

## PATHOGENICITY OF FUNGI ASSOCIATED WITH THE COCOYAM ROOT ROT BLIGHT COMPLEX IN SOUTH-EASTERN NIGERIA.

**Wokocha, R. C. and Aduo, B. C.**

Dept. of Plant Health Management, Michael Okpara University of Agriculture,  
Umudike, P.M.B. 7267, Umuahia Abia State, Nigeria

### ABSTRACT

*Disease surveys were carried out in major cocoyam (*Xanthosoma sagittifolium* (L) Schott) growing communities of Igbariam, Lowa and Umudike in the humid tropics of South-eastern Nigeria in 2009 and 2010 cropping seasons. The highest mean disease incidence of 46.7% and severity of 2.85 occurred in Lowa while the lowest figures of 33.3% and 2.33, respectively were recorded in Umudike. Isolations of associated fungi made from infected cocoyam root tissues and rhizosphere soils yielded eight different fungal isolates. These were *Aspergillus niger* Thom and Raper, *Botryodiplodia theobromae* Pat., *Fusarium solani* (Mart.) Sacc., *Pythium myriotylum* Dresch., *Rhizoctonia solani* Kuhn, *Rhizopus nigricans* Ehhrenb., *Trichoderma harzianum* Rifai and *Sclerotium rolfsii* Sacc. The results showed that *P. myriotylum* was the most frequently isolated organism in infected cocoyam roots in the three locations with percentage frequency of occurrence of 82–90% and 60–72% abundance in infected soils. Pathogenicity tests on susceptible cocoyam variety – *Xanthosoma sagittifolium* cv *ede ocha* (NXS001), using all fungal isolates, showed that only *P. myriotylum* was pathogenic on the test crop. The results of this investigation showed that the cocoyam root rot disease was incited by a single organism viz: *Pythium myriotylum* and not by a complex of organisms as suggested by earlier workers.*

**Keywords:** *Cocoyam root rot blight complex; cocoyam root rot disease; Pythium myriotylum, Cocoyam, Xanthosoma sagittifolium*

### INTRODUCTION

Cocoyam (*Xanthosoma sagittifolium* (L) Schott) is an important tropical root crop cultivated in rural parts of America, the Caribbeans, the Pacific and West Africa (Arene and Ene, 1987; Tambong *et al* 1999). It is a herbaceous monocotyledonous plant of the family Araceae, characterized by the presence of underground stem (corm), net-veined, palmately divided broad leaves with long petiole, spadix inflorescence and superficial but fibrous root system (Purselove, 1972; Dutta, 2005).

Cocoyam is primarily adapted to moist environment. The Mean annual temperature for its cultivation is about 24°C with variations ranging from 13°C to 29°C (Kay, 1987). Optimal yield takes place in deep well drained rich soils with pH of 5.5-6.5 (Arene and Ene, 1987 and Adiobo *et al.*, 2007). Nigeria is the largest producer of cocoyam in the world with estimated annual production of 5.45 million metric tons (FAO, 2007). Cocoyam is grown popularly in the south-eastern Nigeria. Cocoyam

has become an endangered crop on the verge of extinction in the last four decades. One reason for this is that it yields low financial returns and so there is the notion that it is a poor man's crop (Mbanaso *et al.*, 2008). But cocoyam corms and cormels are rich sources of carbohydrate. The protein content is higher in total essential amino acids and the sulphur bearing amino acids than other staple foods such as yam, cassava, etc. (Kay, 1987).

Cocoyam production in Nigeria dropped by 11% between 2000-2004 due to several field and storage diseases (FAO, 2001; FAO 2004) Fungal diseases of cocoyam include Cocoyam Corm Rot incited by *Sclerotium rolfsii*, White Leaf spot caused by *Leptosphaerulina trifolli* and Anthracnose disease caused by *colletotrichum gloeosporiodes*. The most important fungal disease of cocoyam (*Xanthosoma spp.*) is the Cocoyam Root Rot Disease (Pacumbaba *et al* 1992, Tambong *et al* 1999). Other cocoyam diseases include bacterial

leaf spot or blight caused by *Xanthomonas campestris* pv *aracearum* and *X. axonopodis* pv *dieffenbachiae*. Insect pest and Nematodes attack growing tubers while viral diseases of cocoyam result in leaf distortion, vein chlorosis but not in Africa (Adiobo *et al* 2007). The cocoyam root rot disease was reported by Okeke (1982) and Arene and Ene (1987) as caused by a complex of organisms viz: *Botryodiplodia theobormae*, *Fusarium solani*, *Rhizoctonia solani*, *Fusarium moniliforme*, *Sclerotium rolfsii*, *Pythium* spp and *Phytophthora* spp. Characteristic symptoms of cocoyam root rot disease include premature leaf yellowing, reduced leaf size and numbers, root decay, root pruning and severe reduction of the root system (Tambong *et al* 1999). According to Pacumbaba *et al* (1992) severe infection of *Pythium myriotylum* incited the root rot disease and resulted in yield reduction as high as 90% in Cameroon. The etiology of the disease in Nigeria has, however, remained uncertain. Earlier workers (Okeke, 1982; Arene and Ene 1987 and Mbanaso *et al* 2008) have identified the disease as cocoyam root rot blight complex (CRBC) caused by a number of organisms. The objective of this study was to isolate and identify fungi associated with the cocoyam root rot disease and to determine the pathogenicity of these fungi on cocoyam.

## MATERIALS AND METHODS

Disease surveys were carried out on naturally infected farmers' fields in three locations in major cocoyam growing areas in the humid tropics of south-eastern Nigeria. These locations were: Umudike (Abia State), Lowa (Imo State) and Igbariam (Anambra State). South-eastern Nigeria (04°15'N - 07° 00'N, 05° 34'E - 09° 24'E; Alt.122 MSL, Unamma *et al.*, 1985). It has mean annual temperatures of 27-32°C, and mean annual rainfall 1,600mm – 4,338mm. The soil type is tropical ultisol, with pH of 5.0-6.5 (NRCRI, 2011).

### Disease Survey

A total of five farms each 20 x 50 sq. m in size within each location were surveyed during the wet season (June – September) of 2009 and 2010. In the farms, two diagonal transects were taken across and cocoyam plants on every one meter distance along the transect were sample for disease occurrence according to Umechuruba and Elenwo (1997). Disease assessment was carried out visually by noting characteristic symptoms. Disease incidence on a particular field was estimated by counting the number of plants infected within the sampled area of the field and expressing this figure as a percentage of the total number of plants

sampled;

Disease incidence =  $\frac{\text{No. of infected plants}}{\text{Total No. of plants sampled}}$

Disease severity in each field was assessed by giving each diseased plant a numerical rating similar to that used by Wokocho and Nwaogu (2008) thus:

		1=1-20% of plants infected
2	=	21 – 39% of plants infected
3	=	40 – 50% of plants infected
4	=	51 – 69% of plants infected
5	=	70% of plants infected.

### Collection And Handling Of Field Samples From Diseased Cocoyam roots

A total of five infected cocoyam plants randomly selected from each field were carefully dug up (with a clean spade), placed in separate clean specimen bags and taken to the laboratory for analysis. The materials were stored briefly in the refrigerator before use.

### From the soil.

Before collection of soil sample, the top 6mm of soil around the infected cocoyam plants was cleared to remove organic debris. With a clean trowel, about 100g of the infected rhizosphere soil was removed at a depth of 6 cm and placed inside a clean polyethylene bag and carried to the laboratory for analysis.

Five soil samples from each field were bulked together and air-dried inside a sterile tray covered with muslin cloth for 12 hours. Dried samples were temporarily stored in the refrigerator before use.

### Isolation Of Associated Fungi

#### Isolation from infected cocoyam roots.

Infected roots were freed from soil by washing in sterile water to remove soil particles and other debris. The washed roots were cut with sterile scalpel into sections 3-5cm long, surface disinfected in 1% commercial bleach (sodium hypochlorite for 30 seconds, rinsed in three changes of distilled water and dried between sheets of sterile filter paper. The root sections were plated on fresh potato dextrose agar (PDA) in 9.00cm Petri dishes. The dishes were incubated at  $28 \pm 2^{\circ}\text{C}$  for five days. Pure cultures of the isolates were obtained by successive subculturing on PDA medium and stored as stock cultures on PDA slants inside McCartney bottles in the refrigerator at  $15^{\circ}\text{C}$ .

### Isolations from Rhizosphere Soil Sample

One gram of the bulked soil sample was weighed out and mixed with 9 ml of sterile distilled water in a test tube and the suspension shaken together manually for 10 minutes before allowed to settle. From this stock a ten fold serial dilution was made by transferring 1ml of the suspension into a blank 9 ml of sterile distilled water until a concentration of  $10^{-3}$  was obtained according to Martin (1991). One milliliter of this soil suspension was aseptically transferred into a sterile Petri dish before 15ml of molten PDA medium was poured into the dish. The mixture was gently swirled to achieve even dispersion of soil particles. The plates were incubated at  $28 \pm 2^\circ\text{C}$  for five days. Pure cultures of the fungal isolates were obtained by subculturing into sterile PDA plates and preserved inside PDA slants in McCartney bottles in the refrigerator at  $15^\circ\text{C}$ .

### Microscopic Examination And Identification Of Fungal Isolates

Wet mounts of pure cultures were viewed under a binocular compound microscope at X40 magnification. Identification of the isolates was made by reference to CMI Description of Pathogenic Fungi and Bacteria, Compendium of Soil Fungi (Waterhouse and Waterson, 1966) and Barnett and Hunters, (1987).

### Soil sterilization.

Soil used in this experiment was garden soil collected from the National Root Crops Research Institute (NRCRI) research farm, Umudike. The soil was heat-sterilized in a cut metal drum heated with firewood at  $100^\circ\text{C}$  for 3 hours covered and allowed to cool for 12 hours. The sterilized soil was dispensed into 30 cm diameter plastic pots and wooden trays – 0.5' x 3' x 5' in size. These trays were used as cocoyam seedling nursery

### Multiplication of cocoyam seedlings.

Healthy cocoyam (*Xanthosoma sagittifolium* cv. ede ocha (NXS001) cormels obtained from the Umuahia main market were used. The cormels were first surface disinfected by immersion in 1% commercial bleach (sodium hypochlorite) for 5 minutes and then rinsed in three changes of sterile water before being used. The cormels were planted in the wooden trays at the rate of 30 cormels per tray and watered daily for two weeks to achieve uniform sprouting.

### Preparation of inoculum suspension.

Fungal inocula used in inoculations in this experiment were prepared according to Wokocha *et al* (1986). With sterile spatula the fungal mycelia of ten plates of 5-7 day old PDA cultures of each fungal isolates grown at  $28 \pm 2^\circ\text{C}$  were carefully scrapped into a Waring blender. The materials were blended in 200ml of sterile distilled water and homogenized for one minute at low speed. The resultant suspension of each inoculum was filtered through three layers of muslin cloth. The fungal mat remaining on each filter was washed in several changes of sterile distilled water to remove traces of stalling material. The population of spore and hyphal bits was determined using Hawksley haemocytometer and a tally counter.

### Green House Experiment

Experiments were conducted in the Plant Pathology greenhouse of the National Root Crops Research Institute, Umudike under the mean temperature of  $28^\circ\text{C}$  and relative humidity of 70%.

### Cocoyam seedling transplant and inoculation in pathogenicity test.

Four-week old cocoyam seedlings from the nursery trays were transplanted into 30cm plastic pots at the rate of one seedling per pot. Seedlings were inoculated seven days after transplanting. Just before inoculation, soil around each seedling was gently removed to a depth of 5cm, and 100ml of each fungal suspension was introduced into the opening and the removed soil replaced. Seedlings in the control pots were similarly treated with 100ml of sterile distilled water. The seedlings were watered daily and observations made over a period of 14 days. The experiment was laid out in a simple completely randomized design (CRD) replicated 3 times..

### Statistical Analysis.

In the analysis of data, reference was made to Obi (1995). Data were then subjected to analysis of variance (ANOVA) using Genstat Discovery Edition 3 and treatment means were compared by using Fisher's Least Significance difference (LSD) at 5% level of significance.

## RESULTS AND DISCUSSIONS

Results (Table 1) show that the highest mean disease incidence and severity of 46.7% and 2.85 were recorded for cocoyam in Lowa respectively and lowest disease figures of 33.3% and 2.33 respectively occurred in Umudike. No significant ( $P>0.05$ ) difference occurred among the disease figures in the three locations (Lowa, Igbariam and Umudike) in the 2009 and 2010 cropping seasons. The high disease incidence in Lowa could be due to the marshy wetlands

which exist in the area during most of the rainy season. Waterhouse and Waterson (1966) observed that *Pythium* spp incited root rot diseases with heavy losses under excessive waterlogged conditions with reduced oxygen levels. The sampled locations in the South-eastern Nigeria have similar environmental conditions enabling the development of cocoyam root rot disease. Adiobo *et al.*, (2007) reported differing cocoyam root rot disease incidences and severity in some soil types in Cameroon.

**Table 1: Mean disease incidence and severity of the cocoyam root rot disease on rainfed cocoyam in three locations in South-eastern Nigeria during two successive years: 2009 and 2010.**

Location	2009		2010	
	*D.I(%)	*D. Severity	D.1(%)	D. Severity
Lowa	42.5	2.40	46.7	2.85
Igbariam	33.7	1.97	39.1	2.47
Umudike	33.5	1.83	33.3	2.33
Grand Mean	36.6	2.07	39.7	2.55
LSD	24.18	0.776	25.22	1.122
P	NS	NS	NS	NS

\* D.I. = Disease Incidence

\* D.S. = Disease Severity

**Table 2: Fungi associated with the cocoyam root rot disease (CRRD) in Igbariam, Lowa and Umudike in South-eastern Nigeria and their percentage frequencies of occurrence.**

	Fungi				Location	
	Igbariam		Lowa		Umudike	
	Root	Soil	Root	Soil	Root	Soil
<i>Aspergillus niger</i>	-	66%	-	60%	-	52%
<i>Botryodiplodia theobromae</i>	-	58%	-	-	-	-
<i>Fusarium solani</i>	40%	64%	62%	38%	72%	-
<i>Pythium myriotylum</i>	82%	62%	90%	60%	88%	72%
<i>Rhizoctonia solani</i>	30%	64%	-	60%	-	55%
<i>Rhizopus nigricans</i>	-	67%	-	72%	-	60%
<i>Sclerotium rolfsii</i>	-	80%	-	72%	-	80%
<i>Trichoderma harzianum</i>	-	70%	-	70%	-	75%

- = Absent

**Table 3 The pathogenicity of eight fungi associated with the cocoyam root rot disease on seedlings of cocoyam (*Xanthosoma sagittifolium* cv. ede ocha (NXS001) in the greenhouse at temperature of  $28 \pm 2$ oc and relative humidity of  $70 \pm 10$ %.**

Fungi	Disease Figures	
	Incidence (%)	Severity
<i>Aspergillus niger</i>	Non pathogenic	Non pathogenic
<i>Botryodiplodia theobromae</i>	Non pathogenic	Non pathogenic
<i>Fusarium solani</i>	Non pathogenic	Non pathogenic
<i>Pythium myriotylum</i>	Pathogenic (75%)	Pathogenic (5%)
<i>Rhizoctonia solani</i>	Non pathogenic	Non pathogenic
<i>Rhizopus nigricans</i>	Non pathogenic	Non pathogenic
<i>Sclerotium rolfsii</i>	Non pathogenic	Non pathogenic
<i>Trichoderma harzianum</i>	Non pathogenic	Non pathogenic

A total of eight different fungi were isolated from infected cocoyam root tissues and rhizosphere soils from the three locations (Table 2). These were *Aspergillus niger* Thom and Raper, *Botryodiplodia theobromae* Pat., *Fusarium solani* (Mart.) Sacc., *Pythium myriotylum* Dresch., *Rhizoctonia solani* Kuhn, *Rhizopus nigricans* Ehhrenb., *Sclerotium rolfsii* Sacc. and *Trichoderma harzianum* Rifai. In all the locations, a higher percentage (52-80) of the isolates were obtained from soil than from infected root samples (30-90%). All fungi isolated from the roots were also present in the soil. *Fusarium solani*, *S. rolfsii*, *R. solani* and *P. myriotylum* were consistently isolated from infected root tissues.

The result (Table 3) of the pathogenicity test of all the eight fungal isolates, (Table 3), only *P. myriotylum* was pathogenic on cocoyam seedlings. It produced characteristic necrotic roots and chlorotic leaves on the five-week old cocoyam 10 days after inoculation. *P. myriotylum* was reisolated from the infected roots of the cocoyam. The observation of the characteristic root rot symptoms and the re-isolation of the pathogen from infected cocoyam seedlings showed that *P. myriotylum* incited the root rot disease of cocoyam in this study. The other fungi, particularly those from diseased root tissues may have played a synergistic role in the development of the disease.

*Botryodiplodia theobromae*, *Fusarium solani*, *Rhizoctonia solani*, *Fusarium moniliforme*, *Sclerotium rolfsii*, *Pythium spp* and *Phytophthora spp* as causal organisms of the cocoyam disease called cocoyam root rot blight complex (Okeke 1982; Arene and Ene 1987). The results of this investigation, however, showed that the cocoyam root rot disease was incited by the organism, *Pythium myriotylum* and not by a complex of organism as suggested by earlier workers.

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