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Abstract: Myiasis is considered a serious medical condition in animals and humans that occurs predominantly in tropical countries. *Cochliomyia hominivorax* is one of the main fly species that causes myiasis and the actual geographic distribution includes the Caribbean and all of South America, except Chile. The use of synthetic insecticides and endectocides in livestock for the myiasis treatment has resulted in insect resistance, while also causing residuals in the environment and contamination of animal food products. Biologically active compounds are considered an alternative for the sustainable management of insects, as they are biodegradable and less damaging to the environment. This study developed a nanoemulsion containing the essential oil of *Baccharis dracunculifolia* and evaluated its *in vitro* effects on *C. hominivorax* larvae. High pressure homogenization was used to develop the nanoemulsion. Larvicidal tests were carried out with the tested products using 15 larvae/repetition, for a total of 150 larvae per treatment. Five different concentrations of 5, 7.5, 10, 13.5, and 15% (w/v) we found mortality of 28, 48, 70, 84, and 97%, respectively, while for the stored formulation, we found mortality of 17, 36, 51, 81, and 92%. The larvicidal effect was similar, except for the concentrations of 5 and 10% (w/v) (p < 0.05). Our results show that the natural based *B. dracunculifolia* nanoemulsion can be considered a promising alternative for the treatment and control of myiasis caused by *C. hominivorax*.

Key words: Natural products, insecta, phytotherapy, larvicidal activity, myiasis.

Abbreviations

EOs	essential oil
GC-MS	gas chromatography-mass spectrometry
LD	lethal dose
PDI	polydispersity index
TEM	transmission electron microscope

1. Introduction

The species Cochliomyia hominivorax, of the

Calliphoridae family, is one of the main flies that cause myiasis in the Neotropical region [1]. The actual geographic distribution of *C. hominivorax* includes the Caribbean (Cuba, Dominican Republic, Haiti, Jamaica, Trinidad and Tobago) and all of South America, except Chile [2].

Unlike most other fly species, *C. hominivorax* does not place its eggs in decomposing tissue. Rather, it deposits its eggs on live tissue that has been damaged naturally or accidentally, such as wounds caused by ticks and parasites (i.e., larvae of *Dermatobia hominis*), and livestock management practices, such as

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vaccination, dehorning, and castration, among others [1, 3].

Because of the significant economic costs associated with C. hominivorax in agriculture, it has been eradicated in North and Central America through the use of sterile insects [4]. However, this species still causes substantial losses in South America and regions of the Caribbean [5]. Coronado and Kowalski estimate that the costs to control the fly (mainly through the use of larvicides) in Venezuela reached US\$2 million/year [6]. In Brazilian agriculture, the costs incurred due to C. hominivorax are approximately US\$ 336.62 million/year [7]. The main losses are related to mortality of new-born calves, reduced leather quality, and diminished milk and meat production [8].

In South America, the treatment indicated for the control of myiasis consists of synthetic insecticides applied directly to the larvae and/or endectocides [9]. These products are organophosphates, pyrethroids and macrocyclic lactones [10]. Ivermectin is a broad spectrum, antiparasitic drug traditionally used in veterinary medicine. There are reports that the use of ivermectin increases aspartate transaminase (AST) and alanine transaminase (ALT) in rabbits [11, 12]. Teratogenic effects were also associated with the use of ivermectin in mice, rats, and rabbits [13]. In addition, a recent study demonstrated potential damage to DNA from the use of ivermectin in zebu cows [14]. Increase in the levels of diptera resistance to insecticides, along with environment and public health concerns associated with the continued use of some neurotoxic insecticides, has stimulated a search for alternative treatments and controls [9, 15, 16].

Botanical insecticides, or natural products developed for the management of insects based on vegetal material, have been indicated as a potential alternative to substitute conventional synthetic products. Natural products have less impact on the environment and human health, while also causing less damage to organisms and ecosystems [17, 18]. The majority of plant-based insecticides are biodegradable, providing another positive reason for their use. There are more than 1,500 known plant species that possess insecticide activity and many more that are yet to be discovered [19]. Essential oils (EOs) have a complex chemical composition, often presenting more than 100 different terpenic compounds. As such, EOs have been associated with a variety of biological effects, such as antimicrobial, antifungal, antiviral, pest control, and insect repellent [20]. Recently, due to their increasing popularity in relation to organic products and consumers concerned about the environment, the use of EOs as low-risk insecticides has grown considerably [21]. Today, EOs are being included in the development of phytosanitary products [21, 22].

The genus Baccharis includes more than 500 species that are distributed from the United States to Argentina, with a predominance in South America. There are approximately 120 species in the Southwest region of Brazil [23]. Baccharis dracunculifolia is a plant native to Brazil popularly known as field rosemary-of-field [24]. This species has been recognized for its diverse pharmacological properties, such as antifungal [25], anticarcinogenic [26, 27], antimicrobial [28, 29], antioxidant [30], antileishmanial and antiplasmodial [31] as a tick insecticide [32], and as an insecticide and repellent [33]. It is traditionally used for the treatment of liver problems and inflammation. Furthermore, it is the main botanical source for green propolis production, which is widely known for its antimicrobial and anti-inflammatory properties [34].

Despite the insecticide potential of EOs, characteristics such as high volatility, low solubility in water, and easy oxidation, have impeded their use as an alternative product in pest control [35]. The encapsulation of these products in nanoformulations can address these issues as the active compounds are protected from deterioration and loss due to volatility. The controlled liberation of the active compounds and their ease of use are also important [36].

A typical nanoemulsion is composed of oil, water, and an emulsifying agent, with droplets of up to 200

nm [37, 38]. The emulsifying agent is composed of tensioactive molecules, which are arranged on the surface of the droplets during dispersion in distinct phases, forming a superficial layer that minimizes the interfacial tension and the aggregation of droplets due to coalescence [38, 39]. Various nanoemulsion formulations have been developed for different purposes, such as antimicrobial [40], tick insecticide [41], insecticide [42], anti-larvae and mosquito activity [43, 44], and activity against species that cause myiasis, such as *Lucilia sericata* [45].

Currently, there is a significant concern with ethics and animal well-being in scientific experiments. This has impeded the development of *in vivo* tests to evaluate the effectiveness of new therapeutic alternatives in situations that expose animals to suffering, such as through the infliction of wounds or lacerations. As such, *in vitro* tests can offer a reliable approximation that is ethically acceptable [46].

Thus, the aim of this study was to develop a nanoemulsion containing essential oil of *B*. *dracunculifolia* and evaluate its *in vitro* effects on pre-pupae mortality of *C*. *hominivorax*, in light of its potential use as an alternative to synthetic products for the treatment of myiasis.

2. Material and Methods

2.1 Cochliomyia hominivorax Colony

Adult insects were kept in 35×35 cm² cages with controlled temperature (33 ± 4 °C) and ambient humidity. Feeding consisted of pieces of raw cane sugar and water was supplied through a damp cloth. A petri dish containing fresh meat brushed with blood was placed in the cage to stimulate oviposition until successful. The maintenance of the colony followed the methodology described by Mastrangelo [47]. Experiments were realized with larvae obtained after the second generation (F2).

The egg mass was carefully removed and transferred to containers with a suitable diet for development that consisted of ground beef added to water (2 L), red blood cells (6%), egg powder (4%), milk powder (4%), and formalin (2%). The eggs were kept for 48 hours in an incubator at 37 °C and 60% humidity to reach the first larval stage (L1). After this period, the L1 was transferred to larger vessels, again containing the larval diet, for another three days, when they reached their final larval stage (L3).

At the end of the final larval stage, larvae were separated and placed in glass containers with sterile vermiculite and covered by tulle. The larvae were kept in the incubation chamber with a temperature of 26 °C throughout the pupation process, until adult insect emergence.

2.2 Baccharis dracunculifolia Essential Oil

The oil used in the tests was purchased from a commercial establishment (Harmonia Natural, Canelinha, Santa Catarina, Brazil). According to the producer's specification, the plants were grown in an organic agroforestry system.

The chemical characterization of the essential oil was carried out through gas chromatography-mass spectrometry. For this analysis, 200 µL of the essential oil was solubilized in 400 µL of hexane and injected into a gas chromatograph coupled to a mass spectrometer (GC-MS, Shimadzu, QP-5000), operating at 70 eV, on a fused silica capillary column DB -5 (30 m × 0.25 mm × 0.25 µm). Helium was used as the entrainment gas (1.7 mL/min). The settings included the injector at 240 °C, detector at 230 °C, and the following temperature sequence: 60-95 °C, 3 °C/min; 95-130 °C, 8 °C/min; 130-190 °C, 3 °C/min; 190-240 °C, 10 °C/min; split: 1/50; flux: 1 mL/min [48].

Identification of the chemical compounds was realized through a comparative analysis of the mass spectrometry with database 43 of the GC-MS system (Nist 62.lib), the literature [49], and the retention index [50].

2.3 Development of B. dracunculifolia Nanoemulsion

A high-pressure homogenization technique was used

to prepare the *B. dracunculifolia* oil nanoemulsion. Initially, 15 g of *B. dracunculifolia* oil and 2 g of the surfactant Tween 80 were weighed and mixed under magnetic stirring. Purified water (83 mL) was added, with continuous stirring for another 15 minutes. This pre-emulsion was transferred to a high-pressure homogenizer (Homolab FBF Italy, Sala Baganza (Parma), Italy) and subjected to three cycles of homogenization at 500 bars. The formulation was prepared in triplicate.

For comparison, an inert oil nanoemulsion (white nanoemulsion) was prepared under the same conditions. For this, we used 15 g of medium chain triglycerides (MCT), 2 g of Tween 80, and 83 mL of purified water.

2.4 Assessment of Particle Size, Polydispersity Index, and Zeta Potential

Particle diameter and polydispersity index were determined by photon correlation spectroscopy and zeta potential was assessed by Laser Doppler anemometry using a Zetasizer Nano Series (Malvern Instruments, Worcestershire, UK). The nanoemulsions were previously diluted in purified water and measurements were taken at 25 °C. The analysis of size was performed at a fixed scattering angle of 90°. For measurements of zeta potential, the samples were placed in electrophoretic cells, where the potential of ± 150 mV was established.

2.5 Determining pH

The pH of the nanoemulsion was determined using a pHmeter (HANNA hi2221, São Paulo, Brazil) previously calibrated with buffer solutions of pH 4.0 and 7.0.

2.6 Morphological Analysis

The morphological assessment of nanoemulsions was done by transmission electron microscopy (TEM) using a Jeol JEM-1011 microscope (Jeol Ltd., Tokyo, Japan). An aliquot (10 μ L) of each nanoemulsion, previously diluted in purified water, was deposited in

carbon-coated copper grids. The samples were then stained with 2% (w/v) uranyl acetate.

2.7 Stability Analysis

To evaluate the physical stability of the *B*. *dracunculifolia* nanoemulsion, samples were placed in amber bottles and stored at room temperature for 120 days. The particle diameter, polydispersity index, zeta potential, and pH of the samples were measured at 0, 7, 14, 21, 30, 60, 90, and 120 days.

2.8 Oil Content and Encapsulation Efficiency

The encapsulation efficiency of the essential oil was determined using the ultrafiltration/centrifugation technique. The nanoemulsions were placed in an ultrafiltration device (Amicon Centrifugal Filter Devices) containing Ultracel-100 membrane (100 kDa, Millipore Corp., Billerica, MA) and subsequently centrifuged at 2,350 g for 30 minutes to separate the free oil from the encapsulate. To determine the amount of oil incorporated into the nanoemulsions, we calculated the difference between the oil content present in the formulation, determined by the dissolution of nanoemulsions in methanol, and the free oil present in the ultrafiltrate. The oil content in the nanoemulsion after adding methanol and ultrafiltrate was determined by UV-Vis spectrophotometry (BEL photonics) at 253 nm using an external calibration curve (y = 87.904x + 0.0142, R² = 0.999). The encapsulation efficiency was expressed as a percentage and the tests were performed in triplicate.

2.9 Larvicidal Activity of the Essential Oil Nanoemulsion

To evaluate the larvicidal effect of the *B*. *dracunculifolia* nanoemulsion, 15 third instar larvae (L3) of *C*. *hominivorax* were selected and placed in glass vial (9 cm height \times 4 cm diameter), containing vermiculite and filter paper saturated with 1 mL of the product to be tested. The nanoemulsion was diluted with purified water, yielding five different

experimental concentrations: 5, 7.5, 10, 13.5, and 15% (w/v). Each vial containing 15 larvae was considered a repetition of the experiment. For each concentration, i.e.. 10 replicates were performed, 150 larvae/nanoemulsion concentration. In addition to the nanoemulsion, the following negative controls were included: (a) white nanoemulsion without essential oil; distilled water: and (c) treatment. (b) no Organophosphate, a commercial product with the active ingredient Trichlorfone (Neguvon®; Bayer, São Paulo, Brazil), was used as a positive control. For the purpose of comparing the efficacy of the nanoemulsion, the test was also performed with the essential oil dispersed in Tween 80 at 2% (w/v). The same concentrations of oil as those used for the nanoemulsion were tested: 5, 7.5, 10, 13.5, and 15% (w/v).

After application of the treatments in the filter paper containing the larvae, the vials were topped with tulle to facilitate aeration and maintained at 27 ± 1 °C in different incubation chambers. The effect of essential oil, nanoemulsion, and controls were assessed based on adult insect emergence rate, which was evaluated after nine days.

The emergence or mortality of adult insects was counted and the results presented as a mean with standard deviations. A morphological evaluation was conducted during the tests, in which deformities found in pupae and adult insects were recorded.

2.10 Statistical Analysis

The evaluation of the percentage of the larvicidal effect and the nanoemulsion characteristics, such as polydispersity index, particle diameter, zeta potential, and pH, were analyzed using descriptive statistics expressed as mean \pm standard deviation, followed by analysis of variance with adjustment for Tukey's test, through the program Sisvar version 5.3. The effects were considered statistically significant for values of *p* < 0.05.

Lethal doses were calculated with a Probit analysis

through the Biostat Software version 5.9.8.

3. Results

3.1 Chemical Composition of B. dracunculifolia Oil

Chromatographic analysis of the *B. dracunculifolia* essential oil revealed the presence of 39 chemical compounds of the terpene class (Table 1). The main monoterpene components were β -pinene (11.38%) of the composition, followed by limonene at 9.13%, and α -pinene (6.06%). The main sesquiterpenes were spathulenol (14.84%), nerolidol (10.17%), caryophyllene (8.53%), bicyclogermacrene (7.68%) and isoaromadendrene epoxide (6.26%).

3.2 Characterization of Nanoemulsion with B. dracunculifolia Oil

The macroscopic analysis revealed that the nanoemulsion containing 15% of *B. dracunculifolia* essential oil was homogeneous without signs of phase separation, presenting a white, milky color, with a blueish tint and odor characteristic of the essential oil (Fig. 1).

The particle diameter, polydispersity index, zeta potential, and pH of the nanoemulsions are shown in Table 2.

The image obtained by TEM (Fig. 2) revealed that the particles containing the *B. dracunculifolia* oil are spherical in shape, with a similar size between replicates. In addition to the nanoparticles containing the essential oil, the presence of micelles was also observed which are likely the result of an excess of surfactant in the formulation.

3.3 Stability Studies

Throughout the stability analysis period, the formulations showed no changes in appearance or signs of phase separation. These results suggest that the method used to develop the nanoemulsions was effective. The other characteristics of the formulations are described below (Table 3).

The particle diameter of the formulation ranged from

Compounds	RT (minutes)	Relative area (%)
Monoterpenes		
α-tujene	5.837	0.13
α-pinene*	6.048	6.06
Sabinene	7.158	0.51
β-pinene*	7.303	11.38
β-mircene	7.641	0.68
ρ-cimene	8.794	0.29
Limonene*	8.989	9.13
β-linanool	11.566	0.15
Cis-p-menta-2,8-dien-1-ol	12.372	0.04
Trans-pinocarveol	13.038	0.11
β-fenchol	14.619	0.18
Myrtenol	14.792	0.12
Sesquiterpenes		
α-cubebene	18.780	0.25
α-copaene	19.528	0.28
β-elemene	19.950	1.05
Caryophyllene*	20.813	8.53
Isogermacrene-D	21.038	0.24
Aromadendrene	21.355	1.91
α-humulene	21.778	1.52
α-amorphene	22.419	1.05
Germacrene-D	22.587	1.94
β-selinene	22.750	0.14
Bicyclogermacrene*	23.064	7.68
Eremophila-1(10),8,11-triene	23.370	0.64
γ-muurolene	23.560	1.43
δ-cadinene	23.817	3.98
Fokienol	24.105	0.42
β-naftalene- β-hexahydro- β-isopropyl,isopropyl-4,7 dimethyl	24.261	0.20
β-calacorene	24.430	0.18
Nerolidol*	25.031	10.17
Globulol	25.251	0.62
Spathulenol*	25.613	14.84
Isoaromadendrene epoxide*	25.778	6.26
1,1,4,7-tetramethyldecahydro-1h-ciclopropa e azulen-4-ol	26.037	2.80
Globulol	26.380	1.32
Epicubenol	27.113	0.53
Isospathulenol	27.427	1.17
β-guaiene	27.653	0.56
α-cadinol	27.941	1.51

Table 1	Chemical	composition	of <i>B</i> .	dracunculif	olia	essential oil.
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*principal compounds

Compounds were identified by comparing the mass spectra with database 43 in the GC-MS system (Nist 62.lib), the literature [48], and retention index [49].

RT = retention time in minutes.



Fig. 1 Nanoemulsion with B. dracunculifolia essential oil.

 Table 2
 Particle diameter, polydispersity index (PDI), zeta potential, and pH of the formulation developed in high pressure homogenization.

Diameter (nm)	PDI	Zeta potential (mV)	pH
178.60 ± 6.75	0.226 ± 0.03	-35.40 ± 0.69	3.49 ± 0.02

*average of three independent repetitions \pm sd.



Fig. 2 Characterization of the nanoemulsion through the image obtained by TEM. The arrow indicates the empty micelles of surfactant.

Days	Diameter	Polydispersity index	Zeta potential (mV)	рH
0	178.60 ± 6.75 a	0.23 ± 0.03 a	-35.40 ± 0.69 a	3.49 ± 0.02 ab
7	173.93 ± 7.34 a	0.26 ± 0.01 a	-36.56 ± 2.04 a	3.43 ± 0.03 bc
14	168.53 ± 9.53 a	0.23 ± 0.04 a	-34.43 ± 1.40 ab	3.54 ± 0.01 a
21	174.26 ± 5.98 a	0.21 ± 0.02 a	-32.40 ± 2.68 abc	$3.26\pm0.05~d$
30	175.73 ± 3.30 a	0.24 ± 0.03 a	-32.90 ± 2.19 ab	3.45 ± 0.02 abc
60	181.70 ± 7.13 a	0.18 ± 0.03 a	-29.90 ± 0.60 bc	$3.39 \pm 0.01 \text{ c}$
90	170.00 ± 9.84 a	0.30 ± 0.08 a	-27.93 ± 1.36 c	$3.36 \pm 0.05 \text{ c}$
120	168.20 ± 1.57 a	0.28 ± 0.04 a	-34.50 ± 0.85 ab	$3.27 \pm 0.02 \text{ d}$

 Table 3 Mean particle diameter, polydispersity index, zeta potential, and pH of the nanoemulsions throughout 120 days of storage at room temperature.

*average of three repetitions \pm sd. Different letters indicate significant differences between measurement days p < 0.05.

168.20 nm to 181.70 nm (Table 3), with no statistical difference over the storage period (Table 3), indicating good formulation stability. A rapid increase in particle size may indicate low stability in the colloidal system [51].

The polydispersity index was below 0.3 throughout the storage period and did not show significant differences between readings. The pH and zeta potential presented significant differences. However, these differences were not correlated with signs of macroscopic destabilization.

The nanoemulsion presented a multimodal distribution, presenting two distinct populations (Fig. 3). The first peak (~10 nm) represents less than 1% of the particles, while the second peak represents 99% of the particles.

3.4 Larvicidal Test on C. hominivorax Pre-pupae

The *B. dracunculifolia* essential oil nanoemulsion demonstrated a larvicidal effect (Fig. 4) according directly to the concentration used. The formulation of 13.5% essential oil (w/v) caused mortality of more than 80% of *C. hominivorax* pre-pupae. While the effects of the formulations containing 15 and 13.5% (w/v) were similar, the formulations of 5, 7.5, and 10% (w/v) differed from each other. The results found for the freshly prepared formulations were similar to the formulations stored for 120 days. Only the 5 and 10% (w/v) formulations showed differences between the fresh and stored formulations.

The LD_{50} of the essential oil nanoemulsion was 7.4%

(w/v), while that of the free essential oil at the same concentration was 12.0% (w/v).

Several morphological abnormalities were found during the tests (Fig. 5). These abnormalities were only found in the groups that came in contact with the *B*. *dracunculifolia* oil essential nanoemulsion. The pupae showed several deformities, such as reduced diameter and a desiccated appearance. In adults, a reduction in size, poorly developed wings, and black coloring were observed across the body of the insect. In addition, emergence was incomplete, and some insects could not completely disengage from the pupa, and these individuals also presented the abnormalities found in adult insects.

4. Discussion

The results obtained are encouraging and demonstrate the potential effects of B. dracunculifolia from Santa Catarina, Brazil, nanoemulsion on C. hominivorax larvae. The use of an essential oil in the control of larvae from a fly species that is extremely important from a veterinary perspective is significant, particularly considering that the common treatment is the use of synthetic products, such as macrocyclic lactones in myasis. In the veterinary clinic, case reports of avermectins intoxication are very common [52]. Many cases cause the death of dogs carrying mutations in the MDR-1 gene [53, 54]. In addition to dogs, cases of intoxication have been reported in several animal species: cats [55], cattle [56], horses [57], snakes [58], lions [59] and falcons [60].

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Fig. 3 Size distribution of nanoemulsion containing *B. dracundulifolia* oil obtained at a scattering angle of 90°.



Fig. 4 Analysis of *C. hominivorax* pre-pupae mortality after application of *B. dracunculifolia* essential oil nanoemulsion at 5, 7.5, 10, 13.5, and 15% (w/v) concentrations. Control groups: no substance, white nanoemulsion (Blank), water and trichlorfon.

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Fig. 5 Morphological abnormalities found in pupae and adult insects of *C. hominivorax* after larvicidal tests with *B. dracunculifolia* nanoemulsion. A—distorted pupae with reduced diameter. B—incomplete emergence of adult insect. C—small, deformed adults with malformed wings and feet.

The chemical profile of the *B. dracunculifolia* essential oil detected the presence of compounds such as nerolidol, spathulenol, δ -cadinene, β -pinene, α -pinene, and limonene. The chemical profile found for the EO is consistent with that of *B. dracunculifolia* plants from different regions of Brazil, including the

Southeast [61, 62], South [63], Center-West [64], and also from other countries, such as samples from the Cochabamba region in Bolivia [65].

The essential oil nanoemulsion presents satisfactory characteristics such as particle size smaller than 200 nm and PDI below 0.3 (Table 2). The average particle

diameter as well as the polydispersity index is important indicators of nanoemulsion quality, stability, and homogeneity [37]. Furthermore, these two parameters are the most important characteristics in nanoparticulate systems, as they influence *in vivo* distribution, biological activity, and toxicity [66]. Absolute values above 30 mV for the zeta potential are good indicators of stability in colloidal suspensions, since the high charge produces a repellent force between oil droplets and the nanoemulsion, thus minimizing aggregation and destabilization [67].

In the analysis of the polydispersity index, the first peak (Fig. 3) observed around 10 nm possibly represents empty micelles of surfactant, which were not adsorbed at the interface of the oil droplets [68, 69]. As the amount of oil to be encapsulated was 15% (w/v), we decided to maintain the surfactant concentration at 2% (w/v) to prioritize encapsulation, since a reduction in the surfactant concentration could affect the oil encapsulation efficiency. The oil essential nanoemulsion encapsulation efficiency was 99%.

Because the nanoemulsions prepared in this study contain a nonionic surfactant (Tween 80), they should have an electric charge close to zero. However, we found a negative zeta potential. A similar result was also reported in other studies using the same emulsifier and essential oils in the development of nanoemulsions with avocado oil [70], ginger oil [71], and thyme, lemongrass and sage oil [72]. This may occur because of the ability of the oil-water interfaces to adsorb hydroxyl ions preferentially from water or due to the presence of anionic substances in the oil [73]. However, the effect of the essential oil nanoemulsion was tested without the addition of essential oil and an emergence rate greater than 95% was found, thus demonstrating that excess surfactant in the formulation did not interfere with biological activity.

The LD_{50} of the essential oil nanoemulsion was 7.4% (w/v), while that of the essential oil dispersed in Tween 80 at the same concentrations of 12.0% (w/v). The lethal dose calculation demonstrates that the essential

oil nanoemulsion improved the bioavailability of the oil, thus improving its efficacy. The essential oil nanoemulsion has nanoscale particles that may be more effective in penetrating the cuticle of the larvae. In addition, the formulation is considered a stable system, thus avoiding the rapid volatilization of the active compounds in relation to the application of the free oil.

In the present study, all larvae were able to complete their larval development until the pupal stage. The essential oil nanoemulsion exposure could have stimulated metamorphosis to protect against morphological damage. Even so the pupae appeared to be empty, with a desiccated appearance, as shown in Fig. 5A. This clinical sign could occur due to penetration of the extract into the body cavity which completely destroyed the contents of the larvae [74]. According to Khater and Khater, some plant extracts can cause anomalies in larvae and pupae of flies, which may be related to a hormonal disorder that interferes in the physiological processes linked to metamorphosis [75].

The morphological abnormalities found in the pupae and adult insects in this study have also been found in studies on other species of myiasis-causing flies, such as *Lucilia sericata*, with the application of oils from lettuce, chamomile, anise, and rosemary [76], myrrh [77], and Fenugreek, celery, radish, and mustard [75]. The species *Chrysomyia albiceps* was also tested with different plant extracts from Southern Egypt, *Artemisia herba-alba*, *Artemisia monosperma*, *Euphorbia aegyptiaca*, and *Francoeuria crispa* [74]. Other studies on *Musca domestica* found these abnormalities in pupae and adult insects exposed to lemongrass oil [78], and extracts of neem seed, black pepper, chicory leaves, and *Conyza aegyptiaca* [79].

The activity of EOs and their insecticidal compounds are not clearly defined. However, it is evident that EOs affect insect physiology in a number of ways, both as a repellent, and as an inhibitor of food intake and growth, thus affecting biochemical processes and deregulating the endocrine equilibrium

of insects. They may be neurotoxic or growth regulators, disrupting the morphogenesis process [19].

The insecticidal potential of terpene compounds from natural products have been the subject of analysis since the 1970s [80]. The main compounds found during the analysis of *B. dracunculifolia* essential oil were the monoterpenes, limonene, α -pinene, and β -pinene, while the main sesquiterpenes were spathulenol and nerolidol. The biological activities of these compounds have been described in the literature and demonstrate their potential against parasites in general.

The use of botanical insecticides (extracts, essential oils, isolated active components) presents some limiting factors such as low physical-chemical stability, high volatility, among others. The use of nanotechnology may offer a way to develop formulations that improve the efficiency of this type of insecticide [81].

The control of insects and pests through the use of essential oils may present several advantages, such as different modes of action, performance in insect nervous system or elsewhere in the body, and low toxicity to mammals (with rare exceptions LD_{50} values in rodents ranging from 800 to 3,000 mg/kg for compounds an \pounds 5,000 mg/kg for formulated products). Because of their high volatility, oils and their compounds are considered environmentally safe (half-life of 24 hours on open air surfaces, soil, and water) [82].

The essential oil nanoemulsion of *B. dracunculifolia* is a final product that has potential for use in the treatment of myiasis as an alternative to synthetic insecticides. As such, further *in vivo* tests are suggested. In agroecological practices, there are significant challenges in the treatment and control of ectoparasites in animal production systems. The use of essential oil nanoemulsions for the treatment of myiasis may offer a low toxicity alternative for animals and humans, that is also environmentally safe.

5. Conclusions

The development of the *B. dracunculifolia* essential oil nanoemulsion in a high-pressure homogenizer resulted in a formulation with desirable characteristics, such as particle diameter, polydispersity index, zeta potential and pH, that remained stable for 120 days at room temperature. The larvicidal effect of the essential oil nanoemulsion was above 80% at concentrations of 13.5 and 15% (w/v) and greater than the essential oil at the same concentrations. The encapsulation of the essential oil improved the bioavailability of the active compounds. Under laboratory conditions, the product developed herein is shown to be a promising, non-synthetic alternative for the control of C. hominivorax larvae. Studies in vivo are required to define the therapeutic uses for B. dracunculifolia essential oil nanoemulsion in animals and humans.

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Conflict of Interest

The authors have nothing to declare.

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