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Full Length Research Article

PROXIMATE VALUES, INCIDENCE OF AFLATOXIGENIC MOULDS AND CONTROL USING NON-TOXIGENIC FUNGI ISOLATED FROM SORGHUM OBTAINED FROM SOME MAJOR MARKETS WITHIN JOS METROPOLIS, NORTH-CENTRAL, NIGERIA

J.R. Wartu* and F.M. Musa

Department of Microbiology, Faculty of Life Sciences, College of Science, Computing and Engineering, Kaduna State University, Kaduna

*Corresponding Author Email Address: reuben.wartu@kasu.edu.ng +2348037348233

ABSTRACT
The development of aflatoxigenic fungi on grains is accountable for adverse health consequences in human and animals. The aim of this study was to determine proximate valves, the incidence of aflatoxigenic moulds and attempt control with nontoxigenic fungi isolated from sorghum. In this study, forty (40) samples of sorghum were purchased from some major markets and the proximate values determined using standard methods. They were evaluated for fungal contaminants using conventional mycological techniques. The aflatoxin production potentials of the fungal isolates were determined on Yeast Extract Sucrose Agar Medium supplemented with 0.3% cyclodextrin and 0.6% Sodium Desoxycholate (YCSD) and also on coconut agar medium. The inhibitory activity of non-toxigenic fungi on the aflatoxigenic *Aspergillus flavus* strain was then determined. While the incidence of aflatoxigenic moulds was also determined, the total aflatoxin from the sorghum was determined using ELISA. Red sorghum presented the least moisture 6.99±80 and highest was white sorghum with 8.27±0.25. Eight moulds and one yeast was isolated from these studies: *Saccharonycetes cerevisae, Penicillium chrysogenum*, *Rhizopus stolinifer*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus fumigatus*, *Fusarium sp. and* mucor spp. The moulds count range was 3.6×10^2 ± 32.7 - 2.4 $\times10^3$ ±70.9 CFU/g in white sorghum. Red sorghum presented maximum count of 8.8×10^2±59.9 - 1.2×10^3 ± 80.3 CFU/g. *Aspergillus flavus* had the incidence of 14/20 and 11/20 for red and white sorghum respectively. *Aspergillus parasiticus* had incidence of 11/20 in red sorghum and 13/20 in white sorghum. Incidence of aflatoxigenic *Aspergillus flavus* recorded 5/20 and 7/20 in red and white sorghum respectively. All the samples had different aflatoxin levels. The highest was 6.3 µg/kg and the least was 1.2 µg/kg. While all the grain samples were within the 10 µg/kg, fifteen percent (15.0%) of the total samples exceeded the 4.0 µg/kg EU recommended limit. There was no inhibition of aflatoxin production by any of the eight non-aflatoxigenic fungi after incubation with aflatoxigenic *Aspergillus flavus*. The occurrence of aflatoxigenic moulds and the detection of aflatoxins in this study emphasize the need for effort to eradicate their occurrence in foods. Awareness on grain handling and aflatoxins is hereby advocated.

Keywords: Sorghum, Aflatoxigenic Moulds, Aflatoxins.

INTRODUCTION

Fungi are found everywhere as plant and animal pathogens and spoilage agents of foods and feedstuffs (Makun *et al*., 2010; Theodora *et al*., 2022). While moulds have multicellular filaments, yeast usually exhibit single-celled growth, referred to as unicellular (Moore *et al*., 2011). They course spoilage of foods and feedstuffs. Certain species produce toxic secondary metabolites referred to mycotoxins such as; ochratoxins, fumonisins, trichothecenes, citrinin, and aflatoxin and patulin (Theodora *et al*., 2022). The infection of food and agricultural products leads to a reduction in yield and wholesomeness. These secondary metabolites, when consumed by animals and human being, could pose a public health threat because they are carcinogenic, immunotoxic, teratogenic and mutagenic and could lead to birth defects (Shephard, 2008; Kyei *et al.,* 2020). Exposure to high levels of aflatoxin could lead to neurological problems and even death. Some diseases in animals and humans can be caused by certain moulds and their products (Empting, 2009). Different moulds species may develop during grain storage in silos (Perrone *et al*., 2014). The grain sorghum is used for the production of bread and porridge, and provides a significant amount of energy and nutrition for humans. They could also be used as animal feed. In addition, sorghum is an important source of fat (Ndjeunga *et al*., 2022). Sorghum grain has immense potential for food security particularly in developing countries ravaged by droughts. However, it could be susceptible to infection by aflatoxigenic moulds. This study was therefore aimed at determining the proximate values of sorghum, incidence of aflatoxigenic moulds and control using non toxigenic fungi isolated from sorghum sold within Jos Metropolis, North-Central, Nigeria.

MATERIALS AND METHODS

Study Area

The study area was plateau state capital. The state has its capital in Jos. The indigenes are predominantly farmers. According to the 2006 census the former recorded a population as high as 3,406,531. Sorghum is one of the most popular grains cultivated in this area.

Sample collection

Samples of red and white sorghum were collected at random from some major markets in Plateau state capital within the metropolis. At least 200g x 40 samples of sorghum grains were bought from some market sellers and aseptically placed in low-density cellophane for proximate, mycological analysis and aflatoxin detection.

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Determination of Proximate Composition of Sorghum
Percentage Moisture Content
The percentage moisture content of samples was determined using the method adopted by Chaelae *et al. (*2002) and Adegoke *et al.,* (2010) as follows: Ten grams (10g) sorghum samples were weighed on pre-weighed moisture plates and placed in a thermostatic oven at 105°C temperature for one hour. This was weighed to constant weight. The moisture was calculated as seen below:

$\frac{W_2-W_3 * 100}{W_1}$ = A

100- A = percentage moisture

W1 = weight of the sample (10g)
W2 = weight of the undried sample (10g) + petri dish
W3 = weight of dried sample + petri dish

Percentage Ash Content
The percentage ash content was determined following the standard methods of the AOAC (1999). A porcelain crucible was washed and dried in an oven at 100°C to a constant weight, cooled in a desiccator, and weighed (W1). Two grams (2.0g) of finely ground wheat flour to an approximately 200µm size sample at each determination were placed in the crucible and reweighed (W2). It was first charred and transferred into a muffle furnace at 550°C for eight hours. The crucibles containing the ash were removed, cooled in a desiccator, and reweighed (W_3). The percentage of ash was calculated on a dry matter basis.
W_1 =weight of empty crucible
W_2=weight of crucible + sample
W_3=weight of crucible + ash
M_1 = percentage moisture content of the sample

$A = \frac{W_3-W_1}{W_2-W_1} \times 100$ = percentage ash

$B = \frac{A}{100- M_1} \times 100$ = percentage ash on dry matter basis

Percentage Crude Fat Content
The percentage fat content of the samples was determined using a Soxhlet apparatus according to AOAC. (1999). Firstly, a round bottom flask was cleaned, dried, and antibumping granules put into it and the flask weighed. An aliquot of 10g of the groundnut kernels was weighed and put into a clean extraction thimble. The extraction thimble containing the sample was placed inside the middle piece of the Soxhlet apparatus. The middle piece was fixed into a round bottom flask that was already filled to 2/3 of its volume with petroleum spirit with a boiling point (40 - 60°C) on the heating element of the Soxhlet apparatus. The cooling system was run, and the electric unit was put on. As the solvent boils, it evaporates, passes up to the condenser containing cooling water, the vaporized petroleum spirit condenses back into the middle piece containing the sample thimble. The middle piece filled up with the condensed petroleum spirit, it dissolved the sample fat and siphoned back into the flask with the dissolved fats. The process was left to run for six hours. The thimble was then removed, and the solvent was recovered. The fat at this stage was left in the flask. Finally, the flask was dried in a thermostatic oven at 90°C and reweighed.
The percentage fat content was computed on a dry matter basis as can be seen below:
W1 = weight of empty flask
W2 = weight of flask + fat
Q1 = quantity of sample
M1 = moisture of sample

$\frac{W_2-W_1}{Q_1} \times 100$ = A (percentage fat as it is)

$\frac{A}{100 - M_1} \times 100$ = percentage fats on dry matter basis

Crude Protein Content

Percentage Protein Content (Nitrogen Determination by MicroKjeldahl)
The method consists of three basic steps :
Digestion of the sample in sulphuric acid with a catalyst, which results in the conversion of nitrogen to ammonia. This is followed by distillation of the ammonia into a trapping solution; and quantification of the ammonia by titration with a standard solution (AOAC 1999).

Digestion
Accurately 10g of well-mixed grounded sample was placed in a Kjeldahl flask, and ten grams (10g) of potassium sulphate and 0.2g of copper sulphate were added together with an antibumping agent. The flask was heated in an inclined position for one hour. In the receiving flask, 20.0mL of standard sulphuric acid was added and diluted to 50.0ml. The flask was placed on a preheated burner and heated until white fumes cleared the bulb of the flask, swirled gently, and continued heating for 90 min. The flask was cooled, then 250 mL distilled water added.

Distillation
A titration flask was prepared by adding an appropriate volume of accurately measured acid standard solution to the amount of water so that the condenser tip is immersed. Two to three drops of the antifoam agent (tributyl citrate) were added to digestion flask to reduce foaming. Another 0.5 to 1.0 g alundum granules were added. Slowly down the side of the flask, sufficient 45% sodium hydroxide solution (approximately 80 mL) was added to make mixture strongly alkali. It was not mixed until after flask was connected to distillation apparatus otherwise ammonia could be lost. Immediately the flask was connected to distillation apparatus and to distill at about 7.5 boil rates (temperature set to bring 250 mL water at 25°C to boil in 7.5 min) until at least 150 mL distillate is collected in titrating flask into 50ml of boric acid. The digestion flask was removed and the titrating flask from the unit and the condenser tube rinsed with distilled water as the flask is being removed.

Titration
Finally, the distillate was titrated with 0.05M H_2SO_4. However, 1.0 mL of 0.05M H_2SO_4 is equivalent to 0.0014g of N_2. For the reagent blank, 1.0 mL of acid was pipetted and approximately 85 mL of water was added. Three 3 drops of methyl red indicator solution were added.
Total N_2 = $\frac{\text{Titre – blank titre x 0.0014 x 100}}{}$

Weight of sample (g)

Percentage Crude Fibre
Percentage crude fibre was determined according to AOAC (1999).

Percentage Carbohydrate Content
The percentage carbohydrate content was estimated as reported by Amoo and Ascore (2006). The percentages of moisture, ash, crude fats, crude fibre and crude protein were summed up and subtracted from 100, as illustrated below.
Percentage carbohydrate = 100 - (% moisture +%ash + %crude fat +% crude fibre + %crude protein).

Isolation and Identification of Moulds
Isolation of moulds
The moulds was cultured and enumerated as follows: Sterile potato dextrose agar was prepared according to the manufacturer's instruction and allowed to cool to roughly 40 - 45^{o}C. The sterile media was then supplemented with 100ppm of chloramphenicol before being poured into sterile petri dishes, and the mixture was allowed to set (Wartu *et al.,* 2019). A serial dilution of the first sample was made up to a fifth dilution. Then aliquots 0.1mL of the dilutions were made and inoculated on sterile PDA by spread plate methods using sterile bent glass rods. All the inoculated culture media plates were aerobically incubated at 27^{o}C for 7 days. The colonies were then counted using a colony counter and expressed as colony forming unit.
The dilution with plate counts of 10-300 colony-forming units (CFU) was used for further enumeration and isolation. The results were then expressed as colony-forming units per gram (CFU/g) of sample as follows.

$$\text{CFU/g} = \frac{\text{colony count x dilution factor}}{\text{Inoculum volume}}$$

Macroscopic Identification of moulds and yeast
Macroscopic identification was carried out using the methods described by Klich (2002). The microscopic characteristics were determined by observing the colony's color and texture. Colonies that showed dark-green colony morphology and rough conidia were tentatively identified as *Aspergillus parasiticus*, while moulds with morphological characteristics of yellowish-green colonies and smooth conidia was identified as *Aspergillus flavus.* A green, colony with broom-like spores was identified as *Penicillium* sp**,** while white colonies with pink colours and characteristic polyphialides, as well as long, slender nonphialides were identified as *Fusarium* spp etc.

Microscopic Identification of Moulds
Microscopic examination of cultures was carried out using Ellis (2006). Firstly, a drop of lactophenol cotton blue was dropped at the center of a microscope slide. The mould cultures were picked with a sterile pointed inoculation loop and placed inside the stain, and covered with a clean cover slip. The slide was mounted on the stage of the microscope and examined by compound light microscope for identification using 10 x and 40 x objectives. The microscopic features and characteristics were determined by examining the spore color, size, structure, conidiophore structure, and vesicle shape.

Screening for Aflatoxigenic Fungi
Polyphasic characterization of aflatoxigenic fungi
Aflatoxigenic moulds were cultured on Yeast Extract Sucrose Agar (YES) supplemented with 0.3% cyclodextrin and 0.6% Sodium Desoxycholate (YCSD) (Alborch *et al.,*2012). Sterile YCSD plates were prepared by adding 0.3% cyclodetrin to YES medium and sterilized at 121^{0}C for 15 min. The modified medium was allowed to cool to roughly 40 - 45^{o}C before adding 0.6% Sodium desocholate. The mixture was poured into sterile plates and allowed to set. A serial dilution of each sample was made, and an aliquot (1.0 mL) of the test samples was inoculated by spread methods using a sterile bent glass rod on the sterile YCSD agar. The inoculated culture media plates were aerobically incubated at 27^{o}C for 3-14 days. All colonies that form visible beige ring and blue fluorescent ring around the colonies under UV light at 365 nm and the incidence determined by the number of times it was detected per number of samples determined.

Aflatoxin Production Ability by aflatoxigenic Fungi and their Frequency of Occurrence from Sorghum
Aflatoxin production abilities of the moulds isolated were confirmed for their ability to produce aflatoxins through the methods of Adebayo-Tayo *et al.* (2015). Briefly, species of the fungi were inoculated at a central point on a 6 cm diameter Petri dish containing 15 mL of coconut agar medium and incubated for 7 days in the dark at 25°C. Using this CAM based medium test, the apparent production of an orange-yellow pigmentation of the mycelium, followed by the presence of blue fluorescence when the CAM plates were viewed under UV light (365 nm) indicates a positive result after 3, 5 and 7 days (Adebayo-Tayo *et al*., 2015). The incidence of aflatoxigenic moulds was determined as the number of times the fungus was positive divided by the total number of samples determined. All the isolates from this work, including *Sacharomycetes cerevisae* were subjected to the aflatoxin production ability test to obtain nontoxigenic fungi.

Determination of Inhibition of production of Aflatoxins by Toxigenic *Aspergillus flavus* by non-aflatoxigenic Fungi.
The moulds *Aspergillus flavus*, Mucor spp, *Penicillium chrysogenum, Rhizopus stolinifer, Aspergillus niger, Aspergillus parasiticus, Fusarium* spp*, Sacharomycetes cerevisae and Aspergillus fumigatus was* screened for aflatoxin producing ability. Those that did not produce aflatoxins were used as non-aflatoxigenic fungi. Some species of *Aspergillus flavus and Aspergillus parasiticus* that produce aflatoxins from the aflatoxin production ability test were used as aflatoxigenic moulds. Inhibition of the production of aflatoxin by aflatoxigenic moulds by any of the non-aflatoxigenic fungi was demonstrated as follows: Fresh sterile CAM plates were prepared. Then straight inoculation loop was sterilized and allowed to cool. It was then used to first pick each of the non aflatoxigenic fungi separately and inoculated them on the sterile CAM. Then the aflatoxigenic moulds were inoculated likewise at the same spot with each of the inoculated non aflatoxigenic fungi. The aflatoxigenic *Aspergillus flavus* and non-aflatoxigenic *A.flavus* was also inoculated separately as control on sterile CAM plates. All the plates were incubated at 27±2^{o}C for 10-14 days in the dark. The plates were viewed for orange-yellow pigmentation of the mycelium.

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Determination of Total Aflatoxin Levels of Sorghum from North-Central Capital Cities for Human Consumption

Total aflatoxin extraction

The total aflatoxin was determined according to Ridascreen™ aflatoxin total manufacturers` instruction as follows, ten grams (10g) of grain samples were ground to powder and aliquot two grams (2g) were weighed and poured into an extraction bottle with cover. Then ten millilitres (10mL) of 70% methanol was added and mixed for 10minute. The sample mixture was filtered using Whatman No.1 filter paper, and 100µl of the filtrate was diluted using 600µl of distilled water.

Total Aflatoxin Quantification using ELISA

The total aflatoxin was detected and estimated by measuring 50µl of enzyme-antibody conjugate using micro pipette and dispense into each well placed on micro well plate.

Another aliquot of 50µl of each of the sample (diluted filtrate) and the standard (0, 0.05,0.15,0.45,1.35 and 4.05µg/l) were added into appropriate test wells, then 50µl of antibody was dispensed into each test well, the plate was shaken gently to mix the content and allowed to incubate at room temperature for 30 minutes. The contents of the wells were poured off and washed with distilled water. The process was repeated five times. Then absorbent paper was placed on the flat surface of the test wells and tapped to drain the wash solution.

Then (100µl) of the substrate was pipetted and dispensed into test well. Gently, the plate was shaken before incubation at room temperature for 10 minute. Aliquot of 100µl of stop solution was pipetted and dispensed into each test well and shaken gently..

The optical density was read at 450 nm using imark™ microplate reader. A standard curve of absorbance percentage against log concentration of the standards was plotted and the concentrations of each sample calculated using an AUTO calculation spread sheet.

Data Analysis

The experiment was conducted and the results was expressed as mean±SD. Data was analysed using one-way analysis of variance (ANOVA) to determine the statistical significance at $p \leq 0.05$ using statistical package for social science (SPSS) version 25.The means were separated using Duncan multiple range test.

RESULTS AND DISCUSSION

The mean proximate values of red and white sorghum sold within Jos metropolis, North-central Nigeria, were studied, and the results are hereby presented in Table 1. The percentage proximate values of sorghum were variable. The percentage moisture for the two types of grains showed no significant difference between the moisture levels. While red sorghum has higher protein (8.96±0.22%), crude fibre (3.53±0.08%), ash (3.59±0.17%) and fats (6.85±0.05%), carbohydrate was higher in white sorghum (74.37±0.40%).. The fluctuating values determined could be due to major market cross-mixing of the grains with others, differences in hybrids, and unwarranted dilutions of the grains during transport at market levels. In addition, differences in unstandardized storage facilities could be one of the reasons for the differences. Open market selling explains the difference due to exposure to environmental factors (Landers and Davis, 1986; Hell and Mutegi, 2011; Akelo *et al*., 2021). The results indicated that the grain sorghum could serve as a good crude medium for proliferation of moulds and when conditions are favourable, they could produce aflatoxins because of the high nutrient value in the grains. While table 2 presents moulds isolated from sorghum sold within major markets across Jos metropolis, North-Central Nigeria, table 3 presents incidence of moulds isolated from sorghum. Eight moulds species and one yeast species were isolated from the sorghum samples; *Aspergillus flavus*, Mucor spp, *Penicillium chrysogenum, Rhizopus stolinifer, Aspergillus niger, Aspergillus parasiticus, Fusarium* spp*, Aspergillus fumigatus and Sacharomycetes cerevisae.*. The incidence of moulds species isolated from sorghum for human consumption within Jos metropolis, North-Centra capital city were as follows: *Aspergillus flavus* has the highest incidence of 14/20 and 11/20 in red and white sorghum, respectively, followed by *Aspergillus parasiticus* which has an incidence of 11/20 and 13/20 in red and white sorghum respectively. Each of the moulds *Rhizopus stolinifer* and *Fusarium* spp had incidence 1/20 in red sorghum. Some of these moulds has been documented to have the ability to produce aflatoxins (Varga *et al*., 2011; Mathew *et al*., 2017) especially the *Aspergillus* species. The isolation of these moulds from this work therefore portrays danger to the consumers of sorghum, either directly or through the products of sorghum. This infection of the grains with these moulds could be due to the market selling points where the grains are infected through dirty and dusty gangway. Some of the grains are heaped on dirty tampolins for sell while the seller stands by the side to measure out to customers. The harvesting pattern is also worrisome because the pattern is not mechanized. Mats or flat mountain surfaces are usually used on which the sorghum is thrashed with sticks before separating the chaff through natural air. Since the moulds are ubiquitous, they are bound to contaminate such open products, hence the infection from this study. This work reports highest incidence by *Aspergillus flavus* than any other fungi. Al-Wadai *et al*. (2013) also reported dominance of *Aspergillus* species. The work of Al-Wadai *et al*. (2013) also observed this in wheat. However later, Al-Kahtani (2014) also reported the dominance of *Alternaria* spp (68.96%) over *Aspergillus* sp in stored wheat from Saudi Arabia. *Aspergillus flavus* was reported to have a higher percentage frequency when compared with other species of fungi isolated in wheat than from other food and feeds (Rashid *et al.,* 2008).. However, this is in contrast with the research of Rodriguez (2009) who reported the dominance of *Aspergillus parasiticus*. These differences in reports could be due to geographical location (Ritter *et al*., 2011), environmental conditions in which the moulds were isolated (Falade *et al*., 2022). The present detection of moulds from this work could directly affect the wholesomeness of the sorghum and its products. It is therefore possible for the aflatoxigenic strains in this study to produce secondary metabolites such as aflatoxins when conditions are adequate. The aflatoxigenic moulds and aflatoxins in food has been reported (Shitu *et al*., 2021) to have aflatoxin production profiles. *Aspergillus flavus* produces aflatoxin B_1 while *Aspergillus parasiticus* produces aflatoxin B_1, B_2, G_1, and G_2. Aflatoxin B_1 in particular has been classified as a group 1 carcinogen (1ARC, 2002). *Fusarium* species were also reported in wheat. This moulds produces deoxynivalenol (DON), trichothecenes and fumonisins (Camelia & Lucian, 2014). The presence of these toxins in grains poses a threat to human health (Al-Kahtani *et al.,* 2014; Ayo *et al*., 2018). Presence of *Rhizopus* sp in grains could

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indicate spoilage and or due to an increase in pH.

Table 4 presents the mean mould counts of Sorghum for human consumption collected from major markets within Jos metropolis, North-Central Nigeria. The moulds count was highest in Farin Gada and Taminus (1.2 x 10^3± 80.3 CFU/g and 1.1 x 10^3±65.1 CFU/g) in red sorghum. The highest count of white sorghum was from Gada biyu (2.4×10^3±70.9) and the least from Bukuru (3.6×10^2 ± 32.7).

The incidence of aflatoxigenic moulds isolated from sorghum sold for human consumption within Jos metropolis is presented in table 5. Only two of the fungi were positive for aflatoxin production. *Aspergillus flavus* presented an incidence of (5/20) in red sorghum and (7/20) in white sorghum. *Aspergillus parasiticus* presented (4/20) only in white sorghum. The presence of this aflatoxigenic species in this work connotes risks of aflatoxin contamination. The safety of sorghum and its products in this case may be threatened and thus the need for strict surveillance and monitoring of grains.

Other research works have been reported on the detection of aflatoxigenic strains of *Aspergillus flavus* and *Aspergillus parasiticus* and the production of aflatoxins in grains (Bergamini *et al*., 2010). Also aflatoxigenic *Aspergillu*s species, *A. flavus* recorded the highest incidence of 68% in fonio millet and 86% in sesame kernels (Ezikel *et al*.,2014). Furthermore, Makun *et al*.,(2010) reported that 68.57% of the fungi tested were toxigenic and were mostly isolates of *Aspergillus, Fusarium, Penicillium, Rhizopus* and *Mucor*

All the aflatoxigenic moulds isolated from this work presented beige rings when the YES agar was supplemented with 0.3% cyclodetrin and 0.6% sodium desocholate. While the control with aflatoxigenic moulds presented beige ring and orange-yellow pigmentation of the mycelium, followed by the presence of blue fluorescence on CAM plates under UV light (365 nm), the non aflatoxigenic control did not. Biological control for Aflatoxin mitigation attempt was also made by Bandyopadhyay *et al*. (2019).The no sign of inhibitory activity by the seven nonaflatoxigenic fungi in this research could be due to lack of competition effects against the toxigenic moulds for growth-limiting factors and or hydrolytic enzymes (Lyagin and Efremenko, 2019)

The minimum level of aflatoxins in both red and white sorghum was 1.2 µg/kg but it had its highest (6.3 µg/kg) in white sorghum and likewise 4.3 µg/kg in red sorghum (Table 6). All the samples had different aflatoxin levels. Fifteen percent (15.0%) of the total samples exceeded the EU 4.0µg/kg recommended limit. Makun *et al*.,(2014) reported that five of the rice samples contained aflatoxin B 1 (37.26-113.2 µg/kg and also in sesame samples with AFB1 (14.71-140.9 µg/kg), with one sample containing (2.61 µg/kg) of aflatoxin G1. Shittu *et al*. (2021) reported a high concentration of aflatoxin (11.04 µg/kg) in millet and a low concentration in sorghum (1.07 µg/kg). This 1.07 % of aflatoxin registered is lower than the result obtained in this study. Mean aflatoxin levels in maize was reported to be high, 128, 517, and 659 µg/kg in Burkina Faso, Mali, and Niger, respectively (Falade *et al*., 2022). These differences in aflatoxin level could be due to different storage structures and environmental conditions to which the grains are exposed to (Ezekiel *et al*., 2012; Jianglin *et al*., 2022). Some of those store owners may not have the knowledge of aflatoxins just like the report where care givers of young children have poor knowledge about mycotoxins contamination (Lmeriai *et al*., 2021).

Table 1: Mean ± SD Proximate values of Sorghum Sold for Human Consumption within Jos metropolis, North-Central Nigeria

Parameters	Sorghum	
	Red	White
Moisture	6.99±0.80^b	8.27±0.25^c
Ash	3.59±0.17^a	3.33±0.25^a
Protein	8.96±0.22^c	8.60±0.10^c
Crude fibre	3.53±0.08^a	3.19±0.17^a
Fat	6.85±0.05^b	5.43±0.21^b
CHO	73.60±0.18^d	74.37±0.40^d

Values are means ± SD and values with different superscript along the row differ significantly. ($P \leq 0.05$)

Table 2: Moulds Isolated from Sorghum Sold in Some Markets Within Jos Metropolis, North-Central Nigeria

S/N	Moulds Species
1	Mucor
2	*Penicillium chrysogenum*
3	*Rhizopus stolinifer*
4	*Aspergillus niger*
5	*Aspergillus flavus*
6	*Aspergillus parasiticus*
7	*Fusarium spp*
8	*Sacharomycetes cerevisae*
9	*Aspergillus fumigatus*

Table 3: Incidence of Moulds species Isolated from Sorghum for Human Consumption Within Jos Metropolis, North-Central Nigeria

Fungi	Sorghum	
	Red	White
Aspergillus flavus	14/20	11/20
Aspergillus parasiticus	11/20	13/20
Aspergillus niger	2/20	3/20
Rhizopus stolinifer	1/20	1/20
Penicillium chrysogenum	2/20	2/20
Fusarium spp	1/20	1/20
Mucor spp	2/20	2/20
Sacharomycetes cerevisae	4/20	5/20
Aspergillus fumigatus	3/20	4/20

Results are presented as number of fungal isolates per total number of samples analyzed

Table 4: Mean Mould Counts (CFU/g) of Sorghum for human Consumption Collected from Some Markets Within Jos Metropolis, North-Central Nigeria (N=40)

Sample Collection Location	Jos	
	Red Sorghum	**White Sorghum**
Bukuru	$8.8\times10^{2}\pm59.9^{a}$	$3.6\times10^{2}\pm32.7^{a}$
Gada Biyu	$1.0\times10^{3}\pm70.2^{a}$	$2.4\times10^{3}\pm70.9^{b}$
Farin Gada	$1.2\times10^{3}\pm80.3^{a}$	$2.2\times10^{3}\pm94.6^{b}$
Taminus	$1.1\times10^{3}\pm65.1^{a}$	$1.8\times10^{3}\pm75.9^{b}$
p-value	0.921	0.000

Values are means of Five different Samples ± SD and values with different superscript along the row differ significantly ($P\leq0.05$)

Table 5: Incidence of Aflatoxigenic Moulds Isolated from Sorghum sold for Human Consumption Within Jos Metropolis

	Sorghum	
	Red	**White**
Aspergillus flavus	5/20	7/20
Aspergillus niger	0/20	0/20
Rhizopus stolinife	0/20	0/20
Penicillium chrysogenum	0/20	0/20
Fusarium spp	0/20	0/20
Sacharomycetes cerevisae	0/20	0/20
Aspergillus parasiticus	0/20	4/20
Aspergillusfumigatus	0/20	0/20
Mucor spp	0/20	0/20

Key: Results are presented as number of fungal isolates per total number of samples analyzed

Table 6: Total Aflatoxin Levels (µg/kg) in Sorghum Collected from Some Markets, within Jos Metropolis, North-Central Nigeria.

S/N	Sorghum	
	Red	**White**
1	3.2	2.3
2	2.4	1.6
3	2.2	2.4
4	1.2	2.2
5	2.2	1.6
6	1.6	2.2
7	2.1	1.2
8	1.9	6.3
9	4.3	3.5
10	3.1	4.6
Mean	2.420±0.89666	2.490±1.58286
Range	1.2 – 4.3	1.2 – 6.3
Minimum	1.2	1.2
Maximum	4.3	6.3

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Conflict of Interests
The authors declare that there is no conflict of interest.

Conclusion
Proximate values were determined, and the values varied. Red sorghum had higher protein, crude fibre, ash and fats, but has lower carbohydrate than white sorghum. Eight moulds and one yeast were isolated from this research. *Aspergillus flavus* had the highest incidence in red and white sorghum, followed by *Aspergillus parasiticus*in red sorghum.
The moulds count was highest in Farin Gada and Taminus in red sorghum, while the highest count of white sorghum was from Gada biyu and the least was from Bukuru.
Only *Aspergillus flavus* and *Aspergillus parasiticus* were positive for aflatoxin production
All the samples had different aflatoxin levels and only fifteen percent (15.0%) of the total samples exceeded the 4 µg/kg EU recommended limit. However, all the samples are within 10 µg/kg

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