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# GROWTH RESPONSE OF *Phytophthora megakarya* AND TWO ASSOCIATED ORGANISMS (*F.decemcellulare,C.ignotum*) FROM COCOA PODS TO SOME NON-SYNTHETIC MEDIA *IN-VITRO*.

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**ABSTRACT**

The effect of seven non-synthetic media assessed on the growth response of the three fungal propagules (*P. megakarya, C. ignotum, F. decemcelluare*) isolated from cocoa pods was tested *in vitro*. Data obtained showed that all the non-synthetic media assayed supported the radial growth of the fungal propagules assayed to varying levels, when compared with the control. Of the eight media assayed, cocoa extract recorded highest mycelial growth of the three fungal pathogens tested. Also, $V_8$ extract media, potato/carrot media, potato extract media and pear extract media significantly supported the mycelial growth of the test fungi. However, maize extract media (MEM) was the least effective in supporting the mycelial growth rate of *F. decemcellulare*, *C. ignotum* and *P. megakarya* giving growth values of (0.6cm), (0.8cm) and (1.8cm) respectively when compared with cocoa extract medium with values of 4.63cm, 2.98cm and 2.23cm for *P. megakary, C. ignotum* and *F. decemcelluare* respectively. Pear extracts and V8 local extracts were comparable with the Potato control extract.

***Keywords:*** *Fungal Propagules, Non-synthetic Media, Mycelial growth, Cocoa, Black Pod Diseases*.


## 1. INTRODUCTION

Epidemics of black pod disease of cocoa (*Theobroma cacao* L) incited by *Phytophthora megakarya* 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* propagules can be initiated by *Phytophthora propagules* and other fungal propagules surviving in soils, pods left on trees, plant debris, cherelles and a vast array of microorganisms such as bacteria, viruses, protozoa, algae (Kellem and Zentemeyer, 2010).

Studies have shown that temperature, light, hydrogen ion concentration (pH), aeration and humidity, are some of the important physical factors which affect soil-borne pathogens. Also sources of carbon, nitrogen, vitamins and trace elements determine the rate of spore development under soil conditions (Ivan *et al*.,2008).Sporangia germinate to produce zoospores. The surface of the pods, later turns brownish and hardens. *Phytophthora spp* attack a wide range of other plants including pineapple,paw-paw,citrus,tomato,onions,beans,peas,banana,coconut,oil palm, castor oil, cassava, cotton, rubber, tobacco and black pepper (Gabriel,1995). *Phytophthora megakarya produces* abundant sporangia in clusters sympodially and the sporangia germinate directly in a nutrient medium by producing germ tubes that develops into mycelia masses (Kellem and Zentemeyer,2010).

Jeffers (2006) in his study on identification of species of *Phytophthora* reported that Corn Meal Agar (CMA) and Carrot Agar are amongst the best media for sporangia and oospore production seven days after incubation. Also Ripe Cocoa Broth Agar (RCBA), Potato Carrot Agar (PCA) and V8 Juice Agar (VJA) assayed on mycelia growth rates of *Phytophthora spp* have been found to be the best media for all the growth parameters and media characteristics studied. Furthermore, Green Cocoa Media Agar (GCMA)

had been effectively used to isolate or detect *Phytophthora spp* from infected cocoa pod tissues.

Several factors are now responsible for making the use of alternative methods more attractive. The limited external reserves and poor exchange rates of the currency of the developing nations limit the quantity of pesticides that can be imported (Oluma and Garba, 2002). The complete removal of subsidies on synthetic herbicides, insecticides and fungicides have made them inaccessible to the majority of farmers in many African countries. Since majority of farmers are illiterate, the misuse of these chemical frequently occur. It is no longer a hidden fact that these chemicals contaminate stored foods commodity, leaving behind harmful residues especially when an application dosages are not properly followed (Anonyme, 2006).

To circumvent or minimize the high cost and potential hazards of these chemicals, farmers need to resort to more creative, easily affordable and sustainable disease management methods to combat disease/pest problems so as to sustain or increase yields and ensure food security (Neyo and Ajata, 2007). Given the array of selective pressures that diseases exert on plants it is obvious that the plant kingdom offers a tremendous diversity of bioactive phytochemicals that can serve as complementary or alternatives to the synthetic chemicals (Pedigo and Rice, 2006).

The objective of this study therefore was to evaluate the growth response of some non-synthetic media on mycelial growth of the three rot pathogens from cacao pods in culture.

## 2. MATERIALS AND METHODS

### 2.1 Isolation of *Phytophthora megakarya* and the other fungi from cacao pods

This study was carried out in the Plant Pathology Laboratory of National Root Crops Research Institute, Umudike. Infected as well as healthy cacao pods were collected from plantations in the seven communities sampled and transported to the laboratory. The cacao pods were washed in several changes of running tap water to remove debris and then surface-sterilezd using 0.1% sodium hypochloride for 3 minutes and then dabbed with Whatman No. 1 filter paper. About 5mm segments of pod husks were cut into 5mm segments including the advancing margins of infection for infected pods. The cut segments were plated on fresh Potato Dextrose Agar (PDA) medium. This was then incubated at $28^{o}\pm2^{o}$C for 5 days (Awuah and Frimpong, 2008). Five (5mm) segments from healthy pods were similarly treated. Series of isolations were made to obtain pure cultures which were maintained by transferring on to fresh PDA slants in McCartney bottles and kept at $5^{o}$C until required.

### 2.2 Pathogenicity Tests

The confirmation of the pathogenicity of isolates from the cacao pods was done using axenic cultures of the isolates. Five millimeter (5mm) - diameter mycelia agar discs of a 4-day old culture were inoculated into three healthy cocoa pods. This was done for the three test pathogens separately. On appearance of symptoms, the tissues at the margins of the healthy and diseased parts were surface sterilized, excised and plated onto PDA for incubation at $28^{o}$C for seven days. At the end of this period, morphological characteristics and growth patterns observed were compared with the ones of the original isolates. Identification was done and confirmed using, Rossman *et al.* (1997), Barnet and Hunter (1999), and International Mycological Institute (I.M.I) monographs on fungal pathogens.

### 2.3 Preparation of the Non Synthetic Media

The different non-synthetic media assayed ; potato carrot extract, pear extract, avocado extract, maize extract, $V_8$ local extract (cucumber, water melon, tomato, paw-paw, banana, carrot, orange, avocado pear), cocoa extract, and potato extract media was prepared by properly cleaning with tap water, peeled and cut into pieces where necessary, weighed and well blended in Oster food blender ,(Sunbeam-Oster Household Products, Milkwankee. Wis) at chop speed for 1 min and whip speed for another 1 min. The juice was then passed through a sieve with a pore size of 1.5 by 1.5 mm to remove seeds and large pieces of tissues. The different media consisted of 20%

juice, 0.004% calcium carbonate (CaCo3), 2% agar (Awuah and Frimpong, 2002).Various juices were preheated to 100°C before adding CaCo3 to prevent aggregate formulation. Radial mycelial growth rates were determined by placing one agar disc 5mm diameter obtained from a 4-day-old culture growing on PDA at the edge of the different test media in a Petri plate (9cm diameter). Each treatment was replicated three times and the experiment laid out in a Completely Randomized Design (CRD). The analysis was done using Genstat Discovery Edition Version 3.

## 3. RESULTS AND DISCUSSION

Various extracts; avocado, potato, cocoa, maize, potato carrot, pear and V8 extracts media were prepared and assessed for clarity and for potential to support mycelia growth and sporulation of *P. megakarya* and two associated organisms (*F. decemcellulare and C.ignotum*) from cacao pods.

The response of the various non-synthetic media sources on the test fungi assayed revealed that the media impacted on the growth of the pathogens to varying degrees (Fig 1.) Cocoa extract (control) and V8 local extract media was the most effective in as mycelia growth media of the three fungi in culture especially *P. megakarya*. Moreso, cocoa extract media (2.8cm) recorded significant (P=0.05) increase in the mycelial growth of *C. ignotum* when compared with maize extract media(0.7cm), potato carrot media (1.0cm) and other media assayed. Similar mycelial growth level was observed of *F. decemcellulare* on cocoa extract media (2.3cm) and potato carrot extract media (2.3cm) as shown in (Fig 1). The similarity between these two media may be due to preference of the nutrient content in the media by the pathogen.

Cocoa juice is one of the chief ingredients of V8 vegetable juice. It is argued that cocoa juice is the main component responsible for the ability of Vj agar to support good growth and sporulation of fungi (Guo and Ko,1993) This result obtained supported the result of a study by Awuah and Frimpong (2002) who reported that Green Cocoa Mucilage Agar (GCMA) was effectively used to isolate / detect *Phytophthora spp* from cocoa pod tissues.

Maize extract gave the least mycelial growth support of the three test fungi evaluated when compared with other non-synthetic extracts tested. The following growth values were obtained. *P.megakarya* (1.8cm), *C ignotum* (1.7cm) and *F. decemcellulare* (0.5cm) respectively. This was followed by avocado extract media *P. megakarya* (3.2cm), *C.ignotum* (1.7cm) and *F. decemcellulare* (0.9cm). This result confirms report by Jeffers (2006) that potato and carrot agar were amongst the best media for identification of species of *Phytophthora*. *Phytophthora megakarya* produces abundant sporangia in clusters sympodially, and the sporangia germinate directly in a nutrient medium by producing germ tubes that develop with mycelia masses (Keller and Zentemeyer, 2010).

Therefore, the result showed that cocoa extract-based media which was the best growth medium for the test organisms can be substituted for the existing growth media in pathological and physiological studies of these three fungi in places or laboratories where synthetic media are not available or difficult to obtain

## REFERENCES


Amandaraji, M. (2011). Outreach project on *Phytophthora, Fusarium* and *Ralstoma* Diseases of Horticultural and Field Crops. Annual Report, *Indian Institute of Spices Research*. 10, 1 – 100.

Anonyme, (2006) New European regulation on maximum residue levels of pesticides in food minimizing the impact on the cocoa sector. In: Proceedings of the 15th International cocoa Research Conference. San Jose (Costa Rica). P 1565-1571.

Awuah, R. T. (2008). Fungitoxic effects of extracts of extracts from some West African plants. *Ann. Applied Biology.,* 115, 451 – 453.

Awuah, R. T. and Frimpong, M. (2002). Cocoa-based media for cutting *P. palmivora* (Butt.) causal agent of black pod diseases of cocoa. *Mycopathologia*, 155(3), 143 – 147.

Barnett, H. L. and Hunter, B. B (1999) Illustrated genera of Imperfect fungi 5th Edition.Burgress Publishing Company.731p.

Bowers, J. H., Bailey, B. A., Hebbar, P. K., Sanogo, S. and Lunsden, R. D. (2001). The Impact of Plant diseases on World Chocolate Production. Published on line. Plant Health Progress. doi: 10.1094/PHP-2001-0709-01-RV.

Dubey, N. K., Shukla, R., Kumar, Singh, P. and Prakash, B. (2010). Prospects of botanical pesticides in sustainable agriculture. *Curr. Sci.,* 98, 479 – 545.

Guo, L. Y and Ko, W. H (1993) Two widely accessible media for growth and reproduction of *Phytophthora spp* and *Pythium spp*. *Mycologia* 59(7), 2323-2325.

Jeffers, S.N.(2006).Identifying species of Phytophthora.*Plant species*. 1:1-8.

Kellam, M. K. and Xentmyer, G. A. (2010). Comparisons of single-oosppore of Phytophora species from naturally infected cocoa pods in Brazil. *Mycologia*. 78 (3), 351 – 358.

Kim,Y. K., Xiao, C. L. and Rogers, J.D.(2005) Influence of culture media and environmental factors on mycelia growth and pycnidial production of *Sphaeropsis pyripurescens*. *Mycologia* 97(1),25-32.

Kulkarni, S. P. (2006) Studies on *Fusarium oxysporum* Schlecht Fr. f.sp. gladioli (Massey) snyd and Hausi causing wilt of Gladiolus M. Sc. (Agric) thesis. Univ. Agric. Sci, Dharwad, India, 245pp.

*Neyo C. A., Ajata, A. E. (2007). Use of indigenous plant extract for the protection of mechanically injured Sweet potato (Ipomea batata: Lam) tuber. Science. Research. Essay*, 2 (5), 167-170.

Oluma, H. O. A. and Garba, I. U. (2004). Screening of Encalyptus globules and *Ocimium grattissimium* for fungitoxic properties against *Pythium apharidermatum*. *Nigerian. Journal. Plant Protection*. 21, 109 – 114.

Opoku, I. Y., Dakwa, J. T. and Ollenmu, L. A. A. (2005). The development of phytophthora canker on different cocoa genotypes infected with cocoa swollen shoot virus. *Tropical Science* 45, 50 – 53.

Pedigo, L. P. and Rice, M. E. (2006). Entomology and pest management. 5th Edition, Prentice Hall, Upper Saddle River, U. S. A. 749Pp.

Rossman,A. Y., Palm, M., and Spielman L.J (1997) A Literature guide for the identification of plant pathogenic fungi.APS Press, Minnesota, USA. 24p

Sharma, R. I., Singh, B. P., Thakur, M. P., Thapak S. K. (2005). Effect of media, temperature, pH and light on the growth and sporulation of *Fusarium oxysporum* f.sp. lini. *Annal. Plant Protection Science*. 13. 172 – 174.

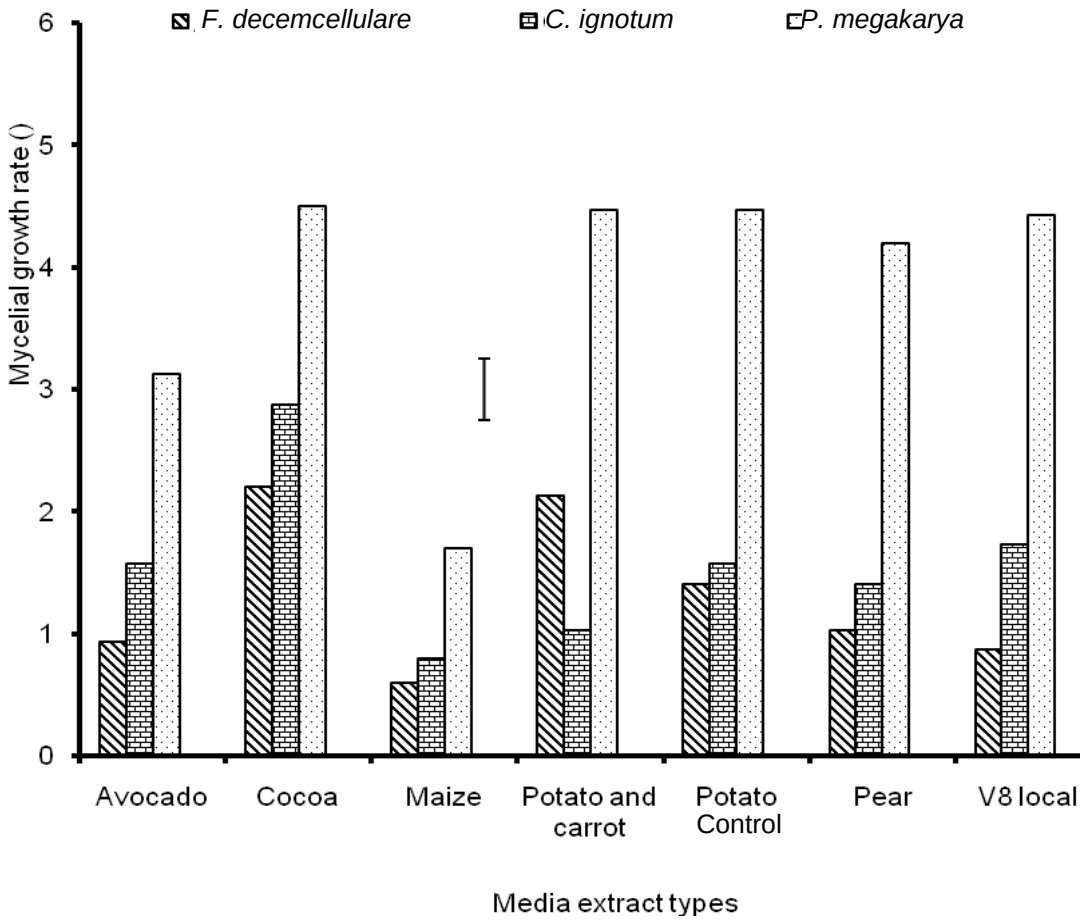


Fig. 1: Assessment of the effect of different non-synthetic media on the mycelia growth rate of three fungal isolates (*F. decemcellulare, C.ignotum* and *P. megakarya* from cocoa pods seven days after inoculation

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