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Peanut (*Arachis hypogea*) accessions differentially accumulate aflatoxins upon challenge by *Aspergillus flavus*: Implications for aflatoxin mitigation

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ABSTRACT

Peanut is among the most important oil crops grown in sub-Saharan Africa for its nutritive value and economic benefit. In this region, *Aspergillus flavus* infects peanuts and contaminates produce with aflatoxins leading to food insecurity through lowered quality. The impact of aflatoxins transcends the food chain causing harm to human and animal health. The selection and production of aflatoxin-tolerant varieties of peanut is a promising strategy to mitigate their potential harm. We studied the resistance of peanut kernels to aflatoxin accumulation in 25 peanut accessions collected from different growing locations in Kenya, Uganda, and Rwanda based on their unique phenotypic characteristics. To obtain sufficient planting material, the peanut accessions were multiplied separately under standard conditions and harvested at their respective maturity dates then dried and stored at 4 °C before further analysis. At the start of the experiment, the kernels were challenged with a laboratory-characterized toxigenic strain (*Aspergillus flavus* 1EM1901) and incubated at 28 °C for 7 days. Afterward, the accessions were analysed (ELISA) for aflatoxins. Of the 25 accessions tested, 60 % (n = 15) exhibited kernel resistance to aflatoxin accumulation while 40 % (n = 10) were susceptible (with aflatoxin levels ranging from 0.54 ppb to 1692.92 ppb). Two of the resistant accessions recorded a decrease in aflatoxin accumulation when challenged with toxigenic *A. flavus*. This differential accumulation of aflatoxins suggests the existence of an inherent trait that can be exploited in breeding programs of peanut varieties with low aflatoxin accumulation when grown in contaminated soils. These findings are further discussed.

1. Introduction

1.1. Importance of peanut

Peanut (*Arachis hypogaea* L.) is an important oil crop globally, playing a vital role in food and nutrition security because of its high protein, fat, and mineral content, as well as being an energy source in human and animal diets [1,2]. Peanut is a key oil crop, covering about 26 million hectares of land area in 120 countries [3]. The Food and Agricultural Organization [FAO] reported that the global production of peanuts in 2021 exceeded 53 million tonnes [4]. Peanut is commonly used as a nutritional supplement when weaning children and in situations of food shortages in special formulations to alleviate malnutrition. Due to its nutritional value and growing demand, peanut is a good source of income for many small-scale farmers in Sub-Saharan Africa [2].

1.2. Contamination of peanut by Aspergillus sp.

While peanut is an important crop, the realization of its benefit is constrained by contaminants chief among them are aflatoxins. The contamination of peanuts by aflatoxins is primarily caused by soil-borne congeneric fungi species *Aspergillus flavus* and *A. parasiticus*. The ubiquitous nature of the fungi in the soil and grain poses an existential threat to food safety and security as well as human and animal health [1]. Since large-scale crop production and soil microhabitat are inseparable, the colonization of kernels by fungi and eventual contamination by aflatoxins is expected. Therefore, aflatoxins are considered inescapable food contaminants [5,6] in many countries the world over, and are present in detectable levels in freshly harvested as well as processed peanuts.

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1.3. Economic and health losses caused by aflatoxins

In Sub-Saharan Africa, including Kenya, aflatoxin contamination of food is associated with significant economic losses. Direct economic losses of aflatoxins are attributed to a reduction in the profitable volume of commodities, a decline in value in national markets, rejection of products in international markets, losses sustained from livestock disease and deaths, and subsequent human morbidity and mortality. Extensive regulations in Europe and Northern America limit the dissemination of aflatoxin-contaminated through market channels for consumer protection, which also results in vast economic losses to farmers who export maize and groundnuts ([7], n.d.). The withdrawal of contaminated food from the supply chain following the high contamination of maize and peanut targeted at local markets [8] or rejection at international trade zones limits food availability have a negative impact on food and economic security.

Aflatoxins are implicated in the deaths of 4.5 million children below the age of 5 in Sub-Saharan Africa (Partnership for Aflatoxin Control in Africa [7], n.d.). The Ugandan government is estimated to spend approximately $910,000 more on annual health expenses [9]. An additional $77 million is lost yearly due to aflatoxin-induced liver cancer cases. The annual economic losses attributed to aflatoxin-related liver cancer in other African countries are estimated at $1599 million (Nigeria), $1100 million (Tanzania), $902 million (Senegal), $392.6 million (Malawi), and $22.5 million (The Gambia) [9]. With these disruptions, growing efforts to mitigate aflatoxin contamination in peanuts are needed to alleviate economic losses and protect human and animal health.

1.4. Aflatoxin mitigation strategies

Various strategies have been developed and deployed to mitigate the adverse effects of aflatoxins but with varying degrees of success. On one hand, pre-harvest practices include biological control, exploiting natural competition between congeneric species of toxin producers and non-producers [10], habitat management using push-pull technology [11,12], modification of abiotic factors such as drought stress [13], and management of biotic factors such as insect control, as well as cultural practices such timely planting and harvesting, and good agronomic practices weed control, adequate fertilization and late season irrigation [14]. On the other hand, post-harvest practices like rapid and proper drying, proper transportation and packaging, sorting, and control of insects have been found promising [15]. However, given the ubiquitous nature of *A. flavus*, it is almost impossible to eliminate the exposure of peanuts to the fungi [16].

For this reason, host-plant resistance to *A. flavus* colonization and accumulation of aflatoxins appears promising [17,18]. Three major constituents of the evaluation of peanuts for resistance include *in-vitro* seed colonization (IVSC), resistance to pre-harvest aflatoxin contamination (PAC), and resistance to aflatoxin production (AP) [17]. Breeding for resistance to seed infection and aflatoxin contamination is an economically viable option for controlling pre-and post-harvest aflatoxin contamination in peanuts [13]. This strategy requires the impartation of resistance to pre-harvest seed infection, in vitro seed colonization, and aflatoxin accumulation [19]. However, these traits are often inherited autonomously [17,18,20], which further complicates the development of a variety with all the desirable traits. Furthermore, the complex interactions between the genes and environment complicate the enhancement of host plant resistance in groundnut ([21]; Tengey et al., 2022).

A number of studies have identified germplasm accession lines with resistance to *in-vitro* seed colonization by *A. flavus* in peanuts [22,23]. Nonetheless, accessions found to be resistant to *in-vitro* colonization have demonstrated susceptibility to contamination under field conditions [20]. Therefore, the focus of research on aflatoxin mitigation in peanut has been redirected to the screening of germplasm for resistance to pre-harvest infection and consequent aflatoxin contamination. However, there is a paucity of data on the resistance to aflatoxin production by peanut genotypes grown and consumed in Kenya as most efforts to combat aflatoxin in food products have been focused on maize. In addition, most data on aflatoxin resistance in peanuts are available for varieties grown in major peanut-producing countries such as China, India, and the United States.

The current study aimed to examine the *in-vitro* resistance to aflatoxin production (AP) and seed-coat barrier of various peanut genotypes available in Kenyan markets. At this level of resistance, *A. flavus* can infect and colonize the seed coat but lead to low or no production of aflatoxins. It is hypothesized that exposure of peanut to toxigenic *A. flavus* should lead to the accumulation of aflatoxins in the kernels. The implications of the findings are further discussed.

2. Materials and methods

2.1. Plant materials

A germplasm set consisting of twenty-five peanut accessions was collected from Kenya, Uganda, and Rwanda. Phenotypic characteristics such as kernel size and seed coat colour of the accessions were documented (Table 1). The accessions were planted in Kakamega County (GPS coordinates 0°18′41.3″ N 34°45′04.1″ E) for multiplication, with all the recommended cultural practices for the crop undertaken [24]. Harvesting was done according to respective maturity dates in this order: EU2, KKMG1, KKMG3, KKMG4, 9991, Uganda Red, RW-TZ, RW-RW, UG-AR, UG-MA, UG-TZ, EUGN-1, RW-LO, and UG-SO at 115 days and BRG1, BRG2, EU1, EUGN2, KKMG2, CG7, ICGV-SM-90704, MG, BS, RW-ML, and UG-ML at 150 days. Harvested pods were sun-dried on gunny bags placed as mats for 7–10 days before hulling. The kernels were stored at 4 °C for 3 months before the initiation of the experiment. Subsequent analyses were conducted at the Mycotoxin Research Laboratory, as well as the Molecular Biology Laboratory at Egerton University.

2.2. Determination of pre-inoculation aflatoxin levels

In order to determine the postharvest (pre-inoculation) aflatoxin levels of contamination of peanut by aflatoxin, a 10-g portion of each

Table 1
Phenotypic characteristics of peanut accession.

Serial No.	Accession	Grain Size	Seed-coat colour
1	BRG1	Large	Brown
2	BRG2	Large	Reddish brown
3	EU1	Very large	Pale yellow to brownish
4	EUGN-2	Large	Deep Red
5	EUGN-1	Very large	Brown
6	EU2	Small	Deep red
7	KKMG1	Small	Deep red
8	KKMG2	Large	Deep red
9	KKMG3	Small	Pale red
10	KKMG4	Small	Pale red
11	9991	Small	Deep red
12	CG7	Large	Deep Red
13	Uganda Red	Small elongated	Deep red
14	ICGV-SM-90704	Large	Pale red
15	MG	Large	Brown
16	BS	Large	Deep red and white spots
17	RW-TZ	Small	Deep red
18	RW-LO	Small	Pale yellowish
19	RW-RW	Small	Pale red
20	RW-ML	Large	Deep red
21	UG-AR	Small	Pale yellowish
22	UG-ML	Large	Deep red
23	UG-MA	Small	Pale red
24	UG-TZ	Small	Deep red
25	UG-SO	Medium	Pale yellowish

accession was ground and extracted in 25 ml of 70 % methanol. The samples were then tested for aflatoxin levels in triplicates using the Enzyme-linked Immunosorbent Assay (ELISA) total aflatoxin kit (Helica Biosystems) according to the manufacturer's instructions. Absorbances were read at 450 nm using a microplate reader (ThermoScientific).

2.3. In vitro inoculation with A. flavus

Kernels from each accession were first surface sterilized under ultraviolet (UV) light for 15 min to preserve testa integrity [20]. Afterward, a 10-g portion of kernels from each accession was inoculated with a toxigenic species of *A. flavus* isolate (1EM19-01) whose toxigenic potential had been demonstrated (Lagat et al., 2021). The fungal inoculum was prepared by growing the fungi on PDA medium in petri dishes for 7 days at 28 °C. Conidia were suspended in 0.01 % Tween 20 solution and conidia concentration was estimated using a haemocytometer and adjusted to a concentration of 1000 conidia/ml. The rationale for this concentration was to avoid overwhelming the peanuts with fungi [20]. A 1000 μl of conidial suspension was used to inoculate each portion of kernels by dispensing the suspension and mixing the contents of the plate for even distribution of the inoculum. Infected kernels were incubated at 28 °C for 7 days. Afterward, visual inspection for visible growth of *A. flavus* was conducted with scoring based on percentage coverage where observable mould = 0 % coverage, moderate kernel coverage by fungi = 50 % coverage; and total kernel coverage by fungi = 100 % coverage. Thereafter, the infected kernels were ground and their toxin levels were determined using an ELISA kit (Helica Biosystems). The mean pre-inoculation and post-inoculation aflatoxin concentrations for each accession was recorded. Accessions with low aflatoxin concentrations post-inoculation were classified as aflatoxin resistant. The experiment was laid out in a Completely Randomized Design with 3 replicates.

2.4. Statistical analyses

Statistical analysis was conducted using the Statistical Package of Social Sciences (SPSS) version 25. Descriptive statistics such as means and standard error of means of total aflatoxin concentrations pre- and post-inoculation were calculated. A one-tailed paired *t*-test was used to compare the mean aflatoxin pre- and post-inoculation in the same accession. Accessions with significant mean differences ($P < 0.05$) were classified as susceptible, whereas accessions with non-significant mean differences ($P > 0.05$) were classified as resistant. A one-tailed Pearson bivariate correlation was conducted to determine the relationship between *A. flavus* density and resistance to aflatoxin accumulation. Analyses were done at $p < 0.05$ level of significance.

3. Results

Twenty-five peanut accessions with diverse attributes (Table 1) were collected and tested. The aflatoxin levels post-inoculation with *A. flavus* 1EM19-01 were generally higher compared to the pre-inoculation states (Table 2). A statistically significant increase in aflatoxin levels after inoculation was considered as susceptibility to aflatoxin accumulation while an insignificant increase in aflatoxin was considered resistance to aflatoxin accumulation. Of the 25 accessions that were tested, 15 (60 %) were classified as resistant to aflatoxin accumulation. These included EUGN-2, KKMG2, KKMG4, 9991, CG7, Uganda Red, ICGV-SM-90704, MG, BS, RW-TZ, RW-LO, RW-RW, RW-ML, UG-ML, and UG-TZ (Table 2). In contrast, 10 (40 %) were susceptible. They included BRG1, BRG2, EU1, EUGN-1, EU2, KKMG1, KKMG3, UG-AR, UG-MA, and UG-SO. A reduction in aflatoxin level was observed in accessions BS and UG-ML after inoculation. The following trend was observed: some accessions had high fungal density but were resistant; some had high fungal density and were susceptible; some had low fungal density (no fungi) but were resistant, and others had low fungal density but were susceptible (Plate 1). A weak negative correlation between the density of *A. flavus* on peanut kernels and resistance to aflatoxin accumulation was obtained $r(25) = -0.244$, $p > 0.05$, implying that aflatoxin resistance increases as fungal density decreases and vice versa. However, this correlation was not statistically significant.

4. Discussion

Aflatoxigenic *Aspergillus* species in the environment colonize peanuts leading to aflatoxin accumulation in pods and kernels prior to, during,

Table 2
Mean aflatoxin content in peanut kernels pre- and post-inoculation.

		Before		After				
SN	Accession	Mean ± SE (ppb)		Mean ± SE (ppb)		Fungal coverage (%)	P-value	Resistant/Susceptible
1	BRG1	0.88	±0.54	2.77	±0.37	0	0.048**	S
2	BRG2	0.47	±0.35	2.19	±0.09	100	0.031**	S
3	EU1	1.18	±0.41	26.37	±12.07	100	0.090	S
4	EUGN-2	1.15	±0.35	1.69	±0.16	50	0.187	R
5	EUGN-1	0.54	±0.17	2.65	±0.41	0	0.012**	S
6	EU2	0.68	±0.38	1.99	±0.10	50	0.034**	S
7	KKMG1	0.92	±0.53	3.74	±0.68	100	0.037**	S
8	KKMG2	0.93	±0.15	1.51	±0.27	50	0.104	R
9	KKMG3	1.27	±0.90	1692.92	±456.83	100	0.033**	S
10	KKMG4	1.43	±0.47	4.70	±1.34	100	0.097	R
11	9991	0.77	±0.33	1.23	±0.31	0	0.060	R
12	CG7	1.94	±0.98	2.52	±0.18	50	0.323	R
13	Uganda Red	1.95	±0.09	2.52	±0.83	50	0.262	R
14	ICGV-SM-90704	1.13	±0.30	2.68	±1.35	0	0.144	R
15	MG	3.62	±0.41	4.90	±0.60	50	0.108	R
16	BS	2.36	±0.53	1.54	±0.37	0	0.060	R
17	RW-TZ	3.03	±1.03	4.39	±1.19	0	0.187	R
18	RW-LO	2.88	±0.39	4.66	±1.39	50	0.109	R
19	RW-RW	2.53	±0.20	3.89	±1.24	50	0.174	R
20	RW-ML	3.55	±1.00	5.42	±0.35	0	0.107	R
21	UG-AR	4.32	±0.15	5.75	±0.20	50	0.026**	S
22	UG-ML	3.88	±1.26	1.99	±0.35	0	0.181	R
23	UG-MA	2.65	±0.33	4.82	±0.56	50	0.040**	S
24	UG-TZ	1.61	±0.06	5.17	±1.85	50	0.093	R
25	UG-SO	2.32	±0.95	3.83	±0.61	50	0.030**	S

Means ± SE across the columns followed by ** are significantly different, paired *t*-test (P = 0.05).

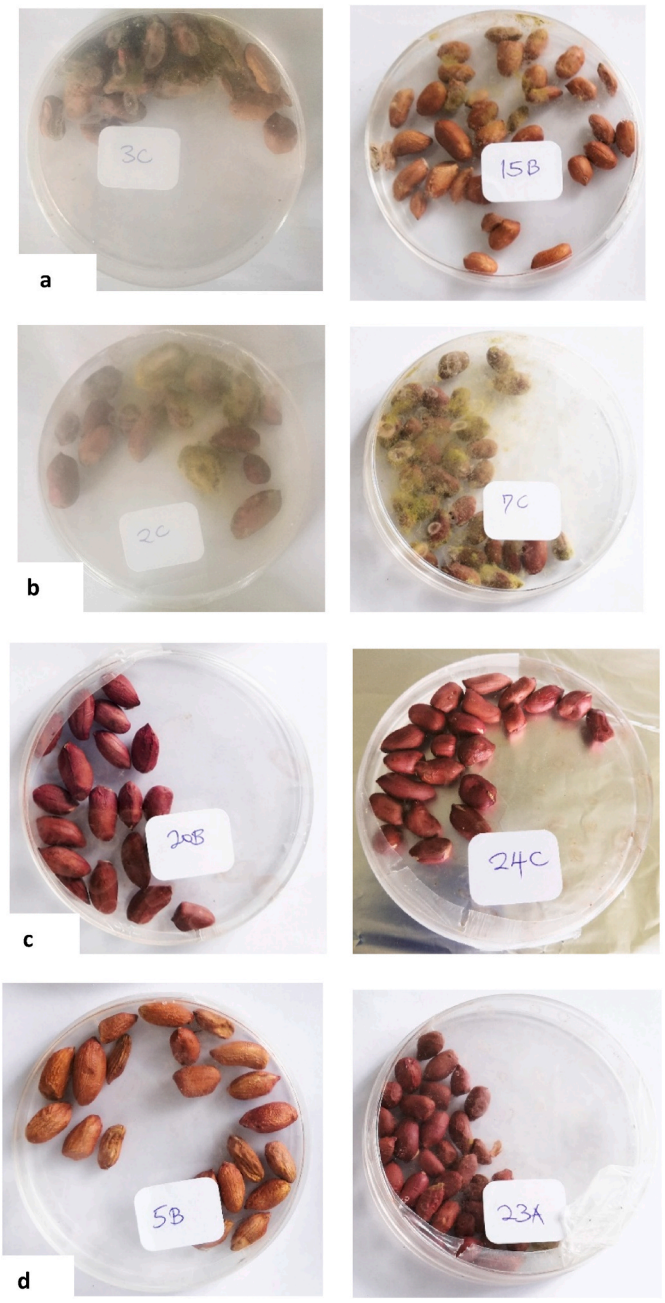

Plate 1. Fungal densities of various peanut accessions and resistance to aflatoxin accumulation 1a) High fungal density, resistant; 1b) high fungal density, susceptible; 1c) low fungal density, resistant; 1d) low fungal density, susceptible.

and after harvest [25], which explains why low levels of aflatoxin were detected in the accessions even before inoculation with the toxigenic *Aspergillus* isolate (Table 2). Following infection, toxigenic *A. flavus* infiltrates the seed coat to colonize the cotyledon surface as it obtains nutrition and produces aflatoxin. This could explain the observation that all peanut accessions except BS and UG-ML had higher aflatoxin levels post-inoculation, which was consistent with the expectation that colonization of kernels with *A. flavus* leads to aflatoxin production [17]. The aflatoxin levels after harvest were lower than the cut-off thresholds of 10 ppb indicating that significant aflatoxin contamination did not happen prior to and during harvest but rather the build-up was post-harvest as reported by Bediako et al. [2]. Additionally, for most accessions except EU1 and KKMG3, post-inoculation aflatoxins levels did not reach or exceed the 10-ppb limit, showing resilience against *A. flavus*. These findings indicate a large number of resistant peanut accessions that can be used in pre-breeding studies for aflatoxin resistance, disease resistance, and other desirable agronomic traits using more advanced biotechnology tools [13].

The period of incubation, the concentration of inocula, and the aggressiveness of *A. flavus* strain are some factors influencing the extent of in-vitro accumulation of aflatoxin peanut kernels as well as post-harvest accumulation [26–28]. Wang et al. [26] recorded the accumulation of more than 20,000 ppb of aflatoxin after inoculating the peanut with 4.0×10^6 CFU/ml spore concentration and incubating for 10 days, whereas Muller et al. [27] used a concentration of 1×10^4 spores/ml for 3 days and recorded aflatoxin levels of 169 ppb. In a separate study, Soni et al. [18] reported 43,989.6 ppb after 7 days in a resistant peanut variety JL24. The current study used an inoculum concentration of 1.0×10^3 spores/ml. The differences in accumulated aflatoxin levels between the current and previous studies can also be accounted for by the use of different toxigenic *A. flavus* inocula whose aflatoxigenic potential varies based on inherent attributes [29] geographic origin, and substrate characteristics [30]. These differences show that what may be considered a resistant variety when exposed to a specific *A. flavus* isolate can be susceptible to aflatoxin accumulation when exposed to a different *A. flavus* isolate at a different concentration and aggressiveness. These spore concentrations used in the inoculation studies are significantly lower than what can be found in a single colony of *Aspergillus* isolated from freshly harvested material but substantially higher than the spore concentration in the air from air sampling studies [31].

The observed variations in aflatoxin accumulation across the peanut varieties can be explained by differences in mechanisms of resistance. The response to aflatoxin contamination can be divided into resistance to pod infection, which is determined by shell structure, seed coat barrier, and aflatoxin production in the cotyledon [18]. Inhibition of aflatoxin production after infection with *A. flavus* has been attributed to the presence of the amino acid tryptophan, which interferes with the expression of three genes involved in the aflatoxin biosynthetic pathways: aflD/norA, aflE/nor-1, and aflO/omtB [25]. However, tryptophan does not lead to a substantial change in fungal mycelial mass, which explains why some peanut accessions such as EU1, KKMG1, and MG had significant mycelial and spore density after inoculation with toxigenic *A. flavus* but did not have high levels of aflatoxin after ELISA. On the other hand, the amino acid proline is associated with high levels of aflatoxin production [25]. Phytoalexins, which are antimicrobial agents produced by plants, are known to inhibit the production of aflatoxins in plants after colonization. Wound-induced stilbene phytoalexins in peanut kernels inhibit the germination of spores and hyphal extension of *A. flavus* [32]. Some resistant accessions such as 9991, ICGV-SM-90704, RW-ML, and UG-TZ did not have any visible fungi growing on the kernels after inoculation with toxigenic *A. flavus* (Plate 1), suggesting the involvement of phytoalexins as reported in the literature. Therefore, further studies are needed to determine the levels of the amino acid tryptophan and proline as well as phytoalexins in these peanut accessions to ascertain the precise mechanism for aflatoxin resistance.

A notable observation in the current study was that there was a reduction in aflatoxin levels in accessions BS and UG-ML after inoculation, indicating a high level of resistance to aflatoxin accumulation. The literature shows that the mechanism of resistance to *A. flavus* infection and aflatoxin accumulation is quantitative due to the interaction of different parameters such as genetic, morphological, physiological, and environmental factors controlled by numerous genes in peanuts [25, 33–35]. Therefore, this observation could be explained by (i) physiological factors, (ii) morphological factors, and (iii) environmental factors and how the inherent genetic composition of the accessions regulates these factors. The likely physiological factor involved is the production of substances that degrade aflatoxins, for example, phytoalexins, which have been reported to promote the enzymatic degradation of aflatoxin or inhibit the production of aflatoxins in plants after

colonization by *Aspergillus* [32,36]. However, the direct involvement of phytoalexins has not been confirmed experimentally in this study. Therefore, future studies should look into the involvement of phytoalexins in this phenomenon. Morphological factors include seed coat characteristics, for example, the presence of polyphenols such as flavonoids, phenolic acid, and coumarins in the seed coat, which may also confer antifungal properties and inhibit the growth of fungi [25]. Nayak et al. [37] reported that peanut kernel skin colour strength is positively correlated with total polyphenol level, which provides antioxidant properties. Peanuts with darker skin colour had higher total polyphenol content compared to the lighter-coloured genotypes (Nayak et al., 2021). In the current study, the accessions BS and UG-ML had dark-coloured seed coats, which suggests the presence of high quantities of polyphenols that contributed to resistance. Seed coats with wax and cutin layers also provide a physical barrier against *A. flavus* infection [25,32]. However, seed coat composition was not investigated in this study. The involvement of environmental factors is ruled out in this study because all 25 accessions were grown under similar conditions. At present, there are no studies that describe or explain this behaviour in peanuts. Therefore, future studies are needed to unravel the mechanism behind this observation and confirm the involvement of phytoalexins, polyphenols, and composition of the seed coat.

Seed maturity [38], pre-harvest, and post-harvest practices (Bediako et al., 2017) are other factors that influence the susceptibility of peanuts to *Aspergillus* colonization and subsequent accumulation of aflatoxins. Aflatoxin contamination is inversely proportional to the maturity of seeds [38]. Seed maturity also corresponds to the production of phytoalexins, which have been shown to have a protective effect on aflatoxin production [25]. To minimize or standardize the impact of maturity, pre-harvest, and postharvest practices, all peanut varieties in this study were grown in a common field under standard conditions and harvested at their respective optimum maturity dates.

The weak negative correlation between the density of *A. flavus* on peanut kernels and resistance to aflatoxin accumulation implies that aflatoxin resistance increases as fungal density decreases and vice versa, indicating the possible involvement of resistance mechanisms that prevent fungal growth and toxin production. These observations also corroborate the findings of Commey et al. [39] that peanut seed coat acts as a physical and biochemical barrier against infection by *A. flavus*. Genotypes with thicker seed coats and smaller hilum, in addition to compact seed coat structures, demonstrated increased resistance to infection by *A. flavus* ([40]; Mendu et al., 2021). Though differences in testa thickness were not evaluated in the current study, the integrity of the physical barrier of all peanut accessions was ensured by using UV light for surface sterilization as opposed to other techniques such as sodium hypochlorite and 70 % ethanol [20]. Therefore, any resistance to aflatoxin arising from differences in seed coat thickness could be a result of genetic variations in the accessions.

The insignificance of the weak negative correlation could be explained by the inconsistent behaviour of some peanut accessions and the lack of a distinct relationship between fungal density on kernels and aflatoxin resistance. For example, peanut accessions such as EU1, KKMG4, and MG, which were dark-coloured had substantial fungal density on kernels, yet were resistant to aflatoxin accumulation (Plate 1a). This observation could be attributed to the role of peanut seed coat in resistance to colonization by *A. flavus* and aflatoxin production and possibly ([25]; Nayak et al., 2021). In contrast, accession EUGN1 did not have a high fungal density on the kernels post-inoculation but was susceptible to aflatoxin accumulation. Overall aflatoxin resistance is a factor of the interaction of numerous parameters such as genetics, environment, morphology, or physiology [41,42]. Given that seed coat colour, phenolic compound composition, thickness, and kernel size are genetically determined and vary by genotype [25], this could explain why the negative correlation between the density of *A. flavus* on peanut kernels and resistance to aflatoxin accumulation was not statistically significant. Although these parameters were not measured in this study, future studies could explore their role in the observed resistance to aflatoxin accumulation in various peanut accessions.

5. Conclusion

Of the 25 accessions that were tested, more than half exhibited kernel resistance to the accumulation of aflatoxin. Two accessions BS and UG-ML exhibited an unusual behaviour where the toxin levels were reduced upon challenge by toxigenic *A. flavus*, which could be attributed to their dark-coloured seed coats and likely involvement of polyphenols. These outcomes are promising in the quest to determine peanut genotypes with aflatoxin resistance. Future studies should explore the aflatoxin resistance of these genotypes under field conditions as well as the gene expression profiles of the peanut during colonization and aflatoxin production. This information would give more insights into the mechanisms of aflatoxin resistance in peanuts and inform breeding endeavours.

CRediT authorship contribution statement

Elsie Nyangweso Salano: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Richard Mwanza Mulwa:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Meshack Amos Obonyo:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors did not use any AI or AI-assisted tools. The authors prepared from scratch, reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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