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Pathogens Associated with Cutrus Fruit Rots in Imo State of Nigeria

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Abstract: The study of pathogens associated with the rot of citrus fruits in some parts of South Eastern Nigeria presented four pathogens identified as *Phytophthora citrophthora* (*Butler*), *Fusarium oxysporum schlecht*, *Fusarium equiseti* (*Corda*) Sacc and *Botryodiplodia theobromae* (pat). The study showed that the rot caused by *B. theobromae* was more severe, followed by *P. citrophthora* rot, *F. oxysporum* rot and *F. equiseti* rot. Some disease control measures were recommended.

Key words: Pathogens, citrus, fruits, rots, *Fusarium oxysporum*, *Botryodiplodia theobromae*, *F. oxysporum*, *P. citrophthora*

INTRODUCTION

Citrus is a tropical plant cultivated extensively in the sub-tropics with a Mediterranean climate (Mumoz, 2003). According to Micole (2000), the fruits are used in making fruit salad and fruit juice. Mumoz (2003) recorded that their juice are palatable and can be taken as mild laxatives in constipation. Their essential oils are used in the cosmetic and pharmaceutical industries (Frazier and Westhofe, 1978).

In the whole world, the post-harvest rots of these fruits have been extensively studied. Fatemi (1972) reported *Citrus* fruits decline in Iran due to fruit rot incited by *Pythium aphanidermatum* (edson) fitz and *Phytophthora citrophthora* (Butler). Graham and Timmer (1994) also described *Phytophthora nicotianae* and *P. citrophthora* as the most common causal organisms of brown rot of *Citrus* fruits in Florida. Timmer and Menge (2000) implicated *P. citricola* Sawada, *P. citriophthora* (R.E. Sm and E.H. Sm) Leonian, *P. hibernalis* Carne and *P. nicotianae* Breda de Haan var. parasitica (Dastur) as causal agents of brown rot of fruits. They also described *Botryodiplodia theobromae* (Pat.) Griffon and Maubl as stem end rot pathogen. *Fusarium oxysporum* Schlechtend Fr. F. sp. citri was also described by them as a causal organism of dry fruit rot of *Citrus*.

In Nigeia, Adisa and Fajola (1982) implicated *Penicillum digitalum* and *P. citrinum* as pathogens of *Citrus fruit rots* in South Western Nigeria. They also considered *B. theobromae* as the most important fruit pathogen in South Western Nigeria. Nzekwe (1996) found out that *Fusarium*, *Curvularia*, *Aspergillus* and *Penicillum* sp. cause *Citrus fruit* rots in Abia State of Nigeria. Dim (2004) isolated and recorded *Aspergillus* sp., *B. theobromae*, *Botrytis cinerea Pers. Fr.*, *Fusarium* sp., *Phytophthora* sp., *Rhizopus stolonifer* (*Ehrenh fr*) Vuill, *Syncephalastrum racemoses*, *Gloesporium nervisequum* and *Mucor racemosus* in some harvested *Citrus* fruits in Imo State of Nigeria.

From the look of things and available materials, it seems that no investigation appears to have been carried out on the extent of fruit rots or the epidemiology of fruit rots in South Eastern Nigeria.

This study therefore, looked into the incidence and severity and extent of citrus rots in some parts of South Eastern Nigeria with a view to suggesting some control measures.

MATERIALS AND METHODS

Rotted fruits were collected from the plantations and markets between September and October, 2008 in clean and labeled polyethylene bags to the laboratory where isolations were carried out by swabbing diseased fruits with 0.01% $HgCl_2$ and rinsed with sterile distilled water. Discs (3 mm thick) of rotted tissues were then cut under aseptic conditions and plated on Sabourands Dextrose Agar (SDA) and incubated at 30°C. Isolates were identified using Bannet and Hunter (1987) and Paul *et al.* (1983).

In the pathogenicity tests, pure isolates of each type of rot were inoculated into healthy fruits at the same stage of ripening. The healthy fruits were surface sterilized with 0.01% $HgCl_2$ and washed in changes of sterile distilled water. A cork borer (5 mm in diameter) was driven to a depth of 4 mm into the fruits making sure that the bored tissues were not removed after withdrawing the cork borer.

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Two drops of a spore suspension (5×10^{4} mL^{-1}) of each isolate were deposited around the wound outline made on the healthy fruits. In the controls, two drops of sterile distilled water were used. For each isolate, 10 treatments and 10 controls were set up. Each inoculated fruit was vaselined at the point of inoculation and placed in a micro-humidity chamber at 25°C for the first seven days. Regular observations were made and re-isolation of any pathogenic fungi was done for comparism with the original isolate.

During this period, the extent of rot caused by each isolate was measured at interval with ruler (in cm) and also recorded. The data obtained was subjected to analysis using Analysis of Variance at 5% level of significance between the extent of rots caused by the four isolates.

$$F = \frac{MSB}{MSW}$$

RESULTS AND DISCUSSION

During the study, four organisms (pathogens) were isolated, characterized (Table 1), identified as *Fusarium oxysporium* Schlecht, *Phytophthora citrophthora* Butler,

Table 1: Characterisation and identification of isolates

Isolate	Colony features	Microscopic features	Remark
1st	Isolate/organism was seen in a culture growth rate was slow. Mycelium was in a white colony, delicate with purple tinge, sparse and sometimes abundant (Fig. 1)	Under the microscope, the organism was seen to have conidia of varied sizes. Micro conidia borne on simple conidiosphore arising laterally on the hyphae. Microconidia generally abundant, variable, oval, ellipsoid, cylindrical, straight to curved structure. Macroconidia sparse and thin walled, generally 3-5 septate and pointed at both ends. There was presence of chlamydospore (Fig. 1)	Isolate identified as *Fusarium schlechti*
2nd	Colony growth was rapid with dense aerial growth. Mycelium white but turned tan to brown colour with age. Underneath surface brownish	Most of macro conidia strongly septate present, sickle shaped with distinctive curvature. Apical cell more elongated in curvature and basal cell foot shaped. Chlamydospore abundant and thick walled (Fig. 2)	Isolate identified as *Fusarium equiseti* (Corda) Sacc.
3rd	Isolate seen as white dense fast growing culture, extensive in growth but gradually formed dirty white to black (Fig. 3)	Short simple conidiosphore was seen, conidia dark, ovoid to elongate. Mature conidia 2-celled, intercalary chlamydospore shown in Fig. 3	Identified as *Botryodiplodia theobromae* Pat.
4th	Colony whitish culture, gradually spreading not profuse but flat and depressed. Underneath colour is milk to yellowish. Aerial mycelium abundant, colony rapid growth (Fig. 4)	Mycelium highly branched and non-septate when young but an old cultures mycelium became septate bearing reproductive bodies (sporangia). The sporangium lemon shaped was borne symbolically on short sporangiosphores. Sporangium ovoid narrow at base produced singly. Sporangia production is sparse, few chlamydospheres	Isolate identified as *Phytophthora palmivora* (Butler)

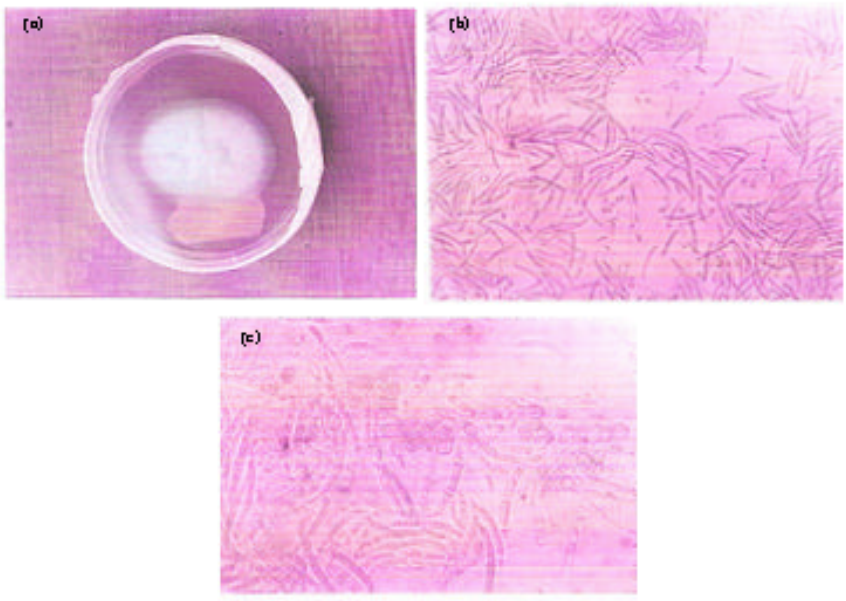

Fig. 1: a) Culture, b) Conidia and c) Chlamydospores of *Fusarium oxysporum*

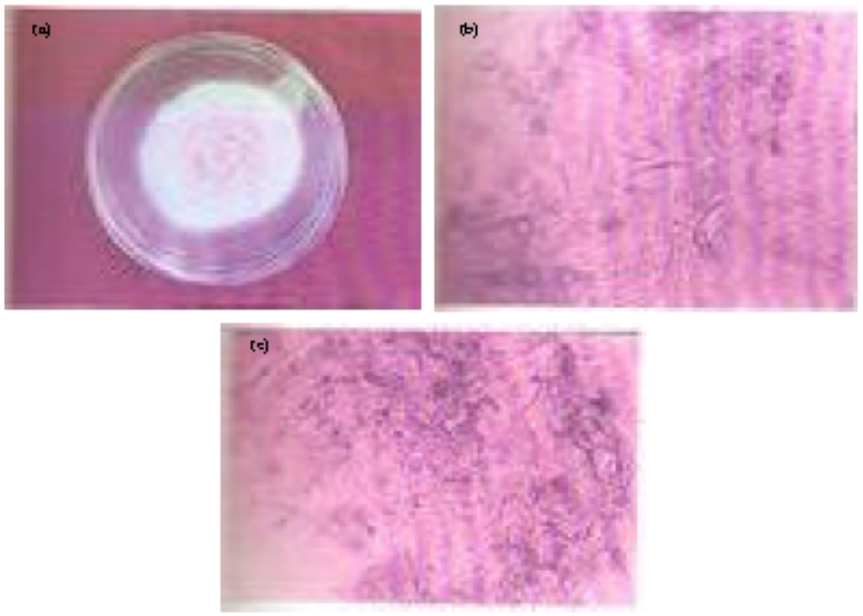

Fig. 2: a) Culture, b and c) Hyphae, conidia and chlamydospores of *Fusarum equiseti*

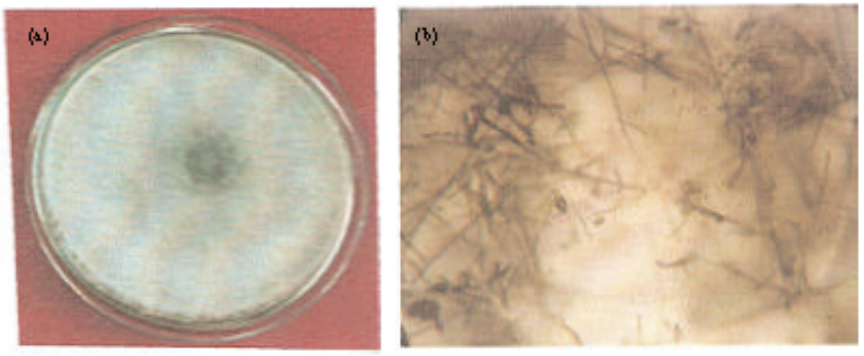

Fig. 3: a) Hyphae, b) Conidium and chlamydospores of *B. theobromae*

Botryodiplodia theobromae Pat and *Fusarium equiseti* (Corda) (Fig. 1-4). Pathogenicity studies confirmed them to be responsible for citrus fruit rot.

During the pathogenicity test, *P. citrophthora* was found to be associated with the fruits infected by browb rot, *B. theobromae* associated with those infected with brown leathery rot and stem end rot and *Fusarium* sp. were found in association with fruits infected with dry rot. This confirms the works of Graham and Timmer (1994) and Timmer and Menge (2000) that *Phytophthora* sp. cause brown rot of *Citrus* sp. and also that *B. theobromae* caused stem end rot of *Citrus* fruits. They also implicated *Fusarium* sp. as causal organisms of dry fruit rots.

The extent of the rot caused by *B. theobromae* seems to be more severe (Table 2). This means that the rot it caused advanced considerably within 5 days. This confirms the findings of Adisa and Fajola (1982) that *B. theobroamae* is the most important *Citrus* fruit rot in the South Western Nigeria. It was also observed that the extent of rot caused by *P. citrophthora* (5.33 cm within 5 days) as well as the incidence and severity of the rot it causes (brown rot) were all high during the study. This finding is in line with the findings of Timmer and Menge (2000) that *P. citrophthora* as the causal agents of brown rot diseases of *Citrus* fruits. The extent of rots caused by the two *Fusarium* sp. were moderate and the incidence and severity of the types of rot they cause (dry rot) was also moderate. This agrees with the research of Dim (2004) who mainly isolated and recorded *Fusarium* sp. as been associated with diseased *Citrus* fruits in Imo State of Nigeria.

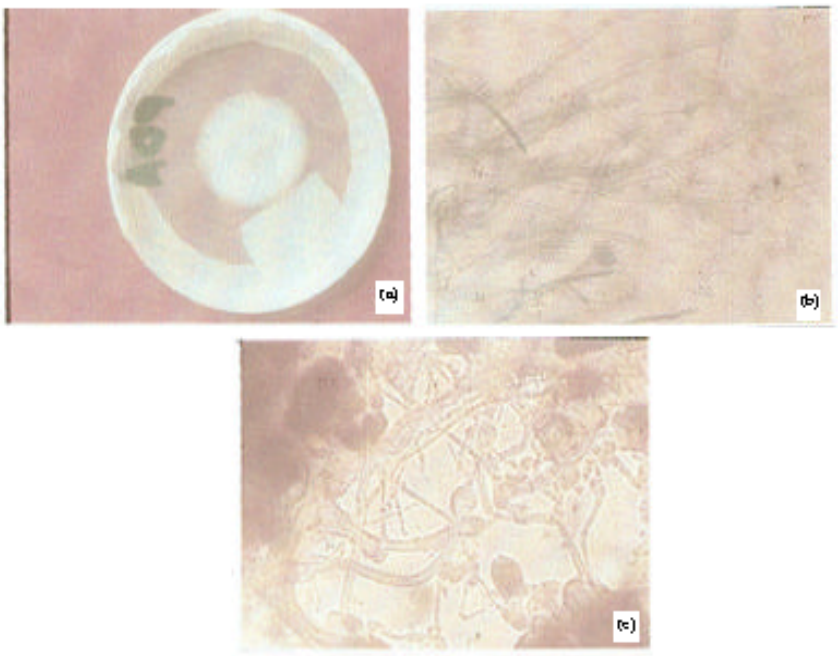

Fig. 4: Culture, a) Hyphae, b) Sprangia of *P. citrphthora*

Table 2: The extent of rots caused by the four fungi isolates (0.05)

Isolates	3rd day (cm)	4th day (cm)	9th day (cm)	Average (cm)
P. citrophthora	2.90	5.10	8.00	5.33
B. theobromae	3.00	4.90	9.20	5.70
F. oxysporium	0.50	1.10	2.70	1.43
F. equiseti	0.10	0.20	0.60	0.30

Data on average of three determinations from isolations

CONCLUSION AND RECOMMENDATIONS

The results of this study showed a high incidence and severity of *Citrus* fruit rot caused by fungal pathogens like *B. theobromae*, *Fusrium* and *Phytophthora* sp. cause fruit rots in the areas studied.

Care should be taken as a disease control measure to avoid wounds on the citrus fruits especially during harvesting and transportation of citrus fruits since wounds encourage easy entrance of the pathogens. Fruits should not be plucked with sticks but be done by climbing the citrus trees with jute bags hung on the tree which is lowered with rope when full. The harvesting is better done when the fruits are mature green. Harvested fruits should be wrapped in papers in baskets during transportation to avoid wounds on the fruits. Most post harvest diseases of citrus fruits are infections that established before harvest. Therefore, pre-harvest treatment or control of the pathogens with fungicides are necessary.

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