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## Studies of some isolated rot-causing mycoflora of yams (*Dioscorea* spp.)

Ogunleye, Akin O.<sup>1,\*</sup>, Ayansola, Oyeronke T.<sup>2</sup>

<sup>1</sup>Department of Microbiology, Lead City University, Ibadan, Nigeria, P.O. Box 23101 U.I. Postoffice, Ibadan, Nigeria

<sup>2</sup>Department of Microbiology, Lead City University, Ibadan, Nigeria

### Email address

aklagok@yahoo.com (Ogunleye, A. O.), ronkay2008@yahoo.co.uk (Ayansola, O. T.)

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### Abstract

This study was carried out on the storage rot of yam tubers *Dioscorea* spp in Ibadan, Nigeria. The *Dioscorea* spp examined were *Dioscorea rotundata* (white yam) and *Dioscorea alata* (water yam) which were the common species available at the time of study. The study area was visited six times in three months for collection of rotted yams which were selected randomly at a particular barn. The symptoms of the diseases were described and their associated organisms isolated and identified. The diseases include dry rot and soft rot which accounted for 54.22% and 45.78% of post-harvest diseases of the yams respectively. Out of 60 yam samples examined 52 yam samples had fungi isolates. The fungi isolated include *Aspergillus niger*, *Lasiodiplodia theobromae*, *Rhizopus stolonifer*, *Fusarium oxysporum*, *Aspergillus flavus*, *Aspergillus ochraceus*, *Aspergillus lilacinum*, *Penicillium* sp, *Pythium* sp., *Sclerotium rolfsii*, *Allomyces arbuscula* and *Rhodotorula* sp. The fungal isolates had the following percentage frequencies of occurrences respectively: 19.29%, 12.14%, 14.29%, 12.85%, 13.57%, 2.14%, 2.86%, 6.43%, 3.57%, 7.86%, 3.57%, and 1.43%. Pathogenicity test carried out confirmed the following organisms as the pathological agents of the yam rot: *Lasiodiplodia theobromae*, *Aspergillus niger*, *Sclerotium rolfsii*, *Rhizopus stolonifer*, *Allomyces arbuscular*, *Fusarium oxysporum*, *Penicillium* sp. and *Aspergillus flavus*. Aflatoxin levels of all *Aspergillus* isolates were determined. The potential hazards posed by the aflatoxins are discussed.

### 1. Introduction

Yam (*Dioscorea* spp.) is a tuber crop belonging to the family of *Dioscoreaceae*. Yam is widely cultivated in west and central Africa, in Asia and south American countries (Coursey, 1967; Orkwor *et al.*, 1998; FAO, 2007). In West African, yam is the most important tuber crop in terms of area coverage and a key staple food, particularly in Nigeria, Ghana, Ivory Coast, Benin and Togo. More than 90% of the global yam production (40 million tons fresh tubers/year) is produced in West Africa (FAO, 2007). Yam can be stored longer than most other tropical fresh products, and therefore stored yam represents stored wealth. Furthermore, yam has been identified as a crop with potential for increased commercial exploitation and processing. However production is adversely affected by pests and pathogenic diseases (Wilson, 1982; Degras, 1993). Fungi causing rot are of particular importance because they reduce vigour and subsequently cause a reduction in tuber yield and quality (Coursey,

1967; Amusa *et al.*, 2003). Yam was first described by Linnaeus (1737) as a monocotyledon plant in the *Dioscoreales* order. The family *Dioscoreaceae* has ten genera: *Dioscorea*, *Higinbothamia*, *Borderea*, *Epipetrum*, *Rajania*, *Tamus*, *Stenomeris*, *Tricuopus*, *Avetra* and *Petermannia* (Knuth, 1924). The genus *Dioscorea* is the largest of the ten genera and consists mostly of tropical plants. Many edible yam species have a large number of cultivates that have yet to be systematically characterized and the distinctions between species are not always evident (Orkwor *et al.*, 1998). About 600 species of yam have been identified (Coursey, 1967; Burkill, 1960).

Mycoflora simply means microorganisms of the fungal species belonging to the fungus kingdom. A fungus is a member of a large group of eukaryotic organism that includes microorganisms such as yeasts and molds, as well as the more familiar mushrooms. Microbiologists use the term fungus (Pl., Fungi; Latin: fungus, mushroom) to describe eukaryotic organisms that are spore-bearing, have absorptive nutrition, lack chlorophyll, and reproduce sexually and asexually. (Willey, *et al.*, 2008).

Mycotoxins are toxic secondary metabolites produced by organism of the fungus kingdom. They are toxic chemical products produced by fungi that readily colonized crops (Turner, *et al.*, 2009). They are toxic chemical products produced by fungi that readily colonize crops. One mould species may produce many different mycotoxins and / or the same mycotoxin as another species. Where conditions are right, fungi proliferate into colonies and mycotoxin levels become high (Fox and Howlett, 2008). The production of toxins depends on the surrounding intrinsic and extrinsic environments and the toxins vary greatly in their severity, depending on the organism infected and its susceptibility, metabolism, and defence mechanisms. Temperature treatments, such as cooking and freezing do not destroy mycotoxins (Fox and Howlett, 2008). Major groups of mycotoxins include: aflatoxins (produced by *Aspergillus* species of fungi), Ochratoxins, Citrinins ( both produced by *Penicillium* and *Aspergillus* species), Patulin (produced by *Aspergillus*, *Penicillium*, and *Paecilomyces* fungal species.), beauvercin and enniatins butenolide, equisetin and fusarins (produced by *Fusarium* species.) (Desjardins and Proctor, 2008), The umbrella term aflatoxin refers to four different types of toxins produced, which are B1, B2, G1 and G2 (Yin, *et al.*, 2008). Yam is prone to infection right from the seedling stage through harvesting and even after harvesting in storage (Amusa *et al.*, 2003). The causes of storage losses of yam tubers include: sprouting, transpiration, respiration, rot due to mould and bacteriosis, insects nematodes and mammals. One major constraint to yam production worldwide is post harvest rot which may be either physiological (Noon, 1978; Noon and Colhoun, 1981) or microbial (Otoo, *et al.*, 2001). Ricci, *et al.* (1989) estimated microbial post harvest losses in yam at 40% while Okigbo and Ikediugwu (2000) indicated that between 20 and 39-5% of stored tubers may

be lost to decay. The principal factors responsible for yam losses during storage are: the natural metabolic processes of the dormant tubers, which result in the conversion of starch of the tuber into carbon (iv) oxide and water; evaporation of water from the tuber; sprouting; and infection by various fungi which decay the tuber (Coursey, D.G., 1965). Losses in yams in storage mostly to rot are considered to be heavy in Nigeria. These losses are pathological problem of yam tubers brought about by bacteria, fungi and nematodes (Ayensu and Coursey, 1972; Coursey, 1967). The losses were estimated to be 10-15% in the first three months (Ekundayo, *et al.*, 1972) while F.A.O (1998) estimated the loss to be 50% and 56% respectively after 6 months in the yam barn. Most of the pathogens of yam tuber are soil-borne, but manifestations of the tuber disease are observed mostly during storage. It has been estimated that an average of over 25% of the yield of yam is annually lost due to disease and pest (Ezeh, 1998).

Agents causing disease reduce the quality of yam produce and its quality as well making it unappealing to consumers (Okigbo, 2005). The entry of pathogens occurs through wounds or cuts and natural openings on the surface of the tubers. (Okigbo, 2004). Though yam tuber naturally has a periderm microorganisms cannot breach, it is easily wounded by rodents, nematodes and man during weeding, harvesting and post-harvest handling (Ogaraku, *et al.*, 2008). Such wound facilitate the penetration and development of rot microorganisms (Noon, 1978). Microbial agents causing rot of yam are mainly fungi (Nwankiti and Arene, 1978; Ogundana and Demis, 1981; Ikotun, 1983, Osai, *et al.*, 1996, Cornelius and Oduro, 1999). Several pathogenic fungi have been found associated with yam, causing diseases such as anthracnose, leaf spots and blight, as well as rotting of yam tubers (IITA, 1975).

Many fungal pathogens have been associated with deterioration of yam during storage. The implications of *Fusarium oxysporium*, *Fusarium solani*, *Penicillium* and *Aspergillus* species as common pathogens in yam storage have earlier been reported. (Morse, *et al.*, 2000; Okigbo, 2003). The main objectives of this research work are to:

- isolate fungi causing rot in yam.
- identify the isolates from rotten yam
- test for degree of pathogenicity
- test for mycotoxins produced by fungi in rotten yam.

## 2. Materials and Methods

### 2.1. Sampling

Two varieties of yam tubers *Dioscorea rotundata* (White yam) and *Dioscorea alata* (Water yam) showing typical symptoms of infections (Dry rot and soft rot) were obtained locally from Bodija market in Ibadan metropolis at an interval of two weeks for a period of three months. In the selected area, a regular yam tuber retailer was visited two times in each month. The average of the samples from the

two visits in each month represented a replication, hence in 3 months, there were 6 replicates. On each day of the visit five tubers of rotten yams of each variety were randomly picked and sampled for the incidence of disease types. Sampling was done based on the presence or absence of disease symptoms.

## 2.2. Sterilization of Materials

All glass wares used in this study were washed with detergent, rinsed and sterilized in a dry ventilated oven (Gallenkamp, Model NYC-101) at 160 °C for 2 hours. All media were sterilized by autoclaving at a temperature of 121 °C and 15 psi for 15 minutes. The scalpel, cork borer, inoculating needle were sterilized by dipping them into 70% ethanol and passing them over a spirit lamp flame until red hot.

## 2.3. Preparation of Culture Medium

Throughout the study, the assayed culture medium employed was LAB M Potato Dextrose Agar (PDA). The medium was used for the growth and maintenance of the fungal isolates.

The preparation of Potato Dextrose Agar (PDA) was done according to the manufacturer recipe (39grams in 1 litre of water). The medium was brought to boil for 15 minutes to dissolve completely (homogenisation) and sterilized by autoclaving at 121 °C and 15psi for 15 minutes.

The medium was allowed to cool to temperature between 42 – 45 °C. 2.5ml of sterile 10% streptomycin sulphate B.P was added to every 250ml of sterile cooled PDA so as to adjust the pH to about  $5.6 \pm 0.2$  (Green, 1994) and to prevent bacteria growth. Approximately 15ml of the cooled amended PDA was poured into sterile petri dishes of 9cm diameter to solidify. Similarly, McCartney bottles were approximately half filled with the molten Potato Dextrose Agar, autoclaved and kept in a slanted position until it solidified. These were used to preserve the isolates obtained inside a refrigerator at 4 °C temperature.

## 2.4. Sterilisation of Samples

Whole Yam tuber and sliced rotten tissue pieces were surface - sterilised to remove contaminants by dipping completely in 0.1% of Mercury II chloride solution for 2 minutes and rinsed three different times, each for one minute in sterile distilled water. The tuber and rotten tissue pieces were placed on sterile Whatman filter paper, number 9, in the inoculating chamber beside lighted spirit lamp to dry for 10 minutes.

## 2.5. Isolation of Fungi

The isolation technique used by Onyike and Maduewesi (1985) was employed in this study. A small section of the yam tissue containing the advancing margin of rot and adjoining healthy tissue were cut using sterilized scalpel and cork borer and surface - sterilized according to section 2.4 mentioned above. The peeled portions of the yam taken

by cork borer were placed on the solidified agar. 3 peeled portions were placed per plate with equal distance between them. 3 replicate plates for each of the rotten portion of site were made for each yam variety. The plates were incubated at  $27 \pm 2$  °C for 72 hours. Fungi associated with the yam rot affected tissue were observed and the frequency of isolation determined using method of Okigbo and Ikediugwu (2000).

## 2.6. Identification of Fungal Isolates

Sub-culturing of the isolates was made to obtain pure culture. The colonies growing on the plates were identified macroscopically and microscopically. Colony colour, type (compact, loose, aerial hyphae), texture (velvety, cottony, coarse) shape and growth pattern were observed. Direct observation of culture under the light microscope (low power) by careful preparation of slides, staining with cotton blue-in-lactophenol was done. Detailed drawings of the diagnostic features and identification manual and guides according to Alexopoulos, (1962); Nelson, *et al.*, (1983); Rippon, (1958); Samson, *et al.*, (1984); and Snowdon, (1991) were used.

## 2.7. Preparation of Pure Culture

The method of Green (1994) was adopted in order to suppress bacterial contamination by adding 2.5ml of 10% streptomycin sulphate to every 250ml of sterile cooled PDA prior to pour-plating. After the medium has solidified, fungal isolates were sub-cultured on to it and incubated at  $25 \pm 2$  °C. The streptomycin sulphate added effectively eliminated bacterial growth.

## 2.8. Pathogenicity Test

The pathogenicity test was carried out to establish which of the fungal isolates caused the rot and to determine whether they could induce similar symptoms on inoculation and be re-isolated, thus fulfilling Koch's postulates. The method of Okafor (1966), Okigbo and Ikediugwu (2000) were adopted for the pathogenicity study. The pure fungal isolates obtained from infected yam tubers of *D. rotundata* and *D. alata* for the period of the collection were used for inoculation.

Healthy looking yam tubers of variety *D. alata* and *D. rotundata* were surface-sterilized as described in section 2.4. Each healthy tuber was bored into about 1cm deep, with a sterile 6mm diameter cork borer at three different points on the yam tuber surface (proximal, middle and distal regions). Another 6mm sterile cork borer was used to cut about 5mm of mycelia disc from edge of a 48-hours old culture of each fungus isolate. The mycelia discs were used to inoculate the holes created by scooping out the yam tissue. The scooped-out tissue of the yam tuber was replaced after 5mm pieces had been cut off to compensate for the thickness of the fungal culture. Nine whole tubers were inoculated per fungus isolate (3 replicates for each tuber variety and the control). The control set up consist of tubers that were similarly bored into and inoculated with sterilized PDA agar discs.

The wounds were sealed with petroleum jelly and inoculated tubers were placed in transparent polythene bags whose inside has been moistened with cotton wool soaked in sterile distilled water to maintain a high humidity. The inoculated tubers were kept in the laboratory at room temperature for about 7 to 10 days.

The tubers were assessed for rot development by cutting through the points of inoculation where rots developed. The pathogens were re-isolated as described in section 2.5; and their cultural and morphological characteristics were compared with those of the original isolates.

## 2.9. Test of Mycotoxin

According to the method of AOAC, (1990), aflatoxin was tested as follows:

- A diluents of water and methanol was prepared to a litre of ratio 9:11 respectively
- 60ml of sample was then mixed with 40ml of diluents solution.
- This was then filtered through whatman No 1 filter paper into test tubes arranged on test tube rack.
- The filtrate was extracted three times each with 20ml of petroleum ether at a boiling point of about 80 °C in separating funnel to remove any lipids fractions. This procedure was repeated 3 times.
- The aqueous methanol extract was then diluted with 25ml chloroform to extract the aflatoxin in Mac Cartney bottles.
- The pooled chloroform extract was then passed through a bed of anhydrous NH<sub>2</sub> NO<sub>3</sub>.
- The filtrate was collected into an antibody coated bottle and incubated in water bath for 15 minutes at 37 °C to allow for colour change (from blue to yellow).
- The deeper the colour the deeper the level of aflatoxin in the sample.
- Quantitative result of aflatoxin present was then determined using ELISA reader or spectrophotometer while aflatoxin typing was done by using standard sample on Thin-Layers Chromatography against known aflatoxin standard. B1, B2, G1 and G2. (Supplied by Aldrich Chemicals Milwaukee, Germany)

## 3. Results

During the isolation and identification of the causative organisms of the rotted yam tuber, 12 fungi were identified according to the standards of Barnet and Hunters (1972), Booth (1971) and Alexopolus (1962). The species of fungi isolated and identified from white and water yam samples obtained from result were *Aspergillus niger*, *Lasiodiplodia theobromae*, *Rhizopus stolonifer*, *Fusarium oxysporum*, *Aspergillus flavus*, *Aspergillus ochraceus*, *Aspergillus lilacinum*, *Penicillium sp.*, *Pythium sp.*, *Sclerotium rolfsii*, *Alloymyces arbuscula* and *Rhodotorula sp.* (Table 1). The frequency of occurrence showed that *Aspergillus niger* has the highest occurrence of 19.29% while *Rhizopus stolonifer*,

*Aspergillus flavus*, *Fusarium oxysporum* and *Lasiodiplodia theobromae* follow with 14.29%, 13.17%, 12.85% and 12.14% respectively (Table 2). The result established the susceptibility of yam tubers and invasion by fungal rot at the various time of collection of infected tubers in Bodija market which is the largest yam depot in Ibadan metropolis. The survey revealed prevalence of two major post-harvest diseases of white and water yam which are dry rot and soft rot. This accounted for 54.22% and 45.78% respectively (Table 3). The percentage infection of yam tubers artificially inoculated with fungi isolated from diseased tubers is presented in table 5. Four out of the eight isolates artificially inoculated on the yam tubers were highly pathogenic while the rest were moderately pathogenic. Aflatoxin level of the fungal isolates shows that *Aspergillus flavus* has the highest level with 1.71mg/kg. *Aspergillus ochraceus*, *Aspergillus lilacinum* and *Aspergillus niger* have 0.23mg/kg, 0.63mg/kg and 0.51mg/kg levels respectively.

Table 1. Fungi associated with yam tubers rot in Bodija, Ibadan.

Fungal Isolates	<i>D. rotundata</i> (White yam)	<i>D. alata</i> (Water Yam)
<i>Aspergillus niger</i>	+	+
<i>Lasiodiplodia theobromae</i>	-	+
<i>Rhizopus stolonifer</i>	+	+
<i>Fusarium oxysporum</i>	+	+
<i>Aspergillus flavus</i>	+	+
<i>Aspergillus ochraceus</i>	+	+
<i>Aspergillus lilacinum</i>	+	-
<i>Penicillium sp.</i>	+	+
<i>Pythium sp.</i>	-	+
<i>Sclerotium rolfsii</i>	+	+
<i>Alloymyces arbuscula</i>	+	+
<i>Rhodotorula sp.</i>	+	-

Key: + = Present, - = absent.

Table 2. Frequency of occurrence of fungi isolates in the different yam species.

Fungal Isolates	<i>D. rotundata</i> (White yam)	<i>D. alata</i> (Water Yam)	Percentage (%) Total
<i>Aspergillus niger</i>	19	8	19.29
<i>Lasiodiplodia theobromae</i>	Nil	17	12.14
<i>Rhizopus stolonifer</i>	7	13	14.29
<i>Fusarium oxysporum</i>	14	4	12.85
<i>Aspergillus flavus</i>	10	9	13.57
<i>Aspergillus ochraceus</i>	2	1	2.14
<i>Aspergillus lilacinum</i>	4	Nil	2.86
<i>Penicillium sp.</i>	6	3	6.43
<i>Pythium sp.</i>	Nil	5	3.57
<i>Sclerotium rolfsii</i>	9	2	7.86
<i>Alloymyces arbuscula</i>	4	1	3.57
<i>Rhodotorula sp.</i>	2	Nil	1.43

Table 3. Frequency of occurrence of the different disease condition.

Disease condition	Frequency of occurrence ( <i>D. rotundata</i> )	Frequency of occurrence ( <i>D. alata</i> )	Percentage occurrence
Dry rot	40	5	54.22
Soft rot	8	30	45.78
Total	48	35	100

**Table 4.** Macro and micro features of the fungi isolates.

Isolates	Colony characteristic	Microscopy
<i>Aspergillus niger</i>	Growth on PDA is rapid. Colonies are black or dark brown.	Non-septate conidiophores arising from thick-walled foot cells. Each conidiophore ends in a terminal enlarged spherical swellings. Conidia borne by phialides arising from a terminal swelling on the conidiophores. It has 'mop-like' head of conidia.
<i>Lasiodiplodia theobromae</i>	Dirty white with black underneath.	Mycelium are septate, chlamydo spores are intercalary and terminal.
<i>Rhizopus stolonifer</i>	Rapid and fast growth of about 36mm after 24 hours which soon spread and cover the plates. Growth appear as black pin-head, similar to cotton wool (white in colour).	Mycelium of non-septate hyphae of large diameter. The sporangiophore bears a terminal black spherical sporangium. The sporangiophores are erect and may be branched or unbranched each bearing a single globose sporangium.
<i>Fusarium oxysporum</i>	Growth on PDA is rapid. White aerial mycelium tinged with pink purple colour.	Micro and macro conidia are present, macro conidia slightly sickle-celled with apical cell and foot shaped basal cell, chlamydo spores are present, single and some in pairs.
<i>Aspergillus flavus</i>	Orange green in colour surrounded by a clear zone.	Non-septate conidiophores arising from thick-walled foot cells. Each conidiophore ends in a terminal enlarged spherical swellings. Conidia borne by phialides arising from a terminal swelling on the conidiophores.
<i>Aspergillus ochraceus</i>	Growth on PDA is rapid and fast. Powdery brown colour almost covering the plates after 72 hours.	Non-septate conidiophores arising from thick-walled foot cells. Each conidiophore ends in a terminal enlarged spherical swellings. Conidia borne by phialides arising from a terminal swelling on the conidiophores.
<i>Aspergillus lilacinum</i>	Pink colour, raised colony, velvet with clear zone.	Non-septate conidiophores arising from thick-walled foot cells. Each conidiophore ends in a terminal enlarged spherical swellings. Conidia borne by phialides arising from a terminal swelling on the conidiophores.
<i>Penicillium sp.</i>	The colony after 48-72 hours of incubation produced powdery blueish grey colour with a clear zone around it.	The conidiophores is branched to form a brush-like head. The conidia are globules resembling glass beads.
<i>Pythium sp.</i>	Colonies appear compact which is grey-black in colour and surrounded by a clear white zone.	Mycelium consists of slender, non-septate hyphae. The sporangia are globose and are terminal on somatic hyphae. It has a large regularly shaped, branched sporangia.
<i>Sclerotium rolfsii</i>	Colony is brown to black, globose and compact	Mycelium lack conidia. Mycelium usually light and straight.
<i>Alloymyces arbuscula</i>	Colony on PDA is powdery colourless, velvet (female gametangia). Surrounding a dirty orange (reddish brown) male gametangia. About 26mm in diameter after 72 hours of incubation.	It has enlarged hyphae that is non-septate which elongates into branches, colourless zoosporangia called sporothallus (female gametangia) on which a thick walled reddish brown sporangia (male gametangia) that bears zoospores is borne.
<i>Rhodotorula sp.</i>	Colony swarming on PDA. The colonies are brightly coloured and yellow.	Consist of budding cells. Budding cell may occur singly, not in chains or may form hyphae-like structure.

**Table 5.** Percentage infection of marketed yam tubers artificially inoculated with fungi isolated from rotten yam.

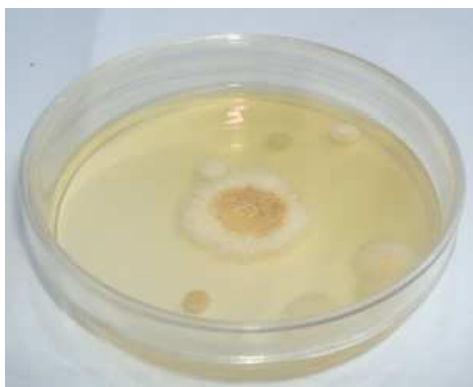
Fungi Isolate	No of tubers Inoculated	% Infection after 14 days	Symptoms of Infection	Pathogenicity
<i>Lasiodiplodia theobromae</i>	6	100	Dry rot	+++
<i>Aspergillus niger</i>	6	100	Dry rot, soft rot	+++
<i>Sclerotium rolfsii</i>	6	88.89	Dry rot	+++
<i>Rhizopus stolonifer</i>	6	61.11	Soft rot	++
<i>Alloymyces arbuscular</i>	6	50	Soft rot	++
<i>Fusarium oxysporum</i>	6	77.78	Dry rot	+++
<i>Penicillium sp.</i>	6	55.56	Dry rot	++
<i>Aspergillus flavus</i>	6	66.67	Dry rot	++

+++ = Highly pathogenic (&gt; 50mm in diameter)

++ = moderately pathogenic (&gt;10 &lt;50 mm in diameter)

**Table 6.** Aflatoxin test result:

Fungal isolates	Aflatoxin level (mg/kg)	Aflatoxin type
<i>Aspergillus ochraceus</i>	0.23	B1
<i>Aspergillus niger</i>	0.51	B2
<i>Aspergillus flavus</i>	1.71	B1
<i>Aspergillus lilacinum</i>	0.63	G1



**Figure 1.** Growth of *Allomyces arbuscular* on Potato Dextrose agar.



**Figure 2.** Growth of *Rhodotoria* sp on Potato Dextrose agar.



**Figure 3.** Growth of *Aspergillus ochraceus* on Potato Dextrose agar.



**Figure 4.** Growth of *Aspergillus lilacinum* on Potato Dextrose agar.



**Figure 5.** Growth of *Lasiodiplodia theobromae* on Potato Dextrose agar.



**Figure 6.** Growth of *Rhizopus stolonifer* on Potato Dextrose agar.



**Figure 7.** Growth of *Fusarium oxysporum* on Potato Dextrose agar.

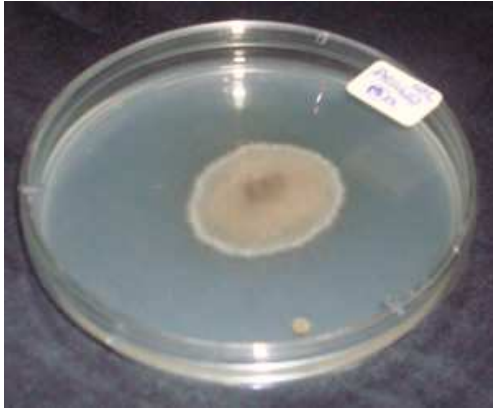


Figure 8. Growth of *Pythium* sp. on Potato Dextrose agar.



Figure 9. Growth of *Sclerotium rolfsii* on Potato Dextrose agar.



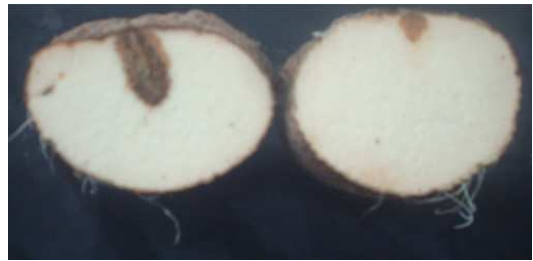
Figure 10. Growth of *Aspergillus niger* on Potato Dextrose agar.



Figure 11. Growth of *Penicillium* sp. on Potato Dextrose agar.



Figure 12. Growth of *Aspergillus flavus* on Potato Dextrose agar.



(A) *D. rotundata*



(B) *D. alata*

Figure 13. Rot caused by *Aspergillus flavus*



(A) *D. rotundata*



(B) *D. alata*

Figure 14. Rot caused by *Lasiodiplodia theobromae*



(A) *D. rotundata*



(B) *D. alata*

**Figure 15.** Rot caused by *Fusarium oxysporum*



(A) *D. rotundata*



(B) *D. alata*

**Figure 16.** Rot caused by *Rhizopus stolonifer*.



(A) *D. rotundata*



(B) *D. alata*

**Figure 17.** Rot caused by *Aspergillus niger*.



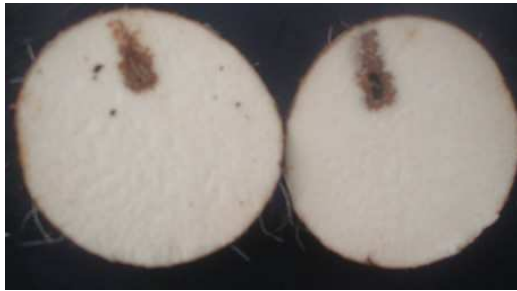
(A) *D. rotundata*



(B) *D. alata*

**Figure 18.** Rot caused by *Sclerotium rolfsii*



(A) *D. rotundata*(B) *D. alata***Figure 19.** Rot caused by *Penicillium* sp.(A) *D. rotundata*(B) *D. alata***Figure 20.** Rot caused by *Allomyces arbuscular*(A) *D. rotundata*(B) *D. alata***Figure 21.** Control

#### 4. Discussion

This study has revealed that a wide range of fungi are responsible for the storage rot of yams in Bodija market, Ibadan Oyo state. Several fungi were isolated from different yam rots (Soft rot and dry rot) in two varieties of yam (*D. alata* and *D. rotundata*) sampled in this study. A total of twelve species of fungi were isolated and identified from rotted yam tuber samples got from Bodija market in Ibadan ( as shown in Table 1). These includes; *Lasiodiplodia theobromae*, *Aspergillus ochraceus*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus lilacinum*, *Sclerotium rolfsii*, *Rhizopus stolonifer*, *Pythium* sp., *Allomyces arbuscular*, *Rhodotorula* sp., *Fusarium oxysporum*, *Aspergillus glaucus* and *Penicillium* sp. The

high frequency of occurrence of *Aspergillus niger*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Aspergillus flavus* were observed in both *Dioscorea alata* (Water yam) and *Dioscorea rotundata* (White yam) while *Lasiodiplodia theobromae* was isolated from *D. alata* only (Table 1). *Sclerotium rolfsii* and *Allomyces arbuscular* was isolated from both *D. alata* and *D. rotundata* while *Rhodotorula* sp and *Pythium* sp from *D. rotundata* and *D. alata* respectively. The survey revealed the prevalence of two major post-harvest diseases of yams, these include dry rot and soft rot. This accounted for 54.22% and 45.78% respectively (Table 3). It was also observed during the study that both soft rot and dry rot symptoms of infection in water yam revealed numerous fungi in which *Rhizopus stolonifer* and *Lasiodiplodia theobrome* were the most predominant fungal

isolates in all batches of the yam samples. The data collected was analyzed using t-test and it was deduced that the null hypothesis is true. This could be attributed to the high water level, the water activity ( $a_w$ ), in this specie of yam to aid the growth of the organisms. The value obtained for frequency of occurrence of fungi (Table 3) could also be attributed to the poor harvesting technique, the poor storage facilities adopted and the prevailing weather condition (Morris, 1977). These fungal species isolated and identified in this study corroborate those reported by Ogaraku and Usman, (2008).

The incidence of decay varied with the species and also with the type of storage. The markedly higher incidence of decay in the yams piled in heaps on the floor of the hut over that of yams tied up on the stakes in a barn might have resulted from visible physical damage resulting from rodent attack as well as the possible humid conditions resulting from stack-piling of tubers on the floor. Apart from damage due to type of storage, damage resulting from bruising and careless cuts during harvesting or pre - harvest nematode attack on the tubers facilitates the invasion of the tubers by decay organisms. During storage, the tubers are subject to losses of up to 50% of the fresh matter. Here, the losses due to microbial attack play a predominant role. The fungal pathogens penetrate through wounds in the tubers and infect the inner tubers tissue. Such wounds are caused by insects, nematodes and poor handling before, during and after harvest (Morris, 1977).

Pathogenicity tests revealed that *Lasiodiplodia theobromae*, *Aspergillus niger*, *Aspergillus flavus*, *Sclerotium rolfsii*, *Rhizopus stolonifer*, *Allomyces arbuscular*, *Fusarium oxysporum*, and *Penicillium sp* caused rot. *Lasiodiplodia theobromae*, *Aspergillus niger*, *Fusarium oxysporum* and *Sclerotium rolfsii* are highly pathogenic while *Rhizopus stolonifer*, *Allomyces arbuscular*, *Aspergillus flavus*, and *Penicillium sp* are moderately pathogenic (Table 4). *Aspergillus ochraceus*, *Aspergillus lilacinum*, *Pythium sp* and *Rhodotorula sp* caused no decay under experimental conditions. They may be secondary organisms colonizing the tuber following decay by the primary organisms.

The nature of rot varies between the inoculated yam tubers with various selected fungi. *Lasiodiplodia theobromae* shows wet – rotting, *Sclerotium rolfsii* caused rot with dirty white colour, *Penicillium nigricans* caused brown to olive rot, *Aspergillus niger*, *Aspergillus flavus*, caused purple - brown rot, *Fusarium oxysporum* and *Allomyces arbuscular* caused soft rot with light to dark brown rot and *Rhizopus stolonifer* caused rot with greyish colour.

None of the fungal isolates were able to penetrate whole yam tubers. This is an indication that for these fungi to infect tubers in storage and market there must be opening. These openings may be due to cuts, bruises, breaks, sun scald injury, abrasion or nematode, pests and insects wound. Bridge (1972) reported that nematodes predisposes yam to infection by fungi especially the species of *Scutellonema bradys* and *Medoidogyne sp*. Morse, *et al.* (2000) also

described the importance of insect pest control as a means of limiting yam tubers rot storage.

Rotting in storage probably starts in the soil and progresses in storage. In most cases, microorganisms gain access into yams through natural openings and wounds that occur during harvesting and transportation from the field to storage barn (Ogundana, *et al.*, 1970). Osagie (1992) confirmed that the soil adhering to the harvested tubers contain many microorganisms that could be pathogenic to the tubers.

The magnitude of rot varies with the infecting microorganisms. Ikotun, (1989) declared that fungi caused 57 – 77% of all rots of yam tubers in Nigeria, and 30 different fungi were isolated from stored yam. These findings were similar to that of Amusa and Baiyewu (1999) who reported *Aspergillus*, *Penicillium* and *Rhizopus* as very important pathogens associated with stored and marketed yam tubers from the tropical forest region of South Western Nigeria. These results agrees in line with the report of Adeniji (1970), Ogundana *et al.*, (1970) and Ikotun (1989) who reported that *Aspergillus niger* and *Lasiodiplodia theobrome* to be very severe in causing yam decay in Nigeria.

The presence of the above mentioned diseases is of economic importance. Some fungal pathogens produce mycotoxins in their infected products as shown in Table 5. *Aspergillus niger*, *Aspergillus lilacinum*, *Aspergillus ochraceus* and *Aspergillus flavus* with the following levels and types respectively: 0.23mg/kg B1, 0.51mg/kg B2, 0.63mg/kg G1 and 1.71mg/kg B2. *Aspergillus flavus* produces aflatoxins with the highest level (1.71mg/kg).

Mycotoxins are hazardous to human and animal health (WHO, 1983). *Fusarium species* produce *Fusarium* toxins such as Trichothecenes, diacetoxyscirpenol, nivalenol and Zearalenone, these cause skin diseases, gastroenteritis, rectal hemorrhage, vomiting and several other disease (Krogh, 1988). *Aspergillus flavus* and *Aspergillus parasiticus* produce aflatoxins (B1,B2,G1,G2) of which aflatoxin B1 is highly carcinogenic causing hepatoma (WHO, 1983).

## 5. Conclusion and Recommendation

Different genera of fungal isolates were identified in causing yam rot. The invasion of the fungi pathogen can be through natural opening or wound that occurs during harvesting, transportation and storage. Not all the fungal isolates actually caused the rot as shown in pathogenicity test. The thick layering on the surface of the control tuber in the pathogenicity studies was probably a healing process in reaction to a cut since the tissues were still living and performing physiological functions. This is supported by Ekundayo and Naqvi (1972). Some fungal pathogens produced mycotoxins which are dangerous to both human and animal health causing cancer and several other diseases.

The disease causing agents reduce the quantity of yam produced and also reduced the quality by making them

unappealing to the consumers. Yam should be handled with care to avoid mechanical injuries which may pave way for the entry of pathogens.

Knowledge of the fungi responsible for yam rot will help in finding effective control measures to extend the life span of yams in storage. Therefore, further research can also be carried out on other species of yam, other microbial pathogens causing rot in yam and mycotoxins produced by the isolated pathogens. Measures should be taken through genetic engineering to improve on the storage capacity of yams.

## References

- [1] Adeniji, M. O. (1979). Fungi associated with storage decay of yam in Nigeria. *Phytopathology*. 60 (4) 590 – 592.
- [2] Alexopoulos, C. J. (1962). Introductory Mycology. 2<sup>nd</sup> Edition. John Wiley and Sons, Inc., New York London. Sydney, pp: 119 – 159.
- [3] Amusa, N. A. and Baiyewu, R. A. (1999). Storage and Market Disease of Yam Tubers in southwestern Nigeria. *Ogun Journal of Agriculture Research Nigeria*. 11: 211 – 225.
- [4] Amusa, N. A., Adegbite, A. A., Mohammed, S. and Baiyewu, R. A. (2003). Yam disease and its management in Nigeria. *App J. Biotechnol*. 2:497 – 502.
- [5] AOAC, (1990). Association of Official Agricultural Chemist. Official Method of Analysis, New York, Williams and Williams.
- [6] Ayensu, E.S. and Coursey, D.G. (1972). Guinea Yams. *Econ. Bot.* 26:301-318.
- [7] Barnet, H. L. and Hunter, B. B.(1972). Illustrated Genera of Imperfect Fungi. 4<sup>th</sup> edition. New York, Macmillan Inc., pp 33 – 40.
- [8] Booth, C. (1971). The Genus *Fusarium*, Common Wealth Agric. Bureaux, Central Wales, Famham Royal Bucks, England, pp: 81 – 167.
- [9] Bridge, J. (1972). Nematode Problems with Yam (*Dioscorea spp.*) in Nigeria. Pest Articles and News Summaries *PANS*, (1): 89-91.
- [10] Burkill, I. H. (1960). Organography and evolution of *Dioscoreaceae*, the family of yams. *Botanical Journal of Linnean Society*, 56: 391 – 412.
- [11] Cornelius, E. W. and Oduro, K. A. (1999). Storage diseases of white yam (*Dioscorea rotundata*), causes, varietal susceptibility and control. *Journal of the Ghana Science Association*. 3: 45 – 52.
- [12] Coursey, D. G. (1965). The role of yams in West African Food economics. *World Crops*, June 1965.pp. 229 – 243.
- [13] Coursey, D. G. (1967). Yam storage I. A. review of storage practices and information on storage losers. *Journal of stored product Research*, 2: 227 – 244.
- [14] Degras, L. M. (1993). The yam: A tropical root crop. The Technical Centre for Agricultural and rural cooperation (CTA). The McMillan Press, London. pp 408.
- [15] Desjardins, A. E. and Proctor, R. H. (2007). Molecular biology of fusarium mycotoxins. *Int. microbial*. 119 (1-2): 47 – 50.
- [16] Ekundayo, J.A. and Naqvi, S.H.Z. (1972). Preharvest Microbial Rotting of Yams (*Dioscorea spp.*) in Nigeria. *Trans. Br. Mycol. Soc.* 58(1):15-18.
- [17] Ezeh, N. O. (1998). Economics of production and Post-harvest Technology In: Orkwor, G. C., Asiedu, R. and Ekanayake, I. J. (eds) Food yam Advances in research IITA and NRCRI, Nigeria, pp. 187 – 214.
- [18] Food and Agriculture Organisation.(1998). FAOSTAT Agriculture Data base –Agriculture Production crops Primary, Fao@www.fao.org.
- [19] Food and Agricultural Organisation. (1998). Food and Agriculture Organization of the United Nations, Year book 48.
- [20] Food and Agricultural Organisation. (2007). Food and Agriculture Organization Production Year Book. FAO Rome.
- [21] Fox, E. M. and Howlett, B. J. (2008). Secondary metabolism: regulation and role in fungal biology. *Curr. Opin microbial*. 11(6): 481 – 7.
- [22] Green, K. R. (1995). Studies on the epidermiology and control of yam anthracnose. Ph.d thesis. University of Readings. pp: 44 - 50.
- [23] Harris, S. D. (2008). Branching of Fungal hyphae: regulation, mechanisms and comparison with other branching systems. *Mycologia* 100 (6): 823 – 832.
- [24] Hawksworth, D. L. (2006). The Fungal dimension of biodiversity: Magnitude, Significance and conservation. *Mycological Research*. 95(6): 641 – 655.
- [25] Ikotun, T. (1983). Postharvest microbial rot of yams in Nigeria. *Fitopatologia Brasileira* 8 (1): 1- 7.
- [26] Ikotun, T. (1986). Microbial rot of tubers of Chinese yams (*Discorea esculenta*) in storage. *Fitopatol, Bras.* 11: 241 – 244.
- [27] Ikotun, T. (1989). Diseases of Yam tubers. *Int. J. Trop plants Dis.* 7: 1 – 21.
- [28] International Institute of Tropical Agriculture (IITA) (1995). Annual Report. Ibadan Nigeria IITA.
- [29] Knuth, R. (1924). *Dioscoreaceae*. In: Engler A, editor. *Das pflanzenreich* 87(IV-43): 1-387.
- [30] Krogh, P. (1988). Adverse Effects of Mycotoxins on Human Health. In: Proceedings of the CTA Seminar. Mathur S. B., and Johns, J. eds. pp 22 – 25.
- [31] Linnaeus, C. (1737). *Dioscoreaceae*. Amsterdam: George Clifford. pp 33
- [32] Morse, S.M, Acholo, M.C and Oliver, R. (2000). Control of Storage Insect as a Means Limiting Yam Tuber Fungal Rots. *Journal of Stored Product Research*. (36): 37-48.
- [33] Nelson, P. E., Tousson, T. A. and Marassas, W. F. O. (1983). *Fusarium species, an illustrated manual for identification*. The Pennsylvania state press, University park and London, pp:193.

- [34] Noon, R.A. (1978). Storage and market disease of yam. *Trop Sci.* (20), 177-188.
- [35] Noon, R.A. and Colhoun, J. (1981). Observations on the nature of Chilling injury in yam. *Phytopathologische Zeitschrift*. 100(1): 10-18.
- [36] Nwankiti, A.O and Arene, O.B. (1978). Diseases of Yam in Nigeria. *PANS* . 24 (4): 486-494.
- [37] Ogaraku, A. O. and Usman, H. O. (2008). Storage Rot of some yams (*Dioscorea* spp) in Keffi and Environs, Nassarawa State, Nigeria. *J. PAT* . 4(2): 22 – 27.
- [38] Ogundana, S. K., Naqvi, S. H. Z. and Ekundayo, J. A. (1970). Fungi Associated with soft Rot of yams (*Dioscorea* spp.) in Nigeria. *Trans. Br. Mycol. Soc.* 58(1), 15 – 18.
- [39] Ogundana, S. K. and Dennis, C. (1981). Assessment of fungicides for the prevention of storage rot of yam tubers. *Pesticide Science* . 12(5): 490 – 494.
- [40] Okafor, N. (1966). Microbial rotting of stored yam (*Dioscorea* spp) in Nigeria. *Expl. Agric.* 2: 179 – 182.
- [41] Okigbo, R. N. and Ikediugwu, F. E. O. (2000). Study on biological control of post harvest rot of yam (*Dioscorea* ) with *Trichoderma Viridae*. *J. Phytopatal.* (148), 351 – 353.
- [42] Okigbo, R. N. (2003). Fungi Associated with Peels of Post Harvest Yams in Storage. *Global Journal of Pure and Applied Sciences*. 9(1): 19 – 23
- [43] Okigbo, R.N. (2004). A Review of Biological Control Methods for Post Harvest Yams (*Dioscorea* spp.) in Storage in South Eastern Nigeria, *KMITL Science Journal*. 4(1): 207 – 215.
- [44] Okigbo, R. N. (2005). Biodegradation of white yam (*Dioscorea rotundata* poir) and water yam (*Dioscorea alata* L.) Slices dried under different conditions. *KMITL sd. Tech. J.* 5(3).
- [45] Oniyike, R. C. I. and Maduwesi, J. N. C. (1985). Variability in pathogenicity of isolates and sensitivity of dithane M – 45 and PCNB on cocoyam rot Fungi. *Nigerian journal of plant protection*. 9: 74 – 81
- [46] Orkwor, G. C., Asiedu, R. and Ekanayake, (1998). The importance of yams. In food yams. *Advances in Research*. I. J., eds. IITA and NRCRI, Nigeria. pp: 249.
- [47] Osagie, A. U. (1992). The yam tuber in storage. Postharvest Research Unit. University of Benin, Benin City, Nigeria. pp: 247.
- [48] Osai, E. O., Ikotun, T., Asiedu, R. and Hahn, S. K. (1996). Susceptibility of yam cultivars to minisett rot pathogens. *Tropical Science* . 36(4): 201 – 205.
- [49] Otoo, J. A., Okoli, O. O. and Liona, P. (2001). Improved production of seed yam. Research Guide 63. International Institute of Tropical Agriculture, Ibadan, Nigeria. pp. 20
- [50] Ricci, P., Coleno, A. and Fevre, F. (1978). Storage problems in the cush-cush yam II. Control of *Penicillium oxalicum* rots. *Annales de Psychopathologies* 10(4): 433 – 440.
- [51] Rippon, J. N. (1988). The pathogenic fungi and pathogenic Actinomycetes. In: *Medical mycology*, 3<sup>rd</sup> edition. Publ. Sanders Co. Philadelphia, pp: 163.
- [52] Samson, R. A., Hockstra, E. S. and Van-Orschot, A. N. (1984). *Introductory to food borne fungi*. The Netherlands academy of arts and science, pp: 1-25.
- [53] Snowdon, A. L. (1991). *A Colour atlas of postharvest diseases and disorders of fruits and vegetables* UK. Vol 2, pp: 416.
- [54] Turner, N. W., Subramanian, S. and Piletsky, S. A. (2009). Analytical methods for determination of mycotoxins: a review. *Anal. Chim. Acta*. 632(2): 168 – 80.
- [55] Willey, J.M., Sherwood, L.M. and Woolvertton, C.J. (2008). Prescott, Harley and Klein's *Microbiology*. 7<sup>th</sup> Edition, McGraw Hill companies, inc.: 629-635.
- [56] Yin, Y. N., Yam, L. Y., Jiang., J. H. and Ma, Z. H. (2008). Biological control of aflatoxin contamination of crops. *J. Zhejiang univ. sci. B* 9(10): 787 – 792.