Referência

MORAES, Marcílio M. de *et al*. Essential oil composition of Eugenia langsdorffii O. Berg.: relationships between some terpenoids and toxicity against Tetranychus urticae. **Journal of the Brazilian Chemical Society**, *[S. l.]*, v. 23, n. 9, p. 1647-1656, 2012. DOI: https://doi.org/10.1590/S0103-50532012005000029. Disponível em: https://www.scielo.br/j/jbchs/a/db68GnTsthmBPL6vZYMSyzJ/?lang=en#. Acesso em: 08 set. 2022.

J. Braz. Chem. Soc., Vol. 23, No. 9, 1647-1656, 2012.
Printed in Brazil - ©2012 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

Essential Oil Composition of *Eugenia langsdorffii* O. Berg.: Relationships Between Some Terpenoids and Toxicity Against *Tetranychus urticae*

Marcílio M. de Moraes,[a] Claudio A. G. da Camara,*,[a] Maria L. dos Santos[b] and Christopher W. Fagg[c]

[a]*Departamento de Ciências Moleculares, Universidade Federal Rural de Pernambuco, 52171-900 Recife-PE, Brazil*

[b]*Instituto de Química, Universidade de Brasília, 70910-900 Brasília-DF, Brazil*

[c]*Faculdade UnB Ceilandia, Universidade de Brasília, 72220-140 Brasília-DF, Brazil*

Óleos essenciais das folhas e frutos de *Eugenia langsdorffii* foram analisados por CG-FID (cromatografia gasosa com detecção através de ionização por chama) e CG-EM (cromatografia gasosa com detecção por espectrometria de massas) e exibiram alta percentagem de sesquiterpenos. *epi*-Longipinanol (13,6 ± 0,1%) e γ-eudesmol (12,3 ± 0,2%) foram os componentes principais do óleo das folhas, enquanto que 10-*epi*-γ-eudesmol (35,7 ± 0,3%) e 1,10-di-*epi*-cubenol (15,6 ± 0,3%) foram majoritários no óleo dos frutos. Estudo comparativo da toxicidade por fumigação e contato residual desses óleos foi conduzido. O óleo das folhas (dose letal média, LC_{50} = 1,7 μL L^{-1} de ar) foi 1,7 vezes mais tóxico do que o óleo dos frutos por fumigação, e 1,8 vezes menos tóxico por contato residual. O controle positivo (eugenol) foi muito mais tóxico no bioensaio de fumigação do que os óleos de *Eugenia*. Entretanto, o efeito de contato residual do óleo dos frutos (LC_{50} = 12,25 μL mL^{-1}) foi apenas 6,7 vezes menor do que o do eugenol. A função exercida por terpenóides na propriedade acaricida dos óleos de *E. langsdorffii* também foi discutida.

Leaf and fruit essential oils of *Eugenia langsdorffii* were analyzed by GC-FID (gas chromatography with flame ionization detection) and GC-MS (gas chromatography with mass spectrometric detection), featuring a high percentage of sesquiterpenes. *epi*-Longipinanol (13.6 ± 0.1%) and γ-eudesmol (12.3 ± 0.2%) were the principal components of the leaf oil, whereas 10-*epi*-γ-eudesmol (35.7 ± 0.3%) and 1,10-di-*epi*-cubennol (15.6 ± 0.3%) were the major constituents of the fruit oil. A comparative study to assess fumigant and residual contact toxicities of the oils was conducted. The leaf oil (lethal concentration average, LC_{50} = 1.7 μL L^{-1} of air) was 1.7 times more toxic than the fruit oil and 1.8 times less toxic by residual contact. The positive control (eugenol) was much more toxic in the fumigation bioassay than *Eugenia langsdorffii* oils. Nevertheless, the residual contact effect of the fruit oil (LC_{50} = 12.25 μL mL^{-1}) was just 6.7 times smaller than that of eugenol. The role of terpenoids in the acaricidal property of the *E. langsdorffii* essential oils was also discussed.

Keywords: *Eugenia langsdorffii*, essential oil composition, acaricidal actitivity, spider mite

Introduction

The cerrado biome occupies a large latitudinal gradient of over 20 degrees in central Brazil, with the Federal District in its centre, and is rapidly being replaced by agriculture (principally soyabeans) and cultivated pastures. It consists of a diversity of landscapes made up of different vegetation formations, from grasslands through savannas to forests either forming galleries along the rivers or dry seasonal forests on richer soils. This diversity of landscapes makes the cerrado the richest savanna in the world, and one of the hotspots of biodiversity,[1] with over 12000 plant species recorded for this biome.[2] Myrtaceae is the eighth most diverse flowering plant family in the cerrado biome with 344 species in 21 genera, and can be found in all vegetation types, principally in the forest and savanna. Throughout Tropical America, *Eugenia* is the largest genus of the Myrtaceae family with around 1009 species.[3] Some plants from this genus are used in folk medicine, mainly in the treatment of wounds and intestinal infections[4] or as repellents

*e-mail: camara@dq.ufrpe.br

or insecticides against domestic and agricultural pest.[5] *Tetranychus urticae* is considered one of the major plagues in the world.[6] It occurs in regions of tropical and temperate climate, in both greenhouses and fields. The reduction of this plague has been made with repeated applications of conventional acaricides. Due to their high volatility, essential oils could be used as eco-friendly fumigants to replace the conventional acaricides in pest control, which produce hazardous wastes for humans and the environment. A review of the literature revealed that essential oils from *Eugenia* species have been reported for their insecticidal[7] and acaricidal properties, particularly for human medicine[8,9] and veterinarian interests[10]. Several oils have been also assayed for various biological activities such as antioxidant,[11] cruzian inhibitory,[12] citotoxity and antimicrobial.[13,14] One of the most representative species found in the cerrado biome is *Eugenia langsdorffii* O. Berg (a low bush to 40 cm high, with many shoots growing from an underground xylopodium) that can be found principally in cerrado *sensu strito* (savanna) and cerradão (dense savannas). This is the first time that the essential oil composition of *E. langsdorfii* and its acaricidal effect is reported.

The biological potential of essential oils has been studied by several researcher groups in Brazil and foreign countries as an alternative to the synthetic pesticides for the control of agricultural pests.[15] Basically, such investigations have been mostly directed to the activities of the essential oil and, occasionally, the activities are attributed to the major isolated constitutes tested.[16] Taking this into account, the aim of the present study was to determine the chemical composition of *E. langsdorffii*, evaluate its fumigant and residual contact action against *Tetranychus urticae*, as well as, investigate the relationship between toxicities of β-pinene, *p*-cymene, valencene, aromadendrene and caryophyllene oxide and their blends.

Experimental

Collection of plant material

The fresh leaves and fruits of *Eugenia langsdorffii* O. Berg. were collected early in the morning on April 2011 in the University of Brasilia (UnB, Brasília, Federal District, Brazil) in a cerrado *sensu strito* vegetation at an altitude of around 1030 m. The plant was identified by the botanist Dr. Carolyn Proença from the Department of Botany at UnB. The voucher specimen was deposited at the UnB herbarium under code J. E. Q. Faria Jn. & Fagg C. W. 918 and a duplicate was also sent to HUEG herbarium of the Universidade Estadual de Goiás (Goiás State, Brazil).

Extraction of essential oils

The essential oils from fresh leaves and fruits (100 g) were extracted using a modified Clevenger-type apparatus and hydrodistillation for 2 h. The oil layers were separated and dried over anhydrous sodium sulfate, stored in hermetically sealed glass containers, and kept under refrigeration at 5 ºC until the acaricidal assays and analysis. Total oil yields were expressed as percentages (g *per* 100 g of fresh plant material). All experiments were carried out in triplicate.

Chemicals

Monoterpenes and sesquiterpenes used in the identifications of volatile components and control positive (eugenol) in the bioassay were purchased from Sigma-Aldrich (Brazil).

Gas chromatography

Quantitative GC analyses were 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 × 0.25 mm × 0.25 μm film thickness) (J&W Scientific). The oven temperature was programmed from 50 to 250 °C at a rate of 3 °C min^{-1} for integration purposes. Injector and detector temperatures were at 250 °C. Hydrogen was used as the carrier gas at a flow rate of 1 L min^{-1} and 30 psi inlet pressure in split mode (1:30). The injection volume was 0.5 μL of diluted solution (1:100) of oil in n-hexane. The amount of each compound was calculated from GC peak areas in the order of DB-5 column elution and expressed as a relative percentage of the total area of the chromatograms. Analyses were carried out in triplicate.

Qualitative GC-MS analysis was performed in an Hewlett-Packard GC-MS (CG: 5890 SERIES II/GC-MS: MSD 5971) system operating in the EI mode at 70 eV fitted with the same column and temperature program as that for the GC experiments, with the following parameters: carrier gas of helium, flow rate of 1 mL min^{-1}, split mode of 1:30, injected volume (diluted solution (1:100) of oil in n-hexane) of 1 μL.

Identification of components

The identification of the components was based on the GC retention indices with reference to a homologous series of C_{11}-C_{24} n-alkanes calculated using the Van den Dool and Kratz equation[17] and by computer matching against the

mass spectral library of the GC-MS data system (NIST 98 and Willey) and co-injection with authentic standards, as well as, other published mass spectra.[18] The area percentages were electronically obtained from the GC-FID response without the use of an internal standard or correction factors.

Optical rotation

Measurements of the optical rotation of the orange peel oils were performed with a digital polarimeter (A. Krüss model Px800, West Germany) at 589 nm and 26 °C as a solution in dichloromethane.

Acaricidal assay

The mite *T. urticae* used for the bioassay was reared on plants of *Canavalia ensiformes* (jack bean) at 25 ± 5 ºC, relative humidity of 65 ± 5% and a 12 h photophase.

Fumigant assay

The fumigant method was the same used by Pontes *et al*.[19] Glass recipients with a capacity of 2.5 L were used as test chambers. Female spider mites on *C. ensiformes* leaf disks of 2.5 cm (in diameter) were exposed to the leaf and fruit oils of *Eugenia langsdorffii*. A fine brush was used to transfer the mites onto the leaf disks. In order to maintain the turgor of the disks and avoid the escape of mites, the leaf disks were placed onto filter paper disks saturated with water in Petri dishes (9 cm). The experiments were performed in triplicate. One replicate consisted of 30 specimens of *T. urticae* placed on 3 leaf disks (10 mites *per* disk) in a Petri dish. The oils were applied with an automatic pipette on a piece of filter paper (5 × 3 cm) attached to the underside of the recipient lid. The oil amounted ranged from 0.5 to 50 µL, representing 0.2 to 20 µL L^{-1} of air, while for the positive control ranged from 1.6×10^{-4} to 3.0 µL, representing 6.4×10^{-5} to 1.2 µL L^{-1} of air. For the positive control amount 1.6×10^{-4}, 8.0×10^{-4} and 4.0×10^{-3}, it was used aliquots of 0.1, 1.25 and 6.25 µL from hexane solution of 0.64 µl mL^{-1}, respectively. For these aliquots of positive control, hexane was applied to the control glass recipient, and for the other one, no material was used in the control glass recipient. Mortality was determined after 24 h. Following exposure, the Petri dishes with spider mites were then removed from the recipients, and the mites were lightly touched with a brush in order to determine mortality. Those with no sign of movement were considered dead. The mortality data for each of the *Eugenia* oils and eugenol were submitted to analysis of variance, with mean values compared by Tukey's test ($p \leq 0.05$) using the SAS software.[20] The same data were also analyzed with the Probit model using the POLO-PC software for the determination of the lethal concentration average (LC_{50}) values, with 95% confidence levels determined for all experiments.[21]

Contact assay

Leaf disc painting method described by Miresmailli *et al*.[22] with modifications was used to test the action of essential oil, eugenol and compound form oil by contact toxicity. Tests were conducted in Petri dishes (10 cm diameter). The concentrations ranged from 5 to 46 µL mL^{-1}, using a spreader sticker adjuvant (Tween-80, 0.1%) diluted in distilled water. Leaf discs (2.5 cm diameter) were cut from leaves of greenhouse-grown jack bean. 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. In order to observe the possible action of the volatile compounds of the oils and compounds tested, experiments were also performed with open Petri dishes. 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 observations were analyzed using the SAS software[20] for analysis of variance (ANOVA). Tukey's test ($p \leq 0.05$) was used to compare means. Probit analysis was used to determine LC_{50} determination with 95 percent confidence level for all experiments, using the POLO-PC software.[21] Robertson and Preisler[23] methodology was used for the calculation toxicity ratio on fumigation and residual assay.

Comparative toxicity of compounds

In order to investigate the role of some terpenoids and the observed toxicity of *E. langsdorffii* essential oils, the compounds with commercial availability, aromadendrene, valencene, caryophyllene oxide, *p*-cymene, β-pinene and (*R*)-limonene (representing 19.8% of the leaf oil composition), and caryophyllene oxide and β-pinene (representing 8.8% of the fruit oil) were selected and their fumigant activity and residual contact evaluated, individually and in the form of blends at the same proportion identified by GC-MS.

The toxicities of these compounds were evaluated in the same concentration used for the *E. langsdorffii* oils which promoted ≥ 96.0% of mortality. This means for fumigation 12 and 20 μL L^{-1} of air for leaf and fruit oils, respectively, and for residual contact 46 and 31 μL mL^{-1} for leaf and fruit oils, respectively. First, a complete mixture of the selected constituents was prepared from the leaf and fruit oils. To the selected constitutes of leaf and fruit oils, new blends were prepared to identify the contribution of each compound to the toxicity of the whole mixture. The preparation of blends was based on the toxicity level of individual compounds and by means of experiments with removal of one constituent of the total mixture. The combinations among selected constituents for blends used in the fumigation and residual contact tests are scheduled in Figure 1. All blends were prepared with the compounds in the same proportion found by GC-MS to leaf and fruit oils.

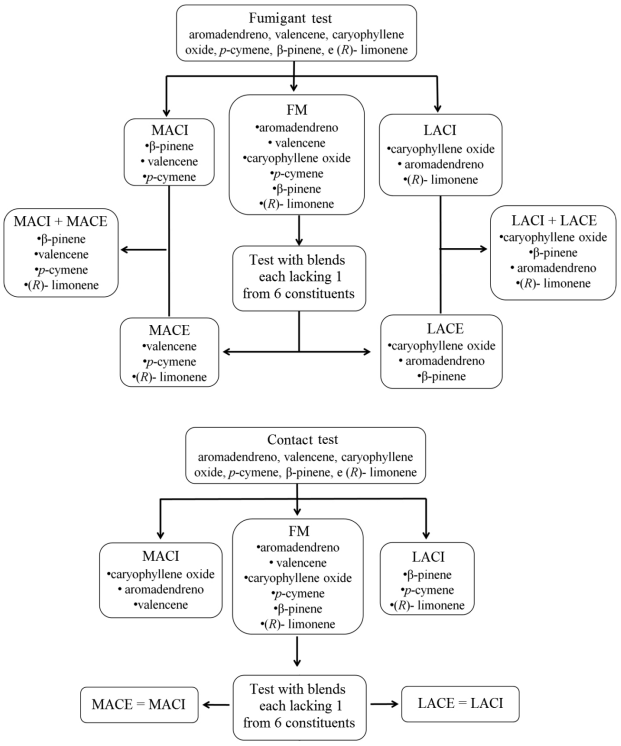

Figure 1. Selected constituents from essential oil of *E. langsdorffii* used in the evaluated blends for fumigation and residual contact against *Tetranychus urticae*. FM: full mixture of constituents, MACI, LACI and MACE, LACE: most and less active constituents selected from test with isolated constituents and component elimination assay from FM, respectively, MACI + MACE: sum of all most active constituents and LACI + LACE: sum of all less active constituents.

Results and Discussion

Table 1 shows the chemical compositions by GC and GC-MS analyses, yield percentage and specific rotation of the leaf and fruit essential oils from *E. langsdorffii*. The yields of oils obtained by hydrodistillation technique from leaves (0.05 ± 0.01%) and fruits (0.06 ± 0.01%) did not significantly differ from each other. The optical rotation indicated that the essential oils are levorotatory and the leaf oil has a larger angle of deviation than the fruit oil. The GC and GC-MS analyses of essential oil from different parts of *E. langsdorffii* identified 56 components represented by mono and sesquisterpenes.

Thirty seven components were identified in the leaf oil, which represented about 93.4 ± 0.8% of the total composition of the oil. *epi*-Longipinanol (13.6 ± 0.4%) followed by γ-eudesmol (12.3 ± 0.2%), (*R*)-limonene (11.8 ± 0.1%) and 10-*epi*-γ-eudesmol (10.6 ± 0.2%) were the major components of the leaf oil. The tricyclic sesquiterpene alcohol maaliol (6.2 ± 0.2%) was also found in appreciable amounts in leaf oil. Nineteen constituents representing 95.1 ± 1.5% were identified in the fruit oil, among which 10-*epi*-γ-eudesmol (35.7 ± 0.3%), 1,10-di-*epi*-cubennol (15.6 ± 0.3%), *epi*-longipinanol (7.3 ± 0.2%), caryophyllene oxide (7.5 ± 0.1%), isolongifolan-7-α-ol (7.1 ± 0.1%) and γ-eudesmol (4.0 ± 0.1%) were the major ones. To the best of our knowledge, there is no chemical investigation for the *E. langsdorffii* essential oil. Previous report on chemical investigations of the *Eugenia* species showed that essential oils are characterized by predominance of sesquiterpenes, along with monoterpenes as the minor fraction.[24] In fact, these oils were characterized by high percentages of sesquiterpenes (72.2 ± 0.9% in leaves and 92.2 ± 0.2% in fruits), followed by monoterpenes. The monoterpne α-pinene and the sesquiterpenes germacrene D, bicyclogermacrene, β-caryophyllene and β-elemene, which were found in *Eugenia* species as principal components,[24-29] were not detected in the leaf and fruit oils of *E. langsdorffii*. Moreover, none of the *Eugenia* species listed in recent review by Stefanello *et al.*[24] showed the presence of *epi*-longipinanol and 10-*epi*-γ-eudesmol, the major components indentified in the leaf and fruit oils of *E. langsdorffii*, respectively. On the other hand, the second major component of fruit oil (1,10-*epi*-cubenol (15.6 ± 0.3%)) was identified in the leaf oil of *E. neonitida*[25] and *Plinia trunciflora*[26] in percentage lower than 0.5%.

Caryophyllene oxide and (*R*)-limonene found in appreciate amounts in the fruits and leaves of *E. langsdorffii* oils, respectively, were reported as major components in the oil from other *Eugenia* species. Caryophyllene oxide was identified as principal compound in the fruit oil of *E. brasiliensis* purple variety (22.2%), *E. pyriformis* (16.2%)] and leaf oil of *E. rocana* (57.7%) and *E. plicato-costata* (25.7%), while the limonene was the main component found in the leaf oil of *E. speciosa*

Table 1. Percentage composition, yield and optical rotation of leaf and fruit essential oils from *Eugenia langsdorffii*

Compound	IR[a]	IR[b]	*Eugenia langsdorffii*		Method of identification
			Leaf	Fruit	
a-Thujene	924	924	0.5 ± 0.1	0.8 ± 0.1	RI, MS
Sabinene	969	969	0.5 ± 0.1	0.9 ± 0.0	RI, MS
β-Pinene	979	974	2.5 ± 0,1	1.3 ± 0.3	RI, MS, CI
p-Cymene	1018	1020	2.4 ± 0.1	–	RI, MS, CI
(*R*)-Limonene	1024	1024	11.8 ± 0.1	–	RI, MS, CI
Dihydro-linalool	1129	1131	0.4 ± 0.0	–	RI, MS
2-Methyl soborneol	1178	1178	0.5 ± 0.1	–	RI, MS
trans-Carveol	1210	1215	0.7 ± 0.0	–	RI, MS
Carvone	1234	1239	0.4 ± 0.1	–	RI, MS
Caren-3-one	1240	1244	0.7 ± 0.1	–	RI, MS
α-Terpinen-7-al	1279	1283	0.8 ± 0.1	–	RI, MS
α-Cubebene	1350	1345	0.6 ± 0.0	–	RI, MS
Silphiperfol-6-ene	1375	1377	2.1 ± 0.1	–	RI, MS
β-Longipinene	1400	1400	1.1 ± 0.2	–	RI, MS
Longifolene	1410	1407	0.5 ± 0.2	–	RI, MS
β-Dupreziene	1418	1421	0.8 ± 0.1	–	RI, MS
Aromadendrene	1439	1439	0.6 ± 0.1	–	RI, MS, CI
trans-Muurola-3,5-diene	1454	1451	0.6 ± 0.1	–	RI, MS
Ishwarane	1467	1465	1.0 ± 0.2	–	RI, MS
γ-Gurjunene	1474	1475	1.4 ± 0.2	1.5 ± 0.1	RI, MS
α-Amorphene	1479	1483	1.4 ± 0.2	–	RI, MS
trans-Muurola-4(14),5-diene	1490	1493	2.7 ± 0.1	–	RI, MS
Valencene	1496	1496	0.7 ± 0.0	–	RI, MS, CI
γ-Cadinene	1512	1513	0.4 ± 0.1	1.5 ± 0.3	RI, MS
α-dehydro- ar-Himachalene	1517	1516	0.6 ± 0.1	–	RI, MS
Silphiperfol-5-en-3-ol B	1529	1534	1.1 ± 0.1	–	RI, MS
Liguloxide	1538	1534	1.2 ± 0.1	1.3 ± 0.1	RI, MS
Selina-3,7(11)-diene	1545	1545	–	0.5 ± 0.0	RI, MS
Ocidentalol	1552	1550	–	1.0 ± 0.1	RI, MS
epi-Longipinanol	1559	1562	13.6 ± 0.4	7.3 ± 0.2	RI, MS
Maaliol	1562	1566	6.2 ± 0.2	1.9 ± 0.0	RI, MS
Zierone	1570	1574	3.1 ± 0.2	–	RI, MS
Spathulenol	1578	1577	1.3 ± 0.1	1.3 ± 0.1	RI, MS
Caryophyllene oxide	1583	1582	1.8 ± 0.1	7.5 ± 0.1	RI, MS, CI
Guaiol	1596	1600	–	2.3 ± 0.1	RI, MS
β-Atlantol	1603	1608	–	1.6 ± 0.0	RI, MS
Isolongifolan-7-α-ol	1614	1618	–	7.1 ± 0.1	RI, MS
1,10-di-*epi*-Cubenol	1614	1618	3.5 ± 0.1	15.6 ± 0.3	RI, MS
10-*epi*-γ-Eudesmol	1019	1022	10.6 ± 0.2	35.7 ± 0.3	RI, MS
γ-Eudesmol	1630	1630	12.3 ± 0.2	4.0 ± 0.1	RI, MS
Pogostol	1654	1651	1.1 ± 0.1	–	RI, MS
neo-Intermedeol	1662	1658	–	2.3 ± 0.2	RI, MS
Aglomerone	1694	1698	1.7 ± 0.1	-	RI, MS
Monoterpenes			21.2 ± 0.2	2.9 ± 0.3	
Sesquiterpenes			72.2 ± 0.9	92.2 ± 0.2	
Total			93.4 ± 0.8	95.1 ± 1.5	
Yield ± standard deviations / %			0.05 ± 0.01	0.06 ± 0.01	
$[\alpha]_D^{22}$ (*c* 1, CH_2Cl_2)			–9.0°	–3.5°	

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

(33.7%), *E. cristata* (45.0%), *E. brasiliensis* (13.9%) and *E. uruguayensis* (17.6%).[24]

Table 2 presents the toxicity of oils against *T. urticae* in testing of fumigation and residual contact. The toxicity of the oils varied according to the plant part studied and the applied method.

In general, the mites were more susceptible to the oils by fumigation than the residual contact. Linear regression analysis to obtain LC_{50} of oils revealed that fumigation of leaf oil (LC_{50} = 1.8 μL L^{-1} of air) was about 1.7 times more toxic than the fruit oil (LC_{50} = 3.0 μL L^{-1} of air). Opposite behavior was observed on the residual contact test, wherein fruit oil (LC_{50} = 12.2 μL mL^{-1} air) was 1.8 times more toxic than leaf oil (LC_{50} = 21.9 μL mL^{-1}), see Table 2. Comparing the oil toxicities with the positive control (eugenol), the fumigant action of the latter was greater than by residual contact. The leaf oil, which presented better result in the fumigation, was about 450 times less toxic than the eugenol (LC_{50} = 0.004 μL L^{-1} of air), while the fruit oil (LC_{50} = 12.25 μL mL^{-1}) in the residual contact test was only 6.7 times less toxic (Table 2). These results suggest that the oils of *E. langsdorffii* present two miticide action modes and that are more efficient through the vapor penetration by the respiratory system of dust mites than by ingestion and/or penetration through the tarsi of the mites.

To confirm the fumigant action observed for these oils, experiments were repeated with fumigation open chambers at the same concentration that the oils promoted ≥ 96.0% in mortality and experiments with closed chambers (12 μL L^{-1} for leaf oil and 20 μL L^{-1} of air for fruit oil).

Under these conditions, both oils showed a dramatic reduction in mortality (26.6 ± 1.1% for the leaf oil and 30.8 ± 2.2% for the fruit). As expected, in these concentrations that were tested on the *E. langsdorffii* oils, eugenol promoted 100% in mortality in experiment with closed chambers. However, in experiments with open chambers (at 12 μL L^{-1} of air), eugenol did not exhibit any fumigation toxicity and the average mortality at 20 μL L^{-1} of air did not reach 10%. These results suggest that the miticide action of eugenol in such conditions is basically by fumigation.

The mites were more susceptible to constituents by fumigation than by residual contact. Table 3 shows toxicity data of individual constituents by residual contact and fumigation. The available oil constituents identified in the fruit (β-pinene and caryophyllene oxide) presented high fumigation toxicity at 20 μL L^{-1} of air (Table 3). Comparing the toxicities between the positive control and those obtained for individual compounds at 20 μL L^{-1} of air, only the toxicity of β-pinene (100.0 ± 0.0% mortality) was equivalent to the eugenol and higher than that obtained for the fruit oil (97.7 ± 2.2%), suggesting that the latter can be responsible for the observed activity by oil. However, the complete mixing between these constituents prepared in the same proportion identified by GC-MS in the fruits oil (1.3 ± 0.3 to β-pinene and 7.5 ± 0.1 for caryophyllene oxide) (Table 1) reduced the mortality to 71.1%, indicating that in this proportion, the caryophyllene oxide exerts an antagonistic effect on β-pinene since the level of toxicities of β-pinene was significantly larger when individually tested (Table 3).

Table 2. Toxicity by fumigation (LC_{50} of air) and residual contact (LC_{50}) of *Eugenia langsdorffii* oils against *Tetranychus urticae*

Oil[a]	N	df	Slope	Fumigation LC_{50} / (μL L^{-1}) (CI 95%)	χ^2	TR_{50} (CI 95%)
Leaf	720	6	1.86	1.79 (1.32-2.34)	12.07	510.0 (123.2-835.7)
Fruit	900	8	1.99	3.06 (2.47-3.69)	13.12	870.1 (212.1.6-1175.4)
Eugenol	580	5	0.84	0.004 (0.002-0.008)	2.50	–
Oil[a]	N	df	Slope	Residual contact LC_{50} / (μL L^{-1}) (CI 95%)	χ^2	TR_{50} (CI 95%)
Leaf	174	5	3.50	21.90 (18.65-25.37)	4.62	12.40 (10.01-15.35)
Fruit	149	4	3.32	12.25 (10.02-14.43)	2.04	6.93 (5.49-8.75)
Eugenol[b]	199	5	2.15	1.80 (1.29-2.63)	5.68	–

[a]Essential oil of *Eugenia langsdorffi*; [b]positive control; N: number of mites *per* dose; df: degrees of freedom; CI: confidence interval; χ^2: chi-squared; TR: toxicity ratio.

Table 3. Mortality (%, ± standard deviation) caused by individual constituents of the *Eugenia langsdorffii* oils against *T. urticae* applied at the same concentration of leaf and fruit oils that showed mortality ≥ 96.0%

Constituent / oil		Fumigation		Residual contact	
		Leaf oil (12 μL L^{-1} of air)	Fruit oil (20 μL L^{-1} of air)	Leaf oil (46 μL mL^{-1})	Fruit oil (31 μL mL^{-1})
Caryophyllene oxide		61.3 ± 2.9b	77.7 ± 2.9c	80.0 ± 1.7c	72.1 ± 4.8b
β-Pinene		91.1 ± 1.1c	100.0 ± 0.0d	38.4 ± 3.0d	24.0 ± 4.8c
Valencene		86.6 ± 1.9d		88.0 ± 2.2c	
p-Cymene		84.4 ± 2.9e		40.7 ± 2.9d	
(*R*)-Limonene		71.1 ± 2.9f		64.0 ± 2.9e	
Aromadendrene		61.3 ± 2.2b		96.0 ± 2.8ab	
FM[a]		98.8 ± 1.6a	71.1 ± 1.1b	92.0 ± 1.8b	71.1 ± 4.8b
Leaf	CFC	96.6 ± 1.9a	97.7 ± 2.2a		
	OFC	26.6 ± 1.1	38.8 ± 2.2		
Eugenol[b]	CFC	100.0 ± 0.0	100.0 ± 0.0		
	OFC	100.0 ± 0.0	100.0 ± 0.0		

[a]Full mixture of constituents prepared at same percentage composition identified by GC and GC-MS analyses of leaf oil; [b]positive control; CFC and OFC are fumigant experiments done with close and open chamber, respectively; mortality means (± standard deviation) with the same letter into the same column did not significantly differ by the Tukey's test ($p \leq 0.05$).

Unlike what was observed for fumigation, in residual contact toxicity, none of the constituents presented the toxicity level observed for the fruit oil (96.0% mortality) in the residual contact. The observed toxicity for the β-pinene (24.02 ± 2.9%) was much smaller than that for the caryophyllene oxide (72.1 ± 1.8%). Comparing the two methods used to evaluate the toxicity against *T. urticae*, it was observed that the β-pinene was more toxic than the caryophyllene oxide in fumigation test. These data can be justified by the increased volatility of β-pinene due to its lower molecular weight and weaker chemical interactions than the caryophyllene oxide in an aqueous solution.

The result by residual contact test of blends from these two constituents suggests that in this case, β-pinene antagonistically interacts with caryophyllene oxide, accounting for only 8.8% of the components identified in the fruit oil and is not the only responsible for contact and residual fumigant action observed for that oil. The results obtained for fumigation and residual contact of these constituents should not be extended to the oil, even though the isolated β-pinene showed a high toxicity. In this case, other oil components (approximately 86.3% of oil) exert a significant contribution to the toxicity observed for fruit oil through the synergistic interactions between them and the two constituents here investigated.

For the leaf oil, the fumigation tests with individual constituents at 12 μL L^{-1} of air revealed three compounds with high toxicity (β-pinene, *p*-cymene and valencene, the supposedly most active), while aromadendrene, (*R*)-limonene and caryophyllene oxide presented toxicity ranging from low to moderate (supposedly less active). No tested constituent presented mortality equal to or greater than the leaf oil. On the other hand, the mortality percentage of mites found by complete mixing of the six constituents prepared in the same proportion that was identified by the analysis by GC-MS of oil (2.5 ± 0.1 for β-pinene, 2.4 ± 0.1 to *p*-cymene,11.8 ± 0.1 for (*R*)-limonene, 0.6 ± 0.1 to aromadendrene, 0.7 ± 0.0 for valencene and 1.8 ± 0.1 for caryophyllene oxide, see Table 1) was greater than that obtained by the oil from the leaves, but did not significantly differ among themselves. Similar results showing likely synergistic interaction between the main chemical constituents of essential oils have been reported in the literature, but representing around 80-99% of the total oil composition.[22,30] However, not always toxicity observed for an oil is attributed to its main components. Jiang *et al.*[31] reported that the mix of the main oil components from *Litsea cubeba* (representing approximately 89.9% of the total toxicity of the oil) was significantly less than that obtained for the oil on the third instar larvae of *Trichoplusia ni*. The results obtained by complete mixing of selected constituents (representing only 19.8% of the oil) showed toxicity similar to the leaf oil of *E. langsdorffii*.

The preliminary conclusion from the results of individual toxicity of the oil constituents and initial mixture from *E. langsdorffii* is that the toxicity observed for the blend is due to synergistic interaction between supposedly more active constituents with the lesser active, as observed in individual tests (Table 3).

In order to investigate the level of contribution of these constituents for toxicity of the complete mixture, blends with the selected constituents from the leaf

oil were evaluated by the fumigation method. The toxicities of blends of more active constituents (β-pinene, *p*-cymene and valencene) and less active (aromadendrene, (*R*)-limonene and caryophyllene oxide) selected from the individual tests were below the levels observed for the leaf oil and complete mixture (Figure 2).

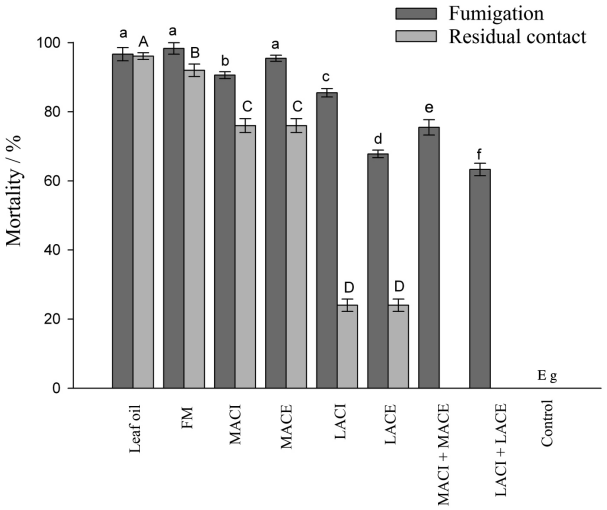

Figure 2. Mean toxicity caused by the oil, full mixture and selected blends of constituents of *Eugenia langsdorffii* leaf oil to *Tetranychus urticae* applied at levels equivalent to the leaf oil which showed mortality ≥ 96.0% compared with the control. Error bars represent the standard error of the mean of 90 mites. Means corresponding to each treatment with different letters are significantly different from each other by the Tukey's test ($p \leq 0.05$). FM indicates a blend of 6 constituents, whereas all others indicate mixture of compounds selected according to scheme shown in Figure 1.

Despite β-pinene having a higher toxicity on its own, the average mortalities among this constituent and the blend with more active constituents did not significantly differ. On the other hand, the toxicity presented by blend of the less active was surprisingly higher than the toxicities of their individual constituents, suggesting that the components of the blends in proportion found in leaf oil synergistically interact with each other (Table 3 and Figure 2).

On the basis of the toxicity results of blends obtained from the experiments with removing one of the components from the complete mixture, it was possible to select other constituents that supposedly contribute more to the toxicity of the mixture. The results of these blends presented in Figure 3 indicate that the absence of some compounds, like *p*-cymene, valencene or (*R*)-limonene, caused a significant reduction in mortality (73.3, 75.5 and 85.5%, respectively) which leads to assume that these constituents are the most active.

On the other hand, the absence of the components β-pinene, aromadendrene and caryophyllene oxide little interfered with the toxicities of the blends, and were

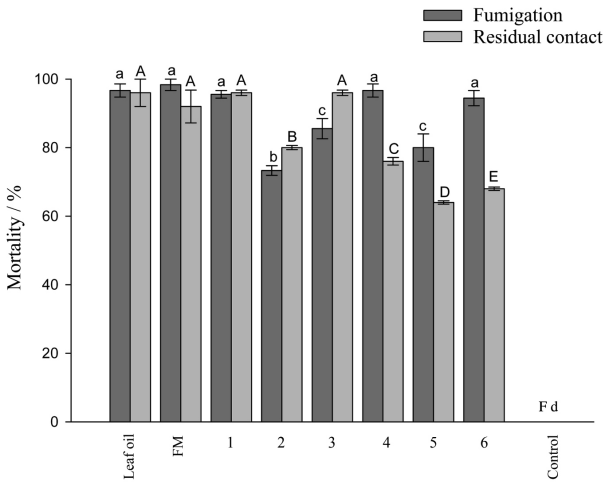

Figure 3. Mean toxicity caused by the oil, full mixture and selected blends of constituents of *Eugenia langsdorffii* leaf oil to *Tetranychus urticae* applied at levels equivalent to the leaf oil that showed mortality ≥ 96.0% compared with the control. Error bars represent the standard error of the mean of 90 mites. Means corresponding to each treatment with different letters are significantly different from each other by the Tukey's test ($p \leq 0.05$). FM indicates a blend of 6 constituents; whereas all others indicate full mixture missing the constituent noted (1) caryophyllene oxide, (2) β-pinene, (3) valencene, (4) *p*-cymene, (5) (*R*)-limonene and (6) aromadendrene.

selected as the least active. As expected, the mites were more susceptible to blends of more active constituents and the average mortality was greater than that of leaf oil, but was not significant between themselves. Comparing the toxicities by fumigation of all blends prepared as the scheme of Figure 1, only the most active constituent blend selected by removal experiments of a compound from complete mixture surpassed the level of toxicity observed for the leaf oil. This suggests a synergistic interaction between the constituents of these blends since the level of toxicity observed for it is greater than those of isolated constituents tested. The toxicity of blends formed by the sum of the most active constituents (MACI + MACE) and the sum of less active (LACI + LACE) was smaller than the toxicities of these separate blends (Figure 2), suggesting an antagonist interaction between the constituents from the sum of the blends.

Through detailed analysis of the relationship between the constituent blends and their toxicities by fumigation, it can be noted that except for the complete mixture, all other blends prepared with β-pinene, the mites mortality rate was less than by this terpene alone. In fact, for the β-pinene, which was the most active constituent individually tested, the toxicity of blends obtained from its removal from complete mixture did not dramatically changed. On the other hand, for the blends consisting of (*R*)-limonene in the absence of β-pinene, the toxicities were higher compared to that of (*R*)-limonene individually. This fact is corroborated

by the results obtained for the interaction between the sesquiterpenes: caryophyllene oxide, aromadendrene with the (*R*)-limonene (LACI), which was more toxic than the interaction of sesquiterpenes with β-pinene (LACE). Similar results were observed for the interactions between valencene, *p*-cymene with (*R*)-limonene and β-pinene in the blends of more active constituents, MACE and MACI, respectively (Figure 2). From these data, it can be can concluded that among the blends tested with the constituents in the same proportions found in *E. langsdorffii* leaf oil, those that contribute the most to the fumigation toxicity of the complete mixture were prepared with (*R*)-limonene, *p*-cymene and valencene.

The constituents evaluated by the method of fumigation revealed different toxicities for the residual contact test. The percentage of mortalities of individual constituents for this test at 46 μL mL^{-1} are shown in Table 3. Among the constituents, only the aromadendrene presented toxicity similar to leaf oil, and the average mortality rate obtained for the positive control (eugenol: 100% mortality) was greater than that of aromadendrene, but did not significantly differ between themselves (Table 3). The mortalities presented by sesquiterpenes were above 80% (aromadendrene, valencene and caryophyllene oxide), being selected as the supposedly more active constituents, while the monoterpenes (β-pinene, *p*-cymene, and (*R*)-limonene) were considered the least active. Blends of these constituents were evaluated by residual contact and the criterion used in the preparation was the same used for fumigation and the constituents were selected as the scheme of Figure 1. The mite mortalities produced by blends in residual contact tests are shown in Figure 2. As expected, the less active constituent blends presented low toxicity and the mortality ranged from 38 to 64% (β-pinene, p-cymene and (*R*)-limonene). However, an unexpected result was obtained for the most active constituent blend (aromadendrene, valencene and caryophyllene oxide). The toxicity presented by this blend was lower than that obtained for the sesquiterpenes individually (Table 3 and Figure 2).

This suggests that these sesquiterpenes are antagonistic to each other. Similar result was obtained for the complete mixture between the constituents. The average mortality of this blend (92.0 ± 1.8%) was lower and significantly differed from the average obtained for the leaf oil (96 ± 1.0%). To confirm the role of these constituents in toxicity presented by oil from the leaves, blends prepared from removing a constituent of the full mixture were subjected to the residual contact test. The results of the toxicity of these blends are presented in Figure 3. These data corroborate the toxicities observed for individual constituents in which the sesquiterpenes were supposedly more active. In fact, the removal of anyone of the sesquiterpenes from the complete mixture reduced the toxicity and the average mite mortalities, ranging between 64 to 76% (Figure 3). The results obtained for these blends revealed that the level of toxicity observed for leaf oil is only achieved when the β-pinene or (*R*)-limonene is missing from complete mixture (Figure 3). On basis of the results obtained for the blends prepared using β-pinene in the proportions found in the leaf oil, the interaction observed between this monoterpene and other constituents was antagonistic, independent of the bioassay used, if by fumigation or residual contact.

Conclusions

The study of the essential oil from *Eugenia langsdorffii* that occurs in the cerrado biome revealed a chemical profile with large amounts of sesquiterpenes, characteristic of species of this genus. The results presented in this work about toxicity of individual constituents and their blends revealed the difficulty to unequivocally establish the active principle of a complex mixture as an essential oil. Tests with the blends prepared using a small sample of the constituents of the oil allowed to identify the relationship between the constituents tested and toxicity, as well as to assume, according to the method used in bioassays, the primary action mode of the miticide blends. The properties observed for these blends are intrinsically related to the proportions of the components used in the mixture and the method employed.

According to the residual contact and fumigant properties demonstrated in this work, the essential oil of *E. langsdorffii* seems to be a promising miticide. However, further studies are needed to establish the cost-benefit ratio to be used as the active principle in the formulation for an integrated management of *T. urticae*.

Supplementary Information

The total ion chromatograms of leaf and fruit essential oils from *Eugenia langsdorffii* are available free of charge at http://jbcs.org.br as a PDF file.

Acknowledgments

Our thanks to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE) for scholarship and financial support (CNPq Proc. No. 476503/2009-4 and FACEPE-APQ No. 0520-55.01/08)

References

1. Mittermeier, R. A.; Myers, N.; Mittermeier, C. G.; *Hotspots: Earth´s Biologically Richest and Most Endangered Terrestrial Ecoregions*; Conservation International-CEMEX: New York, 1999.
2. Mendonça, R. C.; Felfili, J. M.; Walter, B. M. T.; Silva-Júnior, M. C.; Rezende, A. V.; Filgueiras, T. S.; Nogueira, P. E.; Fagg, C. W. In *Cerrado: Ecologia e Flora*; Sano, S. M.; Almeida, S. P.; Ribeiro, J. F., eds.; Embrapa Cerrados: Brasília, 2008.
3. Govaerts, R.; Sobral, M.; Ashton, P.; Barrie, F.; Holst, B. K.; Landrum, L. R.; Matsumoto, K.; Mazine, F. F.; Lughadha, E. M.; Proença, C.; Soares-Silva, L. H.; Wilson, P. G.; Lucas, E.; *World Checklist of the Myrtaceae*; Royal Botanic Gardens: Kew, 2008.
4. Adebajo, A. C.; Oloke, K. J.; Aladesanmi, A. J; *Phytother. Res.* **1989**, *3*, 258.
5. Menezes, E. L. A.; *Inseticidas Botânicos: Seus Princípios Ativos, Modo de Ação e Uso Agrícola*, 1st ed.; Seropédica: Rio de Janeiro, 2005.
6. Flamini, G.; *Nat. Prod. Commun.* **2006**, *21*, 1151.
7. Mora, F. D.; Avila, J. L.; Rojas, L. B.; Ramirez, R.; Usubillaga, A.; Segnini, S.; Carmona, J.; Silva, B.; *Nat. Prod. Commun.* **2010**, *5*, 965.
8. Pasay, C.; Mounsey, K.; Stevenson, G.; Davis, R.; Arlian, L.; Morgan, M.; Vyszenski-Moher, D.; Andrews, K.; McCarthy, J.; *PLoS One* **2010**, *5*, e12079.
9. Wu, H. Q.; Li, J.; He, Z. D.; Liu, Z. G.; *Parasitology* **2010**, *137*, 975.
10. Fichi, G.; Flamini, G.; Giovanelli, F.; Otranto, D.; Perrucci, S.; *Exp. Parasitol.* **2007**, *115*, 168.
11. Salvador, M. J.; de Lourenco, C. C.; Andreazza, N. L.; Pascoal, A. C. R. F.; Stefanello, M. E. A.; *Nat. Prod. Commun.* **2011**, *6*, 977.
12. Stokes, S. L.; Cole, R. A.; Rangelova, M. P.; Haber, W. A.; Setzer, W. N.; *Nat. Prod. Commun.* **2007**, *2*, 1211.
13. Moriarity, D. M.; Bansala, A.; Cole, R. A.; Takaku, S. H.; William, A.; Setzer, W. N.; *Nat. Prod. Commun.* **2007**, *2*, 1263.
14. Werka, J. S.; Boehme, A. K.; Setzer, W. N.; *Nat. Prod. Commun.* **2007**, *2*, 1215.
15. Isman, M. B.; *Annu. Rev. Entomol*. **2006**, *51*, 45.
16. Isman, M. B.; Miresmailli, S.; Machial, C.; *Phytochem. Rev*. **2011**, *10*, 197.
17. Van den Dool, H.; Kratz, P. H.; *J. Chromatogr., A* **1963**, *11*, 463.
18. Adams, R. P. In *Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Apectroscopy*, 4th ed.; Allured Publishing Corporation: Carol Stream, 2007**.**
19. Pontes, W. J. T.; de Oliveira, J. C. S.; da Camara, C. A. G.; Lopes, A. C. H. R.; Godim-Júnior, M. G. C.; de Oliveira, J. V.; Schwartz, M. O. E.; *J. Essent. Oil Res*. **2007**, *19*, 379.
20. SAS Institute Inc.; *SAS/User's Guide: Statistics*, version 9.0, 7th ed.; SAS Institute Inc.: Cary, 2002**.**
21. LeOra Software; *POLO-PC: A User's Guide to Probit Logit Analysis*; LeOra Software, Berkely, 1987.
22. Miresmailli, S.; Bradbury, R.; Isman, M. B.; *Pest. Manag. Sci*. **2006**, *62*, 366.
23. Robertson, J. L.; Preisler, H. K.; *Pesticide Bioassays with Arthropods*; CRC Press: California, 1992
24. Stefanello, M. E. A.; Pascoal, A. C. R. F.; Salvador, M. J.; *Chem. Biodivers.* **2011**, *8*, 73.
25. Defaveri, A. C. A.; Sato, A.; Borré, L. B.; Aguiar, D. L. M.; San Gil, R. A. S.; Arruda, R. C. O.; Riehl, C. A. S.; *J. Braz. Chem. Soc*. **2011**, *22*, 1531.
26. Lago, J. H. G.; Souza, E. D.; Mariane, B.; Pascon, R.; Vallim, M. A.; Martins, R. C. C.; Baroli, A. A.; Carvalho, B. A.; Soares, M. G.; dos Santos, R. T.; Sartorelli, P.; *Molecules* **2011**, *16*, 9827.
27. Oliveira, R. N.; Dias, I. J. M.; Camara, C. A. G.; *Rev. Bras. Farmacogn.* **2005**, *15*, 39.
28. Zoghbi, M. G. B.; Guilhon, G. M. S. P.; Sarges, F. N.; Pereira, R. A.; Oliveira, J.; *Biochem. Syst. Ecol.* **2011**, *39*, 660.
29. Raj, G.; George, V.; Sethuraman, M. G. J.; *J. Essent. Oil Res*. **2011**, *23*, 55.
30. Attia, S.; Grissa, K. L.; Lognay, G.; Heuskin, S.; Mailleux, A. C.; Hance, T.; *J. Econ. Entomol*. **2011**, *104*, 1220.
31. Jiang, Z.; Akhtar, Y.; Bradbury, R.; Zhang, X.; Isman, M. B.; *J. Agric. Food Chem*. **2009**, *57*, 4833.

Submitted: December 2, 2011
Published online: August 22, 2012