Patho-physical Characteristic Studies of Varieties of *Dioscorea Rotundata* (yam) In Storage.

Abdulkadir, R.[1], *Suberu, H. A.[2], Abubakar, A.[1], Bello, I. M.[1]
[1]Department of Biological Sciences, Federal University of Technology, P. M. B. 65, Minna, Niger State, Nigeria.
[2]Department of Biological Sciences, Federal University, Lokoja. Kogi State, Nigeria.

Abstract

Storage rot at the 'top', 'middle', and 'bottom' portions of four (4) varieties of Dioscorea rotundata ('Giwa', 'Suba', 'Kpako', and 'Dindiyam') cultivated in Niger State, Nigeria was investigated on the bases of tissue hardness and moisture content, to establish their comparative rotting subjectivity. Yam tubers stored in an empty room on a polythene material for 30 days were each divided along the length into three equal portions of 'top', 'middle', and 'bottom'; which were further subdivided into many pieces in search for symptom(s) of infection. The rotting was more than twice as high in the bottom (54.2%) as the middle (22.9%) and the top (22.9%). Isolated fungi from the infections were Aspergillus parasiticus and Fusarium accuminatum. A. parasiticus was most pathogenic on 'Suba' variety (13.69±0.75mm) and least on Giwa (11.09±0.48mm), and it is a more potent pathogen of rotting than F. accuminatum, which showed a rotting range of 2.22±0.32 to 3.49±0.46mm. Bottom portions of the yam varieties were most subjective to pathogenicity. In all the varieties, except Dindiyam, the bottom portion contained significantly (P?0.05) higher moisture than the middle or the top. All portions of the yam varieties had significantly different (P?0.05) hardness; ranging from bottom to top. Pathogenicity of A. parasiticus is probably favoured with the optimal moisture and softness of the bottom portion.

1.0 Introduction

Over 90% of the world annual production of yam (*Dioscorea rotundata*) is obtained from West Africa (Okigbo, 2002). Nigeria is the largest yam producer in the world; with about thirty five (35) metric tonnes (FAO, 2008). The most widely cultivated species in Nigeria are *D. rotundata* (White yam), *D. cayenensis* (Yellow yam), and *D. alata* (Water yam) (Amusa, 1999). Inspite of the demand for yam tubers, Nigeria has always exceeded its supply. However, it has been estimated that an average of over 25% of the yield is lost annually to diseases and pests (Arene, 1987; Ezeh, 1998; FAO, 1998). Infact, Onayemi (1983) puts the percentage annual loss of yam in Nigeria at over 50%, especially in storage; due to microbial effects. The microbial infection probably took place in the field

Corresponding Author: hasuberu@yahoo.com

and increased in storage (Okigbo and Ikediugwu, 2000; Eze and Ugwuoke, 2010).

The major fungal pathogens causing diseases in yams penetrate through wounds caused by insects, nematodes and poor handling before, during and after harvest. Reported fungi include *Aspergillus flavus, A. niger, Botryodiplodia theobromae, Penicillium oxalicum, Trichoderma virdae,* and *Rhizopus nodosus* (Adeniji, 1970; Morse *et al.,* 2000; Okigbo, 2004). *Fusarium* species were also reportedly associated with dry rot in yam tubers in Nigeria (Morse *et al.*, 2000; Okigbo and Emeka, 2010). Also, it has been established that the storage environments; relative humidity and aeration contribute to onset and or rate of spoilage of farm produce (Robinson *et al.,* 1975).

Yam belongs to the genus "Dioscorea" and family "Dioscoreaceae" (Kay, 1987 and Ezeibekwe *et al.*, 2009), with over 600 species, out of which only few are cultivated for food or medicine in the tropics (Amusa, 1999). Nutritionally, yams are mainly carbohydrate food, but contain about 1 – 2% dietary protein (Coursey, 1967; Ekefan *et al.,* 1999). Yams are therefore, able to provide a good proportion of protein requirement of man when consumed in large quantities (Coursey, 1967; Odurukwe, 1980). In Nigeria, yams can be eaten as boiled, pounded yam, yam pottage, fired yam, roasted yam and as yam flour for preparing *amala* (Yoruba) (Ogaraku and Usman, 2008).

Large quantities of yams are harvested annually from Niger State, Nigeria. Storage efforts are local which last only few months. This study will identify the fungi causing rotting of yam in storage and determine the physical factors of the yam tubers that enhance the pathogenicity of the fungi.

2.0 Materials And Methods

2.1 Sample collection

Twenty tubers of clean and uninjured *Dioscorea rotundata* commonly cultivated in Niger State were obtained from different farms in Minna, Niger State. The selected tubers, made up of four (4) varieties, were kept in a well ventilated room for one month (31 days) on top of a clean polythene laid on the floor, after which they were taken to Biological Sciences Laboratory, Federal University of Technology, Minna for microbial (fungal) infections.

2.2 Sample Preparation

The yam tubers were aseptically cut into three equal parts; the top, middle, and the bottom with sterilized sharp knife. The knife was in each instance passed over a Bunsen flame until red hot, cooled by dipping it inside 70% ethanol. Each part was cut into small pieces of approximately 8 × 8mm and examined for spoilage. The spoilt spots of the different regions were counted, recorded and the percentage spoilage was calculated.

2.3 Isolation and Identification of Fungi

Rotted yam pieces were surface sterilized with 70% ethanol and rinsed with sterilized distilled water (Okigbo and Nmeka, 2005). The pieces were further cut into smaller bits (approximately 2mm) with sterilized dissecting knife and inoculated on sterilized Potato Dextrose Agar (PDA) plates. The plates were labelled according to the yam sections (top, middle, and bottom), and incubated on bench top at room temperature (37± 2^0C). Pure isolates of the fungi were obtained through subculturing from the mixed growth colonies (Ezeibekwe *et al.*, 2009). Identification of the fungi was carried out using cultural and morphological features in accordance with Domsch *et al.* (1980); Samson *et al.*(1984) and Rippon(1958).

2.4 Fungal Pathogenicity Study

Ten uninfected tubers of each of the varieties; '*Giwa*,' '*Zuba*,' '*Dindiyam*,'and '*Kpako*.' of *Dioscorea rotundata* were obtained from different farms. The tubers were washed under running tap water and externally disinfected with 95% methylated spirit. The length of the tubers was measured and marked into three equal parts (top, middle and bottom). Five tubers of each variety were set out for inoculation, with either *Aspergillus parasiticus* or *Fusarium accuminatum*. The parts of each tuber were bored with sterile cork borer, and aseptically infected with innoculum of either of the isolated fungi. Points of inoculation were sealed off with petroleum jelly (Okigbo and Nmeka, 2005). The treated tubers were kept in sterilized polyethylene bag, and preserved on laboratory bench (Oyeyipo, 2012). After five days, each tuber was cut through the point of inoculation and the pathogenicity determined as measure of the visible area of discoloration and the extent of spoilage, with the aid of sterilized transparent ruler (mm) along three dimensions from the point of inoculation.

2.5 Measurement of moisture.

Cork borer (5mm size) was used to bore out tissue, after peeling, from the three sections (top, middle, and bottom) of each variety of tubers of *D. rotundata*. The cylindrically bored tissues were cut into five millimetre length, and three such pieces were obtained per section. Each was weighed fresh, and after drying in oven at 70°C. Weighing was done at interval of 24 hours until a constant weight was attained. Average weight difference between fresh and dried tissue was recorded for moisture content (AOAC, 2004; Adegunwa *et al.*, 2011)

2.6 Determination of Hardness (gram).

Approximate 5mm cube shaped yam tissue (three pieces) were obtained from the sections of each tuber, of the varieties of *D. rotundata*. They were put to boil in water for 30mins, and air dried for 10mins. Each was placed on top loading

balance, and depressed with the thumb. The reading on the balance, that is pressure of crushing, was taken as an expression of the hardness of the tissue.

2.7 Data Analysis:

All data collected were subjected to Analysis of variance (ANOVA) according to the procedure for CRD experiment, with the group means compared by Duncan Multiple Range Test (DMRT) using the Statistical Package for Social Science (SPSS) version 16.0.(2007). Average mean and standard error of means (SE) were recorded.

3.0 Results

Percentage infection of the fungi showed that bottom (54.2%) section of the tubers were most prone to rotting; with the percentage of infection more than double that of the middle (22.9%) and the top (22.9%) sections (Table I).

Two species of fungi of the genera; *Aspergillus* and *Fusarium* were isolated and identified from the four varieties of *D. rotundata*, using standard morphological and physiological characteristics under light microscopy (×400) (Table II). The two species of fungi isolated were *Aspergillus parasiticus* and *Fusarium accuminatum*.

3.1 Yam Tuber Tissue Hardness of the Varieties of *D. rotundata*

The hardness of various parts of all the four varieties of *D. rotundata* showed same pattern; with the tops been the hardest followed by the middle while the bottom the least. Statistical analysis showed that there was significant difference in the sections of the varieties with *Dindiyam,* been the hardest; ranged (687.00 ± 3.51)g - (382.00 ± 1.15)g and *Giwa* the least; ranged (346.33 ± 3.18)g – (180.00 ± 2.89)g; top to bottom (Table III).

3.2 Moisture Content of the Varieties of *D. rotundata*

The moisture content of the middle portion of the four varieties of *D. rotundata* was the same statistically: 1.08±0.02g (*Giwa*), 1.13±0.01 (*Kpako*), 1.18±0.11 (*Suba*), and 1.19±0.02 (*Dindiyam*) (Table IV). The bottom portions of *Kpako* (1.25±0.01) and *Suba* (1.25±0.02) are the same statistically, but different from those of *Giwa* (1.12±0.01) and *Dindiyam* (1.10±0.03) which are the same. *Suba* had the highest (p?0.05) moisture content at the *top* portion (1.26±0.02), followed by *Kpako* (1.16±0.02), and *Dindiyam* (0.95±0.03) and *Giwa* (0.92±0.02).

3.3 Rotting Effects of the Pathogens on the Varieties of *D. rotundata.*

Pathogenicity effects of *Aspergillus parasiticus* on varieties of *Dioscorea rotundata* ranged between 9.73±0.55mm and 16.27±0.47mm (Table V), and *Fusarium accuminatum* ranged between 1.40±0.12mm and 5.07±0.68mm. The bottom portions were most affected than the middle or top portions. However, general average rotting effect with *A.*

parasiticus was most pronounced in the 'Suba' variety (13.69±0.75mm), followed in 'Kpako' (13.20±1.56mm), 'Dindyam' (13.04±0.68mm), and 'Giwa' (11.09±0.48mm). There was no significant difference between the varieties with *A. parasiticus* as the pathogen. The average rotting effects of *F. accuminatum* were much less than *A. parasiticus*. 'Kpako' variety was most affected (3.49±0.46mm), followed by 'Suba' at 2.98±0.34mm, 'Giwa' (2.76±0.23mm) and 'Dindiyam' least affected at 2.22±0.32mm (Table 6).

4.0 Discussion

Hycenth (2008) reported in his that *Aspergillus* species and *Fusarium* species are among other fungi that are involved in the storage rotting of tubers of *Dioscorea rotundata*. Okigbo (2002, 2005) had also isolated *Aspergillus* species and *Fusarium* species from rotting yam in storage where 50% fresh matter was lost. The isolation of *Aspergillus parasiticus* and *Fusarium accuminatum* in the present study is in agreement with their findings. The results of pathogenicity study confirm the work of Eze and Ugwuoke (2010). They reported that the pathogens isolated from decaying yam tuber and re-inoculated into two species of stored yam varied significantly in their activities on the stored yams with *A.* niger being the most virulent. Okigbo and Emeka (2010) reported that the rotten pathogenic activities of fungi inoculated in the yam tubers was due to the ability of the pathogen to utilize the nutrient of yam as a substrate for growth and development. Similar to the result of this study Belli *et al.* (2004, 2005) reported that optimum water activity (*aw*) for growth of fungi (*A. carbonarius)* in most cases was 0.98, its growth rate increased with increasing *a*w and maximum growth rate being between 0.95 and 0.99 *a*w. The pathogenic growth of the fungi recorded in this study for each variety of the tubers increased from the top to the bottom region in proportion with increase in water content of the region. The least rot was recorded for *Aspergillus* species (9.73 ± 0.55mm) in *Giwa* with water content of 0.92 ± 0.02 and for *Fusarium* species (1.40 ± 0.12mm) in *Dindiyam* with water content of 0.95 ± 0.03. The low pathogenic activities of these fungi at the top region could be attributed to the low water contents of the part in each variety. This was in agreement with the statement of Carlile and Watkinson (1996). They reported that moisture control is the best and most economical means to control the environment, to prevent mould growth and mycotoxin production. They also stated that moisture requirements of food borne moulds are relatively low; most species grow at a 0.85 *a*w or less which is below the minimum water content recorded in this study for all the varieties in all the regions.

Table I. **Percentage (%) Infection of Different Sections of *D. rotundata***

SECTIONS	PERCENTAGE
TOP	22.9%
MIDDLE	22.9%
BOTTOM	54.2%
TOTAL	100

Table II. **Characteristics of Isolated Fungi**

Isolated Fungi	Colony Colour	Hyphae and conidia Characteristics
Aspergillus parasiticus	Black-mould appearance	Uniseriate conidial heads, conspicuously rough walled conidia and septated hairy hyphae
Fusarium accuminatum	Dirty white mycelium on PDA	Macroconidia, strongly curved, sickle shaped, slender, 5 septate cells.

Table III. **Hardness (g) of Portions of Different Varieties of *D. rotundata***

SAMPLE	TOP	MIDDLE	BOTTOM
KPAKO	480.67 ± 0.67^{a}	338.33 ± 9.28^{a}	194.67 ± 2.40^{a}
SUBA	591.00 ± 0.58^{b}	428.67 ± 7.69^{b}	341.00 ± 0.58^{b}
GIWA	346.33 ± 3.18^{c}	255.00 ± 2.89^{c}	180.00 ± 2.89^{c}
DINDIYAM	687.00 ± 3.51^{d}	481.67 ± 0.88^{d}	382.00 ± 1.15^{d}

**Values are Means ± S.E of triplicate weight of various sections of D. rotundata.. Values followed with the same letters in the same column are not significantly different at ($P<0.05$) according DMRT.*

Table IV. **Moisture Content (g) of Sections of Different Varieties of *D. rotundata***

SAMPLE	TOP	MIDDLE	BOTTOM
KPAKO	1.16 ± 0.02^{b}	1.13 ± 0.01^{a}	1.25 ± 0.01^{a}
SUBA	1.26 ± 0.01^{a}	1.19 ± 0.02^{a}	1.25 ± 0.02^{b}
GIWA	0.92 ± 0.02^{c}	1.08 ± 0.02^{a}	1.12 ± 0.01^{c}
DINDIYAMRIN	0.95 ± 0.03^{c}	1.18 ± 0.11^{a}	1.10 ± 0.03^{c}

**Values are Means ± S.E. of triplicate weight of various sections of D. rotundata. Values followed with the same letters in the same column are not significantly different at ($P>0.05$) according to DMRT.*

Table V. **Pathogenicity(mm) of** *Aspergillus parasitucus* **on Sections of Different Varieties of** ***D. rotundata*** .

SAMPLE	TOP	MIDDLE	BOTTOM	AVERAGE
KPAKO	10.47 ± 2.29^{a}	14.27 ± 2.77^{a}	14.87 ± 3.27^{a}	13.20 ± 1.56^{a}
SUBA	11.67 ± 0.84^{a}	13.13 ± 0.55^{a}	16.27 ± 0.47^{a}	13.69 ± 0.75^{a}
GIWA	9.73 ± 0.55^{a}	10.93 ± 0.47^{a}	12.60 ± 0.40^{a}	11.09 ± 0.48^{a}
DINDIYAM	12.60 ± 0.40^{a}	13.07 ± 1.09^{a}	14.87 ± 0.82^{a}	13.04 ± 0.68^{a}

**Values are Means ± S.E. of triplicate rot length of various sections of D. rotundata.. Values followed with the same letters in the same column are not significantly different at ($P<0.05$) according to DMRT*

Table VI. **Pathogenicity(mm) of** *Fusarium accuminata* **on portions of Different Varieties of** ***D. rotundata***

SAMPLE	TOP	MIDDLE	BOTTOM	AVERAGE
KPAKO	2.27 ± 0.13^{a}	3.13 ± 0.07^{a}	5.07 ± 0.68^{a}	3.49 ± 0.46^{a}
SUBA	2.20 ± 0.20^{a}	2.73 ± 0.47^{ab}	4.00 ± 0.53^{ab}	2.98 ± 0.34^{ab}
GIWA	1.93 ± 0.13^{a}	2.87 ± 0.17^{ab}	3.47 ± 0.07^{ab}	2.76 ± 0.23^{ab}
DINDIYAM	1.40 ± 0.12^{b}	2.00 ± 0.20^{b}	3.27 ± 0.48^{b}	2.22 ± 0.32^{b}

**Values are Means ± S.E. of triplicate rot length of various sections of D. rotundata. Values followed with the same letters in the same column are not significantly different at ($P<0.05$) according to DMRT.*

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