BOLETIN LATINOAMERICANO Y DEL CARIBE DE PLANTAS MEDICINALES Y AROMÁTICAS
© / ISSN 0717 7917 / www.blacpma.ms-editions.cl

Articulo Original / Original Article

Chemical composition and larvicidal activity of the essential oil of *Pimenta dioica* leaves

[Composición química y actividad larvicida del aceite esencial de hojas de *Pimenta dioica*]

Paulo Roberto Barros Gomes[1], Silvio Carvalho Marinho[2], Gustavo Oliveira Everton[2], Eduardo Fonseca Silva[2], Maria Alves Fontenele[3], Wellington da Silva Lyra[4], Adriana Crispim de Freitas[3], Virlane Kelly Lima Hunaldo[3], Rômicy Dermondes Souza[5]*, Hilton Costa Louzeiro[6], Maria do Livramento de Paula[7], Jonas Batista Reis[2], Andréa Vasconcelos Melo[2] & Victor Elias Mouchrek Filho[2]

[1]Federal Institute of Education, Science and Technology of Pará - Campus Paragominas, Pará, Brazil
[2]Chemistry Technology Department, Federal University of Maranhão - Campus São Luís, Maranhão, Brazil
[3]Food Engeniering Departament, Federal University of Maranhão - Campus Imperatriz, Maranhão, Brazil
[4]Chemistry Department, Federal University of Paraíba - Campus João Pessoa, Paraíba, Brazil
[5]Bahia's Southwest State University – Campus Itapetinga, Bahia, Brazil
[6]Coordination of Degree in Natural Sciences, Federal University of Maranhão - Campus Pinheiro, Maranhão, Brazil
[7]Pharmacy Department, Federal University of Maranhão - Campus São Luís, Maranhão, Brazil

Reviewed by:
Maria Emilia Carretero
Universidad Complutense de Madrid
Spain

Suelen Pereira Ruiz
Universidade Paranaense
Brazil

Correspondence:
Romicy **D. SOUZA**
romicyds@hotmail.com

Section Biological activity

Received: 20 October 2020
Accepted: 14 March 2021
Accepted corrected: 20 April 2021
Published: 30 March 2022

Citation:
Gomes PRB, Marinho SC, Everton GO, Silva EF, Fontenele MA, Lyra WS, de Freitas AC, Hunaldo VKL, Souza RD, Louzeiro HC, de Paula ML, Reis JB, Melo AV, Filho VEM. Chemical composition and larvicidal activity of the essential oil of *Pimenta dioica* leaves
Bol Latinoam Caribe Plant Med Aromat
21 (2): 207 - 214 (2022).
https://doi.org/10.37360/blacpma.22.21.2.12

Abstract: In this study, we investigated the main constituent, the predominant class and biological activity of the essential oil extracted from the leaves of *Pimenta dioica* and the pattern of the major constituent against larvae in the third stage of *Aedes aegypti*. For this reason, we extracted the oil by hydrodistillation, identified its components by gas chromatography coupled with mass spectrometry (GC/MS) and calculated the lethal concentration (LC_{50}) of the larvicidal activity using the Reed-Muench method. The results show that the oil consists mainly of eugenol, in which the phenylpropanoid class predominated and the lethal concentration, LC_{50}, was 38.86 µg mL^{-1} at a confidence level of 2.25 µg mL^{-1}, while the eugenol standard presented LC_{50} 79.75 µg mL^{-1} at a confidence level of 2.10 µg mL^{-1}. Given the facts, we conclude that the oil is more active than the standard and that it has the potential to replace chemical larvicides.

Keywords: *Aedes aegypti*; Eugenol; Monoterpenes; Volatile compounds, Reed-Muench method.

Resumen: En este estudio, investigamos el constituyente principal, la clase predominante y la actividad biológica del aceite esencial extraído de las hojas de *Pimenta dioica* y el patrón del constituyente principal contra las larvas en la tercera etapa de *Aedes aegypti*. Por este motivo, extrajimos el aceite por hidrodestilación, identificamos sus componentes mediante cromatografía de gases acoplada a espectrometría de masas (GC/MS) y calculamos la concentración letal (CL50) de la actividad larvicida mediante el método Reed-Muench. Los resultados muestran que el aceite está constituido principalmente por eugenol, en el que predominó la clase fenilpropanoide y la concentración letal, CL50, fue de 38,86 µg.mL-1 a un nivel de confianza de 2,25 µg.mL-1, mientras que el estándar de eugenol presentó CL50 79,75 µg.mL -1 a un nivel de confianza de 2,10 µg.mL-1. Dados los hechos, concluimos que el aceite es más activo que el estándar y que tiene el potencial de reemplazar los larvicidas químicos.

Palabras clave: *Aedes aegypti*; Eugenol; Monoterpenos; Compuestos volátiles; Método Reed-Muench

INTRODUCTION

The *Aedes aegypti* mosquito is a vector of four diseases (dengue, yellow fever, zika, and chikungunya) and in recent years it has arouse the interest of the scientific community that is looking for ways to control it (Pereira *et al*., 2018). In the last years, it there is an increase in the number of cases of diseases transmitted by this mosquito (Gould *et al.*, 2017). Between 2015 and 2016, 1.65 million cases of dengue were recorded in Brazil (Donalisio *et al.*, 2017), 38,499 cases of chikungunya and 215.319 cases of zika (Teich *et al.*, 2017). The advance of these diseases is related to the intensive growth of global transport, adaptation of the mosquito to urbanization, inefficiency in controlling the mosquito population and environmental changes (Gould *et al.*, 2017).

There are reports in the literature of fighting the *Ae. aegypti* mosquito, whether in the larval or adult stages, using various methods: fish predation (*Trichogaster trichopteros* and *Astyanax fasciatus*) (Cavalcanti *et al.*, 2007) insect growth regulators, microbial control by *Bacillus thuringiensis* H-14 and chemical insecticides based on carbamates, pteroids, and organophosphates (Rocha Voris *et al.*, 2018). It is a fact that among all the methods used, the most effective is the control by chemical insecticides, due to the simultaneous action on larvae and mosquitoes (Rocha Voris *et al.*, 2018). However, there are reports that its use has caused attacks on non-target organisms, increased the resistance of the mosquito population and has caused damage to the environment in the short and long term (Maestre-Serrano *et al*., 2014). Thus, the search for insecticides, preferably of plant origin, emerges as a viable alternative.

In this manner, essential oils extracted from aromatic plants have stood out as a potential substitute for chemical larvicides. In the literature, studies show that these compounds are easily biodegradable, prevent oviposition, inhibit the growth or reproduction of several mosquito species and have prolonged action when compared to chemical insecticides (Pushpanathan *et al.*, 2006; Bedini *et al.*, 2018).

Among the various compounds identified in several essential oils with proven larvicidal activity is eugenol. A plant that is widely used in cooking and has a high amount of this component is *Pimenta dioica*. Belonging to the Myrtaceae family and grown in Central America and India, this plant is valued for its fruits and oils in the manufacture of Chartreuse and Benedictine liqueurs (Kumar *et al.*, 2016). In addition to its culinary applications, this plant also has anesthetic, analgesic, antiseptic, carminative, bactericidal, fungicidal, antioxidant properties (Charles, 2013), molluscicide (Everton *et al.*, 2018) and larvicide against *Ae. aegypti* (Rocha Voris *et al.*, 2018). We emphasize that larvicidal activity observed for this plant was obtained from the fruits, but with low potency. We know that biological activities of different plants have differences in lethality (Aguiar *et al.*, 2015).

Therefore, because of the above explained, in this study, we will investigate the main constituent, the chemical type and the larvicidal activity against third-stage larvae of *Ae. aegypti* of the essential oil extracted from the leaves of *Pimenta dioica* and the isolated eugenol, as it is the main component.

MATERIAL AND METHODS

Plant material and eugenol standard

The present Leaf collection was carried out at the Santa Elisa experimental farm, of the Agronomic Institute of Campinas (22°53'30.3"S 47°03'52.5"W), in the state of São Paulo, Brazil, and certified by the Herbarium of the Federal University of São Carlos number 3652. After collection, the leaves were placed to dry in a dry room for seven days. The eugenol standard with 99% purity was purchased by Sigma Aldrich.

Essential oil extraction

The essential oil was extracted by hydrodistillation and the average yield was calculated from the density and weight measurements of the crude material. For the extraction, we weigh 30 grams of the samples that were previously ground in an electric mill of the Technal Te-340 model and mixed in 300 mL of distilled water. Then we put this mixture in a 1000 mL round bottom flask and attached it to the Clevenger extractor under 100°C heating in an electric blanket for 3.5 hours. After that time, the extracted oil was collected and dried by percolation an anhydrous sodium sulfate solution. We perform these operations in triplicates and store the samples in amber glass ampoules under refrigeration to avoid possible losses of volatile constituents. A density pycnometer was used to measure density (Gomes *et al*., 2016).

Gas chromatography - mass spectrometry (GC-MS) analysis

The essential oil components were identified by gas

chromatography coupled to mass spectrometry (GC/MS) in a Shimadzu gas chromatography, coupled to an electron impact mass spectrometer and Varian 2100 ion trap analyzer, using helium as carrier gas with a flow in the 1.0 mL.min^{-1} column; injector temperature: 270ºC, split 1:50; 100% methylsiloxane capillary column (30 m x 0.25 mm x 0.25 mm) and oven temperature programming from 60ºC to 200ºC with heating rate of 8ºC min^{-1}, and from 200 to 290ºC with heating rate of 15ºC min^{-1}. In the Mass Spectrometer, the temperatures of the manifold, trap ion, and transfer line were 50ºC, 190ºC, and 200ºC, respectively. We injected aliquots of 1 µL (automatic injector CP - 8410) of the samples diluted in the proportion of 20 µL in 1.5 mL of hexane. We identified the components by comparing their retention index with the data obtained from authentic substances existing in NIST02 reference libraries.

Capture and obtention of Aedes aegypti

The larvae were obtained from ovitramps. Ovitramps are prepared from the addition of water and two eucatex straws in polyethylene buckets with a capacity of 500 mL, where eggs are expected to be deposited by mosquito females. After hatching, the larvae in the 3th stage were kept at room temperature 25 ± 2ºC and relative humidity of 70 to 80%, being fed with dog food.

Larvicidal Bioassay

Then, to perform the toxicity test, we selected the larvae in the third stage and transferred them to a beaker, containing 20 mL of mineral water (26-28ºC), capturing them with a Pasteur pipette. Each test was performed in quintuplicate for each concentration tested (20, 50, 70, 90, 100 µg·mL^{-1}) for both essential oil and eugenol standard. Negative controls were performed with 20 mL of mineral water (26-28ºC) containing 0.04% Tween. The larvae were exposed to the solutions for 24 hours, being monitored hourly. At the end of the periods, we recorded mortality. So, to prepare the test solution, we weighed 20 mg of the essential oil in a container (type Eppendorf), for each milliliter of the test solution, and then a drop of solvent of type Tween 80 was added over the oil, and then homogenization. Next, we use an automatic pipette, add 1 mL of distilled water and mix again. This solution was then transferred to the beaker containing the separated larvae for the test, according to the pre-established concentrations after initial tests.

Statistical analysis

Data statistical analysis was performed according to Reed-Muench method (Reed *et* Muench, 1938), which assumes if an animal survives to a certain dose must survive to any lower dose than that, consequently, the animal that is dying to a certain dose must die to larger doses. From a table containing the mortality data for each concentration tested, a graph was constructed showing dead animals accumulation curve at each concentration, and another curve for survivors' accumulation. The intercession point between the curves is 50% Lethal Concentration (LC_{50}), at this point the surviving animals' number is equal to dead animals' number (Colegate & Molyneux, 2007). The reliability (Pizzi, 1950) in which a graph constructed with the percentage of dead versus log (log) of the dose. Next, the value of -R., which is the difference between the log of killing dose for 75% larvae and the log of killing dose for 25% of larvae, is determined. The variable -h calculated is the mean of differences between log doses values. With this data, the standard error log (SE) is determined by the following formula: $(SE)^2 = 0.79 \times h \times R / 20$. The values, 0.79 (found in the dividend) and 20 (located in the divisor), in the quotient of the above relation refers to conversion factors required to calculate the standard error established in the samples. Finally, the confidence interval value is equal to $2x10^{SE}$ (Gomes *et al.*, 2016).

RESULTS

Oil extraction and chromatographic GC/MS analysis

To determine the composition, we initially extracted the essential oil by hydrodistillation and then analyzed it by gas chromatography coupled to the mass spectrometer. The extraction result revealed an average yield and density, respectively, of 2.94% (mm^{-1}) and 0.980 g mL^{-1}, while the result of the chromatographic analysis showed us the presence of 16 compounds (Table No. 1), in which the main abundant four were eugenol (74.06%), β-pinene (6.51%), 5-indanol (6.06%), and limonene (3.94%) and the predominant class was the 74.06% phenylpropanoid and 12.72% Hydrogenated monoterpenes.

Larvicidal activity

In this manner, we investigate the larvicidal activity, we subjected ten larvae to concentrations ranging from 20 to 100 µg·mL^{-1} of essential oil and the

eugenol standard during a period of 24 h. Then we

Table No. 1
Identification of the components present in the essential oil

Compounds	Retention time (s)	Retention Index	Theoretical retention index[a]	Relative area (%)
1-Octen-3-ol	2.17	980	974	1.38
β-Pinene	2.35	975	974	6.51
α-Pinene	2.49	930	932	0.30
o-Cymene	2.68	1025	1022	1.97
Limonene	2.76	1028	1024	3.94
Linalool	3.58	1096	1095	1.71
Cis-Sabinene hydrate	4.65	969	1065	0.21
α-Terpineol	4.81	1192	1186	0.17
5-Indanol	5.83	1203	1338	6.06
Eugenol	7.38	1362	1356	74.06
α-Cubebene	8.20	1349	1345	0.41
Caryophyllene	8.62	1415	1417	0.08
γ-Muurolene	9.24	1478	1478	0.27
α-Cadinene	9.57	1515	1537	0.17
α-Muurolene	9.75	1499	1500	0.24
δ-Cadinene	9.87	1525	1522	1.74
Hydrogenated monoterpenes				12.72
Oxygenated monoterpenes				2.09
phenylpropanoid				74.06
Hydrocarbon sesquiterpenes				2.91
Phenol				6.06
Alcohol				1.38

[a]Adams Library Retention Index (2017)

calculate the LC_{50} using the Reed-Muench method. From this analysis, we observed that the essential oil extracted from the leaves of *Pimenta dioica* (Table No. 2) and eugenol standard (Table No. 3) showed larvicidal activity, in the concentration of 70 and 100 $\mu g \cdot mL^{-1}$ and LC_{50}, respectively, 38.86 $\mu g\ mL^{-1}$ at a confidence level of 2.25 $\mu g\ mL^{-1}$ and 79.75 $\mu g\ mL^{-1}$ at a confidence level of 2.10 $\mu g\ mL^{-1}$. As shown in the LC_{50} values, we observed that the essential oil exerts a higher lethality than standard eugenol.

Table No. 2
Larvae mortality with essential oil after 24 hours of exposure

Concentration ($\mu g\ mL^{-1}$)	Log concentration	Dead	Alive	Accumulated dead	Accumulated live	Mortality rate (%)
120	2.0791	10	0	48	0	100
100	2.0000	10	0	38	0	100
90	1.9542	10	0	28	0	100
70	1.8451	10	0	18	0	100
50	1.6989	6	4	8	4	60
20	1.3010	2	8	2	12	20

Table No. 3
Mortality of larvae with eugenol standard after 24 hours of exposure

Concentration (µg mL^{-1})	Log concentration	Dead	Alive	Accumulated dead	Accumulated live	Mortality rate (%)
120	2.0791	10	0	30	0	100
100	2.0000	9	1	20	1	90
90	1.9542	8	3	11	4	80
70	1.8451	2	7	3	11	20
50	1.6989	1	9	1	20	10
20	1.3010	0	10	0	30	0

DISCUSSION

In this way, public authorities are looking for ways to combat the vector mosquito *Aedes aegypti* in larval or adult form. Currently, one of the main means used in this control is the larvicides or chemical insecticides. However, studies have shown that the use of these substances provoked resistance from mosquitoes and caused damage to the environment (Sá *et al.*, 2019). Thus, the use of larvicides based on essential oils turns out to be a sustainable alternative to combat the vector mosquito. Given the facts, in this study, we identified the main constituent, the predominant class of essential oil extracted from the leaves of *P. dioica* and evaluated whether the essential oil and its main components have biological activity against larvae in the third stage of *Ae. aegypti*. In this work, we showed that the essential oil is composed mainly of eugenol in which the predominant class was phenylpropanoid and we demonstrated that both the essential oil and the eugenol standard have larvicidal activity against *Ae. aegypti* in a 24 h exposure time. Another important point is that this study is in line with previous studies (Neves *et al.*, 2017; Carneiro *et al.*, 2021) which has reported on the larvicidal activity of plants of the family Myrtaceae.

In the first finding, eugenol, the main component of the oil extracted from the leaves of *P. dioica*, was identified by gas chromatographic analysis coupled to the mass spectrometer with a content of 74.06%. Although this result is within the range expected for this plant (65.9 - 71.4%) (Mérida-Reyes *et al.*, 2020), which must be greater than 70%, we observed divergences from our result with previous studies, in which the values varied, approximately, from 82 to 89% (Oliveira *et al.*, 2009; Tenne & Karunaratne, 2018). Generally, some factors, such as time of extraction, temperature, intensity of solar radiation, the age and development of plants (Gobbo-Neto & Lopes, 2007), seasonality (Silva *et al.*, 2019), parts of plants (Aguiar *et al.*, 2015), among others are responsible for causing differences in the composition of essential oils.

In the second found, we demonstrated that the essential oil *P. dioica* and the eugenol standard have larvicidal activity against *Ae. aegypti* in the third stage. These findings are confirmed from the values obtained by the LC_{50} and compared with the standards determined by Cheng *et al*. (2003), who consider an active essential oil when the LC_{50} is less than or equal to 100 µg·mL^{-1}, and Dias & Moraes (2014), that consider an essential oil or strong constituent when the LC_{50} is less than or equal to 50 µg·mL^{-1} or moderate when the LC_{50} is in the range of 50 to 100 µg·mL^{-1}. Taking into account these two results and the results of our study for essential oil and eugenol, we conclude that the essential oil shows a strong activity, while isolated activity is moderate. This finding is in line with a study by Dias & Moraes (2014), in which they report that the biological activity of essential oils is greater than the isolated compounds.

Other finding relevant it is in the identification of the predominant class in larvicidal activity. In our study, this class was phenylpropanoid, with eugenol as its representative, and this was followed by hydrocarbon and oxygen monoterpenes. The activity found in these classes is in line with previous studies (Santos *et al.*, 2011; Lucia *et al.*, 2013; Hong *et al.*, 2018), in which they demonstrated larvicidal activity. Thus, we highlight that the various compounds found in the plant, alone or in combination, present not only differences in toxicity, but also behavioral and physiological efficacy (Hong *et al.*, 2018).

The result obtained for the larvicidal activity of the leaves in this study show a higher potency

when compared to the fruits of *P. dioica*, which LC_{50} was 104.4 µg·mL^{-1} (Rocha Voris *et al.*, 2018). This discovery is according to previous study (Sarma *et al.*, 2019) in which it demonstrates the differences in the larvicidal activities of essential oils from different parts of the same plant, due to their chemical composition (Pandey *et al.*, 2014). In addition, a factor that influences the differences in activities is the season. Previous studies (Fernandez *et al.*, 2014; Fernandez *et al*., 2018) have shown that the larvicidal activity assessed in the spring and autumn seasons is greater compared to winter and summer.

Therefore, we presented that the essential oil distilled from the leaves of *P. dioica*, together with eugenol has potential larvicidal activity. However, field and toxicity studies against non-target organisms are needed to attest to the effectiveness of this larvicide.

CONCLUSION

To sum up, the essential oil distilled from the leaves of *Pimenta dioica* is mainly composed of phenylpropanoids and monoterpenes, in which the predominant compound is eugenol. The study of biological activity showed that both essential oil and eugenol were active against larvae in the 3rd stage of *Aedes aegypti*, but the essential oil was more lethal. Given the facts, we conclude that the distilled essential oil has potential biological activity, being able to replace the synthetic larvicides.

REFERENCES

Adams RP. 2017. **Identification of essential oil components by gas chromatography/mass spectrometry.** Ed. Allured Publishing Corporation, Carol Stream, Illinois, USA.

Aguiar RWS, dos Santos SF, Morgado FS, Ascencio SD, Lopes MM, Viana KF, Didonet J, Ribeiro BM. 2015. Insecticidal and repellent activity of *Siparuna guianensis* Aubl. (Negramina) against *Aedes aegypti* and *Culex quinquefasciatus*. **PloS One** 10: e0116765. **https://doi.org/10.1371/journal.pone.0116765**

Bedini S, Flamini G, Ascrizzi R, Venturi F, Ferroni G, Bader A, Girardi J, Conti B. 2018. Essential oils sensory quality and their bioactivity against the mosquito *Aedes albopictus*. **Sci Rep** 8: 1 - 10. **https://doi.org/10.1038/s41598-018-36158-w**

Carneiro VCDS, Lucena LBD, Figueiró R, Victório CP. 2021. Larvicidal activity of plants from Myrtaceae against *Aedes aegypti* L. and *Simulium pertinax* Kollar (Diptera). **Rev Soc Bras Med Trop** 54. **https://doi.org/10.1590/0037-8682-0092-2020**

Cavalcanti LPG, Pontes RJS, Regazzi ACF, Júnior FJP, Frutuoso RL, Sousa EP, Filho FFD, Lima JWO. 2007. Efficacy of fish as predators of *Aedes aegypti* larvae, under laboratory conditions. **Rev Saude Publ** 41: 638 - 644. **https://doi.org/10.1590/s0034-89102006005000041**

Charles DJ. 2013. **Antioxidant Properties of Spices, Herbs and Other Sources.** Springer-Verlag, New York, USA. **https://doi.org/10.1007/978-1-4614-4310-0**

Cheng SS, Chang HT, Chang ST, Tsai KH, Chen WJ. 2003. Bioactivity of selected plant essential oils against the yellow fever mosquito *Aedes Aegypti* larvae. **Bioresour Technol** 89: 99 - 102. **https://doi.org/10.1016/S0960-8524(03)00008-7**

Colegate SM, Molyneux RJ. 2007. **Bioactive natural products: detection, isolation, and structural determination**. CRC Press, Boca Raton, USA. **https://doi.org/10.1201/9781420006889**

Dias CN, Moraes DFC. 2014. Essential oils and their compounds as *Aedes Aegypti* L. (Diptera: Culicidae) Larvicides: Review. **Parasitol Res** 113: 565 - 592. **https://doi.org/10.1007/s00436-013-3687-6**

Donalisio MR, Freitas ARR, Zuben APB. 2017. Arboviroses emergentes no Brasil: desafios para a clínica e implicações para a saúde pública. **Rev Saude Publ** 31: 10 - 15.

Everton GO, Teles AM, Mouchrek AN, Filho EMV. 2018. Aplicação do óleo essencial de *Pimenta dioica* Lindl como moluscicida frente ao caramujo transmissor da esquistossomose. **Rev Proc Quím** 12: 85 - 93. **https://doi.org/10.19142/rpq.v12i23.433**

Fernandez CMM, Barba EL, Fernandez ACM, Cardoso BK, Borges IB, Takemura OS, Martins LA, Cortez LER, Cortez DAG, Gazim ZC. 2014. Larvicidal activity of essential oil from *Tetradenia riparia* to control of *Aedes aegypti* larvae in function of season variation. **J Essent Oil-Bear Plant** 17: 813 - 823. **https://doi.org/10.1080/0972060x.2014.892841**

Fernandez CMM, Rosa MF, Fernandez ACAM, Lorenzetti FB, Raimundo KF, Cortez DAG, Gonçalves JE, Simões MR, Colauto NB, Lobo VS. 2018. Larvicidal activity against *Aedes aegypti* of essential oil of *Laurus nobilis* leaves obtained at different seasons. **J Essent Oil Res** 30: 379 - 387.

https://doi.org/10.1080/10412905.2018.1473294

Gobbo-Neto L, Lopes NP. 2007. Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. **Quim Nova** 30: 374 - 381. **https://doi.org/10.1590/s0100-40422007000200026**

Gomes PRB, Silva ALS, Pinheiro HA, Carvalho LL, Lima HS, Silva EF, Silva RP, Louzeiro CH, Oliveira MB, e Filho VEM. 2016. Avaliação da atividade larvicida do óleo essencial do *Zingiber officinale* Roscoe (gengibre) frente ao mosquito *Aedes aegypti*. **Rev Bras Plant Med** 18: 597 - 604. **https://doi.org/10.1590/1983-084x/15_214.**

Gould E, Pettersson J, Higgs S, Charrel R, De Lamballerie X. 2017. Emerging arboviruses: why today?. **One Health** 4: 1 - 13. **https://doi.org/10.1016/j.onehlt.2017.06.001**

Hong TK, Perumalsamy H, Jang KH, Na ES, Ahn YJ. 2018. Ovicidal and larvicidal activity and possible mode of action of phenylpropanoids and ketone identified in *Syzygium aromaticum* bud against *Bradysia procera*. **Pestic Biochem Physiol** 145: 29 - 38. **https://doi.org/10.1016/j.pestbp.2018.01.003**

Kumar CMS, Jacob TK, Devasahayam S, D'Silva S, Nandeesh PG. 2016. Characterization and virulence of *Beauveria bassiana* associated with auger beetle (*Sinoxylon anale*) infesting allspice (*Pimenta dioica*). **J Invertebr Pathol** 139: 67 - 73. **https://doi.org/10.1016/j.jip.2016.07.016**

Lucia A, Zerba E, Masuh H. 2013. Knockdown and larvicidal activity of six monoterpenes against *Aedes aegypti* (Diptera: Culicidae) and their structure-activity relationships. **Parasitol Res** 112: 4267 - 4272. **https://doi.org/10.1007/s00436-013-3618-6**

Maestre-Serrano R, Gomez-Camargo D, Ponce-Garcia G, Flores AE. 2014. Susceptibility to insecticides and resistance mechanisms in *Aedes aegypti* from the Colombian Caribbean Region. **Pestic Biochem Physiol** 116: 63 - 73. **https://doi.org/10.1016/j.pestbp.2014.09.014**

Mérida-Reyes MS, Muñoz-Wug MA, Oliva-Hernández BE, Gaitán-Fernández IC, Simas DLR, Ribeiro da Silva AJ, Pérez-Sabino JF. 2020. Composition and antibacterial activity of the essential oil from *Pimenta dioica* (L.) Merr. from Guatemala. **Medicines** 7: 59. **https://doi.org/10.3390/medicines7100059**

Neves IDA, Rezende SRDF, Kirk JM, Pontes EG, Carvalho MG. 2017. Composition and larvicidal activity of essential oil of *Eugenia candolleana* DC. (Myrtaceae) against *Aedes aegypti*. **Rev Virtual Quim** 9: 2305 - 2315. **https://doi.org/10.21577/1984-6835.20170138**

Oliveira RA, Reis TV, Sacramento CK, Duarte LP, Oliveira FF. 2009. Volatile chemical constituents of rich spices in eugenol. **Rev Bras Farmacogn** 19: 771 - 775. **https://doi.org/10.1590/s0102-695x2009000500020**

Pandey AK, Singh P, Tripathi NN. 2014. Chemistry and bioactivities of essential oils of some Ocimum species: an overview. **Asian Pac J Trop Biomed** 4: 682 - 694. **https://doi.org/10.12980/apjtb.4.2014c77**

Pereira TN, Rocha MN, Sucupira PHF, Carvalho FD, Moreira LA. 2018. Wolbachia significantly impacts the vector competence of *Aedes aegypti* for Mayaro virus. **Sci Rep** 8: 1 - 9. **https://doi.org/10.1038/s41598-018-25236-8**

Pizzi M. 1950. Sampling variation of the fifty percent end-point, determined by the Reed-Muench (Behrens) method. **Hum Biol** 22: 151 - 190.

Pushpanathan T, Jebanesan A, Govindarajan M. 2006. Larvicidal, ovicidal and repellent activities of *Cymbopogan citratus* Stapf (Graminae) essential oil against the filarial mosquito *Culex quinquefasciatus* (Say) (Diptera: Culicidae). **Trop Biomed** 23: 208 - 212.

Reed LJ, Muench H. 1938. A simple method of estimating fifty per cent endpoints. **Am J Epidemiol** 27: 493 - 497. **https://doi.org/10.1093/oxfordjournals.aje.a118408**

Rocha Voris DG, Dias LS, Lima JA, Lima KSC, Lima JBP, Lima ALS. 2018. Evaluation of larvicidal, adulticidal, and anticholinesterase activities of essential oils of *Illicium verum* Hook. f., *Pimenta dioica* (L.) Merr., and *Myristica fragrans* Houtt. against Zika virus vectors. **Environ Sci Pollut Res** 25: 22541 - 22551. **https://doi.org/10.1007/s11356-018-2362-y**

Sá ELRD, Rodovalho CDM, Sousa NPRD, Sá ILRD, Bellinato DF, Dias LDS, Silva LC, Martins AJ, Lima JBP. 2019. Evaluation of insecticide resistance in *Aedes aegypti* populations connected by roads and rivers: the case of Tocantins state in Brazil. **Mem Inst Oswaldo Cruz** 114. **https://doi.org/10.1590/0074-02760180318**

Santos SRL, Melo MA, Cardoso AV, Santos RLC, Sousa DP, Cavalcanti SCH. 2011. Structure–activity relationships of larvicidal monoterpenes and derivatives against *Aedes aegypti* Linn. **Chemosphere** 84: 150 - 153. **https://doi.org/10.1016/j.chemosphere.2011.02.018**

Sarma R, Adhikari K, Mahanta S, Khanikor B. 2019. Insecticidal activities of *Citrus aurantifolia* essential oil against *Aedes aegypti* (Diptera: Culicidae). **Toxicol Rep** 6: 1091 - 1096. **https://doi.org/10.1016/j.toxrep.2019.10.009**

Silva PT, Santos HS, Teixeira AMR, Bandeira PN, Holanda CL, Vale JPC, Pereira EJP, Menezes JESA, Rodrigues THS, Souza EB. 2019. Seasonal variation in the chemical composition and larvicidal activity against *Aedes aegypti* of essential oils from *Vitex gardneriana* Schauer. **South Afr J Bot** 124: 329 - 332. **https://doi.org/10.1016/j.sajb.2019.04.036**

Teich V, Arinelli R, Fahham L. 2017. *Aedes aegypti* e sociedade: o impacto econômico das arboviroses no Brasil. **J Braz Health Econ** 9: 267 - 276. **https://doi.org/10.21115/jbes.v9.n3.p267-76**

Tenne PCRK, Karunaratne MMSC. 2018. Phytochemical profile and bioactivity of essential oil from *Pimenta dioica* leaves on cowpea beetle, *Callosobruchus maculatus* (F.)(Coleoptera: Bruchidae): A farmer friendly solution for postharvest pest management. **Open Agric J** 3: 301 - 309. **https://doi.org/10.1515/opag-2018-0033**