



# Effects of Extracts of Some Botanicals against Three Species of Fungi Isolated from Cocoa (*Theobroma cacao* L.) Pods

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## ABSTRACT

Cocoa is an important cash crop in the tropics and sub-tropics. However, its production is reported to be immensely constrained due to black pod disease caused by fungal agents. This study evaluated *in vitro* the efficacy of ethanolic and aqueous extracts of purging nuts (*Jatropha curcas* L.), bitter leaf (*Vernonia amygdalina*), lemon grass (*Cymbopogon citratus*), pods of guinea pepper (*Xylopiya aethiopica*), seeds of black pepper (*Piper guineense*), rhizomes of ginger (*Zingiber officinale*) and stem ash of *Acioa bacteri* at varying concentrations (10, 20, 30, 40 and 50%) against the fungi associated with pod rot of cocoa. Results obtained from the study showed that the ethanolic extracts of black pepper (*Piper guinense*), wood ash (*Acioa bacteri*) and ginger roots (*Zingiber officinale*) strongly inhibited the radial growth of *Phytophthora megakarya* and *Colletotrichum ignotum* by (84-100%), while PDA media modified with warm water extract of black pepper (*Piper guinense*), bitter leaf (*V. amygdalina*), wood ash (*A. bacteri*) and ginger (*Z. officinale*) roots showed varying levels of inhibition (71-100%) of the radial growth of three test fungi. Of all the extracts, *F. decemcellulare* was only sensitive to water extracts of *V. amygdalina* recording (84-100%) inhibition. However, irrespective of concentration and extracting solvent purging nut (*Jatropha curcas*) *A. aethiopica* and *C. citratus* were not effective in minimizing the growth of the assayed fungi in culture. Hence, extracts of ginger, black pepper, bitter leaf and *A. bacteri* can be exploited for control of pod rot of cocoa caused by *P. megakarya* and *C. ignotum*.

## INTRODUCTION

Cocoa (*Theobroma cacao* L.) belongs to the family Euphobiaceae. It is a major economic cash crop cultivated on over 700,000 km<sup>3</sup> of farmland worldwide especially in most tropical countries of Africa. Nigeria ranks as fourth largest producer of the crop in Africa producing about 400,000 metric tonnes annually (Fagbohun and Lawal, 2011). However, besides mechanical injury; yield and production of cocoa have been seriously affected by many pests and diseases caused by bacteria, virus, parasitic plants and fungal agents including *Phytophthora* spp. at various stages of its growth; resulting in loss of above 40 % potential yield of the crop especially in Nigeria (Agrios, 2005; Grant and Dickson, 2005; Asogwa and Dongo, 2009).

Attacks of black pod of cocoa (*Phytophthora megakarya*) have been reported as a major constraint towards increased and profitable cocoa production in Nigeria (Opoku *et al.*, 2005). Though the disease attacks all above ground parts of the cocoa plant, major economic losses stems from infection of the pods; and its control has been difficult (Aragaki and Uchida, 2001).

Control of this disease has been attempted with bourdeaux mixture and ridomil amongst other synthetic chemical measures (Adejumo, 2005). However, the danger of heavy metal accumulation in the rhizosphere and disruption of the food chain due to such interventions have been documented (Asogwa and Dongo, 2009; Enyiukwu and Awurum, 2012). Exploitation of natural products of plant origin with antifungal properties in plant pathology has been variously reported in recent times and these could potentially play prominent roles in eco-friendly plant disease management (Stoll, 2003; Okigbo and Ogbonna, 2006; Awurum *et al.*, 2015).

Strong fungitoxic activities of ethanolic and aqueous extracts of *Piper guineense* (black pepper), *Xylopiya aethiopica* (guinea pepper), *Azadirachta indica* (Neem), *Zingiber officinale* (ginger), *Carica papaya* (Paw-paw) against crop devastating fungal pathogens such as *Colletotrichum destructivum*, *C. lindemuthianum*, *Rhizoctonia solani* and *Fusarium* spp. have been reported. These extracts acted by inhibiting the spores germination and retarded the mycelial growth of the pathogenic mycoflora (Amadioha, 2003; Okereke and Wokocho, 2006; Okwu and Ukanwa, 2009; Enyiukwu and Awurum, 2011; 2012). This work therefore evaluated *in vitro* the fungitoxic effects of different concentrations of aqueous and ethanolic extracts of some medicinal plants (purging nut leaf, bitter leaf, lemon grass, guinea pepper pods, black pepper seeds, ginger rhizome and *Acioa bacteri* stem ash) against three mycoflora isolated from cocoa pods.

## MATERIALS AND METHODS

### Source and Preparation of Plant Materials

This experiment was conducted at the Plant Pathology Laboratory of the National Root Crops Research Institute (NRCRI) Umudike. The following plant materials purging nuts (*Jatropha curcas* L.) leaves, bitter leaf (*Vernonia amygdalina*), lemon grass (*Cymbopogon citratus*) leaves, guinea pepper (*Xylopiya aethiopica*) pods, black pepper (*Piper guinense*), ginger (*Zingiber officinale*) rhizome and *Acioa bacteri* stem were used for the study. All the plant materials were purchased from Umuahia Main Market in Abia State, except lemon grass which was obtained from the fallowed farm site in the neighbourhood of the Institute.

The plant materials (leaves, stems, pods and seeds) were thoroughly washed with clean tap water, and air-dried to constant weight and then milled to powder using a Thomas Willey machine (Model: T500). However, the dried stem (wood) of *Acioa bacteri* was burnt in fire to obtain its ash. All the milled plant materials and the wood ash were stored in air-tight bottles and kept in clean, dry shelves cupboards until required.

### Extraction of Plant Materials

Fifty (50) grams of each ground plant materials and the wood ash were soaked separately in pre-weighed 250ml sterile conical flask containing 100ml sterile deionized water to give 10%, 20%, 30%, 40% and 50% concentrations of the plant extracts respectively (Okereke and Wokocho, 2006 and Varaspasad *et al.*, 2009). Each suspension was hand shaken intermittently and allowed to stand for 12 h before filtering into a fresh 250 ml flask using four layers of muslin cloth. Each plant material was separately and exhaustively extracted in ethanol for 12 h with a soxhlet apparatus maintained at a temperature of 69°C by means of a heat mantle to obtain the ethanolic extracts. The extracts were then separately and serially diluted to obtain the 10 %, 20 %, 30 %, 40 % and 50 % concentrations of the plant extracts respectively (Wokocho and Okereke, 2006, Varaspasad *et al.*, 2009). Both the aqueous and ethanolic extracts were then separately and aseptically dispensed into differently amber-coloured vials and kept in the refrigerator at 5°C until required.

### Isolation and Identification of Causal agents

Infected as well as healthy improved cocoa pods (Var.: Amazon) were collected from the major cocoa growing communities of Abia State, Nigeria and transported to the Plant Pathology Laboratory of the Institute (NRCRI). The cocoa pods were washed in several changes of running tap water to remove adhering debris, and then the pod husks were cut into 5 mm segments towards the advancing margin of lesions on infected pods. The segments were surface-sterilized in 10 % sodium hypochlorite solution for 3 minutes, rinsed and then dried in between sheets of Whatman No. 1 sterile filter paper.

Then 39.5 g of dehydrated potato dextrose agar (PDA) (Oxoid™ ThermoScientific Product, England, UK) was dissolved in 1 litre of sterile distilled water contained in a 2 litre flat bottomed flask and stoppered with foiled cotton wool. Then the flask with its content was autoclaved at 120°C and 151 mmHg for 30 minutes. The infected tissues were then plated on potato dextrose agar (PDA) medium thus prepared, and then incubated at 28° ± 2°C for five days (Awuah and Frimpong, 2008; Enyukwu and Awurum, 2012). Fungal growths from the incubated specimen were purified by repeated transfers onto fresh PDA slants in McCartney bottles and kept at 5°C until required. The identity of the isolates from the infected pods were confirmed with reference to the descriptive monographs of fungal pathogens by the International Mycological Institute (IMI) and Reference manuals by Barnett and Hunter (2003) and Pfenning (2010).

### Pathogenicity tests

The matured healthy improved cocoa pods harvested from the farmers' fields in the major cocoa growing locations in Abia State were surface sterilized with 10 % sodium hypochlorite and then placed on sterile paper towels in the Laminar Air flow cabinet (Environmental Air Control, Inc., USA) and allowed to dry for 5 minutes. A 5 mm diameter flame sterilized cork borer was used to cut a disc from the skin of the healthy cocoa pods and a flame sterilized inoculation needle was used to collect 5 mm disc of each seven day-old pure cultures of the different fungal organisms (*Phytophthora megakarya*, *Colletotrichum ignotum* and *Fusarium decemcellulare*) consistently isolated from the cocoa pods, and then aseptically inoculated into the hole on the healthy cocoa pods and covered with petroleum jelly. Pathogenicity of the isolated organisms on the inoculated pods was determined after 14 days. At the end of the fortnight, all the isolates produced lesions on the healthy pods similar to those observed on the pods in the farmers' farms.

### Antimycotic Evaluation of the Plant Materials

One millilitre of each concentration (10 %, 20 %, 30 %, 40 % and 50 %) of the plant extracts were pipetted separately and aseptically onto 10 ml of cooled molten potato dextrose agar (PDA) medium in sterile Petri dishes and thoroughly mixed by gentle circular rotations in order to achieve uniform dispersal of the extracts. The medium was allowed to solidify after 24 h, and the plates were inoculated by placing at the centre a 5 mm disc taken from the advancing edge of a 7 day old pure culture of the test fungi (*P. megakarya*, *F. decemcellulare*, *C. ignotum*). Similarly, PDA plates without extracts which were inoculated with mycelia disc of the isolates served as control. All PDA plates were incubated at 28°C for 7 days in a Gallenkamp incubator (Model: IH-100, England). The experiment was laid out in a Completely Randomized Design (CRD) consisting of eighteen (18) treatments which were replicated three (3)

times. The radial growth of each isolate was determined by measuring the diameter of the fungus daily for 7 days using a transparent meter rule. The percentage inhibition of radial growth of the test fungi was calculated by a modification of the formula by Wokocho and Nneke (2011) as:

$$\% \text{ inhibition} = \frac{dc - dt}{dc} \times \frac{100}{1}$$

Where:

dc = is the average diameter of fungal colony in control.

dt = the average diameter of fungal colony in treatment.

### STATISTICAL ANALYSIS

Data collected from the study were analyzed by Analysis of Variance (ANOVA) using GenStat 2008 version. Means were separated and compared using Fisher's Least Significant Different (LSD) at 5% probability level.

### RESULTS

The result presented in Table 1 showed that the ethanol extract of black pepper (*P. guineense*) at 10-50 % concentrations significantly ( $P \leq 0.05$ ) strongly inhibited the radial growth of *P. megakarya* by 100 %. Similarly, ginger rhizome (*Z. officinale*) at increasing concentrations effectively reduced the mycelial growth of *P. megakarya* by 90-95%. This was in contrast with the inhibitions recorded with purging nut (*J. curcas*) 12 %, bitter leaf (*V. amygdalina*) 20 %, and lemon grass (*C. citratus*) 12 %, which did not significantly ( $P \geq 0.05$ ) reduce the mycelial growth of the pathogen (*P. megakarya*). The result also obtained from 20-50% showed that (*A. bacteri*) at increasing concentrations had a strong inhibitory effect on the radial growth of *C. ignotum* by 100 % followed by black pepper (*P. guineense*) at the application rate of 50 % concentration and ginger rhizome (*Z. officinale*) (89 %) recorded at 30 % concentration.

Furthermore, ethanol extract of ginger roots (*Z. officinale*) at 20 % and 30 % concentrations showed strong inhibition by 90 % against *F. decemcellulare*, followed, by black pepper (*P. guineense*) at 10 % and 50 % concentrations which significantly ( $P \leq 0.05$ ) showed high potency in reducing the radial growth of *F. decemcellulare* in modified PDA media. However, when compared with that obtained from the control, bitter leaf extract (*V. amygdalina*) and purging nut (*J. curcas*) gave 20 % and 12 % inhibition respectively when applied at 50 % concentrations and recorded mean inhibition of 27.5 % and 29.0 % respectively across all the assayed concentrations of the pathogen (*F. decemcellulare*) in culture. Therefore, the result in Table 1 showed that ethanol extracts of black pepper (*P. guineense*), wood ash (*A. bacteri*), ginger roots (*Z. officinale*) at 10-50% concentration were found to be the most potent extracts

with strong inhibitory effects against the three pathogenic fungi from cocoa pods evaluated in this study.

In Table 2, the result also revealed that the warm water extract of bitter leaves (*V. amygdalina*) reduced strongly the radial mycelial growth of *P. megakarya* by 97% at increasing concentrations, this was followed by wood ash (*A. bacteri*) 95 % and black pepper (*P. guineense*) 91% against the same pathogen. However, 10-50 % concentration of warm water extracts of Guinea pepper (*X. aethiopica*), purging nut (*J. curcas*), and Lemon grass (*C. citratus*) significantly ( $P \leq 0.05$ ) supported the radial mycelial growth of *P. megakarya*.

Furthermore, warm water extracts of bitter leaves (*V. amygdalina*) at 10-50 % concentrations significantly ( $P \leq 0.05$ ) showed high potency in inhibiting by 96 % the radial mycelial growth of *C. ignotum*, followed by wood ash (*A. bacteri*) 82 % and ginger (*Z. officinale*) rhizome 78 %.

Also, lemon grass (*C. citratus*) at 10-50 % concentrations significantly supported the mycelial

growth of *C. ignotum* in culture by (3-25%), followed by purging nut (*J. curcas* (24-37%) and Guinea pepper (*X. aethiopica*) (9-41%) respectively.

In summary, this result, indicated that warm water extracts of bitter leaves (*V. amygdalina*) was the most effective in significantly ( $P \leq 0.05$ ) reducing the radial growth of *F. decemcellulare* by 100 % followed by wood ash (*A. bacteri*) 92 % at increasing concentrations whereas up to 82 % inhibition was recorded with extract of ginger (*Z. officinale*) rhizome applied at 30 % concentration. However, warm water extracts of purging nuts (*J. curcas*), lemon grass (*C. citratus*), and Guinea pepper (*X. aethiopica*) showed no significant ( $P \geq 0.05$ ) effects against *F. decemcellulare in-vitro* at all levels of application. Therefore, the warm water extracts of bitter leaves (*V. amygdalina*), wood ash (*A. bacteri*) and ginger roots (*Z. officinale*) were the most effective in inhibiting the radial mycelia growth of the three mycoflora isolated from cocoa pods at the various concentrations of the treatments.

**Table 1: Growth inhibition of three mycoflora isolated from cocoa pods using different concentrations of ethanolic extracts (in-vitro)**

| Pathogens           | Extract Conc. | <i>C. citratus</i> | <i>P. guineense</i> | <i>X. aethiopica</i> | <i>A. bacteri</i> | <i>V. amygdalina</i> | <i>Z. officinale</i> | <i>J. curcas</i> | Control (ethanol) |
|---------------------|---------------|--------------------|---------------------|----------------------|-------------------|----------------------|----------------------|------------------|-------------------|
| <i>P. megakarya</i> | 10%           | 12                 | 100                 | 41                   | 46                | 20                   | 66                   | 15               | 39                |
|                     | 20%           | 25                 | 100                 | 47                   | 75                | 22                   | 90                   | 12               | 40                |
|                     | 30%           | 14                 | 100                 | 68                   | 60                | 22                   | 95                   | 15               | 41                |
|                     | 40%           | 28                 | 100                 | 75                   | 95                | 22                   | 91                   | 15               | 45                |
|                     | 50%           | 31                 | 100                 | 71                   | 84                | 22                   | 95                   | 15               | 45                |
| <i>C. ignotum</i>   | 10%           | 61                 | 84                  | 56                   | 43                | 43                   | 71                   | 56               | 39                |
|                     | 20%           | 47                 | 89                  | 63                   | 100               | 31                   | 78                   | 50               | 38                |
|                     | 30%           | 46                 | 92                  | 61                   | 100               | 41                   | 89                   | 53               | 39                |
|                     | 40%           | 56                 | 89                  | 45                   | 100               | 24                   | 70                   | 51               | 37                |
|                     | 50%           | 48                 | 100                 | 56                   | 100               | 31                   | 84                   | 50               | 39                |
| <i>F.d</i>          | 10%           | 47                 | 70                  | 44                   | 32                | 38                   | 61                   | 38               | 34                |
|                     | 20%           | 37                 | 69                  | 42                   | 41                | 21                   | 90                   | 20               | 33                |
|                     | 30%           | 48                 | 63                  | 51                   | 51                | 21                   | 90                   | 33               | 33                |
|                     | 40%           | 54                 | 63                  | 57                   | 30                | 39                   | 75                   | 42               | 33                |
|                     | 50%           | 54                 | 71                  | 51                   | 60                | 20                   | 68                   | 12               | 35                |
| <b>LSD (0.05)</b>   |               | <b>30</b>          | <b>30</b>           | <b>30</b>            | <b>30</b>         | <b>30</b>            | <b>30</b>            | <b>30</b>        | <b>30</b>         |

\**F.d* = *F. decemcellulare***Table 2: Growth inhibition of three mycoflora isolated from cocoa pods using different concentrations of aqueous extracts (in-vitro)**

| Pathogens           | Extract Conc. | <i>C. citratus</i> | <i>P. guineense</i> | <i>X. aethiopica</i> | <i>A. bacteri</i> | <i>V. amygdalina</i> | <i>Z. officinale</i> | <i>J. curcas</i> | Control (Sterile water) |
|---------------------|---------------|--------------------|---------------------|----------------------|-------------------|----------------------|----------------------|------------------|-------------------------|
| <i>P. megakarya</i> | 10%           | 21                 | 71                  | 11                   | 91                | 95                   | 50                   | 20               | 40                      |
|                     | 20%           | 21                 | 91                  | 12                   | 86                | 95                   | 59                   | 22               | 42                      |
|                     | 30%           | 22                 | 86                  | 21                   | 88                | 97                   | 68                   | 22               | 44                      |
|                     | 40%           | 22                 | 86                  | 22                   | 95                | 97                   | 66                   | 22               | 45                      |
|                     | 50%           | 23                 | 84                  | 22                   | 93                | 97                   | 66                   | 22               | 45                      |
| <i>C. ignotum</i>   | 10%           | 23                 | 44                  | 41                   | 82                | 88                   | 58                   | 32               | 34                      |
|                     | 20%           | 08                 | 64                  | 23                   | 73                | 91                   | 76                   | 32               | 34                      |
|                     | 30%           | 03                 | 50                  | 31                   | 71                | 93                   | 78                   | 28               | 32                      |
|                     | 40%           | 15                 | 45                  | 09                   | 72                | 96                   | 74                   | 24               | 33                      |
|                     | 50%           | 25                 | 57                  | 14                   | 80                | 94                   | 74                   | 37               | 35                      |
| <i>*F. d</i>        | 10%           | 38                 | 41                  | 38                   | 74                | 92                   | 64                   | 23               | 39                      |
|                     | 20%           | 15                 | 43                  | 35                   | 84                | 84                   | 74                   | 15               | 39                      |
|                     | 30%           | 14                 | 38                  | 25                   | 80                | 91                   | 82                   | 5                | 35                      |
|                     | 40%           | 10                 | 37                  | 27                   | 89                | 100                  | 78                   | 27               | 37                      |
|                     | 50%           | 15                 | 41                  | 33                   | 92                | 87                   | 76                   | 20               | 39                      |
| <b>LSD (0.05)</b>   |               | <b>30.4</b>        | <b>30.4</b>         | <b>30.4</b>          | <b>30.4</b>       | <b>30.4</b>          | <b>30.4</b>          | <b>30.4</b>      | <b>30.4</b>             |

\**F. d* = *F. decemcellulare*



## DISCUSSION

The present study assessed the antifungal activity of different concentrations of ethanol and warm water extracts of seven selected plants, based on their availability in the cocoa producing communities and their ethno-botanical uses against human fungal pathogens. It was found that the potency of the different plant extracts against the three test fungi varied at different concentrations of the different plant materials.

Findings from this study, showed that the three fungal pathogens were consistently associated with cocoa pod rot in this study but the main causal organism of cocoa pod rot *P. megakarya* could be controlled in culture by increasing concentrations of the ethanolic extracts of black pepper (*P. guineense*) and wood as (*A. bacteri*). This is in agreement with submissions of Amadioha and Markson (2007) and Channya and Chimbekujoo (2002) who reported that both extracts of *P. niigrum* and wood ash treatments proved effective in the control of fungal pathogens responsible for the rot of banana and cassava, effectively reducing the disease severities from the rot causing organisms.

Furthermore, ethanol extract of ginger roots (*Z. officinale*) at 20 % and 30 % concentrations showed strong inhibition by 90 % against *F. decemcellulare*, followed, by black pepper (*P. guineense*) at 10% and 50% concentrations which significantly ( $P \leq 0.05$ ) showed high potency in reducing the radial growth of *F. decemcellulare* in modified PDA media.

Amadioha and Markson, 2007 and Enyiukwu and Awurum (2011) reported that extracts of seeds of black pepper (*P. guineense*) ranked high in fungitoxic activity against *Botrydipodia sp.* and *Colletotrichum destructivum* which cause rot in yam tubers and anthracnose of cowpea respectively, and therefore protected stored yam and cowpea against rot and anthracnose. Yeni (2011) in a study of the inhibitory effects of *Z. officinale* and *Ocimum gratissimum* reported reduction in the mycelial growth of *Rhizopus stolonifer*, *Aspergillus niger*, *Fusarium spp* and *Botrydipodia theobromae* associated with rot of white yam (*Discorea rotundata*) by extracts of the two tropical spice plants. Okigbo and Nneka (2005) and Amienyo and Ataga (2007) in similar manners used phytochemicals obtained from *Z. officinale* to protect yam and mechanically injured sweet potato tubers in storage. Furthermore, Hussain *et al.* (2010) reported extracts of ginger rhizome to exhibit strong antifungal effects against mycotoxigenic *Aspergillus flavus*, *A. parasiticus* (potent producers of the carcinogen aflatoxin) and *Drechslera oryzae*. The findings from this work where both the aqueous and ethanol extracts of the test plants effectively reduced the radial growth of the assayed mycoflora therefore agree with the reports of these investigators. Also, Findings in this study where ethanol extracts of bitter leaf and purging nut which enhanced the growth of *C. Ignotum* in culture as well as warm water extract of lemon grass and guinea pepper

promoted the growth of *P. megakarya in vitro* conform with the reports of Akinbode and Ikotun (2008) who found that extracts of *Riccimus cumminis* significantly promoted the growth of *C. destructivum in vitro*.

Besides the factors of concentration of application of extract, type of plant species, age at harvest of the plant, and the plant part(s) used in the study; the levels of the plant-derived bioactive fungitoxic compounds according to Okigbo and Nneka (2005) and Prekh *et al.* (2005) could be influenced by solubility in the extracting solvent, method of extraction, length of the extraction method, pH of the solvent, temperature, particle size of the plant tissue and the solvent to sample ratio. Differences in solubility of the active ingredients in the extracting solvents may be the reason for the differential toxicity of test extracts to the different pathogens in this study. More so, since water is a universal and effective solvent that can be used to extract plant products with antimicrobial activity with minimal changes to their functional properties, it could be comparative to ethanol for use by the resource-poor farmers in extracting the plant materials (Prekh, *et al.*, 2005; Onispe *et al.* 2008)

## CONCLUSION

This study revealed that extracts of the test materials plants evaluated significantly inhibited the growth of the assayed myco-pathogens. The fungitoxicity of the plant materials varied significantly with extracting solvents and concentration. Therefore extracts of *P. guineense*, *Z. officinale*, *A. bacteri* and *V. amygdalina* are viable candidates of biopesticides for control of fruit rot of cocoa caused by *P.megakarya* and *C. ignotum* in tropical plantations and should be tried out in further studies against these pathogens in the field so as to increase cocoa production. Extracts of higher plants are easily bio-degradable, non-selective, locally available, sources of cost-effective fungicides for farmers who cannot afford expensive synthetic pesticide in the tropics.

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