

Full Length Research Paper

Larvicide and repellent activity of *Hyphenia irregularis* (Benth.) Harley in the alternative control of mosquito *Aedes aegypti*

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Dengue is an emerging epidemic disease and among those transmitted by the *Aedes aegypti* mosquito, it is the one with the highest number of cases and cases with death. With the wide dissemination of the beneficial effects of medicinal plants and the use in agriculture as a pesticide, studies on the prospection of plants with toxic activity have been increasing, highlighting extracts and essential oils. The objective of this study was to evaluate the effects of *Hyphenia irregularis* essential oil extracted by hydrodistillation, in a Clevenger-type device modified against 3rd instar larvae and adult mosquitoes of *Aedes aegypti*. The chemical composition of the oil was determined using the Gas Chromatography/mass spectrometer (GC/MS) technique. The major compounds were: 2, 5-dimethoxy-p-cymene, thymol, o-cymene, phenol-3- (1, 1-dimethylethyl)-4-methoxy and humulene with 27.0, 21.36, 15.56, 8.89 and 5.01%, respectively. For all the tests, were applied concentrations ranging from 0.007 to 0.13 $\mu\text{l.ml}^{-1}$, for the larvicidal test was denoted LC_{50} 0.037 $\mu\text{l.ml}^{-1}$ and CL_{95} 0,122 $\mu\text{l.ml}^{-1}$, for the time response required to achieve 95% mortality (TL_{95}) was 27.412 min. In the oviposition bioassay it was observed that the higher the concentration of essential oil used (0.2 $\mu\text{l ml}^{-1}$), the lower the number of eggs deposited, the less eggs being deposited as compared to the untreated tests. In addition, the action of *H. irregularis* oil showed better results than the commercial insect repellent composed of N, N-diethyl-m-toluamide (15 %) in 135 min of exposure to mosquitoes.

Key words: Dengue, essential oil, medicinal plant, natural insecticide.

INTRODUCTION

Dengue is an emerging epidemic disease and it is among those transmitted by the *Aedes aegypti*, it is the one with the highest number of cases with death (Brasil, 2016).

The mosquito *A. aegypti* is the main transmission vector of dengue fever, yellow fever, zika virus and chikungunya (Jansen and Beebe, 2010; Brasil, 2016).

The large majority of the world's population lives in countries where the incidence of dengue fever is high, and between 50 and 100 million infections are estimated each year (Brady et al. (2012), in Brazil in 2016, according to the Brasil (2016), 1,438,624 probable cases of dengue were registered until the Epidemiological Week (SE), being these all the reported cases, except those already discarded. The mosquitoes vectors of this disease, the *A. aegypti* and *A. albopictus*, are highly adapted to the social dynamics and the environment of the cities, which makes dengue an illness typical of urban areas with specific characteristics (Johansen et al., 2014).

Currently, the most common form of combat to the mosquito is the use of synthetic insecticides, composed of organophosphates and pyrethroids or even other compositions that could be highly toxic. The frequent and intensive use and the increasing doses of these products show the main problems regarding the use of insecticides, creating mosquito populations that are resistant to these products, altering the chronological cycle of the environment and allowing the direct reach of the species that corroborate with the environmental balance, leading to adverse effects (David et al., 2018).

Essential oils are volatile compounds present in various plant organs. They are considered oils due to their lipophilic composition, even though they are chemically different from vegetable oils and fats. And they are called essential because of the association with the defense mechanisms and attraction of pollinators in the plants, functions considered essential for plant survival. Resin, leaf, flower, fruit and seed are organs that accumulate these substances (Siani et al., 2000).

The exploitation of natural resources in the production of insecticides prioritizes several beneficial factors, not only the vector restraint, but also for biodegradable reasons and to be fully derived from renewable resources. This would interpolate the non-resistance of insects, since their composition added to several active principles occurs in a prolonged way. Besides, plants just like insects are species that coevolve, provoking in mosquitoes diverse effects like repellency, inhibition of oviposition and feeding, developmental disorders, deformations, infertility and mortality (Dequech et al., 2009) Figure 1.

H. irregularis is a species regionally known in Jalapão as Cerrado's Rosemary and belongs to the family *Lamiaceae*, subfamily *Nepetoideae*, tribe *Ocimeae* and sub tribe *Hyptidinae*. *Hyptidinae* is widely confined in the Cerrado's central-brazilian region, and is endemic in the central and northern South America's savannas. Despite the great diversity of species living in these areas, the

composition of the essential oil is only known from flowers' samples (Wannes et al., 2010).

Therefore, in the search for renewable and biodegradable natural products to control the transmission of dengue that can be used as an active ingredient in insecticidal and repellent formulations, the essential oil extracted from leaves of *H. irregularis* were tested against larvae and adult mosquitoes of *A. aegypti*.

MATERIALS AND METHODS

Collection of plant material

H. irregularis species, regionally known as Cerrado's Rosemary, was collected in Jalapão-Tocantins, (Latitude: 09°57'46" S and longitude: 47°40'38" W) located in the northern region of Brazil. Branches containing leaves and flowers of *H. irregularis* were collected for taxonomic identification. The assortment was carried out in the months of January and February of 2018.

Extraction and analysis of the essential oil

The extraction was performed by hydrodistillation in a modified Clevenger type apparatus, following the methodology of Guimarães et al. (2008). The *H. irregularis*'s leaves were collected and dried in the shade and after that period were cut into small fragments using a pair of scissors. Subsequently, 1000 ml of distilled water and approximately 300 g of the plant were added to a round bottom flask.

Breeding of larvae and mosquitoes

The breeding of *A. aegypti* was established in the integrated pest management laboratory at the Federal University of Tocantins (Campus-Gurupi), according to the methodology of Aguiar et al. (2015). Mosquitoes of *A. aegypti* were originally collected in Gurupi-Tocantins, Brazil (Latitude: 11°43'45" S and longitude: 49°04'07" W), male vectors were fed with a 10% sucrose solution and female with rodent blood from alive Wistar (*Rattus norvegicus albinus*). Thus, no insecticide or derivative was used to control the mosquitoes. The larvae grew in plastic containers (35 cm x 5 cm) where they were fed with a sterilized diet (80/20 chow chick/yeast mixture). All bioassays were performed in photo period 12 h light / dark at 27 ± 1°C, 65.0 ± 6% RH.

Larvae bioassay

The larvicidal test was performed according to the methodology described by Cheng et al. (2003), with some modifications. Solutions containing water, 1.7% DMSO (Dimethyl sulfoxide) and essential oil were prepared in order to reach the following concentrations: 0.007 to 0.13 µl ml⁻¹. 30 ml of distilled water and twenty five larvae of *A. aegypti* in the 3rd instar (which was appropriate for the research) were added in disposable cups with a capacity of up to 100 ml. For higher average precision and the

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Figure 1. *Hypenia Irregularis*.
Source: Author (2019).



Figure 2. The beginning of the repellency tests for positive and negative controls.
Source: Author (2019)

reduction of statistical errors, triplicates of the 25 insects was performed for each recurrence and concentration applied, since the analysis of the number of dead insects were performed 24 h after the experiments started.

Response time

For the creation of concentration-related response curves, each developmental stage and vector mortality were considered. The test consisted of the same methodology previously used, since the lethal time for 50% of the deaths was considered from the LC_{50} and consequently 95% from the LC_{95} . Mortality was cataloged in the first tests at 15 minutes and then after 60 minutes of exposure to the essential oil concentrations of *H. irregularis*, the results being later described as LT_{50} and LT_{95} , in minutes.

Test of repellency activity

The repellent activity of *H. irregularis* essential oil was tested according to the methodology described by Nério et al. (2010) and WHO (2015) with some modifications. Three acrylic boxes ($24 \times 24 \times 24 \text{ cm}^3$) were prepared for the bioassay, using a total of

one hundred and fifty female *A. aegypti* with four to seven days of age. Solutions were also prepared with the essential oils, which were dissolved in 99.8% ethanol, with the final concentrations being from 0.0033 to $0.167 \mu\text{l.cm}^{-2}$. For the validation of the tests, eight volunteers were requested, and for each product tested fifty females were used per test in five replications.

Before each test the forearms of the volunteers were sanitized with 70% ethanol. After drying, an area of 300 cm^2 was measured and the remaining area was covered by latex gloves, because the sweat particles contain lactic acid, making it attractive to females. After these steps, the volunteers instead their forearms into the acrylic boxes for 3 min (Figure 2). The test was performed at intervals of 30 min and continued until the first bite was recorded or, consequently, 135 min, being it the end of the planned time. Afterwards, the number of bites was counted to calculate the results.

Oviposition test

The effect of the essential oil on the *A. aegypti*'s egg depositing was determined following the methodology described previously. Twenty-five females fed with mouse blood (1 to 3 days) and fifty males were inserted containing different concentrations and

incubated at $28 \pm 2^\circ\text{C}$. The cages contained up to six replicates in plastic cups (100 ml) at appropriate concentrations (0.0833 to $0.2 \mu\text{l}\cdot\text{ml}^{-1}$) in 30 ml of ethanolic solution with 1.7 % of the essential oil of *H. irregularis*. The test was repeated four times. The numbers of eggs were counted daily during the addition of the cups and summed at the end of the seven days.

The percentage of viability for both ovicide and oviposition tests was calculated by the following equation: $\%V = (T-l) / T \times 100$, where % V is the viability percentage of the egg, T is the number of viable eggs in the treatment of control without application of essential oil and E is the number of viable eggs after the treatment with essential oil.

Gas chromatography coupled to mass spectrometer

The chemical composition of the oil was determined in the Analytical Center of the Institute of Chemistry of the University of São Paulo (IQUSP) using the Gas Chromatography/mass spectrometer (GC / MS) technique. The analysis was performed on Shimadzu GC-2010 equipment, equipped with a selective mass detector, model QP2010Plus.

In Gas Chromatography (GC) the compounds were subjected to analysis using Shimadzu GC-2010 instrument, equipped with RTX-5MS fused silica capillary column ($30 \text{ m} \times 0.25 \text{ mm} \times 0.25 \mu\text{m}$ film thickness); with the following temperature planning in the column: 60 to 240°C ($3^\circ\text{C}/\text{min}$); Injector temperature: 220°C ; gas carrier helium; injection with a split rate (1: 100) with an injected volume of $1 \mu\text{l}$ of a 1: 1000 hexane solution. For the mass spectrometer (MS), the following conditions were applied: impact energy of 70 eV ; temperature of the ion source and the interface: 200°C .

Statistical analyzes

The method adopted for the analysis of the results was the use of statistical and non-parametric techniques, that is, mathematical models in which the larvae possessed exponential phases, where, depending on the concentration, they obtained the fastest death, and consequently in greater numbers.

Mortality concentration curves were estimated using the PROBIT procedure using POLO PLUS statistical software (LeOra Software Berkeley, CA, USA). Residual Activity Charts were plotted using the SIGMA PLOT 11.0 software (Systat Software, Inc. San Jose, USA). The results of the oviposition despersuasion and repulsion activity were submitted to variance analysis (ANOVA) followed by the Tukey's test performed in Graph Pad Prism software v.5.03 (San Diego, California, USA). Differences were considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

The *H. irregularis* essential oil showed a high toxicity against 3rd instar *A. aegypti* larvae (Table 1). The tests were performed with concentrations ranging from 0.007 to $0.13 \mu\text{l}\cdot\text{ml}^{-1}$ for the oil studied, by comparing it with other oils studied before. To quantitatively and qualitatively analyze the present compounds of the oil in question, the gas chromatography coupled to the mass spectrometer was performed (Table 1).

The most widely used methods for quantitatively and qualitatively analyzing essential compounds according to European Pharmacopeia (2002), is the gas chromatography coupled to mass spectrometry because

it is more precise and efficient.

18 constituents of *H. irregularis* essential oil, listed in order of elution (Table 1), were identified. The major compounds were: 2, 5-dimethoxy-*p*-cymene, thymol, *o*-cymene, phenol-3-(1, 1-dimethylethyl)-4-methoxy and humulene, being 27.0, 21.36, 15.56, 8.89 and 5.01%, respectively.

Loziene et al. (2003) evaluated twenty-five samples of aerial parts of *Thymus pulegioides* L. and the essential oil composition's main constituents found were: thymol (0.2 to 26%), geraniol (0 to 31%), carvacrol (1.5 to 25%), *p*-cymene (0.1 to 16%), γ -terpinene (21.4% traces), β -caryophyllene (5 to 14%). Silva (2012), using the essential oil of *Lippia gracilis*, showed components similar to *H. irregularis* as Carvacrol 44.43%, *o*-chymen 9.42%, 2-isopropyl-5-methylanisole 5.85% and Thymol 3.83%. Following the parameters of larvicidal activity, it showed a LC_{50} of $98.06 \mu\text{l}\cdot\text{ml}^{-1}$.

Pereira et al. (2014), tested the essential oils of species such as *Pimenta dioica* and *Anibaduckei* against *A. aegypti* and concluded that the major components, eugenol and linalool are capable of providing a greater larvicidal effect to the insect. It is important to note that linalool is one of the non-major components of *H. irregularis* essential oil, but it may cause interactions between the other constituents and potentialize the toxic and larvicidal effect.

The 2,5-dimethoxy-*p*-cymene constituent, or only *p*-cymene, is one of the main responsible for the antimicrobial activity already detected in studies with the use of essential oils tested in *Staphylococcus aureus*, for example. In addition, it is one of the main constituents of the *Alpinia zerumbet* essential oil (false cardamom) in according with the works of Castro et al. (2016).

As already observed by Carvalho et al. (2003), which evaluated the larvicidal property of *Lippia sidoides* essential oil, thymol was also considered as one of the main toxic active ingredients against *A. aegypti* larvae, being able to induce 100% mortality in 90 minutes in the concentration of $0.017 \mu\text{l}\cdot\text{ml}^{-1}$. Brito et al. (2015) also performed studies evaluating these properties, finding thymol (84.95%) as the major constituent of the essential oil of this same species, followed by *p*-cymene (5.33%) and methyl carvacrol ether (3.01%).

According to Silva (2012), comparing the analyzed parameters values obtained in the essential oil tested with those in the literature, we can observe the presence of similarities. The small differences in the values found can be attributed to factors such as collecting period, storage time, genetic factors, edaphoclimatic factors and even different soil types, and according to Barros et al (2009), enzymatic actions can also be influenced by climatic conditions, and may lead to changes in certain secondary metabolites.

According to Cheng et al. (2003), LC_{50} properties of less than $0.1 \mu\text{l}\cdot\text{ml}^{-1}$, based on their effectiveness, are defined as possible larvicidal agents.

Table 1. Relative percentage (Area %), obtained by gas chromatography coupled to mass spectrometry detector, of the components of the *H. irregularis*' dried leaves' essential oil.

NC	Components	RT	RR	(%)
1	Diacetone alcohol	2.93	2.9	4.91
2	Bicyclo [3.1.0] hex-2-ene, 2-methyl-5- (1-methylethyl)	4.01	3.98	0.64
3	α -pinene	4.13	4.1	0.57
4	Mircene	4.87	4.83	2.04
5	o-Cymene	5.43	5.38	15.6
6	Linalool	6.51	6.47	1.43
7	Bicyclo [3.1.0]hex-3-en-2-ona,4-methyl-1-(1-methylethyl)	7.6	7.56	0.65
8	Terpinen-4-ol	7.78	7.75	0.98
9	Benzene, 2-methoxy-4-methyl-1-(1-methylethyl)	8.44	8.4	4.2
10	Benzene, 2-methoxy-1-methyl-4-(1-methylethyl)	8.58	8.53	3.32
11	Thymol	9.28	9.23	21.31
12	Copaene	10.6	10.5	0.48
13	2,5-dimethoxy-p-cymene	10.9	10.9	27
14	Cariofhylene	11.2	11.1	2.08
15	Bicyclo [3.1.1] hept-2-ene, 2,6-dimethyl-6- (4-methyl)	11.3	11.2	0.49
16	Humulene	11.7	11.6	5.01
17	Phenol, 3- (1,1-dimethylethyl) -4-methoxy	11.8	11.7	8.9
18	1,5,5,8-Tetramethyl-12-oxabicide	13.5	13.5	0.41
Total				100 %

NC= Number of components; RT= Retention time; RR= Calculated retention rate; (%) = Percentage of each component.

Table 2. Values of LC₅₀ and LC₉₅ of *H. irregularis* essential oil against 3rd instar larvae of *A. aegypti*.

Oil	Slope \pm SEM	LC50 ($\mu\text{L.mL}^{-1}$)	LC95 ($\mu\text{L.mL}^{-1}$)	χ^2	P
<i>H. irregularis</i>	2.535 \pm 0.280	0.037 (0.020-0.048)	0.122 (0.079-0.221)	8.9667	0.471

SEM: Standard deviation; LC50 / 95: Lethal Concentration; CI: 95% confidence interval; χ^2 : value of the chi-square test.

Therefore, considering the LC₅₀ 0.037 $\mu\text{L.mL}^{-1}$, the *H. irregularis* essential oil's presented results lower than this value, thus reinforcing the viability of its use as a larvicidal for *A. Aegypti*'s larvae, especially considering that the essential oil has a low cost of production, easy acquisition, cultivation and high yield, considering that in the extraction process, with 300 g of *H. irregularis*' dried leaves, 1.25 ml of oil were extracted, yielding 0.35%.

Complications are noticed when performing the comparative analysis with *H. irregularis* oil due to being a recently examined plant, for this purpose, making the literature associated with its studies inaccurate, however, in the tests highlighted, some related studies that also demonstrated good results in relation to lethal concentrations were referenced.

In the larvicidal test, it was possible to verify that the concentrations were, respectively, 0.037 and 0.122 $\mu\text{L.mL}^{-1}$ for determination of LC₅₀ and CL₉₅ of *H. irregularis* essential oil (Table 2). In the literature, Assunção (2013) calculated that the LC₅₀ of the *Citrus sinensis* essential oil

against *Ae. aegypti* was 99.014 $\mu\text{L.mL}^{-1}$, although it has a larvicidal effect, their study shows a concentration needed of almost 3000 times greater than the *H. irregularis* oil to reach the LC₅₀ effect. Senthilkumar and Venkatesalu (2012) tested the *A. calamus*' rhizome's essential oil for its larvicidal effect in *Culex quinquefasciatus* larvae and found the LC₅₀ value of 63.43 $\mu\text{L.mL}^{-1}$ and CL₉₀ of 145.95 $\mu\text{L.mL}^{-1}$.

In the studies by Ríos et al. (2017), eleven essential oils were tested against *A. aegypti* and all of them reached LC₅₀ values lower than 115 $\mu\text{L.mL}^{-1}$, the lowest LC₅₀ value was attributed to the *Thymus vulgaris* plant with 45.73 $\mu\text{L.mL}^{-1}$ of oil and the highest LC₅₀ was attributed to the *Cymbopogon martinii* plant with 114.65 $\mu\text{L.mL}^{-1}$. In addition, the main components of oils with higher larvicidal activity were thymol (42 %) and p-cymene (26.4%), compounds similar to those found in the present study.

According to Pavela (2015), LC₅₀ values lower than 0.05 $\mu\text{L.mL}^{-1}$ are considered effective in the larvicidal

Table 3. LT₅₀ and LT₉₅ values for *H. irregularis* essential oil against 3rd instar larvae of *A. Aegypti*.

Oil	Slope±SEM	LT50 (min)	LT95 (min)	χ ²	P
<i>H. Irregularis</i>	0.747±0.210	20.791 (12.9-39.0)	27.412 (12.5-34.5)	0.934	0.093

SEM: Standard deviation; LC50/95: Lethal Concentration; CI: 95 % confidence interval; χ^2 : value of the chi-square test.

effect. The *H. irregularis* oil reached a value close to this, 0.037 $\mu\text{l.ml}^{-1}$, so it is within the standard of efficiency demonstrated in studies that tested more than 122 species of plants' essential oil as larvicide and repellent against different types of mosquitoes.

Cole (2008), tested the larvicidal activity of the extracts of the fruits of *Schinus terebinthifolius* against *A. aegypti*, and obtained an LC₅₀ result of 117.34 $\mu\text{l.ml}^{-1}$. In another case, Nunes (2017) determined the values of CL₅₀ and CL₉₅ regarding the action of the extract of *L. sidoides* and *H. crenata* for larvae of the 3rd instar of *A. aegypti*, resulting in *L. sidoides* CL₅₀ of 123 $\mu\text{l.ml}^{-1}$ and CL₉₅ of 434.379 $\mu\text{l.ml}^{-1}$, and from *H. crenata* oil the values obtained were LC₅₀ of 0.035 $\mu\text{l.ml}^{-1}$ and CL₉₅ of 0.113 $\mu\text{l.ml}^{-1}$.

Silva et al. (2014) evaluated the *Croton linearifolius* species obtaining an LC₅₀ of 13.3 $\mu\text{l.ml}^{-1}$, Costa et al. (2005) presented an CL₅₀ of 0.0083 $\mu\text{l.ml}^{-1}$ using the *Piper marginatum* oil, and Oliveira (2012), using *Piper aduncum*, that had its oil extracted using organic solvents, obtained an LC₅₀ of 0.29 $\mu\text{l.ml}^{-1}$. Thus, *H. irregularis* oil becomes viable in the face of studies already published, being an economical possibility to formulate repellents and other insect control strategies in general.

To determine the lethal times (LT₅₀ and LT₉₅) in *A. aegypti* 3rd instar larvae using *H. irregularis* essential oil, pre-defined concentrations correlated within 24 hours of total test duration were used. Therefore, in view of the obtained results, it can be inferred that the amount of essential oil related to the concentration can influence the result in the larval and / or adult phases or with differences in the metabolic detoxification.

In addition, there are no studies that demonstrate data on the characteristics and effects of *H. irregularis* essential oil in a complete way, which makes it an interesting alternative for future studies and the development of biological control products for insect vectors for tropical diseases and even microorganisms of agricultural and medical interest.

When evaluating the response times of *H. irregularis* oil (Table 3), it was possible to conclude that the LT₅₀ is reached in 20.79 min and the LT₉₅ in 27.41 min. Considering that the value of p (0.093) < χ^2 (0.934), there is a significant difference between LT₅₀ and LT₉₅. Regarding the literature, Aguiar et al. (2015) analyzed the essential oil of *Siparuna guianensis*, where the response time was also obtained using the concentrations of LC₉₅ determined for the third stage of *A. aegypti*, and

concluded that the time required to reach 50 % was 21 minutes and 95 % mortality (LT₉₅) was 29 min. When comparing it with the studies of *H. irregularis* the former showed superior results, considering that the LT₅₀ was 20.791 and LT₉₅ was 27.412 min.

In addition to toxicity in larvae, *H. irregularis* oil was shown to be very promising as a repellent (Table 3). The concentrations used above 0.167 $\mu\text{L cm}^{-2}/\text{skin}$ were the ones that had the best protection time during the 135 min in 100% (Figure 4). In addition, repellent activity from essential oil concentrations was greater than that of the commercial product generally sold in Brazil as an insect repellent (with 15% N, N-diethyl-m-toluamide (DEET) in its active formulation), which was used as a positive control, and had a maximum protection of 69 min (Figure 3).

According to the literature, Aguiar et al. (2015), 0.00045 $\mu\text{L cm}^{-2}$ obtained 100% of repellency for *A. aegypti*. From these results, *H. irregularis* essential oil has the potential to be good candidate for a possible mosquito repellent formulation.

According to Pacheco (2013), *Melaleuca alternifolia* oil has a repellent potential of up to 98% in only 15 min of testing, however, such oil needs to be better formulated to be a commercial repellent candidate, with better fixation efficacy in the aromatic compounds of the skin and increased performance and duration of the repellent effect. In contrast, *H. irregularis* were able to demonstrate 100% repellency in the 135 min tested. Nevertheless, studies on the improvement of its fixation should be performed, taking into account all the formulations necessary to maintain the tested efficiency and increase its time of action in the skin.

It has been noted that the essential oil can also be dissuasive to oviposition. The percentage of oviposition of the essential oil of *H. irregularis* was calculated (Figure 4), where the vertical axis corresponds to the percentage of eggs deposited and the vertical axis refers to concentrations tested with the oil. Thus, it is observed that the higher the concentration of essential oil used (0.2 $\mu\text{l ml}^{-1}$) the less the deposited eggs (20%) when compared to the untreated tests. At the lowest concentration tested (0.08 $\mu\text{l ml}^{-1}$), the percentage of eggs deposited reaches almost 74%.

At first, the oil showed high inhibition of the mosquito ovipositor, this can be explained due to its major components and also due to its concentrations compared with the control solution. It is also noticeable that in the

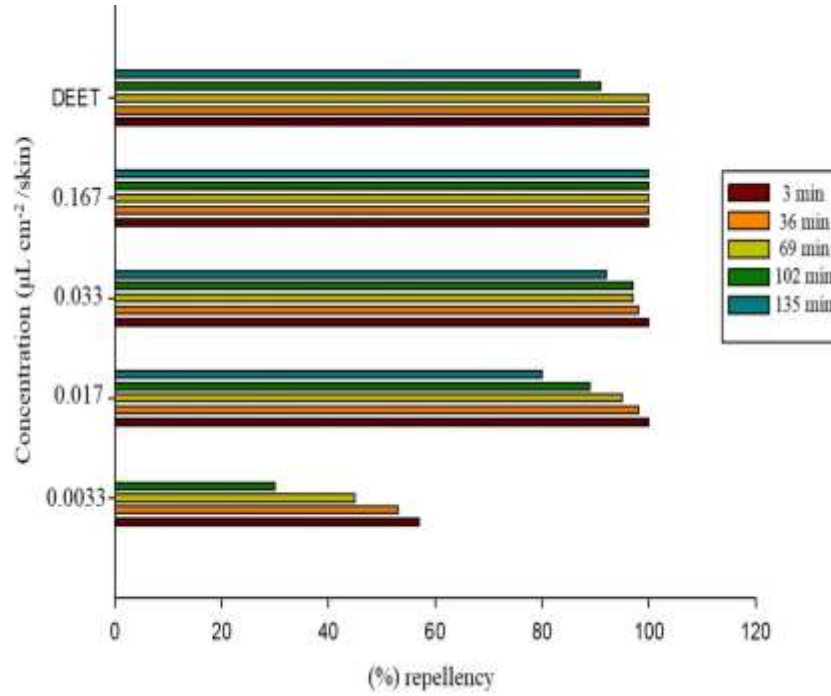


Figure 3. Protective activity of *H. irregularis* essential oil against adult *Aedes aegypti*.

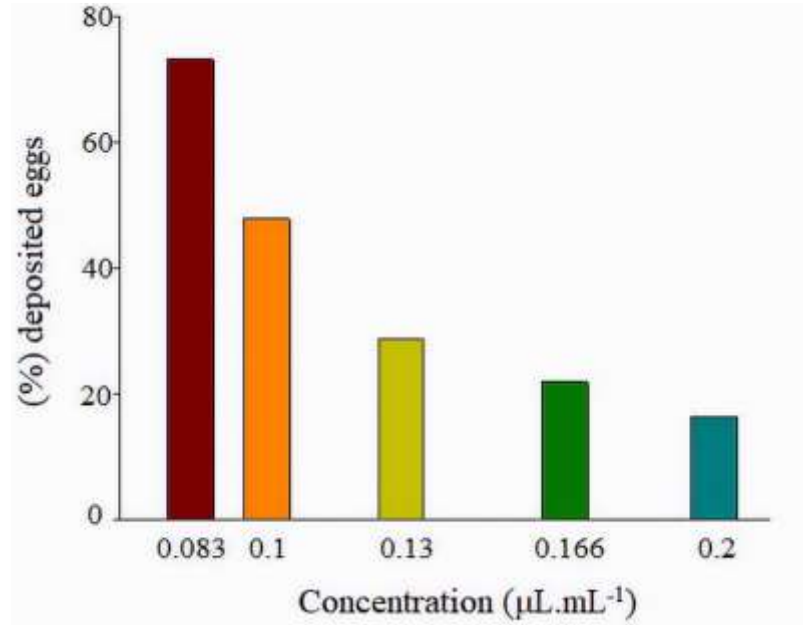


Figure 4. Percentage of oviposition of *h. irregularis* essential oil against 3rd instar larvae of *aedes aegypti*.

course of the days the essential oil volatilization occurred, a variation in the values of the tests when compared to the control, however this factor was not limiting, because even then, a satisfactory inhibitory activity was still noted

until the fourth day.

Silva (2012) tested the species *Etilingera elatioros* and concluded that the major components of the essential oil represented a retarding action that would soon be

analogous to the essential oil at a concentration of 0.05 $\mu\text{l ml}^{-1}$ where there was a significant amount of death of more than 70% of the eggs deposited in the containers. It is important to note that *A. aegypti* females were not attracted only by clean water sources, indicating that the insect has some adaptability in the acceptance of laying substrates that vary in quality (Beserra et al., 2010). For Santos et al. (2017), the use of the essential oil of *Syagrus coronata* was also efficient regarding the deterrent effect in the pregnant females of *A. aegypti*. Their results indicated that the detergent activity may be linked to the presence of octanoic acids in the composition of the essential oil tested.

Thus, in addition to the larvicidal and repellent effect, the *H. irregularis* oil is able to stop the proliferation of insects, by destroying their eggs or impeding their oviposition and occlusion, including *A. aegypti* in several stages of its development, facilitating its control and, consequently, the control of diseases to which the insect is a vector.

Conclusion

The *H. irregularis* essential oil was shown to be effective in the larvicidal effect with an LC_{50} of 0.037 $\mu\text{l ml}^{-1}$ and an LC_{95} of 0.122 $\mu\text{l ml}^{-1}$, for the response time LT_{50} of 20.791 minutes and LT_{95} of 27.412 minutes. In the oviposition bioassay it was observed that the higher the concentration of essential oil used (0.2 $\mu\text{l ml}^{-1}$), the lower the number of eggs deposited, when compared with the untreated tests. The repellency test was 100% approved at a concentration of 0.167 $\mu\text{l cm}^{-2}/\text{skin}$ and was more efficient than the commercial DEET repellent used as a positive control in 135 minutes of exposure to *A. aegypti*. Therefore, the results observed in the present study contribute strongly to the basis of possible new formulations.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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