Abstract

Fungal decay of the yam cultivar Dioscorea dumentorum Pax sold at Nsukka Markets in Nigeria was investigated. The diseased and sound yam cultivar tubers were used to isolate and identify some fungi implicated in post harvest and storage decay leading to economic losses. This cultivar is not as popular as the Dioscorea rotundata and D. cayanensis but it is eaten in many parts of Nigeria in spite of its unpalatable bitter taste. A total of five fungi namely Botryodiplodia theobromiae, Aspergillus sp, Aspergillus niger, Fusarium sp and Syncephalastrum sp were isolated from the tubers. The frequency of isolation was recorded and the most frequently isolated organism was Aspergillus sp. Both Aspergillus sp were the most pathogenic while Fusarium sp was the least pathogenic. Pathogenicity was proved for all the fungal isolates and each of the isolate was associated with the decay. The fungus Syncephalastrum sp was however implicated for the first time. Inoculated tubers were examined visually and by the application of slight pressure and the nature of rot was varied.

Key words: Fungi, yam cultivar, isolation, isolates, inoculation and pathogenicity

Introduction

Yam which belongs to the genus Dioscorea and Family Diocoraceae is a tuber crop with economic and socio-cultural importance in Tropical countries of the world especially (Jova et al., 2005). There are many cultivars of yam that are cultivated in Africa which are Dioscorea rotundata, (white yam) D. cayanensis (yellow yam), D. alata (water yam) and D. dumentorum (cluster yam) with the first two cultivars
being the most popular ones. The cultivar *Dioscorea dumentorum* Pax is commonly known as “cluster yam”, “bitter yam”, “wild yellow yam”, trifoliolate (three leaf) yam respectively. The yam is underutilized in Nigeria but is grown in South West and South East parts of the country. The probable reasons for the underutilization of bitter yam is widely held belief among consumers and farmers of the unpalatable bitter taste and high rate of post-harvest hardening of the tubers especially during storage (Medoua *et al*., 2005). The problem of inadequate food supply in Sub Saharan Africa and the attendant malnutrition and even with the potential of using bitter yam in bakery and pharmaceutical industries are presently the right stimuli that have contributed immensely to its increased cultivation in various parts of Nigeria (Musieba *et al*., 2009. Ukpabi 2008). Unlike the other yam cultivars it lacks known industrial application especially in Nigeria. In South West Nigeria, cluster yam is used in the treatment of malaria in combination with other herbs suggesting a widespread ethnomedicinal importance (Dike *et al*., 2009). Yams are reserved in various ways such as through storage and in the form of yam flour and presently, Nigeria remains the world’s largest exporter of the commodity which has immensely boosted her foreign exchange earnings in addition to crude oil and natural gas.

Coursey (1971) observed that of all the food crops of the tropics few are closely associated with a particular cultural areas as are the yams with West African peoples.

Yams are stored in Nigeria in a variety of ways such as being left unharvest in the ground during the dry season, stacked in heaps on the floor preferably on shelves or racks, in circular or rectangular trench dug in the ground and in a yam barn (Adeniji, 1970).

Kochhaar (2011) noted that the annual world production of yam is estimated to be above 90 million tonnes in 1994 of which some 22 million tons are produced in Nigeria, 2.8 million tons in Cote D’Ivoire and about 1.3 million tons in Benin with Ghana and Togo accounting for other less production. Food and Agricultural Organization (FAO) in 2008 said that Nigeria produced 35 million metric tons of
yam followed by Cote d’Ivoire with 6.9 million metric tons while Colombia, Brazil and Haiti combined produced 50 million metric tons in 2008.

Production of yam is constrained by high cost and availability of planting materials which constitute over 33% of the cost of yam production. As many as 30% of the previous harvest which should have been sold are eaten or reserved as seed yam for next cropping season (Orkwor and Asadu, 1997).

Some researchers such as Okafor (1966), Adeniji (1971) and Ogundana et al. (1970) attributed much losses in yams to microbial rotting after long periods of storage. Adeniji (1970) using tubers of D. rotundata studied degree of decay caused by three storage pathogens namely Penicillium oxalicum, Aspergillus niger, and Botryodiplodia theobromae which pathogenicity was proved for the isolates. According to Anwadike (2009) and Cornelius (1998) a wide range of micro-organisms were associated with the rot of white yams and this is expected because the seed yams used for planting are potential sources of yam rot organisms. Cornelius (1998) identified 12 micro-organisms associated with the rotting of D. rotundata of Poir variety in storage. These fungi include Aspergillus niger, Botryoplodia theobromae, Fusarium curnasium, F. oxysporum, Penicillium brevi-compactum, Penicillium sp, Scutelimma bradys and Erwinia sp. These observations were confirmations of the earlier observations and discovery of microbial rot organisms in storage trials in the United Kingdom by Noon and Colhoun (1979) while Ezebekwe and Ibe (2009) obtained similar results with white yams in Owerri, Imo State of Nigeria.

Although microbial rotting of white yam have been extensively studied in many parts of the tropics including Nigeria but the rot of bitter yam has been given little or no attention hence the paucity of literature on it. This reason has greatly necessitated the preliminary investigation on the fungal decay of the tubers of D. dumentorum which remains underutilized in Nigeria at large so as to know the fungal organisms implicated in the tuber rot of this cultivar and the probable best horticultural and storage practices that will reduce post harvest losses of tubers.
The objective of this research is to isolate and identify the fungi responsible for the postharvest decay of bitter yam tubers bought from Nsukka markets and to prove pathogenicity of the isolates on sound yam tubers.

**Materials and Method**

Bitter yam tubers sold in Nsukka markets were used for the investigation. Poor rotted yam tubers were identified by visual examination and by exerting slight pressure with the fingers and then taken to the laboratory for fungal isolation and study. Affected portions were sliced radially and used for the work.

**Isolation and Identification of Fungal Isolates from Decayed Yam Tubers**

Sliced portions of the tubers were surface sterilized by washing in 70% Ethanol and then rinsed in sterile distilled water. Small portion (about 2 mm in diameter) of the advancing area of decay of the yam were removed with a flame sterilized scalpel and placed aseptically into petri dishes of water agar with 5 pieces per plate. They were incubated on the laboratory bench at the room temperature of 25± 2°C for five days and fungal growths were sub cultured to Potato Dextrose Agar (PDA) plates. Pure cultures were obtained through repeated sub culturing and fungal isolates were labelled A –D.

Slides preparation of the isolates were made by placing the fungi on clean glass slides and stained with brilliant cresyl blue solution and the slides preparation of the isolates were examined under the microscope for identification of the rot organisms. Identification of the isolates was accomplished with the aid of a textbook on Mycology by Barnett (1962) and confirmation sought from expert mycologists.
**Pathogenicity Test with Fungal Isolates**

To confirm that the fungal isolates were responsible for the decay signs observed in the naturally infected tubers, Robert Koch’s postulate was investigated. This involved the inoculation of sound yam tubers with the fungal isolates and the occurrence of identical signs as observed in the naturally infected tubers and re-isolation of similar fungi.

The isolates were tested for pathogenicity by the inoculation of healthy yam tubers according to the method described by Okafor (1966). To study the effects of the various fungal isolates, cylindrical cores of 1.5mm in diameter were taken from the “head”, “middle” and “tail” portions of each yam tuber with a 5 mm diameter cork borer. Four millimeters of 7 day old fungal isolates were placed with fungus end first into the holes made in the sound tubers and covered with the yam cores. It was then sealed with Vaseline (petroleum jelly) and disc of non-inoculated PDA was used as the control. The inoculated tubers with each of the fungal isolate as the experiment was replicated thrice.

The inoculated yam tubers were left on the laboratory shelves at room temperature for four weeks as described by Okafor (1966) after which they were sliced through the site of inoculation. Decayed areas were measured in the “head”, “middle” and “tail” portions of the tubers.

**Results**

The fungi isolated from the decayed portions of the tubers were identified using growth pattern, mycelia colors and morphological features as parameters for identification. Slides of the isolated fungi were prepared from pure cultures microscopically studied. The fungal isolates were labelled A, B1, B2, C and D.
Table 1 shows the types of fungi and frequency of isolation from decayed *Dioscorea dunctorum*.

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Frequency of isolation</th>
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</thead>
<tbody>
<tr>
<td><em>Botryodiplodia theobromae</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Aspergillus sp</em></td>
<td>3</td>
</tr>
<tr>
<td><em>Fusarium sp</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Syncephalastrum sp</em></td>
<td>1</td>
</tr>
</tbody>
</table>

Specimen B1 was identified as *Aspergillus niger* because it formed a black mycelium and showed coenocytic conidiophores which arose from the foot cells. It has a black vesicle with sterigmata bearing numerous blackish conidia.

Specimen B2 formed a brownish black mycelium which is coenocytic and hyaline. The conidiophores arose directly from the foot cells and the sterigmata bore numerous conidia which were bluish in color. It was identified to be *Aspergillus* sp.

Isolate C formed a whitish cottony mycelium and microscopically showed three types of conidia; a cone shaped, slightly curved at the end with 2–3 septations, microconidia with one septation and variable shapes. Both the micro and macroconidia were borne on short conidiophores and chlamydiospores were borne at intercalary positions. Isolate C was identified as species of *Fusarium*. 

Isolate D formed a greenish mycelium and showed a merosporangia radiating from the vesicle with merospores in each merosporangium that were borne on sporangiospores. The specimen was identified as a species of *Syncephalastrum*.

**Table 2: Effect of inoculating sound *D. dumentorum* tubers with isolated fungi.**

<table>
<thead>
<tr>
<th>Isolated Fungi</th>
<th>Average length</th>
<th>Nature and color</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Botryodiplodia. Theobromae</em></td>
<td>1.4</td>
<td>brown, wet, soft</td>
</tr>
<tr>
<td><em>Aspergillus. niger</em></td>
<td>1.5</td>
<td>Purple brown, soft</td>
</tr>
<tr>
<td><em>Aspergillus sp</em></td>
<td>1.5</td>
<td>Purple, brown, soft</td>
</tr>
<tr>
<td><em>Fusarium sp</em></td>
<td>0.1</td>
<td>light, brown, soft</td>
</tr>
<tr>
<td><em>Syncephalastrum sp</em></td>
<td>0.3</td>
<td>brown, hard</td>
</tr>
</tbody>
</table>

From Table 2 above the maximum distance of decay was observed with both species of *Aspergillus* followed by *B. theobromae* while the least decay was associated with *Fusarium* sp.
Fig. 1. Petri dish culture of isolated fungi
(1) Botryodiplodia theobromae; (2) Aspergillus sp; (3) Trichoderma sp; (4) Penicillium sp; (5) Syncephalastrum sp; (6) Aspergillus niger & Sclerotium sp; (7) Fusarium sp; (8) Fusarium sp; (9) Sclerotium sp
Plate 1: Photomicrograph of *Botryodiplodia theobromae* from slide mount: A = Mature 2-celled conidium, B = immature 1-celled conidium (x 100)
**Plate 2**: Photomicrograph of *Aspergillus* sp from slide mount - Vesicle, B = Conidia C = Conidiophore (x100)
Plate 3: Photomicrograph of *Penicillium* sp from slide mount:
i = Conidiophore, (ii) = Sterigma, (iii) = Conidia (x100)
Plate 4: Photomicrograph of *Syncephalastrum* sp from slide mount. 
A = Vesicle   B = Merosporangia  C = Sporangiophere (x100)

Plate 5: Photomicrograph of *Aspergillus niger* from slide mount 
A = Conidiophore   B = Conidia   C = Vesicle. (x100)
Plate 6: Photomicrograph of *Fusarium sp* a from slide mount.

A = Chlamydospore;  B = Macroconidia;  C = Microconidia  
D = Conidia;  E = Conidiophore. (x100)

Discussion

The results of the study revealed that all the fungal isolates showed positive pathogenicity with the sound tubers after inoculation. The tubers were bought from the open markets in Nsukka Town in Nigeria. Majority of the tubers were transported to the markets from the Northern part of the country in open Lorries while very few were locally grown within the neighboring communities. The tubers are tightly packed together under poor ventilation and high sun intensity which is conducive for the pathogenic fungal growth. The rot fungi isolated and pathogenicity proved above have been reported in other yam cultivars such as *D. rotundata* Poir by Adenuji(1970), Okafor(1966), Noun and Coulhoun(1979), Ezebekwe and Ibe(2010). The most frequent isolated fungus was *Asergillus sp* while the rest organisms were at ar in frequency of isolation and it
was observed that the most pathogenic were Aspergillus niger and Aspergillus sp with Fusarium sp implicated as the least pathogenic Probable yam rot disposing factors include bruises on tubers tubers during harvest and transportation which destroy the protective periderm and exposes them to portal entry and infection by pathogens. The isolated pathogens predominantly showed brownish discoloration on the decayed portions of the tubers. Ogundana et al.(1971) also attributed the decay to color to the ability of the pathogens to produce cellulosic and pectin lytic enzymes which degraded the middle lamella of the cell wall.

**Conclusion**

In conclusion pathogenicity was proved for all the fungal pathogens associated with postharvest fungal decay of the yam cultivar in storage and a host of factors pre disposes them to such. This can cause large scale economic losses to farmers and households and thus a major threat to food availability and security in Nigeria and the world at large

**Recommendation**

From the findings it can be recommended that healthy practices associated with postharvest storage such as the avoidance of wounding tubers during harvest and storage should be avoided. Equally high temperature exposure of tubers during transportation and sales should be minimized by transporting tubers in shaded Lorries and pick- up with adequate ventilation. Sales of tubers in open markets under the sun and high humidity should be minimized by selling the tubers under shades and storing them in well ventilated stores. Yam powder production can be done and packaged as a form of post harvest storage to reduce yam losses in storage.
References


