

# Fungal Microflora of Cocoa Soils, Sporulation Density and Mycelial Biomass of Three Fungal Isolates from Cocoa Pods in Abia State, Nigeria

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**Abstract** – Soil samples from seven cocoa plantation soils in Abia State viz: Itunta, Elemaga, Isiala-Ibere, Ndume, Etitulo, Bende and Ajata, were collected, bulked and analyzed for fungal microflora content. Different fungal pathogens isolated from cocoa plantation soils within the seven cocoa growing communities studied showed that *T. harzianum* (80%) was the most abundant fungal pathogen isolated from cocoa plantation soils in Bende and it significantly ( $P \leq 0.05$ ) differed from *F. oxysporum* (70%) in Ndume, *R. solani* (56%) in Isiala-Ibere and *A. niger* (55%) in Ndume respectively. *F. oxysporum* and *P. expansum* were isolated from six out of seven cocoa growing communities studied, also *T. harzianum* were isolated only from two cocoa growing communities while *B. theobromae* were from four communities. The spore counts showed that *P. megakarya* (395spores/ml) had the highest spores followed by *F. decemcellulare* (110spores/ml) and *C. ignotum* (10spores/ml) which had the least number of spores. Also, the mycelial biomass of the three fungal isolates showed that *P. megakarya* (0.9g) had the highest weight of mycelial biomass followed by *F. decemcellulare* (6.3g) and *C. ignotum* (0.2g) which had the lowest.

**Keywords** – Fungal Mycoflora, Cocoa, Spore Density, Mycelia Biomass.

## I. INTRODUCTION

Cocoa (*Theobroma cacao* L) is one of the major export crops and an important non-oil foreign exchange earner in Nigeria (ICCO, 2009). In Abia State, annual production capacity was above 100,000 metric between the years (2000 – 2008) and has declined in 2009 (SCDC, 2009). Production has however declined in recent times due to poor soil quality. Previous records on soil survey within the cocoa belt of Nigeria showed over 65% of Nigeria cocoa are grown on good or fairly good soils (Gbadamosi and Sunday, 2010). As in the production of any other crops, soil with adequate mineral and organic nutrition is important in cocoa production. Crop production involves a complex interaction between the environment, soil parameters, pathogens and nutrient dynamics (Slater and Winpenny, 1998).

Epidemics of black pod disease of cocoa (*Theobroma cacao* L) incited by *Phytophthora megakarya* and other fungal pathogens in cocoa plantations is a limiting factor in cocoa production (Bowers, et al., 2001). The disease has been described by some cocoa farmers in Nigeria as a “strange” disease. (Opoku, et al., 2000) apparently

because of the extreme virulence and the heavy losses associated with the pathogen. *Phytophthora megakarya*, *Fusarium spp*, *Rhizoctonia solani* etc are soil borne pathogen amongst other fungal pathogens and it survives or persist in soil for years in the absence of host tissue, but less than 10 months in soil as saprophyte depending on the ground cover (Guest, 2007).

Epidemics of black pod disease of cocoa (*Theobroma cacao* L) incited by *Phytophthora* propagules can be initiated by *Phytophthora propagules* and other fungal pathogens surviving in soils, pods left on trees, plant debris, cherelles and a vast array of microorganisms such as bacteria, viruses, protozoa, algae (Kellam and Zentmyer, 2010). It has been discovered that microorganisms play an important role in the fertility of the soil, their microbial load or abundance and existence is solely hung on the environmental soil condition (Ozer, et al; 2009). ). *P. megakarya* and *P. palmivora* do not produce chlamydospores in cocoa orchard soils but *P. megakarya* has been shown to produce chlamydospores under laboratory conditions (Macarren, et al., 2005).

The search for isolation of fungal pathogens from cocoa plantation soils, evaluation and identification of these organisms would allow the reduction of the inoculum pressure, as well as reduce level of yield losses of cocoa caused, by fungal pathogens (e.g. )*P. megakarya* *P. palmivora*, *B. theobromae* and others (Mpika, et al., 2009). Knowledge of this isolates will be an additional element of the strategy of integrated disease control (Bowers, et al., 2001). Traditionally *Colletotrichum* species have been identified based on morphological characters (Cai et al., 2008) several identifying features have been utilized by taxonomists, including the size and shape of conidia and aspersoria, the presence of setae and sclerotia, acervuli form and telemorph characters (Abang et al., 2009). Cultural characters such as colony, growth rate and texture have also being utilized (Prihastuti et al., 2009, Yang et al., 2009).

The objective of this study therefore was to assess the amount of fungal microflora occurrence, sporulation density, and mycelial biomass of the three fungal isolates from cocoa plantation soils of the seven major producing communities in Abia State.

## II. MATERIALS AND METHOD

Soil samples were collected with clean sample materials from the cocoa plantations of the seven communities namely; Itunta, Elemaga, Isiala- Ibere, Ndume, Ajata, Bende and Etitulo. These communities are located on longitude 07° 33'E, latitude 05° 29'N, altitude 122m above mean sea level. Soil samples were taken from 0 – 15cm depth in rhizosphere with a hand trowel along the diagonals after removal of litter, and also at different locations within the cocoa plantations which were then bulked together to form a composite sample. These soil samples were processed by drying at room temperature and then sieved with 2mm sieve. The sieve samples were properly labeled and taken to the Plant Pathology Unit of the National Root Crops Research Institute, Umudike.

### **Assessment of the Fungal Microflora Occurrence from Cocoa plantation Soils Studied.**

One (1) gramme of each processed soil sample from all the seven studied communities was transferred into 10mls of sterile distilled water contained in an Erlenmeyer 25mls test tubes. The mixture was shaken for 30 minutes using a mechanical shaker and then allowed to stand for two (2) hours. Ten (10) fold serial dilutions (10-1 to 10-10) were made using sterile test tubes, but 10-3 to 10-7 was used for the experiment. After shaken again, one millilitre of the soil suspension was aseptically transferred into a sterile Petri-dish containing 15mls of cool molten PDA medium and gently swirled to disperse the suspension. The plates were incubated by successive subculturing at a temperature of 28t-20c for five (5) days in a humid chamber. The percentage occurrence of the fungi per plate was counted daily and recorded. It was calculated thus:

$\% \text{ Occurrence of each soil fungal microflora (in-vitro)} = \frac{\text{No of each fungal microflora counted/plate/day}}{x100/1}$

Total no of fungi counted/plate/day.

The identification of the fungi was done using International Mycological Institute (IMI) monographs of fungal pathogens and also Barnett and Hunter 1999(5<sup>th</sup> Edition). Each experiment were all replicated three times and the analysis was done using Gen-Stat Discovery Edition 3.

### **Determination of Mycelial Biomass of The Three Fungal Isolates**

#### ***Phytophthora megakarya, F. decemcellulare and C. ignotum in-vitro***

In this study, 100g fresh potato tubers was weighed, peeled, washed and boiled for 20 minutes. The potato broth was obtained by draining out the liquid with a clean muslin cloth. Five (5) grams of dextrose was added into 250mls sterile conical flask and then shaken mechanically. The potato media was autoclaved at 151psi for 1 hour. After cooling, 5mm mycelia discs of 7 days old pure cultures of each test fungi were collected using a sterile cork borer. Each mycelial disc were inoculated into sterile conical flasks containing the liquid medium (potato broth).

These isolates were incubated at 28±2<sup>0</sup>C for 7 days. Mycelia of each isolates/test fungi were placed in pre-weighed Whatman filter paper, oven dried at 60<sup>0</sup>C and weighed again to record mycelial biomass in milligrams. The experiment was replicated three times in a completely randomized design (CRD). The mycelial biomass was calculated using the formula;

$$\text{WFP MB} = \text{WFP} - \text{WmB}$$

Where WFPmB = Weight of filter paper + weight mycelial biomass of the pathogen

WFP = Weight of dry filter paper

WmB = Mycelial biomass of the pathogen (Onifade, 2000).

### **Determination of The Sporulation Density of The Three Test Fungi Isolated From Cocoa Pods (*P. megakarya, F. decemcellulare, C. ignotum*) GROWN ON PDA MEDIA At 28±2<sup>0</sup>C**

The three pure fungal isolates obtained from the cocoa pods were grown on cool molten PDA media. Seven (7) days after incubation, a 5mm mycelial disc of each pure culture was cut from the center portion of the plate and placed in a sterile 10mls test tubes containing sterile water. This was shaken mechanically so that the spores were dislodged. The suspension of each test fungi was filtered through three layers of muslin cloth. One ml of each was placed in a Hawksley haemocytometer and the fungal spores counted after viewing with a compound microscope. The number of spores/ml of each isolate tested were counted ten times. These were estimated using Duncan and Torrance formulation;

$$S = \frac{Nv}{V}$$

Where S = Number of spores/ml

N = Mean number of spores in co large squares

V = 1m = 11,000m<sup>3</sup>

V = volume of spores suspension under cover glass = 0.00 4mm<sup>3</sup>

## III. RESULTS AND DISCUSSION

The result in Fig. 1 showing the occurrence of the fungi isolated from cocoa plantation soils in different cocoa growing communities of Abia State showed that *T. harzianum* (80%) was the most abundant fungal pathogen isolated from cocoa plantation soil in Bende, this figure was significantly (P≤0.05) different from the incidence figures in other locations. This was followed by *F. oxysporum* (70%) in Ndume, *R. solani* 56% in Isiala-Ibere and *A. niger* 55% in Ndume respectively. This agrees with Ozer, *et al*; (2009) who stated that microorganism play an important role in the soil, their microbial load or abundance and existence is solely hung on the environmental conditions of the soil such as moisture. Also that pathogens present in and spreading through the soil are generally unable to cause sudden or widespread epidemics but often cause local slow spreading disease of considerable severity. The percentage occurrence of *A. niger* (4%) from Etitulo and Isiala-Ibere cocoa plantations were similar as shown from the result in Fig. 1. However,

*A. niger* and *A. flavus* were absent in Bende cocoa soils, but the two fungal isolates had the higher percentage occurrence of 55% in Ajata and Etiulo (18%) than other communities. *Aspegillus niger* for instance has high sporulating nature and this is also coupled with their ability to grow well on laboratory media (Oyeyiola and Husseni, 1992), and also agrees with a study by Ekundayo (2004) that the greater the number of propagules, the more severe the disease produced

*Fusarium oxysporum* and *P. expansum* were isolated from six out of seven cocoa plantation soils of the communities studied. The result also indicated that *T. harzianum* were isolated only from two cocoa growing communities while *B. theobromae* were isolated from four communities as shown in Fig .1.

According to Milgroom and Peevent (2003), the greater the number of pathogen propagules (fungal spores and sclerotia etc) within a near field of host plants, the more inoculum reaches the host plant and at earlier time, thereby increasing the chances of an epidemic greatly. It is also noted that some soil borne fungi, produce their inoculum on infected plant parts in the soil, within which the inoculums disperse slowly and present little danger for sudden or widen spread epidemics but often cause local, slow spreading disease of considerable severity. When such primary soil fungi, however, also produce wind disseminated spores, the letter can spread to considerable areas (Magarey, 2001). The result therefore, showed that eight different fungal pathogens were isolated from cocoa plantation soils of the cocoa growing communities sampled.

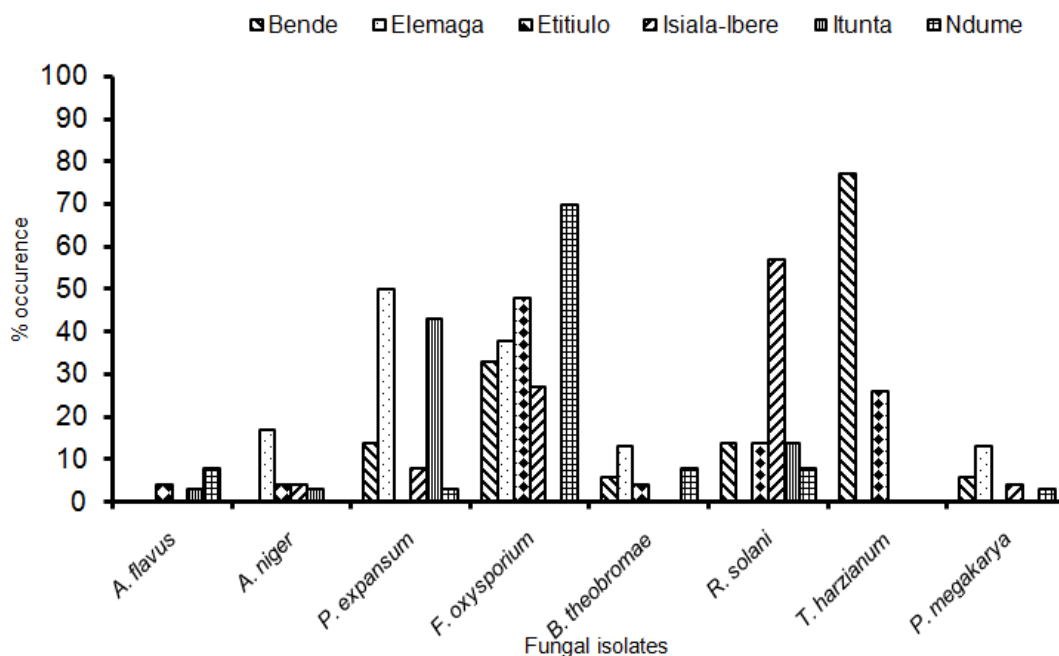


Fig. 1. Percentage (%) occurrence of fungal isolates from the cocoa plantation soils of the seven major cocoa producing areas of Abia State, cultured on PDA media

Furthermore, the result in Fig. 2 showed that isolates of *P. megakarya* significantly ( $P \leq 0.05$ ) gave the highest sporulation density of (395 spores $\mu$ /ml) followed by *F. decemcellulare* with (110 spores $\mu$ /ml) sporulation density higher than that for *C. ignotum* isolates (10 spores $\mu$ /ml) which had the lowest sporulation density at 28 $\pm$ 0C. This result confirmed that *P. megakarya* is the major fungal

pathogen which predisposes cocoa pods to the black pod disease and it had highest production of spores which is an infective part against its host, while *F. decemcellulare* and *C. ignotum* were associated organisms. Many species of *Colletotrichum* spp, infect more than one host, and more than one *Colletotrichum* species may be present on the host (Roberts *et al.*, 2001).

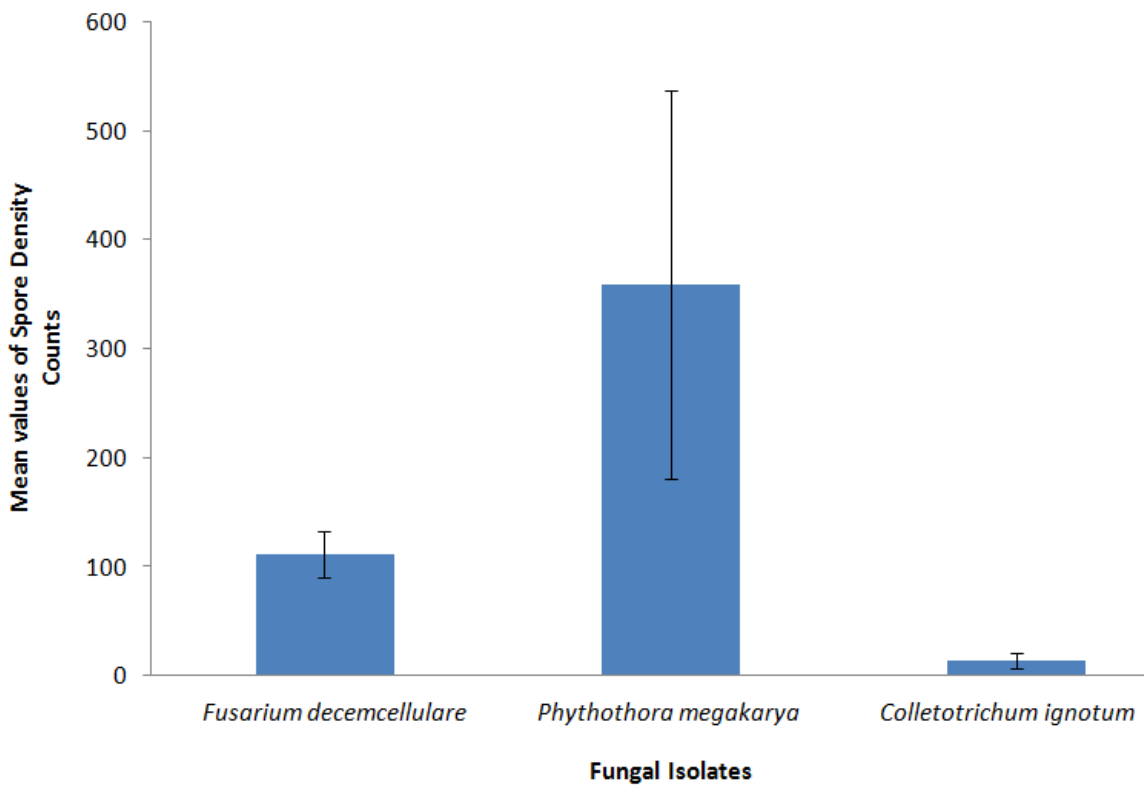


Fig. 2. The sporulation density of the three fungal isolates; *P. megakarya*, *C. ignotum* and *F. decemcellulare* which incites pod rot in cocoa

The evaluation of the mycelia biomass of the three fungal isolates tested as shown in Fig. 3 indicated that *P. megakarya* (0.98g) significantly ( $P \leq 0.05$ ) had the highest weight of mycelial biomass, when compared to *F. decemcellulare* (0.28g) and *C. ignotum* 0.23g which had the lowest weight of the mycelial biomass. These results

are similar to that obtained by Kulkarni (2006) who reported that, the maximum dry mycelial weight of fungus was obtained in potato dextrose broth (PDB) than in other media assessed and the media usually supports the growth of *Phytophthora spp in-vitro*.

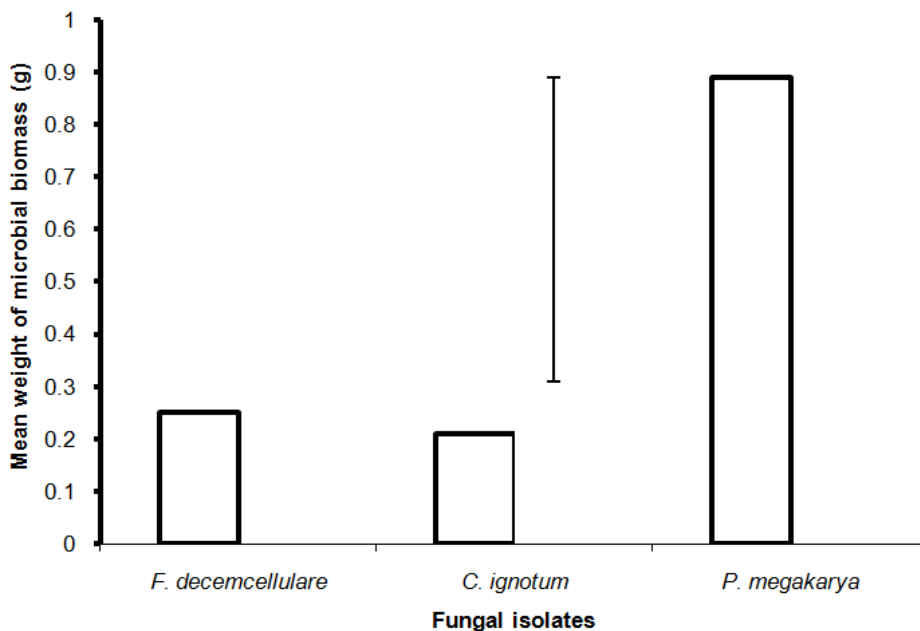


Fig. 3. Evaluation of mycelial biomass of the major isolates from the cocoa pods of the cocoa producing communities of Abia State

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