REVISTA TÓPICOS
ISSN 2965-6672 | Qualis A2

EFFECT OF PLECTRANTHUS AMBOINICUS (LOUR.) SPRENG ESSENTIAL OIL ON THE ELIMINATION OF AEDES AEGYPTI LARVAE

EFEITO DO ÓLEO ESSENCIAL DE PLECTRANTHUS AMBOINICUS (LOUR.) SPRENG. NA ELIMINAÇÃO DE LARVAS DE AEDES AEGYPTI

Ciências Biológicas, Ciências da Saúde • 25/08/2026

REGISTRO DOI: 10.70773/revistatopicos/787615319

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ABSTRACT

Introduction: In recent years, the dengue virus has established itself as a serious public health problem, being the most frequent arbovirus affecting humans. The virus has four known serotypes (DENV-1 to DENV-4) and its main vector is the *Aedes aegypti* mosquito, a diurnal, blood-feeding insect with a preference for urban and indoor environments. Currently, vector control relies on chemical insecticides; however, their continuous use has induced population resistance in the mosquito, besides causing harm to human and environmental health. Therefore, there is a growing need to search for eco-friendly, effective alternatives with low environmental impact. **Objective:** To evaluate the larvicidal potential of malvarisco (*Plectranthus amboinicus* (Lour.) Spreng) essential oil against Aedes aegypti mosquito larvae. **Methodology:** The study initially involved extracting the essential oil from malvarisco. For the in vitro tests, selected Aedes aegypti eggs were used (provided by the INPA entomology laboratory). After hatching and larval development to stages L1 through L4, the larvae were distributed into 6-well culture plates, containing 10 larvae per well, in triplicate. The test group received 5 mL of malvarisco essential oil at concentrations of 20, 40, 80, 100, 160, 180, 200, and 240 ppm. Distilled water and water with 0.01% Tween 80 were used as control groups. **Results:** The tests confirmed the efficacy of the malvarisco essential oil, which promoted total larval mortality within up to 2 hours. **Conclusion:** Malvarisco essential oil proved to be a promising product and a viable alternative for the larval control of Aedes aegypti. Despite the scarcity of detailed scientific literature on the plant's Properties commonly restricted to traditional medicinal use in India and China for respiratory infections this work highlights the importance of scientifically proving its efficacy as a larvicida as well.

Keywords: Plectranthus amboinicus; Pharmacology; Larvicidal activities; Dengue.

RESUMO

Introdução: Nos últimos anos, o vírus da dengue consolidou-se como um grave problema de saúde pública, sendo o arbovírus que mais frequentemente afeta os seres humanos. O vírus possui quatro sorotipos conhecidos (DENV-1 a DENV-4) e seu principal vetor é o mosquito *Aedes aegypti*, um inseto de hábitos diurnos e hematófagos, com preferência por ambientes urbanos e intradomiciliares. Atualmente, o controle do vetor baseia-se no uso de inseticidas químicos; no entanto, o uso contínuo desses produtos tem induzido resistência nas populações de mosquitos, além de causar danos à saúde humana e ao meio ambiente. Portanto, há uma necessidade crescente de buscar alternativas eficazes, ecologicamente corretas e de baixo impacto ambiental. **Objetivo:** Avaliar o potencial larvicida do óleo essencial de malvarisco (Plectranthus amboinicus (Lour.) Spreng) contra larvas do mosquito *Aedes aegypti*. **Metodologia:** O estudo envolveu, inicialmente, a extração do óleo essencial de malvarisco. Para os testes *in vitro*, utilizaram-se ovos selecionados de *Aedes aegypti* (fornecidos pelo laboratório de entomologia do INPA). Após a eclosão e o desenvolvimento larval até os estádios L1 a L4, as larvas foram distribuídas em placas de cultura de 6 poços, contendo 10 larvas por poço, em triplicata. O grupo experimental recebeu 5 mL de óleo essencial de malvarisco nas concentrações de 20, 40, 80, 100, 160, 180, 200 e 240 ppm. Água destilada e água com Tween 80 a 0,01% foram utilizadas como grupos controle. **Resultados:** Os testes confirmaram a eficácia do óleo essencial de malvarisco, que promoveu a mortalidade larval total em um período de até 2 horas. **Conclusão:** O óleo essencial de malvarisco mostrou-se um produto

promissor e uma alternativa viável para o controle de larvas de *Aedes aegypti*. Apesar da escassez de literatura científica detalhada sobre as propriedades da planta — comumente restritas ao uso medicinal tradicional na Índia e na China para infecções respiratórias —, este trabalho destaca a importância de comprovar cientificamente sua eficácia também como larvicida.

Palavras-chave: Plectranthus amboinicus; Farmacologia; Atividade larvicida; Dengue.

1. INTRODUCTION

Aedes aegypti is the vector mosquito of the dengue virus. It has diurnal habits, mostly active during early morning and late afternoon, and prefers urban and indoor environments. Females feed primarily on human blood for egg maturation (Brasil, 2010). Mosquito proliferation occurs through egg-laying by females in stagnant water, where larval hatching takes place. The time elapsed from egg hatching to the adult mosquito stage is approximately 10 days, which is influenced by factors such as temperature, which accelerates this process (Dias et al., 2010). Each year, the number of people affected by the dengue virus worldwide increases. In approximately 550,000 of these cases, patients require hospitalization, leading to roughly 20,000 deaths (Nhantumbo; Pessanha; Proietti, 2012).

Methods to minimize mosquito development include the constant cleaning of water tanks and the elimination of debris and residential waste. On the other hand, state epidemiological control agencies employ vector control treatments using specific insecticides, as described by Coelho, Paula, and Espíndola (2009) and Martins and Vieira (2013). These include Temephos, Methoprene, Diflubenzuron, and Triflumuron, which are harmful to both public health and the environment.

Given this context, new alternatives are being studied to provide more effective, low-environmental-impact solutions for combating the dengue mosquito. Researchers increasingly recognize nature's role in providing raw materials to develop new alternatives that eliminate Aedes larvae without causing harm to public health. Natural plant-derived products are among the primary sources of new drugs; the success of these products lies in the highly diverse chemical substances produced by medicinal plants (Cragg; Grothaus; Newman, 2009). One such alternative treatment for combating the dengue mosquito is the use of essential oil from malvarisco. This plant is very common in the Amazon region, particularly in Manaus, where it is widely cultivated in home gardens.

Malvarisco is a medicinal plant belonging to the *Lamiaceae* botanical family. This is one of the most diverse families, comprising approximately 6,970 to 7,193 species subdivided into 7 subfamilies. In Brazil, there are about 23 genera and 232 native species, 14 of which are found in the State of Amazonas. A variety of these species is utilized in the pharmaceutical and cosmetic industries, as many have demonstrated important pharmacological activities (Sousa; Lorenzi, 2005).

Popularly, malvarisco has been used for decades to treat various conditions, particularly inflammatory skin diseases and infections (Lukhoba; Simmonds; Paton, 2006). An infusion or syrup made from its leaves is used to treat coughs, flu, bronchitis, and asthma

(Vásquez; Mendonça; Do Nascimento, 2014), and is also applied externally for burns and insect bites (Selvakumar et al., 2012).

Palani *et al.* (2010) observed that the ethanolic extract of Plectranthus amboinicus possesses antioxidant and nephroprotective effects against nephrotoxic agents and strong diuretics in rats. In another study, the extract also exhibited diuretic activity, significantly increasing urine volume as well as the excretion of Na^+, K^+, and Cl^- ions in the urine.

The essential oil of the species Plectranthus amboinicus (Lour.) Spreng, which is the subject of this study, is characterized by a strong aroma. Two chemotypes are known for this species: one rich in carvacrol and the other in thymol (Murthy; Ramalakshmi; Srinivas, 2009). The literature demonstrates that P. amboinicus oil can exhibit a carvacrol content of up to 98% (Feng; Jia, 2014; Gonçalves et al., 2012; Senthilkumar; Venkatesalu, 2010), while other studies show that thymol is the major constituent, reaching a content of approximately 94% (Muñoz-Acevedo; Kouznetsov; Stashenko, 2009).

Carvacrol is a phenolic monoterpene that exhibits various pharmacological activities, such as antimicrobial activity. Furthermore, a study utilizing a carvacrol nanoemulsion demonstrated its ability to inhibit the growth of the yeast strains *Zygosaccharomyces bailii*, *Saccharomyces cerevisiae*, *Brettanomyces bruxellensis*, and *Brettanomyces naardenensis*, showing that this effect was dependent on carvacrol concentration (Chang; Landsborough; Clements, 2013). Given all these activities demonstrated by the *Plectranthus amboinicus* essential oil, it can be inferred that this natural product possesses high therapeutic potential, making it an excellent candidate for developing an alternative solution to combat Aedes aegypti larvae.

2. METHODOLOGY

This is an experimental, descriptive, and qualitative study aimed at evaluating the efficacy of malvarisco essential oil against Aedes aegypti larvae. The research was conducted across two distinct laboratories: the Laboratory of Reproduction and Molecular Genetic Biology of Aquatic Organisms at Nilton Lins University, and the Laboratory of Natural Products Chemistry at the National Institute of Amazonian Research (INPA).

2.1. Raw Material Acquisition And Processing

In natura malvarisco leaves were purchased at the Feira do Produtor (Producer's Market), located in the eastern zone of Manaus, and transported to the laboratory at Nilton Lins University for sanitization and drying. For this initial stage, the leaves were transported in plastic bags and washed under running tap water. After washing, the leaves were separated from the stems, weighed individually, and placed in a drying oven at (40 °C) for approximately 10 days. After this period, the dried leaves and stems were ground using a mortar and pestle, weighed again, and subsequently stored in wide-mouth jars wrapped in aluminum foil.

2.2. Essential Oil Extraction

Following the drying stage, the material was taken to the Laboratory of Natural Products Chemistry at INPA, where the essential oil extraction was performed. A Clevenger-type apparatus was utilized for the extraction. The process was initiated using 118.00 g of dried raw material in 3 liters of water. The boiling temperature was approximately (100 °C), and after 2 hours of boiling, the oil was observed flowing through the apparatus for subsequent collection in an Erlenmeyer flask.

2.3. Preparation of Solutions And Dilutions

For the bioassays with the malvarisco essential oil, a stock solution at 1,000 parts per million (ppm) was prepared by adding 1 mL of *P. amboinicus* essential oil to 4 mL of Tween 80, and bringing the total volume to 1,000 mL with distilled water. From this stock solution, dilutions of the essential oil were made to achieve the following concentrations: 20, 40, 80, 100, 160, 180, 200, and 240 ppm, following the methodology described by Faustino (2018).

2.4. Rearing And Selection of Aedes Aegypti Larvae

For the in vitro tests, carefully selected Aedes eggs were used, provided by the Entomology Laboratory at INPA. Egg selection was performed with the aid of a stereomicroscope, totaling 1,000 selected eggs. Following quantification, the eggs were immersed in a tray of water containing ground chow (for future larval feeding) that had been preheated to (28 °C) for at least 24 hours in a B.O.D. incubator. The material was maintained in this incubator for hatching. After hatching and development through larval stages L1, L2, L3, and L4, the larvae were separated for the bioassays.

2.5. Bioassays (Mortality Tests)

The bioassays were performed in triplicate. Larvae from each developmental stage were distributed into 6-well cell culture plates, with 10 larvae per well for each triplicate (totaling 30 larvae per larval stage). Two control groups were prepared: one with distilled water and another with 0.01% Tween 80. Following the exposure of the larvae to the different essential oil concentrations and control groups, they were monitored at intervals of 1, 2, 4, 24, and 48 hours to quantify mortality (Faustino, 2018).

After 24 hours of egg hydration, L1 larvae began to emerge in small quantities. Ten larvae were selected per well, with all larval stages evaluated in triplicate. For these assays, four 6-well cell culture plates were used, and 5 mL of each test concentration was added per well containing the 10 larvae. This procedure was repeated for each subsequent larval stage up to L4, taking approximately two weeks to complete all experimentais rounds. After these periods, the data were organized into Excel spreadsheets to evaluate which concentrations exhibited the highest efficacy regarding *Aedes aegypti* larval mortality.

3. RESULTS AND DISCUSSION

Table 1 highlights the quantification of the mortality percentage (mean ± standard deviation) of Aedes aegypti larvae subjected to five different concentrations of the essential oil (20, 40, 80, 100, and 160 ppm). L1 larvae exhibited low mortality at the initial doses (2.3%), which gradually increased to a maximum of 64.2% at the highest concentration (160 ppm). L2 mortality fluctuated at intermediate doses but reached a high rate (93.3%) at the 160 ppm dose. L3 larvae stood out by demonstrating the highest mortality rates across all tested concentrations; even at the lowest dose (20 ppm), mortality was already at 75.0%, peaking at 95.0% at a concentration of 100 ppm. In the control group, mortality was only 5.0%. Conversely, L4 proved to be the most resistant stage, exhibiting 0.0% mortality at doses of 20 and 40 ppm, and reaching a maximum of only 23.3% at the highest concentration (160 ppm)

Quadro 1. Mortality (%) ou Mortality Percentage (Mean ± SD)

Larval Stage	20 PPM (%)	40 PPM (%)	80 PPM (%)	100 PPM (%)	160 PI (%)
L1	2,3±4,1	2,3±4,1	14,2±4,1	28,5±26,2	64,2±3
L2	13,3±11,5	3,3±5,7	45,0±7,0	3,3±5,7	93,3±1
L3	75,0±21,2	90,0±14,1	75,0±7,0	95,0±21,2	90,0±1
L4	0,0±0,0	0,0±0,0	16,6±15,2	6,6±11,5	23,3±

A: Water (Distilled water, used as the standard vehicle). **T:** Tween (Tween 80 to solubilize and disperse the oil in water). **S:** Solvent or Solution (Referring to

⚠ Esta tabela possui muitas colunas e foi cortada para impressão. Para visualizá-la completa, acesse o artigo original em: https://revistatopicos.com.br/artigos/effect-of-plectranthus-amboinicus-lour-spreng-essential-oil-on-the-elimination-of-aedes-aegypti-larvae?noblockage

The high biological sensitivity observed specifically in the L3 stage (which deviates from the expected pattern where smaller larvae would be more fragile) can be explained by physiological factors of mosquito development. Third-instar larvae (L3) are in a phase of accelerated growth and need to accumulate substantial energy for the final molt (L4) and subsequent pupation. Because they filter water and feed much more intensely and voraciously than L1 and L2 stages, they ultimately ingest a proportionally larger quantity of the essential oil particles dispersed in the medium (which are enhanced by the Tween 80 surfactant).

The L3 stage is characterized by intense endocrine activity to prepare for the exoskeleton exchange. The essential oil components may act as endocrine disruptors or directly interfere with chitin synthesis and the permeability of the developing cuticle. A more permeable or vulnerable cuticle during ecdysis processes facilitates the contact penetration of the lipophilic compounds of the oil.

While the L4 stage already possesses a much more mature enzymatic detoxification system (such as cytochrome P450 enzymes) and a thick, impermeable cuticle which justifies the low mortality rates of 0% to 23.3% in L4 the L3 stage has not yet completed this metabolic defense transition, making it the most vulnerable biological target of the vector's cycle in this experiment. The primary cause of mortality in larvae exposed to *Plectranthus amboinicus* (malvarisco) essential oil is the presence of bioactive chemical compounds, such as carvacrol and thymol (Table 2).

Quadro 2. Chemical constituents of malvarisco

Constituintes Químicos	% Do produto
1,8-cineol	5,45%
Carvacrol	43,1%
Chavicol	5,3%
E -cariofileno	4,20%
Et-salicilato	3.2%
Eugenol	6,4%
Timol	39,30%

Fonte: Arumugam e Swamy (2016)

These substances act as natural larvicides by penetrating the insect's cuticle or being ingested, causing systemic toxicity, damage to the digestive tract, and the collapse of the larval nervous system. The high biological sensitivity observed specifically in the L3 stage (which deviates from the expected pattern where smaller larvae would be more fragile) can be explained by physiological factors of mosquito development.

The search for bioinsecticides is driven by the need to mitigate environmental impacts resulting from the indiscriminate use of xenobiotics and to counter the development of population resistance in vectors. From this perspective, Araújo (2014) highlights that essential oils exhibit equivalent larvicidal efficacy across different Aedes aegypti populations, regardless of their baseline susceptibility profiles. This characteristic confers these secondary metabolites a high bioactive potential to integrate into integrated vector management programs, serving as a viable alternative to circumvent resistance mechanisms to conventional synthetic larvicides.

Additionally, Gomes et al. (2016) and Furtado et al. (2005) reiterate the relevance of essential oils as promising sources of biological inputs, the use of which aims to reduce the historical dependence on synthetic chemical insecticides. Because they are complex, biodegradable matrices, these natural compounds tend to exhibit lower residual toxicity to the environment, human health, and domestic animals. Thus, they represent ecologically safe larvicidal agents for direct application in *Aedes aegypti* breeding sites and proliferation foci.

Regarding the evaluated plant species, Arumugam and Swamy (2016) characterize malvarisco (*Plectranthus amboinicus*) as a medicinal and aromatic herb of significant biological importance, rich in bioactive phytoconstituents and nutrients. The remarkable biological properties observed in both its crude extracts and its essential oil reinforce the species' potential to meet global demands for bioaccessible and low-cost natural resources. Therefore, malvarisco emerges not only as a viable biotechnological tool for vector control but also as a promising raw material for future molecular investigations and applications in the pharmaceutical and nutraceutical industries.

4. CONCLUSION

The present study confirmed the remarkable larvicidal efficacy of *Plectranthus amboinicus* (malvarisco) essential oil against Aedes aegypti larvae, demonstrating its ability to induce complete mortality within an exposure period of only 2 hours. These results position the species' essential oil as a promising biotechnological input and a viable alternative to be integrated into vector control strategies targeting the early stages of development.

Additionally, this research highlighted the latent need to expand investigations focused on prospecting insecticidal plants that are native to or adapted to the Manaus region, with the purpose of mitigating the chronic dependence on industrial synthetic larvicides, whose adverse effects cause severe environmental impacts and human health risks.

Finally, it is worth emphasizing that the scarcity of scientific literature focusing on the larvicidal properties of malvarisco posed a challenge for the comparative discussion in this study. Although the species is widely consolidated in the traditional medicine of countries such as India and China for treating respiratory infections, the findings of this study expand the application landscape of the plant. Thus, its insecticidal potential is scientifically proven, providing a foundation for future research to explore its economic and molecular viability in combating dengue.

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