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Microoganisms Associated With Post Harvest Diseases Of Yams (*Dioscorea* Species) In Barns

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Abstract

A total of 105 tubers of yam which included *Dioscorea rotundata* (White yam), *D. cayenensis* (yellow yam), *D. alata* (Water yam) and *D. esculenta* (Chinese yam) in barns in Rivers State were investigated for microorganisms associated with post-harvest disease. This is aimed at providing basic information on post-harvest and storage diseases of yams (*Dioscorea*) in barns and suggesting ways of controlling losses due to post harvest disease. Standard microbiological techniques and pathogenicity test were used for the analyses. Mean values of fungal counts ranged from 1.7×10^6 $SFUg^{-1}$ to 6.1×10^8 $SFUg^{-1}$. Frequency of fungal isolates was *Aspergillus niger* (30.56%), *Fusarium oxysporum* (12%), *Penicillium citrinum* (11.1%), *Penicillium* spp (20%), and *Rhizopus* spp (25%). All fungal isolates occurred in all the *Dioscorea* spp except *D. esculenta* which had no incidence of *Fusarium oxysporum* and *Penicillium*. Incidence of *Aspergillus*, *Penicillium*, and *Rhizopus* was highest in *D. rotundata* and lowest in *D. esculenta*. Mean values of viable bacterial counts ranged from 3.22×10^8 $CFUg^{-1}$ to 1.06×10^9 $CFUg^{-1}$. Frequency of bacteria was *Corynebacterium* spp (6.2%), *Pseudomonas* spp (20.0%), *Staphylococcus* spp (10.5%), *Bacillus* spp (14.0%), *Enterobacter* spp (12.2%), *Micrococcus* spp (8.8%), *Enterococcus* spp (12.2%), *Escherichia coli* (14.0%) and *Proteus* spp (6.6%). *Pseudomonas* occurred in all the *Dioscorea* species while *E. coli* occurred only in *D. rotundata. Staphylococcus* spp, *Enterococcus* and *Proteus* species occurred only in *D. cayenensis*. *D. esculenta* (Chinese yam) is more resistant to fungal attack while *D. rotundata* (white yam) is more susceptible to fungal attack. On the other hand, the white yam is more resistant to bacterial attack while *D. cayenensis* (yellow yam) is more susceptible to bacterial attack. Dry rot disease, soft rot and wet rot accounted for 49.5%, 39.0% and 11.4% of spoilage respectively. Pathogenicity test revealed that *Aspergillus niger* was highly pathogenic. Species of *Aspergillus, Fusarium* and *Penicillium* are known to produce mycotoxins which have varying implications for health and the economy. *E. coli* an indicator of faecal contamination and all the bacteria isolated also have various health implications. The fungi, bacteria and various rots reported in the *Dioscorea* species is attributed to faecal contamination of farm soil and poor handling before, during and after harvest.

Keywords: Yams, *Dioscorea*, tubers, barns, fungi, bacteria, rot, *E. coli*

Introduction

Yams (*Dioscorea* species) constitute an economically important staple food in tropical and subtropical regions of the world. Production and consumption of yam is predominant in West and Central Africa and it is the second most important root tuber crop in Africa after cassava, with the production of cassava being about 22% more than that of yam (Otegbayo *et al*., 2005).

Nigeria is the largest producer of yams in the world with over 35 million metric tonnes (Ekunwe and Orewa, 2007). Of particular significance are the white Guinea yam (*Dioscorea rotundata*), the water yam (*Dioscorea alata*), the yellow yam (*Dioscorea cayenensis*) and the Chinese yam (*Dioscorea esculenta*). *D. rotundata* and *D. alata* are the most important varieties of yam cultivated in West Africa today. However, *D. rotundata* is the most important variety of yam cultivated for human nutrition not only in Nigeria, but throughout the world (Ogaraku and Usman, 2008).

Tubers of *Dioscorea* have high carbohydrate mainly starch but with varying levels of proteins, lipids, minerals and most vitamins except vitamin C (Kouassi *et al*., 2009). *Dioscorea* provide around 110 calories per 100grams. It is high in potassium, manganese and dietary fibre while being low in saturated fat and sodium. A product that is high in potassium and low in sodium is likely to produce good potassium – sodium balance in the human body and so protects against Osteoporosis and heart disease. *Dioscorea* products generally have a lower glycemic index than potato products and therefore provide more sustained form of energy and give better protection against obesity and diabetes. It is also known to replenish fast – twitch fibres and West Indians use it as a way of recovering after sprinting (Brand-Miller *et al*., 2003).

Yams of Africa species must be cooked to be safely eaten because various natural substances in raw yams can cause illness if consumed. Excessive skin contact with uncooked yam fluid can cause the skin to itch. If this occurs, a quick cold bath or application of palm oil to the affected part of the body will stop the itching. Yams are consumed in various ways but are usually boiled and eaten. The boiled yam can also be pounded to create thick starchy dough known as pounded yam "*Iyan*", as well as "*Fufu*". The raw yam pieces can be sun-dried (turns dark brown in colour) and then milled to create a powder known as "*Elubo*" in Western Nigeria. The brown powder can be prepared with boiling water to create thick brown starchy dough known as "*Amala*" which is also consumed with the local stews and sauces (Otoo and Asiedu, 2009).

Post-harvest handling and storage of yams (*Dioscorea*) is an essential aspect of economic development. In Nigeria, Yam tubers are harvested mostly between June and September and stored in different storage facilities depending on the cultural and tradit
onal values as well as the technological advancement of the people of such area until consumption, sale, or replanting (Nwankiti, 1989; FAO, 1990).

Accurate figures on yam (*Dioscorea*) production in Nigeria are hard to come by. However, some of the losses which can occur during the storage of fresh yams are endogenous, i.e. physiological. These include transpiration, respiration and germination.

Infection of *Dioscorea* by microorganisms could be at any stage in its growth from seedling stage through to post harvest. Most of the pathogens are soil-borne, but manifestations of the tuber disease are observed mostly during storage when tubers could be subject to losses of up to 50% of the fresh matter (Amusa *et al*., 2003). The losses due to microbial attack play a predominant role. Fungi, bacteria, and other pathogens penetrate through wounds or cuts and natural openings on the surface of the tubers and infect the inner tissues. Such wounds are caused by insect pests, nematodes, rodents and poor handling before, during and after harvest.

The aims and objectives of this study are to isolate and characterize the fungi and bacteria associated with spoilage of yams (*Dioscorea*) during storage in barns, to investigate the incidence of these microorganisms, and to conduct pathogenicity tests to confirm the spoilage organisms. This is to provide basic information on post harvest and storage diseases of yams (*Dioscorea*) in barns and to suggest ways of controlling losses due to post harvest disease.

Materials and Methods

Sample Collection and Processing of Dioscorea species

A total of one hundred and five (105) tuber samples of various *Dioscorea* species; *D. rotundata* (White yam), *D. cayenensis* (yellow yam), *D. alata* (Water yam) and *D. esculenta* (Chinese yam) were obtained from different barns in Igwuruta town in Rivers State of Nigeria. Some of the barns are shown in Plate 1 below.

Plate 1: *Dioscorea* (Yam) barns in Igwuruta town, Port Harcourt, Nigeria

The rotted diseased tubers were packaged in sterile polyethene bags and transported in cool boxes to the laboratory where they were analyzed for the presence of micro-organisms.

Each tuber was rinsed in distilled water and sterilized with 70% ethanol and cut open with a sterile knife. The cells of the rotted parts were disrupted using sterile mortar and pestle and 1.0g of each sample was added to separate 9.0mls of diluent and serial dilutions of each sample was made (Ogaraku and Usman, 2008; Okigbo and Ikediugwu, 2000).

Cultivation of fungi and bacteria, Enumeration, Characterization and Identification

Sabouraud dextrose agar (SDA) and Nutrient agar was prepared according to the manufacturer's instruction and sterilized by autoclaving at 121^{o}C for 15 minutes. The agar media were allowed to cool to about 45^{o}C. Tetracycline was added to the SDA medium to prevent bacterial growth and permitted selective isolation of yeasts and moulds (Walker and Colwell, 1976; Paul and Clark, 1988; Harrigan and McCance, 1990). The media were aseptically poured into separate sterile Petri dishes and allowed to set. An aliquot (0.1ml) of 10^{-1} dilution of each *Dioscorea* species was separately plated onto SDA plates and incubated at 27.5^{o}C in an inverted position for 5 days for the cultivation of fungi. The discrete colonies that developed on SDA plates were counted and the mean of replicate plates were recorded. The counts were also computed into spore forming units of fungi per gram (SFUg^{-1}) of yam (*Dioscorea*) sample. Fungal cultures were observed while still on plates and after wet mount in lactophenol on slides under the compound microscope. The observed characteristics were recorded and compared with the established identifications keys of Malloch (1997).

On the other hand, 0.1ml aliquot of 10^{-3} and 10^{-4} dilutions was plated onto nutrient agar plates for the cultivation of bacteria. These dilutions were plated out for bacteria because the dilution of 10^{-1} gave a confluent growth during a preliminary investigation. Cultured plates were inverted and incubated at 28^{o}C for 24 hours after which the plates were examined for growth. The discrete colonies which develop were counted. The averages for duplicate cultured plates were recorded as colony forming unit per gram (CFUg^{-1}) of sample and as the total viable aerobic heterotrophic bacteria in the sample (Obire and Abuba, 2009).

Discrete bacteria colonies which developed were sub-cultured to obtain pure cultures of the isolates that were subjected to various characterization procedures. The bacteria were identified on the basis of their cultural, morphological, and physiological characteristics as described by Cruickshank *et al*. (1975). Further identification was made by comparison of their cultural, morphological and physiological characteristics with those of known taxa and with reference to Holt (1997).

Pathogenicity Test

Healthy tubers of the different *Dioscorea* species were washed with tap water and distilled water and thereafter sterilized with 70% ethanol. Cylindrical discs (4mm) were removed from each tuber with a sterile 4mm cork borer and the test organism was inoculated into the bore. The cylindrical discs (4mm) were replaced after the inoculation of the tubers and then sealed with Vaseline jelly to make it air tight. The tubers were thereafter incubated for 14 days at room temperature (Ogaraku and Usman, 2008; Okigbo and Ikediugwu, 2000). The test result revealed that *A. niger* was highly pathogenic, causing severe dry rot while the rest were mildly pathogenic except for *Penicillium spp* that caused moderate rot. The bacteria spp were also able to cause rot.

Result

The result of the mean value of total viable count of fungi and of bacteria isolated from the different *Dioscorea* (yam) species is shown in Table 1 and Table 2 respectively. *Dioscorea rotundata* (white yam) recorded the highest mean value of fungi of 6.1×10^8 spore forming unit per gram ($SFUg^{-1}$) while *D. esculenta* (Chinese yam) recorded the lowest of 1.7×10^6 $SFUg^{-1}$. Mean values of total bacteria count was highest (1.06×10^9 $CFUg^{-1}$) in *D. cayenensis* (yellow yam) and lowest (3.22×10^8 $CFUg^{-1}$) in *D. rotundata* (White yam). The fungi isolated from the yams were *Aspergillus niger*, *Fusarium oxysporum*, *Penicillium citrinum*, *Penicillium* spp and *Rhizopus* spp. However, *Penicillium citrinum*, and *Rhizopus* spp were not isolated from *D. esculenta*. Generally, the frequency of occurrence of fungal isolate in all the *Dioscorea* species was *Aspergillus* spp (30.56%), *Penicillium* (23.61%), *Rhizopus* spp (20.83%), and *Fusarium* (25.0%). The frequency of each fungal isolate in the various *Dioscorea* species is shown in Figure 1. The frequency of each fungal isolate was highest in *D. rotundata* and lowest in *D. esculenta* (Chinese yam). Generally, the bacteria isolated and frequency of occurrence were *Corynebacterium* spp (6.2%), *Bacillus* spp (14.0%), *Pseudomonas* spp (20.0%), and *Micrococcus* spp (8.8%), *Enterococcus* spp (12.2%), *E. coli* (14.0%), *Enterobacter* spp (12.2%), *Staphylococcus* spp (10.5%), and *Proteus* spp (6.6%).

The three major post harvest diseases of yams observed during the investigation were dry rot, soft rot and wet rot. These accounted for 49.52%, 39.05% and 11.43% of diseased tuber of yam respectively.

Table 1: Mean value of total viable count of fungi isolated from *Dioscorea* species

***Dioscorea* species**	**Total count ($SFUg^{-1}$)**	**Fungi isolated**
D. rotundata	6.1×10^8	*Aspergillus niger*, *Fusarium*, *Penicillium citrinum*, *Penicillium* spp, *Rhizopus*
D. cayenensis	5.6×10^8	*Aspergillus niger*, *Fusarium sp*, *Penicillium citrinum*, *Penicillium spp*
D. alata	1.9×10^7	*Aspergillus niger*, *Rhizopus*, *Fusarium* spp *Penicillium citrinum*, *Penicillium* spp,
D. esculenta	1.7×10^6	*Aspergillus niger*, *Penicillium* sp, *Rhizopus*,

Table 2: Mean value of total viable count of bacteria isolated from *Dioscorea* species

***Dioscorea* species**	**Total count ($CFUg^{-1}$)**	**Bacteria isolated**
D. rotundata	3.22×10^8	*Bacillus* spp, *Escherichia coli*, *Corynebacterium* spp, *Pseudomonas* spp
D. cayenensis	1.06×10^9	*Staphylococcus* spp, *Pseudomonas* spp, *Enterobacter* spp, *Enterococcus* spp, *Proteus* spp
D. alata	7.68×10^8	*Bacillus* spp, *Corynebacterium*, *Pseudomonas* spp, *Enterobacter* spp
D. esculenta	5.92×10^8	*Bacillus* spp, *Pseudomonas* spp, *Corynebacterium*, *Micrococcus* spp

It was discovered that *Pseudomonas* spp and Bacillus spp occurred in all except in yellow yam in which *Bacillus* was absent. The fungal specie commonly isolated in all yam species was *Aspergillus*. Pathogenicity revealed that *Aspergillus niger* was highly pathogenic while the others were mildly pathogenic except for *Penicillium* spp that caused moderate rot. The bacterial species were also able to cause rot.

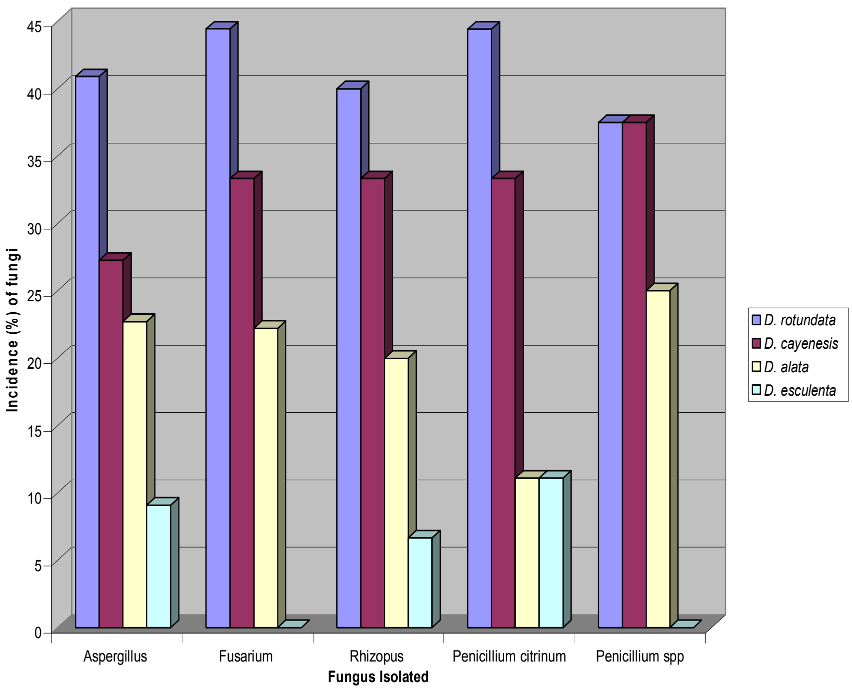

Fig. 1: Frequency of occurrence (incidence) of each fungus in *Dioscorea* (yam) species

Discussion

This present study has revealed that a wide range of fungi are responsible for the post harvest and storage rot of yams (*Dioscorea* species) in barns. The genera of fungi identified were Aspergillus, *Fusarium*, *Penicillium* and *Rhizopus*. The species of fungi isolated and identified in this research corroborate those reported by Gnonlonfin *et al*., (2008) and Ogaraku and Usman (2008). *Aspergillus, Fusarium* and *Penicillium* have been reported to be the most important mycotoxigenic genera of fungi. They are important in food safety due to their production of mycotoxins which have varying implications for health and the economy especially in developing countries. *Fusarium spp* produce *Fusarium* toxins such as Trichothecenes, Discetoxy, Scirpenol, Nivalenol and Zearalenone, these Mycotoxins causes skin diseases, gastroenteritis, rectal hemorrhage, vomiting and several other disease (Ogaraku and Usman, 2008). *Aspergillus flavus* and *Aspergillus parasiticus* produce aflatoxins (B1, B2, G1, G2) of which aflatoxin B1 is highly carcinogenic causing hepatoma (Ogaraku and Usman, 2008). The organisms may even secrete substances harmful to humans (John *et al*., 2015).

Some of the isolated bacteria e.g. *Escherichia coli*, are indicators of faecal contamination probably from the farms where the yams (*Dioscorea*) were harvested and from handlers. Passersby must have urinated or deposited faeces while passing by the farm. Similar result of faecal contamination was reported by Somorin *et al*., (2011) who stated that the faecal contamination from the yam (*Dioscorea*) chips and flour could be during the parboiling stage and handling of the yam chip and flour or from the practice of spreading yam chips on bare grounds and thus contact with the soil during drying. The other bacteria such as *Corynebacterium*, *Bacillus*, *Pseudomonas*, *Micrococcus*, *Enterococcus*, *Enterobacter*, *Staphylococcus*, and *Proteus* isolated in this study are also of serious health implications.

Pseudomonas was found present in this research which could be as a result of low temperature due to the presence of materials like straw, palm leaves and trees situated in and around the barns. More so, *Pseudomonas* species have the ability to survive in vast number of habitats. *Bacillus* was isolated during this study and it has been found that species of *Bacillus* produce cereulide which is heat stable and produces a vomiting – type syndrome (Hoton *et al*., 2009). Food poisoning cases involving vomiting and seizures soon after the consumption of yam flour meals have been reported in families in Nigeria with high severity in children (Adeleke, 2009). In recent times, food poisoning with similar symptoms due to consumption of yam flour meals have been on the increase in Nigeria. Similar cases of outbreaks of food poisoning involving families have been reported (Dierick *et al.,* 2005).

Pathogenicity test during this study revealed that *Aspergillus niger* was highly pathogenic and it caused rot after 14 days. *Fusarium oxysporum* was mildy pathogenic, while *Penicillium* caused moderate rot. *Rhizopus stolinifer* is a secondary invader or saprophyte and its potential to cause rot only increases in association with other fungi such as *Fusarium solani* and *Fusarium oxysporium.*

This research also showed that there are three types of post-harvest and storage rot disease of yams (*Dioscorea*) in barns. These are the dry rot, soft rot and wet rot. The dry rot disease had the highest incidence in the tubers of yams studied.

These diseases have been reported in other parts of Nigeria by Amusa *et al*., (1999). These rot diseases may be as a result of poor handling before, during and after harvest (Ogaraku and Usman, 2008).

The present study has revealed that, *Aspergillus* species and *Corynebacterium* species had the highest incidence for fungi and bacteria respectively in the *Dioscorea* species. More bacteria species were also isolated from *D. cayenensis* which recorded the highest mean value of total bacteria count. The frequency of each fungal isolate was highest in *D. rotundata* (white yam) and lowest in *D. esculenta* (Chinese yam). This shows that the Chinese yam is more resistant to fungal attack while the white yam is more susceptible to fungal attack.

On the other hand, the white yam is more resistant to bacterial attack while *D. cayenensis* (yellow yam) is more susceptible to bacterial attack. The result of this study therefore shows that, different *Dioscorea* specie responded differently to different microbial attack.

Conclusion

Spoilage fungi and bacteria have been shown to be associated with post-harvest and storage yams (*Dioscorea*) in barns. Yam barn shows the best result in view of reducing losses and long storage in comparison to other storage systems wide spread in West Africa. Better storage systems should be adopted and improved seedlings of yams (*Dioscorea*) that are resistant to both fungal and bacterial attack should be distributed to farmers for planting. Moreso, yams (*Dioscorea*) should be handled with care before planting, during and after harvest as to avoid mechanical injuries which may pave way for the entry of pathogens. Nematodes extraction should also be carried out as to know the incidence of rot causing nematodes and their various health implications. Good sanitary practices and hygiene should be maintained around the farms and barns and during handling of yams. These will greatly reduce the health hazards associated with the improper handling of *Dioscorea* species and the huge economic loss sustained by the farmers and traders due to diseases and damages caused by fungi and bacteria.

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