Acarologia
Open Science in Acarology

Bioactivity of essential oils for the management of *Tetranychus urticae* Koch and selectivity on its natural enemy *Neoseiulus californicus* (McGregor): A promising combination for agroecological systems

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Original research

ABSTRACT

The two-spotted spider mite, *Tetranychus urticae*, causes damage to crops grown in northeast Brazil. The adoption of biological control methods and curative methods (plant-based insecticides) is an essential practice for pest management in agroecological systems. Therefore, the aim of the present study was to investigate the chemical properties, toxicity, and ovicidal activity of essential oils (EOs) from *Lippia sidoides*, *Croton rhamnifolioides*, *Croton grewioides*, *Citrus sinensis*, *Citrus limon*, *Citrus aurantiifolia* and *Piper divaricatum* for the control of *T. urticae* and determine the selectivity of these EOs regarding the predator mite *Neoseiulus californicus*. The chemical analysis (gas chromatography–mass spectrometry) of the EOs enabled the identification of 98 compounds. The major constituents were carvacrol (*L. sidoides*), ß-caryophyllene (*C. rhamnifolioides*), (*E*)-anethole (*C. grewioides*), limonene (*Citrus* spp.), safrole and methyl eugenol (*P. divaricatum*). All oils exhibited satisfactory toxicity to the eggs and females of *T. urticae* and were even more toxic than the commercial product Azamax. The *L. sidoides* oil exhibited greater toxicity compared to the other oils, with LC_{50} values of 0.05 and 0.09 µL mL^{-1} for females and eggs, respectively. All oils tested were selective to *N. californicus*, with RS values ranging from 3.61 to 23.28 for *C. aurantiifolia* and *C. grewioides*, respectively. Therefore, the use of products based on the EOs studied in combination with the natural enemy *N. californicus* is a viable option in agroecological systems for the management of *T. urticae*.

Keywords two-spotted spider mite; plant-based acaricide; *Neoseiulus californicus*; selectivity; agroecological systems

Received 19 January 2021
Accepted 11 June 2021
Published 17 June 2021

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Academic editor
Tsolakis, Haralabos

DOI

ISSN 0044-586X (print)
ISSN 2107-7207 (electronic)

Introduction

Brazilian agriculture suffers frequent losses due to the attack of pests. The two-spotted spider mite, *Tetranychus urticae* Koch, causes damage to diverse crops grown in the state of Pernambuco, such as beans, cotton, papaya, grapes and ornamental plants (Ferreira *et al.* 2015; Monteiro *et al.* 2015), the latter of which is often grown in protected environments

How to cite this article Fidelis de Santana M. *et al.* (2021), Bioactivity of essential oils for the management of *Tetranychus urticae* Koch and selectivity on its natural enemy *Neoseiulus californicus* (McGregor): A promising combination for agroecological systems. **Acarologia 61(3): 564-576; DOI**

(greenhouses). The losses caused by agricultural pests are both direct (effects on the crop) and indirect (costs related to the purchasing of pesticides and the consequent environmental contamination and harm to health) (Oliveira *et al.* 2014). Moreover, the indiscriminate use of these products inevitably leads to resistant pest populations. Indeed, *T. uritice* is the agricultural pest with resistance to the largest number of conventional acaricides (526 cases of resistance to 96 different active ingredients) (APRD 2020).

The main form of controlling the two-spotted spider mite is through conventional acaricides (van Leeuwen *et al.* 2010; Rincón *et al.* 2019). However, these products are not permitted in agroecological communities or organic farming activities. Azamax (active ingredient: azadirachtin) is the only plant-based acaricide registered used in agroecological systems in protected environments in the state of Pernambuco. A preventive and curative option for the management of this pest is through biological control and the use of plant-based insecticides (Brzozowski and Mazourek 2018). In Brazil, the predator mite *Neoseiulus californicus* (McGregor) is used for the biological control of *T. urticae*, especially in protected environments (Barbosa *et al.* 2017).

The use of formulations whose active ingredient is derived from plants, such as essential oils (EOs), has been widely investigated due to the broad action on different types of arthropods as well as biodegradability, low toxicity to mammals and the absence of contamination of the environment (Isman 2020). Moreover, these oils are complex mixtures generally made up of terpenes and phenylpropanoids, which makes the development of resistance in the target pest a much slower process, as demonstrated by Feng and Isman (1995) for the green peach aphid, *Myzus persicae* Sulz., as a mixture of active constituents, including neem, mitigated the development of resistance in comparison to a single active ingredient (azadirachtin). Although there are no reports of the resistance of *T. urticae* to azadirachtin (APRD 2020), the frequent use of this active ingredient in agroecological communities of northeast Brazil could favor the resistance of this pest.

Among EOs with recognized acaricidal properties, species belonging to the genus *Lippia* (*L. sidoides*), *Croton* (C. *rhamnifolioides*) and *Citrus* (C. *aurantiifolia*, *C. limon* and *C. sinensis*) stand out (Júnior *et al.* 2010; Cavalcanti *et al.* 2010; Camara *et al.* 2017; Ribeiro *et al.* 2019). However, there are few reports on the selectivity of these EOs for the predator mite *N. californicus*.

In the search for plant-based substances for use as active ingredients in acaricidal formulations, the aim of the present study was to determine the chemical composition of EOs from the leaves of *Lippia sidoides, Piper divaricatum*, *Citrus sinensis*, *C. limon*, *C. aurantiifolia*, *Croton rhamnifolioides* and *C. grewioides* and evaluate toxicity to the eggs and adults of *T. urticae*. A further aim was to investigate the effects of these oils on the predator mite *N. californicus.* The results were compared to those achieved with a plant-based acaricide (Azamax) used as the positive control.

Material and methods

Collection of plant material

The plants collected were identified by Botanist Dra. Maria F.A. Lucena. Voucher of samples were mounted and deposited no Herbário da Universidade Federal de Pernambuco, under number: (46254) *Croton rhamnifolioides* Pax and Hoffm. (Euphorbiaceae), (42193) *Croton grewioides* Baill (Euphorbiaceae), (48734) *Citrus aurantiifolia* (Christm.) Swingle (Rutaceae), (48736) *Citrus limon* (L.) Burm.f. (Rutaceae) and (48739) *Citrus sinensis* Osbeck var. mimo (Rutaceae). *Lippia sidoides* Cham (Verbenaceae) (genotype LISID4) and *Piper divaricatum* (Piperaceae) (Kato-1063) oils were donated by Prof. Alves, PB from Federal University of Sergipe and Prof. Ramos, CS from Chemistry Departament of UFRPE, respectively.

Chemicals

All monoterpenes (α-pinene, β-pinene, α-phellandrene, limonene, 1,8-cineole, *p*-cymene, citronellal, camphor, terpinen-4-ol, terpinolene, linalool e α-terpineol), sesquiterpenes (β-caryophyllene, aromadendrene, α-humulene, germacrene D, bicyclogermacrene, spathulenol and caryophyllene oxide) and phenylpropanoid ((*Z*)-anethole, eugenol, methyl eugenol, safrole) used for chemical constituent identification were purchased from Sigma-Aldrich - Brazil.

Essential oils extraction and GC-FID analysis

The EOs from fresh leaves (100 g) of *C. rhamnifolioides, C. grewioides, C. aurantiifolia*, *C. limon*, *C. sinensis* were separately isolated using a modified Clevenger-type apparatus and hydrodistillation for 2h. The oil layers were separated and dried over anhydrous sodium sulfate, stored in hermetically sealed glass containers and kept at low temperature (-5 °C) until analysis. Total oil yields were expressed as percentages (g/100 g of fresh plant material). All experiments were carried out in triplicate. Quantitative GC (500 GC, PerkinElmer Clarus, Shelton, CO, USA) analysis were carried out using a apparatus equipped with a flame ionization detector (FID) and a non-polar DB-5 fused silica capillary column (30 m x 0.25 mm x 0.25 µm) (J & W Scientific). The oven temperature was programmed from 60 to 240 °C at a rate 3 °C min^{-1}. Injector and detector temperatures were 260 °C. Hydrogen was used as the carrier gas at a flow rate of 1 mL min^{-1} in split mode (1:30). The injection volume was 0.5 µL of diluted solution (1/100) of oil in *n*-hexane. The amount of each compound was calculated from GC-FID peak areas in the order of DB-5 column elution and expressed as a relative percentage of the total area of the chromatograms. Analyses were carried out in triplicate.

GC-MS analysis

The qualitative Gas Chromatography-Mass Spectrometry (GC-MS) (220-MS IT GC, Varian, Walnut Creek, CA, USA) analysis were carried out using a system with a mass selective detector, mass spectrometer in EI 70 eV with a scan interval of 0.5 s and fragments from 40 to 550 Da. fitted with the same column and temperature program as that for the GC-FID experiments, with the following parameters: carrier gas = helium; flow rate = 1 mL min^{-1}; split mode (1:30); injected volume = 1 µL of diluted solution (1/100) of oil in n-hexane.

Identification of components

Identification of the components was based on GC-MS retention indices with reference to a homologous series of C8-C40 *n*-alkanes calculated using the Van der Dool and Kratz equation (Van den Dool and Kratz 1963) and by computer matching against the mass spectral library of the GC-MS data system (NIST 11 and WILEY 11th) and co-injection with authentic standards as well as other published mass spectra (Adams 2017). Area percentages were obtained from the GC-FID response without the use of an internal standard or correction factors.

Rearing of *Tetranychus urticae* and *Neoseiulus californicus*

Specimens of *T. urticae* were originally collected in 2008 from grapevine (*Vitis vinifera* L.) in the municipality of Petrolina in the state of Pernambuco, Brazil (09°12′43.9″ S, 40°29′12.7″ W) and then maintained in the laboratory on jack bean (*Canavalia ensiformes* L.) at 25 ± 1 °C, 65 ± 5% relative humidity and a 12-h photoperiod without any exposure to acaricides. The predator mite *N. californicus* was collected from the municipality of Bonito in the state of Pernambuco, Brazil (08°28′13″ S, 35°43′43″ W) on chrysanthemum (*Dendranthema grandiflora* Tzvelev.) and bred in the laboratory since 2010 with no exposure to acaricides. The breeding method of *T. urticae* and *N. californicus* was according to methodology used by Born *et al.* (2018). The predator mite was reared in plastic arenas (25 cm diameter) maintained in B.O.D. at a mean temperature of 27 °C and a 12-h photoperiod. Jack bean leaf was placed with the margin

surrounded by moistened hydrophilic cotton to avoid the escape of the mites. Cotton fibers were placed on the jack bean leaves to stimulate oviposition. As a food source, *T. urticae* and castor bean pollen (*Ricinus communis* L.) were offered every 2 days.

Residual contact assay

The leaf disc painting method described by Araújo *et al.* (2020) was used to test the action of *C. aurantiifolia*, *C. limon*, *C. sinensis* var. mimo, *L. sidoides*, *C. rhamnifolioides*, *P. divaricatum*, *C. grewioides* and positive control (Azamax) by contact toxicity. The experiments were performed with open Petri dishes (10 cm diameter). Leaf discs (5 cm diameter) were cut from leaves of greenhouse-grown jack bean (*C. ensiformes*). Test solutions were prepared by diluting the EO in water and DMSO (Dimethylsulfoxide) (0.5%) (negative control). The concentration used in the bioassays ranged from 0.009 to 5.40 µL mL^{-1} for the EO. The concentration of the botanical and conventional insecticides used as positive control ranged from 0.009 to 10 µL mL^{-1} for Azamax. Leaf discs (5 cm diameter) were immersed in solutions for 30s. Control mites were held on leaf discs immersed in the water and DMSO. Each leaf disc was infested with 15 adult females of *T. urticae*. Five replicates were used in each bioassay and repeated 2× on different dates using a completely randomized design, totaling 150 mites per concentration. Mortality was determined under a dissecting microscope 24 h after the onset of treatment. Mites were considered dead if the appendages did not move when prodded with a fine paintbrush. The residual contact assays were performed at 25 ± 1 °C, 65 ± 5% RH and a 12-h photoperiod.

Fifty adult females of *T. urticae* were placed on leaf discs (8cm diameter) for 24 hours to effect oviposition. After that period, *T. urticae* were removed. The leaf discs with *T. urticae* eggs were immersed in the concentrations of EO, Azamax and control (water and DMSO) (adaptated from Esteves-filho *et al.* 2013). Subsequently were placed to dry for 30 minutes at room temperature. Each leaf disc 300 eggs were left, which served as contaminated food for *N. californicus*. Each leaf disc was infested with 15 adult females of *N. californicus*. Five replicates were used in each bioassay and repeated 2× on different dates using a completely randomized design, totaling 150 mites per concentration. Mortality was determined under a dissecting microscope 48 h after the onset of treatment. Mites were considered dead if the appendages did not move when prodded with a fine paintbrush. The residual contact assays were performed at 25 ± 1 °C, 65 ± 5% RH and a 12-h photoperiod.

Ovicide assay

The methodology used in this test was adapted from Esteves-Filho *et al.* (2013). Leaf discs (5 cm diameter) were cut from leaves of greenhouse-grown jack bean (*C. ensiformes*). Leaf discs were infested with 15 adult females of *T. urticae*, which were maintained for 24 hours for oviposition. Then leaf discs with eggs of *T. urticae* were immersed in the concentration of each oil, azamax and control, as bioassays described above. Subsequently were placed to dry for 30 minutes at room temperature. Each leaf disc 50 eggs were left. Each bioassay and repeated 3× on different dates using a completely randomized design, totaling 150 mites per concentration. Evaluation was performed after 96 hours of application of oil, azamax and control, which is recorded the number of emerged larvae.

Statistical analysis

For the determination of the lethal concentration necessary for a 50% mortality rate (LC_{50}) and 90% (LC_{90}) of the mite population in the residual contact tests, the mortality data were analyzed using the Probit model implemented in the POLO-Plus 2.0 (LeOra Software 2005) program, with the calculation of 95% confidence levels. Toxicity ratios (TR) and RS (Relative Selectivity) were determined based on the method described by Robertson and Preisler (2017).

Results

Yield and chemical profile of essential oils

The GC-MS of the *Croton* spp., *Lippia sidoides, Piper divaricatum* and *Citrus* spp. oils enabled the identification of 98 compounds (Table 1). The greatest yield of EO was achieved with *C. grewioides* (2.30 ± 0.18%), followed by *C. sinensis* (0.78 ± 0.05%), *C. limon* (0.46 ± 0.06%), *C. rhamnifolioides* (0.17 ± 0.03%) and *C. aurantiifolia* (0.17 ± 0.05%).

The *C. rhamnifolioides* and *C. grewioides* oils had a predominance of compounds belonging to the classes of sesquiterpenes (66.3 ± 0.6%) and phenylpropanoids (75.7 ± 0.5%), respectively. β-Caryophyllene (33.3 ± 0.6%) was the major component of the *C. rhamnifolioides* oil and (*E*)-anethole (55.5 ± 0.4%) was the major component of the *C. grewioides* oil. The predominance of compounds belonging to the chemical class of phenylpropanoids (84.6 ± 0.5%) was also found in the *P. divaricatum* oil, the major constituents of which were safrole (49.3 ± 0.5%) and methyl eugenol (31.0 ± 0.2%).

The *L. sidoides* had a predominance of monoterpenes (92.9 ± 1.0%), with carvacrol (59.5 ± 1.0%) as the major component. An abundance of monoterpenes was found in the *Citrus* oils, with limonene identified as the major component in the *C. limon* (68.2 ± 0.5%), *C. aurantiifolia* (57.7 ± 0.9%) and *C. sinensis* (90.1 ± 1.1%) oils.

Residual contact and ovicidal assay

The relative toxicities of the oils to adult females and the eggs of the two-spotted spider mite and its natural enemy, *N. californicus*, are displayed in Table 2. Toxicity varied with the type of oil, development stage of the pest and species (pest and natural enemy).

Adult females were more susceptible to the oils than the eggs. For a better classification of toxicity to the adult females of *T. uticae* based on the LC_{50} estimates for the oils, the relative toxicities were divided into three groups ranging from most toxic (Group 1) to least toxic (Group 3). Group 1 comprised only the *L. sidoides* oil. Group 2 was formed by the *P. divaricatum*, *C. limon*, *C. rhamnifolioides* and *C. grewioides* oils. Group 3 was formed by the *C. aurantiifolia* and *C. sinensis* oils. Regarding relative toxicity to the eggs, two groups were formed: Group 1 comprised only the *L. sidoides* oil and Group 2 was composed of the *C. grewioides*, *C. rhamnifolioides*, *C. sinensis*, *C. limon*, *C. aurantiifolia* and *P. divaricatum* oils.

Comparing the relative toxicities of the substances tested to the two forms of development of the pest, all oils were more efficient than the positive control (Azamax). The *L. sidoides* oil stood out in this comparison, which was 9.6-fold and 3.4-fold more toxic to the females and eggs of *T. urticae*, respectively.

Based on the LC_{50} estimates and respective confidence intervals, the essential oils were divided into three groups from the most toxic to the least toxic to *N. californicus*. Group 1 was composed of the *L. sidoides* and *C. aurantiifolia* oils. Group 2 was composed of the *C. rhamnifolioides* oil and Group 3 was composed of the *C. grewioides, C. sinensis, C. limon* and *P. divaricatum* oils.

Comparing the toxicity of the oils between species, the oils were more toxic to the pest than the predator, as demonstrated by the LC_{50} estimates, which were higher for *N. californicus*. Based on the relative selectivity (RS) calculated for the oils investigated (Table 2), most oils were more selective than the plant-based acaricide (Azamax). The only exception was the *C. aurantiifolia* oil, which had the same RS as Azamax.

Discussion

Yields and chemical profile of essential oils

The yields of the essential oils from the species analyzed are compatible with those described in previous studies on *C. rhamnifolioides* (Camara *et al.* 2017), *C. grewioides* (Silva *et al.* 2008),

Table 1 Chemical composition (% ± DP) of essential oils from leaves of *Lippia, Piper* and *Croton*, and peels of *Citrus* species.

Compound	RI[a]	RI[b]	*Croton rhamnifolioides*	*Croton grevioides*	*Lippia sidoides*	*Piper divaricatum*	*Citrus limon*	*Citrus aurantiifolia*	*Citrus sinensis*	Method of identification
α-Thujene	921	924	1.2±0.0	-	-	-	0.4±0.0	0.8±0.0	0.2±0.0	RI, MS
α-Pinene	928	932	1.3±0.0	-	0.4±0.0	-	2.9±0.0	3.0±0.2	1.3±0.1	RI, MS, CI
α-Fenchene	948	945	0.2±0.0	-	-	-	-	-	-	RI, MS
Camphene	949	946	0.6±0.0	-	-	-	-	-	-	RI, MS
Sabinene	966	969	-	-	-	-	0.6±0.1	-	1.0±0.0	RI, MS
β-Pinene	971	974	0.8±0.0	-	1.9±0.1	-	-	-	1.9±0.1	RI, MS, CI
Myrcene	988	988	-	-	-	-	4.5±0.1	7.8±0.7	-	RI, MS
α-Phellandrene	1004	1002	1.5±0.1	-	-	-	-	-	-	RI, MS, CI
α-Terpinene	1014	1014	2.5±0.0	-	-	-	2.5±0.1	1.6±0.1	-	RI, MS
p-Cymene	1020	1020	0.9±0.0	-	9.5±0.7	-	-	1.4±0.1	-	RI, MS, CI
o- Cymene	1022	1022	3.0±0.1	-	-	-	-	-	-	RI, MS
Limonene	1024	1024	-	-	-	0.8±0.0	68.2±0.5	57.7±0.9	90.1±1.1	RI, MS, CI
β-Phellandrene	1025	1025	0.1±0.0	-	-	-	-	-	0.1±0.0	RI, MS
Sylvestrene	1025	1025	-	-	0.4±0.0	-	-	-	-	RI, MS
1,8-Cineole	1030	1026	10.5±0.6	1.1±0.1	-	-	-	-	-	RI, MS, CI
(Z)-*β*-Ocimene	1031	1032	-	-	-	-	7.5±0.4	15.5±0.3	0.3±0.0	RI, MS
(*E*)-*β*-Ocimene	1044	1044	-	-	0.4±0.0	-	-	-	-	RI, MS
γ-Terpinene	1055	1054	1.5±0.1	-	6.1±0.2	-	1.0±0.1	0.9±0.0	0.4±0.0	RI, MS
Dihydro myrcenol	1072	1069	3.0±0.2	-	-	-	-	-	-	RI, MS
m-Cymenene	1085	1082	0.2±0.0	-	-	-	-	-	-	RI, MS
Terpinolene	1088	1086	-	-	0.4±0.0	-	-	-	0.4±0.0	RI, MS, CI
p-Cymenene	1092	1089	0.2±0.0	-	-	-	-	-	-	RI, MS
Linalool	1092	1095	0.4±0.0	0.2±0.0	-	-	1.2±0.1	-	0.2±0.0	RI, MS, CI
cis-*β*-Terpineol	1139	1140	-	-	-	-	-	-	0.4±0.0	RI, MS
Camphor	1140	1141	-	0.8±0.1	-	-	-	-	-	RI, MS, CI
Citronellal	1145	1148	-	-	-	-	1.6±0.1	-	0.1±0.0	RI, MS, CI
Myrcenone	1141	1145	0.3±0.0	-	-	-	-	-	-	RI, MS
δ-Terpineol	1162	1162	0.1±0.0	-	-	-	0.8±0.0	-	-	RI, MS
Borneol	1170	1165	0.1±0.0	-	-	-	-	-	-	RI, MS
Terpinen-4-ol	1174	1174	1.2±0.0	-	1.6±0.1	-	-	-	-	RI, MS, CI
(*E*)-Isocitral	1175	1177	-	-	-	-	-	-	0.2±0.0	RI, MS
α-Terpineol	1192	1186	0.3±0.0	0.5±0.0	-	-	-	-	-	RI, MS, CI
Methyl chavicol	1196	1195	-	1.9±0.1	-	-	-	-	-	RI, MS
γ-Terpineol	1202	1199	0.7±0.0	-	-	-	-	-	-	RI, MS
p- Anisaldehyde	1250	1247	-	0.5±0.0	-	-	-	-	-	RI, MS
(*Z*)-Anethole	1251	1249	-	4.6±0.1	-	-	-	-	-	RI, MS, CI
(*E*)-Anethole	1280	1282	-	55.5±0.4	-	-	-	-	-	RI, MS, CI
Safrole	1285	1285	-	-	-	49.3±0.5	-	-	-	RI, MS, CI
Thymol	1289	1289	-	-	11.7±0.4	-	-	-	-	RI, MS, CI
Bornyl acetate	1290	1287	0.6±0.0	-	-	-	-	-	-	RI, MS
Carvacrol	1299	1298	-	-	59.5±1.0	-	-	-	-	RI, MS, CI
δ-Elemene	1331	1335	0.5±0.0	-	-	-	-	-	-	RI, MS
α-Cubebene	1342	1345	0.1±0.0	-	-	-	-	-	-	RI, MS
Eugenol	1356	1356	-	-	-	3.1±0.1	-	-	-	RI, MS, CI
α-Copaene	1369	1374	0.2±0.0	2.1±0.1	-	-	-	-	-	RI, MS
β-Cubebene	1387	1387	0.8±0.0	-	-	0.6±0.0	-	-	-	RI, MS
β-Elemene	1389	1389	0.3±0.0	1.0±0.0	-	-	-	-	-	RI, MS
β-Longifolene	1398	1400	0.7±0.0	-	-	-	-	-	-	RI, MS
Methyl eugenol	1401	1403	-	10.6±0.3	-	31.0±0.2	-	-	-	RI, MS, CI
Cycloseychellene	1406	1406	-	-	-	0.2±0.0	-	-	-	RI, MS
β-Caryophyllene	1415	1417	33.3±0.6	4.5±0.1	2.0±0.1	0.4±0.0	2.0±0.0	1.4±0.0	0.1±0.0	RI, MS, CI

Table 1 Contined.

Compound	RI[a]	RI[b]	*Croton rhamnifolioides*	*Croton grevioides*	*Lippia sidoides*	*Piper divaricatum*	*Citrus limon*	*Citrus aurantiifolia*	*Citrus sinensis*	Method of identification
β-Copaene	1433	1430	0.1±0.0	-	-	-	-	-	-	RI, MS
trans-*α*-Bergamotene	1435	1432	-	0.3±0.0	0.4±0.0	-	1.1±0.1	2.3±0.0	0.1±0.0	RI, MS
β-Humulene	1439	1436	0.5±0.0	-	-	-	-	-	-	RI, MS
6,9-Guaiadiene	1443	1442	0.5±0.0	-	-	-	-	-	-	RI, MS
cis-Prenyl-limonene	1446	1443	-	-	0.3±0.0	-	-	-	-	RI, MS
(*Z*)-Methyl isoeugenol	1451	1451	-	2.9±0.1	-	-	-	-	-	RI, MS
α-Humulene	1453	1452	0.8±0.0	-	-	-	-	-	-	RI, MS, CI
9-*epi*-(*E*)-Caryophyllene	1467	1464	5.1±0.2	-	-	-	-	-	-	RI, MS
γ-Gurjunene	1474	1475	-	-	-	-	2.9±0.1	1.7±0.0	0.9±0.0	RI, MS
Amorpha-4,7(11)-diene	1474	1479	0.2±0.0	-	-	-	-	-	-	RI, MS
γ-Muurolene	1480	1478	0.1±0.0	-	-	-	-	-	-	RI, MS
γ-Himachalene	1481	1481	-	-	-	1.2±0.0	-	-	-	RI, MS
Germacrene D	1484	1484	-	0.4±0.0	-	-	-	-	-	RI, MS, CI
β-Selinene	1489	1489	-	-	1.1±0.0	-	-	-	-	RI, MS
(*E*)-Methyl isoeugenol	1400	1491	-	6.7±0.1	-	-	-	-	-	RI, MS
δ-Selinene	1495	1492	0.5±0.0	-	2.0±0.1	-	-	-	-	RI, MS
trans-Muurola-4(14),5-diene	1497	1493	0.3±0.0	-	-	-	-	-	-	RI, MS
Bicyclogermacrene	1500	1502	0.9±0.0	-	-	-	-	-	-	RI, MS, CI
(Z)-*α*-bisabolene	1507	1506	-	-	-	0.3±0.0	-	-	-	RI, MS
Germacrene A	1512	1508	0.2±0.0	-	-	-	-	-	-	RI, MS
δ-Amorphene	1514	1511	0.1±0.0	-	-	-	-	-	-	RI, MS
γ-Cadinene	1517	1513	0.1±0.0	-	-	-	-	-	-	RI, MS
7-*epi*-*α*-Selinene	1520	1520	-	-	-	0.3±0.0	-	-	-	RI, MS
δ-Cadinene	1521	1522	-	1.3±0.0	-	7.8±0.1	-	-	-	RI, MS
10-*epi*-Cubebol	1535	1533	-	-	-	1.2±0.1	-	-	-	RI, MS
α-Cadinene	1540	1537	1.5±0.1	-	-	-	-	-	-	RI, MS
α-Copaen-11-ol	1543	1539	0.1±0.0	-	-	-	-	-	-	RI, MS
α-Calacorene	1544	1544	0.2±0.0	-	-	-	-	-	-	RI, MS
Elemol	1549	1548	-	-	-	-	-	2.6±0.0	-	RI, MS
Germacrene B	1556	1559	1.0±0.1	-	-	-	-	-	-	RI, MS
β-Calacorene	1562	1564	0.2±0.0	-	-	-	-	-	-	RI, MS
(*Z*)-Isoeugenol acetate	1566	1566	-	-	-	1.2±0.0	-	-	-	RI, MS
Maaliol	1566	1566	-	-	-	1.4±0.1	-	-	-	RI, MS
α-Cedrene epoxide	1569	1574	0.1±0.0	-	-	-	-	-	-	RI, MS
Spathulenol	1572	1577	5.9±0.1	1.6±0.0	-	-	-	-	-	RI, MS, CI
Caryophyllene oxide	1580	1582	5.8±0.6	2.8±0.1	-	-	-	-	-	RI, MS, CI
cis-*β*-Elemenone	1594	1589	0.4±0.0	-	-	-	-	-	-	RI, MS
Viridiflorol	1596	1592	1.6±0.1	-	-	-	-	-	-	RI, MS
Ledol	1606	1602	0.5±0.0	-	-	-	-	-	-	RI, MS
Humulene epoxide II	1613	1608	1.3±0.1	-	-	-	-	-	-	RI, MS
epi-*α*-Cadinol	1639	1638	0.1±0.0	-	-	-	-	-	-	RI, MS
Hinesol	1643	1640	1.3±0.1	-	-	-	-	-	-	RI, MS
α-Muurolol	1645	1644	0.5±0.0	-	-	-	-	-	-	RI, MS
Cubenol	1645	1645	0.1±0.0	0.5±0.0	-	-	-	-	-	RI, MS
α-Eudesmol	1656	1652	0.2±0.0	-	-	-	-	-	-	RI, MS
14-hydroxy-(*Z*)-caryophyllene	1668	1666	0.9±0.0	-	-	-	-	-	-	RI, MS
β-Bisabolenenal	1765	1768	-	-	-	-	-	1.9±0.0	-	RI, MS
Total			97.4±0.8	98.8±0.5	98.6±1.1	98.5±0.5	97.1±0.6	98.3±0.9	97.5±1.1	
Monoterpenes			31.2±0.7	9.6±0.1	92.9±1.0	0.8±0.0	91.2±0.5	88.6±0.9	96.4±1.1	
Sesquiterpenes			66.3±0.6	14.5±0.0	5.8±0.1	13.3±0.1	5.9±0.1	9.7±0.0	1.1±0.0	
Phenylpropanoids				75.7±0.5	-	84.6±0.5	-	-	-	

C. aurantiifolia, *C. limon* (Ribeiro *et al.* 2019) and *C. sinensis* (Júnior *et al.* 2010) collected in different localities in the state of Pernambuco, Brazil.

The chemical profiles determined for the oils from the species of *Croton*, *Lippia*, *Piper* and *Citrus* are in agreement with data previously reported for these species and/or their congeners. For example, β-caryophyllene and (*E*)-anethole, which were respectively the major compounds identified in the *C. rhamnifolioides* and C. *grewioides* oils, were also the main constituents of the oils from these same species collected in Pernambuco (Camara *et al.*, 2017; Silva *et al.*, 2008). Carvacrol (59.5 ± 1.0%), which was the major constituent of the *L. sidoides* oil in the present study, was also found to be the major component in the leaf oil of this species collected in different localities in Brazil in the states of Minas Gerais, Ceará and Pernambuco (Cavalcanti *et al.* 2010; Guimarães *et al.* 2015). The phenylpropanoids safrole and methyl eugenol found to be the major constituents of the *P. divaricatum* oil were also reported for this species in different localities of Brazil and the world (Barbosa *et al.* 2012; Souto *et al.* 2012; de Oliveira *et al.* 2019; Vilhena *et al.* 2019). Limonene was the major constituent of the *Citrus* oils, with proportions ranging from 57.7 ± 0.9% to 90.1 ± 1.1%, which is compatible with data reported in previous studies of these species collected in the state of Alagoas, Brazil (Júnior *et al.* 2010; Ribeiro *et al.* 2020).

Residual contact and ovicidal assay

The use of EOs combined with biological control is an ecologically and agronomically compatible practice to control pest populations, leaving the use of synthetic acaricides as the last option (Barzman *et al.* 2015; Pretty *et al.* 2018). For pests with a history of resistance to synthetic products, such as *T. urticae*, the use of EOs is an excellent alternative, as the complex mixture of monoterpenes, sesquiterpenes and phenylpropanoids, which affect different sites in the pest, favors the slower development of resistance (Koul and Walia 2009).

The EOs tested in the present study exhibited greater toxicity to *T. urticae* than the positive control (Azamax [active ingredient: azadirachtin]). Although there is no evidence of the resistance of *T. urticae* to azadirachtin, the high LC_{50} of this positive control demonstrates its lower effectiveness regarding the mortality of females and lower ovicidal effect compared to all oils tested. Azadirachtin is the only chemical insecticide/acaricide registered for organic

Table 2 Toxicity by residual contact (LC_{50} = µL mL^{-1}) of essential oils from leaves of *Lippia sidoides*, *Croton grewioides*, *Croton rhamnifolioides*, *Citrus sinensis*, *C. limon*, *C. aurantiifolia* and *Piper divaricatum* against *Tetranychus urticae* (Adults and Eggs) and *Neoseiulus californicus*.

Treatments	*Tetranychus urticae*					*Neoseiulus californicus*				
	Stage	N[a]	χ^2 (df) [b]	Slope ± SE[c]	LC_{50}(95% CI)[d]	N[a]	χ^2 (df) [b]	Slope ± SE[c]	LC_{50}(95% CI)[d]	RS [e]
Lippia sidoides	Adults	1350	12.80 (6)	0.88±0.05	0.05 (0.03 – 0.07)	1500	12.11 (8)	0.94±0.05	0.78 (0.65 – 0.93)	15.60 (10.93 – 21.62)
	Eggs	1200	7.16 (6)	0.96±0.06	0.09 (0.08 – 0.11)	-	-	-	-	-
Croton grewioides	Adults	1200	3.25 (4)	1.19±0.06	0.14 (0.12 – 0.17)	1200	10.75 (6)	1.29±0.10	3.26 (2.48 – 4.85)	23.28 (17.32 – 28.82)
	Eggs	1350	2.43(7)	1.22±0.06	0.18 (0.16 – 0.21)	-	-	-	-	-
Croton rhamnifolioides	Adults	1350	9.27 (5)	1.01±0.05	0.12 (0.08 – 0.16)	1050	6.62 (5)	1.17±0.07	1.14 (0.95 – 1.37)	9.50 (7.31 – 12.13)
	Eggs	1350	8.72 (7)	1.17±0.06	0.15 (0.13 – 0.18)	-	-	-	-	-
Citrus sinensis	Adults	1200	4.84 (6)	1.14±0.06	0.28 (0.23 – 0.33)	1050	2.29 (5)	0.63±0.07	3.80 (2.52 – 6.79)	13.57 (8.17 – 22.90)
	Eggs	1200	10.52 (6)	0.96±0.06	0.15 (0.12 – 0.18)	-	-	-	-	-
Citrus limon	Adults	1200	2.81(6)	1.05±0.06	0.13 (0.10 – 0.15)	1350	11.76 (7)	1.37±0.09	2.26 (1.70 – 3.30)	17.38 (13.39 – 22.84)
	Eggs	1200	3.31 (6)	1.13±0.06	0.19 (0.16 – 0.23)	-	-	-	-	-
Citrus aurantiifolia	Adults	1200	5.70 (6)	0.95±0.06	0.21 (0.18 – 0.26)	1350	9.79 (7)	0.90±0.06	0.76 (0.64 – 0.94)	3.61 (2.69 – 4.64)
	Eggs	1050	2.79 (5)	1.07±0.06	0.15 (0.13 – 0.18)	-	-	-	-	-
Piper divaricatum	Adults	1350	10.52 (7)	1.12±0.06	0.11 (0.09 – 0.15)	1200	3.94 (6)	0.91±0.07	1.79 (1.40 – 2.44)	16.27 (11.19 – 21.22)
	Eggs	1350	2.48 (7)	1.20±0.06	0.13 (0.12 – 0.15)	-	-	-	-	-
Azamax	Adults	1650	2.08 (8)	0.99±0.04	0.48 (0.38 – 0.63)	1350	3.49 (7)	0.76±0.05	2.03 (1.59 – 2.66)	4.22 (3.05 – 5.64)
	Eggs	1650	14.65 (9)	0.93±0.04	0.31 (0.26 – 0.37)	-	-	-	-	-

farming in Brazil (Agrofit 2020). However, the product is expensive for small farmers, demonstrating the need for economically viable alternatives for producers.

The EOs tested herein were extracted from cultivated plants as well as some native to the Atlantic Forest and *Caatinga* biomes of Brazil and are easily found in agricultural niches distributed throughout the northeast region of the country. Among these oils, *L. sidoides* had the greatest yield (4.80 ± 0.23%) as well as the greatest ovicidal action and toxicity by residual contact to *T. urticae* females.

The genus *Lippia* is recognized for its acaricidal properties by both fumigation and residual contact (Santos *et al.* 2019; Tabari *et al.* 2020). The residual toxicity for *L. sidoides* found in the present study is compatible with that described by Cavalcanti *et al.* (2010) for *L. sidoides* collected in the state of Sergipe, Brazil. The authors also demonstrated this oil has a fumigant effect. Born *et al.* (2018) recently reported that the oil from the leaves of *Lippia gracilis* Schauer collected in the state of Pernambuco, which had the same major component at that identified in the *L. sidoides* oil (carvacrol), exhibited fumigant and residual contact action (LC_{50} = 29.70 µL mL^{-1}) against *T. urticae*. However, the residual toxicity found for the *L. sidoides* oil analyzed in the present investigation was 594 times greater than that of the *L. gracilis* oil reported by Born *et al.* (2018). These results suggest that the major component is not always the active ingredient of the oil and that other factors should be taken into consideration, such as qualitative and quantitative aspects and multiple (synergistic, additive and/or antagonistic) interactions that can be established among the chemical constituents of an essential oil (Moraes *et al.* 2012; Neves and Camara 2016).

A previous investigation of the biological properties of EOs from species of the genus *Piper* revealed action against several types of arthropods, including mites of importance to veterinary medicine – *Rhipicephalus (Boophilus) microplis* (Vinturelle *et al.* 2017) – and agriculture – *Dolichocybe indica* Mahunka (Pumnuan and Insung 2016) and *T. urticae* (Ribeiro *et al.* 2016; Araújo *et al.* 2020). However, studies addressing the effect of the oil from *P. divaricatum* on arthropods are restricted to the investigation of the insecticidal potential against stored grain pests – *Tribolium castaneum* Herbst (Jaramillo-Colorado *et al.* 2015) – and general pests – *Solenopsis saevissima* (Smith) (Souto *et al.* 2012).

Based on the LC_{50} estimates, the *P. divaricatum* oil was 53 times more toxic by residual contact than the oil from the leaves of *Piper aduncum* L. (Araújo *et al.* 2020) to *T. urticae* adults. The differences in toxicity may be explained by qualitative and quantitative differences in the chemical composition of these *Piper* oils.

Ferraz *et al.* (2010) reported the acaricidal action of oils from the leaves of *Piper mikanianum* (Kunth) Steud. and *Piper xylostaeoides* on *Rhipicephalus microplus* larvae. Comparing these results to those obtained in the present investigation, the *P. divaricatum* oil was 21 and 56 times more toxic to *T. urticae* than the *Piper* oils tested on ticks. Besides differences in the chemical profiles of the oils tested, the greater activity found for the *P. divaricatum* oil may be attributed to morphological differences among mites/ticks.

Citrus is a widely studied genus due to its toxic (Dutra et al. 2016; Papanastasiou *et al.* 2017; Farias *et al.* 2020) and repellent (Camara *et al.* 2015; Ribeiro *et al.* 2019) activity against arthropods. The acaricidal activity of *Citrus* against *T. urticae* has previously been demonstrated by residual toxicity, fumigation and repellent action (Júnior *et al.* 2010; Ribeiro *et al.* 2019). Regarding residual toxicity, the present study reports much lower LC_{50} values for *C. limon* (0.13 µL mL^{-1}) and *C. aurantiifolia* (0.21 µL mL^{-1}) than those reported by Ribeiro *et al.* (2019), which were 25.18 µL mL^{-1} and 106.14 µL mL^{-1}, respectively. This divergence may be explained by variations in populations of *T. urticae*, methodological differences and the percentages of different chemical constituents found in the *Citrus* oils. For instance, the major component (limonene) was identified in higher proportions in the present study (*C. limon*: 68.2% and 40.7% in the present investigation and the study by Ribeiro *et al.* 2019, respectively; *C. aurantiifolia*: 57.7% and 37.7% in the present investigation and the study by Ribeiro *et al.* 2019 , respectively).

A previous study on the potential of EOs from species of the genus *Croton* revealed that these oils are promising due to their activities against stored grain pests (Silva *et al.* 2008; Santos *et al.* 2019; Ribeiro *et al.* 2020), pests of interest to human medicine (Carvalho *et al.* 2016) and synanthropic pests (Brito *et al.* 2020). Recently, EOs from four *Croton* species (*C. pulegiodorus*, *C. conduplicatus*, *C. grewioides* and *C. blanchetianus*) were found to be promising in the control of a tick of interest to veterinary medicine (*Rhipicephalus microplus*) (Castro *et al.* 2019; Rodrigues *et al.* 2020). Despite reports that *Croton* oils can cause toxicity to the red spider mite by contact, fumigation and repellence (Neves and Câmara 2011; Camara *et al.* 2017), to the best of our knowledge, no previous studies have evaluated the acaricidal action of the oil from *C. grewioides* against *T. urticae*.

Comparing the LC_{50} estimates for the *C. grewioides* and *C. rhamnifolioides* oils to those from species of *Croton* reported in the literature regarding toxicity to *T. urticae* by contact, the oils investigated herein were 20 times more toxic than the oil from *C. rhamnifolioides* collected in the municipality of Buique, Pernambuco (Camara *et al.* 2017).

Investigations of substances derived from plants for the control of *T. urticae* are generally directed at assessing the toxicity of EOs to larvae and/or adults. With the exception of the *C. aurantiifolia* oil, which exhibited ovicidal action by fumigation (Pavela *et al.* 2016), none of the oils analyzed in the present study has previously been investigated with regards to its ovicidal potential against *T. urticae*.

While no significant differences in the susceptibilities of the eggs and females were found among the *C grewioides*, *C. rhamnifolioides*, *C. aurantiifolia* and *P. divaricatum* oils, the *L. sidoides* and *C. limon* oils were more toxic to the adult females and the *C. sinensi*s oil was more toxic to the eggs. These results may be explained by several factors: a) the nature of the EOs (qualitative, quantitative and physicochemical aspects); b) the inherent susceptibility of the forms of development investigated (egg and adult); and c) the method used for the evaluation of the oils (direct contact for the eggs and residual contact for the females).

Although there are no records of ovicidal action by direct contact of the oils tested on *T. urticae*, Lima *et al.* (2013) reported the toxicity of a commercially acquired *L. sidoides* oil (thymol chemotype) to the eggs of *Aedes aegypti*. The ovicidal action found in the present investigation indicates that the *L. sidoides* oil tested (carvacrol chemotype) was 737 times more toxic than the commercial *L. sidoides* oil. This greater toxicity may be explained by qualitative differences between the oils as well as morphological differences between the eggs of the two target species.

Acaracides that are selective for natural enemies are highly advantageous to integrated pest management programs. Selectivity is defined as the capacity of a product to control the target pest while exerting the lowest possible impact on beneficial organisms, such as predators, parasitoids and pollinizers (Ripper *et al.* 1951). This selectivity is one of the requirements for natural acaricides to be considered economically viable (Vieira *et al.* 2007). While few studies have investigated the selectivity of EOs for predator mites, the literature offers promising results for the oils of *P. aduncum, Melaleuca leucadendra* L., *Schinus terebinthifolius* Raddi (Araújo *et al.* 2020) and *L. gracilis* (Born *et al.* 2018), which were more selective than the oils tested in the present investigation.

The lower selectivity of the oils in comparison to data reported in the literature may be explained by the method employed in the experiments to assess toxicity to the predator mite. In the present study, we offered leaf disks and eggs of *T. urticae* coated with the oils, whereas Araújo *et al.* (2020) and Born *et al.* (2018) only used leaf disks. Thus, there was both a residual effect and toxic effect by ingestion in the present study, causing greater toxicity to the predator. Nonetheless, based on the calculation of relative selectivity (RS), the oils investigated herein can be considered selective for *N. californicus* (Table 2).

The present results show that *L. sidoides* is the most promising among all oils tested for the management of *T. urticae*, as it exhibited the greatest toxicity to the pest and was also selective for *N. californicus*. Due to its abundance and availability, *L. sidoides* can be a viable option for the preparation of a plant-based insecticide for the management the red spider mite

in agroecological systems in the state of Pernambuco. However, further studies are needed, such as field bioassays, for the cost-benefit analysis of a formulation based on essential oils.

Acknowledgements

The authors are grateful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (PQ-2 302735/2019-4), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (N° 88882.436540/2019-01; 88887.474239/2020-00) and Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE N° IBPG-0584-1.06/20; IBPG-0715-1.06/18; APQ-0398-1.06/19; APQ-0476 -1.06/14) for scholarship and funding this study.

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