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Fungal Organisms Associated with Yam (*Dioscorea rotundata*, Poir) Rot at Owerri, Imo State of Nigeria

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Abstract: Pathological investigations were carried out at Owerri, Imo State, Nigeria to find out the fungal organisms associated with yam rot. The following organisms were isolated and identified as *Fusarium oxysporium* Schlech ex. Fr., *Rhizopus oryzae* Went, *Botryodiplodia theobromae* Pat. and *Fusarium solani* Mart Sacc. Pathogenicity test carried out confirmed these organisms as the pathological agents of the yam rot.

Key words: Fungal, organisms, yam, rot, Owerri, Imo State

INTRODUCTION

Yam is the common name for some species in the genus *Dioscorea* (family Dioscoreaceae). These are perennial herbaceous vines cultivated for the consumption of their starchy tubers in West Africa, Asia, Latin-America, Oceania islands of the South Pacific, South East, Asiaindia, the Caribbean and parts of Brazil. Nigeria alone accounts for considerably more than half of the world's total production of yam (Kay, 1987). Yam tubers can grow up to 2.5 m in length and weigh up 70 kg (150 pounds).

Dioscorea rotundata, the white yam is the most important cultivated yams and mainly found in Africa. There are over 200 cultivated varieties between them. They are large plants, the vines can be as long as 10-12 m. The tubers most often weigh about 2.5-5 kg (6-12 pounds). After 7-12 months growth, the tubers are harvested. In Africa, they are pounded into paste to make traditional dish fufu (Kay, 1987).

Yams can be cooked in various ways by boiling, roasting and frying. Yam in Nigeria is also produced into various staple intermediate end product (Okaka *et al*., 1991) which are used for direct consumption by animals used as basic ingredients for snacks or made into flour. Yam is of very high value as food where it is a major source of carbohydrates, minerals, vitamins B6 and C and dietary fibre (Coursey, 1983).

Onayemi (1983) reported that over 50% of the yam tubers produced and harvested in Nigeria are lost in storage. The disease causing agents reduce the quantity of yam produced and also reduce the quality by making them unappealing to the consumer. Yam is prone to infection right from the seedling stage through harvesting and even after harvesting in storage (Amusa *et al*., 2003). Yams are subjected to several diseases. There are different genera of fungi that have been reported in association with storage deterioration in yam tubers (Noon, 1978; Okigbo and Ikediugwu, 2000).

Knowledge of the fungi responsible for yam rot will be of tremendous help in finding effective control measures to extend the life span of yam in storage.

MATERIALS AND METHODS

Sample collection: About 10 yam tubers with symptoms of rot were obtained from Eke-Ukwu market in Owerri, Imo State. The diseased yam tubers were packaged in polyethylene bags and taken to the laboratory of Imo State University, Owerri where they were assessed for microbial (fungal) presence.

Media preparation: The medium used was Potato Dextrose Agar (PDA) prepared according to the manufacturer's instruction. About 39.6 g of powdered PDA medium was dissolved in 1 L of sterile distilled water and sterilized by autoclaving at 121°C at 15 psi for 15 min and allowed to cool before pouring carefully into 20 sterile Petri dishes.

Two drops of lactic acid were added to the solution to inhibit any bacterial growth. The Petri dishes that contained the medium were incubated for 24 h at room temperature (28°C) to check for sterility before use as described by Cheesebrough (2004).

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Isolation of pathogens: Rotted yam tubers were rinsed in distilled water, surface sterilized with 70% ethanol and cut open (Fig. 1) with a sterilized knife. About 20 pieces (3 mm in diameter) of the infected yam tissues were picked from the point of advancement of rot with a flamed sterilized forcepsand inoculated on a solidified Potato Dextrose Agar (PDA) medium. Two replicates were made for each of the 10 yam tuber samples and the 20 plates inoculated on. The inoculated plates were incubated at room temperature (28°C) and observations made daily for possible fungal growth. Subculturing was done to obtain pure cultures of the isolates. Stock cultures were prepared using slants of Potatoe Dextrose Agar (PDA) in McCarteny bottles and stored in a refrigerator at 4°C. Cultural characteristics of the fungi were observed and recorded. Occurrence of the organisms was recorded as follows:

$$\frac{\text{Total number of figures}}{\text{Total number of fungal occurrence}} \times 100$$

Identification of organisms: The identification of the isolates was done by examining the isolates macroscopically and microscopically. The colony characteristics, spores, mycelium either septate or not, conidium were taken note of. These structural features were matched with standards in Barnet and Barry (1972) and Booth (1971).

Pathogenicity studies: Ten fresh healthy tubers of yam were washed with tap water and distilled water, respectively and thereafter sterilized with 70% ethanol. Cylindrical discs (4 mm) were removed from the tubers with a sterile 4 mm cork borer. About 4 mm discs of 5 days old cultures of the isolates were used to plug the holes created in the tubers, respectively. The discs of the tuber in the cork borer was replaced and then sealed with vaseline jelly to make it air tight. Sterile PDA disc used in place of the culture discs served as the control. This was done for all the isolates obtained in pure culture (Okigbo and Ogbonna, 2006).

Two replicates were made for each organism. The inoculated tubers and the control were each enclosed in a sterile polyethylene bag and incubated for 8 days at room temperature (25-37°C). A micro-humid environment was provided by enclosing a sterile water soaked aseptic cotton wool in each set up.

Fig. 1: Rotted yam: Source of innoculum

Measurement of rot: The extent of rot was determined by using a sterilized and flamed knife to cut open the inoculated yam tuber from the point of inoculation to obtain identical halves. A sterilized transparent ruler was used to measure the depth of rot. The actual depth was determined by substracting the initial depth (2 cm) from the final depth (i.e., final depth-initial depth = actual depth. Also in determining the degree of rot in diameter, the initial diameter (4 mm) was subtracted from the final diameter of rot to get the actual diameter of rot. The area of rot was calculated as follows:

Πdl = Area of diameter
D = Diameter
L = Depth
Π = 22/7 (constant)

The spread of dry or wet rot was calculated for both lengths, depth and width i.e., diameter and recorded. Reisolation was carried out using a sterilized and flamed scapel.

Small pieces of rotted tissue about 1 mm from the advancing edge of the rot were removed from the infected tuber and asceptically inoculated into PDA media in Petri dishes and were incubated for 72 h at room temperature. Subculturing was done to get pure cultures of the organisms. The colony and microscopic features of the organisms identified according to the standards of Barnet and Barry (1972) and Booth (1971).

Experimental design: Experimental design used involved Randomized Complete Block Design (RCBD). Analysis of Variance (ANOVA) to separate the means using Least Significance Difference (LSD) to determine levels of significance.

RESULTS AND DISCUSSION

During the isolation and identification of the causative organisms of the rotted yam tuber, 4 fungi were identified according to the standards of Barnet and Barry

Table 1: Macro and micro features of the fungi (isolates)

Isolates	Colony characters	Microscopy	Identified organism
A	Aerial mycelium, dense was sparse, white/grayish with tinge of bluish brown colouration	Many micro and macro conidia present Chlamydospores present, abundant and oval, terminally and intercalary positioned	*Fusarium solani* (Fig. 2)
B	Fast and rapid growth. Whitish with black spores and milky underneath	Coenocytic (non-septate) has sporangia head (sporangium), flat collumella and spores are single celled	*Rhizopus oryzae* (Fig.3)
C	White to dirty white, black underneath	Mycelium are septate, chlamydospores are intercalary and terminal, conidia are 2 celled	*Botryodiplodia theobromae* (Fig. 4)
D	Growth on PDA is rapid. White aerial mycelium tinged with pink purple colour	Micro and macro conidia are present, macro conidia slightly sickle celled with apical cell and foot shaped basal cell, chlamydospores are present, single and some in pairs	*Fusarium oxysporium* (Fig. 5)

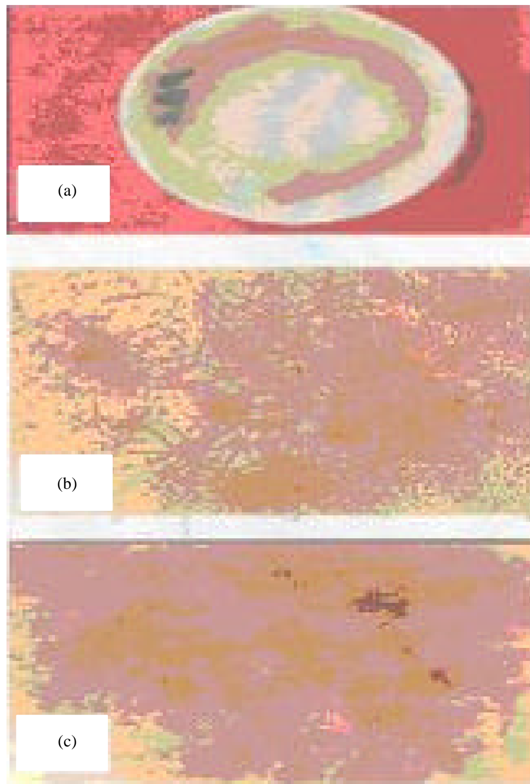

Fig. 2: (a) Culture figure of *Fusarium solani*; (b) shows the micro and macro spores; (c) shows chlamydospores and large macrospores

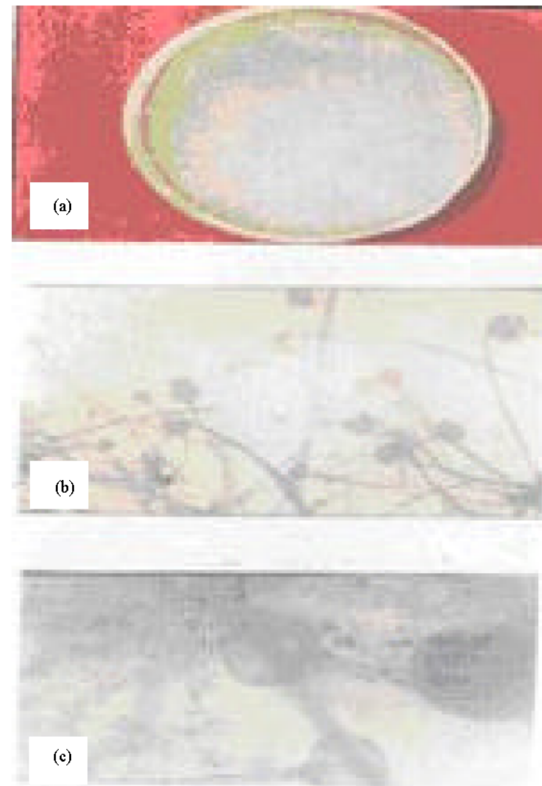

Fig. 3: (a) Culture figure of *Rhizopus oryzae*; (b) shows mycelium and sporangium; (c) shows the columella, sporangium and spores

(1972) and Booth (1971) as *Fusarium solani* Mart sacc. *Rhizopus oryzae* Went, *Batryodiplodia theobromae* Pat. and *Fusarium oxysporium* Schlechr Ex Fr. (Table 1 and Fig. 2-5). The percentage occurrences of the isolates (Table 2) showed that *Fusarium oxysporium* and *Botryodiplodia theobromae* occurred more than others, 33.3% each. The mean diameters of rots exhibited by the respective fungi were shown in Table 3 and Fig. 6-9, *Rhizopus oryzae* exhibited a wider rotted area (25.37 cm) in the pathogenicity tests followed by *Botryodiplodia theobromae* (17.20 cm). *Fusarium solani* (12.65 cm), *Fusarium oxysporium* (1.38) and control (0.0 cm).

The *Fusarium* species produced dry rots light brown to orange brown in colouration. *Rhizopus oryzae* produced extensive wet rot light brown in colour while *B. theobromae* also produced wet rot coffee to black colouration but not as extensive as that of *R. oryzae*. The control produced a brownish thickened layer around the cut area probably due to healing process in response to the cut. The organisms associated with the rot of white yam in this study were *Fusarium solani*, *Rhizopus oryzae*,

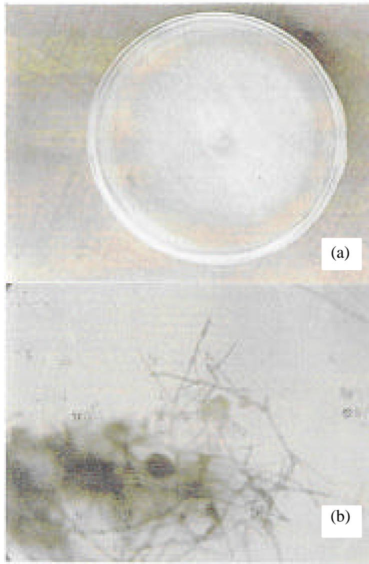

Fig. 4: Culture (a) and (b) hyphae, conidium and chlamydospores of *B. theobromae*

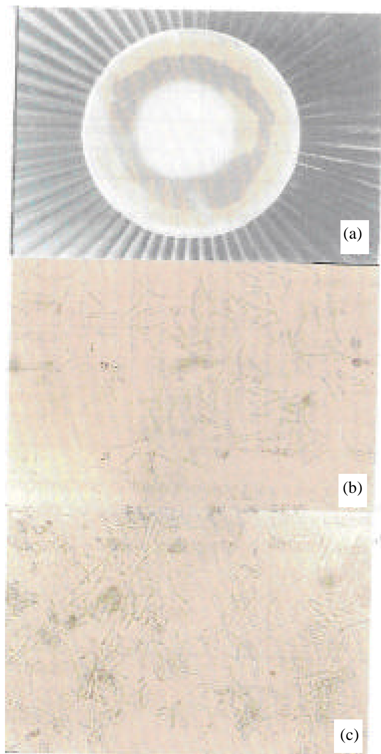

Fig. 5: (a) Conidia (b) Hyphae (c) chlaymydospores of *Fusariyum oxysporum*

Fusarium oxysporium and *Botryodiplodia theobromae.* These fungi have been associated with post harvest rots (Ogundana *et al.*, 1970; Okigbo, 2004). Rotting in storage probably starts in the soil and progresses in storage. In

Table 2: Percentage occurrence of isolated fungi in the rotted yam

Isolates	Occurrence (%)
Fusarium solani	33.33
Rhizopus oryzae	33.33
Botryodiplodia theobromae	16.66
Fusarium oxysporum	16.67

Table 3: Rot measurement of the pathogenicity tests

Isolates	Mean length of rot (cm)	Mean diameter of rot (cm)	Area of rot (cm)
Fusarium solani	2.3	1.75	12.65
Rhizopus oryzae	1.9	4.25	25.37
Botryodiplodia theobromae	1.5	3.65	17.20
Fusarium oxysporum	0.4	1.10	1.38
Control	0.0	0.00	0.00

Mean replication±SD (mm)

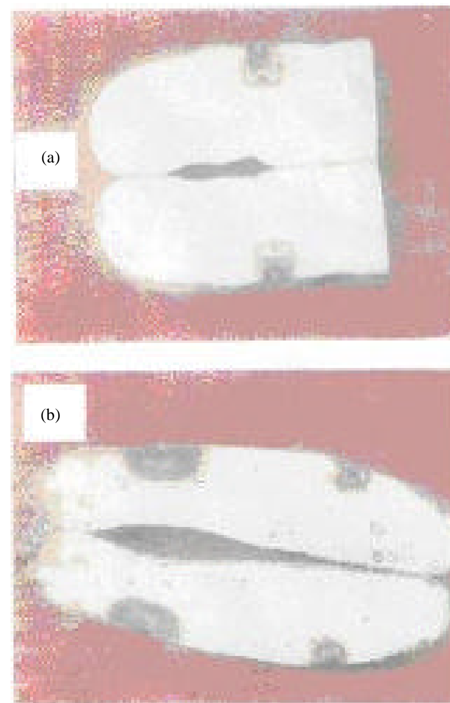

Fig. 6: (a) Rot caused by *Fusariyum oxysporum* (b) Rot caused by *Botrodiplodia theobromac*

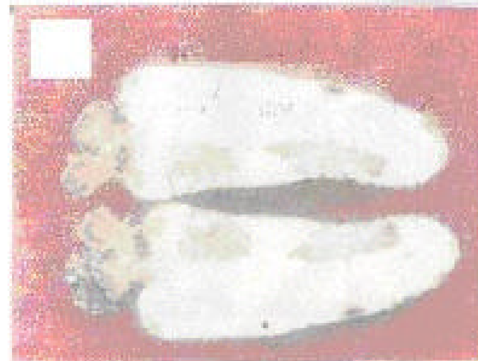

Fig. 7: Rot caused by *Rhizopus stolorifer*

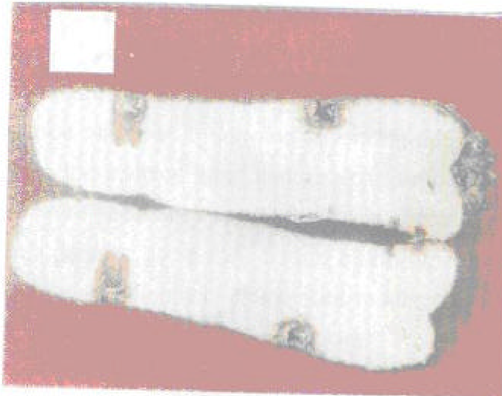

Fig. 8: Rot caused by *Fusarium solani*

Fig. 9: Control tube with no organism

most cases, microorganisms gain access into yams through natural openings and wounds that occur during harvesting and transportation from field to storage barn (Ogundana *et al*., 1970). The soil adhering to the harvested tubers contain many microorganisms that could be pathogenic to the tubers. Osagie (1992) and confirmed this ascertion.

CONCLUSION

The thick layering on the surface of the control tuber in the pathogenicity studies was probably a healing process in reaction to the cut since the tissues were still living and performing physiological functions supported by Ekundaya and Naqvi (1972).

REFERENCES

Amusa, N.A., A.A. Adegbite, S. Mohammed and R.A. Baiyewu, 2003. Yam disease and its management in Nigeria. Afr. J. Biotechnol., 2: 497-502.

Barnet, H.L. and B.H. Barry, 1972. Illustrated Genera of Imperfect Fungi. 2nd Edn., Macmillan Publishing Coy. 886 Third avenue, New York pp: 216.

Booth, C., 1971. The Genus Fusarium, Common Wealth Agric. Bureaux, Central Wales, Farnham Royal Bucks, England, pp: 81-167.

Cheesebrough, M., 2004. Distinct Laboratory Practices in Tropical Countries. Cambridge University Press, New York, USA., pp: 50.

Coursey, D.D., 1983. Post Harvest Losses in Perishable Food in the Developing World. In: Post Harvest Physiology and Crop Preservation, Usherman, M. (Ed.). Plarnium Publication Cooperation, USA., pp: 56-72.

Ekundaya, J.A. and S.H.Z. Naqvi, 1972. Pre harvest microbial rotting of yams (*Dioscorea* sp.) in Nigeria. Trans Brmycol. Soc., 58: 15-18.

Kay, D.E., 1987. Root Crops Tropical. Development and Research Institute, London, pp: 205-206.

Noon, R.A., 1978. Storage and market diseases of yams. Trop. Sci., 20: 177-188.

Ogundana, S.K., S.H.Z. Naqvi and J.A. Ekundayo, 1970. Studies on soft rot yams in storage. Trans. Br. Mycol. Soc., 56: 73-80.

Okaka, J.C., P.A. Okorie and O.N. Ozon, 1991. Quality evaluation of sundried yam chips. Trop. Sci., 30: 265-275.

Okigbo, R.N. and F.E.O. Ikediugwu, 2000. Studies on biological control of post harvest rot of yam (*Dioscorea* sp.) with *Trichoderma viride*. J. Phytopathol., 148: 351-355.

Okigbo, R.N. and U.O. Ogbonna, 2006. Antifungal effects of two tropical plant leaf extracts (*Occimum gratissimum* and *Afromonum meleguata* on Post harvest yam (*Dioscorea* sp.). Afr. J. Biotech., 5: 727-731.

Okigbo, R.N., 2004. A review of biological control methods of post harvest yam (*Dioscorea* sp.) in storage in south eastern Nigeria. KMITL Sci. J., 4: 207-215.

Onayemi, O., 1983. Observation on the dehydration characteristics of different varieties of yam and cocoya. Proceedings of the 6th Syposium of the Iternational Society for Tropical, February 1983, Peru, pp: 252-270.

Osagie, A.U., 1992. The Yam Tuber in Storage. Post Harvest Research Unit, Nigeria, pp: 247.