Research Paper

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Anti-Fungal Activity of Some Botanicals On Helminthosporium Infestans, Causal Agent of Leaf Spot Disease in Eggplant, (Solanum Aethiopicum L.)

Agriculture

KEYWORDS : Eggplant, Inhibition, Phytotoxicity, Plant extracts,Protectants

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ABSTRACT

The effect of ethanolic extracts of Carica papaya and Azadirachta indica leaves, Zingiber officinale stem and Garcinia cola seeds at 0.030g/ml, 0.060g/ml, 0.120g/ml and 0.250g/ml concentrations on the leaf spot disease in eggplant (Solanum aethiopicum L.) caused by Helminthosporium infestans was tested in the laboratory using Potato Dextrose Agar (PDA). All the plant extracts assayed inhibited the growth of the fungus at different degrees when compared with the untreated control 14 days after inoculation. At 3 days of inoculation, the highest anti –fungal activities were observed for seed extracts of G. kola (90%) at 0.0120g/ml followed by leaf extracts of C. papaya (59.54%) at 0.250g/ml, then 0.030g/ml (59.15%) and 0.060g/ml (55.80%) of G. kola. At these concentrations, leaves and seeds of G. cola 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 plant extracts were significantly (P<0.05) more efficacious in inhibiting the growth of H. infestans than the fungicide; Kocide and Ridomil at 7 days of inoculation. The seed extracts of G. kola still proved more toxic than Kocide even after 14 days. The result of this study therefore showed that these plant extracts could be explored as an eco-friendly alternative to synthetic fungicides in the control of H. infestans on African eggplant.

INTRODUCTION

African eggplant (Solanum aethiopicum L.) known as garden egg, añara, etc. is one of the important vegetable crops grown almost worldwide. The name eggplant is derived from the shape of the fruits of some varieties which are white and have the shape of chicken eggs. 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 herb branching in habit with a height of 0.5-1.5m. The fruit 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 et al., 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 or offered to visitors as kola.

However, this species is affected by several fungal diseases which inflict heavy losses in its production. One of such fungal disease is the leaf spot disease, caused by Helminthosporium infestans. 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 to 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. 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). Also, a natural plant product with fungicidal properties could be more environmental friendly than synthetic fungicides.

Medicinal plant materials have been successfully used for the treatment of fungal and bacterial infections in humans (Akinyosoye and Oladummoye, 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 et al., 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 Onoyemi, 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 attacked their crops.

Therefore, this research aimed to determine the anti-fungal activity of extracts of Neem leaves (Azadirachta indica), Ginger stems (Zingiber officinale), Paw paw leaves (Carica papaya) and Bitter Kola seeds (Garcinia cola) and some synthetic fungicides on H. Infestans, causal agent of the leaf spot disease in African eggplant (S. aethiopicum L.)

MATERIALS AND METHOD

One laboratorial experiment was carried out at the Department of Crop Science, University of Nigeria, Nsukka. Nsukka is located in the derived Savannah Zone (06o 52' N, 070 24'E and altitude of 447.26 meters above sea level).

Bioassay

Sources of plant materials

Fresh leaves (the lower leaves)of A. indica was obtained from botanical garden, Department of Botany, 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 of the tested vegetable species were washed separately under tap water, rinsed with sterile distilled water and allowed to dry in a glass house. The dried leaves, stems and seeds were mashed and ground using electric milling machine to a fine powder.

Ethanol extraction

Each powder (200g) was soaked in 600 ml of analytical ethanol .These mixtures were left to stand for 24 hours after which were filtered with cheese cloth and the supernatant obtained were concentrated to dryness in an oven (100^0 C to 105^0 C). The dry supernatant of each was used as the crude plant extracts

Assay of Plant extracts

The efficacy of the tested extracts against the fungi; *H. infestans* followed the hyphal growth inhibition technique (Palanichamy *et al*., 1990).

Then, 1 g (100 mg) of each test 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, 0.0625, 0.125 and 0.25g/ml) were obtained from each extract respectively.

Aliquots of 1ml 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. The mixture was poured on the sterilized 9 cm petri dishes and allowed to stand for 24 hours. Discs (5mm), 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 with 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'' 80% WP (MANCOZEB) (Contact fungicide- 80% mancozeb)
(3) Total 5% S.C (Systemic fungicide-hexaconazole)
(4). Kocide 2000 (contact fungicides- Copper hydroxide 53.8%)

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, 4ml of distilled water was used to dissolve 1 g (100 mg) of Ridomil Plus 66WP, Conti-zeb "5" 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 remains 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 28oC. Data on mycelial growth in terms of colony diameter of the pathogenic fungus were taken after 3, 6, 7,9,10 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 inhibition was determined using the formula adopted by Amadioha (2003, 2004) as follows:

Percentage growth inhibition

$$= \frac{dc-dt}{dc} \times \frac{100}{1}$$

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

The experimental design was a 9x4 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, (√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 ANOVA

RESULT

Plant extracts and synthetic fungicides assay after 3 days of inoculation

The effect of ethanolic extracts of C. papaya and A. indica leaves, Z. officinale stem and G. kola seeds at 0.030g/ml, 0.060g/ml, 0.120g/ml and 0.250g/ml concentrations (Table 1) showed that there were significance effects ($P<0.05$) on the pathogen. All the plant extracts assayed inhibited the growth of the fungus to varying degrees when compared with the untreated control 3 days after inoculation. Anti –fungal activity of H. infestans was highest with seed extracts of G. cola (90.00%) at 0.0120g/ml concentrations followed by leaf extracts of C. papaya (59.54%) at 0.250g/ml concentrations, then, 0.060g/ml (59.80%) and 0.030g/ml (59.15%.) concentrations of G. kola. At these concentrations, seeds and leaves of G. cola 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 0.030g/ml and 0.060g/ml concentrations, respectively were also significantly higher ($P<0.05$) than the untreated control (17.66%). The fungicides; total fungicide (90.00%) and contizeb (90.00%) were very effective at all concentrations.

Table 1: Percentage inhibition after 3 days

	Concentration				
Treatment	0.03	0.06	0.12	0.25	Treatment means
G. kola	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)
Kocide	57.9(49.54)	100.0(90.00)	82.5(65.27)	70.2(56.91)	77.6(61.68)
Ridomil	73.7(59.15)	79.0(62.72)	66.7(54.76)	100.0(90.00)	79.8(63.36)
Total fung.	100.0(90.00)	100.0(90.00)	100.0(90.00)	100.0(90.00)	100.0(90.00)
Conti-zeb	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) =6.55	68.3(55.73)	68.9(56.11)

F-LSD for comparing any 2 treatment means
=8.13

F-LSD for comparing any treatment x concentration means
=11.54

F-LSD for comparing any 2 comparing concentration means

The mean separation were done based on the transformed data

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Figures in parenthesis are the transformed data

Plant extracts and synthetic fungicides assay after 7 days of inoculation

The results indicate that the different plant extracts used in this study (Table 2) showed significant difference (P≤0.05) in the mean percentage inhibition of mycelia growth of the fungus at 7 days after inoculation. Although, all the plant extracts tested had anti- fungal action on the pathogen H. infestans, the seed extracts of G.cola had the highest phytoxic effect (59.08%), followed by the stem extracts of Z. officinale(56.35%). These two extracts were also observed to be more toxic than the fungicide ridomil (53.13%). The result also revealed that the four plant extracts were more effective (p≤0.05) than the fungicide; kocide (51.06%). No significant effect was seen on the different concentrations. The fungicides total (90.00%) was the best

Table 2: Percentage inhibition after 7 days

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)
Kocide	61.00(51.35)	61.00(51.35)	61.00(51.35)	59.00(50.18)	60.50(51.06)
Ridomil	60.00(50.77)	61.00(51.35)	61.00(51.35)	74.00(59.34)	64.00(53.13)
Total Fung.	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
Conti-zeb	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 for comparing any 2 treatment means
=6.29

F-LSD for comparing any 2 comparing concentration means
=5.23

F-LSD for comparing any treatment x concentration means
=8.91

The mean separation were done based on the transformed data

Figures in parenthesis are the transformed data

Plant extracts and synthetic fungicides assay after 14 days of inoculation

The results indicate that the different plant extracts used in this study (Table 3) 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 Kocide (49.31%) even after 14 days and the need for regular application of the plant extracts at interval of 8-10 days because as the days increases, the phyto toxicity of the plant extracts decreases. The total (0.707) was the best treatment even after 14 days of inoculation. Fungicide total (0) has 100 percentage inhibitions. The inhibitory effect of the fungicide Ridomil on mycelia growth increased with increase in concentration.

Table 3: Percentage inhibition after 14 days

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)
Kocide	58.00(49.60)	58.00(49.60)	58.00(49.60)	56.00(48.45)	57.50(49.31)
Ridomil	59.67(50.59)	59.67(50.67)	59.00(50.18)	74.00(59.34)	63.08(52.53)
Total Fung.	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
Conti-zeb	56.00((48.45)	89.00(70.63)	67.00(54.94)	80.00(63.44)	73.00(58.69)
Control	0.00(0.00	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-LSD for comparing any 2 treatment means
=7.05

F-LSD for comparing any 2 comparing concentration means
=5.65

F-LSD for comparing any treatment x concentration means
=9.81

The mean separation were done based on the transformed data

Figures in parentheses were the transformed data

Discussion

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 (Tovingan et al., 2001; Salako, 2002 and Schmutterer, 1990). 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) 53.8 % 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) 53.8% even after 14 days. This observation of high phytotoxicity response by G. cola 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 consist;linalool, imonene, zingeberl, zingerene, camphene, oleoresin (gingerol and shogoal), phenol (gingerol and zingibain), vitamin B6 and vitamin C, calcium, magnesium, phosphorus, and potassium and linoelic acid (Kikuzaki et al.,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 Garcinia cola, the active compounds responsible for anti-microbial, anti-viral and anti-inflammatory properties were bioflavonoids, xanthenes and benzophenomes (Amadioha, 2003). It was also observed from the quantity phytochemical analysis done in this study showed that Garcinia cola had the highest quantity of flavonoid (1.70 mg/100g) than the other plant extracts tested. The antifungal activity observed in Carica papaya may be dues 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) and Akueshi et al., (2002) 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 antifungal properties of crude leaf extracts of C. papaya in paw-paw fruits. Several researchers (Amadioha, 1998; Wokocha and Okereke, 2005 and 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.

Conclusion

This study therefore showed that the plants' crude extracts possess some inhibitory components which cause significant reduction in mycelia growth of the pathogen. The result also revealed the need for regular application of these plant extracts at interval of 8-10 days as the phytotoxicity depreciates overtime.

To summarize, G. kola elicited the highest phytotoxicity response to H. infestans which incite the leaf spot found on the leaves of eggplant. It may be possible therefore to use G. cola as fungicide to control leaf spot in eggplant fields because of their availability and eco –friendliness. Further studies on greenhouse control using these plant extracts should be explored

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