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Phytochemical screening and control of fungal diseases of cocoa (*Theobromae cacao* L.) pod using extracts of plant origin

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ABSTRACT

The antifungal activity of the ethanolic leaf extracts of *Dioscorea dumetorum* and *Moringa oleifera* on the fungal pathogens isolated from infected cocoa pods were investigated *in vitro* and *in vivo*. The pathogens were *Botryodiplodia theobromae* and *Fusarium moniliformes*. For the *in vitro* assay, 5ml of various concentrations of the extracts ranging from 10g/200ml, 20g/200ml, 30g/200ml, 40g/200ml and 50g/200ml were separately added to PDA media. The fungal pathogens were separately inoculated into the media and incubated for seven days. For the *in vivo*, healthy cocoa pods were properly surfaced sterilized using 99% ethanol and Sodium hypoclorite (bleach). The sterile cocoa pods were then sprayed with the extracts at different concentrations two hours prior to inoculation with the fungal pathogens. Each cocoa pod after being treated with different concentration of the extracts at 10g/200ml, 20g/200ml, 30g/200ml, 40g/200ml and 50g/200ml were dipped into beakers containing dissolved spores of each pathogen and incubated for twenty eight days. Results of the *in vitro* studies showed that at 10g/200ml, 20g/200ml, 30g/200ml, 40g/200ml and 50g/200ml concentrations, ethanolic leaf extract of *D. dumetorum* and *M. oleifera* completely inhibited the radial growth of *B. theobromae* and *F. moniliformes* after seven days observation period while results of the *in vivo* studies showed that the extracts had a significant effect ($p \leq 0.05$) on the mycelial growth of the fungal pathogens at all the concentrations tested. Phytochemical screening of the plant extracts showed that cardiac glycosides, anthraquinones, and reducing compounds were highly present in *D. dumetorum* and *M. oleifera* extracts while saponnins and phlobatannins were absent.

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1. INTRODUCTION

Theobroma cacao also known as the cacao tree and cocoa tree, is a small (4 to 8m) (13 to 26ft) tall evergreen tree in the family Malvaceae [1] native to the deep tropical regions of central and South America.

Its seed, cocoa beans, are used to make cocoa Mass, cocoa powder and chocolate [2]. The fruit or cocoa pod is in ovoid shape, 15 to 30cm (5.9 to 11. 8 inch) long and 8 to 10cm (3.1 inch) wide, ripening yellow to orange and weighing about 500g (1.111b when ripe). The pod contains 20 to 60 seeds, usually called “bean”, embedded in a white pulp. The seeds are the main ingredients of chocolate, while the pulp is used in some countries to prepare refreshing juice, smoothies, jelly and natal [3]. Each seeds contains significant amount of fat (40 to 50%) as cocoa butter. Their most noted active constituent is Leobromine a compound similar to caffeine.

Cocoa is cultivated on roughly 17, 000,000 acres (27,000 Sq., 69,00km^2 worldwide [4]. Cocoa production has increased from 1.5 million tonnes in 1983 -1984 to 3.5 million tonnes in 2003 – 2004, almost entirely due to the expansion of the production area rather than to yield increases. Cocoa is grown both by large agro industrial plantations and small

procedures, the bulk of production coming from millions of farmers who have a few trees each [4].

A cocoa tree begins to bear when it is four or five year old. A mature tree may have 6,000 flowers in a year yet develop only about 20 pods. About 1, 200 seeds (40 pods) are required to produce Ikg (2.2Ib) of cocoa paste.

Cocoa bean is the dried and fully fermented fatty bean of *Theobroma cacao* from which cocoa solids and cocoa butter are extracted [4]. They are the basis of chocolate, as well as many Mesoamerican foods such as mole sauce and legate. Cocoa butter has been described as the world's most expensive fat, used rather extensively in emollient "lullet" used for haemorrhoids [5]. A cocoa pod (fruit) has a rough and leathery rough about 3cm thick (these vary with the origin and variety of pod). It is fitted with sweet, mucilaginous pulp inclosing 30 to 50 seeds that are fairly soft and white to a pale lavender colour. While seeds are usually white, they become violet or reddish brown during the drying process [4].

Over the years there have been reports of fungal attack on cocoa pods rendering the seeds (beans) unfit for human consumption. Fungi such as *Phytophthora palmivora, P. Capsici, P. Kevea* and *Lasiodiplodia theobromae* causative agents of black pod rot and Lasiodiplodia pod rot, *Macrophoma spp (*Macrophoma pod rot*), Phytophthora Cirropthora* and *P. megarkarya* (Phytophthora pod rot) have been reported to cause depletion of pods/seeds nutritional value in the field [6].

These diseases are controlled mainly by the application of Agrochemicals. However, the worldwide trend towards environmentally safe methods of plant disease control in sustainable agriculture calls for reducing the use of these synthetic chemical fungicides. In an attempt to modify this condition, some alternative methods of the control have been adopted. Recent efforts have focused on developing environmentally safe, long lasting and effective biocontrol methods for the management of plant diseases [7]. Natural plant products are important sources of agrochemicals for the control of fungal pathogens [8-13].

Furthermore, botanical pesticides are non-phytotoxic, systemic, and biodegradable and contain multiple bioactive metabolites [14-16]. Plant products are now known to reduce populations of fungal pathogens and control the diseases development [17].

Phytochemical screening of the two plant extracts used in this study was carried out to determine their exact phytochemical contents. Phytochemicals, as compounds which occur naturally in plants, form part of plants defense mechanisms against diseases [18]. They are classified into primary and secondary, based on their activity in plant metabolism. The primary ones comprise of sugars, amino acids, proteins and chlorophyll [19], while secondary ones include the phenolic compounds such as tannins, flavonoids, alkaloids, saponins, anthraquinones, phlobatannins, proanthocyanidins, etc. [20]. These phenolic compounds have been reported to possess considerable antimicrobial properties, which is attributed to their redox properties [21,22]. Thus the antimicrobial properties of plants have been attributed to the presence of these secondary metabolites [23].

In view of the adverse effect of fungi infection on cocoa pods which depletes the nutritional contents of the seeds (beans) and seed viability, this research work, therefore, was carried out to isolate and identify the fungal pathogens associated with cocoa pod diseases in the field as well as evaluate the antifungal effect of ethanolic *Dioscorea dumetorum* and *Moringa oleifera* leaf extracts in controlling the isolated fungal pathogens both *in vitro* and *in vivo.* This research is a continuation of our earlier work titled: Mycoflora associated with cocoa pods obtained in the field and their effect on seed nutritional contents.

2. MATERIALS AND METHODS

2.1 Sources of materials

Infected and uninfected cocoa pods were obtained from major Cocoa Farms at Ajasor in Ikom and Agbokim in Etung Local Government Areas of Cross River State, Nigeria and wrapped in sterile cellophane bags and transported to the Laboratory. *Dioscorea dumetorum* and *Moringa oleifera* leaves were obtained from Ugep in Yakurr Local Government Area of Cross River state, Nigeria. The research was carried out in the Laboratory of the Department of Botany, University of Calabar, Calabar, Cross River State, Nigeria.

2.2 Isolation of fungal pathogens and morphological identification

Cut sections of the diseased cocoa pods were surface sterilized with 70% sodium hypochlorite (bleach) solution for 1min and rinsed quickly in 3 changes of sterile distilled water, blotted dry on Whatman's No.1 filter paper and placed on Potato Dextrose Agar (PDA) in Petri dishes. Four (4) sections were inoculated per Petri dish. The plates were incubated at 28 ± 1°C until fungal growth was noticed. After 7 days, the different isolates were subcultured on freshly prepared PDA to obtain their pure culture. Isolated fungi were microscopically (Olympus optical, Phillipines) identified as far as possible using the identification guides of the International Mycological Institute, Kew and of [24, 25].

2.3 Pathogenicity test and Koch's postulates

Pathogenicity test was carried out using the method of [26]. Healthy cocoa pod were washed

in distilled water and surface sterilized with 70% Sodium hypochlorite solution. A 5mm diameter cork borer was used to cut disc from the fruits (three discs per fruit) and cultures of the isolates (four days old) were introduced into pods and replaced with the discs. The discs portions were sealed with Vaseline to prevent entry by other pathogens. They were kept for 24-48 hours wrapped in sterilized cellophane bags. A sterile cotton wool was soaked with distilled water and dropped inside the cellophane bags to enable the organism to breath (respire). The inoculated fruits established symptoms after 24 hours, tissue segment from the healthy and infected fruit was excised, surface sterilized and plated into freshly prepared PDA and incubated at 28±1^{o}C for four days. Pure cultures of the isolate were identified.

2.4 Preparation of plant extracts

Leaves of *Moringa oleifera* and *Dioscorea dumetorum* obtained were washed with distilled water and oven dried at a temperature of 80^{o}C for 24 hours grounded into fine powder and extracted separately using 200 ml of 99% concentration of ethanol.

2.5 Susceptibility test

The extracts percentage concentrations were prepared at 10g/200ml, 20g/200ml, 30g/200ml, 40g/200ml, and 50g/200ml with ethanol as solvent.

2.6 *In vitro* antifungal assay

5ml of each concentration was first poured into different Petri dishes using sterile syringe. The sterile Potato Dextrose Agar (PDA) was also poured into the plates containing the solvent extracts after which the plates containing the solvent extracts were gently swirled to ensure mixing. The media was allowed to solidify and with a sterilized No.2 cork borer of 5.5mm in diameter, a disc of the matured culture was punched out and inoculated at the centre of plates and incubated at room temperature of (28±1^{o}C) for 7days. As a control, the dishes were inoculated on PDA instead of extracts-agar mix. Three (3) control plates were prepared for each extract. Measurement of the mycelia radial growth was done daily for seven days [27].

2.7 *In vivo* antifungal assay

In vivo antifungal assay of the plant extracts was carried out using the method described by [28] with some modifications. Whole healthy cocoa pods were properly surfaced sterilized using 99% ethanol rinsed with distilled water and allowed to dry for 2-3minutes. The sterile pods were then sprayed with the extracts at different concentrations, two hours prior to inoculation with the fungal pathogens. Each pod after being treated with different concentrations of the extracts of 10g/200ml, 20g/200ml, 30g/200ml, 40g/200ml and 50g/200ml were dipped into beaker containing dissolved spores of each pathogen. The pods were then allowed to dry for 2-5minutes, after which each pod was incubated at room temperature (28±1^{o}C) for twenty-eight days. The control which was also dipped in beakers containing the dissolved spores of each pathogen was not treated with any of the extracts. Growth rate were measured after two weeks of inoculation and was repeated after every four days for four times.

2.8 Phytochemical screening of plant extracts

Phytochemical screening of the plant extracts was carried out in the Department of Biochemistry, University of Calabar, Cross River State using standard methods of [29] and [30].

3. RESULTS AND DISCUSSION

3.1 Identified fungal pathogens

The fungal pathogens isolated and identified as the causative agents of cocoa pod diseases from this study and used were *Botryodiplodia theobromae* and *Fusarium moniliformes*.

3.2 Pathogenicity test and Koch's postulates

Result from the pathogenicity confirmed that the two fungal isolates *Fusarium moniliformes* and *Botryodiplodia theobromae* were responsible for the diseases of cocoa pods obtained from Ikom and Etung L.G.A. in Cross River State, Nigeria. Pathogenicity was established 24 - 48 hours after inoculation (Plates 1 and 2).

3.3 *In vitro* effect of the plant extracts *on* the radial growth of *B. theobromae* and *F. moniliformes* at the different concentrations

The *in vitro* effect of the plant extracts at the different concentrations on the radial growth of the fungal isolates is presented in (Tables 1 - 4). Results showed that, at 10g/200ml, 20g/200ml, 30g/200ml, 40g/200ml and 50g/200ml *D. dumetorum* and *M. oleifera* extracts completely inhibited the radial growth of *Botryodiplodia theobromae* and *Fusarium moniliformes* at the different concentrations tested when compared with the control.

3.4 *In vivo* effect of the plant extracts on the growth and sporulation of *B. theobromae* and *F. moniliformes* at the different concentrations

The *in vivo* effect of the plant extracts at different concentrations on the growth and sporulation of the fungal isolates is presented in (Tables 5-8). Results from the study showed that extracts of *D. dumetorum* and *M. oleifera* had significant effect on the isolated pathogens at all concentration levels (10g/200ml, 20g/200ml, 30g/200ml, 40g/200ml and 50g/200ml) tested when compared with the control. Results (Table 5) showed that at 20g/200ml and 30g/200ml concentrations the

Plates 1 & 2. Infected cocoa pods × 400 showing symptoms of rot and mycelia growth of *Fusarium moniliformes* and *Botryodiplodia theobromae* after ten days post inoculation (dpi).

Table 1. *In vitro* effect of ethanolic *D. dumetorum* leaf extract on the radial growth of *B. theobromae* (cm).

Concentrations	Days of incubation						
	1	2	3	4	5	6	7
10g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
40g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
50g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Control	0.60	0.90	4.40	4.50	4.50	4.50	4.50

Table 2. *In vitro* effect of ethanolic *D. dumetorum* leaf extract on the radial growth of *F. moniliformes* (cm).

Concentrations	Days of incubation						
	1	2	3	4	5	6	7
10g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
40g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
50g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Control	0.40	0.60	0.90	1.50	2.20	3.00	3.80

Table 3. *In vitro* effect of ethanolic *M. oleifera* leaf extract on the radial growth of *B. theobromae* (cm).

Concentrations	Days of incubation						
	1	2	3	4	5	6	7
10g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
40g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
50g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Control	0.60	0.90	4.40	4.50	4.50	4.50	4.50

Table 4. *In vitro* effect of ethanolic *M. oleifera* leaf extract on the radial growth of *F. moniliformes* (cm).

Concentrations	Days of incubation						
	1	2	3	4	5	6	7
10g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
40g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
50g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Control	0.60	0.90	1.80	2.90	3.20	3.50	3.90

extract completely inhibited the mycelia growth of *F. moniliformes* in the first and second day of incubation. However, at 40g/200ml and 50g/200ml concentrations, extract of *D. dumetorum* completely inhibited the mycelia growth of *F. moniliformes* in the first three days when compared with the control. While result (Table 6) showed that *D. dumetorum* extract completely inhibited the growth of *B. theobromae* at 30g/200ml, 40g/200ml and 50g/200ml concentrations on only the first day of incubation. Results (Table 7) showed that *M. oleifera* extract completely inhibited the mycelia growth of *F. moniliformes* at 40g/200ml and 50g/200ml concentrations in the first, second and third day of incubation. Result (Table 8) also showed that *M. oleifera* extract completely inhibited the mycelia growth of *B. theobromae* at 40g/200ml and 50g/200ml concentrations only in the first day of incubation. Results (Table 5-8) showed that *D. dumetorum* leaf extract was more effective on the growth of *F. moniliformes* and *B. theobromae* than those of *M. oleifera.*

Table 5. *In vivo* effect of ethanolic *Dioscorea dumetorum* leaf extract on the growth of *Fusarium moniliformes* (cm).

Concentrations	Days of incubation			
	4	8	12	16
10g/200ml	I.02±0.25	3.19±3.09	3.06±4.27	3.00±5.27
20g/200ml	0.00	2.53±1.11	3.08±2.25	5.05±1.65
30g/200ml	0.00	0.00	2.90±3.01	5.10±0.91
40g/200ml	0.00	0.00	0.00	4.34±1.12
50g/200ml	0.00	0.00	0.00	3.55±2.21
Control	3.09±0.04	9.45±1.14	17.36±2.09	26.80±0.31

Note: Values are means of three replicates ± standard error.

Table 6. *In vivo* effect of ethanolic *Dioscorea dumetorum* leaf extract on the growth of *Botryodiplodia theobromae* (cm).

Concentrations	Days of incubation			
	4	8	12	16
10g/200m	2.11±0.08	5.00±0.06	8.32±1.15	15.14±3.09
20g/200ml	3.34±1.75	4.52±2.75	6.10±2.19	16.11±1.17
30g/200ml	0.00	2.23±0.09	4.35±0.78	8.20±2.78
40g/200ml	0.00	1.23±2.11	3.21±2.11	6.15±3.71
50g/200ml	0.00	1.27±0.76	2.00±0.32	4.34±4.23
Control	5.52±0.10	16.09±2.06	28.01±3.16	45.02±1.07

Note: Values are means of three replicates ± standard error.

Table 7. *In vivo* effect of ethanolic *Moringa oleifera* leaf extract on the growth of *Fusarium moniliformes* (cm).

Concentrations	Days of incubation			
	4	8	12	16
10g/200ml	4.16 ±3.21	8.32 ±1.29	14±0.76	17±3.19
20g/200ml	3.06 ±1.29	6.09 ±0.09	10±2.25	13±2.17
30g/200ml	3.40 ±0.01	6.17 ±0.10	9±0.03	12±1.10
40g/200ml	0.00	0.00	4±0.02	6±0.19
50g/200ml	0.00	0.00	0.00	3±0.11
Control	5.15 ±0.76	12±2.25	20±2.31	32±0.16

Note: Values are means of three replicates ± standard error.

Table 8. *In vivo* effect of *Moringa oleifera* leaf extract on the growth of *Botryodiplodia theobromae* (cm).

Concentrations	Days of incubation			
	4	8	12	16
10g/200ml	6.02 ±2.21	10.11±0.09	14.0 ±1.13	18.31±2.20
20g/200ml	4.10 ±0.01	9.02 ±2.23	12.19 ±2.17	15.18 ±3.19
30g/200ml	4.23 ±0.98	8.23 ±0.19	9.30 ±3.19	11.00 ±0.09
40g/200ml	0.00	3.03 ±3.25	5.12 ±2.61	8.20 ±1.12
50g/200ml	0.00	2.45 ±1.16	3.01±3.02	5.11 ±1.76
Control	9.01 ±0.88	20.05 ±1.25	35.32 ±0.65	48.00 ±1.78

Note: Values are means of three replicates ± standard error

3.5 Phytochemical Screening

Phytochemical screening of *Dioscorea dumetorum* and *Moringa oleifera* leaves in this study showed that they contain secondary metabolites like cardiac glycosides, anthraquinones, and reducing compounds as presented in (Table 9).

Table 9. Phytochemical constituents of ethanolic *Dioscorea dumetorum* and *Moringa oleifera* leaf extracts.

Phytochemicals	*D. dumetorum*	*M. oleifera*
Alkaloids	++	++
Cardiac glycosides	++++	++++
Saponnins	-	-
Tannins	++	++
Flavonoids	+++	+++
Reducing compounds	++++	++++
Polyphenol	+++	-
Phlobatannins	-	-
Anthraquinonones	++++	++++
Hydroxylmethyl anthraquinones	-	++

Note: (++++) strongly present (+++) moderately present (++) highly present (-) absent.

Pod rot disease of cocoa is a major limiting factor in the production of cocoa worldwide. Crop losses and cost of controlling this disease constitute a significant burden in agricultural enterprise and has serious socio-economic and environmental consequences. Consequently, there is an urgent need for effective and sustainable control of this disease. The current trend in the effective and sustainable management of plant diseases requires the use of botanical pesticides which are non-phytoxic to human and are environmentally friendly. In this study, the fungal pathogens isolated as the causative agents of pod rot disease of cocoa were *Fusarium moniliformes* and *Botryodiplodia theobromae.* Results of this study revealed that these fungi were responsible for pod rot of cocoa obtained from Ikom and Etung L.G.A of Cross River State as evidenced by the pathogenicity test. The fungal spores of these pathogens could be spread by water, ants and other insects and can land on susceptible cocoa pods [31]. The pathogens (*Phytophthora palmivora, Pythium spp, Collectotricum gloesporoides*, *Collectotrcium capsici* and *Pestaloptiopsia spp)* were reported by [32] to cause pod rot of cocoa. [33] reported *Phytophthora magarkarya* as the causative agent of pod rot of cocoa, which is in disagreement with the finding of this study.

Phytochemical screening of two plant extracts used in this study was carried out to determine the exact phytochemical contents. Phytochemical screening of the plant extracts (Table 9) showed that alkaloids, cardiac glycosides, tannins, flavonoids, polyphenols and anthraquinones were present in ethanolic leaf extract of *D. dumetorum* and *M. oleifera.* These findings are similar to those of [34] who reported the presence of alkaloids in the plant extract of *Aframomum melegueta*. [35] also reported the presence of polyphenols, flavonoid, alkaloids, tannins, saponnin, phylobatannins, phenols, terpenoids, steroids and glycosides in the extracts of higher plants. In this study, the antifungal activity of the two plant extracts was tested *in vitro* and *in vivo* on fungi isolated from cocoa pod obtained from the field. Results (Tables 1-4) showed that, the plant extracts significantly ($p \leq 0.05$) inhibited the mycelia growth of the fungal isolates at all the concentrations levels tested. However, results (Tables 5-8) showed that, the extracts significantly ($p \leq 0.05$) inhibited the mycelial growth of the fungal pathogens at the different higher concentrations tested and the rate of inhibition differed from one extracts to the other. The differences in the fungitoxic potentials between these plant extracts may be attributed to the susceptibility of the fungal pathogens to the different concentrations of the plant extracts. This agrees with the work of some workers like [36-42] who reported that some essential oil and extracts of various plant have shown remarkable antifungal effect exhibited by retardation in mycelial growth and sporulation of fungal pathogens. Results showed that, the plant extracts differed significantly in their potential to inhibit the growth of these fungal pathogens. Complete inhibition of all the pathogens was achieved with ethanolic leaf extract of *D. dumetorum* and *M. oleifera* at 20g/200ml, 30g/200ml, 40g/200ml and 50g/200ml concentrations. Also all the tested extracts concentrations inhibited significantly the mycelial growth of the pathogens.

It was observed that at all the concentration tested, ethanolic leaf extract of *D. dumetorum* was more effective than *M. oleifera* extract. The inhibitory potency of the extract may be attributed to the phytochemical compounds like cardiac glycosides, reducing compounds, polyphenols and anthraquinones. This agrees with work of [43, 44] and [45] who reported the bioactive compounds of plant extracts in the inhibition of spores, mycelial elongation,

development and spread of fungal pathogens. The inhibitory effect of ethanolic leaf extracts *M. oleifera* and *D.dumetorum* were evaluated in order to develop the cheaper methods of controlling pod rot of cocoa. The greater efficiency of *M. oleifera* and *D. dumetorum* may be due to the high contents of cardiac glycosides, polyphenols, flavonoids, alkaloids and hydroxylmethyl anthraquinones they contain, since these compounds are ranked as the most therapeutically significant plant substances in the control of fungal pathogens [46, 47].This results is in disagreement with the work of [48] on the use of *Lippia spp* in the control of pod rot of cocoa. Their result revealed that, the growth of fungal pathogen was completely inhibited at a higher concentration than at a lower concentration.

4. CONCLUSION

The fungal pathogens isolated and identified from this study as the causative agents of pod rot diseases of cocoa obtained from Ikom and Etung L.G.A.in Cross River State were *F. moniliformes* and *B. theobromae*. Pathogenicity test confirmed that these pathogens actually caused pod rot disease of cocoa. The efficacy of the plant extracts (*M. oleifer*a and D. *dumetorum*) against cocoa pod rot fungi was tested *in vitro* and *in vivo*. The in vitro results showed that the extracts significantly inhibited the mycelia growth of the fungal pathogens at all the concentrations tested when compared with the control. However, in vivo assay, the plant extracts significantly ($p \leq 0.05$) inhibited the mycelia growth of the fungal isolates at the higher different concentrations tested and the rate of inhibition differed from each other. The extracts showed significant effect on the growth and sporulation of *B. theobromae* and *F. moniliformes* both *in vitro* and *in vivo* at all the concentration levels tested and as such can be used in the control of pod rot diseases of cocoa in the field.

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AUTHOR CONTRIBUTIONS

API designed the study, wrote the protocol, and wrote the first draft of the manuscript. OEN and UEJ performed the statistical analysis as well as managed the analyses of the study. OJU managed the literature searches. All authors read and approved the final manuscript."

REFERENCES

1. Juan C, Motamayor PL, Jay WI, Da Silva EM, Reg L, David NK, Steven, BJ, Raymond, JS. Geographic and Genetic population differentiation of the Amazonian chocolate tree (*Theobroma cacao*). PLos ONE 2008;3(10):1371-76.
2. Copetti M., Lamanka B., Frisvad J., Pereira J. Taniwaki M. Mycobiota of cocoa from farm and chocolate. Food Microbiol. 2011; 28:1499-1504.
3. Terry GP, Jeffery WH, Mark Del Carmen R, PoncianoOrti, C, Michael, B, David, C, Michael, DC, Hodgson, JG. Oldest chocolate in the new World. Antiq. 2007;81:314-18.
4. Henderson JS. Chemical and archaeological evidence for the earliest Cacao beverage. Proceedings of National Acad. Jour. of Sci. 2007;104:18937-40.
5. Van Duyn MA, Pivanka R. Overview of the health benefits of fruit and vegetable consumption of the dietics professional. Jour. of Amer. Dietics Assoc. 2000;100:1511-21.
6. APS. Diseases of Theobroma cacao in Florida. American Phytopathlogical Society, St Paul Minnesota: APS Press, 2011.
7. Kagale S, Marimuthu T, Thayumanavan BN, Samiyappan R. Antimicrobial activity and induction of systemic resistance in rice by leaf extract of Daturametel against *Rhizoctonia solani* and *Xanthomonas oryzaepv*. Orzae. Physiolog. and Molec. Plant Pathol. 2004;65:91-100.
8. Gupta R, Khokhar, MK, Lai R. Management of black mould of onion. Journal of plant pathol. and Microbiol. 2012;3(5):3-5.
9. Okigbo RN, Ramesh, P, Achusi, CT. Post-Harvest Deterioration of Cassava and its Control using extracts of *Azadirachta indica* and *Aframomum melegueta*. E- Jour. of Chem. 2009; 6(4): 1274-80.
10. Amadioha AC. Evaluation of some plant extracts against *Colletotrichum lindemuthianum* in cowpea. Acta Phytopathol. and Entomol. 2003;38 (3-4): 259-65.
11. Amadioha AC. Fungitoxic effects of *Azadiractha indica* against *Cochliobolus myabeanus* causing brown spot disease of rice. Acta Phytopatholpflanzo. 2002;35: 37-42.
12. Amadioha AC. Fungicidal activity of some plant extracts against *Rhizoctonia solani* in cowpea. Archives of Pythopatholpflanzo. 2001;33: 509 - 17.
13. Amadioha AC. Fungitoxic effects of some leaf extracts against *Rhizopus oryzae* causing tuber rot of potato. Archives of phytopatholplflanzo, 2000;34:1-9.
14. Enyiukwu DN, Awurum AN. Effects of phytochemicals from *Carica papaya* roots and seeds and *Piper guineense* seeds on the germination of spores of *Colletotrichum destructivum*. Continental Jour. of Biol. Sci. 2011;4(20):55-9.
15. Opara EU, Obana FT. Performance of some plant extracts and pesticides in the control of bacterial spot disease of Solanum. Medwell Agricul. Jour. 2010;5(2):45-9.
16. Awurum, AN, Okwu DE, Onyekoro, PO. In vitro and in vivo control of *Colletotrichum destructivum* in cowpea using some plant extracts. Jour. of Sci. and Technol. 2005;5(1-2):52-7.
17. Bowers JH, Locke JC. Effect of formulated plant extracts and oils on population density of *Phytophthora nicotianae* in soil and control of Phytophthora blight in the green house. Plant Dis. 2004;88:116 – 21.
18. Eleazu CO, Eleazu KC, Awa E, Chukwuma SC. Comparative study of the phytochemical

composition of the leaves of five Nigerian medicinal plants. Jour. of Biotech. and Pharm. Res. 2012;3:42-6.
19. Krishnaiah DR, Sarbatly, BA. Phytochemical antioxidants for health and medicine-A move toward nature. Biotech. and Mol. Biol. Rev. 2007;1: 97-104.
20. Eleazu, CO, Iroaganachi MA, Okoronkwo JO. Determination of the physico-chemical composition, microbial quality and free radical scavenging activities of some commercially sold honey samples in Aba, Nigeria: 'The effect of varying colours'. Inter. Jour. of Biomed. Res. 2013;4: 1 – 4.
21. Molan AL, Faraj AM. The effects of condensed tannins extracted from different plant species on egg hatching and larval development of *Teladorsagia circumcincta* (Nematoda: Trichostrongylidae). Folia Parasitol. 2010;57: 62-8.
22. Zongo C, Savadogo K, Somda JM, Koudou J, Traore AS. In vitro evaluation of the antimicrobial and antioxidant properties of extracts from whole plant of *Alternanthera pungens* H.B. & K. and leaves of *Combretum sericeum* G. Don. Inter. Jour. of Phytomed. 2011;3: 182-91.
23. Prakash G, Hosetti, BB. Investigation of antimicrobial properties of *Dioscorea pentaphylla* from Mid-Western Ghats, India. Sci. World, 2010;8: 91- 6.
24. Barnett HL, Hunter BB. Illustrated genera of imperfect fungi. 4th ed. St. Paul Minnesota: APS Press. 1998.
25. Dugan FM. The identification of fungi. St Paul Minnesota: APS Press. 2006.
26. Okigbo RN, Ramesh P, Achusi, CT. Post-Harvest deterioration of cassava and its control using extracts of *Azadirachta indica* and *Aframomum melegueta*. E- Jour. of Chem. 2009; 6(4): 1274-80.
27. Udo SE, Madunagu BE, Isemin CD. Inhibition of growth and sporulation of fungal pathogens in sweet potato and yam by garlic extracts. Nig. Jour. of Bot. 2001;14: 35 –9.
28. Poppe L, Vanhouytte S, Hofte M. Mode of action of *Pantoea agglomerans* CPA-2, an antagonist of postharvest pathogens on fruits. Euro. Jour. of plant pathol. 2003;109: 963 – 73.
29. Koruthu DP, Manivarian NK, Gopinath A, Abraham A. Antibacterial evaluation reducing power assay and phytochemical screening of *Moringa oleifera* leaf extract: effect of solvent polarity. Inter. Jour. of Pharma. Sci and Res. 2011; 2(11): 2991- 5.
30. Shahrian MI, Hossan MI, Bahar ANM, Akhter S, Haque MA, Bhyiyan MA. Preliminary phytochemical screening: *In vitro* antioxidant and cytotoxic of five different extracts of *Moringa oleifera* leaf. Jour. of Applied Pharma. Sci. 2012;2(5): 65 – 9.
31. Evans HC, Stalpers JA, Sampson RA, Beny GL. On the taxonomy of Moniliophthora roreri an important pathogen of Theobroma cacao in South American. Can. Jour. of Bot. 2002;56:2528-32.
32. Nazihah MS, Meon S, Ismail, IS. Antifungal activity of *Cosmus caudatus* extract against seven economically important plant pathogens. Inter. Jour. of Agric. and Biol. 2014;15(5):1814-9596.
33. Ambang Z, Ndong B, Essono G, Ngoh JP. Control of leaf spot disease caused by *Ceratospora spp* on groundnut (*Arachis hypogaea*) using methanolic extract of yellow oleander (*Thervetia peruvania*) seed. Austral. Crop Sci. 2011;5(3):227-232.
34. Okwu DE, Awurum AN, Okoronkwo IJ. Phytochemical composition and *in vitro* activity screening of extracts from Citrus plants against *Fusarium oxysporum* of okra plant. Pest Tech. 2007;1:145-8.
35. Enyiukwu DN, Awurum AN, Ononuju CC, Nwaneri JA. Significance of characterization of secondary metabolites from extracts of higher plant in disease management. Inter. Jour. of Advan. Agric. Res. 2014;2:8-28.
36. Umesh PM. Efficacy of leaf extracts against the post harvest fungal pathogens of cowpea. Biol. Sci. Discov. 2013;4(1):39-42.
37. Tian J, Ban XQ, Zeng H, He JS, Huang B, Wang YW. Chemical composition and antifungal activity of essential oil from *Cicutan virosa* L. var. latisecta Celak. Inter. Jour. of Food Microbiol. 2011;145:464-70.
38. Soylu EM, Kurt S, Soylu S. *In vitro* and *in vivo* antifungal activities of the essential oils of various plants against tomato grey mould disease agent *Botrytis cinerea*. Inter. Jour. of Food Microbiol. 2010; 143: 183–189.
39. Amadioha AC. Reducing food losses through sustainable methods of plant disease management: An imperative for actualization of food security. A paper presented at the13th Inaugural Lecture of Sciences, 2010;1(2):33-47.
40. Tzartzakis NG, Economarkis CD. Antifungal activity of lemon grass (*Cympopogon citrates* L.) essential oil against key postharvest pathogens. Innovative Food Science Emerging Technology, 2007; 8: 253–8.
41. Okigbo RN, Nmeka IA. Control of yam tuber rot with leaf extracts of *Xylopia aethiopica* and *Zingiber officinale*. Afric. Jour. of Biotech. 2005;4(8):804-7.
42. Ilondu EM. Evaluation of some aqueous plant extracts used in the control of pawpaw fruit (*Carica papaya L*.) rot fungi. Jour. of Appl. Biol. Sci. 2001;37:81-5.
43. Amadioha AC, Obi VI. Fungitoxic activity of extracts from *Azadirachta indica* and *Xylopia aethiopica* on *Colletothrichum lindemuthianumin* cowpea. Jour. of Herbs, Spices and Med. Plts, 1999;6(2):33-40.
44. Enyiukwu DN, Awurum AN. Effects of phytochemicals from *Carica papaya* roots and seeds and *Piper guineense* seeds on the germination of spores of *Colletotrichum destructivum*. Continent. Jour. of Biol. Sci. 2011;4(20):55-9.
45. Gujar J, Talwankar, D. Antifungal potential of crude plant extracts on some pathogenic fungi. Wld. Jour. of Sci. and Tech. 2012;2(6):58-62.
46. Okwu DE., Awurum AN, Okoronkwo IJ. Phytochemical composition and *in vitro* activity screening of extracts from Citrus plants against *Fusarium oxysporum* of okra plant. Pest Tech. 2007;1:145–8.
47. Yadav RNS, Agarwala M. Phytochemical analysis of some medicinal plants. Jour. of Phytopathol. 2011;3(12):10-14.
48. Betty SL, Laura H, Aide, PJ, Elena S, Escobar P. *In vitro* effect of essential oils of three Lippia species on Monilphythora. Acad. Jour. of phytopathol. 2012;23:47-58.