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ISOLATION AND IDENTIFICATION OF FUNGI ASSOCIATED WITH POSTHARVEST DETERIORATION OF BANANA (*Musa paradisiaca* L.)

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Abstract

This work was designed to isolate and identify the fungi associated with post- harvest decay of banana fruits sold in local markets in Sokoto metropolis. A total of 6 clusters of rotted fruits samples were collected each from Kasuwar Daji and Tsohuwar Kasuwa markets, small pieces of mouldy parts or lesions were surface- sterilized with alcohol and inoculated on prepared plates of Potato dextrose agar (PDA). After 48-62 hours of incubation, when fungal growth from the tissue was visible, fungi were sub cultured onto PDA to obtain pure cultures. Pure isolated fungi were identified according to the recommended references. Seven fungal species were isolated as agents associated with post-harvest decay of banana. Colletotrichum musae *Aspergillus niger* and *Fusarium*sp had the highest percentage occurrence among the isolates from Tsohuwar Kasuwa while *Colletrotrichum musae* was the most frequently occurring isolate from Kassuwar Daji, Banana fruits being succulent are liable to damage and deterioration during harvesting, transportation, marketing, storage and consumption, if not properly handled. Bananas stand in greater peril to fungal damage than the majority of other fruits because they are sterile, and seedless.

Keywords: *banana, fungi, Fusarium* sp, *Aspergillus niger.*

Introduction

Fungi are considered as an essential post-harvest losses agent of different fruits, based on cultivar, season and production area amid other factors (Valiuskaite et al., 2006; Ewekeye et al., 2016). The postharvest losses are often more harsh in developing countries due to lack of storage and transportation facilities. Fruit infections by fungi may appear during the growth period, harvesting, handling, transportation and post-harvest stockpile and marketing conditions, or after procuring by the consumer. Fruits incorporate high levels of nutrients element and sugars and their low pH values make them exceptionally desirable to fungal decay (Abdullah et al., 2016). Banana fruit is grown in more than 100 countries, mainly in sub-tropical areas (Stover and Simmonds, 1987).). Fruits at the post-harvest stage are exposed to the unnatural conditions which predispose the fruits for the attack of fungi. Pathogenic fungi establish themselves predispose the fruit for attack on fungi. Pathogenic fungi establish themselves Fruits have wide distribution in nature. The relatively short shelf-life period provoked by pathogens is one of the most important limiting factors that impact the economic value of fruits. Approximately 20-25% of the harvested fruits are deteriorated by pathogens during post-harvest handling even in advanced countries (Droby, 2006; Zhu, 2006). For the fresh bananas to reach the consumer in the right condition, it must be marketed properly, bearing in mind the application of most suitable temperature and humidity as well as appropriate packaging and handling methods. Good handling during harvesting can minimize mechanical damage and reduce subsequent wastage due to microbial attack (Wills et al., 1998). The aim of this study is to isolate and identify the fungi species associated with post-harvest deterioration of banana fruit in Sokoto metropolis. The specific objectives were to;

i. Isolate various fungi from a deteriorating banana fruit.

ii. Identify the fungal isolates using comparative morphological analysis.

iii. Determine the percentage occurrence of the different fungal isolates.

Methods

The study was conducted at the Mycology laboratory, Department of Biological Sciences, Usmanu Danfodiyo University Sokoto.

Sample Collection

Three banana clusters each with moderate to severe lesions were collected from Kasuwan daji market and Tsohuwar Kasuwa Market all in Sokoto metropolis. The diseased fruits were collected separately in polythene bags to avoid contamination. The symptoms were carefully noted; completely rotten fruits were avoided for isolation as they contained mostly secondary pathogens. The collected fruits were transferred to mycology department, Usmanu Danfodiyo University, Sokoto for further analysis.

Materials used

The glass wares used in this research include; Petri-plates, conical flask (1000ml), measuring cylinder (1000ml), glass slides and coverslide. Chemicals used include; Potato dextrose agar (PDA), ethanol and streptomycin. Other materials used are; microscope autoclaves, hot plate, aluminum foil, distilled water, masking tape, Bunsen burner, face mask, spatula, weighing balance, inoculating needles, hand gloves and cotton wool.

Sterilization of glass wares

All the glass wares were first washed with tap water and detergent solution. They were then rinsed with distilled water and air dried. The glass petri-dishes were wrapped with aluminium foil and autoclaved at 160OC for one hour (1hr). They were allowed to drop for 30 minutes before usage to avoid cracking.

Media preparation

The PDA media was prepared according to manufacturer's instructions (3.9g for each 100ml of distilled water). 23.4g of PDA was dissolved in 600ml of distilled water and 0.6g streptomycin was added as a bacteriostatic agent to inhibit the growth of bacteria in the medium. The mixture was then heated on a hot plate to obtain a homogeneous mixture. The mixture was then autoclaved with a pressure sterilizer at 121OC for 15 minutes. It was then allowed to cool down to 100OC (0OC on calibrator) before the valves were opened and the lid was removed. After 15 minutes, the media was removed and allowed to cool down to 45OC at room temperature.

Inoculation and incubation

The affected tissues were surfaced-sterilized with 10% ethanol using a cotton wool. Four small pieces from the margin of lesion of each sample were directly inoculated aseptically on prepared plates of Potato

dextrose agar (PDA) and incubated at 28OC for 3-5 days.

Sub culturing

When fungal growth from the tissue was visible, fungi were sub cultured onto PDA to obtain pure cultures for identification. Fungi were continuously sub cultured until pure isolates were obtained. The pure fungal cultures were stored safely in the refrigerator at 4°C to prevent any fungal growth in the plates.

Identification of the isolates

The fungal isolates were subjected to certain comparative morphological studies by an image and analysis system using published descriptions in a mycological atlas contained in the Mycology Laboratory of Department of Biological Sciences Usmanu Danfodiyo University Sokoto. This was followed by a slide mount of each isolate. The characteristics observed were matched with those available in the aforementioned mycological atlas. They were then identified accordingly.

Determination of percentage occurrence of isolates

This was done to determine the percentage occurrence of the different fungal isolates. Isolations were made from four different rotted banana fruits and were cultured differently. The number of occurrence for each of the isolates in the four different samples were recorded and calculated as a ratio of occurrence andwas then expressed as a percentage. The Formula of Muhammad et al. (2004) was followed

Percentage colonization = Number of colonies for a pathogen/Total number of colonies X 100

Results

The result shown in Table one (1) indicates the morphological characteristics of the isolated fungal species involving both the macroscopic and microscopic features in both sample markets from Sokoto metropolis. A total of seven species were identified belonging to five genera Table two (2) indicates the result of the various fungal species isolated from each of the two markets. *Colletrotrichum musae*, *Fusarium*spp, Three *Aspergillus* species and Saccharomyces cerivisae were isolated from both markets. The only exception is Rhizopus spp, which was isolated only in Kasuwar Daji Market. Table three (3) shows the result of percentage occurrence of the isolated fungi from the two markets. *Colletrotrichum musae* is the most frequently occurring isolate from Kasuwar Daji while C. musae, A. *niger* and *Fusarium*spp have been found to be the three most frequently occurring isolates from Tsohuwar Kasuwa Market..

Discussion

Discussion The study reveals the isolation and identification of fungi associated with postharvest deterioration of banana fruit using two markets in Sokoto metropolis as a case study.

A total of seven fungi species were isolated in Kasuwar Daji Market with Tsohuwar Kasuwa having six representatives from the isolates. The only difference was Rhizopus stolonifer, which was isolated in Kasuwar Daji only. *Colletrotrichum musae* was found to be the most frequently occurring fungi isolated from the samples obtained representing 35.71% in Kasuwar Daji and 20% in Tsohuwar Kasuwa. These indicate that my findings are partially in concordance with a similar research conducted by Abdullahi et al (2016) whose result shows a total of seven (7) species isolated from banana fruit. However, only two (2) species isolated in this study (*Colletrotrichum musae* and *Fusarium*spp) are part of the seven (7) isolates. Acremonium sp., Curvularia sp., Alternaria alternata, Colletrotrichum lunata and Ulocladium botrytis are the exceptions. However, the result also showed C. musae to have the highest percentage occurrence (42%). Raut and Ranade (2004) and Ranasinghe et al. (2005) reported that, banana suffer from serious post-harvest losses caused by fungal infections, especially C. musae. Sulali et al. (2004) mentioned that from nine localities in Sri Lanka the fungal pathogen isolated from the anthracnose lesions of banana was identified as C. musae. In Taiwan, Chuang and Yang (1993) reported that banana anthracnose induced by C. musae was an important post-harvest disease and caused serious loss during transport. Similar findings were also reported in Sri Lanka. Nath et al (2015), in a study, "management of banana fungal disease using fungicides", reported lasiopladia theobromae to be the isolate with the most percentage occurrence of banana rot. This was however, not isolated in this study.

Conclusion

Isolation of fungi were made from deteriorating banana fruits by tissues isolation and associated fungi were identified as *Colletrotrichum musae, Fusariumsp., Aspergillus niger, Aspergillus fumigatus, Aspergillus Flavus, Saccharomyces cerevisae, and Rhizopus*

stolonifer which were confirmed by comparative macroscopic and microscopic morphological analyses. Postharvest diseases of fruits in general and banana in particular are of huge economic importance. In this study, all banana fruits from the markets showed symptoms of postharvest rots. The fruits may be attacked by secondary invaders or primary agents of rots during packaging, transportation or storage.

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Table 1: Morphological identification of the isolated fungi.

Isolate	Macroscopic features	Microscopic features
Colletotrichum musae	The fungus grew fast and produced white aerial mycelium on PDA. Acervuli developed abundantly on culture plates after incubation for 8 days. Pinkish conidial masses were produced on the acervuli, which mostly coalesced together.	Conidia were aseptate, hyaline, straight and ellipsoid to globose
Fusariumspp	Growth recognizable within three days. Rapidly growing, wooly to colt, lemon or yellow in colouration	Multicellular distinctive sickle shaped conidia which were septate.
Aspergillus niger	Growth is recognizable within few days, from velvety to glacy surface due to sporulation and appears to be black in colour	The hyphae are septate. Consisting of a compact white or yellow basal felt with a dense layer of dark brown to black conidiosphores. Conidial heads radiate, tending to split into loose columns with age.

Aspergillus fumigatus	Growth is recognized within three days and it consists of dense felt of dark green colouration.	Consisting of dense felt of dark green conidiosphores intermixed with aerial hyphae bearing conidiosphores. Conidial heads typically columnar. Conidiosphores short, smooth, particularly in the upper part.
Aspergillus Flavus	Growth is recognized within three days Consisting of dense felt or yellow-green colouration.	Consisting of septate hyphae. Consisting of dense felt or yellowgreen conidiosphores. Conidial heads typically radiate, later splitting into several loose columns, yellow-green becoming dark yellowgreen.
Saccharomyces cerevisae	Growth is observed within few days. Appear creamyyellowish green.	Appear in green flat circular colony. Spherical spores often in group of fours. Appear spherical or ovoid in shape. Have simple pseudohyphae.

Rhizopus stolonifer	Growth is recognizable within few days. Appear whitish in colour.	Colony whitish becoming grayishbrown due to brownish sporangiosphores and brown-black sporangia, often over 20mm high. Sporangioshores 1.5- 3(4) mm tall, solitary or in groups of 2-7(usually 3-4) from the almost colourless to darkbrown.

Table 2: Distribution of the isolated fungi in the two sample markets.

	C.musae	S.cerevisae	*A.niger*	*A.flavus*	*A.fumigatus*	*F.semitectum*	*R.stolonifer*
Kasuwar Daji	+	+	+	+	+	+	+
Tsohuwar Kaasuwa	+	+	+	+	+	+	-

Table 3: Percentage occurrence of fungal isolates.

	Kasuwar Daji		Tsohuwar Kasuwa	
Isolates	No of colonies	Frequency (%)	No of colonies	Frequency (%)
C. musae	5	35.71	3	20.00
A. niger	2	14.29	3	20.00
A. fumigatus	2	14.29	2	13.33
A. flavus	1	71.14	2	13.33
Fusarium spp.	2	14.29	3	20.00
R. stolonifer	1	7.14	0	0.00
S. cerevisae	1	7.14	2	13.33