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ISOLATION AND IDENTIFICATION OF FUNGAL SPECIES FROM SPOILED FRUITS IN UTAKO MARKET, ABUJA, NIGERIA

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ABSTRACT

Fruits inherently harbour a diverse range of microorganisms, including various pathogens like fungi..Food spoilage is a complex process, and excessive amounts of food are lost due to microbial spoilage, even with modern-day preservation techniques. This research investigated the different types of fungal species associated with fruit spoilage at Utako market in Abuja, Nigeria. A total of one hundred fruits—seventy spoiled and thirty healthy—were collected from the market and brought to the Biology Lab, Faculty of natural and applied science Baze university ,Abuja . A sterile blade was used to cut thin slices of the rotten fruits, which were then inoculated onto PDA media and incubated for five days at 27°C.*Aspergillusniger, A. flavus, A. fumigatus, Aspergillus* spp., *Rhizopus*spp., *Mucor spp*. and *Alternaria* spp. were the seven distinct fungal species isolated from the spoilt fruits. *Alternaria* was the least common species(3.12%), whereas *Aspergillusniger* (37.50%)was the most common species. However, *Alternaria* has the lowest pathogenicity (20mm), whereas *Rhizopus*spp was most pathogenic. Some of these fungal species have strains that are known to produce toxins and have the potential to cause serious food poisoning.

Keywords: Abuja, Isolates, Fungi, Utako

INTRODUCTION

Fruits and vegetables provide the body with all the vitamins, lipids, minerals, and oils required to sustain human growth and development in the proper amounts. However, there are significant obstacles to the survival of fruits and vegetables, such as shifting climatic conditions, pests, insufficient water, and fungalattacks (Akinro *et al.,* 2015). Fruits are fertilized ovaries that served as vital sources of nutrient to man (Akinmusire, 2011). The importance of fruit in human nutrition cannot be overestimated as it provides vitamins and minerals necessary for proper body metabolism (Buah, *et al.,* 2024). The Food and Agriculture Organization of the United Nations states that since there are now 7.5 billion people on the planet, there must be an adequate supply of safe and nourishing food. Minimizing waste and guaranteeing safety are becoming more important as the demand for plant food keeps growing. Microbial food deterioration is a worldwide problem that leads to food waste and unhappy consumers (Alegbeleye, 2022). Food spoilage is a metabolic process that results in changes in sensory qualities that make foods unsafe for human ingestion. Foods that have been spoiled may be safe to consume—that is, they may not be harmful due to the absence of infections or toxins—but they are rejected due to changes in texture, taste, smell, or appearance. According to some ecologists, these offensive odours are made by microorganisms to deter large predators and preserve the food source for themselves (Rawat, 2015).Fruit quality and safety are both important since fruits are vulnerable to microbial deterioration due to their nutritional composition (Zhao *et al.,* 2022) .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 besides those associated with the fruit surface and those from adjacent infected fruits (Baiyewu*et al.,* 2007; Chukwuka*et al.,* 2010). The different kinds of microorganisms that cause crops to deteriorate constitutea number of microbial problems (Akinmusire, 2011). As a group, fungi are among the most resistant spoilage germs and can evade the food industry's control measures. Different fungal propagules can spread quickly through the air and water, endure harsh environments, and grow in biomass over time. It is known that certain fungal species can withstand the harshest physicochemical conditions and thermal processing regimens employed in the manufacturing of commercial food (Snyder and Worobo 2018). Fungi can produce mycotoxins, which are dangerous to human health when consumed, hence their presence in spoiled fruits is a serious worry. Fungal contamination can also shorten the shelf life of fruits and hasten their deterioration (Mukhtar, 2023). Even during refrigeration, some microbes, such as moulds and other fungi, produce mycotoxins of various typesthat are harmful to consumers. These mycotoxins are low molecular weight 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. However, the pathogenic microbes cause infections or allergies (Tournas, 2005). Spoilage microorganisms can be introduced to the crop on the seed itself, during crop growth in the field, during harvesting and post-harvest handling or during storage and distribution loading and offloading (Barth, *et al*. 2009). Fungal toxin contamination of food products can cause acute or chronic intoxications, leads to reduced life expectancy; exacerbate disease conditions in humans leading to 40% loss of economic productivity, Over the years, there has been an increase in the need to identify and isolate fungi associated with their spoilage. This paper therefore aimed at isolating and characterizing the various fungal flora associated with 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

Collection of Sample

The fruit samples were randomly bought from both the wholesalers and retailers from Utako market, Abuja, Nigeria. A total of one hundred samples comprising seventy (70) spoiled and thirty (30) healthy fruits (for pathogenicity test)

were obtained. The fruits were placed in a sterile plastic bag and labeled appropriately. The healthy fruits were kept at room temperature (25–30 ℃) for six days for fungal growth. The spoiled fruits were identified by morphological examination using the method of Bukar *et al*. (2009). The samples were kept in the refrigerator at 4^0 C before laboratory mycological analysis.

Culture Media Preparation

Potato Dextrose Agar (PDA) containing Chloramphenicol (30 mg/l) was used. The culture medium was prepared according to the manufacturer's recommendations. The quantities of the appropriate medium or base mediumwas weighed. This was followed by suspending the weighed amount of the medium in 400ml of distilled water. The medium was heated to boil over a Bunsen flame until the agar melted. The molten agar medium was allowed to cool to 450 ℃ – 500 ℃, and the pH was adjusted according to the manufacturer's recommendation. The medium was cotton-plugged and wrapped with aluminum foil and was autoclaved at 121^0C at a pressure of 15 pounds per square inch for 15 minutes. After sterilisation, the medium was aseptically dispensed in 20ml aliquots into sterile Petri dishes and 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.* (1992) and Balali *et al*. (1995). The infected fruits were surface sterilized with cotton wool soaked in 0.1% mercury chloride (HgCl) for 2 minutes, then rinsed three times with distilled water. A sterile blade and forceps were used to cut a small section of the tissue containing both the healthy and the rotten portion (3mm diameter) and plated on solidified Potato Dextrose Agar (PDA) containing Chloramphenicol (30 mg/l) to prevent bacterial growth. The inoculated plates were incubated at ambient room temperature (25–30 ℃) for seven 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 (1995). The Fungal isolates were subcultured onto 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 (Oyeleke and Manga, 2008). 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 24-48hours, after which it was viewed under the light microscope first with low resolution objective of 10x and then with high resolution objective of 40x to detect spore, hyphae and other special structures. The Morphological characteristics and appearance of the fungal isolates from the rotten fruits used in this study were confirmed and authenticated.

Pathogenicity Test

This was carried out as described by Baiyewu *et* al. (2007) and Chukwuka *et al*. (2010. A pathogenicity test was performed to determine whether the fruits' deterioration was actually caused by the isolated fungi. 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 300C, 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 the irrespective fruit.

Statistical Analysis

The data obtained were analyzed using Analysis of Variance, with least significant difference to separate the means. The data for the prevalence of fungal isolates was analyzed using frequency table.

RESULTS AND DISCUSSION

Seven (7) distinct fungal species were found among the spoiled fruits, according to the results of the characterization of the fungal flora obtained in Utako market (Table 1). *Aspergillusniger* was discovered to be present in all of the fruits in the current study, except onion and pawpaw, and seven fruit-poisoning fungus species were isolated and characterised. On the other hand, *Aspergillusflavu* were found in bananas, pineapples, peppers, tomatoes, and onions. On the other hand, pepper, tomato, watermelon, pumpkin, and pawpaw contain *Rhizopus* spp. *Aspergillus* species were limited to pineapple, pepper, and pawpaw, whereas *Mucors* species were limited to tomato and pawpaw. The outcome also demonstrated that *Aspergillus fumigatus* was found in pineapple and pawpaw, while *Alternaria* spp.was found in onions.

Table 1: Microscopic Characterization of Fungal Isolates from Spoilt Fruits IN Utako Market Abuja

S/N	Samples	Microscopic Characteristics	Fungi Identified
1	PI1a	Thick septatehyphae,chain of conidia on sterigmata	*Aspergillusniger*
2	PI1b	Irregular-sized hyphae with septation	*Aspergillus spp*
3	PI1c	Hyphae isseptate and is small in size	*Aspergillus fumigates*
4	PI1d	Green conidiospores with septate hyphae	*Aspergillusflavus*
5	PE1a	Thick septatehyphae,chain of conidia on sterigmata	*Aspergillusniger*
6	PE1b	Non septate hyphae and irregular in size	*Rhizopus spp*
7	PE1c	Green conidiaspore with septate hyphae	*Aspergillusflavus*
8	PE1d	Thick septatehyphae,chain of conidia on sterigmata	*Aspergillusniger*
9	TO1a	Thick septate hyphae, chain of conidia on the sterigmata	*Aspergillusflavus*

S/N	Samples	Microscopic Characteristics	Fungi Identified
10	TO1b	Thick septatehyphae,chain of conidia on sterigmata	*Aspergillusniger*
11	TO1c	non septate hyphae and irregular in size	*Rhizopus spp*
12	TO1d	Thick non septate hyphae with dark conidiospore	*Mucor spp*
13	WM1a	Thick septatehyphae,chain of conidia on sterigmata	*Aspergillusniger*
14	WM1b	Thick septatehyphae,chain of conidia on sterigmata	*Aspergillusniger*
15	WM1c	non septate hyphae and irregular in size	*Rhizopus spp*
16	WM1d	Thick non septate hyphae with dark conidiaspore	*Rhizopusspp*
17	PU1a	Thick septatehyphae,chain of conidia on sterigmata	*Aspergillusniger*
18	PU1b	Thick septatehyphae,chain of conidia on sterigmata	*Aspergillusniger*
19	PU1c	Thick non septate hyphae with dark conidiaspore	*Rhizopus spp*
20	PU1d	Thick septatehyphae,chain of conidia on sterigmata	*Aspergillusniger*
21	O1a	Erect conidiophores, septate hyphae with cylindrical conidia	*Alternaria spp*
22	O1b	Green conidiospores with septate hyphae	*Aspergillusflavus*
23	O1c	Green conidiospores with septate hyphae	*Aspergillusflavus*
24	O1d	Green conidiospores with septate hyphae	*Aspergillusflavus*
25	B1a	Thick septatehyphae,chain of conidia on sterigmata	*Aspergillusniger*
26	B1b	Thick septatehyphae,chain of conidia on sterigmata	*Aspergillusniger*
27	B1c	Thick septatehyphae,chain of conidia on sterigmata	*Aspergillusniger*
28	Bid	Thick septate hyphae, chain of conidia on the sterigmata	*Aspergillusflavus*
29	PA1a	Unseptate hyphae are irregular in size and ribbon like	*Mucor spp*
30	PA1b	Septate hyphae with conidiospores born on the conidia	*Aspergillus fumigates*
31	PA1c	Irregular hyphae small in size and septate	*Rhizopus spp*
32	PA1d	Hyphae is small and regular with cross septation	*Aspergillus spp*

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

More so, the result for the occurance of fungal isolates on the spoilt fruits of Utako market is shown in Table 2. *Aspergillusniger* was the most dominant fungal species with 37.50% occurrence, while *Alternariaspp* had the lowest occurrence of 3.12%.

Table 2: Ocurance of Fungal Isolates on the Spoilt Fruits from Utako Market

S/N	Fungal Isolate	Frequency n=100	Percentage (%)
1	*Aspergillusniger*	12	37.50
2	*Aspergillus spp*	2	6.25
3	*Aspergillus fumigates*	2	6.25
4	*Aspergillusflavus*	7	21.88
5	*Rhizopus spp*	6	18.75
6	*Mucors pp*	2	6.25
7	*Alternaria spp*	1	3.12
	Total	**32**	**100**

In Table 3, *Rhizopus spp* had the highest pathogenicity, producing largest rotten area of 52 mm in diameter. While *Aspergilus spp* and *Mucor spp* had almost similar pathogenecity.*Alternariaspp*had the least pathogenicity of 20 mm in diameter rotten surface.

Table 3: Pathogenicity of Fungal Isolates on Fresh Healthy Apple from Utako Market

S/N	Fungal Isolate	Diameter of Rot (mm)
1	*Aspergillusniger*	30
2	*Aspergillus spp*	26
3	*Aspergillus fumigates*	32
4	*Aspergillusflavus*	38
5	*Rhizopus spp*	52
6	*Mucor spp*	25
7	*Alternaria spp*	20

Discussion

According to Droby (2005), postharvest fruit spoilage caused by several fungal species poses a danger, particularly in developing nations like Nigeria. Seven distinct fungal species linked to fruit deterioration were identified in the present study. The findings of Ewekeye *et al.* (2013) among the spoiled fruits sold in certain Lagos markets, and Mailafia *et al*. (2017), who isolated and reported *Aspergillus niger* as the most prevalent mycological flora associated with fruit spoilage, are consistent with the presence of Aspergillus spp. and Rhizopus spp. as fruit spoilage agents in this study. This result is also consistent with the findings of Baiyewu *et al.* (2007) and Chukwuka *et al.* (2010), who isolated *R. stolonifer* and *A. niger* from pawpaw in Nigeria. The results are also consistent with the findings of Gadgile and Chavan (2010) regarding the isolation of fungal pathogens from fruits that are preserved and marketed. According to Okereke *et al*. (2010), *Alternaria* species contain *A. niger*. The spoiled mangoes

were used to isolate *Colletotrichum gloeosporioides* and *Botryodiplodia theobromae*. However, the value obtained for the prevalence of A. niger, which produces strong mycotoxins called ochratoxins that can be harmful to humans and animals and causes a disease called black mold on some fruits, is higher than the highest occurrence of 38% reported by Mailafia *et al.* (2017).

The majority of the fungi found in this study were crucial in the spoilage of fruit. *Rhizopus nigricans*, *A. flavus*, *A. niger*, *Fusarium* spp., and *Mucor* spp. were found in pawpaw fruit spoilage from a farm in Oyo state, Nigeria, according to Chukwuka *et al.* (2010). Furthermore, the presence of four distinct Aspergilus species in the spoiled fruits—*Aspergilus niger*, *Aspergilus flavus*, *Aspergillus* spp., and *Aspergilus fumigatus*—indicated the level of pathogenicity that the Aspergillaceae family conferred in causing fruit deterioration. This result aligns with that of Bukar *et al.* (2009), who found that soft rots of orange fruits in Nigeria were caused by *Aspergillus* species, *Mucor*, and *Rhizopus* spp. All of the fruits were impacted by the fungal isolates, according to the pathogenicity tests, but further data is required to compare how the isolates affected the fruits' sugar and nutritional value.

CONCLUSION

It was concluded that *Aspergillus*, *Rhizopus*, and *Mucor* were responsible for spoilage of fruits in the Utako market. This resulted in significant losses for retailers and a serious risk of food poisoning for the customers.

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