Advanced Research Journal of Microbiology ISSN: 1718-3499, Vol. 5 (3), pp. 114-119. April, 2018. Available online at www.advancedscholarsjournals.org © Advanced Scholars Journals

*Full length Research paper*

# Effect of storage on the antimicrobial potencies of some botanicals against *Phytophthora megakarya*, causal pathogen of black pod disease of cocoa in Nigeria

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**Accepted 31 January,2018**

**ABSTRACT**

**Varying concentrations of *Piper guineense, Ocimum gratissimum,* and *Cymbopogon citratus* leaf extracts, at different periods of storage were tested against moderately aggressive strain of cocoa pod rot pathogen-*Phytophthora megakarya*. Percentage inhibitions exhibited by the extracts and chemical fungicide used as standard were calculated and recorded with respect to their storage time. Percentage mycelia inhibitions exhibited by the freshly prepared extracts against the pathogen ranged between 27.73-51.58% (*P. guineense*), 26.99-34.77% (*C. citratus*) and 24.49-48.46% (*O. gratissimum*), while the synthetic fungicide (standard) gave 44.25%. The inhibitions exhibited after seven days of storage dropped to 5.12-15.67%, 4.41-20.72%, 11.23-24.38% and 20.58% for *P. guineense, C. citratus,O. gratissimum* and fungicide respectively. Percentage inhibitions of the pathogen after 14 day storage partly followed a similar trend. The antimicrobial potencies of plant extracts and fungicide used in this study clearly decreased with storage time and as such, could be best applied when freshly prepared to control black pod disease of cocoa.**

**Keywords:** *Phytophthora*, extracts, inhibitions, *Piper, Ocimum,Cymbopogon*, cocoa, potency, storage time.

## INTRODUCTION

Cacao is a crop of economic importance around the world. Although mostly grown in the tropics, its annual yield is often affected by several diseases. Fungal diseases are a principal constraint to world cocoa production and on a global scale, the greatest losses result from black pod rots. Depending on where cocoa is grown, one or more of diseases namely black pod (caused by *Phytophthora*spp.), witches broom (caused by *Crinipellisperniciosa*), frosty pod rot (caused by *Moliniophthorararoreri*) and die back, may reach epiphytotic proportions that cause devastating losses (Adejumo, 2005).

Pathogens of the Straminipile genus, *Phytophthora* cause significant disease losses to global cocoa production. *Phytophthoramegakarya* causes significant pod rot and losses due to canker in West Africa, whereas *P. capsici* and *P. citrophthora* cause pod rots in Central and South America. The global and highly damaging *P. palmivora* attacks all parts of the cacao tree at all stages of the growing cycle. This pathogen causes 20 to 30% pod losses through black pod rot, and kills up to 10% of trees annually through stem cankers. *P. palmivora* has a complex disease cycle involving several sources of primary inoculum and several modes of dissemination of secondary inoculum. This results in explosive epidemics during favorable environmental conditions (Guest, 2007).

Nigerian farmers have devised various means of curbing the effect of the pathogen, prominent among which is the sometimes indiscriminate use of synthetic fungicides which had led to the exposure of both the farmers and the latter consumers of the crop to various health hazards. The ecosystem is also being negatively

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affected and the crop which takes-up part of the chemicals applied eventually becomes contaminated with heavy metals.It therefore becomes imperative to search for effective, viable and environmentally friendly alternative(s), one of which is the use of extracts of tropical plant-source which has been observed to be eco-friendly, bio-degradable, cheaper, available and safe (Ojo and Olaifa, 2011).

To reduce the risks and losses associated with the continuous use of chemical pesticides on food and cash crops, a lot of research has and is being carried out on the use of botanicals which are less phytotoxic, but more systemic and easily biodegradable. They also offer cheap and safer control for those categories of farmers who cannot afford the present increasingly high cost of synthetic pesticides (Adejumo, 2005; Enyiukwu and Awurum, 2011; Pipi and Omodamiro, 2014). The plant extracts may be applied immediately after preparation or stored for future use.

Recent research findings have however led to the discovery of the effects some storage conditions may have on certain chemical constituents of botanicals/plant extracts. Del-Toro-Sanchez *et al.* (2015) opined that high temperature and exposure to light decreased the antioxidant value of *Anemopsiscalifornica*extracts. There were also consistent reductions in the total phenol and flavonoid contents of extracts obtained from various parts of the plant as storage time increased (Del-Toro-Sanchez *et al.*, 2015).

In another research, there were marked increase in the phenolic, flavonoid and iridoid contents of *Ocimumbasilicum*and *Sennapetersiana* plant parts (earlier oven-dried at 50$^{o}$C, ground and stored in airtight containers), when compared with their respective fresh samples (Laher*et al.* 2013).

This study focused on determining the effect of storage on the antimicrobial effectiveness and potency of some commonly used botanicals in the control of cocoa black pod disease caused by *Phytophthoramegakarya* in Nigeria.

## METHODOLOGY

Fresh leaves of *Piper guineense, Ocimum gratissimum,* and *Cymbopogon citratus* were obtained from the field in 2015/2016 and brought to the laboratory for proper identification and processing. Leaves of each of the botanicals were surface sterilized with 2% sodium hypochlorite (NaOCl) solution and rinsed thrice with sterile distilled water. One hundred, 75, 50 and 25g of each of the botanicals were separately weighed and crushed in 100ml of sterile distilled water with the aid of sterile mortar and pestle to give 100, 75, 50 and 25%w/v concentrations.

The crushed botanicals were filtered through sterile muslin clothes after which filtrates obtained were sterilized at 121$^{o}$C for 15 minutes along with a prepared solution of synthetic fungicide approved by the Nigerian government for use on cocoa. The fungicide was used as standard. Parts of the sterilized extracts and fungicide were carefully stored in the laboratory at room temperature for up to fourteen days.

Aliquot (1ml) of each of the sterilized extracts was pour-plated with freshly sterilized but cooled (45$^{o}$C) potato dextrose agar (PDA) in triplicates. The plates were allowed to set, after which disc culture of a moderately aggressive *Phytophthoramegakarya*strain cut with the aid of cork borer No. 4 was inoculated into each of the prepared plates. The plates were then incubated at 22±4$^{o}$C for seven days and observed on daily basis.Similar procedure was repeated for the extracts and fungicide after seven and fourteen days of storage.

Mycelia growth diameters of the pathogen on each treatment were measured and their percentage inhibitions calculated using the formula below (Ogundeji and Olufolaji, 2016).

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

Where:
*dc* = Mycelia growth diameter in control
*dt* = Mycelia growth diameter in treatment

Overall average percentage mycelia inhibitions exhibited by each of the botanicals and fungicide against the pathogen, with respect to each of the storage days (0, 7 and 14) were determined. These were used to extrapolate their antimicrobial potencies.

### Statistical analysis:

The experimental design employed in this research was Completely Randomized Design (CRD). Results obtained were subjected to one-way Analysis of Variance (ANOVA) at 5% level of probability and with the aid of Statistical Analysis System (SAS) 9.1 statistical package. The mean values were separated using Duncan's Multiple Range Test (DMRT).

## RESULTS

The antimicrobial effect of freshly prepared extracts of *Piper guineense, Cymbopogon citratus,* and *Ocimumgratissimum*against *Phytophthoramegakarya*, causative agent of cocoa black pod disease is as shown in Table 1.

The percentage mycelia inhibitions observed ranged between 27.73-51.58% (*P. guineense*), 26.99-34.77% (*C. citratus*) and 24.49-48.46% (*O. gratissimum*), while the fungicide used as standard gave 44.25%. Absolute concentrations (100%w/v) of the three extracts and the standard gave the highest inhibitions. Percentage inhibitions exhibited against the pathogen by *P. guineense* extracts increased with concentration.

Results of the antimicrobial efficacy of seven day old extracts of *P. guineense, C. citratus* and *O. gratissimum*

**Table 1:** Effect of freshly prepared plant extracts (botanicals) on the growth of *P. megakarya*

| Extracts/standard | Extract concentrations (%) | Percent inhibition (%) |
|---|---|---|
| ***Piper guineense*** | | 27.73$^{bc}$ |
| | 25 | |
| | 50 | 28.64$^{abc}$ |
| | 75 | 33.34$^{abc}$ |
| | 100 | 51.58$^{a}$ |
| ***Cymbopogon citratus*** | 25 | 34.31$^{abc}$ |
| | 50 | 26.99$^{bc}$ |
| | 75 | 28.66$^{abc}$ |
| | 100 | 34.77$^{abc}$ |
| ***Ocimum gratissimum*** | 25 | 24.49$^{c}$ |
| | 50 | 27.66$^{bc}$ |
| | 75 | 23.31$^{c}$ |
| | 100 | 48.46$^{ab}$ |
| **Standard** | | 44.25$^{abc}$ |

*Means followed by the same superscripts along the same column are not significantly different at P =0.05 according to Duncan Multiple Range Test (DMRT)*

**Table 2:** Effect of 7-day old stored plant extracts on the growth of *P. megakarya*

| Extracts/standard | Extract concentrations (%) | Percent inhibition (%) |
|---|---|---|
| ***Piper guineense*** | | |
| | 25 | 12.40 |
| | 50 | 13.05 |
| | 75 | 5.12 |
| | 100 | 15.67 |
| ***Cymbopogon citratus*** | | 9.41 |
| | 25 | |
| | 50 | 4.14 |
| | 75 | 20.72 |
| | 100 | 16.86 |
| ***Ocimum gratissimum*** | | 11.23 |
| | 25 | |
| | 50 | 21.47 |
| | 75 | 12.17 |
| | 100 | 24.38 |
| **Standard** | | 20.58 |

*Means are not significantly different at P =0.05 according to Duncan Multiple Range Test (DMRT)*

against *P. megakarya* are as depicted by Table 2. The stored *P. guineense, C. citratus*and *O. gratissimum* exhibited mycelia inhibition ranges of 5.12-15.67%, 4.41-20.72% and 11.23-24.38% respectively, while the standard exhibited 20.58% inhibition. Although 100 and 75% concentrations of the extracts gave the highest inhibitions, there were generally no significant difference (s) (P=0.05) in the inhibitions exhibited by the extracts irrespective of the concentration (Table 2). Their values were however clearly lower than those of the freshly prepared extracts of the same sets of botanicals (Table 1).

Figure 1 shows the antimicrobial potencies of *P. guineense, C. citratus*, *O. gratissimum* and the synthetic

**Table 3:** Effect of 14-day old stored plant extracts on the growth of *P. megakarya*

| Extracts/standard | Extract concentrations (%) | Percent inhibition (%) |
|---|---|---|
| ***Piper guineense*** | | |
| | 25 | 16.09 |
| | 50 | 15.39 |
| | 75 | 10.93 |
| | 100 | 22.08 |
| ***Cymbopogon citratus*** | | |
| | 25 | 14.49 |
| | 50 | -5.09 |
| | 75 | 19.09 |
| | 100 | 16.57 |
| ***Ocimum gratissimum*** | | |
| | 25 | -6.67 |
| | 50 | 7.62 |
| | 75 | 13.57 |
| | 100 | 30.84 |
| **Standard** | | 22.86 |

*Means are not significantly different at P =0.05 according to Duncan Multiple Range Test (DMRT)*

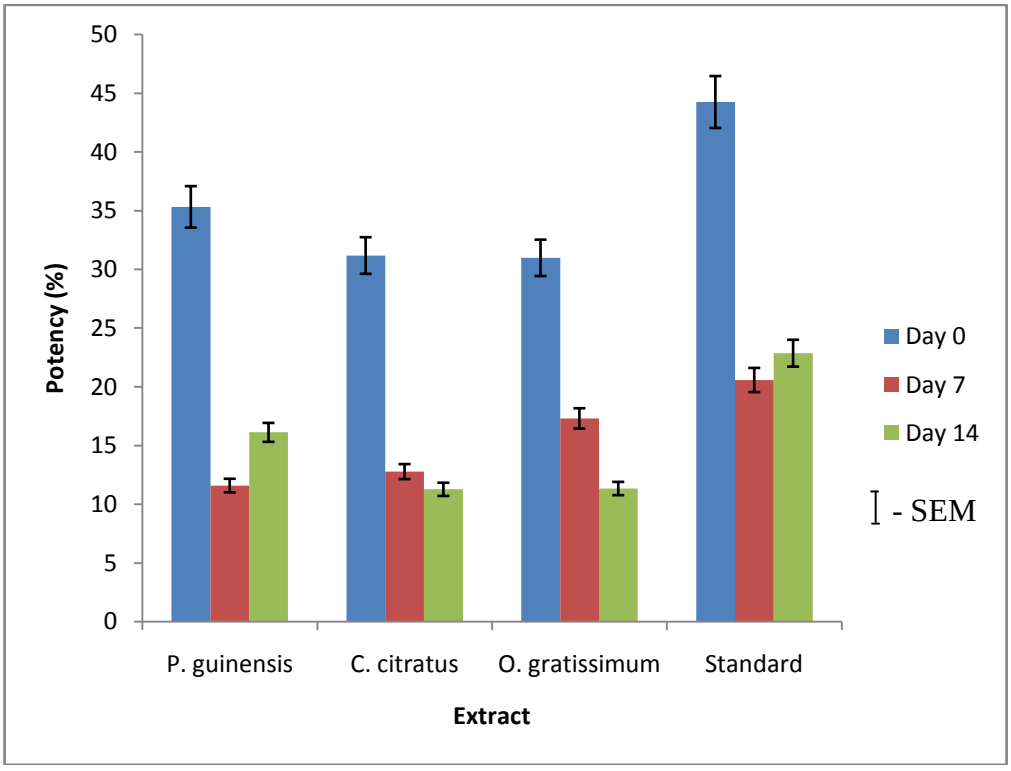


Figure 1: Effect of storage on the antimicrobial potency of *Piper guineense*, *Cymbopogoncitratus*, and*Ocimumgratissimum*

fungicide (standard) used in the study against cocoa black pod pathogen *P. megakarya.* Within the fourteen-day storage period, the antimicrobial potencies of *P. guineense, C. citratus*, and *O. gratissimum* ranged between 11.59-35.32%, 11.27-31.18%, and 11.34-30.98% respectively, while the fungicide used asstandardgave a range of 20.58-44.25%. All of the botanicals as well as standard had their highest potencies on storage day 0 (i.e. when freshly prepared).

Their potencies however dropped after seven days of storage. At storage day 14, slight increase in the antimicrobial potencies of *P. guineense*and the standard were observed. There was however further drop in the potencies of *C. citratus*, and *O. gratissimum*at the same storage time (Figure 1).

## DISCUSSION

Unlike the other botanicals used, it can be observed that percentage inhibition exhibited by *P. guineense*extracts consistently increased with concentration. This is in line with the findings of Nweke (2015)who discovered that the inhibitory effect of plant (*C. aurantifolia*) extract on mycelial growth (and spore germination) of some plant pathogens increased with increasing concentration of the extract. The situation was however slightly different with *C. citratus*and *O. gratissimum*as 50% concentration of the former and 75% of the latter gave the least mycelia inhibitions (though not significantly different at P=0.05). There was also no noticeable significant difference (P=0.05) between the inhibitions exhibited by the chemical fungicide and the extracts (Table 1).

The generally lower percentage inhibitions exhibited by the seven and fourteen day-old aqueous extracts of *P. guineense, C. citratus*and *O. gratissimum* compared with their freshly prepared counterparts against the test pathogen clearly disagrees with the findings of El Shafie and Almahy (2012) which opined that the efficacy of plant extracts stored at room temperature increases with storage time. Inhibitions produced by the 14 day old extracts were however generally much lower than those of the freshly prepared extracts, but slightly lower (in some cases) than those of their 7 day old counterparts (Tables 2 and 3).

A careful examination of the percentage inhibitions exhibited by the freshly prepared and stored plant extracts gave a clue to their antimicrobial potency/stability throughout their period of storage. Antimicrobial potencies of the freshly prepared *P. guineense, C. citratus*and *O. gratissimum*extracts were significantly higher (P=0.05) than those of their respective counterparts stored for seven days. Potencies of fourteen day old *C. citratus*and *O. gratissimum*further reduced to 11.27 and 11.34% respectively, while those of *P. guineense*and the test fungicide slightly increased to 16.12 and 22.86% respectively (Figure 1). Findings of ElShafie and Almahy (2012) disagree with the trend observed on the potencies of *C. citratus*and *O. gratissimum*but partially agree with those of *P. guineense* andthe standard.

## CONCLUSION

Virtually all the extracts used, particularly 25, 75 and 100%w/v concentrations of *C. citratus*and *P. guineense*competed favourably with the synthetic fungicide (standard) and as such, can be used in place of the chemical. The effectiveness/potencies of both the plant extracts and chemical fungicide clearly decreased with storage time. This implies that the plant extracts and fungicide used in this research are in their best forms and so, could be best applied when freshly prepared, to control black pod disease of cocoa.

## ACKNOWLEDGEMENTS

Special thanks to the Management of Cocoa Research Institute of Nigeria, the custodian of most of the facilities used for this research.

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