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Full Length Research Article

EFFECTS OF *MORINGA OLEIFERA* INTERCROPPING AND ITS ROOT EXTRACT ON YIELDS AND AFLATOXIN CONTENTS OF GROUNDNUT (*ARACHIS HYPOGAEA* L.) SEEDS UNDER *ASPERGILLUS FLAVUS* INFECTION

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ABSTRACT
Aflatoxins produced by *Aspergillus flavus* pose serious health risks and commonly contaminate groundnut (*Arachis hypogaea* L.), creating the need for sustainable control methods. This study evaluated the effects of *Moringa oleifera* root pulp extract and intercropping on aflatoxin contamination and yield of groundnut varieties artificially infected with toxigenic *A. flavus*. The experiment was conducted using a Randomized Complete Block Design with cement sacks containing sterilized loamy soil and organic manure (3:1). Treatments included *A. flavus* infection alone, Moringa root extract application, and Moringa intercropping. Aflatoxin levels were quantified using ELISA, and yield parameters (pods and seeds per plant) were statistically analyzed. Results showed significant varietal differences in yield, with treatment–variety interactions significantly influencing pod production ($p \leq 0.05$). Aflatoxin levels ranged from 0.022 to 0.060 ppb, with most varieties showing moderate contamination (0.023–0.025 ppb). The highest aflatoxin level was observed in AFMRE C (0.060 ± 0.003 ppb), while the lowest occurred in AFMS E (0.022 ± 0.005 ppb). Moringa intercropping generally reduced fungal colonization and aflatoxin accumulation more effectively than root extract application. The effectiveness of Moringa-based treatments depends largely on groundnut varietal traits, with genetic resistance being a key factor. Integrating resistant varieties with Moringa intercropping is recommended as an eco-friendly strategy for managing *A. flavus* infection and aflatoxin contamination in groundnut, and further field-based research is advised.

Keywords: *Moringa oleifera*, *Arachis hypogaea*, *Aspergillus flavus*, intercropping, aflatoxin.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.), is an annual legume of significant global importance, cultivated across tropical, subtropical, and warm temperate regions. It is grown on approximately 25.2 million hectares worldwide, producing 35.9 million metric tons annually, with Asia and Africa contributing 66% and 25% of global production, respectively (FAO, 2023). Nigeria ranks first in Africa and fourth globally in groundnut production, producing 1.55–1.65 million metric tonnes annually (NBS, 2024).
Aflatoxins are toxic, polyketide-derived secondary metabolites produced predominantly by *Aspergillus flavus* and *Aspergillus parasiticus*. While *A. flavus* is more prevalent in Africa, *A. parasiticus* dominates in the Americas (Xue, 2020). These fungi contaminate groundnuts both pre- and post-harvest, resulting in substantial yield losses, grain downgrading, and serious health risks to consumers. Prolonged aflatoxin exposure is associated with impaired immunity, malnutrition, stunted growth, liver cirrhosis, hepatocellular carcinoma, and synergistic interactions with hepatitis B and C infections (Bediako *et al.,* 2019). Consequently, aflatoxin contamination is recognized as a major barrier to food security, nutrition, and international trade, prompting many countries to establish maximum permissible limits in food and feed. In response to the growing challenge of aflatoxin contamination, increasing attention is being directed toward eco-friendly management strategies, particularly the use of bioactive compounds from plants. *Moringa oleifera*, commonly referred to as the "miracle tree," has emerged as a promising candidate due to its broad-spectrum antimicrobial and antifungal properties. Native to South Asia but now widely cultivated across tropical and subtropical regions, *M. oleifera* is valued for its nutritional, medicinal, and industrial uses (Pareek *et al.,* 2023). Its leaves, bark, roots, flowers, and seeds are rich in bioactive compounds with reported efficacy against fungal pathogens and their mycotoxins (Abdel-Razek *et al.,* 2019; Chaudhari *et al.,* 2021). Beyond nutritional benefits, local farming practices and oral traditions suggest that intercropping groundnut with *M. oleifera* may reduce postharvest storage problems associated with *Aspergillus* species (Chile and Gwa, 2021).

Despite anecdotal evidence, there remains a paucity of systematic research investigating the antifungal and anti-mycotoxigenic potential of *M. oleifera* in groundnut production systems. Considering the pressing need for sustainable and accessible aflatoxin management strategies, exploring the role of *M. oleifera* in mitigating fungal infection and toxin contamination is both timely and necessary. This study therefore determined the anti-aflatoxigenic efficacy of *M. oleifera* root extracts and intercropping on yield and aflatoxin content of groundnuts challenged with toxigenic *Aspergillus flavus* strain.

MATERIAL AND METHODS

Study Area

The study was carried out at the Nigerian Defence Academy Postgraduate School, Unguwan Kanawa located in Kaduna North Local Government Area of Kaduna State in Nigeria. The vegetation cover of Kaduna State is Sudan Savannah type, characterized by scattered short trees, shrubs and grasses. The soil is mostly loamy to sandy. A substantial amount of clay is found also.

Experimental Design

The design consists of four treatments. Treatments 1 - 4 representing the various combinations, the details of which are as follows:

Group 1: Groundnut seedling only (Control)
Group 2: Groundnut seedling + *Aspergillus flavus*
Group 3: Groundnut seedling + *Aspergillus flavus* + *Moringa oleifera* seedling
Group 4: Groundnut seedling + *Aspergillus flavus* + *Moringa oleifera* root extract

All the treated groups were planted in sacks and allowed to germinate. Treatment with Moringa oleifera root extract started after the seedling emergence and continues after every 2 weeks for a period of six months.

Collection and Characterization of *Aspergillus flavus* Culture

This was done according to the method described by (Alexander and Street, 2001). A well-characterized, toxigenic strain of *Aspergillus flavus* was obtained from the culture collection of the Department of Microbiology, Ahmadu Bello University (ABU), Zaria, Nigeria. The isolate had been previously identified and confirmed for its aflatoxin-producing potential using standard morphological and molecular characterization techniques. The culture was maintained on potato dextrose agar (PDA) slants and stored at 4 °C prior to use to preserve its viability and toxigenic properties. Fresh sub-cultures were prepared 5–7 days before seed inoculation to ensure optimal fungal viability and consistent aflatoxin-producing capacity. All handling of the fungal isolate was carried out in a biosafety level 2 (BSL-2) laboratory following standard microbiological safety procedures, including the use of personal protective equipment (laboratory coats, gloves, and face masks) and work within a laminar airflow cabinet to prevent environmental contamination and personnel exposure. The isolate was selected due to its regional relevance and prior use in aflatoxin-related research in northern Nigeria, thereby ensuring ecological suitability and reproducibility of the findings

Groundnut Seeds Collection

The groundnut seeds varieties were obtained from the Institute for Agricultural Research (IAR) in Zaria, and purchased from Kawo market Kaduna State in Nigeria. 50 kg of each variety of the groundnut seeds were obtained in clean polythene bags,

Collection of Moringa Seedlings

Moringa seedlings were purchased from Teku Farms in Kaduna North local Government area of Kaduna State. The plant samples were taken to the Department of Biological Sciences, Nigerian Defence Academy, Kaduna for Authentication of *Moringa oleifera* L. Identity of *Moringa oleifera* L. seedlings were identified at the Herbarium, Department of Biological Sciences, Nigerian Defence Academy, Kaduna Nigeria. After authentication the plant was issued the voucher numbers as follows: *Moringa oleifera* L.: NDA/BIOH/202512, Local Nut: NDA/BIOH/202513, Samnut 23: NDA/BIOH/202514, Samnut 24: NDA/BIOH/202515: Samnut 25: NDA/BIOH/202516, and Samnut 28: NDA/BIOH/202517, they were all deposited in the Herbarium unit for future reference.

Preparation of *Moringa oleifera* Root Pulp Extract

Fresh and healthy *Moringa oleifera* roots are collected, authenticated, thoroughly washed under running tap water and rinsed with distilled water to remove soil and debris, surface-sterilized with 70% ethanol and rinsed again with sterile distilled water, peeled and chopped into small pieces, ground using a sterile mortar and pestle or blender to obtain a fine pulp, mixed with sterile distilled water in a 1:5 (w/v) ratio, agitated on a rotary shaker for 24 hours at room temperature to allow extraction of bioactive compounds, filtered first through muslin cloth and then Whatman No.1 filter paper, concentrated in a water bath at 40–45 °C to reduce volume, and finally transferred into sterile, labeled amber bottles and stored at 4 °C until use (Doughari *et al.*, 2007).

Greenhouse Study

The experiment was conducted in a Greenhouse-type environment using a Randomized Complete Block Design (RCBD) to minimize the effects of environmental variability within the setup. The planting units used were cement sacks, which served as containers for growing groundnuts under controlled conditions. Standard-sized, used cement sacks (approximately 50 kg capacity) were used. Each sack was cleaned and punctured at the base to allow for proper drainage. The sacks were filled with homogenized, sterilized loamy soil mixed with organic manure in a 3:1 ratio to ensure adequate nutrient availability and soil aeration. Each sack was treated as an individual experimental unit and planted with a specific combination of groundnut variety and treatment.

Inoculation of *Aspergillus flavus* Culture

Groundnut seeds were artificially inoculated with toxigenic strains of *Aspergillus flavus* by preparing a nutrient broth culture of the fungus, which was diluted with sterile water and decanted into sterile containers. The suspension was dispensed onto sterilized Petri plates containing groundnut seeds and incubated for 5 days to allow fungal colonization. Seed surface colonization was assessed using a 1–4 severity rating scale according to Thakur *et al.* (2000). Successfully colonized (infected) seeds were then selected and planted for subsequent experiments. Additionally, *A. flavus* spore suspensions were prepared under sterile conditions and directly inoculated into the soil at planting for treatment groups 2, 3, and 4 to ensure consistent pathogen pressure.

***Moringa oleifera* Seedling Intercropping Procedure**

Each planting sack was sown with three groundnut seeds spaced approximately 15 cm apart in a triangular arrangement to optimize light interception and nutrient use. For treatment group 3, one healthy *Moringa oleifera* seedling (2–3 weeks old) was transplanted at the center of each sack, and the groundnut seeds were planted equidistantly around it. Sacks within each replicate were spaced 30 cm apart to ensure adequate aeration and ease of maintenance. A 1-meter distance was maintained between replicate blocks to reduce edge effects and minimize cross-contamination, particularly from *A. flavus*.

***Moringa oleifera* Root Extract Application Method**

For treatment group 4, an aqueous root extract of *Moringa oleifera* was prepared by macerating fresh roots and filtering the mixture through sterile muslin cloth. The filtrate was applied to the soil at a measured dose on a biweekly basis, beginning after groundnut seedling emergence and continued for a period of six months. Post-harvest, all replicates with the same treatment were bulked to form twenty composite samples, and 15 g of each sample was packaged and submitted to the National Agency for Food and Drug Administration and Control (NAFDAC) for aflatoxin analysis.

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Agronomic Management

All planting sacks were watered manually twice a week using watering cans to maintain consistent soil moisture without causing waterlogging or encouraging excessive fungal growth. Pest control was achieved through the application of a broad-spectrum systemic insecticide (Dress Force) at three-week intervals according to the manufacturer's recommended dosage. Spraying was carried out early in the morning or late in the evening to minimize photodegradation and evaporation, and care was taken to prevent contact between the insecticide and *Moringa oleifera* seedlings or root extract treatment zones. The experiment was conducted using three replicates, with each replicate block separated by 1-meter alleys to ensure clear demarcation, facilitate movement, and minimize cross-treatment contamination; each treatment was represented in every replicate to allow for reliable statistical analysis.

Harvesting and Collection of the Groundnut Sample

Groundnut pods were harvested after six months of sowing, based on physiological maturity indicators. Maturity was determined when more than 50% of the plants exhibited visible signs of senescence, such as yellowing and browning of leaves, hardening of the seed coat, and a noticeable reduction in seed moisture content, which are widely accepted markers of harvest maturity in groundnut (Swathi *et al.*, 2025). The haulms were uprooted and left to dry on top of their sacks to avoid mixing of varieties. Drying took approximately about two weeks to complete thoroughly. The pods were now carefully collected and transferred into labelled polythene bags. The pods were weighed, and the weights recorded as shown in Table 1. Subsequently, the pods were then threshed, and the seeds were carefully placed into another sterile, labelled polythene bags to avoid contamination. The seeds were then weighed, and the weights were recorded as shown in table 1.

Determination of Pod and Seed Yields

The determination of pod and seed yields was carried out following standard agronomic procedures in which plants were harvested at physiological maturity, the harvested pods were separated from haulms, cleaned to remove debris, and weighed to obtain the fresh pod weight; thereafter, pods were air-dried and oven-dried at 60–70 °C to constant weight before threshing to separate seeds, which were similarly cleaned and weighed to obtain seed weight, while moisture content was determined using the AOAC (2019) oven-drying method at 105 °C, after which pod and seed yields (kg ha^{-1}) were calculated by converting dry weights from the measured plot area to a hectare basis as described in the FAO (2000) Seed Production Manual and the IITA (2012) Standard Operating Procedures for seed handling and yield evaluation.

Aflatoxin Extraction and Quantification

The samples were extracted according to the general scheme of the ELISA method as described by Hurburg (2005). A small quantity of the sample was transferred to a vessel and combined with the extraction solvent. For the groundnut cake and paste, 10 g of the sample was used for analysis. Also 20 cm^3 of groundnut oil was used for assay. After mixing with 100-200 cm3 of 80 % acetonitrile for 1 min, the samples were centrifuged, and the supernatants were collected for analysis. The ELISA was performed according to the manufacturer's instruction (Helica Biosystems Inc, Santa Anna, CA). All reagents for ELISA were equilibrated to room temperature before use. About 200 µL of the sample was pipetted into the appropriate wells and mixed. Thereafter, 100 µL of the mixture was transferred to the appropriate antibody-coated wells in triplicate and incubated at ambient temperature for 30 mins. The wells were washed with PBS-T and tapped dry. About 100 µL of aflatoxin HRP- conjugate was added to each antibody coated well and incubated at room temperature for 30 min afterward washed three times with PBS-T buffer and tapped dry. In addition, 100 µL of the TMB substrate was added to each microwell and the plate was incubated at ambient temperature for 10 min. Finally, about 100 µL of the stopped solution was added to each well. The optical density (OD) of each of the microwell was read at 450 nm on a StatFax 2100 spectrophotometer using a differential filter of 630 nm.

Data Analysis

Analysis was performed in triplicate. The data was presented as mean ± standard error of the mean (SEM). Analysis of variance (ANOVA) and visualising data in SPSS version 23.0 were used for statistical analysis, and values with $p \leq 0.05$ were used for statistical significance difference.

RESULTS

Pods and Seeds Yields of Ground nut Varieties Treated *Moringa oleifera* Root Extract and intercropping

These parameters were estimated at harvest by weighing the pods and seeds per plant (standard) of the varieties as shown in Table 1. These parameters were estimated at harvest by weighing the pods and seeds per plant (standard) of the varieties. Test results showed significant differences between the test varieties. CNT A and CNT B, AFMS B and CNT B, AFMS A and CNT D, AFMS A and AFMS B were significantly different ($p \leq 0.05$) for the average number of pods. There were statistically significant differences between CNT B and CNT A, CNT C, AF C, AFMS A, AFMS C, and AFMRE B, while CNT C and AFMS B were also significantly different ($p \leq 0.05$).

Aflatoxin Content of Groundnut seeds varieties Treated with Toxigenic *Aspergillus flavus* strain

The aflatoxin levels evaluated from groundnut varieties, as shown in Table 2, ranged from 0.022 to 0.060 ppb, with the highest concentration recorded in AFMRE C (0.060 ± 0.003 ppb), corresponding to a percentage of 94.9%, and the lowest in AFMS E (0.022 ± 0.005 ppb) at 79.3%. Most varieties, including CNT B, D, E; AF A, D; and all AFMS varieties except AFMS C which had aflatoxin levels around 0.023–0.024 ppb indicating no statistically significant differences ($p \leq 0.05$) between the varieties. However, AF B (0.037 ± 0.000 ppb), AF C (0.032 ± 0.001), and AFMS C (0.032 ± 0.001 ppb), AFMRE B (0.025 ± 0.000), AFMRE C (0.060 ± 0.003), and E AFMRE C (0.031 ± 0.000) showed relatively higher levels but did not significantly differ from all others.

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Table 1: Effect *Moringa oleifera* Root Extract and Seedling Intercropping on Pod and Seed Yields of *Arachis hypogaea* Varieties

Treatment	G. nut Variety	Weight of Pods (g/plant)	Weight of Seeds (g/plant)	Seed: Pod Ratio (%)
Control (CNT)	Samnut 23 (A)	57.00 ± 5.29^{a}	28.67 ± 3.79^{a}	50.3
	Samnut 24 (B)	19.00 ± 20.66^{ab}	10.00 ± 9.54^{abcdef}	52.6
	Samnut 25 (C)	48.33 ± 16.50	29.00 ± 7.55^{b}	60
	Samnut 28 (D)	24.67 ± 6.35^{c}	16.00 ± 7.21	64.9
	Local (E)	37.33 ± 16.50	22.33 ± 11.24	59.8
A. flavus only (AF)	Samnut 23 (A)	38.67 ± 17.04	18.00 ± 7.55	46.6
	Samnut 24 (B)	40.00 ± 28.69	23.33 ± 14.57	58
	Samnut 25 (C)	42.00 ± 18.74	27.67 ± 14.36^{c}	65
	Samnut 28 (D)	32.00 ± 11.36	19.00 ± 7.21	59.3
	Local (E)	29.33 ± 5.69	23.00 ± 1.00	78
A. flavus + Moringa Seedlings (AFMS)	Samnut 23 (A)	62.00 ± 45.03^{bcd}	28.33 ± 19.14^{d}	45.7
	Samnut 24 (B)	20.67 ± 19.22^{ad}	12.67 ± 11.68	61.3
	Samnut 25 (C)	47.67 ± 2.52	27.33 ± 1.53^{e}	57.3
	Samnut 28 (D)	42.33 ± 47.16	17.33 ± 14.01	40.9
	Local (E)	35.67 ± 10.50	22.67 ± 6.81	63.6
A. flavus + Moringa Root Extract (AFMRE)	Samnut 23 (A)	51.33 ± 7.02	26.67 ± 7.10^{f}	51.9
	Samnut 24 (B)	26.00 ± 12.49	16.00 ± 8.72	61.5
	Samnut 25 (C)	40.67 ± 15.37	26.00 ± 9.64	63.9
	Samnut 28 (D)	34.00 ± 13.08	21.33 ± 6.66	62.7
	Local (E)	32.00 ± 16.37	21.00 ± 10.15	65.6

Key**:** Values represent mean ± standard deviation (SD). Means followed by the same superscript letter within a column are **not significantly different** ($p < 0.05$; $LSD_{0.05}$).

CNT = Control (Groundnut only); AF = *A. flavus*-infected only; AFMS = *A. flavus* + *Moringa* seedlings; AFMRE = *A. flavus* + *Moringa* root extract. A = Samnut 23; B = Samnut 24; C = Samnut 25; D = Samnut 28; E = Local variety.

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Table 2: Aflatoxin levels (ppb) of seed of groundnut varieties evaluated

Varieties	Aflatoxin levels (ppb)	Percentage
CNT A	0.028 ± 0.001 ^{c}	96.6
CNT B	0.024 ± 0.000 ^{b}	82.8
CNT C	0.027 ± 0.002^{ac}	93.1
CNT D	0.024 ± 0.001 ^{b}	79.3
CNT E	0.024 ± 0.001 ^{b}	79.3
AF A	0.024 ± 0.001 ^{b}	82.8
AF B	0.037 ± 0.001	97.6
AF C	0.026 ± 0.001 ^{c}	93.1
AF D	0.025 ± 0.001 ^{b}	86.2
AF E	0.026 ± 0.001	89.7
AFMS A	0.024 ± 0.001 ^{b}	82.8
AFMS B	0.023 ± 0.001 ^{b}	75.9
AFMS C	0.032 ± 0.001	86.9
AFMS D	0.023 ± 0.001 ^{b}	82.8
AFMS E	0.022 ± 0.001	79.3
AFMRE A	0.026 ± 0.001 ^{c}	89.7
AFMRE B	0.025 ± 0.001	86.2
AFMRE C	0.060 ± 0.001	94.9
AFMRE D	0.027 ± 0.001 ^{ac}	93.1
AFMRE E	0.031 ± 0.001	90.4

Values are expressed as mean ± STD. Statistical significance mean difference was considered at $p<0.05$ and $LSD_{0.05}$ comparison test was used for post hoc analysis. Values bearing the same superscripts under the same column are not significantly different.
CNT= Control- Groundnut seedling only; AF= Groundnut seedling + *Aspergillus flavus* only; AFMS= Groundnut seedling + *Aspergillus flavus* + Moringa seedlings; AFMRE= Groundnut seedling + *Aspergillus flavus*+ Moringa root extract; A= Samnut 23 variety; B= Samnut 24 variety; C= Samnut 25 variety; D= Samnut 28 variety; E= Local groundnut.

Seed Colonization by *Aspergillus flavus* Detected in the Groundnut Varieties

The findings in Table 3 indicate clear differences in susceptibility to Aspergillus flavus infection among the groundnut varieties evaluated. Most varieties including CNT B, CNT D, CNT E, AF A, AF D, AFMS A, AFMS B, AFMS D, AFMS E, AFMRE B, AFMRE D, and AFMRE E showed moderate susceptibility, with aflatoxin levels ranging from 0.022 to 0.025 ppb. A second group consisting of CNT A, CNT C, AF B, AF C, AF E, AFMS C, and AFMRE A exhibited higher aflatoxin concentrations (0.026–0.037 ppb) and were classified as susceptible, showing little difference from the untreated control varieties (CNTs). The variety AFMRE C recorded the highest aflatoxin level (0.060 ppb) and was identified as very susceptible, as neither moringa seedling intercrop nor moringa root extract conferred protection. Overall, the results highlight significant varietal differences and suggest that biocontrol effectiveness depends on the combined influence of plant–soil ecological interactions rather than extract application alone.

Table 3: Effect *Moringa oleifera* Root Extract and Intercropping on Aflatoxin Content of *Arachis hypogaea* Seeds challenged with Toxigenic *Aspergillus flavus* strain

Treatment	G. nut Variety	Aflatoxin (ppb, mean ± SD)	% Seed Contamination	% Reduction Aflatoxin	Level of Resistance
CNT	Samnut 23 (A)	0.028 ± 0.001 c	96.6	–	Susceptible
	Samnut 24 (B)	0.024 ± 0.000 b	82.8	–	Susceptible
	Samnut 25 (C)	0.027 ± 0.002 ac	93.1	–	Susceptible

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	Samnut 28 (D)	0.024 ± 0.001 b	79.3	–	Moderately Susceptible
	Local (E)	0.024 ± 0.001 b	79.3	–	Moderately Susceptible
AF	Samnut 23 (A)	0.024 ± 0.001 b	82.8	14.30	Moderately Susceptible
	Samnut 24 (B)	0.037 ± 0.001	97.6	−54.2	Very Susceptible
	Samnut 25 (C)	0.026 ± 0.001 c	93.1	3.70	Susceptible
	Samnut 28 (D)	0.025 ± 0.001 b	86.2	4.20	Susceptible
	Local (E)	0.026 ± 0.001	89.7	8.30	Susceptible
AFMS	Samnut 23 (A)	0.024 ± 0.001 b	82.8	14.30	Susceptible
	Samnut 24 (B)	0.023 ± 0.001 b	75.9	4.20	Moderately Susceptible
	Samnut 25 (C)	0.032 ± 0.001	86.9	−18.5	Very Susceptible
	Samnut 28 (D)	0.023 ± 0.001 b	82.8	4.20	Susceptible
	Local (E)	0.022 ± 0.001	79.3	8.30	Moderately Susceptible
AFMRE	Samnut 23 (A)	0.026 ± 0.001 c	89.7	7.10	Susceptible
	Samnut 24 (B)	0.025 ± 0.001	86.2	4.20	Susceptible
	Samnut 25 (C)	0.060 ± 0.001	94.9	−122.2	Most Susceptible
	Samnut 28 (D)	0.027 ± 0.001 ac	93.1	12.50	Moderately Susceptible
	Local (E)	0.031 ± 0.001	90.4	−29.2	Very Susceptible

Values are mean ± SD. Values with the same superscript within a column are not significantly different ($p < 0.05$, LSD post hoc test). Moderately Susceptible ≤ 0.023 ppb; Susceptible 0.024–0.030 ppb; Very Susceptible > 0.030 ppb; Most Susceptible > 0.055 ppb (Guchi & Ayalew, 2023). CNT = Control (groundnut only), AF = + Aspergillus flavus, AFMS = + A. flavus + Moringa seedlings, AFMRE = + A. flavus + Moringa root extract.

DISCUSSION

The study demonstrated that Moringa oleifera root extract and seedling intercropping mitigated the negative effects of *Aspergillus flavus* infection on the pod and seed yields of *Arachis hypogaea*, though the degree of improvement observed was distinctly variety dependent. In general, the application of root extract (AFMRE) offered more consistent yield protection than intercropping alone, confirming its value as a biocontrol intervention under aflatoxigenic stress conditions. Six months after sowing, harvested haulms were dried and processed, and the resulting seed-to-pod ratios fell within the normal ranges reported in groundnut production under standard agronomic practices (Appah, 2011), indicating that plant growth and yield formation were not physiologically compromised by the treatments themselves.

The aflatoxin analysis revealed relatively low aflatoxin concentrations across all treatments (<5 ppb), indicating a generally effective suppression of aflatoxin biosynthesis. With the exception of one variety (C), which exhibited very high susceptibility (0.060 ppb), all Moringa seedling intercrop treatments produced aflatoxin levels classified as only "susceptible," making them the best-performing intervention for aflatoxin reduction in this study. Importantly, disease escape was not possible, as all varieties were deliberately and uniformly inoculated with *A. flavus* before planting. This method ensured consistent pathogen pressure and allowed differences in disease response to be attributed solely to treatment and varietal characteristics a widely accepted approach in host–pathogen evaluation (Mehan *et al.*, 1986; Horn & Dorner, 1999; Waliyar *et al.*, 2006).

While root extract treatments were expected to perform strongly, their inconsistent effectiveness in some varieties may be attributed to leaching of bioactive compounds from the soil surface into deeper layers with irrigation or rainfall, reducing their persistence and limiting anti-fungal activity in the rhizosphere (Agyare *et al.*, 2013). By contrast, Moringa seedling intercropping provided more stable results, likely due to continuous root-zone interactions, allelopathy, and modification of the microbial environment shared with groundnut a mechanism supported by earlier studies (Foidl *et al.*, 2001; Singh *et al.*, 2020; Guchi, 2015; Boaz *et al.*, 2017). The varieties showing the highest susceptibility including AFMRE C, AFMRE E, AFMS C and AF B were those whose root extract treatments were most likely influenced by phytochemical leaching, supporting the importance of treatment delivery method.

The broader literature affirms that aflatoxin contamination in groundnut arises from a complex interplay of environmental and biological factors, including drought stress, insect damage, pod cracking, soil inoculum levels, and postharvest handling (Hell *et al.*, 2000; Cotty & Jaime-Garcia, 2007). High soil temperatures and low soil moisture are particularly associated with increased aflatoxin accumulation (Guo *et al.*, 2009). Insect activities such as those of *Elasmolomus sordidus* and *Helicoverpa armigera* have also been shown to facilitate entry of *Aspergillus* spp. into pods (Widstrom *et al.*, 2001). The biosynthesis of aflatoxin is regulated by a complex

gene cluster influenced by environmental stressors (Yu *et al.*, 2004; Roze *et al.*, 2007), with aflatoxin B1 being the most toxic (IARC, 2002; Klich, 2007). The health implications of exposure remain serious, contributing to liver cancer, immunosuppression, and child growth impairment (Turner *et al.*, 2007; Wild & Gong, 2010), with documented fatal outbreaks linked to aflatoxin-contaminated food (Lewis *et al.*, 2005).

Natural plant extracts, including those from Moringa, garlic, and ginger, have previously shown promise in suppressing aflatoxigenic fungi (Tripathi & Dubey, 2004; Ogundero, 2011). Evidence from this study aligns with such findings, as Moringa demonstrated clear antifungal activity. The variability in extract efficacy across varieties is consistent with reports that phytochemical concentrations determine the potency of antifungal extracts (Amadioha, 2003; Gwa & Richard, 2018; Ekefan *et al.*, 2018). The activity of Moringa extracts has been linked to high levels of flavonoids, terpenes, tannins, glycosides, saponins, and coumarins (Qusti *et al.*, 2010), all of which are known to inhibit fungal growth.

The observed varietal differences in aflatoxin accumulation can also be explained by seed testa and pod characteristics, which act as primary barriers to fungal invasion. Previous studies report that intact seed testa with compact palisade layers, high wax content, and tannin accumulation significantly restrict colonization by *A. flavus* (Wotton & Strange, 1987; Kushalappa *et al.*, 1979; Gradziel & Wang, 2016; Laprade *et al.*, 2018; Awuah & Ellis, 2019). Damage to the testa through mechanical abrasion or harvesting is known to increase fungal penetration, which explains why some varieties presented higher aflatoxin levels.
Overall, the findings indicate that Moringa seedling intercropping (AFMS) generally provided the greatest reduction in aflatoxin levels, with the highest improvement observed in Samnut 23 (14.3%). In contrast, root extract treatments produced more variable outcomes, with certain varieties such as Samnut 25 and Local E displaying increased aflatoxin levels relative to the control. These results highlight the importance of varietal characteristics in determining the success of Moringa-based interventions. Samnut 23 emerged as the most resistant variety, maintaining low aflatoxin levels across all treatments.

Conclusion

The study demonstrates that *Moringa oleifera*-based interventions particularly seedling intercropping significantly mitigate the impact of *Aspergillus flavus* infection on groundnut pod and seed yields, although their effectiveness is strongly variety-dependent. While both Moringa treatments reduced aflatoxin accumulation, seedling intercropping consistently outperformed root extract application, likely due to more stable rhizosphere interactions and reduced loss of bioactive compounds. Aflatoxin levels across most treatments remained below 5 ppb, confirming substantial suppression of aflatoxin biosynthesis even under deliberate pathogen inoculation. The observed differences in varietal responses highlight the role of intrinsic traits such as testa integrity, pod permeability, and biochemical defenses in determining susceptibility to fungal invasion. Samnut 23 emerged as the most resistant variety, maintaining low aflatoxin levels across all treatments, whereas AFMRE C showed extreme susceptibility despite intervention. Overall, the study establishes that integrating Moringa seedling intercropping with tolerant groundnut varieties offers a practical, ecologically sustainable, and biologically effective strategy for reducing aflatoxin contamination in groundnut production systems.

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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.

REFERENCES

Abdel-Razek, A. G., Shehata, M. G., Badr, A. N., Gromadzka, K., & Stępień, L. (2019). The effect of chemical composition of wild *Opuntia ficus-indica* by-products on its nutritional quality, antioxidant, and antifungal efficacy. *Egyptian Journal of Chemistry, 62*(Special Issue), 47–61.

Agyare, W. A., Asare, I. K., Sogbedji, J. & Clottey, V. A. (2013). Challenges to maize fertilization in the forest and transition zones of Ghana. *African Journal of Agricultural Research, 9*(7), 593–602.

Alexander S. and Street D. (2001). In microbiology aphotographic atlas for the laboratory. Benjamincummings, an imprint of Addison Wesly longman, pp.51-52.

Amadioha, A. C. (2003). Evaluation of some plants leaf extracts against *Colletotrichum lindemuthianum* in cowpea. *Acta Phytopathologica et Entomologica Hungarica, 32*(2), 141–149.

AOAC (2019). *Official Methods of Analysis of the Association of Official Analytical Chemists*. 21st Edition. AOAC International, Gaithersburg, Maryland, USA.

Appah, J. (2011). Biofungicidal activity of selected plant products in the management of late leaf spot of groundnut (*Arachis hypogea* L.) incited by *Phaeoisariopsis personata* (Berk & Curt) v. Arx. PhD Thesis, Ahmadu Bello University, Zaria-Nigeria. Pp 117.

Awuah, R. T., & Ellis, W. O. (2019). Effects of some packaging methods and protection with *Ocimum* and *Syzygium* powders on kernel infection by fungi. *Mycopathologia, 154*(1), 29–36.

Bediako, K. A., Ofori, K., Offei, S. K., Deziedzienyo, D., Asibuo, J. W. & Amaoh, R. A. (2019). Aflatoxin contamination of groundnut (*Arachis hypogaea* L.): Predisposing factors and management interventions. *Food Control, 98*, 61–67.

Boaz, N., Wachira, P., Kagot, V. & Okoth, S. (2017). Susceptibility of locally cultivated groundnut (Arachis hypogaea) varieties to aflatoxin accumulation in Homa Bay County, Kenya. *African Journal of Microbiology Research*, 11, 1329-1337.

Chaudhari, A. K., Singh, V. K., Das, S. & Dubey, N. K. (2021). Nanoencapsulation of essential oils and their bioactive constituents: A novel strategy to control mycotoxin contamination in food system. *Food and Chemical Toxicology, 149*, 112019.

Chile, D. D. & Gwa, V. I. (2021). Aflatoxigenic contamination of groundnut (*Arachis hypogaea* L.) seed and its management using seed extract of *Moringa oleifera* Lam. and rhizome of *Zingiber officinale* Rosc. in Katsina State, Nigeria. *Journal of Mycology and Mycological Sciences, 4*(2), 000141.

Cotty, P. J., & Jaime-Garcia, R. (2007). Influences of climate on aflatoxin producing fungi and aflatoxin contamination.

International Journal of Food Microbiology, 119(1–2), 109–115.

CSA (Central Statistical Agency). (2019). Agricultural Sample Survey, *2015/2016 (2008 E.C.), Report on Area and Production of Major Crops, Volume I*. Addis Ababa, May 2016.

Doughari, J. H., Pukuma, M. S., & De, N. (2007). Antibacterial effects of *Moringa oleifera* leaf extracts. *African Journal of Biotechnology*, 6(10), 1426–1430.

Ekefan, E. J., Nwankiti, A. O., & Gwa, V. I. (2018). Comparative assessment of antimicrobial potency of some selected plant extracts against seed-borne pathogens of germinating yam setts. *Journal of Plant Microbiology, 9*, 444.

FAO (2000). *Seed Production Manual*. Food and Agriculture Organization of the United Nations, Rome, Italy.

FAO (2023). *Agricultural production statistics* 2000–2022. FAOSTAT Analytical Briefs, No. 79. Rome. https://doi.org/10.4060/cc9205en

Foidl, N., Makkar, H. P. S. & Becker, K. (2001). The potential of *Moringa oleifera* for agricultural and industrial uses. In L. J. Fuglie (Ed.), *The miracle tree: The multiple uses of Moringa* (pp. 45–76). CTA and CWS.

Gradziel, T. M. & Wang, D. (2016). Susceptibility of California almond cultivars to aflatoxigenic *Aspergillus flavus*. *Horticultural Science, 29*(1), 33–35.

Guchi, E. (2015). Aflatoxin Contamination in Groundnut (*Arachis hypogaea* L.) Caused by Aspergillus Species in Ethiopia. 3, 11-19.

Guo, B., Chen, Z. Y., Lee, R. D. & Scully, B. T. (2009). Drought stress and preharvest aflatoxin contamination in agricultural commodities: Genetics, genomics, and proteomics. *Journal of Integrative Plant Biology, 51*(8), 763–773.

Gwa, V. I. & Nwankiti, A. O. (2018). In vitro and in vivo antimicrobial potency of selected plant extracts in the control of postharvest rot-causing pathogens of yam tubers in storage. *Global Journal of Pests, Diseases and Crop Protection, 6*(1), 276–287.

Gwa, V. I. & Richard, I. B. (2018). Susceptibility of white yam (*Dioscorea rotundata* Poir) tuber to rot fungi and control with extracts of *Zingiber officinale* Rosc., *Azadirachta indica* A. Juss. and *Piper guineense* Schumach. *Journal of Plant Pathology & Microbiology, 9*, 452.

Hell, K., Cardwell, K. F., Setamou, M. & Poehling, H. M. (2000). The influence of storage practices on aflatoxin contamination in maize in four agroecological zones of Benin, West Africa. *Journal of Stored Products Research, 36*(4), 365–382.

Horn, B. W. & Dorner, J. W. (1999). Regional differences in production of aflatoxin B1 and cyclopiazonic acid by soil isolates of Aspergillus flavus along a transect within the United States. *Applied and Environmental Microbiology*, *65*(4), 1444–1449.

Hurburg, C. R. (2005). Sampling and testing for aflatoxin in corn (Contribution of Dr. Charles R., Professor of Agricultural Engineering, Iowa State).

IITA (2012). *Standard Operating Procedures for the Evaluation of Germplasm and Seed Handling*. International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

International Agency for Research on Cancer (IARC). (2002). *Aflatoxins* (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 82). International Agency for Research on Cancer.

Klich, M. A. (2007). Environmental and developmental factors influencing aflatoxin production by *Aspergillus flavus* and *Aspergillus parasiticus*. *Mycoscience, 48*(2), 71–80.

Kushalappa, A. C., Bartz, J. A., & Norden, A. J. (1979). Susceptibility of pods of different peanut genotypes to *Aspergillus flavus* group fungi. *Phytopathology, 69*(2), 159–162.

Laprade, J. C., Bartz, J. A., Norden, A. J., & Demuynk, J. T. (2018). Correlation of peanut seed coat wax accumulation with tolerance to colonization by *A. flavus*. *Journal of American Peanut Research and Education Association, 5*(2), 89–94.

Lewis, L., Onsongo, M., Njapau, H., Schurz-Rogers, H., Luber, G., Kieszak, S., Nyamongo, J., Backer, L., Dahiye, A. M., Misore, A., DeCock, K. & Rubin, C. (2005). Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in eastern and central Kenya. *Environmental Health Perspectives, 113*(12), 1763–1767.

Liang, X. Q., Luo, M. & Guo, B. Z. (2006). Resistance mechanisms to *Aspergillus flavus* infection and aflatoxin contamination in peanut (*Arachis hypogaea* L.). *Plant Pathology Journal, 5*(2), 115–124.

Mamkaa, D. P. & Gwa, V. I. (2018). Effect of *Moringa oleifera* and *Vernonia amygdalina* leaf extracts against *Aspergillus flavus* and *Botryodiplodia theobromae* causing rot of cowpea (*Vigna unguiculata* (L.) Walp) seeds. *Applied Science Research Review, 5*(1–2), 1–7.

Mehan, V. K., McDonald, D. & Rajagopalan, K. (1986). Resistance of groundnut genotypes to seed invasion by Aspergillus flavus in field trials in India. *Plant Disease*, *70*(8), 720–722.

Moyo, B., Masika, P. J. & Muchenje, V. (2012). Antimicrobial activities of *Moringa oleifera* Lam leaf extracts. *African Journal of Biotechnology, 11*(11), 2797–2802.

Ogundero, V. W. (2011). Antifungal activities of leaf extracts of *Moringa oleifera* on *Aspergillus flavus* and *Aspergillus niger* isolated from stored groundnuts. *Journal of Mycology, 4*(2), 22–28.

Pareek, A., Pant, M., Gupta, M. M., Kashania, P., Ratan, Y., Jain, V., Pareek, A., & Chuturgoon, A. A. (2023). *Moringa oleifera*: An updated comprehensive review of its pharmacological activities, ethnomedicinal, phytopharmaceutical formulation, clinical, phytochemical, and toxicological aspects. *International Journal of Molecular Sciences, 24*(3), 2098. https://doi.org/10.3390/ijms24032098

Qusti, S., Khatwa, A. N. A. & Lahwa, M. A. (2010). Screening of antioxidant activity and phenolic content of selected food items cited in the Holy Quran. *European Journal of Biological Sciences, 2*, 40–51.

Roze, L., Beaudry, R., Arthur, A., Calvo, A. & Linz, J. (2007). *Aspergillus* volatiles regulate aflatoxin synthesis and asexual sporulation in *Aspergillus parasiticus*. *Applied and Environmental Microbiology, 73*(22), 7268–7276.

Singh, A. K., Rana, H. K., Tshabalala, T., Kumar, R., Gupta, A., Ndhlala, A. R. & Pandey, A. K. (2020). Phytochemical, nutraceutical and pharmacological attributes of a functional crop *Moringa oleifera* Lam.: An overview. *South African Journal of Botany, 129*, 209–220.

Thakur, R. P., Rao, V. P., Reddy, S. V., & Ferguson, M. (2000). Evaluation of wild *Arachis* germplasm accessions for in vitro seed colonization and aflatoxin production by *Aspergillus flavus*. *International Arachis Newsletter, 20*, 44–46.

Tripathi, P., & Dubey, N. K. (2004). Exploitation of natural products

as an alternative strategy to control postharvest fungal rotting of fruit and vegetables. *Postharvest Biology and Technology, 32*(3), 235–245.

Turner, P. C., Sylla, A., Gong, Y. Y., Diallo, M. S., Sutcliffe, A. E., Hall, A. J., & Wild, C. P. (2007). Reduction in exposure to carcinogenic aflatoxins by postharvest intervention measures in West Africa: A community-based intervention study. *The Lancet, 365*(9475), 1950–1956.

Vabi, M. B., Eche, C. O., Mukhtar, A. A., Kunihya, A., Alabi, O., Adobe, K., Ekefan, E. J., Haruna, S. G., Motagi, B. N. & Ajeigbe, H. A. (2016). *Understanding and managing aflatoxin contamination in the groundnut value chain in Nigeria* (p. 9). International Crops Research Institute for the Semi-Arid Tropics.

Waliyar, F., Traoré, A., Bandyopadhyay, R.. & Reddy, S. V. (2006). Importance of mycotoxins in groundnut trade and methods for their control in Africa. *SAT eJournal*, *2*(1), 1–11.

Widstrom, N. W., Guo, B. Z., Wilson, D. M., Huddleston, R. J. & Lynch, R. E. (2001). Peanut aflatoxin contamination: Its development, problems, and solutions. *Peanut Science, 28*(3), 118–122.

Wild, C. P. & Gong, Y. Y. (2010). Mycotoxins and human disease: A largely ignored global health issue. *Carcinogenesis, 31*(1), 71–82.

Wotton, H. R. & Strange, R. N. (1987). Increased susceptibility and reduced phytoalexin accumulation in drought-stressed peanut kernels challenged with *Aspergillus flavus*. *Applied and Environmental Microbiology, 53*(2), 270–273.

Xue, Q. (2020). *Evaluation of peanut (Arachis hypogaea L.) germplasm for resistance to aflatoxin production by Aspergillus flavus* [Doctoral dissertation, North Carolina University].

Yellagoni, S., Panuganti, R., Jella, S., Uppala, M., Guntha, A., Vemula, A. & Sudini, H. (2025). Effect of sub-optimal moisture levels on the quality of groundnut (*Arachis hypogaea* L.) during storage in triple-layer hermetic storage bags. *Frontiers in Sustainable Food Systems, 7*, 1275133.

Yeni, I. J. (2011). Evaluation of antifungal effects of extracts of *Allium sativum* and *Nicotiana tabacum* against soft rot of yam (*Dioscorea alata*). *Journal of Agricultural Research, 3*(1), 1–5.

Yu, J., Chang, P. K., Ehrlich, K. C., Cary, J. W., Bhatnagar, D., Cleveland, T. E., Payne, G. A., Linz, J. E., Woloshuk, C. P. & Bennett, J. W. (2004). Clustered pathway genes in aflatoxin biosynthesis. *Applied and Environmental Microbiology, 70*(3), 1253–1262.