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*), β-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\textsubscript{50} values of 0.05 and 0.09 µL mL\textsuperscript{-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

**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.
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. urticae* 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. greuwioide* 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 greuwioide* 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-terpinol), 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. ensiformis*). 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 (8 cm 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) (adapted 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. ensiformis*). 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 (LC₅₀) and 90% (LC₉₀) 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. urticae based on the LC₅₀ 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 LC₅₀ 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 LC₅₀ 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** | **IR** | **IR** | **Croton humifusa** | **Croton guayacan** | **Lippia alba** | **Piper cubeba** | **Citrus limon** | **Citrus aurantium** | **Citrus sinensis** | **Method of identification** |
| α-Thujene | 921 | 924 | 1.2±0.0 | - | - | - | 0.4±0.0 | 0.8±0.0 | 2.0±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 | 6.4±0.0 | - | - | - | - | - | - | RI, MS |
| Sabinene | 966 | 969 | - | - | - | 0.4±0.0 | 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 |
| 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 | - | - | - | - | 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 | - | - | - | 8.4±0.0 | - | - | - | RI, MS |
| γ-Terpineol | 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-Cymene | 1085 | 1082 | 0.2±0.0 | - | - | - | - | - | - | RI, MS |
| Terpinolene | 1088 | 1086 | - | - | 0.4±0.0 | - | - | - | 0.4±0.0 | RI, MS, CI |
| p-Cymene | 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-Terpinol | 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 |
| Myrcene | 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-Cubeene | 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-Cubeene | 1387 | 1387 | 0.8±0.0 | - | 0.6±0.0 | - | - | - | - | RI, MS |
| δ-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 |
| Cyclohexene | 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 Continued.

| Compound                        | RIa | RIb | Croton rhamnifolios | Citrus amarantifolios | Citrus limon | 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         | RI, MS                   |
| **β-Humulene**                  | 1439| 1436| 0.5±0.0             | -                     | -            | -               | RI, MS                   |
| 6,9-Guaiaadiene                 | 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         | RI, MS                   |
| Amorpha-5(7)-dione              | 1474| 1479| 0.2±0.0             | -                     | -            | -               | RI, MS                   |
| γ-Murolene                      | 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         | 1490| 1491| -                   | 6.7±0.1               | -            | -               | RI, MS                   |
| δ-Selinene                      | 1495| 1492| 0.5±0.0             | -                     | 2.0±0.1      | -               | RI, MS                   |
| *trans*-Murola-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-Amorphone                     | 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                   |
| Maalol                          | 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-Elemeneone            | 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±0.0             | -                     | -            | -               | RI, MS                   |
| *epi*-α-Cadinol                 | 1639| 1638| 0.1±0.0             | -                     | -            | -               | RI, MS                   |
| Himesol                         | 1643| 1640| 1.3±0.1             | -                     | -            | -               | RI, MS                   |
| **α-Murolol**                   | 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-Bisabololenal**             | 1765| 1768| -                   | -                     | -            | 1.9±0.0         | RI, MS                   |
| Total                           | 79.4±0.8| 99.8±0.5| 98.6±1.1| 98.5±0.5| 97.1±0.6| 98.3±0.9| 97.5±1.1| RI, MS |
| 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| RI, MS |
| 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| RI, MS |
| Phenylpropanoids                | 75.7±0.5| - | 84.6±0.5 | - | - | - | - | RI, MS |
C. aurantifolia, 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. grewioioides 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 agriculture.

| Treatments | Stage | N* | $\chi^2$ (df) | Slope ± SE | LC$_{50}$ (95% CI) | N* | $\chi^2$ (df) | Slope ± SE | LC$_{50}$ (95% CI) | RS* |
|------------|------|----|-------------|------------|-------------------|----|-------------|------------|-------------------|-----|
| 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) |
| Croton grewioioides | Adults | 1350 | 6.62 (5) | - | - | - | - | - | - | - |
| Croton rhamnifolioides | Adults | 1200 | 2.26 (1.70 – 3.30) | 0.15 (0.13 – 0.18) | 1650 | 2.29 (5) | 0.63±0.07 | 3.80 (2.52 – 6.79) | 13.57 (8.17 – 22.90) |
| Citrus sinensis | Adults | 1200 | 2.31 (10) | 0.96±0.06 | 0.15 (0.12 – 0.18) | - | - | - | - |
| Citrus limon | Adults | 1200 | 1.17±0.06 | 0.15 (0.13 – 0.18) | 1050 | 6.62 (5) | 1.17±0.07 | 1.14 (0.95 – 1.37) | 9.50 (7.31 – 12.13) |
| Citrus aurantifolia | Adults | 1200 | 2.81±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) |
| Piper divaricatum | Adults | 1050 | 2.79 (5) | 1.10±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 – 22.21) |
| 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) |

Fidelis de Santana M. et al. (2021), *Acarologia* 61(3): 564-576; DOI 571
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 (LC50 = 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) microplus (Vinturelle et al. 2017) – and agriculture – Dolichocybe indica Mahunka (Pummuang 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 LC50 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 LC50 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. grevioides* 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. grevioides* against *T. urticae*.

Comparing the LC$_{50}$ estimates for the *C. grevioides* 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. grevioides, 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. sinensis* 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.

Acaricides 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.

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