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Bio activity of some botanicals on *Helminthosporium infestans* L. and *Solanum aethiopicum* L. (host)

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Some botanicals were assayed in two laboratory trials for their relative toxicities to African eggplant (*Solanum aethiopicum* L.) and *Helminthosporium infestans* causing leaf spots disease at the Department of Crop Science, University of Nigeria, Nsukka. In the first experiment on the toxicity of the protectants to *S. aethiopicum*, three concentrations (10, 20 and 30 g/ml) were used. Four concentrations (0.03, 0.06, 0.12 and 0.25 g/ml) were used in the second experiment on the toxicity of the botanicals (*Azadirachta indica* leaves, *Gongronema latifolia* leaves, *Garcinia kola* seeds, *Zingiber officinale* stems and *Carica papaya* leaves) to *H. Infestans*. Distilled water served as control in each case. *Solanum* seeds dressed with *G. kola* germinated faster and attained 100% germination 14 days after incubation (DAI). On the other hand, *Z. officinale* inhibited the seed germination most, resulting in the least germination being recorded on seeds treated with *Z. officinale*. Similarly, all the plant extracts assayed inhibited the growth of *H. Infestans* to varying degrees relative to check at 3 days after inoculation. Anti-fungal activity of extracts on *H. Infestans* decreased in the following order: *G. kola* at 0.12 g/ml (90.00%) > *C. papaya* at 0.25 g/ml (59.54%) > *G. kola* at 0.06 g/ml (59.15%) > *G. kola* at 0.03 g/ml (55.15%) > *A. Indica* at 0.06 g/ml (53.67%) > *Z. officinale* at 0.03 g/ml (52.65%) > control (7.71%). The seeds extracts of *G. kola* at 0.12 g/ml therefore proved more effective in the suppression of *H. infestans* relative to other botanicals and concentrations and were be recommended as a good alternative in its control in the field.

Key word: African eggplant anti-fungal, inhibition, protectants, toxicity.

INTRODUCTION

The African eggplant (*Solanum aethiopicum* L.*)*, known as garden egg (Hausa: Dauta; Igbo: añara; Yoruba: Igbagba) is one of the important vegetable crops grown almost worldwide and are highly valued constituents of Nigerian foods and an indigenous medicine.The name eggplant is derived from the shape of the fruits of some varieties which are white and have the shape of chicken eggs. It is commonly consumed almost on daily basis by both rural and urban families (Tindall, 1965). Eggplant can be grown in all parts of Nigeria all the year round. It is grown commercially as an annual crop; the plant is a short- lived perennial branching herb with a height of 0.5 to 1.5 m. It forms part of the traditional sub-sahara African culture are said to represent blessings, fruitfulness and are offered as a token of goodwill during visits, marriages and other social events (Osei et al., 2010)

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.The fruits can be eaten in various forms without the need for an elaborate preparation. It is eaten raw, cooked or used to season other foods. Eggplant supplements starchy foods. It is also a cheap source of protein, minerals and vitamins (Lombin and Yayock., 1988). The tender green leaves of some species are also used as vegetables or eaten raw in African salads, *ugba*. It can be eaten as appetizer.

The African eggplant (*S. aethiopicum* L.*)* is affected by several fungal diseases which inflict heavy losses in its production. One of such fungal disease is the leaf spot disease. The disease can infect seedlings but generally is a problem of older plants. Lower leaves are attacked first and then the disease progresses upwards. Dark-brown spots with concentric rings develop on the leaves, which give target board effect, the most characteristic symptom of the disease. In humid weather, affected areas coalesce and form dark-brown patches. In severe attacks, affected leaves shrivel and fall down pre-maturely resulting in early defoliation. On the leaves, the spots appear as small angular scattered, light-brown in colour, progressing between veins towards the leaflet margin. The severely infected leaflets curl and dry out pre-maturely. Subsequently, the pathogen invades the adjoining healthy leaflets and gradually progresses on the foliage upwards from the infected leaves. Elongated dark brown lesion also appears on the stem and branches (Gupta and Barnerjee, 1970). Severely infected leaves drop off prematurely resulting in the reduction of yield. Due to environmental concerns, great emphasis has been laid on alternative measures other than chemicals, to control this fungal disease. The use of botanicals and antimicrobial agents of plant origin is a time –honored practice for control of plant diseases and pests. The necessity to develop a non-toxic, safe and biodegradable alternative to synthetic fungicides has in recent years led to a concerted effort at developing new control measures from plant parts. The humid especially the rainforest ecological zones are endowed with abundant flora of families of plants and herbs with untapped pesticides potentials (Amadioha, 2003, 2004). Stoll (2000) listed an array of plant families and genera possessing antimicrobial properties, amongst which were *Azadirachta indica, Zingiber officinale, Garcinia kola, Carica papaya, Gongronema latifolium* and host of others. Amadioha (2003), Kumar and Pamar (1996) and Prakash and Roa (1997) listed some of the advantages of plant extracts over synthetic chemicals to include possession of low mammalian toxicity, minimal health hazards and environmental pollution. There is practically no reported risk of developing pest resistance to these products when used in their natural forms. Also, no side effect on plant growth, seed viability or food quality has been reported. Botanicals are less expensive and easily available because of their natural occurrence. Synthetic fungicides are expensive and inaccessible to indigenous farmers who are the bulk producers of eggplant in Nigeria (Amadioha, 1998; Onuegbu et al., 2001). A natural plant product with fungicidal properties could be more environmental friendly than synthetic fungicides.

Aqueous extracts of some plants have been used in laboratory bioassays (John and James, 2004). These plants include *Allium cepa* (onion), a biennial herb of Liliaceae family used commonly as spice for flavoring food. *Allium sativum* L. (garlic), another biennial herb of Liliaceae family and the second most widely use *Allium* after *A. cepa;* it is used as condiments for flavoring foods. Stoll (1998) reported the bactericidal properties of *Azadirachta indica* A Juss (neem), a fast growing tree of the family Meliaceae and also a medicinal plant with insecticidal, nematicidal, antifungal and bactericidal properties. It occupies a foremost status among all the plants exploited so far for bio-efficacy against pests and diseases (Kumar and Pamar, 1996). The primary antimicrobial constituents are Azadirachtin A and B. In addition, Neem contains a good number of other chemical substances which include Salannin, Meliantriol, Azadirachtannin A, Cinnamoyl, Isoazadirohide, Nimbin/Nimbidin, which seem to have anti- viral effects as well as Vilasinim as isolated from the leaf and Azadirone from the seed. *Garcinia kola* Henkel (bitter cola) is a perennial tree in the family Guttiferae with whorled leathery leaves. The seeds are chewed as stimulants and for other various medicinal values. Traditionally, the seeds are believed to repel snakes. *Zingiber officinale* Rose (Ginger) is rhizome of the family Zingiberaceae. The rhizome yields essential oil, oleoresin, consisting 1 to 3% volatile of which serve as the active ingredient against microorganisms and pests (Benjilali et al., 1984).

Medicinal plant materials have been successfully used for the treatment of fungal and bacterial infections in humans (Akinyosoye and Oladunmoye, 2000), suggesting that some plant materials may also possess antifungal and antibacterial constituents which are useful in controlling plant diseases (Amadioha, 1998). Previous reports (Akpomedaye and Ejechi, 1998; Ejechi and Ilondu, 1999; Ejechi and Akpomedaye, 1999) show that spices, herbs and other plant materials possess antifungal activity. Akinyosoye and Oladunmoye (2000) have reported the antifungal efficacy of stem and leaf-extracts of *Mirabilis jalapa* L. in reducing mycelia growth of four different strains of fungi. The legendary medicinal qualities of the neem tree have been known for a long time and the aqueous leaf extract have systemic action (Egunjobi and Onoyerni, 1981; Sowunmi and Akinusi, 1983). The toxic effects of some plant extracts on fungal activities is an indication that such plants could be used as fungicides especially by the peasant farmers who cannot afford the costly synthetic agrochemicals to control fungal diseases that attack their crops.

The research was therefore to determine the bio-activity of plant extract on eggplant and disease organism (*Helminthosporium infestans*).

MATERIALS AND METHODS

Two laboratory experiments were carried out at the Department of Crop Science, University of Nigeria, Nsukka. Nsukka is located in the derived Savannah Zone (06° 52' N, 07° 24'E and altitude of 447.26 m above sea level).

Determination of the bio activity of plant extract on eggplant

In this experiment, 5 locally available plants were evaluated for their ability to inhibit the growth of the fungus responsible for the leaf spot found on the leaves of the eggplants. The 5 test plants selected for the assessment were as follows (*A. indica* leaves, *G. kola* seeds, *Z. officinale* stems, *G. latifolia* leaves and *C. papaya* leaves). Distilled water served as a control.

To determine if botanicals could be harmful to plants at certain concentrations thought to be toxic to the pathogens, bio activity test was carried out. The botanicals selected for use as antimicrobial agents were assessed for bio-activity effect at germination stage of the plant.

Sources of plant materials

The fresh leaves of *A. indica* was obtained from the botanical garden, Department of Botany, *G. latifolia* and *C. papaya* were obtained from Department of Crop Science farm, University of Nigeria, Nsukka while *Z. officinale* stems and *G. kola* seeds were bought from Ogige Main Market, Nsukka.

Preparation of the plant extracts

Fresh leaves (the lower leaves) of the tested vegetable species were washed separately under tap water, rinsed with sterile distilled water and allowed to dry in a glasshouse. The dried leaves, stems and seeds were mashed and ground using electric milling machine to a fine powder.

Hot water extraction

Dried fine powder of each plant materials (5 g) were put in beaker separately and 200 ml of distilled water was added. The mixtures were heated on a hot plate with continuous stirring at 30 to 40°C for 20 min, allowed to cool and filtered through cheese cloth. The filtrates obtained were used for the phytochemical analysis. The water extract was kept in refrigerator for further use.

Ethanol extraction

Each powder (200 g) was soaked in 600 ml of analytical ethanol. These mixtures were left to stand for 24 h after which they were filtered with cheesecloth and the supernatant obtained were concentrated to dryness in an oven (100 to 105°C). The dry supernatant of each was used as the crude plant extracts.

Germination test

Twenty seeds of Eggplant were placed in a sterile Petri dish with sterile filter paper (Whatman No.1) and replicated 3 times so that each treatment had 20 seeds. Using sterile syringe, 1 ml each of the 5 plant extracts was applied at different concentrations (10, 20, 30 100 g/L). The filter paper was previously moistened with sterile water before adding the extracts. The extracts where applied in the morning. There was a control, which was treated with distilled water. The Petri dishes had their lids covered and incubated for 14 days at room temperature. At 7 and 14 days of incubation, percentage seed germination was recorded.

In vitro control of the organism using plant extracts and synthetic fungicides

Assay of plant extracts

The efficacy of the tested extracts against the fungi; *H. Infestans* followed the hyphal growth inhibition technique (Palanichamy and Nagarajan., 1990). Then, 1 g (100 mg) of each tested extracts was dissolved in 4 ml of dimethysulfoxide (DMSO) and mixed thoroughly to obtain the test solution (250 mg/ml per each extracts). From the test solution, serial 2-fold dilution were made using three different test tubes and concentrations (0.03125 mg/ml, 0.0625 mg/ml, 0.125 mg/ml and 0.25 g/ml) were obtained from each extract respectively. Aliquots of 1 ml of each concentration of the different plant extracts were separately and asceptically introduced into the conical flasks containing 19 ml of cool, sterilized PDA. Two drops of streptomycin were added to the mixture, which was gently swirled to obtain even distribution of the plant extracts, PDA and the antibiotics. This mixture was poured on the sterilized 9 cm Petri dishes and allowed to stand for 24 h. Discs (5 mm), taken from the advancing margins of a pure culture of *H. infestans*, with the aid of a sterilized spatula were placed on the centre of each Petri dishes.

Assay of synthetic fungicides

The fungicides used were bought from an agrochemical dealer and they were;

(1) Ridomil Plus 66 – WP [(both systemic and contact fungicide) - 12% metalaxyl- M and 60% copper (1) oxide)]
(2) Conti-zeb “5” to 80% WP (Contact fungicide – 80% mancozeb)
(3) Total 5% SC (Systemic fungicide – 5 % hexaconazole)
(4).Kocide 2000 (contact fungicide - 53.8% Copper hydroxide)

The same procedures and set ups employed in the plant extracts assay were used here except that the DMSO used in dissolving the plant extracts was replaced with distilled water. Thus, 4 ml of distilled water was used to dissolve 1 g (100 mg) of Ridomil Plus 66-WP, Conti-zeb “5’’ to 80% WP, Kocide 2000 and Total 5% SC to obtain 250 mg/ml concentration. Two fold dilutions were also obtained and concentrations (0.03125, 0.0625, 0.125 and 0.25 g/ml) were obtained, respectively. Every other procedure remained the same as that of the plant extracts assay. Cultured plate with neither plant extract nor synthetic fungicides is the control. All inoculated plates were incubated at 28°C. Data on mycelial growth in terms of colony diameter of the pathogenic fungus were taken after 3, 7 and 14 days after inoculation. The percentage growth inhibition or the minimum inhibitory rate were assessed and recorded. Growth inhibitions were obtained by measuring the colony growth diameter, taken as mean of the widest and the shortest diameter. The percentage growth inhibitions were determined using the formula adopted by Amadioha (2003, 2004) as follows:

$$\text{Percentage growth inhibition} = \frac{dc-dt}{dc} \times \frac{100}{1}$$

Where, dc = colony diameter of control. dt = colony diameter of treated plates.

Table 1. Effect of plant extracts concentrations on mean days to seed germination.

Plant extracts	Concentration (g/L)			Plant extracts means
	10	20	30	
G. kola	4.67	4.67	4.67	4.67
Z. officinale	5.00	12.67	7.33	8.33
A. indica	5.33	5.33	6.67	5.78
C. papaya	6.33	7.00	14.67	9.33
G. latifolia	9.00	8.33	7.67	8.33
Water	5.33	5.33	5.33	5.33
Conc. Means	5.94	7.22	7.72	6.96

F-LSD $_{(0.05)}$ for comparing any 2 plant extract means =1.95; F-LSD $_{(0.05)}$ for comparing any 2 comparing concentration means = NS; F-LSD $_{(0.05)}$ for comparing any 2 plant extract x concentration means = 3.37.

Table 2. Effects of plant extract concentrations on mean days to 50 percent seed germination.

Plant extracts	Concentration (g/L)			Plant extract means
	10	20	30	
G. kola	7.67	8.33	8.00	8.00
Z. officinale	17.33	21.00	16.67	18.33
A. indica	8.00	11.00	14.00	11.00
C. papaya	12.33	11.00	16.67	13.33
G. latifolia	10.67	11.00	11.33	11.00
Water	7.33	7.33	7.33	7.33
Conc. Means	10.56	11.61	12.33	11.50

F-LSD $_{(0.05)}$ for comparing any 2 plant extract means =3.34; F-LSD $_{(0.05)}$ for comparing any 2 comparing concentration means= NS; F-LSD $_{(0.05)}$ for comparing any 2 plant extract x concentration means = 5.78.

The experimental design was a 9 × 4 factorial (four plant extracts + four fungicides + distilled water x four concentrations) in a completely randomized design (CRD). Data on the colony growth diameter were transformed to their respective square root value prior to statistical analysis, as the residuals were not normally distributed$\sqrt{X+0.5}$; where X is the colony growth diameter (Bartlett, 1937). Means were later compared using Fisher's LSD procedure as outlined by Obi (2002). Data were analyzed using GENSTAT 5.0 Release 4.23 DE (GENSTAT, 2003). Percentage growth inhibitions were angular transformed (Arc Sin √ Percentage) before analysis of variance (ANOVA).

RESULTS

Bio activity of the different plant extracts on eggplant seeds

Plant extracts concentrations on mean days to seed germination and 50 percent seed germination

The effect of the three concentrations (10, 20, 30 g l^{-1}) of the 5 plant extracts and distilled water on days to seed germination showed that there was significant effect ($p<0.05$) on the plant extracts and the interaction between plant extracts and concentrations (Table 1). The data revealed that *G. kola* (4.67 days) treated seeds germinated faster than other plant extracts and distilled water at all concentrations, those treated with while *C. papaya* (12.67 days) at 20 g/L concentration delayed seed germination. No statistical significant effect was obtained in different concentrations. The effect of the three concentrations (10, 20, 30 g l^{-1}) of the 5 plant extracts and distilled water on days to 50% seed germination showed that the plant extracts and their combinations with the various concentrations significantly ($p<0.05$) influenced 50% seed germination (Table 2). The result revealed that seeds soaked with distilled water (7.33 days) and *G. kola* (8.00 days) were statistically similar ($p<0.05$) in seed germination when compared to other plant extracts. The seeds took 7 to 8 days to attain 50% germination while those treated with *Z. officinale* took up to 18 days to reach 50% seed germination. On the interactions, *G. kola* at 10 g/L concentration took 8 days to reached 50% seed germination, while *Z. officinale* at 20 g/L concentration took 21 days to attain 50% seed germination. However, no significant effect was seen on the different concentrations.

Table 3. Effects of plant extract concentrations on mean days to 100% seed germination.

Plant extracts	Concentration (g/L)			Plant extract means
	10	20	30	
G. cola	12.00	12.67	12.00	12.22
Z. officinale	19.00	21.00	19.00	19.67
A. indica	12.00	13.00	16.00	13.67
C. papaya	18.67	14.00	21.00	17.89
G. latifolia	16.67	14.33	18.00	16.33
Water	12.00	12.00	12.00	12.00
Conc. Means	15.06	14.50	16.33	15.30

F-LSD $_{(0.05)}$ for comparing any 2 plant extract means = 2.40; F-LSD $_{(0.05)}$ for comparing any 2 comparing concentration means = NS;F-LSD $_{(0.05)}$ for comparing any 2 plant extract x concentration means = 4.16.

Table 4. Effects of plant extract concentrations on mean days to leaf formation.

Plant extracts	Concentration (g/L)			Plant extract means
	10	20	30	
G. kola	7.67	7.67	7.67	7.67
Z. officinale	7.00	15.00	9.67	10.56
A. indica	8.00	7.67	8.33	8.00
C. papaya	8.33	9.33	16.67	11.44
G. latifolia	11.33	10.00	9.33	10.22
Water	7.00	7.00	7.00	7.00
Conc. Mean	8.22	9.44	9.78	9.15

F-LSD $_{(0.05)}$ for comparing any 2 plant extract means = 1.66; F-LSD $_{(0.05)}$ for comparing any 2 comparing concentration means = NS; F-LSD $_{(0.05)}$ for comparing any 2 plant extract x concentration means = 2.87.

Plant extract concentrations on mean days to 100 percent seed germination and leaf formation

The effect of the three concentrations (10, 20, 30 g l^{-1}) of 5 plant extracts and distilled water on days to 100 percentage germination showed that plant extracts significantly ($p<0.05$) affected the days on 100% seed germination (Table 3). There were 100% germination on the plates treated with *G. kola* (12.22 days) and distilled water (12.00 days) at the 12th day of incubation. It took up to 20 days for the whole seeds in the ginger plates to germinate. No statistical effect was obtained on the different concentrations. Seeds treated with *G. kola* and distilled water was statistically the same ($p\leq0.05$). On the interactions, *G. kola* at 10 g/L concentration took 12 days to attain 100% seed germination while *Z. officinale* at 20 g/l concentration took 21 days to attain 100 seed germination.

The effect of the three concentrations (10, 20, 30 g l^{-1}) of the 5 plant extracts and distilled water on days to leaf formation showed that that the plant extracts and their combinations with the various concentrations significantly ($p<0.05$) influenced leaf formation (Table 4). The first leaf formation was seen on the seeds soaked with distilled water (7.00 days) at all concentrations. Although, it was significantly the similar with those treated with *G. kola* (7.67 days). Seeds in *C. papaya* extracts were the last to form leaves (11.44 days). On the interactions, *Z. officinale* at 20 g/L concentration delayed leaf formation (15.00 days). No significant effect was seen on the different concentrations.

Plant extract concentrations on mean percentage seed germination at 7 and 14 days of incubation

The effect of the three concentrations (10, 20, 30 g l^{-1}) of the 5 plant extracts and distilled water on percentage germination at 7 days (Table 5) showed that that the plant extracts and their combinations with the various concentrations significantly ($p<0.05$) influenced the percentage seed germination at 7 days of incubation. Distilled water (68.30%) and *G. kola* had the highest percentage germination (53.30%) at all the concentrations. The distilled water and *G. kola* were significantly higher ($p<0.05$) than other plant extracts.

Table 5. Effects of plant extract concentrations on mean percentage seed germination at 7 days of incubation.

Plant extracts	Concentration (g/L)			Plant extract means
	10	20	30	
G. cola	53.30	46.70	53.30	51.10
Z. officinale	5.00	0.01	20.00	8.30
A. indica	46.07	15.00	10.00	23.90
C. papaya	5.00	3.30	1.70	3.30
G. latifolia	26.70	3.30	31.70	20.60
Water	68.30	68.30	68.30	68.30
Conc. mean	34.20	22.80	30.80	29.30

F-LSD $_{(0.05)}$ for comparing any 2 plant extract means =20.47; F-LSD $_{(0.05)}$ for comparing any 2 comparing concentration means = NS.F-LSD $_{(0.05)}$ for comparing any 2 plant extract x concentration means = 35.46.

Table 6. Effects of plant extract concentrations on mean percentage seed germination at 14 days of incubation.

Plant extracts	Concentration (g/L)			Plant extract means
	10	20	30	
G. kola	100.00	100.00	100.00	100.00
Z. officinale	41.70	11.70	45.00	32.80
A. indica	100.00	88.30	63.30	83.90
C. papaya	58.30	86.70	25.00	56.70
G. latifolia	73.30	91.70	68.30	77.80
Water	100.00	100.00	100.00	100.00
Conc. Mean	78.90	79.70	66.90	75.20

F-LSD $_{(0.05)}$ for comparing any 2 plant extract means =19.11; F-LSD $_{(0.05)}$ for comparing any 2 comparing concentration means= NS;F-LSD $_{(0.05)}$ for comparing any 2 plant extract x concentration means = 32.10.

Also, the interaction between the plant extracts and concentrations showed significant difference ($p<0.05$). Seeds treated with *G. kola* at 10 g/L concentration had the highest seed germination (53.30%) while seeds treated with *Z. officinale* (0.01%) at 20 g/L concentration had the lowest seed germination. No significant effect was seen on the different concentrations. The effect of the three concentrations (10, 20, 30 g l^{-1}) of the 5 plant extracts and distilled water on percentage germination at 14 days of incubation (Table 6) showed that the plant extracts and their combinations with the various concentrations significantly ($p<0.05$) affected percentage seed germination at 14 days of incubation. Distilled water (100%) and *G. kola* (32.80%) attained 100% seed germination at all concentrations on the 14th day of incubation while *Z. officinale* recorded the lowest percentage germination. The distilled water and *G. kola* at 10 to 30 g/L concentrations were significantly higher ($p<0.05$) than other plant extracts. The interactions between the plant extracts and the concentrations showed significant effects. Only 12 seeds germinated on the plate treated with *Z. officinale* at 20 g/L concentration. No statistical effect was seen on the different concentrations.

Number of dormant seeds after 14 days of incubation

The effect of the three concentrations (10, 20, 30 g l^{-1}) of the 5 plant extracts and distilled water on number of dormant seeds at 14 days of incubation (Table 7) showed that there was significant effect ($p<0.05$) among the plant extracts on the dormant seeds. The number of dormant seeds after 14 days were highest in seeds treated with *Z. officinale* extracts (13 .44). No dormant seed was seen on the plates treated with *G. kola* and distilled water at all concentrations after 14 days of incubation. No significant effect was seen on the different concentrations.

Table 7. Effect of plant extracts concentrations on the mean number of dormant seeds at 14 days of incubation.

Plant extracts	Concentration (g/L)			Plant extract mean
	10	20	30	
G. kola	0.00 (0.71)	0.00 (0.71)	0.00(0.71)	0.00(0.71)
Z. officinale	11.67(3.41)	17.67(4.26)	11.00(3.39)	13.44(3.73)
A. indica	0.00(0.71)	2.33(1.68)	7.33(2.80)	3.22(1.93)
C. papaya	7.33(2.80)	2.67(1.76)	15.00(3.94)	8.33(2.97)
G. latifolia	5.33(2.41)	1.67(1.47)	6.33(2.61)	4.44(2.22)
Water	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)
Conc. Mean	4.06(2.14)	4.06(2.14)	6.61(2.67)	4.91(2.33)

F-LSD $_{(0.05)}$ for comparing any 2 plant extract means = 3.78; F-LSD $_{(0.05)}$ for comparing any 2 comparing concentration means = NS F-LSD $_{(0.05)}$ for comparing any 2 plant extract x concentration means=6.54 Figures in parentheses were square root transformed data and mean separation were based on them.

Table 8. Mean percentage inhibition of the plant extracts and synthetic fungicides 3 days after inoculation.

Treatment	Concentration				Treatment means
	0.03	0.06	0.12	0.25	
G. cola	68.4(55.80)	73.7(59.15)	100.0(90.00)	59.6(50.53)	75.4(60.27)
Z.officinale	63.2(52.65)	57.8(49.49)	57.8(49.49)	61.4(51.59)	60.1(50.83)
A. indica	57.9(49.54)	64.9(53.67)	52.6(46.49)	49.1(44.48)	56.1(48.50)
C.papaya	61.4(51.59)	52.6(46.49)	57.9(49.54)	74.3(59.54)	61.6(51.71)
Cu $(OH)_2$	57.9(49.54)	100.0(90.00)	82.5(65.27)	70.2(56.91)	77.6(61.68)
Metalaxyl-M	73.7(59.15)	79.0(62.72)	66.7(54.76)	100.0(90.00)	79.8(63.36)
Hexaconazole	100.0(90.00)	100.0(90.00)	100.0(90.00)	100.0(90.00)	100.0(90.00)
Mancozeb	100.0(90.00)	100.0(90.00)	100.0(90.00)	100.0(90.00)	100.0(90.00)
Control	1.75(7.71)	17.5(24.73)	17.5(24.73)	00.00(0)	9.21(17.66)
Conc. Means	64.9(53.67)	71.7(57.23)	70.6(57.17)	68.3(55.73)	68.9(56.11)

F-LSD $_{(0.05)}$ for comparing any 2 treatment means = 8.13; F-LSD $_{(0.05)}$ for comparing any 2 comparing concentration means = 6.55; F-LSD $_{(0.05)}$ for comparing any treatment x concentration means = 11.54; Mean separation were based on transformed data; Figures in parentheses were the arc-sine transformed data.

The mean percentage (%) inhibition of the plant extracts and synthetic fungicides 3 days after inoculation

All the plant extracts assayed inhibited the growth of the fungus to varying degrees when compared with the untreated control 3 days after inoculation (Table 8). Anti-fungal activity of *H. infestans* was highest with seed extracts of *G. kola* (90.00%) at 0.0120 g/ml concentrations followed by leaf extracts of *C. papaya* (59.54%) at 0.250 g/ml concentrations, then, 0.060 g/ml (59.15%) and 0.030 g/ml (55.80%) concentrations of *G. kola*. At these concentrations, leaves and seeds of *G. kola* and *C. papaya* respectively were significantly ($p<0.05$) more toxic to *H. infestans* than the other plant extracts tested. The result also indicated that these extracts were significantly ($p<0.05$) more efficacious in inhibiting the growth of *H. infestans* than the other plant extracts evaluated. The stem extracts of *Z. officinale* (52.65) and leaf extracts of *A. indica* (53.67) at 0.030 and 0.060 g/ml concentrations, respectively were also significantly higher ($p<0.05$) than the untreated control (17.66%). The fungicides; Hexaconazole (90%) and Mancozeb (90%) were very effective at all concentrations.

Mean percentage inhibition of the plant extracts and synthetic fungicides 7 days after inoculation

The data obtained showed that all the plant extracts inhibited the growth of the pathogen *H. infestans* to varying degree. The results indicate that the different plant extracts used in this study (Table 9) showed

Table 9. Mean percentage inhibition of the plant extracts and synthetic fungicides 7 days after inoculation.

Treatment	Concentration (g/ml)				Treatment mean
	0.030	0.060	0.120	0.250	
G. kola	75.00(60.00)	75.00(60.00)	76.00(60.67)	68.00(55.55)	73.50(59.08)
Z.officinale	66.00(54.33)	79.00(62.72)	63.00(52.53)	69.00(56.17)	69.25(56.35)
A. indica	64.00(53.13)	64.67(53.55)	65.33(53.91)	60.67(51.18)	63.67(52.95)
C.papaya	67.00(54.94)	58.33(49.78)	66.67(54.70)	75.00(60.00)	66.75(54.82)
CU (OH) $_2$	61.00(51.35)	61.00(51.35)	61.00(51.35)	59.00(50.18)	60.50(51.06)
Metalaxyl	60.00(50.77)	61.00(51.35)	61.00(51.35)	74.00(59.34)	64.00(53.13)
Hexaconazole	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
Mancozeb	67.00(54.94)	89.00(70.630	70.00(56.79)	80.00(63.44)	76.50(61.00)
Control	0.00(0.0)	0.00(0.0)	0.00(0.0)	0.00(0.0)	0.00(0.0)
Conc. Mean	62.22 (52.06)	65.33(53.91)	62.56(52.30)	65.07(51.94)	63.80(53.01)

F-LSD $_{(0.05)}$ for comparing any 2 treatment means = 6.29; F-LSD $_{(0.05)}$ for comparing any 2 comparing concentration means = 5.23; F-LSD $_{(0.05)}$ for comparing any treatment x concentration mean = 8.91; Mean separation were based on transformed data; Figures in parentheses were the arc sine transformed data.

significant difference ($p \leq 0.05$) in the mean percentage inhibition of mycelia growth of the fungus at 7 days after inoculation. The highest (62.72%) mean percentage inhibition rate were recorded on the stem extracts of *Z. officinale* at 0.06 concentrations. Also the seed extracts of *G. kola* had higher inhibitory effects (60.67%) at 0.12 and 0.06 (60.00%) concentrations, followed by the concentrations of 0.30 to 0.060 of the same material. The stem extracts of *Z. officinale* (56.35%) and the leaf extracts of *A. indica* (52.95%) and *C. papaya* (54.82%) at all concentrations also inhibited the growth of pathogen and were more effective than the fungicide; $Cu(OH)_2$ (51.06%).

Mean percentage inhibition of the plant extracts and synthetic fungicides 14 days after inoculation

The results indicate that the different plant extracts used in this study (Table 10) showed significant difference ($p<0.05$) in the mean percentage inhibition of mycelia growth of the fungus at 14 days after inoculation. The data revealed that the highest mean percentage inhibition of mycelia growth of the fungus (51.88%) were seen on the plates treated with *G. kola* while *A. indica* has the lowest percentage inhibition (30.26%). It also proved that the seeds extracts of *G. kola* (51.88%) was still very effective than fungicide Cu $(OH)_2$ (49.31%) even after 14 days. On the interactions, *G. kola* at 0.3 g/ ml concentration had the highest phytotoxic effect while *A. indica* at 0.03 g/ml concentration was the least. The fungicide hexaconazole (0.0) was the best treatment even after 14 days of inoculation, it had 100 percentage inhibitions. The inhibitory effect of the fungicide metalaxyl on mycelia growth increased with increase in concentration.

DISCUSSION

The effect of plant extracts concentration (10, 20, 30 g/ml) and distilled water on eggplant seeds showed no significant difference on the concentrations. However, differences existed among plant extracts and their interactions. Seeds treated with *G. kola* germinated faster and attained 100% seed germination at the 14th day of incubation, while seeds with *Z. officinale* had the least seed germination percentage. This could be attributed to high levels of flavonoid in *G. kola* which are health promoting in action (Ferguson, 2001). Again, *G. kola* gave the best protection against the pathogen *H. infestans* and that may also be the reason why it germinated faster than the other extracts tested. This observation of high germination percentage by *G. kola* agreed with the findings by Opara and Wokocha (2008) on the phytotoxicity test of the plant extracts on tomato seedlings. The use of plant extracts in disease control is eliciting much interest in developing countries due to high cost of synthetic pesticides and their hazardous effects on the environment (Schmutterer, 1990, Tovingan et al., 2001 and Salako, 2002). In the *in vitro* control experiment, the 4 plant extracts tested inhibited the growth of the pathogen, *H. infestans* to varying degree when compared with the untreated control. The anti-fungal activities to *H. infestans* were highest with the seed extracts of *G. kola* followed by the stem extracts of *Z. officinale* then leaf extracts of *C. papaya* and *A. indica*. The result also indicated that these plant extracts assayed were significantly ($p<0.05$) more toxic and more active than the fungicide; Cu(OH) $_2$ between 3 and 7 days of inoculation. The seed extracts of *G. kola* still proved more phytotoxic and effective than the other three plant extracts and fungicide $Cu(OH)_2$ even after 14 days. This observation of high phytotoxicity response by *G. kola*

Table 10. Mean percentage inhibition of the plant extracts and synthetic fungicides 14 days after inoculation.

Treatment	Concentration (g/ml)				
	0.030	0.060	0.120	0.250	Treatment means
G. kola	75.00(60.00)	61.000(51.35)	53.00(46.72)	58.67(50.010	61.92(51.88)
Z. officinale	44.00(41.55)	61.00(51.35)	52.00(46.15)	40.00(39.23)	49.33(44.60)
A. indica	0.00(0.0)	33.00(35.06)	48.00(43.85)	20.67(26.99)	25.42(30.26)
C. papaya	48.67(44.25)	35.67(36.69)	42.67(40.80)	56.00(48.45)	45.75(42.59)
Cu $(OH)_2$	58.00(49.60)	58.00(49.60)	58.00(49.60)	56.00(48.45)	57.50(49.31)
Metalaxyl	59.67(50.59)	59.67(50.67)	59.00(50.18)	74.00(59.34)	63.08(52.53)
Hexaconazole	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
Mancozeb	56.00((48.45)	89.00(70.63)	67.00(54.94)	80.00(63.44)	73.00(58.69)
Control	0.00(0.0)	0.00(0.0)	0.00(0.0)	0.00(0.00	0.00(0.0)
Conc. Mean	49.04(44.43)	55.26(48.04)	53.30(46.89)	53.96(47.24)	52.89(46.66)

$F\text{-}LSD_{(0.05)}$ for comparing any 2 treatment means =7.05; $F\text{-}LSD_{(0.05)}$ for comparing any 2 comparing concentration means = 5.65; $F\text{-}LSD_{(0.05)}$ for comparing any treatment x concentration means = 9.81;Mean separation were based on transformed data; Figures in parentheses were the arc –sine transformed data.

is consistent with the findings by Okereke and Wokocha (2006) on the effect of some tropical plant extracts, *Trichoderma harzianum* and Captan on the damping off of tomato induced by *Sclerotium rolfsii*. Amadioha (2002) reported that the differences in toxicity of different plant extracts were due to the presence of different active compounds in the plant materials. The active material in *A. indica* is *azadiratin*; *Z. officinale* consisted of linalool, imonene, zingeberl, zingerene, camphene, oleoresin (gingerol and shogoal), phenol (gingerol and zingibain), vitamin B_6 and vitamin C, calcium, magnesium, phosphorus, and potassium and linoelic acid (Kikuzaki and Nakatani.,1993). Sridhar et al. (2002) reported that the stem extracts of *Z. officinale* ground into paste and mixed with water and soap, sprayed thoroughly on the infected plant parts were effective in the control of American boll worm, aphids, plant hoppers and thrips. In *G. kola*, the active compounds responsible for anti-microbial, anti-viral and anti-inflammatory properties were bio-flavonoids, xanthenes and benzophenomes (Amadioha, 2003). The quantity phytochemical analysis done in this study showed that *G. kola* had the highest quantity of flavonoid (1.70 mg/100 g) than the other plant extracts tested. The anti-fungal activity observed in *C. papaya* may be due to the action of proteolytic enzyme papain which is the major component of paw-paw latex. These enzyme acts in adverse manner of the protein components of the fungal cell wall thereby hindering growth of these fungi. Igboko (1983) reported the presence of several chemical compounds (steroids, flavonoids, glucosides and protein) known to perform physiological activities against micro-organisms in *G. kola*. Ilondu (2011) observed the anti-fungal properties of crude leaf extracts of *C. papaya* in paw-paw fruits rot. Several researchers (Amadioha, 1998; Wokocha and Okereke, 2005; Wokocha, 2006) also reported the fungicidal activities of extracts of *A. indica, Z. officinale, C. papaya, G. kola* and other plant materials on *Erysiphe cichoracearum*, *Collectotrichum capsici* and *Protomycopsis phaseoli,* which compared favourably with the chemical pesticides; benlate and ridomil. Akpa et al. (1991) reported a significant inhibitory property of *A. indica* extracts on mycelia growth of *Collectotrichum graminicola*. Nzanza and Mashela (2012) in their field experiments with tomato found out that fermented plant extracts of neem and wild garlic, alone or in combination, have insecticidal properties to maintain lower population densities of whitefly and aphid.

To summarize, *G. kola* seeds extracts gave the highest phytotoxicity response to *H. infestans* which incites the leaf spot found on the leaves of eggplant. Again, they were seen to germinate faster and attained 100% seed germination at the 14^{th} day of incubation. It may be possible therefore to use *G. cola* as fungicide to control leaf spot in eggplant fields because of their availability, eco-friendliness and high levels of flavonoid that promotes health.

Conflict of Interest

The authors have not declared any conflict of interest.

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