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RESEARCH ARTICLE

PHYSICOCHEMICAL ALTERATION OF TISSUES OF WHITE YAM (*Dioscorea rotundata* Poir) TUBERS INCITED BY *Botryoiplodia theobromae* Pat

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ABSTRACT

Investigations on the anatomical aberrations and biochemical alterations of white yam incited by *Botryodiplodia theobromae* was carried out. Inoculated and uninoculated head, middle and tail portions of white yam were treated, sectioned weekly for five weeks and photomicrographs taken. The plates revealed massive cell wall macerations and depletion of starch grains in the three portions of yam assessed. Less damage was evident in the head portion especially after one week of infection compared with the middle and tail portions. However, there was general progressive tissue destruction and starch grains depletion with weeks. Inoculated and uninoculated yam samples were also analyzed for the carbohydrate content weekly for five weeks. Results showed that there was a reduction for carbohydrate with increase in weeks of infection. Values of 24.60 mg, 25.1 mg and 23.9 mg carbohydrate per 100 g of edible portion of white yam were recorded after one week of infection for the head, middle and tail portions respectively. 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. From the third week, there was no tangible reduction in the carbohydrate content especially in the head portion, though appreciable tissue maceration was evident from the photomicrograph sections, particularly, in the tail portions where there was total breakdown and collapse of cell wall boundaries.

INTRODUCTION

Yams (*Dioscorea spp*) are annual or perennial climbing plants with edible underground tubers. About 600 yam species are known. Of these, the white yam (*Dioscorea rotundata*) is the widely cultivated, consumed and has the shortest period of maturity (Burkill, 1985). *Dioscorea rotundata* commands the highest market value owing to the superior suitability of its tubers to the preferred food use in West Africa. It also plays an important role in the sociocultural life of a wide range of small holder households (IITA, 2000). 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.

Nutritionally, yam is rich. A 100 g edible portion of yam (*Dioscorea spp*) is given as; 69 ml water; 119 calories; 1.99 protein; 0.2 g fat; 27.8 g carbohydrate; 0.8 g fibre; 52 mg calcium; 61 mg phosphorus; 0.8 mg iron; 10 µg B-carotene equiv.; 0.11 mg thiamine; 0.02 mg riboflavin; 0.3 mg niacin and 6 mg ascorbic acid (FAO, 1998).

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Yam is medicinally valuable due to its diosgenin content. Diosgenin (a kind of sapogenin) is known to be a prominent source of hemi synthesis of birth control pills (with progesterone and estrogen as well as similar hormones) and corticosteroids (Burkill, 1985).

In spite of its numerous uses, many insect pests and microorganisms attack yam both in the field and in storage. The susceptibility of yams to microbial attacks in storage is a function of their high moisture content (70-80 %). The major storage disease of yam is rot. The organisms implicated in rot diseases include bacteria, nematode and fungi. Of these, fungi are the most virulent agents and the most common cause of rot diseases (Isaac, 1992). Among the numerous fungal agents of rot diseases is *Botryodiplodia theobromae,* the causal agent of the dry black rot of yam (IITA, 1993). Losses due to rot (wet, soft and dry) diseases have been reported to be as high as 30-60 % during the course of 3-6 months storage period (Arinze, 2005). Losses are in the form of reduction in the quality of the tubers through breakdown of tissues resulting in anatomical aberrations and depletion of the nutrient component such as protein, lipids and the major one – starch (Arinze, 2005). Information on the gravity of

damage of tissues and the amount of nutrients (starch) lost within a set period of infection is scanty. This paper is therefore aimed at bridging this gap.

MATERIALS AND METHODS

Sample collection and pathogen identification

Symptomatic and asymptomatic tubers of white yam (*Dioscorea rotundata* Poir) were sampled from open market stalls in three markets in Calabar urban. The markets were Akim, Marian and Watt. Tissues about 5 mm in diameter from the symptomatic and asymptomatic white yam tubers were removed 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. Cultures that were suspected to be *Botryodiplodia theobromae* based on morphological characteristics were transferred to new PDA-containing plates, from where axenic cultures were generated (Gevens *et al*., 2008). Cultures were positively identified as *B. theobromae* 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 a*l, (1997). Confirmation was made by comparing with a culture identified by International Mycological Institute, Egham, UK. (IMI 347961).

Koch's postulates and pathogenicity test

To confirm the pathogenicity of *B. theobromae* isolates from white yam, axenic cultures of *B. theobromae* isolate were used to inoculate three white yam minisetts with 5-mm-diameter mycelial agar plugs of a 4-day-old culture. 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 organism was identified as *B. theobromae* and confirmation of pathogenicity on white yam was completed.

Preparation of tissue sections of inoculated and uninoculated yam.

Sectioning was done by first fixing the inoculated and the uninoculated slices with F.A.A (1:1:18) for 24 – 28 hrs then washed with distilled water. Tissue sectioning was done at 10 mm depth using Reichert Rotary microtome. The sections were then stained with safranin for 2 – 3 minutes and dehydrated using pure xylene. These dehydrated sections were then mounted in Canada balsam on a glass slide. The slide was dried over a hot plate at 35 – 40^0C. Photomicrographs of the prepared slides were taken using Leitz Weitzler Ortholux microscope fitted with a Vivitar – V335 camera.

Determination of total carbohydrate

Determination of starch was carried out adopting the method described by A.O.A.C (1995). 0.5 g powder of the inoculated and uninoculated yam tubers were weighed separately into the extraction tube and 100 ml of 80 % ethanol was added and shaken for 30 mins. The sample was centrifuged and 5 ml of the supernatant pippetted into a 100 ml volumetric flask and 10 ml of anthrone reagent added and made up to the mark with distilled water. This was heated over a water bath for 10 mins and allowed to cool, then read in a spectrophotometer at 620 nm wavelengths. A standard concentration of 0 ppm, 20 ppm, 40 ppm, 60 ppm and 80 ppm were prepared and read at 620 nm wavelengths. This was used in the preparation of the calibration curve for the extrapolation of the results. This procedure was carried out on the inoculated and uninoculated samples weekly for five weeks.

Anatomical studies of the yam

The method used was the modification of the method described by Arinze *et. al.* (1975) to determine the mode of entry by the pathogens into the host tissues. Two yam tubers were peeled and surface-sterilized by dipping them in calcium hypochlorite (3 % available chloride) for 3 mins. Rinsed with several changes of distilled water and allowed to dry naturally. With a sterilized kitchen knife about 1 cm thick slices were obtained. These were placed in sterilized glass Petri dishes and inoculated with spore (conidia) suspension. The spore (conidia) load was estimated using haemacytometer.

The inoculum load was determined using the formula.

$$\text{Spore Load} = \frac{N \times v}{V}$$

Where , N = mean number of spores counted in the chosen square (total mount of spores counted: number of squares).

V = volume of the mounting fluid (sterile distilled water)

v = volume of the mounting solution between the cover glass and above square counted (area of square × depth of the chamber).

A spore (conidia) load of 5.0×10^4/ml of sterile distilled water were used in all the experiments except when otherwise stated. Inoculated slices of tubers in the Petri dishes were incubated at 30^0 C and then sectioned after a week, then at interval of every other week for a period of 5 weeks. Uninoculated slices (control) were similarly sectioned.

RESULTS AND DISCUSSION

Sample collection and pathogen identification

Following the results obtained from isolations of rot-causing pathogens of yam, *Botryodiplodia theobromae* was the most frequently isolated after *Rhizopus stolonifer* (which is known to be a weak parasite) and the most virulent. Cultures of this fungus on PDA were initially white, fluffy and feathery, becoming grey and eventually black. The growth was radial in pattern from the centre of the plate outwards. Literature on identification of pathogenic fungi (Rossman *et al*., 1997) corroborates this observation and the appearance of this fungus fitted the description of *Botryodiplodia* Pat. (=*Lasiodiplodi theobromae* (Pat.) Griff and Maubl.) given by Marley (1998) and confirmation was made by comparing with a culture identified by International Mycological Institute, Egham, UK. (IMI 347961).

Koch's postulates and pathogenicity test

The *B. theobromae* isolate was pathogenic on the three white yam minisetts used for the test. Symptoms of decay (rot) caused by this fungus is known to be dry black rot.

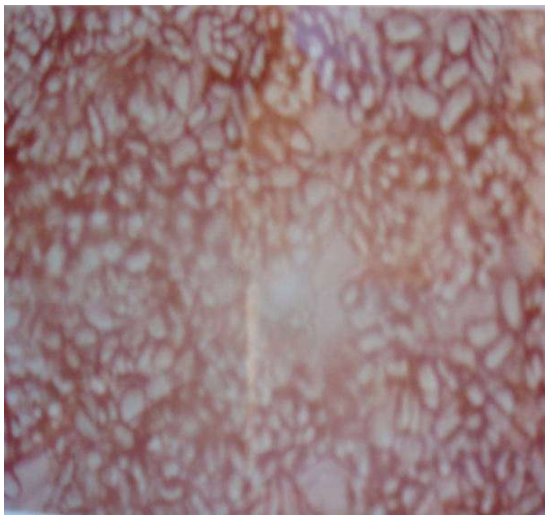


Plate 1a

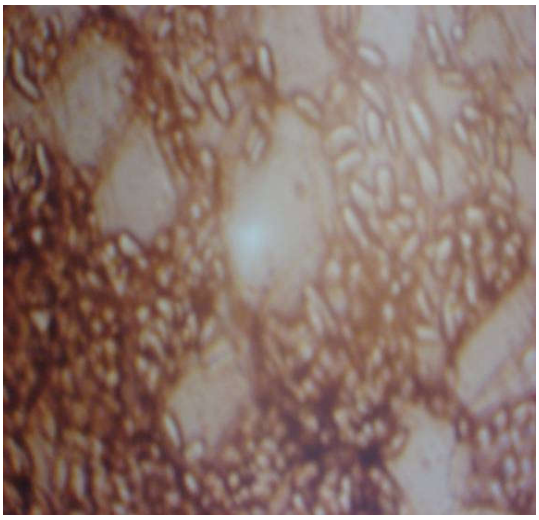
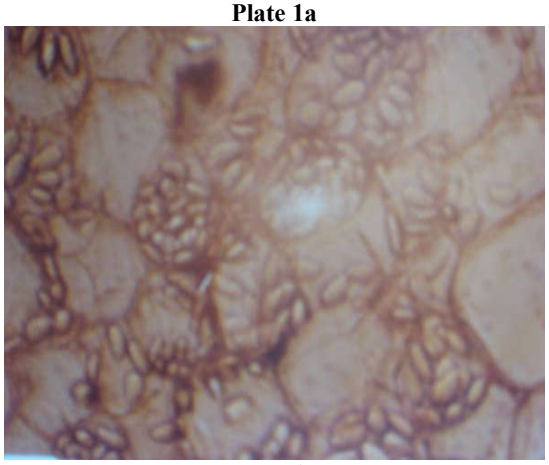


Plate 1b



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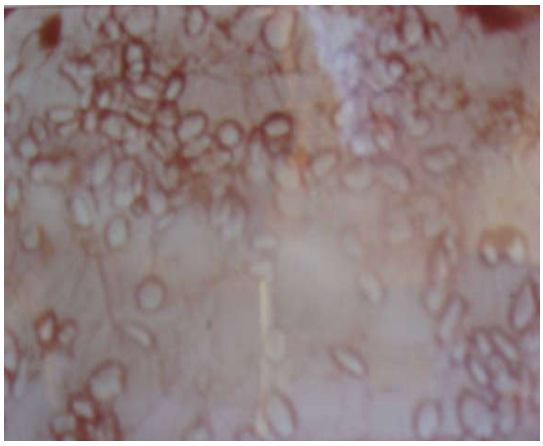

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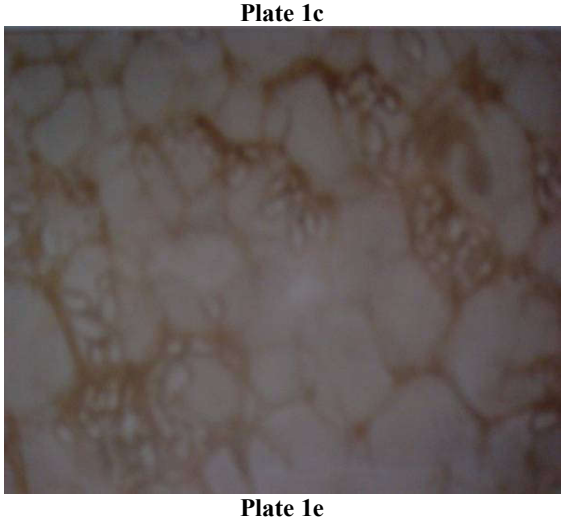

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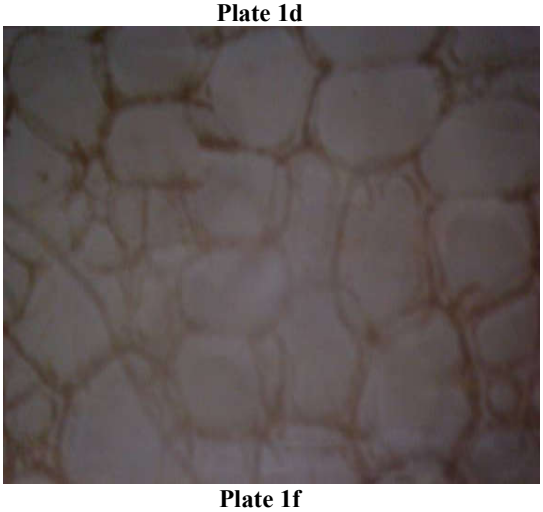


Plate 1f

Plate 1*(a) Fresh uninoculated head portion of yam (Dioscorea rotundata) showing intact cells fully and tightly packed with starch grains (X250).* ***(b)*** *Head portion of yam, 1 week after inoculation with B. theobromae showing some cells depleted of starch grains by the invading pathogen (B. theobromae) (X250).* ***(c)*** *Head portion of yam 2 weeks after inoculation with B. theobromae showing more cells without starch grains as depleted by the test pathogen (B.* ***(d)*** *Head portion of yam, 3 weeks after inoculation with B. theobromae showing massive cell wall maceration and depletion of starch by the test pathogen (B. theobromae) (X250).* ***(e)*** *Head portion of yam, 4 weeks after inoculation with B. theobromae revealing collapse of cell walls and near total depletion of starch grains by the invading test pathogen (B. theobromae) (X250).* ***(f)*** *Head portion of yam 5 weeks after inoculation with B. theobromae showing empty cells totally depleted of starch (X250).*

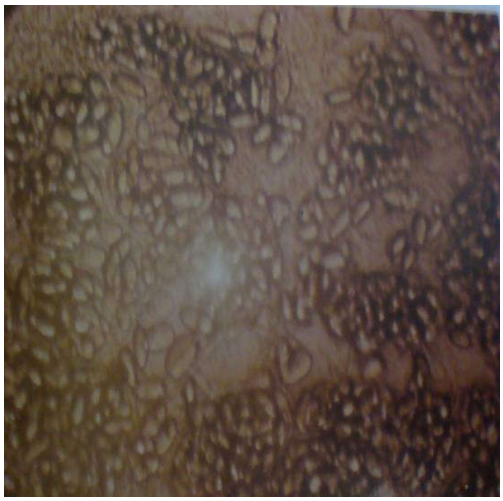


Plate 1

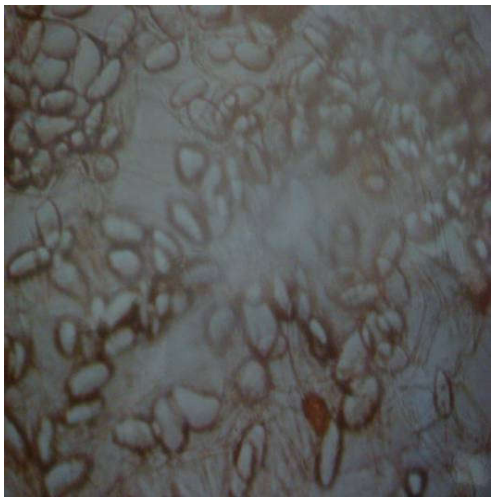
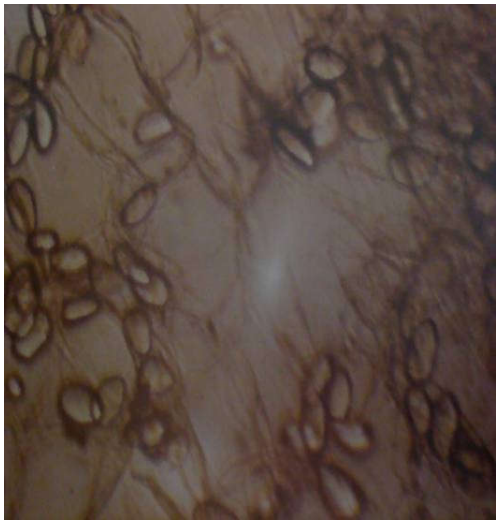


Plate 2b

Plate 2c



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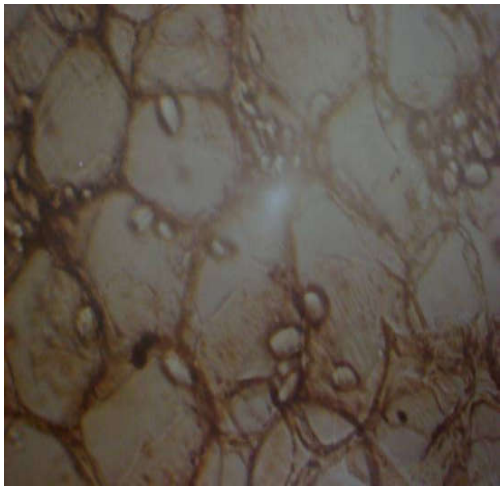

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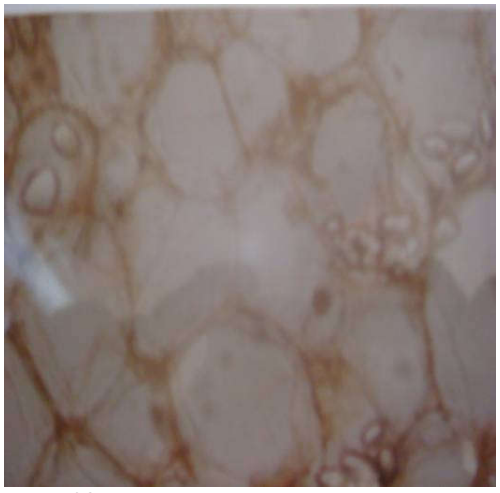


Plate 2f

Plate 2 (a) *Fresh uninoculated middle portion of Dioscorea rotundata revealing cells loosely packed with starch grains (X250).* ***(b)*** *Middle portion of yam, 1 week after inoculation with B. theobromae showing cells fully ramified with fungal hyphae depleting starch grains (X250).* ***(c)*** *Middle portion of yam, 2 weeks after inoculation with B. theobromae indicating further depletion of starch grains and cell wall collapse (X250).* ***(d)*** *Middle portion of yam, 3 weeks after inoculation with B. theobromae showing progressive depletion of starch and cell wall maceration (X250).* ***(e)*** *Midddle portion of yam, 4 weeks after inoculation with B. theobromae revealing almost total depletion of starch grains (X250).* ***(f)*** *Middle portion of yam, 5 weeks after inoculation with B. theobromae showing total collapse of cell wall boundaries and removal of starch grains(X250).*

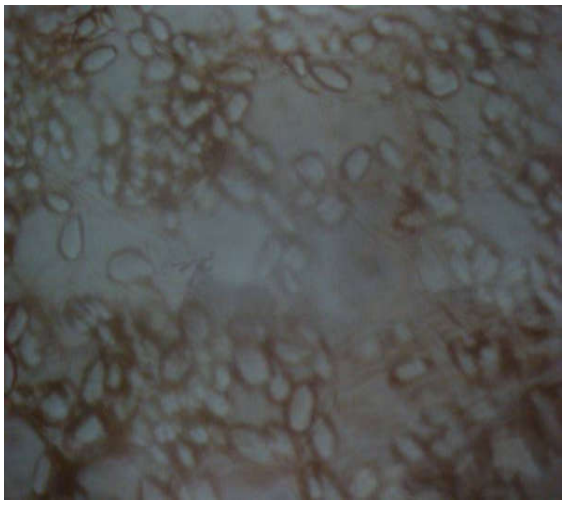


Plate 3a

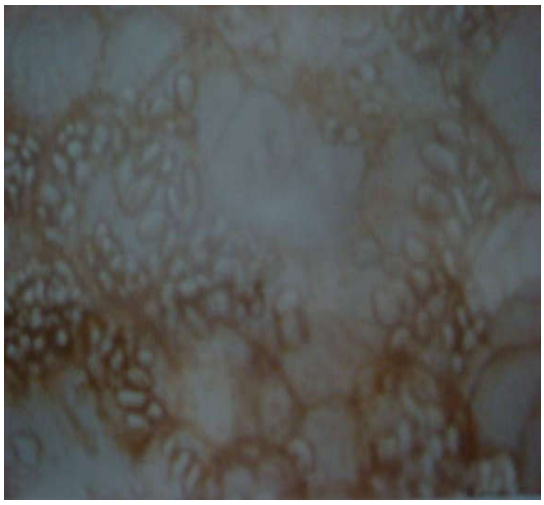
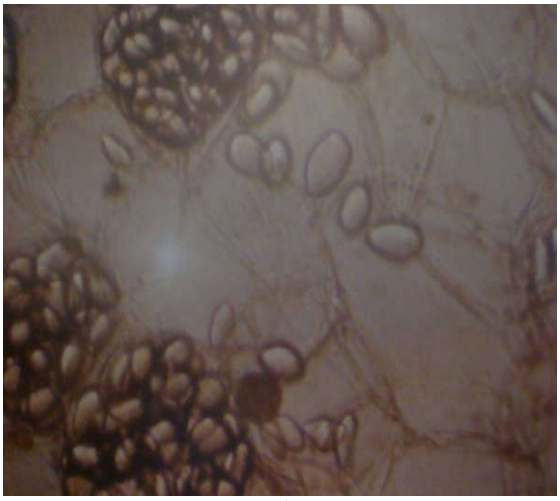


Plate 3b



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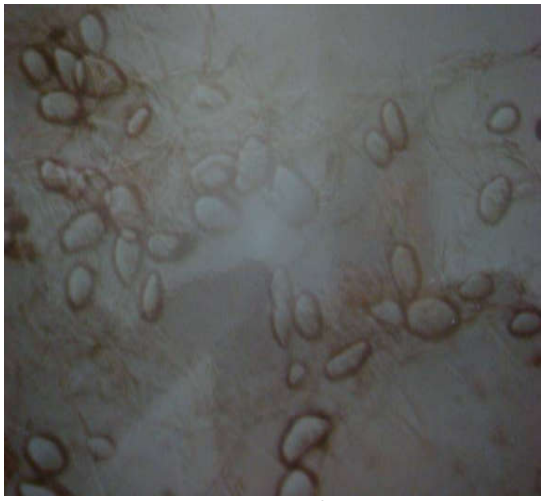

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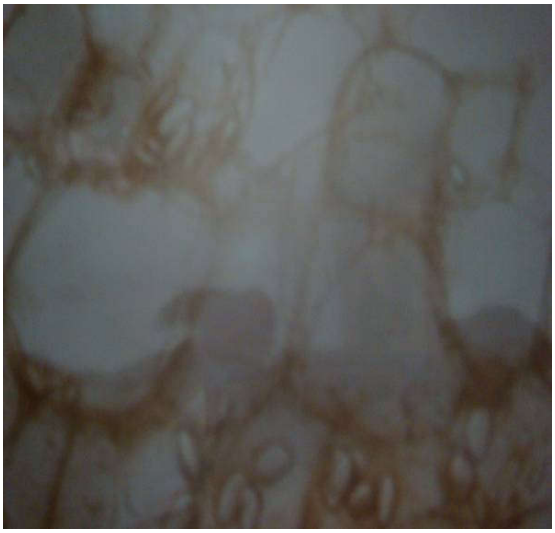

Plate 3e

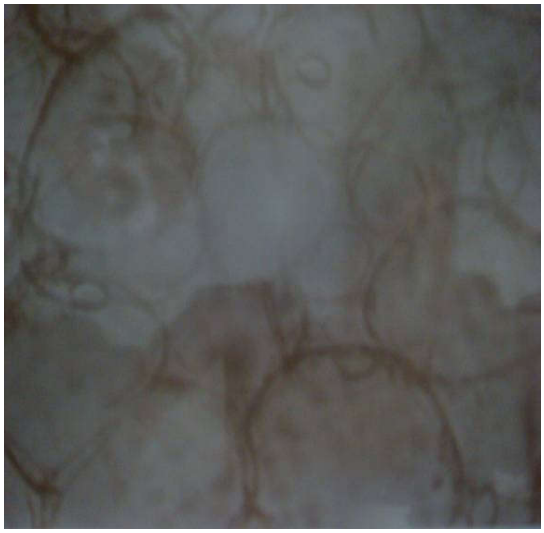

Plate 3f

Plate 3 (a) *Fresh uninoculated tail portion of Dioscorea rotundata revealing a loosely packed arrangement of starch grains (X250).* ***(b)*** *Tail portion of yam, 1 week after inoculation with B. theobromae showing early collapse of cell walls (X250).* ***(c)*** *Tail portion of yam, 2 weeks after inoculation with B. theobromae revealing much loss of starch grains and greater cell wall collapse leaving pockets if intact cells (X250).* ***(d)*** *Tail portion of yam, 3 weeks after inoculation with B. theobromae revealing total collapse of cell walls and massive removal of starch grains by the invading test pathogen (X250).* ***(e)*** *Tail portion of yam, 4 weeks after inoculation with B. theobromae showing few cells with starch grains and a near total disintegration of cells (X250).* ***(f)*** *Tail portion of yam, 5 weeks after inoculation with B. theobromae clearly revealing clearing and collapse of cell wall boundaries.*

Infection commenced with discoloration of the tuber from white to brown, which later become grey and then black. After weeks of infection, the tuber became pulverized breaking into small dry particles. The yams produced symptoms identical to those known for *Botryodiplodia* – infected yam tubers. IITA (1993) gave similar description of symptoms in yam infected with *B. theobromae.* Re-isolation from the inoculated yam tubers produced cultures identical to the original isolate, appearing whitish and fluffy exhibiting a radial pattern of growth.

Determination of total carbohydrate

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. In the middle and tail portions, reductions in the amounts of total carbohydrates were however visible between the third and the fifth week. About 16.90mg and 16.80mg carbohydrate per 100g edible portion of white yam tuber were recorded in the fifth week compared with 20.10mg and 20.09mg recorded in the second week of infection in the middle and tail portions respectively (Fig. 1).

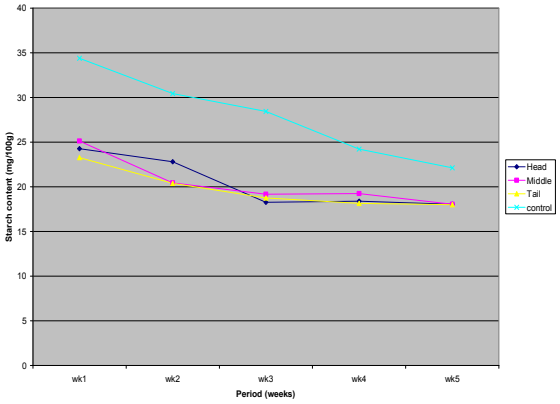

Fig. 1: Effect of infection by ***Botryodiplodia theobromae*** on the carbohydrate (starch) content of tuber of ***Dioscorea rotundata*** (Poir)

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

Results in Table 1 shows the progressive depletion of starch content of the head, middle and tail portions of yam within the incubation period. The amount of carbohydrate lost from these three portions of yam assessed were significantly different ($P<0.05$) in the first week but in the second week, significant differences were only observed between the head portion and the other two (middle and tail) portions were not significantly different from each other. Between the 3rd and the 5th weeks of incubation, the level of deletion of carbohydrate was not significantly different in the three portions of yam tested. However, the level of depletion in each yam part tested was significantly different from that obtained in the control experiment within the test period (Table 1). The rate of depletion of the carbohydrate content of yam tuber by the pathogen was more between the first and 3rd week of incubation. This may have been due to the ability of the pathogen to secrete carbohydrate – degrading enzymes and their utilization of the nutrient for growth as well as colonization of more cells of the yam. The loss of carbohydrate from the cells of infected yam may also result from higher rate of respiration by the yam tissues in response to the invasion by the pathogen (Prasad et al., 1989) Histological studies on the head, middle and tail of yam tuber to determine the rate at which infection progresses and the extent of tissue maceration and nutrient loss showed clearly the progressive depletion of starch grains in the cells and the massive tissue maceration especially from the third week after infection (Plates 1 – 17).

Table 1: Effect of infection by *Botryodiplodia theobromae* on the carbohydrate (starch) content of *Dioscorea rotundata* (Poir)

Carbohydrate content (mg/100g) and incubation period (weeks					
Yam parts	1	2	3	4	5
Head	24.1	22.6	17.9	17.9	17.9
Middle	25.1	21.0	19.0	19.0	18.1
Tail	23.1	20.8	18.5	17.8	17.6
Control	34.9	31.8	28.5	24.8	22.6
LSD	0.03	0.08	0.09	0.18	0.17

Anatomical studies of the yam

The figures below show the photomicrographs of sectioned head, middle and tail portions of yam tissues (healthy and infected). The infected samples were incubated for five weeks and sectioning carried out after every one week. Results obtained revealed that starch grains were progressively removed from the cells of the yam by the test pathogen. In the fresh uninoculated portions (head, middle and tail - Plates 1a, 2a and 3a respectively), starch grains were seen fully packed within the cells. One week following inoculation, Plate 1b reveals a slight reduction in starch grains and a minimal maceration of the cell walls in the head portion compared with that recorded in the middle (Plate 2c) and tail (Plate 3c) portions. This may be due to the presence of glycoalkaloids in head portions of stored yams (Osagie,1992)

om the third week, greater cell wall maceration was observed in the tail portions compared with those of the head and middle. Progress in infection in the head portion was slow and damage to the cell wall was not as severe as seen in the middle and tail portions subjected to similar treatments within the same span of time. The value and popularity of yam as a staple food crop after rice is mainly a function of its carbohydrate content. The role of carbohydrate in human and animal nutrition and health is immense. Pest and microbial attacks result in the reduction of the quality of crops through depletion of nutrients in them.

The insight given by this paper into the massive anatomical aberrations and the colossal nutritional losses resultant from the invasion of yam tuber tissues by *Botryodiplodia theobromae* serves to highlight the extent and gravity of losses incurred by farmers due to microbial attacks that may not have been considered and quantified. The knowledge of these losses will serve to sensitize and re- orientate farmers towards adopting good crop protection practices to evade microbial attacks of their valuable crops such as yam which serve as staple food for millions of people worldwide (IITA, 2000). With this, the cycle of food scarcity especially during the planting seasons can be broken, as yam can remain viable in storage across seasons.

REFERENCES

AOAC 1995. Official Method of Analysis. Association of Official Analytical Chemists. Washington D.C.

Arinze, A.E. 2005. Plant Pathology and Post –harvest Food Loss. *An Inaugural Lecture Series, University of Port Harcourt, Nigeria, No*. 43 P.29-72.

Arinze, A.E., Naqvi, S. H. Z. and Ekundayo, J. A. 1975. Storage of sweet potato (*Ipomoea batatas*) and the effect of fungicide on extra cellular cellulotic and pectolytic enzymes of the causal organisms. *International Biodeterioration Bulletin*, 11(2):41-47

Burkill, H. M. 1985. The Useful Plants of Tropical Africa. Royal Botanical Gardens, Kew.

Ekundayo, C. A. and Okigbo, R. N. 1991. The effects of pathogenic fungi on the nutritional value of the black Plum (*Vitex doniana*). *Nig. J. Plant Protection.*4: 127-134.

F.A.O. 1998. Food and Agricultural Organization. Year Book. FAO, Rome.

Gevens. A. J., Donahoo, K. H. L. and Hausbeck, M.K. 2008.. Characterization of *Phytophthora capsici* causing foliar and pod blight of snap bean in Michigan. *Plant Disease*. 92(2): 198-320.

IITA 1993. Crop improvement Division/ Tuber root improvement program. Archival reports (1989-1993).Part III yam (*Dioscorea spp*) Ibadan, Nigeria. *Fito Pathologia Bras*. 8:1-7.

IITA 2000. Annual report 2000 of project 14 – impact, policy and systems analysis. IITA, Ibadan, Nigeria.

Isaac, S. 1992. Fungal-Plant Interactions. Chapman and Hall, London.

Marley, P. S. 1998. Dieback of pigeon pea (*Cajanus cajan*) caused by *Botryodiplodia theobromae* Pat. *Nig. J. Plant Protection.* 17: 1-68.

Osagie, A. U. 1992. Post Harvest Research. Benin City, Nigeria. Post harvest Research Unit, University of Benin, Nigeria.

Prasad, B.K., Singh, R. N. and Narayan, N. 1989. Biochemical changes in nitrogen and carbohydrate in Coriander infected with *Protomyces macrocarpus*. *Indian Phytopathology*. 42: 369-387.

Rossman, A. Y., Palm M. E. and Spielman L. J. 1997. A Literature Guide for the Identification of Plant Pathogenic Fungi. Minnesota, USA, APS Press.

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