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Multi-stage insecticidal activity of *Myrciaria floribunda* (H.West ex Willd.) O.Berg (Myrtaceae) essential oil against *Aedes aegypti* (Linnaeus, 1762) (Culicidae): an integrated chemical, morphological, and *in silico* approach

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Aedes aegypti is an important vector of dengue, Zika, chikungunya, and yellow fever, highlighting the need for effective vector control. This study evaluated the insecticidal activity of essential oil (EO) from *Myrciaria floribunda* leaves against eggs, larvae, and adult females of *A. aegypti*, integrating chemical characterization, ultrastructural analysis, and in silico ADMET predictions. Hydrodistillation yielded 0.57 ± 0.05% EO, with 1,8-cineole (18.85%), *trans*-caryophyllene (14.60%), *α*-pinene (6.71%), *β*-elemene (6.15%), and alloaromadendrene (5.40%) as major constituents. The EO exhibited concentration-dependent ovicidal activity, with IC_{50} and IC_{90} values of 45.27 and 135.15 µg/mL, respectively. Scanning electron microscopy revealed egg structural damage, including fissures, ruptures, exochorionic disruption, and operculum displacement. Larvicidal LC_{50} values were 6.46, 6.56, and 5.16 µg/mL at 24, 48, and 72 h, respectively, accompanied by larval morphological alterations. Against adult females, LC_{50} and LC_{90} values were 0.70 and 7.10 µg/mL, respectively; however, the LC_{50} was extrapolated below the lowest concentration tested. *In silico* ADMET predictions indicated high intestinal absorption of the major constituents and no predicted inhibition of the evaluated CYP450 enzymes. Predicted skin sensitization was identified for 1,8-cineole, *trans*-caryophyllene, and *β*-elemene. These findings demonstrate activity across multiple developmental stages and support further investigation of *M. floribunda* EO as a plant-derived mosquito control agent.

Keywords: *Aedes aegypti*; Botanical insecticide; Mosquito control; Morphological alterations; ADMET.

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1. Introduction

Dengue remains a major public health concern, particularly in tropical and subtropical regions, where its increasing incidence and geographic expansion have been associated with factors such as climate change, rapid and unplanned urbanization, and the widespread distribution and adaptability of its primary vector, *Aedes aegypti* (Linnaeus, 1762) (Culicidae)[1,2]. This epidemiological burden is particularly evident in Brazil, where 1,643,215 dengue cases were reported in 2025, followed by 442,759 cases reported through August 2026[3].

The control of *A. aegypti* populations has historically relied on synthetic insecticides; however, their effectiveness has been increasingly compromised by the widespread development of insecticide resistance[4]. Resistance in *A. aegypti* populations involves multiple mechanisms, particularly enhanced metabolic detoxification associated with increased activity or expression of enzymes such as cytochrome P450

monooxygenases and esterases, and target-site alterations, including knockdown resistance (*kdr*) mutations in the voltage-gated sodium channel (VGSC), which are primarily associated with pyrethroid resistance[5,6].

This scenario, together with concerns regarding the environmental impacts of conventional insecticides, their potential adverse effects on non-target organisms, and the development of insecticide resistance, has intensified the search for more sustainable control strategies within integrated vector management[7–9]. In this context, botanical insecticides have attracted increasing attention as potential alternatives or complementary tools to conventional chemical insecticides[10]. Among these, essential oils (EOs) are complex mixtures of volatile secondary metabolites, commonly dominated by monoterpenes and sesquiterpenes, and have demonstrated insecticidal activity against *A. aegypti*[11].

The activity of EOs may involve multiple molecular and physiological targets, including neurophysiological pathways and cellular energy metabolism, with studies in *A. aegypti* reporting effects on acetylcholinesterase activity and mitochondrial electron transport[12]. Their biological effects may also extend across different stages of the mosquito life cycle, encompassing ovicidal, larvicidal, adulticidal, and repellent activities[13,14]. Morphological analyses can complement mortality-based assays by providing evidence of structural alterations associated with EO exposure in different developmental stages[15,16].

Despite their insecticidal potential, the application of EOs in vector control also requires consideration of the pharmacokinetic and toxicological properties of their individual constituents. In this context, *in silico* ADMET approaches can provide an initial computational screening of pharmacokinetic characteristics and potential

toxicological liabilities, supporting the prioritization of compounds for subsequent experimental validation[17].

Within this context, Myrtaceae has received particular attention as a source of EOs with insecticidal properties, with activity against *A. aegypti* reported across different genera, including *Eucalyptus*[18], *Eugenia*[19], *Myrcia*[20], and *Algrizea*[21], encompassing larvicidal, adulticidal, and oviposition-related effects and highlighting the potential of this family as a source of bioactive compounds for mosquito control.

Among the species of this family, *Myrciaria floribunda* (H.West ex Willd.) O.Berg is a shrub or tree widely distributed throughout tropical America and occurring across different Brazilian phytogeographic regions[22]. Its EO exhibits considerable chemical variability among plant organs and populations, with profiles dominated by either monoterpenes or sesquiterpenes and compounds such as 1,8-cineole, terpinolene, α-phellandrene, nerolidol, and β-selinene reported among the major constituents[23,24]. More recently, larvicidal activity against *A. aegypti* has been reported for the leaf EO[25] and for an EO based nanoemulsion[26].

However, the effects of *M. floribunda* EO across multiple life stages of *A. aegypti* remain insufficiently explored. Therefore, this study evaluated the ovicidal, larvicidal, and adulticidal activities of the EO, integrating chemical characterization, morphological analyses, and *in silico* ADMET predictions to provide a broader assessment of its potential for mosquito control.

2. Materials and Methods

2.1. Reagents and Reference Compounds

The bioassays utilized the following standards: spinosad (Spinosyn A, Spinosyn D), Cielo ULV (imidacloprid 3.0% w/w, and prallethrin 0.75% w/w). For ultrastructural analyses and fixation, 2.5% (v/v) glutaraldehyde in 0.1 M sodium phosphate buffer (Na-

Pi) (pH 7.2), analytical ethanol, liquid carbon dioxide (CO_2), and gold-palladium (Au-Pd) alloy were employed. Dimethyl sulfoxide (DMSO), analytical grade (≥99.0–99.9% purity, P.A.), was used as a solubilizing agent for the EO (Sigma-Aldrich®).

2.2. Plant Material

The collection of *M. floribunda* leaves was authorized by the Brazilian Biodiversity Authorization and Information System (SISBIO; permit Nº. 93798-1). Plant material was collected in September 2023 at the Adolpho Ducke Forest Reserve (2°55′47.26″S, 59°58′30.61″W), Manaus, Amazonas, Brazil. Taxonomic identification was performed at the Herbarium of the National Institute of Amazonian Research (INPA) by morphological comparison with a previously identified voucher specimen deposited in the herbarium under accession Nº. 185616, with assistance from herbarium technician Nory Daniel de Carvalho Erazo. Access to Brazilian genetic heritage was duly registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen; registration Nº. ADC693C). Plant collection and research activities complied with applicable Brazilian biodiversity legislation. Relevant international conservation guidelines were also considered, including the International Union for Conservation of Nature (IUCN) Policy Statement on Research Involving Species at Risk of Extinction and the provisions of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), where applicable.

2.3. Essential oil extraction

Dried leaves of *M. floribunda* were subjected to three independent hydrodistillation procedures using a Clevenger-type apparatus for 3 h, with 250 g of dried plant material used for each extraction. Following hydrodistillation, the EO was separated from the aqueous phase, dried over anhydrous sodium sulfate, transferred to amber glass vials, and stored at − 4 ± 1 °C until analysis[27].

The EO yield was determined independently for each extraction as the ratio between the mass of EO obtained and the initial dry mass of plant material used for extraction and expressed as a percentage (w/w), according to the following equation: EO yield (%) = [mass of EO (g) / mass of dry plant material (g)] × 100[28].

2.4. Chemical characterization (GC–MS and GC–FID)

Qualitative and quantitative analyses of the EO were performed by gas chromatography–mass spectrometry (GC–MS) and gas chromatography–flame ionization detection (GC–FID), respectively. The EO was diluted in ethyl acetate (1 mg/mL), and 1 μL of the solution was injected into each chromatographic system.

GC–MS analysis was performed using a Trace GC Ultra system coupled to an ISQ mass spectrometer (Thermo Scientific), equipped with a DB-5/MS capillary column (30 m × 0.25 mm × 0.25 μm). Helium was used as the carrier gas, with a split ratio of 1:50. The oven temperature was programmed from 60 to 240 °C at 3 °C/min, with electron ionization at 70 eV and mass acquisition in the *m/z* 40–600 range. GC–FID analysis was performed using an Agilent G-1860 Plus system equipped with an HP-5 capillary column (30 m × 0.32 mm × 0.25 μm), using hydrogen as the carrier gas at a flow rate of 4 mL/min and the same oven temperature program.

Retention indices (RIs) were calculated using a homologous series of *n*-alkanes (C_8–C_{30}) according to Van den Dool and Kratz[29]. EO constituents were identified by comparing their mass spectra and retention indices with literature data and reference libraries such as NIST[30] and the in-house library.

2.5. ***Aedes aegypti*** **colony maintenance**

Mosquitoes were maintained at the Malaria and Dengue Laboratory of the National Institute of Amazonian Research (INPA) under controlled conditions of temperature (27 ± 2 °C) and relative humidity (75 ± 5%)[31,32]. Eggs from the laboratory

149 colony were immersed in distilled water for hatching, and larvae were fed a feline diet
150 (Whiskas®) until reaching the developmental stages required for the bioassays. The
151 remaining larvae were maintained until pupation, after which pupae were transferred to
152 entomological cages (model T-1; 30 × 30 × 30 cm) for adult emergence.

153 The use of *Mesocricetus auratus* (Cricetidae) as blood-meal hosts for female
154 *Aedes aegypti* was approved by the Animal Ethics Committee of the National Institute
155 for Amazonian Research (INPA), Brazil (Protocol No. 100/2023, SEI-INPA). All
156 procedures involving animals were conducted in accordance with the applicable
157 institutional guidelines, the Brazilian National Council for the Control of Animal
158 Experimentation (CONCEA) guidelines, and Brazilian Law No. 11,794/2008. The
159 animals were obtained from the INPA Central Animal Facility (Laboratório Temático
160 Biotério Central). Before blood feeding, hamsters were anesthetized by intraperitoneal
161 administration of ketamine hydrochloride (10%) and xylazine hydrochloride (2%), at
162 reported doses of 20 mg/kg and 2 mg/kg, respectively. The anesthetic mixture was
163 administered at a reported volume of 0.2 mL and a 3:1 ketamine-to-xylazine ratio.
164 Anesthetized animals were carefully positioned on entomological cages (model T-1; 30
165 × 30 × 30 cm) containing female mosquitoes for blood feeding. Following the procedure,
166 the animals were returned alive to the INPA animal facility, with access to water and food
167 and appropriate bedding. No euthanasia was performed.

168 Adult mosquitoes were maintained on a 10% (w/v) sucrose solution, with blood
169 meals provided by the anesthetized hamsters to support colony reproduction.

170 **2.6. Bioassays**

171 All bioassays were conducted at the Malaria and Dengue Laboratory of INPA, under
172 controlled conditions of temperature (27 ± 2 °C) and relative humidity (75 ± 5%).

173 **2.6.1 Ovicidal and morphological analysis**

The ovicidal activity of the EO was evaluated using a concentration–response bioassay based on previously described procedures[15,33]. Groups of 20 *A. aegypti* eggs were exposed to EO concentrations of 31.25, 62.5, 125, 250, and 500 µg/mL in plastic containers (300 mL) containing 100 mL of distilled water. EO solutions were prepared in 1% DMSO, which was also used as the negative control, while Spinosad (1 µg/mL) served as the positive control. Each treatment was performed in quintuplicate, with a total of 100 eggs per concentration. After 120 h of exposure, ovicidal activity was determined based on the percentage of hatching inhibition, calculated as [(total number of eggs − number of hatched eggs) / total number of eggs] × 100.

For morphological analysis, control and EO treated eggs were fixed in glutaraldehyde, dehydrated through a graded ethanol series, subjected to CO_2 critical-point drying, and examined by scanning electron microscopy (SEM; JSM-5800LV) to assess alterations in chorionic morphology[34,35]. Eggs treated with EO at 125 µg/mL were selected for SEM analysis as representative of the tested concentration range, considering the time and resources required for sample preparation.

2.6.2 Larvicidal and morphological analysis

Larvicidal activity was evaluated following the World Health Organization[36] protocol, with minor modifications. Briefly, third-instar *A. aegypti* larvae were exposed to five EO concentrations of 3.90, 7.81, 15.62, 31.25, and 62.50 µg/mL, previously prepared in 1% DMSO. For each concentration, 10 larvae were placed in 300-mL plastic containers containing 100 mL of water. DMSO (1%) was used as the negative control and Spinosad (1 µg/mL) as the positive control, under the same experimental conditions.

Each treatment was tested in quintuplicate, with the bioassay independently repeated three times, totaling 750 larvae exposed to the treatments. Mortality was assessed after 24, 48, and 72 h of exposure, and larvicidal activity was expressed as

199 percentage mortality, calculated by dividing the number of dead larvae by the total
200 number of exposed larvae and multiplying by 100[37].

201 To investigate treatment-induced morphological alterations, larvae exposed to the
202 EO at 31.25 µg/mL for 24 h were compared with those from the negative and positive
203 control groups. After exposure, larvae were immobilized by cooling and examined under
204 a Zeiss SteREO Discovery V20 stereomicroscope. Images were acquired using focal
205 stacking (Z-stack) to increase the depth of field and facilitate the assessment of
206 morphological alterations in selected structures, including the digestive tract, respiratory
207 siphon, and anal papillae.

208 **2.6.3 Adulticidal Activity**

209 The adulticidal activity was evaluated using a bottle bioassay based on the World
210 Health Organization standard operating procedure for testing insecticide susceptibility in
211 adult mosquitoes[38]. The EO was diluted in 1% acetone to prepare test solutions at
212 concentrations of 2, 5, 10, 40, and 50 µg/mL. For each concentration, 1 mL of the
213 corresponding EO solution was introduced into a glass bottle (250 mL) and distributed
214 over the entire inner surface to ensure uniform impregnation. The bottles were maintained
215 for 12 h to allow complete evaporation of the acetone before mosquito exposure.

216 Three independent replicates were performed for each concentration, with 15 non-
217 blood-fed female *A. aegypti* mosquitoes per replicate (n = 45 per concentration). After
218 solvent evaporation, mosquitoes were introduced into the impregnated bottles, and
219 mortality was recorded at 15 min intervals throughout the 90 min exposure period. Bottles
220 treated with acetone alone served as the negative control, whereas Cielo® insecticide at
221 0.00626 µg/mL was used as the positive control.

222 Mosquitoes were considered dead when they were unable to stand or fly
223 following gentle stimulation. Adulticidal activity was expressed as percentage mortality,

calculated as the number of dead mosquitoes divided by the total number of exposed mosquitoes and multiplied by 100.

2.7. *In silico* ADMET prediction

The pharmacokinetic and toxicity-related properties of the major identified constituents of *M. floribunda* EO and selected reference compounds were predicted using the pkCSM platform[17]. Canonical SMILES representations were retrieved from PubChem and used as molecular inputs. The major EO constituents were evaluated alongside imidacloprid and prallethrin, whereas spinosyn A and spinosyn D were assessed as individual molecular structures. The evaluated endpoints included human intestinal absorption, skin permeability, P-glycoprotein (P-gp) substrate status, cytochrome P450 (CYP450) inhibition, AMES mutagenicity, hepatotoxicity, skin sensitization, and minnow toxicity. Compliance with Lipinski's Rule of Five was also recorded as a drug-likeness descriptor. Numerical predictions were interpreted according to the endpoint-specific units and definitions provided by the prediction platform. All results were treated as computational predictions rather than experimental evidence of pharmacokinetic behavior or toxicity.

Compound	**SMILES**
1,8-Cineole	CC1(C2CCC(O1)(CC2)C)C
trans-Caryophyllene	C/C/1=C\CCC(=C)[C@H]2CC([C@@H]2CC1)(C)C
α-Pinene	CC1=CCC2CC1C2(C)C
β-Elemene	CC(=C)[C@@H]1CC[C@@]([C@@H](C1)C(=C)C)(C)C=C
Alloaromadendrene	C[C@@H]1CC[C@H]2[C@@H]1[C@H]3[C@H](C3(C)C)CCC2=C
Spinosyn A	CC[C@H]1CCC[C@@H]([C@H](C(=O)C2=C[C@H]3[C@@H]4C[C@@H](C[C@H]4C=C[C@H]3[C@@H]2CC(=O)O1)O[C@H]5[C@@H]([C@@H]([C@H]([C@@H](O5)C)OC)OC)OC)C)O[C@H]6CC[C@@H]([C@H](O6)C)N(C)C

Compound	SMILES
Spinosyn D	CC[C@H]1CCC[C@@H]([C@H](C(=O)C2=C[C@H]3[C@@H]4C[C@@H](C[C@H]4C(=C[C@H]3[C@@H]2CC(=O)O1)C)O[C@H]5[C@@H]([C@@H]([C@H]([C@@H](O5)C)OC)OC)OC)C)O[C@H]6CC[C@@H]([C@H](O6)C)N(C)C
Imidacloprid	C1CN(/C(=N/[N+](=O)[O-])/N1)CC2=CN=C(C=C2)Cl
Prallethrin	CC1=C(C(=O)CC1OC(=O)C2C(C2(C)C)C=C(C)C)CC#C

Table 1. SMILES (*Simplified Molecular Input Line Entry System*) representations of the major constituents of *M. floribunda* EO nd the reference insecticides used in the *in silico* analyses. SMILES strings and stereochemical descriptors were obtained from PubChem. Spinosyn A and spinosyn D were analyzed as individual molecular structures in the in silico analyses, whereas spinosad was used as a positive control in the biological assays.

2.8 Statistical Analysis

Concentration–response data were analyzed by Probit regression according to Finney[39], using IBM SPSS Statistics version 26.0. The median inhibitory concentration (IC_{50}) was estimated for egg-hatching inhibition, whereas the median and 90% lethal concentrations (LC_{50} and LC_{90}, respectively) were estimated for the larvicidal and adulticidal bioassays. Concentration estimates were reported with their corresponding 95% confidence intervals, together with the slope ± standard error (SE) and chi-square (χ^2) values obtained from the Probit models. Significant differences among groups were assessed by one-way analysis of variance (ANOVA), followed by Tukey's post hoc test, using GraphPad Prism version 8.0. Statistical significance was set at $p < 0.05$[40].

3. Results

3.1 EO yield and chemical profile

Hydrodistillation of *M. floribunda* leaves yielded 0.57 ± 0.05% EO, corresponding to 1.42 g of EO obtained from 250 g of plant material. GC–MS and GC-FID analyses

identified 27 constituents, accounting for 81.97% of the total EO composition (Table 2). The major constituent was 1,8-cineole (18.85%), followed by *trans*-caryophyllene (14.60%). Other relatively abundant constituents included *α*-pinene (6.71%), *β*-elemene (6.15%), and alloaromadendrene (5.40%). Sesquiterpene hydrocarbons represented the predominant chemical class (42.41%), followed by oxygenated monoterpenes (23.47%) and monoterpene hydrocarbons (9.44%). The remaining 18.03% corresponded to chromatographic signals that were not included in the definitive compound assignment.

N°	Constituent	RIcalc	RIlit	Area (%)	Identification
1	1,4-Pentadiene, 2,3,3-trimethyl	835	845	0.27	RI, MS
2	*α*-Thujene	924	930	2.47	RI, MS
3	*α*-Pinene	935	939	6.71	RI, MS
4	1,8-Cineole	1031	1033	18.85	RI, MS
5	(E)-β-Ocimene	1047	1050	0.26	RI, MS
6	Linalool	1085	1095	1.32	RI, MS
7	*trans*-Sabinene hydrate	1097	1098	0.15	RI, MS
8	*β*-Fenchol	1114	1118	2.73	RI, MS
9	Terpinen-4-ol	1176	1177	0.19	RI, MS
10	Nopol	1289	1300	0.23	RI, MS
11	*α*-Copaene	1376	1377	0.78	RI, MS
12	Cycloisolongifolene	1380	1385	1.79	RI, MS
13	β-Elemene	1391	1392	6.15	RI, MS
14	*trans*-Caryophyllene	1418	1420	14.60	RI, MS
15	Calarene	1432	1439	3.96	RI, MS
16	*α*-Bergamotene	1435	1436	0.81	RI, MS
17	*α*-Humulene	1454	1455	1.17	RI, MS
18	Alloaromadendrene	1460	1461	5.40	RI, MS
19	Germacrene D	1482	1485	1.57	RI, MS
20	*β*-Selinene	1490	1492	0.50	RI, MS
21	Ledene	1492	1493	0.68	RI, MS
22	α-Muurolene	1498	1500	0.48	RI, MS
23	*δ*-Guaiene	1501	1502	4.52	RI, MS
24	Nerolidol	1563	1564	0.36	RI, MS
25	(−)-Caryophyllene oxide	1582	1583	3.23	RI, MS

N°	Constituent	RIcalc	RIlit	Area (%)	Identification
26	*β*-Eudesmol	1651	1652	2.45	RI, MS
27	*α*-Bisabolol	1685	1686	0.34	RI, MS
	Aliphatic hydrocarbons			0.27%	
	Monoterpene hydrocarbons			9.44%	
	Sesquiterpene hydrocarbons			42.41%	
	Oxygenated monoterpenes			23.47%	
	Oxygenated sesquiterpenes			6.38%	
	Total identified			**81.97%**	
	Unassigned chromatographic signals			**18.03%**	

Table 2. Chemical composition of the EO from *M. floribunda* leaves. RIcalc, retention index calculated using the retention times of n-alkanes (C_8–C_{30}); RIlit, retention index reported in the literature; MS, mass spectra compared with reference data from the literature. The identified constituents accounted for 81.97% of the total chromatographic area, while the remaining 18.03% corresponded to unassigned chromatographic signals.

3.2 Ovicidal and morphological analysis

The EO from *M. floribunda* caused concentration-dependent inhibition of *A. aegypti* egg hatching across the tested concentration range (31.25–500 µg/mL). Egg-hatching inhibition increased from 38.8 ± 4.8% at 31.25 µg/mL to 100 ± 0% at 500 µg/mL. No egg-hatching inhibition was observed in the negative control DMSO, whereas the positive control, Spinosad (1 µg/mL), completely inhibited egg hatching (100 ± 0%). Probit analysis estimated an IC_{50} of 45.27 µg/mL and an IC_{90} of 135.15 µg/mL (Table 3).

Concentrations (µg/mL)	Inhibition (%)	IC_{50} (µg/mL) (95% CI)	IC_{90} (µg/mL) (95% CI)	X^2 (df)
31.25	**38.8 ± 4.8**	**45.27 (30.83 – 56.35)**	**135.15 (118.12 – 162.25)**	**2.17 (3)**
62.50	**64.3 ± 2.5**			
125	**85.0 ± 1.8**			
250	**100 ± 0**			
500	**100 ± 0**			

Table 3. Ovicidal activity of *M. floribunda* EO against *A. aegypti* eggs. IC_{50}, median inhibitory concentration; IC_{90}, inhibitory concentration required to achieve 90% inhibition; 95% CI, 95% confidence interval; χ^2, chi-square statistic; df, degrees of freedom.

SEM revealed preserved chorionic integrity and continuous exochorionic ornamentation in eggs from the negative control (1% DMSO), with no evident surface disruption (Figure 1A). In contrast, EO exposed eggs (125 µg/mL) exhibited fissures, ruptures, surface discontinuities, disruption or detachment of the superficial exochorionic layer, deformation of the egg capsule, changes in opercular configuration, partial or complete displacement of the operculum, opening of the anterior region, and localized collapse or disorganization of the external chorionic architecture (Figures 1B–D).

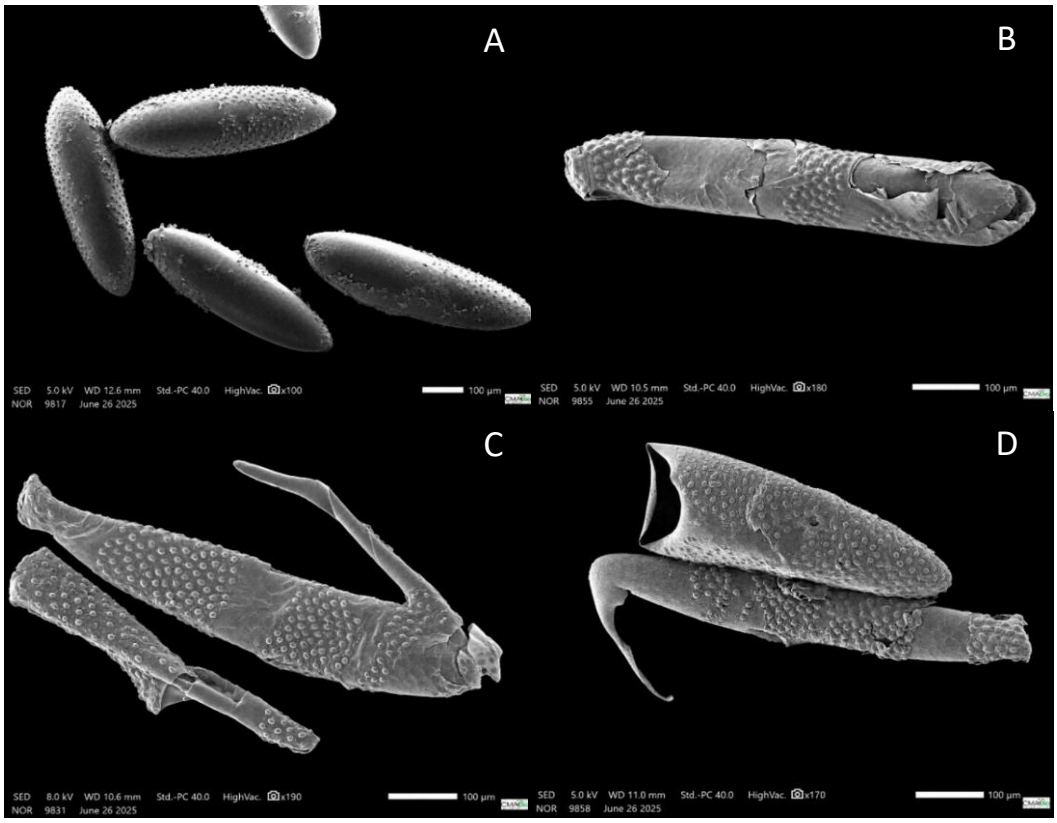


Figure 1.

3.3. Larvicidal and morphological analysis

The EO from *M. floribunda* exhibited larvicidal activity against third-instar *A. aegypti* larvae throughout the 72-h exposure period. Mortality increased with increasing EO concentration, ranging from 32% at 3.90 µg/mL to 100% at 62.50 µg/mL after 24 h, while values at 72 h ranged from 38% to 100% at the same concentrations (Figure 2). The estimated LC_{50} values were 6.46, 6.56, and 5.16 µg/mL at 24, 48, and 72 h, respectively, whereas the LC_{90} values were 23.42, 22.42, and 19.36 µg/mL at the respective exposure times (Table 4). The negative control (DMSO) showed 0% mortality throughout the assay, whereas the positive control (Spinosad) caused 100% mortality within 24 h.

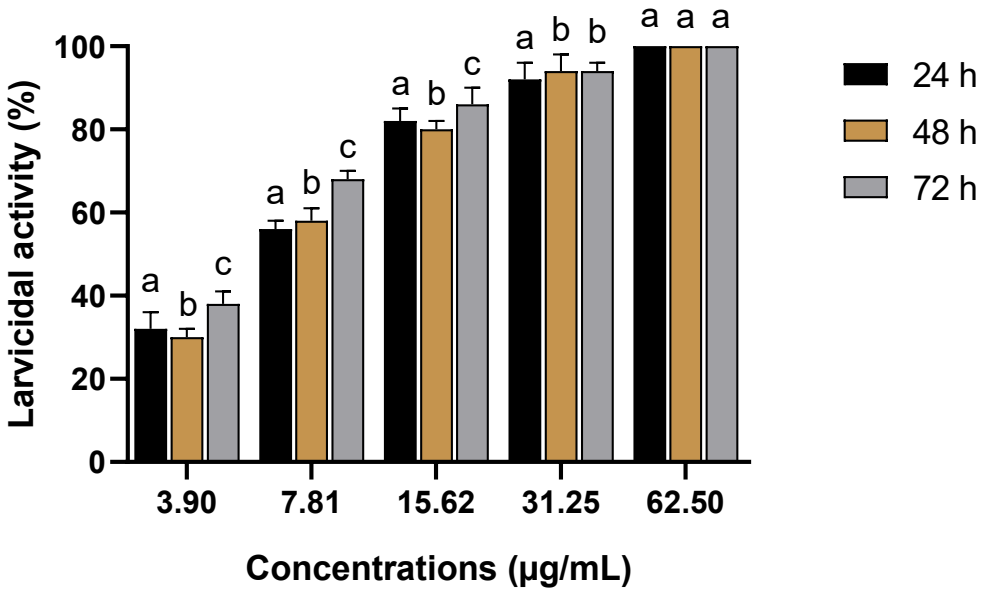


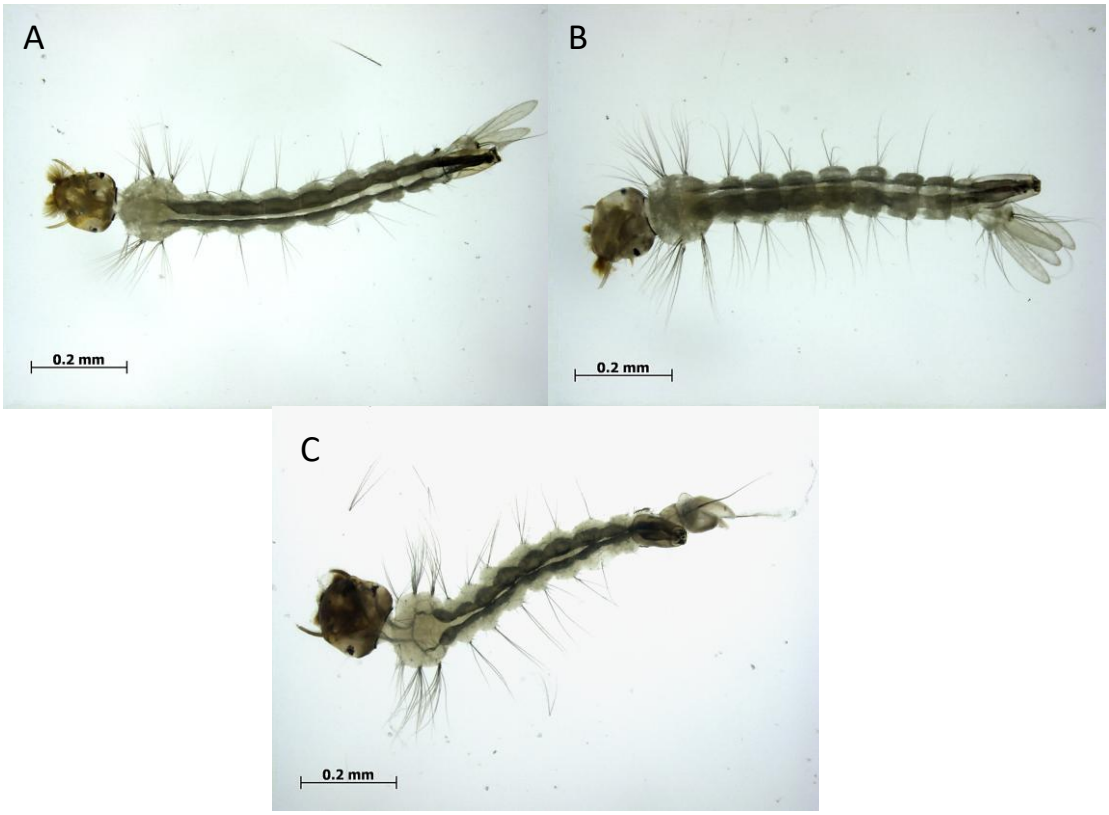
Figure 2.

Time	LC_{50} (µg/mL) (95% CI)	LC_{90} (µg/mL) (95% CI)	χ^2 (df)
24 h	6.46 (4.99 – 7.91)	23.42 (18.20 – 33.94)	1.14 (3)
48 h	6.56 (5.14 – 7.96)	22.42 (17.57 – 32.03)	0.66 (3)
72 h	5.16 (3.77 – 6.47)	19.36 (15.04 – 28.25)	1.05 (3)

Table 4. Larvicidal activity of *M. floribunda* EO against third-instar *A. aegypti* larvae. LC_{50} and LC_{90}, lethal concentrations required to cause 50% and 90% mortality,

304 respectively, estimated by Probit analysis; 95% CI, 95% confidence interval; χ^2, chi-
305 square statistic; df, degrees of freedom.

306 No morphological differences were observed between the negative control (1%
307 DMSO) and positive control (Spinosad at 1 µg/mL) groups. In both groups, the larvae
308 maintained their normal body shape, abdominal turgidity, and transparency, with no
309 apparent morphological abnormalities (Figure 3A, B). In contrast, larvae exposed to the
310 EO at 31.25 µg/mL exhibited marked longitudinal body curvature, reduced segmental
311 turgidity, abdominal contraction, and darkening of the midgut (Figure 3C).



312 **Figure 3.** Morphological alterations in third instar *A. aegypti* larvae after exposure to EO
313 from *M. floribunda*. (A) Negative control (DMSO); (B) positive control (Spinosad); (C)
314 EO exposed larva. Stereomicroscopic images. Scale bar = 0.2 mm.

315 **3.4. Adulticidal Activity**

316 The EO exhibited pronounced adulticidal activity against non-blood-fed female
317 *A. aegypti*, with LC_{50} and LC_{90} values of 0.70 and 7.10 µg/mL, respectively (Table 5).

Mortality increased progressively with increasing EO concentrations, ranging from 66.6% at the lowest concentration tested to 100% at the highest concentration. This concentration-dependent response was consistent with the Probit analysis, which estimated an LC_{50} below the lowest experimental concentration tested. No mortality was observed in the negative control (1% acetone), confirming the absence of a detectable effect attributable to the solvent. In contrast, the positive control (Cielo® at 0.00626 µg/mL) produced 100% mortality under the same experimental conditions.

Concentrations (µg/mL)	Mortality (%)	LC_{50} (µg/mL) (95% CI)	LC_{90} (µg/mL) (95% CI)	χ^2 (df)
2	**66.7 ± 1.2**			
5	**91.1 ± 1.7**			
10	**95.6 ± 1.5**	**0.70 (0.13 – 1.44)**	**7.10 (4.53 – 13.13)**	**6.10 (3)**
40	**100 ± 0**			
50	**100 ± 0**			

Table 5. Adulticidal activity of *M. floribunda* EO against adult *A. aegypti*. LC_{50} and LC_{90}, lethal concentrations required to cause 50% and 90% mortality, respectively, estimated by Probit analysis using mortality data recorded after 90 min of exposure; 95% CI, 95% confidence interval; χ^2, chi-square statistic; df, degrees of freedom. The LC_{50} estimate was extrapolated below the lowest experimental concentration tested (2 µg/mL) and should be interpreted with caution.

3.5. *In silico* ADMET prediction

All evaluated compounds except spinosyn A complied with Lipinski's Rule of Five; the status of spinosyn D was not reported (NR). Predicted intestinal absorption of the major constituents of *M. floribunda* EO ranged from 94.359% for *β*-elemene to 96.505% for 1,8-cineole. Values for imidacloprid, prallethrin, spinosyn A, and spinosyn D were 80.782%, 98.554%, 47.303%, and 100%, respectively. P-glycoprotein substrate

activity was predicted for 1,8-cineole, spinosyn A, and spinosyn D. None of the compounds was predicted to inhibit the CYP450 enzymes assessed.

AMES mutagenicity was predicted for imidacloprid but not for the major essential oil constituents, spinosyn A, or spinosyn D. Hepatotoxicity was predicted only for spinosyn D, whereas skin sensitization was predicted for 1,8-cineole, *trans*-caryophyllene, and *β*-elemene. These findings are computational predictions, not evidence of experimentally confirmed toxicity.

Predicted minnow toxicity values ranged from 0.118 to 1.735 log mM among the five major essential oil constituents, with *β*-elemene showing the lowest value and 1,8-cineole the highest. Values for spinosyn A, spinosyn D, imidacloprid, and prallethrin were 6.027, 1.952, 1.629, and 0.361 log mM, respectively. None fell below the cited pkCSM threshold for high acute toxicity (< −0.3 log mM). However, these predictions alone cannot establish environmental safety because they do not account for exposure, mixture effects, or the behavior of the complete essential oil. The results therefore provide preliminary screening data rather than a substitute for experimental toxicity testing (Table 6).

Compound	Lipinski's Rule of Five	Intestinal absorption (%)	Skin permeability (log Kp)	P-glycoprotein substrate	CYP450 inhibition	Mutagenicity (AMES)	Hepatotoxicity	Skin sensitization	Minnow toxicity (log mM)
1,8-Cineole	Yes	96.505	−2.437	Yes	No	No	No	Yes	1.735
trans-Caryophyllene	Yes	94.845	−1.580	No	No	No	No	Yes	0.504
α-Pinene	Yes	96.041	−1.827	No	No	No	No	No	1.159
β-Elemene	Yes	94.359	−1.279	No	No	No	No	Yes	0.118
Alloaromadendrene	Yes	95.302	−1.828	No	No	No	No	No	0.429
Spinosyn A	No	47.303	−2.735	Yes	No	No	No	No	6.027
Spinosyn D	NR	100	−2.759	Yes	No	No	Yes	No	1.952
Imidacloprid	Yes	80.782	−2.751	No	No	Yes	No	No	1.629
Prallethrin	Yes	98.554	−2.788	No	No	No	No	No	0.361

Table 6. *In silico* prediction of ADMET properties of the major constituents of *M. floribunda* EO and reference insecticides. Lipinski's Rule of Five assesses compliance with criteria for molecular weight, hydrogen-bond donors and acceptors, and lipophilicity. For Lipinski's Rule of Five, "Yes" and "No" indicate compliance and non-compliance, respectively; for other categorical endpoints, they indicate positive and negative predictions, respectively. CYP450 inhibition refers to predicted inhibition of CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4. AMES, Ames bacterial reverse-mutation test; P-gp, P-glycoprotein substrate status; NR, not reported. Minnow toxicity is expressed as log mM. All ADMET endpoints represent in silico predictions.

4. Discussion

Chemical variability in EOs is commonly reported and may arise from geographic origin, environmental conditions, seasonality, plant organ, and extraction procedures[41]. This variability is also evident among *M. floribunda* EOs. Costa et al.[23] reported distinct chemical profiles among samples, with one characterized mainly by terpinolene (23.1%), *α*-phellandrene (17.7%), and *γ*-terpinene (8.7%), whereas another showed a predominance of *γ*-cadinene (17.5%). Similarly, de Moraes et al.[24] described a monoterpene-rich profile in which 1,8-cineole (23.30%), terpinolene (22.23%), and *α*-phellandrene (22.19%) were the major constituents. The chemical profile identified in the present study, therefore, adds to the reported variability of *M. floribunda* EOs and may contribute to differences in their biological activities.

The *M. floribunda* EO exhibited concentration-dependent ovicidal activity against *A. aegypti*, indicating an effect on egg viability and/or embryonic development. Ovicidal effects have also been reported for other Myrtaceae species, including *Syzygium aromaticum* (L.) Merr. & L.M.Perry and *Eucalyptus citriodora* Hook., against *A. aegypti*, *An. dirus*, and *Cx. quinquefasciatus*[42].

The morphological alterations observed in *M. floribunda* EO treated eggs are consistent with structural damage previously reported in *A. aegypti* eggs exposed to isolated plant-derived compounds. Moungthipmalai et al.[15] observed disruption of the exochorionic cuticle after exposure to d-limonene, geranial, and *trans*-cinnamaldehyde, including damage to the exochorionic meshwork and structures associated with aeropyles. Such alterations may compromise the protective and respiratory functions of the chorion and potentially affect embryonic development. These structural changes may therefore contribute to the ovicidal effect observed for *M. floribunda* EO, although the underlying mechanisms remain to be experimentally elucidated. The observed ovicidal

activity may reflect additive or synergistic interactions among the constituents of the EO; however, the contribution of individual compounds cannot be established from the present data.

The LC_{50} values obtained for *M. floribunda* EO were lower than those reported for EOs from other Myrtaceae species against *A. aegypti*, including *Eugenia stipitata* McVaugh (LC_{50} = 340 μg/mL) [19], *Eugenia piauhiensis* Vellaff. (LC_{50} = 230 μg/mL), and *Psidium myrsinites* DC. (LC_{50} = 292 μg/mL)[43].

Beyond larval mortality, the morphological alterations observed after exposure to *M. floribunda* EO provide additional evidence of its effects on *A. aegypti* larvae. Lobato et al.[44] reported deformation of the anal papillae and epithelial tissue peeling in third instar larvae exposed to *Ayapana triplinervis* (Vell.) R.M.King & H.Rob. EO nanoemulsions. Similar alterations in the anal papillae have also been described following exposure to plant-derived compounds. As these structures are involved in ion and water transport and contribute to larval osmoregulation[45,46], the alterations observed in the present study may interfere with these physiological processes and contribute to the larvicidal effect of *M. floribunda* EO.

For the adulticidal assay, Santos et al.[21] reported an LC_{50} of 5.49 μg/mL for the EO from *Algrizea minor* Sobral, Faria & Proença (Myrtaceae) against adult *A. aegypti* after 120 min of exposure, compared with an LC_{50} of 0.70 μg/mL for *M. floribunda* EO in the present study. Silva et al.[47] reported an LC_{50} of 12.5 μg/mL for the EO from *Bocageopsis multiflora* (Mart.) R.E.Fr. (Annonaceae) against adult female *A. aegypti* after 90 min of exposure. The isolated compound eudesmol also exhibited adulticidal activity, with LC_{50} values of 1.82, 1.14, and 1.00 ppm after 24, 48, and 72 h, respectively[48]. Although differences in exposure time, chemical composition, and

experimental procedures limit direct comparisons among studies, the LC_{50} obtained for *M. floribunda* EO was lower than those reported in these studies.

The predicted ADMET profiles varied among the selected constituents of *M. floribunda* EO, reflecting differences in their predicted pharmacokinetic and toxicological properties. Because EO constituents may interact through additive, synergistic, or antagonistic effects, these predictions should be interpreted as compound-specific information rather than as a direct representation of the biological or toxicological profile of the whole EO[49,50]. Moreover, pkCSM predictions are based on molecular structure and previously established datasets and therefore provide an initial screening of potential pharmacokinetic and toxicological properties rather than a substitute for experimental assessment[17]. Further experimental studies are needed to determine whether the predicted effects occur under relevant exposure conditions and to clarify the contribution of individual constituents to the overall activity and safety profile of *M. floribunda* EO.

5. Conclusion

The EO from *M. floribunda* demonstrated insecticidal activity against different developmental stages of *A. aegypti*, including ovicidal, larvicidal, and adulticidal effects. The morphological alterations observed in eggs and larvae support the occurrence of structural damage following EO exposure. The *in silico* ADMET analysis provided additional information on the predicted pharmacokinetic and toxicological profiles of its major constituents, although these predictions require experimental validation. Overall, the findings support further investigation of *M. floribunda* EO as a potential botanical insecticide.

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623 **CRediT authorship contribution statement**

624 **Arley Faria José de Oliveira:** Conceptualization, Methodology, Investigation, Data
625 curation, Writing – original draft. **Maria Luiza Lima da Costa:** Methodology, Formal
626 analysis, Writing – original draft. **André Correa de Oliveira:** Methodology, Formal

analysis, Writing – review & editing. **Rosemary Aparecida Roque:** Supervision, Funding acquisition. **Jefferson Rocha de Andrade Silva:** Supervision.

Data availability

The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request

Declaration of Competing Interest

The authors confirm that there were no conflicts of interest in performing this study.

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Figure captions

Figure 1. Ultrastructural alterations in *A. aegypti* eggs after exposure to EO from *M. floribunda*. Scanning electron microscopy (SEM) images of eggs from the negative control (DMSO) (A) and EO exposed groups (B–D), showing alterations in the chorionic and opercular regions. Scale bars are indicated in each panel.

Figure 2. Larvicidal activity of *M. floribunda* EO against *A. aegypti* larvae at concentrations ranging from 3.90 to 62.50 µg/mL at 24, 48, and 72 h exposure times. Data are presented as Mean ± Standard Deviation (SD). Distinct letters above the bars for the same concentration indicate statistically significant differences between exposure times by Tukey's test ($p < 0.05$).

650 **Figure 3.** Morphological alterations in third instar *A. aegypti* larvae after exposure to EO
651 from *M. floribunda*. (A) Negative control (DMSO); (B) positive control (Spinosad); (C)
652 EO exposed larva. Stereomicroscopic images. Scale bar = 0.2 mm.
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