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Original article

Laboratory evaluation of the effects of essential oil of *Myrciaria floribunda* leaves on the development of *Dysdercus peruvianus* and *Oncopeltus fasciatus*

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ABSTRACT

Myrciaria floribunda (H. West ex Willd.) O. Berg, Myrtaceae, is popularly known as "*camboim-amarelo*" and was collected at Restinga de Jurubatiba (RJ, Brazil). Leaves from this species were submitted to hydrodistillation to extract its essential oil. Monoterpenes were the main compounds found (53.9%), and 1,8-cineole was the major constituent (38.4%). Studies were carried out to evaluate the effects of this essential oil on the development of two species of agricultural pests (*Oncopeltus fasciatus* and *Dysdercus peruvianus*). The essential oil was considered effective against *D. peruvianus* and *O. fasciatus*, causing mortality in both insects. The LD_{50} values (μg/insect) observed were 112.44 μg/insect (*O. fasciatus*) and 309.64 μg/insect (*D. peruvianus*) after one day of treatment, and 72.18 μg/insect (*O. fasciatus*) and 94.42 μg/insect (*D. peruvianus*) after 22 days of treatment. The present study reports for the first time the bioinsecticidal activity of essential oil of *Myrciaria floribunda* leaves, and provides important data regarding the use of essential oils in complementary programs for pest control.

Introduction

During the last decades, several substances have been studied in order to discover new pesticides with selective activity against invertebrates, as insects and arachnids, innocuous to non-target vertebrate species. This concept is important, since most of the products with commercial relevance, such as organophosphates and carbamates have a negative impact on mammals, fish, fowl and other species (Rattan and Sharma, 2011). Alternative sources of potential suitable bioinsecticides include natural products, which may act as antifeedants or growth regulators, and have an additional advantage associated to low environmental persistence

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(Isman, 2006). Furthermore, several compounds of plant origin, such as flavonoids, terpenoids, alkaloids, steroids and phenols, have been documented to have insecticidal properties (Castillo-Sánchez et al., 2010).

The Myrtaceae family consists of about 144 genera and 4,630 species, distributed mainly in tropical and subtropical regions of the world (Judd et al., 2009) and is recognized by its essential oils (Apel et al., 2006). In Brazil, 24 genera and 986 species are found (Sobral et al., 2013), including *Myrciaria floribunda* (H. West ex Willd.) O. Berg. This species is popularly known as "*camboim*", "*jabuticabinha*", "*murta*", "*duque*", "*goiabarana*" and "*araçazeiro*" (Lorenzi, 2009). *M. floribunda* is widely distributed in the north, northeast, mid-west, southeast and south of Brazil (Sobral et al., 2013). Studies revealed that *M. floribunda* is a good source of essential oils able to inhibit bacterial growth (de Ramos et al., 2010) and inhibit acetylcholinesterase enzyme (Tietbohl et al., 2012).

Several studies have reported the effectiveness of essential oils and their constituents as bioinsecticides (Roman, 2005; Nesci et al., 2011; Kumar et al., 2012; Zandi-Sohani et al., 2013). This activity has been associated to the inhibition of acetylcholinesterase, recognized as an important mechanism of insecticidal action (Rattan, 2010; López and Pascual-Villalobos, 2010).

The present study aimed to investigate the biological effects of the essential oil of *Myrciaria floribunda* leaves on the development of the cotton stainer bug *Dysdercus peruvianus* (Hemiptera: Pyrrhocoridae), which causes severe losses in cotton plantations, and against the milkweed bug, *Oncopeltus fasciatus* (Hemiptera: Lygaeidae), which is used as an insect model for insecticidal activity studies.

Materials and methods

Plant material

Three specimens of *Myrciaria floribunda* (H. West ex Willd.) O. Berg, Myrtaceae, were collected at Restinga de Jurubatiba National Park (RJ), Brazil, in the Clusia scrub vegetation (22°12'58.2"S - 41°35'00.0"W, 22°13'3.3"S - 41°35'14.4"W, 22°13'00.1"S - 41°35'01.0"W) during the day on April 3rd, 2011. This species was identified by the botanist Dr. Marcelo Guerra Santos and a voucher specimen was deposited at the herbarium of the Faculdade de Formação de Professores (Universidade do Estado do Rio de Janeiro, Brazil) under the registration number RFFP 13.789.

Extraction of the essential oil

Leaves of the three specimens collected were turbolized with distilled water. The material was placed in a 5l round-bottom flask and brought to boil to allow hydrodistillation for 4h in a Clevenger-type apparatus. The oil was collected and stored at 4°C for further analyses. This procedure was previously described by Tietbohl et al. (2012).

Gas chromatography/mass spectrometry analysis

The essential oil recovered was analyzed using a GCMS-QP5000 (Shimadzu) gas chromatograph, equipped with a mass spectrometer using electron ionization. Gas chromatographic (GC) conditions were as follows: injector temperature, 260°C; FID temperature, 290°C; carrier gas, Helium; flow rate, 1 ml/min and split injection with split ratio 1:40. Oven temperature was initially 60°C and then risen to 290°C at a rate of 3°C/min. One microliter of each sample, dissolved in dichloromethane (1:100 mg/µl), was injected into a DB-5 column (0.25 mm I.D, 30 m in length, 0.25 µm and film thickness). Mass spectrometry (MS) electron ionization was 70 eV and scan rate was 1 scan/s. Retention indices (RI) were calculated by extrapolating the retention times of a mixture of aliphatic hydrocarbons (C9-C30) analyzed under the same conditions (Van Den Dool and Kratz, 1963). Identification of substances was performed by comparing their retention indices and mass spectra with those reported in literature (Adams, 2007). MS fragmentation pattern of compounds was also compared with NIST mass spectra libraries. Quantitative analysis of the chemical constituents was performed by flame ionization gas chromatography (CG/FID), under the same conditions of GC/MS analysis. Percentages of these compounds were obtained by FID peak-area normalization method. This procedure was previously described by Tietbohl et al. (2012).

Insect colonies

Oncopeltus fasciatus and *Dysdercus peruvianus* colonies were established in the Laboratory of Insect Biology of the Universidade Federal Fluminense as described by Fernandes et al. (2013).

Insect bioassays

In this study, the bioinsecticidal activity of the essential oil of *Myrciaria floribunda* leaves at different concentrations against fourth instar of *D. peruvianus* and *O. fasciatus* was evaluated. One microliter of pure essential oil, or its dilutions in ethanol (500, 250, 125 and 62.5 mg oil/ml), were applied topically to the ventral cuticle of insects. The doses administered were 0.5 mg/insect (500 µg/insect), 0.25 mg/insect (250 µg/insect), 0.125 mg/insect (125 µg/insect) and 0.0625 mg/insect (62.5 µg/insect). For the pure essential oil, the dose was 1000 µg/insect. The untreated group did not receive any product. The control group received 1 µl of solvent (ethanol).

Biological evaluation of different treatments was performed during the time required for the development from the fourth instar nymphae to the adult stage (22 days). Acute toxicity (mortality during 24 h immediately after treatment), lethality (mortality during 22 days of treatment) and metamorphosis were evaluated. All experiments were performed in triplicate with groups of fully engorged insects (n = 10), from the day after topical application on fourth instar nymphae (1st day) to the last day of observation (22th day). *Dysdercus peruvianus* were reared at 24-25°C (Milano et al., 1999), 75% relative humidity and a 16:8 h light-dark cycle. Insects were kept in transparent glass pots, covered with screen tissue, fed with cotton seeds (*Gossypium hirsutum*) and had free access to water stored in glass flasks inside the pots. Seeds were placed inside the pots during the mating and laying period, until the first instar. *Oncopeltus fasciatus* were maintained under similar conditions

to *Dysdercus peruvianus*, at 24-25°C (Stanisçuaski et al., 2005), with relative humidity of 70-75% and a 16:8 h light-dark cycle, but fed with sunflower seeds (*Helianthus annuus*) (Feir and Beck, 1963). All experiments were carried out in an incubator with a constant temperature of 25°C.

Statistical analysis

Statistical analysis was performed by one-way ANOVA and Tukey's test, using Stats Direct Statistical Software, version 2.2.7 for Windows 98 (Armitage et al., 2002). Differences were not considered statistically significant when $p > 0.05$. DL50 was evaluated using Combi Stats v5.0 (EDQM, Council of Europe). Probabilities are specified within the text and the tables.

Results and discussion

The chemical analysis of constituents of the essential oil from *Myrciaria floribunda* (H. West ex Willd.) O. Berg, Myrtaceae, allowed the identification of 24 compounds. Monoterpenes constituted the main group, corresponding to 53.9% of relative composition of this essential oil; and 1,8-cineole the major constituent found, corresponding to 38.4 % of total relative composition. Bioinsecticidal evaluation was performed just after extraction. Information regarding chemical composition of this essential oil can have been reviewed elsewhere (Tietbohl et al., 2012).

Topical treatment with essential oil from *M. floribunda* leaves induced a significant increase in the mortality of *D. peruvianus* fourth instar nymphae, as shown in Table 1. The group treated with 1000 µg/insect of pure essential oil presented 100% of mortality. Control group (treated with ethanol) exhibited 16.66 ± 5.70% ($p > 0.05$) of mortality, while the untreated group showed 13.30 ± 5.70% of mortality at the end of the experiments. It was also observed that insects treated with essential oil at the dose of 500 µg/insect in ethanol presented 66.67 ± 25.17 % ($p < 0.001$) of mortality at 24 h (toxicity) and 93.33 ± 5.77% ($p < 0.0001$) 22 days after treatment. Application of essential oil at a dose of 0.25 mg/insect in ethanol induced 30 ± 17.30 % ($p < 0.01$) and 90.0 ± 0.0% ($p < 0.0001$) of mortality after 24 h and 22 days, respectively. The observed mortality rates of insects treated with essential oil at the dose of 0.125 mg/insect in ethanol were 23.30 ± 5.70% ($p < 0.01$) and 76.67 ± 5.70% ($p < 0.001$) after 24 h and 22 days, respectively. The lowest essential oil dose (0.0625 mg/insect) induced 23.33 ± 11.50% of mortality only at the end of the experiment (22 days).

Regarding metamorphosis, approximately 86.70% ($p > 0.05$) of control group and untreated group insects achieved adult stage. Also, about 6.67 ± 5.70% ($p < 0.0001$), 10 ± 5.70% ($p < 0.0001$), 23.33 ± 5.70% ($p < 0.001$) and 76.67 ± 11.50% of the insects reached adult stage, when respectively treated with 0.5, 0.25, 0.125, 0.0625 mg/insect of essential oil from leaves of *M. floribunda* (Table 1).

In Table 2 are listed the results regarding topical treatment with essential oil from *M. floribunda* causing significant increase in the mortality of *O. fasciatus* fourth instar nymphae. Untreated group and control group (treated with ethanol) exhibited 16.60 ± 5.70% of mortality only at the end of the experiments (22 days). Pure essential oil (1000 µg/insect) and dilution at the dose of 500 µg/insect in ethanol induced 100% of mortality immediately after application. A dose of 0.25 mg/insect in ethanol induced 93.33 ± 5.70% ($p < 0.0001$) of mortality after one day of treatment, reaching 100% at the end of the experiment. The mortality rates after one day of observation were 70.0 ± 10.0% ($p < 0.001$), and 86.67 ± 5.70% ($p < 0.001$) after 22 days, with essential oil at a dose of 0.125 mg/insect. Topical treatment with essential oil at a dose of 0.0625 mg/insect in ethanol only induced 30.0 ± 10.0% of mortality at the end of the experiment.

No insects reached adult stage on groups treated with pure essential oil and its dilutions at a dose of 0.5 and 0.25 mg/insect

Table 1
Analysis of mortality and metamorphosis after topical treatment of *Dysdercus peruvianus* fourth instar nymphae with essential oil of leaves from *Myrciaria floribunda*.

Groups	% Mortality ± SD		% Metamorphosis ± SD	
	1st day	22th day	1st day	22th day
Untreated group	0	13.30 ± 5.70 ns	0	86.70 ± 5.77 ns
Control group	0	13.30 ± 5.70 ns	0	86.67 ± 5.77 ns
Pure essential oil	100.00[a]	-	0	0
0.5 mg/insect	66.67 ± 25.17[b]	93.33 ± 5.77[a]	0	6.67 ± 5.70[a]
0.25 mg/insect	30.00 ± 17.30[c]	90.00 ± 0.00[a]	0	10.00 ± 5.70[a]
0.125 mg/insect	23.30 ± 5.70[c]	76.67 ± 5.70[b]	0	23.33 ± 5.70[b]
0.0625 mg/insect	0	23.33 ± 11.50[d]	0	76.67 ± 11.50[d]

The data are presented as the average ± standard deviation (SD) in each group.
Statistical significance test for comparison was done by one-way ANOVA, followed by Tukey's test (n = 10).
ns = No significance: $p > 0.05$.
a – $p < 0.0001$; b – $p < 0.001$; c – $p < 0.01$; d – $p < 0.05$ when compared against control group.
Untreated group did not receive any product. Control group received 1 µl of solvent (ethanol).

Table 2
Analysis of mortality and metamorphosis after topical treatment of *Oncopeltus fasciatus* fourth instar nymphae with essential oil of leaves from *Myrciaria floribunda*.

Groups	% Mortality ± SD		% Metamorphosis ± SD	
	1st day	22th day	1st day	22th day
Untreated group	0	16.67 ± 5.77 ns	0	83.34 ± 5.77 ns
Control group	0	16.67 ± 5.77 ns	0	83.34 ± 5.77 ns
Pure essential oil	100.00[a]	-	0	0
0.5 mg/insect	100.00[a]	-	0	0
0.25 mg/insect	93.33 ± 5.77[a]	100.00[a]	0	0
0.125 mg/insect	70.00 ± 10.00[b]	86.67 ± 5.70[b]	0	13.33 ± 5.77[a]
0.0625 mg/insect	0	30.00 ± 10.00[d]	0	70.00 ± 10.00[c]

The data are presented as the average ± standard deviation (SD) in each group.
Statistical significance test for comparison was done by one-way ANOVA, followed by Tukey's test (n = 10).
ns = No significance: $p > 0.05$.
a – $p < 0.0001$; b – $p < 0.001$; c – $p < 0.01$; d – $p < 0.05$ when compared against control group.
Untreated group did not receive any product. Control group received 1 µl of solvent (ethanol).

in ethanol by the end of the experiments. Only 13.33 ± 5.77% ($p < 0.0001$) of insects reached adult stage at a dose of 0.125 mg/insect, while 70.0 ± 10.0% ($p > 0.01$) of insects reached adult stage after treatment at dose of 0.0625 mg/insect (22 days).

The toxicity of the essential oil of *M. floribunda* leaves was also expressed as the concentration at which 50% of test insects were killed in a specified time (first day and 22th day), being referred as lethal dose (LD_{50}). Table 3 shows LD_{50} (µg/insect) values were 112.44 µg/insect for *O. fasciatus* and 309.64 µg/insect *D. peruvianus* after one day of treatment, and LD_{50} values of 72.18 µg/insect for *O. fasciatus* and 94.42 µg/insect for *D. peruvianus*, after 22 days of treatment.

Considering the risks associated with synthetic insecticides, such as organophosphates and carbamates, causing environmental and health issues to mammals (Prates et al., 1998), bioinsecticides appear as a promising tool to control agricultural pests (Restello et al., 2009). In this context, essential oils are known to cause the death of many insects (Simas et al., 2004). Bioinsecticidal activity and repellency modulated by essential oils has been associated to terpenoids found in these complex volatile mixtures (Simas et al., 2004; Isman, 2000; Rattan, 2010; Dos Santos et al., 2010; López and Pascual-Villalobos, 2010).

Table 3
Determination of median lethal dose (LD_{50}) of *Myrciaria floribunda* essential oil on *Oncopeltus fasciatus* and *Dysdercus peruvianus*.

Insect	LD_{50} (µg/insect) (lower limit - upper limit)	
	1st day	22th day
Oncopeltus fasciatus	112.44 (92.61 - 135.52)	72.18 (57.74 - 88.59)
Dysdercus peruvianus	309.64 (256.24 - 377.79)	94.42 (77.10 - 114.34)

Procopio et al. (2003) evaluated the repellency of several plant species on adults of *Sitophilus zeamais* and found that *Eucalyptus citriodora* (Hook), a species from the family Myrtaceae, was the most active. The most efficient insecticides are mainly active by contact and/or ingestion (Prates and Santos, 2000). Monoterpenes, considered to be the main constituents of essential oil of the leaves from *M. floribunda*, are lipophilic substances with potential to elicit a toxic interference of basic biochemical processes, leading to physiological and behavioral consequences in insects (Viegas Júnior, 2003; Coitinho et al., 2006; Tarelli et al., 2009; López and Pascual-Villalobos, 2010). These substances exert toxic effects after penetration in the body of insects via respiratory system (fumigant effect), through the cuticle (contact effect) and digestive tract (ingesting effect) (Restello et al., 2009).

Chagas et al. (2002) demonstrated that three essential oils from *Eucallyptus* species (Myrtaceae) that had as main constituent 1,8-cineole exhibit insecticidal activity. This monoterpene was the major substance found in the essential oil of *M. floribunda* leaves and, as found in literature, this substance showed effects on the lesser grain borer, *Rhyzopertha dominica* and the red flour beetle Tribolium castaneum (Herbst), responsible for major economic losses caused by damage to stored cereals (Prates et al., 1998).

The monoterpene 1,8-cineole is the principal substance found in the essential oil of the leaves from *M. floribunda*, corresponding to 38.4% of its relative composition. Regarding literature data we can observe that this substance has been previously recognized for its insecticidal activity. Thus, 1,8-cineole may be contributing, at least partially, to the insecticidal activity of *M. floribunda* leaves essential oil. Moreover, synergic effects, which can occur in complex mixtures of substances, may be relevant to this biological activity. The present study reports for the first time the bioinsecticidal activity of the essential oil of *Myrciaria floribunda* leaves and provides important data regarding the use of essential oils as complementary programs for pest control.

Authors' contributions

LACT run the laboratory work; TB contributed to the biological studies; CPF drafted the manuscript and English review; MGS collected the plant material, performed taxonomic identification and confection of herbarium vouchers; FPM contributed to the laboratory work; KTS contributed to drafting of the manuscript; CBMN and MSG did the statistical analysis and interpretation of data; HPA carried out Combi Stats tests to determine LD_{50}; DF and LR critical reading of the manuscript and supervising of the laboratory work. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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