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Bio-fungicides in *Allium sativum* (L) had significant inhibition on *Phytophthora megakarya* (Brasier & Griffin) and cocoa black pod rot disease

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Abstract
Aqueous extracts from three plants (*Chromolaena odorata*, *Zingiber officinale* and *Allium sativum*) were evaluated *In vitro* and *In vivo* for the management of cocoa black pod rot disease caused by *Phytophthora megakarya*. Four concentrations (20, 40, 60 and 80%) of hot (70º C) and cool (35º C) aqueous extracts were obtained from fresh and dry samples of each plant. Efficacy of extracts was determined by inhibitions of mycelial growth of *P. megakarya* (*In vitro*) and disease progression on cocoa pods (*In vivo*). Treatments were replicated four times, and incubated at 28º C ± 2º C and 75% relative humidity. Data were collected on mycelial growth of pathogen on artificial media and radial growth of infection on cocoa pods. Statistical analysis of data collected showed significant differences among the concentrations of aqueous extracts. Inhibition of mycelial growth increased with increasing concentration of extracts but decreased with increasing days after inoculation. *A. sativum* was the most effective *In vitro*. Mycelial growth inhibition of 100% (40% concentration and above) was recorded at 3 days after inoculation. *C. odorata* was the least effective, as its highest percentage mycelial growth inhibition was 54.12% (at the highest concentration of 80%). *A. sativum* was also the most effective *In vivo*. It inhibited infection by 66. 10% and 79.03% (60% and 80% concentrations respectively) at 11 days after infection. *A. sativum* can be recommended for use in the management of cocoa black pod rot disease.

Keywords: aqueous extracts, poison food technique, antifungal properties, cocoa black pod rot, *Phytophthora megakarya*

1. Introduction

Global annual yield loss of cocoa resulting from cocoa black pod rot disease (CBPRD) has been projected to be around 30% (Guest, 2007) [16]. In Africa, 100% yield loss has been reported in farmers' fields in Ghana (Opoku *et al*, 2008) [29] and Nigeria (Oluyole and Lawal, 2008) [24] with devastating social and economic implications (Tijani, 2005; Aikpokpodion and Adeogun, 2011) [30].

Phytophthora spp. are known to incite CBPRD. *P. capsici* and *P. citrophthora* are the causative organisms of the disease in South America, while *P. palmivora,* with the reputation of infecting cacao trees in addition to the pods, was the predominant pathogen of CBPRD in West Africa for a very long time, until 1979 when *P. megakarya* was described for the first time (Brasier and Griffin, 1979) [8]. Today, *P. Megakarya* is the dominant cause of CBPRD in West Africa (Guest, 2007; Akofi, 2015; Ali *et al*, 2017) [16, 5, 6]. It differs from *P. palmivora* by being more virulent, having larger nuclei in its gamentagia, less number of chromosomes and longer sporangia (Samson *et al*, 1975; Froster *et al*, 1990) [14]. Heavy and continuous rain along with high relative humidity and warm temperature are some of the pre-disposing environmental conditions for infection and pathogenesis. Man, rodents, birds, ants as well as rainwater running down the stems of infected cocoa trees all acts as agents dispersing pathogen's inoculum.

The process of developing cultivars of cocoa that are resistant to *P. megakarya* and making such available to cocoa farmers is still at its infancy (Thevinin *et al*, 2004; Paulin *et al*, 2008) [29, 26], hence the management of CBPRD in Africa and most tropical countries, where cocoa is cultivated, has mainly been through the use of synthetic chemicals. Copper/Copper and Metalaxy based fungicides are the most commonly used (Agbeniyi and Oni, 2014) [2]. Unfortunately, however, these chemicals are extremely toxic. In addition, the residue of synthetic chemicals, the organochlorines in particular, have been found in cocoa produce, processed products and by-products (Aikpokpodion *et al*, 2013; Okoffo *et al*, 2016 and Ibigbami and Adebawore, 2017) [4, 22, 17]. To prevent these chemical residues from attaining damaging levels with serious implications on public health, there is an urgent need for alternative management strategies for CBPRD.

This study was designed to evaluate aqueous extracts from three plants (*Chromolaena odorata*, *Zingiber officinale* and *Allium sativum*) for antifungal properties against *P. megakarya.* The test crop was CRIN TC-2 Cocoa cultivar developed by the cocoa research institute of Nigeria (CRIN). It has many desirable agronomic characteristics but is susceptible to the cocoa black pod rot pathogen (CBPRP).

2. Materials and methods

2.1 *In vitro* studies

2.1.1 Collection of infected cocoa pods

Infected cocoa pods were collected in August, a period characterised by high incidence and severity of CBPRD, due to the high humidity and warm temperature associated with this period. Five pods, at various stages of infection, were obtained from a cocoa farm in Idanre, a popular town known for cocoa production in Ondo State, Nigeria. The pods were transported to the pathology lab of The Department of Crop, Soil and Pest management (CSP), Federal University of Technology, Akure (FUTA), (a distance of about 30 km) in sterile glass jars covered with aluminium foil.

2.1.2 Preparation of artificial growth medium

Phytophthora megakarya is an Oomycete, requiring specialised artificial medium like V8 agar for rapid growth. An improvised medium was prepared as a result of the scarcity of V8 agar. It consisted of 100 ml of tomato juice, prepared following the method described by Oluyemi *et al*, (2014) [23] and the same volume of potato infusion (obtained from 20 g Irish potato in 100 ml of distilled water). Four (4) g each of dextrose sugar and agar were added to obtain potato/tomato juice agar (PTJA). Sterilisation was done at 121^{O}C for 15 minutes in a Surgifriend (2010 model) autoclave. Prepared PTJA was amended with 0.4 g of Streptomycin antibiotic to prevent bacterial growth. Pour plating, 20 ml PTJA/sterile Petri-dish, was done when PTJA cooled to 45^{O} C.

2.1.3 Isolation and identification of *P. megakarya* from infected cocoa pods

Isolation of *P. megakarya* was done about 1 hr after collection of infected pods. Small segments, about 2 mm wide, from the active zones of infections were obtained with the aid of sterile scalpels. The segments were surface sterilised in 70% ethanol for 20 seconds, rinsed in 4 changes of sterile distilled water and pat-dried with sterile blotting paper. Inoculation of the segments was done on prepared PTJA after gelling. A total of 10 Petri-dishes were inoculated and each had two pod segments placed at opposite ends, about 8 cm apart. Incubation was done at 28^{O} C ± 2^{O} C. Sub-culturing was done at 48 hours after inoculation and re-incubated as described above. Identification was through visual observation of colony in culture, microscopy, aided by standard text, (Dugan, 2006) and pathogenicity test.

2.1.4 Collection of plant materials

Fresh *Allium sativum,* garlic (bulb), *Zingiber officinale,* ginger (rhizome) and dry (pulverized) of both plants were purchased from Isinkan market in Akure, Ondo state, Nigeria. *Chromolaene odorata,* (Siam weed) leaves were obtained from an abandoned farmland beside the old crop type museum of CSP Department, FUTA. The dry (pulverized) leaves of *C. odorata* was obtained after air drying for 8 days.

2.1.5 Preparation of aqueous extracts

Fresh samples of the three plants were ground separately into pulp in a blender, while extraction of the phytochemical constituent was done using hot (70^{o} C), and cool (35^{o} C) water as the solvent. In each extraction process, 100 ml of solvent was added to 100g of fresh ground or dry pulverized plant sample in a sterile beaker. Gentle shaking, after covering with aluminium foil, was done to obtain a slurry. Each slurry was allowed to stand for 24 hours before filtering with sterile double-layered muslin cloths. From the filtrates (100% conc. w/v), 15 ml each of 20%, 40%, 60% and 80% conc. were prepared by dilution with sterile distilled water as appropriate. A total of 48 concentrations were prepared from fresh and dry plant materials as shown in (Table 1).

Table 1: Botanicals evaluated and their concentrations

Botanicals Fresh/Dry	Solvent/Concentrations (%)							
	Hot water (70^{o} C)				Cool water (35^{o} C)			
Chromolaena odorata	20	40	60	80	20	40	60	80
Allium sativum	20	40	60	80	20	40	60	80
Zingiber officinale	20	40	60	80	20	40	60	80

2.1.6 Preparation of PTJA/Aqueous extract complex and assessment of its anti-fungal properties against *P. megakarya*

A PTJA/Aqueous extract complex was achieved by adding 3 ml of extract to 12 ml of hot (70^{o} C) PTJA in a Peri-dish with gentle swerving to allow for proper mixing of the two. This procedure was repeated for the 48 concentrations. The standard check had aqueous extract replaced with 3 ml of Copper Hydroxide (Kocide 2000) at the manufacturer's recommended rate, while the control had 3 ml of sterile distilled water. The PTJA/Aqueous extract complexes were allowed to cool and gel. Thereafter, 5 mm agar disc each from one-week-old pure culture of *P. megakarya* were punched out with the aid of sterile cork borers and transferred, upside down, to the centre of each PTJA/Aqueous complexes with the aid of a sterile wire loop. A total of 50 treatments, standard check and control inclusive, were evaluated. Each treatment was replicated four times and was laid out in a completely randomized design (CRD). Incubation was done for 9 days at 28^{o}C±2^{o}C.

2.2 *In vivo* studies

2.2.1 Collection/selection of healthy cocoa pods

Ripe and unripe cocoa pods (Plate 1a) were obtained from a cocoa field beside the new crop type museum of CSP Department, FUTA and transported in sterile polythene bags to the pathology lab of the same department immediately after collection. The exocarp of each pod was examined carefully with a 2 MP, 1000 magnification digital microscope/endoscope camera and only healthy pods were selected for the *In vivo* studies.

2.2.2 Evaluation of aqueous extracts for inhibition of infection on detached cocoa pods

2.2.2.1 Preparation of inoculum/ aqueous extract complex

Mycelium and spores were harvested from the one-week-old culture of *P. megakarya*, using a sterile spatula and distilled water (20 ml/Petri-dish). Filtration was done to remove pieces of agar discs and tangled mass of mycelium. A small quantity of the filtrate (0.2 ml), which consisted mostly of chlamydospores and fragments of mycelia, was mixed with an equal volume of the most effective extract concentration from ginger and garlic, in the *In vitro* experiment, to form inoculum/aqueous extract complex. Thirteen treatment, standard check and control inclusive, were evaluated (Table 2).

Table 2: Aqueous extracts and concentrations evaluated in the *In vivo* experiment

Conc.	Aqueous extract/denotation
40%	1. Hot water extract from dry *Z. officinale* (DHZ)
	2. Cool water extract from fresh *A. sativum* (FCA)
	3. Hot water extract from fresh *A. sativum* (FHA)
60%	1. Hot water extract from dry *Z. officinale* (DHZ)
	2. Cool water extract from dry *Z. officinale* (DCZ)
	3. Cool water extract from fresh *A. sativum* (FCA)
	4. Hot water extract from fresh *A. sativum* (FHA)
80%	1. Hot water extract from dry *Z. officinale* (DHZ)
	2. Cool water extract from dry *Z. officinale* (DCZ)
	3. Cool water extract from fresh *A. sativum* (FCA)
	4. Hot water extract from fresh *A. sativum* (FHA0
Control	(Sterile distilled water)
Standard check	Copper hydroxide (Kocide 2000) 10g/15 l of water)

2.2.2.2 Infection of healthy cocoa pods with inoculum/aqueous extract complex

The inoculum/extract complex was introduced into 0.80 cm (width) x 0.40 cm (depth) hole, punched out with a sterile corn borer, on the mesocarp of a healthy cocoa pod (Plate 1b). The thickness of the punched out segment was reduced to prevent spilling of inoculum/aqueous extract complex during coverage (Plate 1c). This procedure was repeated for the selected extract concentrations of *A. sativum* and *Z. officinale.* The standard check had 0.2 ml of Copper hydroxide (Kocide 2000) at the recommended rate of 100 g/15 l (0.007 g/ml), while the control had 0.2 ml sterile distilled water. Each treatment was replicated thrice, on the same cocoa pod, and the replicates were spaced out evenly, depending on the length of each pod.

Plate 1: a. Healthy pods, **b.** Pod with punched out mesocarp, **c.** Pod with mesocarp replaced after inoculation with inoculum/extract complex

Infected pods were arranged randomly on metal stands and suspended above 2 l of water in large plastic bowls. The bowls were covered to create warmth and humidity, both of which are essential for infection and pathogenesis. The moist condition also prevented the cocoa pods from drying out. The set up was allowed to stand for 11 days.

3. Data collection and statistical analysis

Data were collected on the following;

i. Radial growth of *P. megakarya* in Petri-dish. The values obtained were converted to percentage inhibition of mycelial growth with the formula;

$$img = \frac{mc - mt}{mc} x\, 100$$

Where;
img = inhibition of mycelial growth
mc = mycelial growth in the control plate
mt = mycelial growth in the treated plate

ii. Radial growth of infection on a cocoa pod. The values obtained were converted to percentage inhibition of infection from *P. megakarya* on cocoa pods with the formula;

$$ic = \frac{rc - rt}{rc} x\, 100$$

Where;
ic = inhibition of cocoa black pod rot infection
rc = radial growth of infection in the control
rt = radial growth of infection in the treated cocoa pod.

Data collected were subjected to analysis of variance (ANOVA) with the use of MINITAB version 17 software, and means were separated using Tukey's test. Correlation and regression analysis was also carried out to determine the relationship between increasing concentrations and inhibition of mycelial growth.

4. Results

4.1 Isolation and identification of *P. megakarya*

The rate of growth of *P. megakarya* on PTJA was moderate and it took about 9 days for an 8.5 cm Petri-dish to be fully covered. Microscopic examination reveals hyaline and coenocytic mycelium (Plate 2a). Also, chlamydospores were the predominant spores produced and became visible at 4 days after inoculation (Plate 2b). Few sporangia and zoospores were also observed (Plates 2c, and 2d).

4.2 Inhibition of mycelial growth of *P. megakarya* by cool water extracts from dry samples of *Z. officinale*, *A. sativum* and *C. odorata*

Inhibition of mycelial growth (IMG) of CBPRP was significantly different among the extract concentrations. The general trend observed was that IMG increased with increasing concentrations of extracts from the three botanicals (Table 3). The inhibitory attribute of each extract, however, decreased with increasing days after inoculation of *P. megakarya*. At 3 days after inoculation, cool dry extract of *Z. officinale* (CDZ) gave the best values of mycelial growth inhibition (MGI) at 20% and 60% concentrations, while extracts of *A. sativum* gave the best MGI at 40% and 100%. At 6 days after inoculation, IMG of the pathogen had reduced drastically and only the aqueous extract of *A. sativum* was able to exhibit 100% inhibition (at 80% concentration). By the 9th day after inoculation, 20% and 40% extract concentrations of the three botanicals recorded 0% inhibition. Aqueous extract of *A. sativum* gave the best inhibition percentage (62.35%) at this stage (Table 3).

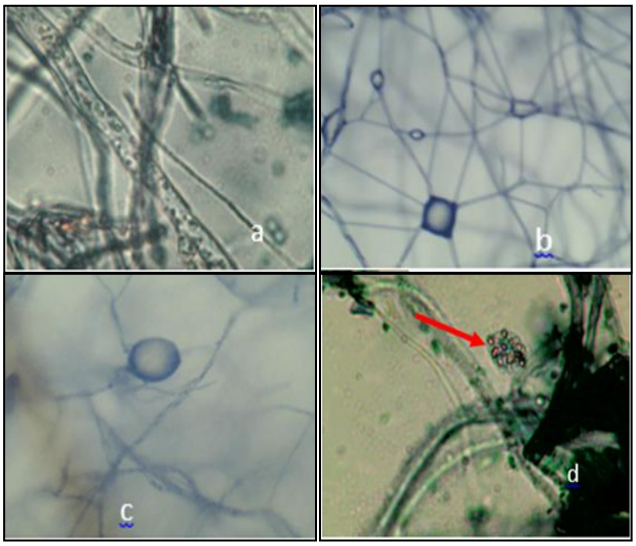

Plate 2: (a). Coenocytic and hyaline mycelium (b). Chlamydospores (c). Sporangium (d). Zoospores

4.3 Inhibition of mycelial growth of *P. megakarya* by hot water extracts from dry samples of *Z. officinale, A. sativum* and *C. odorata*

Mycelial growth inhibition by the different concentrations of hot water extracts was also significantly different. IMG followed the same pattern of increasing inhibition percentage with increasing concentration. Inhibition percentage, however, decreased with increasing days after inoculation (Table 4). On the 3rd day after inoculation, *A. sativum* gave 100% MGI, the same value that was recorded for the standard check. By the 9th day however, only 7.06% and 13.91% MGI were recorded for ginger and garlic respectively (Table 4). Siam weed gave the poorest inhibition percentage.

Table 3: Inhibition of mycelial growth of *P. megakarya* by cool water extracts from dry samples of *Z. officinale, A. sativum* and *C. odorata*

Conc. / Botanicals		Inhibition of mycelial growth/ days after inoculation		
		3	6	9
20%	Co	13.89i	0.00h	0.00e
	Zo	12.98i	10.21g	0.00e
	As	8.15j	0.00h	0.00e
40%	Co	25.00h	0.00h	0.00e
	Zo	43.61f	27.84e	0.00e
	As	49.41e	8.23g	0.00e
60%	Co	36.11g	8.23g	0.00e
	Zo	60.53c	48.23c	27.39d
	As	53.80d	27.84d	0.00e
80%	Co	51.11de	15.29f	0.00e
	Zo	74.47b	58.43b	48.23c
	As	100.00a	100.00a	62.35b
Kocide 2000		100.00a	100.00a	87.94a

Note: Means in the same column followed by the same alphabets are not significantly different ($p < 0.05$) according to Tukey's test.
Legend Co: *Chromolaene odorata.* As: *Allium sativum.* Zo: *Zingiber officinale*

Table 4: Inhibition of mycelial growth of *P. megakarya* by hot water extracts from dry samples of *Z. officinale, A. sativum* and *C. odorata*

Conc. / Botanicals		Inhibition of mycelial growth/ days after inoculation		
		3	6	9
20%	Co	5.12i	0.00f	0.00d
	Zo	16.48g	0.00f	0.00d
	As	2.71j	0.00f	0.00d
40%	Co	9.74h	0.00f	0.00d
	Zo	65.94c	20.00e	0.00d
	As	19.74f	0.00f	0.00d
60%	Co	17.94fg	0.00f	0.00d
	Zo	85.62b	31.76d	0.00d
	As	38.03d	18.33e	0.00d
80%	Co	25.13e	0.00f	0.00d
	Zo	100.00a	51.76b	7.06c
	As	100.00a	49.80c	13.91b
Kocide 2000		100.00a	100.00a	87.94a

Note: Means in the same column followed by the same alphabets are not significantly different ($p < 0.05$) according to Tukey's test.
Legend: Co: *Chromolaene odorata.* As: *Allium sativum.* Zo: *Zingiber officinale*

4.4 Inhibition of mycelial growth of *P. megakarya* by cool water extracts from fresh samples of *Z. officinale, A. sativum* and *C. odorata*

Inhibition of mycelial growth of CBPRP by cool water extract from the three botanicals was significantly different. The general trend was also the same as was observed for cool and hot water extracts from dry samples, except for *A. sativum*. It must be pointed out that garlic extract was outstanding in its IMG. At 40% concentration, 81.52%, 58.04% and 15.68% MGI were obtained at 3, 6 and 9 days after inoculation respectively (Table 5). By day 9, garlic had 100% IMG at 60 and 80% concentrations. This value was significantly higher than 88.95% which was recorded for the standard check. Siam weed extract once again recorded the least values of MGI (Table 5).

4.5 Inhibition of mycelial growth of *P. megakarya* by hot water extracts from fresh samples of *Z. officinale, A. sativum* and *C. odorata*

Hot water extracts from fresh samples showed significant difference in their IMG of CBPRP (Table 6). Inhibitory properties increased with increasing concentrations but decreased with increasing days of exposure for *C. odorata* and *Z. officinale*. *Allium sativum*, however, maintained 100% IMG at 60% and 80% concentration throughout the period of evaluation. It was once again the stand out performer, as it gave significantly highest percentage MGI (all the concentrations evaluated) at 3 and 6 days after inoculation. At 9 days after inoculation, 100% IMG was recorded at 60% and 80% concentrations, comparing favourably with Kocide 2000. Ginger was second most effective while Siam weed was once again the least effective (Table 6).

Table 5: Inhibition of mycelial growth of *P. megakarya* by cool water extracts from fresh samples of *Z. officinale, A. sativum* and *C. odorata*

Conc. / Botanicals		Inhibition of mycelial growth/ days after inoculation		
		3	6	9
20%	Co	5.63g	0.00i	0.00e
	Zo	15.97f	14.60f	0.00e
	As	17.04f	10.58g	0.00e
40%	Co	17.94f	0.00i	0.00e
	Zo	54.76d	30.98e	0.00e
	As	81.52b	58.04b	15.67c
60%	Co	49.41e	0.00i	0.00e
	Zo	55.45d	43.92d	0.00e
	As	100.00a	100.00a	100.00a
80%	Co	54.12d	7.01h	0.00e
	Zo	59.56c	54.90c	3.13d
	As	100.00a	100.00a	100.00a
Kocide 2000		100.00a	100.00a	88.95b

Note: Means in the same column followed by the same alphabets are not significantly different ($p < 0.05$) according to Tukey's test.
Legend: Co: *Chromolaene odorata.* Zo: *Zingiber officinale.* As: *Allium sativum*

Table 6: Inhibition of mycelial growth of *P. megakarya* by hot water extracts from fresh samples of *Z. officinale, A. sativum* and *C. odorata*

Conc. / Botanicals		Inhibition of mycelial growth/ days after inoculation		
		3	6	9
20%	Co	0.00h	0.00g	0.00d
	Zo	18.28e	9.41f	0.00d
	As	18.30e	20.78e	0.00d
40%	Co	3.59g	0.00g	0.00d
	Zo	54.78d	20.00e	0.00d
	As	100.00a	58.04c	0.00d
60%	Co	9.74f	0.00g	0.00d
	Zo	71.80c	49.40d	0.00d
	As	100.00a	100.00a	100.00a
80%	Co	19.48e	0.00g	0.00d
	Zo	86.16b	69.40b	8.23c
	As	100.00a	100.00a	100.00a
Kocide 2000		100.00a	100.00a	88.95b

Note: Means in the same column followed by the same alphabets are not significantly different ($p < 0.05$) according to Tukey's test.
Legend: Co: *Chromolaene odorata.* Zo: *Zingiber officinale.* As: *Allium sativum*

4.6 Correlation and regression analysis between concentrations of aqueous extracts and inhibition of mycelial growth of *P. megakarya*

The result from the simple linear correlation analysis and regression of increasing concentrations of crude extracts of botanicals (X) against the inhibition of mycelial growth (Y) showed positive relationships for all the extract concentrations from the three botanicals (Table 7). Very strong correlations were recorded, especially at 3 and 6 days after inoculation. Hot water extract from both fresh and dry *C. odorata* had 0% inhibition of mycelial growth at 6 days after inoculation, while the same result was obtained for all the extracts at 9 days after inoculation. Consequently, no correlation/regression values were obtained for them (Table 7).

Table 7: Correlation and regression analysis between increasing concentrations of crude extracts (x) and inhibition of mycelial growth (y) (n = 4)

Botanicals/ Extraction method		Correlation coefficient (r)/regression equation/days after inoculation					
		3		6		9	
		Correlation coefficient (r)	Regression (predictor) equation	Correlation coefficient (r)	Regression (predictor) equation	Correlation coefficient (r)	Regression (predictor) equation
C. odorata	(HWD)	0.994	Y= 8.036 + 2.898X	NILL	NILL	NILL	NILL
	(HWF)	0.978	Y= 25.71 + 2.961X	NILL	NILL	NILL	NILL
	(CWD)	0.945	Y = 15.39 + 0.9261X	0.947	Y = 30.51 + 3.315X	NILL	NILL
	(CWF)	0.964	Y = 16.64 + 1.050X	0.775	Y = 40.00 + 5.706X	NILL	NILL
Z. officinale	(HWD)	0.957	Y = 4.60 + 0.6774X	0.995	Y = 19.31+ 1.186X	0.775	Y = 4.00 + 5.666X
	(HWF)	0.973	Y = 0.435 + 0.8582X	0.986	Y = 15.58 + 0.9290X	0.775	Y = 40.00 + 4.860X
	(CWD)	0.982	Y = 4.138 + 0.9575X	0.992	Y = 6.864 + 1.192X	0.948	Y = 30.24 + 1.045X

	(CWF)	0.831	Y = 1.20 + 1.051X	0.999	Y = 9.335 + 1.608X	0.775	Y = 4.00 + 12.75X
A. sativum	(HWD)	0.943	Y = 26.98 + 0.5739X	0.922	Y = 32.74 +1.013X	0.775	Y = 40.00 + 2.876X
	(HWF)	0.775	Y = 11.04 + 0.4896X	0.946	Y = 5.35 + 0.6406X	0.894	Y = 30.00 + 0.4000X
	(CWD)	0.962	Y = 15.06 + 0.6612X	0.907	Y = 32.50 + 0.5143X	0.775	Y = 40.00 + 0.6415X
	(CWF)	0.877	Y = 7.10 + 0.5748X	0.940	Y = 11.71 + 0.5701X	0.926	Y = 25.95 + 0.4460X

Note: Means in the same column followed by the same alphabets are not significantly different ($p < 0.05$) according to Tukey's test.
Legend HWD: Hot water extract from dry sample, HWF: Hot water extract from fresh sample, CWD: Cool water extract from dry sample CWF: Cool water extract from fresh sample

4.7 Inhibition of infection on cocoa pods by extracts from *Z. Officinale* and *A. sativum* in the *In vivo* experiment.

Ginger and garlic extracts gave the best percentage IMG of *P. megakarya* in the *In vitro* experiment. Consequently, cool and hot water extracts of the most promising concentrations of the two plants were evaluated. The results obtained showed that inhibition of infection was significantly different among the different concentrations across the days for which data were collected (Table 8). The general trend was a steady increase in the percentage inhibition of infection up to the 9th day after inoculation, thereafter, a decline was noticed. Only the standard check deviated from this observation (Table 8). At 5 days after inoculation, the hot water extract from dry ginger gave 42.08% and 43.04% inhibition of infection at 60% and 80% concentrations respectively. The two values were significantly higher than the other concentrations. Cool water extract from dry ginger at 60% concentration gave the poorest percentage inhibition of infection, 17.90%, (Table 8). On the 7th days after infection, hot and cool water extracts from ginger and garlic all compared favourably, producing inhibition of infection above 70%. The Percentage inhibition of infection at 9 days after inoculation was 41.40% for cool water extract from garlic at 40% concentration. This was significantly the lowest at this period. The highest value, 82.83%, was recorded for hot water extract from ginger at 80% concentration (Table 8; Plate 3b). This value was however not significantly different from that recorded for hot water extract of fresh garlic (at the same concentration) and the standard check. On the 11th day after inoculation, the hot water extract from fresh garlic (80% concentration) inhibited infection by 79.03%. It was significantly the highest among the concentrations of extracts evaluated. Only the standard check was significantly higher (Table 8; Plate 3c). Cool water extract from Dry ginger (60% concentration) was the poorest, inhibiting infection by 1.87% (Table 8).

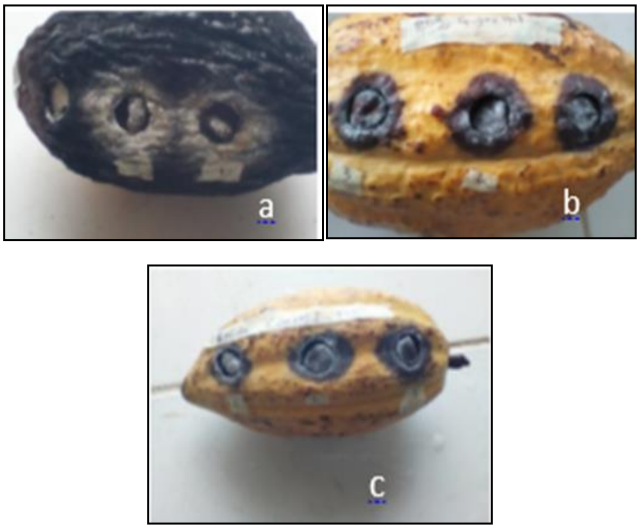

Plate 3: (a). Control at 9 days after infection **(b).** Hot water extract of ginger at 9 days after infection **(c).** Standard check at 11 days after infection.

Table 8: Inhibition of infection of cocoa pod rot pathogen on cocoa pods by aqueous extracts from *Z. officinale* and *A. sativum*

Conc. / Botanicals		Inhibition of infection on cocoa pod/days after inoculation			
		5	**7**	**9**	**11**
40%	HDZ	34.02b	67.08d	58.62g	15.12i
	CFA	21.06e	62.31d	41.40i	5.94k
	HFA	28.05d	68.04cd	50.99h	11.02j
60%	HDZ	42.08a	73.69bc	74.35d	35.01g
	CDZ	17.90e	63.01d	70.15e	1.87lk
	CFA	32.18bcd	68.12cd	64.43f	60.94e
	HFA	27.74d	73.53bc	67.09ef	66.10d
80%	HDZ	43.04a	73.71bc	82.83ab	45.51f
	CDZ	31.52cd	73.89bc	78.53c	24.47h
	CFA	37.45ab	74.73ab	77.25cd	71.04c
	HFA	32.90bcd	73.34bc	80.38bc	79.03b
Kocide 2000		36.77abc	80.11a	85.35a	85.41a

Note: Means in the same column followed by the same alphabets are not significantly different ($p < 0.05$) according to Tukey's test.
Legend HDZ: Hot water extract from dry *Z. officinale.* CFA: Cool water extract from fresh *A. sativum* HFA: Hot water extract from fresh *A. sativum.* CDZ: Cool water extract from dry *Z. officinale*

5. Discussion

Results obtained from the *In vitro* studies showed that aqueous extracts from the three plant samples differ in their IMG of CBPRP. The state of the plant materials (fresh or dry) and the method of extraction (hot or cool water) was also a very important determinant of their efficacy. Mohammed (2014) reported a similar finding on the inhibition of cold and hot water extract of parsley plant on some bacteria belonging to Enterobactericae family. On a general note, percentage mycelial growth inhibition increased with the increasing concentration of aqueous extracts from all the plant samples, regardless of their state or methods of extraction, but decline with the increasing number of days after inoculation, except for *A. sativum* at higher concentrations. This agrees with the findings of Mukherjee *et al*, (2011) [20] and Ajayi and Oyedele (2016) [7]. Aqueous extracts from *C. odorata* were the least effective. The highest percentage of mycelial growth inhibition recorded for it was just a little above 50%, even at the highest concentration of 80%. Adeyemo *et al*, (2018) [1] worked on extracts of *C. odorata* against *P. megakarya* and reported a similarly poor performance of the plant. Ginger and garlic, at high concentrations, compared favourably with the standard check, especially at three days after inoculation. As the number of days after inoculation increased however, the inhibitory effect of ginger decreased and it became less effective compared to *A. sativum*, which was the stand out performer. It exhibited complete inhibition of mycelial growth of *P. megakarya* at 40% concentration (hot water extract from the fresh sample) and above. It was also able to inhibit mycelial growth of the cocoa black pod rot pathogen by 100% at 9 days after inoculation, a feat that not even the standard check could match. The antimicrobial efficacy of crude extracts from garlic against fungal pathogens of plants and humans has been reported by various authors (Daniel *et al*, 2015; Burian *et al*, 2017) [12, 10]. The phytochemical constituent of the three plants, as well as their mode of action, has been well researched (Gruhlke *et al*, 2010; Khashan, 2014; Sefu *et al*, 2015 and Chiejina, 2016) [15, 18, 28, 11].

The result from the *In vivo* studies presented a clear increase in the percentage inhibition of infection of *P. megakarya* on cocoa pods at day 7 (above what was recorded at day 5) for all the extracts evaluated. A decline however set in after this period, and by the 11th day after inoculation, the decline was sharp and obvious for almost all the extracts evaluated. The standard check, (kocide 2000) was the only exception. A decreasing inhibition of infection with an increasing number of days after inoculation is a common feature that has been reported for some plant extracts by researchers (Breda *et al*, 2016; Obaid *et al*, 2017) [9, 21]. This may be a confirmation of their fungistatic rather than fungicidal properties. Extracts from plants, being organic matter, are easily degradable. As they degrade, their antifungal property wane. This may be a blessing, in that accumulation of phytochemicals in the soil and produce is ruled out. There is however a need for frequent re-application. Extract from fresh garlic was the most effective in the *In vivo* study, having the highest percentage inhibition of infection at 11 days after inoculation.

6. Conclusion and recommendation

This research work has thrown light on the potentials of *Z. officinale* and *A. sativum* in the management of CBPRD. Garlic was particularly impressive, due to the high percentage inhibitions of mycelial growth and infection in the *In vitro* and *In vivo* experiments. There is a need for further studies with a view to developing ready to use fungicide from both plants. Such plant-based fungicides will go a long way at mitigating the negative effect associated with synthetic chemicals which are currently in use in the management of CBPRD.

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