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Full Length Research Paper

Inhibition of *Colletotrichum gloeosporioides* (Penz) Sac. causal organism of rubber (*Hevea brasiliensis Muell*. Arg.) leaf spot using plant extracts

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***In vitro* and *in vivo* antifungal evaluations were carried out on twenty-one plants selected from fourteen families on a folial pathogen of para rubber, *Colletotrichum gloeosporioides* (Penz). Sac. Extracts of *Ocimum basilicum* L. and *Allium sativum* L. exhibited total inhibitory effects on the mycelial growth of *C. Gloeosporioides*. An evaluation of concentration effects was carried out using four concentrations of five selected plants: *A. sativum, Jatropha curcas, O. Basilicum, Vernonia amygdalina* and *Ageratum conyzoides*. *A. sativum* and *O. basilicum* totally inhibited conidial germination 24 h after inoculation, and conidial germination in liquid amended media was recorded only in the *A. conyzoides* amended liquid media. An *in vivo* evaluation showed that treatment with 100% *O. basilicum* resulted in disease index (D.I) of 31.7% which was significantly lower than the control 65% D.I at 5% level of probability.**

Key words: *Colletotrichum gloeosporiodes*, *Hevea brasiliensis*, plant extracts, inhibition.

INTRODUCTION

Colletotrichum gloeosporioides (Penz) Sac, causal organism of *Colletotrichum* leaf spot disease is common foliar pathogens of rubber both in the nursery and in the field in Nigeria. Nursery rubber seedlings are greatly affected by this leaf disease (Rao, 1975; Begho, 1990). Infection of rubber by these foliar pathogens results in retarded growth, secondary leaf fall (SLF), dieback and death of trees both in the nursery and in the field, as well as the reduction of latex in mature rubber trees in plantations (Otoide, 1978; Webster and Baulkwill, 1989; Waller, 1992; Begho, 1995; Sabu et al., 2000).

In laboratory tests and limited field trials, benomyl has been found to be most effective in control, but only if weekly spraying is extended for several months (www.irrdb.com/IRRDB/NaturalRubber/Diseases/clfd.html). This is a very high cost, and it would be difficult for any farmer in the developing country to keep up with this very expensive regime.

This study seeks to find possible plants whose extracts have fungicidal effects on *C. gloeosporioides* with the evaluation of (21) plants selected from 14 families based on reports about them in literature (Akobundu and Agyakwa, 1998).

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MATERIALS AND METHODS

Origin of *C. gloeosporioides* culture

C. gloeosporioides culture was isolated from infected leaves of rubber grown in the nursery of the Rubber Research Institute of Nigeria (RRIN), Benin City, Nigeria.

Screening of plants for inhibitory effects

For rapid screening, 100 g of disease free leaves of the 21 plants to be screened were ground in 100 ml of sterile distilled water. The extracts were filtered using cheesecloth, and 3.9 g of PDA per 100 ml of extract was added before sterilization. The sterilized leaf extract PDA (LEPDA) were dispensed into Petri plates and seeded with 1 cm^3 plug of *C. gloeosporioides*.

Concentration effect

The effects of four concentration levels of each of the plant extracts selected from the 21 screened above were evaluated. The four levels were obtained by grinding 10, 25, 50 and 100 g samples of the five selected plants in 100 ml of sterile distilled water.

Effects of extracts on conidial germination and mycelial growth

Four concentrations of extracts at 10, 25, 50 and 100% were used in the assessment of effects. Conidium was considered to have germinated when the germ tube was equal in length to or more than the conidium. Percentage inhibition of mycelial growth was evaluated using the poisoned food techniques (PFT), and calculated using the formula:

$$\% \text{ Inhibition} = \frac{100 \text{ (Control - Treatment)}}{\text{Control}}$$

***In vivo* evaluation**

In vivo inoculation of 1 ml of conidial suspension containing 2 x 10^3 cfu was carried out in the nursery. An assessment of disease infection was carried out 3 weeks after inoculation using the disease score-rating chart (RRIM, 2000). Disease index (D.I.) was calculated using the formula:

$$\text{Disease Index (D.I.)} = \frac{(0^* a) + (1^* b) + (2^* c) + (3^* d)}{a + b + c + d} \times \frac{100}{X}$$

where:

0, 1, 2, 3 = Infection categories,
a, b, c, d = No of leaves/ plant that falls into the infection categories, and
X = Maximum no of infection categories.

Infection category was determined using the disease score rating below:

0 = No infected leaves
1 = Less than 10% of leaves infected
2 = 10-50% of leaves infected
3 = More than 50% of leaves infected (RRIM, 2000)

Experimental design

Experimental design used for the *in vitro* and *in vivo* studies were complete randomised design and randomised complete block design, respectively. All data were subjected to analysis of variance and treatment means separated by the use of the least significant difference.

RESULTS

Screening of the 21 plants against mycelial growth of *C. gloeosporioides*

Six of the plants evaluated promoted the growth of the pathogen *C. gloeosporioides*. These plants; *Tridax procumbens*, *Mitracarpus scaber*, *Ficus elegans*, *Euphorbia hirta, Emilia coccinea* and *Cassia alata* promoted growth by 5% and 40%. Of all the extracts screened, *A. sativum* and *O. basilicum* resulted in 100% inhibition of mycelial growth of the pathogen, and three other plants, *A. conyzoides, J. curcas* and *V. amygdalina* resulted in mycelial growth inhibition of 56.83%, 49.90% and 48.32%, respectively (Table 1). As such, these five plants were selected for further evaluation on the effects of different concentrations on mycelial growth and conidial germination.

Concentration effects of selected plant extracts

Extracts of *A. sativum* and *O. basilicum* exhibited a high inhibitory effect on the mycelial growth of *C. gloeosporioides* and these were significantly lower than the control at 5% level of probability (Table 2). An increase in the concentration of both extracts increased the level of inhibition of mycelial growth (Table 2).

Treatment in *J. curcas* at 10 and 25%, and *V. amygdalina* at 10% induced a significant increase in the mycelial diameter of the pathogen. With an increase in the concentrations to 50% and 100% (*J. curcas*); and 25-100% for *V. amygdalina* significant inhibition of mycelial growth was observed (Table 2).

Assessment of conidial germination in extract amended liquid medium

The four concentrations of extracts *A. sativum, J. curcas, and O. basilicum* and *V. amygdalina* totally inhibited conidial germination of *C. gloeosporioides*. At 10% concentration of *A. conyzoides*, some conidial germination was recorded at the 12th, 18th and 24th h of observation. Conidial germination was however inhibited in all the other concentrations (Table 3).

Conidial germination on solid media

Extracts of *A. sativum* and *O. basilicum* gave 100% inhibition of conidial germination of *C. gloeosporioides* at all concentration levels. *A. conyzoides* extracts at 50% and 100% concentrations also resulted in total inhibition of conidial germination. Some percentage germination (5% and 2.7%) was recorded with treatment in *A. conyzoides* at 10% and 25% extract concentrations, respectively. Conidial germination was observed at 10%, 25% and 50% on *J. curcas*, while on *V. amygdalina*-amended media there was conidial germination from all concentration levels (Table 4).

***In vivo* inoculation in the nursery**

The results of the disease index of conidia inoculation of the pathogen on intact plants in the nursery are summarised in Table 5. The D. I. was observed to decrease with increase in extract concentration for all the five extracts used. The lowest D. I. of 31.69% was recorded in the treatment with 100% *O. basilicum* extract. The other three extracts gave D. I. higher than that recorded in the control except at extract concentrations of 100% in

Table 1. The inhibitory effects of the 21 plant extracts on mycelial growth of *Colletotrichum gloeosporioides* 5 days after inoculation.

Plant	Mycelial growth (SD)	Percentage Inhibition
Acalypha wilkesiana Muell. Arg.	4.80 (0.78) ^{de}	4.95
Ageratum conyzoides *L.	2.18(0.29) ^{k}	56.83
Allium sativum *L.	0.00(0.00) ^{l}	100.00
Azadirachta indica A. Juss	3.15 (0.27) ^{h}	34.62
Carica papaya L.	3.63 (0.64) ^{f}	28.12
Cassia alata L.	5.55 (0.40) ^{b}	-9.90
Centrosema pubescence Bth	4.73 (0.42) ^{de}	6.34
Chromolaena odorata(L.) K.R.	2.95 (0.13) ^{hi}	41.58
Emilia coccinea(Sims) G. Don	5.57 (0.50) ^{b}	-10.30
Euphorbia hirta.L.	5.58 (0.55) ^{b}	-10.30
Ficus elegans (Miq.) Mig	5.33 (0.71) ^{bc}	-5.55
Jatropha curcas *L.	2.53 (0.31) ^{jk}	49.90
Melanthera scandens (Schum.&Thonn.) Roberty	4.48 (0.13) ^{e}	1.29
Mitracarpus scaber Zucc.	5.60 (0.64) ^{b}	-10.89
Ocimum basilicum *L.	0.00 (0.00) ^{l}	100.00
Peperomia pellucida (L.) H. B. and K	3.78 (0.40) ^{f}	25.15
Portulaca oleracea L.	2.90 (0.22) ^{hij}	42.58
Solanum. torvum Swart	2.70 (0.55) ^{ij}	46.54
Synedrella nodiflora (L.) Gaertn	4.78 (1.33) ^{de}	5.55
Tridax procumbens L.	7.15 (0.27) ^{a}	-40.58
Vernonia amygdalina *L.	2.61 (0.25) ^{ij}	48.32
Control	5.05 (0.90) ^{cd}	

$LSD_{extract}$ = 0.38; CV = 6.17%; SD = Standard deviation,* = Selected plant extract.
Values followed by common letters are not significant at 5% level of probability.

Table 2. Effect of concentrations of the five selected plant extracts on mycelial growth of *Colletotrichum gloeosporioides* 5 days after inoculation.

Plant extracts	Concentrations (%)			
	10	25	50	100
Ageratum conyzoides	4.23(0.17)*^{d}	4.18 (0.24) ^{d}	3.20 (0.22) ^{h}	2.98 (0.17) ^{l} cm
Allium sativum	1.68 (0.40) ^{l}	0.70 (0.41) ^{n}	0.05 (0.06) ^{p}	0.00 (0.00) ^{p}
Jatropha curcas	4.83 (0.10) ^{b}	4.73 (0.33) ^{c}	3.73 (0.22) ^{e}	3.33 (0.54) ^{g}
Ocimum basilicum	1.55 (0.06) ^{m}	0.55 (0.06) ^{o}	0.00 (0.00) ^{p}	0.00 (0.00) ^{p}
Vernonia amygdalina	4.95 (0.10) ^{a}	3.55 (0.17) ^{f}	2.85 (0.45) ^{j}	2.68 (0.47) ^{k}
Control	4.17 (0.35) ^{d}	4.17 (0.35) ^{d}	4.17 (0.35) ^{d}	4.17 (0.35) ^{d}

$LSD_{concentration}$ = 0.18; $LSD_{extract^{*}concentration}$ = 0.08; CV = 5.31%; * = Mean value in cm (Standard deviation).
Values followed by common letter are not significantly different at 5% level of probability.

both *A. conyziodes* and *J. curcas* respectively and in extract of *V. amygdalina* at concentrations of 25 to 100%.

DISCUSSION

In this study, the twenty one (21) plants extracts screened showed differing effects on *C. gloeosporioides*. Some of the extracts inhibited mycelial growth of the pathogen whilst six of them promoted mycelial growth. The fungitoxic effects of aqueous plant extracts of different plant species indicate the importance of many plant species as a possible natural source of fungicidal materials. Many workers have reported antifungal activeties of different plant species and stressed the importan-

Table 3. Percentage conidial germination of *Colletotrichum gloeosporioides* in plant extract amended liquid media at 6 h interval for 24 h.

Plant extract	Conc. (%)		Periods (h)		
		6	12	18	24
Ageratum conyzoides	10	0.00 ^{d}	5.56 ^{c}	8.53 ^{b}	9.25 ^{b}
	25	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}
	50	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}
	100	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}
Allium sativum	10	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}
	25	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}
	50	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}
	100	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}
Jatropha curcas	10	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}
	25	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}
	50	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}
	100	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}
Ocimum basilicum	10	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}
	25	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}
	50	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}
	100	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}
Vernonia amygdalina	10	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}
	25	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}
	50	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}
	100	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}	0.00 ^{d}
Control	0	5.56 ^{c}	6.12 ^{c}	10.50 ^{a}	10.56 ^{a}

$LSD_{concentration}$ = 0; $SD_{extract^{*}\ concentration}$ = 1.24; CV = 45.45%.
Values followed by common letter are not significantly different at 5% level of probability.

Table 4. Percentage conidia germination of *Colletotrichum gloeosporioides* at 24 h after inoculation on solid media.

Plant extract	Concentration (%)	Percentage germination (%)
Ageratum conyzoides	10	5.00 ^{bcd}
	25	2.67^{cdf}
	50	0.00 ^{f}
	100	0.00 ^{f}
Allium sativum	10	0.00 ^{f}
	25	0.00 ^{f}
	50	0.00 ^{f}
	100	0.00 ^{f}
Jatropha curcas	10	5.67 ^{abc}
	25	4.00 ^{cd}
	50	1.67 ^{df}
	100	0.00 ^{f}
Ocimum basilicum	10	0.00 ^{f}
	25	0.00 ^{f}
	50	0.00 ^{f}
	100	0.00 ^{f}
Vernonia amygdalina	10	8.33 ^{ab}
	25	8.33 ^{ab}
	50	5.00 ^{bcd}
	100	3.00 ^{cdf}
Control	0	9.00 ^{a}

$LSD_{concentration}$ = 1.53; $LSD_{extract^{*}\ concentration}$ = 3.42; CV = 64.39 %.
Values followed by common letter are not significantly different at 5% level of probability.

Table 5. Disease index of disease infection screening of *Colletotrichum gloeosporioides* in the nursery after 3 weeks of inoculation.

Plant extracts	Concentrations(%)	Disease index(%)
Ageratum conyzoides	10	75 [a]
	25	75 [a]
	50	72.22 [b]
	100	50 [j]
Allium sativum	10	59.72 [f]
	25	50 [j]
	50	45 [l]
	100	41.67 [m]
Jatropha curcas	10	75 [a]
	25	75 [a]
	50	68.33 [c]
	100	63.33 [c]
Ocimum basilicum	10	56.67 [g]
	25	46.67 [k]
	50	36.67 [n]
	100	31.69 [o]
Vernonia amygdalina	10	75 [a]
	25	55 [h]
	50	53.33 [i]
	100	36.67 [n]
Control	0	65 [d]

$LSD_{concentration}$ = 0.69; $LSD_{extract^* concentration}$ = 1.55; CV = 1.96%. Values followed by common letter are not significantly different at 5% level of probability.

ce of plants as a possible source of natural fungicides (Tewari and Dath, 1984; Lakshmanan, 1990; Tewari, 1995; Philip and Sharma, 1997; Ogbebor and Adekunle, 2005).

In this study, the selected five plants; *A. conyziodes, A. sativum, J. curcas, O. basilicum* and *V. amygdalina,* inhibited mycelial and conidial growths of *C. gloeosporioides in vitro* and *in vivo*. Others have found *A. sativum* and *O. basilicum* to be quite effective in the control of plant pathogenic organisms. Upadhyaya and Gupta (1990) reported the control of *Curvularia lunata* with extracts of *Ocimum sanctum*. Singh et al. (1993) reported the effectiveness of aqueous extracts of *O. sanctum* and *Azadirachta. indica* in the control of disease development in banana. Lakshmanan (1990) demonstrated the high antifungal properties of *A. sativum*.

Concentration of extract used was significant (Table 2) and mycelial inhibition was significantly higher with the higher concentrations for the five plant extracts. Extracts of *O. basilicum* (50% and 100% concentrations) and *A. sativum* (100% concentration) resulted in 100% mycelial inhibition of *C. gloeosporioides.* D'Aulerio et al. (1996) reported the significance of concentration effect on control of some plant pathogens using aqueous extracts of garlic.

The five extracts in liquid media on *C. gloeosporioides* gave total inhibition of conidial germination except at 10% extract concentration for *A. conyzoides,* which recorded conidial germination of 5.6% at 6 h and increase to 9.3% after 24 h incubation period. In the solid media total inhibition of conidial were recorded in the four concentrations of extracts of both *O. basilicum* and *A. sativum.* Total conidial inhibition of the pathogen by *C. gloeosporioides* conidial inhibition by extracts of *A. cony-zoides* was at 50% and 100% concentration and by *J. curcas* extracts at 100%. It has been reported that con-tact with solid substrate surface induces germination of conidia of *Colletotrichum truncatum*, and this apparently is the case with conidia of *Corynespora cassiicola* as well (Egley, 1994).

In the nursery, the disease index (DI) of disease infection screening showed that extract of *O. basilicum* recorded the lowest D.I. The results obtained with *O. basilicum* in this study confirmed the importance of *Ocimum* species as earlier reported by Tewari (1995) as plant exhibiting antifungal properties.

More conidia germinations were observed in the solid media than in the liquid media where conidia germinations were only observed in extract of *A. conyzoides* at 10% concentration. This is consistent with the findings of Egley (1994), which indicated that contact with solid subs

trate surface induces germination of conidia of *Colletotrichum truncatum.*

This study has demonstrated the possibility of using extracts from some plants to control mycelial growth as well as conidial germination of *C. gloeosporiodes*. The effectiveness of *O. basilicum* was clearly the highest of the five plants tested, followed by *A. sativum*. These two plants appear to be promising alternatives for the control of C. *gloeosporiodes* infection on Rubber.

The cost of applying botanical fungicides had been reported to be less than that of the synthetic fungicides in South Africa, RS375/ha of *O. sanctum* compared with RS1430/ha for Ediphenphos or RS1580/ha for Carbendazim. Therefore, the exploitation of one or more of these botanical fungicides for the control of one or more of these pathogens may be less expensive, safer for the applier and the ecosystem and could serve as a good alternative to synthetic fungicides. Further studies to test the efficacy of formulations and application methods will be carried out.

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