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Inhibition of *Drechslera heveae* (Petch) M. B. Ellis, causal organism of Bird's eye spot disease of rubber (*Hevea brasiliensis* Muell Arg.) using plant extracts

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ABSTRACT: The *in vitro* and *in vivo* antifungal potency of twenty-one plant extracts selected from fourteen families were evaluated for their botanical fungitoxicants on *Drechslera heveae*. Extracts of *Ageratum conyzoides, Allium sativum, Azadirachta indica, Jatropha curcas*, and *Ocimum basilicum* were selected for evaluation of concentration effects on *D. heveae*. Treatment with *Ocimum basilicum* resulted in the lowest mycelial growth at 100% extract concentration. The four concentrations (10%, 25%, 50% and 100%) of extracts of *Ageratum conyzoides, Allium sativum* and *Ocimum basilicum* gave total conidial inhibition of the pathogen in liquid media. Extracts of *Allium sativum*, and *Ocimum basilicum* at concentrations of 25% - 100% resulted in 100% conidial germination in the solid media. An *in vivo* evaluation showed that treatment with 100% *O. basilicum* gave disease index (D.I) of 33.33% which was significantly lower than the control 50% D.I at 5% level of probability.

Key Words: *Drechslera heveae, Hevea brasiliensis*; Plant extracts, Inhibition.

Introduction

Pathogens and parasites cause the major diseases of Rubber, the infective agent being mostly fungi (Igeleke 1988, Begho 1990). In Nigeria, approximately 65% of Rubber diseases are caused by fungal pathogens (Begho 1995). *Drechslera heveae* (Petch) M.B. Ellis, causal organism of Bird's eye spot is a common foliar pathogens of rubber both in he nursery and in the field (Rao 1975, Begho 1990). Infection of trees 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 (Webster and Baulkwill 1989, Begho 1995, Jayasinghe 2000) in plantations.

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The successful use of chemicals in the control of rubber diseases has been extensively reported by various scientists (Peries *et al.* 1963, Anon 1994, Jayasinghe *et al.* 1995, Jayasuriya *et al.* 1996). However, the high cost of chemical fungicides limits its availability and use by small - scale farmers.

Several workers have investigated the use of plant extracts as fungicides (Khan and Wassilem 1986, Tewari 1995, Qasem *et al.* 1995, Tewari 1995, Ogbebor and Adekunle 2005, Ogbebor *et al.* 2007). Khan and Wassilem (1986) worked on the effects of raw materials from neem tree (*Azadirachta indica*), neem oil and neem extract on fungi pathogenic to humans. The use of extracts from *Ocimum sanctum* L as a botanical fungicide against rice blast has been investigated (Tewari 1995). Tewari (1995) observed that essential oil from *O. sanctum* retained its fungitoxicity on heat treatment up to 100^0C and noted that the effect of the leaf extract was comparable to that of the chemical ediphenphos and carbendazim.

This study seeks to find possible plants whose extracts posses fungitoxic effects on *D. heveae* with the evaluation of 21 plants selected from 14 families based on report about them in literatures (Gill 1992, Akobundu and Agyakwa 1998).

Materials and Methods

Origin of Colletotrichum gloeosporioides culture

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

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 25g; 50g; 75g and 100g 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 according to giving by Vincent (1927), Ogbebor and Adekunle (2005), Ogbebor *et al.* (2007).

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

In vivo Evaluation

In vivo inoculation of 1ml of conidial suspension containing 2×10^3 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:

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

where:-

0, 1, 2, 3 = Infection categories

a, b, c, d = No of leaves/ plant that falls into the infection categories

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

Rapid screening of the 21 selected plants for their inhibitory effects.

Eight of the plants evaluate promoted the growth of *D. heveae*. These plants; *Cassia alata, Emilia coccinea, Melanthera scandens, Mitracarpus scaber, Portulaca oleracea, Solanum torvum Synedrella nodiflora,*and *Tridax procumbens* promoted growth by 0.2% and 13.33%. Of the extracts screened, *Ageratum conyzoides* (3.30%), *Allium sativum* (1.98%), *Azadirachta indica* (3.55%), *Jatropha curcas* (3.73%), and *Ocimum basilicum* (0.11%) gave the least inhibition of the mycelial growth of the pathogen (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

The concentration effects of the five selected plant extracts on mycelial growth of *D. heveae* are summarised in Table 2. *Allium sativum* and *O. basilicum* extracts exhibited high inhibitory effects on the mycelial growth of *D. heveae* with *O. basilicum* giving a total inhibitory effect at 100% extract concentration. Increase in the concentrations of the five extracts resulted in reduction in the mycelial growth of the pathogen. Treatment in *J. curcas* induced a significant increase in the mycelial diameter of the pathogen at all levels of concentration.

Assessments of Conidial germination in extract amended liquid media.

The four concentrations of extract of *A. conyzoides, A. sativum* and *O. basilicum* totally inhibited conidial germination of *D. heveae.* At 10% extract concentration of *A. indica* some conidial germination were recorded at the 12^{th}, 18^{th} and 24^{th} h experimental period. Similarly in extract of *J. curcas* at 10% and 25% extract concentrations conidial germinations were observed from the 6^{th} h to 24^{th} h experimental period except at 25% concentration by 6^{th} h period (Table 3).

Concentration effects on Conidia germination on solid media

Extracts of *A. conyzoides* at 100% concentration and 25%, 50% and 100% extract concentrations in both extracts of *A. sativum* and *O. basilicum* respectively gave 100% inhibition of conidial germination of the pathogen. The four concentrations of extracts of *A. indica* and *J. curcas* gave conidial germination of 20% to 65.33% and 33.33% to 85% respectively. Extract of *A. conyzoides* at 10% to 50% concentration also recorded conidial germination of *D. heveae* (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 on for all the extracts used. The D. I. in the control experiment was significantly different from those recorded in the treatments except in extract of *J. curcas* at 10% and 25% extract concentration. Extract of *O. basilicum* (33.33%) recorded the lowest D. I. while the highest D. I. was recorded in extract of *J. curcas* (48.33%).

Table 1. The Inhibitory effects of the 21 plant extracts on mycelial growth of *D. heveae* 5days after inoculation.

Name of plant	Mycelial growth (SD) cm	Percentage Inhibition (%)
Acalypha wilkesiana	4.78 (0.25)^{g}	3.43
Ageratum conyzoides *	3.30(0.21) ^{l}	33.33
Allium sativum *	1.98 (0.13) ^{m}	60
Azadirachta indica *	3.55 (0.38) ^{j}	28.28
Carica papaya	4.78 (0.16) ^{g}	3.43
Cassia alata	5.61 (0.16) ^{b}	-13.33
Centrosema pubescence	4.46 (0.24) ^{n}	9.9
Chromolaena odorata	4.09 (0.05) ^{I}	17.37
Emilia coccinea	5.14 (0.61) ^{de}	-8.84
Euphorbia hirta	4.80 (0.53) ^{g}	3.03
Ficus elegans	4.90 (0.35) ^{fg}	1.01
Jatropha curcas *	3.73 0.33) ^{j}	24.65
Melanthera scandens	5.29 (0.27) ^{cd}	-6.87
Mitracarpus scaber	5.51 (0.45) ^{bc}	-11.31
Ocimum basilicum *	0.11 0.14) ^{n}	97.98
Peperomia pellucida	4.15 (0.11) ^{I}	16.16
Portulaca oleracea	5.13 (0.14) ^{def}	-3.64
Solanum torvum	5.01 (0.09) ^{efg}	-3.03
Synedrella nodiflora	5.95 (0.17) ^{a}	-0.2
Tridax procumbens	6.15 (0.09) ^{a}	-4.14
Vernonia amygdalina	4.80 (0.30) ^{g}	3.03
Control	4.95 (0.81)^{efg}	0

$LSD_{extract}$= 0.23; CV = 3.25 %; SD = Standard deviation; * = Selected plant extract; Values followed by common letter are not significantly different at 5% level of probability.

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

Plant extracts	Concentration (%)			
	10	25	50	100
Ageratum conyzoides	4.20(0.25)* ^{g}	4.09 (0.16) ^{gh}	3.28 (0.28) ^{ij}	2.73 (0.23) ^{l}cm
Allium sativum	2.99 (0.14) ^{k}	2.70 (0.15) ^{l}	2.31 (0.34) ^{m}	0.73 (0.46) ^{o}
Azadirachta indica	4.83 (0.43) ^{f}	4.05 (0.88) ^{gh}	3.97 (0.77) ^{h}	3.12 (0.32) ^{jk}
Jatropha curcas	6.52 (0.08) ^{ab}	6.62 (0.03) ^{a}	6.35 (0.18) ^{bc}	6.22 (0.08) ^{c}
Ocimum basilicum	1.94 (0.13) ^{n}	0.94 (0.05) ^{p}	0.10(0.39) ^{q}	0.00 (0.07) ^{q}
Control	5.82 (0.21) ^{d}	5.82 (0.21) ^{d}	5.82 (0.21) ^{d}	5.82 (0.21) ^{d}

$LSD_{concentration}$ = 0.25; $LSD_{extract* concentration}$ = 0.22; CV = 5.10; *= Mean value in cm (Standard deviation). Values followed by common letter are not significantly different at 5% level of probability.

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

Plant extract	Conc. (%)	Periods (h)			
		6	12	18	24
Ageratum conyzoides	10	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}
	25	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}
	50	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}
	100	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}
Allium sativum	10	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}
	25	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}
	50	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}
	100	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}
Azadirachta indica	10	0.00 ^{e}	0.47 ^{d}	0.47 ^{d}	0.49 ^{d}
	25	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}
	50	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}
	100	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}
Jatropha curcas	10	1.14 ^{c}	1.15 ^{c}	1.23 ^{c}	1.32 ^{c}
	25	0.00 ^{e}	0.43 ^{d}	0.47 ^{d}	0.49 ^{d}
	50	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}
	100	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}
Ocimum basilicum	10	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}
	25	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}
	50	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}
	100	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}	0.00 ^{e}
Control	*0*	0.00 ^{e}	1.79 ^{b}	2.27 ^{a}	2.42 ^{a}

$LSD_{concentration}$ = 0.00; $LSD_{extract\ concentration}$ = 0.34; CV = 74.85
Values followed by common letter are not significantly different at 5% level of probability.

Table 4. Percentage conidial germination of *Drechslera heveae* at 24 h after inoculation on solid media.

Extract	Concentration (%)	Percentage germination (%)
Ageratum conyzoides	10	80.00[c]
	25	65.00 [ef]
	50	63.33 [f]
	100	0.00 [l]
Allium sativum	10	1.67 [l]
	25	0.00 [l]
	50	0.00 [l]
	100	0.00 [l]
Azadirachta indica	10	65.33 [e]
	25	56.67 [g]
	50	53.33 [h]
	100	20.00 [j]
Jatropha curcas	10	85.00 [b]
	25	70.00 [d]
	50	65.00 [ef]
	100	33.33 [I]
Ocimum basilicum	10	9.00 [k]
	25	0.00 [l]
	50	0.00 [l]
	100	0.00 [l]
Control	0	96.33[a]

$LSD_{concentration}$ = 0.86; $LSD_{extract\ concentration}$ = 1.92; CV = 4.75%
Values followed by common letter are not significantly different at 5% level of probability.

Discussion

Extracts of the 21 selected plants tested showed varied antifungal potential on *D. heveae.* Thirteen of the extracts inhibited mycelial growth of the pathogen while eight of the extracts promoted mycelial growth. Many workers have reported antifungal activities of different plant species and stressed the importance of plants as possible sources of natural fungicides (Tewari 1995, Lakshmanan 1990, Singh *et al.* 1993, Ogbebor and Adekunle 2005, Ogbebor *et al.* 2007).

In this study, extracts of *A. conyzoides, A. sativum A. indica, J. curcas* and *O. basilicum* showed varied antifungal potentials when tested. Extracts of *A. sativum* and *O. basilicum* demonstrated good inhibitory effect on the pathogen. Concentrations of the extracts used were significant (Table 2) and mycelial inhibition was significantly higher with the extract of *A. sativum* and *O. basilicum.* Fungitoxic activities of *Ocimum* species and *A. sativum* against plant pathogens have been reported (Tewari and Dath 1984, Lakshmanan 1990, Tewari 1995, Ogbebor and Adekunle 2005, Ogbebor *et al.* 2007). Results obtained with *O. basilicum* and *A. sativum* in this study confirmed the importance of this plant specie as plants exhibiting antifungal properties both in the *In vitro* and *In vivo* experiment. Lakshmanan (1990) reported the effectiveness of antifungal properties of *A. sativum.* Ogbebor *et al.* (2007) demonstrated high antifungal properties of aqueous extracts of *O. basilicum* and *A. sativum* on *Colletotrichum gloeosporioides*.

In the liquid media total conidial germination were recorded in all concentrations of the five extracts tested except at 10% in extract of *A. indica* from 12th h to 24th hand 10% and 25% in extract of *J. curcas* at 6th h – 24th h and 12th h to 24h h respectively (Table 3). In the solid media total inhibition of the conidial were recorded in extract of *A. conyzoides* at 100% and at 25% to 100% in both extracts concentrations of *A. sativum* and *O. basilicum* (Table4). More conidial germination was recorded in the solid media than the in the liquid media. This is consistent with the findings of Egley (1994), which demonstrated that contact with solid substrate surfaces induces germination of conidial of *Colletotrichum truncatum*. The least D. I of the extracts tested *In vivo* were recorded in extract of *O. basilicum* and *A. sativum* (Table5).

Table 5. Disease index of disease infection screening of *Drechslera heveae* in the nursery after 3 weeks of Inoculation.

Plant extracts	Concentrations (%)	Disease index (%)
Ageratum conyzoides	10	45 ^{cd}
	25	43.33 ^{d}
	50	38.33 ^{e}
	100	35 ^{fg}
Azadirachta indica	10	46.67 ^{bc}
	25	46.67 ^{bc}
	50	45 ^{cd}
	100	43.33 ^{d}
Allium sativum	10	45 ^{cd}
	25	43.33 ^{d}
	50	38.33 ^{e}
	100	35 ^{fg}
Jatropha curcas	10	48.33 ^{ab}
	25	48.33 ^{ab}
	50	46.67 ^{bc}
	100	45 ^{cd}
Ocimum basilicum	10	43.33 ^{d}
	25	36.67 ^{ef}
	50	35 ^{fg}
	100	33.33 ^{g}
Control	0	50 ^{a}

$LSD_{concentration} = 0.75$; $LSD_{extract* concentration} = 1.68$, CV = 2.87 %
Values followed by common letter are not significantly different at 5% level of probability.

The results showed that extracts of the different plants species are substantially varied in their antifungal potentials. These differences are to be expected since plants vary in their chemical constituents, habitats and stages at which they were collected. Differences in the natures and concentration of inhibitory material even between different plant parts have been reported elsewhere (Al – Abed 1992).

Many workers have compared the effectiveness of the use of biological control to chemical control in the management of plant pathogens (Tewari 1995, Eksteen *et al.* 2001). Tewari (1995) demonstrated lesser cost of application of O. *sanctum* (RS 375/ha) compared to synthetic fungicide of Ediphenphos (RS 1430/ha) or Carbendazim (RS 1580 /ha).

There is a large reservoir of natural fungicide in plants, which with continued research would provide safer and effective alternative to synthetic fungicides. Further studies to test the efficacy of formulation and application methods will be carried out.

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