Determination of Diversity of Mold Species and Aflatoxins in Local Traditional Cereal Based Brews Within Nkubu Municipality, Meru County, Kenya

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Abstract

Background: Molds produce mycotoxins such as Ochratoxins, Deoxynivalenol, and Aflatoxins which contaminate more than 25% of the global food produced annually. Most traditional brews consumed in Kenya are produced from cereals contaminated with molds and mycotoxins. This poses a public health concern since it exposes consumers to the risk of nephrotoxicity, cancer, teratogenicity and immune-toxicity. However, data on local brew mycotoxin contamination in Nkubu municipality, Meru County remains limited. This study sought to determine the diversity of molds and aflatoxins in cereal based local brews in Nkubu municipality, Meru County, Kenya.

Methods: A total of sixty-two (62) Marua, seventeen (17) Busaa, and five (5) Chang'aa samples were randomly collected from different brewers in the study area and shipped in sterile containers to the Kenya Methodist University Mycology laboratory for isolation and identification of molds. Molds were isolated using culture method while Aflatoxins were detected using ELISA method.

Results: Marua brew 62/84 (73.8%) was the most common brew in Nkubu followed by Busaa 17/84 (20.2%), and chang'aa 5/84 (6.0%). Co-occurrence of *Aspergillus*, *Penicillium*, and yeast was reported in all the cereal based brews. *Aspergillus* plus yeast was the most abundant mold species in all the three types of brews: Marua (69.4%), Busaa (70.6%), and Chang'aa (60.0%). The co-existence of molds in Marua and Busaa varied significantly (P<0.001). Busaa had a marginally higher aflatoxin mean concentration, and range (6.40±2.45, [1.12-25.70] ppm) compared to Marua (5.74±3.29, [1.12-25.70] ppm) and chang'aa (2.35±3.07, [1.41-3.55] ppm), though this was not statistically significant (P=0.195). 21/62 (33.9%) and 6/17 (35.3%) of Marua and Busaa samples, respectively had aflatoxin levels > 10 ppb.

Conclusion: Occurrence of molds and Aflatoxins in cereal based brews in Nkubu municipality beyond the tolerable levels recommended by the Kenya Bureau of Standards warrants mycotoxicological quality control of cereal based brews to avoid exposing the consumers to high aflatoxins.

Keywords: *Aspergillus*, *Penicillium*, yeast, Aflatoxins, Marua, Busaa, Chang'aa

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1. Introduction

Molds are naturally occurring microscopic fungi, with roots like structures called hyphae, and they grow on either plant or animal matter (Blake, *et al*, 2019). There are different types of molds, namely; *Alternaria, Acremonium, Stachybotrys, Aureobasidium, Chaetomium, Fusarium, Mucor, trichoderma, ulocladium,* and *Penicillium*. *Aspergillus* and *Penicillium* have been shown to be the most common occurring mold genera in sub-Saharan Africa (SSA) (Lizarraga-Paulin *et al*., 2011). These Molds can produce toxic chemicals (metabolites) as waste product of metabolism which are toxic in nature and are referred to as mycotoxins. Different mycotoxins exist, namely; Ochratoxins, Deoxynivalenol (DON), Zearalenone (ZEA), T-2 toxins, Fumonisins (FUMs), and, Aflatoxins (Omara *et al*., 2021). Of the six, Aflatoxins and ochratoxins are the main mycotoxins of health significance. Molds grow on various foodstuffs such as fresh food and stored grains, for example cereals, dried fruits, nuts and spices (Hosseini, & Bagheri, 2011). Mold growth occur earlier before harvest or later after harvest, or on/in the processed cereals often in warm, damp and humid conditions (Sardella, *et al.* 2016).

Inevitably existing mycotoxins termed as aflatoxins develop in a large group of fungi belonging to the genus *Aspergillus* (Klich, 2007; Pickova et al., 2021). It is the most potent mycotoxin of biological importance affecting both human and animal. They are and remains global socioeconomic and life-threatening toxins, especially in SSA, despite being identified in 1960 (Massey *et al*., 1995). High temperature with high humidity in SSA favors the growth of molds and proliferation of mycotoxins (James & Zikankuba, 2018). The four major types of aflatoxins are B1, B2, G1, and G2. They are characterized basing on their fluorescence under UV light (blue or green) and relative chromatographic mobility during thin-layer chromatography. Of the four, Aflatoxin B1 is the most potent natural carcinogen known and is usually the major aflatoxin produced by toxigenic strains (Voth-Gaeddert., *et al.*, 2018).

About 25% of the world's crops are affected by Aflatoxins (Klingelhöfer *et al*., 2018). Global and SSA estimates of aflatoxin contamination in cereals which forms part of the staple

diet is 35%, and 21%, respectively (Talaam *et al*., 2015). Consumption of aflatoxin contaminated products exposes human to health risks such as hepatocellular carcinoma, aflatoxicosis, suppressed immunity, neurotoxicity, and stunted growth. Over 26 000 deaths occur in SSA due to liver related conditions associated with aflatoxins (Ranajit *et al.*, 2019). According to Omara and colleagues, about 200 deaths occur annually in Kenya as a result of exposure to aflatoxins (Omara *et al*., 2021). High temperature with high humidity in Sub-Sahara Africa (SSA) favors the growth of molds and proliferation of mycotoxins (James & Zikankuba, 2018).

Approximately, fifty-one percent (51.6%) of the entire volume of alcoholic drinks consumed in SSA are the traditional cereal based brews (WHO, 2014). Traditional based brews in Kenya are prepared from maize, millet, and sorghum. Traditional brews despite having the nutritional value (Kirui *et al*., 2014), they serve as alternatives for low-income consumers who cannot afford industrially processed finer alcoholic products. They are also used to mark very important traditional ceremonies such as weddings, circumcision, funerals, bumper harvests, rain making, and naming (Kirui *et al*., 2014; Tafere, 2015). The type of traditional brews processed varies from Country to Country (Ezekiel *et al*., 2018). In Kenya, Busaa, Chang'aa, and Muratina are the most common cereal based brews (Gitari *et al*., 2021). In Meru County, Kaanga and Marua, Busaa, and Chang'aa remain the most common consumed traditional brews (Gitari *et al*., 2020).

However, Aflatoxins occurrence has been reported in traditional brews beyond tolerable levels recommended by KEBS (>10 ppm) in different studies in Kenya (Kenji *et al*., 2003; Kirui *et al*., 2014; Salami *et al*., 2019). According to Kirui and colleagues (2014), Busaa samples collected from Kericho County were found to be contaminated with aflatoxins and other mycotoxins. Also another study conducted earlier by Kenji and colleagues in Nairobi County reported the presence of aflatoxins in Busaa collected from Nairobi County (Kenji *et al*., 2003). This is because the brews are prepared from cereals that are contaminated with molds and aflatoxins, or the processes of preparation environment may enhance growth of molds and their metabolites. Despite Marua, Busaa, and chang'aa being the most consumed traditional brews in Nkubu municipality, Meru County, data on their quality, mold and aflatoxin safety is limited and this poses a public health concern. This study sought to fill this gap by determining the diversity of mold species and aflatoxins in selected traditional cereal based brews (Busaa, Chang'aa and Marua) in Nkubu municipality, Meru County.

This study findings firstly, establish the prevalence and levels of molds and aflatoxin contaminations in Marua, Busaa, and Chang'aa in Nkubu municipality, Meru County. Secondly, they provide knowledge to fill the existing gap, and underscore the need of public health education and guide policy makers in the county to come up with appropriate interventions that will help in mitigating the effects of molds and aflatoxins to the public, and lastly, it will also point out the need for mycotoxicological quality control of traditional brews which will guarantee the public of mycotoxicological safety.

2. Materials and methods

2.1 Study site

The study was conducted in Nkubu municipality, Meru County. The county lies between 0° 6′ North and 0° 1′ South and between latitudes 37° West and 38° East. It boarders Isiolo County to the North, Tharaka Nithi County to the East, Nyeri County to the South West, and Laikipia County to the West. It occupies a total area of 7,006 Km^2, with an eighth (972.3Km^2) being gazetted as forest. The following are the nine sub counties found in Meru County; Buuri, Igembe North, Igembe South, Igembe Central, Imenti North, Imenti South, Imenti Central, Tigania East, and Tigania West. The county has a similar climatic condition as that of the coast especially the eastern slopes where the altitude ranges from 300 m to 5,199 m above sea level. Agriculture is the main economic activity in Meru County, with the locals depending on waters from Tana and Ewaso Nyiro rivers for irrigation and domestic use. The county's average temperature ranges between 8°C and 32°C, with annual rainfall ranging between 300 mm to 2500 mm annually in the South East. The county has two rain seasons; the long rains between mid-March and May and short rains between October and December. The county has a total population of 987,653 (489,691 are males, 497,942 females, and 20 intersex persons). There are approximately 244,669 households with an average household size of 4.0 persons per household (KBS census, 2019). ECD centers are 1,437 ECD, Primary schools 1,030 and 372 secondary schools. The county has 14 post high school education centers and TVETs, 6 special schools and over 7,000 adult education centers. There are 460 health facilities, scattered throughout the county, with a total bed capacity of 995. The study covered areas of Nkubu town villages (Figure 1).

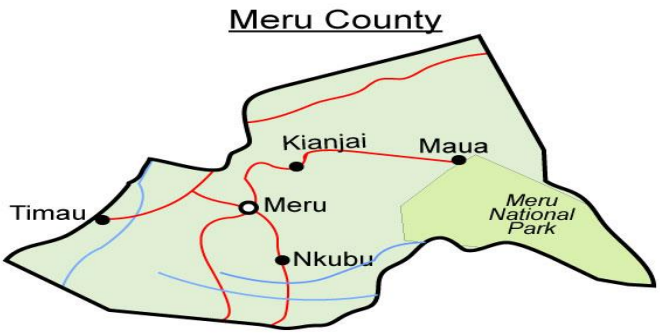

Figure 1: Map of Meru County showing the study

2.2 Study design and sample collection

This was a cross-sectional prospective study where a total of 84 samples: 62 Marua, 17 Busaa, and 5 Chang'aa were randomly collected from different households in Nkubu municipality after consenting between April and December 2023. All the samples were stored in sterile containers and transported to Mycology Laboratory at KEMU for processing, analysis, and storage.

2.3 Inclusion criteria and exclusion inclusion criteria

Households whose heads consented were legible for cereal based brew samples collection and were included into the study, while

those whose heads refused to consent were excluded from the study.

2.4 Isolation of the molds

Fungi isolation was carried out in sixty-two (62) Marua, seventeen (17) Busaa, and five (5) Chang'aa brew samples as described previously by Owuor *et al*. (2018) and Wagacha *et al*. (2016). A total of 100 µL of each brew aliquots were 10-fold serially diluted using 1 mL of sterilized distilled water pH 7 and kept on ice. The setup was replicated thrice for each brew sample. The dilutions of up to 10^{-6} were then spread plated on potato dextrose agar (PDA) supplemented with 100 µg/mL of streptomycin, penicillin, and ampicillin. The plates were incubated at 28°C for 5 days. Counts of the total number of fungal colonies per plate were done. The fungal colonies were further sub-cultured on potato dextrose agar and incubated at 30^{o}C for 7 days to get pure cultures.

2.5 Identification and characterization of mould isolates

The resulting fungal pure cultures were identified to species level based on cultural and morphological characteristics such as colony diameter, colony colour on agar and reverse, colony texture and zonation (Sharma & Pandey, 2010). Morphological features were studied under the light microscope while taking into consideration the key outstanding microscopic features such as conidiophores, conidial shape, phialides and metulae, presence and shape of vesicles (Diba *et al.,* 2007). Contemporary diagnosis of the *Aspergillus* species was based on the descriptions and keys by Klich (2002).

2.6 Quantification of total aflatoxin in the brew samples

Enzyme-Linked Immunosorbent Assay (ELISA) kit (Helica Biosystems, Inc®, Santa Ana, CA, USA) was used to quantify the levels of aflatoxin in the brews according to the manufacturer's instructions. For ELISA analysis, a bottle containing deionized water was placed in water bath set at 40 °C and pre-warmed for 1 hour. 5 ml of the sampled brew was measured into an extraction cup and two capsules of Hydro extraction buffer was added into the cup. 25 mL of warm deionized or distilled water was added and shaken vigorously for 2-3 minutes. The samples were then centrifuged for 1 minute and 100 µL of the supernatant pipetted into a clean tube containing 700 µL of water. The assay protocol for microplate reader analysis began with equilibrating all reagents and samples to room temperature for consistency. Phosphate buffered saline (PBS) -Tween buffer was prepared by reconstituting it with distilled water, followed by dispensing 200 µL of conjugate into designated wells. Standards and samples (100 µL each) were then added to specific wells, mixed thoroughly, and transferred to antibody-coated wells for incubation at room temperature. After incubation, wells were washed with PBS-Tween wash buffer, excess buffer was removed, and substrate reagent (100 µL) added to initiate the reaction. Following a 5-minute incubation, stop solution (100 µL) was added to halt the reaction. Optical density (OD) was measured at 450 nm using a microplate reader (Mindray® Inc. Nanshan, Shenzhen, China), and standard curves were generated using known concentrations of standards.

2.7 Data Analysis

Descriptive statistics: mean, standard deviation, and range, and percentages for aflatoxin levels, and proportions for molds were calculated. Data was presented in frequencies and percentages using tables and graphs. Chi-square was used to test for significance among qualitative data. Differences in mean aflatoxin levels between the three cereal based brews (marua, busaa, and chang'aa), and study sub-locations were compared using one Way ANOVA with Turkey's multiple comparison tests post-hoc analyses. All the statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS) for Window version 17.0 (SPSS Inc., Chicago, Illinois). Two-sided P value < 0.05 was considered statistically significant.

2.8 Ethical approval

This study was approved by the Mount Kenya University Ethical Review Committee (MKUERC) and National Commission for Science, Technology and Innovation (NACOSTI) under protocol numbers #1896 and # NACOSTI/P/23/27764, respectively.

3. Results

3.1 Isolation and morphological identification of fungi from local traditional brews: Marua, Busaa, and Chang'aa

Out of the three brews collected from the study area, marua 62 (73.8%) was the most common brew found in Nkubu municipality, Meru county, followed by busaa 17 (20.2%), and chang'aa 5 (6.0%) (Additional file: Figure S1). Different types of fungi were successfully isolated from the selected brews through culturing and further identified using both culturing and morphological features. The most common fungal types found in the sampled local brews were *Aspergillus*, *Penicillium*, and yeast. *Aspergillus* plus yeast was the most abundant mold species in all the three types of brews: marua (69.4%), Busaa (70.6%), and chang'aa (60.0%). By employing a taxonomic key and species descriptions provided by Klich (2002), all the *Aspergillus* fungi isolated were from the *Aspergillus* section *flavi*. When cultured on potato extract agar (PDA), *Penicillium* species displayed robust growth on PDA media after a 7-day incubation at 25°C. The colonies had a velutinous to floccose texture, appearing green with a white edge hem and corrugation. Clear copious exudate droplets were present, and the reverse colony color was light yellow-brown on PDA. Conidiophores were terverticillate with rough-walled stripes, and phialides were cylindrical tapering to a distinct column. Conidia varied between smooth-walled or rough-walled, globose to subglobose in shape (Figure 2A). In contrast, *A. flavus* displayed colonies with olive-green conidia, which were prominent in the appearance of the colony. These colonies typically exhibited flat borders with a raised center. Under microscopic observation, the conidiophores of *A. flavus* isolates appeared colorless with thick walls, bearing vesicles that were either globose or sub-globose in shape. The cells were arranged mainly in two rows, with phialides growing on the metulae. The metulae covered the surface of the vesicles and extended outward in all directions. The conidia were globose, possessing thin walls and a slightly rough texture (Figure 2B). Yeast fungi exhibited colony morphologies ranging from off-

white to cream, appearing butyrous, dry, or rough on the surface. Colony shapes varied from circular to irregular, with entire or undulate margins. Elevations ranged from flat to cratered (Figure 2C).

3.2 Isolation Frequency of *Penicillium*, *Aspergillus*, and Yeast, in different types of local brews: Marua, Busaa, and Chang'aa

A total of 62 (73.8%) marua samples collected from the study area were successfully cultured on PDA culture media. Different types of molds were isolated from the brew at varying frequencies either as single (isolate with one type of mold) or mixed strains (isolate with two or more types of molds). Yeast in combination with *Aspergillus* (yeast + *Aspergillus*) were the majority, 43 (69.4%), followed by yeast 18 (29.0%), and yeast combined with *Aspergillus* and *Penicillium* (yeast + *Aspergillus* + *Penicillium*) 1 (1.6%). However, there was no yeast and *Penicillium* (yeast + *Aspergillus* + *Penicillium*) molds that were depicted in the Marua isolates (Table 1, Additional file: Figure S2). Similarly, the distribution of molds in Busaa depicted the same trend, as follows: (yeast + *Aspergillus* 12 (70.6%), yeast 2 (11.8%), yeast + *Aspergillus* + *Penicillium* 2 (11.8%), and *Aspergillus* + *Penicillium* 1 (5.9) (Table 1, Additional file: Figure S3). Yeast + *Aspergillus* 3 (60%) was the most common mold isolated from chang'aa, followed by yeast 2 (40%). Conversely, yeast + *Aspergillus* + *Penicillium* and *Aspergillus* + *Penicillium* molds were not shown in Chang'aa brew from the study area (Table 1, Additional file: Figure S4). Chi-square test showed that the frequencies of the four molds in marua and busaa varied significantly, ($P < 0.001$). However, no statistically significant difference was shown in chang'aa isolates, (P=0.655) (Table1).

3.3 Quantification of Aflatoxins produced by molds isolated from selected traditional brews

Aflatoxin metabolites were successfully analyzed in marua (n=62), busaa (n=17), and chang'aa (n=5) using ELISA method. The mean, standard deviation, and range for aflatoxins concentrations in marua, busaa, and chang'aa were 5.74±3.29, (1.12-25.70) ppm, 6.40±2.45, (1.12-25.70) ppm, and 2.35±3.07, (1.41-3.55) ppm, respectively (Table 2). Busaa had a marginally higher aflatoxin mean concentration (6.40±2.45 ppm) compared to Marua (5.74±3.29 ppm) and chang'aa (2.35±3.07 ppm) (Table 2), though the difference was not statistically significant (P=0.195) (Table 2).

3.4 Distribution of aflatoxins in different types of local brews per study sub-location

Aflatoxin levels in each brew were also compared among the study sites/sub-locations from which the samples were collected. The distribution of aflatoxin concentrations in marua were diverse among the study sub-locations, though not statistically significant, (P= 0.567) (Table 3). The mean, standard deviation, and range for aflatoxins concentrations in marua for each sub-location was as follows: Igogi West; 13.05±2.23, n=5, (5.01-42.72) ppb, Kariene; 6.91±1.16, n=4, (5.90-8.24) ppb, Tita; 4.19±2.11, n= 16, (4.19±2.11) ppb, Igogi Central; 5.81±2.50, n=5, (1.27-44.49) ppb, Nkuene; 6.19±3.70, n=15, (6.19±3.70) ppb, and Mitungu; 4.17±5.04, n=17, (0.24-38.03) ppb (Table 3). The mean aflatoxin concentrations of Busaa among the sub-locations were; Igogi West; 9.64±4.05, n=2, (3.58-25.94) ppb, Kariene; 5.07±2.14, n=5, (1.82-12.68) ppb, Tita; 6.76±3.40, n=6, (1.13-21.74) ppb, and Igogi Central; 4.62±1.05, n=2, (4.46-4.78) ppb (Table 3). Apparently, this did not conclude whatsoever statistical noteworthy variation in aflatoxin dispersion in Busaa among the sub-locations, (P=0.951) (Table 3). Chang'aa was successfully collected from three of the six sub-locations; Kariene (n=1), Mitungu (n=3), and Igogi East (n=1) (Table 3). The mean aflatoxin concentration for chang'aa in Mitungu sub-location was 7.43±1.47, (5.01-10.88) ppb (Table 3). The distribution of aflatoxins in the three cereal based brews did not vary significantly across all the study sub locations ($P > 0.05$) (Table 3).

3.5 Assessment of the aflatoxin safety levels of Marua, Busaa, and Chang'aa collected from Nkubu municipality, Meru County

Aflatoxin levels were categorized into three safety levels base on the KEBS (>10 ppm) recommendations, namely; very safe; concentration less than 2 ppb; Safe; concentration between 2-10 ppb and Unsafe; concentration greater than 10 ppb. Out of the 62 marua samples collected from Nkubu municipality, 17 (27.4%) were very safe for human consumption, 24 (38.7%) were safe, and 21 (33.9%) were unsafe. The safety levels for Busaa brewed by the study population were: very safe; 3 (17.6%), safe (47.1%), and unsafe 6 (35.3%). Additionally, all the chang'aa samples collected from the study area were within the safety levels; very safe 2 (40%), and safe 3 (60%) (Figure 3).

Table 1. Isolation Frequency of Yeast, *Aspergillus*, and *Penicillium* in different types of brew collected from Nkuba Municipality, Meru County.

Type of brew	Type of mold/fungi	Number of isolates	Isolation Frequency (%)	*P* value
Marua (n=62)	Yeast	18	29.0	< 0.001*
	Yeast + *Aspergillus*	43	69.4	
	Yeast + *Aspergillus* + *Penicillium*	1	1.6	
	Yeast + *Penicillium*	0	0	
Busaa(n=17	Yeast	2	11.8	< 0.001*
	Yeast + *Aspergillus*	12	70.6	
	Yeast + *Aspergillus* + *Penicillium*	2	11.8	
	Yeast + *Penicillium*	1	5.9	
Chang'aa (n=5)	Yeast	2	40	0.655 NS
	Yeast + *Aspergillus*	3	60	
	Yeast + *Aspergillus* + *Penicillium*	0	0	
	Yeast + *Penicillium*	0	0	

*Statistically significant difference between different mold isolates in busaa ($P < 0.001$) by Chi-square test, NS-no statistically significant difference between different mold isolates in marua and chang'aa ($P > 0.05$).

Figure 2: Colonies of different fungi isolated from selected local brews collected from Nkubu municipality, Meru County. **A**- *Penicillium* mold (front, reverse and microscopic view), **B**- *Aspergillus* section *flavi* species (front, reverse and microscopic view), **C**-Yeast (front, reverse and microscopic view).

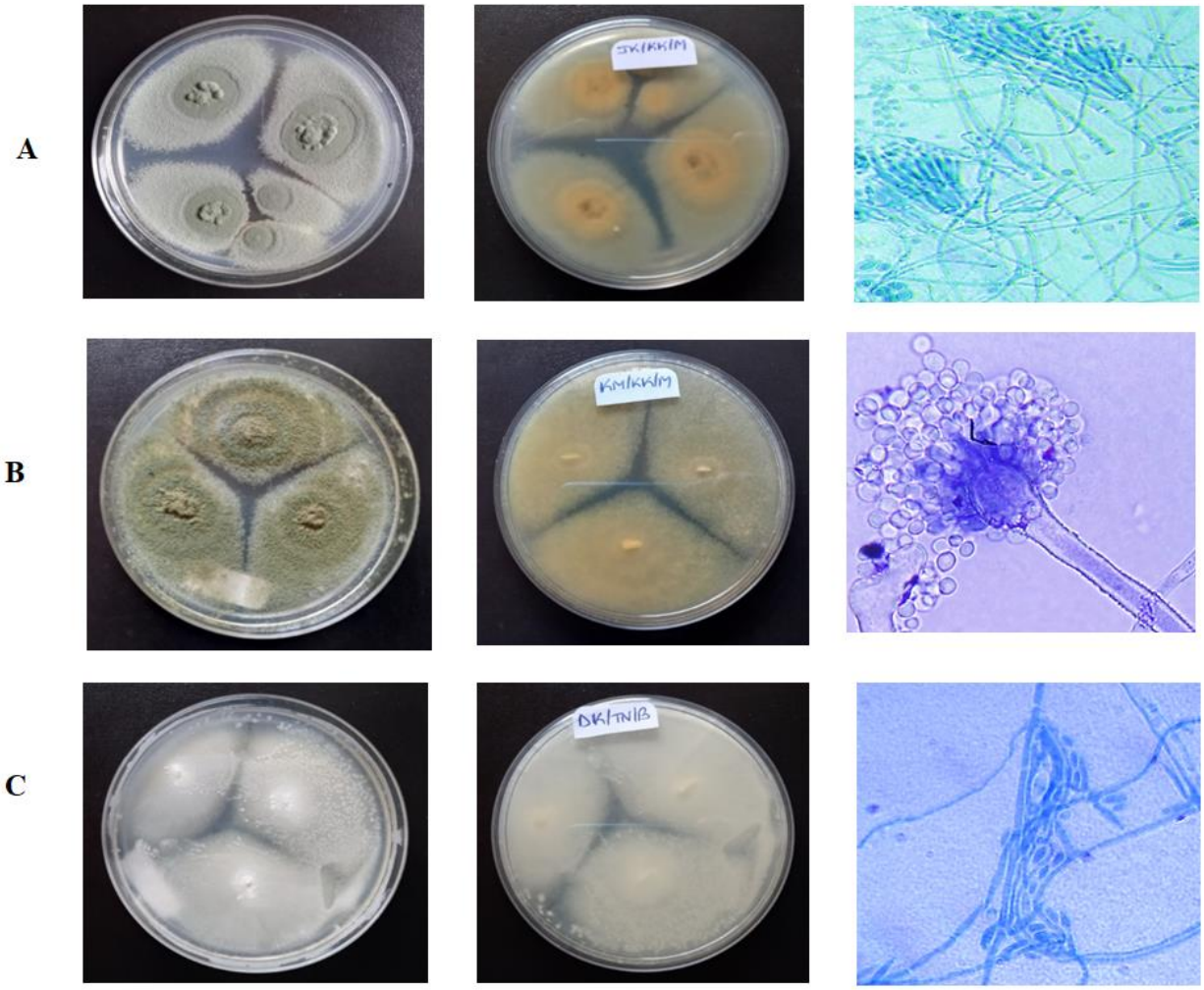

Table 1. Aflatoxin concentration/levels in different types of local brews

Type of Brew	**Aflatoxin concentration** ppm)		
	Mean±SD	**Range (Min-Max)**	***P*-value**
Marua (n=62)	5.74±3.29	1.12-25.70	0.195 **NS**
Busaa (n=17)	6.40±2.45	1.12-25.70	
Chang'aa (n=5)	2.35±3.07	1.41-3.55	

ppb- Parts per billion, SD- Standard deviation, Min- minimum, max-maximum, NS- no statistically significant difference in Aflatoxin concentrations/levels between different types of brews (marua, busaa, and chang'aa) (P=0.195) by one-way ANOVA.

Table 2. Aflatoxin concentration in different types of local brews per study sub-location

Type of brew	**Study site/sub-location (n)**	**Aflatoxin concentration (ppb)**		
		Mean±SD	**Range (Min-Max)**	***P*-value**
Marua	Igogi West (n=5)	13.05±2.23	5.01-42.72	0.567 **NS**
	Kariene (n=4)	6.91±1.16	5.90-8.24	
	Tita (n=16)	4.19±2.11	1.39-7.11	
	Igogi Central (n=5)	5.81±2.50	1.27-44.49	
	Nkuene (n=15)	6.19±3.70	1.40-42.5	
	Mitungu (n=17)	4.17±5.04	0.24-38.03	
Busaa	Igogi West (n=2)	9.64±4.05	3.58-25.94	0.951 **NS**
	Kariene (n=5)	5.07±2.14	1.82-12.68	
	Tita (n=6)	6.76±3.40	1.13-21.74	
	Igogi Central (n=2)	4.62±1.05	4.46-4.78	
	Nkuene (n=1)	N/A	N/A	
	Mitungu (n=1)	N/A	N/A	
Chang'aa	Kariene (n=1)	N/A	N/A	N/A
	Mitungu (n=3)	7.43±1.47	5.01-10.88	
	Igogi East (n=1)	N/A	N/A	

ppb- Parts per billion, SD- Standard deviation, Min- minimum, max-maximum, NS- no statistically significant difference in Aflatoxin concentrations between different types of brews per study sub-location by one-way ANOVA at $P < 0.05$, N/A-Not applicable.

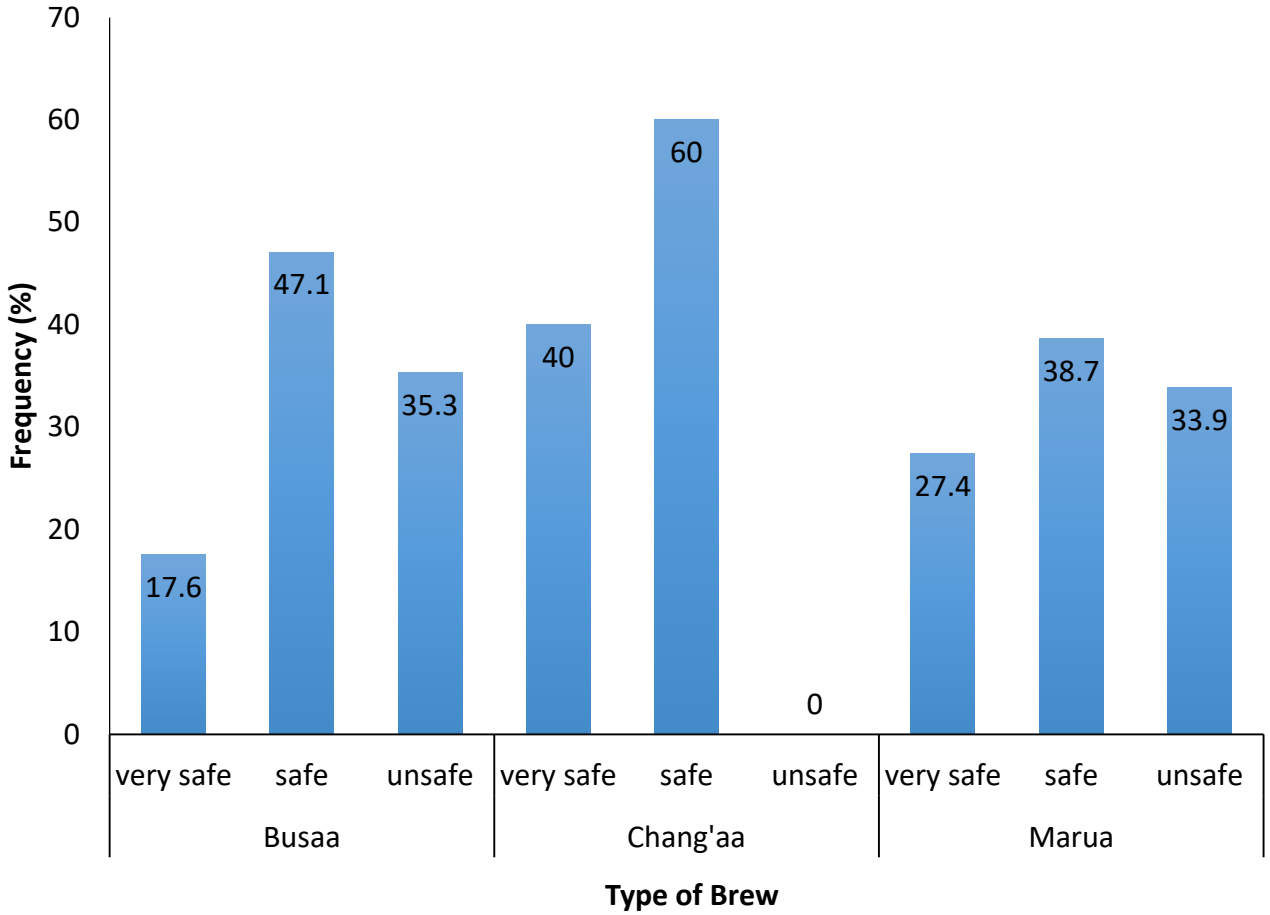

Figure 3: Frequency of Aflatoxin levels in different types of local brews.

Safety levels were categorized as follows: very safe; aflatoxin levels < 2 ppb, Safe; aflatoxin levels 2-10 ppb, and unsafe; aflatoxin levels > 10 ppb. The percentages represent number of examined samples per brew; Busaa (N=17), Chang'aa (N=5), and Marua (N=62)

4. Discussion

This study focusing of the determination of diversity of mold species and aflatoxins in local traditional brews within Nkubu municipality, Meru County, Kenya revealed that *Penicillium spp*, *Aspergillus flavus*, and yeast were the most common mold species found in the local traditional brews in Nkubu municipality. The three common molds were co-occurring with each other in the traditional brew samples. These findings were in line with what Lawrence and colleagues found out in South Africa in a study focusing on analyzing the aflatoxins content in commercially produced traditional beers and traditional beers raw materials (sorghum, sorghum malt, and maize) and (Lawrence, 1988). Another study by Salami and others focusing on the detection of aflatoxins in locally produced and imported beers in Nigeria also reported similar findings, where yeast, *Aspergillus flavus*, and *Penicillium spp* were the common mold species found in the beers under investigation (Salami *et al*., 2019). This is suggestive that raw materials/grains (millet, sorghum, and maize) contaminated with aflatoxins are the cause of mold contamination in locally produced traditional brews in Nkubu municipality. It has been previously argued that aflatoxin contaminated raw materials are font of transferring the contamination to the products such as beer, and traditional brews (Kirui *et al*., 2014; Lawrence *et al*., 1988; Okaru *et al*., 2017; Salami *et al*., 2019). The presence of molds in the traditional brews from Nkubu municipality could be due to the favorable environmental conditions of semi humid to semiarid in Eastern Kenya that favors the growth of fungi in molded raw materials (millet, sorghum, and maize) as earlier argued (Mugure *et al*., 2022; Omara *et al*., 2021), since most of the brewers use molded grains for brewing so that they can sell the products at a low price (Mugure *et al*., 2011; Okaru *et al*., 2017).

Marua and Busaa depicted co-occurrence of all the three species of molds; *Aspergillus flavus*, *Penicillium spp*, and yeast while chang'aa contained only *Aspergillus flavus*, and yeast. The difference in the mold co-occurrence in the three brews could be attributed to the preparation methods of the brews. Previous studies have shown that some preparation methods such as boiling reduce mold contamination while others such as malting increase the contamination (Matumba *et al*., 2015b; Okeke *et al*., 2015). *Aspergillus flavus* was the

dominant fungi from the *Aspergillus* genus isolated from the local brews because it is the main fungi found in agricultural products (Lizarraga-Paulin *et al*., 2011).

Aflatoxins were detected in all the three types of brews at different levels. This outcome was in agreement with findings from earlier studies aiming at determining aflatoxins in Busaa collected from different parts of Kenya, and local brews from African Countries (Kenji *et al*., 2003; Kirui *et al*., 2014; Salami *et al*., 2019; Omara *et al*., 2021). This further supports previous findings that traditional methods of preparing local brews does not detoxify aflatoxins (Kang'ethe *et al*., 2017). The aflatoxin means for busaa was marginally higher than that of marua, and chang'aa, though this was not statistically significant. This is because busaa was majorly maize based while marua and chang'aa were sorghum, and millet based. Previous studies in Kenya, and other African countries have found maize to contain higher aflatoxin concentrations compared to sorghum, and millet (Falade *et al*., 2022; Sirma *et al*., 2016). The aflatoxin mean concentration in busaa, marua, and chang'aa were comparable to previous findings reported by Kirui and colleagues in 2014, and later on by Okaru and others in 2017, in studies investigating total mycotoxins in busaa in Bomet County, Kenya, and beers from different counties in Kenya, respectively (Kirui *et al*., 2014; Okaru *et al*., 2017). Another study analyzing the aflatoxin concentration in busaa from Nairobi County by Kenji and others found out that 68% and 17% of the samples had aflatoxin concentrations of > 5 ppb and 50 ppb, respectively (Kenji, 2003). On the contrary, our findings showed that 52.9% of busaa samples had aflatoxin concentration higher than 5 ppb and no sample had aflatoxin concentration higher than 50 ppb. The difference in occurrence of aflatoxins in the local brews and earlier studies could be attributed to the variation in seasonality or agro ecological zones where the raw materials were sourced from as earlier stated (Bandyopadhyay *et al*., 2016). The distribution of aflatoxins in the three types of local brews across all the study sites was comparable suggesting a uniform distribution of aflatoxins in the study area.

Kenya Bureau of Standards (KEBS) has set 10 ppb as the maximum permissible levels of aflatoxin in food for human consumption (Nyaguthie, 2020). This study assessing the aflatoxin safety levels of local brews revealed that 100% of all the local brews tested had detectable levels of aflatoxins. About 33.9% and 35.3% of marua and busaa samples, respectively were unsafe for human consumption because they had aflatoxin levels above the allowable maximum regulatory limit of KEBS (10 ppb). Despite the local brews having detectable levels of aflatoxin, none of the chang'aa samples reported levels above the recommended maximum limit (10 ppb). This observation collaborates with earlier findings from Kenya and African countries. The minimal presence of aflatoxins in Chang'aa can be attributed to the distillation process involved in its preparation. A study by Kirui and colleagues in 2014 investigating total mycotoxins in busaa in Bomet County, Kenya recorded aflatoxins and other mycotoxins in a local brew (busaa) (Kirui *et al*., 2014). Another study by Okaru and others focusing on assessing for aflatoxins in local produced beers from different counties in Kenya also found aflatoxin in these samples at detectable levels (Okaru *et al*., 2017). Kenji and colleagues in a study analyzing the aflatoxin concentration in busaa from Nairobi County found out that 68% and 17% of the samples had aflatoxin concentrations of > 5 ppb and 50 ppb, respectively (Kenji, 2003). A review paper by Ezekiel and colleagues on mycotoxins existence and contact assessment in African traditional alcoholic drinks, 16 papers reported the existence of different mycotoxins in local processed brews and their raw materials across Africa (Ezekiel *et al*., 2018). These findings further imply that despite the local traditional brews: having the nutritional values, serving as alternatives to low income consumers who cannot afford industrially processed finer alcoholic products, and used to mark very important ceremonies traditionally such as weddings, circumcision, funerals, bumper harvest, rain making, and naming (Kirui *et al*., 2014; Tafere, 2015), they are health hazards that exposes the consumers to the deadly carcinogenic toxins (aflatoxins). Local brews in Nkubu municipality poses a public health concern to the consumers hence measures have to be instituted to control and manage aflatoxin levels so as to improve their quality.

Occurrence of unsafe levels of aflatoxin in busaa and marua, and not in chang'aa could be explained by the different methods of preparation. Marua and Busaa are normally processed by germination of raw materials (maize, millet or sorghum flour)**,** frying/roasting, addition of water and malted finger millet, fermentation, and filtration (Katongole, 2008; Kirui *et al*., 2014). Chang'aa is processed by mixing of water and molasses, subsequent addition of yeast to the mixture, and fermentation followed by distillation (Carey *et al*., 2015). It has also been stated previously that some processing methods reduces mycotoxins concentrations in the raw materials and subsequently in the final products (Ezekiel *et al*., 2015; Matumba *et al*., 2015b). Kabak, 2009 argued that high temperatures (≥ 160°C) distillation decomposes mycotoxins such as aflatoxin B1 in alcoholic drinks thus decreasing their content in the final products (Kabak, 2009). However, malting step in the brewing process has been found to increase mycotoxin levels by 3-fold in sub Saharan Africa since the moistening stage provides a conducive environment for mycotoxin growth (Ezekiel *et al*., 2018; Matumba *et al*., 2011). Our finding further suggests that the extra distillation step in Chang'aa processing plays a role in reducing the levels of aflatoxins in this brew produced in Nkubu municipality, Meru county, Kenya.

Conclusion

The presence of *Penicillium spp*, *Aspergillus flavus*, and yeast in the cereal based brews suggest that brews in Nkubu municipality are prone to different mold contamination depending on the type of raw material used. Detection of aflatoxin at varying levels in marua, busaa, and chang'aa collected suggest that traditional brew preparation methods do not detoxify the brews from aflatoxin. Presence of aflatoxins in Busaa and marua brews at levels more than the maximum

permissible levels set by KEBS exposes the frequent consumers in Nkubu municipality to health hazards impacting negatively on their health and safety. Co-occurrence of molds and aflatoxins in traditional cereal based brews warrants mycotoxicological quality control of traditionally processed brews in Nkubu municipality to guarantee public mycotoxicological safety in Nkubu municipality, Meru County.

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Conflicts of interest
The authors declare that there are no competing interests.

Abbreviations
PDA; potato dextrose agar, ANOVA; analysis of variance; ELISA; enzyme-linked immunosorbent assay

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