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Identification and Prevalence of Fungal Species in Stored Sorghum Grains Surveyed at Bodija and Oyo Town Markets in Oyo State and Ikire and Iwo Town Markets in Osun State, Western Nigeria

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ABSTRACT: This study investigated the identification and prevalence of fungal species in stored sorghum grains surveyed at Bodija and Oyo town markets in Oyo State and Ikire and Iwo town markets in Osun State, Western Nigeria using appropriate standard methods. Six distinct fungal species were isolated from the sorghum grains and identified through macroscopic and microscopic examination. These species included *Aspergillus flavus*, *A. niger*, *A. tamarii*, *A. terreus*, *Fusarium* sp., and *Rhizopus stolonifer*. The prevalence of fungal contamination was found to be higher in the markets of Oyo State compared to those in Osun State. Additionally, *Aspergillus flavus* and *A.niger* were the most prevalent fungal species, while Rhizopus stolonifer was the least prevalent.

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Sorghum (*Sorghum bicolor* (L.) Moench), originating from Africa and member of the Poaceae family, is the fifth most significant cereal crop in the world, preceded by wheat, maize, rice, and barley (Gunu and Musa, 2021). Known in English as millet, guinea corn, broom corn, and sweet sorghum, this crop is called Okababa in Yoruba, dawa/jero in Hausa, and soro in Igbo (Adedeji, 2020). It is a key staple for millions, particularly in South Asia and sub-Saharan Africa (Khalifa and Eltahir, 2023). Sorghum is grown in nearly 110 countries worldwide, predominantly in Africa and Asia, but also in the Americas, Europe, and Oceania (Baloch *et al*., 2023). The top producers of sorghum include the USA, which produces 8.7 million tons annually from 2.0 million hectares; Nigeria, with 6.9 million tons from 5.4 million hectares; Ethiopia, producing 5.3 million tons from 1.9 million hectares; and Sudan, which yields 3.7 million tons from 6.8 million hectares (Ahmad *et al*., 2022).

Sorghum is renowned for its robust resilience and adaptability to various environmental challenges. Its

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widespread cultivation is attributed to its versatility for culinary uses, animal feed, biofuel production, and forage. Sorghum is more resilient to harsh environmental conditions than many other cereals, performing well under limited water and temperature extremes, particularly in marginal lands (Khalifa and Eltahir, 2023). Sorghum grain is increasingly used for human consumption because it is gluten-free and offers health benefits due to its phenolic compounds. It contains bioactive phenolic compounds like ferulic acid, gallic acid, and vanillic acid, which have antioxidant and anti-inflammatory properties (Xu *et al*., 2021). In Africa, sorghum is used to make foods such as sorghum ball "Fura," tuwo, gruel, and beverages like the alcoholic pito and burukutu, as well as the non-alcoholic "kunu zaki" (Abah *et al*., 2020). The nutritional composition of sorghum includes protein (6.2% to 14.9%), carbohydrates (54.6% to 85.2%), fat (1.3% to 10.5%), ash (0.9% to 4.2%), and fiber (1.4% to 26.1%) (Adebo, 2020).

Food crop losses mainly result from inadequate post-harvest handling and storage conditions. In sub-Saharan Africa (SSA), post-harvest losses are a critical issue that exacerbates food insecurity (Mohammed *et al*., 2022). Sorghum grains, if not properly dried and stored, are highly susceptible to mold growth (Terna *et al*., 2019). Fungi such as *Aspergillus, Penicillium,* and *Fusarium* spp. can contaminate improperly stored sorghum, resulting in the production of mycotoxins. *Aspergillus* speciesproduce aflatoxins B_1, B_2, G_1, and G_2, while *Fusarium* species produce mycotoxins such as fumonisins, trichothecenes, and zearalenone. *Penicillium* species produce the mycotoxin isofumigaclavine. These mycotoxins are associated with human diseases such as cancer and hepatitis, and trichothecenes are also recognized as potential bioterrorism agents. The impact of mycotoxins on humans and animals can vary depending on their toxicity, which includes effects like carcinogenicity, disruption of the endocrine system, teratogenicity, mutagenicity, hemorrhaging, estrogenic effects, liver toxicity, kidney toxicity, and immune system suppression (Kange *et al*., 2015). The objective of this paper is to identify and evaluate the prevalence of fungal species in stored sorghum grains surveyed at Bodija and Oyo town markets in Oyo State and Ikire and Iwo town markets in Osun State, Western Nigeria.

MATERIALS AND METHODS

Sample Collection: Sorghum samples were gathered from two markets in Oyo State (Bodija and Oyo Town) and two markets in Osun State (Ikire and Iwo). In each market, five sorghum grain warehouses were chosen at random, and from each warehouse, 200 grams of sorghum grains were collected and placed in a sterile polythene bag. These five 200-gram samples were then combined into one 1000-gram sample, which was labeled with the market name. Consequently, four 1000-gram samples, each representing one of the markets, were prepared (Ajadi and Olahan, 2023). These samples were transported to the Plant pathology laboratory, Department of Botany, University of Ibadan, for further analysis.

Potato Dextrose Agar Preparation, Fungal Isolation and Morphological Identification of the Fungal Isolates: The Potato Dextrose Agar (PDA) culture medium was prepared following the manufacturer's guidelines. Thirty-nine grams of PDA powder were dissolved in one liter of distilled water, heated until fully dissolved, and sterilized in an autoclave at 121°C for 15 minutes. After cooling to 47°C, 1 ml of streptomycin BP was added to prevent bacterial growth as described by Ajadi and Olahan (2023).

To surface sterilize the samples (grains), one gram of each was immersed in a 1% hypochlorite solution for one minute, then washed three times with sterile distilled water. Fungi isolation was conducted using the direct plating method outlined by Hussaini *etal*. (2009). The surface-sterilized samples (in triplicates) were individually plated on PDA plates supplemented with streptomycin and then incubated at room temperature for three days to facilitate the growth of seed-borne fungi. Fungal colonies on each plate were counted, and pure cultures of the isolates were obtained by inoculating them onto fresh PDA plates for subculture. The morphological characteristics of the mycelium, including both macroscopic and microscopic features, were documented following the guidelines provided by Navi *etal*. (1999), Fawole and Oso (2007) and Kidd *et al*. (2023). The percentage occurrence of fungal species was determined using the following formula:

$$\%\,\text{OFS} = \frac{\text{Number of fungal species}}{\text{Total number of fungi isolated}} \times 100 \quad (1)$$

Where % OFS is percentage occurrence of fungal species

RESULT AND DISCUSSION

Six distinct fungal species were isolated from sorghum grains and identified through macroscopic and microscopic examination as *Aspergillus flavus*, *A. niger*, *A. tamarii*, *A. terreus*, *Fusarium* sp. and *Rhizopus stolonifer* (Table 1). The percentage of fungal species associated with the sorghum grain samples across the four markets is shown below (Fig.

1). The highest incidence rate (38%) was observed in samples from Oyo town, followed by Bodija (34%). Samples from Ikire and Iwo had lower contamination levels sharing 14% each, likely due to the poor hygienic conditions of storage facilities in the Oyo town and Bodija markets.

Table 1: Identification of the fungal isolates based on their morphology

S/N	Macroscopic morphology	Microscopic morphology	Probably organism
1	By day three, there was a lemon-green powdery growth of mycelia.	Green conidiospores along with septate hyphae	*Aspergillus flavus*
2	Powdery with dark brown to blackish flatty spread on the agar plate	Septate and branched hyphae having conidia that are in chain. Conidiophore are typically unbranched	*Aspergillus niger*
3	Surface color of greenish-yellow to olive with a woolly texture	Globose vesicle, biseriate and radiate conidial head	*Aspergillus tamarii*
4	Brown colonies with pyriform vesicles	Conidial heads in two rows and conidia arranged in columns	*Aspergillus terreus*
5	Fast growing, white cottony and dark purple undersurface	Oval to kidney shaped microconidia and terminal chlaymdospores in short chains	*Fusarium* sp.
6	Brownish grey coloured colony	Short rhizoid, sporangiophore unbranched, hyphae is coenocytic	*Rhizopus stolonifer*

Sorghum grains are susceptible to contamination by various fungal species. The most commonly found genera include *Aspergillus*, *Fusarium*, *Curvularia*, *Colletotrichum*, *Rhizopus*, and *Alternaria* species (Terna *et al*., 2019; Mohammed *et al*., 2022). These fungi are likely contributors to grain deterioration both before and after harvest. Fungal infections in sorghum grain can be complex and vary throughout its growth, harvest, and storage periods. The findings of this study are consistent with earlier research. Sajjan *et al*. (2014) isolated *Aspergillus* sp., *Fusarium* sp., and *Rhizopus* sp. from Rabi sorghum (*Sorghum bicolor* (L.) Moench) genotypes. Yassin *et al*. (2010) reported *Aspergillus terreus* among the fungi found in sorghum grains in Saudi Arabia. Panchal and Dhale (2011) also identified *Aspergillus terreus* among the seed-borne fungi of sorghum (*Sorghum vulgare* Pers.) in India. Olotu *et al*. (2022) isolated *Aspergillus fumigatus*, *Aspergillus niger*, *Fusarium* sp., *Penicillium* sp., and *Rhizopus* sp. from dried sorghum seeds stored for five months. In a similar study, Danazumi *etal*. (2015) recovered *Aspergillus flavus*, *Aspergillus niger*, *Fusarium* sp., and *Penicillium chrysogenum* from local landrace sorghum seeds sold in the Sabon Gari market, Kaduna State, Nigeria. However, our research did not recover *A. fumigatus* and *Penicillium* sp. Regardless of the prevalence of various fungal species, *A. flavus* and *A. niger* were the most dominant, followed by *A. terreus*, while Rhizopus stolonifer was less common (Fig. 2). Similarly, in a related study, *A. flavus* was the most prevalent species infecting sorghum grain, while *A. niger* was less frequent in a study conducted in Ethiopia (Weledesemayat *et al*., 2016).

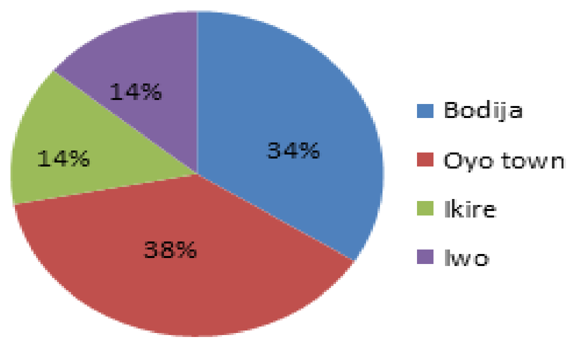

Fig. 1: Occurrences of infected fungal species in the surveyed markets

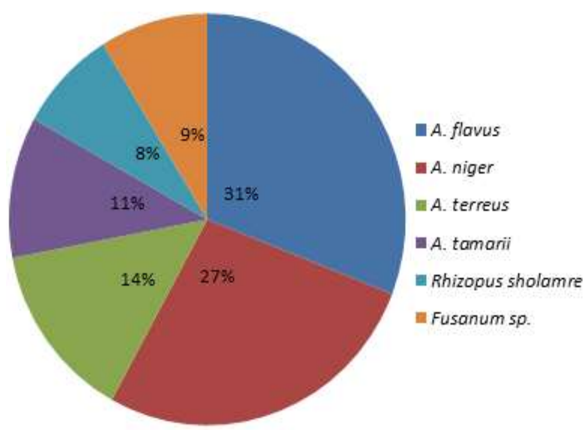

Fig. 2: Prevalence of fungal contamination in sorghum grain samples.

Contrarily, *A. niger* was found to be second dominanting fungus in this study. Dania and Oge (2018) reported that *Aspergillus flavus* was the most prevalent on stored sorghum grains across surveyed markets in Oyo State. This observation is consistent with previous studies implicating this pathogen in the spoilage of stored produce, particularly cereals (Fakruddin *et al*., 2015; Rosie *et al*., 2023; Haggag *et al*., 2024). The higher prevalence of *A. flavus* compared to other fungi may stem from its bioecology as a soil-borne pathogen (Nji *et al*., 2023). This suggests that soil and plant debris serve as viable sources of inoculation for infecting sorghum seeds planted in fields, ultimately transferring to harvested grains during storage.

Conclusion: Aspergillus flavus, *A. niger*, *A. tamarii*, *A. terreus*, *Fusarium* species, and *Rhizopus stolonifer* were reported in this study. The identification of *Aspergillus flavus* and *A. niger* as the most prevalent fungi highlights the importance of monitoring and managing fungal contamination in sorghum storage facilities. The higher contamination levels observed in Oyo State markets suggest a need for improved storage practices and hygiene measures in these areas. Future research could focus on exploring the factors contributing to the differences in fungal contamination between the two states and developing strategies to mitigate fungal infestation in sorghum grains during storage.

Declaration of conflict of interest: The authors declare no conflict of interest.

Data Availability Statement: Data are available upon request from the corresponding author.

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