Crop Protection 125 (2019) 104913

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Bioactivity of essential oil from *Lippia gracilis* Schauer against two major coconut pest mites and toxicity to a non-target predator

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ARTICLE INFO

Keywords:
Aceria guerreronis
Raoiella indica
Thymol
Acaricidal activity
Monoterpenes

ABSTRACT

The coconut mite, *Aceria guerreronis* Keifer (Acari: Eriophyidae), and the red palm mite, *Raoiella indica* Hirst (Acari: Tenuipalpidae), are serious pests of commercial coconut plantations worldwide. We assessed the toxicity and sublethal effects of the essential oil from *Lippia gracilis* Schauer on these two pests, and its toxicity against *Amblyseius largoensis* (Acari: Phytoseiidae), a predatory mite that is mainly associated with *R. indica*. Chemical analyses revealed that the essential oil of *L. gracilis* accession LGRA106 contains thymol as a major compound (52.41%). Based on concentration-mortality bioassays, the toxicity of the oil differs from that of thymol for *A. guerreronis* (LC_{50} = 4.28 mg/mL, $LC_{50thymol}$ = 5.34 mg/mL) and for *R. indica* (LC_{50}=4.99 mg/mL, $LC_{50thymol}$ = 9.03 mg/mL). The LC_{50}, as estimated for *R. indica*, proved to be toxic to the predatory mite *A. largoensis*, killing roughly half of its population. In addition, the essential oil of *L. gracilis* and thymol, at their LC_{50} for each mite species, repelled *A. guerreronis* and reduced the survival and oviposition of *R. indica*. The potential of *L. gracilis* as a natural pesticide on coconut plantations and their effects on beneficial mites are discussed herein.

1. Introduction

Plant essential oils are natural products synthesized by ca. 17,500 aromatic species mainly belonging to a limited number of botanical families (Regnault-Roger et al., 2012). Chemically, these are complex mixtures of a few or up to a hundred compounds. Major constituents are mono- and sesquiterpenes, which belong to the most diverse and abundant group of natural compounds, the terpenoids. They are lipophilic and highly volatile compounds (Dudareva et al., 2013). Plant essential oils have been widely studied for their biological activity and applied in a wide range of industrial fields. Over the past 40 years, research focusing on their potential for developing natural pesticides has shown the biocide and repellent effects of essential oils on a variety of arthropods (for reviews, see Isman, 2000; Renault-Roger et al., 2012; Camilo et al., 2017). Biological effects stem from individual compounds and/or the interaction between them, as result of synergism, additivity and antagonism. Further advantages in the use of essential oils in pest management are their rapid degradation due to their high volatility, which reduces environmental contamination and side effects on non-target organisms (Isman, 2006). In addition, compounds present in the essential oils can act at different sites of action, consequently reducing the chances of arising resistant arthropod biotypes (Bomford and Isman, 1996).

The genus *Lippia* L., is the second largest within the Verbenaceae, and includes ca. 200 species of herbs, shrubs and small trees, concentrated mainly in Central and South America and tropical Africa. *Lippia gracilis* Schauer is an endemic aromatic plant of semiarid caatinga, the main biome of the Brazilian Northeast. The leaves contain large quantities of essential oils with proven bioactivity against fungi, insects, mites and ticks (Albuquerque et al., 2006; Silva et al., 2008; Cruz et al., 2013; Born et al., 2018). The monoterpene thymol is one of the major components of the essential oil of this species (Santos et al., 2016; Born et al., 2018) and

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https://doi.org/10.1016/j.cropro.2019.104913
Received 2 May 2019; Received in revised form 30 July 2019; Accepted 2 August 2019
Available online 3 August 2019

is bioactive against insects (Cruz et al., 2013; Masoumi et al., 2016 and references therein). Therefore, the essential oil of *L. gracilis* and their major compounds are promising candidates to develop biopesticides.

Coconut (*Cocos nucifera* L.) palms are of great socioeconomic importance for Brazil, particularly in the Northeast region, that concentrates more than 70% of the national production (EMBRAPA, 2018). Among pest mites species associated with coconut plantations, two are considered of agricultural relevance. The red palm mite *Raoiella indica* Hirst (Acari: Tenuipalpidae) is one of the most important pests of coconut, banana, and other ornamental palms in different parts of the world (Peña et al., 2009; Carrillo et al., 2012; Vásquez et al., 2012; Otero-Colina et al., 2016). This pest causes severe yellowing of leaves, which may further become necrotic and die as a result of heavy infestation (Melo et al., 2018). The coconut mite *Aceria guerreronis* Keifer (Acari: Eriophyidae) is another important coconut pest in America, Asia and Africa. Attacked fruits develop triangular-shaped yellowish-white spots that turn necrotic. Apart from the aesthetic damage, severe attacks lead to premature fruit fall and distortion, reduced fruit size and weight as well as decreased water content and yield (Návia et al., 2013). In Brazil, the control of these pests relies on prophylactic acaricide sprayings at monthly or even shorter intervals potentially leading to a variety of problems such as resistance, food and environmental contamination, outbreaks of secondary pests and mortality of natural enemies (Desneux et al., 2007; Geiger et al., 2010; Guedes et al., 2016).

In order to develop efficient and environmentally friendly control strategies for coconut growers, this study focused on the acaricidal activity of the essential oils of *L. gracilis* and its major compound towards two key coconut pest mites and the predatory mite *Amblyseius largoensis* (Muma) (Acari: Phytoseiidae). This predatory mite is mainly associated with *R. indica* (Peña et al., 2009; Bowman and Hoy, 2012; Carrillo and Peña, 2012), although it may also feed on *A. guerreronis* (Galvão et al., 2007). One of the major gaps in studies assessing the bioactivity of essential oils and major compounds on pests are their side effects on non-target organisms (Pavela and Benelli, 2016). The use of essential oils could hamper biological control by showing toxic or nonlethal effects.

2. Materials and methods

2.1. Living material

Leaves of *L. gracilis* from the accession LGRA106 were collected from plants of the Active Germplasm Bank of Medicinal and Aromatic Plants of the Federal University of Sergipe, located in the municipality of São Cristóvão (11°00′S; 37°12′W), state of Sergipe, Brazil.

Teliochrysalids and adults of *R. indica* were collected from leaves while adults of *A. guerreronis* were taken from fruits of a green dwarf coconut plantation located in the city of Aracaju (10°57′S; 37°03′W), state of Sergipe, Brazil. To standardize the age of the mites used in bioassays, coconut leaflets containing individuals of *R. indica* were left for 48 h in transparent plastic tubes (52 cm long x 5.5 cm diameter) placed in glass jars filled with water. This kept leaflet turgor, and ensured food for mites until the emergence of adult females. For *A. guerreronis*, we collected individuals from colonies in early stage of oviposition (Oliveira et al., 2017). A colony of the predatory mite *A. largoensis* was started with individuals collected from green dwarf coconut leaves at the same collection place of the pest mites. Individuals were identified using taxonomic keys and a voucher sample was deposited in the collection of the Maranhão State University (UEMA), in São Luís, state of Maranhão, Brazil. Predatory mites were kept in the laboratory (27 ± 3 °C, 70 ± 10% RH and natural photoperiod) in PVC-arenas (22 cm × 12 cm) placed over a piece of polyurethane foam (24 cm × 14 cm x 3 cm) soaked in distilled water and placed in a plastic tray (24 cm × 14.5 cm x 5.3 cm). A layer of wet cotton wool surrounded the arena to prevent the predator from escaping. Cotton fibers under a piece of thin transparent plastic (18 × 18 mm) were placed on the arena to provide shelter and oviposition sites for *A. largoensis*. Mites were fed every other day with pollen of *Typha domingensis* (Typhacea), *R. indica* and diluted honey (10%).

2.2. Essential oil

Freshly collected leaves of *L. gracilis* were dried in a forced air circulation oven, at 40 °C, for five days. The essential oils were extracted by hydrodistillation, using a modified Clevenger apparatus. Samples consisted of 75 g of dry leaves, distilled for 140 min (Ehlert et al., 2006). Three replications were carried out and the extracted oils were stored in amber vials at −20 °C until analyses.

The chemical composition of the essential oil was analyzed using a GC-MS/FID (QP2010 Ultra, Shimadzu Corporation, Kyoto, Japan), equipped with an autosampler AOC-20i (Shimadzu). Separations were accomplished using an Rtx®-5MS Restek fused silica capillary column (5%-diphenyl–95%-dimethyl polysiloxane) of 30 m × 0.25 mm i.d., 0.25 mm film thickness, at constant helium (99.99%) flow rate of 1.2 mL/min. A volume of 0.5 μL (5 mg/mL) was injected with a split ratio of 1:10. The oven temperature was programmed from 50 °C (isothermal for 1.5 min), with an increase of 4 °C/min to 200 °C, then 10 °C/min to 250 °C, ending with a 5 min isothermal at 250 °C.

The MS and FID data were simultaneously acquired by employing a Detector Splitting System; the split flow ratio was 4:1 (MS:FID). A 0.62 m × 0.15 mm i.d. restrictor tube (capillary column) was used to connect the splitter to the MS detector; a 0.74 m × 0.22 mm i.d. restrictor tube was used to connect the splitter to the FID detector. The MS data (total ion chromatogram, TIC) were acquired in full scan mode (m/z of 40–350), at a scan rate of 0.3 scan/s, using the electron ionization (EI), with an electron energy of 70 eV. The injector temperature was 250 °C, and the ion-source temperature was 250 °C. The FID temperature was set to 250 °C, and the gas supplies for the FID were hydrogen, air, and helium at flow rates of 30, 300, and 30 mL/min, respectively. Quantification of each compound was estimated by FID peak-area normalization (%). Compound concentrations were calculated from the GC peak areas, and they were arranged in order of GC elution.

Identification of individual compounds of the essential oil of *L. gracilis* was carried out by computerized matching of the acquired mass spectra with those stored in NIST21, NIST107 and WILEY8 mass spectral library of the GC-MS data system. A mixture of hydrocarbons was injected under the same conditions, and compounds were identified by comparing the spectra obtained with those of the equipment data bank, and by the retention index, calculated for each compound, as previously described (Adams, 2007). Retention indexes were obtained using the equation proposed by Van den Dool and Kratz (1963).

2.3. Contact toxicity

The essential oil and its main compound thymol were sprayed through a Potter Tower (Burkard, Rickmansworth, United Kingdom) at a pressure of 0.34 bar (34 kPa) with 1.7 mL aliquot for *A. guerreronis* or 9.3 mL spray aliquot for *R. indica* and thymol. Procedures resulted in a residue of 1.8 ± 0.1 mg/cm^2 in accordance with the guidelines developed by the IOBC/WPRS (Hassan et al., 1994). For *R. indica* and *A. guerreronis* respectively, the arenas (experimental units) consisted of 1.8-cm-diameter discs of coconut palm leaflets and 1-cm-diameter discs of the perianth of young coconut fruits. In both experiments, the arenas were maintained in Petri dishes (100 × 15 mm) and immersed in a mixture of 5% agar, 0.3% methylparaben (Nipagim®) as fungicide, and distilled water. The arenas were opened with the help of a mold to expose the plant tissue. The concentrations of the essential oil and thymol were selected after preliminary bioassays, and conducted across a broad range, allowing the selection of the highest concentration that did not kill and the lowest concentration that did kill all the mites. Five (2.6, 4.5, 6.3, 8.1 and 11.7 mg/mL) and seven (0.9, 2.7, 4.5, 5.4, 6.3, 9.0

and 10.9 mg/mL) concentrations of the essential oil of *L. gracilis* were used for *R. indica* and *A. guerreronis*, respectively. The toxicity of thymol was assessed as described above using five (6, 9, 12, 16 and 18 mg/mL) and six (0.5, 2, 6, 10, 15 and 17 mg/mL) concentrations for *R. indica* and *A. guerreronis,* respectively. The essential oil was dissolved in tween 20 (0.5% v/v) and distilled water for *A. guerreronis* or in acetone for *R. indica*. Thymol (Merck, 99% purity) was diluted in acetone (Vetec, 99.9% purity). Control arenas were sprayed with tween 20 (0.5% v/v) or acetone, depending on the bioassay.

Sprayed discs were dried in the open air for 30 min before 20 1- to 2-day-old females of *R. indica* or 30 adults of *A. guerreronis* were placed in each. Six replicates for *R. indica* or eight for *A. guerreronis* were included totaling 120 or 240 mites per concentration, respectively. Arenas of *R. indica* were covered with a transparent PVC film. For the *A. guerreronis* experiment, arenas were covered with a black fabric, to simulate the place where mite colonies are located on the fruit, beneath the bracts. Petri dishes were kept in a controlled chamber at 27 °C ± 3 °C, with relative humidity of 70 ± 10%, and 12 h photoperiod.

The numbers of living and dead mites were recorded 24 h after sprayings. Mites were considered dead when they did not move after prodding with a fine paintbrush (*A. guerreronis*) or were not able to move the distance equivalent to their body size (*R. indica*) (Stark et al., 1997).

2.4. Repellency effect on A. guerreronis

The approach to assess the repellency against *A. guerreronis* is described elsewhere (Oliveira et al., 2017). Briefly, the arenas were prepared according to description above, except that only half of each was sprayed. The untreated area was covered with two layers of impermeable tape during spraying; the tape was removed after spraying (Oliveira et al., 2017). Arenas were sprayed with the LC_{50} of the essential oil of *L. gracilis* or thymol, previously estimated for this mite species. The discs were dried in open air for 30 min before adults of *A. guerreronis* were individually and centrally placed onto a dried glue spot (1 × 1.0 × 0.5 mm) in the middle of each disc. The position of the mites on the sprayed and unsprayed disc halves was recorded after 1 h of exposure. Each treatment comprised 3 replicates and each replicate had 20 mites (60 mites per treatment). The mites were maintained in a chamber at 28 °C ± 2 °C, under 80 ± 10% relative humidity and a 12 h photoperiod. A previous bioassay revealed that two disc halves of a single treatment were equally chosen by *A. guerreronis* ($P > 0.05$).

2.5. Effects on survival and oviposition of R. indica

Survival curves were constructed assessing the mortality of *R. indica* females exposed to the LC_{50} of the oil. The experiment was conducted using the same methodology previously described for the toxicity bioassays. Ten replicates with 20 females per arena were used for each treatment. Mortality of *R. indica* was recorded every 3 h during the first 24 h, every 6 h for the following 48 h and every 12 h, thereafter until the end of the experiment (100% mortality in control arenas). The sublethal effects of the oil on the oviposition was further assessed using 50 *R. indica* females individually placed onto the arenas and exposed to the LC_{50} or acetone as control. Every 12 h, the numbers of eggs laid by each female were counted and recorded over a period of 12 days.

2.6. Toxicity against the predatory mite

Since *A. largoensis* is mainly associated with *R. indica* on leaves, the toxicity of the essential oil against this predator was assessed using the LC_{50} estimated for *R. indica* in a completely randomized experiment with six replicates. The LC_{50} estimated for *R. indica* was sprayed using a Potter Tower as previously described on arenas made of PVC discs (4.5 cm diameter) onto which 10 eight- to-ten-day-old females were previously placed. Arenas were kept on a polyurethane foam (4.5 cm diameter x 2.5 cm thick) soaked with distilled water inside a plastic pot (5 cm diameter x 4 cm deep). Females were fed pollen of *T. domingensis* provided on pieces of transparent PVC (0.5 cm^2) to avoid contact with the oil. Mites were kept in controlled chambers (27 ± 3 °C, 70 ± 10% RH and 12 h of photoperiod) for 24 h when mortality was assessed as described in the toxicity bioassay.

2.7. Statistical analyses

Mortality data of *R. indica* and *A. guerreronis* were corrected in relation to control using the Abbott's formula (Abbott, 1925). Dose-mortality response curves were calculated through Probit analyses to determine the lethal concentrations (LC) of the essential oil and thymol necessary to kill 50% of the individuals (LC_{50}) of *A. guerreronis* and *R. indica* populations using the SAS procedure Proc PROBIT (SAS Institute, 2013). LC values were determined with 95% confidence intervals (CI). The LC_{50} value of thymol was divided by the LC_{50} obtained from the oil, and the 95% confidence limits of these toxicity ratio estimates differed significantly if they did not include the value 1 (Robertson et al., 2017). Mortality of the predatory mite was analyzed by performing Student's *t*-test ($P < 0.05$). Survival was analyzed with the Kaplan-Meier estimate and log-rank test curves using SigmaPlot v. 12.0 (Systat Software, 2008). The total number of eggs/female and number of eggs/day of *R. indica* exposed to LC_{50} were compared to the control (acetone) by applying the non-parametric Mann-Whitney *U* test. Mortality data of the predatory mite and oviposition data were analyzed using the Statistica 7.0 software (StatSoft Inc., 2004).

Repellency index (RI) was calculated by the formula: RI = 2G/(G + P), adapted from the Preference Index (PI) of Kogan and Goeden (1970) (Pontes et al., 2007), in which G is the number of mites in the treatment, and P is the number of mites in the control. The safety interval used to consider whether the essential oil or the major compound was repellent was obtained from the mean of the RI and its respective standard deviation (SD), according to the following criteria: mean of RI < 1 - SD, the essential oil or the compound is repellent; mean of RI > 1 + SD, the essential oil or the compound is attractive; and if 1 – SD < mean of RI < 1 + SD, the essential oil or the compound is neutral.

3. Results

3.1. Chemical composition

Thirty-five compounds were identified in the essential oil of *L. gracilis* accession LGRA106, with thymol as the major compound (52.41%), followed by methylthymol (10.82%), and *E*-caryophyllene (8.35%) (Table 1).

3.2. Toxicity

For *A. guerreronis*, the LC_{50} for the accession LGRA106 of *L. gracilis* was estimated to be 4.28 mg/mL, which was significantly smaller than the LC_{50} of its major compound thymol (LC_{50} = 5.34 mg/mL) based on the calculated toxicity ratio (confidence intervals did not include the value 1.0). Similarly, the LC_{50} of the oil to *R. indica* (4.99 mg/mL) was significantly smaller than that of thymol (9.03 mg/mL) (Table 2).

3.3. Repellency effect on A. guerreronis

The LC_{50} of the essential oil of *L. gracilis* and the monoterpene thymol repelled adults of *A. guerreronis* after 1 h of exposure (Table 3).

3.4. Effects on survival and oviposition of R. indica

The survival of females of *R. indica* was affected by the LC_{50} concentration of the essential oil of *L. gracilis* (log-rank: $\chi^2 = 281.39$; d. f. = 1; $P < 0.001$) (Fig. 1). The total number of eggs/female (CL_{50}: 3.04 ± 1.72) was lower compared with the control (11.90 ± 2.41)

Table 1
Chemical composition of the essential oil of leaves of *Lippia gracilis* accession LGRA106.

Compounds	RRI-l[a]	% peak area
α-Thujene	924	1.18
α-Pinene	932	0.49
Sabinene	969	0.21
β-Pinene	974	0.21
Myrcene	988	3.20
α-Phellandrene	1002	0.11
3-δ-Carene	1008	0.13
α-Terpinene	1014	1.26
ρ-Cymene	1020	7.16
Limonene	1024	0.53
1,8-Cineole	1026	2.91
(*Z*)-β-Ocimene	1032	0.14
γ-Terpinene	1054	5.56
(*E*)-4-Thujanol	1065	0.33
Linalool	1095	0.52
Terpinen-4-ol	1174	0.67
α-Terpineol	1186	0.22
Methyl thymol	1232	10.81
Thymol	1289	52.41
Carvacrol	1298	0.87
δ-Elemene	1335	0.11
Thymol acetate	1349	0.10
(*E*)-caryophyllene	1417	8.36
(*E*)-α- Bergamotene	1432	0.12
Aromadendrene	1439	0.17
α-Humulene	1452	0.43
Bicyclogermacrene	1500	0.61
Spathulenol	1577	0.15
Caryophyllene oxide	1582	0.52

[a] RRI-l: relative retention index - literature.

(N = 50; U = −8.6; P < 0.0001). Likewise, the number of eggs/day (CL_{50}: 0.25 ± 0.14) was significantly lower when compared with the control (0.99 ± 0.20) (N = 50; U = −8.6; P < 0.0001).

3.5. Contact toxicity against the predatory mite

The LC_{50} (4.99 mg/mL) of the essential oil, as estimated for *R. indica*, was toxic to *A. largoensis* (LC_{50}: 48.33 ± 3.07%; control: 3.33 ± 2.10; t = 12.07; d.f. = 1; P < 0.0001).

4. Discussion

Essential oils of leaves from species belonging to the genus *Lippia* are known to share several compounds such as thymol, carvacrol, p-cymene, γ-terpinene and caryophyllene, but quantitative variations in the chemical profile is genotype dependent (Cavalcanti et al., 2010; Martínez-Velázquez et al., 2011 and references therein, Cruz et al., 2013). Although in some species such as *L. graveolens* thymol and carvacrol can be found in similar concentrations (Martínez-Velázquez et al., 2011), the genotypes investigated so far in *L. gracilis* contain either thymol or carvacrol as the major compound (Matos et al., 1999; Cruz et al., 2013; Born et al., 2018). For instance, Lemos et al. (1992) found that the essential oil of *L. gracilis* leaves collected in the northeastern state of Ceará, Brazil, presented thymol (30.6%), carvacrol (11.8%) and ρ-cymene (10.7%) as major compounds. In contrast, Matos et al. (1999) reported that the essential oil of plants collected in neighboring Piaui state presented carvacrol (47.7%), ρ-cymene (19.2%) and methylthymol (6.2%) as major compounds. In this study, chromatographic analyses revealed that *L. gracilis* LGRA106 accession had over 50% of thymol, which is in line with the study from Cruz et al. (2013) who reported 59.26% of thymol in extractions from the same genotype collected in Sergipe state.

Plants of the genus *Lippia* have been proven to be toxic to a variety of pest arthropods, including insects (Carvalho et al., 2003; Cavalcanti et al., 2004; Gleiser and Zygadlo, 2007), mites (Cavalcanti et al., 2010; Born et al., 2018) and ticks (Cruz et al., 2013; Peixoto et al., 2015). Such biological effects are generally ascribed to major compounds thymol and carvacrol (Cruz et al., 2013). In this study, the essential oil of the tested accession and thymol differed in their toxicity based on LC_{50} values. Little is known on the mode of action of thymol on mites. In insects, this

Table 3
Repellency of essential oil of *Lippia gracilis* accession LGRA106 (LC_{50}) and its major compound, thymol, to the coconut mite after 1 h exposure.

	RI[a]	1 + SD[b]	1-SD	Classification
L. gracilis	0.367	1.153	0.847	Repellent
Thymol	0.633	1.153	0.847	Repellent

[a] RI = Repellency index (RI = 2G/(G + P)); where G = number of mites in the treatment, P = number of mites in the control (Pontes et al., 2007).
[b] SD = Standard deviation.

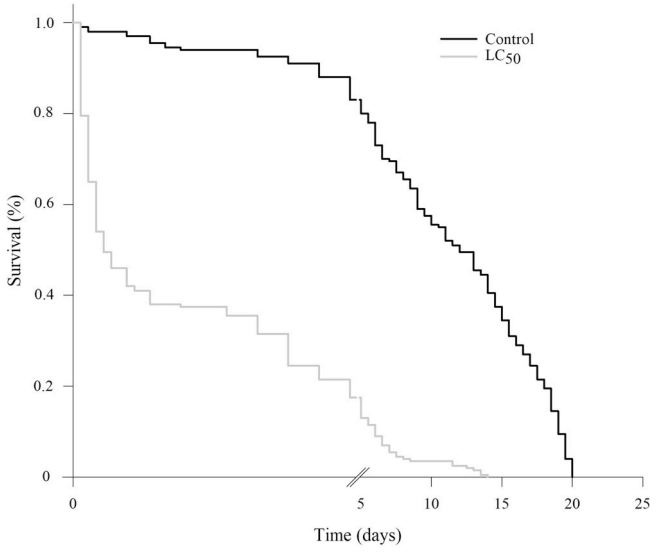

Fig. 1. Survival curves of adult females of *Raoiella indica* exposed to LC_{50} of the essential oil of leaves of de *Lippia gracilis* accession LGRA-106 (log-rank test, $P < 0.001$).

Table 2
Estimated lethal concentration (LC_{50}) of the essential oil (EO) of leaves of *Lippia gracilis* accession LGRA-106 and thymol against *Aceria guerreronis* and *Raoiella indica* after 24 h of exposure.

Mite species		LC_{50} (95% CI[a]) mg/mL	Slope	χ^2[b]	P	TR[b] (95% CI)
A. guerreronis	EO	4.28 (3.95–4.52)	5.24	1.58	0.544	
	Thymol	5.34 (4.51–6.28)	2.27	1.84	0.600	1.24 (1.06–1.43)[c]
R. indica	EO	4.99 (4.63–5.36)	3.74	5.986	0.112	
	Thymol	9.03 (8.38–9.64)	4.13	3.853	0.277	1.81 (1.75–1.86)[c]

[a] CI = confidence interval.
[b] TR = Toxicity ratio.
[c] TR significant (confidence interval does not include the value 1.0).

monoterpene can inhibit the activity of acetylcholinesterase, and consequently cause an overstimulation of neurons (Jukic et al., 2007; Anderson and Coats, 2012). In addition, this compound acts as an allosteric modulator for GABA (γ-aminobutyric acid) receptors in insects. GABA regulates chloride channels, inhibiting the insect nervous system (Tong and Coats, 2010). The RDL receptors activated by GABA play an important role, as inhibitory neurotransmitter and in insecticidal activity, but the mechanisms of how this compound regulates responses are not fully understood (Price and Lummis, 2014). In relation to the mechanism of action of essential oils on mites, despite being little known, its rapid effect would indicate neurotoxic action (Isman, 2006). It is possible that essential oils have more than one site of action because they are complex mixtures of compounds. Moreover, compounds may interact positively or negatively (Tak et al., 2016; Tak and Isman, 2017; Wu et al., 2017). Previous studies have shown that minor compounds can enhance the action of thymol. For instance, thymol alone has proven to have a slower effect on the survival of larvae of *Diaphania hyalinata* (Lepidoptera: Pyralidae) and a higher LC_{50} against *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae) than the essential oil of *L. gracilis* (Cruz et al., 2013; Melo et al., 2018).

This study showed that survival of *R. indica* decreases when individuals are exposed to the essential oil of *L. gracilis.* Similar effects have been reported for the larvae of *D. hyalinata* (Melo et al., 2018). In addition to lethal toxicity, sublethal effects on individuals that survive pesticide exposure play a key role in pest control (Desneux et al., 2007). Such effects include reduced longevity and oviposition as well as altered orientation (attraction and repellence) (Silva et al., 2013; Pavela et al., 2016; Tak and Isman, 2017). In addition to survival, the estimated LC_{50} of the essential oil of *L. gracilis* drastically reduced the number of eggs laid by females. Considering that *R. indica* has a very high reproductive potential, averaging 160 eggs per female in their lifespan (Moutia, 1958; Flores-Galano et al., 2010; Carrillo and Peña, 2012), compounds that interfere in their reproduction performance may be important to reduce population buildup.

The repellent activities of plant oils against pests have been extensively investigated (Nerio et al., 2010). Repellency combined with mortality is an important feature to consider when choosing a pest control product. For controlling *A. guerreronis*, such effect is particularly important, considering that the colonies are located under the perianth of the fruits. Therefore, acaricidal residues can act on *A. guerreronis* adults when they leave the protection of the perianth for dispersion (Monteiro et al., 2012). In this study, the LC_{50} of either the essential oil or its major monoterpene, proved to be repellent to *A. guerreronis*. Although little is known about the repellent effect of *L. gracilis* on mites, *L. junelliana* and *L. turbinata* were shown to repel *Varroa destructor* Anderson & Trueman (Acari: Varroidae) (Ruffinengo et al., 2005). Also, thymol has been extensively studied and proved to be repellent towards a large number of insects (for a review Nerio et al., 2010) and mites (Perrucci et al., 1995; Tabari et al., 2017).

One of the gaps when assessing the effect of essential oils is their impact on non-target organisms. Selectivity of essential oils to predatory mites has been reported in the literature. For example, *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) showed to be more tolerant to essential oils from various plants than its prey *Tetranychus urticae* Koch (Acari: Tetranychidae) (Han et al., 2010; Ribeiro et al., 2016). In this study, in contrast, the LC_{50} of the essential oil of *L. gracilis*, as estimated for *R. indica*, resulted in increased mortality of the predatory mite *A. largoensis.* In addition to the composition of the essential oil (Momen et al., 2001; Choi et al., 2004), susceptibility of predatory mites is species dependent. For example, Momen et al. (2001) observed that essential oil of peppermint is more toxic than essential oil of mint to various predatory mites. Moreover, adult females of *Amblyseius yousefi* (Acari: Phytoseiidae) are less susceptible than *Typhlodromus athiasae* Porath & Swirski (Acari: Phytoseiidae) when exposed to essential oils of mint. Various species of predatory mites inhabit coconut palms (Moraes et al., 2004). Therefore, assessing the effect of *L. gracilis* on other species of phytoseiids occurring in coconut plantations is needed for a comprehensive evaluation of the selectivity of this oil to beneficial organisms. However, because of the low persistence of essential oils, it may be compatible with biological control if a proper monitoring schedule of pest and predator is taken into account. As *A. largoensis* occurs mainly on coconut palm leaves, applications directed to the fruits for reducing populations of *A. guerreronis* may decrease risk of mortality of the predatory mite as well.

Acknowledgments

The authors would like to thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Apoio a Pesquisa e à Inovação Tecnológica do Estado de Sergipe (FAPITEC/SE), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Financiadora de Estudos e Projetos (FINEP) for financial support.

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