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New sources of botanical acaricides from species of Croton with potential use in the integrated management of *Tetranychus urticae*

[Nuevas fuentes de acaricidas botánicos de especies de Croton con uso potencial en el manejo integrado de *Tetranychus urticae*]

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Abstract: Essential oils from the leaves and stems of *Croton adenocalyx*, *C. grewioides*, *C. heliotropiifolius* and *C. blanchetianus* obtained through hydrodistillation were analyzed by GC-MS. We then investigated the lethal and sublethal effects of the Croton oils and 15 major constituents against *Tetranychus urticae*. β-Caryophyllene was the major component in the leaf and stem oils from *C. heliotropiifolius* and *C. adenocalyx*. Spathulenol and (E)-anethole were the major constituents identified in the leaf and stem oils of *C. blanchetianus* and *C. grewioides*, respectively. The oil with the greatest lethal and sublethal effects was those from *C. adenocalyx*. Among the constituents β-caryophyllene and spatulenol were the most toxic to the mite, whereas eugenol and methyl eugenol were the most repellent. The toxicity and repellency of the Croton oils, particularly the oils from *C. adenocalyx*, demonstrate that these oils constitute a promising alternative to synthetic acaricides for use in the control of *T. urticae*.

Keywords: *Croton* ssp.; Essential oil; Botanical acaricide; Two-spotted spider mite.

Resumen: Los aceites esenciales de las hojas y tallos de *Croton adenocalyx*, *C. growioides*, *C. heliotropiifolius* y *C. blanchetianus* obtenidos mediante hidrodestilación fueron analizados a través de GC-MS. Se investigaron los efectos letales y subletales de los aceites de Croton y 15 componentes principales contra *Tetranychus urticae*. El β-cariofileno fue el componente principal en los aceites de hojas y tallos de *C. heliotropiifolius* y *C. adenocalyx*. El espatulenol y el (E)-anetol fueron los principales componentes identificados en los aceites de hojas y tallos de *C. blanchetianus* y *C. growioides*, respectivamente. El aceite con los mayores efectos letales y subletales fue el de *C. adenocalyx*. Entre los componentes, el β-cariofileno y el espatulenol fueron los más tóxicos para el ácaro, mientras que el eugenol y el metil eugenol fueron los más repelentes. La toxicidad y la repelencia de los aceites de Croton, particularmente los aceites de *C. adenocalyx*, demuestran que estos aceites constituyen una alternativa prometedora respecto a los acaricidas sintéticos para uso en el control de *T. urticae*.

Palabras clave: *Croton* ssp.; Aceite esencial; Acaricida botánico; Ácaro araña con dos manchas.

INTRODUCTION

According to the reports of farmers in the main family farming niches in the semiarid region of the state of Pernambuco, Brazil, significant losses of tomato and cucumber crops in protected environments are common due to the attack of several types of pests, particularly the two-spotted spider mite (*Tetranychus urticae* Kock). The main form of controlling this pest is the use of commercial acaricides, such as Ortus®. However, the indiscriminate use of these products has led to populations of *T. urticae* resistant to the active ingredients (Van Leeuwen *et al*., 2010). A low-cost alternative that causes less harm to the ecosystem than these synthetic acaricides is the use of formulations containing natural products derived from plants, such as essential oils.

Among the plants with broad occurrence in the *Caatinga* biome of northeast Brazil, specie of the genus *Croton* L stand out due to their previously investigated acaricidal potential (Neves & da Camara, 2011; da Camara *et al*., 2017). This genus belongs to the subfamily Crotonoideae and comprises around 1300 species of herbs, shrubs and trees distributed in tropical and subtropical regions throughout the world (Berry *et al*., 2005). Among the 350 species of *Croton* recorded for Brazil (Van Ee *et al*., 2011), 35 are found in Pernambuco, where some are considered endemic (Silva *et al*., 2010). Plants of this genus are sources of bioactive compounds with a variety of biological properties, including insecticidal (Cruz *et al*., 2017; Silva *et al*., 2018) and acaricidal (da Camara *et al*., 2017; Castro *et al*., 2019) activity, which are attributed to secondary metabolites pertaining to the diterpene, alkaloid and terpene classes, the latter of which are found in essential oils.

The species *C. adenocalyx*, *C. grewioides*, *C. heliotropiifolius* and *C. blanchetianus* are native to the semiarid region of the state of Pernambuco and are broadly distributed around agricultural niches in the region, used by local communities in the form of tea for the treatment of gastrointestinal diseases and to relieve headache or fever (Hiruma-Lima *et al*., 2000; Macêdo *et al*., 2015). There are no previous reports in the literature on the biological activity of the essential oil from *Croton adenocalyx*. Although a previous investigation evaluated the antimicrobial, antinociceptive and insecticidal action of *C. grewioides*, *C. heliotropiifolius* and *C. blanchetianus*, no studies have addressed the acaricidal effect of the essential oils from these species on *T. urticae*.

Employing the acaricidal properties of aromatic flora for the preparation of more environmentally benign and less harmful formulations could be a viable approach for use in the control of *T. urticae* within the concept of integrated pest management. Thus, the aim of the present study was to investigate whether the essential oils from the leaves and stems of *C. adenocalyx*, *C. grewioides*, *C. heliotropiifolius* and *C. blanchetianus* have effects on *T. urticae* through acaricidal and repellant mechanisms. We also investigated the acaricidal properties of 15 chemical compounds identified as the major constituents of the essential oils. The results were compared to those achieved with the commercial acaricides Ortus® and Azamax® used as positive controls.

MATERIAL AND METHODS

Collection of plant material

The fresh leaves and stems of *Croton adenocalyx* Baill, *C. grewioides* Baill, *C. heliotropiifolius* Kunth and *C. blanchetianus* Baill were collected in the Vale do Catimbau, a Brazilian national park in the state of Pernambuco-Brazil. The plants were identified by Botanist Dra. Maria F.A. Lucena. Voucher of both samples were mounted and deposited in the herbarium of the Federal University of Pernambuco, under number: (30491) *C. heliotropiifolius,* (49390) *C. adenocalyx,* and herbarium of the Federal Rural University of Pernambuco, under number: (48219) *C. blanchetianus* and (42193) *C. grewioides.*

Isolation of Essential Oils

Essential oils from the leaves and stems (100 g) of *C. adenocalyx*, *C. blanchetianus*, *C. grewioides* and *C. heliotropiifolius* were obtained by hydrodistillation using a modified Clevenger apparatus for 4 h. The oil layers were separated and dried over anhydrous sodium sulfate, stored in hermetically sealed glass containers and kept at a low temperature (-5ºC) until the bioassays and analyses. Total oil yields were expressed as percentages (g/100 g of fresh plant material). All experiments were carried out in triplicate.

Chemicals

The constituents used as standards in the identification of volatile compounds in the oils were purchased from Sigma-Aldrich (Brazil). Monoterpenes (*α*-pinene, *β*-pinene, *p*-cymene, 1,8-cineole, camphor, *β*-phellandrene, *α*-phellandrene), sesquiterpenes (*β*-caryophyllene, bicyclogermacrene, spathulenol and caryophyllene oxide) and phenylpropanoid (methyl eugenol, eugenol, (*E*)-anethole, eugenyl acetate) were selected for the

bioassays due to the fact that these compounds were identified in the *Croton* oils as a major constituents and are available commercially. Fenpyroximate (Ortus® 50 g i.a./L SC Arysta Lifescience) and azadirachtin (Azamax® 12 g i.a./L EC E.I.D. Parry) were acquired from the local market and used as positive controls.

Gas chromatography FID analysis

Gas Chromatography (GC) identification was performed using a Hewlett-Packard 5890 Series II 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 and W Scientific). The oven temperature was programmed from 60 to 240°C at a rate 3°C/min. Injector and detector temperatures were 260°C. Hydrogen was used as the carrier gas at a flow rate of 1 mL/min in split mode (1:30). The injection volume was 0.5 μL of diluted solution (1/100) of oil in n-hexane. The percentage of each compound was obtained from GC-FID peak areas in the order of the DB-5 column elution and expressed as the relative percentage of the area of the chromatograms. The analysis was conducted in triplicate.

Gas chromatography-mass spectrometry analysis

The GC-MS analysis of the essential oils was 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; 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 C_8-C_{40} n-alkanes calculated using the Van Den Dool and Kratz equation (Van Den Dool & Kratz, 1963) and by computer matching against the mass spectral library of the GC-MS data system (NIST version 14) 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.

Acquisition and rearing Tetranychus urticae.

Specimens of *Tetranychus urticae* (Acari: Tetranychidae) was originally collected from grapevine (*Vitis vinifera* L.) in the municipality of Petrolina-PE (09º12′43″ S; 40º29′12″ W), and since then maintained in laboratory at the Agronomy Department of the Federal Rural University of Pernambuco, Brazil. *T. urticae* were rearing at a temperature of 25 ± 1ºC, relative humidity of 65 ± 5% and a 12 h photoperiod and without any exposure to acaricides. The breeding method was adapted from Ribeiro *et al*. (2016).

Contact assay

Leaf disc painting method described by Moraes *et al*. (2012) was used to test the action of *Croton* oils and positive control by contact toxicity. Experiments were performed with open Petri dishes (10 cm diameter). Leaf discs (2.5 cm diameter) were cut from leaves of greenhouse-grown jack bean. The concentrations of *Croton* ranged from 0.10 to 2.50 μL/mL (leaves) and 0.50 to 4.50 μL/ml (stems) for *C. adenocalyx*, 10.0 to 100 μL/mL (leaves and stems) for *C. heliotropiifolius*; 1.00 to 8.00 μL/mL (leaves) and 5.0 to 40.0 μL/mL (stems) for *C. blanchetianus*; 5.00 to 40.0 μL/mL (leaves) and 10.0 to 80 μL/mL (stems) for *C. grewioides*. The concentration of botanical and conventional insecticides used as positive control vary from 0.05 to 0.80 μL/mL for Azamax® and from 0.01 to 0.40 μL/mL for Ortus® 50 SC. 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 in the bottom of a Petri dish atop, a 10 cm diameter disc of filter paper wetted with distilled water. Five adult female mites were introduced into each Petri dish. Mortality was determined under a dissecting microscope 24 h after the treatment. All mites were considered dead if appendages did not move when prodded with a fine paintbrush. Control mites were held on leaf discs painted with the carrier solvent alone. All treatments were replicated five times. Mortality was recorded and submitted to Probit analysis. To determine LC_{50} determination with 95 percent confidence level.

Repellency assay

The repellent effect of the *Croton* oils were evaluated using area preference method described by Nascimento *et al*. (2012). Test areas consisted of disks of pig bean leaves (*C. ensiformis*) cut in half (9.81 cm^2). Test solutions were prepared by diluting 10 mg of *Croton* oils or Ortus® 50 SC or Azamax® as a positive control in 2 mL water plus 0.1% of spreader sticker adjuvante (Tween-80). The

concentrations of *Croton* ranged from 0.02 to 0.50 µL/mL (leaves and stems) for *C. adenocalyx*, 1.0 to 25.0 µL/mL (leaves) and 1.0 to 50.0 µL/mL (stems) for *C. heliotropiifolius*; 0.05 to 0.50 µL/mL (leaves) and 0.50 to 4.0 µL/mL (stems) for *C. blanchetianus*; 1.00 to 13.0 µL/mL (leaves and stems) for *C. grewioides*. While the concentration Azamax®, insecticides used as positive control were ranged from 0.01 to 0.30 µL/mL. Each concentration was uniformly applied to a half-leaf disk using a micropipette. The other half leaf was treated with ethanol alone and used as control. Treated and control disks were air-dried for 10 min to evaporate the solvent completely. Each disk was placed into a 10 cm Petri dish. Ten adult mites were released at the center of the disk and the Petri dishes were covered. The treatments were replicated 15 times. The number of mites on the control and treated areas of the disks was recorded after 2 h. To estimate the concentration that repelled 50% and 90% of the mites (RC_{50}) exposed to each oil, the number of mites on the control and treated areas was recorded and submitted to probit analysis.

Statistical analysis

The mortality and repellence data were subjected to Probit analysis at $p>0.05$ using PROC PROBIT (Finney, 1971). The concentrations used were calculated based on the logarithmic series proposed by Robertson *et al*. (2017).

RESULTS

Hydrodistillation of the different parts of *C. heliotropiifolius*, *C. adenocalyx*, *C. blanchetianus* and *C. grewioides* produced yellowish oils with a pleasant aroma. The greatest yields were achieved with the oils from the leaves and stems of *C. grewioides* (leaf oil = 2.40 ± 0.02%; stem oil = 0.10 ± 0.01%), followed by *C. blanchetianus* (leaf oil = 0.50 ± 0.03%; stem oil = 0.03 ± 0.00%), *C. adenocalyx* (leaf oil = 0.36 ± 0.01%; stem oil = 0.01 ± 0.00%) and *C. heliotropiifolius* (leaf oil = 0.21 ± 0.02%; stem oil = 0.01 ± 0.00%).

GC-MS analysis of the oils enabled the identification of 94 compounds (Table 1). The composition of the *C. heliotropiifolius* (leaf oil = 91.79 ± 0.44%; stem oil = 87.76 ± 0.87%), *C. adenocalyx* (leaf oil = 90.22 ± 0.73%; stem oil = 91.64 ± 0.76%) and *C. blanchetianus* (leaf oil = 90.56 ± 0.93%; stem oil = 97.93 ± 0.78%) oils predominantly consisted of sesquiterpenes. In contrast, the leaf and stem oils from *C. grewioides* had higher percentages of phenylpropanoids (75.7 ± 0.48% and 75.8 ± 0.41%, respectively).

Table No. 1A
Percentage compositions of the essential oils from Croton species

Compound	RI[a]	*C. heliotropiifolius* (%±SE[b])		*C. adenocalyx* (%±SE[b])	
		Leaves	Stems	Leaves	Stems
	Yield %	0.21 ± 0.02	0.01 ± 0.00	0.36 ± 0.01	0.01 ± 0.00
α-Pinene[c]	929	-	-	4.65 ± 0.09	0.25 ± 0.00
β-Pinene[c]	973	-	-	3.12 ± 0.10	1.38 ± 0.11
δ-3-Carene[d]	1005	-	-	-	-
o-Cymene[d]	1022	1.23 ± 0.01	2.10 ± 0.07		
Limonene[c]	1021	-	-	-	0.24 ± 0.00
1,8-Cineole[c]	1031	-	-		
Linalool[c]	1092	-	-	-	0.53 ± 0.00
trans-Sabinene hydrate[d]	1098	-	-	-	0.81 ± 0.00
Myrcenol[d]	1113	-	-	-	2.64 ± 0.03
Camphor[c]	1144	-	-	-	-
Isoborneol[d]	1156	-	3.34 ± 0.11	-	-
α-Terpineol[d]	1192	-	-	-	-
Methyl chavicol[d]	1196	-	-	-	-
p-Anisaldehyde[d]	1250	-	-	-	-
(*Z*)-Anethole[d]	1251	-	-	-	-
(*E*)-Anethole[d]	1280	-	-	-	-
Bornyl acetate[d]	1285	-	0.91 ± 0.01	-	-
δ-Elemene[d]	1330	-	-	-	-

α-Cubebene[d]	1341	-	-	-	-
α-Copaene[d]	1369	-	-	-	-
3,4-Dihydro-coumarin[d]	1376	-	3.51 ± 0.15	-	-
(*Z*)-β-Damascenone[d]	1381	-	-	-	-
β-Cubebene[d]	1383	-	-	1.66 ± 0.10	1.13 ± 0.09
β-Bourbonene[d]	1384	-	-	1.72 ± 0.17	0.43 ± 0.00
iso-Longifolene[d]	1385	-	-	-	3.23 ± 0.11
β-Elemene[d]	1389	6.81 ± 0.15	17.28 ± 079	9.34 ± 0.34	3.46 ± 0.13
Cyperene[d]	1398	-	1.36 ± 0.10	-	-
Methyl eugenol[c]	1401	-	-	-	-
β-Caryophyllene[c]	1414	20.82 ± 0.38	8.05 ± 0.22	15.64 ± 0.62	12.21 ± 0.72
β-Cedrene[d]	1416	-	-	0.57 ± 0.01	0.55 ± 0.01
β-Ylangene[d]	1417	-	-	0.52 ± 0.00	0.53 ± 0.00
β-Duprezianene[d]	1418	-	-	0.58 ± 0.00	0.48 ± 0.00
Lavandulyl isobutanoate[d]	1419	-	-	-	1.05 ± 0.01
Linalol butanoate[d]	1421	-	-	5.00 ± 0.20	5.13 ± 0.14
Dictamnol[d]	1428	-	-	1.48 ± 0.05	0.77 ± 0.02
cis-Thujupsene[d]	1429	-	-	0.31 ± 0.02	-
β-Copaene[d]	1429	-	-	11.45 ± 0.32	8.45 ± 0.28
β-Gurjunene[d]	1430	-	-	1.40 ± 0.03	6.27 ± 0.20
trans-α-Bergamotene[d]	1430	-	-	-	-
γ-Elemene[d]	1432	0.93 ± 0.00	-	14.80 ± 0.84	6.85 ± 0.12
β-Dihydro-ionone[d]	1434	-	-	3.44 ± 0.07	0.87 ± 0.02
α-Guaiene[d]	1436	-	-	0.71 ± 0.01	0.57 ± 0.05
Aromadendrene[c]	1436	-	-	1.90 ± 0.14	2.91 ± 0.12
α-Himachalene[d]	1446	-	-	-	-
neo-α-Clovene[d]	1448	-	-	-	-
α-Humulene[c]	1449	3.92 ± 0.02	1.87 ± 0.05	3.08 ± 0.23	1.15 ± 0.13
α-Patchoulene[d]	1453	-	-	0.47 ± 0.00	1.85 ± 0.11
allo-Aromadedrene[d]	1458	-	-	2.24 ± 0.10	4.93 ± 0.15
(*E*)-Methyl isoeugenol[d]	1459	-	-	-	-
Dehydro-aromadedrene[d]	1459	-	-	3.28 ± 0.07	4.43 ± 0.13
9-*epi*-(*E*)-Caryophyllene[d]	1464	-	-	1.70 ± 0.01	3.08 ± 0.22
γ-Gurjunene[d]	1472	-	-	1.10 ± 0.05	4.27 ± 0.17
β-Chamigrene[d]	1474	-	-	0.72 ± 0.01	2.44 ± 0.13
γ-Muurolene[d]	1477	-	-	-	-
γ-Curcumene[d]	1478	-	-	0.84 ± 0.01	1.76 ± 0.06
γ-Himachalene[d]	1480	-	-	-	2.84 ± 0.02
Germacrene D[c]	1484	-	-	1.94 ± 0.08	3.56 ± 0.11
β-Selinene[d]	1486	-	-	1.35 ± 0.10	-
δ-Selinene[d]	1491	-	-	2.98 ± 0.16	4.56 ± 0.13
Viridiflorene [d]	1494	-	-	-	1.88 ± 0.16
(*E*)-Methyl isoeugenol[d]	1499	-	-	-	-
Bicyclogermacrene[d]	1500	6.59 ± 0.00	2.43 ± 0.07	-	-
β-Bisabolene[d]	1503	0.93 ± 0.02	1.68 ± 0.06	-	-
α-Bulnesene[d]	1506	-	-	-	-
δ-Cadinene[d]	1519	-	1.16 ± 0.05	-	-
δ-Cadinene[d]	1520	-	-	-	-
(*Z*)-Nerolidol[d]	1534	0.70 ± 0.00	-	-	-
α-Calacorene[d]	1544	-	-	-	-
Elemol[d]	1549	0.83 ± 0.01	2.02 ± 0.04	-	-
Elemicin[d]	1554	-	1.01 ± 0.08	-	-

Germacrene B[d]	1556	9.33 ± 0.26	2.83 ± 0.07	-	-
Geranyl butanoate[d]	1557	-	-	-	-
β-Calacorene[d]	1560	-	-	-	-
Spathulenol[c]	1572	16.37 ± 0.55	1.43 ± 0.05	-	-
Caryophyllene oxide[c]	1582	8.34 ± 0.22	2.06 ± 0.01	-	-
Globulol[d]	1589	-	-	-	-
Viridiflorol[d]	1590	-	-	-	-
Rosifoliol[d]	1599	-	-	-	-
Guaiol[d]	1602	4.99 ± 0.08	18.38 ± 0.78	-	-
β-Himachalene oxide[d]	1610	0.99 ± 0.01	-	-	-
β-Acorenol[d]	1631	-	-	-	-
epi-*α*-Muurolol[d]	1641	-	1.53 ± 0.07	-	-
α-Muurolol[d]	1645	-	0.91 ± 0.01	-	-
Cubenol[d]	1645	-	2.49 ± 0.03	-	-
β-Eudesmol[d]	1649	1.61 ± 0.01	2.62 ± 0.04	-	-
Himachalol[d]	1650	1.57 ± 0.02	-	-	-
α-Cadinol[d]	1654	-	-	-	-
Valerianol[d]	1657	3.66 ± 0.11	10.62 ± 0.67	-	-
Bulnesol[d]	1666	1.42 ± 0.10	5.84 ± 0.11	-	-
Cadalene[d]	1670			-	-
Foeniculin[d]	1677	1.99 ± 0.06	3.26 ± 0.07	-	-
Khusinol[d]	1679			-	-
α-Bisabolol[d]	1681	-	-	-	-
Hexadecanoic acid[d]	1955	-	-	-	-
Total		93.02 ± 0.47	98.69 ± 1.01	97.99 ± 0.88	97.49 ± 0.79
Monoterpenes		1.23 ± 0.04	9.86 ± 0.11	7.77 ± 0.10	5.85 ± 0.05
Sesquiterpenes		91.79 ± 0.44	87.76 ± 0.87	90.22 ± 0.73	91.64 ± 0.76
Fatty acid		-	-	-	-
Phenylpropanoids		-	1.01 ± 0.08	-	-

[a]Retention indices calculated from retention times in relation to those of a series of C8 –C40 n-alkanes on a DB-5 capillary column; bStandard Error; cMethod of identification: Retention Index; Mass Spectroscopy; Co-Injection with authentic compounds; dMethod of identification: Retention Index and Mass Spectroscop

Table No. 1B
Percentage compositions of the essential oils from Croton species

Compound	RI[a]	*C. blanchetianus* (%±SE[b])		*C. grewioides* (%±SE[b])	
		Leaves	Stems	Leaves	Stems
	Yield %	0.50 ± 0.03	0.03 ± 0.001	2.40 ± 0.02	0.10 ± 0.01
α-Pinene[c]	929	-	-	-	-
β-Pinene[c]	973	-	-	-	-
δ-3-Carene[d]	1005	-	0.57 ± 0.01	-	-
o-Cymene[d]	1022			-	-
Limonene[c]	1021	-	-	-	-
1,8-Cineole[c]	1031			1.11 ± 0.11	0.50 ± 0.03
Linalool[c]	1092	-	-	0.24 ± 0.02	0.21 ± 0.00
trans-Sabinene hydrate[d]	1098	-	-	-	-
Myrcenol[d]	1113	-	-	-	-
Camphor[c]	1144	-	-	0.83 ± 0.05	1.54 ± 0.01
Isoborneol[d]	1156	-	-	-	-
α-Terpineol[d]	1192	-	-	0.53 ± 0.02	0.92 ± 0.03

Methyl chavicol[d]	1196	-	-	1.92 ± 0.11	0.50 ± 0.00
p-Anisaldehyde[d]	1250	-	-	0.51 ± 0.00	1.44 ± 0.10
(*Z*)-Anethole[d]	1251	-	-	4.60 ± 0.07	0.82 ± 0.01
(*E*)-Anethole[d]	1280	-	-	55.51 ± 0.40	37.80 ± 0.38
Bornyl acetate[d]	1285	-	-	-	-
δ-Elemene[d]	1330	1.25 ± 0.01	-	-	-
α-Cubebene[d]	1341	0.11 ± 0.00	-	-	-
α-Copaene[d]	1369	3.73 ± 0.11	1.35 ± 0.05	2.14 ± 0.12	0.22 ± 0.00
3,4-Dihydro-coumarin[d]	1376	-	-	-	-
(*Z*)-*β*-Damascenone[d]	1381	0.37 ± 0.01	-	-	-
β-Cubebene[d]	1383	0.06 ± 0.00	-	-	-
β-Bourbonene[d]	1384	0.13 ± 0.00	-	-	-
iso-Longifolene[d]	1385	-	-	-	-
β-Elemene[d]	1389	0.35 ± 0.01	-	1.00 ± 0.04	0.32 ± 0.00
Cyperene[d]	1398	-	-	-	-
Methyl eugenol[c]	1401	-	-	10.63 ± 0.34	6.61 ± 0.50
β-Caryophyllene[c]	1414	0.81 ± 0.03	-	4.50 ± 0.04	0.20 ± 0.01
β-Cedrene[d]	1416	-	-	-	-
β-Ylangene[d]	1417	-	-	-	-
β-Duprezianene[d]	1418	-	-	-	-
Lavandulyl isobutanoate[d]	1419	-	-	-	-
Linalol butanoate[d]	1421	-	-	-	-
Dictamnol[d]	1428	-	-	-	-
cis-Thujupsene[d]	1429	-	-	-	-
β-Copaene[d]	1429	-	-	-	-
β-Gurjunene[d]	1430	0.12 ± 0.01	-	-	-
trans-α-Bergamotene[d]	1430	-	-	0.34 ± 0.03	0.64 ± 0.01
γ-Elemene[d]	1432	0.09 ± 0.00	-	-	-
β-Dihydro-ionone[d]	1434	-	-	-	-
α-Guaiene[d]	1436	0.14 ± 0.00	-	-	-
Aromadendrene[c]	1436	-	-	-	-
α-Himachalene[d]	1446	1.73 ± 0.08	-	-	-
neo-*α*-Clovene[d]	1448	0.26 ± 0.03	-	-	-
α-Humulene[c]	1449	0.29 ± 0.02	-	-	-
α-Patchoulene[d]	1453	-	-	-	-
allo-Aromadedrene[d]	1458	-	2.03 ± 0.10	-	-
(*E*)-Methyl isoeugenol[d]	1459	-	-	2.90 ± 0.07	0.44 ± 0.00
Dehydro-aromadedrene[d]	1459	0.23 ± 0.01	1.37 ± 0.09	-	-
9-*epi*-(*E*)-Caryophyllene[d]	1464	-	-	-	-
γ-Gurjunene[d]	1472	0.81 ± 0.06	-	-	-
β-Chamigrene[d]	1474	-	-	-	-
γ-Muurolene[d]	1477	0.69 ± 0.04	-	-	-
γ-Curcumene[d]	1478	-	-	-	-
γ-Himachalene[d]	1480	-	-	-	-
Germacrene D[c]	1484		-	0.41 ± 0.00	0.15 ± 0.02
β-Selinene[d]	1486	-	-	-	-
δ-Selinene[d]	1491	-	-	-	-
Viridiflorene [d]	1494	-	-	-	-
(*E*)-Methyl isoeugenol[d]	1499	-	-	6.70 ± 0.10	31.03 ± 0.33
Bicyclogermacrene[d]	1500	-	-	-	-
β-Bisabolene[d]	1503	-	-	-	-
α-Bulnesene[d]	1506	-	2.65 ± 0.05	-	-

δ-Cadinene[d]	1519	17.86 ± 0.33	6.80 ± 0.20	-	-
δ-Cadinene[d]	1520	-	-	1.32 ± 0.04	0.21 ± 0.00
(*Z*)-Nerolidol[d]	1534	-	-	-	-
α-Calacorene[d]	1544	2.49 ± 0.10	1.77 ± 0.16	-	-
Elemol[d]	1549	-	-	-	-
Elemicin[d]	1554	-	-	-	-
Germacrene B[d]	1556	-	-	-	-
Geranyl butanoate[d]	1557	1.09 ± 0.01	-	-	-
β-Calacorene[d]	1560	0.89 ± 0.02	-	-	-
Spathulenol[c]	1572	24.1 ± 0.28	43.52 ± 1.01	1.63 ± 0.01	0.91 ± 0.02
Caryophyllene oxide[c]	1582	-	-	2.84 ± 0.05	2.53 ± 0.10
Globulol[d]	1589	7.30 ± 0.15	6.06 ± 0.32	-	-
Viridiflorol[d]	1590	3.61 ± 0.09	-	-	-
Rosifoliol[d]	1599	-	3.01 ± 0.16	-	-
Guaiol[d]	1602	-	-	-	-
β-Himachalene oxide[d]	1610	-	-	-	-
β-Acorenol[d]	1631	11.16 ± 0.09	13.38 ± 0.21	-	2.62 ± 0.11
epi-*α*-Muurolol[d]	1641	-	-	-	-
α-Muurolol[d]	1645	-	-	-	-
Cubenol[d]	1645	2.02 ± 0.06	2.59 ± 0.06	0.51 ± 0.00	1.01 ± 0.12
β-Eudesmol[d]	1649	-	-	-	-
Himachalol[d]	1650	-	-	-	-
α-Cadinol[d]	1654	6.73 ± 0.11	7.38 ± 0.19	-	-
Valerianol[d]	1657	-	-	-	-
Bulnesol[d]	1666	-	-	-	-
Cadalene[d]	1670	-	3.13 ± 0.21	-	8.42 ± 0.08
Foeniculin[d]	1677	-	-	-	-
Khusinol[d]	1679	1.21 ± 0.02	2.89 ± 0.07	-	-
α-Bisabolol[d]	1681	0.93 ± 0.03	-	-	-
Hexadecanoic acid[d]	1955	9.02 ± 0.18	-	-	-
Total		99.58 ± 0.97	98.50 ± 0.80	98.80 ± 0.51	98.64 ± 0.57
Monoterpenes		-	0.57 ± 0.01	9.64 ± 0.11	5.82 ± 0.10
Sesquiterpenes		90.56 ± 0.93	97.93 ± 0.78	14.54 ± 0.05	6.13 ± 0.13
Fatty acid		9.02 ± 0.18	-	-	-
Phenylpropanoids		-	-	75.7 ± 0.48	75.8 ± 0.41

[a]Retention indices calculated from retention times in relation to those of a series of C8 –C40 n-alkanes on a DB-5 capillary column; bStandard Error; cMethod of identification: Retention Index; Mass Spectroscopy; Co-Injection with authentic compounds; dMethod of identification: Retention Index and Mass Spectroscop

Twenty-eight compounds were identified in the *C. heliotropiifolius* oils, accounting for 93.02 ± 0.47% and 98.69 ± 1.01% of the leaf and stem oils, respectively. β-Caryophyllene (20.82 ± 0.38%) and spathulenol (16.37 ± 0.55%) were identified as the major constituents of the leaf oil, whereas guaiol (18.38 ± 0.78%), β-elemene (17.28 ± 079%) and valerianol (10.62 ± 0.67%) were the major constituents of the stem oil.

Thirty-seven compounds were identified in the *C. adenocalyx* oils, accounting for 97.99 ± 0.88% and 97.49 ± 0.79% of the leaf and stem oils, respectively. The analysis revealed a highly similar chemical composition between the two oils, with β-caryophyllene (15.64 ± 0.62% / 12.21 ± 0.72%), γ-elemene (14.80 ± 0.84% / 6.85 ± 0.12%) and β-copaene (11.45 ± 0.32% / 8.45 ± 0.28%) as the major constituents of the leaf/stem oil.

Thirty-six compounds were identified in the C. blanchetianus oils, accounting for 99.58 ± 0.97% and 98.50 ± 0.80% of the leaf and stem oils, respectively. Spathulenol (24.1 ± 0.28% / 43.52 ± 1.01%), δ-cadinene (17.86 ± 0.33% / 6.80 ± 0.20%) and β-acorenol (11.16 ± 0.09% / 13.38 ± 0.21%)

were the major constituents of the leaf/stem oil. A significant percentage of hexadecanoic acid (9.02 ± 0.18%) was also found in the leaf oil.

Twenty-two compounds were found in the *C. grewioides* oils, accounting for 98.80 ± 0.51% and 98.64 ± 0.57% of the leaf and stem oils, respectively. Phenopropanoids constituted the predominant chemical class, corresponding to 75.7 ± 0.48% and 75.8 ± 0.41% of the leaf and stem oils, respectively. The two oils had a similar chemical profile. With the exception of β-acorenol (2.62 ± 0.11%) and cadalene (8.42 ± 0.08%), the other compounds were found in the oils from both parts of the plants. The major constituent of the leaf oil was (E)-anethole (55.51 ± 0.40%), followed by methyl eugenol (10.63 ± 0.34%). The major constituent of the stem oil was (E)-anethole (37.80 ± 0.38%), followed by (E)-methyl isoeugenol (31.03 ± 0.33%).

Table No. 2 displays the mean lethal (LC_{50}) and repellency (RC_{50}) concentrations for the essential oils from the leaves and stems of the four Croton species. All oils were toxic to T. urticae and exhibited repellent properties against the mite.

Table No. 2
Toxicity by residual contact and repellence activity of essential oil of four species *Croton* collected in Pernambuco-Brazil against *Tetranychus urticae*

Essential oil		Bioassay	N[a]	DF[b]	Slope ± SE[c]	Activity[d] (CI[e] 95%)	χ^{2}[f]
C. heliotropiifolius	leave	Contact	720	6	2.37 ± 0.14	46.83 (41.84-52.25)	5.08
		Repellence	1200	6	1.46 ± 0.10	9.26 (7.501-11.06)	10.53
	stems	Contact	720	6	2.35 ± 0.14	52.63 (47.07-58.67)	4.29
		Repellence	1200	6	2.15 ± 0.13	27.08 (23.97-30.13)	6.42
C. adenocalyx	leaves	Contact	720	6	0.70 ± 0.04	0.97 (0.61-1.44)	8.84
		Repellence	1200	6	0.70 ± 0.04	0.11 (0.08-0.16)	8.79
	stems	Contact	630	5	0.88 ± 0.05	2.36 (1.63-3.26)	4.81
		Repellence	1200	6	0.71 ± 0.04	0.14 (0.09-0.18)	9.96
C. blanchetianus	leaves	Contact	720	6	1.88 ± 0.11	2.95 (2.58-3.38)	4.52
		Repellence	1200	6	0.71 ± 0.03	0.19 (0.14-0.25)	7.34
	stems	Contact	720	6	2.14 ± 0.13	17.77 (15.67-20.07)	6.38
		Repellence	1200	6	0.90 ± 0.05	1.67 (1.31-2.09)	8.71
C. grewioides	leaves	Contact	720	6	1.42 ± 0.10	21.01 (16.68-25.66)	6.23
		Repellence	1200	6	1.12 ± 0.06	7.05 (5.77-8.48)	6.47
	stems	Contact	720	6	1.37 ± 0.10	28.64 (22.80-34.89)	8.24
		Repellence	1200	6	1.06 ± 0.06	6.70 (5.39-8.15)	9.44
Azamax®		Contact	630	5	2.46 ± 0.19	0.31 (0.28-0.36)	8.42
		Repellence	1050	5	0.97 ± 0.09	0.04 (0.03-0.21)	1.02
Ortus		Contact	810	7	1.04 ± 0.07	0.15 (0.08-0.25)	8.66

[a]number of mites; [b]degree of freedom; [c]standart error; [d] LC_{50} for residual contact (µL mL^{-1}) and RC_{50} for repellence activity (µL mL^{-1}); [e]confidence interval; [f]chi-square.

The susceptibility of the mite and the repellent action of the oils varied according to the species and part of the plant studied. The toxicity of the leaf and stem oils from the same species only differed for *C. adenocalyx* and *C. blanchetianus* and the repellent action of the leaf and stem oils from the same species only differed for C. *heliotropiifolius* and *C. blanchetianus*. The oils with the greatest toxicity toward the mite were those from *C. adenocalyx*, from which the leaf and stem oils were threefold and 7.5-fold more toxic than the oils from *C. blanchetianus*, 21.7-fold and 12-fold more toxic than the oils from *C. grewioides* as well as 48.3-fold and 22.3-fold more toxic than the oils from *C. heliotropiifolius*, respectively.

In the repellency assays, two groups with different levels were found for the leaf oils. Group 1 was formed by the *C. adenocalyx* and *blanchetianus* oils and was approximately nine-fold more repellent than Group 2, which was composed of the *C. grewioides* and *C. heliotropiifolius* oils. The following was the order of repellency for the stem oils: *C. adenocalyx* > *C. blanchetianus* > *C. grewioides* > *C. heliotropiifolius*

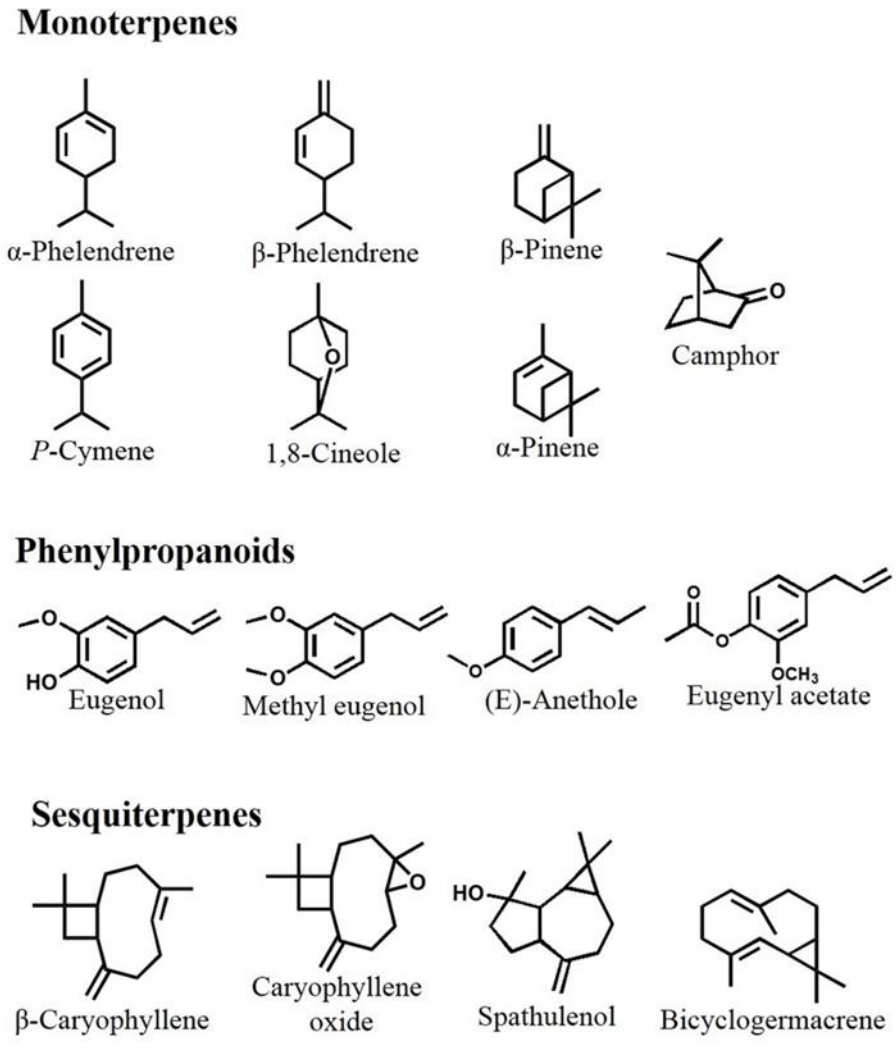

Figure No. 1
Structures of the main compounds found in *Croton* essential oils

In the comparison with the synthetic and plant-based commercial acaricides used as positive controls, none of the oils investigated was more toxic to *T. urticae* than Ortus® and Azamax®. Regarding the repellent action, the C. adenocalyx (leaf and stem) and *C. blanchetianus* (leaf) oils had the same level of repellency as Azamax®.

To determine the relative lethal and sublethal properties of the major chemical constituents found in the *Croton* oils, seven monoterpenes, four phenylpropanoids and four sesquiterpenes were selected for the investigation of toxicity by residual contact and repellent action against *T. urticae*. Figure No. 1 presents the chemical structures of the selected constituents classified according to their biogenetic origins.

Table No. 3
Toxicity by residual contact and repellence of the selected constituents against *Tetranychus urticae*.

Constituents	Bioassay	N[a]	DF[b]	Slope ± SE[c]	Activity[d] (CI[e] 95%)	χ^{2f}
eugenol	Contact	630	5	2.01 ± 0.34	9.13 (7.62-10.12)	7.39
	Repellency	240	6	0.35 ± 0.02	0.001 (0.0009-0.002)	2.19
α-pinene	Contact	810	7	1.55 ± 0.11	26.77 (21.81-33.49)	7.49
	Repellency	240	6	1.78 ± 0.27	2.37 (1.63-3.06)	7.28
β-pinene	Contact	630	5	1.45 ± 0.09	27.80 (23.38-36.13)	1.98
	Repellency	240	6	3.67 ± 0.41	3.04 (2.67-3.43)	2.56
1.8-cineole	Contact	630	5	1.62 ± 0.20	81.80 (72.79-107.84)	4.98
	Repellency	210	5	6.30 ± 0.73	6.07 (5.52-6.57)	1.42
camphor	Contact	720	6	2.02 ± 0.13	10.29 (8.91-11.74)	8.90
	Repellency	240	6	4.50 ± 0.52	5.01 (4.49-5.52)	10.19
(*E*)-anethole	Contact	720	6	1.23 ± 0.07	1.49 (1.19-1.84)	9.98
	Repellency	210	5	1.25 ± 0.15	0.02 (0.01-0.03)	1.45
methyl eugenol	Contact	720	6	2.04 ± 0.15	7.67 (6.82-8.61)	4.00
	Repellency	210	5	1.21 ± 0.14	0.001 (0.0007-0.002)	5.34
β-caryophyllene	Contact	630	5	2.25 ± 0.28	0.74 (0.56-0.91)	1.27
	Repellency	240	6	2.17 ± 0.23	5.07 (4.08-6.21)	1.15
bicyclogermacrene	Contact	720	6	1.48 ± 0.08	4.95 (4.14-5.88)	9.87
	Repellency	240	6	2.25 ± 0.24	18.28 (14.80-22.19)	4.23
spathulenol	Contact	630	5	1.09 ± 0.06	0.70 (0.54-0.90)	8.23
	Repellency	240	6	2.38 ± 0.25	9.51 (7.79-11.43)	2.80
caryophyllene oxide	Contact	630	5	1.97 ± 0.20	105.58 (78.82-125.12)	8.02
	Repellency	240	6	2.54 ± 0.27	13.77 (11.50-16.35)	3.81
ρ-cymene	Contact	810	7	1.45 ± 0.21	28.53 (22.35-35.45)	9.38
	Repellency	240	6	1.92 ± 0.20	0.90 (0.71-1.13)	3.86
eugenyl acetate	Contact	810	7	1.90 ± 0.11	5.67 (5.01-6.45)	0.27
	Repellency	210	5	1.34 ± 0.16	0.002 (0.001-0.004)	7.87
α-phellandrene	Contact	720	6	2.76 ± 0.19	40.80 (37.21-44.69)	3.66
	Repellency	240	6	1.67 ± 0.26	2.57 (1.78-3.34)	7.08
β-phellandrene	Contact	720	6	2.07 ± 0.13	2.67 (2.36-3.02)	5.91
	Repellency	210	5	2.12 ± 0.24	2.13 (1.27-2.73)	3.22

[a]number of mites; [b]degree of freedom; [c]standart error; [d] LC_{50} for residual contact (µL mL^{-1}) and RC_{50} for repellence activity (µL mL^{-1}); [e]confidence interval; [f]chi-square.

Based on the LC_{50} (toxicity) and RC_{50} (repellent effect) values estimated for the selected constituents (Table No. 3), all compounds were toxic by residual contact and acted as repellents to the mite.

Toxicity by residual contact varied according to the chemical class of the compounds. Although the results indicated an increasing order of toxicity (monoterpenes < phenylpropanoids < sesquiterpenes), it was not possible to establish this order for all compounds investigated. For example, while the sesquiterpenes *β*-caryophyllene and spatulenol (with the same level of toxicity) were more toxic to the mite, the sesquiterpene caryophyllene oxide was the least toxic of all the constituents tested. Among the phenylpropanoids, the greatest toxicity was found for (*E*)-anethole, followed by eugenol, eugenyl acetate and methyl eugenol (with the same level of toxicity). Regarding the monoterpenes, *β*-phellandrene exhibited the greatest toxicity, followed by camphor.

In terms of repellency according to the chemical class of the compounds investigated, phenylpropanoids were the most repellent, followed by monoterpenes and sesquiterpenes. The exception was the sesquiterpene *β*-caryophyllene, which had the same level of repellent action as the oxygenated monoterpenes 1,8-cienol and camphor. Among the

phenylpropanoids, eugenol, methyl eugenol and eugenyl acetate (with the same level of repellency) were the most repellent. Non-oxygenated monoterpenes were more repellent that the oxygenated forms, particularly *α* and *β*-phellandrene as well as *α* and *β*-pinene, which had the same level of repellency. Among the sesquiterpenes tested, bicyclogermacrene and caryophyllene oxide had the lowest repellent action (Table No. 3).

DISCUSSION

The *C. grewioides* oils were rich in phenylpropanoids, whereas sesquiterpenes were the predominant chemical class in the *C. heliotropiifolius*, *C. adenocalyx* and *C. blanchetianus* oils. These results are in agreement with data reported for other species of the genus (Dória *et al*., 2010; Filho *et al*., 2017; Castro *et al*., 2019).

The major chemical constituents of the *Croton* species investigated herein have also been reported for these species occurring in other locations of northeast Brazil. For instance, *β*-caryophyllene, which was identified as the major constituent of the *C. adenocalyx* and *C. heliotropiifolius* oils in the present investigation, was also reported as the major constituent of the leaf oil from *C. heliotropiifolius* (28.61 to 46.99%) collected in the state of Pernambuco (Filho *et al*., 2017) as well as *C. blanchetianus* (14.58%) and *C. adenocalyx* (10.23%) collected in the state of Ceará (Pinho-da-Silva *et al*., 2010; De Lima *et al*., 2010). For *C. heliotropiifolius*, *β*-caryophyllene was also found as the major constituent in the oils from the shoots of samples collected in the states of Bahia (23.85 ± 0.36%) (Araújo *et al*., 2017) and Sergipe (35.82%) (Dória *et al*., 2010).

β-Elemene, which was the major constituent of the stem oil from *C. heliotropiifolius*, was also reported for the leaf oil from *C. adenocalyx* (8.31%) occurring in the state of Ceará (De Lima *et al*., 2010). For the *C. blanchetianus* oils, we found the same chemotype [spathulenol] (leaf oil = 24.1 ± 0.28%; stem oil = 43.52 ± 1.01%) described for samples of the species collected in the states of Ceará (38.32%) (Lima *et al*., 2013) and Pernambuco (31.5-10.3%) (Souza *et al*., 2017). The chemical profile found in the present study for the leaf and stem oils from *C. grewioides*, with (*E*)-anethole as the major constituent, is in agreement with data reported by Silva *et al*. (2008) for the same species collected in the state of Pernambuco. On the other hand, Castro *et al*. (2019) investigated two samples collected in the state of Piauí and found the phenylpropanoid methyl chavicol as the major constituent (83.59% in São João do Piaui and 95.38% in Caxingó), which is the isomer of (*E*)-anethole.

As expected, qualitative and quantitative differences were found in the chemical profiles of the essential oils among the species of *Croton*. Another finding of the present study was the similarity in the chemical profile of the oils from the species *C. heliotropiifolius*, *C. adenocalyx* and *C. blanchetianus* to profiles described in previous studies involving these species collected from different locations and in different seasons. This is an important finding for the standardization of a chemical profile that can serve as the basis for the formulation of an acaricide for use in the management of *T. urticae* by family farmers in agricultural niches in the state of Pernambuco.

Plants and their extracts have been used for millennia to protect against or repel arthropods. There are several reports in the literature in which essential oils from different taxa, basically comprising monoterpenes, sesquiterpenes and phenylpropanoids, have consistently shown activity against a variety of arthropods (Nerio *et al*., 2010; Mossa, 2016). Evidence regarding the obtainment of a product formulated on the basis of essential oils for the control of mites and/or insects can be found in the scientific literature, including records of patents with native and cultivated aromatic plants in northeast Brazil (Araújo *et al*., 2015; Melo & da Camara, 2019).

In the present study, the differences in relative toxicity and repellent action against *T. urticae* obtained for the essential oils from *C. heliotropiifolius, C. adenocalyx, C. blanchetianus* and *C. grewioides* can be attributed to quantitative and qualitative differences in the chemical composition, as determined by GC-MS.

With the exception of the *C. grewioides* oil, which was evaluated for its effect on the cattle tick *Rhipicephalus microplus* (Castro *et al*., 2019), previous investigations of the biological properties of *Croton* oils were limited to insecticidal action against *Nasutitermes corniger* (*C. blanchetianus*), an important structural pest in the Neotropics (Lima *et al*., 2013), larvae of the mosquito *Aedes aegypti*, which is the main transmitter of dengue, zika and chikungunya (*C. heliotropifolius* and *C. blanchetianus*) (Morais *et al*., 2006; Doria *et al*., 2010), and the storage grain pest *Zabrotes subfasciatus* (*C. grewioides*) (Silva *et al*., 2008).

A comparison of the acaricidal activity of the *C. grewioides* oil from Caxingó in the state of Piauí (northeast Brazil) against *R. microplus* larvae and

adults (Castro *et al*., 2019) and the activity found in the present study against *T. urticae* adults indicates that the *C. grewioides* oil from the state of Pernambuco, Brazil is much more efficient. This difference may be explained by differences between the arthropods, the chemical composition of the oils and the methods used for the assessment of the acaricidal action.

The present findings reveal that the *Croton* oils are more efficient against *T. urticae* through residual contact than oils from the leaves of *C. rhamnifolioides* from three localities in the state of Pernambuco, Brazil against the same pest. Indeed, the best result in the present study was obtained with the oil from *C. adenocalyx*, which was 2.5-fold more toxic than the oil from *C. rhamnifolioides* collected in the municipality of Buíque in the state of Pernambuco (da Camara *et al*., 2017). These results may be explained by differences in the chemical composition of the oils investigated.

This is the first report of the acaricidal action of *Croton* oils against *T. urticae* and the first report of the biological property of the essential oil from *C. adenocalyx*. None of the oils investigated in this study was more toxic to *T. urticae* than the commercial acaricides use as positive controls. However, as complex mixtures of monoterpenes, sesquiterpenes and/or phenylpropanoids, essential oils can affect different target sites at the same time, thereby delaying the development of resistant pest populations in comparison to a single active ingredient in a conventional insecticide (Koul & Walia, 2009) or even a biopesticide, such as azadirachtin (Feng & Isman, 1995).

The extensive use of acaricidal agents for the control of *T. urticae* in agricultural niches in the state of Pernambuco, Brazil has led to the development of populations resistant to the main classes of acaricides currently recommended and used in Brazil, including fenpyroximate, which is the active ingredient of Ortus® 50 SC (Sato *et al*., 2004) used in the present investigation as one of the positive controls.

Toxicity by residual contact of the chemical constituents found in essential oils against *T. urticae* has been investigated, including the same compounds analyzed in the present study, such as *ρ*-cymene, *β*-caryophyllene, *β*-pinene, *α*-pinene, 1,8-cineole, camphor, bicyclogermacrene and eugenol (Attia *et al*., 2012; Araújo *et al*., 2012; Moraes *et al*., 2017; de Melo *et al*., 2018; Hong *et al*., 2018; Born *et al*., 2018; Cao *et al*., 2019; Abdelgaleil *et al*., 2019;). However, this is the first report of the acaricidal activity against this pest of the constituents (*E*)-anethole, methyl eugenol, spathulenol, caryophyllene oxide, eugenyl acetate, *α*-phellandrene and *β*-phellandrene.

The greater toxicity of *β*-pinene compared to *α*-pinene to larvae of *A. aegypti* has been attributed to the influence of the exocyclic double bond (Lucia *et al*., 2007; Perumalsamy *et al*., 2009). In the present investigation, these monoterpenes had the same level of toxicity by residual contact and the same repellent action. However, the influence of the exocyclic double bond on toxicity was evident in this study for *β*-phellandrene, which was 15.3-fold more toxic to *T. urticae* than its isomer, *α*-phellandrene. On the other hand, the exocyclic double bond of *β*-phellandrene was not a determinant in the repellent activity, as the same level of repellency was found for this compound and *α*-phellandrene.

A previous investigation of the repellent action of the chemical constituents of essential oils demonstrated that phenylpropanoids constitute one of the most efficient chemical classes in terms of repellency to arthropods (Nerio *et al*., 2010). This statement is supported by the present findings, as phenylpropanoids were more repellent to *T. urticae* than monoterpenes and sesquiterpenes. One noteworthy point in the results obtained for the monoterpenes is that, although García *et al*. (2005) found that the presence of the hydroxyl group in terpenes increased the repellency to *Tribolium castaneum*, non-oxygenated monoterpenes were more repellent to *T. urticae* than oxygenated monoterpenes in the present study. These results suggest that it is not possible to generalize the repellent action of a molecule by only considering the functional group; other aspects should be considered, such as the interaction between the molecule and target site, which can vary among pests.

Although we found a relation between the chemical class and repellent action for the compounds selected in the present study, in which phenylpropanoids were more efficient than terpenes, other aspects related to the physical properties of the compounds, such as boiling point and vapor pressure, should be taken into consideration (Paluch *et al*., 2010).

CONCLUSIONS

The chemical analysis using GC-MS in the present study enabled the determination of qualitative and quantitative differences among the essential oils of the four species of *Croton*, independently of the part of the plant from which the oils were extracted. Sesquiterpenes constituted the predominant chemical

class in the leaf and stem oils from *C. heliotropiifolius*, *C. adenocalyx* and *C. blanchetianus*. In contrast, phenylpropanoids were the predominant chemical class in the leaf and stem oils from *C. grewioides*.

Based on toxicity by residual contact and repellent action, the *Croton* oils investigated here, particularly the oils from *C. adenocalyx*, constitute a promising alternative to synthetic acaricides for use in the control of *T. urticae* in family farming activities in the semiarid region of the state of Pernambuco, Brazil. However, prior to their use, further studies are required addressing safety issues for human health and the proper formulation to improve the acaricidal potency.

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