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Acaracidal Property and Repellent Action Against *Tetranychus Urticae* Koch of Essential Oils from Three Species of *Piper* That Occur in Fragments of the Atlantic Forest in the State of Pernambuco, Brazil

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Propriedade Acaricida e Ação Repelente sobre *Tetranychus Urticae* Koch de Óleos Essenciais de Três Espécies de *Piper* que Ocorrem em Fragmentos da Mata Atlântica de Pernambuco-Brasil

Resumo: Os óleos essenciais obtidos por hidrodestilação das folhas de *Piper arboreum, Piper tuberculatum* e *Piper caldense* foram analisados por cromatografia gasosa (CG) e cromatografia gasosa acoplada à espectrometria de massas (CG-EM) e avaliados quanto ao potencial fumigante e ação repelente contra *Tetranychus urticae*, uma importante praga agrícola encontrada nos sistemas irrigados de Petrolina-Pernambuco. As três espécies de *Piper* revelaram sesquiterpenos como a classe de compostos mais abundantes nos óleos. Os principais constituintes identificados nos óleos de *P. arboreum, P. tuberculatum* e *P. caldense* foram biciclogermacreno (17,3 ± 0,1 %) β-cariofileno (10,6 ± 0,4 %) e γ-muroleno (9,6 ± 0,0 %), respectivamente. Entre os três óleos testados, o óleo de *P. tuberculatum* foi o mais tóxico (CL_{50} = 0,50 µL L^{-1} de ar) e repelente (CR_{50} = 6 x 10^{-4} µL mL^{-1}) ao *T. urticae.* Este estudo revelou os perfis químicos dos óleos das folhas de *P. arboreum, P. tuberculatum* e *P. caldense* que ocorrem em Pernambuco e reporta pela primeira vez a ação acaricida desses óleos contra uma importante praga agrícola.

Palavras-chaves: Piper; tetranychus urticae; sesquiterpeno; repelência; fumigação.

Abstract

Essential oils obtained through hydrodistillation of the leaves from *Piper arboreum, Piper tuberculatum* and *Piper caldense* were analyzed using gas chromatography (GC)and gas chromatography coupled to mass spectrometry (GC-MS) and evaluated with regard to their fumigant potential and repellent action against *Tetranychus urticae*, which is an important agricultural pest in irrigated systems in the city of Petrolina, Brazil. Sesquiterpenes were the most abundant class of compounds in the oils. The major constituents identified in the *P. arboreum, P. tuberculatum* and *P. caldense* oils were bicyclogermacrene (17.3 ± 0.1 %), β-caryophyllene (10.6 ± 0.4 %) and γ-muurolene (9.6 ± 0.0 %), respectively. The *P. tuberculatum* oil was the most toxic (LC_{50} = 0.50 µL L^{-1} of air) and repellent (RC_{50} = 6 x 10^{-4} µL mL^{-1}) to *T. urticae.* This paper describes the chemical profiles of the essential oils from the leaves of *P. arboreum, P. tuberculatum* and *P. caldense* that occur in the state of Pernambuco (northeastern Brazil) and reports, for the first time, the acaricidal action of these oils against an important agricultural pest.

Keywords: Piper; tetranychus urticae; sesquiterpenes; repellent action; fumigant.

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Acaracidal Property and Repellent Action Against Tetranychus Urticae Koch of Essential Oils from Three Species of Piper That Occur in Fragments of the Atlantic Forest in the State of Pernambuco, Brazil

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1. Introduction

Brazil has one of the greatest diversities of plants in the world. According to the Brazilian Institute of Geography and Statistics, there are six distinct biomes in the country, two of which (the semiarid *Caatinga* and the Atlantic Forest) are found in the state of Pernambuco.[1] Although greatly reduced and fragmented in comparison to its original state, the Atlantic Forest continues to be of vital importance and home to one of the largest indices of biodiversity in the world.[2] In this biome, species of the genus *Piper* have broad distribution along the entire coast of the state of Pernambuco. This genus is the most representative of the family Piperaceae, with around 2000 espécies,[3] approximately 300 of which are found in Brazil and 27 are recorded for the state of Pernambuco.[4] Species of *Piper* are used as folk remedies for the treatment of intestinal pain, venereal diseases, urinary tract infections and as an insect repellent.[5] Previous investigations of the biological properties of these species against different arthropods have been attributed to the production of secondary metabolites belonging to the terpene, phenylpropanoid, lignan, amide and alkaloid

chemical classes.[6,7] Among the properties revealed by *Piper* species against arthropods, we can mention the acaricidal properties of their essential oils against *Tetranychus urticae*.[7,8]

T. urticae is a cosmopolitan, polyphagous species considered to be one of the major agricultural pests around the world, causing harm to vegetable and fruit crops in both greenhouses and open environments.[9] The occurrence of *T. urticae* in Brazil has been recorded in different states from the northern to the southern regions of the country. In the state of Pernambuco, this pest was first recorded in 1985, following the establishment of irrigated crops in the city of Petrolina.[10] The incidence of this pest in these irrigated systems has led to innumerous crop losses estimated at approximately 30 to 100 % of local production.

Conventional acaricides constitute the main form of controlling *T. urticae*.[11] However, the indiscriminant use of these synthetic compounds is associated with residue that is harmful to both humans and the environment and it can lead to the occurrence of resistant populations. Integrated pest management has been offered as an alternative to conventional pesticides throughout the world. Among the strategies for the alternative control of pests, the use of essential oils is promising due to its proven effectiveness and broad bioactivity against arthropods, such as toxicity (residual contact and fumigation) and sub-lethal effects (deterrence to oviposition, anti-feeding effect and repellency).[7,12,13]

Among the plants recognized for the production of essential oils, species of *Piper* have broad occurrence in the state of Pernambuco, such as *Piper arboreum* Aubl., *Piper caldense* C. DC. and *Piper tuberculatum* Jacq., known locally as *alecrim-de-angola* [Angolan rosemary], *pimenta d'água* [water pepper] and *pimenta de macaco* [monkey pepper], respectively. The antifungal, anti-parasitic, antimicrobial and insecticidal properties of the essential oils from these species have been investigated.[14-17] However, there are no previous reports on the acaricidal activity of these essential oils.

As part of the systematic investigation of the chemical profiles and acaricidal activities of the aromatic flora of the state of Pernambuco by our research group,[13,18,19] this paper reports the chemical composition and acaricidal properties against *T. urticae* of the essential oils from the leaves of *P. arboreum*, *P. tuberculatum* and *P. caldense* that occur in a fragment of the Atlantic Forest in Pernambuco, Brazil.

2. Experimental

2.1. Collection of plant material

Fresh leaves of the *P. caldense* (8°01'10.1"S 34°56'50.4"W), *P. arboreum* (8°01'09.4"S 34°56'46.8"W) and *P. tuberculatum* (8°01'10.4"S 34°56'51.2"W) were collected in the Mata de Dois Irmãos, in Recife, Pernambuco, Brazil. Leaf collections for each specimen were performed at around 9:00 am in the dry season, in February 2009. The plants were identified by botanist Dra. Ângela Maria de Miranda Freitas (University Federal Rural of Pernambuco). Vouchers specimens were mounted and deposited in the Sérgio Tavares Herbarium of the UFRPE under numbers HST 18180 (*P. caldense*), HST 18179 (*P. arboreum*) and HST 18178 (*P. tuberculatum*).

2.2. Isolation of the essential oil

The essential oils from fresh leaves of the *P. caldense*, *P. arboreum* and *P. tuberculatum* (100 g) were obtained by hydrodistillation using a modified Clevenger apparatus for 2 h. The oil layers were separated by density differences and dried over anhydrous sodium sulfate, stored in hermetically sealed glass containers, and kept under refrigeration at - 5 °C until analysis and acaricidal assay. Total oil yields were expressed as percentages (g/100 g of fresh plant material). All experiments were carried out in triplicate.

2.3. Chemicals

All monoterpenes (α-pinene, β-pinene, limonene, 1,8-cineole, linalool, terpinen-4-ol, and α-terpineol), sesquiterpenes (β-caryophyllene, α-humulene, (*E*)-nerolidol and caryophyllene oxide) and phenylpropanoids (dillapiole) were purchased from Sigma-Aldrich, Brazil and used for co-injection to confirm the chemical identification.

The eugenol used as control positive for fumigant and repellency tests was purchased from Sigma-Aldrich, Brazil.

2.4. Gas chromatography – Gas chromatography-mass spectrometry analysis

Quantitative GC analysis were carried out using a PerkinElmer Clarus 500 GC, apparatus equipped with a flame ionization detector (FID) and a non-polar SH-Rtx-5MS [Crossbond (5 %) diphenyl - dimethyl polysiloxane (95 %)] capillary column (30 m x 0.25 mm x 0.25 µm). The oven temperature was programmed from 60 to 240 °C at a rate 3 °C min^{-1}. Injector and detector temperatures were at 260 °C. Hydrogen was used as the carrier gas at a flow rate of 1 mL min^{-1} and 30 p.s.i. inlet pressure 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 peak areas in the order of non-polar SH-Rtx-5MS column elution and expressed as a relative percentage of the total area of the chromatograms. Area percentages were obtained electronically from the GC-FID response without the use of an internal standard or correction factors. All analysis were carried out in triplicate.

The qualitative GC-MS analysis of the essential oils was carried out using a Varian 431 GC 220-MS system with a mass selective detector, mass spectrometer with 70 eV of ionization energy with a scan interval of 0.5 s and fragments from 40 to 550 D fitted with the same column and temperature program as that for the GC 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 the components was based on GC retention indices with reference to a homologous series of C_8-C_{40} *n*-alkanes calculated using the Van den Dool and Kratz [20] equation and by computer matching against the mass spectral library of the GC-MS data system (NIST version 14 and WILEY version 11) and co-injection with authentic standards as well as other published mass spectra.[21]

2.5. Mites

Specimens of *T. urticae* used for the bioassays were reared on jack beans (*Canavalia ensiformes* L.) without any exposure to acaricidal agents at the Agronomy Department of the Rural Federal University of Pernambuco, Brazil. All bioassays were performed at a temperature of 25 ± 1 °C, with relative humidity of 65 ± 5 % and a 12h photoperiod.

2.6. Fumigation bioassays

The fumigant method was the same as that used by Araújo *et al* with modifications.[7] Glass recipients with a capacity of 2.5 L were used as test chambers. Female spider mites on *C. ensiformes* leaf disks 2.5 cm in diameter were exposed to the *Piper* oils. A fine brush was used to transfer the mites onto the leaf disks. In order to maintain the turgor of the disks and avoid the escape of mites, the leaf disks were placed onto filter paper disks saturated with water in Petri dishes (9 cm). The experiments were performed in triplicate. One replicate consisted of 30 specimens of *T. urticae* placed on 3 leaf disks (10 mites per disk) in a Petri dish. The oils were applied with an automatic pipette on a piece of filter paper (5 x 3 cm) attached to the underside of the recipient lid. The concentration of the *Piper* oils used in the bioassays ranged from 0.1 to 2.6 µL L^{-1} air and that of the natural fumigant eugenol ranged from 6.4 x 10^{-5} to 1.2 µL L^{-1}. Negative control, glass recipients contained no essential oil or other products. Mortality was determined after 24 h. Following exposure, the Petri dishes with spider mites were then removed from the recipients and the mites were touched lightly with a brush in order to determine mortality. Those with no sign of movement were considered dead. The mortality data for *Piper* oils and eugenol were analyzed with the Probit model using the POLO-PC software for the determination of LC_{50} values, with 95 % confidence levels determined all experiments.[22] Toxicity ratios were determined based on the method described by Robertson and Preisler.[23]

2.7. Repellency assay

The repellent method was the same as those employed by Araújo *et al*.[7] with modifications. Test consisted of discs of *C. ensiformis* leaves (9 cm diameter) cut in half (9.81 cm^2). Test solutions were prepared by diluting essential oil in ethanol. The concentrations of *Piper* essential oils were prepared to range from 9.8 x 10^{-7} to 15.00 µl mL^{-1} and 2.9 x 10^{-5} to 10.00 µl mL^{-1} for the eugenol (positive control). Each concentration was uniformly applied to a half-leaf disc using a micropipette. The other half leaf was treated with ethanol alone and used as control. Chemically treated and control half discs were air dried for

5 min to evaporate the solvent completely. Each disc was placed into a 10 cm Petri dish and 10 adult mites were released at the centre of the disc, after which the Petri dishes were covered. Treatments were repeated 15x. The numbers of mites present on the control and treated areas of the discs were recorded after 2 h of exposure. To estimate the concentration that repelled 50 % of the exposed mites (RC_{50}) of each assayed sample, the numbers of mites present on the control and treated areas were submitted to Probit analysis using the POLO-PC software.[22]

3. Results and Discussion

3.1. Chemical Composition

Hydrodistillation of the leaves from *P. arboreum*, *P. caldense* and *P. tuberculatum* provided oils of a yellowish color. Table 1 displays the yields and chemical composition of the essential oils. The largest yield was obtained from *P. arboreum*, which was approximately threefold greater than the yield obtained from the leaves of *P. caldense* and *P. tuberculatum*. Similar yields have been reported for *P. arboreum* collected in the city of Brasília (0.3 %) and the state of Minas Gerais, Brazil (0.2-0.4 %)[24, 25] as well as for *P. tuberculatum* collected in the state of Ceará, Brazil (0.03 %) and Venezuela (0.06 %).[26, 27]

The GC-MS analysis of the *P. arboreum*, *P. caldense* and *P. tuberculatum* oils enabled the identification of 65 compounds, representing 98.3 ± 0.4 %, 96.7 ± 0.3 % and 92.6 ± 0.4 % of the oils, respectively. However, only seven compounds (α-copaene, β-bourbonene, β-elemene, β-copaene, bicyclogermacrene, δ-cadinene and α-cadinol) were found in all three species. These data suggest a significant qualitative variation in the chemical profile of the oils from the species of *Piper* investigated.

The *P. caldense* oil was characterized by monoterpenes (9.7 ± 0.1 %), sesquiterpenes (81.7 ± 0.2 %) and phenylpropanoids (0.5 ± 0.0 %), whereas the *P. arboreum* and *P. tuberculatum* oils were composed only of monoterpenes (0.3 ± 0.0/11.7 ± 0.2 %) and sesquiterpenes (98.0 ± 0.4/80.9 ± 0.3 %).

The predominance of sesquiterpenes in these three species of *Piper* that occur in the state of Pernambuco is in agreement with data reported for their essential oils collected in others states of Brazil.[17, 24, 26, 28] In contrast, phenylpropanoids were predominant in the oil from *P. tuberculatum* collected in Mérida, Venezuela.[27]

The major constituents of the oil from *P. arboreum* were bicyclogermacrene (17.3 ± 0.1 %), germacrene D (11.8 ± 0.1 %) and β-duprezianene (10.3 ± 0.1 %). Similar results are reported for samples collected in the states of Ceará[26] and Rondônia[14, 29] as well as the city of Brasília,[24] in which bicyclogermacrene and germacrene D were the major constituents. In contrast, others sesquiterpenes were identified as the major constituents in samples of *P. arboreum* that occur in Brazil, such as caryophyllene oxide (15.2 %) in the state of Minas Gerais,[25] γ- and α-eudesmol (14.61 % and 12.21 %) in the state of Rio de Janeiro[30] and δ-cadinene (25.8 %) identified in a sample collected in Panama.[31]

The major constituents of the oil from *P. tuberculatum* were β-caryophyllene (10.6 ± 0.4 %) and γ-muurolene (9.0 ± 0.0 %). Previous investigations of the chemical composition of the essential oil from this plant from other regions of Brazil and Venezuela report qualitative and quantitative differences. For instance, although β-caryophyllene was the major constituent in the oils from samples collected in the states of Ceará (37.8 %),[26] São Paulo (40.2 %)[17] and Rondônia (26.3 %),[28] γ-muurolene was not found in any of these samples. Other chemotypes found in *P. tuberculatum* oil were reported by Santos *et al*.[32] [germacrene D (36.3 %)] and Mora *et al*.[27] [dillapiole (72.4 %)] in samples from the state of Rio de Janeiro, Brazil, and Mérida, Venezuela, respectively.

The major constituents in the leaf oil from *P. caldense* were γ-muurolene (9.6 ± 0.0 %), caryophyllene oxide (8.9 ± 0.1 %) and α-cadinol (8.4 ± 0.1 %). Analyzing the leaf oil from *P. caldense* also collected in the state of Pernambuco, Rocha *et al*.[16] found α-cadinol (19.0 %) to be the major constituent, whereas the authors found that γ-muurolene corresponded to only 2.0 % of the oil and caryophyllene oxide was not found.

Despite the qualitative and quantitative differences found in the oils from the species of *Piper* collected in the state of Pernambuco, the predominance of sesquiterpenes, followed by monoterpenes and phenylpropanoids (found only in the oil from *P. caldense*) is in agreement

Table 1. Chemical composition and yield of essential oils from leaves of *Piper arboreum, Piper caldense* and *Piper tuberculatum*

Compounds	RI[a]	RI[b]	Relative % ± SD		
			P. arboreum	*P. caldense*	*P. tuberculatum*
Yield (%)± SD			0.25 ± 0.03	0.06 ± 0.01	0.07 ± 0.01
α-Pinene*	928	932	-	4.9 ± 0.1	2.8 ± 0.1
β-Pinene*	979	974	0.2 ± 0.0	-	-
Myrcene	986	988	0.1 ± 0.0	-	-
o-Cymene	1024	1022	-	-	0.2 ± 0.0
Limonene*	1029	1024	-	-	0.4 ± 0.0
1,8-Cineole*	1031	1026	-	-	0.5 ± 0.0
(Z)- β-Ocimene	1038	1032	0.1 ± 0.0	-	-
(E)-β-Ocimene	1050	1044	-	-	0.2 ± 0.0
Sabinene hydrate	1070	1065	-	2.4 ± 0.0	-
Linalool*	1100	1095	-	-	3.9 ± 0.0
trans-Sabinene hydrate	1097	1098	-	0.9 ± 0.0	-
Camphor	1141	1141	-	-	0.3 ± 0.0
Terpinen-4-ol*	1180	1174	-	-	1.6 ± 0.0
α-Terpineol*	1192	1186	-	0.9 ± 0.0	1.7 ± 0.0
δ-Elemene	1341	1335	0.2 ± 0.0	-	-
Cyclolosativene	1365	1369	0.5 ± 0.0	-	-
α-Copaene	1377	1374	3.5 ± 0.0	4.9 ± 0.0	8.4 ± 0.1
β-Bourbonene	1393	1387	0.1 ± 0.0	0.6 ± 0.0	1.4 ± 0.0
iso-Longifolene	1392	1389	-	-	1.3 ± 0.0
β-Elemene	1395	1389	2.7 ± 0.1	1.9 ± 0.0	2.3 ± 0.0
Cyperene	1403	1398	1.1 ± 0.0	-	-
α-Funebrene	1407	1402	-	4.3 ± 0.0	-
α-Gurjunene	1411	1409	0.5 ± 0.0	-	-
β-Caryophyllene*	1415	1417	-	-	10.6 ± 0.4
β-Duprezianene	1426	1421	10.3 ± 0.2	-	-
β-Copaene	1435	1430	0.4 ± 0.0	0.3 ± 0.0	1.4 ± 0.0
α-Guaiene	1440	1437	0.5 ± 0.0	2.4 ± 0.0	-
Aromadendrene	1442	1439	-	-	0.3 ± 0.0
α-Humulene*	1447	1452	-	-	2.0 ± 0.0
Geranyl acetone	1448	1453	-	0.4 ± 0.0	-
Allo-aromadendrene	1460	1458	-	1.6 ± 0.0	3.3 ± 0.0
Linalool isovalerate	1465	1466	9.1 ± 0.0	-	-
γ-Muurolene	1478	1478	-	9.6 ± 0.1	9.0 ± 0.2
Germacrene D	1490	1484	11.8 ± 0.1	-	-
β-Selinene	1487	1489	-	1.5 ± 0.1	3.2 ± 0.0
Bicyclogermacrene	1496	1500	17.3 ± 0.4	4.5 ± 0.0	3.7 ± 0.1
α-Muurolene	1500	1500	-	2.0 ± 0.0	1.6 ± 0.0
β-Bisabolene	1510	1505	-	-	1.2 ± 0.0
γ-Cadinene	1514	1513	-	3.3 ± 0.0	0.8 ± 0.0
δ-Cadinene	1532	1522	4.5 ± 0.0	6.1 ± 0.1	2.4 ± 0.0

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10- *epi*-Cubebol	1534	1533	1.4 ± 0.0	-	-
trans-Cadina-1,4-diene	1540	1533	-	0.3 ± 0.0	-
α-Cadinene	1537	1537	-	0.8 ± 0.0	-
cis-Sesquisabinene hydrate	1546	1542	5.9 ± 0.1	-	-
α-Calacorene	1540	1544	-	0.4 ± 0.0	0.3 ± 0.0
Elemol	1550	1548	0.2 ± 0.0	-	5.5 ± 0.1
(*E*)-Nerolidol*	1563	1561	-	5.1 ± 0.1	5.3 ± 0.0
Geranyl butanoate	1568	1562	5.6 ± 0.1	-	-
Spathulenol	1582	1577	-	-	6.7 ± 0.0
Caryophyllene oxide*	1585	1582	4.3 ± 0.0	8.9 ± 0.1	-
Globulol	1595	1590	-	4.7 ± 0.2	-
Viridiflorol	1597	1592	2.4 ± 0.0	2.3 ± 0.0	-
Rosifoliol	1602	1600	-	2.5 ± 0.0	-
Humulene epoxide II	1612	1608	-	-	2.3 ± 0.1
1,10-di-*epi*-Cubebol	1616	1618	-	0.5 ± 0.0	-
Dillapiole*	1626	1620	-	0.5 ± 0.0	-
1-*epi*-Cubebol	1627	1627	2.5 ± 0.0	1.8 ± 0.1	-
cis-Cadin-4- en-7-ol	1637	1635	-	-	0.5 ± 0.0
epi-α-Muurolol	1641	1640	2.4 ± 0.0	5.5 ± 0.0	-
α-Muurolol	1646	1644	1.2 ± 0.1	1.8 ± 0.0	-
α-Cadinol	1657	1652	5.2 ± 0.0	8.4 ± 0.1	7.5 ± 0.2
(2*Z*, 6*Z*)-Farnesol	1690	1684	3.9 ± 0.1	-	-
Xanthorrhizol	1754	1751	0.3 ± 0.0	-	-
β-Bisabolenol	1788	1789	0.2 ± 0.0	-	-
(5*E*,9*E*)-Farnesyl acetone	1982	1987	-	0.9 ± 0.0	-
Monoterpenes			0.3 ± 0.0	9.7 ± 0.1	11.7 ± 0.2
Sesquiterpenes			98.0 ± 0.4	81.7 ± 0.2	80.9 ± 0.3
Phenylpropanoids			-	0.5 ± 0.0	-
Total			98.3 ± 0.4	96.7 ± 0.3	92.6 ± 0.4

RI[a] = Retention indices calculated from retention times in relation to those of a series C_8–C_{40} of *n*-alkanes on a 30m SH-Rtx-5MS capillary column. RI[b] = Retention indices from the literature. RI = retention index; SD = Standard deviation

with data reported in the literature for the same species. The differences can be attributed to the genetic variability of the species and/or biotic and abiotic factors.[28, 13]

3.2. Acaracidal property of *Piper* essential oils against *Tetranychus urticae*

Using the fumigation method, the essential oils from the leaves of *P. caldense*, *P. tuberculatum* and *P. arboreum* were toxic to *T. urticae* females (Table 2). The susceptibility of the pest varied with the type of oil. The estimated mean lethal concentrations (LC_{50}) indicate that the mite was more susceptible to the *P. tuberculatum* oil (LC_{50} = 0.50 µL L^{-1} of air), which was 1.32-fold more toxic than the *P. arboreum* oil (LC_{50} = 0.66 µL L^{-1} of air) and 2.76-fold more toxic than the *P. caldense* oil (LC_{50} = 1.38 µL L^{-1} of air). However, none of the oils was more toxic than eugenol, which was used as the positive control (LC_{50} = 4 x 10^{-3} µL L^{-1} of air) and it was 140.90 times more effective than the *P. tuberculatum* oil (Table 2).

Table 2 also displays the mean repellent concentrations (RC_{50}) of the *Piper* oils against *T. urticae*. All oils affected the behavior of the mites and repellent action varied among the different species. The *P. tuberculatum* was the most repellent (estimated RC_{50}: 6 x 10^{-4} µL mL^{-1}). Based on the toxicity ratios calculated using the method

Table 2. Fumigant and repellent properties of *P. caldense, P. tuberculatum* and *P. arboreum* essential oils and positive controls against *Tetranychus urticae*

Oil/positive control	n	df	Slope ± SD	Fumigation LC_{50} (µL L^{-1} of air) (IC 95 %)	X^2	TR_{50} (CI 95 %)
P. tuberculatum	710	5	3.22 ± 0.30	0.50 (0.45-0.56)	3.80	140.90* (34.48-575.85)
P. arboreum	622	4	6.23 ± 0.64	0.66 (0.59-0.72)	5.18	183.68* (44.97-750.21)
P. caldense	804	6	5.74 ± 0.50	1.38 (1.29-1.47)	5.95	387.41* (94.81-1583.11)
EU	540	3	0.85 ± 0.08	4×10^{-3} ($2x10^{-3}$-$5x10^{-3}$)	1.52	-
Oil/positive control	**n**	**df**	**Slope ± SD**	**Repellency RC_{50} (µL mL^{-1}) (CI 95 %)**	X^2	TR_{50} (CI 95 %)
P. tuberculatum	858	4	0.34 ± 0.02	6×10^{-4} ($3.0 x10^{-4}$-$9.8x10^{-4}$)	1.01	-
P. caldense	896	4	0.36 ± 0.02	3.6×10^{-3} ($2.0 x10^{-3}$-$6.6x10^{-3}$)	3.93	6.56 (0.43-100.16)
P. arboreum	1047	5	0.46 ± 0.03	2×10^{-2} ($1.0 x10^{-2}$-$2.5x10^{-2}$)	4.74	26.50* (2.19-320.86)
EU	874	4	0.36 ± 0.03	1.6×10^{-2} ($8.5 x10^{-3}$-$2.7x10^{-2}$)	1.88	25.14* (1.92-329.52)

n = number of mites, *df* = degrees of freedom, SD = Standard deviation; CI = Confidence interval; X^2= chi-squared; TR = toxicity ratio; EU = Eugenol as positive control; * = Significant when confidence interval does not include 1

proposed by Robertson and Preisler,[23] the *P. tuberculatum* was 6.56-fold and 26.50-fold more repellent than the *P. caldense* oil (RC_{50} = 3.6 x 10^{-3} µL mL^{-1}) and *P. arboreum* oil (RC_{50} = 2 x 10^{-2} µL mL^{-1}), respectively. In comparison to the positive control (eugenol) (RC_{50} = 1.6 x 10^{-2} µL mL^{-1}), the *P. tuberculatum* and *P. caldense* oils were 25.14-fold and 4.44-fold more repellent, respectively, whereas the repellent action of the *P. arboreum* did not differ significantly from that of positive control.

This is the first report of the repellent action and toxicity by fumigation of the essential oils from *P. tuberculatum*, *P. arboreum* and *P. caldense*. The essential oils of other *Piper* congeners have been evaluated with regard to their acaricidal potential against *T. urticae*. Comparing toxicity by fumigation obtained for the *Piper* oils tested in the present investigation to that described by Ribeiro *et al*.[11] for the oil from the leaves of *Piper marginatum*, the oils from *P. tuberculatum, P. caldense* and *P. arboreum* were respectively 7.54-fold, 5.71-fold and 2.73-fold more toxic to *T. urticae*. In contrast, Araujo *et al*.[7] report an estimated LC_{50} of 0.01 µL L^{-1} of air for the oil from the leaves of *P. aduncum*, which is 50.0-fold, 66.3-fold and 138.0-fold more toxic than the oils from *P. tuberculatum*, *P. arboreum* and *P. caldense*, respectively.

With regard to repellency, the only report in the literature evaluating this property (mean concentration required to repel 50 % of the mite population) investigated the oil from *Piper aduncum*.[7] The mean RC_{50} estimated for the *P. tuberculatum, P. caldense* and *P. arboreum* was respectively 66.6-fold, 11.1-fold and 2.0-fold more repellent to *T. urticae* than the oil from *Piper aduncum*.

The results obtained in the investigation of the repellent property and toxicity by fumigation *Piper* oils against *T. urticae* indicate that the different susceptibilities of the pest may be related to qualitative and quantitative differences in the chemical compositions of the oils. The use of different methods for the evaluation of the lethal and sub-lethal action of different *Piper* oils also suggests that volatile chemical constituents are fundamental to the toxicity and change in behavior of the pest. Indeed, the results indicate that the fumigant property and repellent action of the oils from the *Piper* species, especially *P. tuberculatum*, constitute an advantage when combating *T. urticae*, as these oils could be combined with other control measures in the integrated management of the target pest. However, further studies are needed to evaluate the cost and effectiveness of these oils in the preparation of formulations to be used in the control of *T. urticae* as an alternative to conventional insecticides in irrigated systems in the city of Petrolina, Pernambuco, Brazil.

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