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Evaluation of Groundnut Genotypes for Resistance to Early Leaf Spot Disease and Preharvest Aflatoxin Contamination

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Abstract
Aflatoxin caused by *Aspergillus flavus* is a major field to storage contaminant in groundnut seed production, whereas defoliation diseases in groundnut caused by *Passalora arachidicola* and *Nothopassalora personata* are two of the most yield-limiting biotic stresses. The present study was to identify groundnut genotypes with resistance to leaf spot disease and preharvest aflatoxin contamination. Thirty-three groundnut genotypes originating from crosses between Schubert × 43-09-03-02 and TS32-1 × 60-02-03-02 including three checks: Manipinta, Chinese and ICGV-03401 were evaluated in screenhouse and field studies. In the screenhouse experiment, the groundnut genotypes were arranged in a Complete Randomized Design with three replications. The field experiment was laid in a 9 × 4 alpha lattice design with three replications. Results revealed that *A. flavus* population in the soil increased when the fields were inoculated with the *A. flavus* isolate. Again, results show that these groundnut genotypes namely, L024, L030, L078B, L086A, L092 and L096, showed resistance to leaf spot disease infection. Also, except for L020B, which respectively showed a B_1 and B_2 aflatoxin of 71.5 and 72.0 ppb under field conditions and 132.1 and 131.5 ppb in pots, the remaining genotypes did not accumulate any aflatoxin at the preharvest level. In conclusion, the study identified groundnut genotypes L024, L030, L078B, L086A, L092 and L096 to be resistant to early leaf spot disease and preharvest aflatoxin contamination. These genotypes could be tested in the future for their yield and stability under diverse environments and released as varieties.

Keywords: Aflatoxin; *Aspergillus flavus*; groundnut; inoculation; preharvest

Évaluation des génotypes d'arachide pour la résistance à la maladie des taches foliaires précoces et à la contamination par l'aflatoxine avant la récolte

Résumé
L'aflatoxine causée *par Aspergillus* flavus est un contaminant majeur du champ au stockage dans la production de graines d'arachide, tandis que les maladies de défoliation de l'arachide causées par *Passalora arachidicola* et *Nothopassalora personata* sont deux des stress biotiques qui limitent le plus le rendement. La présente étude vise à identifier les génotypes d'arachide résistants à la maladie des taches foliaires et à la contamination par les aflatoxines avant la récolte.

Trente-trois génotypes d'arachide issus de croisements entre Schubert x 43-09-03-02 et TS32-1 × 60-02-03-02, y compris trois contrôles: Manipinta, Chinese et ICGV-03401 ont été évalués en serre et sur le terrain. Dans l'expérience en serre, les génotypes d'arachide ont été disposés selon un plan aléatoire complet avec trois répétitions. L'expérience sur le terrain a été réalisée selon un plan en treillis alpha 9 × 4 avec trois répétitions. Les résultats ont révélé que la population d' *A. flavus* dans le sol augmentait lorsque les champs étaient inoculés avec l'isolat d'*A. flavus*. De nouveau, les résultats montrent que ces génotypes d'arachide, à savoir L024, L030, L078B, L086A, L092 et L096, ont montré une résistance à l'infection par la maladie des taches foliaires. En outre, à l'exception de L020B, qui a respectivement montré une aflatoxine B_1 et B_2 de 71,5 et 72,0 ppb en conditions de terrain et de 132,1 et 131,5 ppb en pots, les autres génotypes n'ont pas accumulé d'aflatoxine au niveau de la pré-récolte. En conclusion, l'étude a identifié les génotypes d'arachide L024, L030, L078B, L086A, L092 et L096 comme étant résistants à la maladie des taches foliaires précoces et à l'aflatoxine avant la récolte. Ces génotypes pourraient être testés à l'avenir pour leur rendement et leur stabilité dans divers environnements et être homologués en tant que variétés.

Mots clés: Aflatoxine; *Aspergillus flavus*; arachide; inoculation; pré-récolte.

Introduction

Groundnut (*Arachis hypogaea* L.) production is a major source of livelihood for most households, as well as food for humans and feed for livestock (Danso-Abbeam *et al.,* 2015; Variath & Janila, 2017). Nutritionally, groundnut seeds contain high value edible oil (40-50%), consumable protein (20-50%) and carbohydrates (10-20%) with high levels of vitamin E, niacin, riboflavin, thiamine, folic acid, and minerals (Akram *et al.,* 2018; Bonku & Yu, 2020).

Groundnut production faces numerous challenges: soil nutrient deficiencies, cultivation of unimproved varieties, drought, weeds, pests and diseases (Bediako *et al.,* 2019; Wilfred *et al.,* 2020). Furthermore, foliar diseases such as late leaf spot and rust are known to be the most destructive diseases which cause complete defoliation and yield losses of up to 70% in susceptible genotypes (Saleem *et al.,* 2021; Sawadogo *et al.,* 2021). Also, early leaf spot caused by *Passalora arachidicola* and late leaf spot caused by *Nothopassalora personata* diseases are the most yield-limiting biotic stresses in groundnut production worldwide, causing yield losses of up to 50% or even 70% in West Africa (Denwar *et al.,* 2021). Similarly, groundnut seed infected with molds, predominantly *Aspergillus flavus* and *Aspergillus parasiticus* renders ground-nut grain unsafe for consumption due to their potential to produce aflatoxin (Waliyar *et al.,* 2016; Bediako *et al.,* 2019). Aflatoxin-contaminated groundnut seeds have reduced quality, quantity and marketability, thereby having a debilitating effect on the general health and well-being of humans and livestock and the income of farmers (Guchi, 2015; Waliyar *et al.,* 2016; Balendres *et al.,* 2019).

Aflatoxin contamination of groundnut begins in the field and continues during storage, transportation and processing when abiotic conditions are favourable for the aflatoxin-producing fungi (Guchi *et al.,* 2014; Bediako *et al.,* 2019). *A. flavus* is a soilborne fungal pathogen that infects groundnut seeds and is responsible for preharvest and postharvest

aflatoxin contamination (Zhao *et al.,* 2019).

Preventative and curative measures are needed to manage aflatoxin contamination (Guchi, 2015). The development of aflatoxin-resistant cultivars can be an effective preventive strategy that is inexpensive and easily disseminated (Soni *et al.,* 2020). Resistance to preharvest aflatoxin accumulation can be attributed to the structure of the pod, and it has been reported that the first groundnut and *A. flavus* interaction was observed at the pod stage, where the pod serves as a physical barrier for fungal penetration (Wang *et al.,* 2016; Pfliegler *et al.,* 2020). Some groundnut genotypes have been identified under the preharvest aflatoxin contamination (PAC) screening (Waliyar *et al.,* 2016). However, cultivated groundnut is still unavailable for farmers use (Njoroge, 2018). Breeding groundnut genotypes for resistance to leaf spot disease, preharvest aflatoxin buildup and other desirable traits coupled with already existing postharvest management techniques could be a lasting plan for mycotoxin control and yields.

Therefore, the present study aimed at identifying groundnut genotypes with resistance to leaf spot disease and preharvest aflatoxin accumulation.

Materials and Methods

Location of study

The study was conducted at the Council for Scientific and Industrial Research (CSIR) - Savanna Agricultural Research Institute (SARI), Nyankpala, about 16 km west of Tamale. The average monthly atmospheric temperatures range from 26 to 39 °C with an annual mean of 32 °C.

Groundnut genotypes and their sources

A total of 36 genotypes were studied. Thirty-three (33) F_6 genotypes evaluated in this study originated from the crosses between Schubert × 43-09-03-02 and TS32-1 × 60-02-03-02. Both 60-02-03-02 and 43-09-03-02 are interspecific introgression genotypes whilst TS32-1 and Schubert are Spanish groundnut genotypes (Tengey, 2018). The remaining three varieties (i.e. 'Manipinta', 'Chinese' (Kotu *et al.,* 2022) obtained from CSIR-SARI, and a resistant genotype ICGV-03401 (Asare, 2019) obtained from CSIR-Crops Research Institute (CRI), served as checks (Table 1).

Experimental design

Screening for preharvest aflatoxin contamination was done under field and screenhouse conditions. The fields were laid in a 9 × 4 alpha lattice design with three replications while the screenhouse experiment was arranged in a Complete Randomized Design with three replications.

Soil preparation and sterilization for screen house experiment

Samples of sandy loam soil taken from 15 cm depth were collected from experimental sites using a 5 cm diameter soil auger and sterilized as described by Awuku (2017) and Kankam *et al.* (2019) before filling 40 cm diameter pots in the screenhouse.

Planting and agronomic practices observed

Thirty-three groundnut genotypes including two susceptible ('Chinese' and 'Manipinta') and resistant checks (ICGV-03401) were planted in the field and screenhouse on July 30, 2020 and October 10, 2020, respectively. Planting was done in the field at the end of July to expose the groundnut genotypes to terminal drought stress, during which a favourable soil condition is created for rapid growth and development of *A. flavus* around the rhizosphere of the groundnut plant for aflatoxin accumulation. Each plot consisted

Table 1: Advanced groundnut genotypes used for preharvest aflatoxin contamination

No	Genotype	Source	Pedigree
1	L004	T×L164301-4	TS32-1 × 43-09-03-02
2	L006A	T×L164301-6	TS32-1 × 43-09-03-02
3	L007A	T×L164301-7	TS32-1 × 43-09-03-02
4	L009	T×L164301-9	TS32-1 × 43-09-03-02
5	L010A	T×L164301-10	TS32-1 × 43-09-03-02
6	L012	T×L164301-12	TS32-1 × 43-09-03-02
7	L014	T×L164301-14	TS32-1 × 43-09-03-02
8	L015A	T×L164301-15	TS32-1 × 43-09-03-02
9	L020B	T×L164302-20	TS32-1 × 43-09-03-02
10	L024	T×L164302-24	TS32-1 × 43-09-03-02
11	L027B	T×L164302-27	TS32-1 × 43-09-03-02
12	L029	T×L164302-29	TS32-1 × 43-09-03-02
13	L030	T×L164302-30	TS32-1 × 43-09-03-02
14	L034	T×L164303-34	TS32-1 × 43-09-03-02
15	L039	T×L164303-39	TS32-1 × 43-09-03-02
16	L043A	T×L164303-43	TS32-1 × 43-09-03-02
17	L061	T×L164304-61	60-02-03-02 × Schubert
18	L068G	T×L164305-68	Schubert × 60-02-03-02
19	L076J	T×L164305-76	Schubert × 60-02-03-02
20	L078B	T×L164305-78	Schubert × 60-02-03-02
21	L081A	T×L164305-81	Schubert × 60-02-03-02
22	L083	T×L164305-83	Schubert × 60-02-03-02
23	L085B	T×L164305-85	Schubert × 60-02-03-02
24	L086A	T×L164305-86	Schubert × 60-02-03-02
25	L088	T×L164305-88	Schubert × 60-02-03-02
26	L089A	T×L164305-89	Schubert × 60-02-03-02
27	L092	T×L164306-92	Schubert × 60-02-03-02
28	L094	T×L164306-94	Schubert × 60-02-03-02
29	L095	T×L164306-95	Schubert × 60-02-03-02
30	L096	T×L164306-96	Schubert × 60-02-03-02
31	L102	T×L164306-102	Schubert × 60-02-03-02
32	L104B	T×L164306-104	Schubert × 60-02-03-02
33	L106	T×L164306-106	Schubert × 60-02-03-02
34	ICGV-03401	CSIR-CRI	Resistant cultivar
35	Chinese	CSIR-SARI	Susceptible cultivar
36	Manipinta	CSIR-CRI	Susceptible cultivar

AGG-Advanced Groundnut Genotype, RC-Resistant Cultivar, SC-Susceptible Cultivar, SARI-Savanna Agricultural Research Institute, CRI-Crops Research Institute

of 15 plants arranged in a single row spanning a length of 3 m. One seed/hole was planted at a spacing of 20 cm intra-row and 50 cm inter-row. Fields were hand weeded with hoe at four, six and eight weeks after planting. In the screenhouse experiment, the weeds were controlled regularly by handpicking till harvesting.

Field and screenhouse inoculation with *A. flavus*

Preparation of Inoculum

Organic-matrix method (cracked corn) was used to prepare *A. flavus* inoculum (Will, *et al.,* 2009). A 10 day old culture of an aflatoxigenic isolate of *A. flavus* obtained from the Spanish Laboratory, University for Development Studies, Ghana, at a concentration of 1×10^6 spores ml^{-1} determined using a hemocytometer was used to inoculate sterile cracked corn (10 mL/114 g of corn) to produce the organic matrix (Will *et al.,* 2009). Before the inoculation, the cracked corn was sterilised by autoclaving at 15 Ibs (121 °C) for 15 minutes and allowed to cool in the laminar flow. The infected sterilised cracked corn was incubated for 7 days at room temperature to sporulate *A. flavus*. The sterilised and infected cracked corn served as a source of inoculum for *A. flavus,* used for field inoculation.

Inoculation of Groundnut Plants

The soil at the rhizosphere of groundnut plants or pod zones was inoculated with *A. flavus* inoculum to ensure its abundance. Plots were inoculated approximately 60 days after planting (DAP) during the pod development and seed filling stages. A total weight of 60 g of cracked corn infected with *A. flavus* was weighed and applied per 3 m row (maximum 15 plants per row). For the field trial and screenhouse trial, each plant received 4 g of cracked infected corn of *A. flavus* (Holbrook *et al.,* 2000; Will *et al.,* 2009).

Data collection

Population of *A. flavus* in the soil

The *Aspergillus flavus* population in soil samples were enumerated from the screenhouse experiment and field prior to inoculation and after harvest of the groundnut genotypes. Soil samples (1 kg) were taken from the above surface level to about 5 cm deep on the field diagonally for all plots. These samples were labelled and thoroughly mixed for analysis. A similar procedure was followed for the screenhouse experiment. The soil samples were sieved using a 2 mm sieve. A sample of the soil (1 g) was weighed and dissolved in 9 mL of sterile distilled water, serially diluted to 10^{-10}, and then 1 ml of soil solution of 10^{-10} (Odhiambo *et al.,* 2013) was spread on PDA medium. After 7-day incubation at room temperature (25 ± 1 °C), colonies of *A. flavus* (identified as a yellow-greenish mold) were counted.

Colony forming units (cfu) per gram of soil was calculated using the formula according to Arunyanark *et al.* (2009).

$$\text{CFU/g Soil} = \frac{A*B*C}{D*E}$$

where

A = number of *Aspergillus flavus* colonies
B = volume of sterilized water added (mL)
C = dilution factor
D = weight of soil sub-sample (g)
E = volume of soil solution spread (mL)

Assessment of Early Leaf Spot Severity

Since the area is a hotspot for early leaf spot disease (Neindow *et al.,* 2018), the disease severity of the 36 groundnut genotypes was assessed using a modified 9-point scale, where 1 is no leaf spot disease, and 9 is almost all leaves defoliated, leaving bare stems with some leaflets showing severe leaf spots (Subrahmanyam *et al.,* 1995). Disease scoring

began 37 days after planting and was repeated at two-week intervals. Using a modified 9 points scale according to Pooniya *et al.* (2020), the genotypes were further categorized based on their resistance and susceptibility as described where 1 indicates highly resistant at 0% defoliation, 2-3 shows resistance with 1- 20% defoliation, 4-5 represents moderate resistance with 21-50% defoliation, 6-7 indicates susceptibility with 51-70% defoliation and 8-9 represent highly susceptible with 70-100% defoliation.

Visual Rating for Drought Stress

Drought stress on the groundnut genotypes was rated using a modified scale of 1-5, where 1 is healthy plants, no symptoms of drought stress, leaves are raised, turgid, green/bright green and 5 is plants severely wilted and/or nearly dead (Luis *et al.,* 2016). Visual rating was done at two-day intervals between 12:00 p.m. and 1:00 p.m. for field and screen house experiments. The afternoon rating was to assess drought stress as influenced by heat from the sun.

Harvesting of Groundnut Pods

Groundnut plants were carefully dug out, and pods were handpicked at 107 DAP (November 24, 2020, for the field experiment and February 4, 2021, for the screenhouse experiment). The harvested groundnut pods were sun dried for two weeks.

Groundnut Genotype Preparation for Aflatoxin Analysis after Harvesting

After sun drying, pods were hand-shelled while wearing protective gear, including gloves and lab coats to prevent the introduction of external contaminants. Additionally, any equipment that had contact with the previous sample after processing were thoroughly cleaned; grains of each genotype were transferred to Ziplock bags and stored at -20 ºC until further analysis. The samples were kept in an ice chest with ice packs and sent to the Mycotoxins laboratory of the Kwame Nkrumah University of Science and Technology for aflatoxin analysis.

Determination of Aflatoxin Concentration Using HPLC Technique

Sample Extraction

Aflatoxin was extracted using a modified method originally proposed by Sirhan *et al.* (2014). A Preethi Mixer Grinder was used to mill and homogenize the samples. A 2 g sample was weighed into a 15 mL centrifuge tube, 5 mL of distilled water was added, and the tube vortexed for 1 min. The solution was allowed to stand for 5 mins. A volume of 5 mL 1% (v/v) acetic acid in acetonitrile solution was added. The resultant mixture was vortexed using a Genie Vortex machine for 3 mins. A mass of 1.32 g of anhydrous MgSO4 and 0.2 g of NaCl were added to the mixture and vortexed for 1 min. The tube was centrifuged for 5 mins at 4000 rpm, and the upper organic layer filtered through a 0.45 µm nylon syringe before injection. A volume of 50 µL of the filtered extract was injected into the high-performance liquid chromatography (HPLC).

HPLC Analysis

HPLC analysis was carried out based on AOAC Official Method 2005.08 (AOAC, 2006). A Cecil-Adept Binary Pump HPLC coupled with Shimadzu 10A¤L fluorescence detector (Ex: 360 nm, Em: 440 nm) with Sunfire® C18 Column (150 × 4.60 mm, 5 um). The mobile phase used was methanol: water (40:60, v/v) at a flow rate of 1mL/min with column temperature maintained at 40°C. Aflatoxin Mix (G_1, G_2, B_1, B_2) standards (ng/g) were prepared from Romer Labs® aflatoxin standard of 5.02 ng/µL in acetonitrile. Aflatoxins in the samples were detected using the retentions of the standard

solution run and quantification done using the calibration curves of each respective toxin. The Limit of Detection and Limit of Quantification of total aflatoxin were established at 0.5 ng/g and 1 ng/g, respectively.

Statistical Analysis

The data set on the agronomic, leaf spot disease incidence, severity and aflatoxin accumulation in the field and screenhouse were subjected to analysis of variance (ANOVA) using GenStat Discovery (12th Edition). Treatment means were separated using the Tukey HSD test at 5% significant level.

Results

Estimation of *A. flavus* population in the soil

The *A. flavus* population significantly ($P < 0.05$) differed among soil samples from the field and the screenhouse experiments. Soil samples taken after inoculation had a significantly higher *A. flavus* population than those taken before inoculation in both the field and screenhouse experiments. Soil samples from the screenhouse experiment had the highest *A. flavus* population (4752 ± 1218 cfu/g) after inoculation compared with the soil samples obtained from the field experiment (3636 ± 1218 cfu/g). The lowest population of *A. flavus* in the soil before inoculation was 522 ± 1218 cfu/g from the screenhouse experiment. *A. flavus* population in the soil before and after inoculation and in the field and the screenhouse experiment are shown in Figure 1.

Early leaf spot disease scores

Genotypes showed significant differences ($P < 0.05$) to early leaf spot disease at 51, 65, 79, 93 and 107 DAP and area under the disease progress curve values (Table 2). The results indicate that Manipinta and Chinese had the least and highest leaf spot infections, respectively. The genotypes L024, L030, L078B, L086A, L092, L096, and Manipinta showed resistance to leaf spot infection. Similarly, L004, L006A, L007A, L009, L010A, L012, L014, L015A, L020B, L027B, L029, L034, L039, L043A, L061, L068G, L076J, L081A, L083, L085B, L088, L089A, L094, L095, L102, L104B and L106 showed moderate resistance to leaf spot infection whereas ICGV-03401 and Chinese showed susceptibility (Table 2).

Visual drought rating of groundnut genotypes under field and screenhouse experiments

Groundnut genotypes exhibited significant variations ($P < 0.05$) in terms of their visual rating in the field and screenhouse experiments (Table 3). Genotypes L083 and L088 recorded drought stress score of less than 2, whereas Chinese (check), L015A and L095 had the highest drought stress score of 4 under field conditions. In the screenhouse, all groundnut genotypes had a drought stress rating score ranging from 3 to 5.

Preharvest aflatoxin contamination in groundnut genotypes

Except for L020B, aflatoxins B1 and B2 were undetected in the genotypes evaluated in the field and screenhouse experiments (Table 4; Figure 2).

Discussion

The study showed *A. flavus* densities of 24.1% in the field and 9.9% in the soil used in screenhouse experiment before inoculation. Our results agree with the findings of Tédihou *et al.* (2012), who had a condensed natural *A. flavus* population in the soil ranging from 653.1 to 2062.3 cfu/g prior to inoculation. In our study, the physical inoculation of the aflatoxigenic *A. flavus* isolate increased the population level of *A. flavus* in the soil for both the field and screenhouse experiments, which also agrees

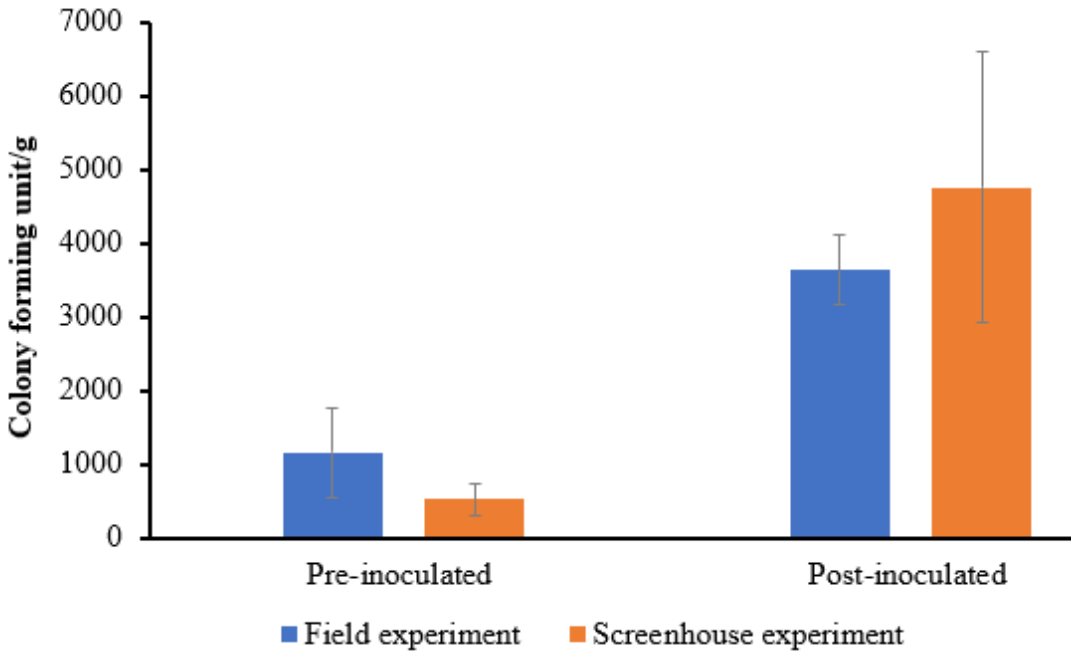

Figure 1: *Aspergillus flavus* population in soil samples before and after inoculation. Error bars indicate standard errors of means

with the findings of Tédihou *et al.* (2012), who reported an increased level of *A. flavus* population in all the inoculated plots. The high *A. flavus* population in the soil after inoculation could be attributed to terminal drought exposure; this agrees with the previous study by Sibakwe *et al.* (2017), who reported significantly higher *A. flavus* population in groundnuts exposed to prolonged drought on the field than the control plants with no drought exposure. Our data showed that the population of *A. flavus* in the screenhouse increased by almost 10-fold compared to about 3-fold in the field experiment. The reduction in the *A. flavus* population in the field experiment compared with the screenhouse experiment might be due to the washing off of conidia by rain or dew or by dispersal of conidia by wind (Rossi *et al.,* 2009). The first interaction between groundnut genotypes and *A. flavus* is at the pod shell in the soil (Wang *et al.,* 2016; Pfliegler *et al.,* 2020). In our study, some of the groundnut genotypes accumulated no aflatoxin at preharvest which could be attributed to the pod structure which serves as a physical barrier to *A. flavus* infection and penetration. As reported earlier resistance of groundnut to aflatoxin contamination and *A. flavus* infection could be attributed to the structure of the pod shell (Wang *et al.,* 2016).

Moreover, the result revealed that aside from the groundnut genotypes being resistant to preharvest aflatoxin accumulation, the genotypes were also resistant to leaf spot

Table 2: Early Leaf Spot (ELS) scores and area under disease progress curve (AUDPC) in the genotypes

Genotype	ELS 37DAP	ELS 51DAP	ELS 65DAP	ELS 79DAP	ELS 93DAP	ELS 107DAP	AUDPC
L004	2.3^{a}	3.0^{a}	3.0^{a}	3.0^{a-d}	3.3^{a-d}	3.7^{a-d}	214.7^{a-f}
L006A	2.3^{a}	2.3^{a}	2.3^{a}	4.3^{b-f}	5.0^{c-e}	5.3^{c-e}	249.7^{b-f}
L007A	2.0^{a}	2.0^{a}	2.3^{a}	3.3^{a-e}	3.7^{a-d}	3.7^{a-d}	198.3^{a-e}
L009	2.0^{a}	2.0^{a}	2.0^{a}	3.0^{a-d}	3.7^{a-d}	4.0^{a-d}	191.3^{a-d}
L010A	2.0^{a}	2.3^{a}	2.3^{a}	3.0^{a-d}	4.0^{a-e}	3.7^{a-d}	203.0^{a-e}
L012	2.3^{a}	3.0^{a}	3.0^{a}	3.7^{a-f}	5.3^{de}	5.3^{c-e}	263.7^{c-f}
L014	2.3^{a}	2.3^{a}	2.7^{a}	3.7^{a-f}	4.7^{b-e}	4.7^{b-e}	235.7^{b-f}
L015A	2.0^{a}	2.3^{a}	2.7^{a}	3.3^{a-e}	4.0^{a-e}	4.0^{a-d}	214.7^{a-f}
L020B	2.0^{a}	2.0^{a}	2.3^{a}	4.0^{a-f}	4.3^{b-e}	5.3^{c-e}	228.7^{a-f}
L024	2.3^{a}	2.7^{a}	2.7^{a}	2.3^{ab}	2.7^{ab}	2.7^{ab}	179.7^{a-c}
L027B	2.3^{a}	2.3^{a}	3.0^{a}	3.7^{a-f}	4.0^{a-e}	4.3^{a-d}	228.7^{a-f}
L029	3.0^{a}	3.0^{a}	3.0^{a}	4.0^{a-f}	4.3^{b-e}	4.3^{a-d}	252.0^{b-f}
L030	2.7^{a}	2.7^{a}	3.0^{a}	3.3^{a-e}	3.3^{a-d}	3.3^{a-c}	214.7^{a-f}
L034	2.3^{a}	2.3^{a}	3.0^{a}	5.3^{ef}	5.3^{de}	5.3^{c-e}	277.7^{d-f}
L039	2.0^{a}	2.3^{a}	2.3^{a}	5.0^{d-f}	5.0^{c-e}	5.3^{c-e}	256.7^{b-f}
L043A	2.3^{a}	3.0^{a}	3.0^{a}	4.7^{c-f}	4.7^{b-e}	4.7^{b-e}	263.7^{c-f}
L061	2.7^{a}	3.0^{a}	3.0^{a}	4.3^{b-f}	4.3^{b-e}	4.3^{a-d}	254.3^{b-f}
L068G	2.0^{a}	2.0^{a}	3.0^{a}	4.0^{a-f}	4.7^{b-e}	5.0^{b-e}	240.3^{b-f}
L076J	2.3^{a}	2.7^{a}	2.7^{a}	3.7^{a-f}	3.7^{a-d}	4.3^{a-d}	224.0^{a-f}
L078B	2.0^{a}	2.0^{a}	2.0^{a}	3.0^{a-d}	3.0^{a-c}	3.3^{a-c}	177.3^{a-c}
L081A	2.0^{a}	2.3^{a}	2.7^{a}	3.7^{a-f}	4.3^{b-e}	4.7^{b-e}	228.7^{a-f}
L083	2.0^{a}	2.0^{a}	2.0^{a}	3.3^{a-e}	4.3^{b-e}	4.3^{a-d}	207.7^{a-f}
L085B	2.0^{a}	2.3^{a}	2.7^{a}	3.7^{a-f}	4.3^{b-e}	4.3^{a-d}	226.3^{a-f}
L086A	2.0^{a}	2.0^{a}	2.0^{a}	2.7^{a-c}	3.0^{a-c}	3.0^{a-c}	170.3^{ab}
L088	2.3^{a}	2.7^{a}	3.0^{a}	4.0^{a-f}	4.7^{b-e}	4.7^{b-e}	249.7^{b-f}
L089A	2.0^{a}	3.0^{a}	3.0^{a}	3.3^{a-e}	3.3^{a-d}	3.7^{a-d}	217.0^{a-f}
L092	2.0^{a}	2.0^{a}	2.0^{a}	2.7^{a-c}	3.0^{a-c}	3.3^{a-c}	172.7^{ab}
L094	2.7^{a}	2.7^{a}	2.7^{a}	5.0^{d-f}	5.3^{de}	5.3^{c-e}	275.3^{d-f}
L095	2.0^{a}	2.3^{a}	2.7^{a}	4.3^{b-f}	4.3^{b-e}	4.3^{a-d}	235.7^{b-f}
L096	2.7^{a}	3.0^{a}	3.0^{a}	3.0^{a-d}	3.0^{a-c}	3.0^{a-c}	207.7^{a-f}
L102	2.3^{a}	3.0^{a}	3.0^{a}	5.0^{d-f}	5.3^{de}	5.3^{c-e}	282.3^{ef}
L104B	2.3^{a}	3.0^{a}	3.0^{a}	4.3^{b-f}	4.3^{b-e}	5.3^{c-e}	259.0^{b-f}
L106	2.3^{a}	2.7^{a}	3.0^{a}	4.0^{a-f}	4.0^{a-e}	4.0^{a-d}	235.7^{b-f}
ICGV-03401	2.3^{a}	2.3^{a}	3.0^{a}	5.7^{f}	6.0^{e}	6.0^{de}	296.3^{f}
Chinese	2.3^{a}	2.3^{a}	3.0^{a}	4.0^{a-f}	5.0^{c-e}	7.0^{e}	266.0^{c-f}
Manipinta	2.0^{a}	2.0^{a}	2.0^{a}	2.0^{a}	2.0^{a}	2.0^{a}	140.0^{a}
P Value	0.232	0.001	0.001	0.001	0.001	0.001	0.001
CV%	18.1	16.7	12.9	18.1	15.6	17.9	12.0

Table 3: Visual rating of groundnut genotypes under field and screenhouse

Genotype	Visual Rating	
	Field Experiment	Screenhouse Experiment
L004	2.3^{abc}	5.0^{a}
L006A	3.0^{cde}	5.0^{a}
L007A	2.3^{abc}	5.0^{a}
L009	3.3^{def}	5.0^{a}
L010A	2.7^{bcd}	5.0^{a}
L012	2.3^{abc}	5.0^{a}
L014	2.7^{bcd}	5.0^{a}
L015A	4.0^{f}	5.0^{a}
L020B	3.0^{cde}	5.0^{a}
L024	2.3^{abc}	5.0^{a}
L027B	2.7^{bcd}	5.0^{a}
L029	3.0^{cde}	5.0^{a}
L030	3.3^{def}	5.0^{a}
L034	3.7^{ef}	5.0^{a}
L039	2.7^{bcd}	5.0^{a}
L043A	2.3^{abc}	5.0^{a}
L061	2.0^{ab}	5.0^{a}
L068G	2.7^{bcd}	5.0^{a}
L076J	3.7^{ef}	5.0^{a}
L078B	2.7^{bcd}	5.0^{a}
L081A	2.0^{ab}	4.7^{ab}
L083	1.8^{ab}	4.7^{ab}
L085B	2.0^{ab}	4.7^{ab}
L086A	2.7^{bcd}	4.7^{ab}
L088	1.8^{ab}	4.3^{bc}
L089A	2.0^{ab}	4.3^{bc}
L092	2.0^{ab}	4.0^{cd}
L094	2.0^{ab}	4.0^{cd}
L095	4.0^{f}	4.0^{cd}
L096	3.7^{ef}	4.0^{cd}
L102	2.7^{bcd}	4.0^{cd}
L104B	3.3^{def}	4.0^{cd}
L106	2.3^{abc}	4.0^{cd}
ICGV-03401	3.3^{def}	5.0^{a}
Chinese	4.0^{f}	5.0^{a}
Manipinta	3.3^{def}	3.7^{d}
P-Value	< 0.001	< 0.001
CV (%)	19.8	7.4

Table 4: Aflatoxin contamination on the field and screenhouse experiment

Genotype	Aflatoxin Contamination Level (ppb)			
	Field		Screenhouse	
	AFB_1	AFB_2	AFB_1	AFB_2
L004	nd	nd	nd	nd
L006A	nd	nd	nd	nd
L007A	nd	nd	nd	nd
L009	nd	nd	nd	nd
L010A	nd	nd	nd	nd
L012	nd	nd	nd	nd
L014	nd	nd	nd	nd
L015A	nd	nd	nd	nd
L020B	71.47	72.01	132.09	131.48
L024	nd	nd	nd	nd
L027B	nd	nd	nd	nd
L029	nd	nd	nd	nd
L030	nd	nd	nd	nd
L034	nd	nd	nd	nd
L039	nd	nd	nd	nd
L043A	nd	nd	nd	nd
L061	nd	nd	nd	nd
L068G	nd	nd	nd	nd
L076J	nd	nd	nd	nd
L078B	nd	nd	nd	nd
L081A	nd	nd	nd	nd
L083	nd	nd	nd	nd
L085B	nd	nd	nd	nd
L086A	nd	nd	nd	nd
L088	nd	nd	nd	nd
L089A	nd	nd	nd	nd
L092	nd	nd	nd	nd
L094	nd	nd	nd	nd
L095	nd	nd	nd	nd
L096	nd	nd	nd	nd
L102	nd	nd	nd	nd
L104B	nd	nd	nd	nd
L106	nd	nd	nd	nd
ICGV-03401	nd	nd	nd	nd
Chinese	nd	nd	nd	nd
Manipinta	nd	nd	nd	nd

Note: ppb-part per billion; AFB_1-aflatoxin B_1; AFB_2-aflatoxin B_2; nd-not detected weather conditions

disease, suggesting multiple disease resistance. Our finding agrees with the previous finding of Saleem *et al.* (2021) who also identified a multiple stress resistant groundnut genotype. Early leaf spot disease resistance of these genotypes confirms a previous selection of these genotypes based on their resistance from an F_6 population (Tengey *et al.* unpublished).

The groundnut genotype that showed the presence of aflatoxin contamination after field and screenhouse inoculation indicates its susceptibility to preharvest aflatoxin contamination. Apart from the aggressiveness of the pathogen as a factor for infection, Pandey *et al.* (2019) reported that the genotype must also be susceptible for it to succumb to aflatoxin contamination.

Most of the groundnut genotypes including susceptible checks, namely 'Chinese' and

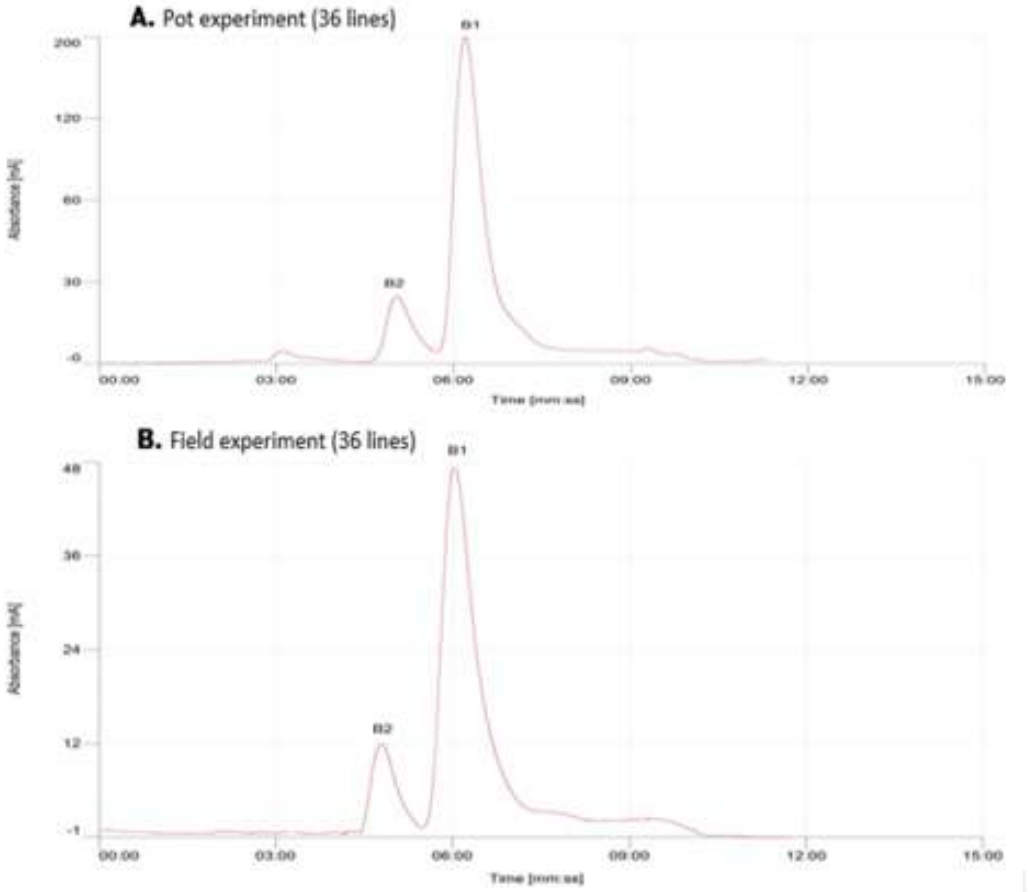

Figure 2: Chromatograms of aflatoxin B_1 and B_2 of groundnut L020B where B_1 and B_2 represent aflatoxins. 2A represents aflatoxin accumulation results from the screenhouse experiment while 2B represents the aflatoxin accumulation results from the field experiment

'Manipinta', showed no aflatoxin contamination at the preharvest level, which corroborates the work of Yeboah *et al.* (2020), who stated that the aflatoxin analysis results of some commonly cultivated groundnut varieties in Ghana such as Chinese had no accumulation of aflatoxin before storage.

The genotype, L020B was moderately resistant to *in vitro A. flavus* infection but showed susceptibility to preharvest aflatoxin contamination for both field and screenhouse experiments, which are above the acceptable limits for human consumption. This non-relationship in the resistance mechanism among preharvest, seed colonisation and aflatoxin accumulation has been reported by Ojiewo *et al.* (2020), who attributed this to different genes involved in resistance mechanisms which are very inconsistent, and self-governing.

In conclusion, L024, L030, L078B, L086A, L092, and L096 were resistant to leaf spot infections and had no aflatoxin contamination at preharvest stage and when coupled with other postharvest aflatoxin management strategies would reduce aflatoxin contamination in the diet of humans and animals that consume them and increase productivity and yield. Therefore, further investigation should be carried out on the genotypes on different storage methods and time.

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Conflict of Interests
The authors declare no conflicts of interest.

Data Availability
The datasets generated and/or analysed during the current study are available in the Mendeley data repository, [https://data.mendeley.com/datasets/43d2t4r2p4/1/files/c804d417-1dbd-444d-aa51-a6de7e102b46]"

Approval for Human Experiments
All the methods performed in the experiment were carried out in accordance with relevant guidelines and regulations.

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