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Full Length Research Paper

Inhibition of conidial germination and mycelial growth of *Corynespora cassiicola* (Berk and Curt) of rubber (*Hevea brasiliensis* muell. Arg.) using extracts of some plants

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Twenty-one plants were screened for fungicidal effects on conidial germination and mycelial growth of *Corynespora cassiicola*. Out of this, 5 plants (*Ageratum conyzoides*, *Centrosema pubescene*, *Emilia coccinea*, *Ocimum basilicum* and *Solanum torvum*) were selected for evaluation of concentration effects. Treatment in *O. basilicum* resulted in the lowest mycelial growth at 100% extract concentration. An *in vivo* evaluation showed that treatment with 100% *O. basilicum* resulted in disease index (D.I) of 43% which was significantly lower than the control 65% D.I at 5% level of probability.

Key words: *Corynespora cassiicola,* Conidial and Mycelial inhibition, plant extracts.

INTRODUCTION

Corynespora cassiicola (Berk and Curt) Weir, causal organism of leaf spot of rubber, was first recorded in 1960 on some iron-deficient nursery plants. It has since been occasionally seen in budwood nurseries on certain clones (Rao, 1975; Ramli et al., 2000). The disease also affects mature fields where it causes severe defoliation of newly matured leaves produced during a dry period, following an earlier wintering in December. The disease is present in Malaysia, India and West Africa (Rao, 1975). The disease is generally severe in areas with high rainfall without any prolong dry period (MCR, 2000). *Corynespora* leaf disease appears to respond to the eco-climatic condition that supposes simultaneous manifestation of refoliation and rain creating a favourable environment for their development (Wahounou, 2000).

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Harinidi et al. (1996) reported that susceptible clones affected by *C. cassiicola* could suffer continuously in such a long period that the crown becomes leafless for the whole year. Folial infection by these pathogens could cause dieback, stunted increment; while on mature trees it could obviously reduce latex production (Awoderu, 1967; Rao, 1975; 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/Natural Rubber/Diseases/clfd.html).

For developing countries, chemical control would be economically crippling especially if repeated spraying is done. As such, we are with this study, carrying out a preliminary evaluation of the inhibitory effects of 21 plants from 14 families on *C. cassiicola*. These selected plants with possible fungitoxic properties were selected from literatures (Gill, 1992; Akobundu and Agyakwa, 1998).

MATERIALS AND METHODS

Origin of *C. cassiicola* culture

C. cassiicola was isolated from infected leaves of rubber grown in the nursery of the Rubber Research Institute of Nigeria (RRIN).

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 was added per 100 ml of extract before sterilization. The sterilized leaf extract PDA (LEPDA) were dispensed into Petri plates and seeded with 1 cm^3 plug of *C. cassicola*.

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 25, 50, 75 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. 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:

% Inhibition = 100 (Control - Treatment)/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 using the formula:

Disease Index (D.I) = 100 [(0*a) + (1*b) + (2*c) + (3*d)] / X (a + b + c + d)

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

Table 1. Effects of extracts of 21 plants on mycelial growth of *C. cassiicola* five days after inoculation.

Name of plant	Percentage inhibition
Acalypha wilkesiana Muell.Arg.	1.63
Ageratum conyzoides L.	23.26
Allium sativum L.	6.28
Azadirachta indica A.Juss.	- 12.79
Carica papaya L.	- 24.12
Cassia alata L.	- 24.12
Centrosema pubescence Bth.	36.98
Chromolaena odorata (L.)K.R	- 3.02
Emilia coccinea(Sims.)G.Don.	24.66
Euphorbia hirta L.	18.84
Ficus elegans (Mig.)Mig.	- 54.66
Jatropha curcas L.	- 27.45
Melanthera scandens Schum.&Thonn.)Roberty	- 48.38
Mitracarpus scaber Zucc.	- 29.77
Ocimum basilicum L.	100.00
Peperomia pullucida (L.)H.B&K	- 8.84
Portulaca oleraceae L.	- 20.47
Solanum torvum Swart.	37.88
Synedrella nodiflora (L.)Gaertn	34.89
Tridax procubens L.	6.98
Vernonia amygdalina L.	- 26.75
Control	**0**

$LSD_{extract}$ = 0.1 at $\propto$ =0.05 ; CV= 2.34%.

RESULTS

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

Of the 21 plants screened, 11 promoted mycelial growth of *C. cassiicola* by between 3-55%. These include; *Carica papaya*, which showed an improvement in mycelial diameter by 55%, *Melanthera scandens* (48%), *Chromoleana odorata* (3%), and *Peperomia pellucida* (9%) (Table 1). Selected for further investigation were the following plants with inhibitory effects on the mycelial growth of *C. cassiicola*. *Ageratum conyzoides* (23%), *Centrosema pubescence* (37%), *Emilia coccinea* (25%), *Solanum torvum* (38%) and *Ocimum basilicum* (100%) (Table 1).

Table 2. Effect of 4 concentrations of five plant extracts on mycelial diameter of C. cassiicola 5 days after inoculation on amended PDA at 28oC.

Plant	Extract Concentration			
	10	25	50	100
Ageratum conyzoides	2.78	2.75	2.12	2.19
Emilia coccinea	2.15	2.18	2.35	2.35
Centrosema pubescence	3.05	3.09	3.14	3.25
Ocimum basilicum	3.68	2.79	1.03	0.84
Solanum torvum	3.03	2.69	2.54	2.44
Control	5.63	5.63	5.63	5.63

LSD (concentration) = 0.20; LSD (extract* concentration) = 0.10; CV = 7.0; ∝ =0.05.

Concentration effects on mycelial growth

Mycelial growth was significantly inhibited by all four concentrations of the five extracts used, and concentration effect was significant at all levels for *O. basilicum* and *S. torvum*. Concentration effects for *A. conyzoides*, *E. coccinea* and *C. pubescence* were not significant between 10 and 25% concentrations (Table 2).

Concentration effects on conidial germination in extract amended liquid medium

There was total inhibition of conidia for the 24-h observation period in both *O. basilicum* and *C. pubescence* amended media at all concentration levels. Conidial germination took place only at 10% extract concentration of *A. conyzoides*, *E. coccinea* and *S. torvum*. Percentage germination in *A. conyzoides* at 24 h (9%) was significantly higher than those from the control (6.4%) and *E. coccinea* and *S. torvum* (5.6% each) at 5% level of probability (Table 3).

Concentration effects on conidial germination on extract amended PDA

Concentration effect was significant though not on all levels (Table 4). The highest percentage germination of 88% was from 10% *S. torvum* and the lowest of zero percent germination from 100% *O. basilicum*. Conidial germination for the control was not significantly different from those treated with 10% *A. conyzoides*, 50% *C. pubescences*, 25 and 50% *E. coccinea* and 100% *S. torvum*.

In vivo evaluation

An *in vivo* evaluation of the four concentrations of 10, 25, 50 and 100% extract concentration resulted in some significant differences in the calculated disease index (DI). The lowest D.I. was 43% from 100% *O. basilicum* treatment, and was significantly lower than those of the control plants and all the other treatments. The second lowest DI of 50% was from 100% *C. pubescences* and 50% *O. basilicum*.

DISCUSSION

In this study, extracts of some common plant species, *A. conyziodes, C. pubescence, E. coccinea, O. basilicum* and *S. torvum,* showed varied antifungal potentials when tested. Jatisatienr and Jatisatienr (2005) testing fungicidal properties of clove and sweet flag, found that the degree of inhibition of hyphal length and percentage germination depended on species of plants extracted. Our results clearly show antifungal properties of some of the screened plants against *C. cassiicola*. *O. basilicum* is the highest on our list followed by *A. conyzoides*. Both of these plants have been showed at various times by others to have antifungal properties against other pathogens(www.hort.purdue.edu/newcrop/proceedings1999/v4-469.html;www.ansci.cornell.edu/plants/medicinal/basil.html). Tewari (1995) reported fungicidal properties of *O. sanctum* in the control of rice blast, when the inhibitory effects on mycelial growth and conidial germination of *Pyricularia grisea* were observed.

There was significant effect of concentration on the inhibitory effects of the extracts used. Al-Abeed (1992) also observed significant effects of concentration levels on the effects of aqueous extracts of varied wild plants on some plant pathogenic organisms. Fabry et al. (1996) also demonstrated the effects of concentration on the control of *Aspergillus* spp. and *Candida* spp. where minimal inhibitory concentrations of as low as 0.006 mg/ml were required for effective control.

Higher percentage of conidial germination was recorded on the solid media, than in the liquid media. It has been reported that contact with solid substrate surface induces germination of conidia of *Colletotrichum truncatum*, and this apparently is the case with conidia of *C. cassiicola* as well (Egley, 1994).

Most promising extracts have been known to originate from those used in traditional medicine. As such, we should also learn from the wide crop of indigenous knowledge available to us and research this large reservoir of knowledge. Eksteen et.al. (2001) worked on crude extracts of *Eucomis autumnalis*, a plant native to South Africa, and it showed significant antifungal activity against seven plant pathogenic fungi comparable to a broadspectrum antibiotic (carbendazim/difenoconazole).

Table 3. Percentage conidia germination of *C. cassiicola* in 4 concentration of extracts amended liquid media at 6-h interval for 24-h incubation period.

Plant	Concentration (%)	Period (h)			
Ageratum conyzoides		**6**	**12**	**18**	**24**
	10	2.50	3.25	4.45	9.09
	25	0	0	0	0
	50	0	0	0	0
	100	0	0	0	0
Centrosema pubsescence					
	10	0	0	0	0
	25	0	0	0	0
	50	0	0	0	0
	100	0	0	0	0
Emilia coccinea	10	2.04	2.75	4.17	5.56
	25	0	0	0	0
	50	0	0	0	0
	100	0	0	0	0
Ocimum basilicum	10	0	0	0	0
	25	0	0	0	0
	50	0	0	0	0
	100	0	0	0	0
Solanum torvum	10	2.33	2.33	3.33	5.56
	25	0	0	0	0
	50	0	0	0	0
	100	0	0	0	0
Control	0	2.22	5.13	5.43	6.38

LSD concentration = 0.00; LSD extract* concentration = 1.42; CV = 65.18 at 5% level of probability.

Table 4. Percentage germination of *C. cassiicola* in 4 concentrations of extracts amended PDA 24 h after inoculation.

Plants	Concentration (%)	Germination (%)
Ageratum conyzoides		
	10	56.67
	25	55.00
	50	46.67
	100	16.67
Centrosema pubsescence		
	10	78.33
	25	55.33
	50	60.00
	100	51.67
Emilia coccinea		
	10	71.67
	25	68.33
	50	73.33
	100	53.33
Ocimum basilicum		
	10	13.33
	25	4.00
	50	2.67
	100	0.00
Solanum torvum		
	10	88.33
	25	81.67
	50	81.67
	100	76.67
Control	0	65.00

Table 4. contd.

LSD concentration= -4.38; LSD extract* concentration = -9.78; CV = 11.48 at 5% level of probability.

There is a large reservoir of natural fungicides in plants and micro-organisms, which with continued research would provide safe and effective alternatives to synthetic fungicides. These compounds would have to be identified

Table 5. Disease index of *C. cassiicola* infection 3 weeks after inoculation and treatment in the extracts at four concentration levels.

Plants	Concentration (%)	Disease Index (%)
Ageratum conyzoides	10	66.67
	25	66.67
	50	60.00
	100	56.67
Centrosema pubsescence	10	68.33
	25	66.67
	50	60.00
	100	50.00
Emilia coccinea	10	65.00
	25	65.00
	50	60.00
	100	56.67
Ocimum basilicum	10	65.00
	25	65.00
	50	50.00
	100	43.33
Solanum torvum	10	63.33
	25	61.67
	50	56.67
	100	56.67
Control	0	65.00

LSD concentration= 0.98; LSD extract* concentration = 2.19; CV = 2.62 at 5% level of probability.

and formulated for application in the control of plant pathogens.

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