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ORIGINAL RESEARCH ARTICLE

Isolation and Identification of Fungal Species Associated with Post-harvest Spoilage of Onions (*Allium cepa*) in the Kafin Hausa Market, Jigawa State, Nigeria

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ABSTRACT
Post-harvest fungal spoilage of onions contributes to significant global economic and food security losses, with up to 30% of harvested bulbs lost annually in developing regions. A study was conducted to isolate fungal species responsible for the spoilage of onions sold at Kafin Hausa Market, Jigawa State, Nigeria. The collected onion samples were cut into pieces of about 5 mm using a sterile razor blade, cultured on potato dextrose agar (PDA), and incubated at room temperature for 5–7 days. Fungal isolates were sub-cultured and purified onto PDA plates. Morphological identification techniques (macro- and micromorphological features) were assessed via lactophenol cotton blue staining and taxonomic keys. A total of 37 fungal isolates were obtained from 12 samples collected across 3 stations (A, B, and C). Result from the determination of frequency of occurrence show that, fungus with the highest frequency was *Aspergillus niger* (40.54%), followed by *Alternaria alternata* (16.22%), *Rhizopus stolonifer* (13.51%), *Fusarium oxysporum* (10.81%), *Aspergillus flavus* (8.11%), *Penicillium digitatum* (8.11%), and *Aspergillus fumigatus* (2.70%) with frequencies differing significantly across sampling stations ($p < 0.05$). This finding provides the first empirical documentation of onion spoilage fungi in the Kafin Hausa region of Jigawa state, Nigeria, identifying *A. niger* as the primary contaminant, and suggests the urgency of improved post-harvest practices (e.g., temperature-controlled storage) to mitigate losses.

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isolation, identification, fungal species, post-harvest, spoilage, onion (*A. cepa*).

INTRODUCTION

Rich in vitamins, minerals, and bioactive substances, such as organosulfur compounds, onions (*Allium cepa* L.) are one of the most popular vegetables in the world and are prized for their distinctively strong flavour as well as their remarkable nutritional and therapeutic qualities. They are associated with a decreased risk of chronic illnesses, including heart disease and several forms of cancer (Sharma, 2023). In addition to being essential parts of the world's diet and important commodities in international trade, onions are also a major source of foreign cash for many impoverished countries. Despite their economic and nutritional importance, onions are highly perishable and suffer from substantial post-harvest losses, diminishing market value and affecting food security.

One of the principal challenges in onion storage is the rapid onset of fungal contamination. The primary causes of post-harvest deterioration are known to be fungi, including *Aspergillus niger*, *Alternaria alternata*, *Rhizopus stolonifer*, and *Fusarium oxysporum* (Samuel & Ifeanyi, 2015; Sharma, 2023). While Samuel & Ifeanyi (2015) provided foundational insights into fungal contamination in Nigerian onion markets, their focus on the Awka market limits generalizability due to geographical and environmental variability. For instance, onions sold in Awka markets predominantly originate from the cultivation regions of northern Nigeria, where production practices, post-harvest handling, and long-distance transportation may uniquely influence contamination risks. Systematic tracking of fungal species from cultivation regions such as Kafin Hausa region of Jigawa state to retail markets is critical to disentangle whether contamination arises from pre-harvest practices (e.g., irrigation, soil management), post-harvest storage, or transport-related stressors. Furthermore, temporal dynamics such as seasonal humidity fluctuations, prolonged storage, or evolving market practices could alter fungal prevalence, suggesting the need for longitudinal studies to address these dynamics. These fungi enter nutrient-rich tissues by taking advantage of damage sustained during harvesting, handling, and transportation-related harm that breaks the protective

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outer scales of the bulbs. Once established, these infections can cause toxic mycotoxins accumulation due to the metabolic activities of the mentioned fungi that are extremely dangerous to human health and speeding up the decay process (Sharma, 2023).

The correlation between storage conditions and fungal growth and mycotoxin formation is quite strong, especially in tropical and subtropical areas with a warm and humid climate. Elevated temperatures have been demonstrated to increase mycotoxin biosynthesis and stimulate fungal growth, leading to 35–40% post-harvest losses for potatoes and onions and several other crops (Gutiérrez-Pozo *et al*., 2024; Subha *et al*., 2024). So, for instance, high temperature and humidity storage of potatoes promotes Fusarium species mycotoxins contamination (Gutiérrez-Pozo *et al*., 2024). Further, Oluwakayode *et al*. (2024) recently observed similar trends occurring in stored wheat (*Triticum aestivum*), where variations in temperature and humidity correlated with mycotoxins, including zearalenone and deoxynivalenol. Such losses economically affect smallholder and commercial farmers, while mycotoxins, which are carcinogenic, also pose significant health risks to the general public (Carvalho *et al*., 2024; Savitha *et al*., 2024). Therefore, storage conditions must be managed appropriately to prevent spoilage and maintain food safety.

To overcome these challenges, several studies have investigated creative post-harvest strategies to reduce fungal growth and prolong onion shelf life. Emerging methods include modified atmosphere packaging, antifungal nanomaterials, and plant-based biofungicides, which have all been promising in controlled trials (Paola *et al*., 2023). As a concrete example, with their use of nanotechnology, either in preventing fungal dissemination or in botanical bio fungicides, these are greener alternatives to synthetics that are being increasingly evidenced for their role in either environmental degradation or for resistant pathogen serovars (Sharma, 2023). In places like Kafin Hausa, where there remain poor storage practices, fungal contaminants pose a risk to farmers with loss of income as well as consumers due to reduced quality of the onions. Therefore, this study aimed to isolate and identify specific fungal species associated with spoiled onion (*A. cepa*) sold across Kafin Hausa Market of Jigawa State. Identification of these fungal species would not only expand scientific knowledge of fungi affecting onions in the region but also serve as a baseline for innovative and sustainable storage development and a guideline for a targeted antifungal development strategy.

MATERIAL AND METHODS

Study Area

The study was carried out in the Kafin Hausa local government area, latitude 12°14' and longitude 9°54' E Jigawa state, Nigeria. Kafin Hausa has a land area of 1,380 KM^2 and a population of 271,053 according to the 2006 census, the population of which continues to increase. The terrain is generally composed of Sudan savannah, which has the potential for both rain-fed and irrigation farming, and the rainy season usually starts from May to September every year. Temperatures of approximately 40°C are common, especially in the months from March to September, but temperatures can be as low as 25°C during the Harmattan period. Agriculture and trading are the most predominant economic activities. It has a major market where people come from nearby villages and other local governments to trade their commodities (Sani *et al.,* 2018).

Sample collection and material sterilization

Because the farmers in the region solely defend on red onion varieties, samples of red onion bulbs with signs of spoilage (such as discoloration, tissue softening, and visible fungal growth) were collected from different positions within the Kafin Hausa Market, Jigawa State, Nigeria. Each of the samples was transported in a sterile polythene bag to the laboratory for sterilization and subsequent fungal isolation (Samuel & Ifeanyi, 2015; Sani *et al*., 2018). All glassware and related materials used were sterilized by autoclaving at 121°C for 15 minutes. This was done to avoid contamination during media preparation as well as sample processing (Sani *et al*., 2018). The visibly affected portions (approximately 5 mm × 5 mm) were excised using sterile razor blades. This is followed by surface sterilization by immersing in a 1% sodium hypochlorite solution for 2 minutes, followed by three sequential rinses with sterile distilled water to reduce the level of surface contaminants while at the same time preserving the mycoflora responsible for the spoilage (Dimkpa & Onuegbu, 2010; Kumar *et al*., 2015).

Media Preparation

Potato dextrose agar (PDA) was used for fungal isolation from the collected onion samples because of its proven effectiveness in cultivating a broad spectrum of fungal species. Thirty-nine grams (39 g) of PDA powder were dissolved in 1 liter of distilled water and allowed to dissolve with gentle shaking. The medium was then autoclaved at 121°C for 15 minutes to ensure complete sterilization. Once cooled to 45–50°C, 4 ml of streptomycin solution (100 mg/ml) was added aseptically to inhibit bacterial growth while allowing fungal development (Sani *et al*., 2018; Diabankana *et al*., 2024).

Isolation and Culturing of Fungal Isolates

Under sterile conditions in a laminar flow hood, small tissue segments were aseptically placed onto the prepared PDA plates via a spread technique. The inoculated plates were then incubated at room temperature (approximately 25 ± 2°C) for 5–7 days under continuous light to promote fungal growth and sporulation. The inoculated plates were observed daily to monitor the colony growth, and emerged colonies were distinctively subcultured onto fresh PDA plates through streak plate inoculation method until pure and morphologically consistent cultures were obtained (Dimkpa & Onuegbu, 2010; Diabankana *et al.,* 2024).

Morphological and microscopic identification

Individually, each of the subcultured and purified fungal isolates was morphologically on the basis of colony morphology by taking into account their color, texture, and growth rate, then followed by microscopic examination. Briefly, 1-2 drops of lactophenol cotton blue stain were added to a small colony fragment on a clean, grease-free microscope glass slide, then covered with a coverslip and examined under a light microscope at 400× magnification. The microscopic identification was through considering the hyphal characteristics, and reproductive features, in line with the preliminary identification guide through standard mycological keys (Louis *et al.*, 1997; Islam *et al.*, 2019).

Determination of the percentage frequency of occurrence

The frequency of the occurrence of different types of isolated fungal contaminants associated with the spoilage of onion was determined. In each case, the percentage of occurrence was determined via the formula adopted by Sani *et al.* (2018), as below:

$$\text{Percentage frequency} = \frac{\text{Number of isolated fungi}}{\text{Total number of isolates}} \text{ X } 100$$

Statistical analysis

As the experiment was conducted in three replications under a Completely Randomized Design (CRD), the recorded data were subjected to statistical analysis using SPSS software (version 20.0). One-way analysis of variance (ANOVA) was performed to determine the significant difference between the frequency of occurrence between the fungal species at a significance level of $p < 0.05$.

RESULT

Table 1 lists the fungal species isolated from spoilt onion bulbs collected at the Kafin Hausa market. The isolates (Figure 1) were grouped by sampling stations (A, B, and C), indicating the diversity of fungi present in the post-harvest environment. The result shows that isolates, specifically *Aspergillus niger, Alternaria alternata, Aspergillus flavus, Rhizopus stolonifer, and Penicillium digitatum* were common in Station A. Contrary to this Station, onions sold in B Station not only indicate the presence of *Aspergillus niger, Alternaria alternata, and Aspergillus flavus* but also indicate the occurrence of *Aspergillus fumigatus* and *Fusarium oxysporum*. Station C exhibited a similar species composition as *Aspergillus niger, Alternaria alternata, Fusarium oxysporum, Penicillium digitatum, and Rhizopus stolonifer.*

The percentage distribution of the fungi in the spoilt onion bulbs is shown in Table 2 below. The percentage distributions were 40.54%, 16.22%, 10.81%, 8.11%, 8.11%, 2.70%, 34.29%, and 13.51% for *Aspergillus niger, Alternaria alternata, Fusarium oxysporum, Aspergillus flavus, Penecillium digitatum Aspergillus fumigatus, and Rhizophus stolonifer,* respectively.

Table 1: Fungal Species Isolated from Spoilt Onion Bulbs across Different Sampling Points at the Kafin Hausa Market, Jigawa State.

Stations	Fungal isolates
station A	*Aspergillus niger, Alternaria alternata, Aspergillus flavus, Rhizophus stolonifer, Penecillium digitatum.*
station B	*Aspergillus niger, Alternaria alternate, Aspergillus flavus, Aspergillus fumigatus, Fussarium oxysporum.*
station C	*Aspergillus niger, Alternaria alternata, Fussarium oxysporum, Penecillium digitatum, Rhizophus stolonifer.*

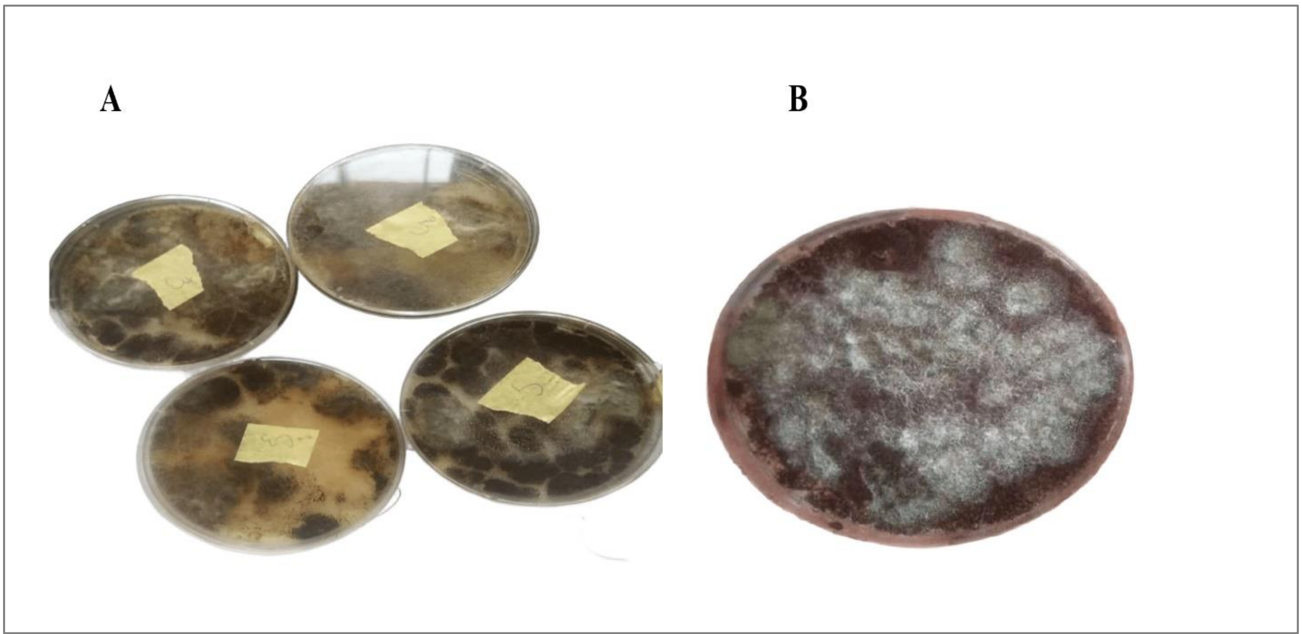

Figure 1: A PDA Plates Showing the Mixed Cultures of Various Fungal Species from the Onion Samples Collected from the Kafin Hausa Market Before Subculturing (Purification) and Identification. (A) Representative Samples from stations A-C. (B) Close view of the PDA plate.

Table 2: Macromorphological and Micromorphological Features of Fungal Species Associated with Spoiled Onions in Kafin Hausa, Jigawa State.

Fungal Species	Macromorphology	Micromorphology
Aspergillus niger	Colonies appeared white Initially, turning black with age. Texture velvety to powdery.	Conidial heads radiate; vesicles are spherical. Phialides cover the entire vesicle. Smooth conidiophores; dark-pigmented, globose conidia.
Alternaria alternata	Colonies woolly, grayish-green to brown. Reverse dark.	Conidia muriform (transverse and longitudinal septa), obclavate, often with a beak-like apex. Chains of conidia in branched acropetal succession.
Fusarium oxysporum	Colonies cottony, white to pink/purple. Pigmentation may vary.	Macroconidia sickle-shaped, 3–5 septate; microconidia oval, single-celled. Chlamydospores present (thick-walled, terminal/intercalary).
Aspergillus flavus	Colonies are granular yellow-green. Reverse pale to golden.	Conidial heads columnar; vesicles globose. Phialides uniseriate, covering the entire vesicle. Conidia are elliptical and rough-walled; some strains produce aflatoxins.
Penicillium digitatum	Colonies velvety, olive-green to gray-green. Often zonate.	Conidiophores symmetrically branched (penicilli). Phialides are flask-shaped, forming chains of spherical, smooth conidia.
Aspergillus fumigatus	Colonies velvety, blue-green to gray. Rapid growth at 37°C.	Conidial heads are compact and columnar. Vesicles are club-shaped; phialides uniseriate, covering the upper half. Conidia globose, green, rough-walled.
Rhizopus stolonifer	Colonies are fluffy, white initially, turning black. Stolons present.	Sporangiophores are brown, emerging from rhizoids. Sporangia black, spherical; columella hemispherical. Sporangiospores are angular or striated.

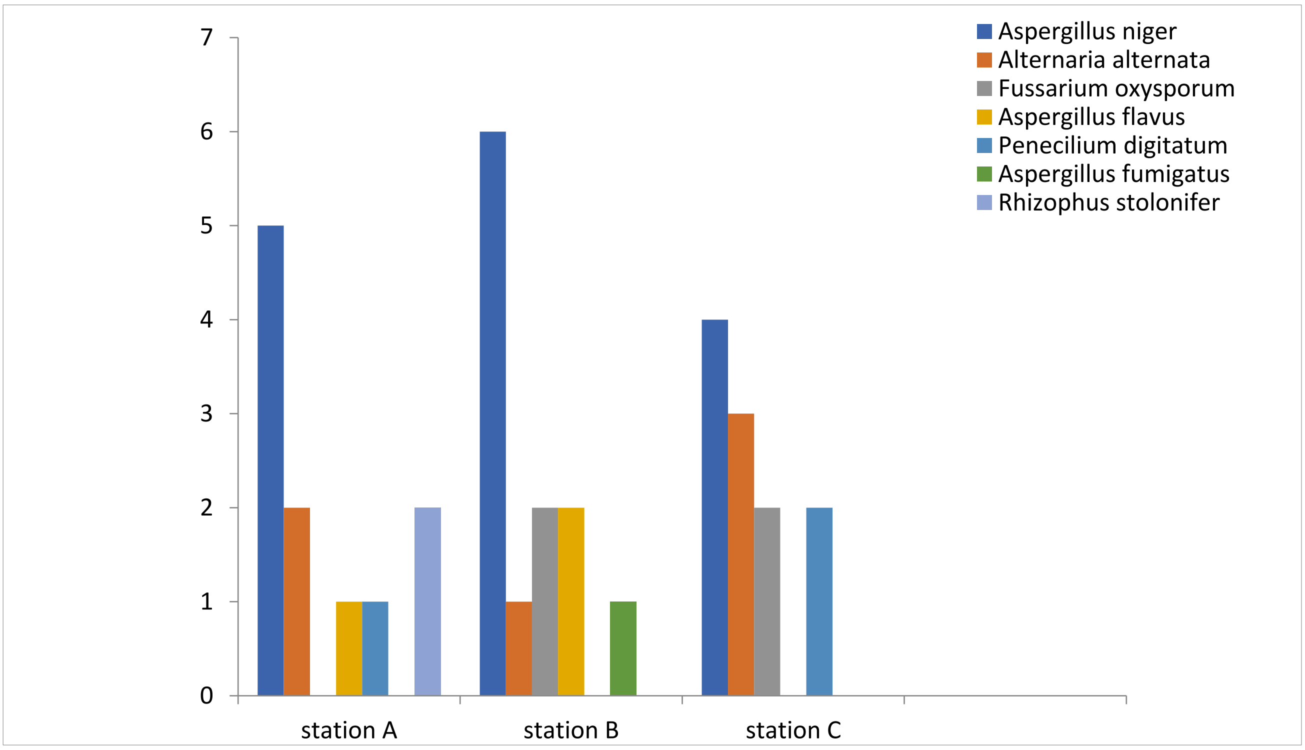

Figure 2: Populations of fungal species isolated from onion samples in relation to the sampling stations.

DISCUSSION

This study identified fungal species linked to post-harvest onion spoilage at Kafin Hausa Market in Jigawa State, Nigeria (Table 1 and 2, Figure 1). The most prevalent fungi, namely Aspergillus niger, *Alternaria alternata*, *Rhizopus stolonifer*, and *Fusarium oxysporum* indicate a significant role in onion deterioration in the region. These results pointed align with earlier studies from Nigeria and other tropical climates, which have similarly documented these fungi in stored onion bulbs (Samuel & Ifeanyi, 2015; Favour *et al.*, 2024; Gathambiri, 2024).

However, the result from our investigation (Table 3, Figures 2 and 3) has out that the increased frequency of these fungal species is closely related to the various post-harvest procedures that are regularly practiced. As such, mechanical damage that occurs during the harvesting process, in addition to the subsequent handling procedure, possibly from farm to market, and the nature of transportation facilities, is likely to contribute to numerous entry points for opportunistic pathogens to infiltrate. For instance, the presence of *A. niger* is well-documented, and its known ability to produce potent mycotoxins could further complicate the qualitative and quantitative losses associated with spoiled produce, including onions (Sharma, 2023). The highest occurrence of *A. niger* in this study is in line with Ikeh *et al.* (2023), that also identified this fungus to be among the highest prevalent (30%) fungi in spoiled onion at the Akwa metropolis in Anambra state, Nigeria. Considering the scope of this study, the clear nonexistence of various fungal species that have been observed in prior studies (Guigui *et al*., 2024; Amaechi & Emmanuel, 2024), such as *Saccharomyces cerevisiae*, *Mucor* and *Candida spp* (Ikeh *et al*., 2023) could suggest a geographical or spatial differences in the practices related to storage and the techniques of handling that are in place, emphasizing the critical requirement for the proposal of localized post-harvest solutions intended to alleviate these concerns.

Table 3. Percent distribution of the fungal species from the split onion bulbs

Species	Number of Isolates	Distribution (Percentage)
Aspergillus niger	15	40.54
Alternaria alternata	6	16.22
Fussarium oxysporum	4	10.81
Aspergillus flavus	3	8.11
Penecillium digitatum	3	8.11
Aspergillus fumigatus	1	2.70
Rhizophus stolonifer	5	13.51
Total (P Value<0.05)	**37**	**100%**

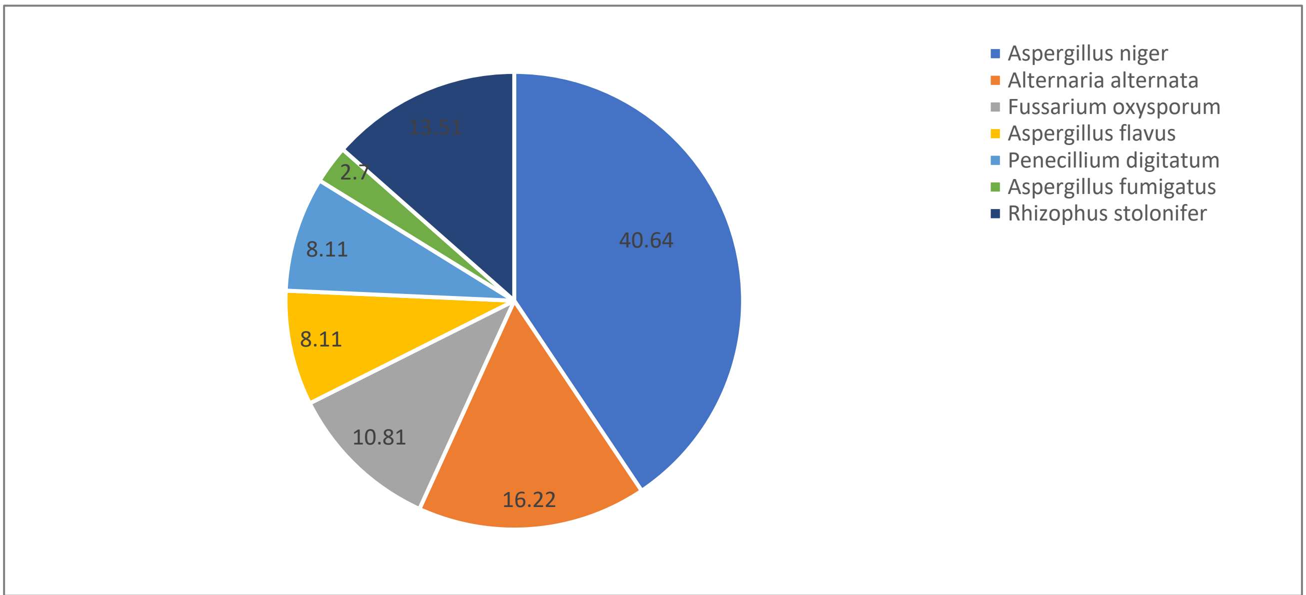

Figure 3: Pie chart showing the distribution (%) of fungal species associated with the spoiled onion in the Kafin Hausa market, Jigawa State.

However, the storage condition greatly influences the establishment of fungal species such as those identified in this study. Fungal development is facilitated by high humidity and temperature range that are ideal for the fungus, which are frequently present in local storage facilities. As clearly indicated in a previous study investigating the post-harvest deterioration of onion, fungal species appeared to be prevalent in all seasons of the year, suggesting *A. niger* as the most common fungus throughout the year, with its highest occurrence in the monsoon season at 36.11% (Rodrigues *et al*., 2021). According to the study, *Aspergillus flavus* was also quite prevalent, especially during the monsoon, where it measures 18.75% and *Botrytis spp* has the lowest prevalence of all the fungi listed in every season. Accordingly, the incidence of onion spoilage-associated fungi can be considerably decreased by effective control techniques all year round and ideal storage conditions, such as improved packing and low-temperature storage facilities (Sharma, 2023); nevertheless, this may require more investigation. Hence, results support this notion; the high frequency of fungal species known to cause post-harvest losses in our samples called for an urgent need for improved storage practices among onion farmers/sellers in Kafin Hausa.

Practically, the significance of these findings lies in the potential to harness them into actionable improvements. Therefore, we recommend that sub-standard post-harvest practices among the farmers or people handling onion in the study area could be the major contributors to exposing onion bulbs to a wide range of fungal species and spoilage; our study reaffirms this view. In order to reduce post-harvest loss due to fungal species identified in this study, economically sustainable protocols that align with the economic and social standards of the Kafin Hausa population could be implemented. These protocols could include the use of fungicides that are of lesser cost, that are harmless to the environment, such as plant-derived, to be precise, especially those that have shown promise and can be utilized as an option. Nonetheless, it may seem beneficial to develop comprehensive sanitation protocols and effective control measures that extend from cultural practices throughout onion production to the market (Paola *et al*., 2023). Recent research, for example, found that combining biofungicides with traditional storage methods might reduce spoiling by up to 30% while avoiding the negative health and environmental effects associated with synthetic fungicides (Sharma, 2023; El-Dawy *et al*., 2024).

Therefore, developing an integrated post-harvest management system that includes advanced storage technologies, improved handling, and sustainable use of antifungal agents designed to manage post-harvest-related diseases is crucial. Furthermore, our findings provide a solid basis for further research. The identification procedure in this study could be improved by using molecular diagnostic tools like ITS sequencing (Islam *et al*., 2019) for more accurate identification of fungal species. These advancements may facilitate targeted, cost-effective, and environmentally sustainable management strategies. Our findings suggest that the fungal species identified could significantly contribute to the post-harvest decay of onions in the Kafin Hausa market, suggesting the need for practical changes and emphasizing the importance of handling and storage conditions. In Kafin Hausa, Jigawa State, stakeholders can enhance onion quality, improve food safety, and significantly reduce losses by adopting innovative antifungal agents and optimizing post-harvest processes.

CONCLUSION

Onions sold at the Kafin Hausa point of vegetables were found to be contaminated with different species of fungi, which favours the spoilage of these vegetables. The findings from this research revealed that onion had the greatest degree of contamination from *A. niger, A. alternata, R. stolonifer, F. oxysporum, P. digitatum, A. flavus and A. fumigatus*.

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