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IN VITRO ANTIFUNGAL EFFECTS OF MEDICINAL PLANTS EXTRACTS ON THE MYCELIA GROWTH OF PHYTOPHTHORA MEGAKARYA CAUSAL AGENT OF COCOA BLACKPOD DISEASE

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ABSTRACT: *Phytophtora megakarya* is the most important fungi pathogen of cocoa the causal agent of blackpod disease of cocoa. Control/Management is majorly done using synthetic chemical fungicides but the report of chemical residue with attendant environmental challenges and adverse effects on the non-target organisms. Medicinal plant metabolites and plant developed pesticides seems to be among the best options because of the little or no environmental effects and eco-friendly attributes compare to synthetic chemical fungicides. In bid to designed eco-friendly, less expensive and easily available control strategy, antifungal assay was done *in vitro* against *Phytophtora megakarya*, extracts of 15 medicinal plants. Three plants viz; *Allium sativum* (77.43%), *Azadirachta indica* (75.17%), *Oxalis latifolia* (71.23%), among the 15 medicinal plants screened showed effective potentiality to inhibit the growth of *Phytophtora megakarya.* Antifungal strength of the 3 medicinal plants was compared with three synthetic chemical fungicides namely; Mancozeb (87.71%), Carbendazim (84.25%) and Copper sulphate (78.45%) at different concentration level. Food poison technique was used for assessing the antifungal actions of the extracts at four concentrations level include; 10%, 20%, 40% and 60% on mycelial growth of *Phytophtora megakarya*. Findings reported in this study revealed that the extracts of medicinal plants portend a good alternative to develop potent bio-fungicides that can be utilized without any adverse effects on the environments in controlling *Phytophtora megakarya*, the causal agent of blackpod disease of cocoa.

KEYWORDS: *Phytophtora megakarya*, *Azadirachta indica* , medicinal plants, blackpod

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1. INTRODUCTION

Cocoa (*Theobroma cacao* L.) is a major commercial crop of the equatorial region which is extensively cultivated in West Africa countries such as Nigeria, Ghana, Côte d'Ivorie, Liberia, Sierra Leone, Togo and Republic of Benin (Opeke, 2003). It is an important foreign exchange earner for these countries (FAO, 2005) and a source of powder used in the confectionary industry for preparing chocolate (Opeke, 2003). Cocoa has made substantial impact on millions of people in West Africa, which supplies about 81 % of the total world production (Sanusi and Oluyole, 2005). Nigeria was ranked the second world largest producer between 1960 and 1969 (Opeke, 2003), but a downward trend was documented shortly after this period (ICCO, 2009). The fall in production output in Nigeria was ascribed to many factors viz; ageing of trees, poor agronomic practices, pests and diseases (Dongo and Orisajo, 2007). Diseases are the most important factors contributing to decline in production in Nigeria. More than 200 different diseases are known to affect cocoa but about 10 of these are of economic importance (Woods and Lass, 1989). Among these diseases are virus and fungi which affect cocoa different stages of growth and development. In economic reality, black pod disease incited by *Phythophtora megakarya.* Blackpoddisease is the most prominent and damaging disease which alter the production output on a global scale. The disease is more devastating in areas of heavy rainfall. Major damage from the disease is the rotting of both small and large pods. Choupons, seedlings (in the nursery) and leaves of trees are attached and killed under specially severe disease conditions following long periods of cool and rainy weather. Babcock *et al* (1992) noted that the pathogen could be managed through the use of chemicals (synthetic pesticides), but their non-biodegradable attributes which poses great threat to the environment coupled with occurrence of fungicide-resistance pathogen, render them unsuitable in developing countries. On the other hand, man has used plants for health care in many countries. Many molecules of medicinal uses were originally derived from plants. However, in the 50(s), with the advance in anti-biotic and particularly the enormous development of synthetic organic chemistry, the use of herbs and herbal products created considerably scientific interests. Responses from traditional society indicate the efficacy of leaf extract of commonly available local plants for combating fungal infections. The antifungal and antiviral property of these plants has been reported by many authors. Consequence upon the above, and the need to source for alternative to chemical control, the role of higher plants as source of fungicides and their importance in controlling different plant pathogens are gaining prominence, in view of the ecological stability and cost effectiveness plant extracts with their biodegradable and environment friendly nature have shown some promise in recent years. The result could add to methods of control used by farmers, thereby reducing reliance on chemical fungicides that are reported to predicate long term harmful consequences on environment, man and other wildlife. The aim of this study is to screen some medicinal plant extracts for their antifungal attributes against

Phytophtora megakarya in vitro.

2. MATERIALS AND METHODS

Collection of diseased pods and calculation of disease severity and percent infection of cocoa blackpod

Assessment of disease severity, collection of disease samples and percentage of infection on cocoa pods in cocoa plantations in Ijebu Igbo was carried out. A plantation was partitioned into groups and 10 trees ineach group were considered and assessed for disease severity and percent infection. Total number of plantations assessed was recorded. Diseased pods were collected for *Phytophthora megakarya isolation*, the causal agent of blackpod disease of cocoa.

Collection of medicinal plants

Fifteen native plants were sampled, collected and used for this study, for their antifungal property. The medicinal plants used are listed in the table 1 below. The plants were from local environment based on the facts that they contain antimicrobial properties according to research findings in literatures, traditional knowledge, readily available in large quantities with very little commercial importance. The selected plants were well adapted to the climatic conditions and were well known for their medicinal attributes among local natives. These plants were obtained from 2 locations (Ijebu Igbo in Ogun State and Akure, Ondo State).

Isolation and identification of the pathogen

The diseased cocoa pod were surface sterilized with 70 % ethanol and immersed in 0.3 % sodium hypochlorite for 10 mins, the sliced portion were rinsed in distilled water and plated on petri dishes contained potato dextrose agar (PDA) medium and incubated in dark at 28 °C for 7 days in the department of Laboratory Science Technology, Abraham Adesanya Polytechnic, Ijebu-Igbo Ogun State. The isolated fungal pathogens were sub-cultured on a fresh PDA medium until a pure culture of Phytophthora megakarya was obtained. Pure cultures were maintained on PDA slants and petri dishes at 4 °C until needed.

Preparation of plant extracts

This was done by taken parts of the collected plants and pound to slurry form using mortar and pestle in distilled water. The macerated lot were kept overnight in culture tubes for the release of bio-chemicals exudates at 4 °C. The lots were filtered using muslin cloth, and were filtrated using Whatman No. 1 filter paper. The filtrate was subjected to centrifugation at 10, 000 RPM for 5 min and the supernatant was sterilized and stored at 4 °C as stock solutions.

Antifungal activity assay of botanical extracts by using poison food technique

Plant extract at different concentrations 10%, 20%, 40% and 60% from each of the stock solution were added to 20 ml of sterilized potato dextrose agar in petri dishes. A 5 mm diameter of the actively growing mycelium disc of the pathogen of 6–7 day old culture was placed in the center of the Petri

dish. Plates without plant extract served as negative control. Plates were incubated at 27 °C. Each treatment were replicated three times. Radial growth of mycelium was measured after seven days of incubation, and the results were compared with negative control. The experiment was repeated three times and mean was recorded for calculations. The percent inhibition of the fungus in each treatment was calculated using following formula following the formulae of Shivapratap*et al* (1996);

$$L = [(C – T)/C] \times 100$$

Where, L is the percent inhibition;

C is the colony radius in control plate and,

T is the radial growth of the pathogen in the presence of plant extracts (Shivapratap et al., 1996).

Evaluation of chemical fungicides by poison food technique on *Phytophthora megakarya.*

Three synthetic chemical fungicides viz; Mancozeb, Carbendazim and Copper sulphate, were evaluated for their efficacy against *Phytophthora megakarya in vitro*. Four concentration levels viz; 10ppm, 20ppm, 40ppm and 60ppm were used for evaluation of their inhibitory actions against the pathogen by poison food technique. The experimental procedure follows the same pattern as the one used for plant extracts.

Data analysis

Statistical analysis of results was performed using SPSS (version 20). Analysis of Variance at $P \leq 0.001$ followed by Tukey test with $P \leq 0.05$ was utilized in determining the significant differences between the results obtained in each of the experiment.

3. RESULTS AND DISCUSSION

Collection of disease samples and calculation of Disease severity and percent infection of *Phytophthora megakarya*of cocoa

Table 1: List of Plant used for screening

S/No	Botanical Name	Family
1	*Tamarindus indicus* L.	Fabaceae
2	*Eucalyptus globulus*Labill	Myrtaceae
3	*Allium sativum*.	Solanaceae
4	*Oxalis latifolia*Kunth.	Oxalidaceae
5	*Agave americana*L.	Asparagaceae
6	*Zingiberofficinale*.	Fabaceae
7	*Tridaxprocumbens*L.	Asteraceae
8	*Partheniumhysterophorus*L.	Asteraceae
9	*Azadirachta indica* A. Juss.	Meliaceae
10	*Ficus religiosa*L.	Moraceae
11	*Ricinuscommunis*L.	Euphorbiaceae

12	*Nerium oleander* L.	Apocynaceae
13	*Cissusquadrangularis*L.	Vitaceae
14	*Artocarpusheterophyllus*Lam.	Moraceae
15	*Allium cepa* L.	Lilliaceae

Table 2: Disease severity and Percent infection of *Phytophthora magakarya*of Cocoa

Ijebu Igbo Farm District	Statistical Analysis
Farm 1	31.00 ± 1.00^{a}
Farm 2	23.85 ± 1.23^{bd}
Farm 3	29.00 ± 1.00^{ab}
Farm 4	21.00 ± 1.00^{d}
Farm 5	23.33 ± 1.53^{d}
Farm 6	16.00 ± 4.00^{d}
Farm 7	33.00 ± 1.73^{a}

Values that have the same superscripts are significantly different at P≤0.05.

Table 3: Antifungal activity of different aqueous extracts at different (10%, 20%, 40% and 60%) concentration showing percent inhibition of mycelia growth

Plant Extract	10 %	20 %	40 %	60 %
Tamarindus indicus	39.33 ± 0.58^{d}	42.33 ± 0.58^{c}	57.33 ± 0.58^{b}	60.00 ± 0.00^{a}
Eucalyptus globulus	40.33 ± 0.58^{d}	45.33 ± 0.58^{c}	47.66 ± 0.58^{b}	57.33 ± 0.58^{a}
Allium Sativum	67.33 ± 0.58^{c}	72.55 ± 1.00^{b}	74.44 ± 0.58^{b}	77.43 ± 0.58^{a}
Oxalis latifolia	55.11 ± 0.58^{c}	56.23 ± 0.58^{c}	65.33 ± 0.58^{b}	71.23 ± 0.58^{a}
Agave americana	42.00 ± 1.00^{d}	44.00 ± 1.00^{c}	48.33 ± 0.58^{b}	54.33 ± 0.58^{a}
Zingiberofficinale	45.33 ± 0.58^{d}	47.66 ± 0.58^{c}	51.33 ± 0.58^{b}	55.66 ± 0.58^{a}
Tridaxprocumbens	35.33 ± 0.58^{c}	44.66 ± 0.58^{b}	44.66±0.58b	48.66±0.58a
Partheniumhysterophorus	57.66±0.58c	61.33±0.58b	63.66±0.58a	64.66±0.58a
Azadirachta indica	51.00±0.58a	60.00±1.00a	72.33±0.58a	75.17±0.00a
Ficus religiosa	32.33±0.58c	40.00±1.00b	40.33±0.58b	55.66±0.58a
Ricinuscommunis	44.00±1.00b	60.00±1.00a	61.00±1.00a	61.66±0.58a
Nerium oleander	24.00±1.00d	54.66±0.58c	57.33±0.58b	60.00±1.00a
Cissusquadrangularis	33.33±0.58c	55.00±1.00b	68.33±0.58a	69.00±1.00a
Artocarpusheterophyllus	32.00±0.58a	53.55±1.00a	55.85±1.00a	57.00±0.58a
Allium cepa	44.33±1.15c	44.66±0.58c	49.33±0.58b	56.33±0.58a
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Values with the same superscripts are significantly different at P≤0.05 within the column.

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Table 4. Evaluation of Chemical Fungicides of poison food technique

Chemical Fungicides	10 %	20 %	40 %	60 %
Mancozeb	75.12±0.83[a]	81.76±1.14[a]	82.42±0.49[a]	87.71±0.57[a]
Copper Sulphate	73.44±0.58[a]	78.34±0.58[b]	79.43±0.58[b]	78.45±0.57[a]
Carbendazim	72.11±1.00[b]	75.74±0.87[c]	75.34±0.58[c]	84.25±2.51[a]
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Antifungal activity assay of plant extracts by using poison food technique

The effects of various extracts of the plants evaluated were studied for percent inhibition of mycelia growth of *Phytophthora megakarya*. 15 plants were tested for antifungal actions at four different concentration levels viz; 10%, 20%, 40% and 60%. Out of the 15 plants extracts tested, three of the plant extracts significantly inhibited growth of *Phytophthora megakarya*, at each of the concentrations level used. The plants extract include; *Solanum indicum*(78.33%), *Oxalis latifolia*(70.33%) and *Azadirachta indica* (75.00%) (Table-3), while the remaining plants extracted tested showed a lesser percentage of inhibition at the same concentration level (Table-3).

Antifungal activity of chemical fungicides on *Phytophthora megakarya*

Three synthetic fungicides were used against *Phytophthora megakarya* at four concentrations in ppm levels viz;10ppm, 20ppm, 40ppm and 60ppm. All the concentrations used showed considerable percentage inhibition of mycelial growth compared to control (table 4). All the synthetic fungicides tested significantly reduced the mycelial growth of the cultured *Phytophthora megakarya*. But from the result gotten, Mancozeb showed to be the most effective among the synthetic fungicide tested in inhibiting mycelial radial growth of the *Phytophthora megakarya*, followed by Copper sulphate and carbendazim (Table- 4).

DISCUSSION

Medicinal plants are full of many phytochemicals that has been reported to a great important defensive roles against pathogens, insects and animals (Duke and Bogenshutz-Godwi, 1999, Fawole*et al.,* 2013). The aqueous form of these plant extract has been documented to inhibit the mycelia growth of plant pathogenic fungi *in vitro* (Senhaji*et al*., 2005; Pak *et al*., 2006; Oyedeji*et al*., 2011). The optimal inhibition of mycelia development recorded in this study inferred that extracts of *A. sativum, A.indica*and*O. latifolia* acted effectively against the *Phythophtora megakarya*. Studies have shown that the antifungal efficacy of extract of *Azardiachta indica, Eucalyptus globules*, *Ocimum sanctum* etc has been tested and proved effective against some vegetable fungal pathogen such as *Fusarium solani*at different concentration level with a significant inhibition in the mycelia growth of the pathogen. Further studies on the biochemical nature of *A. sativum, A.indica*and *O. latifolia ,* coupled with molecular screening of the medicinal plants against fungi pathogens will help

to reveal the active ingredients that inhibit the growth of the mycelia growth of disease causing pathogens. These medicinal plants have been utilized extensively Nigeria because of their importance in trado-medicine, coupled with the level of chemical and biochemical constituents they contain such as polyphenols, flavonoids, phenolic acids, tannins, quinines, coumarins, terpenoids and alkaloids (Ferheen et al., 2005; Omidbeygi et al., 2007; Oyedeji et al., 2011). Flavonoids, phytic acid, tannins and phenols are the chemical constituents found in *A. sativum* contains. *A. sativum* aqueous extract maximally inhibit the mycelia growth of the controlled pathogen. Past studies have revealed that the presence of hydroxyl groups on the phenol groups have relationship in their relative toxicity to microorganisms, this indicated that increase in hydroxylation level results in increased toxicity. Enzyme inhibition of the oxidized compounds are thought to be mechanism responsible for phenolic toxicity to microorganisms, possibly through reaction with sulfhydryl groups through more nonspecific interactions with proteins (Arif*et al.,* 2009).

4. CONCLUSION

The understanding of bio-control agents' compatibility alongside other useful components involve in the development is required to formulate a reliable plant pathogen management strategies. Blackpoddisease of cocoa can cause significant yield losses in cocoa production. It has been concluded from present research that certain botanical extracts are a source of cost effective and non-hazardous bio- fungicide against *P.megakarya* , also they don't have human and environment, health hazard or implications, so same plant extracts such as *A.sativum*, *A. indica*, *O. latifolia*could be a good antifungal agents, which may be use in formulating new, safer and eco-friendly bio-fungicides for the control of *P.megakarya* pathogen causal agent of blackpod disease of cocoa.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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