IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 19, Issue 5 Ser. 1 (May. 2026), PP 30-40
www.Iosrjournals.Org

Prevalence of *Aspergillus flavus* and Aflatoxin levels in stored groundnuts from selected farms in Nyamira County, Kenya

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Abstract
Groundnut (*Arachis hypogaea* L.) is an important legume and oil crop widely consumed in Kenya. It is normally roasted and eaten by many people. However, the peanut seeds are prone to infection by *Aspergillus flavus* while in the field or during the post-harvesting processes as well as in storage. Consumption of groundnuts contaminated with aflatoxins can lead to serious health complications like liver cancer, stunted growth in children and death under severe contamination. This investigated the prevalence of *Aspergillus flavus* and levels of aflatoxins in stored groundnuts from selected farms in Nyamira County. A total of 385 samples were collected based on Fischer's formula, translating to five samples per farm. Groundnuts from the various sample sites in Nyamira county were cultured on Potato dextrose agar (PDA) to determine the percentage prevalence of *Aspergillus flavus* for each variety. Aflatoxins levels were quantified using Enzyme Linked Immunosorbent Assay (ELISA) test and compared with Kenya Bureau of Standards (KEBS) accepted limits for aflatoxin in food products in Kenya (10µg/Kg total aflatoxin and 5µg / Kg aflatoxin B_1 (AFB_1) in peanuts and other food grains. Results showed that Homabay local variety grown in Nyamaiya Division had the highest *A. flavus* prevalence at 46.6%, followed by those grown in Ekerenyo division at 33.30%. Peanut variety CG7 recorded the highest aflatoxin contamination in Nyamusi (184.5ppb) and Ekerenyo (65ppb). In contrast, ICGV 9991 had the lowest aflatoxin levels across all divisions (4.9, 4.7 and 5.3ppb), with ICGV 12991 also showing generally low aflatoxin contamination levels. In conclusion, Peanut variety ICGV 9991 becomes demonstrated greater resistance to aflatoxin contamination and is recommended for safer groundnut production. Based on this research therefore we recommend strengthening extension services to educate farmers on aflatoxin dangers and provide farmers with safe storage practices to reduce peanut aflatoxin contamination levels.

Keywords: Aflatoxins, *Aspergillus flavus*,Groundnuts, Nyamira County, Prevalence.

Date of Submission: 20-05-2026 Date of Acceptance: 30-05-2026

I. Introduction

Peanut (*Arachis hypogeae* L.) is one of the main crops grown in Kenya, primarily for local consumption and export mainly through the World Food Programme in Kenya (Mutegi, 2010; Oywa, 2010). Peanuts rank among the most consumed nuts worldwide, with global consumption reaching 42.6 million metric tons in 2018 (Shahbandeh, 2021). In Kenya, peanuts are mainly cultivated in Western Kenya but are traded and consumed extensively across the country. Production is typically carried out by small-scale farmers under rain-fed conditions (Mutegi *et. al*., 2009). Peanuts are economical to produce since less input cost is incurred in their production hence more affordable than other nuts (Arya SS. *et al*., 2016).

Peanuts are the third most important source of edible oils and the third most used plant protein worldwide. The crop has the ability of fixing atmospheric nitrogen to nitrates, enabling it to grow well in soils with low fertility. The peanuts originated in South America and were introduced to West Africa by the Portuguese (Syamala D. *et al*., 2021). In Kenya, they are commonly roasted, packed in polythene bags of various sizes and sold in most informal markets within the country.

Kenya has been reported as one of the leading countries in terms of incidence and severity of human exposure to aflatoxins over the last four decades (Mehl and Cotty, 2010). Aflatoxicosis occurred in Kenya in 2004, with 317 reported cases and 125 fatalities following consumption of maize contaminated with aflatoxins (Probst *et al*., 2007). Fungal contamination and toxin production in peanuts depend on environmental factors and farmer handling practices, especially during planting, weeding and harvesting. The presence of rain during harvesting promotes mycotoxin production and its accumulation in peanuts (Oliveira *et al.,* 2009*;* Okello *et al*.,

2010). The warm and humid environmental conditions in Africa are suitable for the growth of *Aspergillus flavus*, making aflatoxin contamination in food, including peanuts a major problem in Africa (Gordon, 2003; Bankole *et al*., 2006; Wangacha and Muthomi, 2008). Among the many strategies aimed at controlling aflatoxin producing fungi in crops, biological control appears to be the most promising, safe, economic and environmentally friendly approach for control of *A. flavus* (Dorner, 2009). This study therefore focuses on establishing the prevalence of *A. flavus* on groundnuts and the effects of using *Trichoderma* spp. and *Pseudomonas fluorescens* to manage *A. flavus* in peanuts in Nyamira County, Kenya.

Statement of the Research Problem

Aflatoxins are secondary metabolites produced by *Aspergillus flavus* (Calvo and Cory, 2015). Infection by *A. flavus* increases during drought stress, extreme temperatures and hot, dry weather, and usually affects groundnuts close to harvest (Bhatnagar-Mathur *et al.,* 2015). Increased levels of aflatoxin contamination in foods such as peanuts and the occurrence of aflatoxin related Hepatocellular carcinoma have been reported in Western Kenya (Mutegi *et al*., 2013). Despite contamination of peanuts with high levels of aflatoxins in Western Kenya, this information has not been adequately documented for Nyamira County. The presence of aflatoxins contributes to cancer and other health-related problems (Awuah *et al.*, 2009). Exposure to aflatoxin B_1 leads to liver cancer and is associated with stunting and immune suppression in children (Mossanda, 2015). Moreover, there are scarce reports on the use of *Trichoderma* species and *Pseudomonas* species for the management of aflatoxin-producing fungi in Nyamira County. Therefore, the present study will address this gap.

Justification of the study

Peanuts are consumed almost with every meal of the day in Western Kenya (Wangacha *et al*., 2013). However, cases of peanut contamination with aflatoxin-producing fungi have been reported worldwide and in Kenya. Studies by various researchers have indicated that human exposure to aflatoxins in Western Kenya results in stunting in children following chronic exposure to low levels of aflatoxins over a long period (Bhat and Vashanthi, 2003; Mutegi *et al*., 2009). The climatic conditions in the area are characterized by seasonal rainfall and high temperatures, which favour colonization of peanuts by *A. flavus* (Kaaya and Kyamuhangire, 2006). Related studies have been conducted in other parts of the country, but the information gaps exist on the distribution of *A. flavus* and aflatoxin levels in peanuts in Nyamira County.

Objective

The objective of this study is to establish the prevalence of *Aspergillus flavus* on groundnuts and determine the levels of aflatoxins in stored groundnuts from selected farms in Nyamira County.

General description of groundnuts

Groundnut **(***Arachis hypogaea* L.) is an annual, self-pollinated, wet season legume plant grown in many tropical, sub-tropical and temperate countries (Halima, 2000). It originated in South America but its cultivation has since been practiced widely in many countries including China, India, United States of America and many Sub- Saharan Countries. Developing countries account for 92% of the total global production (Talawar *et al*., 2005). Groundnut is an important legume grown on 19.3 million hectares in about 82 countries worldwide (Reddy *et al.,* 2003). It is highly nutritious, containing protein, fiber, folate, niacin, magnesium, manganese, and other compounds that protect the body against cardiovascular diseases (Azad *et al*., 2020; Tomer, 2018). Peanut is a high-energy food containing 44-56% edible oil and 25-30% protein of the dry seed weight (Reddy *et al.,* 2003; Gachomo *et al.,* 2004). As a legume, peanut has the ability to fix atmospheric nitrogen, thereby improving soil fertility.

Groundnuts can thrive under low rainfall and require few inputs, making them suitable for cultivation by many small scale farmers (Okello *et al*., 2010). The crop grows best in light sandy loam soils and requires five months of warm weather and an annual rainfall of 500 – 1,000 mm (University of Georgia, 2006).

Health Effects of Mycotoxins

Aflatoxins are a group of mycotoxins produced by moulds of the genus *Aspergillus* during the spoilage process of agricultural products. The main forms of aflatoxins include aflatoxin B_1, B_2, G_1 and G_2 (Altomere *et al.*, 2021). Aflatoxins are carcinogenic, immunotoxic and teratogenic to humans and animals (Marshall *et al*., 2020). They have also been linked to reduced immunity, reduced fertility and stunting in children (IARC, 2012).

Aflatoxins have also been reported to suppress immunity in animals (Turner *et al*., 2005). Aflatoxins affect humans and animals of all ages including the developing foetus, children and adults. Studies have shown that in areas where both the healthy and malnourished children have been exposed to aflatoxins, they have been found to have stunted growth (Gong *et al*., 2004).

International Agency of Research on Cancer (IARC) has classified aflatoxins as group one carcinogens. According to the studies, aflatoxins are responsible for 4.6% to 28.2% of entire cases of hepatocellular carcinoma (HCC) in the world and so, it must be inhibited at all costs (Liu and Felicia, 2010). It has been found that aflatoxins not only damage the genetic material of most bacteria but also the cultured cells of humans and animals. Many genetic abnormalities which are caused by aflatoxins include gene mutations, exchange of sister chromatids, formation of micronucleus, mitotic recombination, and the formation of albumin adducts (IARC, 2002).

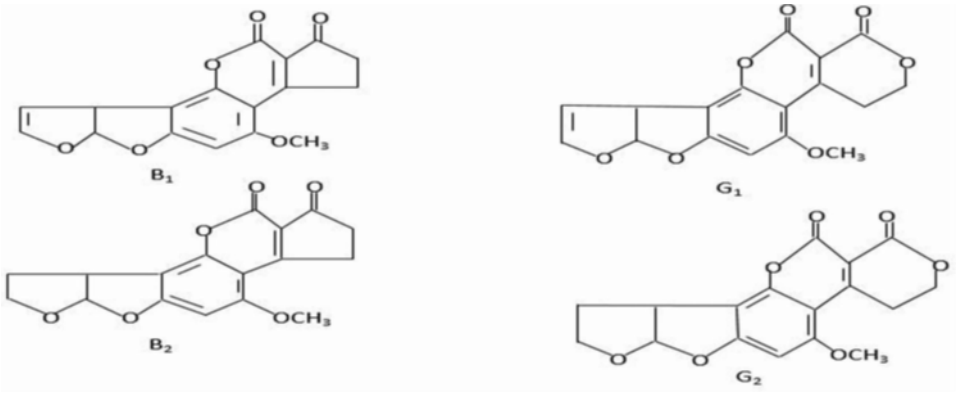

Figure 1: The structure of aflatoxins (Martínez *et al*., 2023)

Factors promoting aflatoxin contamination of grains

Temperature, relative humidity and moisture content are the main factors that determine the ability of *Aspergillus flavus* to grow during storage (Waliyar *et al*., 2015). Peanuts are likely to be contaminated with aflatoxins if they are not dried immediately after harvesting, hence failing to maintain safe moisture content during post-harvest handling. Peanuts are naturally hygroscopic and tend to absorb moisture from the surrounding storage environment (Waliyar *et al*., 2015). When temperature and humidity conditions are favorable, the fungus can invade the agricultural produce at different stages, either in the field or later during drying or storage (Perdocini *et al*., 2019; Joubrane *et al*., 2020).

Drought stress in the field would increase the aflatoxin contamination in peanuts due to over-maturity, reduced seed moisture content, and increased chances of insect and pod damage, which facilitates infection of peanuts by *Aspergillus* spp. (Waliyar *et al*., 2015; Sibakwe *et al*., 2017). Aflatoxins are frequently found in food grown and manufactured in Africa because of factors including excessive heat, high humidity, poor aeration in stores, and insect and rodent damage, which result in the proliferation and spread of fungal spores (Groopman and Kensler, 2005).

Traditional groundnut drying techniques are slow and time consuming, and complicated by persistent rains at harvest and drying times. This makes it difficult to achieve the recommended moisture level, leaving peanuts prone to fungal attack (Kaaya *et al*., 2007; Okello *et al*., 2010). When agricultural commodities are stored incorrectly for long periods, environmental conditions become favourable for fungal growth (Kaaya *et al*., 2000). Insects and mites may damage stored grains and create an avenue for fungal colonization. Toxin producing fungi can infect growing crops as a consequence of insect damage and may produce toxins prior to harvest or during harvesting and storage. During storage, insects, due to their respiratory activities, can increase the temperature of the grains to levels suitable for fungal growth (Hell, 2010). Other factors affecting aflatoxin contamination include pH, fungal strain, substrate and climate change. The pH affects the growth of fungi either through direct action on cell surfaces or indirectly by influencing nutrient availability. Fungi can modify surrounding pH by secreting acids or alkalis; for example, *Aspergillus* spp. can acidify the surrounding by secreting gluconic and citric acid (Vylkova, 2017). Fungal strains vary in toxicity and mycotoxin production. In some cases, mycotoxin production is restricted to specific species (Nicholson, 2004). Osmotic pressure in a substrate affects fungal growth and mycotoxin production as it determines the physiological responses of the fungi, thus influencing biosynthesis of secondary metabolites including mycotoxins (Duran *et al*., 2010).

II. Research Methodology

Study Area

Nyamira County is one of the 47 counties in Kenya. It borders Homabay County to the North, Kisii County to the West, Bomet County to the Southeast and Kericho County to the East. Nyamira County covers an area of 899.4km^2 and lies between latitude 00^o 30’ S and 00^o 45’South and longitude 34^o 45’ E and 35^o 00’ East.

The major soil types in the county are red volcanic Nitosols, which are deep, fertile and well-drained, accounting for 75% of the area. The remaining 25% consists of soils found in valley bottoms and swampy areas suitable for brick making. The county falls within two major agro-ecological zones. The highland zones (LH1 and LH2) cover 82% of the county, while the upper midland zones (UM1, UM2 and UM3) cover the remaining 18%. Annual rainfall ranges between 1200 mm and 2100 mm. The long rains occur from December to June and the short rains from July to November, with no distinct dry spell separating them. The maximum day and minimum night temperatures normally range between 28.7 ^{0}C and 10.1 ^{0} C respectively.

Administratively, Nyamira County is divided into five sub-counties; Nyamira South, Nyamira North, Borabu, Manga and Masaba North. The county is further divided into 14 divisions (Nyamira County Annual Development plan 2018/2019).

Description of the sampling sites and sample size determination

A household survey was conducted in Nyamira County, targeting Nyamusi, Ekerenyo and Nyamaiya divisions. These divisions were selected because they are the major groundnut growing zones in the county. Sampling was carried out among 75 groundnut growing households. Respondents were farm owners who were mapped using a Geographical Information System (GIS) after adjustment on the ground.

The samples size was calculated using the Fischer formula:

Where;

N – Sample size, Z – 1.96 at 95% confidence level, P – estimated proportion of the target population. P=0.5 was used since the proportion was unknown
q =1-p, d= α=0.05, D- design effect =1

Hence sample size, = = 384.16 = 385 samples

Peanut samples were collected after harvesting to determine infection by *Aspergillus flavus* and possible aflatoxin contamination. Samples were collected at multiple points in each farmer's storage bag. Approximately 50g of shelled groundnuts were sampled per bag and the same procedure was repeated for other randomly selected bags to obtain a minimum of 5 sub-samples per household. The collected sub-samples were put in a clean khaki paper bags. Sub-samples per division were thoroughly mixed to make a composite sample. A sub-sample weighing 5 kg was drawn rom the composite for analysis of *Aspergillus flavus* presence and aflatoxin concentration at Kenyatta University Laboratory.

Isolation of *Aspergillus flavus*

Potato Dextrose Agar medium was prepared by dissolving 58.5g of PDA powder in sterile distilled water and making the volume up to 1500ml. The mixture was autoclaved at 121^{0}C and 15psi pressure for 20 minutes to ensure the media was free of contamination. The medium was then allowed to cool to 50^{0}C before it was dispensed to petri-dishes, approximately 20ml per dish.

Isolation of *Aspergillus flavus* from peanuts

A total of 18.7 kg of peanuts were collected from the three divisions and a 5 kg subsample was drawn from the composite for Laboratory analysis. The collected peanuts were disinfected using 1% sodium hypochlorite for five minutes, then rinsed three times in sterile distilled water with each rinsing session lasting 1 minute. The seeds were later dried between clean filter papers (Whatmann, No.1).

Three seeds from each peanut variety were plated on Potato Dextrose Agar (PDA) media with five replications. The use of three seeds per Petri dish was a modification of the protocol by Rajarajan that used five seeds per Petri dish. This modification was done to avoid overlapping of the fungal mycelia on healthy kernels. The plates with the peanut seeds were incubated at 25 ^{0} C for five days. Sub-culturing of mycelia tips was repeated several times to obtain pure cultures, which were preserved on PDA slants awaiting identification (Rajarajan, 2013).

Identification of Aflatoxin producing isolates

Morphological identification of *Aspergillus* section *Flavi* was based on microscopic structures, such as uni- or biseriate conidial heads, production of dark-colored sclerotia by certain species, and yellow-green to brown conidia. *Aspergillus* section *Flavi* includes 33 species, and most of them are natural producers of aflatoxins (Frisvad *et al*., 2019). Macroscopic identification involved observing cultural characteristics of each

species on the plates, such as colony colour. *Aspergillus flavus* is usually orange in colour in *Aspergillus* differential medium.

Microscopic analysis involved observation of prepared cultures under low and medium power objectives of the compound microscope. A wet mount was prepared from the culture plate using lactophenol blue, covered with a cover slip, and then examined under low power. Distinctive features of each species, like the nature of hyphae, conidiophores and conidia, were observed and recorded.

In order to calculate the prevalence of *Aspergillus flavus* fungi on groundnuts, the formula below was used;

Aflatoxin analysis

The sample for aflatoxin analysis were ground to a particle size where 95% passed through a 20 –mesh screen. 70% Methanol was prepared by adding 30ml of distilled water to 70ml methanol for each sample to be tested. Twenty grams of ground sample was weighed and 100ml of extraction solvent was added at a ratio of 1:5 (w/v). The mixture was shaken in a sealed container for a minimum of 2 minutes. The sample particles were allowed to settle and later 5-10 ml of the extract was filtered through Whatman No. 1 filter paper and collected for analysis.

ELISA Test Procedure

Aflatoxin quantification was done using competitive direct ELISA. All the required reagents were brought to room temperature before use. The wash buffer was prepared by reconstituting the PBS-T powder packet by washing out the contents with a gentle stream of distilled water into 1 liter container. One mixing well was placed in a micro well holder for each standard and sample that was to be tested. An equal number of antibody coated micro-titer wells were placed in another micro well holder. Each reagent was mixed by swirling it in a reagent bottle before it was ready to be used. 200 µL of the aflatoxin – HRP conjugate was dispensed into each mixing well. 100µL of each standard and prepared sample were added to the appropriate mixing well containing conjugate using a new pipette tip for each. The contents were incubated at room temperature for 20 minutes. 100µL of contents from each mixing well were transferred using a new pipette tip for each to corresponding antibody-coated micro well. The contents were then incubated at room temperature for 15 minutes. The contents from micro wells were decanted into the discard basin. The micro wells were washed by filling each with PBS-T wash buffer and later the wash was decanted into a discard basin. The washing was repeated for a total of about five washes. The micro wells were tapped while facing down on a layer of absorbent towels to remove residual buffer. The required volume of substrate reagent was measured (1ml/strip or 120 µL /well) and placed into a separate container. 100µL was added to each micro well and then incubated at room temperature for 5 minutes and covered to avoid direct light. The required volume of stop solution was measured (1ml/strip or 120 µL /well) and placed into a separate container. 100µL of the stop solution was added in the same sequence and at the same pace as the substrate as the substrate reagent was added. The optical density of each micro well was read with a micro titer plate reader using a 450nm filter and the results were recorded. The binding percentage for each standard and sample was calculated as a percentage of the zero binding.

After aflatoxins were extracted from the groundnut sample with a solvent, a portion of the sample and a conjugate of an enzyme coupled aflatoxins was added to the micro-titer wells. Any aflatoxin in the sample extract was allowed to compete with the enzyme conjugated protein for the antibody binding sites.

After washing, an enzyme substrate was added and blue colour developed. The intensity of the colour was inversely proportional to the concentration of the aflatoxins in the sample. A solution was added to stop the enzyme reaction. The intensity of the colour in the micro titer wells was measured optically using ELISA reader. The optical densities of the samples were compared with the optical densities of the standards and an interpretive result was determined.

Data analysis

Data on *A. flavus* prevalence and total aflatoxin levels in shelled peanuts were subjected to analysis of variance (ANOVA) using the SAS statistical package version 9.4 (SAS Institute Inc.). One-way ANOVA was performed to test differences in aflatoxin contamination among the three divisions. Treatment means were separated using Fisher's Least Significant Difference test (LSD) at 0.05 probability level. Aflatoxin concentrations were expressed as micrograms per kilogram or parts per billion. The mean aflatoxin levels per division were compared against the Kenyan standard limit of 5µg / Kg aflatoxin B1 (AFB1) in peanuts and other food grains. The total aflatoxin contamination was determined by ELISA test and summarized using Microsoft Excel and calculated as parts per billion (ppb) for each sample. The analysis of variance was done for individual experiments and treatment means determined.

III. Results

Groundnut Varieties grown in Nyamira County

Two main market types of peanut; the runner and Virginia, were grown by farmers in Nyamira county. Of these, four varieties namely; Homa Bay local, ICGV -9991, CG7, and ICGV-12991 were sampled from three divisions in Nyamira county as indicated in Plate 1a-d, and Table 1. Runner-type varieties matured in approximately 100 days, while Virginia-type varieties matured in approximately 70 days.

Plate 1a: Red and Big in size (CG7)

Plate 1b: Red and small in size (ICGV-9991)

Plate 1c: Brown and Very Big (ICGV -12991)

Plate 1d: Brown and Very Big (ICGV -12991)

Table 1: Peanut varieties collected from the three divisions of Nyamira County

Variety name	Family name	Characteristics
Homabay Local	Runner	Brown colour and big in size
ICGV – 9991	Virginia	Red and small in size
ICGV – 12991	Runner	Brown and very big in size
CG7	Virginia	Red and big in size

Prevalence of *Aspergillus flavus* in peanut varieties

The prevalence of *A. flavus* varied significantly (P<0.05) among peanut varieties and divisions in Nyamira county. Homa bay local grown in Nyamaiya division had the highest prevalence of *Aspergillus flavus* at 46.6%, followed by Nyamusi division at 26.6% and Ekerenyo division which had a prevalence of 33.30%. Variety ICGV 12991 grown in Nyamaiya had the highest prevalence of *A. flavus* at 40.0%, followed by those grown in Nyamusi at 26.6% and in Ekerenyo at 20.0%. Variety ICGV 9991 experienced the highest prevalence of *A. flavus* in plants grown in Nyamusi at 20.0%, while variety CG 7 grown in Nyamusi recorded the highest prevalence of *A. flavus* at 33.3%, compared to the other two divisions as indicated on Fig.2.

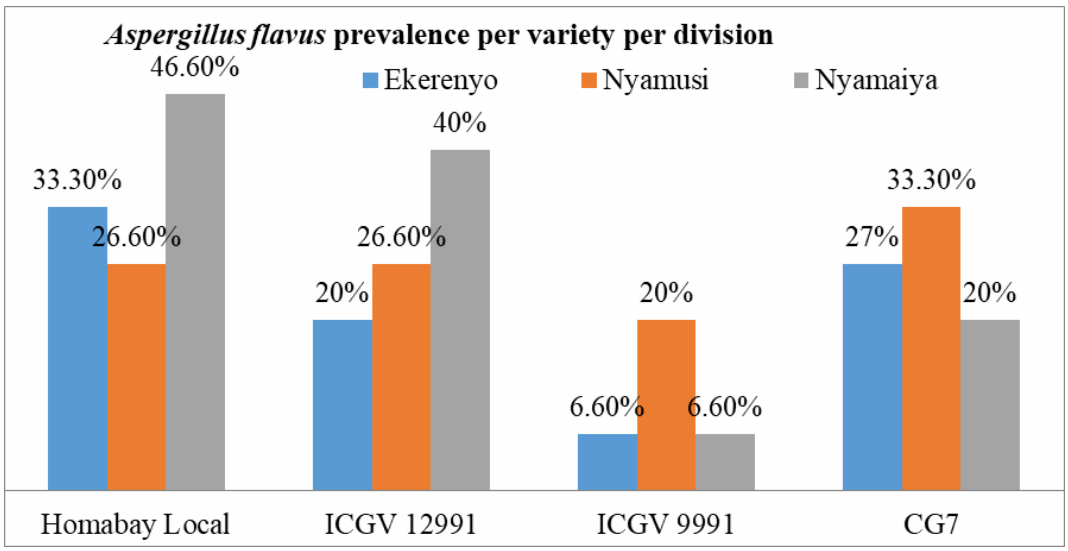

Figure 2: Prevalence of *Aspergillus flavus* per variety per division

Peanut variety grown in Nyamira County

Majority of the farmers in Nyamira county (45.0%) preferred Homa bay local variety since it is sweet and adaptable to the local conditions while variety ICGV-12991 was least grown within the county as shown in fig.3.

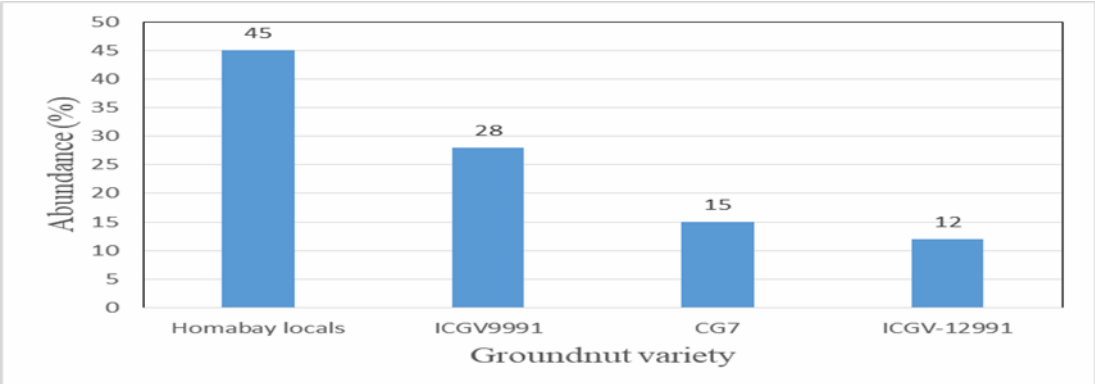

Figure 3: Overall distribution of peanut varieties collected from farmers in Nyamira County

The average farm size under peanut production in Nyamira county ranged from 0.1 - 5.9 acres. Most farmers, 48.3% cultivated peanuts on small plots of 0.1-1.9 acres, indicating that peanut production in Nyamira is dominated by small-scale farmers (Table 2).

Table 2: Farm size under peanut production in Nyamira County

Division	0.1 – 1.9 Acres	2.0 – 3.9 Acres	4.0 – 5.9 Acres	Total
Nyamusi	16 (53.3%)	13 (43.3%)	1 (3.3%)	30
Ekerenyo	7 (70.0%)	3 (30.0%)	0 (0%)	10
Nyamaiya	5 (27.8%)	8 (44.4%)	5 (27.8%)	18
Total	28 (48.3%)	24 (41.3%)	6 (10.3%)	58

Prevalence of *Aspergillus* Species in peanuts per division in Nyamira County

The results showed that most peanuts had been infected by *Aspergillus flavus*. Later, the *Aspergillus flavus* were isolated from the infected peanut seeds and grown on the PDA media as indicated on plate 2 and 3.

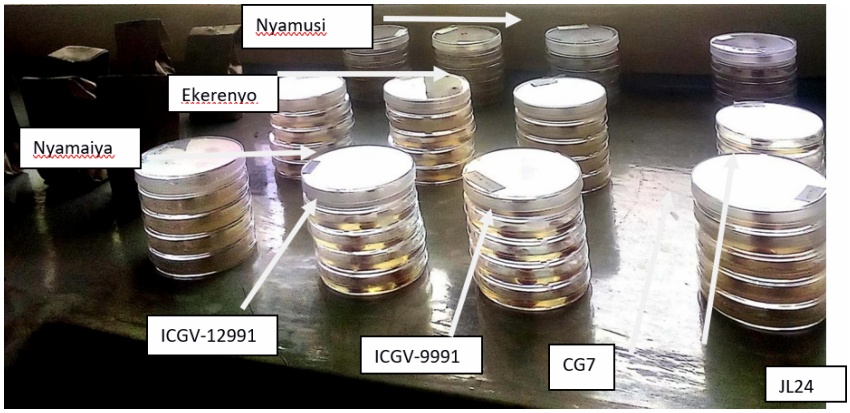

Plate 2: Peanut seeds plated on PDA media in the laboratory in five replications per variety per division

Plate 3a: JL24 in day 5

Plate 3b: ICGV12991 in day 5

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Levels of total aflatoxins in Peanuts

Table 3, shows the levels of total aflatoxins in peanut varieties across the three divisions of Nyamira county. Variety ICGV 9991 consistently recorded the lowest aflatoxin levels across all divisions (Table 3). Homa Bay local and ICGV 12991 had generally low aflatoxin levels, except for a spike in Nyamaiya division where Homa Bay local recorded 16ppb. The variety CG7 had the highest aflatoxin contamination, with 184.5ppb in Nyamusi division and 65ppb in Ekerenyo division being recorded. Nyamaiya division had relatively low levels of aflatoxins across all varieties, except for Homa Bay local at 16.6ppb (Table 3).

Variety and division had a significant effect on aflatoxin levels ($P<0.05$). Three samples exceeded the KEBS maximum limit of 5 µg/kg for total aflatoxins in groundnuts.

Table 3: Levels of aflatoxins in peanuts in parts per billion

Division	Homa Bay local	ICGV 12991	CG7	ICGV 9991
Nyamusi	6.4	6.6	184.5	4.9
Nyamaiya	16.2	7.4	9.2	4.7
Ekerenyo	5.6	9.7	65.5	5.3

IV. Discussion

This study assessed the prevalence of *Aspergillus flavus* and levels of total aflatoxin contamination in groundnuts in Nyamira County. The results were intended to provide important information on whether the peanuts produced and stored by farmers in the area and those traded by registered wholesalers met the safety

standards recommended by the Kenya Bureau of Standards (KEBS) of 5 µg/kg aflatoxin B1. In the field, peanuts can be infected by *Aspergillus parasiticus* and *A. flavus* as a result of insect damage on the developing seed, moisture stress due to drought and high soil temperatures. Agronomic practices carried out by farmers like control of insect pests, use of resistant varieties, late-season irrigation, timely weed control, timely planting and proper harvesting reduce the risk of aflatoxin contamination (Bediako *et al*., 2019). However, 75% of farmers interviewed in Nyamira county did not adopt any pest and disease control strategy on their farms. Only 25% used commercial pesticides, organic pesticides, or cultural practices. This low uptake canbe attributed to inadequate extension services, high cost of agrochemicals, and limited awareness of Integrated Pest Management (IPM) approaches (Okello *et al*., 2020).

Insect damage creates wounds on developing peanut pods, which serve as entry point for *A. flavus* and lead to aflatoxin contamination (Waliyar *et al.*, 2008). This is consistent with studies in East Africa which also indicated that groundnut rosette was among the most damaging viral disease in groundnuts which caused yield losses up to 100% under severe infection (Adugna *et al*., 2020; Monyo *et al*., 2021).

Post harvest handling of peanuts is equally critical in determining their safety, quality and marketability. The peanut seeds should be dried after harvesting to a moisture content of <10% to prevent growth of storage fungi and aflatoxin accumulation (Torres *et al*., 2014). In this study, 94% of farmers dried peanuts for 4 – 6 days while 6% dried them for 3 -4 days. Although most farmers were aware of the need to reduce moisture, the actual drying period depends on weather conditions. The period of drying is a key factor in determining post harvest quality especially with regard to minimizing the growth of grain moulds and subsequent aflatoxin contamination which is common in peanuts stored in poor conditions (Mutegi *et al*., 2019; Waliyar *et al*., 2021). Nyamira county, being a highland region with abundant rainfall and cloud cover, compromises traditional sun-drying and prolongs drying time (Njoroge *et al*., 2022). This increases the risk of *Aspergillus* infection during drying, similar to findings in Ghana where drying on tarpaulins reduced *Aspergillus flavus* spores on peanuts and minimized aflatoxin contamination during storage period. Further, majority of farmers in the study area had not adopted the use of hermetically sealed bags which would prevent entry of moisture into the peanut kernels due to their unavailability and high cost (Yahaya *et al*., 2022).

The assessment of fungal species carried out in the three peanut growing divisions of Nyamira County revealed that all peanut samples were infected with *Aspergillus* spp. *Aspergillus flavus* was the most predominant species isolated at 44%, followed by *A. niger* at 19% ,and the least was *A. parasiticus* at 7%. These findings are in agreement with the study carried out by Menza and Muturi (2018) who reported 60.6% *A. flavus* in peanut samples collected from Kisii and Busia counties. The presence of *A. flavus*, *A. niger* and *A. parasiticus* suggests a widespread contamination problem within the peanut value chain in the region. *Aspergillus flavus* and *A. parasiticus* are most important due to their ability of producing secondary metabolites that are toxic and carcinogenic in nature. These findings are in agreement with the previous studies conducted in Kenya and other Sub- Saharan Africa which reported *A. flavus* as the most common fungi contaminant in peanuts and other legumes (Mutegi *et al*., 2019; Njoroge *et al*., 2022).

Farmers awareness of aflatoxin risks influences post-harvest handling and contamination levels. In this study, 81% of farmers lacked knowledge on whether peanuts could be contaminated with aflatoxins, and this awareness varied from one division to another. Improved knowledge on the good agricultural practices among farmers and stakeholders reduces the prevalence of aflatoxin contamination in peanuts (Martey *et al*., 2020). Similar findings were reported by Seeth *et al*. (2020) who observed that most small-scale peanut farmers in sub-Saharan Africa lacked sufficient knowledge with regard to aflatoxin risks hence leading to unsafe handling and storage practices. This low awareness in Nyamira county may contribute to high contamination observed in some varieties.

Analysis of peanut varieties showed significant variation in susceptibility to *A. flavus* infection. Homa Bay local exhibited the highest infection rate of 35.5%, while improved variety ICGV 9991 showed the lowest prevalence of 11.1%. This suggests that improved varieties offer better genetic resistance to fungal infection. This observation is consistent with breeding efforts by ICRISAT and National research programs that have developed peanut varieties with enhanced resistance to fungal pathogens and reduced aflatoxin contamination (Akinwale *et al*., 2022; Waliyar *et al*., 2021). The high susceptibility of Homa Bay local variety can be attributed to its genetic background, which lacks resistance genes found in improved varieties. Although farmers prefer traditional varieties for taste and adaptability, they pose higher food safety risks.

The prevalence of *Aspergillus flavus* differed from one division to the other. Nyamaiya division recorded the highest prevalence of *A. flavus* at 46.6%, followed by Ekerenyo division with 33.3% and lastly Nyamusi division had 26.6% in Homa bay local variety which proved to be the most susceptible variety. These variations can be attributed to ecological differences across the divisions. Research shows that aflatoxin contamination hotspots are often associated with agroecological zones characterized by high temperatures, insufficient rainfall, and poor soil fertility (Sserumaga *et al*., 2021; Taffa *et al*., 2023).

Analysis of aflatoxin contamination across peanut varieties and divisions showed variations both between genotypes and across divisions. The improved variety ICGV 9991 consistently recorded the lowest aflatoxin levels of 4.9, 4.7 and 5.3 ppb in Nyamusi, Nyamaiya and Ekerenyo divisions respectively. These values fall within the maximum permissible limits set by Kenya Bureau of Standards of 10ppb (KBS, 2019). This indicates that ICGV 9991 is relatively resistant to aflatoxin contamination hence becoming a better option for safe peanut production. In contrast, variety CG7 exhibited extremely high aflatoxin levels with values of 184.5ppb in Nyamusi and 65.5 ppb in Ekerenyo both far exceeding national and international safety thresholds. Previous studies in Kenya and Uganda have reported similarly high aflatoxin levels in susceptible varieties when subjected to drought stress and inadequate postharvest management (Mutegi *et al*., 2019; Sserumaga *et al*., 2021). The Homa Bay local and ICGV 12991 varieties generally showed low aflatoxin concentration except for Nyamaiya which had 16.2 ppb for Homa Bay local. This suggests that environmental conditions such as soil type, rainfall patterns and temperature fluctuations influence fungal growth and aflatoxin accumulation (Wu *et al*., 2019; Omari *et al*., 2023). The data also demonstrates differences across divisions in contamination patterns. Nyamusi division had the highest aflatoxin levels in general while Nyamaiya had moderate contamination and Ekerenyo had a mix of low and moderate contamination. These findings are consistent with reports that aflatoxin hotspots are unevenly distributed across agroecological zones and can vary sharply with short geographic distances depending on microclimatic conditions and agronomic practices within the area (Taffa *et al*., 2023; Njoroge *et al*., 2022). The occurrence of very high aflatoxin contamination in CG7 variety is a public health concern and poses a serious health risk on the consumers within the affected divisions. Chronic exposure to high aflatoxin levels lead to liver cancer, suppression of immunity and stunting in children. (Wu et al., 2019; Omari *et al*., 2023). Therefore, the correlation between high *Aspergillus flavus* prevalence and elevated aflatoxin levels confirms that fungal infection is a key driver of peanut contamination in Nyamira county.

V. Conclusions

This study established that all peanuts samples from Nyamira county were infected with *Aspergillus flavus*. However, percentage infection differed significantly from one area to the other and from one variety to the other. Homa Bay local variety was the most susceptible to *A. flavus* with an average infection rate of 35.5 %, while the improved variety ICGV 9991 was the most resistant at 11%.

Although *A. flavus* was present in all samples, not all strains produced aflatoxin contamination. The *A. flavus* which produce aflatoxins posses specific genes responsible for aflatoxin biosynthesis. By screening the culture under UV light using Coconut Cream Agar (CCA) as the media, aflatoxin producing strains produce a florescent blue pigment. Consequently, all samples were contaminated with aflatoxins, but the concentrations varied widely. Variety ICGV 9991 recorded the lowest aflatoxin levels of 4.9, 4.7 and 5.3 ppb in Nyamusi, Nyamaiya and Ekerenyo respectively. In contrast, variety CG7 exhibited extremely high aflatoxin levels with values of 184.5ppb in Nyamusi and 65.5 ppb in Ekerenyo both far exceeding national and international safety thresholds.

VI. Recommendations

Based on the results from this study, there is need to promote large-scale cultivation of improved peanut varieties such as ICGV 9991, which demonstrated resistance to *A. flavus* and maintained aflatoxin levels within safe limits.

Support breeding programs to develop and distribute more aflatoxin-resistant varieties that also meet farmer preferences for taste, yield and adaptability.

Improved Postharvest Handling and Storage

Train farmers on proper drying practices to achieve ≤10% seed moisture before storage.

Promote use of tarpaulins for drying and hermetic storage technologies to minimize fungal infection and aflatoxin accumulation. Support local innovations to make hermetic bags affordable and accessible to smallholder farmers.

Extension Services and Farmer Training

Strengthen extension services to give more information to farmers about Aflatoxin levels in peanut and bridge the knowledge gap on aflatoxin risks and management practices.

Conduct regular farmer field schools and awareness campaigns on the health and economic risks of aflatoxin contamination.

Policy and Regulatory Interventions

Enforce KEBS aflatoxin standards at farm-gate and market levels to ensure consumer safety and facilitate market access for compliant farmers.

Further Research

Assess socio-economic barriers hindering adoption of aflatoxin management strategies among smallholder farmers.

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