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Assessment of Fungal Species Associated With Banana and Orange Spoilage in Kwali Market Abuja, Nigeria

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ABSTRACT
The research was conducted to determine the fungal species responsible for the spoilage of bananas and oranges in Kwali market, Abuja, Nigeria. Samples of the diseased fruits: Orange (*Citrus sinensis*) and banana (*Musa paradisiaca*) were collected randomly in Kwali market aseptically. The samples were surfaced sterilized and the homogenates were cultured on Potato Dextrose Agar (PDA) and incubated at 28 ^{o}C for 5 days. The pure cultures obtained were identified morphologically using high and low-resolution objectives of a microscope. Data obtained was analyzed in frequency and percentage. The result of isolation is presented in frequency and percentages. The result obtained revealed the presence of five fungal genera represented by eight species associated with the fruit's spoilage. The most predominant fungal species are *Aspergillus niger* and *Rhizopus stolonifer* with 25.00% and 20.83% in orange and 33.33% and 22.22% in banana respectively. The pathogenicity test shows that *Rhizopus stolonifer* and *Aspergillus niger* induce the highest rotten diameter (41 mm) on bananas, however, *C. herbarum* was found to have the lowest rotten diameter of 10 mm in diameter on oranges. Thus, spoilage of bananas and oranges in Kwali market, Abuja, Nigeria was caused by eight fungal species, the most prominent of which are *Aspergillus niger* and *Rhizopus stolonifer*.

Keywords*:* Aspergillaceae; Banana; Fungi; Kwali market; Orange

INTRODUCTION
Bananas (*Musa paradisiaca* L.) and sweet orange (*Citrus sinensis* L.) are the two prominent commercial fruit crops widely eaten fresh or taken as juice which are easily deteriorated by several fungal diseases causing significant losses (Ali *et al.*, 2021). These fruits are vended throughout all the markets in Nigeria. They are mostly cultivated in Nigeria (Karoui and Marzouk, 2013). The fruits are of immense economic value; occupying the top position in fruit production and providing substantial amounts of vitamins essential for healthy living (Atolani *et al.*, 2012), carotenoids, and a wide array of phytochemicals with strong potential for use in drug production or as food supplements (Chede *et al*., 2013; Lawal *et al.*, 2013). The consumption of these fruits is also believed to confer some protection against cardiovascular diseases and cancers. The presence of dietary fiber in these fruits helps to reduce the chance of gastrointestinal problems such as constipation and diarrhea (Uzuazokaro *et al.*, 2018).

Bananas and sweet oranges are susceptible to spoilage by several microbial pathogens causing severe economic losses (Onuorah *et al.*, 2015). Previous studies (Udoh *et al.*, 2015: Imam *et al.*, 2019) have shown that several species of fungi were associated with banana and orange spoilage. The vulnerability of these fruits to fungal attack can be attributed to the high concentration of moisture and relative amount of fiber, crude protein, ash, lipid, and carbohydrate.

Fungal species were found to be the most active microbes as they tend to contaminate the fruits right from the field and in the course of subsequent handling (Kuyu and Tola, 2018). Other sources of postharvest contamination were either through mishandling or lack of proper storage measures as those adopted in the markets. Due to economic constraints, many people choose to go for cheap oranges and banana fruits that are half deteriorated or show decayed symptoms as they are cheap; thereby transferring a high microbial load onto the final consumer with the imminent risk of infection and illness. This study therefore aimed at determining the fungal species associated with the spoilage of these fruits in Kwali market, Abuja, Nigeria.

MATERIALS AND METHODS

Samples Collection

A total of one hundred and twenty (120) spoilt banana and sweet orange samples and forty (40) healthy fruits were randomly procured from the Kwali market. The samples were collected aseptically using hand gloves into sterile polythene bags and labeled accordingly. The samples were taken to the Biology Laboratory, Baze University, Abuja, Nigeria. The spoilt fruits were identified by morphological examination using the method of Mukhtar *et al.* (2019). The samples were kept in the refrigerator at -4 ^{o}C before Laboratory analyses.

Isolation of Fungal Species from Spoilt Fruits

The infected fruits were sterilized with cotton wool soaked in 1% hypochlorite for 2 minutes and then rinsed 3 times with distilled water. A sterile blade was used to cut a small section (2 mm2) of the decayed portions and placed in 9 ml of sterilized distilled water to form the aliquot. The exponential dilution was carried out to the fourth factor. The volume of 0.1 ml of the third factor was aseptically transferred onto Petri dishes containing sterile PDA containing Chloramphenicol (30 mg/l) and spread by using a bent glass rod and incubated at 28° C for 5 days. Colonies observed were sub-cultured on PDA using a similar method as described above.

Identification of fungal Isolates

The identification of the isolates was done by examining the isolates macroscopically and microscopically. For macroscopic characteristics, colony characteristics such as colony appearance, change in medium color, and growth rate were observed. The microscopic examination was carried out using adding 2 drops of Lacto phenol cotton blue on a clean grease-free slide, a little portion of the fungal hyphae was put on the phenol drop and covered with a cover slip. A pure culture of each colony type on each plate was obtained and maintained. The maintenance was done by sub-culturing each of the different colonies onto the PDA slants in McCartney bottles and incubating them at room temperature again for 5 days as described by Kator *et al. (*2018**)**. Fungal colonies observed were microscopically examined under high and low-resolution objectives to confirm the types of fungus in each colony using cultural, and morphological and comparing them with confirmed representatives of the different species as described by Ewekeye *et al.* (2013).

Pathogenicity Test

A pathogenicity or decay test was carried out to know if the isolated fungal species were responsible for the spoilage of apple, banana, orange, and watermelon fruits described by Kator *et al. (*2018). Healthy fruits were surface sterilized with ethanol. Cylindrical plug tissues were cut out from the fruits using a sterilized 2 mm-sized cork borer.

Agar plates containing a week-old fungal culture were aseptically placed in these holes, then covered and sealed off using petroleum jelly. The procedure was repeated separately across each of the fungal isolates. The inoculated samples and the control were placed in sterile polythene bags and incubated in an incubator for 5days. The point of inoculation of each type of fungus was examined and recorded. The diameter of the rotten portion of the orange and banana fruits was measured. The fungi were later re-isolated from the inoculated fruits and compared morphologically with the initial isolates.

Statistical Analysis

The data collected were analyzed statistically using frequency and percentages. Statistical Analysis Software (SAS, 2012) Version 9.0 was used for the analyses.

RESULTS

The result for the morphological characterization of the fungal isolates obtained from the spoilt banana and orange fruits vended in Kwali market Abuja, Nigeria is presented in Table 1. The result revealed the presence of Five genera represented by eight (8) different fungal species associated with the spoilage of these fruits in the Kwali market. The species identified were: *Aspergillus niger, A. fumigatus, A. flavus, Rhizopus stolonifer, R. nigricans, Fusarium oxysporum, Cladosporium herbarum,* and *Alternaria alternata*.

The distribution of the fungal species in the two fruits obtained from the Kwali market is shown in Table 2. The result showed that *Aspergillus niger* is the most predominant fungal species found in bananas with 33.33%. It is followed by *Rhizopus stolonifer* with 22.22%. Two fungal species: *Rhizopus nigricans* and *Cladosporium herbarum* are the least dominant species with 7.41% abundance each.

Table 1: Morphological Characterization of fungal Isolates from Abuja Markets

S/N	Fungal Species	Morphological Characterization	Microscopic Characterization
1	*Aspergillus niger*	Black and white cotton like structure	Thick septate hyphae with conidia borne in chains from the sterigmata
2	*Aspergillus fumigatus*	Gray colour with smooth surface	Septate hyphae with conidiospores
3	*Aspergillus flavus*	White colony with light yellow green, become dark yellow-green	Septate hyphae with Radiate conidial head, coarse rough long vesicular conidia
4	*Rhizopus stolonifer*	Whitish cottony like structure	Aseptate hyphae irregular in size with distinct stolon
5	*Rhizopus nigricans*	Dark colony	Aseptate hyphae with columnar hemispherical aerial sporangia
6	*Fusarium oxysporum*	Pink and white edge cottony growth	Septate hyphae with oval microconidia produced on branched conidiosphore
7	*Cladosporium herbarum*	Thick, velvet colony with lemon shaped conidia variously branched	Septate brown hyphae with erect conidiophores
8	*Alternaria alternata*	Black fluffy growth with white edges	Erect conidiophores, septate hyphae with cylindrical conidia

Table 2: Distribution and Prevalence of Fungal species in Spoilt Banana and orange Fruits in Kwali Market

Fungal Species	Orange	Banana
Aspergillus niger	6 (25.00%)	9 (33.33%)
Aspergillus fumigatus	4 (16.67%)	4 (14.82%)
Aspergillus flavus	2 (8.33%)	-
Rhizopus stolonifera	5 (20.83%)	6 (22.22%)
Rhizopus nigricans	3 (12.50%)	2 (7.41%)
Fusarium oxysporum	2 (8.33%)	4(14.82%)
Cladosporium herbarum	-	2 (7.41%)
Alternaria alternate	2 (8.33%)	-
Total	24 (100%)	27 (100%)

Furthermore, the result of the pathogenicity test (Table 3) shows that *Rhizopus stolonifer* and *Aspergillus niger* induce the highest rotten diameter on banana (41mm). However, *C. Herbarum* was found to have the lowest rotten diameter of 10 mm in diameter on orange. *Aspergillus flavus* and *A. fumigatus* induced a higher degree of spoilage in orange.

Table 3: Pathogenicity of Fungal Isolates on the Selected Fruits showing diameter of rots (mm)

Fungal Species	Orange	Banana
Aspergillus niger	32	41
Aspergillus fumigatus	24	20
Aspergillus flavus	28	12
Rhizopus stolonifera	33	41
Rhizopus nigricans	28	35
Fusarium oxysporum	30	24
Cladosporium herbarum	10	24
Alternaria alternate	21	25

DISCUSSION

Bananas and oranges are two fruits that play a vital role in human nutrition by supplying necessary growth factors such as vitamins and essential minerals in daily diet which help to live a healthy life as stressed by Al-Hindi *et al.* (2011). Besides all these, there is an increasing concern about the use of fruits in diet therapy for certain clinical diseases. However, despite all these tremendous benefits of fruits to human well-being, certain fungal attacks threaten the shelf-life of fruits and degrade their economic value. The present study reported *Aspergillus niger, A. fumigatus, A. flavus, Rhizopus stolonifer, R. nigricans, Fusarium oxysporum,* and *Alternaria alternata* as the species responsible for spoilage of oranges in the study area. This finding agrees with the previous finding of Kator et al. (2018) who reported *Aspergillus niger, A. fumigatus, A. flavus, and Rhizopus stolonifer* as the fungal species responsible for orange spoilage in Buruku Local Government Area of Benue State.

More so, *Aspergillus flavus*, *Rhizopus stolonifer, Aspergillus niger, Aspergillus fumigatus,* and *Fusarium oxysporum* were reported by previous work of Akinro *et al*. (2015) in orange, Similarly, Mailafia *et al*. (2017), Lema *et al*. (2018) and Mukhtar et al. (2019) individually reported *Aspergillus* spp., *Rhizopus* spp., *Fusarium* spp, and *Alternaria* spp among fungal species responsible for orange spoilage.

However, six fungal species: *Aspergillus niger, A. fumigatus, Rhizopus stolonifer, R. nigricans, Fusarium oxysporum,* and *Cladosporium herbarum* were reported to be associated with banana spoilage. This finding is in agreement with that of Kuyu and Tola (2018) who reported similar fungal species among those isolated from rotten banana fruits. A similar finding was reported by Sani and Kasim (2019) who isolated *Aspergillus* spp, *Rhizopus* spp, and *Fusarium* spp from spoilt banana fruits. The presence of a vast array of fungal species associated with orange and banana fruits in the Kwali market is a clear indication of the role played by these fungal species in their spoilage. This is in agreement with the finding of Onyemata and Ibrahim (2018) who reported that fungi are the major cause of fruit spoilage. Fruits contain high levels of sugars and nutrients element and their low pH values make them particularly desirable to fungal infection as stressed by Singh and Sharma (2007).

The presence of *Aspergillus fumigatus, Aspergillus niger, Aspergillus flavus, and Rhizopus stolonifer* as the major dominant fungal species that caused spoilage of bananas and oranges by the present study conforms to the finding of Tafinta *et al*. (2013) who reported similar finding of the fungal species responsible for orange spoilage in Sokoto state. These fungal species produce mycotoxins that are toxic to man and other animals. Similarly, Mwekaven *et al*., (2019) reported these fungal species as the dominant species causing tomato spoilage in Gboko metropolis, Nigeria. Oviasogie *et al*. (2015) also reported *Aspergillus* species and *Alternaria* species as the predominant species causing spoilage of oranges. The presence of these fungal species as spoilage-causing organisms of various fruits was previously reported by researchers such as Mbajiuka *et al*. (2014) and Ezikanyi (2016).

Aspergillus niger is reported by the present study to be more predominant in all the fruits. This finding conforms with the findings of Yaradua *et al*. (2018) who reported a high prevalence of *Aspergillus flavus, Rhizopus stolonifer, and Aspergillus niger* obtained from fruits in Kankara Local Government Area, Katsina State, Nigeria. The presence of *Aspergillus spp*., and *Rhizopus spp*. as fruit spoilage agents in this research conformed with the findings of Ewekeye *et al*. (2013) among the spoilt fruits vended in some selected markets in Lagos. More so, the presence of 3 different species of Aspergillus: *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus fumigatus* among the spoilt fruits indicated the degree of pathogenicity conferred by the members of the family Aspergillaceae in inducing fruits spoilage.

The high prevalence of these fungal species on the fruits may probably be because most of the fruits under study had high acid content, which is a contributing factor to the growth of fungi. This is in line with the work of Barth *et al.* (2009) who reported that fungi can be introduced into the crop on the seed itself, during crop growth in the field, during harvesting and postharvest handling, or storage and distribution and moisture content appeared to be one of the major factors that support fungal growth in most of the fruits under study.

More so, most of these fungal isolates were associated with the market conditions that favor contamination and can be worsened by poor hygiene of the vendors, using microbial unsafe and contaminated containers, poor handling practices, and poor environmental conditions such as sanitarily unsafe marketing environment. This agrees with the finding of Gultie *et al.*, (2013) who asserted that the consequence of the problems could be increased loss of fruit due to microbial spoilage and the existence of some human pathogens stressed by Ahmed *et al*. (2011).

The pathogenicity test reported by this study revealed that all the fungi isolated and identified in the study were pathogenic to the fruits causing decaying of the fruits. Thus, all the fungal isolates were observed positive for causing spoilage of fruits. The result of the pathogenicity test also revealed that the fungi isolated from spoilt fruits of the study were able to induce the same disease symptom present in healthy fruits with the same fungi being re-isolated from the inoculated healthy fruits and thus showing that the fungi were responsible for the spoilage of the fruits. The mycotoxins are not limited to their areas of infections; they diffuse rapidly throughout fruit through its fluid, contaminating all parts and otherwise posing a potential health hazard and less desirable for human consumption.

CONCLUSION

It was concluded from the present study that, *Aspergillus niger* and *Rhizopus stolonifer* are the most predominant fungal species that caused spoilage of bananas and oranges in Kwali market, Abuja, Nigeria. They produced large rotten diameters in the pathogenicity test. The presence of these pathogenic fungi on banana and orange fruits poses a threat to public health besides causing significant loss to retailers and consumers.

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