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## RESEARCH ARTICLE

### ISOLATION AND IDENTIFICATION OF FUNGI AND PATHOGENICITY ASSESSMENT OF SOME SPOILT FRUITS SOLD IN WUSE MARKET, ABUJA, NIGERIA

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#### ABSTRACT

**Aims:** This study was carried out to examine the fungi associated with the spoilage of some fruits in Wuse Market, Abuja. **Materials and Result:** In this study, five different kinds of fruits were collected from Wuse market and examined for Fungi growth using Potato dextrose agar and the pathogenicity of the isolated fungi was confirmed using Koch's postulate. Six fruits spoilage fungi were isolated and identified as follows: *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus niger* complex, *Mucor racemosus*, *Mucor species* and *Fusarium species*. *Aspergillus species* had the highest occurrence in various fruits with the frequency 52% followed by *Mucor species* with frequency 33% and *Fusarium species* had the least occurrence with the frequency of 13%. However, more than one fungus colonies were found associated with some fruits sample like Pineapples and Apples while fruits like oranges and watermelon had just one colony of fungi isolated from them and no fungi were isolated from fruits like tomatoes. *Aspergillus niger*, *A. flavus* and *Mucor species* were isolated from fruits like Apples. *Mucor species* and *Mucor racemosus* from Oranges, *A. niger* complex, *Mucor species*, *Fusarium species* and *A. flavus* from pineapples, and *Fusarium species* was found isolated from one out of three of the watermelon samples. **Conclusion:** The pathogenicity test revealed that each fungus isolate was capable of causing plant diseases in healthy fruits and otherwise poses a potential environmental health hazard and less desirable for human consumption. **Significance and Impact of Study:** The alarming rate of food poisoning in Nigeria necessitates that more awareness is created to mitigate the rise in this malady. This research conducted in the metropolitan market of Abuja serves as a public awareness to the populace and also the regulatory bodies, the possible impact of consumption of unhealthy fruits.

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## INTRODUCTION

The structure of a flowering plant bearing the seed is known as the 'fruit'. Flowering plants are also known as angiosperm consists of the ovary from which a fruit is formed; fruits also play a major role in the dispersal of seed either by man or animals. Man as well as animal relies on fruits as part of their diet and mean of survival deriving nutrient from it (Lewis *et al.*, 2002). In layman's term, "fruits" is referred to as a plant which is made up of seed enclosed by a fleshy edible structure that is usually sweet or maybe sour and consumed in the raw form, for example, watermelons, oranges, apples, strawberries, etc. In botanical term, Fruits may consist of some other structures which are not habitually referred to as fruits, e.g. tomatoes, wheat grains, bean pods, and corn kernels (Schlegel *et al.*, 2003). Fruits serve as the major source of minerals and vitamins in diet and are known to reduce the risk of vitamin A

and C deficiencies; it also helps in prevention of chronic disease. Diet consisting of fruits is important in maintaining overall body health due to phytochemicals which promote health that is present in them. Certain diseases such as cardiovascular diseases, stroke, type 2 diabetes, and cancer can be prevented by eating a diet rich in fruits. Organisms such as yeasts, molds, bacteria, physiological factors as well as environmental factors play important role in fruits spoilage leading to fruit rot which is characterized by the change in texture, flavor, color, in an undesirable way. The fruit environment makes it suitable for a microorganism to inhabit it, they contain high nutrient and water which is required for the growth and reproduction of bacteria and fungi. However, Fruits are acidic in nature making it impossible for most bacteria to thrive in it hence most fruits of spoilage are caused by Fungi (Singh *et al.*, 2007). These microorganisms invade fruits and release their toxins known as mycotoxins into the fruits at the point of contact; this toxin is not limited to the point of invasion but spread rapidly across the fruits making it undesirable for human consumption. The distribution of fungi

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as the source of spoilage of some edible Nigeria fruits abound in various locations and this occurs as a result of exposure to microorganisms present in soil, dust or water which lead to the contamination of these fruits, these microbes are of plants and human pathogens and contaminate fruits through the process of handling during harvest and postharvest period (Eni *et al.*, 2010). A report which is conducted annually claims that, 20% of fruits are lost due to spoilage by Fungi which can be toxigenic or pathogenic (Barth *et al.*, 2009; Thiyam *et al.*, 2013). This study was carried out to isolate and identify fungi associated with spoilt fruits commonly sold in WUSE market, Abuja and verify its pathogenicity using Koch's postulate.

## MATERIALS AND METHODS

**Study area:** The fruits used in this study were collected from Wuse market. This is the main market in Abuja and is located in Zone 5 of Wuse district. This place is situated in Abuja Municipal, Federal capital, Nigeria, with coordinates; 9° 4' 14" North, 7° 28' 3" East. The market is organized and generally clean. The lab work was carried out in the Biological science laboratory, Faculty of Natural and Applied sciences, Nile university of Nigeria, Abuja.

**Collection of Fruits materials:** Five different types of infected fruits (three per type) which include orange (*Citrus sinensis*), watermelon (*Citrullus vulgaris*), apple (*Malus domestica*), pineapple (*Ananas comosus*) and tomato (*Lycopersicon esculentum*) were collected from Wuse market, Abuja.

**Media preparation:** Nutrient agar and potato dextrose agar used in this study were prepared according to the manufacturer's guideline. Agar agar was added to allow the quick solidification of the media. Bacterial contamination was inhibited by adding 500mg of chloramphenicol into 500mls of the agar solution prior to autoclaving and pouring into petri dishes.

**Sterilization of Agar:** Media was placed in the autoclave to allow for homogenization and sterilization. Reliable sterilization with moist heat requires temperatures above that of boiling water. These high temperatures are most commonly achieved by steam under pressure in an autoclave. Steam at a pressure about 15 psi; attaining temperature (121°C) will kill all organisms and their endospores in about 15 minutes. A basic principle of chemistry is that when the pressure of a gas increases, the temperature of the gas increase proportionally.

**Sterilization of work bench:** The working area was sterilized using alcohol swap to prevent contamination.

**Pour plates:** The petri dish were labelled according to the fruits sample that will be inoculate onto them and arranged on the working bench. The conical flask containing the autoclaved hot media was then handled using a heat resistant glove and allow to cool enough to handle with bear hand but not too cool to prevent solidification of media in the container. The media is swirl without introducing bubbles before pouring into plates. The agar plates are then allowed to solidify then turn upside down.

**Isolation of Fungi:** Spoilt fruits surface were washed with distilled water to enhance removal of dirt. A small portion of the spoilt area of the fruits was cut in and out using a sterile scalpel and inoculated onto a freshly prepared nutrient agar

plate. The inoculated plates were incubated at 30°C for 5 days and were observed for fungal growth and later sub-cultured for another 10 days at 30°C on nutrient agar. Resulting colonies were then sub-cultured onto Potato dextrose agar (PDA) until pure isolates were obtained. As a control, some healthy fruits were also selected. A small portion of these healthy fruits was cut using a sterile scalpel and inoculated onto a freshly prepared Nutrient agar. The inoculated plates were then incubated for 5 days to observe for Fungi growth (Iniekong *et al.*, 2015).

**Sub-culturing techniques:** This was done following method by Mailafia *et al.*, 2017.

**Identification of isolated fungi:** The pure fungi isolates were identified using both cultural and morphological characteristics such as Surface texture [glabrous, suede-like, powdery, granular, fluffy, downy, cottony], Surface topography [flat, raised, heaped, folded, domed, radial grooved], Surface pigmentation [white, cream, yellow, brown, pink, grey, black etc.], Reverse pigmentation [none, yellow, brown, red, black, etc.] as well as comparing them with confirmed representatives of different species. (Akintobi *et al.*, 2011) The microscopy identification was carried out by placing a drop of 5% potassium manganese (KMnO<sub>4</sub>) onto a clean slide and a small portion of representative fungi mycelium was removed using a flamed inoculating loop and teased onto the potassium manganese stain using a sterile needle. A coverslip is gently placed on the part of the slide with the stain with little pressure applied to avoid air bubbles. The slide is then mounted and viewed under the microscope with the 10x and 40x objective lens. (Mailafia *et al.*, 2017). Pictures of the Fungi isolates were taken for further characterization and comparison with a documented book of fungi (Sarah *et al.*, 2017) and other representative pictures of fungi species.

**Pathogenicity test:** This was carried out as described by Baiyewu *et al.* (2007) and Chukwuka *et al.* (2010). The healthy fruits were surfaced sterilized with alcohol and hypochlorite and rinsed with distilled water. A sterile hollow metal rod was used to punch out single fruit column from each of the healthy fruits, another sterilized metal rod was used to punch the portion of the mycelium of pure fungi isolate, and a glass rod was then used to remove the punched mycelium from the metal rod to replace the punched portion of the fruits. The inoculated portion was then sealed with petroleum jelly to prevent contamination by other organisms. The inoculated fruits were left at room temperature for 8 days. The spoilage pathogens were isolated from the inoculated fruits after 8 days onto potato dextrose agar and incubated at 30°C, the resulting colonies were sub-cultured to obtain pure isolates. The resulting colonies from pure isolates were compared with the original colonies from which they were isolated. This procedure was carried out using Koch's postulate to determine if the inoculated fungi were responsible for spoilage in their respective fruits. As a control, each of the healthy fruits was surfaced sterilized with alcohol and hypochlorite and rinsed with distilled water. A sterile hollow metal rod was used to punch out single fruit column from each of the healthy fruits and sealed with petroleum jelly and kept at room temperature for 8 days.

## RESULTS

**Results of Fungi isolates:** Nine fruits spoilage fungi were isolated and identified as *Aspergillus niger* (apple, orange,

tomato, watermelon), *Aspergillus flavus* (pineapple, apple), *Fusarium species* (pineapple, tomato, watermelon), *A. niger complex* (pineapple, apple), *Mucor racemosus* (Orange) and *Mucor species* (Oranges, apple, pineapple, tomato), *Rhizopus stolonifer* (orange), *Penicillium chrysogenum* (pineapple), *A. niger niger* (watermelon) as shown in Table-1. However, no growth was observed in the control fruits.

Table 2 shows the Frequency of occurrence of fungal species. *Aspergillus species* has the highest occurrence in various fruits with frequency of 52% followed by *Mucor species* with frequency 33% and *Fusarium spp* had the least occurrence with the frequency of 13%. No growth was observed on the agar inoculated with tomato plates after seven days as well as two of the watermelon plates.

### Pathogenicity test result

In this test, all the fungal isolates were observed positive for causing spoilage in fruits. The Various disease symptoms caused by each of the isolates were observed and recorded as follows; *Aspergillus niger*: fruits appeared water soaked and soft, Developed rapidly through fruit tissue resulting in total rot and exudation of liquids. *Aspergillus flavus*: soft rot spoilage, the infected area turned brown and water soaked. *Fusarium spp*: cause soft rot in the affected area. *Mucor spp*: The lesion produced caused distension of the fruit surface, change in colour in the Infected area. This is shown in Table 9.

### DISCUSSION

Fungi associated with fruits spoilage from Wuse market was isolated and identified. The high prevalence of fungi isolates in the selected fruits showed that Fungi are the major cause of fruit spoilage. This support the previous research where fungi have been found causing fruit and vegetables rot. Fruits contain high levels of sugars and nutrients element and their low pH values make them particularly desirable to fungal infection (Singh *et al.*, 2007). However, no fungi were isolated from spoiled fruits such as tomatoes and two out of the three watermelons used and this could be as a result of physiological factors such as Temperature, Oxygen, or enzymes present in the fruits etc. Previous literature work suggests that the prevalence of fungi as the spoilage organism of some edible fruits and vegetables abound in different locations in Nigeria. Fruits and vegetables are exposed to contamination by microorganisms through direct contact with soil, dust, water and by handling at harvest or during postharvest processing. This makes them harbour a wide range of microorganisms including plant and human pathogens (Eni *et al.*, 2010). This could also cause loss of profit from the fruits to farmers. Earlier researchers such as in the work of Iniekong *et al.* (2015) which studies Fungi playing role in the spoilage of some consumable Fruits and vegetables, he was able to isolate *Rhizopus stolonifer*, *Aspergillus niger*, *Fusarium accuminatum*, *Fusarium oxysporum*, *Fusarium equiseti*, *Fusarium solani*, as well as *Fusarium moniliforme* from spoiled tomatoes while *Fusarium accuminatum*, *Rhizopus stolonifer* and *Aspergillus niger* were found to be responsible for the spoilage of watermelon and Samuel *et al.* (2017) in their study of Fungi assessment in some spoiled fruits sold in Gwagwalada market of Abuja, Nigeria isolated *A. niger*, *F. avanaceum*, *P. digitatum*, *R.stolonifer*, *Saccharomyces species*, *A. flavus*, and *F. solani* with *Aspergillus niger* being prevalent with the percentage of 38% in fruits such as pineapple, watermelon,

oranges, pawpaw as well as tomatoes while *Fusarium avanaceum* had the frequency of 31% in pineapple, watermelon, oranges, pawpaw, and tomatoes *Penicillium digitatum* and *Rhizopus stolonifer* was also isolated from fruits such as tomato and oranges and they have the least occurrence with the frequency of 4%. *Saccharomyces species*, *Fusarium solani*, and *A. flavus* were also isolated in this study with the frequency of 10%, 8%, and 5% respectively.

The Fungi isolated from spoiled fruits in this study include *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus niger complex*, *Mucor species*, *Mucor racemosus*, and *Fusarium species*. *Mucor species* had the highest frequency of 26% in Oranges together with *A. flavus* in Pineapple and Apple while *Mucor racemosus* had the least frequency in Oranges with frequency of 6%. The result of pathogenicity test also revealed that the fungi isolated from spoiled fruits of the study were able to induce the same disease symptom present in healthy fruits with the same fungi being reisolated from the inoculated healthy fruits and thus show 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 pose a potential health hazard and less desirable for human consumption. No growth was observed in the control fruits. *Aspergillus niger*, popularly known as the black mould and several other species cause decay of foodstuffs. *A. flavus* and *A. niger* parasitizes man and animals. They cause a number of diseases grouped under the name Aspergillosis. Most people breathe in *Aspergillus* spores every day without getting sick. However, people with weakened immune systems or lung diseases are at a higher risk of developing health problems due to *Aspergillus*. Health issues related to *Aspergillus* include infection in the organs of the body such as the lung as well as allergic reaction. This infection may also be seen in human ear and is called Otomycosis. *A. flavus* is reported to produce mycotoxin known as Aflatoxin which is a potent carcinogen and has been directly correlated with adverse health effects, such as liver cancer, in many animal species (Martins *et al.*, 2001). Particularly common clinical syndromes associated with *A. flavus* include chronic granulomatous sinusitis, keratitis, cutaneous aspergillosis, wound infections and osteomyelitis following trauma and inoculation. *Aspergillus flavus* outbreak differs from *A. fumigatus* outbreak in that that outbreak that are of *A.flavus* is found to be related to just one strain or closely related strains (Hedayati *et al.*, 2007). Abarca *et al.* (1994) isolated Ochratoxin A Produced by Strains of *Aspergillus niger* var. *Niger OA* contaminates a variety of plant and animal products but is most often found in stored cereal grains. As a nephrotoxin, it has long been of particular importance to the poultry and swine industries. In healthy humans, *Mucor* rarely causes disease, but in the host compromised by immune deficiency, immune suppression, or a serious underlying disease, the incidence of *Mucor* infections is much higher. *Fusarium* is reported to produce trichothecenes, which are most strongly associated with chronic and fatal toxic effects in animals and humans. The adverse effect of fungi in plants and fruits has resulted in the shortage of fruits for consumption. Spoilage of fruits by fungi leads to a shortage of consumption and loss of profits to the farmers and industries whose raw material is fruits. In this study, six fruits spoilage fungi were isolated and identified as follows: *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus niger complex*, *Mucor racemosus*, *Mucor species* and *Fusarium species*. *Aspergillus*

*species* had the highest occurrence in various fruits with the frequency 52% followed by *Mucor species* with frequency 33% and *Fusarium species* had the least occurrence with the frequency of 13%. The isolated fungi are of economic and public health importance. Some species of these fungi have been reported to produce potent mycotoxins called ochratoxins that can be harmful to human beings and animals. *Mucor spp.* rarely causes disease, but in the host compromised by immune deficiency, immune suppression, or a serious underlying disease, the incidence of *Mucor* infections is much higher.

**Conflict of interest:** No conflict of interest.

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## APPENDIX

**Table 1. Fungi isolated from selected fruits**

S/N	Fruit	Fungi Isolate
1	Watermelon	<i>Fusarium species</i>
2	Pineapple	<i>A.niger complex, Mucor species, Fusarium species, A. flavus</i>
3	Orange	<i>Mucor racemosus, Mucor species</i>
4	Apple	<i>Mucor species, A. flavus, A. niger</i>

**Table 2. Frequency of occurrence of fungal species**

Fungi isolate	fruits infected	number isolated	Frequency of occurrences (%)
<i>A. niger</i>	apple	2	13.3%
<i>A. flavus</i>	Pineapple, apple	4	26.6%
<i>Fusarium species</i>	pineapple, watermelon	2	13.3%
<i>A. niger complex</i>	pineapple	2	13.3%
<i>Mucor racemosus</i>	orange	1	6.7%
<i>Mucor species</i>	orange	4	26.6%

\*\*\*\*\*