Isolation and Characterization of Fungi Associated with Orange Spoilage in Maiduguri, Borno State

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Abstract
Fruits play a vital role in human nutrition by supplying essential vitamins and minerals for maintaining good health. This research investigated fungal pathogens associated with orange fruit (*Citrus sinensis*) spoilage sold in four locations in Maiduguri, Borno State, Nigeria. The samples were surface sterilized with ethanol, and the homogenates were cultured on Potato Dextrose Agar and incubated aerobically at room temperature for 7 days at 30 °C. The pure cultures obtained were identified morphologically and microscopically. The investigations revealed that the samples were infected with several fungal species. The most predominant fungi isolated from oranges were *Aspergillus niger* and *Aspergillus* flavus having the highest frequency and distribution from all sampling points (25%). *Aspergillus oryzae* and *Rhizopus stolonifer* had 18.75% occurrences each, while Fusarium species had 12.5%. *Aspergillus niger*, *Aspergillus* flavus, *Rhizopus stolonifer*, and *Aspergillus oryzae* produced the same symptoms and signs as observed in the healthy orange fruits, except for Fusarium species, which were not able to grow and produced spoilage conditions on the inoculated healthy orange fruits after 5 days. *Aspergillus* species produce several toxic metabolites, like aflatoxins and ochratoxins, which are important toxins worldwide because of their hazards to human health. Therefore, precautions should be taken when handling spoilt orange fruits.

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1. Introduction

Fruits play a vital role in human nutrition by supplying essential vitamins and minerals for maintaining good health. Citrus sinensis (L.), commonly known as the sweet orange belonging to the Rutaceae family, is a major commercial fruit consumed widely as fresh fruit and juice due to its high vitamin C content and antioxidant potential [1]. Vitamin C, or ascorbic acid (ascorbate), is crucial for various bodily functions, including the hydroxylation of proline and lysine in collagen. A deficiency in vitamin C leads to scurvy, a disease characterized by the degeneration of connective tissues. Advanced scurvy manifests through symptoms such as as small hemorrhages due to fragile blood vessels, tooth loss, poor wound healing, reopening of old wounds, bone pain, and eventually heart failure. Even mild vitamin C deficiency can cause fatigue, irritability, and increased severity of respiratory tract infections. While most animals synthesize vitamin C from glucose in four enzymatic steps, humans and certain other animals—such as gorillas, guinea pigs, and fruit bats—lack the final enzyme in this pathway and must obtain ascorbate from their diet [2].

Citrus sinensis is primarily cultivated in tropical and subtropical regions, across more than 137 countries [3]. The global cultivation of oranges is a significant agricultural activity due to their high demand as both fresh fruit and processed juice. The economic importance of oranges is underscored by their contribution to the agricultural economies of many countries, providing

income for farmers and contributing to the gross domestic product (GDP). However, this fruit is vulnerable to various pathogens that significantly affect its quality. In developing countries, inadequate protection and handling of fresh fruit lead to losses that can exceed 50% of the harvested crop during transit and storage [4]. These losses are attributed to various factors, including poor infrastructure, a lack of cold storage facilities, and inefficient supply chain management. Even in developed countries, one of the limiting factors affecting the economic value of oranges is their relatively short shelf-life due to post-harvest pathogen-induced spoilage [5].

Spoilage microorganisms can be introduced at various stages: on the seed itself, during crop growth, harvesting, post-harvest handling, or during storage and distribution [6]. Post-harvest losses and decay of oranges are often traced to infections occurring between flowering and fruit maturity or during harvesting and subsequent handling and storage. Fungi, such as yeast and mold, are commonly associated with diseased and deteriorated citrus fruits [7]. These fungal pathogens not only cause direct damage to the fruit, reducing its market value, but also pose significant health risks through the production of mycotoxins. Beyond the immediate issue of mycotoxin contamination, the presence of fungi can lead to disease development in the field when infected seeds are planted, perpetuating the cycle of crop loss and quality degradation. This study was aimed at isolating and characterizing the fungi responsible for the spoilage of *Citrus sinensis* within the Maiduguri metropolis.

Materials and Methods

Collection of Spoiled and Fresh, Healthy Oranges

Spoiled oranges were collected from four different locations, namely Post Office, Tashan Bama, Monday Market, and Jiddari Bus Stop (4 oranges in each location) in Maiduguri metropolis, and healthy oranges (4 oranges per location) were later obtained for the pathogenicity test after the isolation of the fungi. All the samples collected were placed in sterile polythene bags, labeled separately, and transported to the laboratory for fungal isolation and analysis.

Isolation of Fungi

Fungal isolation was done as described by previously [8]. Serially diluted samples were introduced into the center of the appropriate Petri dishes. Sabouraud Dextrose Agar (SDA) was poured into the plates enough to cover the solution in them. The agar also had streptomycin (to prevent the growth of bacteria). The medium was allowed to solidify and then incubated in an inverted position at room temperature for 5 days for the development of colonies. The fungal colonies that emerged were continuously sub-cultured to obtain a pure culture of fungal isolates.

Characterization of fungi

The fungal isolates were identified by observing their macromorphological characteristics. This included examining the colony growth and colour, the presence or absence of aerial mycelium, the presence or absence of wrinkles and furrows, and the presence or absence of pigmentation under a microscope [9]. This was done by placing a drop of lactophenol blue stain on a glass slide that was free of grease. Then a sterile inoculating wire loop was used to pick up the mycelium from the mold culture and spread it evenly on the side. The mycelium was then separated through teasing to create a homogenous mixture. After gently covering the mixture with coverslips, it was allowed to sit for a few seconds before being observed under a microscope with an x40 magnification lens. The microscope examination focused on structures that bore spores and the presence or absence of septa in the actively growing mold [10].

Frequency of Occurrence and Distribution

The identity of each isolated fungus was recorded, along with the location where the sample was obtained. The frequency of occurrence was determined by dividing the number of samples in which the particular fungus is present by the total number of samples analyzed.

Pathogenicity Test

The pathogenicity or decay test was carried out as described by previously [11] to determine if the isolated fungi were responsible for the spoilage of orange fruit. Healthy oranges were surface sterilized with ethanol. Cylindrical plug tissues were cut out from the fruits using a sterilized 3 mm-sized cork borer. A plate containing a one-week-old fungal culture was inoculated into these holes, then covered and sealed off using petroleum jelly. The procedure was repeated separately for each of the fungal isolates. The inoculated samples and the control were placed in sterile polythene bags and incubated in an oven for 5 days. The point of inoculation of each type of fungus was examined and recorded. The diameters of the rotten portions of the oranges were measured. The fungi were later re-isolated from the inoculated fruits and compared with the initial isolates.

Results and Discussion

Table 1 showed that *Aspergillus niger* and *Aspergillus* flavus were the most prevalent, each with a 25% occurrence across all four locations: Post Office, Tashan Bama, Monday Market, and Jiddari Bus Stop, respectively. *Aspergillus oryzae* and *Rhizopus stolonifer* were next in prevalence, each accounting for 18.75% of the isolates. *Aspergillus oryzae* was found in Tashan Bama and Jiddari Bus Stop, while *Rhizopus stolonifer* was present in the Post Office, Monday Market, and Jiddari Bus Stop. Fusarium species were the least frequently isolated, with a 12.5% occurrence. They were found at the Post Office, Tashan Bama, and Monday Market but were absent at the Jiddari Bus Stop. The study revealed the presence of several fungal species associated with orange spoilage in different locations within the Maiduguri metropolis. These fungi were consistently isolated from all sampled sites, indicating their widespread distribution and their significant role in orange spoilage. This distribution suggests that, while *Aspergillus oryzae* and *Rhizopus stolonifer* were also common, they may have more specific environmental or geographical preferences compared to *Aspergillus niger* and *Aspergillus* flavus. The lower frequency and limited distribution of Fusarium species suggest they may be less competitive or less adapted to the conditions in some of the sampled locations. Some of these fungi have been isolated from other fruits in Nigeria [12, 13]. Fungal fruit infection may occur during the growing season, harvesting, handling, transport, post-harvest storage and marketing conditions, or after purchase by the consumer. Orange fruits contain high levels of sugars and nutrients, and their low pH makes them particularly desirable for fungal decay [14]. In a similar study [15], *Aspergillus niger*, Candida

tropicalis and Alternaria nees was identified in spoilt orange, Fusarium incarnatum, Mucor fragilis, and *Rhizopus stolonifer* was found in spoilt banana while *Aspergillus niger*, Colletoricum gloeosporioides, and Penicillium chrysogenum were found in spoilt mango, oranges and banana. Some of these fungi species have been reported to contain potential mycotoxins which can lead to health complications for both humans and animals [16].

The pathogenicity test (Table 2) showed the progression of rot in fresh, healthy orange samples inoculated with the different fungal isolates. The diameter of rot over 5 days provides insight into the aggressiveness and spoilage potential of each fungus. *Aspergillus niger* exhibited the highest pathogenicity, with a rot diameter of 62 mm by Day 5. The onset of rot was observed by Day 2, and the rapid progression indicates that *Aspergillus niger* is highly virulent and capable of causing significant spoilage in a short period. *Aspergillus* flavus also showed considerable pathogenicity, with a rot diameter of 45 mm by Day 5. Similar to *Aspergillus niger*, the rot was first noted on Day 2, highlighting its ability to quickly infect and degrade the fruit. *Aspergillus oryzae* and *Rhizopus stolonifer* displayed moderate pathogenicity. *Aspergillus oryzae* produced a rot diameter of 30 mm by Day 5, while *Rhizopus stolonifer* resulted in a 24 mm diameter. The onset of rot for both fungi was observed on Day 3, indicating a slightly slower progression compared to *Aspergillus niger* and *Aspergillus* flavus. Interestingly, Fusarium species did not induce any rot within the 5 days, suggesting that under the conditions of this study, they may be less pathogenic or require different conditions to manifest their spoilage potential. These findings align with those of other researchers [11], where *Rhizopus stolonifer* had the highest diameter of 45mm after 14 days of incubation. Citrus fruits, due to their low pH, high moisture content and nutrient composition, are very susceptible to attack by pathogenic fungi, which in addition to causing decay, may also make them unfit for consumption by producing mycotoxins [17].

Conclusion

In conclusion, the rapid progression of rot caused by these fungi underscores the need for effective management strategies to control their spread and minimize post-harvest losses. Given the moderate pathogenicity of *Aspergillus oryzae* and *Rhizopus stolonifer***,** targeted interventions may be necessary to address the specific environmental conditions that favor their growth. The limited pathogenicity of Fusarium species suggests that they may pose a lesser threat under typical storage conditions; however, their presence still warrants monitoring to prevent potential spoilage under varying conditions. Therefore, precautions should be taken when handling spoilt orange fruits.

Table 1: Occurrence and distribution of fungal isolates from spoiled oranges

Fungal Isolate	Location				Percentage of Occurrence (%)
	Post Office	Tashan Bama	Monday Market	Jiddari Bus Stop	
Aspergillus niger	+	+	+	+	25
Aspergillus flavus	+	+	+	+	25
Aspergillus oryzae	-	+	-	+	18.75
Rhisopus stolonifer	+	-	+	+	18.75
Fusarium species	+	+	+	-	12.5

+ represents presence, while – represents absence.

Table 2: Pathogenicity of fungal isolates on fresh, healthy orange samples

Fungal Isolate	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Diameter of Rot (mm)
Aspergillus niger	-	-	+	+	+	+	62
Aspergillus flavus	-	-	+	+	+	+	45
Aspergillus oryzae	-	-	+	+	+	+	30
Rhisopus stolonifer	-	-	-	+	+	+	24
Fusarium species	-	-	-	-	-	-	-

+ = isolates grow with similar growth characteristics to the original diseased samples.
- = isolates were not able to grow on the sample.

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