



Isolates of Some Rotting Fruits Collected at Yankaba Market, Kano, Nigeria

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Abstract: Studies on fungi isolates were carried out over a period of 4 weeks with different rotting fruits that were collected from sellers in Yankaba market, Kano. The fruits are sweet orange, apple, pineapple, watermelon, banana, pawpaw, coconut and wild bush mango were collected in clean sterile polythene bags separately. Each of the samples was cultured and isolated under room temperature (37°C). Materials used in culturing included Petri-dishes with potato dextrose agar as the media. Methylene blue was used in mounting and slide staining. It took a period of 7 days to incubate and isolate the fungi namely *Aspergillus spp.*, *Sclerotium spp.*, *Trichoderma spp.*, *Gleosporium spp.*, *Rhizopus spp.* and *Rhizoctonia spp.* were responsible for post-harvest deterioration of fresh fruits and vegetables. *Rhizopus spp.* had the highest frequency of occurrence.

Key Words: Fruits, Fungi, isolates, *Rhizopus*, pawpaw.

1. Introduction

Fungi are major causes of plant disease for about 70% of all the major crop diseases. Some of these fungal plant pathogens are termed biotrophic because they established an intricate feeding relationship with living host cells while others are termed necrotrophic killing the host cells to obtain nutrients (Rop *et al.*, 2009). The fungi that cause major damage to stored fruits and vegetables are necrotrophic pathogens (Ainsworth *et al.*, 1973). Examples of pathogens that are common include apple rotting fungi *Penicillium expansum* and *Monilinia fructigena*. The common 'anthracnose' fungus of bananas *Colletotrichum musae*. The common gray mould of strawberry and other soft fruits *Botrytis Cinerea*. *Fusarium spp.* is responsible for the *Fusarium* rot disease that affects cucurbit fruits. It causes lesions internally with dry, brown, spongy rot and a white halo. *Rhizoctonia species* are another group of fungi which were isolated from strawberry in Israel (Naqri, 2004). The most common causes of citrus fruits decay throughout the world are the penicillium rots due to *Penicillium italicum* and *Penicillium digitatum* (Chaube and Pundir, 2005). Another very important fungus affecting common fruits is *Aspergillus flavus*. This is mostly found in rotting sweet orange (*Citrus sinensis*). The *Rhizopus species* cause *Rhizopus* rot of harvested and over-ripe stone fruits (Ammirati *et al.*,

2009). Defense mechanism in fruit appears to be highly effective against nearly all fungi as only a relatively few genera and species are able to invade and cause serious losses, some of these are highly specialized pathogens attacking one of two kinds of fruits (Dennis, 1983). Fruits become increasingly susceptible to fungal invasion during ripening and when the fruit wall is broken by abrasion, falling, claw marks, tooth trials, partial consumption (by birds, insects, etc). Invasion requires damage to skin tissues which readily occurs in modern bulk handling systems. Decay spreads by contact from fruit to fruit. The spoilage of fruits, in particular apples, is associated with the production of mycotoxins which can represent a hazard to public health from either acute or chronic toxicity. Patulin-like aflatoxin has been reported to exhibit carcinogenic, teratogenic and mutagenic effects (Singh, 1998).

Worldwide postharvest fruit losses are as high as 30% to 40% and even much higher in some developing countries like Nigeria. Too much of the world harvest is lost to spoilage and infestations on its journey to the consumer (Dhingra and Idhare, 1971). The research is aimed to study and identify the different types of fungi present on the various rotting fruits and vegetables collected from Yankaba market, Kano. Identifying the pathogen would help to maintain fruit quality and reduction of spoilage by the pathogens via enhanced storage facilities such as fiberboard boxes or cartoons

used for citrus fruits, polythene bags, modified atmosphere packaging (Anon, 1990).

2. Materials and Methods

2.1 Sampling Site and Collection

The study area was Yankaba market, Kano, a suburb on the eastern axis of Kano metropolis on Longitude and Latitude of 12⁰02'N and 08⁰30'E respectively. The market was first temporarily situated at Yandaru and later relocated to its present site called Yankaba. The market stocks virtually anything eatable in the Northern part of the country, ranging from grains, vegetables, fruits, spices etc. The fruits collected are sweet orange, pineapple, watermelon, wild bush mango, coconut, pawpaw, apple and banana were purchased from sellers in Yankaba market separately in clean sterile containers.

2.2 Isolation Method

2.2.1 Culture Media: Most fungi grow well on media having a pH of 6.5, any rich carbohydrate source supported fungal growth and the most commonly used media was potato dextrose agar (PDA). In this study PDA was used in the isolation and identification of the fungi. A medium too rich in nutrients tends to produce too much mycelium at the expense of fructifications. It is better to grow fungi on nutritionally weak media such as potato dextrose agar (Singh, 1998).

2.2.2 Experimental Design: The different rotting fruits (wild bush mango, sweet orange, pawpaw, apple, pineapple, coconut, watermelon and banana) were collected and each sample was treated with 0.1% sodium hypo chloride for 1-2 minutes for surface sterilization, excess sodium hypochlorite soil was washed off with sterile water 3 times.

A small part of each spoiled fruit was teased with a needle and put in Petri-dishes containing the prepared PDA. It was then incubated at 37⁰C (room temperature) for a period of 5-7 days.

Part of the fungal growth was teased onto the slide and stained with methylene blue. The appropriate microscopic magnification was used in the viewing and the identification of the various fungal isolates. A photomicrograph of each fungi isolate was taken for further identification and naming.

2.2.3 Identification of Fungi Isolates: The identification of molds was mainly done by recognizing the diagnostic morphological features of genera and species by macroscopic and microscopic features, although physiological features also play an important role as complementary tools. The classical method used for microscopically examining culture was methylene blue slide mount of a sample of a culture teased apart with needles. This technique has the disadvantage of disrupting the relationship of conidiophores to their

conidia and this requires expert interpretation (Chaube *et al.*, 2002). The macroscopic characteristic features include the diameter, texture, color of the fungal colony. The microscopic characteristics include unique mycelia structure, hyphae and conidia.

3. Result and Discussion

From the result obtained *Rhizopus spp.* has the highest frequency of occurrence, and then followed by *Rhizoctonia spp.* and *Aspergillus spp.* The colonies appeared smooth or woolly (Renata *et al.*, 2011). The colors ranged from greenish, black, whitish and brown. The colony diameter ranged from 0.5cm – 8.9cm. The most blackish colony was either *Rhizopus spp.* or *Rhizoctonia spp.* The colonies of *Rhizopus* grow very rapidly, fill the Petri-dishes and mature in 4 days. The texture was typically cotton-candy like (Denis, 1983).

Fruits	Growth Description	Colony Size Diameter (cm)	Color	Suspected organism
Sweet Orange	Smooth	8.9	Whitish and Black Sclerotium	<i>Aspergillus spp.</i>
Pineapple	Woolly	1.7	Whitish	<i>Trichoderma spp.</i>
Water melon	Smooth	8.5	Whitish and black sclerotium	<i>Aspergillus spp.</i>
Wild bush mango	Smooth	3.3	Whitish	<i>Gleosporium spp.</i> (Micro & Macro Conidia)
Coconut	Smooth	1.3	Greenish and blackish	<i>Sclerotium spp.</i>
Pawpaw	Smooth	1.4	Greenish, brown	<i>Rhizopus spp.</i>
Banana	Smooth	3.8	Blackish	<i>Rhizoctonia spp.</i>
Apple	Smooth	0.9	Blackish	<i>Rhizopus spp.</i>

Table 1: Colony Characteristics of Fungi Isolates of Different Fruits.

The following fungi were isolated, *Rhizopus spp.*, *Rhizoctonia spp.*, *Aspergillus spp.*, *Trichoderma spp.*, *Gleosporium spp.* From the fungi isolated, *Rhizopus spp.* and *Aspergillus spp.* has the highest rate of contamination in the market, followed by *Rhizoctonia spp.* These are the most common contaminant spoilage fungi of fruits in Nigeria. This might be due to either the difference in the origin of the produce, since the modes of handling in the markets are different (Alexopoulos, 1973). The soft rot of banana caused by *Rhizopus spp.* was found to account for about 85% of the post-harvest fruit rot diseases in the Nigerian market. Visits to the markets revealed that fruits were displayed on open stalls and counters or even on bare grounds close to the open gutters. This provides an adequate environment for contact with dangerous microorganisms. One other factor that enhances the penetration of the fruits by these pathogens might be the constant wetting of the fruits by the vendors. This has been reported to render the fruits more susceptible to pathogens (Rop *et al.*, 2009). The water enhances and stabilizes the water activity requirements for growth by the organisms. Chlorination of water used for wetting of fruits reduces the extent of damage done by those contaminations (Dennis, 1983; 14 & 15).



Plate 1a: Spoilt Apple shows fungal infection.



Plate 2b: Cultured Isolate of the fungi *Sclerotium spp.* (b).



Plate 1b: Petri-dish containing Isolate of *Rhizopus spp.* at 7th day incubation.

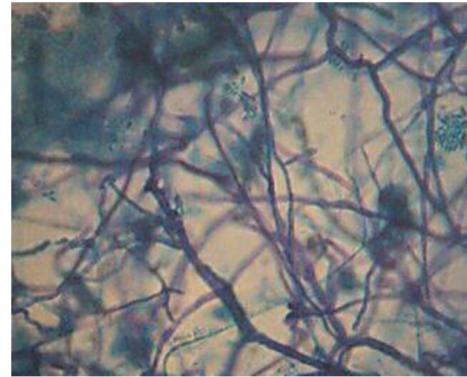


Plate 2c: Photomicrograph of *Sclerotium Spp.* Mg = x 40.



Plate 1c: Photomicrograph of *Rhizopus spp.* Mg = x100
Sporangiophore (c), Sporangium (d).



Plate 4a: Rotting Sweet Orange Fruit showing infected area (a).



Plate 2a: Rotting coconut fruit showing infection (a).



Plate 4b: Petri-dish containing Isolate of *Aspergillus spp.* (b).

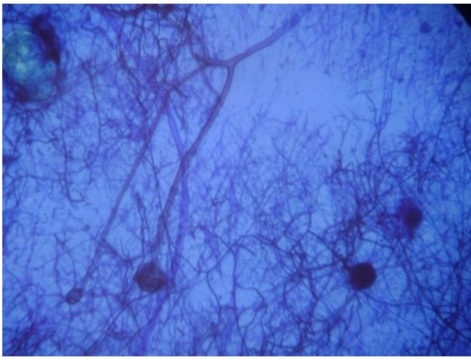


Plate 4c: Photomicrograph of *Aspergillus Spp.* Mg = x40
Sporangium (c), Sporangiphore (d), Stolon (e).



Plate 6a: Rotting Pawpaw showing white lesions from infection from *Rhizopus spp.* showing infected area (a).



Plate 5a: Spoilt Wild Bush Mango showing infected area (a).



Plate 6b: Petri-dish containing Isolate of *Rhizopus spp.*, growth (b).



Plate 5b: Petri-dish containing Isolate of *Gloeosporium spp.* (b).

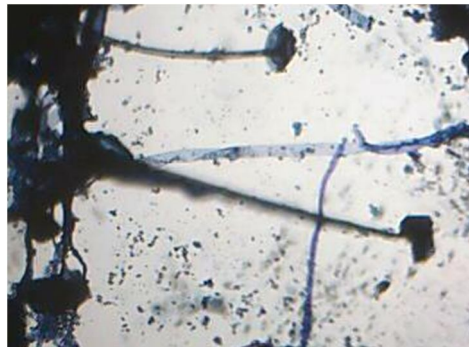


Plate 6c: Micrograph of *Rhizopus spp.* Mg = X40
Sporangiophore (c), Sporangium (d).

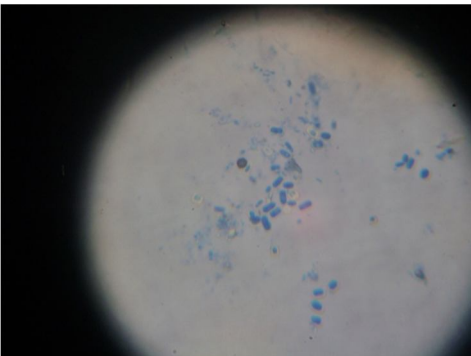


Plate 5c: Photomicrograph of Micro and Macro Conidia of *Gloeosporium spp.* Mg = x40.



Plate 7a: Rotting Pineapple Fruit showing infected area (a).

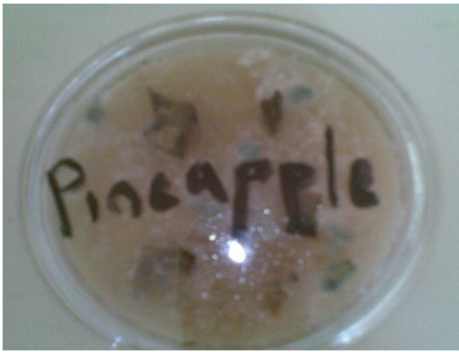


Plate 7b: Petri-dish containing Isolates of *Trichoderma* spp., growth (b).

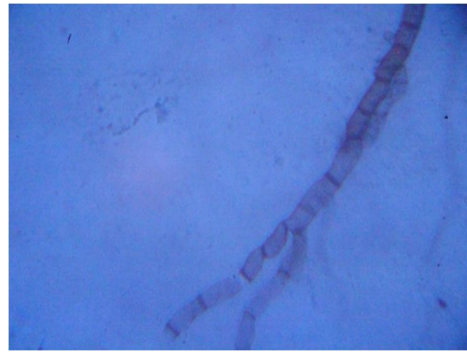


Plate 8c: Photomicrograph of *Rhizoctonia* Spp. Mg = x40.

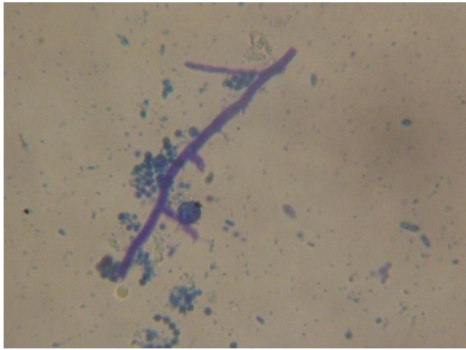


Plate 7c: Photomicrograph of *Trichoderma* Spp. Mg = x40.



Plate 11a: Rotting Water Melon Fruit showing infected area (a).



Plate 8a: Rotting Banana fruit showing infected area (a).



Plate 11b: Petri-dish containing Isolate of *Aspergillus* spp., growth (b).



Plate 8a: Petri-dish containing Isolate of *Rhizoctonia* spp., growth (b).

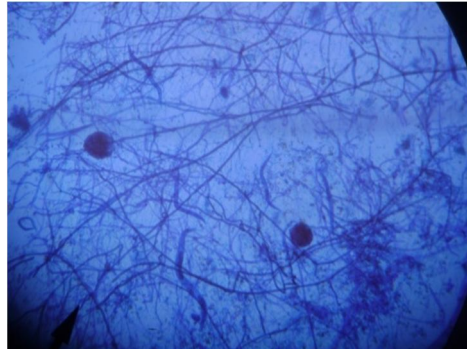


Plate 11c: Photomicrograph of *Aspergillus* spp. isolated from Water Melon. Mg = x40 Sporangiphore (c), Sporangium (d).

4. Conclusion and Recommendation

The result of the study has clearly shown that fungi are found in fruits which have been improperly handled in the markets. This is due to mechanical injury or improper storage facilities. The accurate identification of the causal pathogen is essential before appropriate treatment and improved storage facilities are recommended to control the pathogens. The use of plastic films (box liners, gas tight or perforated bags, pallet covers) is most recommended in packaging. Perforated packages (for apples) allow a certain amount of ventilation, limiting the risk of fermentation and accumulation of carbon dioxide and ethylene. People are discouraged from eating unwashed fruits since some of the molds causing spoilage of these fruits are known to produce toxin e.g. *Rhizopus spp.* and *Aspergillus spp.*

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