Identification of Fungal Species in Rotten Yam Tubers Sold in Makurdi Metropolis

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***Abstract*- This study focused on the identification of fungi species in fresh and rotten yam tubers by inoculating the samples on Potato Dextrose Agar and fungi staining. 16 rotten and 16 fresh yam samples were collected from four different locations, and four (4) different fungi species were isolated and identified. These include *Aspergillusniger, Botryodiplodiatheobromae, Penicilliumspp,* and Yeast. With *Aspergillusspp* having the highest percentage occurrence in fresh (50%) and rotten yam samples (81.25%) followed by Botryodiplodiatheobromae, 62.5% and 25% in rotten and fresh yams respectively. *Penicilliumspp* has 43.7% and 18.75 in rotten and fresh yam samples, with Yeast showing the least percentage occurrence with 62.5% in rotten and 6.25% in fresh yam samples. The presence of these organisms in yam could lead to serious health matters when consumed, therefore should be prevented by proper storage under minimum temperature.**

***Keywords*- Yam, fungi, *Aspergillusniger, Botryodiplodiatheobromae, Penicilliumspp*, Yeast**

I. INTRODUCTION

Yam (*Dioscoreaspp)* belongs to the family *Dioscecoreae.* Itis one of the most important staple foods in the world especially some parts of the tropics and subtropics [1]. Yams are root crops grown for their edible tubers. They provide the staple carbohydrate food in many parts of West Africa; Nigeria alone produces 21,814 million tons of yams per year, making it the world largest yam producer [2], which accounts for over 60% of the world's yam production. In 2005, the Food and Agricultural Organization [3], reported that Nigeria produced about 66.6%(26.6 million metric tons) of total yam production of which Ghana produces only 9.8% (3.9 million metric tons)every year [4] (Tetteh *and* Saakwa,1994). The most cultivated varieties in Nigeria are the *D. Rotundata* (white yam), *D. cayenesis* (yellow yam) and *D. alata* (water yam) [5].The major yam producing states in Nigeria are Benue, Taraba, Adamawa, Nassarawa, Ekiti, Kwara, Kaduna, Ogun, Oyo, Delta, Plateau, Edo, Cross River, Imo, Osun and Ondo states ([6],[7]).

White yam *(Dioscorearotundata*) is the most preferred and cultivated variety found in Africa. White yam (*Dioscorearotundata*) is much preferred to other yam varieties, it constitutes about 80% of the total yam produced in Nigeria [4]. Demand for yam has always been more than the supply. Nutritionally, yams are mainly carbohydrate food. It is one of the most important dietary sources of energy produced in the tropics [8].It contain certain proportion of protein for man when consumed in large quantities [9].Yam has many important cultural values attached to it, especially during weddings and other social and religious ceremonies. In many farming communities in Nigeria and other West African countries, the size of yam enterprise that one has is a reflection of one's social status. Due to the importance attached to yam, many of these communities celebrate yam festival annually [10].Yam can be cooked by boiling and mashing, roasting and frying are most widely used. Yam can also be processed into various staple intermediate and end products in Nigeria [11], which are used for direct consumption by animals, used as the basic ingredient for snacks or made into flower and can be used for making instant puree [11].

The problems associated with yam start from the field. During the period of harvest, most of the tubers are injured or damaged, there by predisposing them to microbial attack which can cause rot during storage [12]. Yam production is constrained by many problems ranging from high cost of production, attack by fungi, nematodes and pests either as sessile or biofilms. These constraints are responsible for field suppression and tuber quality deterioration in storage [5]. The magnitude of these problems has made people express fears that yam production in Nigeria may decline substantially in the nearest future [8].

Lack of good storage and processing facilities cause a lot of wastage of agricultural produce such as tubers, roots, pulses, fruits and vegetable crops. Storage is one of the critical problems limiting yam production [13]. Rot is the major factor limiting the post – harvest life of yams and this result high loss of product. It is estimated that average of 25- 50% of yam tubers produced in Nigeria are lost to pests and diseases [3]. Losses due to post- harvest rot significantly affect farmers' and traders' income, food security and seed yams stored for planting.

II. MATERIALS AND METHODS

Study area

Makurdi Town area of Benue State lies within the middle belt zone of Nigeria, its bearing is 300Km South-East of Abuja and 887Km North-East of Lagos[14]. The area lies between the hot humid zone with little temperature variation throughout the year and experiences two major seasons between November and March, then April and October.

Makurdi has an annual temperature of about 31.5C with relative humidity of 65 and 95%, rainfall varies between 100 and 250cm [14]. Survey was done during dry season to be able to collect both fresh and rotten yam samples.

Sample Collection

Sample of yams with evidence of rot were collected in four different markets (Wadata, High level, Wurukum and North bank). Together with fresh yams which serve as the control. Total of sixteen samples was collected from different sellers in a sterile polythene bags. The experiment was carried out in Biological science laboratory of the University of Agriculture Makurdi. The experiment was carried out from the period of October-December 2015.

Media Preparation

The medium used was potato dextrose Agar (PDA) prepared according to manufacturer's instructions.39.6gof powdered PDA was dissolved in 1000ml of distilled water and sterilized by autoclaving at 121^{o}c at for 15mins and allowed to cool before pouring carefully into sterile Petri dishes. Two drops of streptomycin was added to the solution to inhibit bacterial growth. Petri dishes that contained the medium were incubated for 24hr at room temperature (28^{o}C) to check for sterility before use.

Isolation of fungal organisms

Rotted yam tubers were sterilized with 70% ethanol and cut open with a sterile knife. Small portion of each of the infected yam tissues were picked from the point of advancement of rot with a flamed scalpel and inoculated on a solidified PDA medium using direct inoculation method. Plates were then incubated at 28^{0}C at room temperature. After 24hrs, plates were observed for fungi growth (duration of fungi growth 24-72hrs).Sub-culturing was carried out in order to obtain pure culture

Macroscopic and Microscopic Examination of fungi

Macroscopic examination of fungi was based on cultural characteristics which include the structure of hyphae wall and spores, size and Color. Microscopic examination procedure was used. A drop of Lacto phenol blue and Methylene blue was placed on a clean microscopic slide. Using an inoculating needle, I picked few piece of agar that contained fungi hyphae transferred on the drop of the stain, cover slip was placed on the slide and examined microscopically

*Data analysis***:** TheT- test analysis was used to analyze the results, withsimple percentage to determine the level of occurrence.

III. RESULTS AND DISCUSSION

Table 1 shows the number of fungal positive plates from different rotten yam samples gotten from different markets within the study area. With Wadata market yam samples having the highest number of plates (14), Wurukum market with 10, High level market with 8 and same with North bank market.Table 2 shows the number of positive plates of fungi growth gotten from fresh yam samples gotten from the different markets within the study area. 4 positive plates from Wadata, Wurukum and High level markets and 5 positive plates for North bank market. The cultural characteristics of the different fugal isolates gotten from the samples are represented in Table 3 and their various microscopic examination results.

Table 1: Fungal Isolates from Rotten Yam tubers

Locations		***Aspergillus sp.***	***Penicillium sp.***	***Yeast***	***Botryploida-theobromae***
High level Market	A.	+	–	+	+
	B.	–	+	–	+
	C.	-	-	–	-
	D.	+	–	+	+
Total		2	1	2	3
Wadata Market	A.	+	+	+	-
	B.	+	–	+	+
	C.	+	+	+	+
	D.	+	+	+	+
Total		4	3	4	3
North Bank Market	A.	+	-	+	–
	B.	+	+	–	+
	C.	+	–	–	-
	D.	+	–	–	+
Total		4	1	1	2
Wurukum Market	A.	+	-	+	+
	B.	+	+	+	+
	C.	+	–	–	-
	D.	–	+	+	–
Total		3	2	3	2

Keys: +positive plate - Negative plate

Table 2: Fungal Isolates from fresh Yam tubers

Locations		***Aspergillus sp.***	***Penicillium sp.***	***Yeast***	***Botryploidathe-obromae***
High level Market	A.	+	–	–	–
	B.	–	–	–	–
	C.	+	+	–	+
	D.	–	–	–	–
Total		2	1	0	1
Wadata Market	A.	+	–	+	+
	B.	–	–	–	–
	C.	–	–	–	–
	D.	+	–	–	–
Total		2	0	1	1
North Bank Market	A.	+	+	–	–
	B.	+	–	–	–
	C.	+	–	–	+
	D.	–	–	–	–
Total		3	1	0	1
Wurukum Market	A.	+	–	+	–
	B.	–	+	–	–
	C.	–	–	–	+
	D.	–	–	–	–
Total		1	1	1	1

Keys: + positive plates and – negative plates

Table 3: Cultural Characteristics of Fungal Isolates

Macroscopic examination	Microscopic examination	Organism present
Moist, slimy whitish colonies	Ova, spherical and clustered shape	*Yeast*
Colony colour ranged from greenish-blue colouration	Green spores with whitish hyphae	*Penicillium sp.*
Colony colour range from greyish to dark green colouration	Dark green conidia	*Aspergillus sp.*
Colony colour range from white to black	Septate hyphae	*Botryoplodiatheo bromae*
Black dark brown pigment cottony large mass with whitish hyphae	Conidiosporeswith hyphae & mycelium	*Aspergillusniger*
Dirty green pigment colour on plate	Its spores are produce in chains, conidiophores are with a swollen head or vesicle bearing bottles	*Aspergillus sp.*
Colony colour ranged from white to black	Septate with hyphae	*Botryoplodiatheo bromae*
Colony colour was parrot green to deep green.	Light green conidia and septate hyphae	*Aspergillus sp.*
Colony colour ranged from grayish to dark blue colouration	Green spores with whitish hyphae	*Penicillium sp.*

The research was able to identify four (4) Fungi species in both rotten and fresh yams associated with post-harvest rot of yam. They include *Aspergillusspp*, *Penicillium* species, *Botryoplodiatheobromae*, and Yeast. Two of these fungi species, *Aspergillus*spp and *Penicillium* have been previously linked to post harvest yam rot in other parts of the country ([13], [15]).According to Ogundana *et al.* (1970)[16] rotting in storage probably would have been initiated by microorganism in the soil while the crop is in the field and subsequently manifest in storage condition. He reported that most cases, pathogens gain entry into yams through natural opening and wound that occur mechanically during harvesting and transient from field to storage ban or market.

Umogbai and Satimehin (2004)[17] agreed that fungi infection of yam could be attributed to its storage in underground and mud structures and this was also agreed in the study conducted by Umogbai, (2013)[18] on storage of yam tubers. This was also discovered in this present study. Other studies have shown that fungi rot is the greatest cause of tuber loss during post-harvest rot of yam [19]. *Botryodiplodiatheobromae* and *Penicillium*spp were identified in the study carried out by ([19], [20] [21]), but not Yeast as discovered in this present study, this could be as a result of variation in temperature between the study areas. *Aspergillusniger* with 50% frequency of occurrence was the most commonly occurring, medically important fungi [7]. It also has 50% and 81.25% frequency of occurence for fresh and rotten yam samples in present study. In severe cases of exposure to *Aspergillusniger*, asthma and alveolitis have been known to occur. It is also a major cause of aspergillosis and because of its high percentage of occurrence in that study, people in the Makurdi locality may be at risk of infection with increase in exposure to the spores of *A. Niger* from rotted yams.

Aspergillusniger is one of the major causes of yam tuber rots either in the field or during storage and has led to a high economic loss and reduction in market value of field produce and low income for farmers due to their biodeteriorative abilities. *A. niger* also has the ability to produce certain mycotoxins which are hepatocarcinogenic or nephrogenic in nature [22]. Mycotoxins are hazardous to human and animal health. They are toxic metabolites produced by some group of fungi which can cause serious and sometimes fatal diseases when contaminated foodstuffs containing them are ingested. This poses a great danger for individuals who consume contaminated yam products such as yam flour, yam chips and other unhygienically processed yam products. Ochratoxin A is also produced by *A. niger*[23]. *Penicilliumspp* which was isolated during this study is known to produce the antibiotic penicillin. It is less likely incriminated in yam tuber rots like other species such as the *P. oxalicum*(a major causative agent of yam tuber rots). This means when present in food substrates *(P. chrysogenum)*, may inhibit colonization by bacteria, thus reducing microbial spoilage of food [7].

IV. CONCLUSION

This work was able to analyze the fungal growth in fresh and rotten yam samples and discovered that fresh yams samples do have a measure of fungal contamination but not as much as that of rotten yams. The organisms isolated are pathogenic to consumers; their presence reduces farmers' income, and can lead to wastage and scarcity. Proper storage at a low temperature could be a major factor to limit fungal spoilage of yams as discovered in this study.

REFERENCES

[1]. Okigbo, R.N. and Ogbonnaya,U.O.(2006). Antifungal effect of two tropical plant leaf extract Ocimumgratissimum and Aframomummelegueta on post harvest yam (Dioscoreaspp) rot, Afr. J. Biotech. 5 (9): 727-731

[2]. Okigbo, R. N. (2005). Biological control of post harvest fungal rot of yam (Dioscorea spp.) with Bacillus subtils. Mycopathologia 159(2): 307 – 314

[3]. Food and Agricultural Organization, FAO (2005): food and agricultural organization, Rome, 2005 pp23

[4]. Tetteh, J.P. and Saakwa, C. (1994). Prospects and constraints of yam production in Ghana Tyum press. Union of Food Science and Technology, Raleigh, 3:1-12.

[5]. Amusa, N. (1995): Yam diseases and their management in Nigeria. African Journal of Biotechnology, 2(12), 497-502

[6]. Akanji, B.O., Akpokodje, G. and Ogundele, O.O. (2003). Labour Use Pattern on Farms. NISER, Annual Survey of Crop Production Condition in Nigeria, 3: 34-46.

[7]. Ajayi, A.O. and Olorundare, S.D. (2014). Bacteria and Fungi Species Associated with Yam (Dioscorearotundata) Rot at Akanugba—Akoko, OndoStaste, Nigeria. Applied Science Research Journal, 2, 12-28.

[8]. Ekefan, N. (1999). Diseases of yam tubers, International Journal of Tropical Plant Diseases.3: 19-23

[9]. Odurukwe, S.W.(1980). Yam maize intercropping investigation in Nigeria.Tropical Agriculture. Trindad, 63, 17-21
[10]. Awoniyi, Olubukola, Omonona, Bolarin Titus (2006). Production Efficiency in Yam Based Enterprises in Ekiti State, Nigeria. Central European Agriculture Journal; 7(4):627-636.
[11]. Okaka,J.C.,Anajekwu,B.(1990). Preliminary studies on the production and quality evaluation of dry yam snacks,Trop.Sci.30:67-72
[12]. Taiga, A. (2012) Differential Rate of Dry Rot in Dioscorearotundata (White Yam) along the Tuber Length Due to Rot Causing Fungi. Advances in Microbiology, 2: 452-455.
[13]. Okigbo R.N. (2004). A Review of Biological Control Methods for Postharvest Yams (Dioscorea spp.) in Storage in South Eastern Nigeria. KMTL Science Journal, 4: 207-215
[14]. Eguzo,T. (2000).Climate zone and weather distribution in Nigeria. pp24-30
[15]. Okigbo R. N. and Emeka A. N. (2010). Biological control of rot-inducing fungi of water yam (Dioscoreaalata) with Trichodermaharzianum, Pseudomonas syringae and Pseudomonas Chlororaphis. Journal of Stored Products and Post harvest Research. 1(2): 18-23.
[16]. Ogundana, S.K., Naqui, S.H and Ekundayo, J.A. (1970). Fungi associated with soft rot of yam (Dioscoreaspp) in Nigeria. Trans. Britainia Mycology Society, 54:445-451.
[17]. Umogbai, V. I. and Satimehin, A. A. (2004): development and performance evaluation of modified storage structure for fruits and vegetable. Journal of prospects in science, 7:17-21
[18]. Umogbai, V. I. (2013): design, construction and performance evaluation of an underground storage structure for yam tubers. International journal of scientific and research publication, 3(5): 22-34
[19]. Amusa, N. A., Adegbite, A. A, Muhammed S. and Baiyewu R. A. (2003): Yam diseases and its management in Nigeria. African Journal of Biotechnology, 2 (12), 497-502
[20]. Amusa, N. A. and Baiyewu, A. R. (1999): Storage and market diseases of yam tubers in South-Western Nigeria. Ogun Journal of Agricultural Resources 11:211 – 255
[21]. Okigbo, R. N. (2005). Biological control of post harvest fungal rot of yam (Dioscorea spp.) with Bacillus subtils. Mycopathologia 159(2): 307 – 314
[22]. Ajay, K., Gautum, S.S., Shubhi, A. and Rekha, B. (2011). Diversity, pathogenicity and toxicology of A. niger: An important spoilage fungi. Research journal of Microbiology, 6:270-280.
[23]. Al-Adalall, A. (2009). Production of aflatoxins by A. flavus and A. niger strains isolated from seeds of pulses. Journal of food agriculture environment, 7(2):33