

Isolation and Identification of Toxigenic *Aspergillus* species Associated With Sorghum Grains and Locally Brewed Beer (Burkutu) in Bukuru Metropolis of Jos South Local Government Area, Nigeria.

ABSTRACT

Aim: This study is aimed at Isolating and Identifying the Toxigenic *Aspergillus* species Associated With Sorghum Grains and Locally Brewed Beer (Burkutu) in Bukuru Metropolis of Jos South Local Government Area, Nigeria.

Methodology: Sorghum grains and locally brewed beer (Burkutu) were collected from three different locations; Gyel, Kugiya, and Doruwa Market of Bukuru Metropolis of Jos South Local Government Area, and Screened for their fungal and total aflatoxin level. The grains were blended for 30 seconds using a high-speed blender. 1 gram of the blended sorghum and 1ml of the Burkutu sample (local beer) were cultured into freshly prepared PDA supplemented with 0.5mg/ml of gentamycin and streptomycin to suppress bacterial growth. This was then incubated at a temperature of 37°C for 72 hours after which various fungi colonies were observed and counted with a colony counter. The fungi isolated were further sub-cultured to obtain pure cultures after which they were classified using conidial morphology as obtained from microscopy. Lactophenol cotton blue mount was also carried out to identify the exact fungi with reference to a mycological atlas. The colony-forming unit for each sample was determined, and the Aflatoxin test was carried out using a Mycotoxin kit (the Rida Quick Scan method) to determine the Total aflatoxin level.

Results: The *Aspergillus* species found in both Sorghum grains and Burkutu (local beer) were: *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus fumigatus*. For sorghum grains, *Aspergillus niger* had the highest frequency of occurrence (42.86%) while *Aspergillus flavus* & *Aspergillus fumigatus* occurred equally with (28.57%) respectively. *Aspergillus flavus* had the highest frequency of occurrence in Burkutu (50%), followed by *Aspergillus niger* (33.33%) and the least was *Aspergillus fumigatus* (16.67%). The highest aflatoxin level for grains was detected in Kugiya which had (3.6 parts per billion) followed by the grain sample from Gyel which had (3.0 P_{pb}) and the least was Doruwa (2.8 P_{pb}). Burkutu had the highest total level of aflatoxin (3.1P_{pb}) in the sample from Gyel, followed by Burkutu from Doruwa (2.6 P_{pb}) and the least was from Kugiya (2.4 P_{pb}).

Conclusion: Both the sorghum grains and Locally Brewed Beer (Burkutu) collected from the Bukuru Metropolis of Jos South Local Government Area, Nigeria were significantly contaminated by the toxigenic *Aspergillus* species. This indicates possible health hazards for humans and animals ingesting these substances. However, effective screening of these grains for aflatoxins is advocated to ensure the safety of both grains and their products.

Keywords: Sorghum, Burkutu, *Aspergillus* species, and Toxigenic.

1. INTRODUCTION

Aflatoxins are a family of highly toxic secondary metabolites derived from polyketides [1] produced by certain fungi that are found on agricultural cereal crops such as maize, peanut, cotton, wheat, walnut, and tree nuts [2,3]. The main fungi that produce aflatoxin are *Aspergillus flavus* and *Aspergillus parasiticus* which are abundant in the warm and humid regions of the world [1]. Aflatoxin-producing fungi can contaminate crops in the field at harvest and during storage and it is extremely persistent under the moist condition of storage, handling, and processing. Aflatoxins have been found as a contaminant in agricultural food products, especially in cereals and animal feed [4]. *Aspergillus flavus* is widespread and is most often found when certain grains are grown under certain conditions of stress such as drought. Additionally, Mold occurs in soil, decaying vegetation, hay, and grains undergoing microbiological deterioration and invades all types of organic substrates whenever and wherever the conditions are favorable for its growth. Aflatoxins contamination of food causes various complications to humans like hepatotoxicity, immunotoxicity, and teratogenic effects [5,6,7] associated with childhood stunting, which causes severe economic losses to the country [8]. It is a serious health problem because their presence in food interferes with micronutrient absorption and status in the body, as a consequence, they affect immunity and development. The extent to which mycotoxins affect human health is difficult to quantify but is highly significant as it poisons the body through respiratory routes resulting in overactivation of the inflammatory responses [9]. Aflatoxins are classified into four main groups which are **B₁(AFB₁) and B₂(AFB₂)** produced by *Aspergillus flavus* and **G₁(AFG) and G₂(AFG₂)** produced by *Aspergillus flavus* as well as *Aspergillus parasiticus* [10]. In addition, Aflatoxins are largely associated with different plant materials such as cotton, peanuts, maize, and sorghum [11,12].

Sorghum (***Sorghum bicolor* L.**) is the fifth most economically important cereal crop with 60 million tons annually produced in the world. It is a staple food grain for over 750 million people in Africa, Asia, and Latin America [13]. Nigeria however is the world's second-highest sorghum producer with a total annual grain production of 6.9 million tons and 5.4 million hectares [14]. Nigeria is the leading sorghum producer in Africa followed by Ethiopia in terms of total production [14]. It provides raw materials for the production of locally brewed beer or alcohol also known as Burkutu among other products [15]. Unfortunately, cereals are naturally contaminated with fungi in the field during drying, processing, transportation, and subsequently storage and it may be difficult to completely prevent mycotoxins formation in contaminated commodities, particularly those that are produced in tropical and subtropical climates where high temperature and humidity promote the growth and proliferation of fungi [16]. Several studies on fungi associated with sorghum grains have been carried out and It was observed that ***Aspergillus*, *Alternaria*, *Arthrinium*, *Rhizopus*, and *Fusarium***, were isolated [17]. Sorghum has high phenol and tannin content and this principle makes it resistant to mold infestation, diseases, and damage, however, these grains are often contaminated by molds and they are ideal substrates for these molds growth when poorly dried and stored [18].

Sorghum is however used as an important starchy food for human and animal consumption, particularly in Northern Nigeria. Nigerians produce a local beer called Burkutu which is a traditional fermented beer from sorghum. Burkutu is a popular alcoholic beverage with a vinegar-like flavor consumed in Nigeria and it is produced mainly from the grains of guinea corn of the specie *Sorghum vulgare* and *Sorghum bicolor* [19]. The preparation of the local beer involves steeping, germination, fermentation, and maturation and the resulting product is a cloudy alcoholic beverage called 'Burkutu' [20]. Burkutu contains almost all essential amino acids in the required proportion except cysteine and tryptophan which are destroyed by heat during boiling [21]. Burkutu plays some vital socio-economic roles in Nigeria influencing to a large extent the lives of the people. It is important to most rural Nigerian populations because it is more affordable than commercially brewed beer. The percentage alcohol content of Burkutu is between 3-6 percent [22]. Most people take this beverage to add variety to their diet and for its stimulating effect, this is so because the traditionally brewed beers added from sorghum contain vitamins, iron, magnesium, manganese, phosphorus, calcium, 26.7g starch, and 5.9g of protein per liter [23]. It is a relatively cheap alcoholic drink with a unique flavor which makes it popular among Berom people that consume it in the Bukuru areas of Jos South Local Government Area of Plateau State, Nigeria. It is also consumed at festivals and ceremonies like birthdays,

marriages, and burials. Alcohols such as Burkutu, when consumed in large quantity have been discovered to be poisonous to the body, particularly to organs like the liver, kidney, and brain as it causes liver fibrosis, alcohol liver disease (ALD), and Hepatocellular carcinoma (HCC) [24,25,26].

Based on the foregoing, this study was undertaken to estimate fungal contamination associated with *Aspergillus species* and aflatoxin contamination on sorghum samples and the locally brewed beer collected from Bukuru metropolis of Jos South Local Government Area, Plateau State.

2. MATERIALS AND METHOD

2.1. Experimental Materials

The materials used in this study include the following: Autoclave, Petri dishes, hot plate, spirit lamp, measuring cylinder, conical flask (500ml), Glass slide, coverslip, microscope, micropipette, optional shaker, blender, and Filter papers. The solvent/reagents used are distilled water, methylated spirit, Methanol (70%), absolute ethanol, disinfectants (hypo-bleach), and detergents. Other materials used include Cotton wool, sterile needle, syringe, masking tapes, matches, foil paper, and hand towel.

2.2. Sample Location and Collection

Sorghum and its product (Burkutu) were collected from three geographical locations namely Gyel, Kugiya, and Doruwa market of Bukuru metropolis in Jos South Local Government Area, Nigeria. To test for aflatoxin, Sorghum intended for beer production was collected and 200ml of its beer was also collected from the different locations. The grains were blended for 30 seconds using a high-speed blender. Samples were packaged into transparent nylon bags, labeled accordingly, and stored for subsequent use.

2.3 Media preparation

Preparation of Potato Dextrose Agar (PDA) was carried out. A 100g of potatoes were peeled and chopped into cubes. 100ml of distilled water was poured into a conical flask (250mls) with the potato cubes and heated to a boil. 15g of plain agar and 10g of dextrose sugar were dissolved into 100ml of the potatoes-distilled water solution, shaken well, and heated to dissolve properly. It was then sterilized in an autoclave at a temperature of 121°C for a holding time of 15minutes. It was then poured in an aliquot amount of 20ml per plate of the sterile Petri dishes.

2.4 Innoculation, Fungi Isolation, and Colony Counting

The method described by Cotty[27] was adopted. Potatoe dextrose Agar was freshly prepared as described in media preparation. One gram (1g) of the blended Sorghum sample and 1ml of the Burkutu sample was cultured/plated out into the freshly prepared PDA supplemented with 0.05mg/ml of Gentamycin and streptomycin to suppress bacterial growth. The poured plates were allowed to solidify, after solidification, an inoculum was picked with a sterile wire loop and inoculated aseptically into the solid media in the Petri dishes and incubated at a temperature of 37°C for 72 hours after which the various fungi colonies were counted with the aid of a colony counter. Fungal colonies growing were further subcultured to obtain pure cultures. Isolates were classified based on conidial morphology and colony characteristics. Conidial morphology was done microscopically by each

culture tube under a good source of light. Lactophenol cotton blue mount was carried out on the fungal cultures to identify the exact fungi based on the conidia and hyphal arrangements and fungi were identified with reference to a mycological atlas. This experiment was replicated three (3) times.

2.5 Determination of Colony Forming Unit

The colony-forming unit for each sample was determined by counting the total number of colonies per culture plate, divided by the total number of plates, multiplied by 100. The colony-forming unit for sorghum is measured in grams (Cfu/g) while that of Burkutu is measured in mls (Cfu/ml).

2.6 Detection of Aflatoxins

Total aflatoxins test was carried out using a Mycotoxin kit (the Rida Quick Scan method).

2.6.1. Sample Preparation

2.6.1.1. Sorghum grains

Ten (10) grams of the ground sample (Sorghum) was weighed into a beaker and Twenty (20) mls of methanol (70%) was added. The grains were blended for two minutes and filtered to obtain the filtrate using Whatman's number one filter paper. Fifty (50) μl of the clear supernatant filtrate solution were added to a Hundred (100) μl of the temperate mobile solvent to enhance the flow. It was mixed properly and a Hundred (100) μl was used for the test.

2.7.1.2 Local Beer (Burkutu)

Ten (10) mls of Burkutu were mixed with (70%) methanol to homogenize the mixture. It was filtered to obtain the filtrate using Whatman's number 1 filter paper. Fifty (50) μl of the clear supernatant filtrate solution was added to a Hundred (100) μl of the temperate mobile solvent to enhance the flow. It was mixed properly and a Hundred (100) μl was used for the test.

2.7.2 Test Procedure

A Hundred (100) μl of the sample solution was applied to the application area of the test strip for five minutes, after which the test strip was inserted into the Rida Quick Scan machine (confirmation of the result was carried out after sixteen minutes).

3. RESULTS

The result of this study shows the total number of colonies in each culture plate in various locations for both Sorghum and Burkutu in Tables 1 & 2 respectively. The frequency of occurrence for *Aspergillus species* for both Sorghum and Burkutu is also represented in Tables 3 and 4 respectively. Tables 5 and 6 show the total level of aflatoxin detected from both grains and products (Burkutu).

Table 1. Total number of fungal colonies and their respective colony-forming units for sorghum from the different locations

	S.K	S.D	S.G
R ₁	17	3	19
R ₂	31	3	14
R ₃	11	1	24
CFU (cfu/g)	1966.67	233.33	1900

KEYS= S.K: Sorghum-Kugiya, S.D: Sorghum-Doruwa, S.G: Sorghum-Gyel, R₁, R₂, and R₃: Replicates, CFU: Colony-Forming Unit.

Table 2. Total number of fungal colonies and their respective colony-forming units for Burkutu from the different locations

	B.D	B.K	B.G
R ₁	3	5	11
R ₂	5	6	22
R ₃	19	4	7
CFU (cfu/ml)	900	500	1333.33

KEYS= B.D: Burkutu-Doruwa, B.K: Burkutu-Kugiya, B.G: Burkutu Gyel, R₁,R₂ & R₃: ReplicateS, CFU: Colony-Forming Units.

Table 3. Frequency of occurrence of *Aspergillus species* in Sorghum from the various locations

Fungal isolates	S.K	S.D	S.G	Total	Percentage (%)
<i>Aspergillus niger</i>	+	+	+	03	42.86
<i>Aspergillus flavus</i>	+	-	+	02	28.57
<i>Aspergillus fumigatus</i>	+	-	+	02	28.57
Total	03	01	03	07	100

KEYS= S.K: Sorghum-Kugiya, S.D: Sorghum-Doruwa, S.G: Sorghum Gyel

Table 4. Frequency of occurrence of *Aspergillus* species in locally brewed beer (Burkutu) in the various locations

Fungal isolates	B.D	B.K	B.G	Total	Percentage (%)
<i>Aspergillus niger</i>	+	-	+	02	33.33
<i>Aspergillus flavus</i>	+	+	+	03	50.00
<i>Aspergillus fumigatus</i>	+	-	-	01	16.67
Total	03	01	02	06	100

KEYS= B.D: Burkutu-Doruwa, B.K: Burkutu-Kugiya, B.G: Burkutu-Gyel

Table 5. Total Aflatoxin Level (part per billion ppb) detected from Sorghum samples

Sorghum Samples	Total Aflatoxin Level (ppb)
Sorghum-Doruwa(SD)	2.8
Sorghum-Kugiya(SK)	3.6
Sorghum-Gyel(SG)	3.0

Table 6. Total Aflatoxin Level (part per billion ppb) detected from Burkutu Samples

Burkutu Sample	Total Aflatoxin Level (ppb)
Burkutu-Doruwa(B.D)	2.6
Burkutu-Kugiya(BK)	2.4
Burkutu-Gyel(BG)	3.1

Table 7. Cultural and Morphological Appearances of Fungal Isolates from Sorghum and Burkutu.

Isolates	Colonial	Morphology
<i>Aspergillus flavus</i>	Dark-green in color with a whitish edge	Conidiophores are branched with septate hyphae
<i>Aspergillus niger</i>	Dark-brown to black	Conidial heads are dark-brown to black. Long conidiophore with aseptate hyphae.
<i>Aspergillus fumigatus</i>	Blue-green in colour	The conidia are grey.

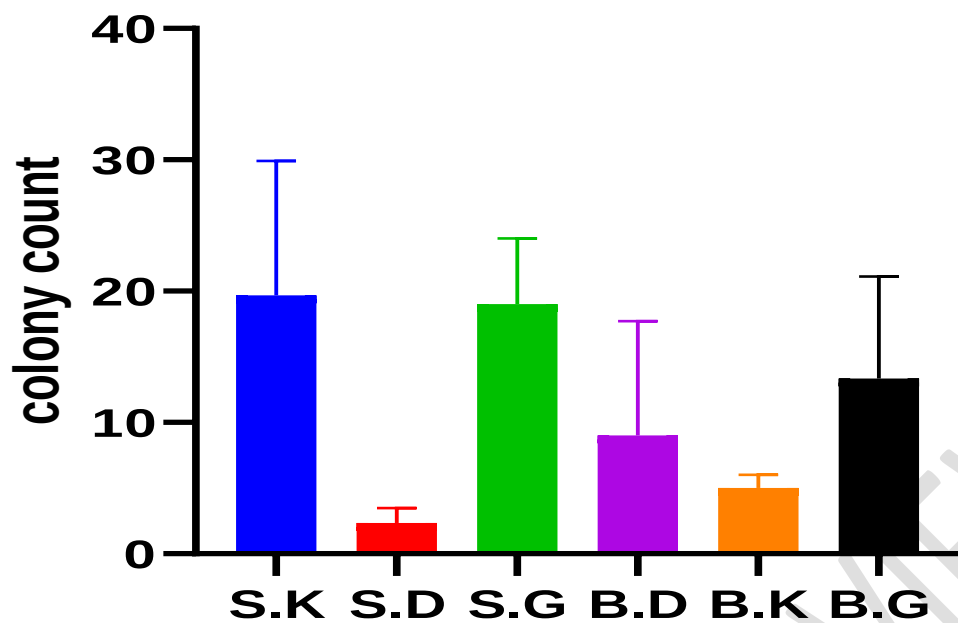


Fig. 1. Level of *Aspergillus* species contamination of the studied sample with.

KEYS= S.K: Soghum-Kugiya, S.D: Sorghum-Doruwa, S.G: Sorghum-Gyel, B.K: Burkutu-Kugiya, B.D: Burkutu-Doruwa, B.G: Burkutu-Gyel.

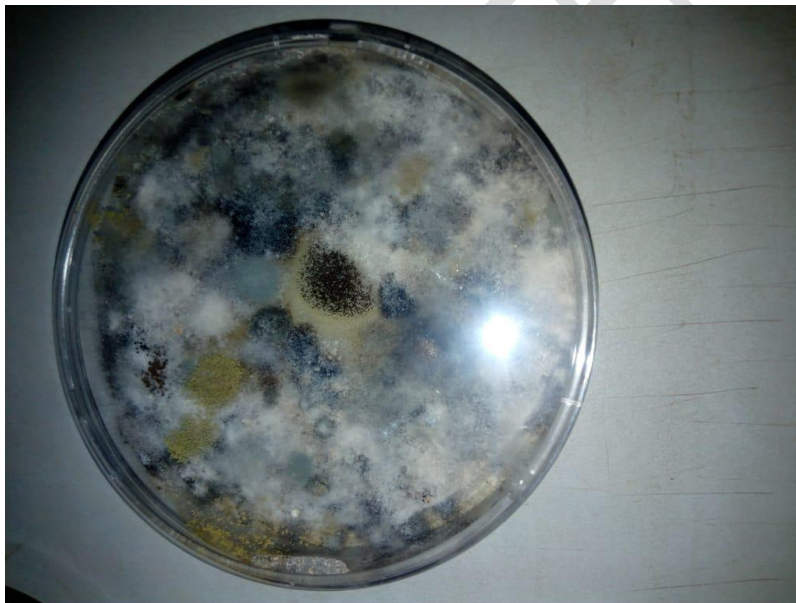


Plate 1. Mixed colonies of *Aspergillus* species in Burkutu from Kugiya.



Plate 2. Mixed colonies of *Aspergillus* species in Sorghum from Gyel



Plate 3. Mixed Colonies of *Aspergillus* species in Burkutu from Doruwa

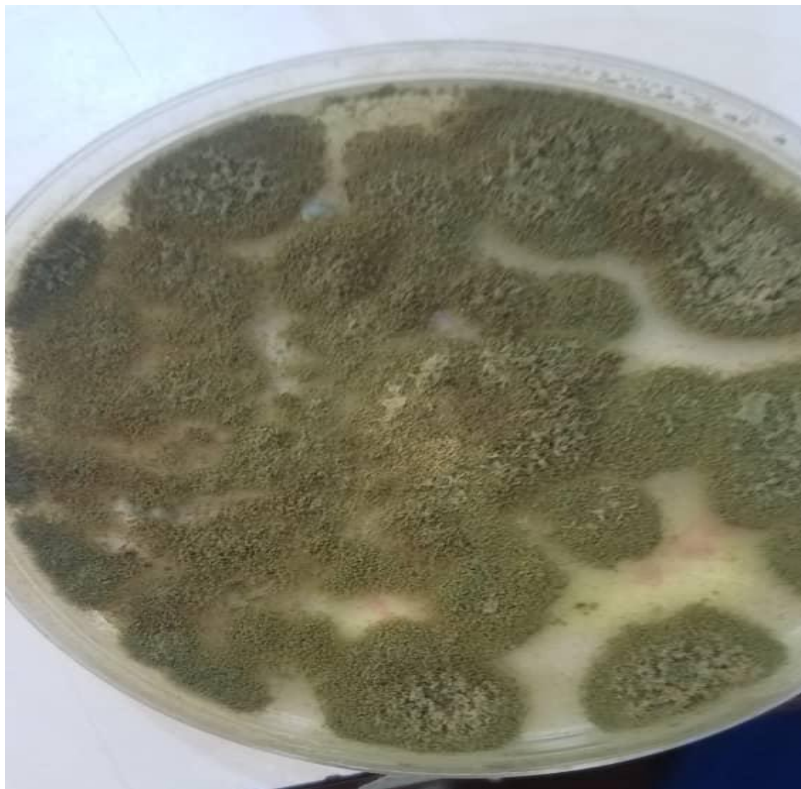


Plate 4. Pure plate of *Aspergillus flavus* in Burkutu from kugiya



Plate 5. Pure Plate of *Aspergillus niger* in sorghum from Gyel

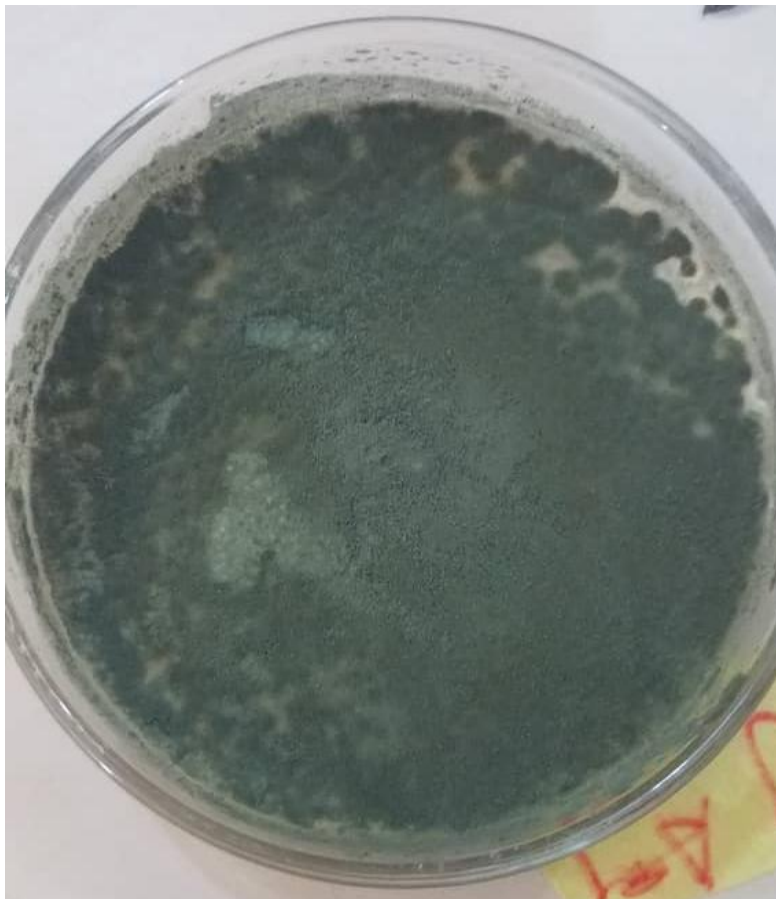


Plate 6. Pure Plate of *Aspergillus fumigatus* in Burkutu from Doruwa

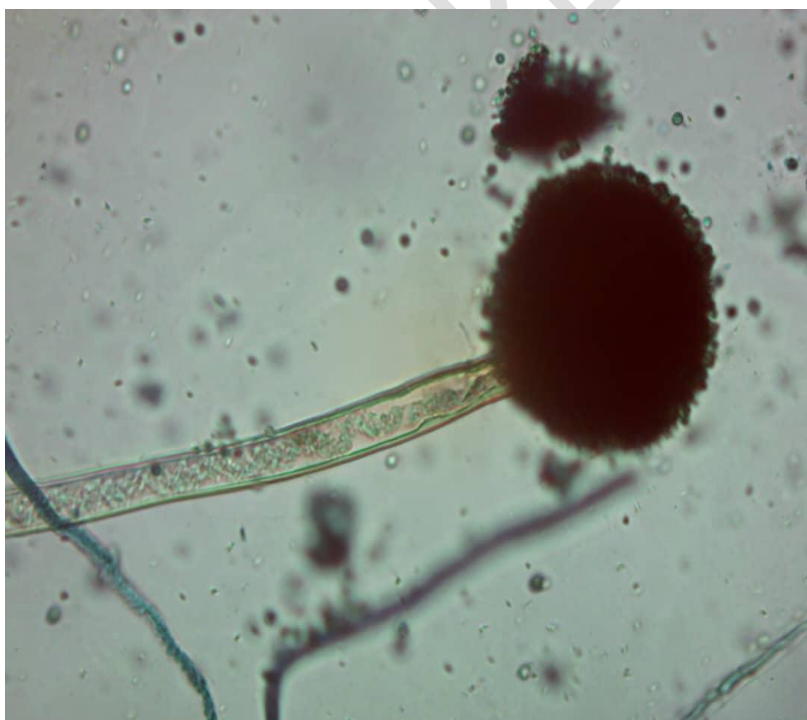


Plate 7. Microscopic View of *Aspergillus niger* (x40) in Sorghum from Gyel

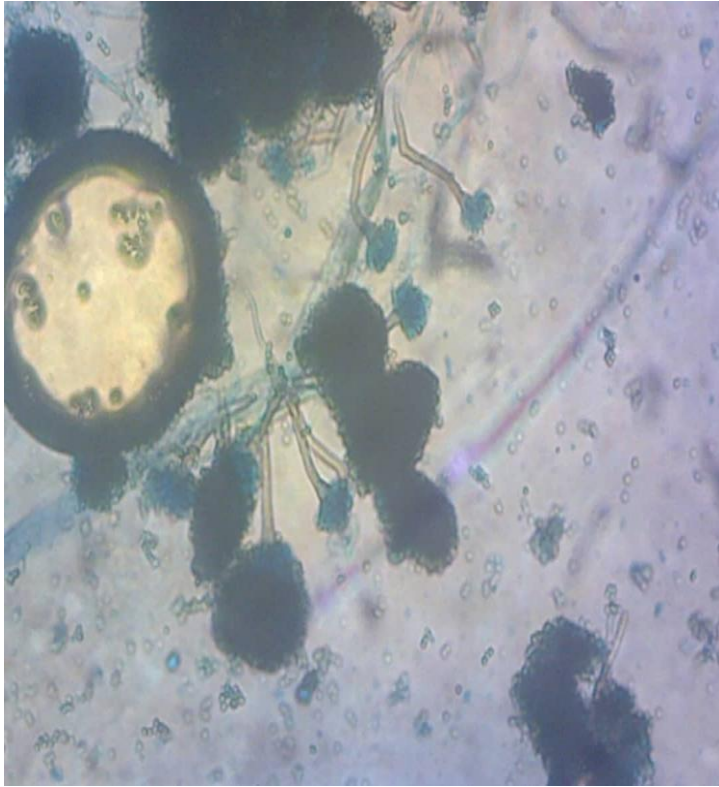


Plate 8. Microscopic View of *Aspergillus fumigatus* (x40) in Burkutu from Doruwa

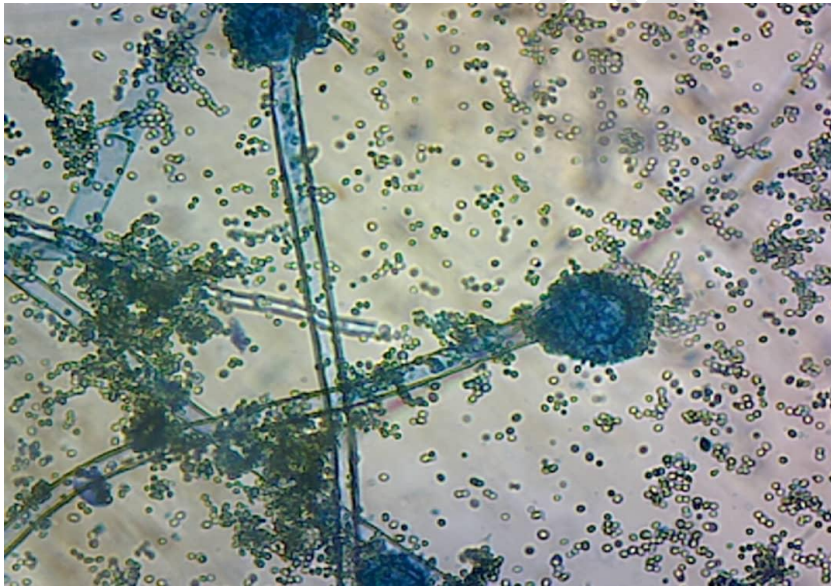


Plate 9. Microscopic View of *Aspergillus flavus* (x40) in Burkutu from Kugiya

4. DISCUSSION

The result of this study showed that both the grains and local beer (Burkutu) samples were contaminated, of which *Aspergillus* species were dominant, and it lead to the isolation and identification of *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus fumigatus*. The predominance of *Aspergillus* species in grains may be due to storage fungi which are widely spread in grains while for the Burkutu, it is probably due to poor sanitary conditions in and around the brewhouse, and possible contamination occurs with a small number of spores when taken for storage under high temperature and moisture after harvesting. *Aspergillus* species are of concern particularly due to their effect on human health. It grows at high moisture content and grows more rapidly than *Fusarium* and *Penicillium* species as these latter two will take a longer time to sporulate. It thus appears from previous works worldwide that out of the many genera of molds involved in food contamination, *Aspergillus* and *Penicillium* species ranked highest among others [28]. As reported by Adebajo and Idowu [29], surveys conducted worldwide showed that these two genera of fungi have consistently been prominent in food samples and animal feed in Egypt, Nigeria, and Argentina respectively.

From the result obtained in table 1, Sorghum had the highest number of colonies with a colony count of (1966.67cfu/g) in Kugiya market. The predominance of *Aspergillus* species in this market area could probably be because the germinated grains were dried on mats around the brewhouse which was unclean and has led to a high level of contamination in this area. In addition, the Sorghum could have been contaminated by the electrical grinding machine that was also used for other food items without prior proper cleaning of the machine. This observation conforms to the reports of Nestor W.E [30] who stated that the source of contamination might be due to humans concerned who had not followed adequate sanitation procedures, such as hand and utensils washing before preparation of the local beverage. Sorghum grains collected from Doruwa and Gyel as seen in table 1 had fewer *Aspergillus* species with a colony count of 1900cfu/g for sorghum from Gyel and 233.33 cfu/g for sorghum from Doruwa. From observations made, the population in these two areas are less compared to that of kugiya which had probably led to a good hygienic system in these area resulting in a low level of contamination.

However, storage fungi are most widely spread in grains probably because contamination occurs even with small quantities of spores in the grains when taken for storage after harvesting. These spores are possibly spread through infection that might have occurred in storage structures and under high temperature and moisture, a small amount of inoculum can increase rapidly in stored structures. The result of these findings agrees with the work of Mclean et al.,[31] who stated that fungal contamination is mostly affected by conditions favoring fungal growth which are numerous and not well understood but it is now obvious that hot humid conditions enhance the development of these organisms in food and feedstuffs. Lacey [32], reported that the spoilage of food and stored products of pre and post-harvest involves a wide range of fungi contamination which differs greatly in their ecological determination. Other factors such as higher insect infestation, mechanical damage, aeration, microbial load, and longer storage period when they occur in grain ecosystem, are likely to cause higher fungal contamination than in grains without these conditions in the ecosystem

Burkutu samples collected from Gyel had the highest number of colonies (1333.33cfu/ml) as seen in table two (2) compared to Kugiya and Doruwa. However, it was observed that at the brewhouse in Gyel, the pots used for the preservation of Burkutu were not often covered, and flies perched on the local beverage and may sometimes fall into it. The sanitary condition in and around the brewhouse was very poor. There was stagnant filthy water in the vicinity of the brewhouse due to a blocked drainage system, unclean calabashes were also used to serve the beverage in a poor hygienic environment, and sometimes the brewing water used is obtained from hawkers whose sources of water are unknown and usually not treated. There were fewer *Aspergillus* species in Burkutu samples from Kugiya and Doruwa . This may be because the brewhouses at Kugiya and Doruwa were kept clean and tidy but then there were few *Aspergillus* species probably because the utensils used for cooking and serving were not properly washed and sometimes rusted. It was also observed that in all the areas, Kugiya, Gyel, and Doruwa, the brewing water were mainly obtained from hawkers whose sources of water are unknown and usually not treated.

The large variety of fungi isolated from the current study is not surprising because of the crude Agricultural practices in Nigeria, thereby rendering many of these grains grossly damaged by primitive tools, coupled with poor drying and storage facilities. Also, many of these grains are spread in the open on the bare floor of the roadside, and on rooftops in an environment with a high level of humidity. *Aspergillus species* are of concern particularly due to their effect on human health. It grows at high moisture content and grows more rapidly than *Fusarium* and *Penicillium* species as these latter two will take a longer time to sporulate.

The total level of aflatoxin was detected using the Rida Quick Scan machine as presented in table 4. Sorghum grains collected from Kugiya had the highest total level of aflatoxin with 3.6 parts per billion followed by sorghum from Gyel with a total of 3.0 parts per billion while sorghum from Doruwa had the least with a total of 2.8(Ppb). The Burkutu sample from Gyel had the highest level of aflatoxin (3.1Ppb) followed by Burkutu samples from Doruwa which had (2.6Ppb) and lastly, the Burkutu samples from Kugiya had the least with a total of (2.4Ppb). It was observed that areas with the highest fungal occurrence had the highest total level of aflatoxins both for the grains and the product (Burkutu); the higher the presence of *Aspergillus species*, the higher the total level of aflatoxins and vice versa.

5. CONCLUSION

From the findings made from this work, three species of fungi of the *Aspergillus* genera were isolated and identified which include *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus fumigatus* from both Sorghum grains and Burkutu in Kugiya, Gyel, and Doruwa in Bukuru Metropolis of Jos South Local Government Area, Nigeria. The result of this study raises major public health concerns due to the total level of aflatoxin found in stored sorghum grains and its product (Burkutu) collected from Kugiya, Gyel, and Doruwa. In addition, the complications that go with liver cirrhosis and other diseases associated with the locally brewed beer (Burkutu) could be traced to the toxicity of this contaminant. The fermentation processes, the vessels used, and raw materials constitute a great source of contamination. In essence, utmost care should be taken to minimize or eliminate postharvest pathogens that produce aflatoxins in stored grains. Proper handling and processing of the various samples should be encouraged. Therefore, there is a need to train farmers, producers, and handlers of local alcoholic beverages on basic hygiene and measures for reducing the risk of contaminating their brews with pathogenic microorganisms such as *Aspergillus spp.*

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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