



ISOLATION AND IDENTIFICATION OF FUNGAL SPECIES ASSOCIATED WITH THE DETERIORATION OF SOME SELECTED FRUITS SOLD IN WUKARI, TARABA STATE, NORTH EAST, NIGERIA



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**Abstract:** Fruits are nutritionally rich and hence support the growth of spoilage microorganisms such as fungi. Hence, this study was carried out to identify fungal species associated with spoiled fruits sold in Wukari metropolis, Taraba State. Thirty-six (36) fruits with evidence of deterioration were selected for the study including Orange, Water melon, Apple, Pineapple, Tomatoes, Mango, Banana, Pawpaw, and Avocado pea. Deteriorated fruit samples were cultured for fungal isolation on potato dextrose agar supplemented with streptomycin (30µg/mL). Fungal identification was done using the Lactophenol cotton blue stain. Pathogenicity test was carried out by inoculating fungal isolates on healthy fruits and observing for evidence of deterioration. Eight (8) fungal deteriorogens were identified from spoiled fruits including *Aspergillus niger* (22.22%), *Rhizopus stolonifer* (16.67%), *Aspergillus flavus* (13.89%), *Aspergillus fumigatus* (2.78%), *Fusarium* sp. (13.89%), *Penicillium* sp. (11.11%), *Mucor* sp. (13.89%), and *Alternaria alternata* (5.55%). Furthermore, all fungal isolates reproduced deterioration in healthy fruits. The result from the different fruit samples showed that the highest number of fungal deteriorogens were isolated from Tomato and Mango with six (6) deteriorogens each while the least number of fungal deteriorogens (2) were isolated from Pineapple. The Presence of these fungi on edible fruits poses a serious threat to the health of the consumers especially humans and animals because some of these fungi, especially *Aspergillus* spp. are associated with the production of mycotoxins such as Aflatoxins which are lethal when consumed. Hence, the need for adequate quality control measures to be put in place during harvest, transportation, storage and sales in other to reduce the risk of contamination by these fungi thus reducing economic losses to sellers and also public health.

**Keywords:** Deterioration, fungal species, frequency of occurrence, fruits, Mycotoxin

## Introduction

Generally, fruits are considered as the edible part of a mature ovary of a flowering plant (Ikhiwili, 2012). They contain sugars, vitamins, mineral elements and small quantities of protein and oil which are necessary for human growth, repair and control of body processes (Zubbair, 2009). Fruits production and consumption has dramatically increased during the past few decades due to increase in consumers' awareness of healthy nutrition (Barth *et al.*, 2009). It is also estimated that about 20% of all fruits and vegetables produced is lost each year due to spoilage, which is usually as a result of several factors among which are: changes in Climatic condition, pests, and microbial attack. Microbial spoilage is one of the major causes of quality loss of fresh fruits and vegetables by formation of off-flavors, fermented aromas, and tissue decay. The shelf-life of many food may be accurately predicted by quantifying the population of microbes present on the food product.

Fruit Spoilage refers to changes in fruit conditions in which the fruit becomes less palatable or toxic in appearance, smell, taste or even texture. The spoilage of fruits may occur during growing season, harvesting, handling, transport and post-harvest storage or after purchase by the consumer (Al-Hindi *et al.*, 2011). Therefore, early intervention measures during crop development and harvesting through the use of good agricultural practices (GAP) will provide dramatic reductions in the yield loss due to deterioration at all subsequent steps in the food (Barth *et al.*, 2009). Moreover, high sugar content and nutrient elements in addition to low pH values in fruits make them particularly desirable to fungal spoilage (Singh and Sharma, 2007). The occurrence of fungal spoilage of fruits is recognized as a source of potential health hazard to man and animal. This is due to their production of mycotoxins which are capable of producing aflatoxin in man, following ingestion or inhalation (Bajuka *et al.*, 2014). Over the years, there has been an increase in the need to identify and isolate microorganisms associated with the spoilage of fruits as a way of finding a means of controlling them (Akinyele and Akinkunmi, 2012). Hence, this study was aimed at isolating

and characterizing fungal pathogens associated with spoiled fruits commonly sold in Wukari metropolis, Taraba State, Nigeria and recommend appropriate control measure.

## Materials and Method

### Study Area

This study was carried out in Wukari, the headquarter of Wukari Local Government Area of Taraba State, North Eastern part of Nigeria and it is located between longitude 7°57' and latitude 9°42' of the equator. Wukari is the home of Jubilee University (Kwararafa University) and Federal University Wukari (Aso *et al.*, 2022). Wukari is a commercial town dominated by farmers, herdsmen, fishermen, traders (Ade *et al.*, 2019).

### Sample collection and physical examination

Randomly selected fruit samples were obtained from different fruit vendors within Wukari metropolis, Taraba State. Fruits were physically examined for evidence of spoilage and deterioration before selection (Balali *et al.*, 1995). Fifty-four (54) fruits with evident deterioration were selected such as Orange (*Citrus sinensis*), Watermelon (*Citrullus lanatus*), Apple (*Malus pumila*), Pineapple (*Ananas comosus*), Tomato (*Solanum lycopersicum*), Mango (*Mangifera indica*), Banana (*Musa acuminata*), Pawpaw (*Carica papaya*), and Avocado pear (*Persa Americana*).

### Culture and Isolation of fungi

The surface of selected fruits were washed with distilled water to remove dirt. Tissue segments (3-5cm) from the margins of lesion of each spoiled fruit was cut using a sterile scalpel. The cut segment of the lesion was disinfected for 2 minutes in 70% ethanol before rinsing three different times in distilled water (Amusa *et al.*, 2002; Bariyewu *et al.*, 2007).

Each excised fruit segment was then inoculated on a freshly prepared potato dextrose agar plate supplemented with streptomycin (30 mg/l). The inoculated plates were then incubated at room temperature (28°C) for 5 days. Distinct fungal colonies were further subcultured on potato dextrose agar supplemented with streptomycin.

**Identification of fungal Isolates**

Fungal isolates were identified using cultural and morphological characteristics such as surface texture, surface topography, surface pigmentation, reverse pigmentation, and comparing them with confirmed representatives of different species. (Klich, 2002; Akintobi *et al.*, 2011). The microscopic identification was carried out by teasing out fungal colonies in a drop of 5% lactophenol cotton blue on a clean grease-free glass slide. A coverslip was gently placed on the part of the slide with the stain with little pressure applied to avoid air bubbles. The slide was then mounted and viewed under the X40 objective lens of a compound microscope.

**Pathogenicity test**

Pathogenicity test was carried out to determine the ability of fungal pathogens to reproduce deterioration in healthy. Eighteen (18) healthy fruits were washed with sterile distilled water and disinfected with 70% ethanol. A Sterile 4mm cork borer was used to bore holes in the different healthy fruits. The fungal isolates were then used to inoculate nine (9) healthy fruits before sealing the bored holes with petroleum jelly. The remaining nine (9) healthy fruits were allowed to stand as controls. The inoculated fruits and controls were placed in individual sterile polythene bags. Each of the fruits was moistened with wet balls of absorbent cotton wool to create a humid condition and were thereafter incubated at room temperature for five days and observed for spoilage (Ogodo *et al.*, 2020). The fungi were re-isolated from the fruits and compared with the original isolates.

**Result**

A total of thirty-six (36) fungal deteriorogens were recovered in association with spoilage in different fruits. On the basis of colonial appearance and microscopic properties, the fungal isolates were identified and classified into 8 different species (Table 1).

*Aspergillus niger* (22.22%) was the most prevalent followed by *Rhizopus stolonifer* (16.67%), *Aspergillus flavus* (13.89%), *Fusarium* sp. (13.89%), *Mucor* sp. (13.89%), *Penicillium* sp. (11.11%), *Alternaria alternata* (5.56%), and *Aspergillus fumigatus* (2.78%) (Table 2). All recovered fungal isolates passed the pathogenicity test as they were able to reproduce spoilage in the associated fruits.

*Aspergillus niger* was recovered from all spoilt fruits except Avocado, *Aspergillus flavus* was recovered from spoilt Apples, Oranges, Pawpaw, Tomato, and Mango while *Aspergillus fumigatus* was only isolated from spoilt oranges. *Penicillium* sp. was associated with spoilage in Apples, Avocado, Mango, and Tomato, *Fusarium* sp. was associated with spoilage in Watermelon, Pineapple, Pawpaw, Tomato, and Avocado while *Alternaria alternata* was associated with spoilt Mango, and Banana. *Rhizopus stolonifer* was associated with spoilt Orange, Watermelon, Pawpaw, Tomato, Mango, and Avocado while *Mucor* sp. was associated with spoilage in Banana, Pawpaw, Tomato, Mango, and Avocado (Table 3).

**Table 1: Colonial appearance and microscopic properties of fungal isolates**

Colony Morphology	Microscopy	Isolated fungi
White hairy growth spreading across the entire petri dish with white reverse	Non-septate hyphae with long and branched sporangiophores. Sporangiophores bear round terminal spore-filled sporangia.	<i>Mucor</i> sp
Deep cottony, at first white to yellow, then turning black with white edges. Reverse white to cream	Dark brown globose and large conidial heads, becoming radiate and splitting into several columns with age. Conidiophore stipes is smooth walled. Conidial heads are biseriolate, and phialides are borne on metula.	<i>Aspergillus niger</i>
White surface which then becomes very powdery and bluish green with a white border. Reverse is white	Septate hyphae with branched conidiophores attached to metula. Flask-shaped phialides bearing unbranched chains of round conidia are attached to the metula.	<i>Penicillium</i> sp
Velvety, yellow to green. Reverse golden to red-brown	Short conidiophores with rough walls. Phialides are both Uniseriate and biseriolate, cover the entire vesicle, and point out in all directions.	<i>Aspergillus flavus</i>
Whitish becoming brown black with age	Aseptate mycelia sporangia with spores directly opposite branched rhizoids. Sporangia are subglobose. sporangiospores are ovoid in shape and columella are subglobose	<i>Rhizopus stolonifer</i>
Velvety white at first white, then dark greenish to gray with age. White reverse white.	Short and smooth conidiophores with round conidia. Phialides are uniseriate, close together, forming only on the upper two-thirds of the vesicle.	<i>Aspergillus fumigatus</i>
At first white and cottony, but quickly became blue-green. The reverse is light	Small, oval microconidia mixed with smaller numbers of crescent-shaped macroconidia	<i>Fusarium</i> sp
White at first, then turn to greenish black with a light border. Reverse black	Chains of pale brown, club-shaped conidia with transverse and longitudinal septa.	<i>Alternaria alternata</i>

**Table 2: Prevalence of fungal isolates from spoilt fruits**

Fungal Isolates	Frequency	Percentage of Occurrence
<i>Aspergillus niger</i>	8	22.22%
<i>Rhizopus stolonifera</i>	6	16.67%
<i>Aspergillus flavus</i>	5	13.89%
<i>Aspergillus fumigatus</i>	1	2.78%
<i>Fusarium</i> sp.	5	13.89%
<i>Penicillium</i> sp.	4	11.11%
<i>Mucor</i> sp.	5	13.89%
<i>Alternaria alternata</i>	2	5.55%
Total	36	100

**Table 3: Correlation of fungal isolates with individual spoilt fruits**

Fruits samples	Fungal species
Apples	<i>Aspergillus flavus</i> , <i>Aspergillus niger</i> , <i>Penicillium</i> sp.
Orange	<i>Aspergillus niger</i> , <i>Aspergillus flavus</i> , <i>Aspergillus fumigatus</i> , <i>Rhizopus stolonifer</i>
Watermelon	<i>Aspergillus niger</i> , <i>Fusarium</i> sp., <i>Rhizopus stolonifera</i>
Banana	<i>Alternaria alternata</i> , <i>Mucor</i> sp., <i>Aspergillus niger</i>
Pineapple	<i>Aspergillus niger</i> , <i>Fusarium</i> sp.
Pawpaw	<i>Aspergillus flavus</i> , <i>Rhizopus stolonifer</i> , <i>Aspergillus niger</i> , <i>Mucor</i> sp., <i>Fusarium</i> sp.
Tomato	<i>Aspergillus niger</i> , <i>Fusarium</i> sp., <i>Aspergillus flavus</i> , <i>Penicillium</i> sp., <i>Rhizopus stolonifer</i> , <i>Mucor</i> sp.
Mango	<i>Alternaria alternata</i> , <i>Aspergillus flavus</i> , <i>Aspergillus niger</i> , <i>Mucor</i> sp., <i>Penicillium</i> sp., <i>Rhizopus stolonifera</i>
Avocado	<i>Fusarium</i> sp., <i>Penicillium</i> sp., <i>Mucor</i> sp., <i>Rhizopus stolonifera</i>

### Discussion

By virtue of their immense nutritional composition, fruits serve as significant media that can support the growth of microorganisms. When these microorganisms metabolize nutrients contained within food substances, they produce secondary metabolites that can inhibit other microorganisms but also act as detriogens. The presence of microorganisms and/or their secondary metabolites can cause the spoilage of food. Hence, our study was carried out to determine fungal species associated with the spoilage of fruits in Wukari metropolis, Taraba State.

The fungal isolates associated with the spoilage of fruits in Wukari metropolis were *Aspergillus niger*, *Rhizopus stolonifer*, *Aspergillus flavus*, *Fusarium* sp., *Mucor* sp., *Penicillium* sp., *Alternaria alternata*, and *Aspergillus fumigatus*. In a similar study carried out in Gwagwalada Abuja, Nigeria, Mailafia *et al.* (2017) reported *Aspergillus niger*, *Fusarium avenaceum*, *Fusarium solani*, *Aspergillus flavus*, *Penicillium digitatum*, and *Rhizopus stolonifer* in association with deterioration of Pineapple, Tomato, Watermelon, Orange, and Pawpaw. Furthermore, Mairami *et al.* (2018) reported *Aspergillus niger*, *Rhizopus nigricans*, *Mucor mucedo*, and *Fusarium oxysporum* in association with the spoilage of different fruits in Bwari market, Abuja, Nigeria. In similar studies involving spoilt orange fruits in Bhubaneswar, India, Parida *et al.* (2020) reported *Penicillium digitatum*, *Alternaria citri*, *Fusarium* spp., *Mucor* spp., and *Aspergillus niger* while Saleh and Al-Thani (2019) reported *Aspergillus* spp., *Rhizopus* spp., *Penicillium* spp., and *Alternaria* spp. in Qatar.

In Jimma town in Southwest Ethiopia, Kuyu and Tola (2018) reported *Fusarium* spp. in association with spoilage of Banana fruits, Ali *et al.* (2021) reported *Aspergillus fumigatus*, *Aspergillus flavus*, and *Aspergillus niger* in Pakistan, and Sani and Kasim (2019) reported *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Fusarium* spp., and *Rhizopus stolonifer* as the fungal deteriorants of Banana in Sokoto State, Nigeria.

Different studies have also reported similar fungal detriogens of Tomato fruits as reported in our study. Abubakar *et al.* (2019a) reported *Aspergillus niger*, *Rhizopus stolonifer*, *Fusarium oxynoporum*, *Alternaria alternata*, and *Penicillium digitatum* in Jega local government area, Kebbi State, Nigeria, Aminu *et al.* (2022) reported *Aspergillus* spp., *Mucor* spp., and *Alternaria alternata* in Kaduna state, Nigeria while Ogodo *et al.* (2020) reported *Aspergillus niger*, *Aspergillus*

*flavus*, *Fusarium* spp., and *Mucor* spp. in Wukari, Taraba State, Nigeria. Furthermore, Abubakar *et al.* (2019b) reported *Aspergillus flavus* and *Aspergillus niger* in association with the spoilage of Watermelons in Jega local government area, Kebbi state, Nigeria while Al-Ziadi *et al.* (2019) reported *Penicillium* sp., *Aspergillus niger*, *Alternaria alternata*, *Fusarium solani*, and *Fusarium oxysporum* as fungal detriogens of Apples in Al-Diwaniya city, Iraq.

Although fungal contamination of fruits can arise via several ways including during the growing season, harvesting, handling, transport, processing, postharvest storage and marketing conditions, fungal spoilage of these fruits is attributable to their water content, nutrient content, environmental factors, state of handling and storage, and the inherent quality of the fruits (Ogodo *et al.*, 2020; Abubakar *et al.*, 2019a). However, the significance of these fungal isolates is in the fact that they are able to reproduce disease in uninfected fruits. Also, these fungi are significant in their ability to produce potent mycotoxins which are detrimental to health (Chukwuka *et al.*, 2010; Akinmusire, 2011). *Aspergillus* spp. are known for their production of aflatoxins, which has been implicated in hepatotoxicity and hepatocellular carcinoma while *Fusarium* spp. have been reported to produce potent mycotoxins (such as fumonisins, zearalenone, moniliformin, and trichothecenes) with significant toxicity effects against human cells and tissues (Nesic *et al.*, 2014). *Rhizopus stolonifer* is an opportunistic fungal pathogen that can only produce fatal disease in immune-suppressed individuals. Ochratoxin A is another potent mycotoxin which is produced by *Penicillium* spp. and *Aspergillus* spp. (Bennett and Klich, 2003).

### Conclusion

Fungi can survive and multiply in favorable environments, including fruits. They are able to metabolize the nutrient compositions of food consequently producing toxins and enzymes which have deleterious effects on food. In this study, eight (8) fungal detriogens were recovered from spoilt fruits such as apples, orange, watermelon, banana, pineapple, pawpaw, tomato, mango, and avocado. The isolated fungal detriogens were *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Mucor* sp., *Fusarium* sp., *Rhizopus stolonifer*, *Penicillium* sp., and *Alternaria alternata*. Fungal contamination of fruits could arise from different sources during planting, processing, transportation, and handling. High prevalence of medically significant pathogens in deteriorated fruits is a significant marker against their ingestion as their ingestion could kickstart a cascade of fungal

disease and/or toxinosis. Hence, farmers and marketers should be properly sensitized on the proper handling of fruits so as to prevent contamination with pathogenic microorganisms.

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