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Evaluation of Fungi Associated With Fungal Diseases of Yam (*Dioscorea* species) Tubers in Storage

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ABSTRACT

Yam (*Dioscorea* species) is among the most important food in the world and contributes to food security in Nigeria. This study was conducted to evaluate fungi associated with fungal diseases (storage rot) of white yam (*Dioscorea rotundata*), yellow yam *(Dioscorea cayenenesis*) and water yam (*Dioscorea alata*) purchased from Ariaria Market Aba, Abia State, Nigeria. Nine deteriorated yam tubers, three each of the *Dioscorea* species were examined and used for the isolation, identification, and characterization of the fungi. Standard mycological and agar well diffusion methods were used to isolate the fungi causing rot development in yam. Also, nine healthy tubers, three each purchased from the same market were also used for the pathogenicity test using fungi which were previously isolated from the deteriorated yam tubers. Pathogenicity test was carried out by inoculating healthy yam tubers at the head and tail with pure culture of each fungal isolate. Six fungi identified included *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Penicillium chrysogenum, Rhizopus stolonifer* and *Fusarium oxysporum.* Fungi with the highest percentage frequency were *A. fumigatus* from *D. alata* (57.1%), *A. flavus* from *D. rotundata* (50%), *A. niger* and *F. oxysporum* from *D. alata* (45.5%), *A. fumigatus* from *D. rotundata* (42.9%), while the least were *A. flavus* from *D. cayenensis* (8.3%), *A. niger* and *F. oxysporum* from *D. cayenensis* (18.2%). Pathogenicity test showed these fungi to be pathogenic agents of the rot disease. Fungal rot worsens post-harvest storage losses in yams by reducing their shelf life, market value or economic loss and palatability. Therefore, yam should be handled with care to avoid mechanical injuries which may pave way for the entry of the pathogens.

Key words: Yam (*Dioscorea* species), food security, *Aspergillus niger*, *Rhizopus stolonifer*, fungal diseases, storage rot.

Introduction

Yam belonging to the genus *Dioscorea* in the family of *Dioscoreaceae* is one of the most important dietary sources of energy within the tropics (Ezeibekwe *et al.*, 2009). Yam is an annual or perennial climbing plant with edible underground tuber. African, Asian and South American countries are the major producers of yam in the world (Okigbo and Ogbonnaya, 2006; FAO, 2013). In Nigeria, yam contributes significantly to food security and its availability in the market throughout the year helps prevent food shortages because it stores longer than other root crops. Before the present economic downturn, yam has long served as the principal source of food and nutrition for many of the world's poorest and under nourished households. About 10 species of yam are commonly cultivated for food, while several others are harvested from the wild in times of food scarcity (Bhandan *et al.*, 2003).

Yam plays an outstanding function in social and cultural lines of some producing regions like the celebrated moon festival and the popular yam festival in west Africa, an act that is well observed. Yam storage in comparison with some ether staple crops has relatively longer life span, so stored tubers symbolize stored wealth.

Studies have shown that fungal rot is the greatest loss in storage (Amusa, 2003). Microbial attacks on yam result into dry rot, soft rot and wet rot (Glover *et al.*, 2013; Taiga, 2012; Anjorah *et al.*, 2014). In dry rot, infected tissues become hard and dry with varying colouration depending on the microorganisms involved. Dry rot is caused by *Fusaruim* spp, *A. niger, A. flavus* and *Pseudomonas aeroginosa* (Glover *et al.*, 2013). Soft rot causes infected tissues to become soft ramified by the fungal mycelia and turn burn. Fungi associated with soft rot include *Mucor* spp,

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Rhizoctonia spp, *Rhizopus* spp and *Penicillium* spp. Microbial deterioration of stored products has been known to reduce the eating quality and market value of yam (Amusa *et al.,* 2003). Rot diseases lead to total loss of tuber carbohydrate by transforming it into inedible coloured mass.

Losses due to rot attack affect availability of food security and revenue of farmers. Research has shown that the process of rotting usually starts at the field and matures during storage. Shukla *et al.* (2012) noted that the high moisture content on yam causes a lot of deterioration by fungal pathogen in storage.

Yam shows nutritional superiority over the other tropical root and tuber crops. However, it is believed that the supply of yam tuber is lower than its high demand, a problem currently facing the country (Okigbo and Ikediegwu, 2000; Okigbo and Einoghere, 2004; Okigbo and Ogbonnaya, 2006). The objective of this study is to contribute information on storage rots of yam by determining the fungi causing spoilage of yam tubers and assessing their pathogenicity.

Materials and Method

Collection of Yam Tubers

Nine deteriorated yam tubers, three each of *Dioscorea rotundata*, *Dioscorea cayenensis* and *Dioscorea alata* showing varying degrees of rots were purchased from Ariaria Market Aba, Abia State, Nigeria. The rotten yam tubers were packaged in sterile polyethylene bags and taken to be laboratory for isolation and identification of fungi. Nine healthy yam tubers (three each of *Dioscorea rotundata*, *Dioscorea cayenensis* and *Dioscorea alata*) were also purchased for pathogenicity test.

Sterilization of Materials

All glass wares used in this study were washed with detergent and 5% sodium hypochlorite (commercial bleach) solution, rinsed with clean water and sterilized in a dry, ventilated oven at 160°C for 1 hour. All media were sterilized in autoclave at a temperature of 121^{o}C and 15 psi for 15 minutes. The scalpel, cork borer and inoculating loop were sterilized by dipping them into 70% ethanol and passing them over a Bunsen burner flame until red hot.

Preparation of Culture Medium

Throughout the study, the assayed culture medium employed was potato Dextrose Agar (PDA). The preparation of potato Dextrose Agar (PDA) was done according to the manufacturer recipe. About 39 g of the agar was dissolved and homogenized in one-liter distilled water and autoclaved at 121°C and 15 psi for 15 minutes for complete dissolution and homogeneity. Thereafter, it was allowed to cool to temperature of 45°C. Streptomycin was added to the sterile cooled PDA to inhibit bacterial growth. Approximately, 15 ml of the cooled PDA was poured into each sterilized Petri dish and allowed to solidify.

Isolation of Fungal Species from Rotten Yam Tubers

A small section of each yam tissue showing advancing margin of rot was-cut using sterilized scalpel. Appropriate 1g of the rotten yam tissue was weighed; surface sterilized in 70% ethanol for one minute, rinsed and allowed to dry. The rotten yam tissue was ground using mortar and pestle into a paste. A ten-fold serial dilution was made and 0.2 ml aliquot of tubes 10^{-3}, 10^{-4} and 10^{-5} were introduced into the prepared culture media using spread plate method. The plates were incubated at temperature of 25^{o}C for five days. After five days incubation, the frequency of each fungal growth from different yam species was recorded. Fungal colonies that developed from the plated yam tissue were sub-cultured onto fresh PDA plates to obtain pure cultures of single species. From these pure cultures, inoculum of the different fungal species isolated was obtained for the pathogenicity tests.

Identification of Fungal Isolates

Characteristics of fungal isolates from rotten yam tuber such as pigment production, spore or conidia-producing structures and spore shapes were documented. The characteristics were observed from fungal tissues grown on PDA for one week, depending on the fungal species. Spore and mycelium characteristics were studied using the compound microscope by careful preparation of slides, staining with lacto-phenol cotton blue. These characteristics were used in identifying the fungal organisms **(**Oyeleke and Manga, 2008).

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Pathogenicity Test

Nine healthy yam tubers (three for each species) were neatly washed using clean tap water to get rid of dirt. 5 % sodium hypochlorite solution was used to further surface-sterile the tubers for up to 2 minutes before the tubers were rinsed in four successive changes of sterile water to remove chemicals (Gwa *et al*., 2018). A five-day old culture of the fungal isolates identified in section 3.7 was used as inoculums for pathogenicity tests. A sterile five-millimeter diameter cork borer was used to remove discs (1 cm thick) from the "head" and "tail" regions of each yam tuber (Okigbo and Ikediugwu, 2000). Another five-millimeter sterile cork borer was used to cut plugs of mycelia discs from the five-day old cultures of the fungal isolates. These fungal plugs were put in the holes created in the yam tubers. A portion of the tuber flesh removed earlier was cut off to give way for inoculum size and the remaining tuber flesh was used to plug the remaining parts of the hole made in the yam tubers. Melted candle wax from a burning candle was used to seal the edges of the replaced yam discs to prevent any external influence on the positioned inoculum. Controls of each species were set up in which the sterile cork borer was used to remove five-millimeter diameter tuber tissue. The disc was used to plug the hole without placing any fungal organisms in the hole, and its edges sealed with melted wax. The yam tubers were kept in the laboratory at room temperature for 10 days. The yams were assessed for rot development by cutting through the point of inoculation where rots developed. A transparent ruler was used to measure the depth of rots and recorded in millimeter (mm).

Results

The result of the macroscopic and microscopic characteristics of the fungi isolated from yam tuber rots are presented in Table 1. Six fungi isolated were identified as *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Penicillium chrysogenum*, *Rhizopus stolonifer* and *Fusarium oxysporum*.

The percentage frequency of isolated fungal pathogens from different yam species indicated isolated pathogens occurred more in *D. alata* (water yam), followed by *D. rotundata* (white yam) and *D. cayenensis* (yellow yam) ass presented in Table 2.

Fungi with the highest percentage frequency of occurrence were *A. fumigatus* from *D. alata* (57.1%), *A. flavus* from *D. rotundata* (50%), *A. niger* and *F. oxysporum* from *D. alata* (45.5%), *A. fumigatus* from *D. rotundata* (42.9%), while the least were *A. flavus* from *D. cayenensis* (8.3%), *A. niger* and *F. oxysporum* from *D. cayenensis* (18.2%).

Result of the pathogenicity tests conducted is presented in Table 3. Pathogenicity at the head and tail regions of the healthy yam species ranged from (7 mm – 16 mm)**.** The result revealed that all the isolates proved more potent at the head region than the tail region except for *P. chrysogenum which was more* potent at the tail region. *in D. cayenensis.* The results shows that the organisms isolated were responsible for the rot (Table 3).

Table 1: Macroscopic and Microscopic Characteristics of Fungal Species Isolated from Yam Tuber Rots

Macroscopy	Microscopy	Isolate
Dark brown to black colonies.	Smooth walled and colourless conidiophores and spores.	*Aspergillus niger*
Dark-green to grey-green colonies with clear white zone.	Radiating, conidial heads and rough, non-septate conidiophores.	*Aspergillus flavus*
Grey colonies with smooth surface.	Small columnousglobuse and smooth conidia.	*Aspergillus fumigates*
Blue to blue-green colonies.	Subglouse to elliptical, smooth-walled conidia arranged in long irregular chains. Asymmetrical with diverging branching penicillia.	*Penicillium chrysogenum*
Whitish cottony and black colonies.	Erect and unbranched sporangiospores with the sporangium containing sporoangiospores.	*Rhizopus stolonifer*
White to dark violet pigment.	Elliptical shaped microconidia and non-septate.	*Fusarium oxysporum*

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Table 2: Percentage Frequencies of Isolated Fungal Pathogens from Different Yam Species

Fungal Isolate	*D. rotundata*	*D. cayenensis*	*D. alata*
Aspergillus niger	36.4	18.2	45.4
Aspergillus flavus	50.0	8.3	41.7
Aspergillus fumigates	42.9	-	57.1
Penicillium chrysogenum	33.3	25.0	4.7
Rhizopus stolonifer	37.5	25.0	37.5
Fusarium oxysporum	36.4	18.2	45.4

Key: *Dioscorea rotundata* (White yam); *Dioscorea cayenensis* (Yellow yam); *Dioscorea alata* (Water yam)

Table 3: Pathogenicity of Healthy Yam Species showing rot Development (mm) at Head and Tail Regions after 10 Days of Incubation

Fungal Isolate	*D. rotundata*		*D. cayenensis*		*D. alata*	
	Head	Tail	Head	Tail	Head	Tail
Aspergillus niger	12	09	08	07	14	11
Aspergillus flavus	13	10	09	07	16	14
Aspergillus fumigatus	10	08	10	08	15	13
Penicillium chrysogenum	12	11	11	12	13	10
Rhizopus stolonifer	12	12	10	09	14	13
Fusarium oxysporum	12	09	11	09	13	10

Key: *Dioscorea rotundata* (White yam); *Dioscorea cayenensis* (Yellow yam); *Dioscorea alata* (Water yam)

Discussion

Pathogenic fungi associated with the rot of *Discorea rotundala* (white yam), *D. cayenensis* (yellow yam) and *D. alata* (water yam) in the present study were *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, P. chrysogenum, R. stolonifer* and *F. oxysporum*. Reports have shown that these fungi constitute the major threat to post harvest deterioration of yam and other crops in different parts of Nigeria (Ogunleye and Ayansola, 2014; Gwa and Nwankiti, 2018). Each type of rot is characteristics of causal organisms. Fungi with consistent higher frequency of occurrence in all yam species include *R. stolonifer, P. chrysogenum, A. flavus, A. niger*, and *F. oxysporum*. This finding agrees with results obtained by Ogunleye and Ayansola (2014); Gwa *et al*. (2018) who reported these fungi as major cause of rot in yam tuber in different parts of Nigeria.

Pathogenicity test showed that all the isolates induced rot at both regions of the different yam species 10 days after inoculation of the tubers with the test pathogens. Tubers that were not inoculated with rot causing fungi did not produce symptoms of rot. *R. stolonifer, P. chrysogenum* and *A. flavus* are the most virulent fungi causing rot in the healthy yam species of *D. rotundata, D. cayenensis* and *D. alata* respectively both at the head and tail regions compared with the less virulent *A. fumigatus, A. niger* and *F. oxysporum* on the three yam species respectively. This study suggests that these fungi pathogens could be the leading cause of post-harvest decay of yam tubers in various part of Nigeria. The results have demonstrated that healthy tubers of yam were more susceptible to rot fungi than the tail regions of the tubers**.** This study is in consonance with results of Awuah and Akrasi (2007) who studied the suppression of yam tuber rot caused by *A. niger* by yam *rhizobacterium* and reported that the rot development was higher at the head region compared with the tail region when inoculated with the fungus.

This study is not in agreement with the work of Taiga (2011) who studied the differential rate of dry rot in white yam along the tuber length caused by rot pathogens and found out that the depth of rot caused by *A. niger* and *R. stolonifer* were higher in tail regions and least in the head regions of yam tuber. *Rhizopus stolonifer* proved most potent on *Dioscorea rotundata* at head and tail region (14mm and 12mm) respectively, while the least virulent was *Aspergillus fumigatus* (10mm and 8mm). *Penicillium chrysogenum* proved most potent on *D. cayenensis* (11mm and 12mm), while the least virulent were *Aspergillus niger* (8 and 7 mm).

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Aspergillus flavus was most potent on *Dioscorea alata* (16mm and 14mm), while the least virulent were *Penicillium chrysogenum* and *Fusarium oxysporum* (13mm and 10mm).

In conclusion, this study revealed that rots of *D. rotundata*, *D. cayenensis* and *D. alata* are caused by different fungal pathogens in storage that occurred in different frequency. The study has demonstrated that these fungal isolates have pathogenic potency and can induce rot at head and tail regions of yams after inoculation. Initiation of rots occurs through injury therefore injuries should be minimized by reducing the physical damage of tubers that creates opening for attacks of pathogens.

References

Amusa, N. A., Adegbite, A. A., Muhammed, S. & Baiyewu, R. A. (2003). Yam diseases and their management in Nigeria. *African Journal of Biotechnology, 2(12),* 497-502.

Anjorin, T.S., Nwokocha, O.V. & Sanni, A.D. (2014). Morphological Characteristics and Incidence of Diseases on White Yam (*Dioscorea rotundata* L. Poir) Tubers in Abuja, Nigeria. *Nature and Science, 12,* 58-65.http://www.sciencepub.net/nature.

Awuah. R. T. & Akrasi, K. O. (2007). Suppression of tuber rots of yam caused by *Aspergillus niger* with a yam *Rhizobacterium*. *Afr. Crop Sci. Conference Proceedings, 8,* 875-879.

Bhandari, M. R., Kasai, T. & Kawabata, J. (2003). Nutritional evaluation of wild yam (*Dioscorea* spp) tubers of Nepal. *Food Chemistry, 82(4),* 619-623.

Ezeibekwe, I. O., Opara, M. I. & Mbagwu. F. N. 2009. Antifungal effect of Aloe vera gel on fungal organisms associated with yam (*Dioscorea rotundata*, Poir) rot. *J. Mol. Genetics, 1,* 11–17.

FAO (2013). FAOSTAT database (online). Available at: http://bit.ly NmQzZf. (Assessed: 10 April 2014).

Glover, A.M., Quansah, J. & Peget, F.M. (2013). Performance and Acceptability of Legume-Fortified Yam Flours. *Food Science and Quality Management, 17,* 14-18.

Gwa, V. 1., Nwankiti, A. O. & Ekefan. E. J. (2018). Antifungal effect of live aqueous plant extracts on mycelial growth of *Penicillium expansum* isolated from rotted yam tubers in storage. *Acta. Scientific Agric*., *2,* 65-70.

Gwa, V. I. & Nwankiti, A. O. (2018). *In vitro* and *In vivo* antimicrobial potency of selected plant extracts in the control of postharvest rot-causing pathogens of yam tubers in storage. *Global J. Pest. Dis. Crop. Protection, 6,* 276-287.

Ogunleye, A. G. (2005). *Macroeconomics: An introductory test*. Oke Ado, Ibadan, Nigeria: Emmaon Educational Publishers, pp. 1-22.Ogunleye, A. O. & Ayansola, O. T. (2014). Studies of some isolated rot-causing mycoflora of yams (*Dioscorea* spp). *Amer. J. Microb. and Biot., 1,* 9-20.

Okigbo, R. N. & Emoghene, A. O. (2004). Antifungal activity of leaf extracts of some plant species on *Mycosphararella fijiensis* Morelet, the causal organism of black sigatoka disease in banana (*Musa acuminata*). *KMITL Sci. J., 4(1),* 20-31.

Okigbo, R. N. & Ikediugwu, F. E. (2000). Studies on biological control of postharvest rot of yam with *Trichoderina viridae*. *J. Phytopathol., 148,* 351-355.

Okigbo, R. N. & Ikediugwu, F. E. (2002). Evaluation of water losses in different regions of yam (*Dioscorea* spp) tuber in storage. *Nig. J. Exp. Appl. Bio., 3,* 320.

Okigbo, R. N. & Ogbonnaya, U. O. (2006). Antifungal effects of two tropical plant extracts (*Ocimum gratissimum* and *Aframonium melegueta*) on postharvest yam rot. *African J. Biotechnology, 5(9),* 727-731.

Oyeleke, A. & Manga, S .B. (2008). *Essentials of laboratory Practice, 3rd edition, Tobest Publishers,* Minna, Niger State, Nigeria, Pp.12-29.

Shukun, A. M., Yadav, R. S., Shashi, S. K. & Dikshit, A. (2012). Use of plant metabolites as an effective source for the management of postharvest fungal pest: A review. *Int. J. Curr. Discoveries Innov., 4(1),* 33-45.

Taiga, A. (2011). Comparative studies of the efficacy of some selected fungicidal aqueous plant extracts on yam tuber dry rot disease. *Ann. Biol. Res*., *2,* 332-336.

Taiga, A. (2012). Differential Rate of Dry Rot in *Dioscorea rotundata* (White Yam) along the Tuber Length Due to Rot Causing Fungi. *Advances in Microbiology*, 2, 452-455.http://dx.doi.org/10. 4236/aim. 2012.24058.

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