PEST MANAGEMENT

Plant Extracts as an Alternative to Control *Leucoptera coffeella* (Guérin-Mèneville) (Lepidoptera: Lyonetiidae)

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Abstract

We evaluated the effects of crude extracts from the plantain *Plantago lanceolata* and the bitter gourd *Momordica charantia* on the oviposition preference and development of the coffee leaf miner *Leucoptera coffeella* Guérin-Mèneville & Perrottet under laboratory and/or greenhouse conditions. The ovicidal effects of these extracts were also studied in a greenhouse. *Plantago lanceolata* and *M. charantia* extracts also underwent fractionation directed by oviposition tests with the coffee leaf miner. The extracts of both plants reduced *L. coffeella* oviposition and egg hatching, apparently as a result of action of plant metabolites on the embryo. Adults originating from eggs treated with the extracts exhibited similar survival rates, but a higher female/male ratio. Fecundity was reduced for females obtained from eggs treated with the *M. charantia* extract. Partial chemical analysis indicated that both extracts produced polar fractions that reduced the oviposition of *L. coffeella* on coffee leaves under laboratory conditions. The extracts of *P. lanceolata* and *M. charantia* have potential for use in the development of new products to control the coffee leaf miner.

Introduction

The coffee leaf miner, *Leucoptera coffeella* (Guérin-Mèneville), is an economically important pest of coffee crops (Pimentel 2007). It is mainly controlled by synthetic insecticides, which contaminate humans and the environment, and cause biological imbalances that lead to the increase of secondary pests and to the selection of insect populations resistant to insecticides (Sharma 2008).

A possible alternative to control the coffee leaf miner is the use of plant secondary metabolites that are active against herbivores (Biere *et al* 2004, Schaller 2008). These substances are intensively studied as an alternative

strategy in insect pest management, and some of them are already commercially available, such as those derived from *Azadirachta indica* (Agboka *et al* 2009, Siddiqui *et al* 2009). However, the diversity of plants and their metabolites requires wider investigation into the role of other plant metabolites for insect control.

Some of the potential plants are the bitter gourd *Momordica charantia* (Cucurbitaceae), which acts as a repellent and a proteinase inhibitor (Boeke *et al* 2004, Telang *et al* 2003); and the plantain *Plantago lanceolata* (Plantaginaceae), which produces large amounts of glycosylated iridoids (Marak *et al* 2000, Tamura & Nishibe 2002) with deterrent or growth-reducing effects against insects (Bowers & Puttick 1988, Nieminen *et al* 2003).

Based on the potential of *M. charantia* and *P. lanceolata* for use in the production of new insecticides, we evaluated their effect on the oviposition and development of *L. coffeella*, and conducted bioactivity-directed fractionation of the extracts.

Material and Methods

Plant extracts

Fresh leaves of *P. lanceolata* (1569.9 g) and *M. charantia* (617.8 g), which were in vegetative and reproductive states (with flowers), respectively, were collected in June, 2005 at the campus of the Universidade Federal de Lavras. The leaves were cut into small pieces and placed in a 1000 ml glass vial containing approximately twice the volume of methanol in relation to the amount of plants. After resting for 48h in methanol, the samples were filtered through cotton wool plugs. The plant material was re-extracted with methanol five additional times. The filtrates were dried in a rotary evaporator and freeze-dried for 24h to obtain 21.30 g and 44.24 g of *M. charantia* and *P. lanceolata* dry extracts, respectively, which were stored at -10°C.

Laboratory oviposition assay

Aliquots (18 mg) of the dry extracts were dissolved in 2.0 ml of an aqueous 1.0% (g/ml) Tween 80 solution, resulting in extract solutions at a concentration determined by preliminary tests. The extracts were spread onto the adaxial surface of coffee leaves, *Coffea arabica* cv. Catuaí, with a soft paintbrush. Control (no treatment) and treated leaves were set on a styrofoam board in a Petri dish. The bottom of the Petri dish was covered with wet cotton wool, and each dish was placed in a polyvinyl chloride cage covered with transparent plastic film. Inside the cage, two pairs (1-3 days old) of *L. coffeella* were released. These adults were reared according to Reis Jr *et al* (2000). Cotton wool soaked with a 10% sucrose solution was offered as food to the adults, which were allowed to oviposit for 72h under controlled conditions (25 ± 1ºC, RH 70 ± 10%, and 14h photoperiod). Then, the eggs on each leaf were counted. The experiment was carried out in a random design, using five replicates per treatment, with each replicate represented by an experimental cage containing one treated and one untreated leaf. Aqueous 1.0% (g/ml) Tween 80 and 0.2% (v/v) Lorsban® (chlorpyrifos, Dow AgroSciences, Indianapolis) solutions were employed as negative and positive controls, respectively. Data were expressed as a percentage [eggs on treated leaf x 100 / (eggs on treated leaf + eggs on untreated leaf)] and submitted to the χ2 test (P ≤ 0.05).

Greenhouse oviposition assay

Six-month-old coffee plants (*Coffea arabica* cv. Catuaí IAC-99) were trimmed to four leaves and sprayed with extracts of *M. charantia* and *P. lanceolata* at 1.0% (g/ml) in 1.0 % (g/ml) Tween 80. The experiment was carried out in a randomized block design with five replicates, using water and aqueous Tween 80 as controls. Each block consisted of a wooden cage containing four coffee plants, in which each plant corresponded to a treatment. Twenty pairs of *L. coffeella* were released in each cage. The eggs were counted 48h and 72h after the moths were released, and their numbers were expressed as a percentage [eggs in each treatment x 100 / total number of eggs per cage] and submitted to the χ2 test (P ≤ 0.05).

Greenhouse ovicidal assay

Coffee plants were exposed to *L. coffeella* for 12h, to allow oviposition. The plants were then removed from the cage and the number of eggs per leaf was counted, leaving five eggs on each leaf. The plants were then sprayed with solutions of *M. charantia* and *P. lanceolata* aqueous extracts 1.0% (w/v) Tween-80 until they were dripping. Each treatment comprised four replicates arranged in a random design. Aqueous 1.0% (g/ml) Tween 80 and 0.2% (v/v) Sumithion® 500 CE (Fenitrothion, IHARABRAS S.A., São Paulo) solutions were used as negative and positive controls, respectively. After seven days, nonviable eggs were counted, and the numbers were expressed as percentages and subjected to analysis of variance (ANOVA) and to the Scott-Knott test (P ≤ 0.05) for comparisons.

Ultrastructural analysis of nonviable eggs

The nonviable eggs obtained from the greenhouse ovicidal trial were prepared for scanning electron microscopy analysis according to Alves (2004). Initially, pieces of coffee leaves containing nonviable eggs were kept in the modified Karnovsky fixative (2.5% glutaraldehyde, 2.5% formaldehyde, 0.05 M sodium cacodylate buffer at pH 7.2, 0.001 M calcium chloride) at 4°C for 24h, and later transferred to 1% osmium tetroxide solution (ml/ml) buffered with 0.05 M cacodylate for 4h at room temperature. Samples were washed with distilled water and dehydrated using a graded series of propanone (25, 50, 75, 90, and 100% - 10 min/each), and subjected to critical-point drying on a Balzers CPD 030 Critical-Point Dryer. Samples were mounted on aluminum stubs using double-sided carbon tape, sputter-coated with gold on a Balzers SCD 050 gold evaporator, and analyzed on a Leo Evo 40 scanning electron microscope using the Leo User Interface software.

Effect of plant extracts on L. coffeella *development*

This experiment was carried out as described for the

greenhouse ovicidal trial. On the fifteenth day after the plants were sprayed with the extracts, leaves containing young miners were collected and maintained in Petri dishes under controlled conditions (25 ± 1ºC, RH 70 ± 10%, and 14h photophase) until pupation. Pupae were individually transferred to glass tubes, where they were kept until the adults emerged, to determine pupae survival and sex ratio. Each pair was released in a polyvinyl chloride cage containing a Petri dish with two coffee leaves. After 48h, the eggs were counted. All data were submitted to analysis of variance (ANOVA) and means were compared by the Scott-Knott test (P ≤ 0.05).

Fractionation of P. lanceolata *extract*

A sample of 9.81 g of *P. lanceolata* extract was washed with hexane (Hex), ethyl acetate (AcOEt), and methanol (MeOH) (5 x 200 ml for each solvent). Each of the resulting solutions was concentrated to dryness in a rotary evaporator and freeze-dried to afford a hexane (2.0147 g), an ethyl acetate (0.3078 g), and a methanol (5.9264 g) soluble fraction, and an insoluble residue (0.7693 g). Aliquots (0.37%) of all fractions (including the residue) were dissolved in 2.0 ml of an aqueous 1.0% (g/ml) Tween 80 solution prior to use.

A sample (1.00 g) of the MeOH soluble fraction was eluted through a 3 x 20 cm column packed with silica gel (230-400 mesh, Merck), using MeOH, distilled water, and 0.1 M HCl (300 ml each) as eluents. After concentration of the resulting eluates, six new fractions were obtained: MeOH-I (0.0042 g), MeOH-II (0.7877 g), MeOH-III (0.0720 g), water-I (0.0678 g), water-II (0.0445 g), and HCl (0.2988 g). Aliquots (2.84%) of these fractions were dissolved in 2.0 ml of a 1% Tween 80 solution, and applied to coffee leaves to assess the effects on the oviposition of leaf miners under laboratory conditions.

Fractionation of the M. charantia *extract*

The *M. charantia* extract (10.2332 g) was treated similarly to the *P. lanceolata* extract, and four fractions were collected (Hex – 0.4398 g, AcOEt – 3.7300 g, MeOH-I – 4.6316 g, all colored dark green color; MeOH-II – 0.9153 g, colorless). A sample (1.0266 g) of the fraction MeOH-I was fractionated as described above, and three new fractions were obtained: MeOH (0.8453 g), water (0.1242 g), and HCl (0.2186 g).

Results and Discussion

Leaves treated with the extracts of *Plantago lanceolata* (χ^2 = 47.21, P < 0.05) and *M. charantia* (χ^2 = 63.71, P < 0.05) were less preferred than control leaves for oviposition by coffee leaf miner adults in a free-choice test, under laboratory conditions (Table 1). Forty-eight hours after the release of insects, the *P. lanceolata* extract at

Table 1 Effect of *Plantago lanceolata* and *Momordica charantia* extracts on *Leucoptera coffeella* oviposition under laboratory conditions.

Experiment	Treatment	Eggs on treated leaves (%)
1	Tween 80 (1%)	46.7 ± 11.23
	P. lanceolata	7.3 ± 4.20*
	Lorsban® (0.2%)	0.0 ± 0.00*
2	Tween 80 (1%)	62.2 ± 15.36
	M. charantia	6.3 ± 3.15*
	Lorsban® (0.2%)	0.0 ± 0.00*

The experiments were conducted in the same way, but at different times. Five replicates were used per treatment, each plot consisting of an experimental cage containing one treated and one untreated leaf. *Means differ significantly according to the χ^2 test, compared with the negative control (P < 0.05).

concentrations of 0.9% (χ^2 = 10.72, P < 0.05) and 1.8% (χ^2 = 14.81, P < 0.05) deterred oviposition by *L. coffeella*. In contrast, the *M. charantia* extract deterred oviposition only at 1.8% (χ^2 = 14.81, P < 0.05). By 72h after the release, only the *P. lanceolata* extract at 0.9% (χ^2 = 13.01, P ≤ 0.05) and 1.8% (χ^2 = 14.48, P < 0.05) reduced oviposition by *L. coffeella* (Table 2).

The eggs of *L. coffeella* treated with extracts of *P. lanceolata* and *M. charantia* had lower viability than eggs under control treatments (Table 3). Scanning electron microscopy observations of nonviable eggs exposed to both extracts (Fig 1) showed alterations on their surfaces

Table 2 Effect of *Plantago lanceolata* and *Momordica charantia* extracts on *Leucoptera coffeella* oviposition in a greenhouse assay.

Experiment	Treatment	Eggs on coffee leaves (%)	
		48h	72h
1	Water	37.1 ± 3.90	28.4 ± 0.87
	Tween 80 (1%)	29.8 ± 4.52	22.5 ± 2.12
	M. charantia (0.9%)	27.4 ± 3.12	32.6 ± 3.78
	P. lanceolata (0.9%)	10.5 ± 3.78*	18.6 ± 3.12*
2	Water	40.3 ± 10.38	28.6 ± 4.87
	Tween 80 (1%)	31.4 ± 9.38	26.1 ± 2.49
	M. charantia (1.8%)	18.2 ± 6.59*	27.4 ± 4.13
	P. lanceolata (1.8%)	10.2 ± 4.68*	18.5 ± 6.44*

The experiments were conducted in the same way, but at different times. Five plants were used for each treatment, and four leaves per treatment were evaluated. The percentage of eggs on each leaf was calculated: total number of eggs in each block/total number of eggs in each treatment. *Means differ significantly according to the χ^2 test (P < 0.05), compared with the negative control.

Table 3 Effect of *Plantago lanceolata* and *Momordica charantia* extracts on egg viability of *Leucoptera coffeella*.

Treatment	Viable eggs (%)
Sumithion® (0.2%)	62.3 ± 14.49 a
P. lanceolata (0.9%)	63.0 ± 18.12 a
M. charantia (0.9%)	68.4 ± 19.05 a
Tween 80 (1%)	88.9 ± 17.04 b
Water	95.5 ± 11.55 b

Four plants were used for each treatment, and four leaves per treatment, with five eggs, on average, for a mean of 20 eggs per plant, were removed for evaluation. Means followed by the same letter do not differ significantly according to the Scott-Knott test (P > 0.05).

that were typical of those observed for nonviable eggs treated with commercial insecticides (Fig. 1 g, h). In all these cases, superficial fractures on the chorionic structure of the eggs could be easily observed.

The treatment of eggs of *L. coffeella* with extracts had no effect on adult emergence, although these treatments produced a skewed sex ratio towards females, which showed reduced fecundity (Table 4).

The methanol fraction from *P. lanceolata* (χ^2 = 24.93, P ≤ 0.05) and the polar fraction MeOH I of *M. charantia* (χ^2 = 35.01, P < 0.05) reduced oviposition by leaf miner adults (Table 5). When fractionated by column chromatography, both fractions yielded new active fractions: MeOH I (χ^2 = 10.61, P < 0.05), MeOH II (χ^2 = 32.2, P < 0.05), MeOH III (χ^2 = 8.83, P < 0.05), Water I (χ^2 = 22.97, P < 0.05), and HCl (χ^2 = 37.98, P < 0.05) for *P. lanceolata*; and MeOH (χ^2 = 41.48, P < 0.05) and HCl (χ^2 = 29.6, P < 0.05) for *M. charantia* (Table 6).

The choice of the host plant for oviposition by the insect depends on a complex set of stimuli and responses (Reudler *et al* 2008, Städler & Reifenrath 2009) that may be mediated by the insect's sensory system, composed of receptors that can perceive plant metabolites, resulting in an increase or decrease in the number of eggs that females can lay on a plant (Schoonhoven *et al* 2005, Navarro-Silva 2009). Our data indicated that both extracts tested contain molecules that can affect the sensory system of *L. coffeella* females used to evaluate the quality of the host plant as a substrate for oviposition.

The non-preference of *L. coffeella* for oviposition on leaves treated with *P. lanceolata* extracts is probably due to the glycosylated iridoids aucubin and catalpol, which are produced by *P. lanceolata* and have shown deterrent activity against several insects (Talsma *et al* 2008). As observed with *L. coffeella* in the present study, the methanol extract of *M. charantia* has also reduced the oviposition of *Liriomyza trifolii* (Burgess) (Diptera:
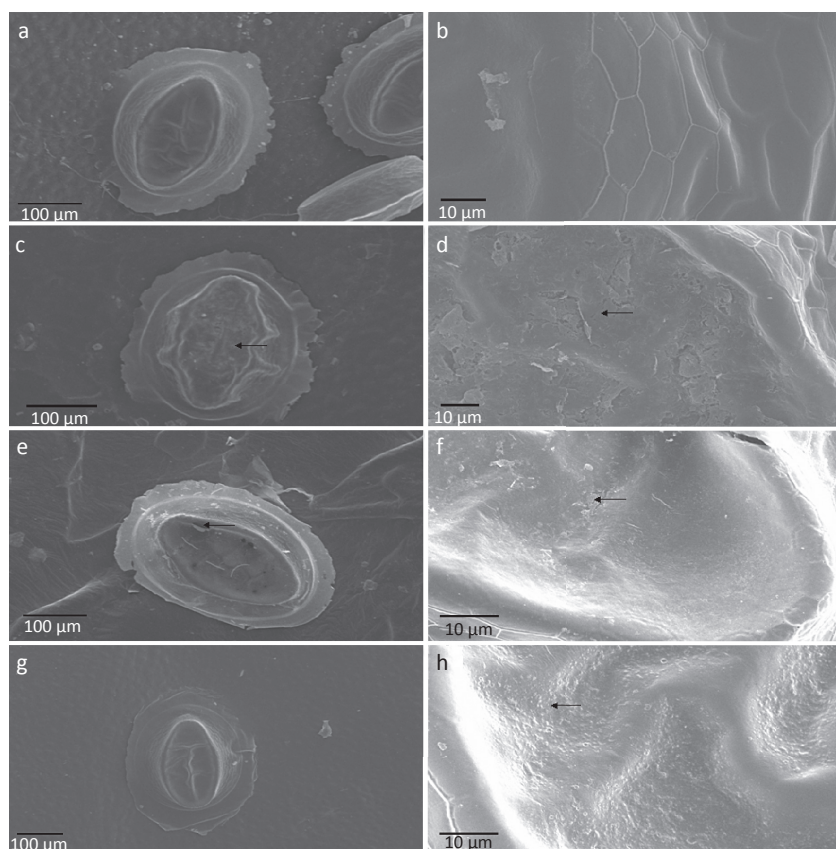

Fig 1 Scanning electron photomicrographs of eggs of *Leucoptera coffeella*. a and b) eggs treated with an aqueous 1.0% Tween 80 solution; c and d) eggs treated with *Plantago lanceolata* extract; e and f) eggs treated with *Momordica charantia* extract; g and h) eggs treated with the commercial insecticide Sumithion®. Changes in the outer surface of eggs are indicated by arrows.

Table 4 Mean (± SE) sex ratio and number of adults emerged from pupae obtained from eggs of *Leucoptera coffeella* exposed to extracts from *Momordica charantia* and *Plantago lanceolata*, and eggs laid by these adults.

Treatment	Emerged adults[1]	Sex ratio[2]	Number of eggs/female
Sumithion® (0.2%)	16.7 ± 7.56 a	1.0 ± 0.00 b	-
M. charantia	60.4 ± 10.83 b	0.8 ± 0.22 b	5.5 ± 1.11 a
P. lanceolata	66.5 ± 10.29 b	0.7 ± 0.17 b	11.0 ± 2.23 b
Tween 80 (1%)	72.0 ± 3.40 b	0.6 ± 0.12 a	8.5 ± 2.06 b
Water	81.5 ± 11.72 b	0.5 ± 0.08 a	9.0 ± 1.87 b

Four plants were used for each treatment and four leaves per treatment, with five eggs, on average, for a mean of 20 eggs per plant that received treatments. Means followed by the same letter, in each column, do not differ significantly according to the Scott-Knott test (P > 0.05). [1]Number of adults x 100/ number of pupae; [2]Number of females/number of adults.

Table 5 Effect on *Leucoptera coffeella* oviposition by fractions of *Plantago lanceolata* and *Momordica charantia* extracts that resulted from washings with hexane, ethyl acetate (AcOEt), and methanol (MeOH).

	Treatment	Eggs on treated coffee leaves (%)
	Tween 80 (1%)	49.4 ± 5.86
	Lorsban® (0.2%)	0.0 ± 0.00*
Extract of *P. lanceolata*	Hexane fraction	33.1 ± 3.11
	AcOEt fraction	30.4 ± 2.30
	MeOH fraction	11.1 ± 1.91*
	Insoluble fraction	52.3 ± 3.85
	Tween 80 (1%)	39.9 ± 4.26
	Lorsban® (0.2%)	0.0 ± 0.00*
Extract of *M. charantia*	Hexane fraction	52.1 ± 5.62
	AcOEt fraction	27.1 ± 7.53
	MeOH I fraction	5.8 ± 3.65*
	MeOH II fraction	40.3 ± 10.63

Five replicates were used per treatment, each plot consisting of an experimental cage containing one treated and one untreated leaf. *Means differ significantly according to the χ^2 test (P < 0.05) for the respective treated leaves.

Agromyzidae) (Mekuria *et al* 2005), but no chemicals were identified by these authors.

The ovicidal effect observed for both extracts is very interesting, because ovicidal activity of both synthetic and botanical products is uncommon (Ofuya 1997, Martinez & Meneguim 2003). Although the mechanisms by which extracts of plantain and bitter gourd kill the embryos of

Table 6 Effect on *Leucoptera coffeella* oviposition by fractions obtained by column chromatography of fractions active against this insect, obtained by solvent washings (MeOH: methanol) of the extracts from *Plantago lanceolata* and *Momordica charantia*.

Treatment		Eggs on treated leaves of coffee (%)
	Tween 80 (1%)	31.1 ± 4.89
	Lorsban® (0.2%)	0.0 ± 0.00*
	MeOH I fraction	22.3 ± 4.63*
Methanol fraction (MeOH) of *P. lanceolata*	MeOH II fraction	0.0 ± 0.00*
	MeOH III fraction	25.1 ± 5.31*
	Water I fraction	12.2 ± 7.20*
	Water II fraction	34.0 ± 10.12
	HCl fraction	4.5 ± 2.91*
	Tween 80 (1%)	38.2 ± 6.78
Methanol fraction (MeOH I) of *M. charantia*	Lorsban® (0.2%)	0.0 ± 0.00*
	MeOH fraction	3.1 ± 1.31*
	Water fraction	36.9 ± 5.63
	HCl fraction	8.7 ± 4.95*

Five replicates were used per treatment, each plot consisting of an experimental cage containing one treated and one untreated leaf. *Means differ significantly according to the χ^2 test (P < 0.05) for the respective treated leaves.

L. coffeella are still unclear, our observations indicate that they may act as disruptors of the egg chorion, since the observed damage to the chorionic structure of the egg may favor dehydration and induce mortality.

Despite the lack of any report on the activity of *M. charantia* against *L. coffeella*, the effects here observed were similar to those reported elsewhere (Neraliya & Srivastava 1996), with the molecules momordicine I and II being associated with the noxious effects induced by extracts of this plant (Chandravadana 1987, Mekuria *et al* 2005, Ling *et al* 2008). Because of the polar nature of momordicine and because only the MeOH I fraction of *M. charantia* reduced the oviposition of *L. coffeella*, it is likely that momordicine I and II are also the molecules that affected this insect. Also the activity of *P. lanceolata* against some insects has been reported and attributed to the production of polar molecules such as aucubin and catalpol (Marak *et al* 2000, Fuchs & Bowers 2004, Harvey *et al* 2005). Furthermore, Bowers & Stamp (1993) demonstrated that catalpol concentration in plants increases under herbivore attack. Consequently, the reduction in the oviposition of *L. coffeella* on leaves treated with the MeOH polar fraction of the *P. lanceolata* extract may be induced by one of these molecules.

Our data indicate that *M. charantia* and *P. lanceolata*

produce active compounds against *L. coffeella*, and may have potential for use in the development of new products for controlling the infestation level of this insect pest in coffee plantations. Therefore, further studies should be carried out to investigate the potential of these plants and their compounds for the control of *L. coffeella*.

Acknowledgments

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