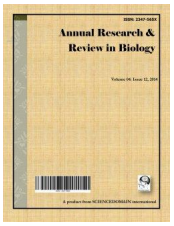

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Fungal Rot of Yam (*Dioscorea alata* Lin.) Sold At Nsukka Markets in Nigeria

B. C. Anwadike[1*]

[1]College of Education, Warri, Nigeria.

Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

Fungal decay of yam (*Dioscorea alata*) popularly known as water yam bought from Nsukka Markets in Nigeria was investigated for the fungi responsible for postharvest rot of tubers in storage. The tubers were used to isolate and identify fungal species that cause rot and deterioration of tubers in storage. A total of seven fungi namely *Botryodiplodia theobromae, Aspergillus sp, Aspergillus niger, Fusarium sp*[a], *Fusarium* sp, P*enicillium* sp and *Trichoderma* sp were isolated from healthy or sound tubers. Pathogenicity test was done and proved for all the isolates with *Botryodiplodia theobromae* and *Penicillium* sp implicated as the most pathogenic while *Trichoderma* sp was the least pathogenic. Inoculated tubers were examined and the nature of the rot varied with the pathogens.

Keywords: Fungi; pathogenicity; isolates; postharvest; yam tubers.

1. INTRODUCTION

Yams which belongs to the genus *Dioscorea* and family Dioscoraceae is an economic crop grown purposely for its tuber. It has a socio – economic and cultural importance especially in the tropical countries of the world such as in Nigeria, Ghana, South America and Asia where it is associated

**Corresponding author: E-mail: anbenj@yahoo.com;*

with feasts and other religious festivals. In Nigeria for instance there is a new yam festival called “Iwu Festival” celebrated annually among the Igbo tribe of South East Nigeria to appease the gods of the land that is responsible for the good harvest. Yams constitute an important part of the diet in many African countries providing carbohydrates and other food nutrients (Jova et al. [1]. This crop provides staple carbohydrates food stuff for most Nigerians from which they derive almost half their calories requirement. According to Dike [2] the species *D dumentorum* has ethnobotanical value and importance being used as an anti malarial plant in the therapy of malaria while Medouia et al. [3] reported that the hard –to cook property of the cultivar has made it an unpopular species among yam growers and consumers. Although grown in all parts of Nigeria, they are widely grown in the Eastern and Middle Belt Regions where cultural and favorable climatic factors enhance their production. Yam has as many as 600 tropical and subtropical species which are grown for their stem tubers. Historically, the crop was introduced into Nigeria from South East Asia where it is believed to have originated. Six species of yams are cultivated in Nigeria and in order of importance are as follows; *Dioscorea rotundata* Poir (white yam*), D. cayanensis* Lam (yellow yam), *D. alata* Lam (water yam), *D. dumentorum* Pax (cluster yam*), D. esculenta* Bourk (chinese yam) and *D. bulbifera* Lam.

Coursey [4] noted that of all the food crops of the tropics few are closely associated with particular cultural areas as are the yams with West African peoples giving a “yam zone”. This implies that the crop is widely grown in West Africa relative to the other parts of the continent

Kochhaar [5] gave the statistics of the annual world production of yam to be above 90million tones 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) [6] 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 has been observed to be constrained by high cost of cultivation and availability of planting materials which constitute over 33% 0f the cost of yam production (Ukpabi [7]. Additionally it was reported that many as 30% of the previous harvest are sold, eaten or reserved as seed yam for next cropping season (Asare –Bediako et al. [8]. However microbial decay of yam is known to contribute significantly to post harvest losses of yam in storage making them unavailable for consumption which have been reported by many authors.

Yams are stored in Nigeria in a variety of ways such as being left unharvested 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, [9]. Some researchers such as Okafor [10], Adeniji [11] Ogundana et al. [12] attributed much losses in yams to microbial rotting after long periods of post harvest storage. Adenili [9 and 11] using tubers of *D. rotundata* studied degree of decay caused by three storage pathogens namely *Penicillium oxalicum, Aspergillus niger*, and *Botryodiplodia theobromae* where pathogenicity were proved. Cornelius [13] and Anwadike [14] among other researchers have identified and reported a wide range of micro-organisms that are associated with the rot of yams and this is expected because the seed yams are potential sources of yam rot organism both in the field and in storage. Cornelius [13] identified and reported 12 micro-organisms associated with the rotting of *D. rotundata* of Poir variety in storage. These fungi include *Aspergillus flavus, A. niger, Botryoplodia theobromae,Fusarium cumasium, F. oxysporum, Penicillium brevi-compactum, Penicillium sp,Scutelimm abradys* and *Erwinia* sp. These observations were confirmed in storage trials in the United Kingdom by Noon and Colhoun [15]. Ezebekwe and Ibe [16] reported similar results with white yam tubers in Owerri, Imo State of Nigeria while Anwadike [14] confirmed some of the same yam rot pathogens while using the same yam cultivar sold in open markets in Warri metropolis .

Although microbial rotting of white yam have been extensively studied in many parts of the tropics including Nigeria but the rot of water yam has been given little or no attention hence the few literature on it. This reason has greatly necessitated the preliminary investigation on the fungal decay and pathogenicity test of the fungal isolates associated with the tubers of *D.* alata which remains an unpopular yam cultivar in Nigeria at large probably due to the nature of the parenchyma which is soft and is not as tasty as white yam and the rest cultivars.

2. MATERIALS AND METHODS

Water yam tubers sold in Nsukka open markets were bought, examined and selected for the investigation. Poor rotted yam tubers were identified by visual examination and by exerting slight pressure with the fingers. Unhealthy tubers with signs of decay were isolated, taken to the laboratory for fungal isolation/identification and study. Affected portions were sliced radially and the advancing areas of decay were removed with a sterilized scalpel. The removed portions were aseptically cultured in the various growth media.

2.1 Isolation, Culturing and Identification of Fungal Isolates from Decayed Yam Tubers

Sliced portions of the advancing areas of decay 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± 2ºC for five days and fungal growths were sub cultured to Potato Dextrose Agar (PDA) plates. Pure cultures were obtained through repeated sub culturing and the pure fungal isolates prepared cultures and slides were labelled A –F.

Slides preparation of the isolates were made by placing the fungi on clean glass slides and stained with brilliant cresyl blue solution. Microscopic observation was done for the identification of the isolates which was accomplished with the aid of a textbook on Mycology by Barnett and Hunter [17] and confirmation sought from expert mycologists.

2.2 Pathogenicity Test with Fungal Isolates

Pathogenicity test for the fungal isolates implicated for the decay signs observed in the naturally infected tubers was done using Robert Koch's postulate. 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 after one month of inoculation was made.

The isolates were tested for pathogenicity by the inoculation of healthy yam tubers according to the method described by Okafor [10]. 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 and incubated on the laboratory bench.

The inoculated yam tubers were left on the laboratory shelves at room temperature for four weeks as described by Okafor [10] after which they were sliced through the site of inoculation. Decayed areas were measured in the "head", "middle" and "tail" portions of the tubers.

3. 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, D,E and F.

Table 1 shows the types of fungi and frequency of isolation from decayed portions of the tubers. Isolate A is identified as *Botryodi[lodia theobromae* having dark pycnidia bearing pycnidiospores which are called conidia which were one and two celled.

Isolate B1 formed a brownish black myceliua and showed a coenocytic hyaline and long conidiophores which arose directly from foot cell with vesicles and sterigmata. It was identified to be *Aspergillus* sp.

Isolate B2 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.

Isolate C formed a white cottony colony with some tinge of pink in the medium. It showed three types of conidia, a cone shaped macro conidia with 2 – 3 septations. Some of the micro and macro conidia were borne on short

conidiophores and chlamidiospores borne in intercalary positions. The isolate was identified as Fusarium sp[a]. Isolate D has two 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 D was identified as species of *Fusarium*.

Isolate D formed a cottony white mycelium with 2 types of conidia, macroconidia which is boat shaped slightly curved at both ends with 2 septations and microconidia which showed variable shape. The specimen was identified as a species of *Fusarium*.

Isolate E formed a blue green mold with septate conidiophores which were more branched near the apex forming finger – like projections. It had sterigmata with chains of oval greenish conidia and was identified as *Penicillium* sp.

Isolate F formed a greenish powdery mycelium with greenish white patches. Microscopic examination revealed greenish conidia borne on long conidiophores with characteristic right angled branching. It was identified as *Trichoderma* sp.

From Table 2 above the maximum distance of decay was observed with both *B. theobromae* and *Penicillium* sp while the least decay was associated with *Trichoderma* sp.

Table 1. Types of fungi and frequency of isolation from decayed *Dioscorea alata*

Fungus	Frequency of isolation
Botrodiplodia theobromae	3
Aspergillus sp	3
Aspergillus niger	2
Fusarium sp[a]	2
Fusarium sp	2
Penicillium sp	3
Trichoderma sp	3

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

Isolated fungi	Average length (cm) of decay	Nature and colour
Botrodiplodia theobromae	2.5	Brown, wet, soft
Aspergillus. sp	1.6	Purple, brown, soft
Aspergillus niger	1.7	Purple,brown, soft
Fusarium sp[a]	1.5	light,brown,soft
Fusarium sp	1.3	brown,hard
Penicillium sp	2.5	brown hard
Trichoderma sp	0.8	brown hard

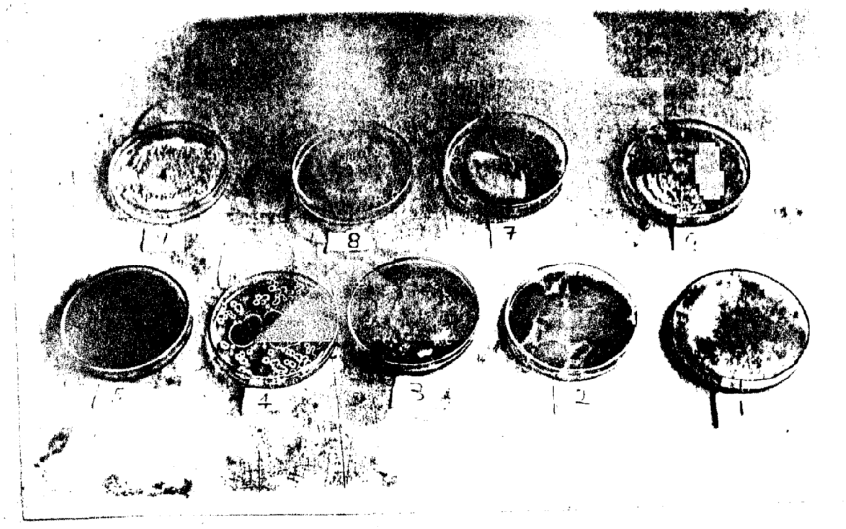

Fig. 1. Petri dish culture of isolated fungi

(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

Fig. 2. Photomicrograph of Botryodiplodia theobrome from slide mount
A = Mature 2-celled condium, B = immature I – celled condium (x 100)

Fig. 3. Photomicrograph of *Aspergillus* sp from slide mount
A = vesicle, B = conidia C = Conidiophore (x100)

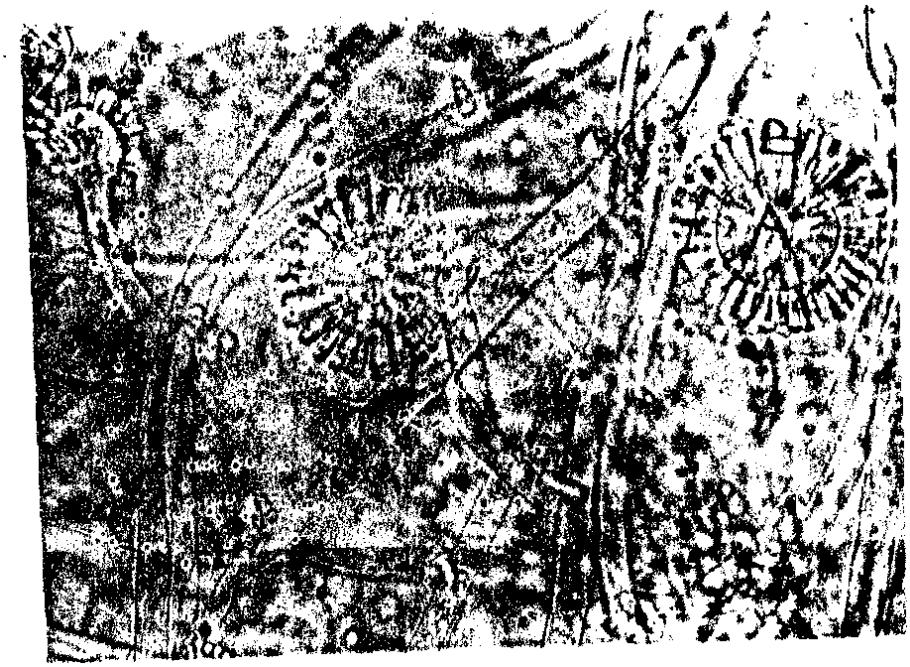

Fig. 4. Photomicrograph of Syncephalastrum as from slide mount
A voside B = merosporangia C = sporangiophoro (x 100)

Fig. 5. Photomicrograph of *Aspergillus niger* from slide mount
A = conidiophores B conidia C = vesicle (x100)

4. 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 [9 and 11], Okafor [10], Noun and Coulhoun [15], Ezebekwe and Ibe [16]. Other probable yam rot disposing factors include bruises on 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

Fig. 6. Photomicrograph of fusarium sp from mount
A = chlamydosporo B = macroconidia C = microconidia D = conidia E = conidiaophore (x100)

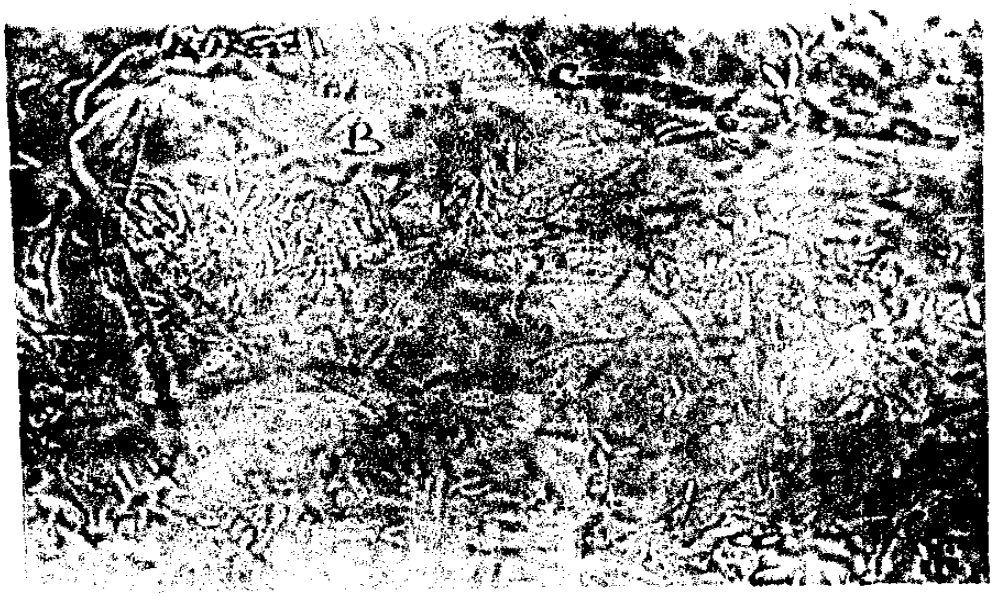

Fig. 7. Photomicrograph of fusarium sp from slide mount
A = macroconidia; B = microconidiun C = Conidiophores

the decayed portions of the tubers. Ogundana et al. [12] 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.

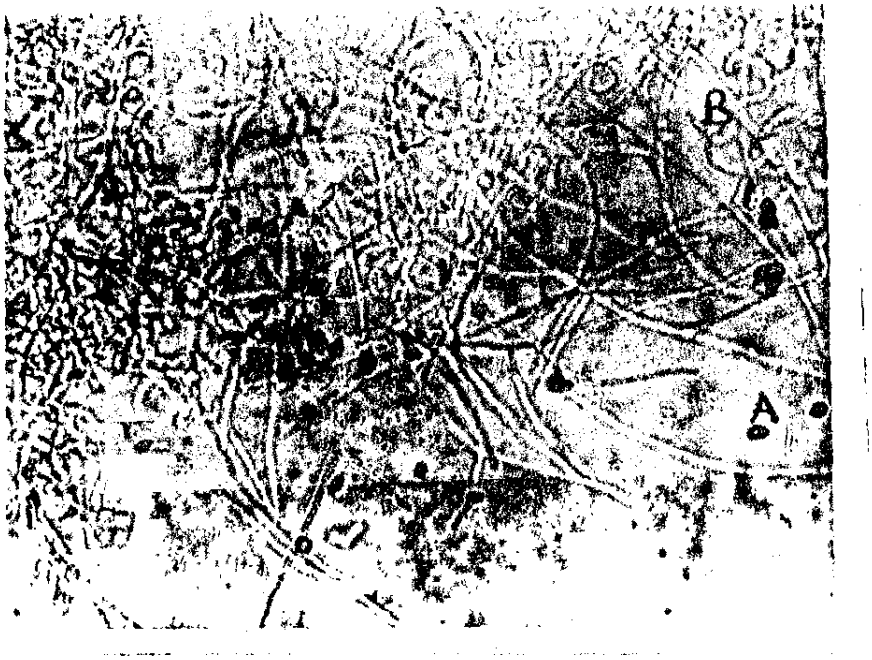

Fig. 8. Photomicrograph of *Trichoderma* sp from slide mount
A = oval conidium, B = conidiophores branching at right angles (x100)

Fig. 9. Photomicrograph of penicilium sp from slide mount
(i) = conidiophore, (ii) sterigma (iii) conidia (x 100)

5. 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.

From the findings it can be recommended that healthy practices associated with postharvest storage such as the avoidance of tubers wounding 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.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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