Research, Society and Development, v. 9, n. 10, e909108424, 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i10.8424

Effects of nanoemulsion and essential oil from the leaves of *Ocotea elegans* against *Dysdercus peruvianus*

Efeitos da nanoemulsão e do óleo essencial das folhas de *Ocotea elegans* contra *Dysdercus peruvianus*

Efectos del nanoemulsión y el aceite esencial de las hojas de *Ocotea elegans* contra *Dysdercus peruvianus*

Received: 09/13/2020 | Reviewed: 09/14/2020 | Accept: 09/17/2020 | Published: 09/19/2020

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Abstract

The *Dysdercus peruvianus* Guérin-Méneville is commonly known as the cotton stainer bug. In this study, it was evaluated the insecticide activity and mode of action of the essential oil from leaves of *Ocotea elegans* Mez and its nanoemulsion against *D. peruvianus*. Leaves of *O. elegans* were extracted by hydrodistillation. The essential oil obtained was analyzed by gas chromatography coupled with electron impact mass spectrometry and flame ionization detector. The essential oil toxicity measured by lethal dose 50 (LD50) and survival rate of insects were recorded. Lastly, an assay was carried out to assess the inhibition of the enzyme acetylcholinesterase to determine a possible mechanism of action of insecticidal activity. The sesquiterpene sesquirosefuran was the major compound detected and corresponds to 92% of the components of the essential oil. The nanoemulsion more stable showed hydrophilic lipophilic balance (HLB 11.74), droplet size 92±1.80 nm, and polydispersity index (PDI of 0.215±0.015). After the topical application of the *O. elegans* essential oil, significant decreases in the survival of *D. peruvianus* occurred in a dose-response manner with LD_{50} = 162.18 µg and the survival rate of the nanoemulsion in *D. peruvianus* was 10.0±5.47, a better value than in pure essential oil. The acetylcholinesterase inhibition presented inhibition concentration (IC_{50} = 1.37mg/mL) and mixed type of inhibition. This indicates that the essential oil of leaves from *O. elegans* and its nanoemulsion are promising candidates for use in integrated pest management programs.

Keywords: Acetylcholinesterase; Cotton stainer; *Gossypium* sp.; Green pesticide; Insect growth regulators; Lauraceae.

Resumo

O *Dysdercus peruvianus* Guérin-Méneville é comumente conhecido como o percevejo do algodão. Neste estudo, avaliou-se a atividade inseticida e o modo de ação do óleo essencial

das folhas de *Ocotea elegans* Mez e sua nanoemulsão contra *D. peruvianus*. Folhas de *O. elegans* foram extraídas por hidrodestilação. O óleo essencial obtido foi analisado por cromatografia gasosa acoplada a espectrometria de massa de impacto de elétrons e detector de ionização de chama. A toxicidade do óleo essencial medida pela dose letal 50 (LD50) e a taxa de sobrevivência dos insetos foram registradas. Por fim, foi realizado um ensaio para avaliar a inibição da enzima acetilcolinesterase para determinar um possível mecanismo de ação da atividade inseticida. O sesquiterpeno sesquirosefurano foi o principal composto detectado e corresponde a 92% dos componentes do óleo essencial. A nanoemulsão mais estável apresentou balanço lipofílico hidrofílico (HLB 11,74), tamanho de gota de 92 ± 1,80 nm e índice de polidispersidade (PDI de 0,215 ± 0,015). Após a aplicação tópica do óleo essencial de *O. elegans*, diminuições significativas na sobrevivência de *D. peruvianus* ocorreram de forma dose-resposta com LD50 = 162,18 µg e a taxa de sobrevivência da nanoemulsão em *D. peruvianus* foi de 10,0 ± 5,47, a melhor valor do que em óleo essencial puro. A inibição da acetilcolinesterase apresentou concentração de inibição (IC50 = 1,37mg / mL) e inibição do tipo mista. Isso indica que o óleo essencial das folhas de *O. elegans* e sua nanoemulsão são candidatos promissores para uso em programas de manejo integrado de pragas.

Palavras-chave: Acetilcolinesterase; Manchador de algodão; *Gossypium* sp.; Pesticida verde; Reguladores de crescimento de insetos; Lauraceae.

Resumen

El *Dysdercus peruvianus* Guérin-Méneville se conoce comúnmente como el insecto manchador del algodón. En este estudio se evaluó la actividad insecticida y el modo de acción del aceite esencial de hojas de *Ocotea elegans* Mez y su nanoemulsión contra *D. peruvianus*. Las hojas de *O. elegans* se extrajeron por hidrodestilación. El aceite esencial obtenido se analizó mediante cromatografía de gases acoplada a espectrometría de masas por impacto de electrones y detector de ionización de llama. Se registró la toxicidad del aceite esencial medida por la dosis letal 50 (LD50) y la tasa de supervivencia de los insectos. Por último, se realizó un ensayo para evaluar la inhibición de la enzima acetilcolinesterasa para determinar un posible mecanismo de acción de la actividad insecticida. El sesquiterpeno sesquirosefurano fue el principal compuesto detectado y corresponde al 92% de los componentes del aceite esencial. La nanoemulsión más estable mostró equilibrio hidrófilo lipófilo (HLB 11,74), tamaño de gota 92 ± 1,80 nm e índice de polidispersidad (PDI de 0,215 ± 0,015). Después de la aplicación tópica del aceite esencial de *O. elegans*, se produjeron disminuciones significativas en la supervivencia de *D. peruvianus* en forma de dosis-respuesta con LD50 =

162.18 µg y la tasa de supervivencia de la nanoemulsión en *D. peruvianus* fue 10.0 ± 5.47, un mejor valor que en aceite esencial puro. La inhibición de la acetilcolinesterasa presentó concentración de inhibición (IC50 = 1,37 mg / ml) y tipo de inhibición mixta. Esto indica que el aceite esencial de hojas de *O. elegans* y su nanoemulsión son candidatos prometedores para su uso en programas integrados de control de plagas.

Palabras clave: Acetilcolinesterasa; Chinche manchador del algodón; *Gossypium* sp.; Pesticida verde; Reguladores del crecimiento de insectos; Lauraceae.

1. Introduction

The cotton crop (*Gossypium* spp.) is one monoculture of great economic and social importance. Cotton is one of the most important raw materials in the production chain of the textile industry. The cotton plant has many applications, especially the core cottonseed and fibers with more than 400 industrial uses (Chaudhry, 2010). Cotton plants are attacked by several plagues including bedbugs, *Horcias nobilellus* Berg, (1883) (Hemiptera: Miridae), and *Dysdercus peruvianus* Guérin-Méneville (1831) (Heteroptera: Pyrrhocoridae) commonly known as the cotton stainer bug (Rafiq et al., 2014). In *Gossypium* spp., the latter pest provokes abnormal development of fruits, falling of new apples, the defective opening of buds, and staining of the fibers caused by insect excrement, reducing crop production by loss of weight, seed quality and oil content (Liu et al., 2014, Schaefer, 2015).

To avoid such economic damage, control methods using chemical pesticides are usually employed, but these have high environmental toxicity due to the non-biodegradable properties (Jemâa, 2014). This factor leads to environmental and public health negative impact (Koul, Walia & Dhaliwal, 2008). The use of large quantities of synthetic chemicals also leads to the appearance of resistant insects (Rattan, 2010).

Natural products have been studied as potential new insecticides, which may act as antifeedants or growth regulators, and have the additional advantage of potential low environmental persistence (Isman, 2006). These include active plant compounds, such as steroids, terpenoids, and alkaloids that have been shown to have insecticidal properties (Castillo-Sanchez et al., 2010). Also, several studies have reported the effectiveness of essential oils and their constituents as potential insecticides (Nesci et al., 2011; Budki et al., 2012, Zandi-Sohani et al., 2012). This activity has often been associated with the inhibition of acetylcholinesterase (AChE) and is recognized as an important mechanism of insecticidal activity (Rattan, 2010, López & Pascual-Villalobos, 2010).

Ocotea elegans Mez (Lauraceae), known as "Canela sassafraz", occurs in the Jurubatiba Sandbank National Park and has a tropical and subtropical distribution (Brotto et al., 2013, Kropf et al., 2015), reaching up to 15 m and was previously described by our group with acaricidal activity against *Rhipicephalus* (*Boophilus*) *microplus* Canestrini (1887) (Figueiredo et al., 2018). The present study, therefore, evaluates the effects of *O. elegans* essential oil and its nanoemulsion on *D. peruvianus* and analyzes its chemical composition and efficacy as an insecticide nanoemulsion.

2. Metodology

2.1 Plant material

Leaves of *O elegans* were collected from different specimens at Jurubatiba Sandbank National Park, Rio de Janeiro State, Brazil (22° 18'32"S, and 41° 66'11"W). Identification of plant material was performed by Dr. Marcelo Guerra Santos, and a voucher specimen was deposited under the registration number RFFP 16.873 at the herbarium of the Faculdade de Formação de Professores (Universidade do Estado do Rio de Janeiro, Brazil).

a. Extraction of the essential oil

The *O. elegans* fresh leaves were turbolized with distilled water, placed in a 5 L round-bottomed flask, and submitted to hydrodistillation for 4 h using a Clevenger-type apparatus. The collected essential oil was dried over with anhydrous sodium sulfate and stored at 4ºC until further analysis (Tietbohl et al., 2014).

Chemical analysis of the essential oils

The *O. elegans* essential oil was analyzed by a GCMSQP5000 (SHIMADZU) gas chromatography equipped with a mass spectrometer using electron ionization. The gas chromatographic (GC) conditions were as follows: injector temperature, 260°C; FID temperature, 290°C; carrier gas (Helium), flow rate 1 mL/min and split injection with split ratio 1:40. The oven temperature was initially 60°C and then rose to 290°C at a rate of 3°C/min. One microliter of each sample was dissolved in n-hexane (1:100 mg/μL) (Chromosolv, Sigma-Aldrich, St Louis, MO) and injected into an RTX-5 column (30 m x 0.32 mm x 0.25 μm) (Agilent Technologies, Santa Clara, CA). The mass spectrometry (MS) conditions were voltage 70 eV and scan rate 1 scan/s. The retention indices (RI) were calculated by interpolation to the retention times of a mixture of aliphatic hydrocarbons (C9-

C30) (Chromosolv, Sigma-Aldrich, St Louis, MO) analyzed in the same conditions. The identification of the substances in the ESSENTIAL OIL was performed by comparison of their retention indices and mass spectra with those reported in the literature (Adams, 2007). The MS fragmentation pattern of compounds was also checked with NIST (National Institute of Standards and Technology) mass spectra and PHEROBASE libraries (El-Sayed, 2019). Quantitative analysis of the chemical constituents was performed by flame ionization gas chromatography (GC/FID), under the same conditions as in the GC/MS analysis and percentages obtained by the FID peak area normalization method (Adams 2007).

b. Nanoemulsion preparation

The nanoemulsion was obtained by low energy method (Ostertag, 2012) using 90% (w/w) of water, 5% (w/w) of *O. elegans* oil, and 5% (w/w) of surfactants. Tween 20 (Polyoxyethylenesorbitan monolaurate) and Span 80 (Sorbitane monooleate) were the surfactant mixture in different proportions used to prepare the nanoemulsions with hydrophilic lipophilic balance (HLB) value between 4.3 and 16.7. For the preparation of the nanoemulsions, the essential oil and the surfactants were homogenized for 30 min by magnetic stirring (800 rpm). After this, the aqueous phase was added to the oily phase and the mixture was stirred at 800 rpm for 60 min. The formulations were analyzed by photon correlation spectroscopy (Zeta-sizer ZS, Malvern, UK), the parameters verify were droplet size and polydispersity index (PDI). Formulations with the lowest droplet size and PDI values indicated the appropriate HLB of the essential oil of *O. elegans*.

c. Insect bioassays

A colony of *D. peruvianus* was established in the Laboratory of Insect Biology (UFF) and kept at a constant temperature (26 ± 1 °C), photoperiod (16L:8D), and relative humidity (60% ± 5) (Fernandes et al., 2013). Genetic biodiversity property was authorized under number (A0E95C4) at National Management System of Genetic Heritage and Associated Traditional Knowledge (SISGEN) of Brazilian Ministry of the Environment. To evaluate the biological effects of the *O. elegans* essential oil, randomly chosen fourth instar *D. peruvianus* were topically applied with the pure essential oil, its dilutions, and the nanoemulsion containing essential oil of *O. elegans*. The essential oil was previously weighed and 1g corresponded to 1.15 mL (1 mL weighed 0.87 g). From 1g of the pure essential oil, a serial dilution in pure acetone (Merck, Darmstadt, FRG) produced final concentrations of 480, 240, 120, 60, and 30 mg/mL of oil. Subsequently, 1 µL of each dilution was applied topically to

the dorsal cuticle of the insects in the experimental groups. Therefore, the dose administered was 0.48, 0.24, 0.12, 0.06 and 0.03 mg per insect respectively. Another experimental group received 1 µL of the pure undiluted oil (Fernandes et al., 2013, Tietbohl et al., 2014). There was an untreated control group and the solvent control group only received a topical application (1 µL) of the acetone diluent. The nanoemulsion contained 5% (w/w) of the essential oil (0.05g/mL). There was an untreated control group and the blank nanoemulsion control group only received a topical application (1 µL) of the nanoemulsion without essential oil.

Biological evaluation of different treatments was performed from the day after topical application of the essential oil (1st day) and during the entire time required for development from the fourth instar nymphs to the adult stage (25 days). Parameters recorded were survival rate of insects and toxicity of the essential oil od *O. elegans*, which was expressed as the concentration at which 50% of test insects were killed in a specified time, referred to as the median lethal dose (LD_{50}). All experiments were repeated three times at least, in groups of 30 insects at a temperature of 26 ± 1 °C (Koul, 2008).

d. Acetylcholinesterase activity

An acetylcholinesterase preparation was made by modification of a technique described (Cunha Bastos et al., 1991, Lima et al., 1996). Briefly, Wistar rats (approximately 200 g) were euthanized with isoflurane (Ohmeda, Liberty Corner, NJ) and the brains were removed. The brains were washed in distilled water and dried on filter paper for removal of the meninges. After that, the brains were chopped and one part of the brain was homogenized in 5.5 volumes (mL) of distilled water (4 ^{o}C) per gram of tissue for 2 min. The homogenate was then centrifuged at 20.000 rpm for 2 h, the sediment resuspended in 2 volumes of 4% Triton X-l00 (Sigma-Aldrich, St Louis, MO) and stirred for l h at 25 °C. After another centrifugation under the same conditions, the supernatant was collected and diluted in 0.2M pH 7.4 phosphate buffer, until the concentration of protein reached 20 mg/mL. Finally, the preparation was aliquoted into 10 mL samples, lyophilized and stored at -80 ^{o} C. All procedures were performed with Swiss Wistar rats provided by the Laboratory Animal Center at UFF – Universidade Federal Fluminense following the ethical principles of the animal experimentation from Brazilian Society of Science using Laboratory Animals (SBCAL) and the Fluminense Federal University Ethics Committee in Animal Use (CEUA- 303).

i. Colorimetric determination of the acetylcholinesterase residual activity

Following adaptation from the Ellman et al. (1961) method, the essential oil was added in different concentrations (10 µg/mL to 500 µg/mL) to each test tubes, then 20 µL of acetylcholinesterase enzyme and 110 µL of 0.2M pH 7.4 sodium phosphate buffer (Sigma-Aldrich, St Louis, MO) were added to each test tube and incubated at 37 º C for 1h. After incubation, 250 µL of dithionitrobenzoate (DTNB) (Sigma-Aldrich, St Louis, MO) 1.0 mM in 0.2M pH 7.4 sodium phosphate buffer (Sigma-Aldrich, St Louis, MO) and 250 µL 1.25 mM of acetylthiocholine iodide (Sigma-Aldrich, St Louis, MO) (substrate) were added. Samples were then analyzed at 412 nm in a spectrophotometer. The increase in absorbance (optical density) was measured every one minute for three minutes and the average absorbance increased per minute was calculated. The control corresponds to 100% of enzyme activity. The enzymatic activity obtained in each concentration was calculated through the difference between the absorbance of control tubes and the absorbance of the experimental tubes.

2.2 Characterization of the inhibition of acetylcholinesterase (Lineweaver-Burk plot)

The type of inhibition was analyzed through a Lineweaver-Burk plot, assessing Km and Vmax values in the presence and absence of the acetylcholinesterase inhibitor. Test tubes containing essential oil from *O. elegans* at concentration 1.28 mg/mL were prepared, then 20 µL of enzyme and 110 µL of 0.2 M sodium phosphate buffer (Sigma-Aldrich, St Louis, MO) were added. After 1 hour incubation at 37 ^{o}C 250 1,0 mM in 0.2 M pH 7.4 sodium phosphate buffer were added. For substrate addition, five acetylcholine iodide solutions (Sigma-Aldrich, St Louis, MO) (substrate) at different concentrations were prepared as follows: 1.25 mM, 0.25 mM, 0.125 mM, 0.0833 mM and 0.0625 mM. Then, 250 µL of the substrate at different concentrations were added to the tubes containing the essential oil and its corresponding controls. After substrate addition, absorbance was measured.

The increase in absorbance (optical density) was measured every one minute during three minutes and the average absorbance increased per minute was calculated. The enzymatic activity was calculated through the difference between the absorbance of control tubes and the absorbance of the treated tubes.

a. Statistical analysis

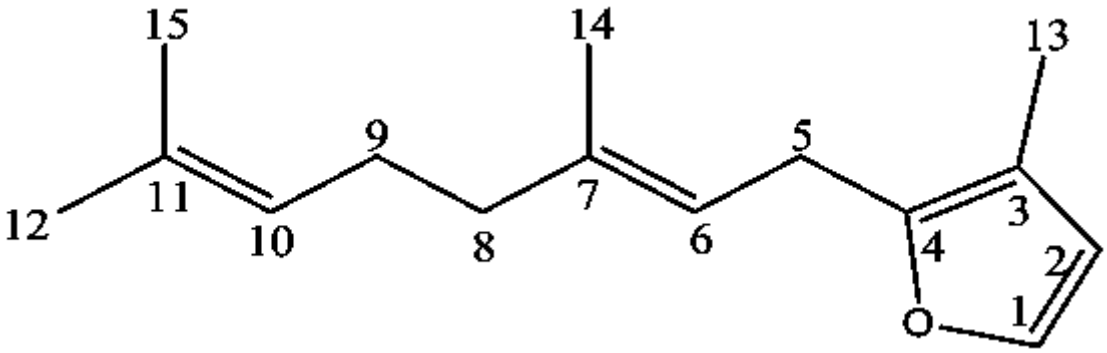
The significance of the results was analyzed using Kaplan-Meier survival curves and Log-rank (Mantel-Cox) test (Bland & Altman, 2004) according to Graphpad Prism version 7.04. The survival data were expressed in mean ± standard deviation (SD) (Armitage et al., 2002), chi-square (x^2), and the probability levels are specified within the text and tables. The LD50 values and its minimum and maximum confidence limit 95% were calculated using the program Statgraphics Centurion XV version 15.1.02.

3. Results

3.1. Chemical analysis

The yield of the hydrodistillation of the fresh leaves of *O. elegans* was 0.4% and the essential oil presented clear light yellow color. In Table 1, four compounds were identified in the volatile oil from *O. elegans* and all were identified as sesquiterpenes. Sesquirosefuran (Figure 1) was the main chemical compound (92.20%) in the essential oil.

Figure 1. Chemical structure of sesquirosefuran from *Ocotea elegans* essential oil.



Source: Author.

Table 1. Relative abundance (%) of the chemical composition essential oils from *Ocotea elegans* leaves.

Compounds	RI_{Lit}	RI_{Exp}	Relative abundance (%)
Farnesene<(*E*)-β->	1454	1451	03.9
Bicyclogermacrene	1500	1491	00.6
Sesquirosefuran	1541	1546	92.1
Methyl farnesoate <(2*E*,6*E*)->	1783	1776	00.3
Hydrocarbon sesquiterpene			96.6
Oxygenated sesquiterpene			00.3
Total identified			96.9

*RI_{Lit}: Retention Indices Literature, RI_{Exp}: Retention Indices Experimental. Source: Authors.

3.2 Nanoemulsion

The criteria used for the selection of the formulation were size < 200 nm; PDI<0.250. The formulation F5 (Table 2) showed mean values of droplet size as 92±1.8 nm and PDI of 0.215±0.015 with 11.74 HLB value. The nanoemulsion exhibited homogeneous and translucent appearance.

Table 2. Size droplet, polydispersity index (PDI), and hydrophilic-lipophilic balance (HLB) values of formulations with essential oil from *Ocotea elegans*.

Formulation	Sizedroplet (nm)	PDI	HLB
F1	283.10±6.5	0.260±0.052	16.70
F2	193.40±5.5	0.175±0.007	15.46
F3	163.60±2.5	0.253±0.013	14.22
F4	101.50±4.2	0.215±0.003	12.98
F5	091.92±1.8	0.215±0.015	11.74
F6	097.47±1.7	0.215±0.002	10.50
F7	119.30±2.3	0.182±0.019	09.26
F8	149.20±1.0	0.179±0.010	08.02
F9	208.00±1.8	0.209±0.034	06.78
F10	266.70±5.5	0.324±0.066	05.54
F11	270.20±8.2	0.212±0.008	04.30

Source: Authors.

3.3 Insect bioassays

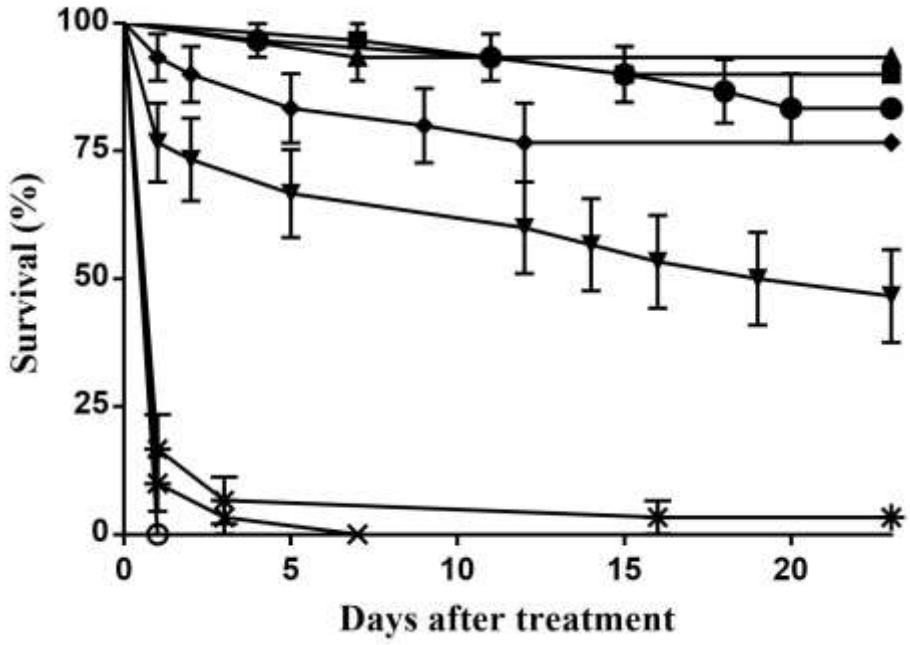
Topical treatment of *D. peruvianus* 4th instar nymphs with essential oil of *O. elegans* and its dilutions induced significant survival rates in a dose-response manner. As shown in Figure 2, insects treated with pure essential oil displayed no survival rates after 24 h (x^2=59, P<0.0001), and the same result was expressed on 0.48 mg per insect dilution (x^2=59, P<0.0001) at seven days of treatment. By the end of observations (23 days), 90.0±5.47% (x^2=0.1915, P=0.6617) of solvent control insects, and 93.3±4.55% of the untreated control insects had survived (Figure 2). In the other groups treated with 0.24, 0.12, 0.06 or 0.03 mg of essential oil per insect, 3.33±3.27% (x^2=59.63, P<0.0001), 46.67±7.72% (x^2=15.74, P<0.0001), 76.67±6.80% (x^2=3.358, P=0.0669), and 83.33±6.20% (x^2=1.355, P=2.444) of insects were survived, respectively, at 23 days after treatment.

Insects that died within 24 hours had the symptoms tremors, convulsions, and paralysis. These responses suggest an anticholinesterase action of the substances in the essential oil of *O. elegans*. Other insects that survived and died later showed deformations in the wings indicating also Insect Grown Regulators (IGR) activity.

For all experimental groups, surviving insects always reached the fifth instar and, subsequently, the adult stage. In the group treated with 0.12 mg of essential oil per insect, all survived insects displayed deformed wings (not shown). The LD50 values recorded (Table 3) were 162.18 µg per insect (151.3 – 174.18) and 90.99 µg per insect (85.31 – 96.82) in the first and 23rd day after treatment, respectively.

As shown in Figure 3, after topical application, nanoemulsion containing 5% of essential oil of *O. elegans*, gradually decreased the survival until 10.0±5.47% (x^2=50.78, P<0.0001) at 20 days of observation. At the same time, the untreated control group exhibited 96.67±3.27% of survival and the solvent control group showed 93.33±4.55% (x^2=0.3448, P=0.5571) of surviving insects.

Figure 1. Survival curve of *Dysdercus peruvianus* fourth instar nymphs after topical treatment with pure essential oil (1 µL) and their different concentrations obtained from the leaves of *Ocotea elegans.*



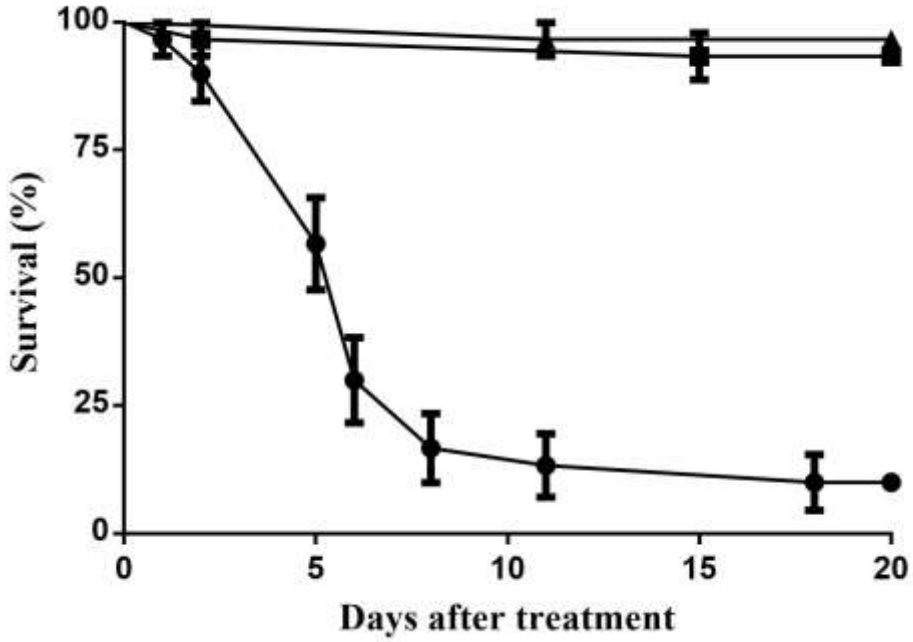
. Untreated control; Solvent (acetone) control; 0.03 mg/insect; 0.06 mg/insect; 0.12 mg/insect; 0.24 mg/insect; 0.48 mg/insect; Pure essential oil. Source: Authors.

Table 3. Determination of median lethal dose (LD) of *Ocotea elegans* essential oil on *Dysdercus peruvianus* at 1st and 23rd days of treatment.

Time	Estimated LD (µg/insect) (lower limit - upper limit)
1st	169.49 (168.3 – 170.68)
23rd	94.91 (93.73 – 96.09)

Sourece: Authors.

Figure 2. Survival curve of *Dysdercus peruvianus* fourth instar nymphs after topical treatment with nanoemulsion of *Ocotea elegans* essential oil.

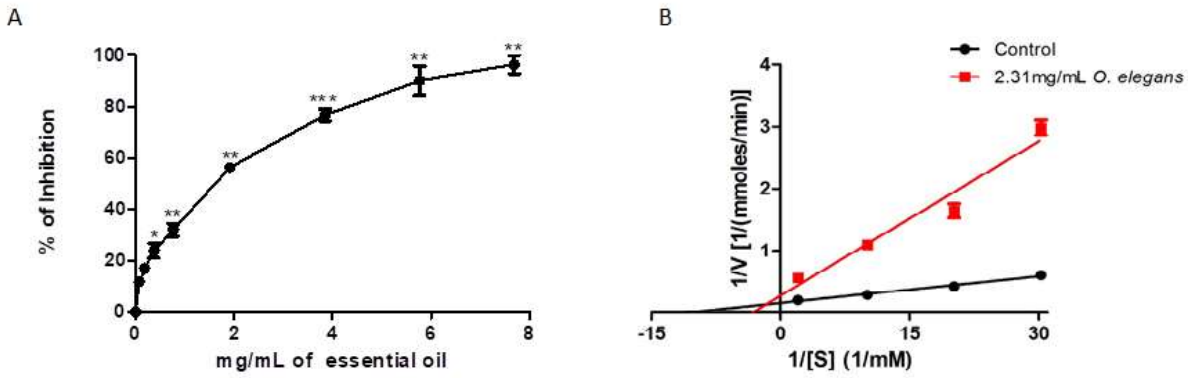


. Nanoemulsion containing 5% of essential oil; Blank Nanoemulsion (Nanoemulsion without essential oil); Untreated control. Source: Authors.

3.4 Acetylcholinesterase activity and inhibition characterization

As shown in Figure 4, the effect of *O. elegans* essential oil on the activity of AChE from rat brain was observed with IC_{50} = 1.37mg/mL and the characterization of the type of inhibition of AChE was mixed.

Figure 4. Acetylcholinesterase activity and inhibition characterization.



A. Effect of essential oil from leaves of *Ocotea elegans* on the activity of rat brain AChE by assaying the enzyme. **B** Characterization of type of inhibition of AChE by *O. elegans* essential oil. Sourece: Authors.

4. Discussion

The search for new insecticide agents results from the need for compounds with lower human toxicity that are more biodegradable and with the possibility of a small scale used in many sectors of agriculture, apiculture, veterinary and human health (Gonzalez et al., 2014). Several plant species produce essential oils with insecticidal activity and which can efficiently be used to control important crop pests of stored grains such as *Zingiber purpureum* Roscoe against *Tribolium castaneum* Herbst (1797) (Coleoptera: Tenebrionidae) and *Lasioderma serricorne* Fabr. (1792) (Coleoptera: Anobiidae) (Wang et al., 2015). *Datura stramonium* L., *Eucalyptus camaldulensis* Dehnh., *Moringa oleifera* Lam. and *Nigella sativa* L. were tested against *T. castaneum, Trogoderma granarum* Everts (1899) (Coleoptera:Dermestidae) and *Cryptolestes ferrugineus* Stephens (1831) (Coleoptera: Laemophloeidae) (Saleem et al., 2014), and essential oil of Orange activity was analyzed against *T. castaneum* (Kim & Lee 2014). Besides, the effects of several essential oil from plants have been recently described, such as those from *Ammi visnaga* (L.) Lam. (Maleck et al., 2013), *Myrciaria floribunda* (H.

West. ex Willd) O. Berg (Tietbohl et al., 2014), *Eugenia sulcata* Spring ex Mart. (Gonzalez et al., 2014) and *Manilkara subsericea* (Mart.) Dubard (Fernandes et al., 2014) on the development of *Oncopeltus fasciatus* Dallas (1852) (Hemiptera: Lygaeidae), an important model of physiological studies.

The HLB created by Griffin in 1969 (Griffin 1949), is a semi-empiric scale to categorize surfactants. A mixture of noninonic surfactants is a tool to determine the HLB of oil. Therefore, the closer the HLB value of the oil is to the HLB of the surfactant blend, the more stable the system will approach. Furthermore, the topical application of the essential oil and nanoemulsion containing 5% of essential oil of *O. elegans* on *D. peruvianus* induced high and significant reduction of insect survival, shortly after treatment and during the long-term development of the bug. Moreover, this effect was expressed in a typical dose-response manner. Interestingly, the concentration of the nanoemulsion (50µL/mL) corresponded to the concentration of essential oil between 0.06 and 0.03 mg per insect. The 0.06 mg per insect of essential oil showed 76.67% of survival insects in 20 days, while the nanoemulsion expressed a 10.0% survival rate at the same time. Deliverance of the active substances is one of de advantages of nanoemulsions containing essential oil. They allow better interaction between the active principles with the biological membranes. The mechanism may occur increasing surface area and passive transport through plasma membrane; fusion of the micelles with membrane cells; sustained release of the essential oil; bioadhesion of the droplet due to electrostatic interaction in the cell membrane. Thus, nanoemulsion formulation seems to increase the effect of the essential oil of *O elegans* on the insects when compared with the pure essential oil (Jaiswal et al., 2015).

Sesquiterpenes appear as main constituents in the essential oil from species of Lauraceae such as *Persea* sp. (Gottlieb, 1972) and *Actinodaphne longifolia* (Blume) Nakai. *Ocotea gomezii* (W.C. Burger) and *O. morae* (Gomez-Laur.) are rich in monoterpenoids and sesquiterpenoids, and the main constituent bicyclogermacrene are also present in minor quantity in *O. elegans* (Gottlieb, 1972).

Also, the major constituent of the essential oil of *O. elegans*, sesquirosefuran, is a furanosesquiterpene present in other Lauraceae species including *Lindera strychnifolia* Vill, *Neolitsea aciculata*, *N. sericea* (Blume Koidz), *N. zeylanica* Merr. (Gottlieb, 1972), *Actinodaphne longifolia* (Blume) Nakai (Hayashi & Komae, 1980), *Litsea coreana* H. Lev that. (Araki & Butsugan, 1982) and described as semiochemical (Petroski et al., 2005). Farnesene <(*E*)-β-> is a sesquiterpene synthesized via a mevalonic acid pathway in aphid species as an alarm pheromone that is released when disturbed, and it was described with

wing induction in *Acyrthosiphon pisum* and insecticidal effects at higher doses (Van et al., 1990; Kunert et al., 2007). Bicyclogermacrene is a bicycle sesquiterpene derived of germacrene and has been found as a potential larvicide against *Anopheles subpictus* Grassi (1899) (Diptera: Culicidae), *Aedes albopictus* Skuse (1894) (Diptera: Culicidae), and *Culex tritaeniorhynchus* Giles (1901) (Diptera: Culicidae), (Govindarajan & Benelli, 2016). Methyl farnesoate <(2E,6E)-> is the methyl ester of farnesoic acid and was described as a juvenile hormone in crustaceans (Homola & Chang, 1997), sex pheromone in stink bugs *Chlorochroa ligata* Say (1832) and *Chlorochroa uhleri* Stål (1872) (Hemiptera: Pentatomidae) wich belongs to the same order (Hemiptera) of *D. peruvianus* (Ho & Millar, 1993) and juvenile hormone in larvae of *Drosophila melanogaster* Meigen (1830) (Diptera: Drosophilidae) (Harshman et al., 2010).

Recent studies have proven that terpenoids could be an alternative to synthetic insecticides against stored-product pests. The inhibition of AChE activity is the mode of action of many terpenes, since the symptoms are similar to those produced after organophosphate and carbamate intoxication, which cause high mortalities when used against insect plague (López & Pascual-Villalobos, 2010).

In the present study, the IC_{50} (1.37 mg/mL) values obtained for the essential oil from *O. elegans* indicate a smaller inhibition than the essential oil from leaves (IC_{50} of 681 μg/mL) or flowers (IC_{50} of 1583 μg/mL) of *Myrciaria floribunda* (Tietbohl et al., 2014) and (IC_{50} of 1798 μg/mL) from leaves of *Eugenia pruniformes*, which were collected in the same locality (Albuquerque et al., 2012). This reduced activity may be attributed to the different major compound sesquirosefuran or the synergistic interaction between one or more sesquiterpenes present in the essential oil of *O. elegans*. The mixed inhibition profile can be associated with the interaction between the sesquiterpenes which compose the essential oil (Miyazawa & Yamafuji, 2006; Arruda et al., 2012). Sesquirosefuran represents more than 90% of the composition of the essential oil of *O. elegans*, and it suggest that this component is responsible for the activity of the essential oil of *O. elegans*. However, the essential oil and the nanoemulsion showed high mortality indicating a different mode of action besides the inhibition of the enzyme acetylcholinesterase.

In this work, it was observed two important effects for the use of this essential oil to combat pests: i) AChE inhibitory activity in the first 24h; ii) some insect growth regulator activity along the insect cycle, resulting in deformed wings. Further studies will be done to understand the effect of the essential oil on the biosynthetic pathway. Due to the constant increase in the use of toxic pesticides, there is a strong public appeal to replace these chemical

agents for less aggressive alternatives. Insecticides derived from plants are biodegradable and may cause less environmental impact and less harm to human health, being a more ecological and sustainable option instead of conventional synthetic insecticides.

5 Final Considerations

In this study, the results indicate that the essential oil of *O. elegans* leaves and its nanoemulsion are promising essential oil-based green pesticides against the 4th instar nymphs of *D. peruvianus*. The nanoemulsion could have possible uses as environmentally friendly in Integrated Pest Management (IPM) programs.

The present study was conducted in laboratory, experimental field work is recommended. Assessment of absence of toxicity to other organisms and to the soil should also be performed

Acknowledgment

The authors thank the Universidade Federal Fluminense for the grants to the scientific initiation students as well as Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Apoio a Pesquisa do Estado do Rio de Janeiro (FAPERJ), and Prociência-Universidade do Estado do Rio de Janeiro for financial support. We also thank Professor Norman Ratcliffe (fellowship E-26/201.787/2017) from Department of Biosciences, Swansea University, Singleton Park, Swansea, for the English revision of the manuscript.

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