Comparative Assessment of Pathogenicity of Storage Rot Causing Fungi of Cocoyams *(Colacasia Esculenta*) (L.) Schott and their Host-Pathogen Interactions

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Abstract

Pure isolates of five storage rot causing fungi of cocoyam (*Colocasia esculenta*) (*L*)Schott) corms were assessed for their potency in causing rot of the corms during storage. The isolates were *Sclerotium rolfsii* Sacc., *Botryodiplodia theobromae* Pat., *Fusarium solanii* (Mart) Sac., *Fusarium* SP. and Rhizopus stolonifer (Ehren ex. Fr) Lind. Each of the fungal organisms were inoculated on five corms of cocoyam free from blemish and were left for two weeks after which the extent of rot was determined by assessing both the area and volume of rot caused by each pathogenic fungus. *S. rolfsii* and *B. theobromae* proved most potent in causing rot with mean percentage volume of 14.50 cm^3 and 10.14cm^3 respectively. The area of rots caused by these two fungal organisms was again significantly higher (P≤0.05) than the rest. The least pathogenic organism was *R. stolonifer* with only 3.10cm^3 mean volume of rot caused and less than 2 cm^2 mean area of rot. Fungal hyphae penetrated the corms of cocoyams from cell to cell both inter-and intra- cellularly in the carbohydrate rich storage parenchyma.

Key words: Comparative Assessment, Pathogenicity, Storage rot fungi, Cocoyam, host-pathogen interactions.

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INTRODUCTION

An appraisal of the major constraints on cocoyam production indicated that it is not due to lack of demand but losses due to field and especially post-harvest deterioration (Ugwuanyi and Obetta, 1996, Okeke, 1981, Lyonga and Nzietchueng, 1987, Nwachukwu and Osuji 2008, Maduewesi and Onyike, 1981). Fungi are the main microbial pathogens that cause storage rot of cocoyam corms (Nwachukwu and Osuji 2008, Ogundana, 1976, Maduewesi and Onyike, 1981). Fungi causing storage rot of cocoyams (*C. esculenta*, *C. antiquorum* and *Xanthosoma sagittifolium*) have been reported in USA, India, Egypt, the Pacific Islands and Nigeria (Onyike and Maduewesi, 1985).

Fungi reported as important rot pathogens include *Fusarium solani* (Mart) Sacc, *F. oxysporium* Schl; *F moniliforme wr. Et* Reg; *F.* avenaceum (Fr.) Sacc; Botryodiplodia theobromae Pat; *Sclerotium rolfsii* Sacc; Botrytis sp; Pythium sp, Phytophthora colocasiae Rac. Rhizoctonia bunoides (Berk and Br.) Sacc. (Onyike and Maduewesi, 1985). In Nigeria, *B theobromae, S. rolfsii, F. salani, F. oxysporium, Fusarium sp;* and *R. stolonifer* have consistently been reported as rot pathogens consistently isolated from corms of *C. esculenta, C. antiquorum* and X. *sagittifolim* during storage. These fungal organisms have also been reported to be the major cause of storage rots of other root and tuber crops which includes yam, cassava and sweet potatoes (Yusuf and Okusanya, 2008, Okigbo et al., 2010; Okigbo and Nwakamma, 2005; Amusa, Adegbite, et al., 2003, Amusa and Baiyewu, 1999, Ogaraku and Usman 2008; Banito, Kpemoua, Bissang and Wydra, 2010; Amienyo and Ataga,2007).

Quantitative pathogenic losses of the stored cocoyams result from the rapid and extensive break down of host tissues by microorganisms especially fungi. The pattern of attack is usually an initial infection normally through wounds caused by harvest bruises and points of detachment from mother corms by one of a few specific pathogenic or saprophytic organisms which grow on the dead moribund tissues remaining from the primary infection (Eze, 1984).

Rot symptoms are characterized by separation of the cells along the line of the middle lamella (Ogundana, 1976). He observed that the rot fungi after penetration, established themselves intracelulary. Arene et al., (1985) observed that growth of the rot pathogen is both inter- and intra-cellulary with resultant complete death of the cells of the parenchymatous tissues. It is well known that for some successful

parasitism of an organ or tissue some chemical barriers in the cells must be overcome. Ogundana, et al., (1971) did establish in their studies on yam that the yam tuber pathogens produce both cellulotytic and pectolytic enzymes extracellularly which are capable of degrading the cellulose microfibrils of the cell wall and the pectin in the lamella of the parenchyma respectively thus breaking the chemical barriers due to these substances to induce the characteristic rot symptom.

For successful control of storage rot pathogen of cocoyam which pose major constraint for their production and utilization, detailed knowledge of the potency and host-pathogen relation of the major storage rot fungi need thorough understanding. This study therefore aims at assessing the potency and the host-pathogen interactions of the major storage rot fungi of cocoyam (*C. esculenta*).

MATERIALS AND METHODS

Collection of materials for the study: Freshly harvested corms of cocoyam (*Colocasia esculenta*) (L.) Schott) cultivated at Onuiyi, Nsukka in Enugu State of Nigeria free from blemish were used for this study. Pure isolates of *Sclerotium rolfsii,* Sacc; *Botryodiplodia theobromae* Pat; *Fusarium solani* (Mart) Sacc; *Fusarium sp* and *Rhizopus stolonifer* (Ehren ex. Fr.) Lind obtained from plant Pathology Laboratory of the Department of Botany, University of Nigeria, Nsukka, Nigeria, were employed for this study. These were fungal organisms which had earlier been established as the major rot pathogens of cocoyam corms during storage.

Pathogenicity assessment studies of fungal organisms: To assess the potency of the fungal organisms causing rots, freshly harvested corms of cocoyam were washed first with ordinary tap water ten times before surface sterilizing with 5% commercial bleach (chlorox) and allowed to dry for three hours. After, cylindrical cores, 15 mm long were removed with a 4mm diameter sterile cork borer from the middle portion of each corm. Discs 4mm of 7-day old fungal cultures of each fungal organism were plugged into the holes in the corms and the cores of cocoyam from the corm were replaced after 2 mm pieces have been cut-off to compensate for the thickness of the agar inoculum. The replaced cores were then sealed with Vaseline under aseptic conditions. Discs of uninoculated potato Dextrose Agar (PDA) were used as controls. Each organism under study was inoculated on ten cocoyam corms described above which served as a replicate and there were three replicates for each of the fungal organism under study. All treated corms were placed on the raised platform in the screen house under natural conditions (28± 2 oC) of storage for two weeks.

The extent of rot was determined by first cutting the inoculated corm longitudinally into two halves with a sterile knife at right angles to the hole through which the inoculum was introduced and examined for rot development. To compare the pathogenicity of each fungal organism under study, the degree of rot development in the inoculated corms was assessed. Measurements were taken along the semi-major axis and semi-minor axis at right angles to each other for both the entire corm and the rotted portion only and their means recorded. From these measurements, the volume of rot caused by each of these known pathogenic fungus was determined by employing the formula for finding the volume of an elliptic cross-section which is:

$V = 2\pi b^2 (1-a/3)$ where v = volume in cm^3

a = measurement of semi-major axis and

b = measurement of semi-minor axis

This formula was adapted from Vygodsky, (1984).

The mean volume of rot for the three replicates for each fungus under study was obtained from the calculations above. The data was analyzed using one-way analysis of variance (ANOVA) and the means were separated using Duncan's Multiple Range Test (DMRT).

Determination of Host-Pathogen Interactions: Two sets of cocoyam (*C. esculenta*) corms consisting of five corms per set were employed for this investigation. One set consisted of corms naturally infected and showing moderate to severe (25-30%) rotting and also exhibiting characteristic symptoms of rot. This set was randomly selected from corms stored in a traditional way of heaping on shaded area. The other set consisted of five freshly harvested corms free from any damage or infection which were artificially inoculated with the above mentioned pathogenic fungi. Inoculations of corms with these fungal organisms were carried out as described above and incubated for a period of 8-days at laboratory room temperature of 28 ± 2^0c

Discs of cocoyam corms showing rot from each of the two sets described above were embedded in candle wax and sectioned with microtome and the sections were observed under light microscope with 10 x 40 magnification after staining with cotton blue in Lactophenol. Some of the sections were stained with Lactophenol in acid Fuschin. Uninoculated discs were similarly sectioned and investigated anatomically after staining as mentioned above.

RESULTS AND DISCUSSION

Differences were observed in the pathogenic potency of the different fungal organisms tested. The fungi that proved most potent in causing rot was *S. rolfsii* (Plate 1 & Table 1). This was followed by *B. theobromae* and *F. salani* which caused severe rotting while *R. stolonifer* caused least rot (Table 1 and Fig 1). However, rot caused by *Fusarium* sp did not differ significantly (P≤0.05) in pathogenicity from *R. stolonifer.* Generally, significant difference occurred (P≤0.05) among the fungal organisms tested both in mean rot severity index scores and mean volume of rot caused (Table 1).

Table1: Mean Volume (cm^3) of rot caused by each pathogenic fungus after 14 days of inoculations.

Fungal Pathogen	Volume of rot (cm^3)
S. rolfsii	14.74[a]
B . theobromae	10.17[a]
F . solani	8.97 [bc]
Fusarium sp	6.84[d]
R . stolonifer	2.73[e]
Control	0.00[f]

Means followed by the same letter in the column are not significantly different (P≤0.05).

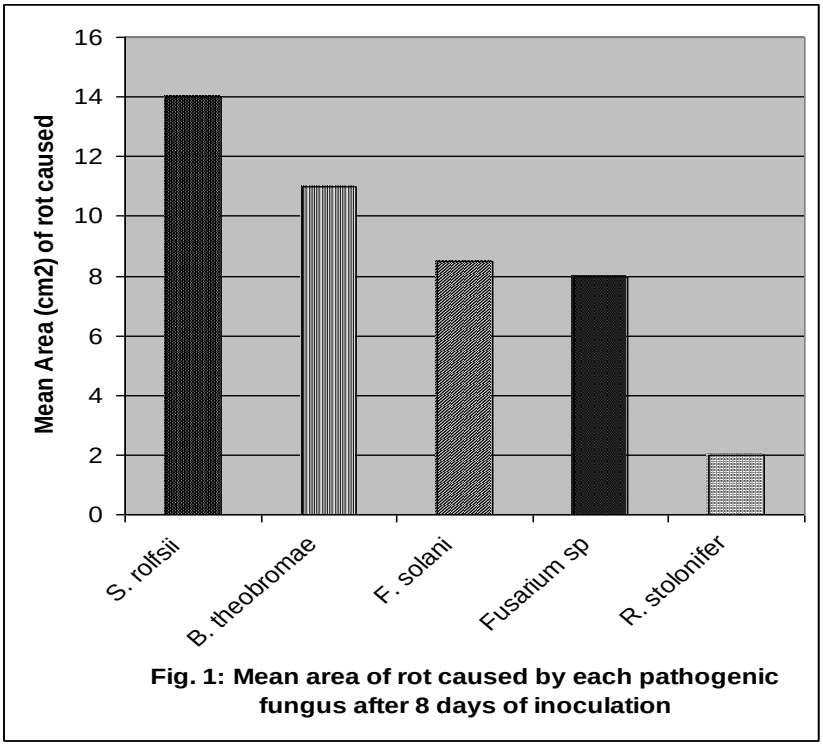

Fig. 1: Mean area of rot caused by each pathogenic fungus after 8 days of inoculation

Earlier workers (Adeniji, 1970a, Okeke, 1981) used linear measurements of either radius or diameter of the rotted tissue to determine the extent/degree of rot caused by the pathogenic microorganisms. These type of measurements gave only the superficial idea of the extent of rot or damage caused but does not actually show the degree/extent of tissue damaged by the microorganisms.

This study therefore presents a better understanding of the true situation of the extent of damage caused by each fungal pathogen on the corms by quantifying the rotted or damaged tissue in relation to the healthy tissues. The use of this volume method in quantifying the degree/extent of damage caused by pathogenic microorganisms in the cocoyam corms and other root and tuber crops is therefore recommended.

Studies on host pathogen interactions showed that fungal hyphae penetrated the cocoyam corm tissue cells both inter and intra cellularly. Fungal hyphae were observed ramifying between and within the

corm tissue cells in the carbohydrate rich storage parenchyma. The penetration from one cell to another was observed to occur through the cell walls. It was also observed that the infected cells disintegrated and the cell walls had dark to dark brown colour.

Previous work on *X. sagittifolium* by Ogundana (1976) showed that the rot fungi after penetration, established themselves intracellular only. However, Arene, et al., (1985) in their studies on yam observed that the growth of the rot pathogen is both inter and intra-cellular with resultant complete death of the parenchymatous tissues.

In conclusion, this study has shown that the degree of rot caused by different fungi associated with storage rot of cocoyam (*C. esculenta*) differ significantly. The study also showed that fungal hyphae penetrated the cocoyam corms from cell to cell both inter and intra-cellularly in the carbohydrate rich storage parenchyma.

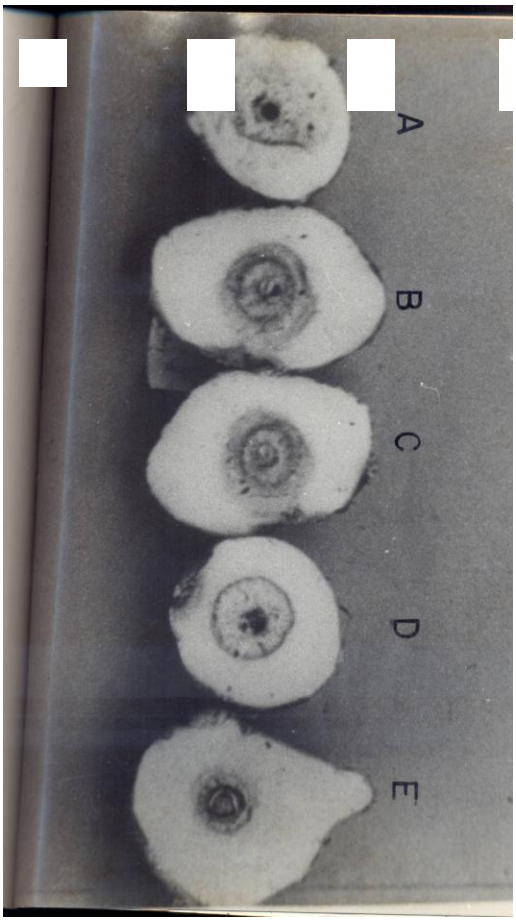

Plate 1: Rot of cocoyam (*C. esculenta*) 8 days after inoculation of corms with indicated fungus: A*, Sclerotium rolfsii; B. Botryodiplodia theobromae; C. Fusarium solani; D. Fusarium sp; E. Rhizopus stolonifer*

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