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Research Article
Isolation and Characterization of Fungal Species from Spoilt Fruits in Utako Market, Abuja, Nigeria

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Abstract

Background and Objective: Fruits are subjected to natural contamination by many different kinds of micro-organisms, including pathogens such as fungi. This research was carried out to investigate the types of fungal flora associated with the spoilage of fruits at Utako market, Abuja, Nigeria. **Materials and Methods:** One hundred fruits comprising of 70 spoilt ones and 30 healthy ones were collected from the market and transported to the laboratory. Thin sections of the rotten fruits were obtained using a sterile blade and inoculated onto PDA media and incubation was carried out at 27°C for 5 days. **Results:** The result obtained indicated the presence of 7 different fungal species: *Aspergillus niger*, *A. flavus*, *A. fumigatus, Aspergillus* sp., *Rhizopus stolonifer, Mucor mucedo* and *Alternaria* sp. However, *Aspergillus niger* was the most predominant species while *Alternaria* sp. was the least common. However, the pathenogenicity of *Rhizopus stolonifer* was higher than that of the remaining fungal species isolated, while that of *Alternaria* sp. was the least. **Conclusion:** It was concluded that, seven fungal species were found to be responsible for the deterioration of fruits in Utako market. Some of these isolated fungal species produce toxins that could cause severe food poisoning and other potential health hazards.

Key words: Rotten fruits, fungal flora, food poisoning, fungal species, pathenogenicity

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fruits are fertilized ovaries that served as vital sources of nutrient to man. They give the body the necessary vitamins, fats, minerals and oil in the right proportion for human growth and development[1]. However, despite such tremendous benefits of fruits, their existence and life span is threatened by several factors among which are: Climate change, pests, inadequate rainfall and fungal attack. Fruits contain high levels of sugars and nutrient elements and their low pH values make them particularly desirable to microbial decay. These fruits are usually displayed on benches and in baskets for prospective customers in the open markets until sold, thereby exposing them to further microbial infection beside those associated with fruit surface and those from adjacent infected fruits[2,3]. Spoilage of fruits refers to various changes in which they becomes less palatable or taste, smell, appearance or texture. Numerous microbial defects of agricultural crops are characterized by the types of micro-organisms responsible for their deterioration[1]. Generally, spoiling microbes are considered toxigenic or pathogenic as a lot of toxigenic fungi have been isolated from spoilt fruits. Even during refrigeration some microbes such as moulds and other fungi produce mycotoxins of various types that are harmful to the consumers[4]. These mycotoxins have low molecular weight and toxic secondary metabolites from some species of fungi. They are dangerous even in minute quantities and present extreme toxicity due to their ability to withstand heat[5]. However, the pathogenic microbes cause infections or allergies[6]. Spoilage micro-organisms can be introduced to the crop on the seed itself, during crop growth in the field, harvesting and post-harvest handling or during storage and distribution loading and offloading[7].

According to this source, fungal toxin contamination of food products can cause acute or chronic intoxication, leads to reduce life expectancy; exacerbate disease conditions in humans leading to 40% loss of economic productivity[5]. Over the years, there has been an increase in the need to identify and isolate the fungi associated with their spoilage. This paper therefore, aimed at isolating and characterizing the various fungal flora associated with the fruits spoilage in Utako market, Abuja Nigeria with the view of providing a firsthand information on the possible dangers associated with the consumption of such fruits.

MATERIALS AND METHODS

Sample collection: The fruit samples (Orange, banana, pawpaw, pineapple, water melon, pumpkin, tomato and pepper) were collected from both the whole sellers and retailers randomly from Utako market, Abuja, Nigeria. A total of 100 samples comprising of 70 spoilt fruits and 30 healthy ones (for pathogenicity test) were collected from April, 2017 to August, 2017. The samples were placed in a sterile plastic bag and labeled, respectively and for the healthy ones were kept at room temperature (25-30°C) for 6 days for fungal growth. The spoilt fruits were identified by morphological examination using the method of Bukar *et al.*[8]. The samples were kept in the refrigerator at 4°C before laboratory mycological analysis.

Culture media preparation: Potato Dextrose Agar (PDA) containing chloramphenicol (30 mg mL^{-1}) were used. The culture media were prepared according to manufacturer's recommendations. The quantities of the appropriate medium or base medium were weighed. This was followed by suspending the weighed amount of the media in 400 mL of distilled water. The media were heated to boil over Bunsen flame until the agar melts. The molten agar media were allowed to cool to 45°C and the pH adjusted according to the manufacturer's recommendation. The media were cotton plugged and wrapped with aluminium foil and were autoclaved at 121°C at a pressure of 15 lbf/in^2 for 15 min. After sterilization, the media were aseptically dispensed in 20 mL aliquots sterile Petri dishes and were allowed to set on the flat then aseptically dispensed into sterile Petri dishes. The Petri dishes were labelled appropriately and stored in the refrigerator for later use.

Isolation of fungi from spoilt fruits: Isolation of the mycological flora followed the method of Dashwood *et al.*[9] and Balali *et al.*[10]. The infected fruits were surface sterilized with cotton wool soaked in 0.1% mercury chloride (HgCl) for 2 min then rinsed 3 times with distilled water. A sterile blade and forceps was used to cut small section of the tissue containing both the healthy and the rotten portion (3 mm diameter) and plated on solidified Potato Dextrose Agar (PDA) containing chloramphenicol (30 mg mL^{-1}) to prevent bacterial growth. The inoculated plates were incubated at ambient room temperature (25°C) for 7 days. Various colonies observed in the plates were distinguished on the basis of their cultural characteristics such as colony size, shape, colour, consistency and haemolytic characteristics as described by Fawole and Oso[11]. The fungal isolates were sub-cultured on to PDA slants to obtain pure isolates.

Identification of fungal isolates: Fungal isolates obtained from the slant were identified based on their gross

morphology such as colony growth pattern, conidial morphology and pigmentation by slide culture techniques[12]. A small portion of the aerial mycelia from the representative culture was picked using a sterile inoculating needle and inoculated on a slide containing a fraction of a prepared solidified potato dextrose agar and incubated for 48 h, after which it was viewed under the light microscope first with low resolution objective of x10 and then with high resolution objective of x40 to detect spore, hyphae and other special structures according to the methods described by Barnett[13]. The morphological characteristics and appearance of the fungal isolates from the rotten fruits used in this study were confirmed and authenticated with the help of mycological atlas of Domsch *et al*.[14].

Pathogenicity test: Pathogenicity test was carried out to know if the isolated fungi were really responsible for the spoilage of the fruits. Healthy samples were surface sterilized with 0.1% HgCl. Cylindrical plug tissues were cut out from the fruits using a sterilized 2 mm sized cork borer. Agar disc containing 1 week old fungal culture were aseptically placed in these holes, then covered and sealed off by means of 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 at 28°C for 14 days. The point of inoculation of each type of fungus was examined and recorded. The diameter of the rotten portion of the fruits was measured and the fungi were later re-isolated from the inoculated samples and compared with the initial isolates[15].

Statistical analysis: The data obtained was analyzed using one way Analysis of Variance with least significant difference to separate the means and the significant level was set at $p<0.05$. The data for the prevalence of fungal isolates was analyzed using frequency table.

RESULTS

Characterization of fungal flora: The characterization of fungal flora from fruits obtained in Utako market was presented in Table 1. The result showed the occurrence of 7 different fungal species distributed among the spoilt fruits. In this study, 7 fruits' spoilage causing fungal species were

Table 1: Microscopic characterization of fungal isolates from spoilt fruits in Utako market, Abuja

Samples	Microscopic characteristics	Fungi identified
PI1a	Thick septate hyphae, chain of conidia on sterigmata	*Aspergillus niger*
PI1b	Irregular size hyphae with septation	*Aspergillus* sp.
PI1c	Hyphae is septate and is small in size	*Aspergillus fumigates*
PI1d	Green conidiospores with septate hyphae	*Aspergillus flavus*
PE1a	Thick septate hyphae, chain of conidia on sterigmata	*Aspergillus niger*
PE1b	Non septate hyphae and irregular in size	*Rhizopus stolonifer*
PE1c	Green conidiaspore with septate hyphae	*Aspergillus flavus*
PE1d	Thick septate hyphae, chain of conidia on sterigmata	*Aspergillus niger*
TO1a	Thick septate hyphae, chain of conidia on the sterigmata	*Aspergillus flavus*
TO1b	Thick septate hyphae, chain of conidia on sterigmata	*Aspergillus niger*
TO1c	non septate hyphae and irregular in size	*Rhizopus stolonifer*
TO1d	Thick non septate hyphae with dark conidiospore	*Mucor mucedo*
WM1a	Thick septate hyphae, chain of conidia on sterigmata	*Aspergillus niger*
WM1b	Thick septate hyphae, chain of conidia on sterigmata	*Aspergillus niger*
WM1c	non septate hyphae and irregular in size	*Rhizopus stolonifer*
WM1d	Thick non septate hyphae with dark conidiospore	*Rhizopus stolonifer*
PU1a	Thick septate hyphae, chain of conidia on sterigmata	*Aspergillus niger*
PU1b	Thick septate hyphae, chain of conidia on sterigmata	*Aspergillus niger*
PU1c	Thick non septate hyphae with dark conidiospore	*Rhizopus stolonifer*
PU1d	Thick septate hyphae, chain of conidia on sterigmata	*Aspergillus niger*
O1a	Erect conidiophores, septate hyphae with cylindrical conidia	*Alternaria* sp.
O1b	Green conidiospores with septate hyphae	*Aspergillus flavus*
O1c	Green conidiospores with septate hyphae	*Aspergillus flavus*
O1d	Green conidiospores with septate hyphae	*Aspergillus flavus*
B1a	Thick septate hyphae, chain of conidia on sterigmata	*Aspergillus niger*
B1b	Thick septate hyphae, chain of conidia on sterigmata	*Aspergillus niger*
B1c	Thick septate hyphae, chain of conidia on sterigmata	*Aspergillus niger*
Bid	Thick septate hyphae, chain of conidia on the sterigmata	*Aspergillus flavus*
PA1a	Unseptate hyphae are irregular in size and ribbon like	*Mucor mucedo*
PA1b	Septate hyphae with conidiospores born on the conidia	*Aspergillus fumigates*
PA1c	Irregular hyphae small in size and septate	*Rhizopus stolonifer*
PA1d	Hyphae is small and regular with cross septation	*Aspergillus* sp.

O: Orange, B: Banana, PA: Pawpaw, PI: Pineapple, WM: Water melon, PU: Pumpkin, TO: Tomato, PE: Pepper

Table 2: Prevalence of fungal isolates on the spoilt fruits from Utako market

Fungal isolates	Frequency	Percentage
Aspergillus niger	12	37.50
Aspergillus spp.	2	6.25
Aspergillus fumigatus	2	6.25
Aspergillus flavus	7	21.88
Rhizopus stolonifer	6	18.75
Mucor mucedo	2	6.25
Alternaria sp.	1	3.12
Total	32	100.00

Table 3: Pathogenicity of fungal isolates on the fruits from Utako market

Fungal isolates	Diameter of rot (mm)
Aspergillus niger	30
Aspergillus spp.	26
Aspergillus fumigatus	32
Aspergillus flavus	38
Rhizopus stolonifer	52
Mucor mucedo	25
Alternaria sp.	20

characterized as *Aspergillus niger* was found to occur in all the fruits except in orange and pawpaw. While, *Aspergillus flavus* occurs in pineapple, pepper, tomatoes and banana. However, *Rhizopus stolonifer* occurred in pepper, tomato, water melon, pumpkin and pawpaw and *Mucor mucedo* occurs in only tomato and pawpaw. The result also showed that *Alternaria* sp. occurred only in oranges and *Aspergilus fumigatus* in pineapple and pawpaw.

Prevalence of fungal isolates: The prevalence of fungal isolates on the spoilt fruits samples from Utako market was shown in Table 2. The result indicated *Aspergillus niger* to be the most dominant fungal species with 37.50% occurrence while *Alternaria* sp. had the lowest percentage occurrence of 3.12%.

Pathogenicity test: The Pathogenicity test was represented in Table 3. The result indicated that, *Rhizopus stolonifer* had the highest pathogenicity producing largest rotten area of 52 mm in diameter while *Aspergillus* spp. and *Mucor* spp. had almost similar Pathogenicity. *Alternaria* sp. had the least pathogenicity of 20 mm in diameter rotten surface.

DISCUSSION

The result of this study revealed the presence of 7 different fungal species from deteriorated fruits obtained from Utako market. These fungal species were confirmed to be causative agents of the spoilage. In view of the threat poses by post-harvest spoilage of fruits induced by several fungal species especially in the developing countries like Nigeria as reported by Droby[16]. This study isolated 7 different fungal species associated with the fruits spoilage. The presence of *Aspergillus* spp. and *Rhizopus* spp. as fruits spoilage agent in this research was in conformity with the findings of Ewekeye *et al.*[17] among the spoilt fruits vended in some selected markets in Lagos. Mailafia *et al.*[18], who isolated and reported *Aspergillus niger* as the most dominant mycological flora that was associated with spoilage of fruits. This finding was also in conformity with that of Baiyewu *et al.*[2] and Chukwuka *et al.*[3] who isolated *A. niger* and *R. stolonifer* from pawpaw in Nigeria. The finding was also in conformity with the findings of Gadgile and Chavan[19] on the isolation of fungal pathogens from fruits stored and sold in the market. Furthermore, Bali *et al.*[20] stated that *A. niger* was the cause of post-harvest spoilage in sweet orange and acid lime at field. Okereke *et al.*[21] reported that *A. niger*, *Alternaria* species, *Botryodiplodia theobromae* and *Colletotrichum gloeosporioides* were isolated from the spoilt mangoes. However, the value obtained for the prevalence of *A. niger* which caused a disease called black mold on certain fruits and produced potent mycotoxins called ochratoxins that can be harmful to human beings and animals, was higher than the one reported by Mailafia *et al.*[18] who reported the highest occurrence of 38%.

Most of the fungal organisms isolated in this study played a great vital role in causing fruits spoilage. Chukwuka *et al.*[3] reported *Rhizopus nigricans*, *A. flavus*, *A. niger*, *Fusarium* spp. and *Mucor* spp. in pawpaw fruit spoilage from a farm in Oyo state, Nigeria. Moreso, the presence of 4 different species of Aspergilus: *Aspergilus niger, Aspergilus flavus, Aspergilus* spp. and *Aspergilus fumigatus* among the spoilt fruits indicated the degree of pathenogenicity conferred by the family Aspergillaceae in inducing fruits spoilage. This finding was in conformity with that of Bukar *et al.*[8] who reported that Aspergillus species, *Mucor* and *Rhizopus* spp. as responsible for the soft rots of orange fruits in Nigeria. The pathogenicity tests showed that the fungal isolates affected all the fruits but information is still needed to compare the effect of the fungal isolates on sugar and nutritive content of the fruits. Based on the finding of this study, some fungi were isolated and various literature indicated that the fungal species isolated were known to produce toxins that could cause severe food poisoning and other potential health hazards, there is a need for proper orientation for the vendors on using hygienic ways of storing fruits and also proper washing of the fruits in saline water before consumption.

CONCLUSION

It was concluded that Aspergillus, Rhizopus and Mucor were responsible for the spoilage of fruits in Utako market

which subsequently inferred a great loss to the retailers and imposed clinical threat to the consumers via severe food poisoning.

SIGNIFICANCE STATEMENT

This study discovered the prevalence of fungi responsible for deterioration of fruits sold in Utako market, Abuja, Nigeria that can be beneficial for providing an insight on fungi causing spoilage and methods of preventing such spoilage. This study will help the researchers to uncover the critical areas of microbial spoilage that many researchers were not able to explore. Thus a new theory on microbial spoilage may be arrived at.

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