

Original Research Article

Sequence of application benomyl and plant extracts in the control of cowpea anthracnose caused by *Colletotrichum lindemuthianum* sensu lato

Abstract

Field experiment was conducted to compare the efficacy of hot water extracts of *Ricinus communis*, *Jatropha gossypifolia* and *Datura stramonium* at three concentrations (65, 50 and 30%) with benomyl in the control of cowpea anthracnose disease. The experiment was laid in a randomized complete block design. The total area of the farm (225m²) was subdivided into three. The first subplot was sprayed with the different concentrations of the extracts and benomyl followed by inoculation of the conidia of the fungus after 48 hours. The second subplot was sprayed with the spore suspension of the fungus followed by application of the extracts and benomyl after 48 hours. The third subplot was sprayed with the conidia of the fungus followed by immediate application of extracts or benomyl, and the control plot in each case was sprayed with distilled water. Result from the experiment shows that all the extracts at the tested concentration reduced the incidence and severity of the disease. *D. stramonium* at 65% concentration compares favourably with benomyl fungicide in reducing incidence and severity of infection. At 65% concentration of *D. stramonium*, incidence of the disease was 22% on pooled mean basis and this was not significantly different from that of benomyl (21%). The percentage of normal seeds recorded at 65% concentration of the extract was 85% and was not significantly different from benomyl (86%). In terms of disease severity, trace infections were observed at 65% concentration of the extract and benomyl. At lower concentrations of all extracts, significant variations were observed on incidence of disease and percentage of normal seeds when compared with that of benomyl. Application of the extracts and benomyl before inoculation reduced disease incidence compared to post inoculation of the fungus. The study therefore shows that extracts of these indigenous plants can be used as a substitute for the benomyl fungicide and that they are more effective when used as a preventive method in the management of anthracnose disease.

Keywords: Cowpea, Plant extracts, *C. lindemuthianum*, Benomyl, Disease incidence.

1.0 Introduction

Cowpea (*Vigna unguiculata*) production in the world, especially Nigeria is low due to anthracnose disease which forms a major biotic factor influencing yield and productivity (Ogu and Owoeye, 2013). The extent of yield loss is determined by the incidence and severity of infection as well as drought, lack of good seed and attacks by pest. Thus, annual output of 2.1 million metric tonnes (MMT) fell short of domestic demand by 0.52 million metric tonnes in Nigeria. (Abubakar and Olukosi, 2008). Anthracnose affects all the stages of the plant but more often in the reproductive stages. Its symptoms include round brownish or purple specks which become darker and enlarge

into lesions (about 2 cm in diameter). The individual lesions are usually lenticular to circular, tan to brown in coloration and the size and distribution depend on the degree of severity (Sharon and Douglas, 2011). The symptoms are most visible on leaves and ripe fruits but the disease also produces cankers on petioles and stems thereby causing defoliation and rotting of fruits. Infected fruits have water-soaked and sunken circular spots (Davis *et al.*, 1991). Severity of infection is affected by water availability to the plant (Dodd *et al.*, 1991) which makes the management of irrigation regimes alongside the use of botanicals an effective integrated approach for its control.

This disease can also be controlled effectively by the use of resistant varieties where they exist and culturally by removing sources of inoculums. The use of synthetic fungicides like Benomyl (Benzimidazole) and Mancozeb (Dithiocarbamate) had proved very effective in time past (Alves *et al.*, 2015). However, due to the increased awareness of side effects of these synthetic pesticides,(increased cost, toxic residue in soil and environment and development of resistance) much attention is being focused on the alternative methods of pathogen control like the use of plant extracts .The extract obtained from such are cheap and compatible with the farming practices (Lowell, 2004) .Leaf extracts of plants such as purple princess (*Cyathula prostrata*), *Diodia scandens*, black pepper (*Piper nigrum*),clove basil (*Ocimum gratissimum*), lemon (*Citrus limon*), lemon grass (*Cymbopogon citratus*) have been tested against fungal infections in cowpea with varying degrees of success (Gideon and Anita 2013; Amadioha and Obi, 1999; Amadioha, 2003). The potentials of botanicals for disease control have been recognized such that there is the need for research into other botanicals for the control of anthracnose in cowpea. Thus the aim of the study was to compare the efficacy of the three plant extracts with that of benomyl fungicide in the management of anthracnose disease in cowpea.

64 2.0 Materials and Methods

65 2.1 Location of experiments

66 The field experiments were conducted at Ekiti State University Teaching and Research farm, Ado
67 Ekiti. 7.7129° N, 5.2523° E, Nigeria.

68 2.2 Collection and preparation of plant leaves

69 Leaves of the three plants namely; *D. stramonium*, *J. gossypifolia* and *R. communis* were
70 collected at Ekiti State University. (Latitude 7 7212°N and longitude 5.2575°E) in south western
71 Nigeria and air-dried at 28°C for 6-8 weeks until each plant had a constant weight. The dried leaves
72 were milled using a blender (Okapi®, Mixer-Grinder), packaged into sealable nylon and refrigerated
73 at 4°C for about 2 weeks until they were required for bioassay. Extracts were prepared by mixing
74 equivalent grams of prepared plant powder (65, 50 and 30) with 100 ml of distilled water at 70°C in
75 500 ml bottles and kept in hot water bath-shaker for 30 minutes. Thereafter, the liquid extract was
76 separated by vacuum filtration and poured inside standard bottles which were refrigerated at 4°C.
77 These extracts were used as the stock solution from which 65%, 50% and 30% of each extract were
78 prepared.

79 2.3 Isolation and identification of *C. lindemuthianum*

80 Cowpea plants showing distinct symptoms of the pathogen were collected from the cowpea
81 fields at Ekiti State University. The leaves were cut into small pieces of about 1-2 cm² and surface
82 sterilized by immersion in 0.2 % NaOCl for two minutes and followed by two rinses in sterile distilled
83 water in a laminar flow cabinet. Three leaf cuttings per plate were placed on PDA. The plates were
84 sealed with parafilm and incubated at 28°C for 5-6 days. Single spore of developing colonies was
85 isolated and sub-cultured to obtain pure cultures. Samples from single spore cultures were used for
86 identification on Malt Extract Agar (MEA) at x400 magnification of a compound microscope
87 (OLYMPUS Binocular) (Zivkovic *et al*, 2010).

2.4 Comparison of plant extracts with benomyl in the control of cowpea anthracnose

The experiment was carried out in September, 2012 and repeated during the same period in 2013. The size of the plot was 2 m² and separated by boarder row of 1m. The total area of the farm was 225 m² and there were 540 stands of cowpea and the plots were sub-divided into three. Cowpea variety, Ife brown susceptible to anthracnose was planted at three seeds hole⁻¹ and later thinned to two per stand. The experiment was a Randomised Complete Block Design (RCBD). Three leaf extracts: *D. stramonium*, *R. communis* and *J. gossypifolia* at three concentrations (30%, 50% and 65%) were sprayed on two weeks old plants in the first subplot and inoculated with the conidia suspension after 48 hours. Benomyl, a conventional synthetic fungicide, was applied at the rate of 0.1 g liter⁻¹ while the control plot was sprayed with distilled water. Plants in the second sub-plot were inoculated with the spore suspension and later sprayed either with the plant extracts or benomyl after 48 hours. In the third plot, the crop was sprayed with the spore suspension followed by immediate application of benomyl or the extracts. In all the experiments, conidia suspension containing 10⁴ conidia ml⁻¹ was used. Thereafter, three plants were tagged in each plot and assessed for disease incidence and severity. Percentage disease incidence was determined using the formula:

$$\% \text{ disease incidence} = \frac{\text{Number of plants infected} \times 100}{\text{Total number of plants}}$$

2.5 Disease Assessment

Assessment of the incidence of the disease was done by using five randomly tagged plants per plot. The number of diseased leaves was counted and expressed as a percentage relative to the total number of leaves tagged per plant. The assessment commenced at three weeks after planting (WAP) and continued till 8 WAP. Disease severity for cowpea anthracnose was assessed at 8 WAP through the count of lesions number and rating of symptom expressed with the aid of a visual scale (Enikuomihin and Peters, 2002). Five plants per plot were selected from each plant and lesion number was counted. Severity of the

disease was assessed through measurement of the size of five randomly selected lesions per plot. After maturity, seeds were harvested and subjected to seed test.

2.6 Seed Health Assessment

Two hundred seeds harvested from each treatment were batched into four replicate samples of 50 seeds each and examined by visual inspection under a stereomicroscope for normal and abnormal seeds. Abnormal seeds were those with malformed shape, wrinkled seed coat or those bearing fungal propagules and data was analysed using ANOVA and treatment means were separated using Turkey significant honest test. Severity of disease was recorded on a scale of 0 – 6 as shown in Table 1.

Table 1. Disease severity index

Scale	Rating	Symptoms of <i>Anthracnose</i> on cowpea
0	No disease	No trace of infection
1	Hypersensitivity	Hypersensitive spot on lower leaves only
2	Trace Infection	Small lesions on lower and upper leaves and stem
3	Slight Infection	Small lesions on lower and upper leaves and stem
4	Moderate infection	Advanced lesions on upper and lower leaves, with or without new infections on stems and petiole
5	Severe infection	Advanced lesions on upper and lower leaves, flowers, buds, stems and petiole and slight infection of pod.
6	Very severe infection	All features of five above with severe infection of pod

Source: Enikuomehin & Peters (2002)

3.0 Results

Table 2 shows the effect of time lag and sequence of application of plant extracts, benomyl and infective conidia of *C. lindemuthianum* on disease incidence. Results from the study shows that disease incidence at all concentrations were significantly ($P \leq 0.05$) lower where extracts and benomyl were applied two days before inoculation of the fungus compared to post application of the extracts and benomyl or concurrent application of extract and benomyl followed by inoculation. Significant variations were also observed among the various concentrations used in the study.

Table 2: Sequence of application of inoculum, benomyl and plant extracts on disease incidence in cowpea anthracnose disease.

Plant extracts	Concentration	2DBI (%)	2DAI (%)	CAEI (%)
<i>D. stramonium</i>	65	20.5 ^d	22.2 ^d	24.4 ^d
	50	22.7 ^c	25.6 ^c	27.8 ^c
	30	23.7 ^c	27.7 ^c	29.5 ^c
<i>R. communis</i>	65	22.8 ^c	24.3 ^c	26.4 ^c
	50	24.3 ^b	26.7 ^b	27.7 ^b
	30	25.8 ^b	27.1 ^b	29.2 ^b
<i>J. gossypifolia</i>	65	23.3 ^c	25.4 ^c	27.2 ^c
	50	25.1 ^b	28.1 ^b	30.1 ^b
	30	26.0 ^b	27.8 ^b	29.8 ^b
Benomyl	0.1g/l	19.7 ^d	19.5 ^d	19.6 ^d
Control		70.4 ^a	72.2 ^a	73.4 ^a

DBI=Days before inoculation, DAI= Days after inoculation

CAEI=Concurrent application extracts followed by inoculation

Means with the same letter are not significantly different using Tukey's (HSD)

Table 3 shows comparative efficacy of benomyl and leaf extract of *D. Stramonium*, *R. Communis* and *J. gossypifolia* on incidence of anthracnose disease. Incidence of the infection was significantly $p \leq 0.05$ lower where higher concentrations of the extracts were used. At 65 50, and 30% concentration of *R. Communis*, incidence of the disease were 24.5, 30.75, and 41.82% respectively in 2012 season, whereas in 2013 season at same concentrations, values were 26.15, 33.06 and

44.30%. The incidence of the disease at 65% concentration of *D. stramonium* extract in 2012 (20.4) was not significantly different from that of benomyl (19.92), but at lower concentrations of the extract, significant variations were observed. Generally disease incidence in 2013 season were higher than those recorded for 2012.

Table 3: Comparative effect of benomyl fungicide and three plant extracts on incidence of cowpea anthracnose disease

Plant extracts	Conc. (%)	2012	2013	Pooled mean
		Incidence of Disease (%)		
<i>D. stramonium</i>	65	20.40 ^d	22.72 ^d	21.56 ^d
	50	28.60 ^c	30.07 ^c	29.34 ^c
	30	43.62 ^b	44.95 ^b	44.29 ^b
<i>R. communis</i>	65	24.50 ^d	26.15 ^d	25.33 ^d
	50	30.75 ^c	33.06 ^c	31.91 ^c
	30	41.82 ^b	44.30 ^b	43.06 ^b
<i>J. gossypifolia</i>	65	28.20 ^d	29.75 ^d	28.98 ^d
	50	32.50 ^c	34.4b ^c	33.50 ^c
	30	43.15 ^b	46.17 ^b	44.66 ^b
Benomyl		19.92 ^a	21.95 ^a	20.94 ^b
Control		65.4 ^a	66.7 ^a	64.5 ^a

Means with the same letter are not significantly different using Tukey's (HSD)

Table 4 shows comparative efficacy of foliar spray with leaf extracts of *D. Stramonium*, *R. Communis* and *J. gossypifolia* and that of benomyl synthetic fungicide on the severity (symptoms rating) of anthracnose disease in Ife Brown variety, in 2012 and 2013 planting seasons. Severity of anthracnose infection during the two seasons were similar. Where extracts of *D. stramonium* and *R. communis* were applied at 65, 50 and 30%, their effects resulted in trace, slight and moderate infections. However, variations were observed with the use of *J. gossypifolia* extracts, such that at 50 and 30% concentrations trace infections were noticeable. The level of infections in the treatments at all the concentrations of the three extracts were significantly lower than the control

Table 4: Comparative effect of benomyl fungicide and three plant extracts on severity of cowpea anthracnose disease

Plant extracts	Conc. (%)	2012	2013	Pooled Mean	Symptom Rating
Disease severity					
<i>D. stramonium</i>	65	2	2	2	Trace
	50	3	3	3	Slight
	30	4	4	4	Moderate
<i>R. communis</i>	65	2	2	2	Trace
	50	3	3	3	Slight
	30	4	4	4	Moderate
<i>J. gossypifolia</i>	65	2	2	2	Trace
	50	3	3	3	Slight
	30	3	3	3	Slight
Benomyl		2	2	2	Trace
Control		6	6	6	Very Severe

Table 5 shows the effect of foliar spray of leaf extracts *D. Stramonium*, *R. Communis* and *J. gossypifolia* and that of benomyl synthetic fungicide on the percentage of normal and abnormal seeds of Ife Brown in 2012 and 2013 planting seasons. The percentage of normal seeds was significantly higher $P \leq 0.05$ than the abnormal seeds for the two planting seasons. The percentage of anthracnose infection on seeds was relatively lower where higher concentration of the extracts were used. At 65, 50 and 30% concentrations of *J. gossypifolia* leaf extracts, the percentage of normal seeds were 80.5, 78.7 and 75.1% respectively. The percentage of normal seeds harvested from plots sprayed with 65% concentration of *D. Stramonium* (85.3) was not significantly different from the plots sprayed with benomyl (86.7) in 2012 season.

Table 5 Comparative effect of benomyl fungicide and three plant extracts on incidence of normal and abnormal seeds

Leaf extract	Conc.	2012		2013		Pooled mean	
		Normal and Abnormal seeds (%)					
		NS	AB	NS	AB	NS	AB
<i>D. stramonium</i>	65	85.3 ^a	14.7 ^d	83.9 ^a	16.1 ^d	84.6 ^a	15.4 ^d
	50	81.5 ^b	18.5 ^c	79.4 ^b	20.6 ^c	80.0 ^b	20.0 ^c
	30	78.4 ^b	21.6 ^b	76.8 ^b	23.2 ^b	77.6 ^b	22.4 ^b
<i>R. communis</i>	65	82.7 ^b	17.3 ^c	81.2 ^b	18.8 ^c	82.0 ^b	18.0 ^c
	50	80.6 ^b	19.4 ^c	78.0 ^b	22.0 ^c	79.3 ^b	20.7 ^c
	30	76.4 ^c	23.6 ^b	74.2 ^c	25.8 ^b	75.3 ^c	24.7 ^b
<i>J. curcas</i>	65	80.5 ^b	19.5 ^c	78.7 ^b	21.3 ^c	79.6 ^b	20.4 ^c
	50	78.7 ^b	21.3 ^b	77.4 ^b	22.6 ^b	78.1 ^b	21.9 ^b
	30	75.1 ^c	24.9 ^b	75.3 ^c	26.5 ^b	74.3 ^c	25.7 ^b
Control		65.4 ^d	34.6 ^a	63.2 ^d	36.8 ^a	64.3 ^d	35.7 ^a
Benomyl	0.1g/l	86.7 ^a	13.3 ^d	84.5 ^a	15.5 ^d	85.6a	14.4 ^d

NS=Normal seed. AB=Abnormal seed

Means with the same letter in each row are not significantly different (P<0.05) (Tukey's HSD)

Values are averages obtained for 2012 and 2013 planting seasons.

NS= Normal seeds; AS= Abnormal seeds

Table 6 shows the comparative effect of benomyl fungicide with three plant extracts namely, *D. stramonium*, *R. communis* and *J. gossypifolia* on seed weight of cowpea. Result of the study shows that yield of cowpea treated with extracts *D. stramonium* at 65% concentration (488 kg/ha) was not significantly different from the yield of benomyl (505 kg/ha). However, significant variations were observed at other concentrations. At 50% concentration, yield of benomyl treated plots (505 kg/ha) was significantly higher than that of *D. stramonium* (407 kg/ha). The yield of the plots treated with *R. communis* was higher (353 kg/ha) than that of plots treated with *J. gossypifolia* (332 kg/ha). Similar results were obtained at 30% concentration, yield of plots treated with extracts and benomyl was significantly higher than the control (P≤0.05)

Table 6: Comparative effect of benomyl fungicide and three plant extracts on threshed pod weight cowpea

Extract Conc.(%)	Threshed seed weight of cowpea (kg ha ⁻¹)			
	<i>D. stramonium</i>	<i>R. communis</i>	<i>J. gossypifolia</i>	Benomyl
65	488.33 ^a	410.00 ^a	397.00 ^a	504.67 ^a
50	406.67 ^b	353.33 ^b	332.33 ^b	504.67 ^a
30	248.67 ^c	208.33 ^c	193.00 ^c	504.67 ^a
0	99.33 ^d	99.33 ^d	99.33 ^d	99.33 ^b

Means with the same letter in each column are not significantly different (P<0. 05) using Tukeys (HSD)

Table 7 shows comparative effect of benomyl fungicide and three plant extracts on seed germination and fungal infection on cowpea seed. At all the tested concentrations of the plant extracts used, the percentage of seed germination was higher and not significantly different from that of benomyl fungicide. However, values obtained for the control were lower. Fungal infection on all the seeds were low at the tested concentration. Significant differences (P≤ 0.05) were observed with the use of benomyl and *D. stramonium* at 65% concentration compared to other extracts used in the study.

Table 7: Comparative effect of benomyl fungicide and three plant extracts on seed germination and fungal infection on Cowpea seeds for 2012/2013 planting season

Plant extracts	Conc.	Seed germination	Fungal infection
<i>D. stramonium</i>	65	97.96 ^a	3.44 ^c
	50	95.66 ^a	4.48 ^b
	30	94.32 ^a	4.91 ^b
<i>R. communis</i>	65	95.85 ^a	4.37 ^b
	50	96.10 ^a	4.10 ^b
	30	95.70 ^a	4.51 ^b
<i>J. curcas</i>	65	94.95 ^a	4.54 ^b
	50	94.62 ^a	4.71 ^b
	30	93.91 ^a	4.78 ^b
Benomyl		98.4% ^a	3.42 ^c
Control		68.2 ^b	28.41 ^a

660 seeds were plated at 20 seeds/ plate each concentration replicated three times

Means with the same letter in each column are not significantly different (P<0. 05) using Tukeys (HSD)

Discussion

In this study, yield of the cowpea plots treated with extracts and benomyl were significantly higher than the control plots. The yield of cowpea plots sprayed with extracts of *D. stramonium* at 65% concentration was not significantly different from the yield of cowpea plots sprayed with benomyl fungicides. However, at lower concentrations of the extracts, (50 and 30%) significant variations were observed such that the yield of cowpea treated with benomyl were higher than those treated with plant extracts. Similarly, the extracts and benomyl reduced incidence and severity of anthracnose disease compared to the control. Severity of infection in extracts and benomyl sprayed plots were characterized with small lesions on lower leaves of cowpea plant while the control plot was characterized with advance lesions on leaves, pod and petiole. This suggest the reason why lower incidence of abnormal seeds were recorded on the treated plots than the control. This is important if the seeds are to be used for planting in subsequent years since it will improve their germination. The benomyl treated plots compared favourably with high concentrations (65%) of the extract of *D. stramonium* in fungitoxicity. This finding agrees with the report of Enyiukwu and Awurum (2014) on the control of cowpea anthracnose caused by *Colletotriuchum destructivum* O. Gara with the extracts of *Carica papaya* roots and seeds, *Piper guineense* seeds alongside the use of benomyl, the study shows that *piper guineense* extract compared favourably ($P \leq 0.05$) with benomyl in fungitoxicity to the pathogen. In contrast, Ogwulumba *et al.* (2008) reported reduction in incidence of foliar disease in groundnut *Arachis hypogeal* and that the synthetic fungicides did not reduce the spread of the disease as effective as the pawpaw and bitter leaf extracts.

In this study, the percentage of seed germination and the number of normal seeds recorded using the extracts of *D. stramonium* at 65% concentration compared favourably with benomyl. However, at lower concentrations of all the extracts (50 and 30%), the percentage of germination and the number of normal seeds recorded with the use of benomyl was higher than that recorded with the use of extracts. Similarly, fungal infections observed on all seeds using bloater test at all concentrations of the extracts and benomyl were low compared to the control. This findings agree

with the work of Jimoh *ital.*, (2016) who reported that incidence of normal seeds was higher than the abnormal seeds when extracts of *Tithonia diversifolia* and *Chromolaena odoratum* were sprayed to control foliar diseases of sesame caused by *Alternaria sesamicola* and *Cercospora sesame* , in addition, there was better outcome in terms of seed yield, seed health and germinability.

In this study, all the extracts of the plants used on the field and benomyl for the control of *C. lindemuthianum* were effective. Pretorius *et al.* (2002) reported that extracts of some indigenous plants were effective in-vitro but failed to control the spread of the pathogen on the field, which contrasts the results of the current study. In the field, abiotic interactions such as temperature and solar radiation may negatively interfere with the performance of active constituents in botanical extracts. The stability of bioactive substances to temperature and ultraviolet radiation may vary with their chemical structure and this may be responsible for the failure reported by Pretorius *et al.* (2002).

In the study, field trials were conducted to evaluate disease incidence where the three extracts and benomyl were applied before inoculation, shortly after inoculation and inoculation of the plant before application of the extracts and benomyl. Disease incidence was significantly lower when extracts and benomyl were applied two days before inoculation of the cowpea plants. The trend common to all the extracts and benomyl when compared to application of extracts and benomyl two days after inoculation or inoculation followed by application of extracts and benomyl within the shortest possible time of 20-30 minutes. This result suggest that extracts and benomyl were more effective when applied as a preventive rather than curative means. This findings agree with the work of Amadioha (1999) who control rice blast caused by the fungus *Pyricularia oryzae* with extracts of *Azadirachta indica*, and carbendazim fungicide, the study shows that both the fungicide and extracts were effective on the field when applied before inoculation.

Similarly, the results of this study are in aggrement with the report of Joycee *ital.*,(2010) who controlled bean anthracnose caused by *Colletotrichum lindemuthianum* invitro and invivo using extracts of thirteen indigenous plants, the study shows that extracts of *M. argrophylla*, *M. fallas*, *O.*

vulgare, *S. arianeae* and *S. pohlili* were the most promising for the inhibition of mycelia growth conidial germination, and reduction in severity of disease on the field. Similarly, Tegegne *et al* (2007) reported that extracts of *Agapanthus africana* and thiram fungicide reduced both loose and covered smut disease of sorghum caused by *Sporisorium sorghi* and *Sporisorium cruentum*, the study shows that both treatments resulted in yield increase, reduced disease incidence and that the extract compared favourably with the fungicide. The present study therefore justifies that extracts of *D. stramonium*, *R. communis* and *J. gossypifolia* can be used as a natural protective pesticide against *C. lindemuthianum* of cowpea thus improving yield.

REFERENCES:

- Abubakar, M. I. and Olukosi, J.O. (2008). Analysis of cowpea production under the national programme on food security in Argungu Local Government Area of Kebbi State, Nigeria. *Nigerian Journal of Basic and Applied Sciences*, 16 (2): 243-247.
- Alves, K. F., Laranjeira, D., Camara, M. P., Camara, C. A. and Michereff, S. J. (2015). Efficacy of Plant extracts for Anthracnose Control in Bell Pepper Fruits under controlled conditions. *Horticultural Brasileira* 33: 332 – 338.
- Amadioha, A. C. (2003). Evaluation of some plant leaf Extracts against *Colletotrichum lindemuthianum* in Cowpea. *Acta Phytopathologica. Hungaria*: 38, 3-6.
- Amadioha, A. C. and Obi, V. I. (1999). Control of Anthracnose disease of cowpea by *Cymbopogon citratus* and *Ocimum gratissimum*. *Acta phytopathol. Entomol* 34: 85-89.
- Dodd, J. C. *et al.*, (1991). The effect of climatic factors on *Colletotrichum gloeosporioides*, causal agent of mango anthracnose, in the Philippines. *Plant Pathology*, 40(4), 568-575.
- Enikuomehin, O. A. and Peters, O. T (2002). Evaluation of Crude extracts from some Nigerian Plants for the Control of Field Disease of Sesame (*Sesamum indicum*). *Journal of Tropical Oil Seeds*. 7: 84-93.
- Enyiukwu, D.N. and Awurum, A.N. (2014). Comparative Fungitoxicity of Benomyl and Extract of *Carica papaya* Roots and Seed and *Piper guineense* Seed on *Colletotrichum destructivum* O Gara. *Journal of Biological Sciences*. 5: 26 – 3

- 306 Gideon, K. O. and Anita (2013) *In vitro* control of Anthracnose disease of cowpea *Vigna unguiculata*
307 L. Walp caused by *C destructivum* with *Cyathula prostrata* L and *Diodia scadens* S. W.
308 *Journal of Intercultural Ethnopharmacology* 2 (1):29-36.
- 309 M. Jimoh, O.A. Enikuomelin, C.G. Afolabi, V.I.O.Olowe and A. Egbontan (2016). Achieving improved
310 control of foliar diseases of sesame (*Sesamum indicum* L.) intercropped with maize (*Zea*
311 *mays* L.) through foliar spray of plant extracts.
- 312 Lowell, J. F. (2004). Producing food without pesticide. Macmillan Publishers
- 313 Ogu, G. E. and Owoeye A. E. (2013). *Invitro* control of anthracnose disease of cowpea (*Vigna*
314 *unguiculata* L Walp) caused by *Colletotrichum destructivum* O Gara with Bioactive
315 Compounds to be used as Nutraceuticals and Food Ingredients. *Journal of International*
316 *Ethnopharmacology*. 2 (1): 29-36
- 317 Ogwulumba, S.I, Ugwuoke, K.I, Iloba, C. (2008). Prophylactic effect of pawpaw leaf and bitter leaf
318 extracts on the incidence of foliar mycopathogens of groundnut (*Arachis hypogeal* L.) in
319 Ishiagu Nigeria. *African Journal of Biotechnology*. 24: 2878 – 2880.
- 320 Sharon, T. and Douglas, M. (2011). Anthracnose Diseases of Trees. Agricultural Experiment Station.
321 Department of Plant Pathology and Ecology.
- 322 Pretorius, J. C., Zietman, P. C., and Eksteen, D. (2002). Fungitoxic Properties of Selected South African
323 Plant Species against Plant Pathogens of economic importance in Agriculture. *Annual*
324 *Applied Biology*. 141: 117 – 1
- 325 Živković, S., Stojanović, S., Ivanović, Ž, Trkulja, N., Dolovac, N., Aleksić, G., & Balaž, J. (2010).
326 Morphological and molecular identification of *Colletotrichum truncatum* from tomato fruit.
327 *Pesticidi fitomedicina*. 25(3): 2 –

