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Chemical composition and acaricidal activity of essential oils and selected terpenes from two species of *Psidium* in the Cerrado biome of Brazil against *Tetranychus urticae*

[Composición química y actividad acaricida del aceite esencial y terpenos seleccionados de dos especies de *Psidium* de Cerrado Biome de Brasil contra *Tetranychus urticae*]

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Abstract: The aim of this study was to investigate the chemical composition and acaricidal effect of two *Psidium* species essential oils and selected compounds on *Tetranychus urticae*. Essential oils from the leaves of *Psidium laruotteanum* and *Psidium myrsinites* were obtained through hydrodistillation, analyzed using CG-FID and CG-MS and evaluated for toxicity to *T. urticae* by fumigation and residual contact. The susceptibility of *T. urticae* to monoterpenes and sesquiterpenes was also investigated. The major constituents of the *P. laruotteanum* oil were (E)-nerolidol (9.6 ± 0.4%) and γ-terpinene (9.4 ± 0.6%) and the major constituents of the *P. myrsinites* oil were β-caryophyllene (21.2 ± 0.9%) and α-humulene (10.3 ± 0.5%). Based on the LC_{50} estimates, no significant differences were found between the two oils regarding toxicity by fumigation or residual contact. β-Caryophyllene and (E)-nerolidol had the highest level of toxicity, independently of the method investigated. The findings indicate that both oils and selected constituents, especially β-caryophyllene and (E)-nerolidol, are promising as natural acaricidal agents that affect *T. urticae* through more than one mode of action.

Keywords: Essential oil; GC-MS; Sesquiterpenes; *Psidium laruotteanum*; *Psidium myrsinites*.

Resumen: Los aceites esenciales de las hojas de *Psidium laruotteanum* y *Psidium myrsinites* se obtuvieron por hidrodestilación, se analizaron por CG-FID y CG-MS, y se evaluaron sus toxicidades por fumigación y contacto residual contra *Tetranychus urticae*. Se investigó también la susceptibilidad del *T. urticae* a monoterpenos y sesquiterpenos. En el aceite esencial de *P. laruotteanum*, (E)-nerolidol (9.6 ± 0.4%) y γ-terpinene (9.4 ± 0.6%) se identificaron como constituyentes mayoritarios, mientras que en el aceite esencial de *P. myrsinites*, β-caryophyllene (21.2 ± 0.9%) y α-humulene (10.3 ± 0.5%) se encontraron como mayoritarios. Con base en las CL_{50} estimadas, no se observaron diferencias significativas entre las toxicidades de los aceites por fumigación, y tampoco por contacto residual. β-Caryophyllene y (E)-nerolidol presentaron las mayores toxicidades, independientemente del método investigado. Estos resultados indican, que los dos aceites, así como los constituyentes seleccionados, entre los que se destacan β-caryophyllene y (E)-nerolidol, son promisores agentes acaricidas naturales por actuar en el *T. urticae* por más de un mecanismo de acción.

Palabras clave: Aceites esenciales; GC-MS; Sesquiterpenos; *Psidium laruotteanum*; *Psidium myrsinites*.

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INTRODUCTION

The Brazilian savanna, which is known as the *Cerrado*, is one of the richest biomes in the world, with about 12,000 species of native plants (Mendonça *et al*., 2008). Covering approximately two million square kilometers and extending through ten states, including the Federal District, the *Cerrado* accounts for 22% of the area of Brazil and is the second largest biome in the country, behind only the Amazon forest (Ratter *et al*., 1997). The plant biodiversity of this biome has stimulated research groups to search for bioactive compounds of economic value for phytotherapeutic products, the food industry and agricultural applications (Baenas *et al*., 2019).

Among the genera most commonly found in the *Cerrado*, *Psidium* L. is represented by approximately 150 species and has wide distribution in all regions of Brazil (Souza *et al*., 2018; Machado *et al*., 2018). The fruits of species of this genus, which include guava, are used for the production of juices and jams (Frazon *et al*., 2009). Plants of the genus are also used in folk medicine in the form of teas as a diuretic and astringent as well as for the control of diabetes and obesity (Rodrigues & Carvalho, 2001). *Psidium* species are rich in phenolic compounds, with high antioxidant, antimicrobial, anti-inflammatory and anti-parasitic activity as well as insecticidal activity against larvae of the mosquito *Aedes aegypti* (Medina *et al*., 2011; Flores *et al*., 2013; Mendes *et al*., 2017; Machado *et al*., 2018). Among the species of *Psidium* with broad distribution in the *Cerrado*, *P. laruotteanum* and *P. myrsinites* are known locally as "*araçá-cascudo*" and "*araçá-bravo*", respectively. A previous investigation of the essential oil from *P. myrsinites* revealed a predominance of sesquiterpenes (Dias *et al*., 2015; Medeiros *et al*., 2015). Recently, Medeiros *et al.* (2018) showed that the essential oil from the leaves of *P. laruotteanum* is rich in monoterpenes.

The *Cerrado* has considerable plant biodiversity that has been explored little in terms of an alternative source of substances with acaricidal potential for the preparation of formulations for use in the integrated management of the *Tetranychus urticae*. In recent years, our research group has been investigating the acaricidal potential of aromatic flora from different biomes of Brazil with the aim of taking advantage of the considerable plant biodiversity for the preparation of formulations that can be used by small farmers for the integrated management of pests (Moraes *et al*., 2012; Nascimento *et al.*, 2012). *T. urticae* is one of the main polyphagous agricultural pests throughout the world, with wide distribution in both hemispheres. This mite causes significant damage to tomato crops and ornamental plants grown in protected farming activities in different regions of Brazil (Vassiliou & Kitsis, 2013). The control of this pest consists mainly of the application of synthetic acaricides, such as Abamectin, but the indiscriminant use of this product has given rise to *T. urticae* populations that are resistant to its active ingredient (Dias *et al*., 2015). Recent studies have demonstrated that essential oils rich in monoterpenes and sesquiterpenes are promising for the management of *T. urticae* in green houses (Born *et al.*, 2018) through both mechanisms of toxicity to the mite and by affecting feeding and egg-laying preferences (Moraes *et al*., 2017).

Giving continuity to the chemical study of aromatic plants in Brazil, the aim of the present study was to determine the chemical composition of the essential oils from the leaves of *P. laruotteanum* and *P. myrsinites* occurring in the *Cerrado* biome of central Brazil and evaluate the toxicity of these oils to *T. urticae*. The acaricidal properties of selected terpenes in the essential oils were also investigated with the aim of contributing to the formulation of an emulsionable acaricide. The results were compared to Azamax® and eugenol used as positive controls.

MATERIALS AND METHODS

Collection of plant material

Leaves of *Psidium laruotteaanum* Cambess and *Psidium myrsinites* DC. were collected from three adult plants of each species during the flowering period in the campus of University of Brasília campus, Brasília, Federal District, Brazil in a cerrado *sensu strito* vegetation. The plant was identified by the botanist Dr. Carolyn Proença from the departament of Botany at UnB. The voucher speciem was deposited at the UnB herbarium under code of J.E.Q. Faria Jn. & Fagg C.W. 932 and 933 with a duplicate sent to HUEG (Herbarium of the Universidade Estadual de Goiás, Goiás State University).

Chemicals

All monoterpenes (*α*-pinene, *β*-pinene, limonene, *p*-cymene, 1,8-cineole, *α*-terpineol and *γ*-terpinene), sesquiterpenes (*β*-caryophyllene, aromadendrene, *α*-

humulene, caryophyllene oxide, valencene and (*E*)-nerolidol) used in the identifications of volatile components and eugenol used as the positive control (fumigation) were purchased from Sigma-Aldrich - Brazil. Azamax® (12 g azadirachtin /L EC E.I.D. Parry) was acquired from the local market and used as positive control (residual contact).

Essential oils extraction and GC-FID analysis

The essential oils from fresh leaves (100 g) 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 (-5ºC) 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 analysis were carried out using a PerkinElmer Clarus 500 GC 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 1.0 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) analysis were carried out using a Varian 220-MS IT GC 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 (Dool & 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, 2007). Area percentages were obtained from the GC-FID response without the use of an internal standard or correction factors.

Acaricidal assay

Specimens of *Tetranychus urticae* (Acari: Tetranychidae) was originally collected from grapevines (*Vitis vinifera* L.) in the municipality of Petrolina-PE (09°12′43.9″ S; 40° 29′12.7″ W) and maintained at the Laboratory for the Chemical Investigation of Natural Insecticides, Department of Agronomy of the Federal Rural University of Pernambuco. The mite *T. urticae* used for the bioassay was reared in plants of *Canavalia ensiformes* at temperature of 25±5ºC, relative humidity of 65±5% and a 12 h photophase, without any exposure to acaricides.

Fumigation and Residual Contact Bioassays of Oils and Selected Compounds

The fumigation and residual contact methods were the same as those employed by Araújo *et al*. (2012). Hermetically sealed glass recipients with a capacity of 1.0 L were used as test chambers. A fine brush was used to transfer female *T. urticae* onto the leaf disks (2.5 cm in diameter). 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 experiment was performed in triplicate and repeated three times. One replicate consisted of 30 specimens placed on three leaf disks (10 mites per disk) in a Petri dish. The oils, constituents and positive control (eugenol) were applied with a pipette on a piece of filter paper (5 x 2 cm) attached to the underside of the lid of the recipient. In the fumigation bioassays, the concentrations ranged from 0.1 to 2.5 μL L^{-1} of air for the oils and 3.2 x 10^{-4} to 26 μL L^{-1} of air for the selected compounds and positive control. Immediately after the application of the oil/compound, the fumigation chamber was closed and covered with PVC plastic wrap. Mortality was determined 24 hours after treatment. Mites with no sign of movement were considered dead.

In the residual contact bioassays, tests were

conducted in Petri dishes (10 cm diameter) and solutions were prepared by diluting essential oil in methanol. A 20 µL aliquot of each concentration was painted on the underside of the disc with a micropipette. After drying at room temperature for 2 min, each disc was individually placed at the bottom of a Petri dish lid on a 10 cm diameter disc of filter paper wetted with distilled water. Ten adult female mites were introduced into each Petri dish. All treatments were replicated tree times. Concentrations ranged from 5.0 to 40.0 µL mL^{-1} for the oils and 0.1 to 675.0 µL mL^{-1} for the selected compounds and positive control. Mortality was determined 24 hours after treatment. Mites with no sign of movement were considered dead. Control mites were held on leaf discs painted with the carrier solvent alone.

Statistical analysis

To estimate the curve slopes, LC_{50} (lethal concentration) of each *Psidium* oils, selected constituents and positive controls, mortality data were submitted to PROBIT analysis using POLO PC (LeOra, 1987). The concentrations used were calculated based on the logarithmic series and toxicity ratios based on the method described by Robertson *et al*. (2017).

Table No. 1
Percentage composition and yield of essential oils from *P. laruotteanum* and *P. myrsinites*

Compounds	RI[a]	RI[b]	*P. laruotteanum*	*P. myrsinites*	Method of identification
Yield (%) ± DP			0.4±0.0	0.4±0.1	
Santolin triene	907	906	-	0.2±0.0	RI, MS
Artemisia triene	918	923	0.5±0.2	-	RI, MS
α-Pinene	928	932	1.8±0.1	-	RI, MS, CI
Sabinene	964	969	-	0.4±0.0	RI, MS
β-Pinene	974	974	1.0±0.0	6.4±0.3	RI, MS, CI
α-Phellandrene	998	1002	1.3±0.0	-	RI, MS
p-Mentha-1(7),8-diene	999	1003	0.5±0.0	-	RI, MS
iso-Sylvestrene	1006	1007	1.1±0.2	-	RI, MS
p-Cymene	1017	1020	2.8±0.2	-	RI, MS
Limonene	1021	1024	2.8±0.0	1.5±0.1	RI, MS, CI
β-Phellandrene	1022	1025	-	0.5±0.0	RI, MS
1,8-Cineole	1024	1026	3.2±0.2	-	RI, MS, CI
(*Z*)-*β*-Ocimene	1034	1032	2.8±0.1	-	RI, MS
γ-Terpinene	1050	1054	9.4±0.6	-	RI, MS, CI
Terpinolene	1082	1086	2.1±0.1	1.7±0.2	RI, MS, CI
Linalool	1091	1095	3.1±0.1	-	RI, MS, CI
Neoiso-3-thujanol	1144	1147	-	0.7±0.0	RI, MS
Terpinen-4-ol	1169	1174	2.3±0.1	-	RI, MS, CI
α-Terpineol	1184	1186	2.1±0.1	-	RI, MS, CI
Cubebene	1350	1345	-	1.0±0.1	RI, MS
α-Copaene	1372	1374	6.6±0.1	-	RI, MS, CI
β-Patchoulene	1383	1379	-	1.3±0.1	RI, MS
β-Caryophyllene	1414	1417	3.4±0.1	21.2±0.9	RI, MS, CI
β-Copaene	1425	1430	-	0.9±0.0	RI, MS
β-Gurjunene	1426	1431	-	4.1±0.1	RI, MS

γ-Elemene	1432	1434	3.2±0.1	-	RI, MS
α-Guaiene	1436	1437	0.9±0.1	-	RI, MS
Aromadendrene	1437	1439	-	1.1±0.1	RI, MS, CI
α-Humulene	1448	1452	-	10.3±0.5	RI, MS, CI
α-neo-Clovene	1450	1452	0.6±0.1	0.5±0.1	RI, MS
allo-Aromadendrene	1453	1458	-	0.8±0.0	RI, MS
cis-Cadina-1(6),4-diene	1464	1461	1.0±0.0	-	RI, MS
Dehydro-aromadendrene	1465	1460	-	6.5±0.1	RI, MS
9-*epi*-(*E*)-Caryophyllene	1466	1464	1.2±0.1	-	RI, MS
β-Chamigrene	1471	1476	1.1±0.1	5.1±0.2	RI, MS
γ- Muurolene	1473	1478	1.0±0.0	-	RI, MS
Amorfa-4,7(11)-diene	1477	1479	1.4±0.0	-	RI, MS
Germacrene D	1486	1484	-	0.7±0.0	RI, MS
cis-*β*-Guaiene	1487	1492	1.5±0.1	1.4±0.1	RI, MS
Valencene	1493	1496	5.7±0.5	-	RI, MS, CI
Epizonarene	1496	1501	0.8±0.0	-	RI, MS
10-epi-Cubebol	1533	1533	-	1.0±0.0	RI, MS
β-Vetivenene	1554	1554	-	6.6±0.2	RI, MS
Germacrene B	1557	1559	-	7.9±0.3	RI, MS
(*E*)-Nerolidol	1558	1561	9.6±0.4	2.3±0.1	RI, MS, CI
Longicanfenilono	1566	1561	-	0.9±0.1	RI, MS
Maaliol	1565	1566	1.5±0.0	-	RI, MS
Himachalene epoxide	1574	1578	1.0±0.0	-	RI, MS
Caryophyllene oxide	1580	1582	7.3±0.1	3.4±0.1	RI, MS, CI
Longiborneol	1597	1599	-	1.5±0.2	RI, MS
Rosifoliol	1601	1600	0.5±0.0	-	RI, MS
Rosifoliol	1603	1600	-	0.7±0.0	RI, MS
Ledol	1607	1602	0.7±0.0	-	RI, MS
Isolongifolan-7-*α*-ol	1615	1618	1.5±0.1	-	RI, MS
epi-Cedrol	1614	1618	0.7±0.1	-	RI, MS
10-*epi*-*γ*-Eudesmol	1622	1622	1.7±0.0	1.5±0.0	RI, MS
trans-Isolongifolane	1626	1625	-	0.5±0.0	RI, MS
γ-Eudesmol	1629	1630	-	0.6±0.0	RI, MS
epi-*α*-Cadinol	1633	1638	-	0.7±0.0	RI, MS
α-Muurolol	1639	1644	4.6±0.0	1.1±0.0	RI, MS
Cubenol	1647	1645	-	1.3±0.0	RI, MS
Pogostol	1647	1651	-	1.5±0.0	RI, MS
α-Cadinol	1649	1652	2.6±0.0	-	RI, MS
α-Eudesmol	1650	1652	0.6±0.0	-	RI, MS
Eudesmol acetate	1675	1680	0.9±0.0	-	RI, MS
Amorfa-4,9-dien-2-ol	1703	1700	0.1±0.0	-	RI, MS

RI[a] =	Sclareolide	2060	2065	0.1±0.0	-	RI, MS
	Monoterpenes			36.6±0.3	11.4±0.2	
	Sesquiterpenes			61.8±0.6	86.4±0.8	
	Total			98.6±0.5	97.8±0.7	

Retention indices calculated from retention times in relation to those of a series C_8–C_{40} of n-alkanes on a 30m DB-5 capillary column. RI[b] = Retention indices from the literature. RI = retention index; MS = mass spectroscopy; CI: Co-injection with authentic compounds.

RESULTS AND DISCUSSION

The yields and compounds identified in the essential oils from the leaves of *P. laruotteanum* and *P. myrsinites* are listed in Table No. 1.

The oil yields were 0.4±0.0% for the *P. laruotteanum* oil and 0.4±0.1% for the *P. myrsinites* oil. These findings are in agreement with yields reported in the literature for other species of *Psidium* (Adam *et al*., 2011; Chalannavar *et al*., 2014; Khadhri *et al*., 2014).

The chemical analysis by GC-MS revealed a total of 68 compounds (Figure No. 1), corresponding to 98.6±0.5% and 97.8±0.7% of the chemical composition of the *P. laruotteanum* and *P. myrsinites* oils, respectively. The oils exhibited a terpene chemical profile (monoterpenes and sesquiterpenes, with a predominance of the latter). This profile is in agreement with data reported for the oils of other species of the genus *Psidium* that occur in different regions of Brazil and the world (Freitas *et al*., 2002; Pino *et al*., 2004; Chen *et al*., 2006; Sartorelli & Correa, 2007; Adam *et al*., 2011; Khadhri *et al*., 2014; Medeiros *et al*., 2015; Dias *et al*., 2015).

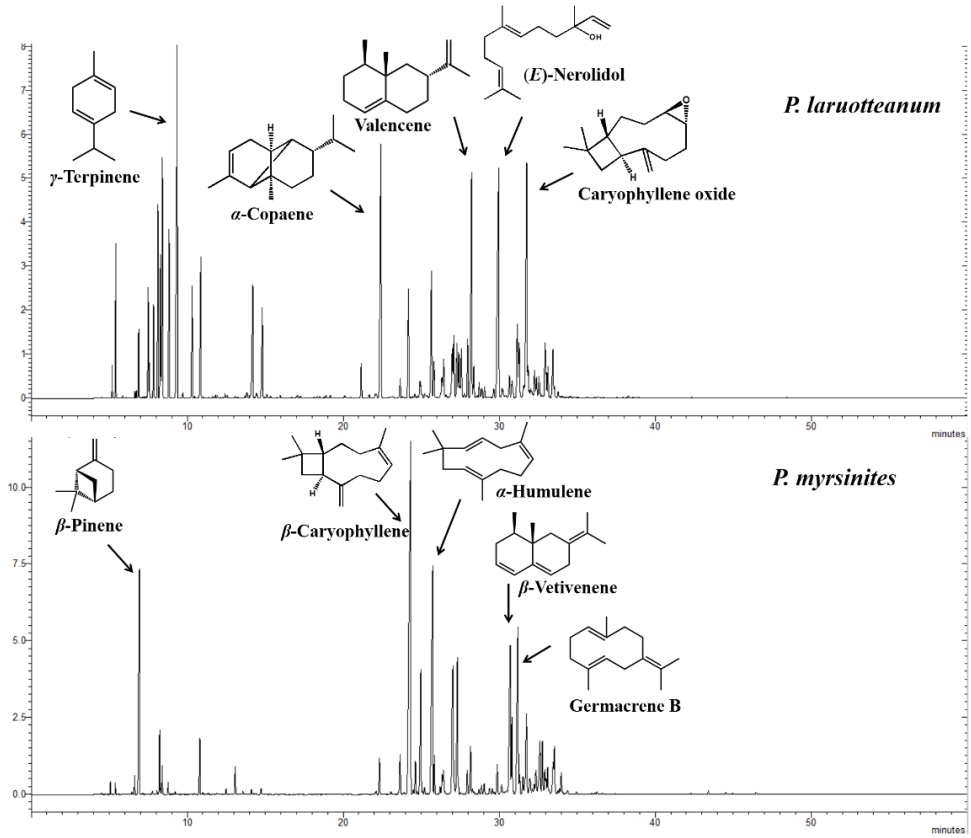

Figure No 1
GC-MS chromatogram of *Psidium laruotteanum and Psidium myrsinites* leaf oil and molecular structures of main constituents.

Forty-one compounds were identified in the essential oil from *P. laruotteanum*. Sesquiterpenes accounted for the largest proportion (61.8±0.6%), with (*E*)-nerolidol (9.6±0.4%) and *γ*-terpinene (9.4±0.6%) as the major constituents. Other constituents were also found in significant percentages, such as *α*-copaene (7.6±0.1%), caryophyllene oxide (7.3±0.1%), valencene (5.7±0.5%) and *α*-muurolol (4.6±0.0%). Medeiros *et al*. (2018) recently reported a chemical profile rich in monoterpenes (89.3 to 92.5%) for three populations of *P. laruotteanum* collected in the *Cerrado*, describing *p*-cymene (19.4 to 34.8%), 1,8-cineole (6.9 to 19.2%) and α-pinene (9.2 to 11.4%) as the major constituents of the essential oils. These compounds were also identified in the present study, but in smaller percentages [*p*-cymene (2.8±0.2%), 1,8-cineole (3.2±0.2%) and *α*-pinene (1.8±0.1%)]. While (*E*)-nerolidol was not found in the populations of *P. laruotteanum* investigated by Medeiros *et al.* (2018), this sesquiterpene has been reported in significant percentages in other congeners that occur in Brazil, such as *P. guajava* collected in the states of Minas Gerais (8.19%) (Lima *et al*., 2008) and Espírito Santo (13.7%) (Souza *et al*., 2017). *γ*-Terpinene was a major constituent in the essential oils described by Medeiros *et al.* (2018) and in the present investigation, but has been found at proportions lower than 1% in the essential oils from other species of *Psidium* (Chen *et al*., 2006; Pino *et al*., 2006; Chen *et al*., 2008; Biegelmeyer *et al*., 2011Adam *et al*., 2011; El-Ahmady *et al*., 2013).

Table No. 2
Fumigation toxicity (LC_{50} at µL L^{-1} of air) and residual contact (LC_{50} at µL mL^{-1}) of the essential oil of *P. laurotteanum* and *P. myrsinites.*

Oil	Bioassay	n	df	slope	LC_{50} (CI 95%)	P-values	$\chi 2$	RT_{50} (CI 95%)
P. laruotteanum	Fumigation	450	4	1.72 (1.59 - 1.85)	0.91 (0.54 - 1.44)	0.08	6.89	258.04 (163.03 - 408.40)
	Contact	540	4	1.36 (1.27 - 1.45)	21.25 (14.88 - 32.76)	0.06	8.99	70.75 (51.79 - 91.28)
P. myrsinites	Fumigation	450	4	1.40 (1.28 - 1.52)	0.82 (0.30 - 1.51)	0.06	6.26	231.80 (137.45 - 390.91)
	Contact	630	5	1.12 (1.05 - 1.19)	16.06 (10.65 - 22.65)	0.05	10.82	51.97 (39.18 - 68.95)
Eugenol	Fumigation	580	5	0.84 (0.72 - 0.97)	0.004 (0.002 - 0.008)	0.68	2.50	-
Azamax®	Contact	630	5	2.46 (2.26 - 2.65)	0.31 (0.26 - 0.37)	0.13	8.30	-

Eugenol and Azamax® used as positive control. n = number of mites/dose; df= degrees of freedom; CI = Confidence interval. $\chi 2$= chi-squared. TR = toxicity ratio.

Thirty-five compounds were identified in the essential oil from *P. myrsinites*, accounting for 97.8±0.7% of the oil. A predominance of sesquiterpenes was found (86.4±0.8%), with *β*-caryophyllene as the major constituent (21.2±0.9%). Other compounds were also found in significant percentages, such as *α*-humulene (10.3±0.5%), germacrene B (7.9±0.3%), (*E*)-*β*-vetivenene (6.6±0.2%), dehydro-aromadendrene (6.5±0.1%) and *β*-pinene (6.4±0.3%). These results are in agreement with data described for the leaf oil from *P. myrsinites* collected in the state of Maranhão, Brazil, in which *β*-caryophyllene (26.5%) and *α*-humulene (23.92%) were the main constituents (Dias *et al*., 2015). Castelo *et al*. (2012) also report *β*-caryophyllene, *α*-humulene, *β*-guaiane and caryophyllene oxide as the major constituents of the leaf oil from *P. myrsinites* collected in Brasília, Brazil. On the other hand, a study on the shoots of *P. myrsinites* found caryophyllene oxide (26.1%) to be the major constituent (Medeiros *et al*., 2015). *β*-Caryophyllene as the major constituent has been reported for several species of the genus *Psidium*, such as *P. guajava* (18.3 to 58.28%) (Chen *et al*.,

2006; Chen *et al*., 2008; Adam *et al*., 2011; El-Ahmady *et al*., 2013; Souza *et al*., 2017; Wang *et al*., 2017), *P. cattleyanum* (22.5 to 31.5%) (Biegelmeyer *et al*., 2011; Adam *et al*., 2011), *P. cattlenium* (59.0%) (Chen *et al*., 2006) and *P. myrsinoides* (22.4%) (Freitas *et al*., 2002).

The results of the fumigation and residual contact bioassays investigating the effect of the *P. laurotteanum* and *P. myrsinites* oils on *T. urticae* are displayed in Table No. 2. The concentration-response curves obtained in the bioassays for *T. urticae* mortality were adjusted to the Probit model (non-significant values of $\chi 2$ and $p>0.05$).Both oils were toxic to the mite independently of the method employed. No significant differences between the oils were found with regard to toxicity by fumigation or residual contact. The oils were more toxic to the mite through fumigation than residual contact, suggesting the greater toxicity of the vapors through the respiratory pathway of the mite than through ingestion or contact with the tarsi (Lorini *et al*., 2015; Enan, 2001). Similar results have been reported for other oils, such as those from *Eugenia langsdorffii* (Moraes *et al*., 2012) and *Vitex agnus-castus* (Neves & da Camara, 2016). Neither oil was more active than eugenol and Azamax® by fumigation and residual contact, respectively.

Table No. 3A
Toxicity by fumigation (LC_{50} at µL L^{-1} of air) and residual contact (LC_{50} at µL mL^{-1}) of majority of the individual compounds selected from oils *P. laurotteanum* and *P. myrsinites* oils against *Tetranychus urticae*.

Compound	% in the oil		Bioassay	n	df	Slope
	1	2				
α-Pinene	1.8±0.1	-	Fumigation	722	5	3.91 (3.53-4.29)
			Contact	150	4	2.16 (1.86-2.45)
β-Pinene	1.0±0.0	6.4±0.3	Fumigation	630	4	2.95 (2.69-3.20)
			Contact	150	4	2.73 (2.39-3.07)
p-Cymene	2.8±0.2	-	Fumigation	630	5	2.11 (1.97-2.25)
			Contact	200	6	2.69 (2.36-3.02)
Limonene	2.8±0.0	1.5±0.1	Fumigation	444	3	7.35 (6.46-8.23)
			Contact	124	3	3.48 (2.91-4.04)
1,8-Cineole	3.2±0.2	-	Fumigation	630	4	8.16 (7.62-8.22)
			Contact	150	4	2.61 (2.21-3.01)
γ-Terpinene	9.4±0.6	-	Fumigation	540	4	10.03 (8.87-11.19)
			Contact	540	4	1.75 (1.49-1.99)
α-Terpineol	2.1±0.1	-	Fumigation	633	4	6.08 (5.20-6.96)
			Contact	123	3	1.70 (1.46-1.94)
β-Caryophyllene	3.4±0.1	21.2±0.9	Fumigation	720	6	0.80 (0.33-1.27)
			Contact	175	5	2.25 (1.97-2.52)
Aromadendrene	-	1.1±0.1	Fumigation	540	4	2.14 (1.98-2.30)

			Contact	175	5	8.00 (6.17-9.83)
α-Humulene	-	10.3±0.5	Fumigation	629	5	1.85 (1.74-1.96)
			Contact	200	6	2.45 (2.12-2.77)
Valencene	5.7±0.5	-	Fumigation	540	4	0.84 (2.64-3.04)
			Contact	540	4	1.55 (1.41-1.69)
(*E*)-Nerolidol	9.6±0.4	2.3±0.1	Fumigation	450	3	8.21 (7.34-9.08)
			Contact	540	4	1.28 (1.19-1.37)
Caryophyllene oxide	7.3±0.1	3.4±0.1	Fumigation	720	6	1.37 (1.29-1.45)
			Contact	630	5	1.97 (1.77-2.17)

Table No. 3B
Toxicity by fumigation (LC_{50} at µL L^{-1} of air) and residual contact (LC_{50} at µL mL^{-1}) of majority of the individual compounds selected from oils *P. laurotteanum* and *P. myrsinites* oils against *Tetranychus urticae*.

Compound	p-values	X^2	Bioassay	TR50(CI 95%)
α-Pinene	0.06	11.04	Fumigation	276.25 (183.07 - 350.01)
	0.60	2.73	Contact	56.95 (36.81-76.17)
β-Pinene	0.06	9.16	Fumigation	111.82 (74.51-182.61)
	0.54	8.10	Contact	69.40 (43.96-82.98)
p-Cymene	0.08	9.83	Fumigation	103.32 (60.53-151.35)
	0.37	6.48	Contact	63.32 (41.85-78.49)
Limonene	0.05	7.60	Fumigation	220.15 (136.06 - 250.91)
	0.68	1.52	Contact	129.36 (87.43-162.94)
1,8-Cineole	0.11	7.76	Fumigation	97.10 (60.06-156.99)
	0.58	1.97	Contact	144.86 (9.31-2254.06)
γ-Terpinene	0.06	7.66	Fumigation	123.78 (82.00-186.20)
	0.05	9.29	Contact	129.74 (86.74-185.72)
α-Terpineol	0.05	7.81	Fumigation	52.98 (19.77 - 111.12)
	0.40	2.94	Contact	78.72 (40.04-98.23)
β-Caryophyllene	0.17	9.12	Fumigation	-
	0.98	0.72	Contact	-

Aromadendrene	0.05	9.28	Fumigation	160.11 (94.00 - 295.29)
	0.09	3.43	Contact	49.94 (34.85-60.57)
α-Humulene	0.14	8.41	Fumigation	55.03 (24.74 - 98.71)
	0.83	2.85	Contact	37.19 (26.98-51.26)
Valencene	0.39	4.08	Fumigation	107.94 (74.11-180.81)
	0.05	9.29	Contact	99.74(66.74-135.72)
(*E*)-Nerolidol	0.91	2.88	Fumigation	9.96 (4.44-22.59)
	0.98	0.38	Contact	5.91 (2.44-10.59)
Caryophyllene oxide	0.10	10.5	Fumigation	136.89 (89.09-212.22)
	0.09	8.02	Contact	198.04 (123.62-258.21)

n = number of mites/dose; df= degrees of freedom; CI = confidence interval; χ2= chi-squared; TR = toxicity ratio.

This is the first report of the acaridical action of the essential oils from *P. laurotteanum* and *P. myrsinites* against *T. urticae*. However, the effects of fumigation and residual contact have been investigated in the essential oil of the congener *P. cattleianum* with regard to its effects on the dust mites *Dermatophagoides farinae* and *D. pteronyssinus*, which cause allergic reactions, asthma, conjunctivitis and allergic rhinitis (Oh *et al*., 2014). The fumigant and residual contact results of the *P. laruotteanum* and *P. myrsinites* oils showed more toxicity than essential oil from *Aristolochia trilobata* (de Melo *et al*., 2018) and *Schinus terebinthifolius* (Nascimento *et al*., 2012). However, similar results were in agreement with data reported for essential oils from the leaves of *Croton jacobinensis*, stems and leaves of *C. muscicapa* by fumigation (Neves & da Camara, 2011) and leaves of *C. rhamnifolioides* by residual contact (da Camara *et al*., 2017).

The results of the fumigation and residual contact bioassays investigating the effects of selected compounds from the *P. laurotteanum* and *P. myrsinites* oils are displayed in Table No. 3a and 3b.

As observed for the oils, the compounds exhibited greater toxicity to the mites by penetration of the vapors through the airways (fumigation) than by residual contact. Among the constituents tested, *β*-caryophyllene and (*E*)-nerolidol exhibited the greatest toxicity, independently of the method employed. Based on the estimated LC_{50} values of the selected compounds, the order of toxicity by fumigation was *β*-caryophyllene > (*E*)-nerolidol > *α*-humulene = *α*-terpineol = *p*-cymene = valencene = *γ*-terpinene = *β*-pinene = caryophyllene oxide = 1,8-cineole > aromadendrene = limonene = *α*-pinene and the order by residual contact was *β*-caryophyllene > (*E*)-nerolidol > *α*-humulene > *p*-cymene = aromadendrene = *α*-pinene = *α*-terpineol = *β*-pinene > valencene = *γ*-terpinene = limonene = 1,8-cineole = caryophyllene oxide. None of the selected compounds was more toxic than eugenol and Azamax® used as the positive control in the fumigation and residual contact bioassays, respectively.

In the comparison of relative toxicity by fumigation among the essential oils and constituents, *β*-caryophyllene was 16-fold and 18-fold more toxic than the oils from *P. myrsinites* and *P. laruotteanum*, respectively, and (*E*)-nerolidol exhibited the same level of toxicity as the oils. In the comparison of relative toxicity by residual contact, *β*-caryophyllene was 25-fold and 33-fold more toxic than the oils from

P. myrsinites and *P. laruotteanum*, respectively, and (*E*)-nerolidol was 10-fold to eight-fold more toxic than the *P. myrsinites* and *P. laruotteanum* oils, respectively. These results suggest that *β*-caryophyllene and (*E*)-nerolidol play an important role in the acaricidal activity of the *Psidium* oils investigated in this study. However, previous investigations on the roles exercised by terpenes in terms of fumigant action and residual contact of essential oils show that the toxicity of an oil cannot only be attributed to the individual toxicity of its constituents; the proportions and possible interactions between the compounds that make up the essential oil should also be taken into consideration (Moraes *et al*., 2012; Neves & da Camara, 2016; Moraes *et al*., 2017).

CONCLUSION

The chemical analysis by GC-MS enabled the identification of a novel chemotype rich in (*E*)-nerolidol (9.6±0.4%) and γ-terpinene (9.4±0.6%) for *P. laruotteanum* in the *Cerrado* biome of central Brazil. This study also confirms the occurrence of a chemotype rich in *β*-caryphyllene for *P. myrsinites* occurring in the same biome.

The present findings indicate that the essential oils from the leaves of *P. laruotteanum* and *P. myrsinites* and selected constituents, especially *β*-caryophyllene and (*E*)-nerolidol, are promising natural acaricidal agents with more than one mode of action (fumigation and residual contact). However, further studies are needed to investigate the effect of these oils and constituents on non-target organisms and establish the cost-benefit ratio for the formulation of an acaricide for use in the management of *T. urticae* in organic and protected farming activities.

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