International Journal of Plant & Soil Science

20(4): 1-8, 2017; Article no.IJPSS.37039
ISSN: 2320-7035

Effect of Time of Exposure on the Antimicrobial Potentials of Some Tropical Plants against Cocoa Pod Rot Pathogen- *Phytophthora megakarya* (B & G) in Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author EAB designed the study, carried-out the research practicals and wrote the protocols/literature searches. Author BAO performed the statistical analysis, managed the analysis, wrote the manuscript and assisted in the research practicals. Author SOA assisted in designing the study and in the general supervision of the research. Author AOA assisted in the research practicals and in managing the research results. Authors BAO and EAB read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2017/37039
Editor(s):
(1) Omer Kilic, Bingol University, Turkey.
Reviewers:
(1) Zakaria A. M. Baka, Damietta University, Egypt.
(2) Clint Magill, Texas A&M University, USA.
Complete Peer review History: http://www.sciencedomain.org/review-history/22220

Original Research Article

Received 28th September 2017
Accepted 14th October 2017
Published 9th December 2017

ABSTRACT

Aims: To determine the effect of time of exposure on the antimicrobial effectiveness of some tropical plants in the control of black pod disease of cocoa caused by *Phytophthora megakarya* in Nigeria.
Study Design: Completely Randomized Design (CRD).
Place and Duration of Study: Plant Pathology Section, Cocoa Research Institute of Nigeria (CRIN), Ibadan, Nigeria, from 2015 to 2016.
Methodology: The antimicrobial effects of aqueous extracts of six selected Tropical plants: *Moringa oleifera, Sida acuta, Piper guineense, Ocimum gratissimum, Chromolaena odorata and Cymbopogon citratus* against *Phytophthora megakarya*, causal agent of black pod disease of cocoa was investigated in an *in vitro* experiment. 200 g leaf samples of each of the plants used

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were surface sterilized, mashed separately and the resultant pastes were soaked in 200 ml of sterile distilled water and filtered. Varied concentrations of the extracts (10, 25, 50 and 75%) were pour-plated with freshly prepared potato dextrose agar (PDA). Disc culture (5 mm) of *Phytophthora megakarya* was inoculated into each of the PDA plates and incubated at 25℃ for 144 hours.

Results: Aqueous extracts of the six plants, at varied concentrations showed different levels of inhibition against the pathogen. *C. odorata* at all tested concentrations consistently reduced the mycelia growth and showed significantly highest percentage inhibition ($P < .05$) against the pathogen, followed by *Sida acuta, P. guineense, O. gratissimum* and *C. citratus* while *M. oleifera* gave significantly lowest inhibitory percentage ($P < .05$).

Conclusion: Findings from this research showed that the antimicrobial effectiveness of all the botanicals used decreased with time contrary to that of the standard (chemical fungicide) used. Aqueous extracts of *C. odorata* having shown some promise, could therefore be used as bio-fungicide in the control of black pod disease of cocoa, but with intermittent application.

Keywords: Antimicrobial; in vitro; aqueous; tropical plants; Phytophthora; inhibition; Moringa; Sida; Piper; Ocimum; Chromolaena; Cymbopogon.

1. INTRODUCTION

Cocoa is cultivated worldwide in countries like Ivory Coast, Ghana, Indonesia, Nigeria, Cameroon, Brazil, etc. on land mass of over 700,000 km^2. The crop is of high economic value in the producing nations and the world at large. Its productivity is however affected by factors such as temperature, rainfall, humidity, altitude, light, shade, soil type, pests and diseases, etc. [1].

In a bid to correct the undesirable yield and productivity reductions caused by pests and pathogens of cocoa, many control measures have been employed in times past. Application of synthetic pesticides is the most widely used. It has been estimated that about 125,000 to 130,000 metric tonnes of pesticides are applied every year in Nigeria [2].

The use of synthetic fungicides to control black pod disease of cocoa has been reported to be effective but not without some attendant problems which include environmental pollution, health hazards, persistency and development of resistance by fungal pathogens [3]. It has therefore become expedient to search for effective, viable and reliable alternative(s). One of such alternatives is the use of phyto-extracts of tropical plant-source which has been observed to be eco-friendly, bio-degradable, cheaper, available and safe [4]. These extracts have been found to be effective in the control of some diseases [5,6]. Adejumo and Otuonye [7] reported the potentials of botanicals in controlling inflorescence blight disease of cashew. Tijani and Omondiagbe [8] also reported the use of Siam weed (C. *odorata*) among cocoa farmers in Osun State for crop protection. Solution made of Siam weed, alum, black soap and water- called Siam weed soap solution (SWSS) was prepared, tested and found to be preliminarily effective against fungus diseases affecting cocoa. The SWSS has also been found to be very effective in the control of cocoa pests with no visible side effects on the crop. Siam weed soap solution has also been reported to have demonstrated prominent advantages over copper sulphate and other innovations such as kerosene soap solution, neem soap solution and tobacco soap solution against the pests and diseases of cocoa in Osun State, Nigeria.

In view of the above, this study sought to assess the efficacy of some tropical plants' aqueous extracts with respect to their time of exposure/duration of application in the *in vitro* control of black pod disease of cocoa caused by *Phytophthora megakarya*.

2. MATERIALS AND METHODS

2.1 Collection of Botanicals

Fresh and matured leaves of some tropical plant species: *Ocimum gratissimum, Sida acuta, Piper guineense, Chromolaena odorata, Cymbopogon citratus* and *Moringa oleifera* were collected within the premises of Cocoa Research Institute of Nigeria (CRIN), Ibadan, Nigeria and taken to the Herbarium Unit for identification and then taken (in well labeled sterile polythene bags) to Plant Pathology laboratories for further analyses.

2.2 Preparation of Plant Extracts

Two hundred grammes of each of the identified plant samples were surface sterilized with 2%

sodium hypochlorite and rinsed twice with sterile distilled water. The leaves were mashed with sterile mortar and pestle, and the resultant pastes were separately soaked in 200 ml sterile distilled water.

The extracts obtained were filtered through sterile Whatman no. 1 filter paper. From the filtrate (stock), different concentrations (10, 25, 50 and 75%) were prepared alongside a synthetic fungicide (Ultimax Plus) which served as standard. These preparations were sterilized at 70℃ for 15 minutes with the aid of water bath.

2.3 *In vitro* Bioassay of Plant Extracts

Aliquot (1 ml) of each extract concentration was incorporated into freshly sterilized but cooled (45℃) potato dextrose agar (PDA) using poisoned food technique and allowed to set. Five (5 mm) disc of *P. megakarya* culture freshly isolated and identified at the Plant Pathology Sectional laboratory, CRIN, was inoculated at the center of each of the poisoned PDA plates and incubated at 25℃ for 144 hours. Each of the treatments was replicated thrice.

Mycelia growth diameters of the pathogen on each of the inoculated plates were taken at 24 hours interval and their respective percentage inhibitions were calculated using the formula below [9]:

$$Percentage\ mycelia\ inhibition = \frac{dc - dt}{dc} x\ 100$$

Where:

dc = Mycelia growth diameter in control
dt = Mycelia growth diameter in treatment

Results obtained from this research work were subjected to Analysis of Variance (ANOVA) at 5% level of probability and with the aid of Statistical Analysis System (SAS) 9.1 statistical package.

3. RESULTS AND DISCUSSION

The antimicrobial effect of *Moringa oleifera* extract on the mycelia growth of *Phytophthora megakarya* as presented in Table 1 showed that the percentage inhibition exhibited by 10% concentration of the extract ranged between 14.85 and 28.61%. The 25, 50 and 75% concentrations of the extract gave inhibition ranges of 5.55 - 16.83%, 7.77 – 21.79% and 10.17 – 16.18% respectively, while the standard (Ultimax Plus) exhibited mycelia inhibitions of between 76.52 - 92.34% against the pathogen.

Throughout the 144 hours of incubation, Ultimax Plus exhibited significantly highest inhibitions ($P < .05$) against the pathogen. This was followed by 10% concentration which exhibited 28.32, 28.44 and 26.57% inhibitions against the pathogen at 24, 48 and 72 hours respectively. 25, 50 and 75% concentrations of the extract, however exhibited the lowest inhibitions at similar periods of incubation (Table 1).

At 96 hours of incubation, concentrations 10 and 50% of the extract gave the highest inhibitions (28.61 and 17.89% respectively), while 25% extract concentration exhibited the significantly lowest mycelia inhibition of the pathogen when compared with others at 144 hours. Percent inhibitions exhibited by this (*M. oleifera*) extract against the pathogen at different concentrations were significantly much lower ($P < .05$) than those exhibited by the standard (Table 1).

Table 2 shows that the percentage inhibitions exhibited by 10% concentration of *Cymbopogon citratus* extract on the mycelia growth of the test pathogen ranged between 12.61 and 20.76%, while those of 25, 50 and 75% extract concentrations ranged between 10.30 – 40.99, 12.64 – 52.03 and 15.42 – 69.72% respectively. However, the mycelia inhibitions exhibited by the chemical fungicide (Ultimax Plus) used as a standard against the pathogen ranged between 76.64 - 91.50% (Table 2).

The inhibition exhibited against the test pathogen by the standard was significantly higher ($P < .05$) than those of the extract concentrations. It was followed by those produced by 75% concentration of the extract while 10% concentration gave the significantly lowest inhibitions at 24 and 48 hours, but at 72 hours of incubation, there was no significant difference ($P < .05$) in the inhibitions produced by extract concentrations 10, 25 and 50%. There was also no significant difference ($P < .05$) in the inhibitions exhibited by the extract concentrations at 96, 120 and 144 hours (Table 2).

The antimicrobial effect of *Sida acuta* extract on the mycelia growth of *Phytophthora megakarya* is as presented in Table 3. The results show that *Sida acuta,* at 10% concentration exhibited the percentage inhibition range of 36.64 – 53.89%, while 25, 50 and 75% concentrations of the extract gave inhibition percentage ranges of 16.52 - 42.96%, 16.68 - 44.49% and 18.37 -

38.385% respectively. The chemical fungicide (standard) exhibited significantly highest inhibition ($P < .05$) throughout the 144 hours of incubation (Table 3).

At 72 hours of incubation, there was no significant difference ($P < .05$) in the inhibition percentages exhibited by concentrations 25, 50 and 75%. Likewise, at 96,120 and 144 hours, the inhibitions exhibited by 25, 50 and 75% extract concentrations were not significantly different ($P < .05$) from one another (Table 3).

Table 4 depicts the results on the effect of *Piper guineense* extract on the mycelia growth of *P. megakarya.* The percentage inhibitions exhibited by 10% extract concentration of the botanical ranged between 10.59 and 37.10%, while those of 25, 50 and 75% concentrations ranged between 13.37 - 36.82, 15.57 - 66.65 and 22.62 – 69.33%. The fungicide used as standard (80.71 - 91.93%) gave significantly highest inhibition ($P < .05$) within 48 to 144 hours, while there was no significant difference ($P < .05$) in the inhibitory activities of the fungicide when compared with extract concentrations 50 and 75% (Table 4).

At 72, 96 and 120 hours of incubation, 75% *P. guineense* extract concentration gave significantly higher mycelia inhibitions ($P < .05$) when compared with those of 10, 25 and 75% concentrations. At 144 hours after incubation, inhibitions produced by the extract concentrations were however not significantly different ($P < .05$) from one another (Table 4).

Table 1. Effect of *Moringa oleifera extract* on the mycelia growth of *Phytophthora megakarya*

Extract conc. (%)	Percentage inhibition at different periods after inoculation (%)					
	24 Hrs	48 Hrs	72 Hrs	96 Hrs	120 Hrs	144 Hrs
10%	28.32^{b}	28.44^{b}	26.57^{b}	28.61^{b}	24.55^{b}	14.85^{b}
25%	6.11^{c}	9.76^{c}	10.02^{c}	13.80^{c}	16.83^{b}	5.55^{c}
50%	7.77^{c}	14.30^{c}	11.97^{c}	17.89^{bc}	21.79^{b}	13.46^{b}
75%	10.17^{c}	16.18^{c}	11.88^{c}	12.05^{c}	16.08^{b}	13 46^{b}
Standard	76.52^{a}	86.06^{a}	89.02^{a}	90.93^{a}	91.49^{a}	92.34^{a}

Values with the same superscripts along the same column are not significantly different at P < .05 using Fisher's LSD Test

Table 2. Effect of *Cymbopogon citratus* extract on the mycelia growth of *Phytophthora megakarya*

Extract conc. (%)	Percentage inhibition at different periods after inoculation (%)					
	24 Hrs	48 Hrs	72 Hrs	96 Hrs	120 Hrs	144 Hrs
10%	20.76 ^{c}	15.46^{c}	12.61^{b}	20.18^{b}	15.87^{b}	16.89^{b}
25%	40.99^{bc}	15.51^{c}	12.61^{b}	10.30^{b}	19.42^{b}	14.03^{b}
50%	52.03^{ab}	19.64^{bc}	12.64^{b}	11.56^{b}	17.12^{b}	17.72^{b}
75%	69.72^{ab}	27.08^{b}	17.82^{b}	15.42^{b}	18.08^{b}	18.19^{b}
Standard	76.64^{a}	86.06^{a}	88.99^{a}	90.37^{a}	91.50^{a}	91.18^{a}

Values with the same superscripts along the same column are not significantly different at P < .05 using Fisher's LSD Test

Table 3. Effect of *Sida acuta* extract on the mycelia growth of *Phytophthora megakarya*

Extract conc. (%)	Percentage inhibition at different periods after inoculation (%)					
	24 Hrs	48 Hrs	72 Hrs	96 Hrs	120 Hrs	144 Hrs
10%	53.89^{b}	45.23^{b}	44.81^{b}	49.14^{b}	40.92^{b}	36.93^{b}
25%	42.96^{bc}	25.43^{c}	18.54^{c}	16.52^{c}	21.63^{c}	22.85^{c}
50%	44.49^{bc}	27.43^{c}	17.87^{c}	16.68^{c}	19.71^{c}	22.90^{c}
75%	38.38^{c}	25.43^{c}	18.37^{c}	19.11^{c}	20.56^{c}	22.86^{c}
Standard	76.64^{a}	86.05^{a}	88.96^{a}	90.37^{a}	91.67^{a}	91.71^{a}

Values with the same superscripts along the same column are not significantly different at P < .05 using Fisher's LSD Test

Table 4. Effect of *Piper guineense* extraction on the mycelia growth of *Phytophthora megakarya*

Extract conc. (%)	Percentage inhibition at different periods after inoculation (%)					
	24 Hrs	48 Hrs	72 Hrs	96 Hrs	120 Hrs	144 Hrs
10%	37.10[b]	19.28[d]	21.44[c]	17.09[c]	10.59[c]	18.54[b]
25%	36.82[b]	18.63[d]	13.91[c]	17.09[c]	13.37[c]	20.98[b]
50%	66.65[a]	27.51[c]	16.62[c]	15.57[c]	16.74[c]	22.08[b]
75%	69.33[a]	45.13[b]	30.54[b]	31.67[b]	26.14[b]	22.62[b]
Standard	80.71[a]	86.87[a]	88.92[a]	90.64[a]	91.86[a]	91.93[a]

Values with the same superscripts along the same column are not significantly different at P < .05 using Fisher's LSD Test

The inhibitory effects of *Chromolaena odorata* on mycelia growth of *P. megakarya* are as shown in Table 5. The percentage inhibitions exhibited by 10, 25, 50 and 75% concentrations of the extract ranged between 29.51 – 64.54%, 30.54 – 62.39%, 39.67 – 68.54% and 46.57 – 79.27% respectively. However, the standard gave mycelia inhibitions ranging between 79.27 and 91.93% (Table 5).

Throughout the period of incubation, the standard exhibited inhibitions which were significantly higher ($P < .05$) than those of the *Chromolaena* extract concentrations except at 24 hours during which no significant difference ($P < .05$) was noticed between the former and the latter. At 48, 72 and 96 hours of incubation, 75% extract concentration gave higher mycelia inhibitions 69.07, 61.62 and 57.13% respectively than other extract concentrations of the botanical. Within the same incubation period, extract concentrations 10 and 25% exhibited mycelia inhibitions that were not significantly different ($P < .05$) from one another (Table 5).

At 120 hours of incubation, the inhibitions produced by extract concentrations 10 and 25% were not significantly different ($P < .05$) from each other, while concentrations 50 and 75% (at the same period of incubation) exhibited higher mycelia inhibitions (43.48 and 46.57% respectively) which were also not significantly different ($P < .05$) from each other. At 144 hours of incubation, 75 and 50% extract concentrations gave the highest mycelia inhibitions (after the standard), and closely followed by 25% concentration, while the significantly lowest inhibition ($P < .05$) was produced by 10% concentration of the same extract (Table 5).

Table 6 shows the inhibitory effect of *Ocimum gratissimum* against the mycelia growth of *P. megakarya*. Percentage inhibitions exhibited by 10% concentration of the extract ranged between 33.09 -56.47%, while 25, 50 and 75% concentrations of the extract exhibited inhibitions ranging between 8.93 – 20.70, 9.16 – 25.69 and 10.21 – 32.01% respectively. However, Ultimax (standard) exhibited mycelia inhibition that ranged between 76.64 and 91.49%.

Throughout the 144 hours of incubation, the mycelia inhibitions exhibited by the standard against the pathogen were significantly higher ($P < .05$) than other concentrations of the (*O. gratissimum*) extract. Mycelia inhibition exhibited by 10% concentration of the extract was not significantly different ($P < .05$) from that of the standard at 24 hours of incubation, while those of 25, 50 and 75% of the former were significantly lower ($P < .05$) than that of the latter (Table 6).

At 48, 72, 96,120 and 144 hours of incubation, extract concentration 10% exhibited inhibitory effects which were significantly higher ($P < .05$) than those of concentrations 25, 50 and 75%. At 24, 48, 72, 96 and 144 hours of incubation, extract concentration 25% exhibited the lowest mycelia inhibition against the pathogen (Table 6).

The antifungal effect of the plant extracts investigated showed that the extracts at varied concentrations of 10, 25, 50 and 75% inhibited the growth of fungi pathogen [10]. The inhibitory effect of these leaf extracts may be as a result of the presence of varied water soluble chemicals in them [11]. Singh et al. [12] reported such chemicals to either dissolve the cytoplasm or render it inactive. Chemicals such as phenol, lignin, terpene and flavonoids in the leaf extracts are capable of penetrating the microbial wall thus complicate microbial metabolic process as feeding deterrent [12].

Table 5. Effect of *Chromolaena odorata* extract on the mycelia growth of *Phytophthora megakarya*

Extract conc. (%)	Percentage inhibition at different periods after inoculation (%)					
	24 Hrs	48 Hrs	72 Hrs	96 Hrs	120 Hrs	144 Hrs
10%	64.54^{a}	32.46^{d}	30.98^{d}	29.51^{d}	30.41^{c}	29.74^{d}
25%	62.39^{a}	39.73^{cd}	33.71^{cd}	30.54^{d}	34.22^{c}	38.68^{c}
50%	68.54^{a}	46.69^{c}	40.22^{c}	39.67^{c}	43.48^{b}	43.16^{bc}
75%	79.27^{a}	69.07^{b}	61.62^{b}	57.13^{b}	46.57^{b}	47.45^{b}
Standard	76.64^{a}	86.06^{a}	89.02^{a}	90.61^{a}	91.50^{a}	91.93^{a}

Values with the same superscripts along the same column are not significantly different at P < .05 using Fisher's LSD Test

Table 6. Effect of *Occimum gratissimum* extract on the mycelia growth of *Phytophthora megakarya*

Extract conc. (%)	Percentage inhibition at different periods after inoculation (%)					
	24 Hrs	48 Hrs	72 Hrs	96 Hrs	120 Hrs	144 Hrs
10%	56.47^{ab}	40.15^{b}	45.56^{b}	39.47^{b}	33.20^{b}	33.09^{b}
25%	20.70^{b}	9.00^{c}	16.75^{c}	13.09^{d}	11.56^{c}	8.93^{d}
50%	25.69^{b}	13.34^{c}	17.14^{c}	16.41^{c}	9.16^{c}	18.92^{c}
75%	32.01^{b}	11.24^{c}	21.66^{c}	25.39^{c}	10.21^{c}	14.92^{cd}
Standard	80.71^{a}	86.63^{a}	90.73^{a}	91.58^{a}	91.50^{a}	91.93^{a}

Values with the same superscripts along the same column are not significantly different at P < .05 using Fisher's LSD Test

The various extract concentrations of *M. oleifera exhibited* generally low mycelia inhibition on the test pathogen particularly when compared with the standard throughout the incubation period (Table 1). The lowest of the extract concentrations (10%) however gave the highest inhibition. The least concentration of *S. acuta* and *O. gratissimum* also gave the highest mycelia inhibition throughout the period of incubation when compared with other concentration (25, 50, and 75%) of the same extract. These findings are in agreement with the report of Adejumo [13] who reported that "Tiwantiwa", a herbal plant mixture containing roots and leaves of four herbal plants at 10% concentration of the extract, developed by a peasant farmer in Nigeria for controlling cocoa pod disease, showed inhibition of fungal growth and the findings of Omorashi et al. [11] which state that the growth rates of *Aspergillus* when controlled with the extract of *O. gratissimum* were consistently inhibited at the lowest concentration than at the highest concentration.

The extract of *C. citratus* showed little inhibitory effect in the control of the mycelia growth of *P. megakarya* when tested at different concentrations. Omorashi et al. [11] also said that extracts from *C. citratus* has minimal antifungal properties in an *in vitro* study.

Chromolaena odorata extracts significantly inhibited the mycelia growth of *P. megakarya.* The highest concentration of 75% rapidly reduced the growth rate of the test pathogen followed by 50% until the least concentration of 10%. This showed that as the concentration of the extract increased, its inhibitory effect also increased. This agrees with the findings of Andiru [14] and Omorashi et al. [11] which state that *C. odorata* at higher concentrations significantly inhibit the radial growth of *Phytophthora palmivora*, *Aspergillus niger, Fusarium monilioformes, Rigidoporus lignosus and Trichoderma spp. in vitro*.

All the tested concentrations of *C. odorata* competed favourably with the standard (Ultimax) in the mycelia inhibition of the test pathogen. This may be as a result of the diverse antifungal components such as phenol, lignin, terpene and the flavonoids it possesses [10,11,12].

P. guineense extract showed inhibition on the test pathogen at the highest concentration (75%) used, but at 50, 25 and 10% concentrations,

there was no significant difference (P<0.05) in the inhibitions displayed. Adejumo [15] reported the potentials of Christmas bush or Siam weed (*Chromolaena odorata*) and African black pepper or Ashanti pepper (*Piper guineense*) in black pod disease control. It was also reported by Ramos et al. [5] that phytochemicals from some tropical plants such as *Azadiracta indica* and *Piper guineense* strongly retarded the germination of spores of *Colletotrichum destructivum.* Findings from this work agree with the above assertions.

4. CONCLUSION AND RECOMMENDATIONS

Results from this research indicate that *C. odorata* was most effective among the botanicals used in the *in vitro* study*. Moringa oleifera* was however practically not effective against the pathogen. It was also discovered that the antimicrobial effectiveness of each of the botanicals used decreased with time. This was not so with the standard (chemical fungicide) used. Out of all the six tropical plants used in this study, *C. odorata* can be used as bio-fungicide in the control of black pod disease of cocoa, although its application need to be intermittent or somewhat frequent in nature, if it would be appropriate as possible replacement for the chemical fungicide. There is however, an urgent need for field trials of the botanicals especially *C. odorata* to confirm the findings of this research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/22220