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Fungitoxic Screening of Some Local Plant Extracts for the Control and Yield Performance of Cowpea (*Vigna unguiculata* L Wasp) Infested with Anthracnose Disease in Southeastern Nigeria

Isreal Philip Eno[1], Godwin Michael Ubi[2*] and Imaobong Sunday Essien[3]

[1]Department of Plant Health Management, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.
[2]Department of Genetics and Biotechnology, University of Calabar, Calabar, Nigeria.
[3]Department of Zoology and Environmental Biology, University of Calabar, Calabar, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author IPE designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors GMU and ISE managed the literature searches, performed the statistical analysis, while all authors discuss the conclusion. All authors read and approved the final manuscript.

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ABSTRACT

Field experiments were conducted in late 2013 and 2014 cowpea cropping seasons in Michael Okpara University of Agriculture. Umudike, to assess the efficacy of leaf extracts of *Gmelina arborea*, *Chromoleana odorata* and *Anacardium occidentale* in the control of anthracnose disease of cowpeas induced by *Collectotrichum lindermuthianum* and to assess the yield performance of the crop. The study was designed to evaluate the effects of the different plant extracts on the disease development, growth and yield performance of cowpea inoculated with anthracnose pathogen, with a view to identifying the most effective extract that can be used in the control of the

**Corresponding author: Email: ubiology.gu@gmail.com;*

disease. Seeds of cowpea (Ife Brown) disinfected and inoculated with spores of *Colletotrichum lindemuthiamum* were used for the study. The field trial was a three factor experiment, (leaf extracts, seed inoculation and seed disinfection) designed in randomized complete block design and replicated three times. Cowpea was spaced at 0.5 m x 0.75 m and sown in plots measuring 3 m x 3 m. Data were collected fortnightly for yield traits performance such as grain yield (kg/ha), number of pods per plant, number of seeds per pod, pod length (cm), 100-seed weight (g) and percent yield losses. Data generated from the study were subjected to statistical analysis using enhanced Genstat Software for Analysis of Variance (ANOVA) and the Fisher's LSD mean separation. Results of data analysis showed that significant (P<0.05) differences existed among the leave extracts used as treatments. Results on disease development showed that disease incidences of anthracnose recorded for cowpea were 71.03% and 18.06% for control and *C. odorata* treated plots respectively. The disease severity indices of 7.17 and 0.59 were recorded for the control and *A. occidentale* extract sprayed plots, respectively. Results on yield traits performance showed significant (P<0.05) differences in the effect of the different plant extracts evaluated. Highest grain yield of 68.18 kg/plot was recorded on *Anacardium occidentale* extract treated plots. Number of seeds per pod was 11.17 for *Chromoleana odorata* with higher yield loss of 66.99% in the control plot. Plots sprayed with benomyl yield loss were 22.39%, while plots sprayed with *A. occidentale* yield loss was 31.83%. Plots sprayed with leaf extracts of *C. odorata* recorded yield losses of 42.81%. Percent yield of cowpea was 43.24% in plots sprayed with leaf extracts of *G. arborea*. The order of efficacy of leave extracts was Benomyl > *Anacardium occidentale* > *Chromoleana odorata* > *Gmelina arborea* > Control. Hence, it is recommended that farmers should use leaf extracts of *Anacardium occidentale* to reduce yield losses from anthracnose infested cowpea and for high yield performance and control of cowpea Anthracnose disease in South Eastern Nigeria.

Keywords: Fungitoxic screening; anthracnose disease; yield performance of cowpea.

1. INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp), one of the most important food legumes in the tropics, belongs to the family Fabaceae of the order fabales [1]. The cultivated cowpeas are grouped under the subspecies *unguiculata* comprising the *unguiculata biflora, textilis* and *sesquipedalis*.

Cowpea is probably a native of Central Africa where almost all the wild forms are found [2]. Though cowpea is thought to have originated from Africa, its cultivation spans across Asia, Europe, and America where the prevailing agroclimatic conditions have best adapted the crop to the regions [3].

Cowpea is an important crop especially to the low-income earners in developing countries. The crop is very unique in that it produces food, cash and fodder. Cowpea has a high potential to increase farmer's and trader's income. Cowpea grains are rich in protein (23-25%) and carbohydrates (50-67%) [4]. The use of cowpea varies and includes its uses as food for humans and feed for livestock. It also serves as a valuable crop that plays an important role in the restoration of soil fertility. Virtually, all the above ground parts are eaten and are highly nutritious [5]. The young leaves and immature pods are used as vegetables, while the grains constitute a major source of various main dishes and snacks. The crop is also valued as a source of essential amino acids, especially lysine that complements high methionine acid and cystine contents of cereals [6] and this improves the nutritional quality of tropical diets which has been predominantly starchy cereals, roots and tubers.

The production of cowpea has not been satisfactory due to some constraints. It has been estimated that about 3.3 million tonnes of cowpea dry grains were produced worldwide in 2000. Nigeria accounted for about 2.1 million tonnes of this, making her the world largest producer of cowpea, followed by Niger Republic which produced 0.65 million tones and Mali with 0.11 million tonnes [7]. Total land area of 9.5 million hectares was estimated to be under cultivation of cowpea in West Africa [8]. World average yield was estimated at 337kg/ha; average yield in Nigeria was 417kg/ha [9].

The production of this valuable crop is threatened and characterized by low yields due to abiotic and biotic constraints, such as unfavourable environmental conditions, insect pests, and other disease causing agents all of which have tremendously reduced the full realization of the productive potentials of the crop in this agroecology [10]. Although, it is generally recognized that cowpea is predominantly a crop of the drier regions, research studies [11] have shown that there are some high productive potentials for cowpea in the humid wet regions when adequate agronomic and cultural practices needed for optimal cowpea production are maintained.

The problem of pests and diseases is the most challenging in agriculture [12]. Various approaches and practices have been adopted, either singly or in combination, in an attempt to effectively tackle these problems [13]. Such practices include; the use of synthetic and non-synthetic pesticides [14], biological control agents [15] and the use of resistant varieties [16]. Although these methods have been successful in different places and systems in controlling pests and diseases of crop plants, and consequently reducing crop yield losses, there are still some attendant problems associated with their use such as seasonal weather and climatic changes, high cost of control, resistance and resurgence of pests and above all, adverse environmental impact associated with the use of synthetic pesticides, which include; birth defect, mutation and cancer, adverse effect on non-target organisms, pollution of underground water and its long residual effect [17].

Cowpea Anthracnose disease caused by *Colletotrichum lindemuthianum* is one of the most important cowpea diseases worldwide. The pathogen infects leaves, stems, pods and caused up to 90-100% yield losses in susceptible cultivars [18,19]. As a seed borne fungal pathogen of cowpea, [20] had advocated the use of seed treatment with chemicals and foliar sprays with synthetic chemicals as remedial measures.

However, with an array of problems associated with the use of synthetic chemicals, it therefore, becomes necessary for an intensive search for alternative control methods. This has led to the evaluation of fungicidal property of many plants products for disease control.

Previous studies have revealed the existence of a vast array of higher plants with fungitoxic potentials [19-22]. Evaluation of antifungal activities of extracts of plant origin *in vitro* has been demonstrated [18]. *Colletotrichum lindemuthianum* has been reported to be tolerant to a wide range of synthetic chemicals used [23].

Development of pesticides of plant origin such as from *Gmelina arborea*, (*Chromoleana odorata*) and (*Anacardium occidentale*) will be cheap and readily available to resource poor farmers who are the major producers of the cowpea consumed in Nigeria (Olayide et al. 1980). There is need to evaluate the fungitoxic potentials of some plant extracts against *C. lindemuthianium.*

The present study was therefore design to evaluate the potentials and efficacy of some local plant extracts to control the pathogen causing Anthracnose disease and improve yield performance of cowpea (*Vigna unguiculata*) in this agro-ecological zone of Nigeria.

2. MATERIALS AND METHODS

2.1 Source of Plant Material

The plant materials used for the study were sourced locally within Michael Okpara University of Agriculture, Umudike. They included the leaves of the following:

i. *Gmelina arborea* (Gmelina)
ii. *Chromoleana odorata* (Siam weed)
iii. *Anacardium occidentale* (cashew)

The leaves of the plants were stripped from their respective trees/shrubs. These were washed thoroughly with running water; air dried for 48 hours, and ground separately on a laboratory mortar and pestle into fine particles.

2.2 Source of Inoculum

The inoculum was a conidial suspension of the fungus *Colletotrichum spp*, isolated from already infested cowpea plants grown in the research farm of Michael Okpara University of Agriculture, Umudike.

2.3 Source of Seeds

Seeds of the cowpea cultivar (Ife brown) were used for the study. These were obtained from the International Institute for Tropical Agriculture [24].

2.4 Preparation of Culture Medium

The culture medium used for the research study was Potato Dextrose Agar (PDA). The medium consisted of broth of boiled Irish potato (200 g), dextrose sugar (20 g) and 20 g of Agar (Technical). Two hundred and fifty mililitres (250 ml) of the antibiotics (Chloramphenicol 125 ml and Gentamycin 125 ml) were added in equal volume to the culture medium to forestall bacterial growth and posible contamination. The content were placed in one litre flask and made up to 1 litre mark by the addition of distilled water. This was autoclaved at 112℃ at a pressure of 760 mmHg for 15 minutes. The molten medium was poured into an Erlenmeyer flask.

2.5 Isolation of Pathogen

Freshly prepared Potato Dextrose Agar (PDA) was allowed to cool to 40℃ before being dispensed into sterilized Petri dishes under aseptic conditions at 20 ml per dish and then allowed to solidify.

The inoculated plates were incubated at room temperature (27±2℃) and observations for the growing fungus were made daily. Isolates from the fungal culture were sub-cultured to obtain a pure culture.

2.6 Pathogenicity Test

Pathogenicity test was carried out using the isolated fungi in accordance with Koch's postulate as described by Awurum [25]. Uninfected cowpea plants growing in the control plots (28-days old) were used for the pathogenicity test. Pure cultures of the isolated fungal species (*Colletotrichum spp.*) were grown on PDA medium amended with streptomycin (PDAs) in Petri dishes for 7 days to sproulate. Plates were kept at room temperature (25℃±2℃) in an incubation chamber at an alternating light and darkness cycles of approximately 12 hours each. Sterile distilled water (20 ml) was poured out on the solid surface of the culture medium in each plate and the surface robbed with the edge of a sterilized glass slide. The suspension was filtered through a 2-layer cheese cloth for proper filtration. A haemocytometer was used for the spore count. The conidial suspension of about $1x10^6$ml conidia/ml of distilled water (haemocytometer reading) was sprayed on the uninfected cowpea seedlings. The potted seedlings were covered with wet polyethylene bag to create humid environment for the fungal pathogen. Cowpea on the control plots were sprayed with distilled water.

2.7 Identification of Pathogen

The establishment of the pathogen according to Koch's postulates confirms that *Colletotrichum spp.* was the pathogen responsible for anthracnose disease of cowpea used for the research study. Identification was achieved by comparing the organism observed under compound microscope with already characterized pathogen by [26].

2.8 Preparation of Plant Extracts

To prepare 30% concentration of each plant extract 30 g of each of the ground dried leaves of *Gmelina arborea*, *Chromoleana odorota*, and *Anacardium occidentale* were soaked separately overnight in 100 ml of cool water in 250 ml conical flasks [27].

The suspension was hand-shaken and filtered into a clean beaker using four folds of cheese cloth. In order to check bacterial contamination, 125 mg/l of streptopenicillin (a mixture of streptomycin (62.5mg) and penicillin (62.5 mg)) was added to each of the plant extracts [28].

2.9 Preparation of Inoculum and Seed Inoculation

Conidial suspensions were prepared by flooding the surface of 8-day old culture plates with sterile distilled water, scrapping the surface with a bent glass rod and filtering the suspension through four fold layers of cheese cloth. The concentration of the pathogen was adjusted to 10^5 spores per ml to conform with the haemocytometer standard as described by [29].

Seeds used for the study were inoculated by thoroughly soaking them in conidial suspension of the pathogen for 30 minutes with inoculum containing 10^5 spore/ml (50 seeds/50 ml) [30].

2.10 Seed Disinfection

Seeds were disinfected before inoculation by dipping the seeds in a solution containing 1.25 litres of household bleach and 3.75 litres of sterile distilled water (5 litres of solution) for 2 minutes. Five (5) litres of the solution was used per 500 g of seeds.

2.11 Study Location

Field studies were conducted during the early cropping seasons of 2013 and 2014 at the research farm of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. Umudike is located in the humid tropical rainforest belt of Nigeria (Lat 5°29N and Long. 7°33E) with mean annual rainfall of 2177 mm per annum and a mean monthly temperature range of 25-29℃. The soil type is predominantly loamy sand tropical ultisol [30].

2.12 Design of Experiment and Layout

The layout was five (plant extracts) by two (inoculated and uninoculated) by two (disinfected and undisinfected) i.e 5 x 2 x 2 factorial in a randomized complete block design (RCBD) replicated three times. The trials were conducted during the early cropping seasons of 2013 and 2014. Factor one hads five (5) levels which comprised of leaf extracts of *Gmelina arborea, Chromoleana odorata and Anarcardium occidentale*, a 0.8 kg ai /ha of benomyl fungicide and the control (sprayed with distilled water).

Factor two had two levels comprising of inoculated and uninoculated seeds while factor three had two levels as disinfected and undisinfected seeds. A total of twenty (20) treatment combinations were replicated thrice to give a total of sixty (60) experimental units each measuring 3 m x 3 m as plot size, distance between plots was 0.5m apart and a total land area of 1025 m^2 with dimensions of 41 m by 25 m. Plant spacing used for the study was 0.5 m x 0.75 m making a total plant population of 48 plants per plot and 26,667 plants/ha.

Agronomic practices and cultural practices which guarantee good cowpea growth and yield were observed and weeding was done twice at the first and second months after planting.

2.13 Treatment Application

Spray applications of extracts and benomyl were carried out at weekly intervals at the onset of cowpea flowering phase when the plant were a month old. The application was done at sunset (evening period) to reduce the rate of solar decomposition of the volatile active ingredients of the plant extracts [30].

2.14 Disease Incidence

Disease incidence was recorded by counting the number of infected/diseased plants and expressed as a percentage of total number of cowpeas plant per plot. This was done according to the formula by [31].

Disease incidence (%) =

$$\frac{\text{No. of plants infected}}{\text{Total No. of plant examined}} \times \frac{100}{1}$$

2.15 Disease Severity

Disease severity was assessed by considering different plant parts which included; leaf, peduncle, stem and pod of each plant. The plant parts were visually assessed and rated according to the 1-10 severity scale based on the percentage covered by necrotic lesions on the plant organ as described by [31].

1. No visible anthracnose symptoms
2. Leaf or stems with a few isolated small lesions covering approximately 1% of the total area
3. Larger lesions covering approximately 5% of the leaf and stem area
4. Larger lesions covering approximately 10% of the area
5. Larger lesions covering 25% or more of the leaf and stem area
6. More than 50% of the plant tissues affected.

2.16 Sampling Area

Twenty four (24) plants growing centrally in each plot were visually examined and rated according to the disease severity scale described above. Disease severity of each plot was evaluated according to the procedures of [31].

This was expressed as the sum of the severity scores recorded on plants. This was calculated using the formula described by [31] as follows:

Disease severity =

$$\frac{\text{Sum of individual disease rating}}{\text{Total No. of plant examined}}$$

2.17 Estimating Percent Yield Loss (%)

Yield loss due to Anthracnose disease infestation on cowpea was determined using the formula according to [32].

$$\text{\% yield loss} = \frac{(\text{Yield of sprayed plots} - \text{yield of unsprayed plots})}{\text{Yield of sprayed plot}} \times \frac{100}{1}$$

3. DATA COLLECTION OF YIELD PERFORMANCE OF COWPEA

3.1 Measurement of Yield Components

Pods were harvested at maturity. In each plot, the yields of the two middle rows were bagged separately from the boarder rows. The pod and grain yields, number of seeds per pod as well as the 100-seed weight were determined and obtained by weighing with weighing balance.

3.2 Data Analysis

Data generated from the study were collected and subjected to analysis of variance using the generalized linear programming model procedure in GENSTAT (ICRAF Version). Treatment means were compared at 5% probability level. Mean separation was done using the Fisher's Least Significant Difference (LSD) test option available in the GENSTAT statistical software.

4. RESULTS

4.1 Results of Effect of Plant Extracts on the Incidence of Cowpea Anthracnose Disease in the Field

Results obtained in the field of cowpea Anthracnose as influence by different plants extracts are presented in Fig. 4.1. The results showed that incidence of Anthracnose disease on cowpea increased with increase in time after planting.

At four weeks after planting (4 WAP), the incidence of Anthracnose disease on cowpea cultivated at Umudike showed significant ($P<0.05$) differences among the different treatments. Incidence of cowpea Anthracnose was highest (71.05%) on cowpea plants in the control, while the least (3.63%) incidence of Anthracnose recorded on cowpea grown on plots whose seeds were sprayed with benomyl fungicide at this stage of growth.

The application of leave extracts of *Gmelina arborea* and *Chromoleana odorata* to cowpea plants after four weeks of growth was effective with 38.24% and 18.06% incidence, respectively, on plots treated with the extracts. Results of effect of plant extracts on the incidence of cowpea Anthracnose in the experimental field at the 6^{th}, 8^{th}, 10^{th}, 12^{th} and 14^{th} weeks after planting showed similar trend with the application of leave extracts of *Gmelina arborea*, *Chromoleana odorata, Benlate fungicide* and *Anacardium occidentale*.

Results as presented in Fig. 4.1 showed that the incidence of Anthracnose disease on cowpea was significantly low in the sprayed plots, but high in the control plots, for weeks four (4 WAP – 14 WAP). The order of efficacy of treatments in the reduction of Anthracnose disease of cowpea in the field was in this order: Benlate > *Anacardium occidentale* > *Chromoleana odorata* > *Gmelina arborea*. This trend was observed from the 4^{th} to 14^{th} weeks after planting.

The incidence of Anthracnose disease on cowpea in Umudike was more on plants grown from seeds that were inoculated and undisinfected, than on plants grown from uninoculated and disinfected seeds.

The incidence of Anthracnose disease on cowpea at Umudike was progressive with increasing weeks after planting assuming higher degree at the 14^{th} weeks after planting and least at the onset of field trial.

4.2 Results of Effect of Plant Extracts on the Severity of Cowpea Anthracnose Disease in Umudike

Results on the severity of cowpea Anthracnose as affected by different plant extracts on cowpea plants in the field are presented in Fig. 4.2. The study showed that the severity of Anthracnose disease of cowpea was progressive with increasing weeks after planting.

The results of the severity of Anthracnose on cultivated cowpeas in the field as shown in Fig. 4.2 showed that after 4 weeks of planting, there were significant ($P<0.05$) differences among the various treatments of leaf extracts applied.

The severity of Anthracnose disease was highest on cowpea plants grown in the control plots with severity index as high as 7.17. The least severity index of 0.36 was obtained on cowpea plants in plots sprayed with Benomyl. Anthracnose severity indices of 0.59, 1.23 and 3.14 were obtained from cowpea plants sprayed with extracts of *Anacardium occidentale*, *Chromoleana odorata* and *Gmelina arborea,* respectively after four (4) weeks of planting.

As shown in the Fig. 4.2 results of the effect of the plant extracts on the severity of cowpea Anthracnose disease in the field after 6^{th}, 8^{th}, 10^{th}, 12^{th} and 14^{th} weeks after planting showed similar trend.

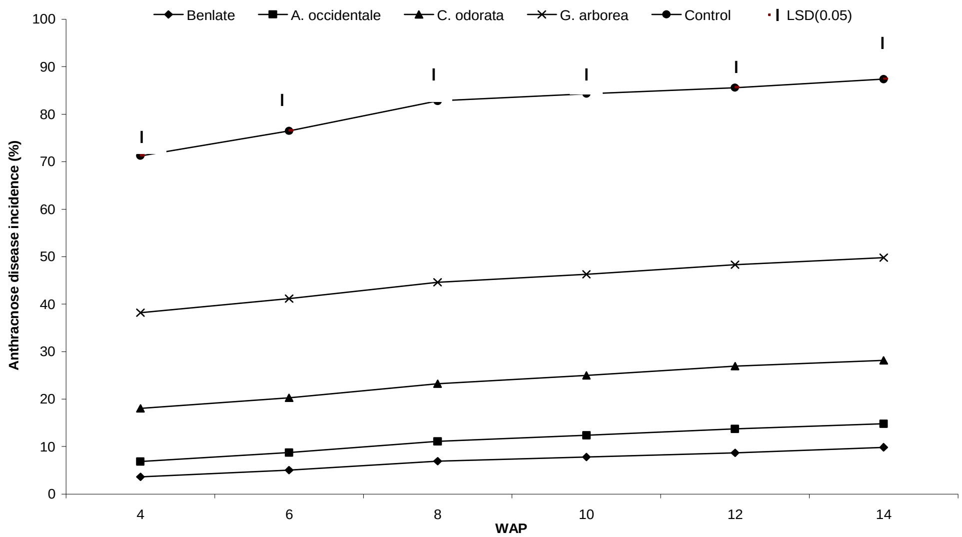

Fig. 4.1. Effect of plant extracts on progress of anthracnose disease incidence on cowpea over 14 weeks period at Umudike

The severity of Anthracnose disease on cowpea was significantly lower on sprayed plots than in the control plots at four (4 WAP) to 14 (WAP). The order of efficacy of leaves extracts in the control of Anthracnose disease severity on cowpea in the field was in this order, Benomyl > *Anacardium occidentale* > *Chromoleana odorata* > *Gmelina arborea*.

The results further showed that the severity of Anthracnose disease on cowpea in the experimental field was more severe with 8.11 severity in plants grown from inoculated and undisinfected seeds in the control plot but comparatively low in plants grown from uninoculated and disinfected seeds.

4.3 Results of Effect of the Different Plant Extracts on the Yield Performance of Cowpeas Infected with Anthracnose Disease

Tables 1 to 2 show results of effect of plant extracts on yield and yield components of cowpea plants cultivated on the field. As presented in Table 1, significant (P<0.05) differences were detected in the grain yield (kg/ha) of cowpea plants among the treatments. As shown, grain yield was least, averaging only 33 kg/plot in the control plots where leave extracts were not applied. Higher grain yield of 82.62 kg/plot was obtained from cowpea plants sprayed with Benomyl. Plots sprayed with leaf extracts of *Gmelina arborea* produced grain yield of 56.76 kg/plot of cowpea per plot and *Chromoleana odorata* extracts produced 57.19 kg/plot of cowpea. Plots sprayed with *A. Occidentale* yielded cowpea grains measuring 68.18 kg/plot.

As shown in Table 1, results of grain yield in kg/ha obtained in 2013 trials were higher than grain yield obtained in the 2014 trials. The results further showed that grain yield (kg/plot) obtained from inoculated seeds were lower than grain yield obtained from uninoculated seeds. Furthermore, the results showed that grain yield (kg/plot) of cowpea obtained from disinfected seeds was higher than grain yield (kg/plot) obtained from undisinfected seeds.

The order of effectiveness or efficacy of the leave extract on the grain yield (kg/plot) of cowpea was in the order of Benomyl > *Anacardium occidentale* > *Chromoleana odorata* > *G. arborea* > Control.

Results of the effect of leaf extracts of *Anacardium occidentale*, *Gmelina arborea, Chromoleana odorata* and Benomyl on pod length (cm) of cowpea in 2013 and 2014 are presented in Table 2.

As shown in the Table, there were no significant (P>0.05) differences in pod length of cowpea treated with various extracts. Cowpea plants treated with Benomyl had longer pods measuring up to 17.23 cm, while plants not treated with any of the extracts and grown in the control plots produced the shortest pods measuring only 6.40 cm. Plants in plots sprayed with *Anacardium occidentale* produced pod with length measuring 14.54 cm while cowpea plants with applications of leaf extract of *Gmelina arborea* and *Chromoleana odorata* yielded pods measuring 12.33 cm and 14.30 cm in length, respectively.

The order of efficacy of the extracts on pod length yield is Benomyl > *Anacardium occidentale* > *Chromoleana odorata* > *Gmelina arborea* > Control. It was also observed from the study that pods were longer in cowpea plants grown from uninoculated and disinfected seeds than in those grown from inoculated and undisinfected seeds.

Results of the effect of leaf extracts on the number of seeds per pod of cowpea in 2013 and 2014 were presented in Table 3. The results showed that the number of seeds per pod of cowpea differed among the various extracts used as treatments. The results also showed that the number of seeds per pod were significantly ($P<0.05$) different among the disinfected and inoculated seeds as well as for the undisinfected and uninoculated seeds used for the trials in the two cropping seasons.

Maximum number of 15.67 seeds was obtained per pod from cowpeas sprayed with Benomyl. Minimum number of 6 seeds per pod was obtained from cowpea grown in the control plots.

Table 4 shows the result of the number of pods per plant of cowpea treated with leave extracts of *Gmelina arborea*, *Anacardium occidentale*, *Chromoleana odorata* and Benomyl in 2013 and 2014. The results shows that the number of pods per plant differed significantly ($P<0.05$) among the different treatments applied.

Cowpea pods harvested from plots sprayed with Benomyl produced the highest number of pods per plant of about 20.25 pods/plants while 9.94 pods were harvested per plant from the control plots. Plots sprayed with leaf extracts of *Chromoleana odorata* produced 14.98 pod/plant while pods sprayed with *Anacardium occidentale* leaf extract produced 14.81 pods/plant. Plots sprayed with leaf extract of *G. arborea* produced 14.04 pods per cowpea plant.

Results of the number of pods per plant obtained from the field trials also showed that number of pods per plant differed significantly ($P<0.05$) among the disinfected/undisinfected and inoculated/ uninoculated seeds used for the trials. Benomyl > C.odorata > A. occidentale > G.arborea > control.

Table 5 shows the result of a 100-seed weight (g) of cowpea seeds obtained from the various treatments of leaf extracts on cowpea in 2013 and 2014.

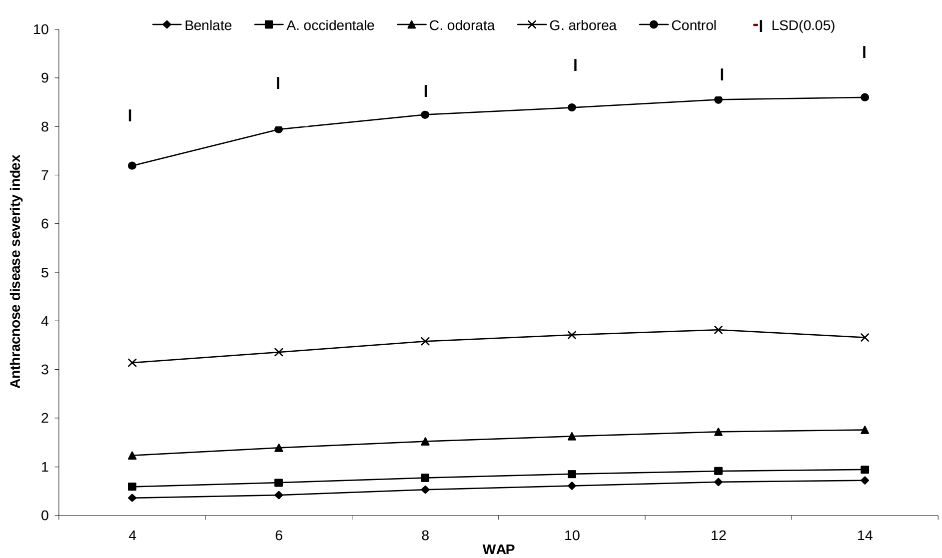

Fig. 4.2. Effect of plant extracts on progress of anthracnose disease severity on cowpea over 14 weeks period at Umudike

Table 1. Effect of plant extract on grain yield (kg/ha) of cowpea infected with Anthracnose disease under field conditions

Year	*Gmelina arborea*				*Chromolaena odorata*				*Anacardium occidentale*				*Benlate fungicide*				*Control*			
	Inoc.		Uninoc.		Inoc.		Uninoc.		Inoc.		Uninoc.		Inoc.		Uninoc.		Inoc.		Uninoc.	
	Dis.	Undis.	Dis.	Undis.	Dis.	Undis.	Dis.	Undis	Dis.	Undis.	Dis.	Undis	Dis.	Undis.	Dis.	Undis	Dis.	Undis.	Dis.	Undis.
2013	68.35	72.74	73.24	64.17	73.80	70.22	71.80	69.66	87.97	82.31	75.55	72.55	92.04	87.13	96.60	95.30	54.57	54.86	42.59	46.42
2014	37.59	40.01	40.28	35.29	40.59	38.62	39.49	38.31	48.38	45.27	41.61	39.90	50.62	47.92	58.63	52.42	30.01	30.17	23.42	22.53
Mean	52.97	56.38	56.76	49.73	57.19	54.42	55.65	53.99	68.18	63.77	58.58	56.23	71.33	67.53	82.62	73.86	42.29	42.52	33.00	34.48

$LSD_{(0.05)}$ Interaction (2013) = 0.33, $LSD_{(0.05)}$ Plant extracts = 0.621, $LSD_{(0.05)}$ Interaction (2014) = 0.42, $LSD_{(0.05)}$ PE X Inoc/Uninoc = 0.042, $LSD_{(0.05)}$ Interaction (Mean) = 0.0965, $LSD_{(0.05)}$ Dis/Undis = 0.121, $LSD_{(0.05)}$ Dis/Undis X Inoc/Uninoc = 0.10, $LSD_{(0.05)}$ PE X Dis/Undis X Inoc/Uninoc = 0.0965

Table 2. Effect of plant extracts on the length of pod (cm) of cowpea infected with Anthracnose disease under field conditions

Year	*Gmelina arborea*				*Chromolaena odorata*				*Anacardium occidentale*				*Benlate fungicide*				*Control*			
	Inoc.		Uninoc.		Inoc.		Uninoc.		Inoc.		Uninoc.		Inoc.		Uninoc.		Inoc.		Uninoc.	
	Dis.	Undis.	Dis.	Undis.	Dis.	Undis.	Dis.	Undis	Dis.	Undis.	Dis.	Undis	Dis.	Undis.	Dis.	Undis	Dis.	Undis.	Dis.	Undis.
2013	11.37	12.02	11.91	11.27	12.43	12.89	14.47	13.60	13.38	14.26	14.84	14.66	17.08	16.82	17.28	16.92	6.95	5.97	6.35	6.11
2014	11.52	12.64	12.49	12.33	14.02	13.27	14.13	14.80	13.69	14.14	14.24	14.24	14.14	16.44	17.22	17.23	7.54	7.47	6.51	6.69
Mean	11.45	12.33	12.20	11.80	13.23	13.08	14.30	14.20	13.54	14.20	14.54	14.40	16.76	17.02	17.23	17.08	7.25	6.72	6.43	6.40

Table 3. Effect of plant extract on the number of seeds per pod of cowpea infected with Anthracnose disease under field conditions

Year	*Gmelina arborea*				*Chromolaena odorata*				*Anacardium occidentale*				*Benlate fungicide*				*Control*			
	Inoc.		Uninoc.		Inoc.		Uninoc.		Inoc.		Uninoc.		Inoc.		Uninoc.		Inoc.		Uninoc.	
	Dis.	Undis.	Dis.	Undis.	Dis.	Undis.	Dis.	Undis	Dis.	Undis.	Dis.	Undis	Dis.	Undis.	Dis.	Undis	Dis.	Undis.	Dis.	Undis.
2013	8.33	8.00	8.00	8.33	9.00	8.33	8.00	8.00	6.33	11.00	9.67	10.00	14.67	15.67	16.00	14.33	7.00	6.33	7.67	7.00
2014	10.00	8.67	11.33	12.33	13.33	9.67	8.67	7.67	9.00	10.00	10.33	10.33	16.00	15.00	15.33	15.67	6.67	5.67	7.00	7.33
Mean	9.17	8.34	9.67	10.33	11.17	9.00	8.34	7.84	7.67	10.50	10.00	10.17	15.34	15.34	15.67	15.00	6.84	6.00	7.34	7.17

$LSD_{(0.05)}$ Interaction (2013) = 0.321, $LSD_{(0.05)}$ Plant extracts = 0.62*, $LSD_{(0.05)}$ Interaction (2014) = 0.531* $LSD_{(0.05)}$ Dis/Undis = 0.31*, $LSD_{(0.05)}$ Interaction (Mean) = 2.97, $LSD_{(0.05)}$ PE X Dis/Undis = 0.44*, $LSD_{(0.05)}$ PE X Inoc/Uninoc = 5.21, $LSD_{(0.05)}$ PE X Dis/Undis X Inoc/Uninoc = 2.97*

**Significant @ 0.05%*

The results show that 100-seed weight of cowpea harvested from the various plots differ significantly (p<0.05) among the various treatments. Plots sprayed with Benomyl produced seeds with highest weight of 23.96 g per 100-seed. The least weight of 8.45 g of 100-seeds was obtained from plants in the control plots. Plots sprayed with leaf extracts of *Anacardium occidentale* produced 100-seeds weighing 19.40 g while plots treated with leaf extracts of *Chromoleana odorata* produced 100-seeds that weighed 18.24 g. Leaf extracts of *Gmelina arborea* sprayed on cowpea produced 100-seeds that weighed 16.11 g.

The results showed that for a 100-seed weight of cowpea, the order of efficacy of the extracts was in the order of Benomyl > *Anacardium occidentale* > *Chromoleana odorata* > *Gmelina arborea* > Control.

The results further showed that the weight of a 100-seed was higher in plots cultivated with disinfected/uninoculated seeds and less in plots sown with undisinfected/uninoculated cowpea seeds.

Table 6 shows the results of percentage yield loss (%) of cowpea treated with leaf extracts of *Chromoleana odorata*, *Anacardium occidentale*, *Gmelina arborea* and Benomyl cultivated in 2013 and 2014.

The results showed that percentage yield loss of cowpea varied significantly (P<0.05) among the various treatments. Plots sprayed with Benomyl recorded the least yield loss of 22.39 percent. Cowpeas in the control plots recorded the highest yield loss of 66.99 percent. Plots sprayed with leaf extracts of *Chromoleana odorata*, *Anacardium occidentale* and *Gmelina arborea* recorded percentage yield losses of 42.81, 31.83 and 43.24 respectively.

In order of efficacy, leaf extracts of Benomyl > *Anacardium occidentale* > *Chromoleana odorata* > *Gmelina arborea* > Control. The efficacy of the treatments was also significantly (P<0.05) different in the yield losses recorded from disinfected/uninoculated seeds and undisinfected/uninoculated seeds, with higher yield losses recorded from plots sown with undisinfected/inoculated seeds compared to lower yield losses recorded from plots seeded with disinfected/uninoculated seeds as shown in Table 6.

5. DISCUSSION OF RESULTS

The major limiting factor for cowpea (*Vigna unquiculata*) production in Nigeria and beyond is its susceptibility to disease pathogens and pests [33]. According to [34] the anthracnose disease of cowpea is a major disease of the crop causing considerable yield loss in many parts of the world.

[34,35] had observed in their studies that the fungitoxic activity of different plant extracts vary, likely due to their different fungitoxic properties. These reports on the variances in the efficacy of plant extracts against fungal pathogens of cowpea agree with the findings of this study.

The present findings showed that the effects of the different plant extracts on the development of anthracnose disease of cowpea varied significantly amongst the various extracts, inoculated/uninoculated and disinfected and undisinfected seeds. Anthracnose development on cowpea was greatly suppressed and reduced by the extracts. *Anacardium occidentale* extract was the most effective in reducing the incidence of cowpea anthracnose compared to *Chromoleana odorata* and *Gmelina arborea*. This finding collaborate with the reports of [36] that the extracts of *Gmelina arborea* possessed good fungicidal properties. Furthermore, the findings is in agreement with the reports of [36,25], who reported that leaf extracts of cashew (*Anacardium occidentale*), possessed high fungitoxic and insecticidal properties against post-flowering insect pests of cowpea and some disease-causing pathogens of the legume.

The *in vitro* investigations of [25,37] indicated the fungicidal potential of leaf extracts of some plant species they worked with.

The early reduction of anthracnose incidence and severity in cowpea crops grown from uninoculated and disinfected seeds suggest the seed borne nature of the pathogen and a further reflection of the potency of the leaf extracts in suppressing the development of anthracnose of cowpea.

The synthetic fungicide, Benomyl showed high degree of efficacy in the suppression of anthracnose disease. However, the persistence of this chemical pesticide on the environment, their effect on humans and their actions on non-target organisms makes its usage less popular.

Table 4. Effect of plant extracts on the number of pods per cowpea plant infected with Anthracnose disease under field conditions

Year	*Gmelina arborea*				*Chromolaena odorata*				*Anacardium occidentale*				*Benlate fungicide*				*Control*			
	Inoc.		Uninoc.		Inoc.		Uninoc.		Inoc.		Uninoc.		Inoc.		Uninoc.		Inoc.		Uninoc.	
	Dis.	Undis.	Dis.	Undis.	Dis.	Undis.	Dis.	Undis	Dis.	Undis.	Dis.	Undis	Dis.	Undis.	Dis.	Undis	Dis.	Undis.	Dis.	Undis.
2013	15.33	15.67	15.67	17	18	18	18.67	18.00	17.67	18.00	18.00	19.33	19.67	21.67	15.67	18.69	12.00	11.67	11.67	10.6 7
2014	12.12	11.10	12.40	9.63	10.11	11.30	11.29	11.61	10.81	11.62	10.66	10.87	12.86	15.11	14.82	13.61	8.10	9.38	8.44	9.21
Mean	13.73	13.39	14.04	13.32	14.06	14.65	14.98	14.81	14.24	14.81	14.33	15.10	16.27	18.39	20.25	16.15	10.05	10.53	10.06	9.94

$LSD_{(0.05)}$ Interaction (2013) = 2.61, $LSD_{(0.05)}$ Plant extracts = 5.31*, $LSD_{(0.05)}$ Interaction (2014) = 3.71*, $LSD_{(0.05)}$ Dis/Undis = 0.72*, $LSD_{(0.05)}$ Interaction (Mean) = 0.92*,$LSD_{(0.05)}$ PE X Inoc/Uninoc = 0.662*, $LSD_{(0.05)}$ PE X Dis/Undis X Inoc/Uninoc = 0.92*

**Significant @ 0.05%*

Table 5. Effect of plant extracts on 100-seeds weight of cowpea plant infected with Anthracnose disease under field conditions

Year	*Gmelina arborea*				*Chromolaena odorata*				*Anacardium occidentale*				*Benlate fungicide*				*Control*			
	Inoc.		Uninoc.		Inoc.		Uninoc.		Inoc.		Uninoc.		Inoc.		Uninoc.		Inoc.		Uninoc.	
	Dis.	Undis.	Dis.	Undis.	Dis.	Undis.	Dis.	Undis	Dis.	Undis.	Dis.	Undis	Dis.	Undis.	Dis.	Undis	Dis.	Undis.	Dis.	Undis.
2006	15.56	15.97	16.27	16.29	17.69	18.32	17.65	17.41	18.52	18.05	19.88	18.27	25.41	25.27	26.77	27.11	10.68	11.12	12.90	9.77
2007	14.86	13.22	14.41	15.93	16.81	14.86	17.55	17.40	17.96	18.22	18.91	17.21	19.20	20.41	21.11	20.81	9.31	8.44	9.44	9.56
Mean	15.21	14.60	15.34	16.11	17.25	16.59	17.60	17.41	18.24	18.14	19.40	17.74	22.31	22.84	23.94	23.96	9.99	9.78	10.88	8.45

Table 6. Effect of plant extracts on percent yield loss (%) of cowpea infested with Anthracnose disease under field conditions

Year	*Gmelina arborea*				*Chromolaena odorata*				*Anacardium occidentale*				*Benlate fungicide*				*Control*			
	Inoc.		Uninoc.		Inoc.		Uninoc.		Inoc.		Uninoc.		Inoc.		Uninoc.		Inoc.		Uninoc.	
	Dis.	Undis.	Dis.	Undis.	Dis.	Undis.	Dis.	Undis	Dis.	Undis.	Dis.	Undis	Dis.	Undis.	Dis.	Undis	Dis.	Undis.	Dis.	Undis.
2013	31.65	27.26	26.76	35.83	26.20	29.78	28.20	30.34	12.03	17.69	24.45	27.45	7.96	12.87	3.40	4.70	45.43	45.14	57.41	53.58
2014	62.41	59.99	59.72	64.71	59.41	61.38	60.51	61.69	51.62	54.73	88.39	60.10	49.88	52.08	41.37	47.58	69.83	76.58	77.47	77.48
Mean	47.03	43.63	43.24	50.77	42.81	45.58	44.36	46.02	31.83	36.21	41.42	43.78	28.67	32.48	22.39	26.14	57.71	57.49	66.99	65.53

$LSD_{(0.05)}$ Plant extracts = 0.052, $LSD_{(0.05)}$ Dis/Undis = 0.91*,$LSD_{(0.05)}$ PE X Dis/Undis = 0.492, $LSD_{(0.05)}$ PE X Inoc/Uninoc = 3.11* $LSD_{(0.05)}$ Dis/Undis X Inoc/Uninoc = 1.40*, $LSD_{(0.05)}$ PE X Dis/Undis X Inoc/Uninoc = 0.63*

**Significant @ 0.05%*

Disinfection of cowpea seeds before planting and foliar spray of extracts of *Anacardium occidentale*, *Gmelina arborea* and *Chromoleana odorata* reduced the incidence and severity of anthracnose of cowpea in the field. These findings confirm the assertions of [8], that timely seed treatment and foliar application of infected cowpea with protectant fungicides reduced disease incidence and severity in the field, but concluded that the effectiveness of foliar fungicides varies with cropping season.

The study revealed that incidence and severity of anthracnose disease of cowpea were very high in the control plots where no extract was applied. Anthracnose disease development was very rapid resulting to percentage incidence and severity being high. This result agrees with earlier reports of [37] who stated that if anthracnose disease of cowpea is not checked, can cause significant cowpea crop losses with severely infected plants showing typical symptoms and eventual death. This assertion was supported by [38].

The effect of the extract on the growth of cowpea showed marked significant differences among various treatment combinations used. The findings showed that the efficacy of extract was in the order of *Anacardium occidentale* > *Chromoleana odorata* > *Gmelina arborea* > Control respectively with *Anacardium occidentale and* Benomyl treated plots having tallest plants and control plots having shortest plants. Similarly, number of leaves per plant was more in *Anacardium occidentale* treated plots and least in control plots. These findings are in consonance with the report of [38], who stated that extracts of cashew (*Anacardium occidentale*) were effective against some pre and post flowering pathogen and pests of cowpea. Similar reports were also given by [38,39].

Effects of plant extracts on yield performance of cowpea infected with anthracnose pathogen was also evaluated and the results revealed that yield components such as pod length and number of pods per plant did not vary significantly among the different treatment combinations, but showed significant differences in grain yield (kg / plot), number of seeds per pod and 100-seed weight. This result is in conformity with the reports of [39] who stated that yield of cowpeas are greatly affected at the later stage of cowpea growth if no remedial measures are adopted. They further stated that infected cotyledon senesce prematurely, while pods develop poorly, with reddish brown to black blemishes.

Reports of [39] also showed that yield loss was very significant and high in undisinfected and inoculated anthracnose affected cowpeas and thus advocated the need for urgent and prompt treatment and curative measures in anthracnose affected cowpeas such as seed treatment and use of botanicals with high fungicidal properties to avert high and significant crop and yield losses.

The call for the use of plant extracts or botanicals with high pesticidal properties in the control of plant diseases was borne out of the environmental hazards and wide range attendant consequences associated with the use of synthetic chemical pesticides in the control of plant diseases such as cowpea Anthracnose disease caused by the pathogen *Collectolrichum lindemuthianum.*

6. CONCLUSION

The present study showed that leaf extracts of cashew (*Anacardium occidentale*), Siam weed (*Chromoleana odorata*) and Gmelina (*Gmelina arborea*) showed great potentials of fungicidal properties and abilities to control anthracnose disease of cowpea in the field. Results of our study further indicated that control of anthracnose disease of cowpea using plant extracts was better in cowpea grown from disinfected seeds and seeds not inoculated with the anthracnose pathogen. Anthracnose incidence and severity on cowpea were greatly reduced by the use of the botanicals on the crops. The action of the botanicals on yield of cowpea was also evident on the yield response of the test crops in terms of days to 50% flowering, number of pods per plant, grain yield, number of seeds per pod, length of pod, 100-seed weight and yield loss. The plants extracts can thus be used for the improvement of yield and genetic enhancement of the cowpea crop for the benefit of farmers, for food security and sustainability for the country and the world at large.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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