


Human Journals

**Research Article**


February 2017 Vol.:5, Issue:4

© All rights are reserved by Elaigwu M et al.

## *In Vivo* and *in Vitro* Activities of Some Plant Extracts on *Macrophomina phaseolina* (Tassi) Goid- the Causal Agent of Charcoal Rot of Sesame in Benue State, Nigeria



**IJSRM**  
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY  
An Official Publication of Human Journals



**Elaigwu M<sup>1</sup>; H.O.A. Oluma<sup>2</sup> and Ochokwunu D.I<sup>3</sup>**

<sup>1</sup> Department of Integrated Science, College of Education, Oju, Benue State, Nigeria.

<sup>2</sup> Department of Biological Sciences, University of Agriculture, Makurdi, Benue State, Nigeria.

<sup>3</sup> Department of Biology Science, College of Education, Oju, Benue State, Nigeria

**Submission:** 2 February 2017  
**Accepted:** 7 February 2017  
**Published:** 25 February 2017



[www.ijsrm.humanjournals.com](http://www.ijsrm.humanjournals.com)

**Keywords:** HPTLC, Flavonoids, *Eugenia uniflora*

### ABSTRACT

Macrophomina root rot is an important disease of *Sesamum indicum* L. causing significant reduction in yield. In present study, the pathogenic fungus was isolated from infected beniseed plant parts and confirmed based on morphological and cultural characters as *Macrophomina phaseolina* (Tassi) Goid. The *in vivo* effectiveness of the plant extracts viz: : *Prosopis africana*, *Azadirachta indica*, *Eucalyptus globulus*, *Vitex doniana*, *Tridax procumbens*, *Mangifera indica*, *Eucalyptus camaldulensis*, *Ocimum gratissimum*, *Cymbopogon citratus*, *Morinda lucida*, *Jasminum dichotomum*, *Citrus aurantifolia*, *Carica papaya*, *Chromolaena odorata*, *Nauclea latifolia*, *Musa sapientum*, *Gmelina arborea*, *Daniella oliveri*, *Psidium guajawa*, *Lophira lanceolata*, *Bridelia ferruginae*, *Gardenia florida*, *Thevetia peruviana*, *Lawsonia inermis*, *Vernonia amygdalina*, *Amaranthus spinosus*, *Delonix regia*, *Annona senegalensis*, *Newbouldia laevis* and *Anacardium occidentale* were tested against the causative agent of root rot of *Sesamum indicum* L. Most of the leaves extract showed significant reduction of the mycelia growth of the pathogen. However, the leaf extracts of *A. occidentale* and *P. africana* were found to be most effective and their efficacies on the development of the disease incidence were further tested in field applications. Seeds soaked in 2ml of the extracts were used in the study. Results of the *in vivo* experiment shows that extracts of both *P. africana* and *A. occidentale* significantly ( $P < 0.05$ ) reduced the incidence on the development of *M. Phasolina* root rot disease both in pre-yield and yield parameters. However, the extract of *Prosopis africana* substantially shows greater reduction by showing the highest number of mean on the growth and yield parameters as compared to the control. Present study concludes that the leaf extracts of *P. africana* and *A. occidentale* may contain natural fungicides which can be exploited for the management of *M. phaseolina*.

## INTRODUCTION

Beniseed (*Sesamum indicum L.*) is an oilseed crop generally cultivated on small holdings by poor resource farmers in the tropics. The crop was first grown in the middle belt of Nigeria in the late 1940s which fall within the Guinea savannah (Olowe, 2007). Sesame has been cultivated for a long time; no significant increase in productivity has been achieved yet and this low productivity, El-Bramawy & Abdul (2006) has been attributed to pests and diseases. The crop suffers from various fungal, bacterial, viral and phytoplasma diseases (Bedigan, 2004). Many synthetic fungicides had shown promise in the control of sesame diseases (Shokalu *et al.*, 2002); however, the high cost of such chemicals forbids their use by ordinary farmers. Furthermore, continuous use of these chemicals may pose ecological problems. According to Babu, *et al.*, (2008) the recurrent and indiscriminate use of fungicides have a serious threat to human health and to the existing human eco-geographical conditions as some of them have already been proved to be either mutagenic, carcinogenic or teratogenic. Keeping in view the drawback of chemical management of plant diseases, the use of plant extracts in the management of plant disease is gaining importance. This, in turn, necessitates the search for alternatives in plant products, many of which have been reported to be effective in the control of several plant diseases (Enikuomehin and Peters, 2002; Okigbo and Emoghene, 2003, Nico *et al.*, 2005; Vimala *et al.*, 2004 and El-Mougy *et al.*, 2004).

Arshad & Rehman, (2011) reported that a significant reduction in fungal biomass was recorded due to different concentrations of the leaf extracts of *E. citriodora* and that organic solvent extracts of allelopathic especially chloroform containing antifungal constituents and can effectively be used for the management of *M. phaseolina*. The leaf crude extracts of *E. citriodora* were effective in suppressing the growth of fungus *Didymella bryoniae* (Fiori, 2000). Similarly, Jabeen and Javaid (2008) reported that the fungal activity of alcoholic and chloroform extracts of leaves of *E. citriodora* against *A. rabiei*. Jabeen *et al.*, (2008) reported antifungal activity of ethanol and chloroform leaf extracts of this tree species against *Ascochyta rabiei*. The antifungal activity of *Melia azedarach* could be attributed to the presence of antifungal compounds namely hydroxycoumarin scopoletin, vanillin, 4-hydroxy-3-methoxycinnamaldehyde and ( $\pm$ ) pinoselinol (Carpinella). Recently, Jabeen *et al.*, (2011) have isolated five compounds namely  $\beta$ -sitosterol,  $\beta$ - amyrin, ursolic acid, benzoic acid, 3-5 dimethoxy benzoic acid from leaves of *Melia azedarach*, which showed antifungal activity against *A. rabiei*.

In Nigeria, lemon grass powder and essential oil have effectively protected melon seeds against toxigenic *Aspergillus flavus* (Banjole & Joda, 2004). Enikuomehin and Peters, (2002) reported that extracts of *Ocimum gratissimum*, *Azadirachta indica* and *Magnifera indica* reduced mycoflora load of sesame seeds through a possible reduction in infection.

Oluma and Garba, (2004) have found that crude extract of *Eucalyptus globulus* and *Ocimum gratissimum* reduced radial growth of *Pythium aphanidermatum* by 44.5-100 percent with *E. globulus* being more potent. Radial growth and dry weight of two well-known rice pathogens, *Helminthosporium oryzae* and *Rhizoctonia solani* have been drastically reduced by the volatile oils from *Eucalyptus citriodora* and its major constituent citronella (Ramezani *et al.*, 2002). Arshad & Hafiza, (2011) reported that the leaf extracts of allelopathic trees especially ethyl acetate and chloroform extracts of *A. indica* contain natural fungicides which can be used for the management of *M. phaseolina*. The extracts of pulverized bark of *prosopis africana* and leaves of *N. latifolia* inhibited both radial mycelia growth and sclerotial formation by 100% of *Macrophomina phaseolina* (Oluma and Elaigwu, 2006).

Keeping the above facts in view, this work was undertaken to evaluate these environmental friendly plants extracts their activities against Sesame, *Macrophomina* root rot of Sesame in Benue state, Central Nigeria.



## MATERIALS AND METHODS

Thirty plants that are known for their medicinal and antimicrobial activities in Nigeria were used for their efficacy against *Macrophomina phaseolina* the causative agent of root rot of *Sesamum indicum*. These plants were selected because they are associated with pest management and disease control practices in several parts of Africa (Stoll, 2001; Adodo, 2000). Using polythene bags, the plant leaves were collected from the environment and labeled properly before taken to the laboratory for further processing.

Taxonomic identification of the plant samples was authenticated at the Department of Biological Sciences University of agriculture Makurdi. Voucher specimens were preserved in the Herbarium collection of the Department of Biological Sciences, University of Agriculture, Makurdi.

## LABORATORY TESTS

The pathogen, *Macrophomina phaseolina* (Tassi) Goid (Syn. *Rhizoctonia bataticola*) (Taub) Butler was selected for this experimental work. The pathogen was isolated from dry root rot of Beniseed (*Sesame indicum* L.) With visible symptom of black charcoal in a commercial farmer's field in Makurdi metropolis and its environs of Benue state, Nigeria. Diseased sample was surface washed three times with sterilized distilled water.

To isolate the organism, sample of infected roots of Beniseed was sterilized in 1% alcohol for 1 minute. The sample was then rinsed in several changes of sterile distilled water and blotted dry with sterile filter paper. Segments of the diseased root tissues were plated on Czapeck's agar (CDA) and incubated for fungi growth at  $28\pm 2^{\circ}\text{C}$ .

Pure cultures of the pathogens were obtained by sub-culturing colonies growing from the plated root tissues on Czapeck's agar (CDA) medium.

The thirty fresh and healthy leaves collected; *Prosopis africana*, *Azadirachta indica*, *Eucalyptus globules*, *Vitex doniana*, *Tridax procumbens*, *Mangifera indica*, *Eucalyptus calmaldulensis*, *Ocimum gratissimum*, *Cymbopogon citrulus*, *Morinda lucida*, *Jasminum dichotomum*, *Citrus aurantifolia*, *Carica papaya*, *Chromolaena odorata*, *Nuclea latifolia*, *Musa sapientum*, *Gmelina arborea*, *Daniella oliveri*, *Psidium guajava*, *Lophira lanceolata*, *Bridelia ferruginea*, *Gardenia florida*, *Thevetia peruviana*, *Lawsonia inermis*, *Vernonia amygdalina*, *Amaranthus spinosus*, *Delonix regia*, *Annona senegalensis*, *Newbouldia laevis* and *Anacardium occidentale* (Table1). The specimens were washed with several changes of sterile distilled water and were later pulverized using pestle and mortar according to the method of Oluma and Garba (2004) The crushed leaves were separately plunged in required quantity of water (1:1 w/v) in a beaker and boiled at  $100^{\circ}\text{C}$  for 10 minutes Crude extracts of the leaves were obtained after 24 hours in the laboratory at  $28\pm 2^{\circ}\text{C}$  by filtering the infusions through cheese cloth which formed a standard plant extracts (100%).

Czapek's agar medium was prepared and autoclaved in 250ml Erlenmeyer flasks. Aqueous plant extracts of 2ml were added to 98ml of aliquots in the flask so as to get the final concentration of 2% of the extracts in the medium. The medium was poured into Petri plates at 15ml/ plate. Czapek's agar without plant extracts served as control.

The CDA-plant extracts were inoculated respectively at the center of the plates with 2mm mycelia discs of the test fungus. The mycelia plugs were obtained with 2mm cork borer lifted from the margin of actively growing culture of the test fungi. These were placed upside down in center of each plate. The inoculated plates were incubated at  $28\pm 2^{\circ}\text{C}$  for 6 days. Plates of medium without plant extracts served as control. Radial growth (cm) of fungus was measured on the 7<sup>th</sup> day.

### ***In Vitro* Experiment**

Plants extract which showed greater inhibitory effects on the test pathogen (*A. anacardium* and *P. africana*) were further tested against the pathogen in the field experiment. The experiment was conducted at the University of Agriculture Makurdi. The experiment was laid in randomized complete block design (RCBD) with three replicates. The treatment methanolic extracts of *Anacardium occidentale* and *Prosopis africana* applied at one level (1) levels (0 and 2ml) and identified as T<sub>0</sub> (control) T<sub>2</sub> (2ml of *A. occidentale*), T<sub>3</sub> (2ml) of *Prosopis africana*. This gives the total of three treatments. The pots were watered twice a day and continued up to pod formation stage. Records were taken of days to germination, number of seeds germinated, number of flowers, length of plant at flowering stage, number of uninfected leaves, number of lesions, number of flower buds, and number of pods formed.

## **RESULTS**

### **Fresh leaves extracts**

Among the selected plants screened, fresh leaves extracts of *Anacardium occidentale* and *Prosopis africana* were found most effective against *M. phaseolina* (Table 1). Highest inhibition in radial mycelia growth (%) induced by *Macrophomina phaseolina* was exhibited by *Anacardium occidentale* (69.2%) followed by *Prosopis africana* (66.1%), *Gmelina arborea* (56%), *Azadirachta indica* (53.2%) *Carica papaya* (44.6%), *Delonix regia* (42.1%) *Eucalyptus globulus* (42.5%), *Nuclea latifolia* (41.8%), *Tridax procumbens* (38.2%), *Ocimum gratissimum* (38.5%), *Citrus aurantifolia* (25.5%), *Morinda lucida* (23.1%), *Lophira lanceolata* (21.5%), *Vernonia amygdalina* (19.5%), *E. camaldulensis* (16.9%), *Lawsonia inermis* (12.6%), *Psidium guajawa* (12%), *Jasminum dichotonum* (11.7%), *Thevetia peruviana* (9.50%), *Vitex doniana* (1.50%), *Gardenia florida* (10.8%), *Annona senegalensis* (10.5%) (Table1).

No inhibition in radial mycelia growth was recorded with the fresh extracts of *Amaranthus spinosus*, *Bridelia ferruginea*, *Chromolaena odorata*, *Cymbopogon citratus*, *Daniellia oliveri*, *Mangifera indica*, *Newbouldia laevis* and the control (Table 1)

**Table 1. Effect of Fresh Extracts of leaves of different plant species on the mycelia growth of *Macrophomina phaseolina***

S. NO.	Scientific name	Family name	Part of the plant used	Mean radial mycelia Growth	Mean % radial Mycelia growth
1	<i>Amaranthus spinosus</i>	Amaranthaceae	Leaves	3.25	0.00 (0.00) i
2	<i>Anacardium occidentale</i>	Anacardiaceae	Leaves	1.00	69.2 (0.14) a
3	<i>Annona senegalensis</i>	Anonaceae	Leaves	2.91	10.5 (0.05) gh
4	<i>Azadirachta indica L.</i>	Meliaceae	Leaves	1.52	53.2 (0.13) b
5	<i>Bridelia ferruginea</i> Benth	Euphorbiaceae	Leaves	3.25	0.00 (0.00) i
6	<i>Carica papaya</i>	Caricaceae	Leaves	1.80	44.6 (0.12) c
7		Asteraceae	Leaves	3.25	0.00 (0.00) i
8	<i>Citrus aurantifolia</i> (christm) swingle	Rutaceae	Leaves	2.42	25.5 (0.08) e
9	<i>Cymbopogon citratus</i> (spreng)	Poaceae	Leaves	3.25	0.00 (0.00) i
10	<i>Daniellia oliveri</i>	Fabaceae	Leaves	3.25	0.00 (0.00) i
11	<i>Delonix regia</i>	Caesalpiniaceae	Leaves	1.88	42.1 (0.11) d
12	<i>E. camaldulensis</i> Dehnh.	Lecythidaceae	Leaves	2.70	16.9 (0.07) f
13	<i>Eucalyptus globules</i> (labill)	Lecythidaceae	Leaves	1.87	42.5 (0.11) d
14	<i>Gardenia florida</i>	Rubiaceae	Leaves	2.90	10.8 (0.05) d
15	<i>Gmelina arborea</i>	Verbenaceae	Leaves	1.42	56.3 (0.13) b
16	<i>Jasminum dichotomum</i> Vahl	Oleaceae	Leaves	2.87	11.7 (0.06) g
17	<i>Lawsonia inermis L</i>	Lythraceae	Leaves	2.84	12.6 (0.06) g
18	<i>Lophira lanceolata</i> Tiegh.ex keay	Ocinnaceae	Leaves	2.55	21.5 (0.08) e
19	<i>Mangifera indica L.</i>	Anacardiaceae	Leaves	3.25	0.00 (0.00) i
20	<i>Morinda lucida</i>	Rubiaceae	Leaves	2.50	23.1 (0.08) e
21	<i>Musa sapientum</i>	Musaceae	Leaves	2.00	38.5 (0.11) d
22	<i>Newbouldia laevis</i> (P.Beauv)	Bignoniaceae	Leaves	3.25	0.00 (0.00) i

23	<i>Nuclea latifolia</i>	Rubiaceae	Leaves	1.89	41.8 (0.11) d
24	<i>Ocimum gratissimum</i>	Silva	Leaves	2.60	38.5 (0.11) d
25	<i>Prosopis africana</i> (Taubert)	Mimosoideae	Leaves	1.10	66.1 (0.14) a
26	<i>Psidium guajawa</i>	Myrtaceae	Leaves	2.86	12 (0.06) g
27	<i>Thevetia peruviana</i> (Pers.) K. Schum	Apocynaceae	Leaves	2.94	9.5 (0.05) gh
28	<i>Tridax procumbens</i> L.	Asteraceae	Leaves	2.01	38.2 (0.11) d
29	<i>Vernonia amygdalina</i>	Asterraceae	Leaves	2.62	19.4 (0.08) e
30	<i>Vitex doniana</i>	Verbenaceae	Leaves	3.20	1.50 (0.02) i
31	Control	Anacardiaceae	Leaves	3.25	0.00 (0.00)

Those figures followed by the same letters are not significantly different ( $p < 0.05$ ) according to Duncan's New Multiple Range Test. Figures in parentheses are arc sin transcribed values,  $\sin \sqrt{x}$ .

#### Air dried leaves extract

Maximum inhibition in radial mycelia growth (%) induced by *Macrophomina phaseolina* was exhibited by *Prosopis africana* (34.2%) followed by *Anacardium occidentale* (32.7%), *Azadirachta indica* (21.1%), *Ocimum gratissimum* (16.0%), *Vernonia amygdalina* (15.2%), *Newbouldia laevis* (12.2%), *Musa sapientum* (11.9%), *Delonix regia* (11.9%), *Nuclea latifolia* (4.97%), *Carica papaya* (3.80%), *Mangifera indica* (2.04%), *Cymbopogon citratus* (2.92%), *Annona senegalensis*, *Bridelia ferruginea* and *Lawsonia inermis* (1.64%) respectively, *E. globulus* and *Thevetia Peruviana* (0.58%), *Gmelina arborea* (0.87%). (Table 2) No inhibition in radial mycelia growth was recorded with the dried leaves extracts of *Tridax procumbens*, *Citrus aurantifolia*, *Morinda lucida*, *Lophira lanceolata*, *E. camaldulensis*, *Psidium guajava*, *Jasminum dichotonum*, *Vitex doniana*, *Gardenia florida*, *Amaranthus spinosus*, *Chromolaena odorata*, *Daniellia oliveri*, and the control (Table 2).

**Table 2. Effect of Extracts of dried leaves of different plant species on the mycelia Growth of *Macrophomina phaseolina***

S. N O.	Scientific name	Family name	Part of the plant used	Mean radial mycelia	Mean % Radial Mycelia growth
1	<i>Amaranthus spinosus</i>	Amaranthaceae	Leaves	3.42	0.00 (0.00)i
2	<i>Anacardium occidentale</i>	Anacardiaceae	Leaves	2.30	32.7 (0.10)a
3	<i>Annona senegalensis</i>	Anonaceae	Leaves	3.37	1.46 (0.02)g
4	<i>Azadirachta indica L.</i>	Meliaceae	Leaves	2.70	21.1 (0.08)b
5	<i>Bridelia ferruginea Benth</i>	Euphorbiaceae	Leaves	3.37	1.46 (0.02)g
6	<i>Carica papaya</i>	Caricaceae	Leaves	3.29	3.80 (0.03)f
7	<i>Chromolaena odorata L. (King &amp; H.E Robins)</i>	Asteraceae	Leaves	3.42	0.00 (0.00)i
8	<i>Citrus aurantifolia (christm)swingle</i>	Rutaceae	Leaves	3.42	0.00 (0.00)i
9	<i>Cymbopogon citratus (spreng)</i>	Poaceae	Leaves	3.32	2.92 (0.03)f
10	<i>Daniella oliveri</i>	Fabaceae	Leaves	3.42	0.00 (0.00)i
11	<i>Delonix regia</i>	Caesalpiniaceae	Leaves	3.01	11.9 (0.06)d
12	<i>Eucalyptus camaldulensis Dehnh.</i>	Lecythidaceae	Leaves	3.42	0.00 (0.00)i
13	<i>Eucalyptus globulus (Labill)</i>	Lecythidaceae	Leaves	3.40	0.58 (0.01)h
14	<i>Gardenia florida</i>	Rubiaceae	Leaves	3.42	0.00 (0.00)i
15	<i>Gmelina arborea</i>	Verbenaceae	Leaves	3.39	0.87 (0.02)g
16	<i>Jasminum dichotomum Vahl</i>	Oleaceae	Leaves	3.42	0.00 (0.00)i
17	<i>Lawsonia inermis L.</i>	Lythraceae	Leaves	3.37	1.46 (0.02)g
18	<i>Lophira lanceolata Tiegh.ex keay</i>	Ocinnaceae	Leaves	3.42	0.00 (0.00)i
19	<i>Mangifera indica L.</i>	Anacardiaceae	Leaves	3.35	2.04 (0.02)g
20	<i>Morinda lucida</i>	Rubiaceae	Leaves	3.42	0.00 (0.00)i
21	<i>Musa sapientum</i>	Musaceae	Leaves	3.01	11.9 (0.06)d
22	<i>Newbouldia laevis (P.Beauv)</i>	Bignoniaceae	Leaves	3.00	12.2 (0.06)d
23	<i>Nuclea latifolia</i>	Rubiaceae	Leaves	3.25	4.97 (0.04)e
24	<i>Ocimum gratissimum</i>	Silva	Leaves	2.87	16.0 (0.07)c
25	<i>Prosopis africana (Taubert)</i>	Mimosoideae	Leaves	2.25	34.2 (0.10)a



26	<i>Psidium guajawa</i>	Myrtaceae	Leaves	3.42	0.00 (0.00)i
27	<i>Thevetia peruviana</i> (Pers.) K. Schum	Apocynaceae	Leaves	3.40	0.58 (0.01)h
28	<i>Tridax procumbens</i> L.	Asteraceae	Leaves	3.42	0.00 (0.00)i
29	<i>Vernonia amygdalina</i>	Asterraceae	Leaves	2.90	15.2 (0.07)c
30	<i>Vitex doniana</i>	Verbenaceae	Leaves	3.42	0.00 (0.00)i
31	Control	Anacardiaceae	-	3.42	0.00 (0.00)i

Those figures followed by the same letters are not significantly different ( $p < 0.05$ ) according to Duncan's New Multiple Range Test. Figures in parentheses are arc sin transcribed values,  $\sin \sqrt{x}$ .

**Table 3. Effect of Seed Soaked in aqueous leaf extracts of *A. Occidentale* and *P. Africana* on the development of *M. Phaseolina* Root Rot Disease in Pre- Yield Parameters.**

(MEAN + S.E)

Treatment Seeds soaked in 2 ml of extract.	No. of seeds germinated after 6 days		No. of plants survived to flowering stage		Height of plants at flowering stage		No. of uninfected leaves	
	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E
<i>A. occidentale</i>	17.67 <sup>a</sup>	1.45	13.00 <sup>a</sup>	3.0 0	32.00 <sup>a</sup>	3.06	15.00 <sup>a</sup>	2.08
<i>P. africana</i>	19.33 <sup>a</sup>	2.96	16.67 <sup>a</sup>	3.0 6	43.33 <sup>b</sup>	1.76	20.67 <sup>a</sup>	1.76
Control	23.67 <sup>a</sup>	1.76	13.67 <sup>a</sup>	1.8 6	27.67 <sup>a</sup>	1.45	15.00 <sup>a</sup>	2.52
F <sub>stat.</sub>	2.06		1.10		13.49		12.33	
P (0.05).	0.21		0.07		0.01*		0.18	

Mean values with same alphabets are not significantly different from each other at

( $p \leq 0.05$ ) in Duncan's Multiple Range Test, each treatment comprised of 3 replicates.

\* = Significant at  $P \leq 0.05$ .

**The number of seeds germinated after 6 days**

With table 1, the treatment means are statistically the same by DMRT at ( $p < 0.05$ ). However, higher germination rate was observed in the control and *P.africana* at (23.67) and (19.33) respectively.

**The number of plants survived to flowering stage**

The mean values are statistically the same at ( $p < 0.05$ ). The highest mean values were observed from *P. africana* at (16.67) and least from *A. occidentale* at (13.00) compared (13.67) of the control.

**The height of plant at flowering stage**

The heights of plant survived are statistically different. The response from *P. africana* is highest at (43.33). *A. occidentale* and the control have the least mean at (32.00) and (27.67) respectively.

**The number of infected leaves**

The treatment means are statistically the same in the number of uninfected leaves. Similarly, the highest mean of un- infected leaves (20.67) is observed from *P. africana* compared with (15.00) for both *A. occidentale* and the control.

**TABLE 4. EFFECT OF THE SEED SOAKED IN 2MLS OF AQUEOUS LEAF EXTRACT OF *A. OCCIDENTALE* AND *P. AFRICANA* ON THE DEVELOPMENT OF *M. PHASEOLINA* ROOT ROT DISEASE IN YIELD PARAMETERS (MEAN + S.E).**

Treatment Seeds soaked in 2ml of fresh extracts.	No. of lesion		No. of flowering buds		No .of plants infected with <i>M. phaseolina</i>		No. of pods formed.	
	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E
<i>A. occidentale</i>	2.33 <sup>a</sup>	0.33	8.00 <sup>a</sup>	1.53	5.33 <sup>a</sup>	2.40	4.00 <sup>a</sup>	0.58
<i>P. africana</i>	1.67 <sup>a</sup>	0.58	13.00 <sup>c</sup>	1.16	3.33 <sup>a</sup>	0.88	7.33 <sup>a</sup>	1.33
<b>Control</b>	4.33 <sup>b</sup>	0.88	12.33 <sup>ab</sup>	1.45	6.00 <sup>a</sup>	1.16	6.33 <sup>a</sup>	1.20
<b>F<sub>stat.</sub></b>	5.78		3.83		0.73		2.47	
<b>P (0.05).</b>	0.04*		0.09		0.52		0.17	

Mean values with same alphabets are not significantly different from each other at ( $p \leq 0.05$ ) in Duncan’s Multiple Range Test, each treatment comprised of 3 replicates

### **The number of lesion (yield parameters)**

The means of the number of lesion developed are statistically significance different. The highest mean of number of lesion (4.32) was observed in the control and (2.33) in *A. occidentale* while the least is (6.67) is from *P. africana*.

### **The number of flowering buds**

The means for the number of flowering buds are statistically different. The highest mean (13.00) was observed from *P. africana* treatment while (12.33) and (8.00) were observed from *A. occidentale* and the control respectively.

### **The number of plants infected with the disease**

There is no significance difference in the means of the number of plants infected before pod formation. However, the least mean of (3.33) was observed in *P. africana* treatment while highest (6.00) was observed in the control.

### **The number of pods formed**

The treatment mean for the number of pods formed from liquid extracts are statistically the same. However, the highest mean of (7.33) was observed from *P. africana* while the least was observed with the *A. occidentale* (4.00).

## **DISCUSSION**

The present study has demonstrated the antifungal properties of the fresh leaf extracts of *Anacardium occidentale* and *Prosopis africana* respectively against the mycelia growth of *Macrophomina phaseolina*. The study has also demonstrated the efficacy of the seeds soaked in leaf extracts of the plants at 2ml for controlling the disease incidence of *Macrophomina phaseolina*, the causal agent of root rot of Beniseed (*Sesamum indicum*, L) indicating their broad range of activity as compared with other plants. This is consistent with the earlier reports that many plant products contain fungitoxic constituents that have the potential to control plant diseases (Tewari and Nayak, 1991; Amadioha and Obi, 1999; Enikuomhin and peters, 2000).

Apart from this indication of antifungal activity particularly, in Nigeria, the decoction of root and stem of *Anacardium occidentale* has been used as anti-inflammatory agent and anti-

diarrhoeal (Mota, *et al.* 1985). Similarly, The antimicrobial activities of *Anacardium occidentale* extracts have been confirmed (Laurens, 1982; Kudi, *et al.*, 1999). Akash, *et al.*, (2009) reported that petroleum ether extract and ethanolic extracts of *Anacardium occidentale* leaves exhibited significant antimicrobial and antifungal activity. Omojasola and Awe (2004) reported the antimicrobial activity of the leaf of *Anacardium occidentale* and *Gossypium hirsutum* against *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhimurium*, *Staphylococcus aureus* and *pseudomonas aeruginosa*.

Among the plant products screened, the fresh leaves extracts of *Amaranthus spinosus*, *Bridelia ferruginea*, *Chromolaena odorata*, *Cymbopogon citratus*, *Daniella oliveri*, *Mangifera indica*, *Newbouldia laevis* had no inhibitory effects on *Macrophomina phaseolina*. The differences in inhibitory effect of these plants extracts on *Macrophomina phaseolina* may be due to qualitative and quantitative differences in the antifungal principle present in them (Sigh and Singh, 1980). The phytochemical screening of extract of *Anacardium occidentale* revealed the presence of high amount of tannins, moderate saponins and trace of free reducing sugars (Okorie, *et al.*, 2010). According to Mendes, *et al.* (1990). Anacardic acids, one of the phytochemical constituents of cashew extract, has been shown to curb the darkening effect of aging by inhibiting tyrosinase activities and kill certain cancer cells. A wide range of chemicals was isolated and identified from cashews such as anacardic acids, anacardol, hydroxybenzoic acid, kaempferol, salicylic acid and tannins. Similarly, it has been reported that the root decoction of *P. africana* is used to treat toothache in Ghana and the bark and root used to treat and relieve tooth decay in Mali (World Agroforestry Centre, 2008). In Ghana the pod ashes of *P. africana* are a source of potash for soap making (Ezike, *et al.*, 2010). *Prosopis africana* is confirmed to contain Alkaloids, Beta-phenethylamine and tryptamine (Tapia *et al.*, 2000). Although the chemical analysis of *Anacardium occidentale* and *Prosopis africana* was not investigated in this work.

The extracts of pulverized bark of *Prosopis africana* and leaves of *N. latifolia* inhibited both radial mycelia growth and sclerotial formation by 100% of *Macrophomina phaseolina* (Oluma and Elaigwu, 2006). Arshad & Rehman, (2011) reported that a significant reduction in fungal biomass was recorded due to different concentrations of the leaf extracts of *E. citrodora* and that organic solvent extracts of allelopathic especially chloroform contain antifungal constituents and can effectively be used for the management of *M. phaseolina*. The leaf crude extracts of *E. citrodora* were effective in suppressing the growth of fungus

*Didymella bryoniae* (Fiori, 2000). Similarly, Jabeen and Javaid (2008) reported the fungal activity of alcoholic and chloroform extracts of leaves of *E. citriodora* against *A. rabiei*. Prince and Prabakaran (2011) demonstrated that *Vitex negundo* showed maximum antifungal activity against the pathogenic fungus *Colletotrichum falcatum*.

Neem products are used in selectively controlling pests in plant helminthic, antifungal, antidiabetic, antibacterial, antiviral, contraceptive and sedatives (Ganguli, 2002). Silva *et al.*, (2010) reported that the essential oil of *Ocimum gratissimum* contains eugenol and shows some evidence of antibacterial activity. This is similar with the earlier reports by Enikuomehin and Peters, (2002) that extract of *Ocimum gratissimum*, *Azadirachta indica* and *Magnifera indica* reduced mycoflora load of sesame seeds loads through a possible reduction in infection. Oluma and Garba, (2004) have found that crude extract of *Eucalyptus globulus* and *Ocimum gratissimum* reduced radial growth of *pythium aphanidermatum* by 44.5-100% with *E. globulus* being more potent. *Tridax procumbens* is known for several potential therapeutic activities like antiviral, antibiotic efficacies; wound healing activity, insecticidal and anti-inflammatory activity. Some reports from tribal areas in India states that the leaf juice can be used to cure fresh wounds, to stop bleeding, as a hair tonic (Saxena & Albert, 2005).

Kanwal *et al.*, (2010) reported that Mangiferin a pharmacologically active flavonoid, a natural xanthone (C-glycoside) is extracted from Mango at high concentrations from the young leaves shows an exceptionally strong antioxidant capacity. It has a number of pharmacological actions and possible health benefits. These include antidiabetic, antioxidant, antifungal, antimicrobial, anti-inflammatory, antiviral, hepatoprotective, hypoglycemic, anti-allergic and anticancer activity. In Indonesia, it was reported that *Chromolaena odorata* is used as a traditional medicine. The young leaves are crushed, and the resulting liquid can be used to treat skin wounds (Fu *et al.*, 2002). According to Shadab *et al.*, (1992) *Cymbopogon citratus* (Lemongrass) oil is used as a pesticide and as preservative. This is consistent with the earlier reports in Nigeria that, lemongrass powder and essential oil have effectively protected melon seeds against toxigenic *Aspergillus flavus* (Banjole & Joda, 2004). Kokwaro, (1976) reported that the root of *Vitex doniana* is used for treating gonorrhoea, and women drink a decoction of it for backaches. The young tender leaves are pounded and the juice squeezed into the eyes to treat eye troubles. Makinde *et al.*, (1985) observed that decoctions and infusions or plasters of root, bark and leaves of *Morinda lucida* are recognized remedies

against different types of fever, including yellow fever, malaria, trypanosomiasis and feverish condition during childbirth.

It was reported that crocetin is a chemical compound found in *Gardenia florida* fruit, when in high concentrations; it has protective effects against retinal damage *in vitro* and *in vivo* (Yamauchi, *et al.*, 2011). *Lophira lanceolata* in traditional medicine is used to treat dermatitis, toothache and muscular tiredness. A concoction prepared from the bark of the roots and trunk is used against pulmonary diseases (Arbonnier, 2000). The root decoction of *G.arborea* is reported in India as used in folk remedies for abdominal tumors (Little, 1983). Phytochemicals in papaya have been studied and may suppress the effects of progesterone (Oderinde *et al.*, 2002). *Musa sapientum* fruits have been reported to prevent anemia by stimulating the production of hemoglobin in the blood. Its role in regulating blood pressure has been associated with the high content of potassium Akinyosoye, (1991).

Kareru *et al.*, (2010) reported that *Thevetia peruviana* contains a milky sap containing a compound called thevetin in its natural form is extremely poisonous, as well as all parts of the plants, especially the seeds. The plant's toxins have tested in experiments for uses in biological pest control. *Thevetia peruviana* seed oil can be used to make paint with antifungal, antibacterial and anti-termite properties. The barks of *B. ferruginea* is reported for wound treatment, gonorrhea infections, antimicrobial potency, gastro-infection treatment, anti-diabetic, anti-inflammatory and radical scavenging activities (Ekanem *et al.*, 2008). Similarly Ogunbosoye & Babayemi, (2010) confirmed that the leaves of *Newbouldia laevis* have antibiotic, bacteriostatic and fungistatic properties. Arshad & Hafiza, (2011) reported that the leaf extracts of allelopathic trees especially ethyl acetate and chloroform extracts of *A. indica* contain natural fungicides which can be used for the management of *M. phaseolina*.

The antifungal activities of the extracts increased as the concentration increased as found in the field experiment conducted. This does not differ from the research findings of Banso and Adeyemo (2007) that the tannins isolated from medicinal plants possess remarkable toxic activity against bacteria and fungi and may assume pharmacological importance in future. Investigations into the active ingredients of the extracts and the mode of action and further studies are in progress in our laboratory to determine the minimum concentration required for maximum disease control as well as the frequency and mode of application of the different plant extracts.

## CONCLUSION

The present findings indicate that plant products particularly extracts of fresh leaves of *Anacardium occidentale*, *Prosopis africana* and seed treatment with fresh extracts of *Prosopis africana* offer a potential and environmentally safe alternative for use as fungicides and could be exploited for effective management of root rot diseases of Beniseed (*Sesamum indicum*) caused by *Macrophomina phaseolina*.

## REFERENCES

1. Adodo, A. (2000). Nature power-A christian Approach to Herbal medicine. St. Benedict Monastery, Ewu Nigeria 288p.
2. Akash, P.D, Vishal, D.J.and Arun B.J. (2009). Antimicrobial screening of different extract of *Anacardium occidentale* Linn. Leaves. Journal of ChemTech Research 1; 856-858.
3. Akinyosoye, V.O. (1991). *Tropical Agriculture*, Macmillan Publishers Limited, Ibadan p. 65 – 68.
4. Amadioha, A.C. and Obi, V.I. (1999). Control of Anthracnose disease of cowpea by *Cymbopogon citratus* and *Ocimum gratissimum*. Acta phytopathol, Hun., 34;85-89.
5. Arbonnier, M., (2000). *Arbres, arbustes et lianes des zones sèches d'Afrique de l'Ouest*. CIRAD, MNHN, UICN. 541 pp.
6. Arshad J. & Rafiza, H. A. (2011). Antifungal activity of leaf extracts of some medicinal trees against *M. phaseolina*. *Journal of Medicinal plants Research*, 5 (13): 2868-2872.
7. Arshad J. & Rehman, H. A. (2011). Antifungal activity of leaf extracts of some medicinal trees against *M. phaseolina*. Journal of Medicinal plants Research vol.5 (13).pp.2868-2872.
8. Babu, J., Muzafar, A. and Vinod, K. (2008). *Bioefficacy of plant extracts to control Fusarium solani F. Sp. Melongenae Incitant of Brinjal Wilt*. Global journal of Biotechnology 3:56-59.
9. Banjole, S.A. and Joda, A.O. (2004). *Effect of lemon grass (Cymbopogon citratus) powder and essential oil on mould deterioration and flatoxin contamination of melon seeds (Colocynthis citrullus L.)*. Afr. J. Biotechnology., 3: 52-59.
10. Banson, A. and Adeyemo, S.O (2007). *Evaluation of antimicrobial properties of tannins isolated from Dichrostachys cinerea*. African journal of Biotechnology, 6(15) 1785-87.
11. Bedigian, D. (2004). *History and lore of sesame in Southwest Asia*. Economic Botany 58(3): 329-35
12. Ekanem, J.T, Kolawole O.M, Abbah O.C (2008). *Trypanocidal potential of Methanolic extracts of Bridelia ferruginae benth bark in Rattus norvegicus*. Afr. J. Biochem. Res., 2(2): 45-50.
13. El-Bramawy M.A.S. and Abdul Wahid O.A. (2006). *Field resistance of crosses of sesame (Sesame indicum L.) to charcoal root rot caused by Macrophomina phaseolina (Tsssi.) Goid. Plant protect. Sci., 42:66-72*
14. El-Mougy, N.S., Abd-El-Karem, F., Nadia, G.E. and Fotouh, Y.O. (2004). *Application of fungicides alternatives for controlling cowpea root rot diseases under greenhouse and field conditions*. Egypt. J. Phytopathol., 32: 23-35.
15. Enikuomhin, O.A. and Peters, O.T. (2002). *Evaluation of crude extracts from some Nigerian plants for the control of field diseases of sesame (Sesamum indicum L.)*. Trop. Oilseeds J., 7: 84-93.
16. Ezike, A.C; Akah, PA; Okoli, CO; Udegbonam, S; Okwume, N; Okeke, C; Iloani, O. (2010). "Medicinal Plants Used in Wound Care: A Study of *Prosopis africana* (Fabaceae) Stem Bark". *Indian J Pharm Sci* 72 (3): 3349.p.
17. Fiori, A.C.G (2000). Antifungal Activity of Leaf Extracts and Essential Oils of some Medicinal Plants against *Didymella bryoniae*. *Journal of Phytopathology*. 148: 483–487. doi:10.1046/j.1439-0434.2000.00524.x
18. Fu, P.P., Yang, Y.C., Xia, Q., Chou, M.C., Cui, Y.Y., Lin G. (2002). "Pyrrolizidine alkaloids-tumorigenic components in Chinese herbal medicines and dietary supplements", *Journal of Food and Drug Analysis*, 10 (4): 198-211. pp.
19. Ganguli, S. (2002). "Neem: A therapeutic for all seasons". *Current Science*. 82(11):1304 P.

20. Jabeen K, Javaid A (2008). Antifungal activity of aqueous and organic solvent extracts of allelopathic trees against *Ascochyta rabiei*. *Allelopathy J.*, 22: 231-238.
21. Jabeen K, Javaid A, Ahmad E, Athar M (2011). *Antifungal compounds from Melia azedarach leaves for management of Ascochyta rabiei, the cause of chickpea blight*. *Nat. Prod. Res.*, 25(3): 264-276.
22. Jabin, M. (2003). *In vitro inhibitory activity of cow urine, cow dung and some medicinal plant extracts against Fusarium semitectum Berk & Rav of CHillsi*. M Sc Thesis, Department of Botany, Chittagong University, Bangladesh. 107 pp.
23. Kanwal Q, Hussain I, Latif S. H, Javaid A. (2010) "*Antifungal activity of flavonoids isolated from mango (Mangifera indica L.) leaves*." *Nat Prod* (20):1907-14
24. Kareru P.G., Keriko J.M., Kenji G.M., Gachanja A.N (2010) "*Anti-termite and antimicrobial properties of paint made from Thevetia peruviana (Pers.) Schum. oil extract*" *African Journal of Pharmacy and Pharmacology* 4:2 (087-089)
25. Kokwaro J. O. (1976). *Medicinal plants of East Africa*. East African Literature Bureau.
26. Kudi A. C, Umoh, J.U. and Eduve, L.E (1999). *Screening of some Nigeria Medicinal plants for antibacterial activity*. *J. Ethnopharmacol.* 69(2) 225-8.
27. Laurens (1982). Study of antimicrobial activity of *Anarcadium occidentale*. *Ann. Pharm. Fr.* 40(2) 143-6.
28. Little, E.L. (1983). *Common fuelwood crops: a handbook for their identification*. McClain Printing Co., Parsons, WV.
29. Loper, J.E., M.D. Henkels, R.G. Roberts, G.G. Grove, M.J. Willett and T.J. Smith. (1991). Evaluation of streptomycin, oxytetracycline, and copper resistance of *Erwinia amylovora* isolated from pear orchards in Washington State. *Plant Disease*, 75: 287-290.
30. Makinde, J.M. & Obih, P.O., (1985). Screening of *Morinda lucida* leaf extract for antimalarial action on Plasmodium berghei berghei in mice. *African Journal of Medicine and Medical Sciences* 14(1-2): 59-63.
31. Mendes N.M., de Oliveira A.B., Guimareas J.E., Pereira J.P., Katz N. (1990). Molluscicide activity of mixture of 6-n-alkyl salicylic acids (anacardic acid) and 2 of its complexes with Copper (II) and lead (II). *Rev. Soc. Bras. Med. Trop.*, 23 (3): 217-24.
32. Mota M.L, Thomas G, Barbosa-filho J.M (1985). Anti-inflammatory actions of tannins isolated from the bark of *Anacardium occidentale* L. *J. Ethnopharmacol.* 13(3): 289-300.
33. Nico, A.I., Mónaco C; Dal Bello G.; Alippi Y.H. (2005). *Efectos de la adición de enmiendas orgánicas al suelo sobre la capacidad patogénica de Rhizoctonia solana. II. Microflora asociada y antagonismo in vitro de los aislados más frecuentes*. *RIA* 34:29-44. *Triterpens from antibacterial Camphora Africans: a preliminary Report*.
34. Oderinde, O; Noronha, C; Oremosu, A; Kusemiju, T; Okanlawon, OA (2002). "Abortifacient properties of Carica papaya (linn) seeds in female Sprague-Dawley rats". *Niger Postgrad Medical Journal* 9 (2): 95-8.
35. Ogunbosoye, D. O.; Babayemi, O. J. (2010). *Potential values of some non-leguminous browse plants as dry season feed for ruminants in Nigeria*. *Afr. J. Biotech.*, 9 (18): 2720-2726.
36. Okigbo, R. N. and Emoghene, A.O. (2003). *Effect of leaf extract of three plant species on Mycosphaerella fijiensis Morelet, the causal organism of Black Sigatoka disease of Banana (Musa acuminata)*. *Niger. J. Pl. Prot.*, 20: 101-110.
37. Okorie, J.M, Okonkwo, T J.N. and Okonta (2010). Sub- chronic hepatotoxicity of *Anarcadium occidentale*. *Indian journal of pharmacology science*. 72(3) 353-357
38. Olowe, V.I.O. (2007). *Optimum planting date for sesame (Sesamum indicum l.) in the transition zone of south west Nigeria*. *Tropical ET subtropica*. 40 (4) 156-164
39. Oluma, H.O.A. and Elaigwu, M. (2006). *Antifungal activity of extracts of some medicinal plants against Macrophomina phaseolina (Tassi). Goid*. *Nigeria Journal of botany* 19:121-128.
40. Oluma, H.O.A. and Garba, I.U. (2004). Screening of *Eucalyptus globulus* and *Ocimum gratissimum* against *pythium aphanidermatum*. *Nigeria Journal of plant protection* 21; 109-114.
41. Omojasola, P.F. and Awe s. (2004). The antibacterial activity of the leaf extracts of *anarcadium occidentale* and *Gossypium hirsutum* against some selected microorganisms. *Bioscience Research Communications*, 60(1):25-58.



42. Prince L. and Prabakaran, P (2011). Antifungal activity of medicinal plants against plant pathogenic fungus *Colletotrichum falcatum*. *Asian Journal of Plant Science and Research*, 1 (1):84-87.
43. Ramemezani, H., Singh, H.P., Batish, D.R., Kohli, R.R. and Dorgan, J.S.(2002). Fungicidal effect of the volatile oils from *Eucalyptus citridorai* and its major citronella. *New Zealand plant protection* 55:3227-330.
44. Reddy, P.S, Jamil K, Madhusdhal P (2001) *Pharma. Biol*, 39, 236-238.
45. Saxena, V. K. & Albert, S. (2005). B-Sitosterol-3-O- $\beta$ -D-xylopyranoside from the flowers of *Tridax procumbens* Linn. *J Chem Sci* 117:3 263-266
46. Shadab, Q., Hanif, M. & Chaudhary, F.M. (1992) *Antifungal activity by lemongrass essential oils*. *Pak. J. Sci. Ind. Res.* 35, 246-249.
47. Shokalu, O., Enikuomehin O.A., Idowu, A.A. and Uwala, A.C. (2002). *Effects of seed treatment on the control of leaf blight disease of sesame*. *Trop. Oilseeds J.*, 17: 94-100.
48. Silva L.L., Heldwein C.G., Reetz L.G.B., Hörner R., Mallmann C.A., Heinzmann B.M. (2010). Chemical composition, antibacterial activity in vitro and brine-shrimp toxicity of the essential oil from inflorescences of *Ocimum gratissimum* L. *Brazilian Journal of Pharmacognosy*, 20(5): 700-705.
49. Singh, H. B. and Singh, U. P (1980). Inhibition of growth and sclerotium formation in *Rhizoctonia solani* by garlic oil. *Mycologia* 72: 1022-1025.
50. Stoll, G. (2000). Natural crop protection in the tropics. *Letting information come to life*. F&T muller bader Fildsttdt publishers, Germany 208p.
51. Suratuzaman, M., Islam, M. R. and Hossain I. (1995). *Effect of plant extracts and chemicals in controlling seed borne fungi of soybean*. *Progress. Agric.*, 1&2: 39-42.
52. Tapia A, Egly F.G, Bustos D, Astudillo L, Theoduloz C, Schmeda-Hirschmann G. (2000). "Biologically active alkaloids and a free radical scavenger from *Prosopis* species". *J Ethnopharmacol* 71 (1-2): 241-6.
53. Tewari, S.N. and Nayak, M. (1999). *Activity of four plant extracts against three fungal pathogens of rice*. *Trop. Agric (Trinidad)*, 68:373-375.
54. Tiwari, S. and Singh, A. (2004). Toxic and sub-lethal effects of oleadriin on biochemical parameters of freshwater air breathing murrel, *Chant punctatus* (Bloch.). *Indian J. Exp. Biol.*, 42: 413-418.
55. Vimala, R., Suriachandraselven, M., Murugesan, N. and RaMalingam, A. (2009). *Management of stem weevil and root rot complex in cotton*. *J. Biopesticides*. 2:18-21.
56. World Agroforestry Centre, 2008 Agroforestry Database.
57. <http://www.worldagroforestrycentre.org/sites/treedbs/aft/speciesPrinterFriendly.asp?Id=1346>.
58. Yamauchi, M; Tsuruma, K; Imai, S; Nakanishi, T; Umigai, N; Shimazawa, M; Hara, H (2011). "Crocetin prevents retinal degeneration induced by oxidative and endoplasmic reticulum stresses via inhibition of caspase activity". *European journal of pharmacology* 650 (1): 110-9
59. Zainab, M., Ahmed, S. and Mariam T. (2009). *Fungicidal potential of some local tree seeds for controlling root rot disease*, *pak. j. bot.*, 41(3): 1439-1444