Anais da Academia Brasileira de Ciências
ISSN: 0001-3765
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Academia Brasileira de Ciências
Brasil

DE MORAES, MARCILIO M.; G. DA CAMARA, CLAUDIO A.; C. DA SILVA, MILENA M.
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Anais da Academia Brasileira de Ciências, vol. 89, núm. 3, julio-septiembre, 2017, pp. 1417-1429
Academia Brasileira de Ciências
Rio de Janeiro, Brasil

Anais da Academia Brasileira de Ciências (2017) 89(3): 1417-1429
(Annals of the Brazilian Academy of Sciences)
Printed version ISSN 0001-3765 / Online version ISSN 1678-2690
http://dx.doi.org/10.1590/0001-3765201720170139
www.scielo.br/aabc | www.fb.com/aabcjournal

Comparative toxicity of essential oil and blends of selected terpenes of *Ocotea* species from Pernambuco, Brazil, against *Tetranychus urticae* Koch

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Manuscript received on February 22, 2017; accepted for publication on March 31, 2017

ABSTRACT

Essential oils from the leaves of two species of the genus *Ocotea* that occur in the Atlantic Forest in the state of Pernambuco, Brazil, were analyzed using gas chromatography-mass spectrometry. The acaricidal activity of these oils as well as 11 selected components and blends were evaluated in fumigation and residual contact tests against the two-spotted spider mite (*Tetranychus urticae*). Sixty-seven constituents were identified, totaling 97.3 ± 0.3% and 97.8 ± 0.5% of the oils from *O. duckei* and *O. glomerata*, respectively. Sesquiterpene was the dominant class. The compounds β-caryophyllene (18.6 ± 0.1%) and aromadendrene (17.3 ± 0.6%) were the main constituents of the oils from *O. duckei* and *O. glomerata*, respectively. Acaricidal action varied depending on the method employed, species and chemical nature of the selected constituents. The mites were susceptible to the oils and chemical constituents using the fumigation method. The *O. duckei* oil was respectively 2.5-fold and 1.5-fold more toxic than the *O. glomerata* oil using the fumigation and residual contact methods. Among the selected constituents, β-caryophyllene was the most toxic, independently of the method employed. The individual toxicity of the selected compounds and their blends as well as the role of these constituents in the overall toxicity of the essential oils are also discussed.

Key words: acaricidal activity, *β*-caryophyllene, essential oil, *Ocotea, Tetranychus urticae*.

INTRODUCTION

Ocotea is one of the most representative genera of the family Lauraceae, with approximately 400 species of plants distributed throughout the American and African continents (van der Werff 1991). It is estimated that between 120 and 160 of the species for this genus occur in Brazil (Baitello 2001), with 52 species recorded for the northeastern region of the country (Quinet et al. 2010) and 11 recorded for the state of Pernambuco within this region (Barreto 1990).

Species of the genus produce a large amount of essential oils and these plants are widely used in civil construction, furniture manufacturing and cooking practices (Moraes 2005). Due to the economic potential, uncontrolled extractivism has led to the decline of natural populations, placing some species at risk of extinction, such as *Ocotea catharinensis* Mez, *Ocotea langsdorffii* (Meisn.) Mez and *Ocotea porosa* (Nees & C. Mart.) Barroso (IBAMA 1992). Plants from the genus *Ocotea* are

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also used in folk medicine for the treatment of infections, headaches, ulcers, menstrual cramps, snake bites, diarrhea, neuralgia, indigestion and pain in general (Coutinho et al. 2006, Moraes 2005, Morais 1998).

There are several reports in the literature on the chemical composition of oils from species of *Ocotea* that occur in various regions of the world. These oils are extracted from different parts of the plant and the main chemical classes found are monoterpenes (Olivero-Verbel et al. 2010), sesquiterpenes, diterpenes (Setzer et al. 2006, Takaku et al. 2007, Yamaguchi et al. 2013) and phenylpropanoids (Oltramari et al. 2004). Studies on the biological properties of these essential oils reveal a broad spectrum of activity, including antimicrobial (Leporatti et al. 2014, Farago et al. 2010), anti-inflammatory, cytotoxic, antioxidant (Destryana et al. 2014, Chaverri et al. 2011) and molluscicidal (Coutinho et al. 2007) properties, including insecticidal action against arthropods of medicinal interest, such as *Aedes aegypti* (Menut et al. 2002), and agricultural interest, such as *Sitophilus zeamais* (Mossi et al. 2013). To date, however, no studies have addressed the acaricidal activity of these oils.

The two-spotted spider mite (*Tetranychus urticae* Koch) is a major agricultural pest throughout the world. In Brazil, this polyphagous, cosmopolitan pest has caused serious damage to bean, tomato and papaya crops as well as ornamental plants grown in the field and in green houses.

The use of natural products for the control of agricultural pests has intensified in recent years (da Camara et al. 2015, Isman and Miresmailli 2011, Isman et al. 2011). Our research group has recently conducted investigations of substances with acaricidal properties as alternatives to conventional pesticides for use in the integrated management of *T. urticae* (Ribeiro et al. 2016, Nascimento et al. 2012, Neves and da Camara 2011). Moreover, few studies are found in the literature on the acaricidal activity of the chemical constituents of essential oils and their role in the biological activity of such oils. Using the acaricidal action of individual chemical constituents, it is possible to prepare artificial blends through the selection of the best combination of compounds for use in the integrated management of the two-spotted spider mite as well as investigate the degree of contribution of each compound in the artificial oil (Neves and da Camara 2016, Moraes et al. 2012, Jiang et al. 2009, Miresmailli et al. 2006).

Ocotea duckei Vattimo-Gil and *Ocotea glomerata* (Nees) Mez are among the species that occur in remaining fragments of the Atlantic Forest in the state of Pernambuco (northeastern Brazil). These species are locally known as *louro pimenta* and *caneleira*, respectively. A bibliographic survey revealed that no previous studies have investigated the chemical and biological properties of the essential oil from *O. glomerata*. Moreover, although there are reports of the chemical characterization and cardiovascular effect of the essential oil from *O. duckei* (Barbosa-Filho et al. 2007), which occurs in the state of Paraíba (northeastern Brazil), the literature offers no studies on the acaricidal activity of this oil against *Tetranychus uticae*.

Therefore, as part of systematic research on the aromatic flora and acaricidal activity of plants from northeastern Brazil, the focus of the present study was on the chemical composition and acaricidal activity against *T. urticae* of essential oils from *O. duckei and O. glomerata* that grow wild in fragments of the Atlantic Forest. The relationship between the toxicity of the selected constituents and their blends was also investigated.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL

Fresh leaves from *Ocotea duckei* and *Ocotea glomerata* were collected in the Mata Senhorzinho Cabral, in Camocim de São Félix in September

2010. The geographical coordinates of the collection point was 07º58'41.2" S 034º 50'21.4"W. The plants were identified by botanist Dr. Maria R. C. S. de Melo (University Federal Rural of Pernambuco). Vouchers of both samples were mounted and deposited in the Vasconcelos Sobrinho Herbarium of the UFRPE under numbers 19951 *Ocotea duckei* and 49645 *Ocotea glomerata*.

OPTICAL ROTATION

Optical rotation of the essential oils was performed with a digital polarimeter (A. Krüss model Px800, Germany) at 589 nm and 25°C in a dichloromethane solution.

CHEMICALS

All monoterpenes (α-pinene, β-pinene, *p*-cymene, limonene, terpinolene, terpinen-4-ol and α-terpineol), sesquiterpenes (β-caryophyllene, aromadendrene, α-humulene and valencene) and eugenol used as control positive for fumigant test were purchased from Sigma-Aldrich, Brazil. Azamax used as a positive control in the residual contact test was acquired from the local market in Recife, Pernambuco, Brazil.

ESSENTIAL OIL EXTRACTION AND GAS CHROMATOGRAPHY FID ANALYSIS

The essential oils from the fresh leaves (100 g) of *Ocotea* species 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 acaricidal assays and analysis. Total oil yields were expressed as percentages (g/100 g of fresh plant material). All experiments were carried out in triplicate. GC identification was carried out 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 film thickness) (J & W Scientific). The oven temperature was programmed from 60 to 240°C at a rate 3°C min^{-1}. Injector and detector temperatures were 260°C. Hydrogen was used as the carrier gas at a flow rate of 1 mL min^{-1} in split mode (1:30). The injection volume was 0.5 μL of diluted solution (1/100) of oil in *n*-hexane. The 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. 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^{-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 C_8-C_{40} n-alkanes calculated using the van Den Dool and Kratz equation (van Den Dool and Kratz 1963) and by computer matching against the mass spectral library of the GC-MS data system (NIST version 14 and WILEY version 11) 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 *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 ± 1ºC, with relative humidity of 65 ± 5% and a 12-h photoperiod.

FUMIGATION AND RESIDUAL CONTACT BIOASSAYS OF OILS, SELECTED COMPOUNDS AND ARTIFICIAL OILS

The fumigation and residual contact methods were the same as those employed by Araújo et al. (2012). The mortality data for the *Ocotea* oils, selected compounds and blends were analyzed using the probit model with the aid of the POLO-PC software program (LeOra 1987) for the determination of the lethal concentration necessary for 50% mortality (LC_{50}) of the mite population, with the calculation of 95% confidence levels. Toxicity ratios were determined based on the method described by Robertson and Preisler (1992). The data were submitted to analysis of variance (ANOVA) and means were compared using Tukey's test, with the level of significance set to 5% ($p < 0.05$). In the fumigation bioassays, the concentrations ranged from 0.08 to 3.6 μL L^{-1} of air for the oils, 3.2 x 10^{-4} to 26 μL L^{-1} of air for the selected compounds and 0.15 to 4.5 μL L^{-1} of air for the blends. In the residual contact bioassays, concentrations ranged from 0.10 to 15.0 μL mL^{-1} for the oils, 0.1 to 675.0 μL mL^{-1} for the selected compounds and 0.10 to 40.5 μL L^{-1} of air for the blends. The results were submitted to descriptive analysis using the Statistical Analysis System program (SAS 2002). The artificial oils were prepared with selected compounds from the *O. duckei* and *O. glomerata* oils at the same proportions as those identified by gas chromatography-mass spectrometry (GC-MS) and gas chromatography with a flame ionization detector (GC-FID), as shown in Table III.

COMPARATIVE TOXICITY OF INCOMPLETE BLENDS

Incomplete blends were prepared from artificial oil to investigate the relationship between the compounds selected from the essential oils of *Ocotea* and their toxicity, with each blend lacking one constituent. The respective fumigation and residual contact activities were evaluated. The toxicity of the incomplete blends was evaluated at the same concentration as that of the *Ocotea* oils that promoted ≥ 96.0% mortality in the fumigation bioassays: 12 and 20 μL L^{-1} of air for the essential oil from *O. duckei and O. glomerata*, respectively; in residual contact bioassays: 46 and 31 μL mL^{-1} for the essential oil from *O. duckei* and *O. glomerata*, respectively.

RESULTS AND DISCUSSION

CHEMICAL COMPOSITION OF *Ocotea* OILS

The oils obtained through hydrodistillation exhibited a light yellow color and citric aroma. No significant differences were found between the yields of oil from the fresh leaves of the two species. Table I displays the yields, specific rotations and compounds identified in the leaf oils from *Ocotea duckei* and *O. glomerata*. In the comparison of the findings with data from the literature, the yield of the oil from *O. duckei* in the present study (1.6 ± 0.0%) was higher than that reported by Barbosa-Filho et al. (2007) for leaves of a specimen collected in the state of Paraíba (0.7%). The specific rotation values for the *Ocotea* oils were levorotary, with large angles for the *O. glomerata* oil and small angles for the *O. duckei* oil.

The GC-MS analysis of the essential oils revealed a total of 67 compounds, corresponding to 97.3 ± 0.3% and 97.8 ± 0.5% of the chemical

TABLE I
Percentage composition, yield and optical rotation of essential oils from *O. duckei* and *O. glomerata*.

Compound	RI [a]	RI [b]	*O. duckei*	*O. glomerata*	Method of identification
Yield (%) ±sd			1.6±0.0	1.8±0.1	
$[\alpha]_D^{25}$(c.=1,CH_2Cl_2)			-8.9°	-19.1°	
artemisia triene	920	923	-	0.6±0.0	RI, MS
α-pinene	928	932	2.5±0.1	6.9±0.1	RI, MS,CI
verbenene	957	961	0.3±0.0	-	RI, MS
sabinene	965	969	0.3±0.0	-	RI, MS
β-pinene	970	974	5.2±0.0	-	RI, MS, CI
myrcene	985	988	5.6±0.0	-	RI, MS
α-phellandrene	999	1002	-	0.4±0.0	RI, MS
iso-sylvestrene	1006	1008	-	1.1±0.0	RI, MS
p-cymene	1017	1020	-	4.9±0.0	RI, MS, CI
limonene	1024	1024	1.1±0.0	1.0±0.0	RI, MS, CI
γ-terpinene	1050	1054	0.7±0.0	6.4±0.0	RI, MS
m-cymene	1081	1082	0.7±0.0	-	RI, MS
terpinolene	1084	1086	-	2.1±0.1	RI, MS, CI
trans-pinene hydrate	1119	1119	0.1±0.0	-	RI, MS
terpinen-4-ol	1168	1174	-	0.4±0.0	RI, MS, CI
α-terpineol	1188	1186	0.3±0.0	-	RI, MS, CI
iso-menthyl acetate	1308	1304	2.0±0.0	-	RI, MS
δ-elemene	1335	1335	-	0.3±0.0	RI, MS
α-cubebene	1340	1345	-	1.2±0.0	RI, MS
α-terpinyl acetate	1350	1346	1.4±0.0	-	RI, MS
ylangene	1370	1373	-	0.3±0.0	RI, MS
α-copaene	1372	1374	1.2±0.0	-	RI, MS
β-cubebene	1388	1387	1.8±0.0	-	RI, MS
β-bourbonene	1390	1387	0.1±0.0	3.1±0.0	RI, MS
β-elemene	1384	1389	-	0.3±0.0	RI, MS
β-caryophyllene	1415	1417	18.1±0.1	14.6±0.3	RI, MS, CI
β-duprezianene	1418	1421	-	0.6±0.0	RI, MS
β-copaene	1428	1430	-	0.3±0.0	RI, MS
β-gurjunene	1431	1431	-	0.5±0.0	RI, MS
aromadendrene	1437	1439	0.5±0.0	17.3±0.6	RI, MS, CI
prezizaene	1443	1444	-	1.9±0.0	RI, MS
α-humulene	1455	1452	2.2±0.0	-	RI, MS, CI
dehydro-aromadendrene	1457	1460	3.0±0.0	-	RI, MS
cumacrene	1466	1470	4.7±0.1	-	RI, MS
germacrene D	1480	1484	-	2.1±0.0	RI, MS
β-selinene	1483	1489	-	2.3±0.1	RI, MS
δ-selinene	1489	1492	1.5±0.0	-	RI, MS

TABLE I (continuation)

Compound	RI [a]	RI [b]	*O. duckei*	*O. glomerata*	Method of identification
Yield (%) ±sd			1.6±0.0	1.8±0.1	
$[\alpha]_D^{25}$(c.=1,CH_2Cl_2)			-8.9°	-19.1°	
γ-amorphene	1490	1495	-	1.6±0.0	RI, MS
valencene	1496	1496	17.6±0.0	-	RI, MS, CI
α-muurolene	1501	1500	1.9±0.0	2.1±0.0	RI, MS
bicyclogermacrene	1504	1500	2.7±0.0	5.8±0.2	RI, MS
β-bisabolene	1507	1505	0.2±0.0	-	RI, MS
δ-amorphene	1511	1511	-	1.1±0.0	RI, MS
δ-cadinene	1520	1522	0.6±0.0	0.6±0.0	RI, MS
zonarene	1525	1528	-	2.0±0.0	RI, MS
α-cadinene	1535	1537	-	4.0±0.1	RI, MS
α-calacorene	1545	1544	-	2.8±0.0	RI, MS
elemol	1550	1548	6.8±0.0	-	RI, MS
trans-dauca-4(11),7-diene	1557	1556	2.4±0.0	-	RI, MS
maliol	1562	1566	-	0.2±0.0	RI, MS
longipinanol	1563	1567	-	0.8±0.0	RI, MS
palustrol	1564	1567	-	0.4±0.0	RI, MS
caryophyllene alcohol	1568	1570	-	0.6±0.0	RI, MS
α-cedrene epoxide	1572	1574	1.0±0.0	-	RI, MS
spathulenol	1574	1577	0.6±0.0	3.6±0.1	RI, MS
tujopsan-2-β-ol	1586	1588	0.6±0.0	-	RI, MS
globulol	1590	1590	1.1±0.0	-	RI, MS
viridiflorol	1595	1592	-	2.8±0.0	RI, MS
rosifoliol	1603	1600	-	0.7±0.0	RI, MS
ledol	1607	1602	0.7±0.0	-	RI, MS
trans-isolongifolane	1626	1625	-	0.5±0.0	RI, MS
α-muurolol	1642	1644	5.6±0.0	-	RI, MS
cubenol	1647	1645	-	1.3±0.0	RI, MS
cadinol	1649	1652	3.6±0.0	-	RI, MS
β-eudesmol	1649	1652	0.6±0.0	-	RI, MS
elemol acetate	1675	1680	0.9±0.0	-	RI, MS
amorpha-4,9-dien-2-ol	1703	1700	0.1±0.0	-	RI, MS
sclareolide	2060	2065	0.1±0.0	-	RI, MS
Monoterpenes			20.8±0.1	25.0±0.1	
Sesquiterpenes			76.5±0.3	72.8±0.7	
Total			97.3±0.3	97.8±0.7	

[a]Retention indices calculated from retention times in relation to those of a series of n-alkanes on a 30m DB-5 capillary column.
[b]Linear retention indices from the literature. RI = retention index, MS = mass spectrum, CI = co-injection with authentic standards.

composition of the *O. duckei* and *O. glomerata* oils, respectively. The oils exhibited a terpene chemical profile (monoterpenos and sesquiterpenes), with sesquiterpenes as the dominant class: 76.5 ± 0.3% in the *O. duckei* oil and 72.8 ± 0.7% in the *O. glomerata* oil. The predominance of sesquiterpenes in the oils of species of *Ocotea* that occur in the state of Pernambuco is in agreement with data reported for oils from other species of the genus that occur in various regions of the world (Ballabeni et al. 2007, Chaverri and Cicció 2007, Coutinho et al. 2007, Setzer et al. 2006, Takaku et al. 2007). With the exception of oils from the fruit and leaves of *O. odorífera* (Mossi et al. 2013, Oltramari et al. 2004) collected in the state of Rio Grande do Sul (southern Brazil) and the leaves of *O. puchury-major* (Leporatti et al. 2014) collected in the state of Amazonas (northern Brazil), in which phenylpropanoides are the predominant chemical class, the abundance of sesquiterpenes found in the oils in the present study is also in agreement with data reported for other species of the genus that occur in different regions of Brazil (Sacchetti et al. 2006, Coutinho et al. 2007, Farago et al. 2010, Garrett et al. 2010 Yamaguchi et al. 2013), including the northeastern region in a sample of *O. duckei* collected in the state of Paraíba (Barbosa-Filho et al. 2007).

Among the 67 compounds identified in the oils analyzed herein, only ten were common to both *O. duckei* and *O. glomerata*. β-caryophyllene (18.1 ± 0.1%), valencene (17.6 ± 0.0%), β-pinene (5.2 ± 0.0%), myrcene (5.6 ± 0.0%), cumacrene (4.7 ± 0.1%), elemol (6.8 ± 0.0%) and α-muurolol (5.6 ± 0.0%) were the major constituents in the *O. duckei* oil. Among these compounds, only β-caryophyllene was also found in the *O. glomerata* oil, suggesting considerable chemical diversity. The major constituents of the *O. glomerata* oil were aromadendrene (17.3 ± 0.6%), β-caryophyllene (14.6 ± 0.3%), α-pinene (6.9 ± 0.1%), *p*-cymene (4.9 ± 0.0%), γ-terpinene (6.4 ± 0.0%), bicyclogermacrene (5.8 ± 0.2%) and α-cadinene (4.0 ± 0.1%).

β-caryophyllene was also the major component in the essential oil from *O. duckei* leaves reported by Barbosa-Filho et al. (2007), corresponding to 60.54% of the oil. The authors identified eight compounds corresponding to 74.7% of the essential oil from *O. duckei* occurring in the state of Paraíba.

The present study shows that the chemical profile of the essential oil from *O. duckei* does not differ significantly based on location (states of Paraíba and Pernambuco). However, the chemical analysis of *O. duckei* and *O. glomerata* enables the inference of qualitative and quantitative differences between the essential oils. The chemical profile rich in monoterpenes and sesquiterpenes characterized in the oils from the species of *Ocotea* that occur in the state of Pernambuco is typical of essential oils from some species of *Ocotea* in South America (Chaverri et al. 2011).

TOXICITY OF ESSENTIAL OILS AND SELECTED CHEMICAL CONSTITUENTS

Table II displays the results of the fumigation and residual contact bioassays with the *Ocotea* oils. Acaricidal activity varied in accordance with the type of oil and method employed. The *O. duckei* oil was 2.5-fold and 1.5-fold more toxic than the *O. glomerata* oil in the fumigation and residual contact bioassays, respectively. Thus, the mites were more susceptible to the penetration of oil vapors in the airways (fumigation) than through the tarsi and/or ingestion (residual contact). These results are in agreement with data on the toxicity of other oils on the same pest (Neves and da Camara 2016, Moraes et al. 2012). However, neither oil was more active than eugenol and azamax, which were used as the positive control in the fumigation and residual contact bioassays, respectively.

The differences in toxicity against *T. urtice* between the *O. duckei* and *O. glomerata* oils may be attributed to the qualitative and quantitative

TABLE II
Fumigation toxicity (LC_{50} at µL L^{-1} of air) and residual contact (LC_{50} at µL mL^{-1}) of the blends of selected constituents and essential oils of *O. duckei* and *O. glomerata*.

Oil/blends	Bioassay	n	df	slope	Fumigation CL_{50} (CI 95%)	χ^2	TR_{50}(CI 95%)
O. duckei	Fumigation	540	4	2.09 (1.94-2.23)	0.52 (0.40-0.67)	5.87	148.78 (74.78 - 196.92)
	Contact	175	5	1.89 (1.64-2.11)	4.68 (3.50-6.20)	4.54	1.17×10^4 (1.01×10^4 -1.31×10^4)
O. glomerata	Fumigation	540	4	3.10 (2.86-3.34)	1.32 (1.00-1.61)	8.68	374.76 (290.76 - 447.36)
	Contact	150	4	2.99 (2.61-3.37)	7.22 (5.89-8.76)	1.96	2.4×10^4 (2.23×10^4 -2.65×10^4)
FMD	Fumigation	630	5	3.28 (3.06-3.50)	0.78 (0.66-0.86)	1.81	192.70 (164.71 - 234.12)
	Contact	150	4	3.06 (2.66-3.46)	5.02 (3.86-7.12)	3.55	1.25×10^4 (1.10×10^4 -1.41×10^4)
FMG	Fumigation	540	4	4.98 (4.58-5.37)	2.47 (2.11-2.69)	8.89	616.08 (587.54 - 666.91)
	Contact	150	4	6.21 (5.40-7.01)	23.99 (21.76-26.34)	1.81	6.00×10^6 (5.71×10^6 -6.35×10^6)
EU	Fumigation	580	5	0.84 (0.72-0.97)	4.0×10^{-3} (2.0×10^{-4}-8.0×10^{-4})	2.50	-
Azamax	Contact	540	4	2.45 (2.25-2.64)	3.0×10^{-4} (2.4×10^{-4}-4.0×10^{-4})	8.04	-

FMD = Full mixture of selected constituents in the oil from *O. duckei*; FMG = Full mixture of selected constituents in the oil from *O. glomerata* (prepared at same percentage composition identified by GC/MS analyses of *O. duckei* and *O. glomerata*); Eugenol (EU) and Azamax used as positive control; n = number of mites/dose; df= degrees of freedom; LC_{50} = Median Lethal Concentration; CI = confidence interval; χ^2= chi-squared; TR = toxicity ratio.

differences in the chemical constituents identified in the oils. Toxicity of the major constituents selected from the two oils also varied in accordance with the method employed (fumigation and residual contact).

To identify variations in the toxicity of the selected constituents determined during the fumigation bioassays, these compounds were divided into six groups from the most toxic to the least toxic based on the intensity of the acaricidal action: Group A - β-caryophyllene; Group B - terpinen-4-ol; Group C - terpinolene and α-humulene; Group D - α-humulene, α-terpineol and *p*-cymene; Group E - *p*-cymene, β-pinene, valencene and aromadendrene; and Group F - aromadendrene, limonene and α-pinene. The same procedure was used for the relative toxicity of the compounds tested using the residual contact method, for which five groups were determined: Group A - β-caryophyllene; Group B - α-humulene, α-pinene, *p*-cymene, α-terpineol, aromadendrene and valencene; Group C - β-pinene, α-pinene, *p*-cymene, α-terpineol, aromadendrene and valencene; Group D - limonene; and Group E - terpinolene and terpinen-4-ol. The findings suggest that β-caryophyllene was the most toxic constituent, independently of the method employed. Terpinen-4-ol was the second most toxic in the fumigation bioassays, whereas terpinen-4-ol and terpinolene exhibited the least toxicity in the residual contact bioassays.

In the fumigation bioassays, β-caryophyllene proved to be 13-fold and 33-fold more toxic than the *O. duckei* and *O. glomerata* oils, respectively. In the residual contact bioassays, this sesquiterpene was seven-fold and 11-fold more toxic than the

O. duckei and *O. glomerata* oils, respectively. Other constituents of the *O. glomerata* oil also demonstrated significant toxicity, such as terpinen-4-ol, which was three-fold more toxic than the oil. The acaricidal activity of the *Ocotea* oils may therefore be attributed to the chemical constituents with greater toxicity. These data also suggest a possible antagonistic interaction of some constituents of the *Ocotea* oils against β-caryophyllene.

The high susceptibility of mites exposed to the *Ocotea* oils and individual compounds using the fumigation method has been reported for other essential oils and their chemical constituents (Moraes et al. 2012, Neves and da Camara 2016). This demonstrates that such products are more toxic to *T. urticae* through the penetration of vapors in the respiratory system (fumigation) than ingestion and/or penetration through the tarsi (residual contact).

TOXICITY OF ARTIFICIAL OILS: BLENDS OF CONSTITUENTS SELECTED FROM *Ocotea* OILS

Table III displays the mean lethal concentrations during the fumigation and residual contact bioassays of the chemical constituents (artificial oils) selected from the *Ocotea* oils at the same proportion in which these compounds were identified by GC-MS. Toxicity of the artificial oil from *O. duckei* was the same as that found for the essential oil. However, the artificial oil prepared from seven constituents of the *O. glomerata* oil was 1.8-fold and 3.3-fold less toxic than the essential oil in the fumigation and residual contact bioassays, respectively. The results suggest that the absence of non-selected constituents did not directly affect the toxicity of the artificial oil derived from *O. duckei*, whereas non-selected constituents were important to the toxicity of the artificial oil derived from *O. glomerata*. Neither of the artificial oils prepared from the constituents of the *Ocotea* oils were more active than eugenol and azamax used as the positive control in the fumigation and residual contact bioassays, respectively.

ATTRIBUTION OF ROLE OF TERPENES IN TOXICITY OF ARTIFICIAL OILS DERIVED FROM *O. duckei* AND *O. glomerata*

Figure 1 shows the mean mite mortality values for the blends prepared with the absence of one of the constituents of the artificial oil derived from *O. duckei* (incomplete blend).

In the fumigation bioassays, the removal of the minor constituents α-pinene (2.5 ± 0.1%), β-pinene (5.2 ± 0.0%), α-terpinenol (0.3 ± 0.0%), aromadendrene (0.5 ± 0.0%) or α-humulene (2.2 ± 0.0%) did not affect the toxicity of the artificial oil and the removal of limonene (1.1 ± 0.0%) exerted a small effect. In contrast, when one of the main constituents [β-caryophyllene (18.1 ± 0.1%) or valencene (17.6 ± 0.0%)] was removed, the toxicity of the incomplete blend was drastically reduced. These findings suggest that β-caryophyllene and valencene contribute most to the toxicity of the artificial oil using the fumigation method. In the residual contact tests involving the incomplete blends, the major constituents β-caryophyllene and valencene as well as the minor constituent *a*-humulene contributed most to the toxicity of the artificial oil derived from *O. duckei*. The findings indicate that β-caryophyllene, which is the main component of the oil and most toxic individually, is the compound that contributes most to the toxicity of the artificial oil using the fumigation and residual contact methods. These results are in agreement with data reported by Miresmailli et al. (2006), who demonstrated the role of the major constituents (1,8-cineole and α-pinene) in the toxicity of the oil from *Rosmarinus officinalis* against *Tetranychus urticae*. However, the results are in disagreement with data described by Neves and da Camara (2016) for 1,8-cineole, which is the main constituent of an artificial oil derived from the leaves of *Vitex agnus-castus*.

TABLE III

Toxicity by fumigation (LC_{50} at µL L^{-1} of air) and residual contact (LC_{50} at µL mL^{-1}) of the individual terpenes selected from *Ocotea* oils against *T. urticae*.

Compound	% in the Oil		Bioassay	n	df	slope	LC_{50} (CI 95%)	χ^2	TR_{50} (CI 95%)
	O. duckei	*O. glomerata*							
α-pinene	2.5±0.1	6.9±0.1	Fumigation	722	5	3.91 (3.53-4.29)	12.46 (9.56-14.77)	11.04	276.25 (103.07 - 440.01)
			Contact	150	4	2.16 (1.86-2.45)	34.13 (26.38-44.81)	2.73	56.95 (36.81-76.17)
β-pinene	5.2±0.0	-	Fumigation	540	4	2.95 (2.69-3.20)	4.25 (3.49-6.16)	9.16	109.12 (43.72 - 272.33)
			Contact	150	4	2.73 (2.39-3.07)	38.93 (31.72-46.91)	8.10	69.40 (43.96-82.98)
p-cymene	-	4.9±0.0	Fumigation	630	5	2.11 (1.97-2.25)	3.69 (2.76-4.71)	9.83	95.77 (30.36 - 220.27)
			Contact	200	6	2.69 (2.36-3.02)	36.95 (28.03-46.49)	6.48	63.32 (41.85-78.49)
limonene	1.1±0.0	1.0±0.0	Fumigation	444	3	7.35 (6.46-8.23)	9.80 (7.05-11.39)	7.60	270.15 (96.06 - 490.91)
			Contact	124	3	3.48 (2.91-4.04)	76.94 (63.19-92.20)	1.52	129.36 (87.43-162.94)
terpinolene	-	2.1±0.1	Fumigation	445	3	2.17 (1.99-2.35)	1.08 (0.62-1.62)	6.92	29.87 (8.80 - 64.71)
			Contact	121	3	3.05 (2.53-3.57)	341.90 (206.90-520.83)	3.78	543.40 (393.56-633.45)
terpinen-4-ol	-	0.4±0.0	Fumigation	450	3	5.64 (5.12-6.16)	0.42 (0.35-0.49)	5.86	11.45 (6.54 - 15.27)
			Contact	149	4	9.47 (7.96-10.98)	366.56 (306.47-421.30)	8.43	593.28 (501.21-690.76)
α-terpineol	0.3±0.0	-	Fumigation	633	4	6.08 (5.20-6.96)	2.39 (1.64-2.76)	7.81	52.98 (19.77 - 111.12)
			Contact	123	3	1.70 (1.46-1.94)	40.43 (27.48-58.72)	2.94	68.72 (40.04-98.23)
β-caryophyllene	18.1±0.1	14.6±0.3	Fumigation	720	6	0.80 (0.33-1.27)	0.04 (0.02-0.08)	9.12	-
			Contact	175	5	2.25 (1.97-2.52)	0.64 (0.49-0.83)	0.72	-
aromadendrene	0.5±0.0	17.3±0.6	Fumigation	540	4	2.14 (1.98-2.30)	6.59 (5.21-7.11)	9.28	170.11 (94.00 - 395.29)
			Contact	175	5	8.00 (6.17-9.83)	29.61 (26.94-32.38)	3.43	49.94 (34.85-60.57)
α-humulene	2.2±0.0	-	Fumigation	629	5	1.85 (1.74-1.96)	1.80 (1.30-2.30)	8.41	55.03 (24.74 - 98.71)
			Contact	200	6	2.45 (2.12-2.77)	23.97 (19.32-28.94)	2.85	37.19 (26.98-51.26)
valencene	17.6±0.0	-	Fumigation	540	4	2.84 (2.64-3.04)	4.45 (3.97-5.06)	4.08	123.76 (86.75 - 165.40)
			Contact	150	4	8.01 (6.47-9.55)	31.21 (27.68-33.93)	1.78	53.43 (36.99-58.39)

n = number of mites/dose; df = degrees of freedom; LC_{50} = Median Lethal Concentration; CI = confidence interval; χ^2 = chi-squared; TR = toxicity ratio.

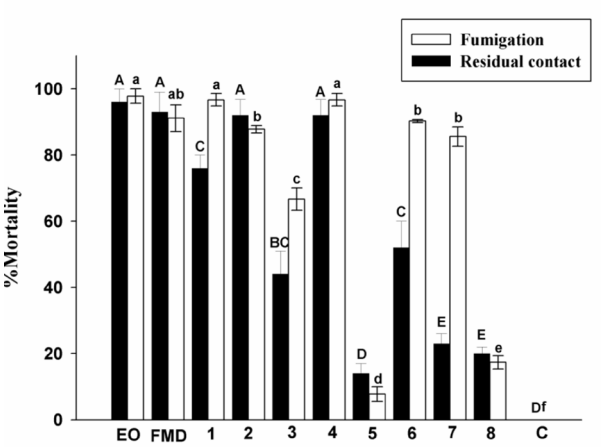

Figure 1 - Mean mortality caused by incomplete blends prepared with the removal of one constituent from the complete artificial blend of *O. duckei* at concentration equivalent to the experiment with the leaf oil that promoted ≥ 96.0% mortality (3.2 μL L^{-1} air for fumigation and 20 μL mL^{-1} for residual contact). Bars with the same uppercase or lowercase not differ significantly by Tukey test ($P \leq 0.05$). EO = essential oil from *O. duckei*, FMD = Full mixture of selected constituents in the oil from *O. duckei*. The numbers indicate the blends with the absence of the labeled compound. 1 = α-pinene; 2 = β-pinene; 3 = limonene; 4 = α-terpineol; 5 = β-caryophyllene; 6 = aromadendrene; 7 = α-humulene; 8 = valencene; C = control.

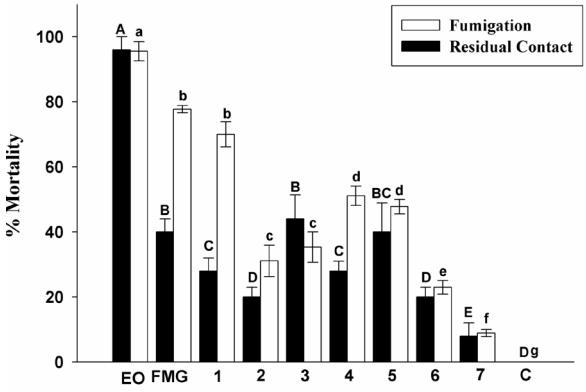

Figure 2 - Mean mortality of mite caused by incomplete blends prepared with the removal of one constituent from the complete artificial blend of the leaf oil of *O. glomerata* at concentration equivalent to the experiment with the leaf oil that promoted mortality ≥ 96.0% (3.6 μL L^{-1} air for fumigation and 22 μL mL^{-1} to residual contact). Bars with the same uppercase or lowercase not differ significantly by Tukey test ($P \leq 0.05$). EO = essential oil from *O. glomerata*, FMG = Full mixture of selected constituents in the oil from *O. glomerata*. The numbers indicate the blends with the absence of the labeled compound. 1 = α-pinene; 2 = *p*-cymene; 3 = limonene; 4 = α-terpinolene; 5 = terpinen-4-ol; 6 = β-caryophyllene; 7 = aromedendrene; C = control.

Figure 2 displays the relative toxicities of the incomplete blends of the artificial oil derived from *O. glomerata*.

The removal of α-pinene (6.9 ± 0.1%) did not affect the toxicity of the artificial oil in the fumigation bioassay and the toxicity of the artificial oil was not reduced by the removal of limonene (1.0 ± 0.0%) or terpinen-4-ol (0.4 ± 0.0%) in the residual contact bioassay. In contrast, the removal of aromadendrene (17.3 ± 0.6%) led to a drastic reduction in mite mortality, indicating that this sesquiterpene contributed most to the toxicity of the artificial oil from *O. glomerata*, followed by β-caryophyllene (14.6 ± 0.3%) and *p*-cymene (4.9 ± 0.0%). Comparing the individual relative toxicities of the constituents that most contributed to the toxicity of the artificial oil from *O. glomerata*, the major component, aromadendrene, exhibited the same degree of toxicity as *p*-cymene, independently of the method employed (fumigation or residual contact), whereas the second major component of the artificial oil, β-caryophyllene, was 170-fold and 50-fold more toxic than aromadendrene in the fumigation and residual contact bioassays, respectively. These results suggest that the contribution of one chemical constituent in a complex blend is not predictable based solely on its individual toxicity or the proportion at which it is found in the blend.

CONCLUSIONS

This study confirmed the terpene nature with a predominance of sesquiterpenes in essential oils from species of the genus *Ocotea*. Although the oils investigated have the same chemical profile, the present study enabled the determination of qualitative and quantitative differences in the chemical composition of the oils from *O. duckei* and *O. glomerata*. The chemotype β-caryophyllene found in the oil from *O. duckei* occurring in a fragment of the Atlantic Forest in the state of

Pernambuco in northeastern Brazil is the same as that found in the oil from *O. duckei* collected from a fragment of the Atlantic Forest in the state of Paraíba in the same region of the country.

The two-spotted spider mite was more susceptible to the oil from *O. duckei* than that from *O. glomerata*, independently of the method employed. Both oils were more toxic when using the fumigation method, which suggests better action of the oils through the penetration of the airways of the mite than through ingestion or contact with the tarsi.

The bioassays with the selected constituents and different blends prepared with these compounds demonstrated that the contribution of a single chemical constituent to the toxicity of the complete blend is not predictable based solely on individual toxicity or the proportion at which the compound is found in the blend. Therefore, possible interactions among these constituents should be taken into consideration.

ACKNOWLEDGMENTS

The authors are grateful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for awarding a grant (CAPES – Proc. N° IBPG-0984-5.01/10; scholarship of productivity CNPq, Nº 312277/2013-0) and funding this study (CNPq Nº 477778/2013-5).

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