# Evaluation of some pesticides of plant origin for control of anthracnose disease (*Colletotrichum destructivum* O'Gara) in cowpea

DAVID NWAZUO ENYIUKWU\*, ANDERSON CHIDI AMADIOHA, CHARLES CHIMEZIE ONONUJU

Department of Plant Health Management, Michael Okpara University of Agriculture. Umudike, KM 10 Umuahia-Ikok Ekpene Road, PMB 7267 Umuahia, Abia State, Nigeria. Tel.: +234(0)09092309790, •email: enyidave2003@gmail.com

Manuscript received: 29 July 2020. Revision accepted: 12 October 2020.

**Abstract.** Enviukwu DN, Amadioha AC, Ononuju CC. 2021. Evaluation of some pesticides of plant origin for control of anthracnose disease (Colletotrichum destructivum O'Gara) in cowpea. Asian J Agric 5: 4-11. Anthracnose is a common disease of cowpea in many bean growing areas of the world. This study evaluated the effects of Alchornea cordifolia, Tabernaemontana pachysiphon, and Lantana camara as low-input biopesticides for control of the disease. The experiment was laid out in randomized complete block design (RCBD) made up of 14 treatments with 4 replications. The results indicated that all the plant materials irrespective of carrier solvent and concentrations of application significantly ( $P \le 0.05$ ) minimized the incidence and severity of the disease, as well as improved the yield and yield parameters of the treated crop than the control. Amongst all evaluated dosages of the plant materials, 50-100 % concentration of *L. camara* gave the best disease control and yield improvement of the crop, followed by full strength of *T. pachysiphon* and *A. cordifolia* was the least. However, comparative to benomyl a standard fungicide, the plant-derived pesticides demonstrated lower fungitoxicity against the pathogen apart from 50-100 % extracts of *L. camara* which were statistically ( $P \ge 0.05$ ) at par with the effects of the fungicide. Therefore, all the plant extracts could be used at higher doses as prophylactics to stem the disease; however, *L. camara* could be applied at lower doses to achieve the same level of control. These plant materials overall could therefore contribute as effective bio-fungicides towards improving productivity of cowpea in the humid tropics.

Keywords: Anthracnose, bio-pesticides, cowpea, fungitoxicity, plant disease, plant extracts

# **INTRODUCTION**

Cowpea (*Vigna unguiculata* L. Walp.), otherwise called southern pea, is an important legume in tropical Africa and some parts of Asia where it is widely consumed as leafy vegetable and grain legume (Nielsen et al. 1997; Enyiukwu et al. 2018a). Anthracnose caused by *Colletotrichum destructivum* O'Gara has been identified as one of the major pathogen-induced challenges affecting its production in the humid tropics (Adegbite and Amusa 2008; Enyiukwu et al. 2014). Owing to the many drawbacks associated with use of synthetic pesticides in agriculture and public health, alternatives from natural sources especially higher tropical plant species are being vigorously sought (Awurum and Enyiukwu 2013; Awurum et al. 2016).

Alchornea cordifolia (Euphorbiaceae) is a staggering evergreen small tree that occurs sporadically in the rain forest and savannas of the tropics. On the other hand, Tabernaemontana pachysiphon (Apocynaecea) is a tropical evergreen tree used occasionally as ornamental plant, while Lantana camara (Verbenaceae) is a prickly invasive shrub found in many countries of Africa and Australia (Enviukwu 2017). These plants have a long history of usage as phytotherapeutic agents against several human bacterial and fungal pathogens such as: Klebsiella sn. Staphylococcus sp. Proteus sp. and Candida sp. (Okwu and Ukanwa 2010; Enyiukwu 2017). Recently methanol extracts of these medicinal plant species demonstrated considerable fungi toxic activity against C. destructivum O'Gara, a destructive plant pathogen responsible for

anthracnose disease of some legumes *in vitro* (Enyiukwu 2017).

Therefore, this work aimed at evaluating the effects of aqueous and methanol extracts of *L. camara., T. pachysiphon,* and *A. cordifolia* against the initiation, development, and spread of anthracnose disease caused by *C. destructivum* O'Gara in cowpea and performance of the treated crop in the screen-house.

#### MATERIALS AND METHODS

#### **Experimental site and location**

The experiment was conducted at the Plant Health Management Laboratory and Greenhouse of the College of Crop and Soil Sciences of Michael Okpara University of Agriculture, Umudike (MOUAU), Nigeria in August to October, during the 2015 cropping season. The environmental parameters of the location during the study months were: rainfall between 276-280.20 mm, a temperature range of 25-30°C, and relative humidity from 74-87.0 %. The soil type was sandy clay with organic carbon recorded at 68000 ppm, while organic matter was 10300 ppm, pH 4.17; phosphorus 26.55 mg/kg and calcium 2.10 Cmol/kg. The location stands at an altitude of 121.08 meters above sea level (GPS Coordinates 2017).

#### Preparation of extracts of the plant materials

Leaves of A. cordifolia T. pachysiphon and L. camara were washed under running tap and rinsed in 500 ml of sterile distilled water, air-dried on the laboratory bench for 3 weeks, and then using a hand milling machine they were separately milled into fine powder (300 g of each specimen) and stored separately in air-tight bottles. About 25, 50, 75, and 100 g of each powdered specimen were weighted out and soaked separately in 100 ml of sterile distilled water or same quantity of 30 % methanol contained in 250 ml conical flasks and allowed to stand for 6 h. Thereafter they were sieved separately through 4-folds of cheesecloth into different 200 ml sterile beakers to obtain the filtrates of 25, 50, 75, and 100 % concentrations of the aqueous and methanol extracts respectively (Amadioha 2003).

### Isolation, identification, and preparation of spore suspension of the causal agent

Culture medium was prepared using dehydrated potato dextrose (PDA) (Oxoid<sup>TM</sup> ThermoScientific Product, England, UK), while the pathogen was isolated from infected cowpea, subjected to pathogenicity tests, and identified. The spores suspension of the isolated pathogen was subsequently prepared and standardized using a hemocytometer counting slide to  $1.0 \times 10^5$  spores/ml of sterile distilled water (generally reported in literature as the concentration of pathogenic fungal spores in experiments; that can effectively initiate infection in susceptible host plants) (Amadioha 2003; Alberto 2013; Nwaoguikpe et al. 2014).

#### Evaluation of the effects of the extracts of the plant materials on infected cowpea in the screen-house

Four weeks after sprouting, healthy cowpea (Var. IAR-48) seedlings growing in pots containing heat-sterilized topsoil (4 kg) were spray-inoculated to run-off with spore suspension of C. destructivum (1x10<sup>5</sup> spores/ml), and subsequently grouped into two groups A and B; which were simultaneously treated with the test bio-pesticides. Seedlings in Group A were sprayed with aqueous suspensions of the different concentrations (25, 50, 75, and 100%) of the plant extracts, while those in Group B were sprayed with corresponding concentrations of methanol extracts of the plant materials. The control experiments were set up in a similar manner but sprayed only with suspension of the fungus and sterile distilled water or benomyl. The experiment was arranged in a randomized complete block design (RCBD) made up of 14 treatments and 4 replicates. The incidence and severity of the disease on the inoculated cowpea seedlings were assessed according to the formula adopted by Amadioha (2003) as follows:

% Incidence =  $\underline{\text{Number of diseased plants}} \times 100$ Total number of plants examined

The disease severity was assessed by visual assessment of the test plants with typical symptoms of the anthracnose disease, using the descriptive scale of 1-10 as outlined by Enyiukwu and Awurum (2013b): Where :

1. no disease;

2. 1-25% of seedling with anthracnose disease;

- 6. 51-75% of seedling with anthracnose disease;
- 8. 76-100% of seedling with anthracnose disease and

10. Stem breakage, girdling or death of seedling due to anthracnose disease.

The fungitoxicity of the extracts was evaluated as reduction in severity of anthracnose disease on the potted seedlings sprayed with spore suspension and the extracts of varying concentrations compared to the control experiments treated with water or benomyl alone (Amadioha 2003).

# Evaluation of effects of extracts of the plant materials on height, number of leaves, and biological yield of cowpea

The growth parameters (height and number of leaves) of the two groups of the inoculated potted cowpea plants after exposure to the various concentrations of the test plant extracts were recorded at weeks 6 and 8 after planting (WAP). However, the effects of the treatments on the biomass accumulation of each treated plant were recorded only at 8 WAP. At this time, the treated cowpea was harvested (uprooted) and oven-dried at 60°C for 3 days and the weight of the dry matter content of the treated plants was measured using a digital balance and recorded according to the protocols adopted by Awurum and Ogbonna (2013) and Awurum et al. (2016).

#### **RESULTS AND DISCUSSION**

# Effects of the extracts on the incidence and severity of anthracnose disease of cowpea in the screen-house

Results of Table 1 indicated that high mean incidence (83%) and severity (approx. 6) of anthracnose disease were recorded on the untreated cowpea (control experiment) compared to nearly 19.00 % and 1.45; 20.40 % and 1.80; 27.00 % and 2.06; and 30.00 % and 2.22 recorded for incidence and severity of the disease on benomyl, L. camara, T. pachysiphon, and A. cordifolia treated cowpea respectively. The result of the effects of the concentrations of the different botanicals revealed that the crude extracts of the test plants significantly inhibited the initiation, development, and spread of the fungus in cowpea (Table 1). The 100 % concentration of the *L. camara* reduced the incidence of C. destructivum induced anthracnose disease in the test cowpea from 55 % and 85 % recorded on the control at 6 WAP and 8 WAP to 10 % and 20 % for the methanol and 10 % and 22 % for water extract respectively. Also, the disease severity indices were reduced from 4.1 and 5.71 recorded on the control experiment to 1.0 and 2.02 for methanol extract (100 %) and 1.0 and 1.90 for water extract of L. camara after 6 and 8 WAP. Both methanol and water extracts of T. pachysiphon and A. cordifolia at 100 % concentration recorded lower disease incidence and severity when compared with the control (Table 1). There was no significant difference between the results recorded with benomyl (benzimidazole) and 75-100% concentration of L.

*camara* extracts in terms of disease severity. There also was no significant difference in fungitoxicity of benomyl when compared with methanol and water extracts of the test plants in disease incidence on cowpea both at 6 WAP and 8 WAP (Table 1).

The toxicity profile of the botanicals was *L. camara*>*T. pachysiphon*>*A. cordifolia*. The results also revealed that irrespective of the type of plant material evaluated, the fungitoxicity increased with the concentration of the test plant extracts. The fungi toxic activity of the aqueous extracts was not statistically (P<0.05) superior to methanol extracts of the plant materials against the target fungus.

The mean effects of the different levels of the biopesticides on the disease progression of the *C. destructivum*-inoculated cowpea (Figure 1 and 2) (see Table 3) indicated that the disease progressed very rapidly, increasing exponentially between 4 and 6 WAP hence the sharpest slope and largest area under the growth curve within this period. However, it increased at a decreasing rate from 6-8 WAP. This suggests that the disease caused by *C. destructivum* was severe within two weeks (6 WAP) having severity index of 1.6 times greater than infection rate at 4 WAP.

# Effects of the plant extract on the growth parameters and biological yield of cowpea

The results presented in Table 2 indicated that the plant extracts had significant effects on the growth and biological yield of cowpea. Amongst the plant extracts, the best dry matter yield of 4.30 g, 4.41 g, and 4.52 g were obtained from *L. camara* treated cowpea at 50 %, 75%, and 100% concentrations respectively. There was no significant

difference in the dry matter yield (4.62 g) obtained from benomyl (benzimidazole) and those recorded with 50-100% concentrations of the methanol extracts of *L. camara.* Also, there was no significant difference with benomyl (4.49 g) when compared with respective yields of 4.19 g, 4.38 g, and 4.41 g from 50, 75, and 100% concentrations of the water extracts of the plant materials on the test cowpea 8 WAP (Table 2). However, the dry matter yields obtained from all the extracts of the test plants irrespective of concentration, and solvent of extraction were statistically (p≤0.05) greater than those recorded for the control experiment.

All the extracts irrespective of concentration and type of plant material significantly (P≤0.05) enhanced the plant height and number of leaves as well as dry matter yield of the crop over the control. In terms of number of leaves, except for T. pachysiphon at 50% concentration, all the assayed plant extracts irrespective of concentration and carrier solvent significantly (P≤0.05) increased the number of leaves of the test crop over the control 8 WAP and compared favorably (P≤0.05) also with those recorded for benomyl treated cowpea at both 6 WAP and 8 WAP. Similarly, all the plant extracts tested at 100 % concentration had a better performance than the control experiment in improving the height of the cowpea at 6 WAP and 8 WAP except A. cordifolia. In general, the best vield and vield attributes were recorded when the crop was treated with L. camara followed by T. pachysiphon. However, in terms of dry matter yield 50%, 75%, and 100% concentrations of methanol and water extracts of L. camara was statistically (P≤0.05) at par with result obtained from benomyl treatment.

 Table 1. Effects of the aqueous and methanol extracts of test plants and benomyl against Collectorichum destructivum in cowpea eight weeks after planting (8 WAP)

	Disease incidence (%), severity, and time of planting (weeks)							
Treatment	6 WAP		8 WAP		6 WAP		8 WAP	
	DI	DS	DI	DS	DI	DS	DI	DS
Alchornea cordifolia								
25%	30	2.0	41	3.23	28	2.0	35	2.20
50%	30	2.0	40	2.84	27	2.0	32	2.20
75%	24	2.0	33	2.45	22	2.0	31	2.17
100%	22	2.0	32	2.41	22	2.0	30	2.08
Tabernaemontana pachysiphon								
25%	25	2.0	35	2.31	23	2.0	30	2.11
50%	23	2.0	33	2.30	21	2.0	30	2.01
75%	23	2.0	32	2.40	20	2.0	29	2.01
100%	20	1.8	30	2.22	20	2.0	27	2.00
Lantana camara								
25%	20	2.0	28	2.18	20	2.0	30	2.10
50%	18	2.0	26	2.05	18	2.0	24	2.05
75%	15	1.5	23	2.03	16	1.0	26	1.95
100%	10	1.0	20	2.01	10	1.0	22	1.90
Benomyl (3g/l)	11	1.2	17	1.69	10	1.0	20	1.91
Sterile water	55	4.1	85	5.71	51	4.0	82.9	5.64
LSD (0.05)	7.3	1.1	4.2	0.78	1.9	0.9	5.02	0.57

Note: Values are means of 4 replicates in two separate experiments; DI: disease incidence (%); DS: disease severity

**Table 2.** Effects of aqueous and methanol extracts of test plants and benomyl on height, number of leaves, and yield of cowpea 6 and 8WAP

	Agronomic parameters and time of planting (weeks)									
Treatment	6 WAP 8 WAP			6 W	6 WAP		8 WAP			
	PH	NL	PH	NL	DM	PH	NL	PH	NL	DM
Alchornea cordifolia										
25%	23	3.01	32	4.04	3.18	22	3.00	30	4.11	3.17
50%	23	3.02	33	4.08	3.24	23	3.05	31	4.14	3.21
75%	27	3.40	37	4.34	3.38	25	3.12	32	4.17	3.49
100%	28	3.40	41	4.41	3.73	30	3.21	35	4.28	3.61
Tabernaemontana pachysiphon										
25%	25	3.00	32	4.22	3.90	24	3.20	33	4.21	3.58
50%	23	3.00	34	3.30	3.90	25	3.20	33	4.22	3.68
75%	24	3.20	33	4.31	4.09	25	3.31	38	4.30	3.78
100%	23	3.40	33	4.40	4.10	27	3.40	39	4.30	3.97
Lantana camara										
25%	23	3.20	36	4.05	4.10	23	3.10	34	4.15	4.12
50%	24	3.41	36	4.18	4.30	23	3.23	34	4.33	4.18
75%	24	3.50	38	4.43	4.41	24	3.28	36	4.38	4.29
100%	26	3.51	40	4.61	4.53	25	3.40	42	4.95	4.38
Benomyl (3g/l)	26	3.45	41	4.57	4.62	25	3.29	38	4.81	4.49
Sterile water	23	2.38	29	3.16	2.05	21	2.48	29	2.64	2.18
LSD (0.05)	2.2	0.25	6.02	0.78	0.38	1.92	0.9	5.1	0.57	0.33

Note:PH: Plant height (cm); NL: Number of leaves; DM: Dry matter content of biological yield of treated whole cowpea plant (grams)

Table 3. Mean effects of different	concentrations of water and methanol	extracts of the plant materials	on anthracnose incidence in
cowpea			

	Extracting solvent and weeks after planting (WAP)						
Plant materials	Week 4	Week	6	Week	8		
	<b>Both solvents</b>	Water	Methanol	Water	Methanol		
Alchornea cordifolia	0	25.00	26.50	32.00	36.50		
Tabernaemontana pachysiphon	0	23.30	2280	30,50	32.50		
T. camara	0	16.00	15.80	25.00	24.30		
Benomyl	0	10.00	11.00	20.00	17.00		
Distilled water	0	51.00	55.00	82.90	85.00		
Total	0.00	125.00	131.10	190.40	195.30		
Mean	0	25.00	26.22	38.08	39.06		

### Discussion

The results of Table 1 showed that high mean incidence and severity of anthracnose disease were recorded on the untreated control experiment. Mohammed (2013) reported that high ambient temperature, rainfall, and relative humidity encourage the initiation, development, and spread of anthracnose disease of cowpea. The high mean incidence (83%) and severity (approx. 6) recorded on the untreated control experiment in this study may have been favored by high ambient temperature (29-31°C), rainfall (276-280.2 mm), and relative humidity (74-87%) which lasted for up to 23 days in the study months (September-October 2015). Well-drained, slightly acidic, sandy clay soil with a pH 5, that was rich in organic matter, organic carbon, and low in salt (sodium) content permits optimal growth and performance of cowpea (Anyanwu et al. 1980; Timko et al. 2007). Similarly, low calcium and phosphorus contribute to poor development of resistance mechanisms in crops (Owolade et al. 2006; King et al. 2012; Boumaaza et al. 2015). Hence, the poor phosphorus (26.55 mg/kg) and calcium (2.10 Cmol/kg) status of the greenhouse soil may have informed the poor resistance of the test crop to C. *destructivum* resulting in the high mean incidence and severity of anthracnose disease recorded on the control experiment in this study.

Table 1 also indicated that irrespective of type of carrier solvent and concentration of application of the botanicals used in this study, all the test extracts performed well in minimizing the incidence and severity of anthracnose disease of cowpea in the field which was statistically greater (P $\leq$ 0.05) than the control. However, benomyl demonstrated a superior fungitoxicity over the botanicals except for 50-75% concentration of *L. camara*. This implies that all of the test plants could be used as prophylactics against anthracnose disease of cowpea since they minimized the development and expression of the disease at 4 weeks after planting in the field.

Several workers have demonstrated the efficacy of pesticides of plant origin against several pathogenic fungi in the field. Field trials in Nigeria and Brazil showed that extracts of Ocimum sanctum, Piper nigrum, Vernonia polyanthus Xylopia aethiopica, Cymbopogon citratus, and C. flexuosus seriously affected the development and spread of anthracnose caused by C. lindemuthianum on cowpea (Amadioha 2001; 2003; Lemos da Silva et al. 2015). Similarly, P. guineense seed extract was reported to effectively minimize C. destructivum-induced anthracnose lesions on treated cowpea in the field (Enyiukwu and Awurum 2013). The effectiveness of these botanicals was found in most evaluations to compare well with the effect of the synthetic fungicides such as benomyl, thiophanatemethyl or Celest® in a dose-dependent manner (Enyiukwu and Awurum 2013; Masangwa et al. 2013). The findings in this study where the extracts of L. camara, T. pachysiphon, and A. cordifolia reduced the incidence and severity of anthracnose disease of cowpea in the field in a dosedependent manner are in agreement with the earlier reports by other scientists as mentioned above.

Botanicals are reported to contain several bioactive chemical groupings such as terpenoids, alkaloids, saponins, steroids, flavonoids, tannins, glycosides, and fatty acids; these have been reported to underpin the bioactivity of these plant materials (Edeoga et al. 2005; Enviukwu and Awurum 2013a). Presence of alkaloids, flavonoids, saponins, tannins, polyphenols, glycosides, and terpenoids have been reported in L. camara, T. pachysiphon, and A. cordifolia used in this study (Duru and Mbata 2010; Pradhan et al. 2012; Nwaoguikpe et al. 2014). Fatty acids including 9,12-Octadecadienoic acid methyl ester, 9-Octadecenoic acid methyl ester, and Dodecanoic acid (1,2,3-propanetryl ester) isolated from these plant materials (Enyiukwu 2017) are known to be antimicrobial compounds (Okwu and Njoku 2009; Akpuaka et al. 2013); suggesting that the fungitoxicity of the plant materials used against the test pathogen in this investigation may be due to these compounds. The high presence of these fatty acids, especially dodecanoic (lauric) acid in L. camara followed by T. pachysiphon, may have informed their higher antifungal activity over A. cordifolia on C. destructivum O'Gara in this study (Enyiukwu 2017).

Earlier field evaluations from Amadioha and Obi (1998; 1999) reported the superiority of fungi toxic activity of extracts of Xylopia aethiopica and Azadirachta indica over benomyl in minimizing Colletotrichum lindemuthianium incited anthracnose lesions in cowpea. Awurum and Ucheagwu (2013) also found Piper guineense in a similar manner to out-perform benomyl in protecting cowpea seeds in storage against Fusarium and Colletotrichum induced deteriorations over a 3-month period. Findings from this study, however, did not agree with these submissions since the extracts of the test plants used in this assay were not as effective as benomyl in arresting the initiation, development and spread of the pathogenic fungus (Table 1). The development of resistance of *Colletotrichum spp.* to benomyl (Tu 1981) and tolerance to its close chemical relatives carbendazim and thiophanate-methyl (Emechebe and Florini 1997) have been reported and this may explain the lower sensitivity of the pathogen to the fungicide as reported by the other investigators.

The disease progress curves (Figures 1 and 2) suggest that the control strategies against cowpea anthracnose disease should be preventive and such measures must be initiated early in the cycle (4 WAP) to stem the disease. High incidence and severity of *Colletotrichum spp.* induced anthracnose, brown blotch, and related fungal diseases following natural or artificial infection of seedling of Allium cepa and cowpea at 2-4 WAI have been reported by Owolade et al. (2004) and Awurum et al. (2016) respectively. These investigators maintained that the diseases were very severe during this period, progressing rapidly and extensively 2-3 weeks post-infection with the pathogens. Data from this study affirm that the attacks and severity of C. destructivum induced anthracnose disease in the test cowpea progressed rapidly and severely at this same period on the test seedlings. Extracts of the botanicals should therefore be applied early in the growth cycle of cowpea on or before the appearance of anthracnose symptoms on cowpea to minimize its spread and damage to the crop. The figures (Figures 1 and 2) further suggest that A. cordifolia and T. pachysiphon may be applied at higher doses to effect good control, unlike L. camara which could be applied at lower doses for effective control. Therefore, the closest alternative and/or substitute for the synthetic fungicide in this study is L. camara.

Frequent synthetic chemical sprays on cowpea and other crops to stem fungal diseases 2-3 WAI has also been reported (COPR 1981). Edema and Adipala (1994) and Oparaeke (2007) reported that weekly sprays with mancozeb or extracts of P. guineense on cowpea 2 WAP until flowering and podding significantly controlled brown rust (Uromyces vignae) and bruchids in the crop. In another study, Bretag (2008) found that for effective control of anthracnose disease of lentil, the fungicide Bravo 500 (50% Chlorothalonil) should be applied prior to onset of the disease and repeated a fortnight later. Awurum et al. (2016) reported that maintaining biweekly sprays of botanicals on onion and Amaranthus significantly checked the initiation, development, and spread of wet rot and anthracnose disease in the crops which corroborates the recommended control period at 4 WAP than 6 WAP for anthracnose of cowpea caused by C. destructivum in this study.

Time of application of chemicals and botanicals prior, simultaneous or after infection affects the efficacy of the product (Bretag 2008). Amadioha (2003) and Falade (2016) reported that the initiation, growth, and spread of anthracnose disease of cowpea caused by Climdemuthianum in cowpea were checked significantly  $(P \le 0.05)$  better with extracts of *P. nigrum, O. sanctum, D.* strammonium, J. gossipifolia and T. procumbens when applied 2 days prior to the inoculation or arrival of the pathogen on the crop than when applied simultaneously or 2 days after infection with the pathogen. Also, X. arthiopica and A. indica demonstrated higher fungitoxicity against the organism at 2 days before inoculation of the pathogen than at other application times (Amadioha and Obi 1998).

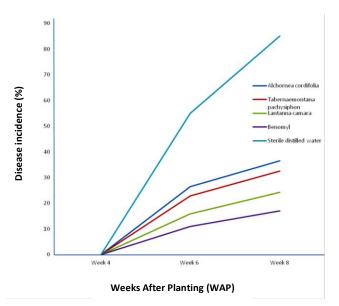


Figure 1. Mean effects of different concentrations of methanol extracts of the plant materials on disease incidence in cowpea 8 WAP

This suggests that plant extracts, besides direct toxicity to the test fungus, seem to stimulate host plant immunity towards resisting the disease and this may explain the higher control effects from the extracts of the test plants when applied at two or more days before than simultaneous or 2 or more days after inoculation of the pathogen on the crop. The less efficacy of the test extracts especially *A. cordifolia* and *T. pachysiphon* recorded in this study where the extracts and the pathogen were simultaneously applied on the test crop collaborate these findings.

The influences of phyto-pesticides in improving crop yields have been reported. Aqueous extracts of Piper guineense and C. papaya in a greenhouse trial on cowpea, besides reducing the development and spread of anthracnose disease caused by C. destructivum on the crop, improved the leaf area index, plant height, and biomass accumulation of test crop (Enviukwu and Awurum 2013b). Also, extracts of Hyptis marrubioides, Aloysia gratissima and Cordia verbenacea significantly improved seedling emergence and dry matter content of treated soybean challenged by the anthracnose disease agent C. truncatum (Da Costa et al. 2012) Similar reports on field trial on Amaranthus cruentus (Amaranth) and Allium cepa L. (onion) exposed to phytochemicals derived from Dennettia tripetala, Azadirachta indica and Spondias mombin noted that besides significantly reducing the damage attendant from C. curcubitarium causing wet rot and *C*. gloeospoirioides incitant of anthracnose disease respectively on the crops, enhanced the yield and yield attributes of the test plants (Awurum and Nwaneri 2011; Awurum et al. 2016). According to Mark et al. (2015) essential oil derived from A. sativum L. effectively improved the number of leaves, leaf area, plant height and stem girth of treated cowpea. Findings from this study whereby the extracts of the test plants (L. camara, T.

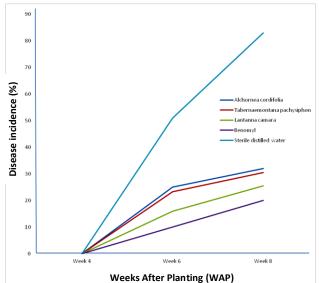


Figure 2. Mean effects of different concentrations of water extracts of the plant materials on disease incidence in cowpea 8 WAP

*pachysiphon* and *A. cordifolia*) significantly ( $P \le 0.05$ ) improved the plant height, number of leaves and dry matter yield of cowpea agree with the foregoing submissions.

The better performance of the methanol extract in improving the biomass content of the test crop over the water extracts (Table 2) may be due to a better extraction and synergism of the active compounds in the methanol, as extracting solvent rather than water. Or it may be that the active principles dissolved more or were more soluble in methanol, as extracting solvent than water which translated to better control of the disease and yield of the crop. It could also be possible that water extracts of the test plant contained more inhibitors to the active principle than the methanol extracts (Amadioha 2001). Similarly, the better performance of benomyl over the plant-derived phytochemicals may have been a result of its longer persistence in the eco-system when compared with the phyto-pesticides which are easily degraded by heat and UV radiation (Enviukwu et al. 2014).

The improvement in the yield and yield parameters of the crop recorded in this study is thought to be brought about by two mechanisms-reduction of the growth and spread of the fungal pathogen causing the anthracnose disease and stimulating or priming of the crop immunity to resist further attacks by the disease-causing organism (Enyiukwu et al. 2016). Biopesticides derived from Gliocladium virens showed strong antibiosis against Alternaria helianthi evidenced by bursting of the hyphae of the pathogen and inhibiting its cellulose, cutinase and chitinase activities (Anitha and Murugesan 2012). Phytochemicals gleaned from Dennetia tripetala, Azadirachta indica and Spondias mombin improved the yield and quality of the treated crops by stemming the fungal attacks on the vegetables. These plant materials contain phenols and phenolic acids. Phenols are aromatic alcohols, which

are constituents of various ranges of pesticides (Okwu and Njoku 2009; Enyiukwu and Awurum 2013a). In biosystems this class of phytochemicals has been reported to block cell division, slow cellular growth and elongation, hamper sporangia formation, spore development and impair a wide array of microbial enzymes (Enyiukwu and Awurum 2013a). Revnoutria sachalinensis a phytopesticide containing the anthraquinone compound and is reported to induce defense mechanism in the host through stimulation and boosting of chitinases production, phytoalexin synthesis, papilla formation, vacuolization of infectious haustoria, inhibiting reactive oxygen species (ROS) and enzyme phenolic pathways (Lehnohof 2007). Likewise, Reboledo et al. (2015) in agreement reported that extracts of Phyacomitrella patens activated defense responses against C. gloeosporioides in susceptible hosts. The phenolic-like compounds of the extract inhibited the advancement of the pathogen by inducing certain genes coding for the formation and incorporation of protein-like polymers that re-inforced the host's cell wall. Also, bioactive fatty acids and essential oils containing functional groups or substituents such as furans, aldehydes, oxides, ketones, phenols, lactones, coumarins, and terpene hydroxides could induce resistance in treated crops through induction of host enzyme peroxidases against invading pathogens (Lemos da Silva et al. 2015). In beans (Phaseolus vulgaris L.), increased enzyme peroxidase activities due to fatty acid-containing phytochemicals have been associated with resistance to anthracnose disease caused by C. indemuthianium in the crop. The mechanism of this action has been linked to ability of extracts of Cymbopogon flexuosus and Vernonia polyanthes to induce host enzyme peroxidases to produce free radicals (H<sub>2</sub>O<sub>2</sub>) toxic to the pathogen in an oxidative burst and participate in lignin synthesis that strengthened host cell wall (Lemos da Silva et al. 2015). Applying phytochemical controls early enough was thought to have supplied phenols or improved the production of infection-fighting phenolics or radicals such as hydrogen peroxide in the onion plants through re-enforcing the structural components of its walls thereby reducing the advancement and damage due to fungal pathogens resulting in improved yield and quality of produce (Awurum et al. 2015). Therefore, the ability of the phytochemicals used in this study to improve the yield and quality of the treated cowpea may be due to these protective mechanisms developed by the host due to the phytochemicals from the test plant materials.

#### REFERENCES

- Adegbite AA, Amusa AN. 2008. The major economic field diseases of cowpea in the humid agro-ecologies of South-Western Nigeria. Afr J Biotech 7 (25): 4705-4712.
- Akpuaka A, Ekwenchi MM, Dashak DA, Dildar A. 2013. Biological activities of characterized isolates of n-hexane extract of *Azadirachta indica* A. Juss (Neem) leaves. Nat Sci 11 (5): 141-147.
- Alberto E. 2013. How to calculate the concentration of fumgal spores suspension. National University of General San Martín-CONICET, Buenos Aires.
- Amadioha AC, Obi VI. 1998. Fungitoxic activity of extracts from Azadirachta indica on Colletotrichum lindemuthianum in cowpea. J Herbs Spices Med Plants 6 (2): 33-40.

- Amadioha AC, Obi VI. 1999. Control of anthracnose disease of cowpea by Cymbopogon citratus and Ocimum gratissimum. Acta Phytopathol Entomol Hung 34 (1-2): 85-89.
- Amadioha AC. 2001. Fungitoxic effect of some leaf extracts against *Rhizopus oryzae* causing tuber rot of potato. Arch Pytopathol Plant Protect 33 (6): 499-507.
- Amadioha AC. 2003. Evaluation of some plant leaf extracts against *Colletotrichum lindemuthianum* in cowpea. Acta Phytopathol Entomol Hung 38: 259-265.
- Anitha R, Murugesan K. 2012. Mechanism of action of *Gliocladium* virens on Alternaria helianthi. Ind Phytopathol 5(4): 449-452.
- Anyanwu AC, Anyanwu BO, Anyanwu VA. 1980. Agriculture for School Certificate (Revised Edition). Africana Educational Publishers, Nigeria.
- Awurum AN, Enyiukwu DN, Odoemena VK. 2016. Influence of plantgleaned compounds on the initiation and development of fungal diseases of onion (*Allium cepa* L.) in the field. J Biol Agric Healthcare 6 (9): 71-80.
- Awurum AN, Nwaneri JA. 2011. Fugitoxic effects of some plants extracts on wet rot of *Amaranthus* induced by *Choanephora cucurbitarium*. Niger J Plant Prot 25 (2): 230-236.
- Awurum AN, Ogbonna MJ. 2013. Field trial on the efficacy of some plant extracts on the control of wet rot of *Amaranthus cruenta* L. induced by *Choanephora curcubitarium*. Cont J Agron 7 (1): 10-17.
- Awurum AN, Ucheagwu PO. 2013. Effects of duration of contact of *Piper guineense*, *Monodora myristica* and *Xylopia aethiopica* on the germination and incidence of seed-borne fungi of stored cowpea (*Vigna unguiculata* L.Walp) seeds. Cont J Biol Sci 6 (1): 37-42.
- Boumaaza B, Benkhelifa M, Belkhudja B. 2015. Effects of two salts compounds on mycelial growth, sporulation and spore germination of six isolates of *Botrytis cinerea* in West North Algeria. Int J Microbiol 2015: 001-008.
- Bretag T. 2008. Lentil anthracnose caused by *Colletotrichum truncatum*-Industry biosecurity plan for the grain industry threat specific contingency plan. Plant Health Australia and Grain Research & Development Corporation, Australia. www.planthealthaustralia.au
- COPR [Centre for Overseas Pest Research]. 1981. Pest Control in Tropical Grain Legumes. London, UK.
- Da Costa CL, Geraldo MRE, Aroteia CC, Kemmelmeier C. 2010. In vitro activity of neem oil on aspergillus flavus growth, sporulation, viability of spores morphology and aflatoxin B1 and B2. Adv Biosci Biotechnol 1: 292-299.
- Duru CM, Anyadoh-Nwadike SO, Okechukwu RI. 2015. Antimicrobial activity and phytochemical analysis of aqueous and ethanolic extracts of the bark of *Tabernaemontana pachysiphon* Stapf. Sci J Publ Health 3 (1-5): 8-13.
- Edema R, Adipala E. 1994. Cowpea diseases (1 and 2). Control of false smut and brown rust in Uganda using benomyl and mancozeb. East Afr Agric J 60 (1): 11-17.
- Edeoga HO, Okwu DE, Mbaebie BO. 2005. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol 4 (7): 685-688.
- Emechebe AM, Florini DA. 1997. Fungal and bacterial diseases of cowpea. In: Singh RS, Morgan-Raj Daswell KE, Jackai LEN (eds) Advances in Cowpea Research IITA.
- Enyiukwu DN, Amadioha AC, Ononuju CC. 2018a. Significance of cowpea leaves for human consumption. Greener Trends Food Sci Nutr 1 (1): 1-10.
- Enyiukwu DN, Amadioha AC, Ononuju CC. 2018b. Biochemical composition, potential food, and feed values of aerial parts of cowpea (*Vigna unguiculata* L. Walp.) Greener Trends Food Sci Nutr 1 (1): 011-018.
- Enyiukwu DN, Awurum AN, Ononuju CC, Nwaneri JA. 2014. Biology and management strategies of cowpea anthracnose disease caused by *Colletotrichum* species. Greener J Biochem Biotechnol 1 (2): 52-65.
- Enyiukwu DN, Awurum AN, Ononuju CC, Nwaneri JA. 2016. Modes of action of potential phyto-pesticides from tropical plants in plant health management. IOSR J Pharm 6 (7): 1-17.
- Enyiukwu DN, Awurum AN. 2013a. Fungitoxic principles and antifungal activity of extracts from *Carica papaya* and *Piper guineense* on *Colletotrichum destructivum*. Cont J Biol Sci 6 (1): 29-36.
- Enyiukwu DN, Awurum AN. 2013b. Fungitoxic effects of *Carica papaya* and *Piper guineense* extracts on *Colletotrichum destructivum* in the glasshouse. Cont J Agric Sci 7 (1): 23-28.
- Enviukwu DN. 2017. Effects of extracts of some medicinal plants on Colletotrichum destructivum O'Gara causing anthracnose disease of

cowpea (*Vigna unguiculata* L. Walp.) in Nigeria. [Dissertation] Department of Plant Health Management, MOUAU, Nigeria.

- Falade MJ. 2016. *In vitro* and *in vivo* control of cowpea anthracnose caused by *Colletotrichum lindemuthianum* using some plant extracts of indigenous plants. Intl J Soil Crop Sci 4 (2): 059-066.
- King BC, Waxman KD, Nenni NV, Walker LP, Bergstorm GC, Gibson DM. 2010. Arsenal of plant cell wall degrading enzymes reflects host preference among plant pathogenic fungi. Biotechnol Biofuel 4 (4): 001-004.
- Lehnohof F. 2007. Mode of action of Milsana (Biofa): a *Reynoutria* sacchalinnensis based-plant extract for preventive control of powdery mildews. www.abim.ch/file admin/edu.../../t\_lehnhof\_abim\_2007./df.
- Lemos da Silva J, Estevao de Souza P, Alves E, Pinto JEBP, Bertoluci SKV, Freilas MLO, Large de Andrade CC, Resende MLV. 2015. Essential oil of *Cymbopogon flexuosus, Vernonia polyanthus* and potassium phosphite in the control of bean anthracnose. J Med Plant Res 3: 243-253.
- Mark WA, Channya KF, Chimbekujwo IB, Bristone B. 2015. Control of *Colletotrichum capsici* (pathogen of brown blotch of cowpea in the savanna) using garlic oil. Intl J Res Agric For 3 (1): 22-29.
- Masangwa JIG, Aveling TAS, Kritziger Q. 2013. Screening of plant extracts for antifungal activities against *Colletotrichum* species of common bean (*Phaseolus vulgaris*) and cowpea (*Vigna unguiculata* L.). J Agric Sci 151 (4): 482-491.
- Mohammed A. 2013. An overview of distribution, biology and the management of common bean anthracnose. J Plant Pathol Microbiol 4 (8): 1-6.
- Nielsen SS, Ohler TA, Mitchell MC. 1997. Cowpea leaves for human consumption: production, utilization and nutrient composition. In:

Singh BB (eds) Advances in Cowpea Research. IITA, Ibadan, Nigeria.

- Nwaoguikpe RN, Braide W, Okwu GN, Anyanwu BO, Ujowundu CO. 2014. The effects of extracts of *Alchornea cordifolia* (Christmas bush) on sickle cell haemoglobin. Br J Pharm Res 4 (1): 001-015.
- Okwu DE, Njoku EE. 2009. Chemical composition and *in vitro* antifungal activity screening of seed and leaf extracts from *Afromonum melenguata* and *Monodora myristica* against *Sclerotium rolfsii* of cowpea plant. Pest Technol 3 (1): 58-67.
- Okwu DE, Ukanwa N. 2010. Isolation, characterization and antibacterial activity screening of anthocyanidin glycoside from *Alchornea cordifolia* leaves. E-J Chem 7 (1): 41-48.
- Oparaeke AM. 2007. Toxicity and spraying schedules of biopesticides prepared from *Piper guineense* against two cowpea pests. Plant Protect Sci 43 (3): 103-108.
- Owolade OF, Akande MO, Alabi S, Adeniran JA. 2006. Phosphorus levels effect on brown blotch disease, development and yield of cowpea. World J Agric Sci 2 (1): 105-108.
- Pradhan RR, Hati DK, Samal S. 2012. Pharmacognostical, phytochemical and antimicrobial studies on the leaves of *Lantana camara* Linn. Pharm Lett 4 (6): 1648-1656.
- Reboledo G, Dee Campo R, Alvarez A, Montesano M, Mara H, De Leon IP. 2015. *Physcomitrella patens* activate defense responses against the pathogen *Colletotrichum gloeosporioides*. Intl J Mol Sci 16: 22280-222298.
- Timko MP, Ehlers JD, Roberts PA. 2007. Cowpea. In Pulses, sugar and tuber crops. Springer, Berlin.
- Tu JC. 1981. Anthracnose (*Collectorichum lindemuthianum*) on white bean (*Phaseolus vulgaris* L.) in southern Ontario: spread of the disease from an infection focus. Plant Dis 65 (6): 477-480.