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## Toxicity and repellency of the essential oil from *Lippia gracilis* to the coconut mite *Aceria guerreronis* (Acari: Eriophyidae)

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### ABSTRACT

The essential oil (EO) from different genotypes of *Lippia gracilis* Schauer (Verbenaceae) present two distinctive chemotypes containing either thymol or its isomer carvacrol as major compounds, both of which have proven bioactivity against several agricultural pests. Recently, we have shown that *L. gracilis* accession LGRA 106 and its major compound thymol are toxic and repellent against the coconut mite, *Aceria guerreronis* Keifer (Acari: Eriophyidae), a key pest of coconut plantations in Asia, Africa and America. Since intraspecific variation affects the chemical composition of EO and hence, its bioactivity, here we assessed the acaricidal and repellent effects of *L. gracilis* accession LGRA109 to *A. guerreronis*. Leaves of this accession contain carvacrol (49.35%) as major compound. The LC<sub>50</sub> of the EO and of carvacrol estimated for *A. guerreronis* were 28.01 and 6.84 mg/mL, respectively. Carvacrol, at its LC<sub>50</sub>, as well as the EO and carvacrol at their LC<sub>99</sub>, repelled the mite. Our results indicated that carvacrol and the EO of *L. gracilis* accession LGRA109 were bioactive against *A. guerreronis*; however, the EO was less effective than its major compound.

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### Introduction

The aromatic shrub, *Lippia gracilis* Schauer (Verbenaceae) is endemic of semi-arid Caatinga, the largest biome of Brazil's northeast. As other Verbenaceae species, the leaves of *L. gracilis* are rich in essential oils (EO). Different genotypes present the monoterpene phenol isomers thymol or carvacrol as major compounds and variable minor quantities of other terpenoids such as limonene,  $\gamma$ -terpinene, p-cymene,  $\beta$ -caryophyllene, camphor and linalool (Teles et al. 2010; Cruz et al. 2013). Based on the chemical composition of the EO, the thymol and the carvacrol chemotypes are distinguished in this species (Melo et al. 2018).

Thymol and carvacrol are toxic or repellent against various agricultural pests (Park et al. 2017; Gong and Ren 2020). Moreover, the lethal and sublethal effects of the EO of *L. gracilis* have been shown towards several noxious organisms (Melo et al. 2018; Santos et al. 2019). Recently, we have shown that the EO of the leaves of *L. gracilis* accession LGRA106 (thymol-chemotype) has toxic and repellent activities against the coconut mite, *Aceria guerreronis* Keifer (Acari: Eriophyidae) (Santos et al. 2019), a major coconut pest in tropical countries worldwide. Colonies of this mite develop beneath the bracts causing necrotic lesions that reduces the value of fruits intended for the fresh market. Moreover, under high infestations, fruits fall before maturing, or present small size and water content (Negloh et al. 2011; Navia et al. 2013). Thus, *A. guerreronis* has serious economic impacts on yields and fruit quality (Negloh et al. 2011). Since intraspecific variation in the chemical composition of the EO of *L. gracilis* affects its bioactivity (Cruz et al. 2013), we investigated here whether a carvacrol-chemotype (LGRA 109) and its major compound are bioactive against the coconut mite.

### Materials and methods

#### Living materials

Leaves of *L. gracilis* accession LGRA109 were obtained from the Active Germplasm Bank of Medicinal and Aromatic Plants of the

Federal University of Sergipe, situated in São Cristóvão (11°00'S; 37°12'W), Sergipe, Brazil. Adults of *A. guerreronis* were collected from colonies in the early stage of oviposition developing beneath the bracts of young coconut fruits of the variety green dwarf, in Aracaju (10°57'S; 37°03'W), Sergipe, Brazil.

#### Extraction and analysis of essential oil

The EO was extracted from 75 g of dry leaves (at 40°C for 5 days) by hydrodistillation, using a modified Clevenger apparatus, for 2 hours and 20 minutes following procedures described in Santos et al. (2019). Extractions were replicated three times. Samples were analysed by a GC-MS/FID (QP2010 Ultra, Shimadzu Corporation, Kyoto, Japan), equipped with an autosampler AOC-20i (Shimadzu) and an Rtx®-5MS Restek fused silica capillary column (5%-diphenyl-95%-dimethyl polysiloxane) of 30 m  $\times$  0.25 mm i.d., 0.25 mm film thickness (Santos et al. 2019). Individual compounds were quantified from the GC peak areas and identified by comparing their acquired mass spectra and retention indexes (Van den Dool and Kratz 1963) with those in NIST21, NIST107 and WILEY8 mass spectral libraries, and in the literature (Adams 2007).

#### Toxicity

The procedures used in this bioassay are described in Santos et al. (2019). Briefly, 1-cm-diameter discs of perianth of young coconut fruits (experimental units) were sprayed with 1.7 mL of the EO or 9.3 mL of carvacrol through a Potter spray tower (Burkard, UK), at a pressure of 5 psi/pol<sup>2</sup>. The discs were immersed in a preparation of 5% agar and 0.3% methylparaben, and kept in Petri dishes (100 mm diameter  $\times$  15 mm depth) to prevent mites from escaping. A mould was used to expose the plant tissue of the arenas. Nine increasing concentrations of the EO of *L. gracilis* (0.9, 4.5, 9.0, 18.1, 22.6, 27.1, 31.6, 40.7 and 45.2 mg/mL) and seven of carvacrol (1, 3, 5, 7, 10, 15 and 20 mg/mL) were selected. The EO was dissolved in tween 20 (0.5% v/v) and distilled water, while

carvacrol (98% purity) in acetone (99.9% purity). Tween 20 or acetone were used as control. Sprayed discs were left to dry for 30 minutes before thirty adult mites were transferred onto each experimental unit. A black fabric covered the Petri dishes to simulate darkness conditions underneath the fruit bracts. The experiment was replicated eight times. Petri dishes were maintained at controlled conditions ( $27^{\circ}\text{C} \pm 3^{\circ}\text{C}$ ,  $70 \pm 10\%$  RH, and 12 h photoperiod) for 24 hours after which the number of living and dead mites was recorded. The toxicity ratio was calculated by dividing the  $\text{LC}_{50}$  for carvacrol by the  $\text{LC}_{50}$  for the EO of *L. gracilis*.

### Repellency

The procedures used in this bioassay followed Santos et al. (2019). In short, two layers of impermeable tape were used to cover half of coconut perianth discs (1 cm diameter) before spraying with the respective  $\text{LC}_{50}$  of the EO of *L. gracilis* or carvacrol, as estimated for *A. guerreronis* in the previous bioassay. The tape was removed and the discs were air-dried for 30 minutes before a single adult mite was gently transferred onto a dried glue spot placed in their centre. The experimental units were kept under controlled conditions ( $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ,  $80 \pm 10\%$  RH and a 12 h photoperiod). The position of the mites on the treated or untreated half of the discs was recorded one and 24 hours after spraying. Each treatment was replicated three times with 20 mites per replicate.

### Statistical analysis

Corrected mortality data (Abbott 1925) were subjected to Probit analyses using SAS Proc PROBIT (SAS 2013) to estimate the lethal concentrations (LC) of the EO and carvacrol.  $\text{LC}_{50}$  and  $\text{LC}_{99}$  values were further used in the repellency bioassay. The repellency index (RI) was determined according to the formula:  $\text{RI} = 2 G/(G + P)$  (Kogan and Goeden 1970), where G and P are the numbers of mites in the treated and in the untreated areas, respectively. The estimated mean and standard deviation (SD) of the RI were considered as the safety interval to classify the EO or carvacrol as repellent (mean of  $\text{RI} < 1 - \text{SD}$ ), neutral ( $1 - \text{SD} < \text{mean of RI} < 1 + \text{SD}$ ) or attractive (mean of  $\text{RI} > 1 + \text{SD}$ ).

### Results

Thirty compounds were identified in the EO of *L. gracilis* accession LGRA109 (Table 1). Carvacrol was the major compound (49.35%), followed by p-cymene (11.29%),  $\gamma$ -terpinene (10.09%) and (E)-caryophyllene (6.79%).

The estimated  $\text{LC}_{50}$  and  $\text{LC}_{99}$  for *L. gracilis* accession LGRA109 to *A. guerreronis* were 28.01 and 49.11 mg/mL, respectively. For carvacrol, the  $\text{LC}_{50}$  and  $\text{LC}_{99}$  to *A. guerreronis* were 6.84 and 45.95 mg/mL, respectively (Table 2). According to LC and toxicity ratio, carvacrol was 4.5-fold more toxic than the essential oil (Table 2).

The  $\text{LC}_{50}$  for the accession LGRA109 of *L. gracilis* was repellent to the coconut mite only after 24 hours of exposure. However, the EO at its estimated  $\text{LC}_{99}$ , and carvacrol at both LC, repelled the mites irrespective of exposure time (Table 3).

### Discussion

The chemical composition reported here matches that reported by Cruz et al. (2013) who quantified carvacrol (48.99%), p-cymene (13.02%) and  $\gamma$ -terpinene (8.55%) as main compounds in the EO extracted from leaves of plants of this accession. Based on LC and the toxicity ratio, the toxicity of the EO was estimated to be about 4.5 times lower than its major component. Likewise, a recent study showed that the EO of *L. gracilis* accession LGRA109 was ca. 5 times less toxic to the melonworm moth, *Diaphania hyalinata* L. (Lepidoptera: Pyralidae) than carvacrol (Melo et al. 2018). Also, the  $\text{LC}_{50}$  of the EO extracted from this accession was estimated to be about 14.5 greater than that for carvacrol against larvae of the cattle

**Table 1.** Chemical profile of the essential oil from leaves of *Lippia gracilis* accession LGRA109.

Compounds	RRI- <sup>1</sup>	% Peak Area
$\alpha$ -Thujene	924	1.20
$\alpha$ -Pinene	932	0.29
Camphene	946	0.14
Sabinene	969	0.10
$\beta$ -Pinene	974	0.10
Myrcene	988	1.58
$\alpha$ -Phellandrene	1002	0.14
3- $\delta$ -Carene	1008	0.10
$\alpha$ -Terpinene	1014	1.79
<b>p-Cymene</b>	<b>1020</b>	<b>11.29</b>
Limonene	1024	0.48
1,8-Cineole	1026	0.36
<b><math>\gamma</math>-Terpinene</b>	<b>1054</b>	<b>10.09</b>
(Z)-Sabinene hydrate	1065	0.47
Linalool	1095	0.65
Camphor	1141	0.45
Terpinen-4-ol	1174	0.48
Methyl thymol	1232	4.40
Methyl carvacrol	1241	0.19
Thymol	1289	3.05
<b>Carvacrol</b>	<b>1298</b>	<b>49.35</b>
$\delta$ -Elemene	1335	0.46
Carvacrol acetate	1349	0.54
<b>(E)-caryophyllene</b>	<b>1417</b>	<b>6.79</b>
$\alpha$ -E-Bergamotene	1432	0.46
Aromadendrene	1439	0.24
$\alpha$ -Humulene	1452	0.40
Bicyclogermacrene	1500	2.46
Spathulenol	1577	0.58
Caryophyllene oxide	1582	0.75

<sup>1</sup>RRI-I: Relative retention index – literature.  
The main compounds are marked in bold.

**Table 2.** Lethal concentrations ( $\text{LC}_{50}$  and  $\text{LC}_{99}$ ) of the essential oil from leaves of *Lippia gracilis* accession LGRA109, and its major compound carvacrol, to the coconut mite *Aceria guerreronis* after 24 hours of exposure.

	$\text{LC}_{50}$ (95% CI) <sup>1</sup> (mg/mL)	$\text{LC}_{99}$ (95% CI) (mg/mL)	Slope	$\chi^2$	P	TR <sup>2</sup>
EO	28.01 (27.03–28.96)	49.11 (45.56–54.31)	9.55	1.09	0.781	4.53
Carvacrol	6.84 (5.99–7.79)	45.97 (34.33–68.57)	2.81	0.42	0.810	

<sup>1</sup>CI: Confidence interval.

<sup>2</sup>TR: Toxicity ratio.

**Table 3.** Repellency of the essential oil from leaves of *Lippia gracilis* accession LGRA109, and its major compound carvacrol, to the coconut mite *Aceria guerreronis* after 1 and 24 h of exposure.

		LC	RI <sup>1</sup>	1+SD <sup>2</sup>	1–SD	Classification
EO	1 h	$\text{LC}_{50}$	0.667	1.379	0.621	Neutral
		$\text{LC}_{99}$	0.800	1.100	0.900	Repellent
	24 h	$\text{LC}_{50}$	0.867	1.115	0.885	Repellent
		$\text{LC}_{99}$	0.900	1.100	0.900	Repellent
Carvacrol	1 h	$\text{LC}_{50}$	0.400	1.100	0.900	Repellent
		$\text{LC}_{99}$	0.367	1.058	0.942	Repellent
	24 h	$\text{LC}_{50}$	0.467	1.058	0.942	Repellent
		$\text{LC}_{99}$	0.600	1.100	0.900	Repellent

<sup>1</sup>RI: Repellency index.

<sup>2</sup>SD: Standard deviation.

tick, *Rhipicephalus microplus* (Canestrini) (Acari: Ixodidae) (Cruz et al. 2013). The toxic effects of carvacrol on mites are well known (Cruz et al. 2013; Born et al. 2018). Removing carvacrol from a synthetic blend of compounds reduced the fumigant and residual contact activity of a synthetic oil against the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) (Born et al. 2018). Nevertheless, there was no relationship between toxicity and carvacrol content in different accessions as shown for *R. microplus* (Cruz et al. 2013).

The mode of action of thymol and carvacrol possibly relies on an inhibitory effect on the activity of acetylcholinesterase enzyme (AChE) (Jukic et al. 2007; Dandlen et al. 2011). Moreover, it has shown that carvacrol is more efficient than thymol, which suggests that the position of the hydroxyl group in the molecular structure of the

compound plays an important role in inhibiting AChE (Aazza et al. 2011). The LC<sub>50</sub> of carvacrol estimated here was slightly higher compared to that of thymol (LC<sub>50</sub> = 5.34 mg/mL) against the same pest (Santos et al. 2019). However, we found that the LC<sub>50</sub> of the carvacrol-chemotype LGRA 109 is about 6-fold higher than the LC<sub>50</sub> previously estimated the thymol-chemotype accession LGRA106 to the same mite species (Santos et al. 2019). In contrast, both accessions had similar contact toxicity against *D. hyalinata* (Melo et al. 2018). Variations in chemical composition due to physicochemical properties of individual compounds in the mixture can influence the biocidal activity of EOs (Peixoto et al. 2015). Therefore, other compounds in the blend may play an important role in the bioactivity of the EO.

Repellence is a behavioural response of an organism to chemical, mainly volatile, stimuli that results in orientated movements away from the odour source, and derives from the avoidance of toxicants without contact or after contact (irritability) (Cordeiro et al. 2010). Except for the LC<sub>50</sub> of the EO of *L. gracilis* after 1 hour of exposure, all the treatments repelled *A. guerreronis*. In contrast, the LC<sub>50</sub> of the thymol-chemotype repelled this mite after 1 hour of exposure (Santos et al. 2019). Similar to toxicity, the responses of arthropods to particular chemotypes are species-specific as shown for two stored grain pests. Whereas the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) was repelled by the EO of *L. alba* genotype LA 57 and its major compound carvone, the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) was not (Peixoto et al. 2015). This highlights the importance of assessing different chemotype-pest species combinations for sustainable application of the oil in pest management.

Plant essential oils may or may not present stronger repellent effects than individual compounds (Silva Lima et al. 2016) due to the volatility of active components or synergism among them (Gillij et al. 2008). Nonetheless, responses of arthropod are complex and vary depending on specific volatile mixtures. According to Jaensson et al. (2005), species seem to have their own chemical niche of positive and negative stimuli, and certain compounds can attract or repel, or even be toxic at certain concentrations. For instance, compounds that repel the black bean aphid, *Aphis fabae* Scopoli (Hemiptera: Aphididae) turn attractive when combined in a blend at a concentration that elicits the greatest repellency (Webster et al. 2010). Subtle differences in concentrations, ratios between compounds, or even presence or absence of minor components in this genotype that are ubiquitous in nature may have decreased the repellent action of carvacrol at the estimated LC<sub>50</sub> of the oil.

In conclusion, we have demonstrated the acaricidal and repellent activities of the carvacrol-chemotype of the EO of *L. gracilis* against a key coconut pest, *A. guerreronis*. Such promising results suggest that it holds potential for pest management purposes. As arthropod responses are species-specific, screening of different genotypes and their effects on different pest species are needed for efficient applications and recommendations.

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## Disclosure statement

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