

## Preliminary screening of mycotoxin-producing fungi of stored groundnuts in Maiduguri, Nigeria

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### Abstract

Mycotoxins produced by filamentous fungi pose significant health risks to humans and animals. This study was carried out to evaluate groundnut (*Arachis hypogea* L.) samples from markets in Maiduguri, Nigeria for mycotoxin-producing fungi using a cost-effective, culture-based approach. Fungal species were isolated via direct plating and identified through morphological and microscopic analysis. Sample moisture content, a key factor in fungal growth, was measured and found to be below 10%. Seven fungal species, namely *Aspergillus ustus*, *A. clavatus*, *A. fumigatus*, *A. niger*, *A. penicilloides*, *Ulocladium chartarum*, and an unidentified white cotton-like isolate, were identified. All the isolates tested positive in the ammonium hydroxide vapor test, indicating mycotoxin production potential. Despite low moisture levels suggesting safe storage, the presence of mycotoxigenic fungi highlights a health risk if contaminated groundnuts are consumed. Given the high cost and complexity of conventional mycotoxin detection methods, this study advocates the ammonium vapor test as a rapid, affordable preliminary screening tool for identifying mycotoxin-producing fungi in food and feed, particularly in resource-constrained environments.

**Keywords:** Ammonium vapor, Fungal isolates, Groundnut, Mycotoxin, Nigeria.

### Introduction

Groundnut is a vital oilseed and food crop native to South America, where it is exclusively found in cultivated farms (Onyimba *et al.*, 2021, Tiwari *et al.*, 2025). It is extensively grown in semi-arid and subtropical regions across nearly 100 countries, with China, India, Nigeria, the USA, and Myanmar leading global production (Ajeigbe *et al.*, 2014). Rich in nutrients, groundnut kernels contain 40–50% fat, 20–50% protein, and 10–20% carbohydrates, along with essential vitamins and minerals (Guchi *et al.*, 2014). Groundnuts are consumed raw, roasted, or boiled, while their oil is used for culinary purposes (Amoniyon *et al.*, 2022). Additionally, groundnut husks, oil cakes, and seeds serve as animal feed, industrial raw materials, and fertilizers, making groundnuts a valuable cash crop for both domestic and international markets (Guchi *et al.*, 2014, Lungaho *et al.*, 2025). However, groundnut seeds are highly susceptible to fungal contamination by species such as *Aspergillus flavus*, *Penicillium* spp., and *Fusarium* spp., which can occur during pre-harvest and post-harvest stages (Aditeya *et al.*, 2017, Akale *et al.*, 2023). These fungi produce secondary metabolites, including mycotoxins, which are toxic compounds that contaminate agricultural products and pose

significant health risks to humans and animals (Olagunju *et al.*, 2019; Agriopoulou *et al.*, 2020). Mycotoxins, produced under favorable climatic conditions, contaminate an estimated 60–80% of global feed raw materials, such as cereals and soybeans, reducing their nutritional value and rendering them unfit for consumption (Eskola *et al.*, 2020).

Mycotoxigenic fungi are a major threat to human and animal health, with potential carcinogenic, teratogenic, mutagenic, and immunosuppressive effects (Awuchi *et al.*, 2022). Their presence in food and feed necessitates intensive research and effective control measures to mitigate their harmful impacts, safeguarding both health and economic interests (Bajwa *et al.*, 2001; Javaid *et al.*, 2005; Zhang *et al.*, 2024). This study aimed to screen stored groundnut samples from markets in Maiduguri for mycotoxin-producing fungi using a simple, cost-effective laboratory method involving exposure to ammonium hydroxide vapor.

### Materials and Methods

#### Study area and sample collection

The study was carried out in Maiduguri, Borno, Nigeria, with the GPS coordinates of 11° 49'

59.9988" N and 13° 9' 0.0000" E. The areas selected for sampling were Monday Market, Custom Area Market, and Muna Market. Groundnut samples were collected from each of the three markets from randomly selected stores. An average weight of 200 g of groundnut per market was considered. The collected groundnut samples were placed in polythene bags and transported to the laboratory of University of Maiduguri, Department of Biological Sciences for the research.

#### Moisture content analysis

Moisture content (based on water loss) was determined by the oven drying method. Two varieties of groundnut were used for the experiment namely Samnut 26, and NC (Nigerian cultivar). These two varieties were collected from each market in triplicates for the determination of moisture content. The samples were ground and dried in the oven at 130 °C for 2 h (Dharmaputra and Retnowati, 1996). The average moisture content for each variety from each market was also determined. The moisture content was determined using the following formula: Moisture content (%) =  $(M_o - M_I) \div M_o \times 100$  Where,  $M_o$  is the initial mass of the test sample.  $M_I$  is the mass of the dry test portion.

#### Isolation and identification of fungi

Nutrient agar plates were used for isolation and identification of colonizing fungi for each variety from each of the three markets. The experimental design involved the introduction of 5 groundnut samples into 5 plates of each of the two varieties from the three different locations translating into 30 plates each inoculated with 5 groundnut samples. The groundnut samples from the three sampling sites were first surface sterilized using 1% sodium hypochlorate before the actual isolation of fungi to ensure that the isolated fungi were not contaminants but originated from the groundnut seeds.

Potatoes dextrose agar (PDA) medium (11.7 g) was added to 300 mL of distilled water and autoclaved at 121 °C for 20 min. It was allowed to cool a little and was poured into 30 Petri dishes. Drops of 0.3% streptomycin sulphate solution was added into the Petri dishes before pouring of the PDA. It was then allowed to solidify before inoculation of groundnut samples. Using a spirit lamp, the inoculation needles were sterilized and used to inoculate the 30 plates each with 5 groundnut samples of the two varieties from different location. The inoculated plates were then incubated at normal ambient temperature of 25 °C for 5–7 days for fungal colony appearance. Number on colonies appearing on each plate were counted. Colony characteristics (color) for different types of fungi that grew were subsequently recorded and tentatively used to separate the fungi into pure cultures by repeated sub-culturing of the different isolates. The fungal

identification was performed using microscopic observations of the hyphal morphology as seen on cotton blue stained-glass slides of the hyphae that were compared with illustrated manuals for fungal identification (Dugan, 2006; Domsch *et al.*, 2007).

#### Ammonium vapor test

Ammonium vapor test was carried out using coconut agar medium as a suitable medium that enhances the production of mycotoxin if present. Coconut powder (11.6 g) was dissolved in 70 mL of distilled water and 1.05 g of agar was added for the mixture to solidify. The coconut agar medium was then autoclaved at 121 °C for 20 min and poured into plates. The pure cultures were inoculated into the plates and incubated in the dark room for seven days. After seven days of incubation, six of the seven plates were inverted over 2 mL of ammonium hydroxide while one plate that was not identified was not exposed to ammonium hydroxide. A change in color of the medium after 10 min indicated that the fungi were mycotoxigenic and marked positive for the test (Yazdani *et al.*, 2010).

#### Data analysis

The results of the total fungal counts were recorded in tables and frequency of each fungus was determined. For the moisture content analysis, it was determined using percentage, and the mean and range of the moisture content were also determined.

## Results

#### Moisture content

The moisture content of the two varieties of groundnut sample from the three markets were relatively low, all falling below 5%. However, the moisture content of the NC variety was lower than that of the Samnut 26 variety in two out of the three markets namely Monday and Muna markets as shown in Table 1. Moisture content for Custom Market showed a higher value for NC variety compared to Samnut 26 variety as also shown in Table 1.

#### Total Heterotrophic fungal plate count

The highest number of fungal isolates with frequency of 41.30% was obtained from Customs Market followed by Muna Market with frequency of 31.74% and the lowest frequency was recorded at Monday Market (26.96%) as shown in Table 2. The number of fungal isolates for the two varieties of groundnut did not show consistent pattern of high and low fungal content. Table 2 also shows the isolated fungal species and their frequencies of occurrence with *Aspergillus clavatus* having the highest frequency of occurrence (31.30%), followed by *Aspergillus ustus* (26.52%), closely followed by *Aspergillus niger* with 27.39% and the least frequency presented by *Ulocladium chartarum*

(0.43%). *Aspergillus* species dominated in the isolation experiment with 5 different species out of the 6 species isolated and identified.

#### Ammonium vapour test

The identified fungal isolates were subjected to ammonium vapor test on coconut agar medium and media changed from white to yellow except the unidentified isolate that was not exposed to ammonium vapor as control. This indicated that all the isolates were mycotoxigenic. Table 3 shows the result of ammonium vapor test indicating that all isolates tested positive to ammonium vapor exposure.

#### Discussion

The study on the fungi isolated from stored groundnuts from three markets in Maiduguri explores the ability of these fungal isolates to produce mycotoxins, which directly impacts the safety of the stored groundnuts for human consumption. The result of this study implies that there are potential risks associated with fungal contamination in groundnuts, particularly in relation to the public health significance of mycotoxin exposure.

The fungal species isolated from the groundnut samples were predominantly *Aspergillus* species, known for their ability to produce mycotoxins, particularly aflatoxins. The high prevalence of *A. niger* (27.39%), *A. ustus* (26.52%), and *A. clavatus* (31.30%) in the samples indicates that these fungi may have a significant role in mycotoxin contamination. Javaid *et al.* (2005) isolated three *Aspergillus* spp. namely *A. niger*, *A. flavus* and *A. fumigatus* from pulses collected from Lahore, Pakistan. Likewise, Shafique *et al.* (2005) reported *A. niger* and *A. fumigatus* from maize. *Aspergillus* species are notorious for producing aflatoxins, which are highly toxic and carcinogenic compounds (Bhat *et al.*, 2017). The ability of these fungi to produce mycotoxins under normal storage conditions means that groundnuts from these markets may pose a public health risk if consumed without proper processing.

The fact that all the isolated fungi showed a positive reaction to exposure to ammonium hydroxide vapor on coconut agar medium suggests that these fungi have the potential to produce mycotoxins under the right environmental conditions. This indicates that these fungi are not only capable of surviving in storage environments but also capable of synthesizing harmful secondary metabolites, which are typically mycotoxins. In this study, the presence of these mycotoxins is a crucial concern, as it demonstrates that stored groundnuts may exceed acceptable mycotoxin limits and thus be unsafe for consumption. Mycotoxins, particularly aflatoxins, are among the most hazardous contaminants in food, known to pose serious public

health risks. Aflatoxins are potent carcinogens, with *A. flavus* and *A. parasiticus* being primary producers. However, other *Aspergillus* species like *A. niger* and *A. clavatus* have also been shown to produce aflatoxins or related mycotoxins (Astoreca *et al.*, 2012). The results from this study suggest that groundnuts from Maiduguri, which have been exposed to these fungi, may contain levels of mycotoxins that exceed acceptable limits set by food safety authorities. For example, the World Health Organization (WHO, 2012) and Food and Agriculture Organization (FAO) set a maximum permissible level for aflatoxin B1 in food products at 4  $\mu\text{g kg}^{-1}$  for groundnuts (FAO, 2004). Aflatoxin contamination of groundnuts can lead to severe health problems, including acute poisoning (aflatoxicosis), which can result in liver damage, immune system suppression, and even death in extreme cases (IARC, 2012). Chronic exposure to aflatoxins, even at lower levels, is associated with an increased risk of liver cancer, particularly in individuals with compromised liver function (Wu *et al.*, 2011). Therefore, the presence of mycotoxins in stored groundnuts from Maiduguri represents a significant public health risk, especially considering the widespread consumption of groundnuts in many households and the fact that the contaminated products may enter the local food chain.

The results of the present study showed that, despite the relatively low average moisture content of the groundnuts (3.38%), fungal growth and potential mycotoxin production still occurred. This observation, although seemingly contradictory to reports that most fungi require moisture levels above 14% to thrive (Proctor *et al.*, 2009), can be explained by several factors. First, the reported moisture value represents an average, which may mask localized variations within individual kernels or microenvironments that support fungal survival and growth. Second, certain species, particularly xerophilic *Aspergillus* spp., are well adapted to low water activity conditions and can remain viable or metabolically active even at reduced moisture levels. Additionally, external factors such as temperature fluctuations during storage and physiological stress conditions may stimulate fungal activity and secondary metabolite (mycotoxin) production even when bulk moisture appears low. Therefore, the presence of fungi under these conditions reflects both the adaptive resilience of the isolates and the limitations of relying solely on average moisture content as an indicator of storage safety.

While the moisture content in this study is relatively low, this finding emphasizes the need for careful management of post-harvest storage conditions. Poorly stored groundnuts, even with relatively low moisture, can still be at risk for fungal growth and mycotoxin production. Bhat *et al.* (2017) highlighted that temperature and humidity are key factors influencing mycotoxin production, with

warmer temperatures and higher relative humidity leading to increased fungal activity and mycotoxin synthesis.

The findings of this study propose simple culture-based method as a first line strategy for monitoring the occurrence of mycotoxin in stored grains and foods. It therefore underscores the need for immediate action to reduce the potential public health risk posed by contaminated groundnuts by Regular screening of groundnut samples for mycotoxin levels that would help identify contaminated batches early and prevent their distribution in the market.

## Conclusion

The findings of this study highlight the significant potential of fungi isolated from stored groundnuts to produce mycotoxins, which pose a serious public health risk. The high prevalence of *Aspergillus* species known for mycotoxin production, combined with the positive reaction to ammonium vapor exposure, suggests that the groundnuts from Maiduguri may exceed safe limits

for mycotoxins, especially aflatoxins. Given the well-documented health risks of mycotoxins, particularly their carcinogenic and toxic effects, this research calls for more stringent food safety measures that adopts inexpensive preliminary culture-based tests like ammonium vapor sensitivity of fungi capable of mycotoxin production and better storage practices to protect public health.

## Contribution of authors

OEC conceived and designed the study. OEC, OAI and OEC performed the experiments and data collection. OIO and NAC contributed to laboratory analysis and data interpretation. AAM and AP assisted in sample collection and experimental procedures. OGC contributed to data analysis and manuscript drafting. All authors read, revised, and approved the final manuscript.

## Conflict of interests

No conflict of interest was declared by the authors.

**Table 1:** Moisture content of groundnut samples.

Location	Variety	Moisture content (%)
Monday market	Samnut 26	3.00
	NC	2.70
Muna market	Samnut 26	4.20
	NC	3.83
Custom market	Samnut 26	2.7
	NC	3.85

**Table 2:** Total heterotrophic fungal plate count and tentative isolates on groundnut samples.

Fungal colony colour	Monday market		Muna market		Custom market		Total	Frequency (%)	Tentative isolate
	Samnut 26	NC	Samnut 26	NC	Samnut 26	NC			
White	3	10	2	19	9	18	61	26.52	<i>Aspergillus ustus</i>
Green	8	9	15	8	18	14	72	31.30	<i>A. clavatus</i>
Black	7	19	18	1	11	7	63	27.39	<i>A. niger</i>
Green/White edge	0	0	5	3	9	1	18	7.82	<i>A. penicilliodes</i>
Dark ash	0	0	0	1	0	2	3	1.30	<i>A. fumigatus</i>
Tea brown	0	0	0	1	0	0	1	0.43	<i>Ulocladium chartarum</i>
White cottony	3	3	0	0	0	6	12	5.22	Unidentified
Total	21	41	40	33	47	48	230	100	
	62 (26.96%)		73 (31.74%)		95 (41.30%)		230		

**Table 3:** Mycotoxin production capacity of the fungal Isolates on CAM (coconut agar medium) using NH<sub>4</sub>OH vapor.

S. No.	Fungal species	CAM color change
1	<i>Aspergillus clavatus</i>	+
2	<i>Aspergillus fumigatus</i>	+
3	<i>Aspergillus niger</i>	+
4	<i>Aspergillus penicilloides</i>	+
5	<i>Aspergillus ustus</i>	+
6	<i>Ulocladium chartanum</i>	+
7	Unidentified	Not exposed to ammonium vapor

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