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Article

Larvicidal Activity of Calcium Alginate Microcapsules Containing Clove Essential Oil Obtained by Microfluidics

Débora T. Condé,[a] Luiza A. Mendes,[a] Guilherme P. Ramos,[a] Rafael R. A. Silva,[b] Alvaro V. N. C. Teixeira,[c] Róbson Ricardo Teixeira,[a] Gustavo F. Martins,[d] Cristiane I. Cerceau[a] and Renata P. Lopes[a,]*

[a]*Departamento de Química, Universidade Federal de Viçosa (UFV), Av. Peter Henry Rolfs, s/n, Campus Universitário, 36570-900 Viçosa-MG, Brazil*

[b]*Departamento de Tecnologia de Alimentos, Universidade Federal de Viçosa (UFV), Av. Peter Henry Rolfs, s/n, Campus Universitário, 36570-900 Viçosa-MG, Brazil*

[c]*Departamento de Física, Universidade Federal de Viçosa (UFV), Av. Peter Henry Rolfs, s/n, Campus Universitário, 36570-900 Viçosa-MG, Brazil*

[d]*Departamento de Biologia, Universidade Federal de Viçosa (UFV), Av. Peter Henry Rolfs, s/n, Campus Universitário, 36570-000 Viçosa-MG, Brazil*

Essential oils (EO) have diverse applications, such as antibacterial and antifungal activity. However, they are susceptible to oxidation in the presence of air, light, and moisture. In addition, they are thermally unstable. In this sense, it is necessary to develop techniques to increase the lifespan of EO. In this work, EO was obtained from cloves and characterized by different techniques. The major compounds found were eugenol (83%), eugenol acetate (9%), and β-caryophyllene (8%). The EO was encapsulated by extrusion using a microfluidic device. The sodium alginate was used as wall material, and the $CaCl_2$ solution was used as a crosslinking agent. The microcapsule presented sizes of 164.7 ± 0.3 μm, with an encapsulation yield of 64 ± 14%. Functional characteristic groups of EO were observed in the microcapsule by infrared and Raman spectroscopies. The microcapsule increased the thermal decomposition of the EO from 162 to 230 °C. Release kinetics of the capsule was performed, with an equilibrium time of 72 h and release of 54% of the EO. Finally, the pure EO and encapsulated EO-microcapsules were applied in the *Aedes aegypti* larvae control, showing mean lethal concentration necessary to eliminate 50% (LC_{50}) values of 74.4 and 96.9 μg mL^{-1} and lethal concentration necessary to eliminate 90% (LC_{90}) of 106.2 and 133.3 μg mL^{-1} for pure EO and encapsulated EO, respectively. Therefore, it was concluded that these microcapsules have the potential for application in the *Aedes aegypti* larvae control.

Keywords: *Aedes aegypti*, natural products, dengue control, microencapsulation

Introduction

The female mosquito of the genus *Aedes aegypti* (Diptera: Culicidae) is responsible for the transmission of dengue, zika, chikungunya, malaria, and yellow fever. Considering the first five months of 2020, over 1.6 million dengue cases were registered in Brazil, making this country one of the most affected in the Americas.[1] The high incidence of these viral diseases has raised concerns of health authorities and society. The increase of the number of cases is mainly due to mosquito proliferation.[2] The control of this vector is relatively difficult due to its ease of adapting to environmental changes. In addition, the mosquito mates, feeds and lays eggs during the day inside and around the people's habitation, where it is often difficult for health officials to verify its existence.[3]

Synthetic chemicals, such as pyrethroids, control larvae and mosquito *Aedes aegypti* proliferation.[4] However, the constant application of these compounds can lead to the development of resistance of the mosquito larval, not to mention the damages to the environmental and human health.[5] Due to these factors, studies[6,7] have been performed to obtain safe alternatives to control this vector. In this

*e-mail: renata.plopes@ufv.br
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sense, natural products with insecticidal and larvicidal effects have become a sustainable alternative.

Essential oils (EO) obtained from plants is a rich source of bioactive compounds that have been evaluated in the *A. aegypti* larva control.[6,7] These substances are less harmful to the aquatic environment due to their biodegradability and low toxicity to non-target organisms such as fish, for example.[8] In addition, some plant species have larvicidal properties, such as *Syzygium aromaticum*, popularly known as clove.[9] The EO obtained from clove flower buds contains mostly eugenol (4-allyl-2-methoxyphenol), with approximately 90% abundance, being effective against *A. aegypti*.[10]

However, EO presents some limitations, such as low water solubility and high volatility, besides being degraded under light, heat, and oxygen.[11] These characteristics make its direct use in an aqueous system difficult. An alternative to minimize EO degradation consists of its microencapsulation. In this technique, droplets, or very small particles of liquid or solid material are involved with a continuous film of the polymeric compound, ceramic, among other materials, obtaining small capsules. Microencapsulation provides environmental protection to the encapsulated compound, controlling its release and availability in the system.[12] Microfluidics is a versatile technique able to produce microcapsules. The advantages of the microfluidics technique include the fine control of size, shape, and composition of the capsules, in addition to providing uniformity of the materials and high yield.

Alginate is a natural anionic polysaccharide derived from brown seaweed.[13] It is found in the sodium salt form, containing α-guluronic acid (G) and β-D-mannuronic acid (M). The G and M acids are grouped into sequential blocks of the G-block, GM-block, and M-block types, due to the bonds between C-1 and C-4 carbons.[14] The alginate forms a gel in the presence of divalent cations such as calcium ions. According to Grant *et al.*,[15] the gelation occurs due to the modification of the linear alginate shape to three-dimensional network in an "eggshell". In view of the above, this work aimed to produce calcium alginate microcapsules containing clove EO by microfluidic technique and evaluate its larvicidal efficiency against *A. aegypti*.

Experimental

Standards and reagents

The reagents and solvents used were of analytical grade, including dichloromethane (99.5%, Química Moderna, Barueri, Brazil), sodium alginate ($(C_6H_7NaO_6)_n$ Dynamica, Indaiatuba, Brazil), calcium chloride (Isofar, Duque de Caxias, Brazil), sodium dodecyl sulfate (SDS) (Merck, Darmstadt, Germany), acetonitrile (99.5%, Merck, Darmstadt, Germany) and sodium hydroxide (Proquimios, Bangu, Brazil). Type 1 water, obtained from the Milli-Q system (Millipore Corporation, Burlington, United States), was used to prepare the solutions.

Extraction of essential oil from clove

The clove was obtained from a local market in Viçosa, Minas Gerais, Brazil and the material was used without pre-treatment. The extraction of essential oil was carried out by hydro distillation using a simple distillation apparatus. Thus, 30.0 g of cloves were weighed in a round bottom flask, and 400 mL of type 1 water was added. The system was heated at 100 °C, without stirring, for 3 h. The generated steam was condensed and collected, obtaining the hydrolate. This one was subjected to three sequences of liquid-liquid extraction using dichloromethane (100 mL for each extraction). Finally, the solvent was removed by a rotary evaporator for 40 min at 22 °C. The essential oil was collected, stored in a glass bottle protected from light, and stored under refrigeration at 4 °C. The EO yield was determined according to equation 1.

$$\text{Yield (\%)} = \frac{m_o}{m_i} \times 100 \quad (1)$$

where, m_0 is the mass of EO obtained, and m_i is the mass of clove.

Physicochemical characterization of the essential oil

The density of the EO was determined by weighing a precise oil volume measured using a micropipette. The assay was made in replicate (n = 4). An analytical balance with precision to the tenths of milligrams was used.

The essential oil was characterized by gas chromatography coupled with mass spectrometry (GC-MS, GC-MSQP2010 Ultra spectrometer, Shimadzu, Kyoto, Japan). The conditions used were: He as the carrier gas, with a flow of 1.80 mL min^{-1}; injector temperature was 220 °C at a split ratio of 1:10; Ultra Alloy® fused silica capillary column (30 m × 0.25 mm) containing Rtx®-5MS stationary phase (0.25 μm film thickness). The oven temperature was programmed as follows: initial temperature of 40 °C, remaining for 2 min. Then, the temperature was increased with a heating rate of 3 °C min^{-1} until reaching 240 °C and remained for 5 min. The total time of the analysis was 73.67 min. The compounds were identified according to the similarity of the library database (Wiley 7, NIST 05,

and NIST 05s). Compounds with a relative area above 1% were identified.

The EO was dissolved in acetonitrile at a concentration of 19.8 g L^{-1} and submitted to UV-Vis analysis using an Evolution Array model spectrophotometer (Thermo Scientific, Waltham, United States). The analyses were performed in scan mode at 190 to 800 nm. A quartz cuvette with a 1 cm optical path was used.

The hydrogen nuclear magnetic resonance (^{1}H NMR) spectra were obtained using a Varian Mercury 300 MHz spectrometer (Palo Alto, USA), using deuterated $CDCl_3$ as solvent and acetonitrile as internal standard. The 45° pulse (pw45) was determined for all samples using the calibration sequence implemented by Agilent Technologies' VnmrJ4.2 program. The longitudinal relaxation time (T1) was experimentally determined by the inversion recovery sequence for all hydrogens in the sample. The longest T1 obtained for validation and quantification of essential oil samples was 4 s. The number of transients (nt) of 16, acquisition time (aq) of 4.55 s, and delay time (d1) was 20 s. The analyzes were performed in replications (n = 3).[16]

$$m_{eug} = \frac{I_{eug}}{I_{acet}} \times \frac{N_{acet}}{N_{eug}} \times \frac{M_{eug}}{M_{acet}} \times m_{acet} \quad (2)$$

where m_{eug}: amount of eugenol (g); I_{eug}: integral of the eugenol signal in the ^{1}H NMR spectrum; I_{acet}: acetonitrile internal standard signal integral; N_{acet}: number of hydrogens of the chosen acetonitrile signal (H = 3); N_{eug}: number of hydrogens of the chosen eugenol signal (H = 3); M_{eug}: molar mass of eugenol (164.204 g mol^{-1}); M_{acet}: molar mass of acetronitrile (41.053 g mol^{-1}); m_{acet}: acetonitrile mass (0.04293 g).

Preparation of EO microcapsules by microfluidics

First, an oil/water emulsion was prepared mixing 150 mg of clove EO and 10 mL of sodium alginate solution (69.4 mmol L^{-1}), containing 1.00 mL of SDS solution (100 mmol L^{-1}). The surfactant SDS was used to stabilize the emulsion. The system was submitted to magnetic stirring for 10 min at room temperature (25 ± 2 °C) resulting in a homogeneous white emulsion. Then, the emulsion was placed in glass syringe and pumped to a microfluidic device using a syringe pump, whose flow rate was set at 1000 µL min^{-1}. The device consisted in a small glass capillary tube where air was flowing at a rate of approximatively 50 mL s^{-1}. The emulsion was pumped through a smaller tube inside the glass tube and, through the shear stress of the flow of air on the emulsion, regular small drops were made. The drops were collected in a container containing 40 mL of calcium chloride (100 mmol L^{-1}) to obtain the microcapsules. To compare, control experiments, i.e., microcapsules without clove essential oil were obtained under the same conditions.

The microcapsules were stored in a freezer at –27.1 °C for 72 h and after lyophilized (Lyophilizer L101 - LIOTOP, São Carlos, Brazil) for 48 h at –51 °C and 80 µHg of pressure.

Characterization of microcapsules

The morphology and size of the microcapsules were determined using an optical microscope (Carl Zeiss, Jena, Germany). The size of the microcapsules was determined by the ImageJ software,[17] wherein 30 spheres (n = 30) from each lot were analyzed.

The thermal decomposition of the materials was evaluated through the thermogravimetric analyzer, model DTG-60H (Shimadzu, Kyoto, Japan). For this characterization, a certain mass of material was weighed in an alumina crucible and heated at 10 °C min^{-1} to 600 °C, under a nitrogen atmosphere with a flow of 50 mL min^{-1}.

The Raman spectra were obtained through the MicroRaman system, InVia Renishaw, Wotton-under-Edge, UK, with 785 nm wavelength laser and 3 mW potency. The spectral resolution was 4 cm^{-1}, and signal-to-noise ratios were obtained with 730 and 3000 cm^{-1} scans. Each sample was added in a 10 mm path length quartz cuvette. The microcapsules obtained by microfluidics analyzed in the surface and also in its interior, by pressed the capsules to their rupture.

Fourier-transform infrared spectroscopy (FTIR) spectra were obtained by attenuated total reflectance (ATR) technique, using a Varian 660-IR equipment (Palo Alto, USA) with a GladiATR accessory with diamond crystal, in transmittance mode with scan from 400 to 4000 cm^{-1}.

Determination of EO encapsulation efficiency

An analytical curve of EO from clove was obtained to evaluate the EO encapsulation efficiency. A stock solution of EO in acetonitrile was prepared at 13.2 µg mL^{-1} to prepare the working solutions between 6.6 to 46.8 µg mL^{-1}. All solutions were prepared in replicate (n = 3). Each solution was analyzed by UV-Vis spectroscopy (Evolution array model of the Thermo Scientific, Waltham, United States). The monitored wavelength was 280 nm. The obtained data were adjusted to linear regression using the least squares method. The quality of fit was evaluated by the coefficient of determination using the Minitab program, version 15.[18]

The analytical curve data of essential oil (EO) is described by equation 3.

$$A = (17.06 \pm 0.34 \text{ mL mg}^{-1})C + (0.013 \pm 0.011) \quad (3)$$

where A is the absorbance and C is the EO concentration.

To quantify the encapsulated essential oil, 0.0400 g of the lyophilized microcapsules were weighed in a beaker, under 2.00 mL of a NaOH solution (40 g L^{-1}) and 18.0 mL of acetonitrile. The system was stirred for 24 h at room temperature (ca. 25 °C). Subsequently, an aliquot of this solution was analyzed by UV-Vis spectroscopy, at 280 nm wavelength and the EO concentration determined by equation 3. The encapsulation efficiency (EE in percentage) was determined according to equation 4.

$$EE\ (\%) = \frac{M_{oe}}{M_{ioe}} \times 100 \quad (4)$$

where M_{oe} is the encapsulated essential oil mass; M_{ioe} is the initial essential oil mass.

Release kinetics of EO from microcapsules

The release of EO from microcapsules was performed according to Babaoglu and co-workers.[19] The lyophilized microcapsules (100 mg) were dispersed in 1.00 mL of NaOH solution (40.0 g L^{-1}), and 200 mL of acetonitrile were added to the system. The system was submitted to constant agitation (580 rpm), and aliquots were taken at different time intervals (0 to 88 h) for UV-Vis analyses. All removed aliquots for analysis were returned to continue the experiment. The EO concentrations were determined through the analytical curve (equation 3).

The nonlinear model was adjusted to an experimental data as described in the equation 5.

$$AR(t) = AR_{max} - (AR_{max} - AR_0)e^{-t/\tau} \quad (5)$$

where AR is the accumulated release, AR_0 and AR_{max} are the initial and maximal (saturation) value of AR, t is the time and τ is the characteristic time of the release of EO.

Finally, the accumulated mass of essential oil removed, expressed as a percentage, was determined according to equation 6.

$$\text{Released EO mass } (\%) = \left(\frac{C \times V_t}{m_{cap}}\right) \times 100 \quad (6)$$

where C is the EO concentration; V_t is the total volume of the system; m_{cap} is the mass of the lyophilized microcapsules.

Application of EO microcapsules in *Aedes aegypti* larvae control

The larvicidal activity of EO from cloves and microcapsules were performed with fourth-instar *A. aegypti* larvae (L4) (PPCampos strain, Campos dos Goytacazes, RJ, Brazil), obtained from a colony of successive generations maintained in the insectary of the Department of General Biology (Universidade Federal de Viçosa). After the eggs hatch, the larvae were kept in dechlorinated tap water, fed daily with turtle food (Reptolife, Alcon Pet, Camburiú, SC, Brazil), and maintained at a controlled temperature (28 °C) for 12 h of photoperiod until L4. This stage is ideal for carrying out larvicidal action trials.[20]

For EO from clove assays, solutions were prepared at concentrations from 10 to 130 μg mL^{-1} in dimethylsulfoxide (DMSO) 1% (v v^{-1}). The negative control was prepared with 1% (v v^{-1}) DMSO in water. For microcapsules assays, suspensions were prepared at dosages from 80 to 180 μg mL^{-1} in water. The negative control was prepared with the microcapsules without the EO in water. Assays were performed with 100 L4 *per* treatment. Larvae were placed in glass vials (150 mL) containing 50 mL of solutions with EO or only DMSO in water (control). For each treatment or control, the total number of larvae (i.e., 100) was divided into four batches (i.e., replications) of 25 larvae. The assays were maintained under controlled temperature (28 °C) for 12 h of photoperiod. The negative controls were performed simultaneously with the positive assays. After 24 h, the dead larvae were counted. Larvae immobile to the touch with a Pasteur pipette were considered dead. To determine the values of mean lethal concentration necessary to eliminate 50% (LC_{50}) and 90% (LC_{90}) of the population, the data were adjusted to Probit model, with normal distribution at 95% significance, through Minitab Program, version 15.[18]

Results and Discussion

Essential oil extraction and characterization

The EO was obtained from the clove flower buds via hydro distillation, with a 10.6% m/m yield, a value that is in agreement with the average yield for eugenol essential oil (11.5%) obtained by hydro distillation.[21] The EO presented a transparent appearance, with a characteristic odor and a density of 1.15 g mL^{-1}. The analysis of the EO by gas chromatography coupled to mass spectrometry showed the presence of three peaks, with retention times of 29.9, 32.2, and 37.0 min, which were attributed to

compounds eugenol (**1**), β-caryophyllene (**2**), and eugenol acetate (**3**) (Figure 1), with a yield of 83, 8, and 9%, respectively. These results are similar to those obtained by other authors.[22-24]

Table S1 in the Supplementary Information (SI) section summarizes the results found in the GC-MS analysis of the clove EO. Besides, in Figures S1-S3 (SI section), mass spectra that confirm the identification of the essential oil components are provided.

The EO obtained was also characterized by ^{1}H NMR. The ^{1}H NMR spectrum of EO showed nine signals attributed to different hydrogens of eugenol, highlighted by letters (A-H) and marked in the structure of the Figure S4. A peak can be observed at δ 3.81 with integration of 227.65. This signal is attributed to the methoxy group of eugenol represented by H letter. Other authors obtained similar results.[25,26] The signal close to δ 2.00 with integrated area equal to 1000, is attributed to the hydrogen of acetonitrile, represented by J letter (inset of Figure S1). The eugenol content was determined according to equation 2, with eugenol content of 88.4 ± 0.1%. This result agrees with GC-MS result, whose content was 83%. In the SI section, the assignments for hydrogens of eugenol, the main component of the essential oil, are provided (Figure S5).

The UV-Vis spectrum of EO solution in acetonitrile is shown in Figure S6 (SI section). Three characteristic bands can be observed in the ultraviolet region. The first one, close to 210 nm, can be attributed to the $\pi \rightarrow \pi^*$ transition due to double bonds present in eugenol. The second one, close to 230 nm, can be attributed to the $n \rightarrow \pi^*$ transition due to the ester group of eugenol acetate. The third one at 280 nm can be attributed to the $n \rightarrow \pi^*$ due to phenol transition. Other authors have reported similar results.[27,28] Such results prove the efficiency of EO extraction from cloves.

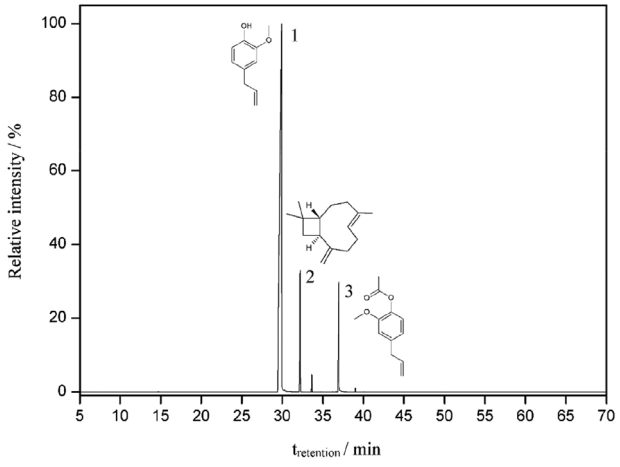

Figure 1. Clove essential oil chromatogram obtained by GC-MS analysis. Eugenol (**1**), β-caryophyllene (**2**), and eugenol acetate (**3**).

Characterization of microcapsules

The EO emulsion stabilized by SDS, after stirring, showed a milky appearance and a significant amount of foam due to the surfactant as shown in Figure 2a. This occurs because of the dispersion of the gas (air) in the liquid (DMSO), indicating an excess of the used emulsifier. To destabilize the foam-type colloid (gas/liquid) produced, the system was kept at rest for 40 min (Figure 2b). Optical microscopy images of the obtained microcapsules are shown in Figure 2c. It is possible to observe spherical microcapsules with good size uniformity, 164.7 ± 0.3 μm. Furthermore, two microcapsule contrast patterns (*i*) dark and (*ii*) slightly gray are visualized. Blocking visible light by dark microcapsules can result from the thicker layer of sodium alginate and the presence of microencapsulated EO. However, in general, few microcapsules are slightly gray, indicating good homogenization in shape and encapsulation.

A scheme of the sodium alginate structure before and after crosslinking forming the microcapsules and trapping the EO is shown in Figure 3.

The thermal decomposition of the EO and the materials obtained by microfluid with and without the EO was determined by thermogravimetric analysis (TGA). The results are shown in Figure 4. For EO, a thermal event is observed between 75-165 °C, with a peak on the DTGA (the first derivative of the TGA curve) curve at 162 °C. This result corresponds to a mass loss of 99.5%, which can be attributed to EO decomposition. Fang and co-workers[29] obtained similar result.

For microcapsules containing EO (Figure 2), there is mass loss of 40% at ca. 100 °C, which can be attributed to humidity present in the sample due to hydrophilic character of calcium alginate.[30] The second thermal event, around 230 °C, with a mass loss of ca. 30%, can be attributed to the formation of sodium carbonate.[31] The third thermal event occurs around 400 °C, which can be attributed to the carbonization of the alginate polymer chains and, therefore, to the decomposition of EO. Pure alginate presented a thermal event around 100 °C due to the loss of mass of ca. 20%, attributed to the humidity of the sample. Another thermal event occurred between 250 and 500 °C, where the total degradation of the alginate is observed. From the TGA results, the increase in the EO decomposition temperature was confirmed, due to the small mass variation (< 2%) of the capsule-EO in the EO decomposition temperature range (100-165 ºC).

The EO and lyophilized calcium alginate microcapsules with and without EO were analyzed by Raman spectroscopy (Figure 5).

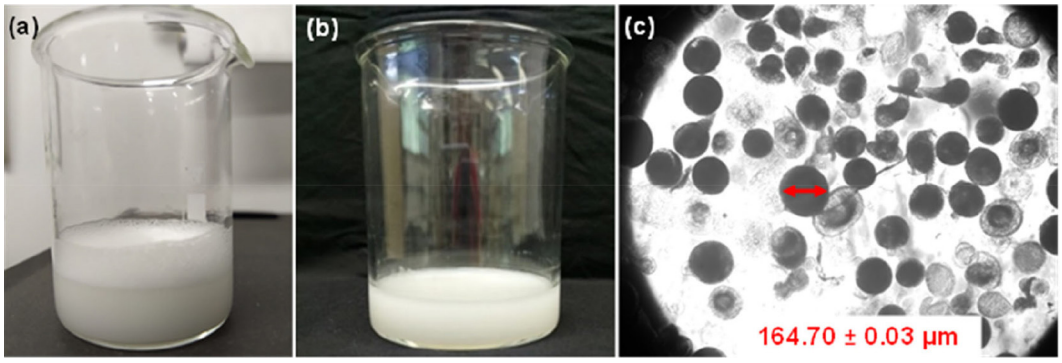

Figure 2. Essential oil emulsion containing SDS (a) after stirring, (b) after keeping the system undisturbed (c) image of EO microcapsules obtained by optical microscopy.

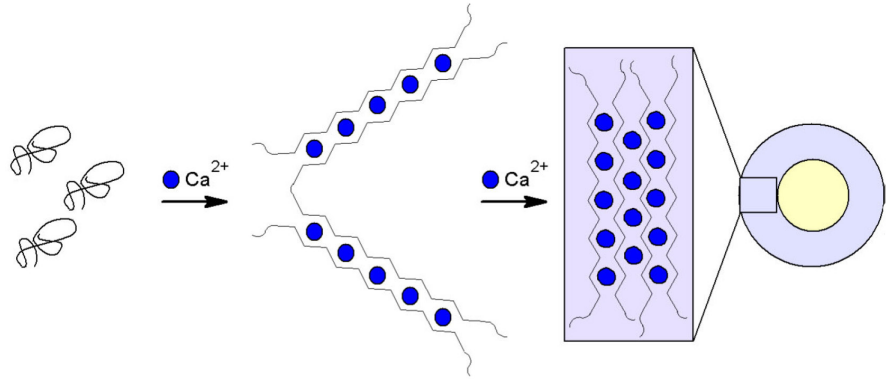

Figure 3. Representation of the structure of sodium alginate before and after cross-linking forming the microcapsules and trapping the EO.

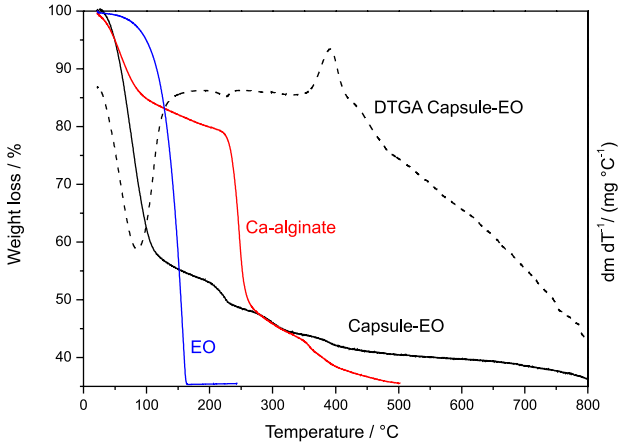

Figure 4. Thermogravimetric analysis of freeze-dried microcapsules without EO (Ca-alginate) in red color, freeze-dried microcapsules containing EO (capsule EO) in black color, essential oil (EO) from clove in blue color and DTGA from capsule-EO in dashed lines in black.

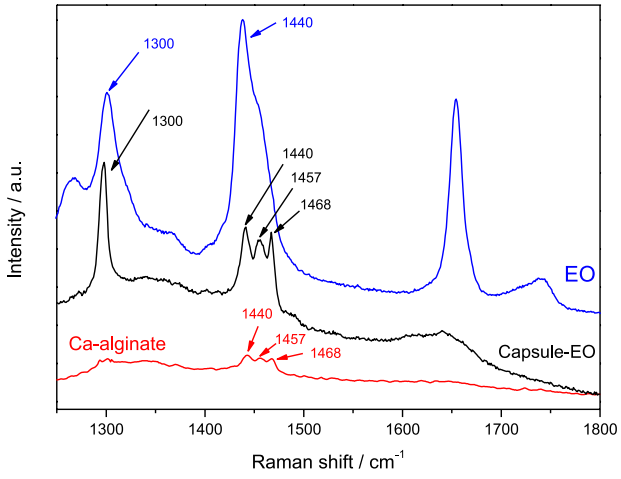

Figure 5. Raman spectra of the essential oil (EO) from clove, freeze-dried microcapsules containing EO (capsule EO), and freeze-dried microcapsules without EO (Ca-alginate).

For EO, the band in 1300 cm^{-1} can be attributed to Ar–O stretching mode (where Ar = benzene ring) of eugenol.[32] Bands in ca. 1440 cm^{-1} can be attributed to bending modes of CH_3 and CH_2 groups.[32] The band in ca. 1650 cm^{-1} can be attributed to C=C stretching mode due to the double bond present in the eugenol.[32] Ca-alginate, without the oil, did not show characteristic bands of the EO. These bands can be attributed to the CH_2 and CH_3 deformation vibration.[33] For microcapsules containing EO, in addition to the characteristic bands of alginate, EO bands can be observed, as in 1300 and 1650 cm^{-1}. Therefore, these results indicate that the oil was encapsulated.

The materials were also analyzed by FTIR and the results are shown in Figure 6. The EO spectrum showed a band at 3516 cm^{-1} that can be attributed to the stretching

vibration of hydroxyl groups (*ν*OH). The bands in 2900 and 2834 cm^{-1} can assign to asymmetrical and symmetrical stretching of C_{sp3}–H bonds (*ν*C–H). The band at 1513 cm^{-1} can be attributed to the C=C stretching of aromatic moiety. The peaks at 1431 and 1265 cm^{-1} can be attributed to CH_2 deformation vibration due to presence of eugenol and eugenol acetate in the EO.[34]

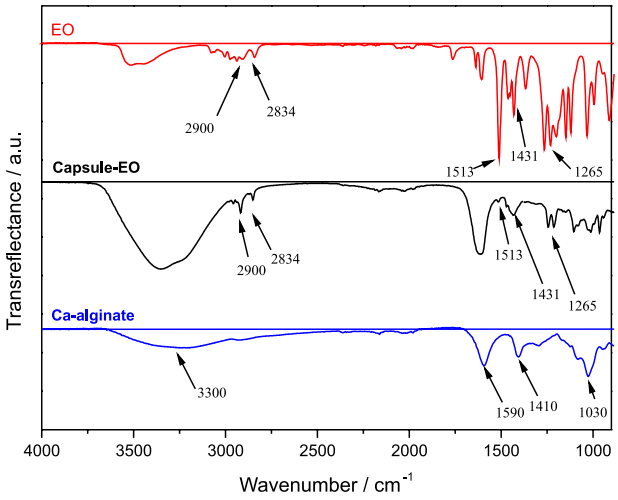

Figure 6. FTIR (ATR) spectra of the essential oil (EO) from clove, freeze-dried microcapsules containing EO (capsule EO), and freeze-dried microcapsules without EO (Ca-alginate).

The lyophilized microcapsules containing EO (Figure 6), showed characteristic EO bands at 2900, 2834, 1513, 1431, and 1265 cm^{-1}. However, the lyophilized microcapsules without EO did not show the EO characteristic bands. For microcapsules without EO, it can be observed that a broad band at 3300 cm^{-1} is attributed to the stretch vibration of hydroxyl groups. Others intense peaks at 1590 and 1410 cm^{-1} can be attributed to the asymmetric and symmetrical stretching of the carboxyl groups. The band at 1030 cm^{-1} can be attributed to symmetrical and asymmetrical vibrations of C–O–C bonds typical of polysaccharide rings.[35] These results indicate the presence of EO in the microcapsules.

The encapsulation efficiency (EE) calculated using the equation 4 was determined using an analytical curve, equation 3. The curve presented a satisfactory linear fit, evaluated by the coefficient of determination ($R^2 = 0.997$). The release profile of the EO encapsulated in the microcapsules is shown in Figure 7. The nonlinear model (equation 5) was adjusted to release kinetics data. A first rapid release step (burst effect) is observed in 30 min with approximately 10%. Possibly, this release refers to the oil present on the surface of the microcapsule. From the fit to the experimental data, it was obtained a maximum of release of ca. 54% ($AR_{max} = 54.1 \pm 1.1$) and a characteristic time of 23 h ($\tau = 23.3$ h). The fit also shown an initial value of AR equals to 3.5% ($AR_0 = 3.5 \pm 2.0$) probably due to the oil present on the surface of the microcapsule that are rapidly liberated when put in solution.

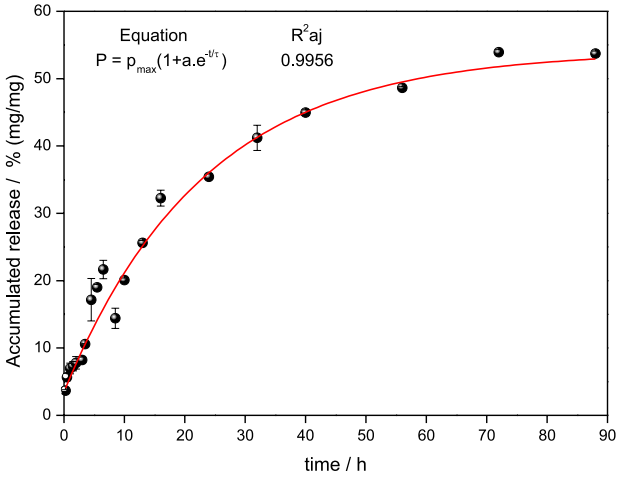

Figure 7. Kinetic release of clove EO from freeze-dried microcapsules.

The slow release of EO in the calcium alginate microcapsules after the first hour can be explained by the rigid polymeric chains.[35,36] The addition of NaOH increases the pH of the system, allowing it to be swollen and disintegrated under mild alkali conditions quickly.[37] The microcapsules release 54% of the EO in 72 h, remaining constant until 88 h. From the obtained data from the EO released, it was possible to determine the encapsulation efficiency, which was (64 ± 14)%.

Application of EO-microcapsules in *Aedes aegypti* larvae control

The pure EO was applied to *A. aegypti* larvae control. The EO in DMSO presented a LC_{50} and LC_{90} of 76.37 (74.13-78.61) and 106.19 (102.88-110.02) μg mL^{-1}, respectively. The negative control, i.e., DMSO solvent, showed no mortality. Therefore, it can be concluded that the EO from clove is high toxic to larvae since it presented $LC_{50} < 100$ μg mL^{-1}, being considered an active mixture against larvae according to literature.[38] Thus, this EO can be considered promising due to its high toxicity. Similar results are described by other authors.[39]

The EO-microcapsules were also applied to *A. aegypti* larvae control. The results are shown in Figure 8. The EO-microcapsules presented an LC_{50} and LC_{90} of 96.89 (93.38-100.40) and 133.27 (128.25-139.27) μg mL^{-1}, respectively. The negative control, i.e., calcium alginate microcapsules without the EO, showed no mortality. Calcium alginate is environmentally friendly since this

material is a food ingredient and a food additive with several reported benefits.[40] Although the lethality control of the EO-microcapsules is lower than EO, unprotected oil can be degraded under environmental conditions and may lose its action in a short period of time. The advantage of the encapsulation consists in the protection of the EO by a natural polymer, allowing a larvicidal efficiency against the *A. aegypti* vector.

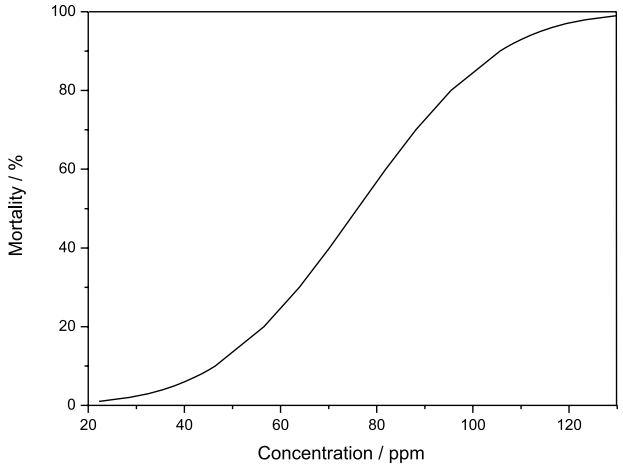

Figure 8. Mortality curve of *Aedes aegypti* larvae by essential oil from calcium alginate microcapsules.

One last aspect deserves comments. Duran *et al.*[41] describe in a very recent review, the advances observed in the field of microcapsulation by droplet microfluidics. It points out to the fact that manufacture of microencapsulated materials can reach industrial-scale production and extend the use and applications of this technique. Therefore, it is possible to envisage that large-scale production of the microencapsulated eugenol essential oil may be possible and the resulting material may be applied at home to control *A. aegypti* larvae.

Conclusions

In view of the results obtained, it can be concluded that the essential oil from clove was successfully obtained and encapsulated using the microfluidic technique, with an encapsulation yield greater than 60%. The EO encapsulation efficiency was confirmed by different techniques, such as TGA, UV-Vis, FTIR, and Raman spectrometry. Furthermore, an increase in the decomposition temperature of the essential oil was observed when microencapsulated. The microcapsules containing EO showed larvicidal activity against *A. aegypti*, demonstrating its potential for application in the fight against dengue. The material produced is environmentally safe, easy processing, and can be considered eco-friendly.

Supplementary Information

Supplementary information is available free of charge at http://jbcs.sbq.org.br as PDF file.

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Author Contributions

Débora T. Condé was responsible for data curation, formal analysis, investigation, methodology, validation, writing original draft; Luiza A. Mendes for formal analysis, investigation, methodology, writing review and editing; Guilherme P. Ramos for methodology; Rafael Silva for formal analysis, investigation, methodology, writing review and editing; Alvaro Teixeira for conceptualization, formal analysis, funding acquisition, investigation, methodology, writing review and editing; Robson Teixeira for investigation, methodology, writing review and editing; Gustavo F. Martins for formal analysis and methodology; Cristiane I. Cerceau for formal analysis, methodology; Renata P. Lopes for conceptualization, data curation, funding acquisition, investigation, supervision, writing-original draft, review and editing.

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