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Repellent activity of the essential oil from *Laurelia sempervirens* (Ruiz & Pav.) Tul. (Monimiaceae) on *Triatoma infestans* (Klug) (Reduviidae)

[Actividad repelente del aceite esencial de *Laurelia sempervirens* (Ruiz & Pav.) Tul. (Monimiaceae) en *Triatoma infestans* (Klug)(Reduviidae)]

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Abstract: *Triatoma infestans* (Klug) is the principal vector of Chagas disease in Bolivia and neighboring countries. The objective of this study was to determine the chemical composition of the EO of the Chilean laurel, *Laurelia sempervirens* (Ruiz & Pav.) Tul. (Monimiaceae) and to evaluate its repellent effect on fifth-instar nymphs of *T. infestans*. The EO from *L. sempervirens* was obtained by hydrodistillation and analyzed by gas chromatography coupled to mass spectrometry (GC-MS). Their main components were *cis*-isosafrole (89.8%), β-terpinene (3.9%), *trans*-ocimene (2.7%) and methyleugenol (2.2%). Repellency was evaluated on a circle of filter paper divided into two equal zones which were impregnated with test substances [EO or *N*,*N*-diethyl-3-methylbenzamide (DEET) as positive control] and acetone as blank control, respectively. Several concentrations of test substances between 4.125 and 132 µg/cm^2 were tested. The EO from *L. sempervirens* produced significant repellency at concentrations equal or above 66.0 µg/cm^2, while DEET repelled starting at 16.5 µg/cm^2. Future works will be oriented to the study of repellent properties of *cis*-isosafrole alone and mixed with β-terpinene, *trans*-ocimene and methyleugenol on *T. infestans*.

Keywords: *Triatoma infestans; Laurelia sempervirens;* Essential oils; Cis-isosafrole; Repellent activity.

Resumen: *Triatoma infestans* (Klug) es el vector principal de la enfermedad de Chagas en Bolivia y los países vecinos. El objetivo de este estudio fue determinar la composición química del AE del laurel chileno, *Laurelia sempervirens* (Ruiz & Pav.) Tul. (Monimiaceae) y evaluar su efecto repelente en ninfas del quinto estadio de *T. infestans*. La AE de *L. sempervirens* se obtuvo por hidrodestilación y se analizó por cromatografía de gases acoplada a espectrometría de masas (CG-EM). Sus componentes principales fueron *cis*-isosafrol (89.8%), β-terpineno (3.9%), *trans*-ocimeno (2.7%) y metileugenol (2.2%). La repelencia se evaluó en un círculo de papel de filtro dividido en dos zonas iguales que se impregnaron con sustancias de prueba [AE o *N*,*N*-dietil-3-metilbenzamida (DEET) como control positivo] y acetona como control en blanco, respectivamente. Se analizaron varias concentraciones de sustancias de prueba entre 4.125 y 132 µg/cm^2. El AE de *L. sempervirens* produjo una repelencia significativa a concentraciones iguales o superiores a 66.0 µg/cm^2, mientras que DEET repelió a partir de 16.5 µg/cm^2. Futuros trabajos serán orientados al estudio de las propiedades repelentes de *cis*-isosafrol solo y mezclado con β-terpineno, *trans*-ocimeno y metileugenol en *T. infestans*.

Palabras clave: *Triatoma infestans*; *Laurelia sempervirens*; Aceites esenciales; *Cis*-isosafrole; Actividad repelente

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INTRODUCTION

Chagas disease, also called American trypanosomiasis, is caused by the protozoan *Trypanosoma cruzi* (Pérez-Molina, 2018). It is transmitted as disease by blood-sucking bugs belonging to the family Reduviidae. The main vector of Chagas in Bolivia is *Triatoma infestans* (Hemiptera: Reduviidae) (Torrico, 2007; Rojas-Cortez, 2007). According to WHO (2015), vector control is considered the most advantageous method for preventing Chagas disease in Latin America. At present, most campaigns of Chagas vector control are based on spraying pyrethroid formulations diluted in water (Vassena *et al.*, 2007). In Bolivia, the National Chagas Program performs controls inside and outside homes to eliminate transmission of the vector (Rojas-Cortez, 2007). Most Bolivian domestic populations of *T. infestans* are resistant to the pyrethroid deltamethrin (Vassena *et al.*, 2007; Lardeux *et al.*, 2010). On the other hand, it is desirable to replace conventional insecticides with alternatives having less environmental impact and less toxicity towards non-pest organisms (Diaz-Barriga, 2002). For these reasons, there is a constant search for new tools to control triatomines as well as for the design of integrated pest management strategies (Lardeux *et al.*, 2010).

Essential oils (EOs) are complex mixtures of monoterpenes and other substances (Isman, 2008) which display a broad spectrum of insecticidal activity (Park *et al.*, 2003; Sfara *et al.*, 2009; Tarelli *et al.*, 2009; Alzogaray *et al.*, 2011; Urzúa *et al.*, 2010; Urzúa *et al.*, 2011a; Urzúa *et al.*, 2011b; Urzúa *et al.*, 2013; Echeverría & Albuquerque, 2019). Lethal as well as sublethal repellency effects of terpenes on insects have been extensively documented (Katz *et al.*, 2008; Sfara *et al.*, 2009; Moretti, 2017).

Laurelia sempervirens (Ruiz & Pav.) Tul. (Monimiaceae) is an evergreen endemic tree of the temperate rainforests of southern Chile, mainly on the coast between the Región del Libertador General Bernardo O'Higgins and Región de los Lagos (Rodríguez *et al.*, 2018). Different extracts from the plant have shown a variety of activities against insects. Thus, the EO from leaves and bark of *L. sempervirens* showed insecticidal and repellent activity towards the confused flour beetle *Tribolium confusum* (Zapata & Smagghe, 2010; Torres *et al.*, 2014; Herrera-Rodríguez *et al.*, 2015; Torres *et al.*, 2015; Ortiz *et al.*, 2017), the greenhouse white fly *Trialeurodes vaporariorum* and its chalcidoid parasitic wasp *Encarsia formosa* (Zapata *et al.*, 2016), and the pea aphid *Acyrthosiphon pisum* (Zapata *et al.*, 2010), while the leaf powder produced lethal and sublethal effects on the maize weevil *Sitophilus zeamais* (Torres *et al.*, 2015; Noranbuena *et al.*, 2016) and the bean weevil *Acanthoscelides obtectus* (Bittner *et al.*, 2008). The aim of this study was to determine the chemical composition of the EO from leaves of *L. sempervirens* and its repellent effect towards fifth-instar nymphs of *T. infestans.*

MATERIAL AND METHODS

Chemicals

N,N-diethyl-3-methylbenzamide DEET (97%) and acetone (analytical grade) were purchased to Sigma Aldrich (Buenos Aires, Argentina) and Merck (Darmstadt, Germany), respectively.

Biological material

The assays were performed with fifth-instar nymphs of *Triatoma infestans* (colony CIPEIN). This colony was maintained at 26 ± 2°C and 60-90% relative humidity (RH); and weekly fed pigeon blood, according to the protocol approved by the Institutional Animal Care and Use Committee of CIPEIN (IACUC/CICUAL - National System of Bioterium Registry Number 1572/155).

Extraction and chemical analysis of the EO from L. sempervirens

Plant material

Leaves from adult trees of *L. sempervirens* (Ruiz et Pav.) Tul. were collected near Valdivia, Chile (39°52.395S, 73°14.597O, 4 m.a.s.l.). Following Vogel *et al.* (1997), care was taken to haphazardly collect leaves from different positions of each of several trees. The material was identified by Sebastián Teillier, Universidad Central de Chile. Voucher specimens (LQE-231) were deposited at the herbarium of the Laboratorio de Química Ecológica, Facultad de Ciencias, Universidad de Chile.

Essential oil extraction

Plant material (ca. 30 g) was air dried, cut into small pieces, and hydrodistilled for 3 h in a Clevenger-type apparatus (Echeverría & Niemeyer, 2017). The yellowish oil was dried over anhydrous sodium sulphate and stored in a glass ampoule at 4°C until

analysed.

Chromatographic analysis

A Hewlett-Packard 5891 gas chromatograph linked to a Hewlett-Packard 5972 mass spectrometric detector (Hewlett Packard, Palo Alto, CA, USA) and provided with a capillary column (SPB-5, 30m x 0.25 mm, film thickness 0.25 µm, Supelco, Deerfield IL, USA).Operating conditions were as follows: on-column injection; injector and detector temperatures, 150°C and 280°C, respectively. The carrier gas was He at a flow of 1.3 mL/min. The oven temperature was programmed as follows: 50 ºC for 10 min, increase to 280 ºC at 5 ºC/min, and then 280º C for 45 min. In the mass detector, ionization was by electron impact at 70 eV; scan time, 1.5 s; and acquisition mass range, 50 to 500 Dalton. Compounds were identified by: i) calculation and comparison of retention indexes (Kovats indexes, KI) with those reported in the literature (Adams, 2012) or with those of available chemicals; and ii) comparison of mass spectra with those in the NIST98 library database. The integrated data system provided relative peak areas which were used as semi-quantitative composition data.

Bioassays

Experiments were carried out in a melamine-coated wooden box (1.0×0.5×0.5 m, length x width x height). The experimental arena was a filter paper disc (110 mm in diameter) (Hangzhou Xinxing Paper Industry & Co. Ltd.) placed centrally on the floor of the box. A video camera was located 20 cm above the arena (HD Webcam C525, Logitech, Lausanne, Switzerland) and connected to a personal computer. The camera has a resolution of 640 × 480 pixels with an acquisition and image processing speed of 30 frames/second. Lighting was provided by a bulb (22 watts; Luxa, Shanghai, China) placed above the paper circle. Temperature and relative humidity were 25 ºC and 80%, respectively.

The circle of filter paper was cut into halves. One half was treated with 0.25 mL of an acetonic solution of DEET or EO, and the other half with 0.25 mL of acetone alone. DEET was used as positive control and also to compare the effect of the EO with that of a regularly used insecticide; acetone was used as blank (Sfara *et al*., 2011). The following concentrations were applied: 4.125, 8.25, 16.5, 33, and 66 µg/cm^2 of DEET; and 8.25, 16.5, 33, 66 and 132 µg/cm^2 of EO. After the solvent had evaporated, both halves were stuck back together with adhesive tape on the underside, and the circle was placed on the horizontal floor of the test box. Then, a glass cylinder (2.5-cm high, 10-cm diameter) was placed around the paper circle to prevent the nymphs from leaving the arena. Finally, a nymph was gently deposited at the center of the arena. The time spent by the nymph on each half of the experimental arena was recorded for ten minutes. The position of the nymph in one or the other half of the paper were determined from playbacks of the recordings. The center of gravity of the insect was used to determine its position. Six independent replicates were performed.

Results were expressed as a Distribution coefficient (DC = (AT-At)/AT; where AT is the total time of the experiment, and At is the time of the nymph spent in the treated half). DC varies from 0 to 1, 0 being the maximum value of attraction and 1 the maximum value of repellency. A value of 0.5 indicates that there is an equal distribution between the treated and untreated areas (Sfara *et al.*, 2011). The results were analyzed using one-way ANOVA followed by Tukey's test.

RESULTS

Table No. 1 shows the chemical composition of the EO from *L. sempervirens.* The 11 compounds identified accounted for 99.88% of the total peak area. The main component was *cis*-isosafrole (89.8%), followed by β-terpinene (3.9%), *trans*-ocimene (2.7%) and methyleugenol (2.2%). Other components were present at relative areas less than 0.7%.

DEET showed a significant, concentration-dependent repellent activity on fifth-instar nymphs of *T. infestans* (ANOVA, F=5.811, DF=5, 16, p=0.003) (Figure No. 1**)**. The minimum concentration of DEET that produced repellency was 16.5 µg/cm^2 (DC=0.82). The EO from *L. sempervirens* also modified significantly the behaviour of the nymphs (ANOVA, F=8.824, DF=5, 18, p<0.001) (Figure No. 2). The repellent effect also depended on the concentration and could be observed at concentrations 66.0 µg/cm^2 (DC=0.91) and above.

DISCUSSION

In this work, the EO from leaves of the Chilean laurel, *L. sempervirens*, showed repellent activity towards fifth-instar nymphs of *T. infestans*. The minimum concentration that produced significant

repellency was four times higher than the minimum concentration required of the positive control (DEET).

Several studies have reported safrole as the major component in the EO from leaves of *L. sempervirens* (Montes *et al.*, 1990; Bittner *et al.*, 2008; Bittner *et al.*, 2009; Zapata *et al.*, 2010; Herrera-Rodriguez *et al.*, 2015). Here we found a very different composition, where *cis*-isosafrole (89.8%) and, to a much lesser extent, *β*-terpinene (3.9%), *trans*-ocimene (2.7%) and methyleugenol (2.2%) were the main components. Recently, Zapata *et al.* (2016) reported α-isosafrole and *β*-isosafrole (33.86%) in the EO of *L. sempervirens*, thus more closely resembling our results.

Table No. 1
Chemical composition of the essential oil of leaves of *L. sempervirens*

Peak	Compound[a]	RI $_{calc.}$ [b]	Relative area (%)[c]	Methods of identification[d]
1	*α*-pinene	939	0.70	MS,RI,ST
2	*β*-pinene	980	0.10	MS,RI,ST
3	*α*-phellandrene	1006	0.20	MS,RI,ST
4	β-terpinene	1035	3.90	MS,RI,ST
5	*trans*-ocimene	1054	2.70	MS,RI
6	linalool	1101	0.03	MS,RI,ST
7	α-terpineol	1196	0.05	MS,RI,ST
8	*cis*-isosafrole	1310	89.8	MS,RI
9	methyleugenol	1411	2.20	MS,RI,ST
10	*β*-caryophyllene	1438	0.10	MS,RI,ST
11	germacrene B	1496	0.10	MS,RI
	Total Identified		99.88	
	Hydrocarbon monoterpenes		7.60	
	Oxygenated monoterpenes		0.08	
	Hydrocarbon sesquiterpenes		0.20	
	Phenylpropanoids		92.0	

[a]Compounds listed in order of elution in SPB-5 column; [b]Retention indexes calculated (RI_{calc}) using a homologous series of *n*-alkanes C_7-C_{40} in SPB-5 column; [c]Peak areas relative to total peak area; [d]Bases for dentification: comparison of mass spectra using the NIST98 MS library and the literature (MS), retention index (RI) and/or authentic compounds (ST).

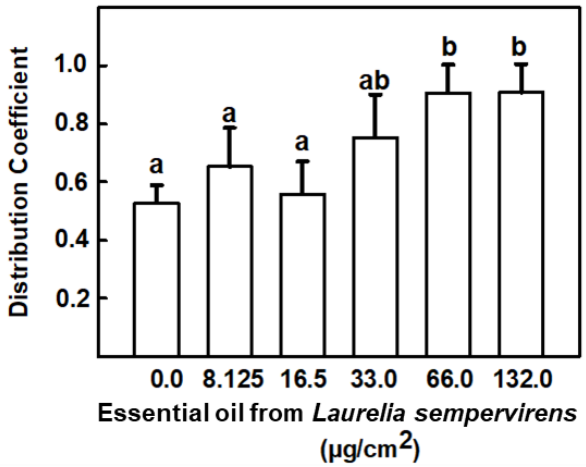

Figure No. 1
Repellent effect of essential oil from *Laurelia sempervirens* leaves on fifth-instar nymphs of *T. infestans*. Each bar is the mean of six independent replicates. Vertical lines are SE. Distribution coefficient (DC) = (AT-At)/AT; where AT is the total time of the experiment, and At is the time of the nymph spent the treated half). Bars marked with different letters are significantly different (p<0.05).

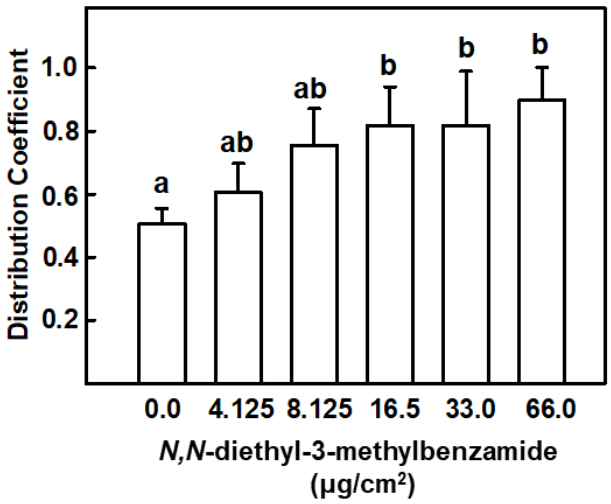

Figure No. 2
Repellent effect of *N,N*-diethyl-3-methylbenzamide (DEET) on fifth-instar nymphs of *Triatoma infestans*. Each bar is the mean of six independent replicates. Vertical lines are SE. Distribution coefficient (DC) = (AT-At)/AT; where AT is the total time of the experiment, and At is the time of the nymph spent the treated half). Bars marked with different letters are significantly different (p<0.05).

The differences in the chemical composition of the EO of *L. sempervirens* may be due to the collection time and also to exogenous factors such as light, precipitation, growing site, soil quality. Seasonal variation and the occurrence of ecotypes and chemotypes may also be expected. Endogenous factors such as age, plant part processed, as well as the extraction techniques can also be responsible for the variations observed (Barra, 2009). Particularly, the seasonal variation of the insecticidal and insectistatic properties of the essential oil of *L. sempervirens* in the control of *S. zeamais* has been demonstrated (Ortiz *et al*., 2017).

In our study, the major component in the EO from *L. sempervirens* was isosafrole (89.8%), a benzenoid that was previously reported with insecticidal and repellent activities against other insects such as the American cockroach, *Periplaneta americana* (Ngoh *et al.*, 1998). On the other hand, safrole and its isomerized regioisomer, isosafrole, have been identified as natural pesticides towards the beetles *S. zeamais* (Coleoptera: Curculionidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Huang *et al.*, 1999). In addition, isosafrole has shown a potent synergistic activity with pyrethroid insecticides towards adult house flies *Musca domestica* (Scott, 1996). Furthermore, methyleugenol has shown important insecticidal properties against various insects (Huang *et al*., 2002; Xu *et al.*, 2015). The high concentration of these compounds suggest their leading role in the repellent effect of the *L. sempervirens* EO.

The repellent activity of the EO of *L. sempervirens* was of the same order of magnitude as that of DEET, a universal repellent of insects whose effect is rarely surpassed by other substances (Zhu *et al.*, 2018). This result should make it worthwhile to study the repellent properties of *cis*-isosafrole alone and mixed with β-terpinene, *trans*-ocimene and methyleugenol on *T. infestans* and other pest insects.

CONCLUSION

The EO from *L. sempervirens* leaves produced an important repellent effect on fifth-instar nymphs of *T. infestans* under laboratory conditions. Testing it under natural conditions would be desirable. The effect was of the same order of magnitude as that of DEET, suggesting the study of the repellent action of *cis*-isosafrole, the main component of the EO from *L. sempervirens* leaves, alone or mixed with other compounds.

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