



DEVELOPMENT OF NANOSTRUCTURED SYSTEMS WITH *SYZYGIUM CUMINI* ESSENTIAL OIL FOR LARVAL CONTROL OF *Aedes Aegypti*

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Abstract: The objective was to develop nanostructured formulations with essential oil from *Syzygium cumini* leaves for larvicidal application against *Aedes aegypti* larvae. The oil was extracted using the hydrodistillation method and incorporated into nanostructured systems, defined from ternary phase diagrams with Procetyl (PPG-5-CETETH-20), water, and the oil phase. Formulations representative of the microemulsion (AJ1), phase transition (AJ2), and liquid crystal (AJ3) regions were selected and characterized by polarized light microscopy. Larvicidal bioassays with L3/L4 larvae demonstrated that the structured formulations significantly reduced the LC₅₀ of pure oil (10 ppm) compared to isolated essential oil. Formulation AJ1 (microemulsion) had the lowest LC₅₀ (6,5 ppm), followed by AJ2 (3,8 ppm) and AJ3 (4,6 ppm). The results highlight the potential of nanostructured systems as a sustainable and effective alternative in vector control.

Keywords: Nanostructured, *Syzygium cumini*, *Aedes aegypti*

Abbreviations: LC₅₀, lethal concentration

1. Introduction

In recent years, there has been a considerable increase in the number of arbovirus cases, human infectious diseases transmitted by arboviruses carried by biological vectors [1]. Among these vectors, *Aedes aegypti* stands out as the primary transmitter of diseases such as dengue, Zika, chikungunya, and yellow fever, particularly in urban areas of tropical regions [2]. According to data from the 2024 Rapid Survey of *Aedes aegypti* Infestation Index (LIRAA), ten municipalities in the state of Sergipe presented high infestation rates of the mosquito, while 48 were classified as medium risk and only 17 as low risk.

The intensive use of synthetic insecticides, such as pyrethroids, although effective, has contributed to the selection of resistant populations and has generated significant

environmental impacts. In this context, there is growing interest in sustainable alternatives with lower environmental toxicity, such as the use of bioactive compounds derived from medicinal plants [3–4].

Syzygium cumini, native to the Indian subcontinent, is recognized for its rich bioactive compounds with multiple applications in traditional medicine and fragrances. Different parts of *S. cumini*, such as seed and leaf extracts, have been extensively investigated due to their medicinal properties [5].

Among the most relevant components of *S. cumini*, its essential oil stands out, being rich in β -farnesene (3.42%), caryophyllenol (3.46%), terpinen-4-ol (3.61%), β -myrcene (3.90%), γ -cadinene (4.09%), fenchol (4.22%), cis- β -ocimene (4.40%), and 1,3,6-heptatriene,



5-methyl- (4.90%)[6]. Some of these compounds exhibit characteristics associated with biological and therapeutic applications, which may enhance the release of active constituents present in the oils.

Nanotechnology applied to the delivery of natural compounds emerges as a promising approach to improve their stability, efficacy, and controlled release. Nanoformulations, such as microemulsions and liquid crystals, offer advantages including enhanced penetration and prolonged action, particularly in antimicrobial and larvicidal applications [4].

Therefore, this study aims to develop nanostructured formulations containing *Syzygium cumini* essential oil, focusing on evaluating their larvicidal activity against *Aedes aegypti*, thus representing an integration of natural resources and innovative technologies for the control of infectious and vector-borne diseases.

2. Materials and Methods.

2.2. Extraction of the essential oil from *Syzygium cumini*

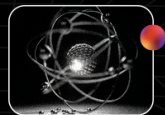
The following analyses were performed in triplicate at the Food Research Laboratory (LPA) located at the Institute of Technology and Research (ITP) of Universidade Tiradentes.

2.2 Construction of the Ternary Phase Diagram

The ternary phase diagram was obtained according to the methodology described by de Jesus Santos *et al.*, (2022) at the Bioprocess Engineering Laboratory, Institute of Technology and Research (ITP/UNIT). The system composition included Procetyl (PPG-5 CETETH-20) as surfactant, moringa oil as the oil phase, and an aqueous phase.

Initially, surfactant solutions with *S. cumini* essential oil were prepared at the following ratios: 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. These solutions were kept under magnetic stirring for 30 min for stabilization. Subsequently, aliquots of 100 μ L of ultrapure water were added until homogenization was achieved according to the predetermined ratios, under magnetic stirring, with appropriate intervals to maintain system equilibrium. During the analysis intervals, system changes were recorded.

Liquid and transparent formulations were classified as microemulsions (MEs); transparent systems with high viscosity as liquid crystals (LCs); and opaque systems as emulsions (EMs) or phase separation (PS). Phase transitions were mapped using OriginPro 8.5 software, and the selected samples for characterization were designated as microemulsion (AJ1), lamellar phase liquid crystal (AJ2), and hexagonal phase liquid crystal (AJ3).



2.3 Polarized Light Microscopy (PLM)

The optical properties of the systems obtained from the ternary phase diagram were identified by polarized light microscopy (PLM) using an Olympus BX-51 microscope equipped with an LC Color Evolution digital camera (PL-A662) and image analysis software (PixeLINK). For measurements, samples were placed on a glass slide, covered with a coverslip, and analyzed. Images were captured at 20× magnification at room temperature after a minimum equilibration period of five days [6].

2.4 Rheological Analysis

The rheological behavior of the formulations was evaluated using a Discovery Hybrid Rheometer (DHR-10, TA Instruments) with a cone-and-plate system, using a stainless steel cone of 50 mm diameter (depending on viscosity) and a 1° angle. Dynamic frequency sweep experiments (oscillatory measurements) were performed within the linear viscoelastic region. The storage (G') and loss (G'') moduli were measured over a frequency range of 0.1–100 Hz at (25.0 ± 0.2) °C, with a constant oscillation stress of 0.5 Pa. Data fitting was performed using OriginPro 8.0 software [6].

2.5 Larvicidal Activity Against *Aedes aegypti* Larvae

To determine the larvicidal activity of *Syzygium cumini* essential oil, 100 mg of the oil was separately dispersed in Tween 80 (0.25 mL) in a 10 mL beaker, followed by the

addition of distilled water (4.75 mL) to prepare stock solutions (20,000 ppm). These solutions were used to prepare 100 mL of aqueous solutions with concentrations ranging from 5 to 100 ppm, along with 20 Rockefeller strain L3/L4 larvae.

3. Results and Discussion

3.1 Ternary Phase Diagram

The phase diagram allowed understanding of the surfactant molecules' behavior in response to variations in the proportions of the aqueous and oil phases, enabling the formation of isotropic homogeneous systems (micellar and/or microemulsified), anisotropic systems (liquid crystals in lamellar, hexagonal, or cubic phases), and emulsified systems [7].

The phase separation (PS) system formulations were formed with a high content of *Syzygium cumini* essential oil, low surfactant percentages, and water ranging from 20 to 80%. This composition indicates weak interaction and compatibility among these components in such proportions, preventing the formation of homogeneous systems. Emulsions were formed at high water concentrations, around 70%, approximately 30% oil, and between 10 to 50% Procetyl, characterizing a thermodynamically unstable and reversible system [4].



Microemulsions were observed in compositions containing 10 to 30% oil, 30 to 70% water, and above 30% Procetyl, characterizing a system with optically stable properties and a stable, homogeneous solution (Jalali-Jivan, Garavand, & Jafari, 2020) [8]. The liquid crystal phase was formed by formulations containing between 40 and 60% water, 20 to 40% oil, and above 40% Procetyl. This can be explained by the increase in the aqueous phase, which facilitates the aggregation of surfactant molecules into micellar aggregates, forming bilayers at the phase interface, leading to the formation of lamellar liquid crystals [9].

Thus, the phase diagram analysis demonstrated the influence of component proportions on the formation of different systems. Among the various mapped points, three formulations were selected for a more detailed analysis of the microscopic characteristics. Points 10, 20, and 30 were chosen to represent the microemulsion, phase transition, and liquid crystal regions, respectively, characterized by Polarized Light Microscopy.

3.2 Polarized Light Microscopy

From the visualization of Image 1, it is possible to observe that this sample presented a dark field when viewed under the microscope. This is a typical characteristic of microemulsions, which possess a single

refractive index and isotropic behavior that allows polarized light to pass without modifying its propagation. This result is confirmed by the presence of an air bubble within the dark field, showing no birefringence signal [10].

Formulation AJ2, on the other hand, besides presenting a dark field due to its isotropic pattern, also shows points of birefringence without specific texture, which may indicate the presence of a system transitioning between microemulsion and liquid crystal, possibly caused by changes in composition and temperature [11].

Formulation AJ3 exhibits a texture of birefringent patterns organized in layers, which are observed in lamellar liquid crystals. This is due to the transition from an isotropic to an anisotropic system upon water addition, enabling binding with polar molecules separated by hydrophobic tails, generating a one-dimensional structure. This results in distinct axes that interact according to the orientation of the incident light, creating an aspect comparable to a Maltese cross and streaks.

3.3 Rheological Analysis

Based on the microscopy analysis, the rheological behavior of the samples was evaluated. Rheology is fundamental in the characterization of nanostructured systems,



such as microemulsions and liquid crystals, whose properties vary according to composition and interactions among aggregates [6]. Figure 2a shows that samples with 10% and 20% aqueous phase exhibited Newtonian behavior, with shear stress and shear rate indicating constant viscosity and good correlation with the expected model for microemulsions, displaying a linear relationship. All formulations showed the same profile, producing linear flow curves formulations with higher amounts of aqueous phase exhibited increased viscosity and structural organization. Figure 2b shows the storage modulus (G') and loss modulus (G'') of sample AJ3. The frequency dependence of G' over G'' at all frequencies may be related to the amount of polymer used; in formulations with higher aqueous phase content, larger polymer quantities can lead to the formation of a more robust internal structure, indicating a typical hexagonal phase structure with gel-like behavior and a high degree of organization [12].

3.4 Evaluation of Larvicidal Activity Against *Aedes aegypti* Larvae

Nanostructured formulations containing *Syzygium cumini* essential oil demonstrated significant larvicidal activity against *Aedes aegypti* L3/L4 larvae, with a marked reduction in LC_{50} values compared to the isolated oil. Formulation AJ1, characterized as an isotropic microemulsion, showed the highest efficacy

with an LC_{50} of 65.5 ppm, followed by formulation AJ2 (3.8 ppm), exhibiting phase transition characteristics, and AJ3 (4.3 ppm), with a liquid crystal structure.

In comparison, the pure oil exhibited an LC_{50} of 10 ppm, evidencing that nanostructured systems enhance the bioavailability and penetration of the active compound into the target organism. The greater efficacy of the microemulsion may be related to its lower viscosity and higher system stability, which allows greater contact between the active ingredient and the larvae. Although liquid crystals have a denser structure, they demonstrated higher efficacy than the isolated oil, possibly due to controlled release and prolonged contact time with the vector. These results confirm the potential of nanostructured systems as an effective and sustainable strategy for *Ae. aegypti* control, reinforcing the potential of such systems as an alternative vector management approach.

4. CONCLUSION

The results demonstrate that nanostructured systems containing *Syzygium cumini* essential oil, classified as microemulsions and liquid crystals, exhibited marked structural differences, as evidenced by polarized light microscopy and rheological analyses.

Larvicidal activity against *Aedes aegypti* revealed greater efficacy of the formulations compared to the isolated essential oil, with emphasis on the microemulsion AJ2, which

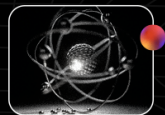


showed the lowest LC_{50} value. This performance can be attributed to higher colloidal stability, better active ingredient dispersion, and increased bioavailability.

Thus, the developed systems represent a promising technological alternative that is effective and environmentally safe for vector control, reinforcing the potential of using natural products in innovative formulations aimed at public health and the fight against arboviruses.

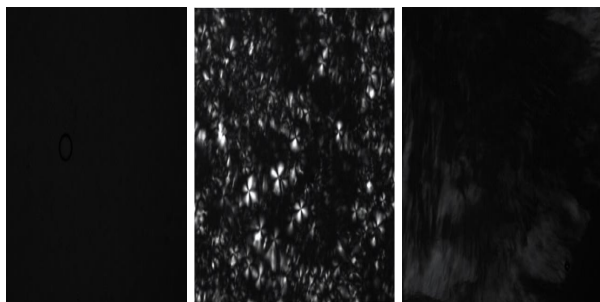
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Tables, figures and graphics

Figure 1. Micrograph at 20,000x magnification of formulations AJ1, AJ2, and AJ3 in a system containing water, Procetyl, and *Syzygium cumini* essential oil.



Graphic 2. (a) Rheogram of samples AJ1 and AJ2 and (b) Elastic modulus (G') and loss modulus (G'') as a function of frequency for sample AJ3.

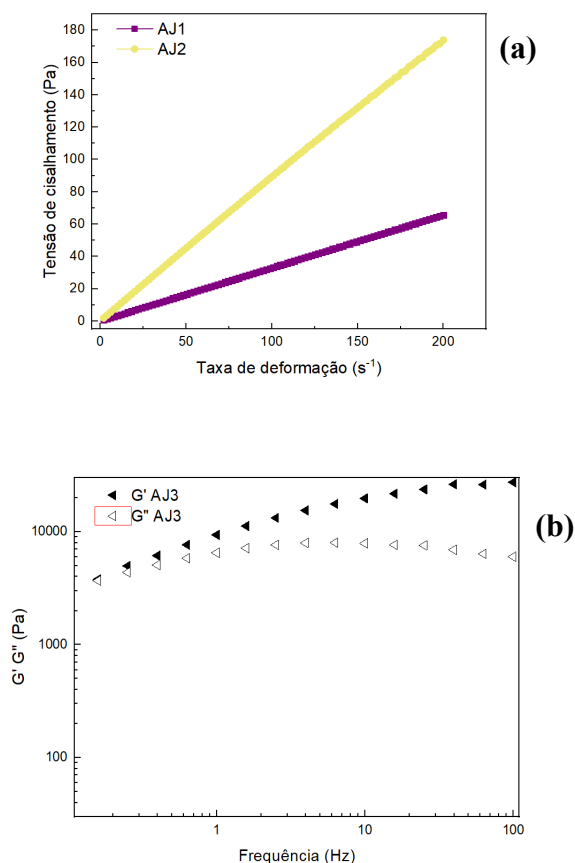


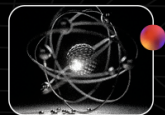
Table 1. LC_{50} values of the oil and formulations against *Ae. aegypti* L3/L4 larvae after 24 h of exposure.

Formulation	LC_{50} (95% IC) ppm
OIL	10,0 (10,3 – 10,6)
AJ1	6,5 (5,8 - 6,9)
AJ2	3,8 (3,0 - 3,3)
AJ3	4,3 (3,8 - 4,9)

95% CI – 95% confidence interval.

LC_{50} – lethal concentration required to kill 50% of the exposed population.

ppm – parts per million.



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