RESEARCH ARTICLE

BIOCHEMICAL ALTERATION OF YAM TUBER TISSUES INCITED BY *Botryodiplodia theobromae* Pat.

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ABSTRACT

Tubers of white yam (*Dioscorea rotundata* Poir) obtained in three markets in Calabar, Cross River State, Nigeria, were found associated with seven microbial pathogens causing dry and wet rot of tissues. The pathogens included; *Aspergillus niger*, *Penicillium expansum, Fusarium oxysporum, Botryodiplodia theobromae, Penicillium sclerotigenum, Fusarium moniliformes, and Rhizopus stolonifer.* Of these *A. niger, P. expansum, F. oxysporium and B. theobromae* were highly pathogenic causing extensive tissue rot compared to the last three. *Botryodiplodia theobromae* effected an appreciable reduction in the amounts of nutrients in the head, middle and tail portions of yam tested within the five-week span of the experiment. One week after inoculation, there was a decline in the carbohydrate content from 34.80mg/100g dry weight in the control samples to 24.60mg, 25.10mg and 23.90mg in the head, middle and tail portions respectively. These values fell to 16.90 and 16.80mg/100g by the fifth week in the middle and tail portions respectively. Protein recorded a slight depreciation after one week of inoculation. This was followed by appreciable increment between week two and four (from 5.25mg/100g to 9.19mg/100g in the tail portion) and then a slight drop one week thereafter. The lipid content of the three portions tested recorded a steady decline throughout the period of the experiment. Amount of oxalate increased steadily in the inoculated tubers within the period of the experiment.

INTRODUCTION

Yam, considered to be among the most primitive monocotyledonous angiospermic plants is a member of the order Liliflorae, family Dioscoreaceae and genus Dioscorea (Burkill, 1985). Yams (*Dioscorea spp*) are annual or perennial climbing plants with edible underground tubers. The tuber is the part of the yam in which the group's (*Dioscorea*) biological originality and agronomic potential are manifested. Yam is cultivated in many parts of the World. More than 90% of the world's yams are currently grown in sub-Saharan Africa, with the remainder grown in West Indies and parts of Asia and South and Central America (Osagie, 1992). Nigeria alone produces about 70% (21,814 million tonnes per year) of the world's total, making it the world's largest producer. Of the 600 yam species known, only six species are cultivated and consumed in Nigeria as food. In addition, of these six, *Dioscorea rotundata* (white yam) commands the highest market value owing to the superior suitability of its tubers to the preferred food use in West Africa.

The tubers have organoleptic qualities that make them the preferred carbohydrate food, hence contributing to 350 dietary calories per person per day for millions of people in the major producing countries. Yam is eaten directly with sauce after boiling, fried in oil or roasted. It is cooked into pottage with added protein sources and oils. In the coastal West Africa, yam is boiled pounded and made into a thick dough popularly called pounded yam, and eaten with soup.

In the Yoruba land in Nigeria, a reconstituted form called 'amala' (another popular dough) is the people's favorite (Coursey, 1983). A few yam-based commercial products developed from yam flakes and flour are produced in Nigeria, Cote d' Ivoire and Ghana for export and sale in the urban centre. The flakes are produced from fresh tubers by peeling, slicing, sulphate bathing, cooking, mashing, drying and flaking followed by packaging. In the animal feed industry, yams play a prominent role. In Nigeria, yam peels are commonly used to feed pigs on family-run farms. Industrially, yam flour mixed with rice is used in making pastries (Oyenuga, 1968).

Yam production, storage and usage have been greatly mitigated by pest and microbial attacks. About 50% of the yams produced are reported subject to losses (Arinze,

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2005). A myriad of microorganisms attack yams both in the field and in storage. These include *Fusarium spp., Rhizoctonia solani, Aspergillus niger, Rhizopus spp., Botryodiplodia theobromae* etc. Losses are eminent through reduction in the quantity and quality of the basic nutrient (carbohydrate, lipids and proteins) components. Many authors (Ekundayo and Okigbo, 1991; Prasad *et al.*, 1989) have given reports highlighting such losses in many important food crops. This study is therefore aimed at highlighting the impact of microbial attack on the nutritional content of yam.

MATERIALS AND METHODS

Sources of healthy and infected yam

Healthy and infected white yam (*Dioscorea rotundata*) tubers used in this study were sourced from three yam markets (Akim, Marian and Watt) in Calabar, Cross River State, Nigeria. Sampling was done between the months of March and May when microbial attacks are usually abundant. The infected samples were collected in sterile cellophane bags and taken to the laboratory for isolation.

Preparation of culture medium

The solid medium that was used in the experiments was potato dextrose agar (PDA). Thirty– nine grammes of PDA was added to one litre of distilled water and amended with 1000mg of chloramphenicol, thoroughly mixed and dispensed into 1000 ml conical flasks and then plugged with cotton wool and capped with aluminum foil before sterilization at 121° C (105 kg/ cm^2) for 15 minutes in an autoclave. About 20 ml of the sterilized medium were dispensed into sterile 9cm diameter disposable Petri dishes.

Isolation and identification of isolates

Yam tissues about 5mm in diameter were removed from infected white yam tubers following surface sterilization with 70 % ethanol for 10 s, blotted dry with sterile paper towel, and plated onto chloramphenicol-amended potato dextrose agar (PDA). After three days of incubation at 28^0 C, microbial growth was assessed by microscopy. Seven isolates were positively identified based on morphological characteristics described in the 1987 illustrated genera of fungi by Barnett and Hunter and with literature on identification of pathogenic fungi by Rossman *et al*, (1997).

Pathogenicity test

To confirm the pathogenicity of isolates from white yam, axenic cultures of each of the isolates were used to inoculate three white yam minisetts. A 5 mm diameter mycelial agar plugs of a 4-day-old culture of each isolate was inserted into the healthy white yam tuber, sealed with petroleum jelly and incubated at 28^0 C. After symptoms developed, 15 to 21 days post inoculation (dpi), tissue at the margin of the healthy and diseased part was excised, surfaced-sterilized, and plated onto PDA for incubation at 28^0 C for four days. Based on the morphological characteristics (as stated above), the organisms were identified and confirmation of pathogenicity on white yam was completed.

Analysis of healthy and infected yams for nutrients

The yam tissues were analyzed for total carbohydrate, crude protein, lipids and an anti-nutritional component- oxalate. Anthrone reagent method was adopted to determine the total carbohydrate. Crude protein was determined using Kjedhal method. The ether extraction method was used in the determination of the amount of lipid in healthy and infected tubers. The anti-nutritional content- oxalates in both healthy and diseased tissue were also determined. All the determinations were carried out according to the procedures of A.O.A.C, (1995).

RESULTS AND DISCUSSION

Isolation and identification of isolates

The seven isolates identified and their frequencies of isolation are as shown in Table 1. Of these, *Aspergillus niger* was not isolated from yams in Watt market and *Fusarium solani* was not found associated with yams in Marian market. Akim market indicated the presence of all the isolates identified.

Pathogenicity test

Results of pathogenicity test showed that all the isolates were pathogenic on the yam (artificially inoculated) (Table 2). Four (*A. niger, P. expansum, F. solani* and *B. theobromae*) of the seven fungi isolated caused the most extensive rot (> 50 mm diameter) under experimental conditions. The remaining three were mildly pathogenic on the yams. Re-isolation from the inoculated yam tubers produced cultures identical to the original isolates. With this, Koch's postulate was completed. Based on the frequency of isolation and level of pathogenicity, *B. theobromae* was chosen and used throughout the course of this experiment as the test pathogen.

Table 1. Frequency of occurrence (isolation) of some fungi ofyam tuber rot in three markets in Calabar

PATHOGENS (FUNGI)	% FREQUENCY OF ISOLATION		
	*MARIAN	*AKIM	*WATT
Aspergillus niger	13	31	0
Penicillium expansum	41	57	10
Penicillium sclerotigenum	3	23	5
Fusarium solani	0	3	25
Botryodiplodia theobromae	42	81	16
Fusarium moniliforme	21	47	10
Rhizopus stolonifer	70	87	42

● - market

Effect of the test pathogen on the nutritional content of white yam

In the fresh and uninoculated yam tuber, about 34.80 mg of carbohydrate per 100 g of edible portion of yam tuber was recorded. However, after a week of infection, values of 24.60 mg, 25.1 mg and 23.9 mg carbohydrate resulted in the head, middle and tail portions respectively (Fig 1). Further decline in the carbohydrate content was observed in the inoculated yam samples two weeks after inoculation. During this period however, only a slight drop (from 34.80 mg to about 31.02 mg) in the carbohydrate content was visible in the control experiment. Between the third and fifth weeks, there was no marked reduction in the total carbohydrate in the head portion. This is presumably due to the hydrolyses of the cell wall components (cellulose and hemicelluloses) and the few starch grains to soluble carbohydrates like glucose, sugars etc. (Ekundayo and Okigbo, 1991; Prasad *et al.*, 1989) by cell wall degrading enzymes produced by the pathogen during pathogenesis (Isaac, 1992). These soluble sugars may have made up for the visibly lost starch grains as revealed by the photomicrographs. It is also likely that it may have been due to the presence of glycoalkaloids in the head portion (Osagie, 1992) that may have been fungistatic hence constituting a delay to the

pathogen in ramifying and colonizing this portion of the yam tuber effectively thereby resulting in minimal reductions for carbohydrates.

Table 2. Microorganisms found associated with stored and marketed yams obtained from the study area and their pathogenicity on white yam (*Dioscorea rotundata* **Poir**)

PATHOGENS	SYMPTOMS OF INFECTION	PATHOGENICITY
Aspergillus niger	Dry rot	+ + +
Penicillium expansum	' '	+ + +
Penicillium sclerotigenum	' '	+ +
Botryodiplodia theobromae	' '	+ + +
Fusarium moniliforme	Soft rot	+ +
Fusarium oxysporum	' '	+ +
Rhizopus stolonifer	' '	+ + +

+ +: Mildly pathogenic (>10<50mm in diameter). + + +: Highly pathogenic (>50mm in diameter).

In the middle and tail portions, reductions in the amounts of total carbohydrates were however visible between the third and the fifth week. About 16.90 mg and 16.80 mg carbohydrate per 100 g edible portion of white yam tuber were recorded in the fifth week compared with 20.10 mg and 20.09 mg recorded in the second week of infection in the middle and tail portions respectively (Fig 1). Effect of infection on the protein content of the tuber did not follow any particular trend. However, there was a decline in protein content in the tuber portions tested one week after inoculation (Fig 2). In the head portion, a slight increment was observed between weeks 3 and 4 with a slight decline in the last week of the experiment.

In the middle portion the decline continued till the third week. There was a sharp increase from 5.25 mg/100 g dry weight to 8.56 mg/100 g in the fourth week. After which a slight decline was observed. Following the initial decline after the first week, there was slight but steady rise in the protein content in the tail portion until a value of 9.19 mg/100 g dry weight was recorded on the fourth week. This then fell to a value of 8.56mg/100g. In the control experiment, there was a slight drop (from 7.88 mg/100 g to 6.72 mg/100 g) in the protein content within the period of the study. In their work on *V. doniana,* Ekundayo and Okigbo (1991) also reported a reduction in crude protein from 72.53 to 58.09 µg/g in three days. This agrees with the results obtained in this work where there was a general decline in protein content in the yam tubers in the first week of incubation.

Results obtained by Prasad *et al,* (1988) on muskmelon (*Cucumis melo* var *mormodica*) infected with *Fusarium semitectum* and *Curvularia lunata* and that by Prasad *et al,* (1989) on *Protomyces macrocarpus*-infected coriander plant revealed that loss in proteins was due to the capability of the pathogens to secrete protease enzymes. Mabadeje and Arohkeshi (1990) made similar observations on guava, mango and pawpaw fruits infected with *Aspergillus niger.* The increase observed in the second week in the head and the middle portions may be attributed to the production of novel proteins in the form of enzymes by the fungus. Increases in insoluble nitrogen and protein of *A. flavus* and *A. parasiticus*-infected wheat seeds has also been reported (Dube *et al,* 1988). Higher levels of enzymes such as L- serine dehydrase, L-arginine

desimidase, L (+)-cysteine desulphurase and L-isoleucine deaminase have been recorded in coriander plant infected
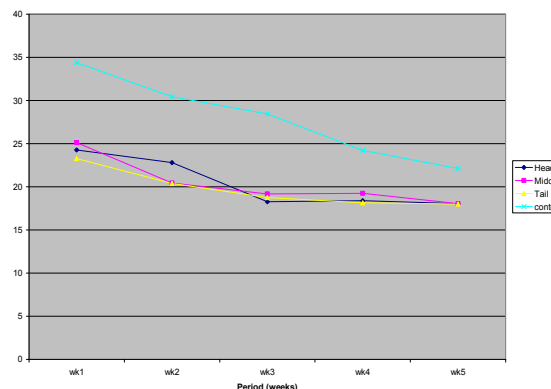

Fig. 1. Effect of infection by *Botryodiplodia theobromae* on the carbohydrate (starch) content of tuber of *Dioscorea rotundata* (Poir).
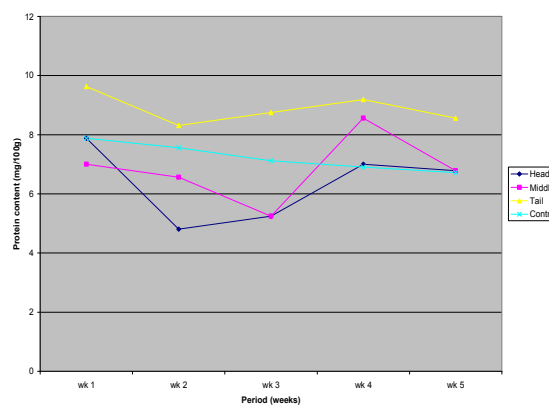

Fig. 2. Effect of infection by *Botryodiplodia theobromae* on the protein content of tuber of *Dioscorea rotundata* (Poir)

of enzymes in the pathogenesis of fungi-incited diseases have also been documented by Arinze (2005) and Isaac (1992) There was a consistent decline in the amount of lipids with increasing period of infection (Fig. 4.8). Similar observation has been made by Amadioha (1995) on Rhizopus and *Rhizoctonia* – induced scab of two cultivars of potato. Results from this study indicated appreciable difference between the oxalate content of the infected and the healthy (control) yam tubers (Fig. 4).

This was highest in the head portion of the tuber when compared with the figures obtained for the middle and the tail portions. Irrespective of the portion of the tuber, there was a steady increase in oxalate content with time (weeks). In the head portion, the oxalate content of the tuber increased from 3.45 at the end of one week to 55.2 mg/g of tuber at the end of the fifth week. In the middle portion, increment was from 3.24 to 54.49 mg/g dry weight and in the tail portion, it increased from 2.97 mg/g to 50.66 mg/g dry weight within the same period. Only a slight increment (from 1.89 to 2.89 mg/g) was observed in the control experiment. Reports on the increments of some antimicrobial components such as phenols, catechuic acid,

oxalates etc in tubers have been reported (Amadioha, 1995; Arinze, 2005, Amusa, 2000; Prasad *et a*l, 1988;
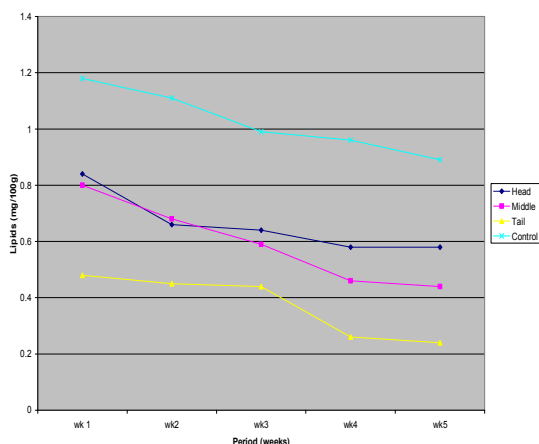

Fig. 3. Effect of infection by
Botryodiplodia theobromae on the lipid
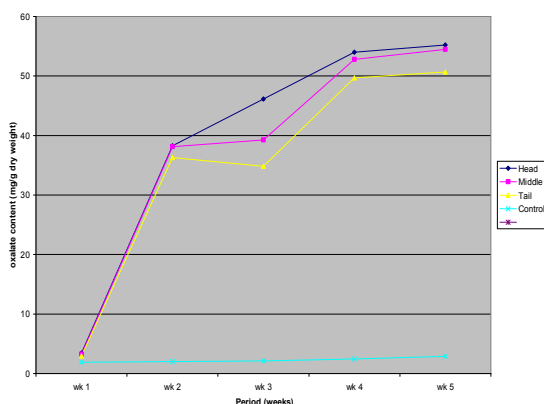content of tuber of ***Dioscorea rotundata*** (Poir)

Fig. 4. Effect of infection by
Botryodiplodia theobromae on the
oxalate content of tuber of ***Dioscorea rotundata*** (Poir)

Dwivedi, 1990). A steady rise in the oxalate content of potato tuber have been recorded by Amadioha (1995) in two cultivars (Red Pontiac and Irish cobbler) infected with *Rhizopus oryzae* and *Rhizoctonia bataticola*. Value as high as 15 mg/g and 52 mg/g were reported after 10 days of infection.

Prasad *et al,* (1988) observed increased levels of phenols in muskmelon fruits infected with *Fusarium semitectum* and *Curvularia lunata* within one week of incubation. Also in a report given by Dwivedi (1990) on *Aspergillus niger* – infected chickpea seeds, a 3 to 4 – fold increases was recorded in the levels of phenolic compounds within one week of storage. Such levels were maintained for three weeks in *Penicillium oxalicum* – infected seeds. Both researchers saw the high levels of phenolic compounds in these infected plant materials as a response of the infected tissue the infection by the pathogens. Since the value of any food crop resides in the quality and quantity of its nutritional content, it is necessary to preserve and conserve these nutrients through protecting the crop from microbial attacks by adopting good crop health management practices.

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