

Full Length Research Paper

***In-vitro* evaluation of plant extracts against *Lasiodiplodia theobromae* causing cashew inflorescent blight**

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Extracts of four plant species, red acalypha (*Acalypha hispida*), siam weed (*Chromolaena odorata*), aidan (*Tetrapleura tetraptera*), and neem (*Azadirachta indica*) were screened *in-vitro* for fungitoxicity against *Lasiodiplodia theobromae* (cashew inflorescence blight pathogen) at four different concentrations viz., 10, 20, 40 and 80%. The result shows that the plants extracts significantly ($P < 0.05$) reduced mycelia growth of the fungus. The fungitoxicity of the plant extracts against *L. theobromae* also varied with concentration. Extract from *T. tetraptera* exhibited maximum efficacy in reducing the mycelia growth of *L. theobromae* to 9.83 mm at 80% concentration while the highest mycelia growth of 59.33 mm was recorded in extract of *A. hispida* at 40% concentration. *C. odorata* at 10% concentration reduced the colony diameter to 50.50 mm while 20% *T. tetraptera* inhibited the pathogen to 42.33 mm. The percentage reduction in colony diameter by each of the phytoextracts ranged between 30.20 and 88.44%. Findings from this study reveal the possibility of utilizing naturally available plant chemicals for controlling *L. theobromae* with the ultimate goal of eliminating pesticide residues in the marketable products of cashew.

Key words: Cashew, *Lasiodiplodia theobromae*, inflorescent blight, plant extracts.

INTRODUCTION

Cashew (*Anacardium occidentale* L.) is a crop of considerable economic importance in Nigeria with a high potential for foreign exchange. The crop can be grown on almost all soil types, including wastelands of low fertility. The nut is highly nutritious, has a pleasant taste and flavour and can be eaten raw or fried, and sometimes salted or sweetened with sugar (Manay et al., 1987).

Nigeria ranks as the sixth largest cashew-producing country in the world, with an annual production estimate of 120,000 tones (Ezeagu, 2002). However, this figure is still below attainable yield. Pests and diseases have been identified as one of the major constraints to optimum cashew production in Nigeria. Several pathogens infect the various parts of cashew plant, including roots, stems,

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twigs, branches, flowers, pseudo-apple and the inflorescence, causing substantial economic losses (Asogwa et al., 2008). Inflorescence blight disease (also referred to as dieback) caused by *Lasiodiplodia theobromae* is a common and widespread disease of cashew in Nigeria (Olunloyo, 1979). The symptoms of infection include withering of petals and other parts of the flower, followed by a progressive dieback of the small peduncles from the tips and downward to the main floral shoots. The disease is spread through insects, which create wounds that predispose the inflorescence axes to infection. Inflorescence blight accounts for 40 to 45% crop losses annually (Olunloyo, 1983).

The use of synthetic fungicides remains the primary means of controlling fungal diseases of crop plants in tropical countries. However, synthetic pesticides leave residue in marketable products of cashew, ultimately reducing the economic value in the international market. Many fungicides have also lost potency and efficacy owing to the development of resistance by plant pathogens (Singh and Dwivedi, 1987). Furthermore, the prohibitive cost, phytotoxicity, deleterious effects on agricultural land, water and soil as well as the associated health problems on man have necessitated the need to search for alternative control measures that are cheap, environment-friendly, ecologically sound and medically safe. The use of natural pesticides has been recognized as a sound alternative to synthetic chemicals in plant disease control.

Plant extracts have found useful place as bio-pesticides in the control of diseases of crop plants. Thus, the present study was aimed at assessing, *in-vitro*, the fungitoxicity potential of extracts of four plants, namely, red acalypha (*Acalypha hispida*), siam weed (*Chromolaena odorata*), aidan (*T.tetraptera*), and neem (*Azadirachta indica*) against *L. theobromae*, causative agent of inflorescence blight disease of cashew.

MATERIALS AND METHODS

Isolation of pathogen

Floral shoots of cashew showing typical symptoms of inflorescence dieback were collected from the Cashew Experimental Plot in Cocoa Research Institute of Nigeria (CRIN), Ibadan, Nigeria. The diseased samples were collected in sterile polythene nylon, made airtight and transferred to the CRIN plant pathology laboratory. The samples were washed thoroughly in tap water to free them of secondary invaders and contaminants. The infected portions were cut into small pieces of 3 to 5 mm thickness, sterilized in 1% sodium hypochlorite solution for 2 min, rinsed thrice in sterile distilled water and dried on sterilized filter paper at room temperature. The cut tissue sections were then placed on potato dextrose agar (PDA) and incubated at 25°C for seven days. Pure culture of *L. theobromae* isolated was then maintained on PDA.

Preparation of plant extracts

Leaves of *A. hispida*, *C. odorata*, *A. indica* and pod of *T. tetraptera*

were collected from the wild, dried under shade and milled separately. The extracts were prepared by weighing 10, 20, 40 and 80 g of milled samples separately into 100 ml sterile distilled water in separate 500 ml flasks, to obtain 10, 20, 40 and 80% concentration, respectively. The resulting sap solution were vigorously agitated and left to stand for 48 h on the bench. The sample mixtures were filtered using sterile Whatman filter paper and the filtrates used as extracts were sterilized in the autoclave at 121°C for 15 min.

In vitro assay of anti-fungal activity

The inhibitory effect of the plant extracts was assessed using the food poisoned technique: two millilitres (2 ml) of each extract was aseptically added to 20 ml of sterilized and cooled PDA in a Petri dish, gently agitated and allowed to solidify, after which a 5 mm mycelia disc from periphery of a 7 day old culture of *L. theobromae* was placed in an inverted position on the extract-amended PDA and incubated at ambient temperature (28±2°C). Each of the treatments was replicated three times while the Petri plates without extracts served as the control. The radial mycelia growth was recorded at seven days after inoculation (DAI), by which time, the upper surface in the control treatment was fully covered with the mycelia of *L. theobromae*. The percentage mycelia growth inhibition was calculated using the formula of Opara and Wokocha (2008):

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

Where dc = average mycelia growth of control, dt = average mycelia growth of treated plates

The collected data were statistically analyzed by using SAS software and means separated by Duncan multiple range test at 5% probability level.

RESULTS AND DISCUSSION

Extracts of four plants species were screened *in-vitro* for fungitoxicity against *L. theobromae* at four different concentrations viz., (10, 20, 40 and 80%). The results of the study show that all the extracts were effective in reducing mycelia growth (colony diameter) of *L. theobromae* at all concentrations. The extract from *T. tetraptera* exhibited maximum efficacy in reducing the mycelia growth of *L. theobromae* (9.83 mm) at 80% concentration when compared with extracts of other plants species and extract concentrations assayed, and also differed significantly from other treatments as well as the control (Table 1). The highest mycelia growth (59.33 mm) was recorded in extracts of *A. hispida* at 40% concentration. However, there was not observed significant difference in the colony diameter of *L. theobromae* with extracts of the four botanicals at 40% concentration. The least effective extract concentrations across the plant materials in reducing the mycelia growth of *L. theobromae* are 40% *A. hispida* (59.33 mm), 10% *C. odorata* (50.50 mm), 20% *T. tetraptera* (42.33 mm) and 80% *A. indica* (57.50 mm) (Table 1). The percentage reduction in colony diameter by each of the phytoextract ranged between 30.20 and 88.44% (Table 2). *T.*

Table 1. Fungitoxic effects plant extracts on mycelia growth of *Lasiodiplodia theobromae*.

Aqueous extract conc. (%)	<i>Acalypha hispida</i>	<i>Chromolaena odorata</i>	<i>Tetrapleura tetraptera</i>	<i>Azadirachta indica</i>
	Mean colony diameter (mm)			
10	49.33 ^{bc}	50.50 ^b	21.33 ^{cd}	45.0b ^c
20	40.33 ^c	31.83 ^c	42.33 ^b	36.50 ^c
40	59.33 ^b	36.67 ^{bc}	39.50 ^{bc}	48.33 ^{bc}
80	49.0b ^c	37.83 ^{bc}	9.83 ^d	57.50 ^b
0 (Control)	85.0 ^a	85.0 ^a	85.0 ^a	85.0 ^a

Means followed by the same letter in each column are not significantly different ($P = 0.05$) according to Duncan's Multiple Range Test (DMRT).

Table 2. Percentage inhibition of *Lasiodiplodia theobromae* growth.

Plants spp.	Extract conc. (%)	Mycelia growth (mm)	Growth inhibition (%)
<i>Acalypha hispida</i>	10	49.33 ^{bcd}	41.96
	20	40.33 ^{cd}	52.55
	40	59.33 ^b	30.20
	80	49b ^{cd}	42.35
<i>Chromolaena odorata</i>	10	50.50 ^{bcd}	40.59
	20	31.83 ^{de}	62.55
	40	36.67 ^{de}	56.86
	80	37.83 ^{de}	55.49
<i>Tetrapleura tetraptera</i>	10	21.33 ^{ef}	74.91
	20	42.33 ^{bcd}	50.20
	40	39.50 ^{cde}	53.53
	80	9.83 ^f	88.44
<i>Azadirachta indica</i>	10	45.0 ^{bcd}	47.06
	20	36.50 ^{de}	57.06
	40	48.33 ^{bcd}	43.14
	80	57.50 ^{bc}	32.35
Control	0	85.0 ^a	0

Means followed by the same letter in each column are not significantly different ($P = 0.05$) according to Duncan's multiple range test (DMRT).

tetraptera at 80% concentration exhibited the highest growth inhibition percentage (88.44%) among all extract concentrations assayed and the control. This was followed by 10% extract concentration of the same plant species (74.91%). The least effective phytoextract against *L. theobromae* was 40% *A. hispida* with 30.20% inhibition percentage.

The presence of antifungal active ingredients in the leaves and pod of selected botanicals was demonstrated in this study. This was shown by their ability to inhibit the growth of the tested pathogen in culture. Several workers have reported antifungal activities of different plant species and stressed the importance of plants as possible sources of natural fungicides. The result obtained in this study is in agreement with previous

studies showing the antifungal activities of leaf extracts of *C. odorata* on *L. theobromae* (Adejumo, 2000b). Similarly, Adejumo and Otuonye (2002) reported that *C. odorata* at 5, 7.5 and 10% concentrations reduced the incidence of inflorescence blight disease in cashew. The fungicidal and fungistatic activities of *C. odorata* and *C. papaya* against *Botryodiplodia theobromae* at 10, 20 and 30% leaf extract concentrations have also been documented in literature (Ilondu, 2011). Slight decrease in the incidence of *B. theobromae* to 1.5, 1.0 and 0.5% in seeds treated with *C. odorata* leaf extract for 1, 6 and 24 h, respectively have also been reported (Adjei, 2011). The differences observed in the fungitoxic activity of the plant extracts could be attributed to variations in the strength of the active ingredients, solubility of the active

compounds in the extraction medium (solvent) and the presence of inhibitors (Qasem and Abu-Blan, 1996; Amadioha, 2000). For instance, Water and ethanol extracts of *A. indica* and *C. odorata* proved to be fungitoxic on *B. theobromae* when used to inhibit its growth in culture (Adjei, 2011).

The presence of antifungal compounds in higher plants has long been recognized as a key factor in disease resistance (Mahadevan, 1982). Such compounds, being biodegradable and selective in their toxicity, are considered valuable in controlling several plant diseases (Singh and Dwivedi, 1987). Exploitation of naturally available chemicals that have the tendency to retard the growth and or reproduction of plant pathogenic fungi would be a more realistic and ecologically sound method of integrated plant disease management. This will ultimately have a prominent role in the development of commercial pesticides for crop protection (Verma and Dubey, 1999). The ban of some synthetic pesticides has stimulated research into novel control strategies of pests and diseases of cashew in Nigeria. The need to minimize pesticide residues in the marketable products (such as cashew nuts) has saddled researchers and chemical companies with the responsibility of developing biologically active plant derived pesticides for crop protection (Yanar et al., 2011).

Conclusion

In-vitro studies shows that the extract from *T. tetraptera* was the most effective in reducing the mycelia growth of *L. theobromae* among others. However, field study is recommended to further evaluate and ascertain the performance of the botanicals to combat cashew inflorescence blight pathogen in natural cashew ecology. Further researches would also unfold exploiting the potentials of these botanicals on other hosts of *L. theobromae*.

Conflict of interests

The authors have not declared any conflict of interests.

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