Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Larvicidal activity and chemical composition of four essential oils against *Aedes aegypti* (Diptera: Culicidae)

Atividade larvicida de óleos essenciais de Myrtaceae e Lamiaceae contra *Aedes aegypti* (Diptera: Culicidae)

I. L. S. Cruz[a,b,c*], M. A. G. Pimentel[d], T. A. Nascimento[a,b], S. P. Alves[c], M. Maleck[b,c,e] and M. M. C. Queiroz[a,b]

[a]Fundação Oswaldo Cruz – FIOCRUZ, Instituto Oswaldo Cruz – IOC, Programa de Pós-graduação em Biodiversidade e Saúde, Rio de Janeiro, RJ, Brasil

[b]Fundação Oswaldo Cruz – FIOCRUZ, Instituto Oswaldo Cruz – IOC, Laboratório Integrado: Simulídeos e Oncocercose & Entomologia Médica e Forense, Rio de Janeiro, RJ, Brasil

[c]Universidade de Vassouras – Univassouras, Laboratório de Insetos Vetores, Vassouras, RJ, Brasil

[d]Embrapa Milho e Sorgo - Embrapa, Sete Lagoas, MG, Brasil

[e]Colégio Pedro II, São Cristóvão, RJ, Brasil

Abstract

The use of botanical insecticides has increased in recent years due to the demand for effective products, particularly against insects resistant to conventional insecticides. Among these is *Aedes aegypti*, a well-adapted mosquito to urban environments that opportunistically feeds on humans and animals, contributing to the spread of virus. We evaluated the potential of essential oils (EOs) extracted from *Eucalyptus citriodora*, *Eucalyptus staigeriana*, *Eucalyptus caryophyllus*, and *Mentha arvensis* in terms of their larvicidal activity against *Ae. aegypti*. EOs' compounds were determined using gas chromatography-mass spectrometry (GC-MS). Bioassays were performed on third instar larvae of *Ae. aegypti* to evaluate the larvicidal effects of EO dilutions in dimethyl sulfoxide (DMSO) at different concentrations. Mortality rates were observed over a 72-hour period to determine the efficacy of the treatments. Citronellal (86.64) predominated in *E. citriodora*, limonene in *E. staigeriana* (41.68), eugenol in *E. caryophyllus* (87.76), and menthol in *M. arvensis* (51.53%). EOs exhibited larvicidal activity from 10 ppm, with notable efficacy at 85 ppm, in which those from *E. staigeriana* and *M. arvensis* caused maximum mortality to *Ae. aegypti* larvae. Results revealed distinct efficacy patterns among EOs, with *E. staigeriana* displaying high toxicity within 24 h, achieving LC_{50} and LC_{95} values of 47.04 ppm and 97.35 ppm, respectively. Larvicidal effects within 1 h were observed for *E. citriodora* and *E. caryophyllus*. This study underscores larvicidal efficacy against *Ae. aegypti*, notably *E. staigeriana*, which had the lowest LC_{50} value. The findings indicate that the tested samples have potential for use as bioinsecticides.

Keywords: *Eucalyptus citriodora*, *Eucalyptus staigeriana*, *Eugenia caryophyllus*, *Mentha arvensis*, larvicide.

Resumo

O uso de inseticidas botânicos tem aumentado nos últimos anos devido à demanda por produtos eficazes, especialmente contra insetos resistentes aos inseticidas convencionais. Entre eles está o *Aedes aegypti*, um mosquito bem adaptado a ambientes urbanos que se alimenta oportunisticamente de humanos e animais, contribuindo para dispersão de vírus. Avaliamos o potencial de óleos essenciais (OEs) extraídos de *Eucalyptus citriodora*, *Eucalyptus staigeriana*, *Eucalyptus caryophyllus* e *Mentha arvensis* em relação à sua atividade larvicida contra *Ae. aegypti*. Os compostos dos OEs foram determinados usando cromatografia gasosa acoplada à espectrometria de massa (GC-MS). Os bioensaios foram realizados em larvas de terceiro instar de *Ae. aegypti* para avaliar os efeitos larvicidas das diluições de OE em dimetil sulfóxido (DMSO) em diferentes concentrações. As taxas de mortalidade foram observadas ao longo de 72 horas para determinar a eficácia dos tratamentos. O componente citronelal (86,64) predominou em *E. citriodora*, limoneno em *E. staigeriana* (41,68), eugenol em *E. caryophyllus* (87,76) e mentol em *M. arvensis* (50,73%). Os OEs exibiram atividade larvicida a partir de 10 ppm, com grande eficácia a 85 ppm, onde os de *E. staigeriana* e *M. arvensis* causaram mortalidade máxima de larvas de *Ae. aegypti*. Os resultados revelaram padrões de eficácia distintos entre os OEs, com *E. staigeriana* exibindo maior toxicidade dentro de 24 horas, alcançando valores de CL_{50} e CL_{95} de 47,04 ppm e 97,35 ppm, respectivamente. Efeitos larvicidas dentro de 1 hora foram observados para *E. citriodora* e *E. caryophyllus*. Este estudo destaca a eficácia larvicida contra *Ae. aegypti*, especialmente *E. staigeriana*, que teve o menor valor de CL_{50}. Concluímos que esses OEs são potencialmente larvicidas para o controle eficaz de *Ae. aegypti*.

Palavras-chave: *Eucalyptus citriodora*, *Eucalyptus staigeriana*, *Eugenia caryophyllus*, *Mentha arvensis*, larvicida.

*e-mail: igorlscruz@gmail.com
Received: February 25, 2024 – Accepted: August 16, 2024

1. Introduction

Aedes (*Stegomyia*) *aegypti* (Linnaeus, 1762) plays a significant medical role as a vector of various arboviruses affecting humans, such as dengue (DENV), chikungunya (CHIKV), yellow fever (YFV), and Zika (ZIKV) (Gubler, 2002; Ferreira-de-Brito et al., 2016; Costa et al., 2017; Dalpadado et al., 2022). These diseases pose an increasing challenge in tropical and subtropical regions worldwide. The rise in temperatures, coupled with climate change, has raised concerns about the exacerbation of these illnesses in endemic areas, increasing the risk of viral transmission, vector survival, and reproduction (Messina et al., 2019; Badolo et al., 2022). This mosquito is well-adapted to urban environments, breeding in a variety of habitats and opportunistically feeding on both humans and animals, contributing to the spread of these diseases. This process is driven by the ongoing increase in urbanization, international trade, and travel, facilitating the dissemination and establishment of *Ae. aegypti* in new areas (Powell and Tabachnick, 2013; Martin et al., 2021; Rose et al., 2023).

In Brazil, various initiatives have been undertaken over the years to manage the spread of DENV. These include public education campaigns, programs for controlling mosquito populations, environmental management practices, and most recently, the rollout of a dengue vaccine. The recent approval of this vaccine represents a significant advancement in combating this mosquito-borne disease (Duarte et al., 2024). Despite this progress, the World Health Organization (WHO) estimates that approximately 2.5 billion people remain at risk of dengue (Khattak et al., 2023). Furthermore, infections with CHIKV and ZIKV can result in permanent sequelae. Although there is an effective vaccine for YFV, it continues to spread into new areas, leading to new epidemics (Chen and Wilson, 2020; Carvalho and Long, 2021).

While vaccination is the most effective prophylaxis against these diseases, controlling *Ae. aegypti* through insecticides, whether chemical or biological, remains necessary due to the persistent risk and impact of these diseases (Benelli, 2015; Silvério et al., 2020).

Chemical strategies have improved vector control but now face the challenge of insecticide resistance. Despite their widespread use, these products are often ineffective and harmful to non-target species and the environment (Bolzonella et al., 2019; Demirak and Canpolat, 2022). Over the past decade, pyrethroids like deltamethrin and permethrin have been increasingly used for dengue control, leading to the selection of resistant individuals in *Ae. aegypti* populations. These populations have shown increased survival and reproduction rates under the pressure of all four classes of insecticides (carbamates, organochlorines, organophosphates, and pyrethroids), posing a threat to the efficacy of control measures (Vontas et al., 2012). Organophosphates and pyrethroids can also affect mammals through ingestion, skin absorption, and inhalation, causing symptoms such as nausea, respiratory issues, and reproductive problems (Zhu et al., 2020; Badr, 2020).

Given the challenges associated with chemical insecticides, targeting different life stages of mosquitoes is an effective technique for controlling their populations. Larvicidal measures, in particular, serve as powerful tools against various disease-causing vectors. Since mosquito larvae are localized and cannot change habitats, unlike adult mosquitoes that can fly and evade control measures, focusing on the larval stage is a more efficient strategy for reducing mosquito populations (Ganesan et al., 2023). Most current larvicides used in vector control are synthetic, such as growth inhibitors like diflubenzuron and methoprene. However, due to widespread resistance and adverse effects on non-target organisms and the environment, biolarvicides are increasingly preferred. These biolarvicides are derived from natural sources such as plants, bacteria, algae, lichen, and fungi, offering a more sustainable and environmentally friendly approach to mosquito control (Milugo et al., 2021).

Botanical larvicides have emerged as a promising alternative to synthetic insecticides due to their high efficacy and environmental safety (Soleimani-Ahmadi et al., 2017). Essential oils (EOs), extracted from various parts of aromatic plants like rhizomes, flowers, leaves, fruits, barks, and stems through hydrodistillation (Santos et al., 2023), exhibit significant antileishmanial, antibacterial, antifungal, and insecticidal properties (Moreira et al., 2019; Teles et al., 2019; Pesavento et al., 2015; Moussii et al., 2020; Hu et al., 2019; D'agostino et al., 2019; Senthil-Nathan, 2020; Spinozzi et al., 2021). They are particularly promising for mosquito larvicidal control due to their selective action and minimal impact on non-target organisms (Esmaili et al., 2021; Azevedo et al., 2023).

Beyond their antibacterial and antifungal properties, EOs are potent natural insecticides. Although developing commercial EO-based products involves challenges like cost and regulatory issues, the demand for natural pesticides is driving progress in this field. EO-based products are already in use in major agricultural regions, demonstrating their potential as safe and effective alternatives to conventional pesticides (Isman, 2020).

Eucalyptus species are cultivated primarily for their wood and the EO derived from their leaves. This EO features a blend of various compounds, including monoterpenes, esters, aldehydes, and sesquiterpenes, playing a central role in the plant's defense mechanism against vectors and microorganisms (Čmiková et al., 2023).

Eugenia caryophyllus (Spreng.) Bullock and S. G. Harrison, commonly known as clove, is a plant whose EO is mainly composed of eugenol. This EO has various biological activities, including antifungal, antibacterial, antioxidant, and insecticidal properties (Hemalatha et al., 2016; Bueno et al., 2023).

Mentha arvensis Linn. is an aromatic plant with leaves that emit a pleasant odor and whose main compound is menthol. Due to their antispasmodic and anti-inflammatory effects, these leaves are widely used in medicine for treating respiratory diseases and addressing digestive tract disorders (Nabi et al., 2020).

In the present study, selective bioassays were conducted with the target organism *Ae. aegypti*, using EOs extracted from the following plants: *Eucalyptus citriodora* Hook, *Eucalyptus staigeriana* F. Muell. ex Bailey, *E. caryophyllus*, and *M. arvensis*. Although some of these species have been previously tested for their insecticidal action, it is recognized that the chemical composition of essential oils can vary significantly due to factors such as geographical origin, climatic conditions, and extraction methods. This variability suggests that a thorough assessment of their larvicidal activity is necessary to understand their potential efficacy. Additionally, these essential oils were selected for their availability on a large scale, cost-effectiveness, and efficiency, aiming to identify products capable of controlling *Ae. aegypti* larvae. Our study aims to evaluate the larvicidal activity of these EOs, considering their chemical variability, and to compare these findings with existing literature, thereby contributing to a more nuanced understanding of their effectiveness in pest control.

2. Material and Methods

The present study was conducted at the Laboratório de Insetos Vetores (LIV), Campus Vassouras, Universidade de Vassouras (UNIVASSOURAS), Vassouras, Rio de Janeiro, Brazil, in collaboration with the Instituto Oswaldo Cruz (IOC), Rio de Janeiro, RJ.

2.1. Essential oils

The EOs of *E. citriodora*, *E. staigeriana*, *E. caryophyllus*, and *M. arvensis* (Table 1) were commercially obtained in partnership with the Empresa Brasileira de Pesquisa Agropecuária (Embrapa) – Centro Nacional de Pesquisa de Milho e Sorgo, Sete Lagoas, Minas Gerais, Brazil. They underwent qualitative analysis by gas chromatography-mass spectrometry at the Central Analítica, Instituto de Química da Unicamp, Campinas, São Paulo, Brazil.

The selection criteria for plants and EOs were based on their widespread availability, ensuring high extraction yields and broad commercial availability. Additionally, we considered the economic aspect, opting for cost-effective options that would not hinder future commercial products. Finally, biological efficacy was a key factor, choosing plants and oils with a proven track record in insect control, supported by dose-response assays.

2.2. EOs' composition: analysis by gas chromatography-mass spectrometry

EO samples were diluted in dichloromethane and analyzed by gas chromatography coupled with mass spectrometry (GC-MS) on a quadrupole linear-type instrument, model 5975C (Agilent). The chromatographic column used was an HP-5MS, with 30 m in length, 0.25 mm in internal diameter, and a film thickness of 0.25 µm. Helium was used as the carrier gas, while the injector temperature was maintained at 290 °C. The temperature started at 60 °C, with a gradual increase of 3 °C/min until reaching 285 °C, where it remained for 20 min. Mass spectra were obtained in scan mode (0.5 sec/scan), with a mass range from 35 to 500 and ionization energy (IE) of 70 eV.

The volatile compounds detected by GC-MS analysis were identified by comparison of the obtained mass spectra and retention indices, which were automatically calculated by the Automated Mass Spectral Deconvolution and Identification System (AMDIS), with those of the National Institute Standard and Technology 11 (NIST11) database. Combination of AMDIS with the NIST 11 database was utilized.

2.3. Mosquito rearing

The eggs of *Ae. aegypti* were obtained from the mosquito colony at LIV/UNIVASSOURAS. A strip of filter paper with eggs was placed in a rectangular polypropylene plastic container (44 cm x 30 cm x 8 cm) with distilled water, aged for 24 h, and preheated to 28 ºC to stimulate larval hatching. The containers with larvae were kept in a Biological Oxygen Demand (B.O.D.) incubator at 27 ± 1 ºC, relative humidity (RH) of 70 ± 10%, and a 12-h photophase, along with a diet composed of brewer's yeast (Lev Life ©). After hatching and larval development, third-stage larvae (L3) were selected for the larvicidal test.

2.4. Larval bioassays

The bioassays were conducted following the methodology of Maleck et al. (2016) and Mituiassu et al. (2021), adapted from the World Health Organization (WHO, 2005), under the same breeding conditions. EOs were diluted in dimethyl sulfoxide (DMSO) and applied at final concentrations of 10, 25, 40, 55, 70, 85, 100, and 115 ppm in hexagonal glass containers (5 cm x 4.5 cm x 5 cm) containing 20 mL of distilled water. Third instar larvae of *Ae. aegypti* (n=20) were used for each experimental group (test, control, and testimony). In the test group, the diluted concentrations of EOs in DMSO were applied; in the control group, there was no presence of EOs or the solvent used in the dilutions; and in the testimony group (DMSO control), EOs were not used, while the dilution solvent was maintained. The positive control was diflubenzuron, as reported by Maleck et al. (2016). Three repetitions of the experiment were conducted, each comprising five replicates for each experimental group. Each group consisted of 100 larvae, distributed in different batches for each repetition, with 20 larvae per replicate. Larval mortality was recorded at 1, 24, 48 and 72 hours post-treatment. Larvae were considered dead when they showed no movement or response to mechanical stimulus, using a disposable Pasteur pipette.

Table 1. Plant information and extraction methods.

Name of plant	CAS Number:	Origin	Extraction
E. citriodora	8000-48-4	Brazil	Steam distillation of the leaves
E. staigeriana	1627700-32-6	Brazil	Steam distillation of the leaves
E. caryophyllus	8000-34-8	Indonesia	Steam distillation of the buds
M. arvensis	68917-18-0	China	Steam distillation of the leaves

2.5. Statistical analysis

Larvae mortality rates and standard deviations were calculated using R Studio (R Core Team, version 1.3.1093). The obtained data underwent probit analysis (dose × mortality) after 1 h and 24 h, with the calculation of LC_{50} and LC_{95} values (PROC PROBIT; SAS INSTITUTE, 2002). Additionally, a chi-square test was performed to assess the homogeneity of the tested population.

3. Results

3.1. GC-MS analysis

The main compounds identified in the EO samples of *E. citriodora* and *E. staigeriana* were citronellal (86.64) and limonene (41.68), respectively. Regarding *E. caryophyllus*, eugenol was detected at the highest concentration, reaching 87.76%. In the case of *M. arvensis*, the predominant compound was menthol, with a concentration of 51.53%. Figure 1 presents the chromatograms, and Table 2 presents the chromatographic profiles and mass spectra of the EOs.

3.2. Larvicidal activity

All EOs demonstrated larvicidal activity starting at the lowest concentration of 10 ppm, with significant efficacy compared to the control (Table 3). Notably, maximum mortality rates were achieved with the EOs of *E. staigeriana* (100 ± 1.7) and *M. arvensis* (100 ± 0.8) at 85 ppm.

The results, expressed through concentration-mortality curve slopes, revealed distinct efficacy patterns for each

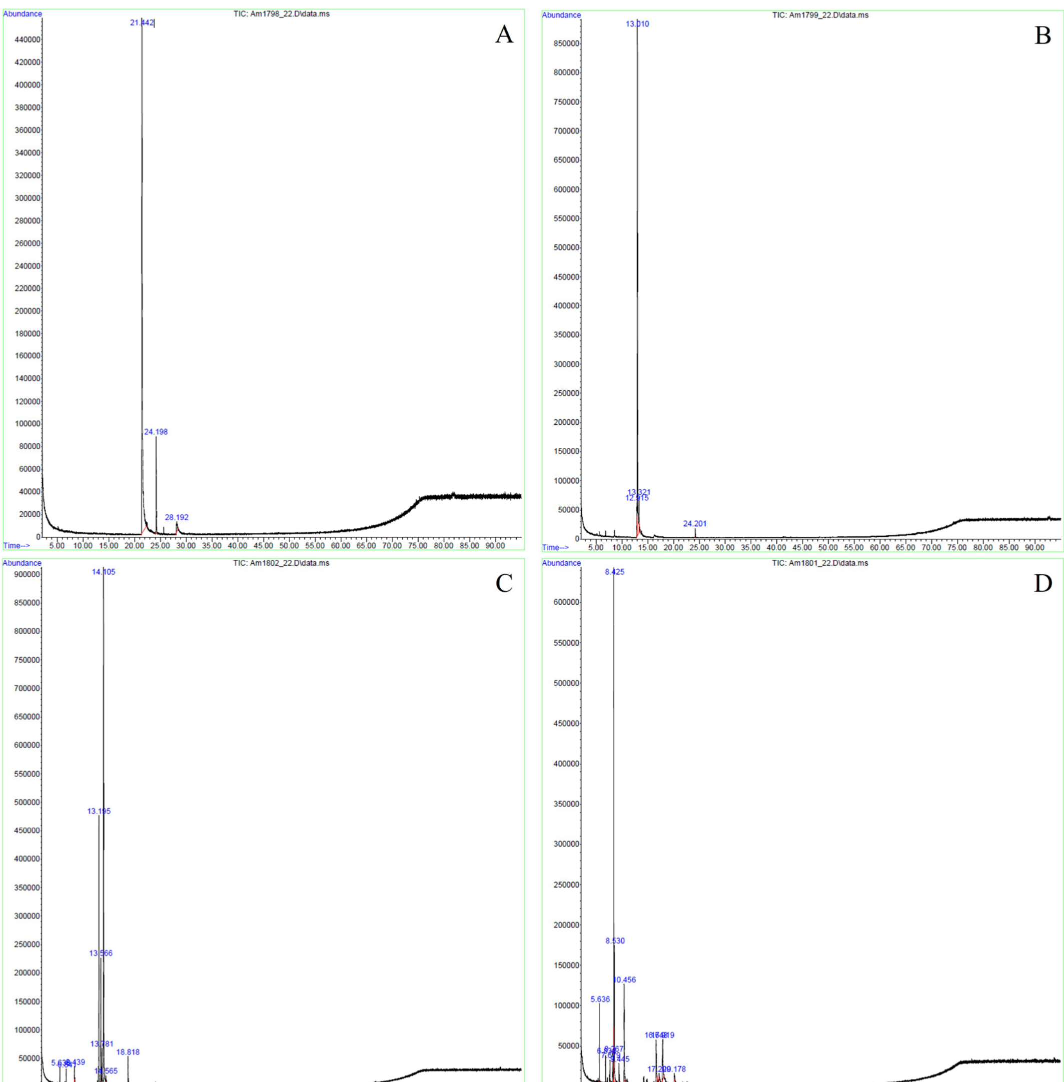

Figure 1. Chromatograms of essential oils obtained from (A) *Eugenia caryophyllus*, (B) *Eucalyptus citriodora*, (C) *Mentha arvensis*, and (D) *Eucalyptus staigeriana*, carried out using an Agilent 5975C gas chromatograph-mass spectrometer with an HP-5MS capillary column. Components were identified based on their retention times (in minutes).

Table 2. Compounds detected in essential oil samples from four different plants.

Essential Oil	Peak	t_R	Compound	%A
Eucalyptus citriodora	1	12.913	isopulegol	4.39
	2	13.009	citronellal	86.64
	3	13.319	iso-isopulegol	7.27
	4	24.204	caryophyllene	1.70
Eucalyptus staigeriana	1	5.638	α-pinene	5.14
	2	6.837	β-pinene	2.10
	3	7.682	phellandrene	2.63
	4	8.265	cymene	3.14
	5	8.425	limonene	41.67
	6	8.532	eucalyptol	13.76
	7	9.447	terpinene	2.41
	8	10.458	terpinolene	11.12
	9	16.646	neral	8.10
	10	17.208	unidentified	0.88
	11	17.919	geranial	7.82
	12	20.176	methyl geranoate	1.23
Eugenia caryophyllus	1	21.442	eugenol	87.76
	2	24.198	caryophyllene	9.41
	3	28.192	eugenol acetate	2.83
Mentha arvensis	1	5.638	α-pinene	1.10
	2	6.842	β-pinene	1.10
	3	8.441	limonene	1.11
	4	13.196	menthone	25.40
	5	13.565	isomenthone	12.26
	6	13.779	neomenthol	3.87
	7	14.105	menthol	51.53
	8	18.817	menthene	3.63

t_R = Retention time of the compound in the column (minutes); %A = Percentage of normalized area, indicating the relative distribution of compounds in the sample.

evaluated EO. Larval mortality was dose-dependent for all tested EOs. Within 24 h, *E. staigeriana* EO exhibited high toxicity against *Ae. aegypti*, reaching 47.04 ppm and 97.35 ppm for LC_{50} and LC_{95}, respectively (Table 4). The observed larvicidal effect at 1 h was positive only for the EOs of *E. citriodora* and *E. caryophyllus*.

4. Discussion

Many of the compounds identified in EOs display insecticidal properties. An example is eugenol, which was detected in the present study through mass spectrometry in *E. caryophyllus* EO and whose efficacy was previously confirmed by Adhikari et al. (2022) and Govindarajan et al. (2016). The effectiveness of this compound, particularly when associated with other synergists, was highlighted by Barbosa et al. (2012), who demonstrated its effectiveness as a larvicide across different generations. Our findings, corroborated by the research of Adhikari et al. (2022), demonstrate that the presence of eugenol can be an effective larvicide over multiple generations without quickly leading to resistance. Their study showed that eugenol induced a dose-dependent mortality in *Ae. aegypti* larvae, maintaining its efficacy for up to 30 generations. Despite fluctuations in LC_{50} values across these generations, the overall sensitivity of the mosquito larvae to eugenol remained relatively consistent, indicating that eugenol's effectiveness can be sustained over time. However, it is important to note that the larvicidal activity observed for *E. caryophyllus* oil in our study cannot be solely attributed to eugenol without further investigation into the roles of other constituents. Barbosa et al. (2012) highlighted that while eugenol is a significant active component, the potential synergistic effects of other minor constituents cannot be disregarded.

Table 3. Mortality rate of each concentration per essential oil (EO) assessed in *Aedes aegypti* larvae at 24, 48, and 72 h.

EO source	Concentration (ppm)*	Mortality rate (% ± DS)**		
		24 h	48 h	72 h
Eucalyptus citriodora	Control	0	0	0
	10	0	0	0
	25	4 ± 0.8	6 ± 0.5	8 ± 0.5
	40	5 ± 0.5	7 ± 0.5	10 ± 0.8
	55	29 ± 2.1	32 ± 0.8	38 ±0.8
	70	70 ± 0.7	75 ± 1.2	76 ± 0.4
	85	74 ± 1.9	80 ± 1.0	82 ± 0.5
	100	79 ± 2.5	85 ± 1.6	87 ± 0.5
	115	88 ± 2.3	90 ± 0.5	94 ± 1
Eucalyptus staigeriana	Control	0	0	0
	10	0	0	0
	25	6 ± 0.8	12 ± 0.4	12 ± 0
	40	40 ± 2.3	49 ± 1.4	53 ± 0.8
	55	78 ± 3	91 ± 1.3	91 ± 0
	70	78 ± 2.1	92 ± 1	92 ± 0
	85	93 ± 2.1	95 ± 0.5	100 ± 1.7
	100	95 ± 1.4	97 ± 0.5	100 ± 0.8
	115	93	± 1.5	100 ± 1.5
Eugenia caryophyllus	Control	0	0	0
	10	0	2 ± 0.5	2 ± 0
	25	0	0	0
	40	0	0	5 ± 0
	55	8 ± 0.5	10 ± 0.5	10 ± 0
	70	29 ± 3.5	43 ± 1.7	48 ± 1
	85	59 ± 4.1	70 ± 2.1	74 ± 0.8
	100	82 ± 1.9	86 ± 0.8	94 ± 1.3
	115	97 ± 0.5	99 ± 0.5	100 ± 0.4
Mentha arvensis	Control	0	0	0
	10	0	0	0
	25	1 ± 0.4	2 ± 0.4	2 ± 0
	40	32 ± 2.8	45 ± 2.4	51 ± 1.0
	55	71 ± 1.9	77 ± 1.3	78 ± 0.4
	70	64 ± 1.3	73 ± 1.4	79 ± 0.4
	85	91 ± 1.9	98 ± 1.1	100 ± 0.8
	100	93 ± 1.6	99 ± 1.7	100 ± 0.4
	115	95 ± 1	98 ± 0.8	100 ± 0.5

*Each dilution was prepared using the DMSO solvent; **Mortality rates and standard deviations were calculated for each concentration assessed (five replicates totaling 100 larvae per group).

Matos et al. (2020), in their study on the chemical composition of *E. caryophyllus* EO, observed significant variations in the proportions of its constituents. Notably, eugenol consistently emerged as the primary component, constituting 74.31% of the composition, followed by eugenol acetate at 13.91%, and caryophyllene at 5.85%. In contrast, our current investigation revealed distinct percentages, with eugenol comprising 87.76%, caryophyllene representing

Table 4. Larvicidal activity of *Eucalyptus citriodora*, *Eucalyptus staigeriana*, *Eugenia caryophyllus*, and *Mentha arvensis* essential oils (EOs) against *Aedes aegypti* in 1 h and 24 h.

Exposure	EO source	No.[a]	Slope ±SEM[b]	LC_{50} (95% FL)	LC_{95} (95% FL)	χ^2	*P*
1 h	*E. citriodora*	600	3.62±0.44	106.57 (97.72-119.40)	302.66 (234.44-456.27)	2.70	0.60
	E. caryophyllus	700	13.77±1.89	99.88 (96.58-104.78)	131.48 (121.12-151.15)	4.15	0.52
24 h	*E. citriodora*	400	2.61±0.41	149.30 (116.71-230.79)	634.65 (359.73-1,848)	3.20	0.20
	E. staigeriana	600	5.20±0.36	47.04 (43.92-50.12)	97.35 (88.73-109.20)	2.65	0.61
	E. caryophyllus	700	6.17±0.56	92.67 (87.89-98.66)	171.13 (151.17-203.63)	4.24	0.51
	M. arvensis	500	4.65±0.42	52.82 (48.11- 57.12)	119.14 (106.46-138.62)	4.39	0.22

[a]No.=Number of insect tested; [b]SEM=Standard error of the mean. LC=lethal concentration; FL= Fiducial limits (lower FL, upper FL); $\chi 2$= chi-square test; *P*= p value.

9.41%, and eugenol acetate making up 2.83% of the oil. The observed monoterpene demonstrated notable inhibitory effects on the mitochondrial membrane potential and complex I in C6/36 cells derived from mosquitoes of the *Aedes* genus. However, it did not exhibit activity in rat PC12 cells. Interestingly, eugenol displayed heightened sensitivity in insect cells while exhibiting improved safety in mammals.

In our study, we observed a predominant presence of menthol (51.53%) in the EO of *M. arvensis*, a finding consistent with the work of Manh and Tuyet (2020), who reported a similar main component composition with menthol (66.04%). This aligns well with earlier investigations by Hussain et al. (2010) and Makkar et al. (2018), where menthol was identified as the primary constituent in essential oils extracted from *M. arvensis*.

Kumar et al. (2011a) emphasized *M. arvensis* as the primary natural source of menthol, focusing predominantly on agricultural studies. Similarly, Pandey et al. (2013) conducted larval mortality tests with *Ae. aegypti*. The authors showed 25.0% mortality when exclusively employing menthol diluted at 200 ppm. In addition to revealing the isolated efficacy of menthol, our findings suggest that the observed activity may be due to the presence of other compounds, particularly menthone, pinene, limonene, iso-menthone, and menthene, present in *M. arvensis* EO.

It is worth highlighting that EOs derived from *Mentha* species, such as that from *Mentha piperita*, are remarkably efficient in controlling *Ae. aegypti*. Kumar et al. (2011b) found an LC_{50} of 98.66 ppm over a 24-hour interval, with a significant response in larval mortality. In contrast, our study found a lower LC_{50} of 52.82 ppm for *M. arvensis* EO, suggesting higher efficiency in controlling mosquito larvae. This lower concentration required in our study could be attributed to differences in chemical composition between the two oils. These differences might contribute to the enhanced effectiveness of *M. arvensis* EO compared to *M. piperita*, underscoring its potential as a more potent larvicidal agent.

The *E. citriodora* EO analyzed by us revealed a composition including the following compounds: isopulegol (4.39%), citronellal (86.64%), iso-isopulegol (7.27%), and caryophyllene (1.70%), with different percentages from those found by Vera et al. (2014), especially regarding citronellal (49.3%). This difference suggests that the larvicidal activity of the EO is not exclusively associated with citronellal. In the present study, a mortality rate of 79% was recorded at 100 ppm after 24 h (LC_{50} = 149.30 and LC_{95} = 634.65 ppm). In contrast, a previous study found a mortality rate of 72% (LC_{50} = 71.22 and LC_{95} = 288.0) at 97 ppm during the same interval (Vera et al., 2014).

Similarly to other essential oil samples analyzed in this study, *E. staigeriana* exhibited a distinct chemical composition. Limonene, the primary constituent, constituted 41.67% of the composition in this study, displaying nearly doubled variations compared to the 72.9% observed in a prior investigation (Ribeiro et al., 2013). Additionally, various other compounds, such as myrcene, α-thujene, o-Cymene, α-terpinolene, and α-Pinene, were identified in the previous study but not detected in the current work. Notably, compounds like cineol (also known as eucalyptol) and cymene, present in our analysis, manifested different concentrations.

The compounds present in EOs, especially phenylpropanoids (eugenol and safrole) and monoterpenoids (citronellal, linalool, menthol, pinene, menthone, and limonene), are highly volatile and easily detected by insect antennae (Maciel et al., 2010). These compounds have the ability to impact insects in various ways, including growth inhibition, interference with diet (appetite suppression), alteration in oviposition (deleterious activity), as well as topical and oral toxic effects (Serdeiro et al., 2023; Viegas Júnior, 2003).

The yield and composition of EOs depend on several factors, as they are subject to variations determined by factors such as the geographical origin of plants, climate, cultural conditions, phenological stage, extraction method, and genotypic differences within the same species. Their chemical composition is shaped by different variables, like the plant's collection site, age, soil type, and environmental conditions (Razzouk et al., 2022). EO extraction from plants results in a complex mixture of organic volatile compounds, with terpenes and phenylpropanoids emerging as the predominant compounds. These secondary metabolites are notably influenced by the factors mentioned above, with a particular emphasis on altitude levels and climatic conditions (García-Díaz et al., 2023; Melito et al., 2016). Limonene, for example, is a monoterpene present in various EOs, and its insecticidal effects have been shown against *Spodoptera frugiperda* (Cruz et al., 2016). The low concentration of limonene, as described by some authors, highlights the efficacy of this compound in combating culicid larvae. Limonene exhibits known anticholinesterase activity (AChE) against some insects, including *Aedes* species (Seo et al., 2014; Botas et al., 2017). Silva et al. (2008) found an LC_{50} of 37 ppm regarding *Ae. aegypti*, emphasizing the importance of compounds present in smaller quantities in EOs and reinforcing the existence of synergistic activity against larvae. These results underscore the potential effectiveness of limonene, even in reduced concentrations, and highlight the complex interaction between different EO compounds in combating mosquito larvae.

It is important to emphasize that even if an EO does not exhibit effective mortality, its biological activity on an insect should still be considered from other angles. Castillo et al. (2017) achieved a protection rate against *Ae. aegypti* of 100% (2 min.) using *E. citriodora* EO, with only a 10% decrease in a 15-minute exposure.

Regarding the composition of constituents in the EO of *E. citriodora*, our study found that citronellal constituted 86.64% of the EO, a notably higher concentration compared to literature values of 52.23% (Batish et al., 2006) and 52.8% (Bossou et al., 2015) reported for EOs obtained from leaves of *E. citriodora* plants originating from India and Benin, respectively. This discrepancy in citronellal content may be attributed to the different geographical locations where the samples were collected.

Studies investigating the insecticidal activity of *E. staigeriana* are still scarce compared to other *Eucalyptus* species. Many of these studies focus on antifungals and the treatment of various plant diseases, as highlighted by Tomazoni et al. (2017), Pedrotti et al. (2022), and Dhakad et al. (2018). In a fumigation test proposed by Gusmão et al. (2013), *E. staigeriana* EO had significant results in mortality rates, demonstrating that small variations in concentration induced expressive responses in this variable. In terms of larvicidal effects, we found a considerable difference in the mortality rate (%) when comparing concentrations of 25 ppm (24 h = 6 ± 0.8; 48 h = 12 ± 0.4; 72 h = 12 ± 0) and 40 ppm (24 h = 40 ± 2.3; 48 h = 49 ± 1.4; 72 h = 53 ± 0.8).

According to the literature, when exposed to limonene treatment, *Culex quinquefasciatus* larvae exhibited a concentration-dependent response, as indicated by LC_{90} values of 53.80 ppm and 32.52 ppm after 24 and 48 hours of exposure, respectively (Kassir et al., 1989). In another study, 2nd and 4th instar larvae of *Cx. quinquefasciatus* were subjected to various concentrations of limonene (1, 5, 10, 50, and 100 ppm), resulting in a progressive increase in mortality rates over time at each tested concentration. This rise in mortality rates was notably significant with prolonged exposure duration for both larval instars, with mortality rates of 80% or higher observed at limonene concentrations of 50 and 100 ppm (Mohsen et al., 1989). In the present study, similar findings were observed, wherein limonene, present in the EO of *E. staigeriana* (41.67%), along with other compounds, demonstrated a mortality rate exceeding 78% within 24 hours, across a concentration range of 55 to 115 ppm.

The present results indicate that clove EO eliminates third-stage larvae of *Ae. aegypti* remarkably quickly, with an LC_{50} of 99.88 ppm in just 1 h. These findings are supported by Pandiyan et al. (2019), who demonstrated the larvicidal potential of an EO extracted from *Syzygium aromaticum* containing eugenol with an LC_{50} of 66.90 mg/L. Such an agreement between these results strengthens the evidence that eugenol can be effectively used as a larvicide and highlights its promising application in mosquito control with minimal degradation to environmental health.

The efficacy of the EOs was evaluated in comparison with a positive control based on data from Maleck et al. (2016), which diflubenzuron demonstrated 100% mortality of L3–L4 larvae between the 1st and 18th days of treatment. For example, the EO of *E. citriodora* showed significant effectiveness, with a mortality rate of 79% at 100 ppm after 24 hours. This is comparable to the positive control in terms of efficacy, but it is important to note that diflubenzuron, as a synthetic larvicide, achieves complete mortality at a lower concentration. Similarly, *E. staigeriana*, showed a mortality rate exceeding 78% within 24 hours, demonstrating its potential as an effective larvicide.

The results at 48 and 72 hours revealed total insect mortality (100%), rendering it impossible to determine LC values for these assessment periods. This outcome underscores the potent larvicidal efficacy of the tested oils against the target species, supporting their potential for effective vector control strategies.

While the effectiveness of these EOs is evident in laboratory tests, many of these studies still lack large-scale field evaluations. The true effectiveness and feasibility of these phytoproducts need to be assessed to determine the efficacy and applicability of mosquito control techniques in the real world (Maciel et al., 2010).

Despite many studies and data confirming the high efficacy of EOs against the larvae of various mosquito species and other insects, few commercial products are available for effective use as larvicides (Koul et al., 2008). This is due to several factors, as outlined by Miresmailli and Isman (2014): EOs are relatively expensive active substances, often derived from plants that cannot be cultivated in large quantities. Additionally, the chemical composition of EOs is highly variable, resulting in fluctuations in their biological efficacy. Large-scale production can also be challenging due to the limited availability of natural resources, requiring adjustments in formulations to compensate for scarcity and the high cost of ingredients, which would affect the product quality. Unlike synthetic insecticides, botanical extracts and essential oils contain lipophilic and volatile components that are susceptible to degradation by oxidation and polymerization, resulting in a loss of efficacy and quality. Exposure to air, light, and high temperatures can further compromise the stability of these compounds, limiting their residual effects, which may be short or even nonexistent in some cases.

One of the most well-known botanical insecticides is neem (*Azadirachta indica*), which has demonstrated insecticidal effects on over 400 pest species and is commonly used in organic farming. According to WHO guidelines, natural insecticides like neem do not harm nontarget species, particularly humans. However, the commercial use of EOs and other botanical extracts remains limited due to challenges such as slow action, variable performance, and shorter shelf life. The effectiveness of EOs can vary significantly based on concentration, with some oils showing toxicity at low doses, while others require higher concentrations to be effective. For example, neem and other EOs like eucalyptus, citronella, and lavender are recognized by the U.S. Environmental Protection Agency (EPA) as biopesticides, but their practical application in pest control, particularly in Brazil, is still emerging. Future perspectives in this field include developing nanosystems to enhance the efficacy of botanical insecticides and achieving selective toxicity against vector insects at low doses (Sá et al., 2023; Assadpour et al., 2024).

Our findings demonstrated the larvicidal efficacy of the EOs against *Ae. aegypti*, with *E. staigeriana* demonstrating the lowest LC_{50}. The swift action of *E. citriodora* and *E. caryophyllus* against *Ae. aegypti* larvae within just 1 h is emphasized. The EOs from *E. citriodora*, *E. staigeriana*, *E. caryophyllus*, and *M. arvensis* have potential as alternative, ecologically sustainable, and safe natural larvicides, representing a promising approach in *Ae. aegypti* control. However, it is crucial to consider the high cost associated with EOs as a potential challenge for their application, associated with plant availability. This integrated approach emphasizes the importance not only of efficacy but also of economic viability and environmental sustainability in implementing these larvicidal agents, with the potential to result in safer insecticides for the environment, specifically targeting mosquitoes and other vector arthropods.

Acknowledgements

This research was funded by PAEF-IOC/FIOTEC (Strategic Actions for Development and Strengthening Accredited Laboratories and Research Support Areas) from The Oswaldo Cruz Foundation (FIOCRUZ) (Process Identification: IOC-023-FIO-18-2-30); Also, to the scholarship received by the first author financed by Personnel Improvement Coordination Higher Level – CAPES (Financial Code: 001). We also acknowledge The National Council of Research and Technological Development - CNPq (Process number: 316254/2021-5). Finally, to The Carlos Chagas Filho Research Support Foundation of the State of Rio de Janeiro - FAPERJ (Process Identification: E-26/201.329/2016; E-26/210.228/2018; E-26/210.982/2021; E-26/200471/2023). Authors gratefully acknowledge the Central Analítica do Instituto de Química da Unicamp (IQ Unicamp) for the qualitative analysis by gas chromatography of the samples and the Fundação Educacional Severino Sombra (FUSVE) for the technical support.

References

ADHIKARI, K., KHANIKOR, B. and SARMA, R., 2022. Persistent susceptibility of *Aedes aegypti* to eugenol. *Scientific Reports*, vol. 12, no. 1, pp. 2277. http://doi.org/10.1038/s41598-022-06302-8. PMid:35145175.

ASSADPOUR, E., KARAÇA, A.C., FASAMANESH, M., MAHDAVI, S.A., SHARIAT-ALAVI, M., FENG, J., KHARAZMI, M.S., REHMAN, A. and JAFARI, S.M., 2024. Application of essential oils as natural biopesticides: recent advances. *Critical Reviews in Food Science and Nutrition*, vol. 64, no. 19, pp. 6477-6497. http://doi.org/10.1080/10408398.2023.2170317. PMid:36728841.

AZEVEDO, F.R., FERREIRA, R.C.A.B., LEANDRO, C.S., ARAÚJO, I.M. and TINTINO, S.R., 2023. Use of essential oils from plants of Araripe National Forest against *Aedes aegypti* (Diptera: culicidae). *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 83, pp. e275062. http://doi.org/10.1590/1519-6984.275062. PMid:38055504.

BADOLO, A., SOMBIÉ, A., YAMÉOGO, F., WANGRAWA, D.W., SANON, A., PIGNATELLI, P.M., VIANA, M., KANUKA, H., WEETMAN, D. and MCCALL, P.J., 2022. First comprehensive analysis of *Aedes aegypti* bionomics during an arbovirus outbreak in West Africa: dengue in Ouagadougou, Burkina Faso, 2016–2017. *PLoS Neglected Tropical Diseases*, vol. 16, no. 7, pp. e0010059. http://doi.org/10.1371/journal.pntd.0010059. PMid:35793379.

BADR, A.M., 2020. Organophosphate toxicity: updates of malathion potential toxic effects in mammals and potential treatments. *Environmental Science and Pollution Research International*, vol. 27, no. 21, pp. 26036-26057. http://doi.org/10.1007/s11356-020-08937-4. PMid:32399888.

BARBOSA, J.D.F., SILVA, V.B., ALVES, P.B., GUMINA, G., SANTOS, R.L.C., SOUSA, D.P. and CAVALCANTI, S.C.H., 2012. Structure–activity relationships of eugenol derivatives against *Aedes aegypti* (Diptera: Culicidae) larvae. *Pest Management Science*, vol. 68, no. 11, pp. 1478-1483. http://doi.org/10.1002/ps.3331. PMid:22674880.

BATISH, D.R., SINGH, H.P., SETIA, N., KAUR, S. and KOHLI, R.K., 2006. Chemical composition and inhibitory activity of essential oil from decaying leaves of *Eucalyptus citriodora. Zeitschrift für Naturforschung. C, A Journal of Biosciences*, vol. 61, no. 1-2, pp. 52-56. http://doi.org/10.1515/znc-2006-1-210. PMid:16610217.

BENELLI, G., 2015. Research in mosquito control: current challenges for a brighter future. *Parasitology Research*, vol. 114, no. 8, pp. 2801-2805. http://doi.org/10.1007/s00436-015-4586-9. PMid:26093499.

BOLZONELLA, C., LUCCHETTA, M., TEO, G., BOATTO, V. and ZANELLA, A., 2019. Is there a way to rate insecticides that is less detrimental to human and environmental health? *Global Ecology and Conservation*, vol. 20, pp. e00699. http://doi.org/10.1016/j.gecco.2019.e00699.

BOSSOU, A.D., AHOUSSI, E., RUYSBERGH, E., ADAMS, A., SMAGGHE, G., DE KIMPE, N., AVLESSI, F., SOHOUNHLOUE, D.C.K. and MANGELINCKX, S., 2015. Characterization of volatile compounds from three *Cymbopogon* species and *Eucalyptus citriodora* from Benin and their insecticidal activities against *Tribolium castaneum. Industrial Crops and Products*, vol. 76, pp. 306-317. http://doi.org/10.1016/j.indcrop.2015.06.031.

BOTAS, G.S., CRUZ, R.A.S., ALMEIDA, F.B., DUARTE, J.L., ARAÚJO, R.S., SOUTO, R.N.P., FERREIRA, R., CARVALHO, J.C.T., SANTOS, M.G., ROCHA, L., PEREIRA, V.L.P. and FERNANDES, C.P., 2017. *Baccharis reticularia* DC. and limonene nanoemulsions: promising larvicidal agents for *Aedes aegypti* (Diptera: Culicidae) control. *Molecules (Basel, Switzerland)*, vol. 22, no. 11, pp. 1990. http://doi.org/10.3390/molecules22111990. PMid:29149027.

BUENO, G.N., FIGUEIREDO, G.D., LOPES, E.S., OLIVEIRA, R.S.R. and SILVA, N.C.S., 2023. Extração e caracterização de óleos essenciais do cravo da índia (*Eugenia caryophyllus*). *Journal of Exact Sciences*, vol. 37, no. 1, pp. 8-10.

CARVALHO, V.L. and LONG, M.T., 2021. Perspectives on new vaccines against arboviruses using insect-specific viruses as platforms. *Vaccines*, vol. 9, no. 3, pp. 263. http://doi.org/10.3390/vaccines9030263. PMid:33809576.

CASTILLO, R.M., STASHENKO, E. and DUQUE, J.E., 2017. Insecticidal and repellent activity of several plant-derived essential oils against *Aedes aegypti*. *Journal of the American Mosquito Control Association*, vol. 33, no. 1, pp. 25-35. http://doi.org/10.2987/16-6585.1. PMid:28388322.

CHEN, L.H. and WILSON, M.E., 2020. Yellow fever control: current epidemiology and vaccination strategies. *Tropical Diseases, Travel Medicine and Vaccines*, vol. 6, pp. 1. http://doi.org/10.1186/s40794-020-0101-0. PMid:31938550.

ČMIKOVÁ, N., GALOVIČOVÁ, L., SCHWARZOVA, M., VUKIC, M.D., VUKOVIC, N.L., KOWALCZEWSKI, P.Ł., BAKAY, L., KLUZ, M.I., PUCHALSKI, C. and KAČÁNIOVÁ, M., 2023. Chemical composition and biological activities of *Eucalyptus globulus* essential oil. *Plants*, vol. 12, no. 5, pp. 1076. http://doi.org/10.3390/plants12051076. PMid:36903935.

COSTA, A.A., NASPI, C.V., LUCIA, A. and MASUH, H.M., 2017. Repellent and larvicidal activity of the essential oil from *Eucalyptus nitens* against *Aedes aegypti* and *Aedes albopictus* (Diptera: culicidae). *Journal of Medical Entomology*, vol. 54, no. 3, pp. 670-676. http://doi.org/10.1093/jme/tjw222. PMid:28399283.

CRUZ, G.S., WANDERLEY-TEIXEIRA, V., OLIVEIRA, J.V., LOPES, F.S.C., BARBOSA, D.R.S., BREDA, M.O., DUTRA, K.A., GUEDES, C.A., NAVARRO, D.M.A.F. and TEIXEIRA, A.A.C., 2016. Sublethal effects of essential oils from *Eucalyptus staigeriana* (Myrtales: Myrtaceae), *Ocimum gratissimum* (Lamiales: Laminaceae), and *Foeniculum vulgare* (Apiales: Apiaceae) on the biology of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Journal of Economic Entomology*, vol. 109, no. 2, pp. 660-666. http://doi.org/10.1093/jee/tow005. PMid:26868417.

D'AGOSTINO, M., TESSE, N., FRIPPIAT, J.P., MACHOUART, M. and DEBOURGOGNE, A., 2019. Essential oils and their natural active compounds presenting antifungal properties. *Molecules (Basel, Switzerland)*, vol. 24, no. 20, pp. 3713. http://doi.org/10.3390/molecules24203713. PMid:31619024.

DALPADADO, R., AMARASINGHE, D., GUNATHILAKA, N. and ARIYARATHNA, N., 2022. Bionomic aspects of dengue vectors *Aedes aegypti* and *Aedes albopictus* at domestic settings in urban, suburban, and rural areas in Gampaha District, Western Province of Sri Lanka. *Parasites & Vectors*, vol. 15, no. 148, pp. 148. http://doi.org/10.1186/s13071-022-05261-3. PMid:35477476.

DEMIRAK, M.Ş.Ş. and CANPOLAT, E., 2022. Plant-based bioinsecticides for mosquito control: impact on insecticide resistance and disease transmission. *Insects*, vol. 13, no. 2, pp. 162. http://doi.org/10.3390/insects13020162. PMid:35206735.

DHAKAD, A.K., PANDEY, V.V., BEG, S., RAWAT, J.M. and SINGH, A., 2018. Biological, medicinal and toxicological significance of *Eucalyptus* leaf essential oil: a review. *Journal of the Science of Food and Agriculture*, vol. 98, no. 3, pp. 833-848. http://doi.org/10.1002/jsfa.8600. PMid:28758221.

DUARTE, G., BRAGA NETO, A.R., KREITCHMANN, R., MENEZES, M.L.B., MIRANDA, A.E.B., TRAVASSOS, A.G., MELLI, P.P.S., NOMURA, R.M.Y., SILVA FILHO, A.L. and WENDER, M.C.O., 2024. Prevention, diagnosis, and treatment protocol of dengue during pregnancy and the postpartum period. *Revista Brasileira de Ginecologia e Obstetrícia*, vol. 46, pp. e-rbgo73. https://doi.org/10.61622/rbgo/2024rbgo73. PMid: 38994458.

ESMAILI, F., SANEI-DEHKORDI, A., AMOOZEGAR, F. and OSANLOO, M., 2021. A review on the use of essential oil-based nanoformulations in control of mosquitoes. *Biointerface Research in Applied Chemistry*, vol. 11, no. 5, pp. 12516-12529. http://doi.org/10.33263/BRIAC115.1251612529.

FERREIRA-DE-BRITO, A., RIBEIRO, I.P., MIRANDA, R.M., FERNANDES, R.S., CAMPOS, S.S., SILVA, K.A.B., CASTRO, M.G., BONALDO, M.C., BRASIL, P. and LOURENÇO-DE-OLIVEIRA, R., 2016. First detection of natural infection of *Aedes aegypti* with Zika virus in Brazil and throughout South America. *Memorias do Instituto Oswaldo Cruz*, vol. 111, no. 10, pp. 655-658. http://doi.org/10.1590/0074-02760160332. PMid:27706382.

GANESAN, P., SAMUEL, R., MUTHEESWARAN, S., PANDIKUMAR, P., REEGAN, A.D., AREMU, A.O. and IGNACIMUTHU, S., 2023. Phytocompounds for mosquito larvicidal activity and their modes of action: a review. *South African Journal of Botany*, vol. 152, pp. 19-49. http://doi.org/10.1016/j.sajb.2022.11.028.

GARCÍA-DÍAZ, J., SOUTO, R.N.P., ESCALONA-ARRANZ, J.C., FERREIRA, R.M.D.A., COSTA, T.S., GONZÁLEZ-FERNÁNDEZ, R., HEREDIA-DÍAZ, Y., CHIL-NÚÑEZ, I., VEGA, J., MONZOTE, L., QUEIROZ, M.M.C. and SETZER, W.N., 2023. Larvicidal and adulticidal activity of essential oils from four Cuban plants against three mosquito vector species. *Plants*, vol. 12, no. 23, pp. 4009. http://doi.org/10.3390/plants12234009. PMid:38068644.

GOVINDARAJAN, M., RAJESWARY, M., HOTI, S.L., BHATTACHARYYA, A. and BENELLI, G., 2016. Eugenol, α-pinene and β-caryophyllene from *Plectranthus barbatus* essential oil as eco-friendly larvicides against malaria, dengue and Japanese encephalitis mosquito vectors. *Parasitology Research*, vol. 115, no. 2, pp. 807-815. http://doi.org/10.1007/s00436-015-4809-0. PMid:26518773.

GUBLER, D.J., 2002. The global emergence/resurgence of arboviral diseases as Public Health problems. *Archives of Medical Research*, vol. 33, no. 4, pp. 330-342. http://doi.org/10.1016/S0188-4409(02)00378-8. PMid:12234522.

GUSMÃO, N.M.S., OLIVEIRA, J.V., NAVARRO, D.M.A.F., DUTRA, K.A., SILVA, W.A. and WANDERLEY, M.J.A., 2013. Contact and fumigant toxicity and repellency of *Eucalyptus citriodora* Hook., *Eucalyptus staigeriana* F., *Cymbopogon winterianus* Jowitt and *Foeniculum vulgare* Mill. essential oils in *Callosobruchus maculatus* (FABR.) (Coleoptera: Chrysomelidae, Bruchinae). *Journal of Stored Products Research*, vol. 54, pp. 41-47. http://doi.org/10.1016/j.jspr.2013.02.002.

HEMALATHA, R., NIVETHA, P., MOHANAPRIYA, C., SHARMILA, G., MUTHUKUMARAN, C. and GOPINATH, M., 2016. Phytochemical composition, GC-MS analysis, in vitro antioxidant and antibacterial potential of clove flower bud (*Eugenia caryophyllus*) methanolic extract. *Journal of Food Science and Technology*, vol. 53, no. 2, pp. 1189-1198. http://doi.org/10.1007/s13197-015-2108-5. PMid:27162398.

HU, F., TU, X.F., THAKUR, K., HU, F., LI, X.L., ZHANG, Y.S., ZHANG, J.G. and WEI, Z.J., 2019. Comparison of antifungal activity of essential oils from different plants against three fungi. *Food and Chemical Toxicology*, vol. 134, pp. 110821. http://doi.org/10.1016/j.fct.2019.110821. PMid:31533060.

HUSSAIN, A.I., ANWAR, F., NIGAM, P.S., ASHRAF, M. and GILANI, A.H., 2010. Seasonal variation in content, chemical composition and antimicrobial and cytotoxic activities of essential oils from four *Mentha* species. *Journal of the Science of Food and Agriculture*, vol. 90, no. 11, pp. 1827-1836. http://doi.org/10.1002/jsfa.4021. PMid:20602517.

ISMAN, M.B., 2020. Commercial development of plant essential oils and their constituents as active ingredients in bioinsecticides. *Phytochemistry Reviews*, vol. 19, no. 2, pp. 235-241. http://doi.org/10.1007/s11101-019-09653-9.

KASSIR, J.T., MOHSEN, Z.H. and MEHDI, N.S., 1989. Toxic effects of limonene against *Culex quinquefasciatus* Say larvae and its interference with oviposition. *Anzeiger für Schädlingskunde, Pflanzenschutz, Umweltschutz*, vol. 62, pp. 19-21. http://doi.org/10.1007/BF01905742.

KHATTAK, A., KHAN, S., ALI, I., GUL, A., KHABIR, M.N., JAVED, B., AYESHA., ADNAN, M., KHAN, S.N. and ATTAULLAH, S., 2023. Burden and distribution of dengue infection in Pakistan (2000-19): a review. *Brazilian Journal of Biology = Revista Brasileira* *de Biologia*, vol. 84, pp. e267982. http://doi.org/10.1590/1519-6984.267982. PMid:37075435.

KOUL, O., WALIA, S., DHALIWAL, G.S. and NAGAR, P., 2008. Essential oils as green pesticides: potential and constraints. *Biopesticides International*, vol. 4, no. 1, pp. 63-84.

KUMAR, P., MISHRA, S., MALIK, A. and SATYA, S., 2011a. Insecticidal properties of *Mentha* species: A review. *Industrial Crops and Products*, vol. 34, no. 1, pp. 802-817. http://doi.org/10.1016/j.indcrop.2011.02.019.

KUMAR, S., WAHAB, N. and WARIKOO, R., 2011b. Bioefficacy of *Mentha piperita* essential oil against dengue fever mosquito *Aedes aegypti* L. *Asian Pacific Journal of Tropical Biomedicine*, vol. 1, no. 2, pp. 85-88. http://doi.org/10.1016/S2221-1691(11)60001-4. PMid:23569733.

MACIEL, M.V., MORAIS, S.M., BEVILAQUA, C.M.L., SILVA, R.A., BARROS, R.S., SOUSA, R.N., SOUSA, L.C., BRITO, E.S. and SOUZA-NETO, M.A., 2010. Chemical composition of Eucalyptus spp. essential oils and their insecticidal effects on Lutzomyia longipalpis. *Veterinary Parasitology*, vol. 167, no. 1, pp. 1-7. http://doi.org/10.1016/j.vetpar.2009.09.053. PMid:19896276.

MAKKAR, M.K., SHARMA, S. and KAUR, H., 2018. Evaluation of *Mentha arvensis* essential oil and its major constituents for fungitoxicity. *Journal of Food Science and Technology*, vol. 55, no. 9, pp. 3840-3844. http://doi.org/10.1007/s13197-018-3291-y. PMid:30150845.

MALECK, M., HOLLANDA, P.O., SERDEIRO, M.T., SOARES, R.O., HONÓRIO, N.A. and SILVA, C.G., 2016. Toxicity and larvicidal activity of Podophyllum-based lignans against *Aedes aegypti* (Diptera: culicidae). *Journal of Medical Entomology*, vol. 54, no. 1, pp. 159-166. http://doi.org/10.1093/jme/tjw130. PMid:28082643.

MANH, H.D. and TUYET, O.T., 2020. Larvicidal and repellent activity of *Mentha arvensis* L. essential oil against *Aedes aegypti. Insects*, vol. 11, no. 3, pp. 198. http://doi.org/10.3390/insects11030198. PMid:32235733.

MARTIN, J.L., LIPPI, C.A., STEWART-IBARRA, A.M., AYALA, E.B., MORDECAI, E.A., SIPPY, R., HERAS, F.H., BLACKBURN, J.K. and RYAN, S.J., 2021. Household and climate factors influence *Aedes aegypti* presence in the arid city of Huaquillas, Ecuador. *PLoS Neglected Tropical Diseases*, vol. 15, no. 11, pp. e0009931. http://doi.org/10.1371/journal.pntd.0009931. PMid:34784348.

MATOS, L.F., BARBOSA, D.R.S., LIMA, E.C., DUTRA, K.A., NAVARRO, D.M.A.F., ALVES, J.L.R. and SILVA, G.N., 2020. Chemical composition and insecticidal effect of essential oils from *Illicium verum* and *Eugenia caryophyllus* on *Callosobruchus maculatus* in cowpea. *Industrial Crops and Products*, vol. 145, pp. 549-556. http://doi.org/10.1016/j.indcrop.2020.112088.

MELITO, S., PETRETTO, G.L., PODANI, J., FODDAI, M., MALDINI, M., CHESSA, M. and PINTORE, G., 2016. Altitude and climate influence *Helichrysum italicum* subsp. microphyllum essential oils composition. *Industrial Crops and Products*, vol. 80, pp. 242-250. http://doi.org/10.1016/j.indcrop.2015.11.014.

MESSINA, J.P., BRADY, O.J., GOLDING, N., KRAEMER, M.U.G., WINT, G.R.W., RAY, S.E., PIGOTT, D.M., SHEARER, F.M., JOHNSON, K., EARL, L., MARCZAK, L.B., SHIRUDE, S., WEAVER, N.D., GILBERT, M., VELAYUDHAN, R., JONES, P., JAENISCH, T., SCOTT, T.W. and REINER JUNIOR, R.C., 2019. The current and future global distribution and population at risk of dengue. *Nature Microbiology*, vol. 4, no. 9, pp. 1508-1515. http://doi.org/10.1038/s41564-019-0476-8. PMid:31182801.

MILUGO, T.K., TCHOUASSI, D.P., KAVISHE, R.A., DINGLASAN, R.R. and TORTO, B., 2021. Naturally occurring compounds with larvicidal activity against malaria mosquitoes. *Frontiers in Tropical Diseases*, vol. 2, pp. 718804. http://doi.org/10.3389/fitd.2021.718804.

MIRESMAILLI, S. and ISMAN, M.B., 2014. Botanical insecticides inspired by plant-herbivore chemical interactions. *Trends in Plant Science*, vol. 19, no. 1, pp. 29-35. http://doi.org/10.1016/j.tplants.2013.10.002. PMid:24216132.

MITUIASSU, L.M.P., SERDEIRO, M.T., VIEIRA, R.R.B.T., OLIVEIRA, L.S. and MALECK, M., 2021. *Momordica charantia* L. extracts against *Aedes aegypti* larvae. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 82, pp. 236498. http://doi.org/10.1590/1519-6984.236498. PMid:33787746.

MOHSEN, Z.H., AL-CHALABI, B.M. and KASSIR, J.T., 1989. Factors influencing the larvicidal activity of limonene against *Culex quinquefasciatus* Say (Dipt., Culicidae). *Journal of Applied Entomology*, vol. 108, no. 1-5, pp. 107-110. http://doi.org/10.1111/j.1439-0418.1989.tb00439.x.

MOREIRA, R.R.D., SANTOS, A.G., CARVALHO, F.A., PEREGO, C.H., CREVELIN, E.J., CROTTI, A.E.M., COGO, J., CARDOSO, M.L.C. and NAKAMURA, C.V., 2019. Antileishmanial activity of *Melampodium divaricatum* and *Casearia sylvestris* essential oils on *Leishmania amazonenses. Journal of the Institute of Tropical Medicine of São Paulo*, vol. 61, pp. e33. http://doi.org/10.1590/s1678-9946201961033. PMid:31269109.

MOUSSII, I.M., NAYME, K., TIMINOUNI, M., JAMALEDDINE, J., FILALI, H. and HAKKOU, F., 2020. Synergistic antibacterial effects of Moroccan *Artemisia herba alba, Lavandula angustifolia*, and *Rosmarinus officinalis* essential oils. *Synergy*, vol. 10, pp. 100057. http://doi.org/10.1016/j.synres.2019.100057.

NABI, A., NAEEM, M., AFTAB, T. and KHAN, M.M.A., 2020. Alterations in photosynthetic pigments, antioxidant machinery, essential oil constituents and growth of menthol mint (*Mentha arvensis* L.) upon nickel exposure. *Brazilian Journal of Botany*, vol. 43, no. 4, pp. 721-731. http://doi.org/10.1007/s40415-020-00649-w.

PANDEY, S.K., TANDON, S., AHMAD, A., SINGH, A.K. and TRIPATHI, A.K., 2013. Structure-activity relationships of monoterpenes and acetyl derivatives against *Aedes aegypti* (Diptera: Culicidae) larvae. *Pest Management Science*, vol. 69, no. 11, pp. 1235-1238. http://doi.org/10.1002/ps.3488. PMid:23908002.

PANDIYAN, G.N., MATHEW, N. and MUNUSAMY, S., 2019. Larvicidal activity of selected essential oil in synergized combinations against *Aedes aegypti. Ecotoxicology and Environmental Safety*, vol. 174, pp. 549-556. http://doi.org/10.1016/j.ecoenv.2019.03.019. PMid:30861442.

PEDROTTI, C., FRANZOI, C., ROSA, M.T.S., TRENTIN, T.R., VILASBOA, J., SCARIOT, F.J., ECHEVERRIGARAY, S.L. and SCHWAMBACH, J., 2022. Antifungal activity of essential oil from *Eucalyptus staigeriana* against *Alternaria alternata* causing leaf spot and black rot in table grapes. *Anais da Academia Brasileira de Ciências*, vol. 94, no. 2, pp. e20200394. http://doi.org/10.1590/0001-3765202220200394. PMid:35319598.

PESAVENTO, G., CALONICO, C., BILIA, A.R., BARNABEI, M., CALESINI, F., ADDONA, R., MENCARELLI, L., CARMAGNINI, L., DI MARTINO, M.C. and LO NOSTRO, A., 2015. Antibacterial activity of Oregano, Rosmarinus and Thymus essential oils against *Staphylococcus aureus* and *Listeria monocytogenes* in beef meatballs. *Food Control*, vol. 54, pp. 188-199. http://doi.org/10.1016/j.foodcont.2015.01.045.

POWELL, J.R. and TABACHNICK, W.J., 2013. History of domestication and spread of *Aedes aegypti* - a review. *Memorias do Instituto Oswaldo Cruz*, vol. 108, suppl. 1, pp. 11-17. http://doi.org/10.1590/0074-0276130395. PMid:24473798.

RAZZOUK, S., MAZRI, M.A., JELDI, L., MNASRI, B., OUAHMANE, L. and ALFEDDY, M.N., 2022. Chemical composition and antimicrobial activity of essential oils from three Mediterranean plants against eighteen pathogenic bacteria and fungi. *Pharmaceutics*, vol. 14, no. 8, pp. 1608. http://doi.org/10.3390/pharmaceutics14081608. PMid:36015234.

RIBEIRO, W.L.C., MACEDO, I.T.F., SANTOS, J.M.L., OLIVEIRA, E.F., CAMURÇA-VASCONCELOS, A.L.F., PAULA, H.C.B. and BEVILAQUA, C.M.L., 2013. Activity of chitosan-encapsulated *Eucalyptus staigeriana* essential oil on *Haemonchus contortus. Experimental Parasitology*, vol. 135, no. 1, pp. 24-29. http://doi.org/10.1016/j.exppara.2013.05.014. PMid:23748159.

ROSE, N.H., BADOLO, A., SYLLA, M., AKORLI, J., OTOO, S., GLORIA-SORIA, A., POWELL, J.R., WHITE, B.J., CRAWFORD, J.E. and MCBRIDE, C.S., 2023. Dating the origin and spread of specialization on human hosts in *Aedes aegypti* mosquitoes. *eLife*, vol. 12, pp. e83524. http://doi.org/10.7554/eLife.83524. PMid:36897062.

SÁ, G.C.S., BEZERRA, P.V.V., SILVA, M.F.A., SILVA, L.B., BARRA, P.B., XIMENES, M.F.F.M. and UCHÔA, A.F., 2023. Arbovirus vectors insects: are botanical insecticides an alternative for its management? *Journal of Pest Science*, vol. 96, no. 1, pp. 1-20. http://doi.org/10.1007/s10340-022-01507-2.

SAS INSTITUTE, 2002. *SAS/STAT User's Guide*. Cary: SAS Institute.

SANTOS, G.R., MOURÃO, D.S.C., HAN, J.S., OSÓRIO, P.R.A., ARAÚJO, S.H.C. and LIMA, L.R., 2023. Potencial fungitóxico dos óleos essenciais de plantas do cerrado no controle dos fitopatógenos *Curvularia lunata* e *Rhizoctonia solani. Peer Review : Emerging Trends and Key Debates in Undergraduate Education*, vol. 5, no. 15, pp. 112-127. http://doi.org/10.53660/751.prw1929.

SENTHIL-NATHAN, S., 2020. A review of resistance mechanisms of synthetic insecticides and botanicals, phytochemicals, and essential oils as alternative larvicidal agents against mosquitoes. *Frontiers in Physiology*, vol. 10, pp. 1591. http://doi.org/10.3389/fphys.2019.01591. PMid:32158396.

SEO, S.M., KIM, J., KANG, J., KOH, S.H., AHN, Y.J., KANG, K.S. and PARK, I.K., 2014. Fumigant toxicity and acetylcholinesterase inhibitory activity of 4 Asteraceae plant essential oils and their constituents against Japanese termite (*Reticulitermes speratus* Kolbe). *Pesticide Biochemistry and Physiology*, vol. 113, pp. 55-61. http://doi.org/10.1016/j.pestbp.2014.06.001. PMid:25052527.

SERDEIRO, M.T., DIAS, T.D., LIMA, N.T.R., BARBOSA-FILHO, J.M., BELATO, R.S., SANTOS-MALLET, J.R. and MALECK, M., 2023. Study on morphological changes and interference in the development of *Aedes aegypti* caused by some essential oil constituents. *Tropical Medicine and Infectious Disease*, vol. 8, no. 9, pp. 440. http://doi.org/10.3390/tropicalmed8090440. PMid:37755901.

SILVA, W.J., DÓRIA, G.A.A., MAIA, R.T., NUNES, R.S., CARVALHO, G.A., BLANK, A.F., ALVES, P.B., MARÇAL, R.M. and CAVALCANTI, S.C.H., 2008. Effects of essential oils on *Aedes aegypti* larvae: alternatives to environmentally safe insecticides. *Bioresource Technology*, vol. 99, no. 8, pp. 3251-3255. http://doi.org/10.1016/j.biortech.2007.05.064. PMid:17662602.

SILVÉRIO, M.R.S., ESPINDOLA, L.S., LOPES, N.P. and VIEIRA, P.C., 2020. Plant natural products for the control of *Aedes aegypti*: the main vector of important arboviruses. *Molecules (Basel, Switzerland)*, vol. 25, no. 15, pp. 3484. http://doi.org/10.3390/molecules25153484. PMid:32751878.

SOLEIMANI-AHMADI, M., SANEI-DEHKORDI, A., TURKI, H., MADANI, A., ABADI, Y.S., PAKSA, A., GOROUHI, M.A. and RASHID, G., 2017. Phytochemical properties and insecticidal potential of volatile oils from *Tanacetum persicum* and *Achillea kellalensis* against two medically important mosquitoes. *Journal of Essential Oil-Bearing Plants*, vol. 20, no. 20, pp. 1254-1265. http://doi.org/10.1080/0972060X.2017.1388752.

SPINOZZI, E., MAGGI, F., BONACUCINA, G., PAVELA, R., BOUKOUVALA, M.C., KAVALLIERATOS, N.G., CANALE, A., ROMANO, D., DESNEUX, N., WILKE, A.B.B., BEIER, J.C. and BENELLI, G., 2021. Apiaceae essential oils and their constituents as insecticides against mosquitoes: a review. *Industrial Crops and Products*, vol. 171, pp. 113892. http://doi.org/10.1016/j.indcrop.2021.113892.

TELES, A.M., ROSA, T.D.S., MOUCHREK, A.N., ABREU-SILVA, A.L., CALABRESE, K.S. and ALMEIDA-SOUZA, F., 2019. *Cinnamomum zeylanicum*, *Origanum vulgare*, and *Curcuma longa* essential oils: chemical composition, antimicrobial and antileishmanial activity. *Evidence-Based Complementary and Alternative Medicine*, vol. 2019, no. 1, pp. 2421695. https://doi.org/10.1155/2019/2421695.

TOMAZONI, E.Z., PAULETTI, G.F., RIBEIRO, R.T.S., MOURA, S. and SCHWAMBACH, J., 2017. In vitro and in vivo activity of essential oils extracted from *Eucalyptus staigeriana, Eucalyptus globulus*, and *Cinnamomum camphora* against *Alternaria solani* Sorauer causing early blight in tomato. *Scientia Horticulturae*, vol. 223, pp. 72-77. http://doi.org/10.1016/j.scienta.2017.04.033.

VERA, S.S., ZAMBRANO, D.F., MÉNDEZ-SÁNCHEZ, S.C., RODRÍGUEZ-SANABRIA, F., STASHENKO, E.E. and DUQUE LUNA, J.E., 2014. Essential oils with insecticidal activity against larvae of *Aedes aegypti* (Diptera: culicidae). *Parasitology Research*, vol. 113, no. 7, pp. 2647-2654. http://doi.org/10.1007/s00436-014-3917-6. PMid:24781026.

VIEGAS JÚNIOR, C., 2003. Terpenos com atividade inseticida: uma alternativa para o controle químico de insetos. *Quimica Nova*, vol. 26, no. 3, pp. 390-400. http://doi.org/10.1590/S0100-40422003000300017.

VONTAS, J., KIOULOS, E., PAVLIDI, N., MOROU, E., DELLA TORRE, A. and RANSON, H., 2012. Insecticide resistance in the major dengue vectors *Aedes albopictus* and *Aedes aegypti. Pesticide Biochemistry and Physiology*, vol. 104, no. 2, pp. 126-131. http://doi.org/10.1016/j.pestbp.2012.05.008.

WORLD HEALTH ORGANIZATION – WHO, 2005 [viewed 4 May 2023]. *Guidelines for laboratory and field testing of Mosquito Larvicides* [online]. Geneva: WHO. Available from: http://www.who.int/iris/handle/10665/69101

ZHU, Q., YANG, Y., ZHONG, Y., LAO, Z., O'NEILL, P., HONG, D., ZHANG, K. and ZHAO, S., 2020. Synthesis, insecticidal activity, resistance, photodegradation and toxicity of pyrethroids (A review). *Chemosphere*, vol. 254, pp. 126779. http://doi.org/10.1016/j.chemosphere.2020.126779. PMid:32957265.