Fumigant Insecticidal Activity and Repellent Effect of Five Essential Oils and Seven Monoterpenes on First-Instar Nymphs of *Rhodnius prolixus*

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ABSTRACT The aim of this study was to evaluate the fumigant and repellent activity of five essential oils (from eucalyptus, geranium, lavender, mint, and orange oil) and seven monoterpenes (eucalyptol, geraniol, limonene, linalool, menthone, linalyl acetate, and menthyl acetate) on first-instar nymphs of the bloodsucking bug *Rhodnius prolixus* Stahl (vector of Chagas disease in several Latin American countries). Fumigant activity was evaluated by exposing the nymphs to the vapors emitted by 100 μl of essential oil or monoterpene in a closed recipient. The knockdown time 50% (KT_{50}) for eucalyptus essential oil was 215.6 min (seven times less toxic than dichlorvos, a volatile organophosphorus insecticide used as a positive control). The remaining essential oils showed a poor fumigant activity: <50% of nymphs were knocked down after 540 min of exposure. The KT_{50} values for monoterpenes, expressed in minutes, were as follows: 117.2 (eucalyptol), 408.7 (linalool), 474.0 (menthone), and 484.2 (limonene). Eucalyptol was 3.5 times less toxic than dichlorvos. No affected nymphs were observed after 540 min of exposure to geraniol, linalyl acetate, or menthyl acetate. Repellency was quantified using a video tracking system. Two concentrations of essential oils or monoterpenes were studied (40 and 400 $\mu g/cm^2$). Only mint and lavender essential oils produced a light repellent effect at 400 $\mu g/cm^2$. Geraniol and menthyl acetate produced a repellent effect at both tested concentrations and menthone only elicited an effect at 400 $\mu g/cm^2$. In all cases, the repellent effect was lesser than that produced by the broad-spectrum insect repellent *N,N*-diethyl-3-methylbenzamide (DEET).

KEY WORDS *Rhodnius prolixus*, essential oils, monoterpenes, knockdown, repellency

The triatomine bug *Rhodnius prolixus* Stahl is an important vector of Chagas disease in several South and Central American countries and is usually controlled with conventional insecticides (e.g., pyrethroids) (Molina de Fernández et al. 2004). However, at the end of the last century, low levels of resistance to pyrethroids were detected in *R. prolixus* from Venezuela and in *Triatoma infestans* Klug, another important Chagas disease vector, from Brazil (Vassena et al. 2000). Recently, high levels of pyrethroid resistance were also found in *T. infestans* individuals sampled in four villages in northern Argentina (Picollo et al. 2005). The emergence of this insecticide resistance in insect pest populations around the world is considered one of the main causes of pest control failure (Stenersen 2004). It is also the reason why there is a constant search for new active ingredients that can be used as alternatives to conventional insecticides. Because of their selectivity (high toxicity for acarids and insects but not for other organisms) and their minimal environmental effects, and because some of them have been shown to be effective against insects resistant to other insecticides (Ahn et al. 1997), essential oils are a potential tool for insect pest control.

Essential oils are the volatile organic constituents of aromatic plant matter and contribute to both flavor and fragrance (Tisserand and Balacs 1995). They are liquid at room temperature and can be extracted by distillation or cold-press (Enan 2001). Many of these oils have an insecticidal effect and hence constitute an important alternative to conventional insecticides. This is largely because of their selectivity (high toxicity for acarids and insects but not for other organisms) and their minimal environmental effects (Isman 2000). Essential oils are a mixture of several compounds, sometimes hundreds. Monoterpenes, the main components of essential oils (Tisserand and Balacs 1995), are lipophilic molecules that can easily penetrate the insect tegument. They are also characterized by a high vapor tension and hence present fumigant toxicity.

The biological activity of essential oils from a number of plants has been evaluated in repellency, knockdown, and mortality assays on insects and acarids. For

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example, oils extracted from *Rosemarinus officinalis* L. and *Thymus vulgaris* L. have proved to be effective mosquito repellents (Choi et al. 2002). Essential oils extracted from the roots of *Vetiveria zizanioides* L. modified the digging behavior of *Coptotermes formosanus* Shiraki termites (Isoptera: Rhinotermitidae), reducing the damage that this insect causes to wood (Maistrello et al. 2001). Essential oils and some of their components have also been effective in protecting stored grains (Shaaya et al. 1991, Regnault-Roger et al. 1993, Huang et al. 1998, Kim et al. 2003, Park et al. 2003). Effective results have been obtained on *Sitophilus zeamais* Motsch fed on corn treated with essential oils extracted from *Ageratum conyzoides* L., *Chromolaena odorata* L., and *Lantana camara* L. (Bouda et al. 2000); on *Callosobruchus maculates* F. exposed to essential oil vapors from plants of the *Ocium* genus (Kéïta et al. 2000); and on *Sitophilus orizae* L. exposed to eucalyptus and lavender oil vapors (Lee et al. 2001).

There are very few studies regarding the toxicity of essential oils or their components on Chagas disease vectors. Fournet et al. (1996) studied the composition and insecticidal properties of essential oils from Bolivian medicinal plants on *T. infestans* and *R. neglectus* Lent. They observed differences in toxicity caused by the application method and species (in general, topical application was more effective than exposure to impregnated filter papers and *R. prolixus* was more susceptible than *T. infestans*). Menthone, iso-menthone, pulegone, eucalyptol, linalool, and limonene were some of the components identified in the tested oils, but their individual toxicities were not evaluated. Laurent et al. (1997) tested the insecticidal activity of 63 essential oils isolated from Bolivian plants on *T. infestans* for ovicidal and larvicidal properties. Twenty oils showed insecticidal activity on nymphs and eggs when applied as impregnated papers.

The possibility of using essential oils or their components as fumigant triatomicides deserves more attention. The use of nonresidual fumigant formulations for Chagas disease vector control has been considered an important alternative to conventional contact insecticides in the surveillance phase of control campaigns (Zerba 1999). Fumigant insecticides have very good penetration in the thatched roofs of Latin American rural dwellings where most triatomines take shelter (Zeledon and Rabinovich 1981). Thus, the aim of this work was to study the fumigant insecticidal activity and repellent effects of five essential oils and seven monoterpenes on first-instar nymphs of *R. prolixus.*

Materials and Methods

Biological Material

One- to 5-d-old, starved first-instar nymphs of *R. prolixus,* extracted from a stable colony reared at the Centro de Investigaciones de Plagas e Insecticidas (CIPEIN, Villa Martelli, Argentina), were used in all the experiments.

Chemicals

Commercial essential oils, namely eucalyptus, geranium, lavender, mint, and orange, were purchased from Swiss-Just (Lomas del Mirador, Argentina; manufactured under the supervision and control of Ulrich Jüstrich AG, Walzenhausen, Switzerland). Monoterpenes (eucalyptol, geraniol, limonene, linalool, linalyl acetate, menthone, and menthyl acetate) were donated by Friztche SAICA (San Fernando, Argentina). Dichlorvos (technical grade) was a gift from Chemotecnica SA (Spegazzini, Argentina). *N,N*-diethyl-m-methylbenzamide (DEET) was purchased from Aldrich (Steinheim, Germany) and acetone from Merck (Darmstadt, Germany).

Evaluation of Fumigant Insecticidal Activity

The insects were exposed to essential oil or monoterpene vapors in plastic containers (4 cm diameter by 4 cm high) closed with plastic lids. The bottom of each container was covered with a circle of filter paper to offer the insects an appropriate support as they are unable to walk properly on plastic surfaces. Groups of 15 insects were placed on the filter paper, and the container was closed. An Eppendorf cap (Eppendorf, Hamburg, Germany) containing 100 μl of essential oil or monoterpene (100% concentration) was hung on the inside of the plastic lid, outside the insects' reach. Control groups were exposed to empty Eppendorf caps.

Insects lying on the filter paper that were unable to walk were considered knocked down. The number of knocked down insects was registered at different times. The same observation times could not be used in all cases because some compounds produced a very fast effect, whereas the activity of others was very slow. Therefore, to obtain an acceptable number of observations to estimate the respective KD values, the results were registered every 2 min for dichlorvos, 10 min for eucalyptol, or 15 min for essential oils and the remaining monoterpenes. In general, each replicate was interrupted when $>$90% of the insects were affected. All the experiments were interrupted after 540 min of exposure, because some compounds produced no effect during this period of time. Eight independent replicates were performed for each bioassay (only two replicates were performed if no affected insects were observed after 540 min of exposure).

Evaluation of Repellent Effect

Recording Equipment. A closed-circuit black and white video camera (VC 1910; Sanyo Electrical Co., Tokyo, Japan) and an image analyzer (Videomex V, Columbus, OH) were used to evaluate repellency. The video camera captured the image of the insects placed on a circular piece of filter paper treated with a putative repellent. The image analyzer converted the analog signal input from the video camera into digital data. Resolution was 256 by 192 pixels, and the acquisition and processing speed was 30 frames/s. In the monitor, the video signal colors were inverted and therefore white objects appeared to be black and vice versa. Thus, the presence of insects in the filter paper was determined by a visual contrast between the individuals (white) and the paper bakcground (dark) and scored as the number of on pixels. The distribution of the insects on the filter paper was recorded using Multiple Zones Motion Monitor for Videomex software that records the occupied area and the movement of multiple objects in multiple zones. The occupied area, expressed as the number of on pixels, allows determination of whether the insects are randomly distributed on the filter paper or whether they prefer staying, for example, in the zone treated only with acetone (repellency). Each set of data was imported and processed in a personal computer.

Bioassays. A circular piece of Whatman N° 1 filter paper (Whatman International, Maidstone, United Kingdom), 11 cm in diameter, was divided into two halves: zone I and zone II. Zone I was treated with 0.25 ml of acetone alone, and zone II was treated with 0.25 ml of essential oil or monoterpene solution in acetone (70 $\mu g/cm^2$). After acetone evaporation, the filter paper was placed on a table, 15 cm below the video camera described in the previous paragraph. A glass ring (high: 3 cm; internal diameter: 11 cm) was used to avoid the insects leave the filter paper. Groups of four nymphs were placed on the filter paper. To determine the distribution of nymphs on the filter paper, the TV field image was divided into two zones using Multiple Zones Motion Monitor for Videomex software (the zones for the TV image were the same as the zones for the filter paper). The area (expressed in pixels) occupied by nymphs in each zone during the experiment was recorded for 30 min. Results were expressed as a repellency coefficient (RC) = A(I)/A(II), where A(I) is the area occupied by insects in zone I and A(II) is the area occupied in zone II (Alzogaray et al. 2000). Six independent replications were performed for each bioassay.

Statistical Analysis

Knockdown time 50% (KT_{50}) values were calculated with their respective 95% confidence intervals (CIs) using the statistical software POLO PC (Le Ora Software 1987). Differences between values were considered significant ($P < 0.05$) if the respective 95% CIs did not overlap. The significance of the RC values was determined using one-way analysis of variance (ANOVA) and Tukey test for post hoc comparisons.

Results

Table 1 shows the KT_{50} values for five essential oils and seven monoterpenes applied as fumigants on first-instar nymphs of *R. prolixus.* The KT_{50} for eucalyptus oil (215.6 min) was significantly higher than the value for dichlorvos (DDVP) (32.2 min, positive control; $P < 0.05$). The remaining essential oils knocked down <50% of the nymphs after 540 min of exposure. The KT_{50} values for monoterpenes, expressed in minutes, were as follows: 117.2 (eucalyptol), 408.7 (linalool), 474.0 (menthone), and 484.2 (limonene). The KD_{50} for DDVP (positive control) was 33.0 min. No affected nymphs were observed after 540 min exposure to geraniol, linalyl acetate, or menthyl acetate.

Table 1. Estimated knockdown time in first-instars nymphs of *R. prolixus* resulting from exposure to essential oils or monoterpenes

Compound	N	Slope ± SE	KT_{50} (min)	95% CI	χ^2
Essential oils					
Eucalyptus	120	8.8 ± 0.5	215.6a	211.3–220.0	10.4NS
Geranium	30		>540		
Lavender	30		>540		
Mint	30		>540		
Orange	30		>540		
Dichlorvos	120	9.7 ± 0.4	32.2b	31.6–32.8	11.6NS
Monoterpenes					
Eucalyptol	120	11.8 ± 0.7	117.2a	114.9–119.3	7.0NS
Geraniol	30		>540		
Limonene	120	8.4 ± 0.7	484.2b	472.2–498.4	7.3NS
Linalyl acetate	30		>540		
Linalool	120	13.5 ± 1.3	408.7c	401.1–417.3	1.5NS
Menthone	120	15.0 ± 1.0	474.0b	467.0–480.6	10.0NS
Menthyl acetate	30		>540		
Dichlorvos	120	14.4 ± 0.7	33.0d	32.5–33.4	8.3NS

In each group (essential oils or monoterpenes), KT_{50} values with the same letter are not significantly different ($P > 0.05$). Each value of KT_{50} was obtained from the data from eight independent replicates (except when no affected insects were observed after 540 min of exposure, in which case only two replicates were performed).

NS, not significant.

Figure 1 shows the RC values for five essential oils and seven monoterpenes on first-instar nymphs of *R. prolixus.* Only essential oils from lavender and mint showed a significant repellent effect when tested at 400 $\mu g/cm^2$ ($P < 0.05$). Geraniol and menthyl acetate produced a significant repellency at the two doses tested ($P < 0.05$). Menthone produced a significant repellency only at 400 $\mu g/cm^2$ ($P < 0.05$).

In all cases, the repellency was lower than that produced by 40 $\mu g/cm^2$ of DEET.

Discussion

We studied the fumigant insecticidal activity and the repellent effect of five essential oils and seven monoterpenes on first-instar nymphs of *R. prolixus.* Eucalyptus essential oil and eucalyptol, the most active among the tested ingredients when applied as fumigants, were 7 and 3.5 times less toxic than the organophosphorus insecticide dichlorvos, respectively. These values represent a good performance considering that, in general, greater differences in toxicity have been reported in previous studies comparing the fumigant activity of conventional versus botanical insecticides in other insects. For example, Rice and Coats (1994a) obtained LC_{50} values between 9.1 and >2,500 $\mu g/cm^3$ for 22 monoterpenes applied as fumigants against the housefly. The lowest of these values was 20 times less toxic than dichlorvos. In the same work, most of the tested compounds were at least

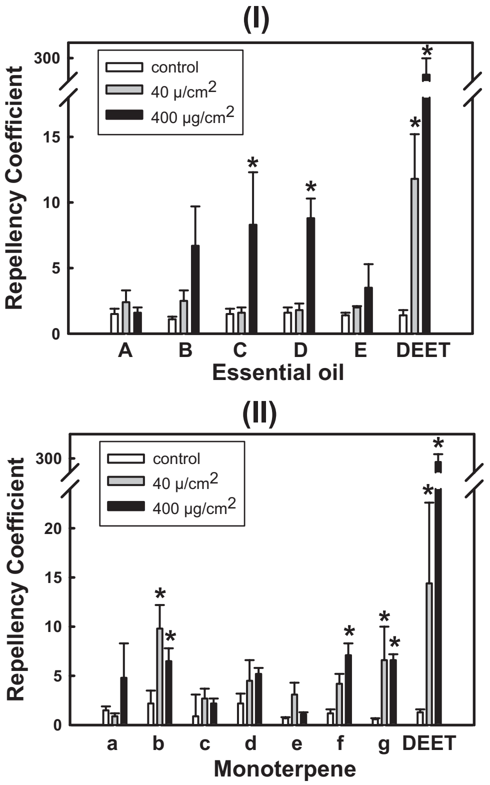

Fig. 1. Repellency produced by essential oils or monoterpenes on first-instar nymphs of *R. prolixus*. (I) Essential oils: A, eucalyptus; B, geranium; C, lavender; D, mint; E, orange. (II) Monoterpenes: a, eucalyptol; b, geraniol; c, limonene; d, linalyl acetate; e, linalool; f, menthone; g, menthyl acetate. DEET, *N,N*-diethyl-3-methylbenzamide (positive control). Each bar represents the mean of six independent replicates ± SE. *Significant difference from the respective control group.

nine times less toxic than dichlorvos when applied to the red flour beetle. In another report, 55 monoterpenes or derivatives were at least 360 times less toxic than dichlorvos to the housefly, and 7 of them were at least 29.1 times less toxic to *Tribolium castaneum* Herbst (Rice and Coats 1994b).

Only lavender and mint essential oils and the monoterpenes geraniol, menthyl acetate, and menthone showed a repellent effect on *R. prolixus* nymphs. In all cases, the effect was lower than that produced by the positive control (DEET). The repellent effect of essential oils and monoterpenes has been less studied than their lethal or knockdown activity. Several monoterpenes showed a repellent effect on mosquitoes (Curtis et al. 1987, Barnard 1999, Ansari et al. 2000, Choi et al. 2002), and human head lice (Toloza et al. 2006). When DEET was used as a positive control, it usually produced a higher repellent effect than the botanical compounds; however, there are some exceptions (Choi et al. 2002).

The five essential oils we studied showed very different values of KT_{50} and RC. This was also true for the seven monoterpenes tested. The toxicity of pure and mixed compounds depends on their physicochemical properties and is the final result of the different toxicokinetic and toxicodynamic steps (penetration, distribution, metabolism, and interaction with the site of action). In exposure to vapors, the main access to the organism is airborne: the volatile substances enter with the air insects inhale through their spiracles as part of their respiratory process (Mill 1985). The substances are transported to different tissues through the network of tracheas and tracheoles, thus reaching their site of action. The toxic effect of volatile substances entering the insect body through the respiratory system is strongly associated with their volatility rate. In fact, Rice and Coats (1994a) detected a polynomial correlation between the volatility and the fumigant toxicity of monoterpenoids applied to the housefly. Furthermore, Toloza et al. (2006) showed a linear correlation between KT_{50} values and the vapor pressure of essential oil components tested on head lice.

Volatility is also an important parameter in the case of repellents. According to McIver (1981), the repellency of DEET is the result of the interaction between vapor phase molecules and neuron membrane lipids of chemosensory sensilla. In accordance with this, a previous study performed at our laboratory showed that the repellent effect of DEET on *T. infestans* occurs when the compound source is outside the insects' reach (Alzogaray et al. 2000).

An additional factor modulating toxicity should be considered in the case of essential oils. Because they are complex mixtures of substances, the toxicological interactions that take place between their components (e.g., synergism or antagonism) cannot be discarded.

In conclusion, eucalyptol showed good performance as a fumigant, and geraniol and menthyl acetate presented an important repellent effect on *R. prolixus.* Based on these results, we consider it worthwhile to perform additional studies on the toxicological properties of these monoterpenes on *R. prolixus* and other Chagas disease vectors.

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References Cited

Ahn, Y. J., M. Kwon, H. M. Park, and C. K. Han. 1997. Potent insecticidal activity of *Gingko biloba* derived trilactone terpenes against *Nilaparvata lugens,* pp. 90–105. *In* P. A. Hedin, R. M. Hollingworth, E. P. Masler, J. Miyamoto, and D. G. Thompson (eds.), Phytochemicals for pest control. American Chemical Society, Washington, DC.

Alzogaray, R. A., A. Fontán, and E. N. Zerba. 2000. Repellency of deet to nymphs of *Triatoma infestans.* Med. Vet. Entomol. 14: 6–10.

Ansari, M. A., P. Vasudevan, M. Tandon, and R. K. Razdan. 2000. Larvicidal and mosquito repellent action of peppermint (*Mentha piperita*) oil. Biores. Tech. 71: 267–271.

Barnard, D. 1999. Repellency of essential oils to mosquitoes (Diptera: Culicidae). J. Med. Entomol. 36: 625–629.

Bouda, H., L. A. Tapondjou, D. A. Fontem, and M.Y.D. Gumedzoe. 2000. Effect from essential oils from leaves of *Ageratum conyzoides, Lantana camara* and *Chromolaena odorata* on the mortality of *Sitophilus zeamais* (Coleoptera: Curculionidae). J. Stored Prod. Res. 37: 103–109.

Choi, W., B. Park, S. Ku, and S. Lee. 2002. Repellent activities of essential oils and monoterpenes against *Cullex pipiens pallens.* J. Am. Mosq. Control Assoc. 18: 348–351.

Curtis, C. F., J. D. Lines, J. Ijumba, A. Callaghan, N. Hill, and M. A. Karimzad. 1987. The relative efficacy of repellents against mosquito vectors of disease. Med. Vet. Entomol. 1: 109–119.

Enan, E. 2001. Insecticidal activity of essential oils: octopaminergic sites of action. Comp. Biochem. Physiol. C 130: 325–337.

Fournet, A., A. Rojas de Arias, B. Charles, and J. Bruneton. 1996. Chemical constituents of essential oils of Muña, Bolivian Plants traditionally used as pesticides, and their insecticidal properties against chagas disease vectors. J. Ethnopharmacol. 52: 145–149.

Huang, Y., S. K. Hee, and S. H. Ho. 1998. Antifeedant and growth inhibitory effects of α-pinene on the stored-product insects, *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* (Motsch). Int. Pest Control 18: 20.

Isman, M. B. 2000. Plant essential oil for pest and disease management. Crop Protect. 19: 603–608.

Kéïta, S. M., C. Vincent, J. Schmit, S. Ramaswamy, and A. Bélanger. 2000. Effect of various oils on *Callosobruchus maculates* (F.) (Coleoptera: Bruchidae). J. Stored Prod. Res. 36: 355–364.

Kim, S. L., C. Park, M. Ohh, H. Cho, and Y. Ahn. 2003. Contact and fumigant activities of aromatic plant extracts and essential oils against *Lasioderma serricorne* (Coleoptera: Anobiidae). J. Stored Prod. Res. 39: 11–19.

Laurent, D., L. A. Vilaseca, J.-M. Chantraine, C. Ballivian, G. Saavedra, and R. Ibáñez. 1997. Insecticidal activity of essential oils on *Triatoma infestans.* Phytother. Res. 11: 285–290.

Lee, B., W. Choi, S. Lee, and B. Park. 2001. Fumigant toxicity of essential oils and their constituent compounds towards the rice weevil, *Sitophilus orizae* (L.). Crop Protect. 20: 317–320.

LeOra Software. 1987. POLO PC: a user's guide for probit or logit analysis. LeOra Software, Berkeley, CA.

Maistrello, L., G. Henderson, and R. A. Laine. 2001. Efficacy of vetiver oil and nootkatone as soil barriers against formosan subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 94: 1532–1537.

McIver, S. B. 1981. A model for the mechanism of action of the repellent DEET on *Aedes aegypti* (Diptera: Culicidae). J. Med. Entomol. 18: 357–361.

Mill, P. J. 1985. Structure and physiology of the respiratory system, pp. 517–593. *In* G. A. Kerkut and L. I. Gilbert (eds.), Comprehensive insect physiology, biochemistry and pharmacology, vol. 3. Pergamon, Oxford, United Kingdom.

Molina de Fernández, D., A. Soto Vivas, and H. Barazarte. 2004. Susceptibilidad a insecticidas piretroides en cepas de campo de *Rhodnius prolixus* Stahl (Hmeiptera: Reduviidae) de Vednezuela. Entomotropica 19: 1–5.

Park, I., S. Lee, D. Choi, J. Park, and Y. Ahn. 2003. Insecticidal activities of constituents identified in the essential oil from leaves of *Chamaecyparis obtusa* against *Callosobruchus chinensis* (L.) and *Sitophilus oryzae* (L.). J. Stored Prod. Res. 39: 375–384.

Picollo, M. I., C. Vassena, P. Santo Orhiuela, S. Barrios, M. Zaidemberg, and E. Zerba. 2005. High resistance to pyrethroid insecticides associated with ineffective field treatments in *Triatoma infestans* (Hemiptera: Reduviidae) from Northern Argentina. J. Med. Entomol. 42: 637–642.

Regnault-Roger, C., A. Hamraoui, M. Holeman, E. Theron, and R. Pinel. 1993. Insecticidal effect of essential oils from Mediterranean plants upon *Acanthoscelides obtecus* Say (Coleoptera: Bruchidae), a pest of kidney bean (*Phaseolus vulgaris* L.). J. Chem. Ecol. 19: 1233–1244.

Rice, P. J., and J. R. Coats. 1994a. Insecticidal properties of several monoterpenoids to house fly (Diptera: Muscidae), red flour beetle (Coleoptera: Tenebrionidae), and southern corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 87: 1172–1179.

Rice, P. J., and J. R. Coats. 1994b. Insecticidal properties of monoterpenoids derivatives to the house fly (Diptera: Muscidae) and red flour beetle (Coleoptera: Tenebrionidae). Pestic. Sci. 41: 195–202.

Shaaya, E., U. Ravid, N. Paster, B. Juven, U. Zisman, and V. Pissarev. 1991. Fumigant toxicity of essential oils against four major stored products insects, J. Chem. Ecol. 17: 499–504.

Stenersen, J. 2004. Chemical pesticides. CRC, Boca Raton, FL.

Tisserand, R., and T. Balacs. 1995. Essential oil safety. Livingstone, London, United Kingdom.

Toloza, A. C., J. Zygadlo, G. Mougabure Cueto, F. Biurrun, E. Zerba, and M. I. Picollo. 2006. Fumigant and repellent properties of essential oils and component compounds against permethrin-resistant *Pediculus humanus capitis* (Anoplura: Pediculidae) from Argentina. J. Med. Entomol. 43: 889–895.

Vassena, C., M. I. Picollo, and E. N. Zerba. 2000. Insecticide resistance in Brazilian *Triatoma infestans* and Venezuelan *Rhodnius prolixus.* Med. Vet. Entomol. 14: 51–55.

Zeledon, R., and J. E. Rabinovich. 1981. Chagas disease: an ecological appraisal with special emphasis on its insect vectors. Annu. Rev. Entomol. 26: 101–133.

Zerba, E. 1999. Susceptibility and resistance to insecticides of Chagas disease vectors. Medicina 59: 41–46.

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