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Isolation and Identification of Fungal Species Associated with Postharvest Spoilage of Watermelon (*Citrullus lanatus*) Fruits in Sokoto Metropolis, Nigeria

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ABSTRACT

Postharvest fungal spoilage represents a major constraint to watermelon (*Citrullus lanatus*) production, marketing, and food safety in sub-Saharan Africa, yet the fungal pathogens responsible for its spoilage in Sokoto metropolis, northwestern Nigeria, remain poorly characterized. This study aimed to isolate and identify fungal species associated with watermelon spoilage, determine their frequency of occurrence across nine market locations, and evaluate their pathogenicity on healthy fruits. Spoiled watermelon fruits were collected from nine sampling points in Sokoto metropolis, and fungal isolates were recovered on Sabouraud Dextrose Agar (SDA), purified by repeated sub-culturing, and identified using macroscopic colony morphology and microscopic examination with lactophenol cotton blue staining; pathogenicity was subsequently assessed following Koch's postulates. Five fungal species were isolated and identified: *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum*, *Rhizopus oligosporus*, and *Mucor hiemalis*. *Aspergillus flavus* recorded the highest frequency of occurrence (28.57%), followed by *F. oxysporum* (23.81%), *R. oligosporus* (19.05%), *A. niger* (14.29%), and *M. hiemalis* (14.29%), and all isolates induced characteristic spoilage symptoms in inoculated healthy fruits, thereby fulfilling Koch's postulates. These findings demonstrate that fungal contamination, particularly by the aflatoxigenic *A. flavus*, constitutes a significant food safety concern for watermelon marketed in Sokoto, and improved postharvest handling, proper sanitation, and adequate cold storage are urgently recommended.

Keywords: *Citrullus lanatus*; fungal spoilage; *Aspergillus flavus*; postharvest disease; mycotoxin; food safety; Sokoto; Nigeria

INTRODUCTION

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai; family Cucurbitaceae) is one of the most widely cultivated and consumed fruits globally, ranking among the top five most important cucurbit crops by production volume (FAOSTAT, 2022). Its high-water content (approximately 92%), combined with a rich profile of vitamins A, B6, and C, lycopene, citrulline, and potassium, makes it an important dietary component and a commercially significant cash crop in many tropical and subtropical countries, including Nigeria (Mandel et al., 2003; Liu et al., 2018).

Despite its nutritional and economic importance, watermelon is highly perishable owing to its thin rind, high moisture content, and susceptibility to mechanical injury. Postharvest losses from microbial deterioration, particularly fungal spoilage, are estimated to range from 20% to 50% in sub-Saharan Africa, depending on the handling conditions and the season (El-Sharouny et al., 2018; Magnani et al., 2019). Such losses severely reduce market availability, reduce growers' incomes, and compromise consumer food safety. Fungal pathogens are among the principal agents responsible for postharvest deterioration of fruits and vegetables worldwide. Genera such as *Aspergillus*, *Fusarium*, *Penicillium*, *Rhizopus*, and *Mucor* have been consistently associated with spoilage of cucurbit and other fresh produce (Pitt & Hocking, 2009; Guan et al., 2017; Liu et al., 2018). These fungi penetrate bruised or wounded tissue, produce a repertoire of cell wall-degrading enzymes—including pectinases, cellulases, and hemicellulases—and cause rapid tissue maceration and accelerated fruit deterioration (Zhao et al., 2018). Beyond economic losses, fungal spoilage raises important public health concerns because several species produce secondary metabolites known as mycotoxins. Aflatoxins (produced by *Aspergillus flavus* and *A. parasiticus*) and ochratoxin A are classified as Group 1 and Group 2B carcinogens, respectively, by the International Agency for Research on Cancer (IARC). In addition to carcinogenicity, these toxins exert hepatotoxic, nephrotoxic, and immunosuppressive effects in humans and livestock (Zhao et al., 2018; Marín et al., 2020). The occurrence of aflatoxigenic fungi on fruits marketed in northern Nigeria is, therefore, a matter of serious public health concern.

Sokoto metropolis is an important commercial hub in northwestern Nigeria, where watermelon is widely cultivated and traded. However, information on the specific fungal species responsible for watermelon spoilage within this metropolis, their distribution across market locations, and their pathogenicity remains scarce. Such baseline data are indispensable for designing evidence-based postharvest management interventions tailored to local conditions. This study therefore aimed to: isolate and identify fungal species from spoiled watermelon fruits collected across nine locations in Sokoto metropolis; determine the frequency of occurrence of each identified species; characterize their macroscopic and microscopic morphological features; and assess their pathogenicity on healthy watermelon fruits.

MATERIALS AND METHODS

Study Area

The study was conducted in Sokoto metropolis, located in the Sudan Savannah agro-ecological zone of northwestern Nigeria, at approximately latitude 13°02′N and longitude 5°14′E, at an elevation of 350 m above sea level. The metropolis encompasses Sokoto North, Sokoto South,

Wamakko, and parts of Kware Local Government Areas. The climate is characterised by a prolonged dry season (October–May) with mean annual temperatures ranging from 28°C to 40°C and a short rainy season (June–September) with mean annual rainfall of approximately 500–700 mm. These conditions favour rapid fungal growth and postharvest deterioration of fresh produce.

Sample Collection

Spoiled watermelon fruits displaying visible signs of fungal decay (surface discolouration, soft rot, mycelial growth, and/or off-odour) were collected from nine purposively selected sampling points across Sokoto metropolis between March and May 2022 (Table 3). The sampling points included major retail markets, roadside vendors, and local farms. A minimum of three fruits per location were collected, transported in sterile polythene bags to the Biological Sciences Laboratory, Sokoto State University, and processed within 24 hours of collection.

Surface Sterilisation and Sample Preparation

The outer surfaces of collected fruits were gently wiped with 70% (v/v) ethanol-soaked sterile swabs and subsequently rinsed twice with sterile distilled water to eliminate surface epiphytes and reduce external contamination. Under a laminar flow cabinet, small tissue sections (approximately 0.5 cm^2) were aseptically excised from the margins of visibly spoiled areas using a sterile scalpel blade.

Culture Media Preparation

Sabouraud Dextrose Agar (SDA; Difco™, Becton Dickinson, USA) was prepared by dissolving 35.5 g of powder in 500 mL of sterile distilled water with continuous heating until complete dissolution. The medium was sterilised by autoclaving at 121°C for 15 minutes at 103 kPa. After cooling to approximately 45°C, the medium was supplemented with chloramphenicol (50 μg mL^{-1}) to suppress bacterial contamination and poured into sterile Petri dishes (90 mm diameter). The solidified plates were incubated at 25°C for 3–5 days as a sterility check before use.

Inoculation and Incubation

Tissue sections were placed aseptically onto the surface of SDA plates (three sections per plate) using sterile forceps. Inoculated plates were sealed with Parafilm®, inverted, and incubated at 28 ± 2°C in a calibrated incubator (Memmert IN 55, Germany) for 5–7 days. Plates were examined daily for the appearance of fungal colonies.

Purification and Preservation

Morphologically distinct colonies were sub-cultured onto fresh SDA plates by the hyphal tip transfer method to obtain axenic (pure) cultures. This process was repeated at least twice to ensure purity. Confirmed pure cultures were maintained on SDA slants at 4°C and on SDA supplemented with 15% (v/v) glycerol at −20°C for long-term preservation.

Morphological Identification

Fungal identification was performed using a combination of macroscopic and microscopic criteria. Macroscopic features assessed included colony colour (obverse and reverse), surface texture (cottony, granular, powdery, or velvety), colony margin, colony diameter, and rate of growth. For microscopic examination, a portion of each colony was mounted on a clean microscope slide in a drop of lactophenol cotton blue (LPCB) stain, covered with a coverslip, gently heated, and observed under a compound microscope (Olympus CX23, Japan) at magnifications of ×40 and ×100. Structures examined included hyphal morphology (septate vs. aseptate), conidial arrangement, conidiophore structure, sporangiophore morphology, presence of rhizoids, and spore ornamentation. Identifications were made with reference to standard mycological keys and descriptions (Pitt & Hocking, 2009; Samson et al., 2004; Ellis, 1971).

Pathogenicity Test

Pathogenicity tests were conducted following the method described by Baiyewu et al. (2007) and Chukwuka et al. (2010), with minor modifications. Mature, healthy watermelon fruits with no signs of injury or decay were thoroughly washed with tap water, rinsed with sterile distilled water, and surface-sterilised with 75% ethanol. A sterile 4 mm cork borer was used to create uniform wounds on each fruit. An agar plug (4 mm diameter) bearing a 5-day-old fungal colony was inoculated into each wound, and the displaced plug was replaced. The inoculation site was sealed with petroleum jelly to maintain humidity and reduce the risk of secondary contamination. Negative controls consisted of fruits wounded with a sterile cork borer but inoculated with a sterile agar plug only. Each treatment was replicated three times. Inoculated fruits were individually enclosed in sterile polythene bags containing moistened sterile cotton wool to maintain a humid microenvironment and incubated at 30 ± 1°C for 5 days. Fruits were inspected daily from Day 0 to Day 5 for the development of spoilage symptoms. To fulfil Koch's postulates, fungi were re-isolated from the margins of symptomatic tissues and compared with the original isolates based on morphological characteristics.

Frequency of Occurrence

The frequency of occurrence (FO %) of each fungal species was calculated using the formula:

FO (%) = (Number of isolations of a species / Total number of all fungal isolations) × 100

RESULTS AND DISCUSSION

Occurrence and Distribution of Fungal Species across Sampling Locations

A total of 21 fungal isolates, representing five distinct species, were recovered from spoiled watermelon fruits collected across the nine sampling locations in Sokoto metropolis. All five species were identifiable at more than one site, indicating their widespread distribution throughout the study area. The distribution of each species across the sampling points is presented in Table 1. *Aspergillus flavus* was recovered from six of the nine locations (A, B, D, F, H, and I), representing the broadest distribution among all identified species. *Fusarium oxysporum* was isolated from five locations (A, B, D, H, and I), while *Rhizopus oligosporus* was recovered from four locations (C, D, F, and G). Both *Aspergillus niger* and *Mucor hiemalis* were isolated from three locations each (A, C, G and C, E, H, respectively).

Table 1: Distribution of Fungal Isolates from Spoiled Watermelon Fruits across Nine Sampling Locations in Sokoto Metropolis

Fungal Species	A	B	C	D	E	F	G	H	I
Fusarium oxysporum	+	+	−	+	−	−	−	+	+
Aspergillus niger	+	−	+	−	−	−	+	−	−
Mucor hiemalis	−	−	+	−	+	−	−	+	−
Rhizopus oligosporus	−	−	+	+	−	+	+	−	−
Aspergillus flavus	+	+	−	+	−	+	−	+	+

Note: + = Species present; − = Species absent. Location codes are defined in Table 3.

Morphological Characteristics of Identified Fungal Species

All five fungal species were characterised on the basis of macroscopic and microscopic morphological features. The observed characteristics were consistent with established taxonomic descriptions (Pitt & Hocking, 2009; Samson et al., 2004) and are summarised in Table 2.

Fusarium oxysporum produced white to pale-pink, woolly colonies with a pink reverse. Microscopically, hyaline septate hyphae (3–8 µm in diameter) with characteristic acute-angle branching were observed, along with falcate macroconidia (three to five septa) and smaller microconidia. *Aspergillus flavus* developed coarsely granular, yellow-green colonies with both uniseriate and biseriate conidial heads on rough-walled conidiophores. *Aspergillus niger* formed jet-black velvety colonies bearing characteristic biseriate conidial heads with dark-brown, rough-walled conidia arranged in radiate columns. *Mucor hiemalis* produced rapidly growing cottony, grey-brown colonies with aseptate (coenocytic) hyphae bearing columellate sporangia but lacking rhizoids, a feature that distinguishes it from *Rhizopus* spp. *Rhizopus oligosporus* was characterised by the presence of distinct stolons and rhizoids, with unbranched sporangiophores arising from nodal points, and ellipsoidal, striated sporangiospores.

Table 2: Macroscopic and Microscopic Morphological Characteristics of Fungal Species Isolated from Spoiled Watermelon Fruits

Fungal Species	Macroscopic Features	Microscopic Features
Fusarium oxysporum	White to pale-pink, woolly/cottony; flat, spreading colony; pinkish reverse	Hyaline, septate hyphae (3–8 µm diam.); acute-angle branching; macro- and microconidia present; falcate macroconidia with 3–5 septa
Aspergillus flavus	Yellow-green surface; coarsely granular/powdery texture; brownish reverse	Septate hyphae; uniseriate and biseriate conidial heads; rough-walled conidiophores; globose to subglobose conidia
Aspergillus niger	Initially white, rapidly turning jet black; velvety surface; pale reverse	Septate hyphae; globose vesicle; biseriate phialides bearing brown-black, rough-walled conidia in radiate columns
Mucor hiemalis	Initially white, becoming grey-brown with blackish sporangia; rapid cottony growth	Aseptate (coenocytic) hyphae; columellate sporangia; no rhizoids; spherical sporangiospores
Rhizopus oligosporus	Whitish to greyish-brown, rapidly spreading woolly colony	Stolons and rhizoids present; unbranched sporangiophores arising from nodes; globose sporangia; striated ellipsoidal sporangiospores

Frequency of Occurrence

Aspergillus flavus was the most frequently isolated species, accounting for 28.57% of all isolations. It was followed by *F. oxysporum* (23.81%), *R. oligosporus* (19.05%), *A. niger* (14.29%), and *M. hiemalis* (14.29%) (Table 3).

Table 3: Frequency of Occurrence of Fungal Species Isolated From Spoiled Watermelon Fruits in Sokoto Metropolis (N = 21 Total Isolations)

Fungal Species	No. of Isolations	Frequency of Occurrence (%)
Aspergillus flavus	6	28.57
Fusarium oxysporum	5	23.81
Rhizopus oligosporus	4	19.05
Aspergillus niger	3	14.29
Mucor hiemalis	3	14.29

Pathogenicity Test

All five fungal isolates successfully induced spoilage symptoms on inoculated healthy watermelon fruits within the 5-day observation period. No symptoms were observed in the negative controls throughout the experiment (Table 4). *F. oxysporum*, *A. flavus*, *A. niger*, and *R. oligosporus* all produced visible symptoms by Day 1, while *M. hiemalis* symptoms appeared on Day 2 and persisted through Day 5. Fungi re-isolated from symptomatic tissues were morphologically identical to the original inocula, thereby fulfilling Koch's postulates. Typical symptoms included

tissue softening, discolouration, surface mycelial growth, and a characteristic musty odour at the inoculation site.

Table 4: Results of Pathogenicity Tests on Healthy Watermelon Fruits Inoculated with Isolated Fungal Species (Incubation at 30 ± 1°C for 5 Days)

Fungal Isolate	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Fusarium oxysporum	−	+	+	+	+	+
Aspergillus flavus	−	+	+	+	+	+
Aspergillus niger	−	+	+	+	+	+
Mucor hiemalis	−	−	+	+	+	+
Rhizopus oligosporus	−	+	+	+	+	+

Note: + = Spoilage symptoms present; − = No symptoms. Controls (wounded but not inoculated) remained symptom-free throughout.

Appendix: Sampling Location Codes

Table 5: Sampling Location Codes and Corresponding Sites in Sokoto Metropolis

Code	Sampling Location
A	Unguwar Rogo
B	Kasuwar Dan Kure Market
C	Kasuwar Daji Market
D	Kofar Marke
E	Kofar Aliyu Jodi
F	Runjin Sambo
G	Kofar Atiku
H	Gawon Nama
I	Guiwa Low-Cost

Discussion

This study isolated and identified five fungal species—*Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum*, *Rhizopus oligosporus*, and *Mucor hiemalis*—from spoiled watermelon fruits marketed in Sokoto metropolis, Nigeria. The recovery of these organisms from multiple sampling locations underscores the ubiquitous nature of postharvest fungal contamination in local market conditions, where temperature control and hygienic handling practices are generally inadequate.

The predominance of *A. flavus* (28.57%) in the present study is consistent with findings from comparable studies in the region. Tafinta (2009) similarly reported *Aspergillus* as the dominant genus in spoiled mango fruits from Sokoto markets, while El-Sharouny et al. (2018) identified *Aspergillus* spp. as major spoilage agents of watermelon across Egyptian markets. The high prevalence of this aflatoxigenic species is particularly alarming from a food safety perspective, as aflatoxin B1 is one of the most potent naturally occurring carcinogens known and can contaminate both the fruit tissue and juice of infected watermelons (Zhao et al., 2018). The warm, semi-arid climate of Sokoto, combined with inadequate storage infrastructure in local markets, likely creates conditions highly conducive to *A. flavus* proliferation and aflatoxin biosynthesis.

Fusarium oxysporum was the second most frequently recovered species (23.81%). This pathogen is a well-documented cause of crown rot, fruit rot, and vascular wilt in cucurbits globally (Guan et al., 2017). *F. oxysporum* has been shown to secrete pectin methyl esterase, polygalacturonase, and cellulase enzymes that degrade the middle lamella and primary cell wall of fruit tissues, leading to progressive tissue maceration and water-soaked lesions (Zhao et al., 2018). The finding of this species in the present study corroborates earlier reports of *Fusarium* spp. from spoiled oranges in Kano (Bukar et al., 2009) and from decaying cucurbit fruits elsewhere in Nigeria.

Rhizopus oligosporus (19.05%), recovered from four locations, is a member of the Mucorales order known to cause soft rot of cucurbits, characterised by rapid water-soaked tissue collapse. The presence of rhizoids facilitates firm anchoring to fruit surfaces and accelerates colonisation. *A. niger* and *M. hiemalis*, both recovered at a frequency of 14.29%, are cosmopolitan saprotrophic fungi commonly associated with the spoilage of various food commodities and fresh produce under warm, humid conditions (Pitt & Hocking, 2009). Although *A. niger* is primarily regarded as a spoilage organism causing black mould rot, it is also capable of producing ochratoxin A under certain conditions, adding a secondary food safety concern (Nogueira et al., 2018).

The pathogenicity tests confirmed that all isolated species were capable of inducing spoilage in healthy watermelon tissues, thereby satisfying Koch's postulates. The earlier symptom onset (Day 1) observed for *A. flavus*, *A. niger*, *F. oxysporum*, and *R. oligosporus* compared with *M. hiemalis* (Day 2) suggests variation in the aggressiveness and enzymatic capacity of these species. These findings are consistent with those of Baiyewu et al. (2007) and Chukwuka et al. (2010), who demonstrated similar pathogenic potential of *Aspergillus* and *Fusarium* species on pawpaw fruits in southwestern Nigeria.

Several factors may have contributed to the high fungal load observed in watermelons from Sokoto markets, including: (i) mechanical damage incurred during harvesting and transportation on unpaved roads; (ii) inadequate sanitation of market displays and equipment; (iii) the absence of cold chain infrastructure; and (iv) prevailing high ambient temperatures that accelerate fungal sporulation and growth. These findings highlight the urgent need for improved postharvest management at multiple points along the watermelon supply chain in the region.

A limitation of the present study is that fungal identification was based solely on classical morphological criteria. Molecular identification using ITS (internal transcribed spacer) rDNA sequencing would provide greater taxonomic

resolution and confirm species-level designations (Liu et al., 2018). Future studies should also include mycotoxin profiling of contaminated fruits to fully quantify the food safety implications of fungal spoilage for consumers in Sokoto metropolis.

CONCLUSION

The present study identified *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum*, *Rhizopus oligosporus*, and *Mucor hiemalis* as the principal fungal species responsible for postharvest spoilage of watermelon fruits marketed in Sokoto metropolis, Nigeria. *Aspergillus flavus* was the most prevalent species and represents a significant food safety hazard due to its aflatoxin-producing capacity. All isolates demonstrated pathogenicity on healthy watermelon fruits, confirming their aetiological role in spoilage. These findings underscore the necessity of adopting improved postharvest practices—including prompt cooling of harvested fruits, careful handling to minimise mechanical injury, enforcement of market hygiene standards, and consumer education—to reduce fungal spoilage losses and safeguard public health. Molecular characterisation of isolates and mycotoxin analyses are recommended as priorities for future research.

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