

CHEMICAL COMPOSITION AND ACARICIDAL EFFECT OF THE *Eugenia uniflora* ESSENTIAL OIL AGAINST TWO *Tetranychus* (Acari: Tetranychidae) SPECIES

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Abstract: *Tetranychus evansi* Baker & Pritchard and *Tetranychus urticae* Koch (Acari: Tetranychidae) are important mites for the economy of many countries. Populations from these species have presented resistance to acaricides of different chemical groups, thus demanding new products with an acaricidal effect. Essential oils have been investigated. The chemical composition and acaricidal effect of the *Eugenia uniflora* L. (Myrtaceae) essential oil were investigated. The essential oil was extracted by hydro-distillation and the chemical composition was identified by Gas Chromatography coupled to Mass Spectrometry (GC/MS). Repellence, fumigation and residual effect tests were performed. The essential oil presented the sesquiterpenes selin-1,3,7(11)-trien-8-one and selin-1,3,7(11)-trien-8-one epoxide. Repellence occurred against *T. urticae* and *T. evansi*. Oil vapors provoked 100% *T. evansi* mortality at all concentrations and time intervals tested with significant mortalities being observed against *T. urticae* after 48 h. *Tetranychus evansi* mortalities greater than 80% due to a residual effect were recorded after 24 h, whereas no significant mortality was observed for *T. urticae*.

Keywords: *Tetranychus urticae*. *Tetranychus evansi*. Repellence. Fumigation. Residual effect.

COMPOSIÇÃO QUÍMICA E EFEITO ACARICIDA DO ÓLEO ESSENCIAL DE *Eugenia uniflora* CONTRA DUAS ESPÉCIES DE *Tetranychus* (Acari: Tetranychidae)

Resumo: *Tetranychus evansi* Baker & Pritchard e *Tetranychus urticae* Koch (Acari: Tetranychidae) são ácaros importantes para a economia em muitos países. Populações dessas espécies têm apresentado resistência a acaricidas de diferentes grupos químicos, demandando por novos produtos com efeito acaricida. Óleos essenciais têm sido investigados. A composição química e o efeito acaricida do óleo essencial de *Eugenia uniflora* L. (Myrtaceae) foram investigados. O óleo essencial foi extraído por hidrodestilação e a composição química identificada por Cromatografia Gasosa acoplada a Espectrometria de Massas (CG/EM). Testes de repelência, fumigação e efeito residual foram realizados. O óleo essencial apresentou como constituintes os sesquiterpenos selina-1,3,7(11)-trien-8-ona e epóxido de selina-1,3,7(11)-trien-8-ona. Houve repelência de *T. urticae* e *T. evansi*. Vapores do óleo provocaram mortalidade de 100% em *T. evansi* em todas as concentrações e intervalos de tempo testados e em *T. urticae* houve mortalidades significativas 48 h após. Mortalidades de *T. evansi* superiores a 80%, por efeito residual, foram registradas a partir de 24 h, já sobre *T. urticae* não houve mortalidade significativa.

Palavras-chave: Controle alternativo. Repelência. Efeito residual. Fumigação.

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Introduction

Essential oils are cellular secondary metabolism products from some plants, presenting complex chemical composition, generally formed by mono and sesquiterpenes. Characterized by volatility and synergism, these are rapidly degraded, which when considering safety criteria and environmental conservation in pest control, hinders the selection of resistant populations (ASLAN et al., 2004; BAKKALI et al., 2008).

Eugenia uniflora L. (Myrtaceae) is a species with a wide distribution in Brazil; its extracts and essential oils have revealed insecticidal activity against *Atta laevigata* Smith and *Sitophilus zeamais* Motschulsky (COUTINHO et al., 2010; JUNG et al., 2013) as well as acaricidal activity against *Tetranychus urticae* Koch (NEVES et al., 2009).

Tetranychus urticae is one of the most important phytophagous mite species for the world's economy. This polyphagous and cosmopolitan mite infests more than 1,200 plant species in more than 100 countries (MIGEON; DORKELD, 2009). Already *Tetranychus evansi* Baker & Pritchard is a species of quarantine importance, attacking mainly Solanaceae (NAVAJAS et al., 2013).

Tetranychus evansi and *T. urticae* have been controlled by chemical method, however the selection pressure established over the years has led to the emergence of resistant populations (SATO et al., 2005; 2007; 2009; NYONI et al., 2011). Problems such as this, environmental contamination and non-targeted organism intoxications have encouraged the search for natural products, which are generally biodegradable and with lower toxicity.

Thus, the objective of this study was to identify the chemical constituents and to investigate the acaricidal activity of the essential oil from fresh *E. uniflora* leaves against *T. evansi* and *T. urticae*.

Material and Methods

Mite acquisition and growth

Tetranychus urticae was collected in *Gerbera* sp. in the municipality of Crato, CE, Brazil (7° 12' S and 39° 30' W); *T. evansi* was obtained from *Solanum americanum* L. plants in the municipality of Barbalha, CE, Brazil (7° 19' S and 39° 24' W). The mite species were kept in the Entomology and Acarology Laboratory of the Universidade Regional do Cariri – LEA/URCA. *Tetranychus urticae* was grown on *Canavalia ensiformes* L. plants and *T. evansi* on *S. americanum*. The biological material collection and the present study development took place from October 2010 to February 2011.

Plant material collection and essential oil acquisition

Eugenia uniflora leaves were collected in Barbalha (7° 19' S and 39° 24' W). An exsiccate for species certification was assembled and deposited in the Herbário Caririense Dárdano de Andrade Lima - HCDAL at URCA under number 6,759.

Fresh leaf samples (300 g) were submitted to hydro-distillation for 3 h in a Clevenger type apparatus (GOTTLIEB; MAGALHÃES, 1960). The essential oil was collected, dried with anhydrous sodium sulfate (Na₂SO₄) and kept under refrigeration until analysis.

GC/MS Analysis

The essential oil was analyzed by Gas Chromatography coupled to Mass Spectrometry (GC/MS) in a SHIMADZU apparatus with a QP5050A selective mass detector, operated with 70eV ionization energy. Component identification was performed by comparing mass spectra with standards recorded in the Wiley 229 library database and compared with literature data (ADAMS, 2001).

Bioassays

Repellence, fumigation and residual effect tests were performed at LEA/URCA and all experiments were kept in an air-conditioned room with a temperature of 25 ± 0.5 °C; 70 ± 6% RH and 12/12 h photoperiod.

Repellence test

The method used was adapted from BRITO et al. (2006a). *Solanum americanum* and *C. ensiformes* leaf discs (7 cm Ø) were used as substrates for *T. evansi* and *T. urticae*, respectively. Each disc was divided into three areas, two lateral ones of the same size and a central band (0.5 cm wide; neutral). One of the lateral areas was treated with the ethanolic essential oil solution at 0.25, 0.50, 0.75 and 1% concentrations while the other, used as a control, was immersed in ethanol and in the neutral range nothing was applied. Subsequently, the leaf discs remained on filter paper at room temperature for five minutes for solvent evaporation. Then, two filter paper disks (8.5 cm Ø) saturated with distilled water were deposited inside Petri dishes (9 cm Ø). Each plate was used as an experimental unit. In the neutral range of each experimental unit, 10 female mites were released. The experimental units were then closed and kept in an

climatized room.

Evaluations were performed 12, 24, 48 and 72 h after the start of the tests. Five replicates for each concentration were made. The Repellence Index (RI) values were calculated according to Kogan e Goeden (1970) by the formula: $RI = 2G/(G + P)$, where G = number of mites in the treatment and P = number of mites in the control. The safety interval was obtained from the relationship between the mean RI and its respective standard deviation (SD). Interpreting the results: if the RI average is less than $1 - SD$, the oil is repellent. If the mean is greater than $1 + SD$, the oil is attractive and if the mean is between $1 - SD$ and $1 + SD$ the oil is considered neutral.

Fumigation test

The method employed was that of Aslan et al. (2004). A 2.5 L glass containers were used as fumigation chambers. An experimental unit was placed inside each chamber. Each experimental unit consisted of a Petri dish (7 cm Ø) with two filter paper disks (6 cm Ø) saturated with distilled water on which a, *S. americanum* for *T. evansi* or *C. ensiformes* for *T. urticae*, leaf disc (5 cm Ø) was deposited. 10 females were released onto the leaf discs. 2, 5, 10, 15, 20 and 25 µL doses of the *E. uniflora* essential oil were autoclaved onto filter paper strips (10 x 2 cm), attached to the inner surface of the chamber lids, which were then closed and taken to the heated room. A control was considered, in which nothing was applied.

Mortality was assessed at 12, 24, 48 and 72 h after the onset of exposure. For each dose and exposure time, three replicates were made. Mites which did not move after a slight touch with a fine bristle brush were considered dead.

Residual effect test

The method used was adapted from Potenza et al. (2005). *Solanum americanum* and *C. ensiformes* leaf disks (7 cm Ø) were used as a substrate for *T. evansi* and *T. urticae*, respectively. The disks were immersed for 5 s in ethanolic *E. uniflora* essential oil solutions at concentrations of 0.25, 0.50, 0.75 and 1%, while in negative and positive controls, ethanol and Abamectin (Vertimec® 1mL/L of water) were used, respectively. The discs were then placed on filter paper for 5 min at room temperature for drying and solvent evaporation.

After drying, each leaf disc was placed onto two filter paper disks (8.5 cm Ø) moistened with distilled water inside a Petri dish (9 cm Ø). Subsequently, 10 females were released on the leaf discs. The

plates were closed and kept in an air-conditioned room. Evaluations were performed at 12, 24, 48 and 72 h after application of the solutions. Three replicates for each treatment and evaluation time were made. The criteria used to consider whether the mites were dead was the same as for the Fumigation test.

Statistical Analysis

Bioassays mortality data were subjected to an analysis of variance with the means compared by Tukey's test at 5% probability using the GraphPad Prism, version 6, program.

Results and Discussion

Chemical composition of the oil

The *E. uniflora* essential oil showed a 0.25% yield of a greenish-green fluid the sesquiterpenes selin-1,3,7(11)-trien-8-one (52.05%) and selin-1,3,7(11)-trien-8-one epoxide (47.95%) being identified as the major constituents. These same sesquiterpenes were also found in *E. uniflora* leaf oils in other studies (COSTA et al., 2009; NEVES et al., 2009; BRUN; MOSSI, 2010; GALLUCCI et al., 2010).

Repellent effect

The *E. uniflora* oil did not present a repellent effect against *T. evansi*. However, the oil repellent action varied with the concentration against *T. urticae*. After 12 h of commencing the tests, the 0.25 and 0.50% concentrations indicated repellent effect. At the 24 and 48 h intervals, all concentrations repelled *T. urticae* with mite repellence percentages ranging from 60 to 80%. At the 72 h interval, only the two highest concentrations continued to repel the mites (Table 1).

Table 1. Repellency by *Eugenia uniflora* essential oil on *Tetranychus urticae* at different concentrations and time intervals under temperature 25 ± 0.5 ° C; relative humidity $70 \pm 6\%$ and photoperiod 12/12 h.

| Time interval (h) | Concentration (%) | Repelled mites (%) | RI \pm SD ¹ | Classification |
|-------------------|-------------------|--------------------|--------------------------|----------------|
| 12 | 1.00 | 68 aA | 0.64 \pm 0.52 | Neutral |
| | 0.75 | 64 aB | 0.72 \pm 0.52 | Neutral |
| | 0.50 | 80 aC | 0.40 \pm 0.24 | Repellent |
| | 0.25 | 80 aD | 0.40 \pm 0.32 | Repellent |
| 24 | 1.00 | 78 aA | 0.44 \pm 0.48 | Repellent |
| | 0.75 | 66 aB | 0.52 \pm 0.39 | Repellent |
| | 0.50 | 80 aC | 0.40 \pm 0.20 | Repellent |
| | 0.25 | 76 aD | 0.48 \pm 0.18 | Repellent |
| 48 | 1.00 | 76 aA | 0.48 \pm 0.36 | Repellent |
| | 0.75 | 60 aB | 0.80 \pm 0.14 | Repellent |
| | 0.50 | 78 aC | 0.44 \pm 0.26 | Repellent |
| | 0.25 | 80 aD | 0.40 \pm 0.00 | Repellent |
| 72 | 1.00 | 74 aA | 0.52 \pm 0.27 | Repellent |
| | 0.75 | 68 aB | 0.64 \pm 0.17 | Repellent |
| | 0.50 | 60 aC | 0.80 \pm 0.24 | Neutral |
| | 0.25 | 68 aD | 0.64 \pm 0.43 | Neutral |

¹ Repellency index \pm Standard deviation; Means within the same time interval followed by the same lowercase letter and averages of the same concentration followed by the same capital letter, do not differ by the Tukey test at 5% probability

The present results are similar to those obtained by Araújo Junior et al. (2010) for *Citrus limon* (L.) Burm oils, and *Citrus reticulata* L. which also repelled *T. urticae*. Commercial products based on *Azadirachta indica* A. Juss, Natuneem (BRITO et al., 2006a), Neemseto (BRITO et al., 2006b) and Organic Neem (CAVALCANTE et al., 2007) also repelled *T. urticae* at the same concentrations 24 h after treatment, as observed for the *E. uniflora* oil in the present study.

Pontes et al. (2007) showed that the *Protium heptaphyllum* (Aubl.) March. fruit oil repelled *T. urticae* after 24 h at the concentrations 0.50, 0.75 and 1%, however at the 0.25% concentration the oil remained neutral, differing from the results obtained for the *E. uniflora* oil which at that concentration and time repelled more than 70% of *T. urticae*.

The absence of repellence after 72 h at the lower concentrations observed here is probably associated with the onset of oil degradation, thus causing the oil to begin to lose its repellent effect.

Fumigating effect

The *E. uniflora* oil vapors caused 100% mortality against *T. evansi* at different concentrations and time intervals while for *T. urticae*, 100% mortality was observed only at the highest time intervals (Table 2).

Table 2. Mean mortality of *Tetranychus urticae* by the fumigant action of *Eugenia uniflora* essential oil in four time intervals under temperature 25 ± 0.5 °C, relative humidity $70 \pm 6\%$ and photoperiod 12/12 h

| $\mu\text{L/L}$ de air | Mean mortality (%) \pm SE ¹ | | | |
|------------------------|--|-------------------|--------------------|--------------------|
| | 12 h | 24 h | 48 h | 72 h |
| 0.0 | 0.0 \pm 0.0 aA | 0.0 \pm 0.0 aA | 0.0 \pm 0.0 aA | 0.0 \pm 0.0 aA |
| 0.8 | 3.3 \pm 0.3 aA | 0.0 \pm 0.0 aA | 53.3 \pm 2.3 abB | 53.3 \pm 2.3 abB |
| 2.0 | 0.0 \pm 0.0 aA | 0.0 \pm 0.0 aA | 73.3 \pm 1.2 bB | 73.3 \pm 1.2 bB |
| 4.0 | 0.0 \pm 0.0 aA | 10.0 \pm 0.6 aA | 90.0 \pm 1.0 bB | 90.0 \pm 1.0 bB |
| 6.0 | 3.3 \pm 0.3 aA | 13.3 \pm 0.9 aA | 86.7 \pm 1.3 bB | 83.3 \pm 0.9 bB |
| 8.0 | 0.0 \pm 0.0 aA | 0.0 \pm 0.0 aA | 86.7 \pm 0.7 bB | 100.0 \pm 0.0 bB |
| 10.0 | 0.0 \pm 0.0 aA | 3.3 \pm 0.3 aA | 100.0 \pm 0.0 bB | 100.0 \pm 0.0 bB |

¹ Mortality \pm Standard error. Means followed by the same lowercase letter in the column and the same capital letter in the row did not differ statistically from each other by the Tukey test at 5% probability

These results differ from those observed by Neves et al. (2009), which registered a significant *T. urticae* mortality when subjected to *E. uniflora* essential oil vapors in the first 24 h.

Araújo et al. (2012) recorded *T. urticae* mortalities greater than 90%, 24 h after treatment with *Piper aduncum* L. and *P. arboreum* Aubl. at 8.0 μL and 20.0 $\mu\text{L cm}^{-2}$ concentrations, respectively.

Residual effect

Eugenia uniflora essential oil residues were not efficient in *T. urticae* control (Table 3). As for *T. evansi*, significant mortalities were obtained 24 h after the beginning of the test. Thereafter, mortalities with 0.75 and 1% concentrations were higher than 83%, not differing statistically from the Abamectin positive control. However, at the lowest concentrations (0.25 and 0.50%), mortality percentages did not differ from the negative control (ethanol), except for the 0.50% concentration after 24 h of exposure, when mortality was greater than 76% (Table 4).

Table 3. Mortality of *Tetranychus urticae* submitted to residual action of *Eugenia uniflora* essential oil at different concentrations and periods of exposure under temperature 25 ± 0.5 °C, relative air humidity $70 \pm 6\%$ and photoperiod 12 / 12 h.

| % Concentration | Mortality (%) \pm SE ¹ | | | |
|---------------------|-------------------------------------|-------------------|-------------------|-------------------|
| | 12 h | 24 h | 48 h | 72 h |
| Ethanol | 0.0 \pm 0.0 aA | 0.0 \pm 0.0 aA | 0.0 \pm 0.0 aA | 0.0 \pm 0.0 aA |
| 0.25 | 0.0 \pm 0.0 aA | 0.0 \pm 0.0 aA | 0.0 \pm 0.0 aA | 6.7 \pm 0.7 aA |
| 0.50 | 0.0 \pm 0.0 aA | 0.0 \pm 0.0 aA | 0.0 \pm 0.0 aA | 13.3 \pm 0.9 aA |
| 0.75 | 0.0 \pm 0.0 aA | 3.3 \pm 0.3 aA | 0.0 \pm 0.0 aA | 23.3 \pm 0.9 aB |
| 1.00 | 0.0 \pm 0.0 aA | 6.7 \pm 0.3 aA | 0.0 \pm 0.0 aA | 20.0 \pm 0.6 aB |
| Abamectin-Vertimec® | 0.0 \pm 0.0 aA | 16.6 \pm 1.5 aA | 50.0 \pm 1.0 bB | 66.7 \pm 1.5 bB |

¹ Mortality \pm Standard error. Means followed by the same lowercase letter in the column and the same capital letter in the row did not differ statistically from each other by the Tukey test at 5% probability

Table 4. Mortality of *Tetranychus evansi* submitted to residual action of *Eugenia uniflora* essential oil at different concentrations and periods of exposure under temperature 25 ± 0.5 ° C, relative air humidity $70 \pm 6\%$ and photoperiod 12 / 12 h

| % Concentration | Mortality (%) \pm SE ¹ | | | |
|---------------------|-------------------------------------|--------------------|--------------------|--------------------|
| | 12 h | 24 h | 48 h | 72 h |
| Ethanol | 3.3 \pm 0.3 aA | 0.0 \pm 0.0 aA | 0.0 \pm 0.0 aA | 1.3 \pm 0.7 aA |
| 0.25 | 33.3 \pm 2.0 aA | 13.3 \pm 0.7 aA | 53.3 \pm 2.6 aA | 36.7 \pm 0.3 aA |
| 0.50 | 36.7 \pm 0.3 aA | 76.7 \pm 0.9 bA | 33.3 \pm 1.4 aA | 46.7 \pm 0.9 abA |
| 0.75 | 50.0 \pm 2.5 aA | 96.7 \pm 0.3 bB | 96.7 \pm 0.3 bB | 86.7 \pm 1.3 bB |
| 1.00 | 40.0 \pm 0.6 aA | 90.0 \pm 0.6 bB | 100.0 \pm 0.0 bB | 83.3 \pm 1.7 bB |
| Abamectin-Vertimec® | 100.0 \pm 0.0 bA | 100.0 \pm 0.0 bA | 100.0 \pm 0.0 bA | 100.0 \pm 0.0 bA |

¹ Mortality \pm Standard error. Means followed by the same lowercase letter in the column and the same capital letter in the row did not differ statistically from each other by the Tukey test at 5% probability

The low toxicity of the *E. uniflora* oil formulations as well as the positive control (Abamectin) observed in the present study for *T. urticae* may be associated with populations from this species which are resistant to chemicals. The high reproductive potential of this mite in conjunction with its short life cycle and associated with selection pressure favored by the continuous use of chemical products with the same mechanism of action contribute to the rapid development of resistant populations.

The resistance of *T. urticae* populations to acaricides with different chemical groups has been investigated in several countries (STUMPF; NAUEN, 2002; HERRON; ROPHAIL, 2003; SATO et al., 2007; 2009). Abamectin is used in Brazil for the control of various insect and mite species, including *T. urticae*, in many cultures. According to Sato et al. (2009), the *T. urticae* abamectin resistance problem is very serious in ornamentals, with 75% of populations with high resistance frequencies. In one of the populations collected from ornamentals in Holambra, SP, Brazil the percentage of resistant mites was 81.3%. The *T. urticae* population used in this study was collected from the municipality of Crato, *Gerbera* sp., which came from the municipality of Holambra, SP. Thus, the low *T. urticae* mortality observed in the present study may be associated with resistance.

Conclusions

The major constituent of the *E. uniflora* fresh leaves essential oil contributed with the acaricidal action and repellent effect observed against *T. urticae* and *T. evansi*.

The *E. uniflora* essential oil displayed potential for its use as a *T. urticae* repellent, even at low concentrations, as well as a fumigant action against the two mite species tested.

The *E. uniflora* essential oil was shown as a promising alternative for *T. urticae* and *T. evansi*

control in atmospheric environments known as greenhouses.

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