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MORPHOLOGICAL IDENTIFICATION OF FUNGI ASSOCIATED WITH SPOILED SWEET ORANGES AND BITTER LEMONS IN KATSINA LOCAL GOVERNMENT, KATSINA STATE

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ABSTRACT

Postharvest spoilage of fruits causes significant food losses globally. This study identified fungal species associated with the spoilage of sweet orange (Citrus sinensis) and bitter lemon (Citrus limon) in Katsina metropolis, Nigeria. Sixteen spoiled fruit samples were purposively collected from markets and farms. After surface sterilization with 1% sodium chloride, infected tissues were cultured on Potato Dextrose Agar (PDA) and incubated at 37°C for 3–7 days. Fungal isolates were identified based on macroscopic (colony morphology and pigmentation) and microscopic (spore and hyphal structure) characteristics. The fungi identified included Penicillium oxalicum, Aspergillus flavus, Rhizopus stolonifer, Fusarium incarnatum, Mucor fragilis, Endomyces, and Curvularia. These were linked to spoilage symptoms such as soft rot, black rot, and brown discoloration. Penicillium oxalicum and Aspergillus flavus were most prevalent, with the latter being notable for its aflatoxin production, posing serious food safety risks. Rhizopus stolonifer was associated with soft rot, while Fusarium incarnatum produced pink-centered colonies. Yeast-like Endomyces appeared in early spoilage of sweet lemons, contributing to fermentation and off-flavors. Differences in fungal colonization were observed: sweet lemons favored yeast and Aspergillus species due to higher sugar content, while bitter lemons, though less penetrable, supported aggressive molds like Rhizopus and Penicillium. These findings highlight the complexity of fungal spoilage and the importance of early detection, proper storage, and handling to prevent postharvest losses and mycotoxin contamination in citrus fruits.

Keywords: Postharvest Spoilage, Citrus Fruits, Sweet Orange, Bitter Lemon, Fungal Species.

1. INTRODUCTION

Microorganisms and fruits have a close, long-term relationship, Symbolic microorganisms in fruit crops (Singh et al., 2020). Microorganisms are associated, in a variety of ways with all the food we eat. They may influence the quality availability, and quantity of our food. Microorganisms live on the surface of fruit (Kumar et al., 2020). Microorganisms lives within the fruits tissue (Liu et al., 2020). The importance of fruit in human nutrition cannot be overestimated as it provides essential growth factors such as vitamins and minerals necessary for proper body metabolism. Fruit consumption reduces cardiovascular disease risk by lowering blood pressure, cholesterol, and inflammation (Aune et al., 2020). Fruit intake reduces age-related muscular degeneration risk, possibly due to antioxidant and anti-inflammatory effects (Zhang et al., 2020). Lemons (Citrus limon) are rich in a variety of phytochemicals—such as polyphenols, terpenes, and tannins—which contribute to their antioxidant and antimicrobial activities. Recent studies, such as that by Daba et al., (2023), have shown that these compounds are present in significant amounts in various lemon varieties and play a role in plant defense mechanisms as well as human health benefits. According to the analysis by Olatunde et al., (2022), lemon juice contains a higher concentration of citric acid—ranging from approximately 3.2 to 44.6 g/L—when compared to lime, grapefruit, and orange juices, making it more acidic and potentially more inhibitory to microbial growth.

Fruits remain a primary source of nutrition for both humans and animals due to their rich composition of sugars, vitamins, minerals, and amino acids. However, as noted by Adegoke and Afolayan (2021), these same properties—combined with their naturally low pH—create ideal conditions for fungal growth and colonization. Spoilage due to fungal contamination remains a significant concern in fruit production, particularly in post-harvest stages. Bhilwadikar et al., (2019) estimate that approximately 30% of fruit and vegetable yield is lost annually to microbial spoilage, especially in regions with poor post-harvest handling and storage systems.

Furthermore, fungal spoilage is not limited to visual and sensory deterioration. Many fungi produce mycotoxins—secondary metabolites that pose severe health risks to humans and animals. For instance, Aspergillus species, commonly found on spoiled fruits, are known to produce aflatoxins and other toxic compounds that are carcinogenic and immunosuppressive (Viegas et al., 2020). In addition, the global rise in temperatures and humidity is expected to exacerbate the spread of toxigenic and pathogenic fungi. Recent reports suggest that fungal threats like Aspergillus fumigatus are adapting to environmental changes and may increasingly affect populations in new regions (New York Post, 2025). This research aimed at identifying and isolating microorganisms responsible for contamination of sweet orange and bitter lemon through the objectives: i. To Isolate and identify the fungal organisms responsible for the post harvest of spoilage of sweet oranges and bitter lemon. ii. To determine the frequency of occurrence of microorganisms.

2. MATERIALS AND METHODS

Study Area

The study took place in the Katsina metropolis, within Katsina Local Government Area, Nigeria, situated at approximately 12.99080°N latitude and 7.60180°E longitude.

Sample Collection

Spoiled fruits of sweet orange and bitter lemon were purposively collected from selected markets and farms located within Katsina Local Government Area, Katsina State, Nigeria. A total of sixteen (16) visibly spoiled citrus fruit samples—comprising both sweet and bitter varieties—were gathered. The fruits were transported to the Biology Laboratory of Umaru Musa Yar'adua University in sterile polyethylene bags to prevent external contamination.

Sample Preparation

Upon arrival at the laboratory, the fruits were first rinsed with distilled water to remove surface debris and then dried using sterile paper towels under aseptic conditions. The fruit surfaces were further disinfected using a 1% sodium hypochlorite (NaCl) solution to eliminate surface microorganisms. Subsequently, the fruits were peeled, and both visibly infected and adjacent apparently healthy tissues were excised and cut into approximately 1 cm² sections for thorough fungal screening.

Culture Medium

Potato Dextrose Agar (PDA) was used as the culture medium for fungal isolation. The medium was composed of the following constituents (Al-Hindi, 2011):

Potato extract – 4.0 g

Dextrose – 20.0 g

Agar – 15.0 g

Distilled water – 1 liter

To prepare the medium, 39 g of PDA powder was weighed and dissolved in 1 liter of distilled water. Additionally, 2 ml of chloramphenicol was added to inhibit bacterial growth. The mixture was sterilized by autoclaving at 121°C for 15 minutes. After cooling to a manageable temperature, the sterilized medium was poured into pre-sterilized 9 cm diameter Petri dishes and allowed to solidify.

Inoculation and Incubation

Tissue samples (1 cm²) were aseptically placed on the surface of solidified PDA in sterile Petri dishes using a sterile scalpel. The inoculated plates were incubated at 37°C for 3 to 7 days to promote the growth of fungi (Oladipo et al.,2022).

Observation and Isolation of Fungi

Fungal colonies emerging from the inoculated tissues were observed daily for morphological changes. Distinct colonies were sub-cultured onto fresh PDA plates using the aseptic transfer method to obtain pure fungal isolates. The protocol for isolating and identifying fungi associated with the spoilage of sweet orange (Citrus sinensis) and bitter lemon (Citrus limon) was adapted from the methodology described by Oladipo et al., (2022) in the Journal of Food and Science Technology.

Microscopic and Macroscopic Characterization

Pure fungal isolates were subjected to both macroscopic and microscopic examination for identification:

Macroscopic features such as colony color, texture, shape, and growth pattern were documented.

Microscopic features were assessed using lactophenol cotton blue staining. Fungal structures, including conidia, hyphae, and spore arrangements, were examined under a compound light microscope.

Fungal identification was achieved by comparing observed features to standard taxonomic keys and fungal identification manuals.

3. RESULT AND DISCUSSION

Physical symptoms observed on sweet orange samples (SWT1–SWT8)**,** which were likely affected by fungal spoilage. Each sample is described by the type of rot; the rot varies in texture-hard, soft, smooth which may suggest different stages of infection or involvement of different fungal species and its appearance. All symptoms involve brown rot**,** indicating a similar type of decay**,** possibly caused by related fungi. Deep brown rot" suggests more aggressive or advanced fungal activity**,** potentially from more invasive fungal pathogens. In morphological identification**,** fungi are often distinguished based on the symptoms they produce on hosts, alongside microscopic features like spore shape, hyphae type, and colony color/texture. Macroscopic examination was reported by (Abubakar et al., 2023) on Citrus sinensis were he reported a similar result that Aspergillus niger, Aspergillu sfugumatus, Aspergillus flavus and Rhizopus stolonifer are black colors with white edges growth recognized within three days and consist of felt green and white coloration, a powdery masses of yellowish green spores on the upper surface and reddish gold on the lower surface, the colonies were white and cottony later became dark brown respectively. (Tafinta et al., 2014) in a similar study conducted on sweet orange (Citrus sinensis) in Sokoto state Nigeria also reported that From the incubated plates the different fungal isolates with different colorations observed includes; (i) Brown (ii) Black (iii) Green and (iv) White which signified the occurrence of different fungal colonies Detailed result is shown in the table 1 below

Table 1: Macroscopic Identification of Fungi Infesting Sweet Orange

SAMPLES	SYMPTOM TYPE	MORPHOLOGICAL CLUES	FUNGAL CANDIDATES
SWT1	Hard brown rot	Dry, firm tissue; likely slow penetration	Phomopsis, Lasiodiplodia
SWT2	Brown soft rot	Watery, mushy tissue; rapid decay	Penicillium, Fusarium, Rhizopus
SWT3	Brown soft rot	Watery, mushy tissue; rapid decay	Penicillium, Fusarium, Rhizopus
SWT4	Smooth brown rot	Even rot without sporulation on surface	Early-stage Alternaria or Colletotrichum
SWT5	Deep brown rot	Extensive internal rot; often blackening	Botryodiplodia theobromae, Alternaria
SWT6	Deep brown rot	Extensive internal rot; often blackening	Botryodiplodia theobromae, Alternaria
SWT7	Hard brown rot	Dry, firm tissue; likely slow penetration	Phomopsis, Lasiodiplodia
SWT8	Brown soft rot	Watery, mushy tissue; rapid decay	Penicillium, Fusarium, Rhizopus

The variety of symptoms observed on the bitter lemon samples indicates the presence of multiple fungal species, each exhibiting distinct morphological traits. This symptom-based data is crucial in guiding laboratory identification through: Colony appearance on growth media (PDA), Microscopic characteristics (spore shape, septation), Pigmentation and sporulation patterns. Such morphological assessments, when paired with symptom observation, form a strong foundation for identifying the causative fungal agents of citrus spoilage. Soft brown/black soft rots; indicate flesh breakdown, often associated with fast-growing fungi like Rhizopus or Fusarium. Hard brown rot indicates a more localized infection, possibly from fungi like Phomopsis or Lasiodiplodia theobromae species that cause dry, firm lesions. Black rots; often linked with darkening of tissues and dry**,** leathery decay. Likely fungal culprits: Alternaria spp., Aspergillus spp., or Guignardia citricarpa (black rot pathogen in citrus). Smooth brown rot suggesting non-sporulating or early-stage infection, where rot is progressing evenly without obvious fungal outgrowth. In a study conducted by (Akinmusire et al., 2022) in Gamboru market Maiduguri, Nigeria the Observed symptoms were (soft, mushy texture, brown or black spots). The detailed is shown in table 2 below

Table 2: Macroscopic Identification of Fungi Infesting Bitter Lemon

SAMPLES	SYMPTOM TYPE	MORPHOLOGICAL CLUES	FUNGAL CANDIDATES
BIT1	Soft rot	Cottony mycelium, fast growth, sporangia/spores	Rhizopus, Fusarium
BIT2	Black soft rot	Wet rot with black color, possibly with dark sporulation	Alternaria, Aspergillus, Cladosporium
BIT3	Soft brown rot	Moist lesions, light pigmentation, curved/spindle spores	Fusarium, early-stage Colletotrichum
BIT4	Hard brown rot	Firm texture, limited spread, dark brown hyphae	Phomopsis, Lasiodiplodia
BIT5	Black rot	Wet rot with black color, possibly with dark sporulation	Alternaria, Aspergillus niger
BIT6	Hard brown rot	Firm texture, limited spread, dark brown hyphae	Phomopsis, Lasiodiplodia
BIT7	Black soft rot	Wet rot with black color, possibly with dark sporulation	Alternaria, Aspergillus, Cladosporium
BIT8	Smooth brown rot	Uniform rot, slow sporulation	Colletotrichum, Botryosphaeria

The morphological characterization of fungal colonies isolated from spoiled bitter and sweet lemons revealed a diverse community of spoilage organisms, including both filamentous molds and yeast-like fungi. These organisms were identified based on their colony appearance and microscopic features, which provide critical insights into their taxonomy and potential roles in fruit spoilage. Among the isolates, Penicillium oxalicum, Aspergillus flavus, and Rhizopus stolonifer were notable for their rapid growth, distinctive colony colors, and clear morphological structures under microscopic examination. Penicillium oxalicum produced velvety, greenish-blue colonies with a brush-like arrangement of phialides and conidia, consistent with its classification in the Penicillium genus. This fungus is commonly associated with postharvest citrus decay and can produce mycotoxins, making it significant not only in terms of spoilage but also food safety. Aspergillus flavus was identified by its fast-growing colonies and characteristic yellow to dark green pigmentation. Microscopically, the presence of globose vesicles and thick-walled conidia confirmed its identity. This species is a major concern in citrus spoilage due to its ability to produce aflatoxins, potent carcinogenic compounds that pose serious health risks. (Buah et al., 2024) also report similar morphological features for Aspergillus flavus with White colony with light yellow-green later becoming dark- yellow-green Rhizopus stolonifer recognized by its cottony white colonies that later turned black due to sporangia development, is a common agent of soft rot in fruits. Its non-septate hyphae and rhizoid structures were consistent with known Rhizopus morphology (Buah et al., 2024) also report similar morphological features for Rhizopus stolonifer with White cottony at first brownish grey then blackish-grey and Yeast-like fungi such as Endomyces and generic budding yeast were also observed. These organisms typically dominate the early stages of spoilage, particularly in sweet lemon, which has a higher sugar content that supports rapid yeast fermentation. Endomyces formed large white colonies with cylindrical arthrospores, while the generic yeast exhibited budding cells with narrow-thread attachments. These traits are typical of fermentative yeasts involved in fruit deterioration and off-flavor development. Other molds identified included Fusarium, Cladosporium, Curvularia, and Mucor. Fusarium inacarnatum presented with pink-centered colonies and sickle-shaped conidia, indicating a potential for mycotoxin production. Cladosporium and Curvularia were slower growing and less aggressive but are often found on stored or overripe fruits. Mucor fragilis. was characterized by its cottony white growth and non-septate hyphae, frequently associated with soft rot and advanced stages of spoilage (Buah et al., 2024) also report similar morphological features for Mucor fragilis with Greenish cotton like, colony like growth spotted with blackish color. The higher sugar content in sweet lemon may favor yeast and Aspergillus growth, while bitter lemon’s tougher rind and lower sugar content could initially limit colonization but still allow infiltration

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by aggressive molds like Rhizopus and Penicillium. Another study conducted by (Akinmusire et al., 2022) revealed that most of the spoilage occurring in sweet oranges in the Gamboru market Maiduguri, Nigeria was caused by Penicillium oxalicum as it was the most prevalent of the four isolates on the sweet oranges. The detailed is shown in the table below

Table 3: Macroscopic of fungal isolates from the fruit samples (Citrus sinensis and Citrus lemon)

ORGANISM IDENTIFIED	COLONY MORPHOLOGY	MICROSCOPIC FEATURES	SIGNIFICANCE IN SPOILAGE
Endomyces	Large white colonies	True mycelium divided into cylindrical arthrospores with round edges	Yeast-like fungi; known to spoil fruit through fermentation and enzymatic breakdown. White colonies may indicate early spoilage.
Penicillium oxalicum	Velvety to powdery, greenish-blue with pale yellow to brown reverse. Rapid growth.	Septate conidiophores, brush-like phialides, elliptical conidia in long chains	Common citrus fruit spoilage agent. Produces mycotoxins. Its rapid growth and colored colonies are easy to detect.
Fusarium inacarnatum	Pink center with white edges	Septate mycelia, sickle-shaped microconidia with multiple septa	Important postharvest pathogen; causes rot and produces harmful mycotoxins (e.g. fumonisins). The pink coloration is a telltale sign.
Mucor fragilis	White cottony colonies, darken as reproductive structures develop	Branched, non-septate hyphae; oval-shaped sporangia	Fast-growing mold; causes soft rot and often seen during advanced stages of fruit decay.
Aspergillus flavus	Rapid spread, yellow to dark green colonies; pear-shaped conidia	Septate, colorless hyphae; thick-walled conidia, globose vesicles	Highly significant due to aflatoxin production. A major concern in food safety.
Curvularia	Green, deeply grown colonies	Swollen conidia, septate horizontally, arranged in triplets	Less common but can still be found on citrus; environmental mold. Conidia arrangement is diagnostic.
Cladosporium	Slow-growing, olivaceous brown colonies	Distinct conidiospores, branched only at apices	Frequently isolated from stored fruits; not usually a major toxin producer, but can cause spoilage and aesthetic damage.
Yeast (generic)	Smooth, white yeast-like colonies	Oval, uninucleate, budding yeast cells; narrow-thread-like attachments	
Rhizopus	Cotton-like white growth with black spots	Sporangia with spores and rhizoids	

4. CONCLUSION

The study on fungal spoilage of sweet and bitter lemons (Citrus sinensis and Citrus lemon) identified a range of fungal species responsible for postharvest deterioration. Through both macroscopic and microscopic analyses, several fungi

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were found to be predominant in fruit spoilage. Endomyces, a yeast-like fungus, was characterized by large white colonies and cylindrical arthrospores. It is typically involved in the early stages of spoilage, especially in sweet lemons, where high sugar content supports rapid yeast fermentation. Penicillium oxalicum was notable for its greenish-blue colonies and rapid growth. This species is commonly associated with citrus spoilage and produces mycotoxins, which pose a serious food safety risk. Fusarium inacarnatum produces pink-centered colonies and is responsible for rot in citrus fruits. Its ability to produce mycotoxins like fumonisins makes it a significant postharvest pathogen. Mucor fragilis, a fast-growing mold, forms white cottony colonies and is associated with advanced stages of soft rot. Its non-septate hyphae and oval-shaped sporangia are indicative of its role in fruit decay. Aspergillus flavus, known for its yellow to dark green colonies and the production of aflatoxins, is a major concern for food safety due to its carcinogenic properties. Curvularia, identified by its green colonies and triplet-conidia arrangement, is less common but can contribute to spoilage in citrus fruits. Cladosporium, a slow-growing mold, is often found on stored fruits and can cause spoilage, though it does not typically produce harmful toxins. Generic yeasts, prevalent in sweet lemon samples, indicated early spoilage with their yeast-like colonies and budding cells. Rhizopus stolonifer, known for its rapid growth and black-spored cotton-like colonies, is a leading agent of soft rot in citrus fruits.

5. RECOMMENDATION

- **Monitoring and Early Detection**: Early detection of fungal pathogens such as Penicillium oxalicum, Aspergillus flavus, and Rhizopus stolonifer is essential to prevent widespread spoilage and mycotoxin contamination. Implementing regular monitoring of citrus fruits for these fungi using both visual and molecular diagnostic tools can help manage postharvest losses.
- **Fungicide Application**: The use of appropriate fungicides, such as those effective against Penicillium, Aspergillus, and Rhizopus, should be incorporated into postharvest handling protocols. This could significantly reduce spoilage and prevent mycotoxin production.
- **Use of Biological Control Agents**: Exploring the use of antagonistic fungi like Trichoderma spp. could provide an eco-friendly alternative to chemical fungicides. These fungi could suppress the growth of spoilage-causing molds in citrus fruits.

CONFLICT OF INTEREST

The author declare no conflict of interest

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