature. Flowering and maturity days were generally shorter for the improved than those of the local varieties. This is also because of its inherent capacity for dormancy as a member of the subspecies hypogaea which generally took more days than the fastigiata species to mature. Upadhaya *et al*. (2009) observed among other parameters, that it took between 19 and 25 days to flower and between 110 and 120 days to mature. Nigam *et al*. (2019) reported that these differences are due to the different market types, and that Valencia and Spanish types flower earlier and mature faster than Virginia types that flower later and mature late. Kamara *et al*. (2011) noted that, differences in days to maturity were due to the different market types and subspecies, which is in agreement with the present study.

The improved varieties *Fayo* (43.67) and *Bulki* (43.33) were performed well in the number of pods per plant, possibly due to the efficient utilization of available phosphorus during the pod filling stage of the crop. It is also likely that they are tolerant to short dry spells of August during the pod filling stages. While the improved varieties *Baha Jido* (25.33) and *Werer-961*(25.33) were the least in the number of pods per plant. The improved variety *Bulki* (89) was the highest in number of seeds per plant, while *Werer-963* (29) was the least compared to other varieties. The improved variety *Bulki* (97g) gave the highest 100 seeds weight and were significantly different ($p \leq 0.05$) than the other varieties evaluated, while the local variety *Sartu* (28g) gave the least in 100 seeds weight. This could be as a result of the short dry spell in August which occurred during the pod filling stage and the thick pods taking a good chunk of the entire pod biomass.

The improved varieties *Bulki* (38.74 qt/ha) gave the highest pod yield and significantly different ($p \leq 0.05$) than the other varieties evaluated, while *NC-343* (20.46 qt/ha), *Sartu* (20.38 qt/ha), *Tole-2* (20.19 qt/ha), *Shulamiz* (19.97 qt/ha) and *Werer-963* (19.94 qt/ha) gave the least pod yield. In the present study, a short dry spell in August may be responsible for the poor pod filling which resulted in generally low pod yield. This is because the crop was in the pod filling stage when the dry spell occurred. Also, the least pod yield was due to termite pest infestation of the field during the crop maturity stage. The present results were in agreement with the report of Alemayehu *et al*. (2014) who reported that *Bulki, Werer-962, NC-343* and *Werer-961* were gave high pod yield at Dara districts in Southern Ethiopia. Vichai & Suteera (2006) observed differences among advanced breeding lines than local accessions that were evaluated, for reason of good soil and uniform rainfall distribution at the early stages of crop growth, which was in agreement with the present findings. Kale *et al.* (2010) observed that, advanced breeding lines out yielded the local check in all qualities assessed, which was in agreement with the present findings. Generally, variations in groundnut varieties responses in flowering, maturity, number of pods per plant, number of seeds per plant, 100 seed weight and pod yield might be attributed to the differences in genetic makeup, as well as environmental factors. Also, relative effects of temperature, relative humidity, and moisture content at the time of sowing may affect the performance of the various varieties. According to Amare & Tamado (2014), the environment had the highest effect (84.7%) of the treatment sum of squares indicating the environments were diverse and caused most of the variation in dry pod yield, which is inagreement with the present study.

The differences in mycelial growth surface coverage were probably attributed to differences in physical and chemical features of the seed-coat, pod-shell thickness and reticulation. Laprade *et al*. (2018) and Liang *et al*. (2017) reported that groundnut resistance to *A. flavus* and subsequent aflatoxin contamination could have been attributed to seed coat thickness, permeability and seed testa constituents. According to Olwari *et al*. (2013) reported that groundnut varieties with the biggest mean ratings of kernels and pods with invisible mycelia or no visible sign of infection and smallest mean ratings of infected pods and kernels could be considered tolerant to *A. flavus* colonization and infection. Wotton & Strange (1987) and Liang et al. (2006) reported that wax and cutin isolated from seed testa play an inhibitory role against *A. flavus* colonization and invasion of groundnut kernels. The smaller mean ratings of infected kernels could have been due to the compact arrangement of palisade-like layers of the seed testa. The compact palisade-like layers of seed testa were reported to have reduced *A. flavus* colonization, invasion and subsequent infection of groundnut kernels (Gradziel & Wang, 2016).

Seeds of certain groundnut (*Arachis hypogaea* L.) genotypes were reported to be resistant to colonization by *A. flavus* based on inoculations of hand- harvested, hand-shelled seed (Kushalappa *et al*., 1979). Resistance was reduced or eliminated by practices that damaged the testa of the seed. Machine harvest (stripping the pods from the plants) and machine shelling decreased resistance, and abrading the testa with Carborundum or pricking it with pins eliminated resistance and seeds without testae had no resistance (Kushalappa *et al*., 1979). Resistant seeds seemed to have greater surface wax accumulations, more compact cells and a greater number of fibers in the testa along with smaller hila, and a greater concentration of tannins (Laprade *et al*., 2018).

All these studies on seed shelled by hand and established that resistance to *A. flavus* and aflatoxin contamination in groundnut seed was a function of the testa, which acts as a barrier to movement of the fungus into the seed. This was because of the intact testa and pods acting both as physical (pod) and chemical barriers (testa) to ward off fungal infection with pathogens and aflatoxins (Awuah & Ellis, 2019). Wounded seed and pod surfaces when left unprotected could also act as easy points of entry for fungi. This may account for why some of the samples had higher aflatoxin levels, which is in agreement with the present study.

Conclusion

A total of 16 groundnut varieties, twelve groundnut varieties released in Ethiopia by the Werer Agricultural Research Institutes, 2 groundnut varieties released in Ethiopia by Haramaya University and 2 local groundnut varieties were evaluated at Babile experimental fields of Haramaya University. Findings

from the field evaluation suggest that the yield performance of the varieties varied significantly at $p \leq 0.05$ level of significance. The improved varieties *Bulki*, performed better than other improved and local varieties. Groundnut varieties in which sporulating growth on less than 15% of the seeds number (improved varieties-*Baha Gudo* (13.70%) and *Sedi* (14.23%), and local variety-*Sartu* (14%)) were resistant to *A. flavus* infection and should be promoted as an aflatoxin management strategy. Moreover, the improved varieties *Baha Gudo* (1.93 ppb) and *Sedi* (3.70 ppb), and local variety *Sartu* (6.40 ppb) were resistant to pre-harvest aflatoxin contamination in which their aflatoxin levels are below 15 ppb. Groundnut seed, when carefully taken care of during growth, harvesting and storage, though may be subject to fungal infection, could limit pathogen infection and aflatoxin production.

Recommendations

Pending confirmation through further assessments, the varieties that showed resistance could form part of an integrated management of aflatoxin contamination in Eastern Ethiopia. Further verification of groundnut varities (Baha Gudo, Sedi and Sartu) that exhibited resistance to field seed colonization and infection by *A. flavus* and pre-harvest aflatoxin contamination across years and locations would generate conclusive results. Further evaluation of resistance of different groundnut varities to *A. flavus* infection and pre-harvest aflatoxin contamination should be done in other areas of the country to come up with full picture at the country level. Also there is a need to elucidate the mechanisms of resistance to *A. flavus* infection and pre-harvest aflatoxin contamination in order to promote dissemination of the varieties.

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