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Mycoflora of postharvest rots of bitter yam *Dioscorea dumentorum* Pax in Nsukka agro ecological zone, Nigeria

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Abstract

This study was done to recover fungi associated with postharvest rotting of bitter yam, *Dioscorea dumentorum* tubers in Nsukka Agroecological Zone, Nigeria. Tubers showing signs of rot were picked from farmers in four randomly selected towns in the zone. The towns included, Nsukka, Orba, Ibagwa and Obollo-Afor. Five (5) samples each were picked from three (3) randomly selected farmers. Samples were packaged in sterile polythene bags, labeled accordingly and moved to the laboratory for studies. The fungal isolates were identified using both cultural and molecular techniques. Results showed that mycoflora of rotted bitter yams in the Zone were, *Aspergillus flavus, Aspergillus niger, Lasiodiplodia pseudotheobromae, Galactomyces candidum* and *Rhizopus delemar*. The percentage frequencies of occurrence of the fungal organisms ranged from 14.03% to 26% with *A. niger* recording the highest (26%) while *A. flavus* recorded the least (14.03%). Pathogenicity tests revealed that the fungi produced rots (diameter) ranging from 1.70 cm to 2.40 cm with *R. delemar* recording the highest rot diameter (2.40 cm) while *A. niger* produced the least (1.70 cm)

Keywords: Bitter yam, mycoflora, postharvest, fungal organisms, Nsukka, rots**.**

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INTRODUCTION

Dioscorea dumentorum Pax is an edible tuberous plant of the family Dioscoreaceae and grows in the tropics, subtropics and some temperate regions of the world (Hamon and Toure 1990; Bai and Ekanayake 1998; IITA 2006; Lebot2008). It is known by many common English names which include, bitter yam, three-leafed yam, wild yellow yam, African bitter yam etc. (Degrass,1993; Cemulak*et al*. 2018; Ukpabi, 2014). In South Eastern Nigeria, African bitter yam has some local/vernacular names that include but not limited to the following, "*Ji una*","*Una*", "*Ji ona*", "*Ona*", "*Ono*", and "*Unu*" (Ukpabi, 2015; Cemulak *et al*. 2018). Degrass (1993) profiled the proximate composition of D. *dumentorum* tubers to contain 17% carbohydrate, 2.78% protein, 0.28% fat, 0.3% fibre, 0.72% ash and 79% moisture. It also

contains 0.8 mg/100 mg niacin, 0.050 mg/ 100 g riboflavin, 0.14 mg/100 g thiamine, 0.020 mg/100 g vitamin A and ascorbic acid ranged from 6.6 mg/100 g- 21 mg/100 g. Bitter yam tubers could be prepared for consumption by boiling, frying, roasting or in dough (Burkil, 1985). It contains a hypoglycemic agent, dioscoretine (Sonibare *et al*. 2010) and as such could be recommended for diabetic patients. It is also reported to be used as a topical anaesthetic (Corley *et al*. 1985). Bitter yam starch is of immense value in tablet and capsule formation because it has smaller granules, higher specific surface area and most is amenable to compression with high crushing force acceptability (Odeku and Picker-Freyer, 2007).

Pathological postharvest deterioration of yam generally is attributed to fungi, nematodes and bacterial organisms (Coursey, 1967; Ayensu and Coursey, 1972; Ogunleye and Ayansola, 2014) and reports on losses have been in the range of 10% to 56% (Ekundayo and Naqvi, 1972; Ezeh, 1998; FAO, 1998). Activities of rot organisms on postharvest yam tubers cause reduction in the quality of the nutrients and their marketability (Okigbo, 2005). Information on postharvest rot organisms is important for control strategies and for public health concerns due to the possibilities of contamination with mycotoxins which are secondary metabolites of certain associated fungi. A good number of fungi have been implicated in the postharvest rottening of yams which included, *Aspergillus niger*,*Botryodiplodiatheobromae*, *Rhizopus nodosus, Fusarium moniliforme, Penicillium sclerotigenum* and *Macrophomina phaseoli* (Okigbo and Ikediugwu 2000). Others are, *Fusarium oxysporum*, *Fusarium solani*, *Penicillium* spp. and *Aspergillus* spp (Morse *et al*. 2000; Okigbo,2003).

The present work, therefore, was undertaken to ascertain the fungi responsible for postharvest rotting of bitter yam in Nsukka Agroecological Zone, Nigeria.

MATERIALS AND METHODS

Sources of Plant Materials and Authentication

Samples of tubers of *D. dumentorum* showing signs of fungal rots were collected from farmers in some randomly selected towns in Nsukka Agroecological Zone, Nigeria. The towns were, Nsukka, Orba, Ibagwa and Obollo-Afor. A total of 5 samples each were collected from three (3) farmers in each town. Samples were packaged in properly sterilized polyethylene bags, labelled and sent to the laboratory for analysis. Authentication of the samples was done in Crop Science Department of the University of Nigeria, Nsukka

Sterilization of Materials

All apparatuses used in this study such as the Petri dishes and other glass wares were autoclaved at 103 KN M^{-2} pressures and 121 ^{o}C for 15 minutes, washed and dried in an oven at 100 ^{o}C for 24 hr. Other metal-based apparatuses were sterilized by immersing them in absolute ethanol and flaming till red hot using spirit lamp.

Preparation of Culture Media

Water agar was prepared by dissolving 20 g of agar into 1000 ml of sterile distilled water. The solution was sterilized in the autoclave at 103 KN M^{-2} pressures and 121 ^{o}C for 15 minutes. About 0.2 ml of Streptomycin sulphate was added to the agar medium to suppress bacterial growth. It was then poured into sterile Petri dishes and allowed to solidify before inoculation.

Potato Dextrose Agar (PDA; Difco, Sparks, MD, USA) medium was also prepared by dissolving 39 g of PDA in 1000 ml of sterilized water. The medium was sterilized in an autoclave at 103 KN M^{-2} pressures and 121 ^{o}C for 15 minutes and was allowed to cool. About 0.2 ml of Streptomycin sulphate was added to the PDA to suppress bacterial growth. The PDA was dispensed into sterilized Petri dishes and was allowed to gel before use.

Isolation of Fungi

The pieces of interface between the healthy and diseased portions of bitter yam tubers were cut using sterilized scalpel and surface-sterilized by soaking in 0.1% Mercury II chloride solution for 1 minute and rinsed twice with sterile distilled water. The surface-sterilized yam pieces were placed on water agar medium using sterile forceps. The inoculated plates were incubated on the laboratory bench at room temperature until fungal mycelia growth was observed.

The fungal mycelia growing on the water agar medium were sub-cultured into the sterile PDA plates using a sterile inoculation needle and allowed to incubate at room temperature. Continuous sub-culturing was done until pure cultures were obtained, and their photographs taken. The cultures were observed daily for fungal growth, records were kept, and the frequencies and relative abundance of the various fungal isolates determined.

Identification of Fungal Isolates

The fungal isolates were identified using procedures and guidelines as provided by (Samson *et al*. 1984; Rippon 1984; De Hoog *et al*. 2000). The percentage frequency of occurrence of each fungus was calculated using the procedure outlined by Okigbo and Ikediugwu (2000).

Fungal DNA Extraction, PCR and Sequencing

The DNAs of all the fungal isolates were extracted from mycelia using the procedures of Cenis (1992) while amplification of extracted DNAs and gene sequencing were achieved using the protocol of White *et al.* (1990). The resulting sequence data were deposited in GenBank (BLAST) and accession numbers assigned.

Pathogenicity Tests

The pathogenicity tests wereconducted using all the fungi isolated to prove their pathogenicity in line with Koch's postulates. The procedure adopted by (Ogunleye and Ayansola, 2014) was used for the pathogenicity test.

Pure cultures of fungal isolates from rottening tubers of *D. dumentorum* were used for the tests. Wholesome tubers of *D. dumentorum* which were surface- sterilized using 0.1%mercuric chloride, rinsed in two changes of sterilized water and allowed to dry in the inoculating chamber were deployed. Each wholesome tuber was bored in two different locations to a depth of 1 cm from the surface using a sterile 6mm diameter cork borer. A 5 mm mycelium disc from the periphery of a 48-hour old culture of each fungus was put in each of the holes bored in the tubers and covered immediately with the tissue scooped from the tubers during the boring. The points of inoculations were sealed with petroleum jelly and the inoculated tubers were put inside sterilized polythene bags containing damp cotton wool to ensure suitable humid condition. The experiment was set up in three replications for all the fungi isolated. The control experiment was conducted similarly using sterile PDA discs. The tubers were kept on the laboratory bench at room temperature (27 ± 2^{o}C) for 10 days after which they were sectioned from the points of inoculation to assess rot development. The fungi were re-isolated and their cultural and morphological identities confirmed to be same with the earlier isolates.

Statistical Analyses

Data collected from pathogenicity tests were statistically analysed using ANOVA on Genstat statistical software for windows version 3.2. Means were separated using Least Significant Difference (LSD) at 5% level of probability.

RESULTS

Results showed that five fungi were associated with postharvest rotting of *D. dumentorum* in Nsukka Agroecological Zone.

They included, *Aspergillus flavus, Aspergillus niger*, *Lasiodioplodia pseudotheobromae, Galactomyces candidum* and *Rhizopus delemar*. The percentage frequencies of occurrence of A. *flavus*, A. *niger*, *L. pseudotheobromae*, *G*. *candidum* and R. *delemar* were, 14.03, 26.32, 16.67, 17.55 and 25.43, respectively (Table 1).

All the isolated fungi caused rot on *D. dumentorum* tubers and they were re-isolated thereby confirming their pathogenicity in accordance with Koch's postulates

Table 1. The Percentage Frequencies of Occurrence of the Fungi isolated from rotted bitter yam

Fungal organisms	Total number of isolations	Percentage frequency
A.flavus	16	14.03
A. niger	30	26.32
L.pseudotheobromae	19	16.62
G. candidum	20	17.55
R. delemar	29	25.43

R. delemar produced the highest rot diameter (2.4 cm) while *A. niger* recorded the least rot diameter (1.70 cm) though there was no significant difference (P≥0.05) among the fungi (Figures 1 C, E, I, L, O; Table 2). However, there was a significant difference (P≤0.05) between the rot diameter caused by the fungi and that of the control.

Table 2: Mean Diameter of Rotten Tissue on Inoculated Tubers of *D. dumentorum*

Fungal organisms	Mean diameter (cm) of rots
A. flavus	2.20
A. niger	1.70
L. pseudotheobromae	1.95
G.candidum	2.10
Rhizopus delemar	2.40
Control	0.73
LSD (0.05)	0.97

Culture Plate description of isolated fungi

The result showed that *A. flavus* grew by 2.9 cm in 24 hr on the PDA at room temperature (27±2 ^{o}C). The colony was olive green on the front view (Figure 1A) while the reverse was light yellow (Figure 1B). *A. niger* grew by 2.5 cm in 24 hr on PDA at room temperature. The colony appeared white initially but quickly turned black (Figure 1D) while the reverse was dirty white (Figure 1E). *G. candidum* grew by 1.5 cm in 24 hr on PDA at room temperature. The colony was concentric and white in colour on both the front and the reverse (Figures 1G, H). *L. pseudotheobromae* grew by 1.6 cm in 24 hr on PDA at room temperature. The mycelium was greyish-white on the front view but dark green on the reverse (Figures 1 J, K). *R. delemar* appeared white initially then became profuse and fluffy and greyish black at maturity (Figures 1M, N). It covered the Petri-dish in 24-48 hr at room temperature. The identities of the isolated fungi were confirmed using the BLAST searches as *Aspergillus flavus, Aspergillus niger*, *Lasiodioplodia pseudotheobromae, Galactomyces candidum* and *Rhizopus delemar* and their corresponding accession numbers (Table 3).

DISCUSSION

Five species of fungi were implicated with postharvest rot of Bitter yam in Nsukka Agroecological zone, Nigeria. They were, *Aspergillus flavus, Aspergillus niger*, *Lasiodioplodia pseudotheobromae, Galactomyces candidum* and *Rhizopus delemar*. The findings showed that all the organisms isolated were widely distributed in the study areas and caused reasonable measures of rotting. This study is probably the first reported study on the fungal flora associated with postharvest rot of bitter yam in Nsukka Agroecological zone. Studies on isolation of such fungal organisms were only centre on other more celebrated and popular yams in the likes of white yam and water yam among others (Ekundayo and Naqvi, 1972; Okigbo, 2003; Ogunleye and Ayansola, 2014; Anwadike, 2018). To our knowledge, the isolation of *L. pseudotheobromae* from bitter yam tuber as a postharvest rot fungus is rare and perhaps the first report in bitter yam, though it has been reported in China where it was found to be pathogenic on *Acacia confusa*, *Mangifera sylvastica*, *Paulownia fortunei* and *Albizia falcataria*(Zhao *et al.,* 2010). It was also reported in China as causative agent of pedicel and peduncle discolouration on grapes (Dissanayake *et al.,* 2015). Similarly, this seems to be the first report of isolation of *Galactomyces candidum* from a postharvest bitter yam tuber or even yam tubers generally. The ability of this fungus (yeast) to induce rots in bitter yam tuber is a strong

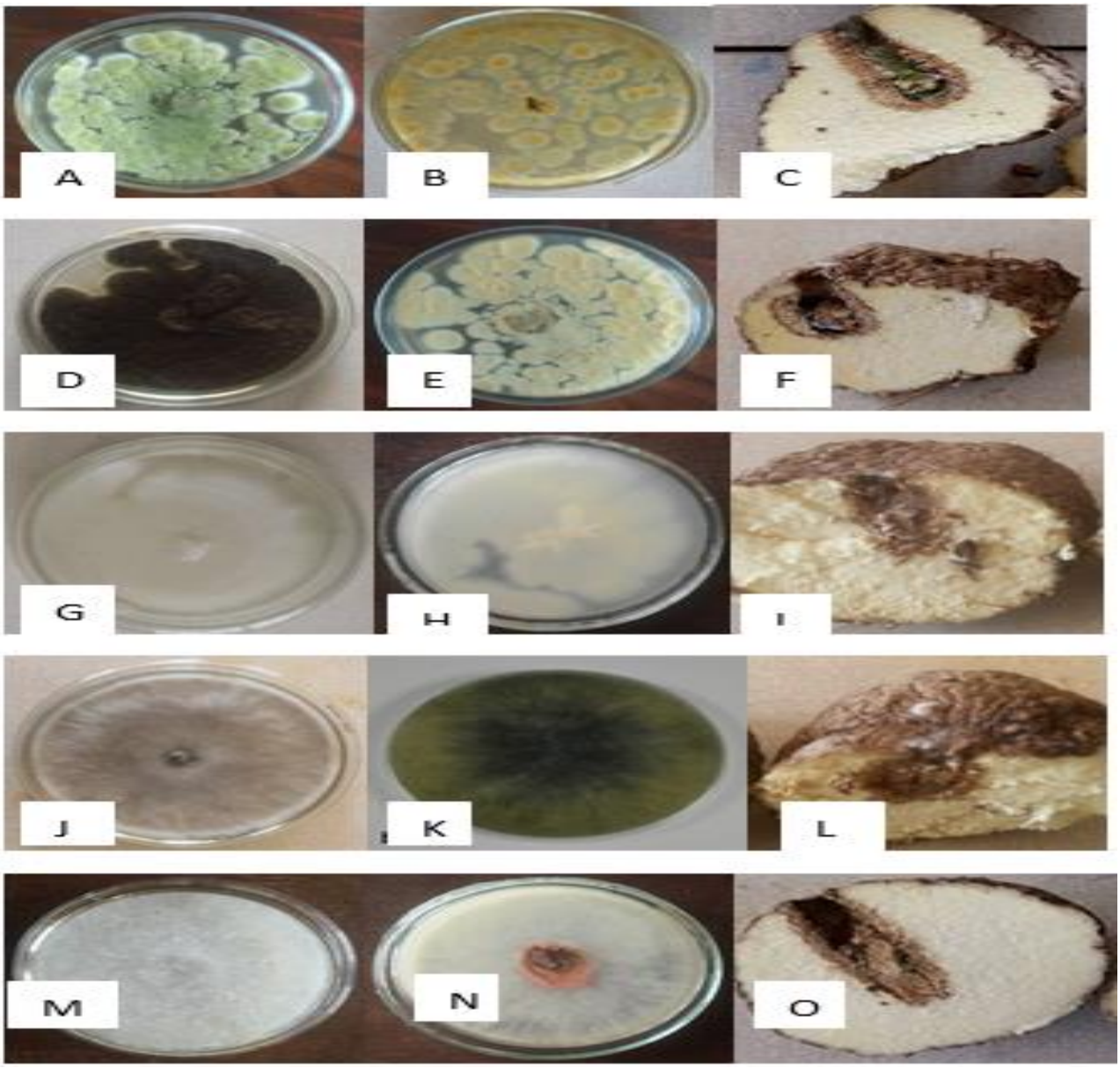

Figure 1: Culture plate photographs of isolated fungal organisms and induced rots by artificial inoculation. ABC, *A. flavus* on PDA (A, front view; B, reverse view), and induced rot (C).DEF, *A. niger* on PDA (D,front view;E,reverse view), and induced rot (F). GHI,*G. candidum* on PDA(G,front view; H, reverse view), and induced rot(I).JKL, *L. pseudotheobromae* on PDA(J, front view; K, reverse view), and induced rot(L). MNO,*R.delemar* on PDA(M, front view; N, reverse view), induced rot(O).

indication of its importance in postharvest yam rot generally. Meanwhile, *Geotrichum candidum,* an anamorph of *Galactomyces* species (De Hoog *et al.*, 2000) has been implicated in postharvest deterioration of fruits (Brown, 1979; Bourret, 2013; Hafeez *et al.,* 2015). Ademoh *et al*. (2017) reported *G. candidum* as one of the postharvest fungi associated with pepper fruits in Kogi State, Nigeria. *A. niger*, *A. flavus* and *R. delemar* have been widely reported as rot agents by past workers (El-Shanshoury *et al.,* 2013;Ademoh *et al.,* 2017).However, the isolation of *A. flavus* is of greater concern given the associated serious health risks due to aflatoxin production by the fungus. Aflatoxins are known carcinogenic and hepatotoxic agents (Wild and Gong, 2010). Mycotoxins, when consumed consistently even at low dosages can induce delayed growth and development, cause immune system malfunction and altered DNA processes (Bryden, 2007).

Table 3: GenBank accession numbers of Isolated fungi, their closest relatives and percentage similarities.

Isolated Fungi	Accession Numbers of isolated fungi	Percentage Similarity	Closest relatives	Accession numbers of closest relatives
A. flavus	MH279384	99.50	*Aspergillus oryzae*	EU030335
A. *niger*	EU440768	99.4	*Aspergillus niger*	HQ170509
L. pseudotheobromae	KC492492	99.20	*Lasiodiplodia theobromae*	MF449518
G. candidum	KT336518	92.50	*Galactomyces geotrichum*	DQ683114
R, delemar	MF445156	99.60	*Rhizopus oryzae*	AB097271

Concerted efforts should be put in place by stakeholders to limit postharvest wastages caused by bio-deterioration agents to ensure food security and safety. Also, with serious health implication is the identification of *R. delemar* which belongs to the ubiquitous members of Mucorales that have been linked to mucormycosis with high death rate (Petrikkos *et al.,* 2012). Mucormycosis is an invasive mycosis contracted when foods contaminated with the pathogen are ingested by an individual (Ibrahim *et al.,* 2014). Anwadike (2018) reported that seven fungal organisms may be responsible for postharvest rot of water yam in Nsukka. They were, *Botryodiplodia theobromae*, *Aspergillus niger*, *Aspergillus* spp., *Fusarium* spp., *Penicillium* sp., and *Trichoderma* sp. It is important to note that both intrinsic and extrinsic properties that are particular to a food crop variety influence the diversity of fungal organisms that are associated with its postharvest deterioration (Ogbo and Agu, 2014). Another factor is the environment where crops are cultivated, harvested and even the harvest procedures.

Conclusion: This study reported fungi that are responsible for postharvest deterioration of bitter yam tubers in Nsukka Agroecological Zone and highlighted possible health and economic implications.

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Declaration of Interest

The authors have no conflict of interest to declare.

References

Ademoh, O.F., Afolabi, A.M., Orisasona, B.A. and Olowolaju E.D. (2017). Isolation and Identification of Rot Fungi on Post-Harvest of Pepper (*Capsicum annuum* L.) Fruits. AASCIT *Journal of Biology* **3**(5) :24-29.

Anwadike, B.C. (2018). Fungal Rot of Yam (*Dioscorea alata* Lin.) Sold a t Nsukka Markets in Nigeria. *Annual Research and Review in Biology* **28**(2): 1-9.

Ayensu, E.S. and Coursey D.G. (1972). Guinea yams. *Economic Botany* **26**:301-318.

Bai, K.V.and Ekanayake I.J. (1998). Taxonomy, morphology and floral biology. In: Orkwor, G., Asiedu, R. and Ekanayake I.J.(Eds.). *Food yams: Advances in Research*, IITA/NRCRI, Nigeria Co-publication, Umudike, Nigeria. Pp: 13-37.

Bhattacharjee, R., Melaku, G., Alieu, S., Otoo, E., Dumet, D., Kikuno, H., Kumar, P.L. and Asiedu R. (2011). *Dioscorea*. In: Kole, C. (eds) Wild Crop Relatives: Genomic and Breeding Resources, Industrial Crops Springer-Verlag, Berlin, Heidelberg Pp 71-76.

Bourret, T.B., Kramer, E.K., Rogers,. JD. and Glawe D.A. (2013). Isolation of *Geotrichum candidum* pathogenic to tomato (*Solanum lycopersicum*) in

Washington State. *North American Fungi* **8**(14): 1-7.

Brown, G.E. (1979). Biology and control of *Geotrichum candidum,* the cause of citrus sour rot. *Proceedings of the Florida State Horticultural Society.* **92**: 186-189.

Bryden, W.L. (2007). Mycotoxins in the food chain, human implications. *Asia Pacific Journal of Clinical Nutrition.* **16**(1):95–101.

Burkil, H.M. (1985). *The Useful Plants of West Tropical Africa*. Royal Botanical Gardens. http//www.aluka.org

Cemaluk, E.A.C., Daniel, N.C.and Nkiru E.O.C. (2014). Effect of soaking prior to oven drying on some nutrients and anti-nutrient properties of bitter yam (*Dioscorea dumentorum). Journal of Nutrition and Food Sciences* **4**: 280.

Cenis, J.L. (1992). Rapid extraction of fungal DNA for PCR amplification. *Nucleic Acids Research* 20:2380.

Corley, D., Tempesta, M.S. and Iwu M. (1985). Convulsant alkaloids from *Dioscorea dumentorum. Tetrahedron letters* **26**(13): 1615-1618.

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.

de Hoog, G.S., Guarro, J., Gene, J.and Figueras M.J. (.2000). *Atlas of clinical fungi* 2nd edition. Centraalbureau voor Schimmelcultures Utrecht, The Netherlands/ Universitat Rovira I Virgili Reus, Spain. 1126pp.

Degras, L. (1993). *The Yam, A Tropical Root Crop*. Macmillan Press, London.

Dissanayake, A.J., Zhang, W., Mei, L., Chukeatirote, E., Yan, J.Y., Li, X. and Hyde K.D. (.2015). *Lasiodiplodia pseudotheobromae* causes pedicel and peduncle discolouration of grapes in China Australasian. *Plant Disease Notes* **10**:21

Ekundayo, J.A. and Naqvi, S.H.Z. (1972). Preharvest and postharvest microbial rotting of yams (*Dioscorea* spp.) in Nigeria. *Transanctions of British Mycological Society* **58**(1): 15-18.

El-Shanshoury, A.R., Bazaid, S.A., El-Halmouch, Y.and Ghafar M. (2013). Control the post-harvest infection by *Aspergillus* spp. to Taify table grape using grape epiphytic bacteria. *Life Sciences Journal* **10**(1):1821-1636.

Ezeh, N.O. (1998). Economics of production and postharvest Technology In: Orkwor, G.C., Asiedu, R. and Ekanayake I.J. (eds.) *Food yam Advances in research IITA and NRCRI, Nigeria,* pp.187-214.

Food and Agriculture Organisation(FAO). (1998). *Food and Agriculture Organization of the United Nation, Yearbook 48*.

Hafeez, R., Ahktar, N., Shoaib, A., Haider, M.S., Bashir.and Awan Z.A. (.2015). First report of *Geotrichum candidum* from Pakistan causing postharvest sour rot in Loquat (*Eriobotrya japonica*). *The Journal of Animal and Plant Sciences* **25**(6): 1737-1740.

Hamon, P. and Toure, B. (1990). The classification of the cultivated yams (*Dioscorea cayenensis-rotundata* complex) of West Africa. *Euphytica* 47:179–187.

Ibrahim, A., Edwards, J.E. and Filler S.G. (2004). *Mucormycosis*. Harcourt Brace, Philadelphia**.**

IITA. (2006). *Yam: Research Review*. International Institute of Tropical Agriculture, Ibadan, Nigeria, pp 1-4.

Lebot, V. (2008). *Crop production science in horticulture.* Volume 17: Tropical root and tuber crops: Cassava, sweet potato, yams and aroids. Wallingford, Oxon: CABI Publishing.

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.

Odeku, O.A. and Picker-Freyer K.M. (2007). Analysis of the material and tablet formation properties of four *Dioscorea* starches. *Starch* **59**:430–444.

Ogbo, C.F. and Agu C.K. (2014). Role of Fungal Rots in Post-harvest Storage Losses in Some Nigerian Varieties of *Dioscorea* Species. *British Microbiology Research Journal* 4(3): 343-350.

Ogunleye, A.O. and Ayansola O.T.(2014). Studies of some isolated rot-causing mycoflora of yams (*Dioscorea* spp.). *American*

Journal of *Microbiology* and *Biotech nology* 1(1): 9-20.

Okigbo, R.N. (2003). Fungi associated with peels of postharvest yams in storage. *Global Journal of Pure and Applied Sciences* **9**(1): 19-23.

Okigbo, R.N. and Ikediugwu, F.E. (2000). Studies on biological control of post-harvest rot of yam (*Dioscorea* spp.) with *Trichoderma viride. Journal of Phytopathology* **148**: 351–355.

Okigbo, R.N. (2005). Biodegradation of white yam *(Dioscorea rotundata* Poir) and bitter yam *(Dioscorea alata* L.) slices dried under different conditions. *KMITL Science and Technology Journal* 5(3):577-586.

Petrikkos, G., Skiada, A., Lortholary, O., Roilides, E., Walsh, T.J. and Kontoyiannis D.P. (2012). Epidemiology and Clinical Manifestations of Mucormycosis. *Clinical Infectious Diseases* **54**(S1):23–34.

Rippon, J.N. (1984). The pathogenic fungi and pathogenic actinomyces. In: *Medical mycology* 3rd edition. Sanders Co. Philadelphia 163pp.

Samson, R.A., Hockstra, E.S.and Van-Orschot A.N. (1984). *Introduction to food borne fungi*. The Netherlands Academy of Arts and Science

Sonibare, M.A., Asiedu, R. and Albach, D.C. (2010). Genetic diversity of *Dioscorea dumentorum* (Kunth) pax using amplified fragment length polymorphism (AFLP) and cpDNA. *Biochemical Systematics and Ecology* **38**: 320-334

Ukpabi, U.J. (2014). Molecular analysis of *Dioscorea dumentorum* in South-East Nigeria using random amplified polymorphic DNA markers. *American Journal of Microbiology and Biotechnology,* **1** (2): 77-82.

Ukpabi, U.J. (2015). Traditional food processing techniques of *Dioscorea dumetorum* in Nigeria. *American Journal of Food Science and Nutrition* **2**(3): 21-30.

White, T.J., Bruns, T., Lee, S. and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A, Gefland, D.H., Sninsky, J.J. and White T.J. (eds.). *PCR Protocols: A Guide to Methods and Applications.* Academic Press, New York; pp315-322.

Wild, C.P.and Gong Y.Y. (2010). Mycotoxins and human disease: a largely ignored global health issue. *Carcinogenesis* **31**:71-82.

Zhao, J.P., Lu, Q., Liang, J., Decock, C. and Zhang X.Y. (2010). *Lasiodiplodia pseudotheobromae*, a new record of pathogenic fungus from some subtropical and tropical trees in southern China. *Cryptogamie. Mycologie 31:431*–439.