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Fungi Species Isolated From Seeds of a Selected Landrace of Sorghum (*Sorghum bicolor* L. Moench) Sold in Sabon Gari Market, Kaduna State, Nigeria

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ABSTRACT

Fungi are ubiquitous and infect cereals particularly during storage resulting in economic loss. This study was conducted to isolate and characterize the fungi associated with varieties of Sorghum seeds (*Sorghum bicolor* (L) Moench). A local landrace "Kaura" was obtained from Sabon Gari market and stored for five months. The fungi were isolated using standard microbiological techniques. Five seeds from the landrace were picked randomly, washed and sterilized using 1% sodium hypochlorite to remove surface contaminants. The seeds were washed thrice with distilled water to remove any residual sodium hypochlorite and placed onto freshly prepared Potato Dextrose Agar Petri plates with three replications each. The cultured plates were incubated at 25-27°C for seven days after which the colonies that appeared were counted and recorded. Each colony was subsequently subcultured to obtain pure isolates. Identification was based on macroscopic, microscopic features and molecular characterization at Centre for Agriculture and Biological Institute Eigham, United Kingdom. Seven different fungi found to be associated with the selected Sorghum and frequency of isolation were; *Aspergillus flavus* (25%), *Aspergillus niger* (25%), *Fusarium oxysporum* (17%), *Fusarium solani* (17%) *Curvularia lunata* (08%), *Penicillium chrysogenum* (05%) and *Didymaria* (03%). Some of these fungi are known potential pathogens and mycotoxin producers. There is therefore the need to devise good storage facilities to prolong the shelf life of sorghum seeds after harvest. Physical damage to seeds should also be avoided since they serve as major points of entry for pathogens. These will help to safeguard the public health.

Keywords: Fungi, *Sorghum bicolor*, post harvest, storage, pathogens, molecular characterization.

Introduction

Sorghum (*Sorghum bicolor* (L) Moench) is the fifth most important world cereal after rice, wheat, corn and barley and an important native cereal in Africa (Muhammad *et al*., 2022). The largest world's sorghum producers are the USA with total annual grain production of 8.7 million tons from 2.0 million hectares, Nigeria (6.9 million tons and 5.4 million hectares), Ethiopia (5.3 million tons and 1.9 million hectares), and Sudan (3.7 million tons in 6.8 million hectares) (FAO, 2019; Muhammad *et al*., 2022). The four leading sorghum producers in Africa are Nigeria, Ethiopia, Burkina Faso and Niger (Acquaah, 2012; Muhammad *et al*., 2022). Sorghum is the largest staple cereal crop accounting for 50% of the total output and occupying about 45% of the total land area devoted to cereal crops production in Nigeria (Ajeigbe *et al*., 2018; FAO, 2019; Mrema *et al*., 2020).

In Nigeria, Sorghum is grown in different ecological zones (Sudan savannah, Northern Guinea savannah and Southern Guinea savannah) due to its adaptability to a wide range of environmental conditions including particularly drought (Ajeigbe *et al*., 2018). The sorghum productivity in the country is 1.23 t ha−1, which is relatively low compared with the world average of 1.45 t ha−1 and the USA with 4.58 t ha−1 (FAO, 2019).

Sorghum is a principal source of energy, protein, vitamins and minerals to the poorest people of the semi – arid tropics. The crop is dried stored and later used to prepare stiff porridge, thin porridge or fried dumpling (FAOSTAT, 2015; Mofokeng *et al*., 2017). It is also used in brewing local beer (Awada, 2016; Mafokeng *et al*., 2017; Catherine *et al*., 2020).). The leaves provide fodder for farm animals and the stalk are used in fencing, roofing, weaving baskets and mats and also as fuel wood (Ogbonna, 2011).

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Sorghum grains are used industrially in the manufacture of items such as wax, starch, syrup, alcoholic and nonalcoholic beverages, dextrose agar, edible oils and gluten feed (Kowieska *et al*., 2011; FAO, 2012). In addition, it is used to manufacture gypsum lath, paper, cloth sizing and adhesives (Delserone, 2007; Magdof and Evans, 2009).

The major biotic constraints of sorghum production are insects, parasitic weeds, birds and diseases (Buntin, 2012). Fungi are important sorghum disease causing organisms (Nadia *et al*., 2009; Stapleton *et al*., 2010). Moulds are ubiquitously distributed in nature and their spores can be found in the atmosphere even at high altitudes, carried and disseminated by wind and air currents, as well as can be spread by insects, rodents, and other animals. Food products, being organic substances and containing essential nutrient, are very suitable substrate for the mould growth (Machio, 2016). Due to their powerful arsenal of hydrolytic enzyme, moulds can cause a high degree of deterioration when present in/on foods/feeds and can be responsible for considerable economic losses (Marie *et al*., 2016).

Besides the possible food decay caused by moulds and ultimate changes in nutritional and organoleptical characters, the moldiness in food stuffs is toxicologically significant since the mould species growing on such products are potentially mycotoxigenic (Gupta *et al*., 2010; Kange *et al*., 2015). Mycotoxins are toxic metabolites produced by filamentous fungi that have been detected in several food commodities (Grenier and Oswald, 2011).

The consumption of mouldy products can cause human or animal mycotoxicoses, and more importantly, some mycotoxins are potent carcinogens (Gupta *et al*., 2010; Marie *et al*., 2016; Abdel *et al*., 2017). Various reports have shown yield losses both quantitatively and qualitatively by fungi of up to 67% (Gupta *et al*., 2010; Mohammed *et al*., 2015). Seed borne inoculums of fungi, have severe implication for yield, seed production, trade, human nutrition and germplasm (Jayashree and Wesely, 2019).

The study aimed to isolate and identify fungal species associated with Sorghum seeds (*Sorghum bicolor* (L) Moench). Landrace, from Sabon Gari market located in Kaduna State, Nigeria, so as to ascertain the health risk associated with infested Sorghum seeds.

Materials and Methods

Study Area

The study was carried out in the Department of Crop protection, Ahmadu Bello University, Zaria located in Kaduna State between latitude $11^0$09N, long 7^0 42E at an altitude of 686 meters above sea level.

Source of Materials

A local landrace ‘Kaura’ was obtained from Sabon Gari market located in Kaduna State between latitude $11^0$723’32” N, long 7043’55.79” E at an altitude of 673 meters above sea level in a sterile polyethene bag which was then transported to the Department of Crop protection, Ahmadu Bello University, Zaria for mycological analysis. The Sorghum seeds were identified as Sorghum (*Sorghum bicolor* (L) Moench) at the herbarium of the Department of Botany, Ahmadu Bello University, Zaria.

Isolation and Identification of Fungi from Sorghum Seeds

Five seeds of the selected sorghum landrace were rinsed in distilled water separately, surface sterilized with 1% sodium hypochlorite solution (NaOCl)) for 3 minutes (Sallam *et al*., 2012) and rinsed with three changes of distilled water to remove residue of NaOCl. to remove surface contaminants. Potato dextrose agar (PDA) was used for culturing the fungi. This was prepared according to manufactures recommendation by suspending 39g of the medium (Potato extract 4g, Dextrose 20g and Agar 15g) in 1 liter of distilled water. The solution was placed in a water bath with frequent agitation and allowed to boil for one minute to completely dissolve the medium. Chloramphenicol (500mg) was added to inhibit bacterial growth. The medium was autoclaved at 121°C for 15 minutes. Five seeds each were picked randomly and placed onto 20 ml potato dextrose agar (PDA) in Petri dishes which was incubated at room temperature (25-27°C) for 5 days (Lagnika *et al*., 2012) and observed regularly for any fungal growth. Each plate containing the medium and the sorghum landrace was replicated three times. After the incubation period, the numbers of colonies that appeared were counted, recorded and each sub cultured into a freshly prepared PDA to obtain pure cultures. Identification of the fungi was done using macroscopic and microscopic features and subsequently sent to Center for Agriculture and Biological Institute (CABI), Eigham, United Kingdom for confirmation.

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Statistical Analysis

Percentage abundance of fungi was reported in a pie chart. The percentage abundance was calculated using the formular of Abu *et al*. (2015).

$$\% \text{ Abundance of Fungi} = \frac{\text{Number of times a fungus is encountered}}{\text{Total number of fungi isolated}} \text{x} 100\%$$

Results

Fungi Isolated and Identified from *Sorghum bicolor* landrace yielded seven different associated fungi namely *Aspergillus flavus*, *Aspergillus niger*, *Curvularia lunata*, *Didymaria* sp *Fusarium oxysporum*, *Fusarium solani*, and *Penicillium chrysogenum*. These were however identified based on macroscopic and microscopic features with further characterization to species level.

Molecular Characterization revealed the following in relation to the fungal isolates.

Aspergillus flavus: DNA was extracted from this organism but failed to amplify. The sample could not therefore be identified by molecular methods. Identification was made by macroscopic and microscopic analysis of subcultures prepared on diagnostic media. The isolate was identified as *Aspergillus flavus*. Morphology of colonies and sporulating material matched species descriptions provided in published taxonomic keys for the identification of common *Aspergillus* Species (Klich, 2002).

Aspergillus niger: The organism was characterized by ITS rDNA sequence analysis using the FASTA algorithm with the Fungus database from EBI. Top matches of >99% were obtained to sequences assigned to members of *Aspergilus niger*, including 100% to *Aspergilus* sp. strain NRRL 5537 [which belongs to the *Aspergillus* species complex (EMBL/GenBank accession number GQ505677)]

Curvularia lunata: The organism was characterized by ITS rDNA sequence analysis using the FASTA algorithm with the fungus database from EBI. The ITS sequence obtained from this sample showed top matches at 99% identity to sequences of *Curvularia* lunata. These included sequences published in peer-reviewed literature e.g sequence KP131956 published in Irinyi *et al*. (2015).

Didymaria sp. The organism was characterised by ITS rDNA sequence analysis using the FASTA algorithm with the Fungus database from EBI. The ITS sequence obtained from this sample showed top matches at 100% identity to members of the genus *Didymaria* mainly to sequences of *Didymaria* including sequence (AF046022) from the type strain of this species (CBS 249.92) published in Heinrichs *et al.* (2012).

Fusarium oxysporum: The organism was characterized by ITS rDNA sequence analysis using the FASTA algorithm with the Fungus database from EBI. Top matches of >99% were obtained to sequences assigned to *Fusarium oxysporum*, including 100% to *Fusarium* sp. FIESC strain CBS 131787 [which belongs to the *Fusarium* species complex (EMBL/GenBank accession number JX162391)].

Fusarium solani: The fungus was characterized by ITS rDNA and partial translation elongation factor 1-alpha (TEF) gene sequence analysis using the FASTA algorithm with the fungus database from EBI. It was confirmed that 99% matches were obtained to authentic type strain sequences of taxa belonging to the family *Fusarium solani* species aggregate, including 99.8% to the TEF sequence of *Fusarium solani* strain.

Penicillium chrysogenum: This sample was identified by ITS and partial calmodulin gene sequence analysis using the FASTA algorithm with the Fungus database from EBI and the BLAST algorithm with the NCBI database, limited to sequences from type strains. The ITS sequence obtained from this sample showed 98-100% identity to members of *Penicillium* section Nidulantes including sequence AY373868. from the type strain of *Penicillium chrysogenum* (NRRL 250).

Identification of this species was confirmed by results of partial calmodulin gene sequencing which produced top matches of >99% identity to multiple sequences of *Penicillium chrysogenum* including 100% identity to the calmodulin marker sequence EF652362.

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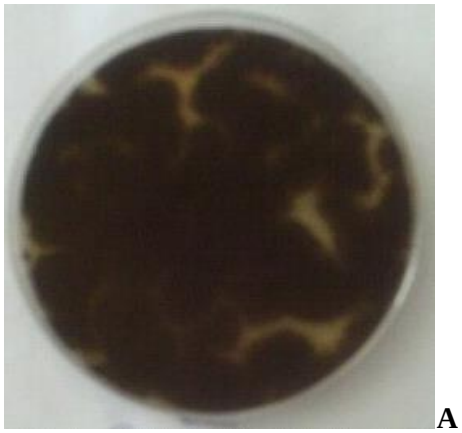
A

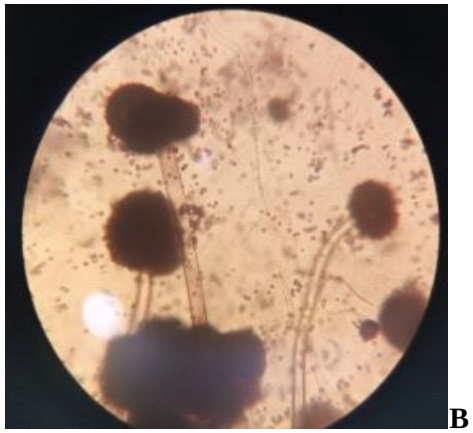
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Plate I: Pure culture of *Aspergillus niger*

A = Macroscopic feature **B = Microscopic feature (mag. x40)**

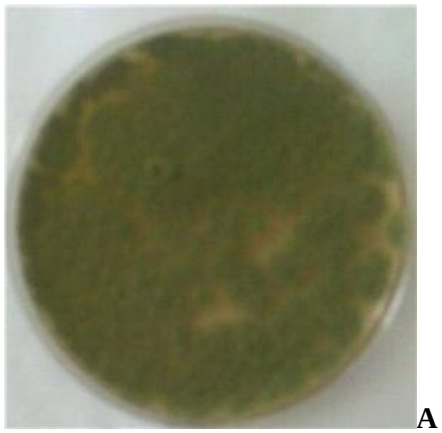
A


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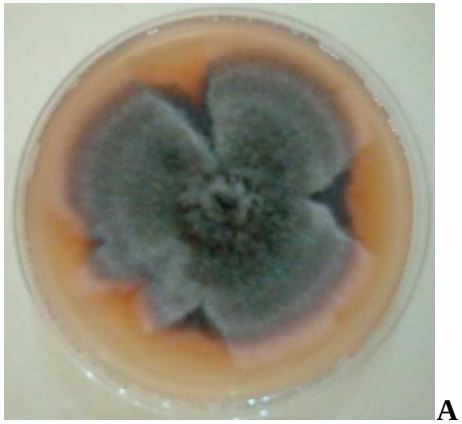
Plate II: Pure culture of *Aspergillus flavus*

A = Macroscopic features **B = Microscopic feature (mag. x40)**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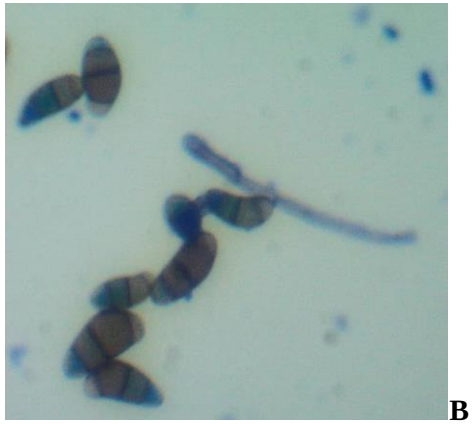
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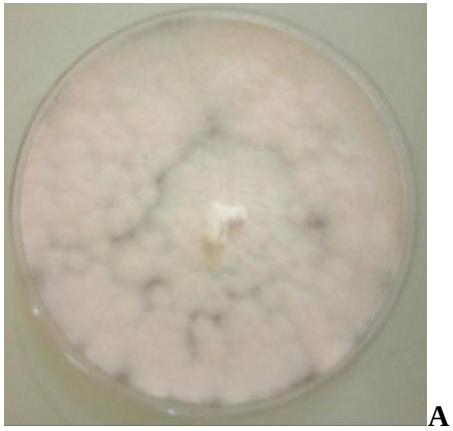
Plate III: Pure culture of *Curvularia lunata*

A = Macroscopic feature **B = Microscopic feature (mag. x40)**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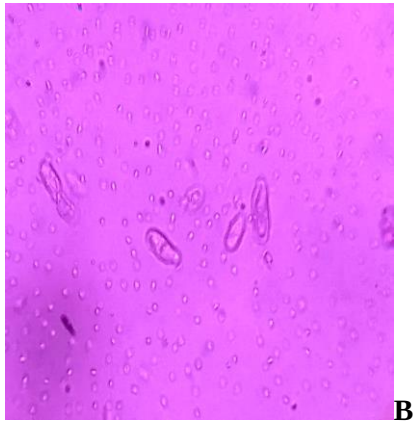
B

Plate IV: Pure culture of *Didymaria* sp.

A = Macroscopic feature **B = Microscopic feature (mag. x40)**

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A

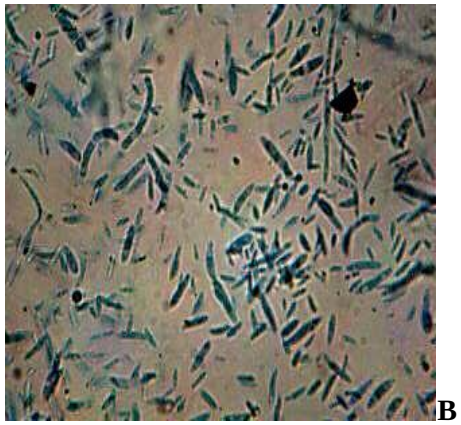

B

Plate V: Pure culture of *Fusarium solani*

A= Macroscopic features **B= Microscopic features (mag. x40)**


A

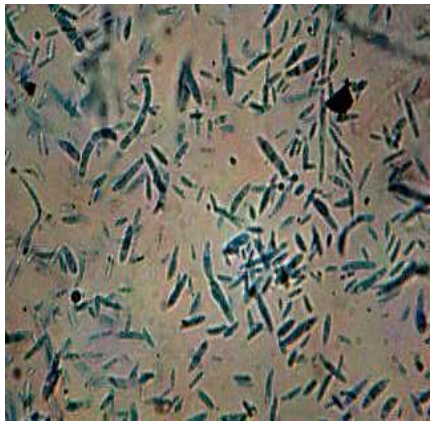
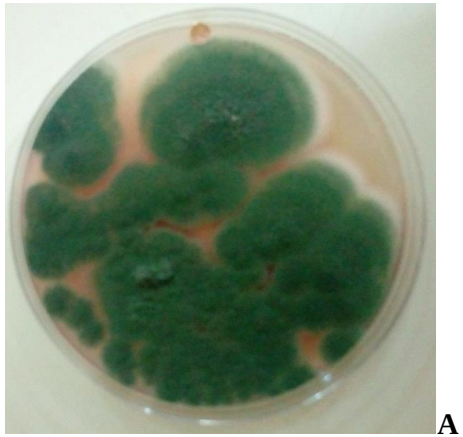

B

Plate VI: Pure culture of *Fusarium oxysporum*

A= Macroscopic features **B= Microscopic feature (mag. x40)**


A

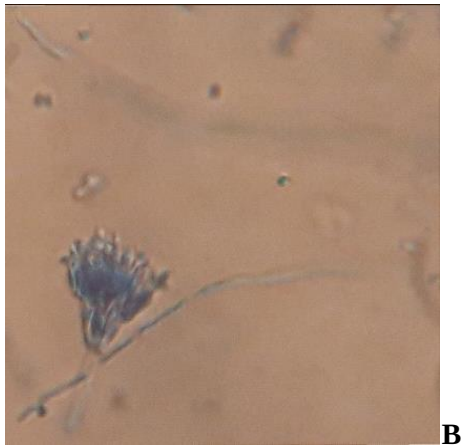

B

Plate VII: Pure culture of *Penicillium chrysogenum*

A= Macroscopic feature **B= Microscopic feature (mag. x40)**

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Percentage Abundance of Fungi Associated with Seeds of Kaura Landrace of *Sorghum*

The percentage abundance of fungi from the seeds of Kaura landrace of sorghum is presented in Figure 1. A total of 35 colonies of fungi developed on isolation from the seeds of Kaura land race of sorghum.

Aspergillus flavus and *Aspergillus niger* had 25% abundance, this was followed by *Fusarium oxysporum* and *Fusarium solani* which recorded 17% abundance each. However, *Curvularia lunata* and *Penicillium chrysogenum* recorded 8% and 5% abundance respectively whereas the least abundance of 3% was recorded by *Didymaria* sp (Figure 1).

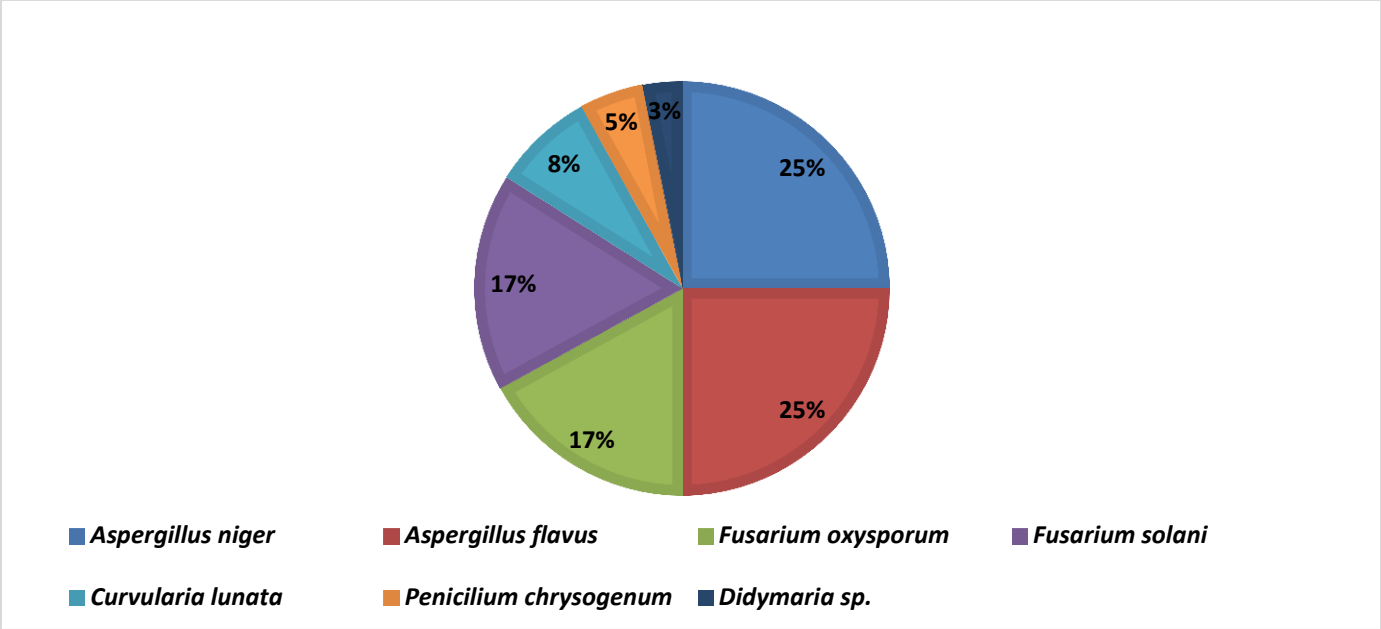

Fig.1: Percentage abundance of fungi from the seeds of Kaura landrace of sorghum

Discussion

Microorganisms especially fungi are known to be the major cause of market and field losses of crops (Jayashree and Wesely, 2019). Different fungal genera such as *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum*, *Fusarium solani*, *Curvularia lunata*, *Didymaria sp.* and *penicillium chrysogenum* were found to contaminate the *Sorghum bicolor* landrace.

These fungi might have colonized the grains during production in the field, transportation or storage (Sultan *et al*., 2022), and many are facultative parasites or saprophytic fungi, as they contribute to pre- and post-harvest deterioration of grains (Pushpavathi *et al*., 2017). All the isolated fungi when compared with already described species by Barnett and Hunter (1972), Larone (2002), Klich (2002) and Samson *et al*. (2004) were found to be similar.

The predominance of *Aspergillus* species from farmers' storage facilities observed in this study conforms to reports of (Abdulsalaam and Shenge 2011; Ismail *et al*. 2012; Kange *et al*., 2015; Olotu *et al*., 2022). The high occurrence of *Aspergillus* species indicates that *Aspergillus* genus possibly acts as either pioneer organisms in seed borne infection of sorghum, or the dominant species found in the tropics. A widespread distribution of *Fusarium* species is attributed to the ability of the fungi to grow on a wide range of substrates and their efficient mechanism of spore dispersal (Olotu *et al*., 2022).

The results have demonstrated the invasion of seeds with mycotoxin producing fungi (*Aspergillus* and *Fusarium* spp) which could pose a risk to consumer health. A significant portion of the agricultural produce in the country and the world become unfit for human consumption due to mycotoxin contamination of grains (Ogbonna *et al*., 2015; Pushpavathi *et al*., 2017).

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The main toxic effects are carcinogenicity, genotoxicity, terratogenicity, nephrotoxicity, hepatotoxicity, reproductive disorders and immuno suppression (Gallo *et al*., 2015; Marie *et al*., 2016).

A similar trend of fungi invasion was reported for stored sorghum by Abdulsalaam and Shenge (2011) who recorded *Aspergillus*, *Fusarium*, *Rhizoctonia* and *Curvularia* species as the most common in washed sorghum grains. A similar trend was reported for stored sorghum grains in Kenya (Kange *et al.,* 2015). Abdel *et al*. (2017), also recorded *Aspergillus*, *Fusarium* and *Curvularia* species associated with Sorghum and Maize grains during storage. The result is also in conformity with the findings of Sultan *et al*. (2022) who recorded *Aspergillus* and *Fusarium* species as the frequently encountered species of fungi associated with stored grains in Rimi and Dawanau Markets, Kano State, Nigeria.

In conclusion, this study revealed that, seven fungal species namely *Aspergillus flavus*, *Aspergillus niger*, *Curvularia lunata*, *Didymaria* sp, *Fusarium oxysporum*, *Fusarium solani* and *Penicillium chrysogenum* were found to contaminate the selected *Sorghum bicolor* seeds. The presence of fungi in the sample seeds is an indication that there is a need for effective monitoring of fungal contamination and raising awareness on the hazards of fungi and their mycotoxin on human and animal health.

Testing seeds health of major crops should be introduced in the national control system, and proper harvesting, packaging and storage conditions should be promoted. Physical damage to seeds should be avoided since they serve as major points of entry to pathogens. The use of resistant cultivars should also be encouraged.

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