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Artigo

# SEASONAL CHEMICAL COMPOSITIONS OF THE ESSENTIAL OILS OF TWO *Eugenia* SPECIES AND THEIR ACARICIDAL PROPERTIES

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The leaf essential oils of *Eugenia lutescens* Cambess and *Eugenia langsdorffii* O. Berg, collected in the rainy (RS) and dry seasons (DS), were extracted by hydrodistillation and then characterized by a gas chromatography-flame ionization detector and a gas chromatography-mass spectrometer. The potential acaricidal activity and oviposition deterrence of these oils were evaluated against *Tetranychus urticae*. The oil yields were higher in the RS for *E. lutescens*, while those for *E. langsdorffii* were higher in the DS. α-Pinene and β-pinene were determined to be the major constituents of the oils from *E. lutescens*, while bicyclogermacrene, spathulenol, and β-caryophyllene predominated in *E. langsdorffii*. Seasonal variations in the oils were primarily related to chemical diversity, and *E. lutescens* was more affected than was *E. langsdorffii*. The *E. langsdorffii* oil collected in the DS was most toxic to the spider mite, while the oils of *E. lutescens* and *E. langsdorffii* collected in the RS drastically reduced its egg quantities. This study successfully determined the periods of greater oil production and acaricidal activity.

Keywords: *Eugenia lutescens*; *Eugenia langsdorffii*; seasonal variations; acaricidal properties; *Tetranychus urticae*.

## INTRODUCTION

With approximately 2 million km$^2$ the Brazilian savanna, hereafter called cerrado, represents about 23% of the country, extending over ten states and the Federal District and features as the second greatest biome after the Amazonian forest.[1] Brazilian cerrado is the richest tropical savanna in the world, with over 12,000 flowering plants recorded,[2] and one of the 25 global biodiversity hotspots for priority conservation,[3] statistics which have been stimulating the prospection of chemical compounds of economic value for medicinal,[4,5] agricultural and other applications.[5]

*Eugenia* species (Myrtaceae) have been found in floristic and phytosociological studies in various forest in cerrado including the southeast of Brazil.[6] Their plants, usually appreciated for its edible fruits, also show high levels of essential oils characterized by chemical diversity (*e.g.* sesquiterpenes, monoterpenes and phenylpropanoids) and a wide range of biological properties.[7] For instance several studies with essential oils from *Eugenia* species have shown therapeutic potential as anticonvulsant,[8] anti-inflammatory,[9] antinociceptive,[9,10] hypothermic,[10] antioxidant and antimicrobial,[11] but none of them have investigated *Eugenia langsdorffii* O. Berg and *Eugenia lutescens* Cambess.

In addition, there are some records associated to the activity of essential oils from *Eugenia* genus against several arthropods. For example, the insecticidal and acaricidal properties of the *E. uniflora* and *E. caryophyllata* essential oils against the maize weevil (*Sitophilus zeamais*);[12] and the dust mite (*Dermatophagoides farinae*)[13] and spotted spider mite (*Tetranychus urticae*).[14]

The spotted spider mite is one of the most important agricultural pests that produce major losses in cultivated crops from North to South Brazil. The principal form of control of this pest involves the indiscriminate use of acaricides, associated with the presence of residues in foods, contamination of the environment and selection of more resistant populations, which then require greater number of applications.

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Aiming to establish a rational control, with low toxicity to mammals and a reduced persistence in the environment, the use of essential oils as an active ingredient in formulations for the control of agricultural pests is an excellent alternative to synthetic pesticides, given the chemical diversity of the oils, which act at the same time in various areas of the insect, reducing the risk of the pest acquiring resistance.[15]

According to a bibliographic survey, no study has been undertaken with the essential oil of *E. lutescens* against spider mite. While our research group has published the chemical composition of the leaf essential oil of *E. langsdorffii* reporting that *epi*-longipinanol (13.6 ± 0.1%) and γ-eudesmol (12.3 ± 0.2%)[16] are the major constituents and promising products for the control of *T. urticae* rather than the fruit oil, no study has been undertaken confirming if the chemical profile of the *E. langsdorffii* leaf oil varies seasonally and whether it affects its acaricidal properties.

Continuing ongoing studies on the chemical and biological potential of aromatic plants that occur in the cerrado of Brazil and looking for new products with acaricidal properties, the purpose of this work is to investigate the seasonal variation in yields and micro molecular composition of leaf essential oils from *E. lutescens* and *E. langsdorffii*, collected in two seasons, and correlating their chemical profile with acaricidal property on the spider mite (*Tetranychus urticae*).

## EXPERIMENTAL

### Collection of plant material

The fresh leaves from *E. lutescens* (15°45'56.2"S 47°51'26.1"W) and *E. langsdorffii* (15°46'23.5"S 47°51'58.2"W) were collected in the morning during two periods of 2012 (March and August) in the Cerrado biome around the campus Darcy Ribeiro of the University of Brasília (UnB), Federal District. The plants were identified by the botanist Jair Faria Jn of the Botany Department, UnB. Voucher specimens were deposited in the UnB herbarium (UB) under the numbers: Fagg CW 2189 (*E. lutescens*) and Faria Jn JEQ & Fagg CW 918 (*E. langsdorffii*).

**Isolation of the essential oil**

The essential oils (OE's) from fresh leaves of *E. lutescens* and *E. langsdorffii* (100 g) were extracted using a modified Clevenger-type apparatus by hydrodistillation for 2 h. The oil layer was separated and dried over anhydrous sodium sulfate, stored in hermetically sealed glass vials, and kept under refrigeration at +5 °C until the acaricidal assays and chemical analysis. Total oil yield was expressed as percentages (g/100 g of fresh plant material). All experiments were carried out in triplicate.

**Chemicals**

Monoterpenes and sesquiterpenes used for identifications of volatile components (β-pinene, α-pinene, limonene, α-terpineol, β-caryophyllene and its oxide, aromadendrene, α-humulene and valencene) and eugenol used in the bioassay as a positive control 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 x 0.25 mm x 0.25 μm film thickness) (J & W Scientific). The oven temperature was programmed from 50 to 250 °C at a rate 3 °C $min^{-1}$ for integration purposes. Injector and detector temperatures were 250 °C. Hydrogen was used as the carrier gas at a flow rate of 1 $L.min^{-1}$ and 30 p.s.i. inlet pressure in split mode (1:30). The injection volume was 0.5 μL of diluted solution (1/100) of oil in hexane. The amount of each compound was calculated from GC peak areas in the order of column elution and expressed as a relative percentage of the total area of the chromatograms. Analyses were carried out in triplicate.

**Gas chromatography-mass spectrometry**

The qualitative GC/MS analyses of essential oils were carried out using a Hewlett-Packard GC/MS (GC: 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 = helium; flow rate = 1 mL $min^{-1}$; split mode (1:30); injected volume = 1 μL of diluted solution (1/100) of oil in hexane.

**Identification of components**

Identification of the components was based on GC retention indices with reference to a homologous series of $C_{11}$-$C_{24}$ n-alkanes calculated using the Van den Dool & Kratz equation[17] and by computer matching against the mass spectral library of the GC/MS data system (NIST 98 and WILEY) as well as other published mass spectra.[18] Area percentages were obtained electronically from the GC-FID response without using an internal standard or correction factors.

**Acaricidal bioassay**

*Rearing of Tetranychus urticae*

The population of *T. urticae* was acquired from the Agricultural Acarology Lab, UFRPE. The rearing was undertaken in the Natural Insecticides Laboratory, Agronomy Department, UFRPE, on jack bean plants (*Canavalia ensiformes* L.) cultivated in 5 L pots containing soil mixed with humus (3:1). Twenty five day old plants were infested with eggs, larvae, nymphs and adults of spider mite. The stock population was not exposed to the acaricides and was maintained at a temperature of 25 ± 1 °C, relative humidity of 65 ± 5 % and 12 h photoperiod.

*Fumigation bioassay*

The methodology of Pontes *et al*.[19] was followed for the fumigation experiments. Fumigation chambers were 2.5 L glass containers. Three leaf discs of 2.5 cm diameters of jack bean were placed equal distance from each other on a 9 cm diameter Petri dish, containing filter paper saturated with distilled water to prevent the migration of the spider mites and maintain leaf turgidity, undertaken in triplicate. On each of the leaf discs 10 adult female spider mites were placed. The petri dishes were put in fumigation chambers, with 30 adult females per chamber (10 spider mites per disc). The OE's were applied with an automatic pipette (5 μL) on strips of filter paper 10 × 2 cm, attached to the internal surface of the fumigation chamber lid. The concentrations used varied from $6.4x10^{-5}$ to 1.2 μL $L^{-1}$ of air, depending on the development activity of the OE's. The sample dilutions were made with dichloromethane, also applied as negative control and eugenol as positive control. All analyses were undertaken in triplicate in different fumigation chambers and evaluated after 24 h of exposition. The spider mites unable to walk after prodding were considered dead.

*Fecundity bioassay*

The *T. urticae* oviposition deterrent effect from *Eugenia* species oils vapors were determined using a fumigation bioassay method adapted from Pontes *et al.*[19] Five jack bean leaves (1.5 cm) were placed equidistant in a Petri dish (10 cm) containing filter paper saturated with water. Each leaf disc was infested with an adult *T. urticae* female, totaling five females per Petri dish. The OE's and pure chemicals were applied to strips of filter paper (10 x 2 cm) attached to the inner surface of the lid fumigation chamber with the aid of an automatic pipette. The concentrations used in the fecundity tests for the leaf oils from the *Eugenia* species was the $CL_{50}$ found to the positive control eugenol (0.001 μL $L^{-1}$ air). No substances were used in the negative control. Immediately after the application of the oil/compound, the fumigation chamber was closed and covered with PVC® plastic wrap. An entirely randomized design was employed, with five replicates, totaling 10 repetitions. The number of eggs in the treatments and controls were recorded after 24 h.

*Statistical analysis*

The fecundity and toxic fumigation bioassays with *T. urticae*, after attending the normality and variance tests (Proc. Univariate and GLM), were submitted to the Probit analysis and the lethal concentrations ($CL_{50}$) estimated using the software POLO-PC.[20] The Robertson e Preisler[21] method was used for the calculation of toxicity ratios, with a 95% confidence interval. The relative percentages of identified compounds in the oils of *E. lutescens* and *E. langsdorffii* from the two collection periods, and the fecundity data were submitted to analysis of variance (ANOVA) with the means compared by the Tukey test ($P < 0.05$) using the statistical program SAS Institute.[22]

**RESULTS AND DISCUSSION**

The average yields and chemical constituents of leaf essential oils of *E. lutescens* and *E. langsdorffii*, collected in the rainy (March) and dry seasons (August), as well as the relative humidity and temperature at the time of collection are presented in Table 1. The oil yields varied according to the species and collection period. *E. langsdorffii* produced the greatest quantity of oil compared to *E. lutescens* independent of the season.

**Table 1.** Chemical constituents identified on the essential oils of two *Eugenia* species harvested in two seasons of the year

| Compound | | | *E. lutescens* | | *E. langsdorffii* | |
|---|---|---|---|---|---|---|
| | | | **Rainy** | **Dry** | **Rainy** | **Dry** |
| | **Date of collection** | | 03/30/2012 | 08/30/2012 | 03/31/2012 | 08/31/2012 |
| | **M/M (%)** | | 0.25 ± 0.01 | 0.21 ± 0.01 | 0.40 ± 0.04 | 0.60 ± 0.03 |
| | **T (ºC)*** | | 25 ± 2 | 28 ± 2 | 23 ± 2 | 29 ± 2 |
| | **RH (%)*** | | 61 ± 5 | 35 ± 5 | 69 ± 5 | 27 ± 5 |
| | RI[a] | RI[b] | % ± SD | % ± SD | % ± SD | % ± SD |
| Santolina triene | 894 | 906 | - | 8.4±0.0 | 7.1±0.0 | - |
| α-Pinene[c] | 932 | 932 | 12.9±0.1 | 12.5±0.1 | 2.0±0.0 | 1.1±0.1 |
| Sabinene | 977 | 969 | - | - | 3.0±0.0 | - |
| β-Pinene[c] | 978 | 979 | 24.0±0.1 | 24.3±0.1 | 5.9±0.0 | 4.9±0.1 |
| Myrcene | 988 | 988 | 0.7±0.0 | - | - | - |
| α-Phelandrene | 1006 | 1002 | - | - | 0.7±0.1 | 1.0±0.1 |
| o-Cimene | 1023 | 1020 | - | - | - | 1.2±0.1 |
| Limonene[c] | 1025 | 1024 | 1.9±0.0 | 3.0±0.1 | 5.4±0.0 | 8.3±0.0 |
| (*E*)-β-Ocimene | 1044 | 1044 | - | - | 1.6±0.1 | 0.9±0.0 |
| α-Terpineol[c] | 1193 | 1186 | - | 3.0±0.0 | - | - |
| δ-Elemene | 1332 | 1335 | 1.4±0.0 | 2.2±0.0 | 3.3±0.1 | 1.8±0.1 |
| α-Copaene | 1375 | 1374 | 0.6±0.0 | - | 1.3±0.0 | 2.5±0.0 |
| β-Elemene | 1386 | 1389 | 0.7±0.1 | 3.2±0.1 | 1.2±0.0 | 2.4±0.1 |
| β-Caryophyllene[c] | 1420 | 1417 | 7.2±0.0 | 8.6±0.1 | 9.1±0.0 | 9.5±0.1 |
| β-Gurjunene | 1426 | 1431 | - | - | 1.1±0.0 | 1.2±0.0 |
| γ-Elemene | 1426 | 1434 | 3.8±0.1 | 5.5±0.1 | - | - |
| Aromadendrene[c] | 1433 | 1439 | 0.8±0.0 | 2.0±0.1 | 1.5±0.0 | 1.8±0.0 |
| α-Humulene[c] | 1450 | 1452 | 1.2±0.0 | 3.5±0.0 | 1.3±0.0 | 1.6±0.0 |
| *allo*-Aromadendrene | 1452 | 1458 | 1.3±0.0 | 1.5±0.0 | - | 2.9±0.0 |
| 9-*epi*-(*E*)- Caryophyllene | 1454 | 1464 | - | - | 2.4±0.0 | - |
| γ-Gurjunene | 1470 | 1475 | 1.7±0.0 | - | - | - |
| *trans*-Cadina-1(6),4-diene | 1470 | 1475 | 3.5±0.0 | - | - | - |
| γ-Muurolene | 1471 | 1478 | 1.0±0.1 | 5.0±0.0 | - | 0.8±0.0 |
| δ-Selinene | 1483 | 1492 | 0.8±0.0 | - | - | - |
| Valencene[c] | 1488 | 1496 | - | - | 1.7±0.1 | 1.6±0.0 |
| Bicyclogermacrene | 1490 | 1500 | 2.0±0.0 | - | 15.0±0.1 | 14.0±0.1 |
| α-Muurolene | 1494 | 1500 | 0.8±0.0 | - | 0.8±0.0 | 1.0±0.0 |
| γ-Cadinene | 1509 | 1513 | 0.5±0.1 | - | 1.1±0.0 | 2.0±0.1 |
| δ-Cadinene | 1518 | 1522 | 1.7±0.0 | - | 4.0±0.0 | 5.8±0.1 |
| Germacrene B | 1553 | 1559 | 0.6±0.0 | - | 4.7±0.0 | 3.4±0.1 |
| Spathulenol | 1572 | 1577 | 5.7±0.0 | - | 8.2±0.1 | 11.0±0.0 |
| Caryophyllene oxide[c] | 1576 | 1582 | 2.9±0.0 | - | 1.4±0.1 | 2.2±0.0 |
| Globulol | 1580 | 1590 | 4.9±0.1 | - | 2.7±0.0 | 2.9±0.1 |
| β-Copaen-4-α-ol | 1587 | 1590 | - | - | 1.0±0.0 | - |
| Carotol | 1590 | 1594 | 1.9±0.0 | - | - | 1.0±0.0 |
| Cubeban-11-ol | 1591 | 1595 | 1.5±0.1 | - | - | - |
| Rosifoliol | 1596 | 1600 | 1.0±0.1 | - | - | - |
| β-Atlantol | 1619 | 1608 | - | 3.8±0.1 | - | - |
| 1-*epi*-Cubenol | 1621 | 1627 | - | - | 2.1±0.0 | 1.1±0.0 |
| Eremoligenol | 1621 | 1629 | 1.0±0.0 | - | - | - |
| Muurola-4,10(14)- dien-1-β-ol | 1624 | 1630 | - | - | - | 1.5±0.1 |
| *epi*-α-Cadinol | 1627 | 1638 | 1.1±0.0 | - | 1.5±0.1 | 2.8±0.1 |
| *allo*-Aromadendrene epoxide | 1627 | 1639 | - | 2.1±0.0 | - | - |
| *epi*-α-Muurolol | 1637 | 1640 | 1.4±0.1 | - | 1.2±0.1 | 4.0±0.1 |
| α-Muurolol | 1639 | 1644 | 0.9±0.0 | - | - | 1.8±0.1 |
| Cubenol | 1639 | 1645 | - | 1.5±0.0 | - | - |
| α-Cadinol | 1650 | 1652 | 2.8±0.0 | 3.8±0.1 | 3.3±0.0 | - |
| Hydrocarbons Monoterpenes | | | 39.5±0.2 | 48.2±0.3 | 25.7±0.2 | 17.4±0.4 |
| Oxigenated Monoterpenes | | | 0.0±0.0 | 3.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| Hydrocarbons Sesquiterpenes | | | 29.6±0.4 | 31.5±0.4 | 48.5±0.3 | 52.3±0.7 |
| Oxigenated Sesquiterpenes | | | 25.1±0.4 | 11.2±0.2 | 21.4±0.4 | 28.3±0.5 |
| **Total** | | | **94.2±1.0** | **93.9±0.9** | **95.6±0.9** | **98.0±1.6** |

[a]RI = retention index calculated from retention times in relation to those of the series *n*-alkanes on a 30 m DB-5 capillary column; [b]RI = retention index citation from literature; [c]identification further confirmed by co-injection with authentic standards; SD = standard deviation; M/M = yields on the oil mass and the fresh weight of plant; (*) T= temperature, RH = relative humidity, data from the National Institute of Meteorology (INMET) records at http://www.inmet.gov.br, during collection dates.

The essential oil yields observed in this study indicated that the species do not respond to the seasons in the same way. The oil yields also differed significantly between seasons in the same species. The oil yield of *E. lutescens* was significantly greater in the rainy season (RS), when the temperature was lower and relative humidity higher, while that of *E. langsdorffii* was greater in the dry season (DS), with higher temperature and lower humidity. Similar yields to these were reported by Moraes *et al.*,[23] who investigated the relationship between water stress and essential oil production in *Protium bahianum* Daly (Burseraceae).

The differences in oil yields from *E. lutescens* and *E. langsdorffii* can be rationalized in term of their predictable genetic variation along with environmental factors, responsible for the production and variability of special metabolites in plants.[24,25] For instance, the soil where the species were collected is a clay type (latosolo) which acts similarly to a sandy soil reducing water retention.[26] According to Ivanauskas *et al*.[27] the water stress also depends on the root system and the cerrado plants with deep rooting do not suffer severe water stress. Studies have shown that species submitted to water deficit biochemically respond raising the oil production to compensate the lack of water.[28,29] Therefore, the detected variances could be justified by the water stress associated with the soil type and plants root systems, which probably differ between the two species here studied.

A total of 47 volatile compounds were identified in the essential oils of *E. lutescens* and *E. langsdorffii* (Table 1). In *E. lutescens* were detected 37 compounds, of which 32 in the RS, representing 94.2% of the oil and 17 (93.9%) in the DS. For *E. langsdorffii* 35 compounds were identified, with 29 in the RS, representing 95.6% of the oil and 30 (98.0%) in the DS. The presence of α-pinene, β-pinene, limonene, δ-elemene, β-caryophyllene, aromadendrene and *allo*-aromadendrene were detected in both seasons and species (Table 1). These compounds also have been found in the essential oils of other *Eugenia* species, for example, β-caryophyllene was found above 20% in *E. punicifolia*[30] and *E. caryophyllata*;[31] limonene (12.4%) was the main component of *E. pyriformis* fruit oil;[32] δ-elemene was found in the leaf oils of *E. cartagensis* (2.0%) and *Eugenia* sp. A (3.0%);[33] aromadendrene (4.7%) and *allo*-aromadendrene (1.1%) found in the leaf oil of *E. zuchowskiae.*[34]

The oils of *E. lutescens* and *E. langsdorffii* are composed of terpenes and terpenoids. The predominance of sesquiterpenes followed by monoterpenes has been reported for other species of *Eugenia*.[35] Stefanello *et al.*[32] reported that the flower (82.5%) and fruit oil (51.3%) of *E. pyriformis* are basically formed by sesquiterpenes. However based on the data presented in Table 1, the *E. lutescens* oil had monoterpenes as the predominant chemical class (51.2%) in the oil collected in the dry season.

Related to the oxidation level of the terpene fraction in both investigated oils, the presence of oxygenated sesquiterpenes were observed independent of the collection period and oxygenated monoterpenes were found only in the dry season in *E. lutescens* (Table 1).

The major compounds identified in the oil of *E. lutescens* were the same in both seasons (see Table 1 and oil chromatograms in Figures 1S, Supplementary Material): α-pinene (12.5% in RS and 12.9% in DS) and β-pinene (24.0% in the RS and 24.3% in the DS). Other compounds found in significant percentages in the oils of this species, with percentages between 2.8-10.0% were limonene, β-elemene, β-caryophyllene, γ-elemene, α-humulene, γ-muurolene and α-cadinol. The major constituents identified in the oil of *E. lutescens* are also reported as majority compounds in the leaf oils of *E. umbelliflora* (α-pinene = 24.7% e β-pinene = 23.5%)[36] and *E. rotundifolia.*[37] β-Pinene (0.4-25.7%) was also the main component in the leaf oil of *E. pyriformis*.[32] The main component identified in the *E. langsdorffii* oil collected in the rainy season was bicyclogermacrene (15.0%) followed by β-caryophyllene (9.1%) and spathulenol (8.2%). The same constituents were found in the dry season, where the quantity of β-caryophyllene was practically the same, while spathulenol increased to 11.0% and bicyclogermacrene reduced to 14.0% (see Table 1 and oil chromatograms in Figure 2S, Supplementary Material).

The relationship between rainy and dry season (RS/DS) percentage of β-pinene (5.9%/4.9%), limonene (5.4%/8.3%) and δ-cadinene (4.0%/5.8%) were identified in significant amounts in the oil of *E. langsdorffii* in both seasons. These results show little variation in the chemical profile of the *E. langsdorffii* oil collected in RS and DS. A previous study of essential oil of *E. langsdorffii* collected in the rainy season from the same population of plants in the Federal District revealed sesquiterpenes as the dominant chemical class, but the chemical composition differed from this study, even in the majority compounds: *epi*-longipinanol (13.6%) and γ-eudesmol (12.3%).[16] On the other hand, bicyclogermacrene, majority constituent in the *E. langsdorffii* oil in the RS and DS, was also the main component identified in the oils of *E. neonitida* (15.2-24.3%)[37] and *E. beaurepaireana* (14.3%).[38]

Our data presented for the chemical composition of the oils of two *Eugenia* species collected in the RS and DS, indicated that the seasons affected more the oil of *E. lutescens* than that of *E. langsdorffii*. For *E. lutescens*, while the percentage of majority compounds (α and β-pinene) identified in the two periods were similar, only 32.4% of the compounds were found in the oils collected in the RS and DS, and that the quantity of compounds identified in the RS (32) was almost double that collected in the DS (17) (Table 1). These data indicate that the chemical profile of this oil varied basically in the diversity of compounds. These results are in accordance with that reported in the investigation of seasonal effects on the chemical composition of the leaf oil of *E. dysenterica*, from the cerrado biome (Planalto Central – DF), which showed an expressive variation in the chemical diversity in different seasons of the year.[39] Chemical analysis of the oils of *E. langsdorffii* revealed little variation in the chemical diversity between the oils collected in the rainy and dry seasons compared with *E. lutescens.* The percentage of compounds found in the oils of *E. langsdorffi* in the RS and DS was 68.6%, with 29 constituents identified in the oil in RS and 30 in the DS (Table 1).

The proportions of major compounds found in the oils of *E. lutescens* and *E. langsdorffii* in both seasons differ from that reported by Hussain *et al.*[40] and Celikatas *et al*.,[41] who found large changes in the different seasons for the majority compounds in *Ocimum basilicum* and *Rosmarinus officinalis*, respectively. While being congeneric species and collected in the same region, this study showed qualitative and quantitative chemical differences between the oils of *E. lutescens* and *E. langsdorffii* that occur in the central highlands of the Federal District. These differences are probably correlated to the intraspecific genetic variation as well as the seasonal interference.

The effect of the oils from *E. lutescens* and *E. langsdorffii* collected in the RS and DS against *T. urticae* are presented in Table 2. The oil vapors were toxic to *T. urticae* and varied according to the species and collection time. Based on the $CL_{50}$ estimates for the oils, the order of toxicity against *T. urticae* was oil from: *E. langsdorffii* DS > *E. lutescens* DS > *E. langsdorffii* RS = *E. lutescens* RS. None of the oils were more toxic than eugenol, used as a positive control.

Comparing the relative toxicity between the oils, from different seasons in the same species, the *E. lutescens* oil from the DS was 1.5 times more toxic than the oil collected in the RS. The same seasonal variation was observed in the oil from *E. langsdorffii*, oil collected in the DS was more efficient against the pest than oil collected in the RS.

**Table 2.** Toxicity by fumigation ($LC_{50}$ in μL $L^{-1}$ air) of essential oils from *Eugenia* species collected in rainy and dry periods against *T. urticae*

| Specie | Period of collection | n | DF | $\chi^2$ | Slope ± SD | $LC_{50}$ (CI) |
|---|---|---|---|---|---|---|
| ***E. lutescens*** | **Rainy** | 627 | 4 | 3.23 | 4.12 ± 0.43 | 3.66 (3.31-3.98) |
| | **Dry** | 617 | 4 | 3.64 | 5.52 ± 0.66 | 2.50 (2.28-2.69) |
| ***E. langsdorffii*** | **Rainy** | 628 | 4 | 3.59 | 4.24 ± 0.43 | 3.34 (3.01-3.64) |
| | **Dry** | 623 | 4 | 4.1 | 3.43 ± 0.30 | 1.58 (1.33-1.83) |
| **EU** | - | 540 | 3 | 1.52 | 0.85 ± 0.08 | $4x10^{-3}$ ($2x10^{-3}$-$5x10^{-3}$) |

n = number of miter *per* dose; DF= degree of freedom; SD = standard deviation; EU = Eugenol (positive control); $LC_{50}$ = average lethal concentration; CI = confidence interval for 95%; $\chi^2$ = chi – squared.

Based on the results obtained for the *E. lutescens* and *E. langsdorffii* oils collected in the RS and DS, and the variations observed in the fumigation activity on the spider mite, could be a result of the variation in quality and quantity of the chemical composition of the oils, that is similar to other results reported for other oils on the same pest.[16,42,43]

The toxicity of the *E. langsdorffii* oil collected in the dry season on the spider mite compared with that reported by Moraes *et al*.[16] for an oil sample of the same species collected in the same locality but in the rainy season, the oil from the dry season was 1.13 times more toxic than that collected in the rainy season, suggesting that acaricidal property is affected by seasonality, and the best time to collect this oil to control spider mite is in the dry season.

In our experiments the toxicity by fumigation with the oils was also evaluated, found that the number of eggs deposited by the spider mites was reduced significantly with increasing oil concentrations compared to the control. In this way, to investigate whether the reduction in egg numbers it is attributed to the action of the oils or due to spider mite death, further biological tests were undertaken to compare the oil action on spider mite fecundity. The average number of eggs deposited by *T. urticae* after a 24 h of exposition to the oils of *E. lutescens* and *E. langsdorffii* collected in both seasons is shown in Figure 1. The oils of *E. lutescens* (collected in both seasons) acted at the same level as eugenol (positive control) on mite fecundity, producing a significantly greater reduction in eggs numbers when compared to the oil of *E. langsdorffii* collected in the DS.

The ability to reduce fertility in *T. urticae* was previously reported for the leaf oils of *Protium bahianum*[44] and *P. heptaphylum*[45] by fumigation bioassays, but without indicating whether the reduction in fertility came from the action of oils or mortality of the mites. The results presented for the oils tested on the spider mite fecundity suggest that the oils, particularly *E. lutescens* collected in the RS and DS and *E. langsdorffii* collected in the RS acted on the fecundity of *T. urticae*, drastically reducing the number of eggs laid.

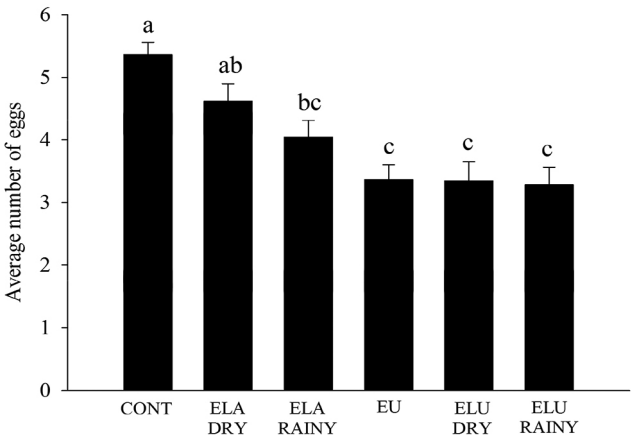


***Figure 1.*** *Mean number of eggs per T. urticae female when submitted to vapors from the Eugenia essential oils after 24 h of exposure. CONT = control; ELA = Leaf oil of E. langsdorffii; ELU = Leaf oil of E. lutescens; EU = Eugenol (positive control). Bars followed by the same letter are not significantly different by Tukey test (P < 0.05)*

## CONCLUSION

This study demonstrated that there was a seasonality influence on the oil production. For *E. lutescens* was higher in the RS (with lower temperature and higher relative humidity), whereas for *E. langsdorffii* it was higher in the DS (higher temperature and lower relative humidity). Chemical analysis by GC-MS showed that the chemical diversity of essential oils of *E. lutescens* collected in the RS was much higher than in DS. For *E. langsdorffii* the seasonality practically did not affect the chemical oil profile.

The chemical composition and acaricidal activity of *E. lutescens* essential oil is reported for the first time. Among the tested oils, *E. langsdorffii* collected in the DS presented a better acaricidal property. However, *E. lutescens* oils and *E. langsdorffii* oil (collected in the RS) drastically reduced the number of eggs laid/spider mite. All this results can be justified by qualitative and quantitative differences in chemical composition between the investigated oils. Regardless of these botanical species, the mites were more susceptible to oils collected during periods of higher temperatures and lower relative humidity (DS). These findings further suggest that the best time to collect this oil for use in spider mite control is in the DS, which also showed higher oil yields.

This investigation revealed the effects of seasonal variations on the yield and chemical profile of the oils from the two species studied, identifying the period of greater oil production and acaricidal activity. However, the use of these oils as active ingredients in a new acaricidal formulation requires further investigations to maximize their potential against the spider mite, evaluate its selectivity on natural enemies, avoid their possible toxicity effects on mammals, and assess their cost benefit.

## SUPPLEMENTARY MATERIAL

The Figures 1S and 2S are available at http://quimicanova.sbq.org.br, as a pdf file, with free access.

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