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Sublethal doses of *Eucalyptus benthamii* essential oil induce overcompensatory responses in *Aedes aegypti* (Diptera: Culicidae)

Doses subletais de óleo essencial de *Eucalyptus benthamii* induzem respostas sobrecompensatórias em *Aedes aegypti* (Diptera: Culicidae)

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ABSTRACT

Managing mosquito populations remains the most effective approach to mitigating arbovirus transmission. However, stressor-induced mortality under determined conditions can trigger compensatory or overcompensatory effects in adult mosquito characteristics (total biomass, individual size). In this study, we evaluated the role of *Eucalyptus benthamii* essential oil as a stressor on *Aedes aegypti* larvae, investigating its effects on larval development, adult size, longevity, and overall adult emergence. First-instar *A. aegypti* larvae were subjected to essential oil concentrations of 8.5 (LD50) and 18.5 ppm (LD90) in controlled laboratory conditions. We assessed pupal mortality, the proportion of pupae that successfully transitioned to adulthood, adult longevity (in days), larval growth rates, wing length, and overall larval mortality. The results revealed that the interaction between time and treatment had a significant effect on larval mortality. Although the essential oil concentration did not affect the number of larvae reaching adulthood, adult longevity and larval development time were notably extended under LD90 and LD50 concentration, respectively. Larval mortality rates were highest during the first week of exposure to the LD90 treatment. Furthermore, males and females produced in microcosms with LD50 and LD90 had significantly larger wings than in the control. These findings suggest that sublethal doses of *E. benthamii* essential oil may enhance certain adult *A. aegypti* population characteristics through overcompensatory mortality.

Keywords: larval density; insecticide; natural compounds; vector control.

RESUMO

O controle populacional de mosquitos ainda é a abordagem mais eficaz para mitigar a transmissão de arbovírus. Todavia, a mortalidade induzida por estressores sob determinadas condições pode desencadear efeitos compensatórios ou sobrecompensatórios nas características dos indivíduos adultos remanescentes (biomassa total, tamanho individual). Avaliamos o papel do óleo essencial de *Eucalyptus benthamii* como estressor em larvas de *Aedes aegypti*, seus efeitos sobre desenvolvimento larval, tamanho do adulto, longevidade e emergência de adultos. Larvas de *A. aegypti* de primeiro instar foram submetidas às concentrações de óleo essencial 8,5 (LD50) e 18,5 ppm (LD90), em condições controladas de laboratório. Avaliamos a mortalidade das pupas, a proporção de pupas que metamorfosearam em adultos, a longevidade dos adultos (dias), as taxas de crescimento das larvas, o comprimento das asas e a mortalidade geral das larvas. Nossos resultados revelaram que tempo (em semanas) e interação entre tempo e tratamento tiveram efeito significativo na mortalidade das larvas. Embora a concentração do óleo essencial não tenha afetado o número de larvas que atingiram a idade adulta, a longevidade dos adultos e o tempo de desenvolvimento das larvas foram notavelmente estendidos sob as concentrações LD90 e LD50, respectivamente. As taxas de mortalidade larvais foram maiores durante a primeira semana de exposição ao tratamento LD90. Machos e fêmeas produzidos em microcosmos com LD50 e LD90 tinham asas significativamente maiores que o controle. Esses resultados sugerem que doses subletais do óleo essencial de *E. benthamii* podem melhorar determinadas características da população de *A. aegypti*, por meio de mortalidade sobrecompensatória.

Palavras-chave: compostos naturais; controle de vetores; densidade larval; inseticida.

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Introduction

Native to sub-Saharan Africa, the mosquito species *Aedes aegypti* (Linnaeus, 1762) was most likely introduced to the Americas between 400 and 550 years ago (Powell et al., 2018). Known as the primary vector for Dengue, Chikungunya, and Zika viruses, this mosquito is also capable of transmitting over 100 different types of arboviruses under laboratory conditions (Silva Ferreira et al., 2020). In this context, controlling mosquito populations remains the most effective approach to reduce human mosquito interactions and immediately brake disease transmission (Cozzer et al., 2025). Current insecticides are still regarded as the most reliable method to reduce mosquito numbers (Collins et al., 2025). Over recent decades, however, control strategies have relied predominantly on synthetic chemical insecticides, whose intensive use poses significant risks to both environmental and human health (Marsaro et al., 2023; Collins et al., 2025).

Mosquito control is at a crossroads, encouraging the search for alternative strategies (Demarque and Espindola, 2021; Collins et al., 2025). Several chemical compounds of natural origin are widely recognized for their efficacy against *A. aegypti*, targeting various life stages including eggs, larvae, pupae, and adults (Demarque and Espindola, 2021). These alternatives include genetically modified organisms, *Wolbachia* symbionts, *Bacillus thuringiensis* var. *israelensis* (Bti) (Alto and Lord, 2016; Carvalho et al., 2025), and natural, plant-derived products (Chellappandian et al., 2018). Essential oils have shown substantial toxicity against mosquito larvae (França et al., 2021; Marques et al., 2021). Not only do they degrade rapidly in the environment reducing residual levels and minimizing impact on non-target species (Vivekanandhan et al., 2020; Demarque and Espindola, 2021; Marsaro et al., 2023) however, their complex blend of multiple active compounds further complicates the development of resistance in mosquitoes (Rattan, 2010; Demarque and Espindola, 2021). Within this context, several *Eucalyptus* (Myrtales: Myrtaceae) species stand out for producing large quantities of essential oils that exhibit broad-spectrum lethality against bacteria, fungi, mites, weeds (Mossi et al., 2011), and insects, including mosquito larvae (Vivekanandhan et al., 2020).

While the non-lethal effects of insecticides are well-documented, research remains limited regarding the overall "quality" of surviving mosquitoes following failed insecticide treatments. Density-dependent factors, such as larval mortality regardless of cause can substantially influence the quality of emerging adults, as the pressure selects for the fittest individuals (Muturi et al., 2011; Cozzer et al., 2025). The populational response can manifest through changes in growth, development, fecundity, vector competence, and, ultimately, abundance (Alto and Lord, 2016; Bellamy et al., 2024; Cozzer et al., 2025). Although one would assume that introducing an external mortality factor into a larval population inevitably decreases its overall density, the actual outcomes sometimes defy these expectations (Hastings, 2013; Cozzer et al., 2023). Under certain conditions, mortality induced by stressors can provoke compensatory (equal) or overcompensatory (greater) production in comparison to an unstressed population, possibly resulting in an effect collectively referred to as the hydra effect, increasing the number of individuals (population size) (Abrams and Matsuda, 2005; Abrams, 2009; Marsaro et al., 2023). Despite the importance of this unexpected relationship between reduced larval density and increased mosquito production, it remains largely underappreciated in global mosquito control strategies (Schröder et al., 2014; Carvalho et al., 2025).

Given the rising use of natural products in mosquito control and the urgent need for alternative strategies, the objective of the present study was to evaluate *Eucalyptus benthamii* Maiden & Cambage essential oil as a potential stressor of *A. aegypti* larvae, investigating its impact on larval development, adult size, longevity, and total adult emergence. The premise is that, when considering the significance of density-dependent processes in mosquito breeding habitats, any control agent that induces partial larval mortality and thus potentially alleviates intraspecific competition should be examined for possible compensatory or overcompensatory outcomes before implementation (Cozzer et al., 2023; Cozzer et al., 2025). We hypothesize that the partial larval mortality induced by sublethal doses of *E. benthamii* essential oil will trigger overcompensatory responses in surviving individuals (larger adult size and greater longevity) compared to controls, potentially leading to an increased adult number, characterizing the hydra effect. If confirmed, this paradoxical scenario would suggest that larval control strategies relying on partial mortality may inadvertently produce fitter adults, complicating conventional approaches to vector management. Unwanted ecological responses to human attempts at vector control should be added to the existing knowledge of clean and environmentally safe technologies in order to formulate more effective solutions aimed at sustainable development and human well-being.

Methods

Eucalyptus sampling, essential oil extraction and chemical analysis

The *E. benthamii* adult plant leaves were collected from the experimental area of the Agricultural Research and Rural Extension Company of Santa Catarina, located in the municipality of Guatambu, in the state of Santa Catarina, Brazil (27°07' 55"S, 52°44'04"W). Eucalyptus essential oil was obtained through hydro distillation using a Clevenger-type apparatus (Amaral et al., 2017). The distillation process was repeated four times, using 200 g of fresh leaves per run and lasting 120 minutes each, resulting in a total of 800 g of leaves (Gallon et al., 2020). The essential oil samples were analyzed by gas chromatography coupled with mass spectrometry (GC-MS) using an Agilent 7890B gas chromatograph connected to a 5977A quadrupole mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). Separation was carried out on an HP-SMS capillary column (5% phenyl methyl siloxane, 30 m × 250 μm × 0.25 μm). Helium was used as the carrier gas at a constant flow rate of 1 $mL.min^{-1}$. The oven temperature was programmed as follows: an initial temperature of 40°C held for 4 minutes, followed by an increase to 240°C at 10°$C.min^{-1}$, and then to 300°C at 40°$C.min^{-1}$, with a final hold for 5 minutes. The injector temperature was set at 280°C. Oil samples were diluted in ethyl ethanol to a concentration of 10 $mg.mL^{-1}$, and 1 μL of the solution was injected with a split ratio of 20:1. The transfer line temperature of the MS was maintained at 150°C, and the ion source was kept at 230°C. Electron ionization was performed at 70eV, with a mass scan range from m/z 40 to 300. Chemical compounds were identified by comparing their mass spectra with those in the NIST 5.01 Mass Spectral Library (Agilent P/N G1033A). The relative abundance of each component was determined based on the peak areas observed in the chromatogram (Gallon et al., 2020).

Laboratory mosquito colony

Eggs of *A. aegypti* were provided by the Laboratory of Ecological Entomology (LABENT-Eco) in Chapecó, Santa Catarina, Brazil. The eggs were hatched in a plastic tray measuring 25 x 30 cm, containing 1 liter of tap water. The larvae were reared at a density of 1,000 larvae per liter and fed with TetraFin® commercial fish food. Rearing conditions occur under controlled temperature (25±3°C), relative humidity (70–80%) and photoperiod (12:12 h) conditions, based on Cozzer et al. (2024). The original *A. aegypti* mosquitoes to start the insectary came from field egg samples, donated by the Epidemiological Surveillance of Chapecó. The genetic variability of the colony was maintained by annually collecting, identifying, and releasing wild strains of the *A. aegypti* mosquito, but there was no generational control.

Lethal Dose Experiment with Essential Oil

To determine the lethal doses (LD) of *E. benthamii* essential oil required to kill 50% (LD50 — intermediate mortality) and 90% (LD90 — high mortality) of *A. aegypti* larvae, a preliminary experiment was conducted using a microcosm approach. Each microcosm consisted of a plastic cup with a total capacity of 80 mL, containing 50 mL of ultrapure water. The essential oil was serially diluted from the stock solution to achieve treatment concentrations of 7.5, 10, 12, and 25 parts per million (ppm) in 0.4% acetone. Each treatment received 200 μL of the respective concentration. Control cups received 200 μL of acetone at 0.4%. Each experimental unit included twenty first-instar *A. aegypti* larvae. Mortality, indicated by motionless larvae, was recorded after 1, 2, 4, and 24 hours, following Gallon et al. (2020). The LD50 and LD90 were estimated based on the resulting mortality curve (Probit Analysis) for *A. aegypti* larvae exposed to the concentrations described above.

Density-dependent larval development experiment

The experiment was carried out using 500 mL glass beakers, each filled with 375 mL of ultrapure water and containing 150 newly hatched first-instar *A. aegypti* larvae, along with 0.15 g/L of TetraFin® fish food following Cozzer et al. (2024). The treatments, based on Probit Analysis, consisted of 8.5 (LD50) and 18.5 ppm (LD90) concentrations of the *E. benthamii* essential oil, whereas the control received acetone alone at a concentration of 0.4% (six replications each). The experiment was maintained under the same conditions as the rearing protocols described above. Experimental units were checked daily, and dead larvae were counted. Pupae were transferred to adult emergence flasks upon formation.

Adult mosquito maintenance

Newly emerged adult mosquitoes were housed in 1,000 cm^3 cages made from cylindrical plastic buckets. The cages were assigned based on the experimental unit and the date of mosquito emergence, ensuring that mosquitoes of different ages were kept separate. During the adult longevity test, no food was provided. Each day, the cages were inspected for dead mosquitoes, which were then removed and sexed. Mosquito size was determined by measuring wing length, an allometric indicator of body size (Gutiérrez et al., 2022). The right wing of each mosquito was carefully removed, photographed under a stereomicroscope equipped with a digital camera (Digital Microscope User), and measured using Meter Size® software (Version 1.1).

Statistical analysis

A factorial GLM with a quasi-binomial distribution was used to assess the effects of *E. benthamii* essential oil treatments on the proportion of larval mortality over time (in weeks). To evaluate the effects of different *E. benthamii* essential oil treatments on *A. aegypti* pupal mortality (adult emergence), larval development time, adult lifespan, and wing length (for both males and females), Generalized Linear Models — GLMs were applied (Crawley, 2007). All models were tested for under or overdispersion by the Half-Normal Plots package and HNP function (Moral et al., 2017) and the GLMs were adjusted accordingly. A one-way GLM with a quasi-binomial distribution (logit link, F-test) was used to analyze the proportion of pupae reaching adulthood. When analyzing count data such as larval development time, adult lifespan, and wing length (for both males and females), a one-way GLM with a Gaussian distribution (identity link, F-test) was employed. For all treatment differences and temporal trends, a orthogonal contrast analysis was performed (Crawley, 2007). In this process, dependent variables were ranked in ascending order and compared pairwise. Sequential pairwise comparisons were performed by incorporating non-significant values into the model and proceeding with the next comparison, following a stepwise model simplification approach (for more details, see chapter 9 of Crawley, 2007).

Results

The main chemical constituents identified in the *E. benthamii* essential oil, along with their relative percentages based on total chromatogram peak areas were α-Pinene (56.89%), Globulol (10.36%), Ledol (2.96%) and D-Limonene (2.38%). The experiment assessing larval development lasted for seven weeks (49 days). Generalized linear model (GLM) analyses indicated that the interaction between time and treatment had a significant effect on mosquito larval mortality (Table 1A). The highest mortality rate occurred during the first week of the LD90 treatment (Figure 1), in which approximately 40% of the mosquito larvae died. However, mortality rates dropped sharply in the second week, and no further larval mortality was recorded from the fourth week onward (Figure 1). In contrast, both the LD50 treatment and the control group exhibited larval mortality rates below 15% throughout the experiment (Figure 1). Notably, the control group experienced the highest mortality rates during the third and fourth weeks (Figure 1).

Larval development time (Table 1B; Figure 2a) was significantly influenced by essential oil concentration, however the essential oil concentrations did not affect the proportion of adults emerging from the exposed larvae (Table 1C; Figure 2b). Adults emerging from the LD90 treatment exhibited longer lifespans (Table 1D; Figure 2c) than those from the LD50 and control groups, whereas larval development time was prolonged in the LD50 treatment in comparison to the LD90 and control groups. Regarding wing size, *A. aegypti* females generally had larger winged than males, and the essential oil significantly affected wing dimensions in both females (Table 1E; Figure 2d) and males (Table 1F; Figure 2e). Statistically, both LD50 and LD90 produced larger wings than the con-

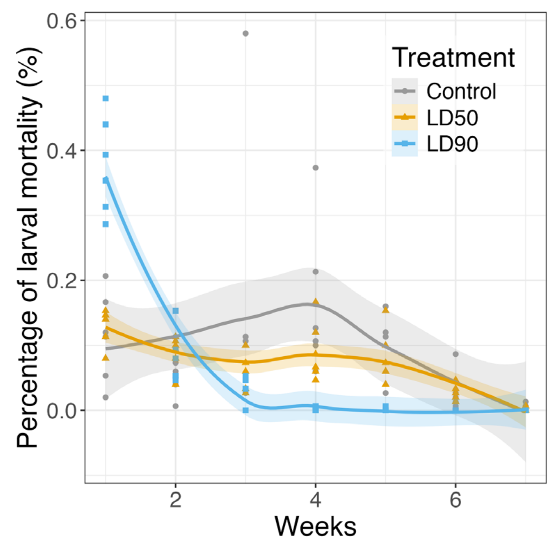

Figure 1 – *Aedes aegypti* larval mortality over a period of seven weeks among *Eucalyptus benthamii* essential oil in distinct treatments in a laboratory test. Lines represent the smoothers of larval mortality and colorful areas the 95% confidence intervals from GLM models.

Table 1 – (A) Factorial and (B, C, D, E and F) One-way GLM of *Aedes aegypti* responses against *Eucalyptus benthamii* essential oil treatments (Control, LD50 and LD90) and time (Weeks), in a laboratory test. Additionally, contrast analysis results for the GLMs are included.. Degrees of freedom (Df), Deviation (Deviance), Residual Degrees of Freedom (Resid. Df), Residual Deviance (Resid. Dev) and Values of F statistics and associated *p* (Pr>F).

GLM	DF	Deviance	Resid. Df	Resid. Dev	F	Pr(>F)	Analysis of contrast
A. Larval Mortality							
Treatment	2	1.02	123	14.926	1.37	0.256	Control=LD50<LD90
Time	1	37.03	122	9.342	99.76	<0.001	W7=W6=W5<W2<W4=W3<W1
Treatment:Time	2	25.11	120	5.558	33.83	<0.001	
Residual			125	15.08			
B. Larval development time							
Treatment	2	17.062	15	6.39	20.03	<0.001	Control=LD90<LD50
Residual			17	23.45			
C. Proportion of produced adult							
Treatment	2	0.087	15	0.71	0.94	0.411	
Residual			17	0.797			
D. Adult lifetime							
Treatment	2	1.357	15	3.5063	2.91	0.049	Control=LD50<LD90
Residual			17	4.863			
E. Wing size (female)							
Treatment	2	0.32	15	1.118	3.61	0.047	Control<LD50=LD90
Residual			17	1.438			
F. Wing size (male)							
Treatment	2	0.424	15	0.903	3.52	0.045	Control<LD90=LD50
Residual			17	1.328			

trol for each sex, without a significant difference between LD50 and LD90 treatments. Nonetheless, the biological trend indicated that female wings tended to be largest in the LD90 treatment, whereas male wings were largest in the LD50 treatment.

Discussion

In the current study, *A. aegypti* larval mortality peaked during the first week under the LD90 treatment. In contrast, the control and LD50 treatments exhibited smaller mortality levels during the first two weeks, leading to higher larval densities that intensified intraspecific competition. This increased competition may have contributed to higher larval mortality in the third and fourth weeks, as density-dependent mortality likely stemmed from food-limited competition, as reported in previous studies (Hastings, 2013; Cozzer et al., 2024; Carvalho et al., 2025). Under the LD90 treatment, the results showed a 36% reduction in the mosquito larval population in the first week (reaching approximately 60% by last week). This reduction likely alleviated intraspecific competition by improving the food-to-larva ratio, thereby enhancing food intake, growth, and development of the surviving larvae (Muturi et al., 2011; Alto and Lord, 2016; Cozzer et al., 2024), which in turn reduced larval mortality in subsequent weeks. Additionally, the high mortality observed in the LD90 treatment during the first week may have indirectly benefited the surviving larvae. The carcasses of dead larvae could have served as an additional source of organic matter, supplementing the available nutrients in the microcosm environment. Previous studies suggest that organic inputs, including decomposing insect carcasses, can influence larval development by increasing resource availability and reducing competition (Muturi et al., 2011; Cozzer et al., 2025).

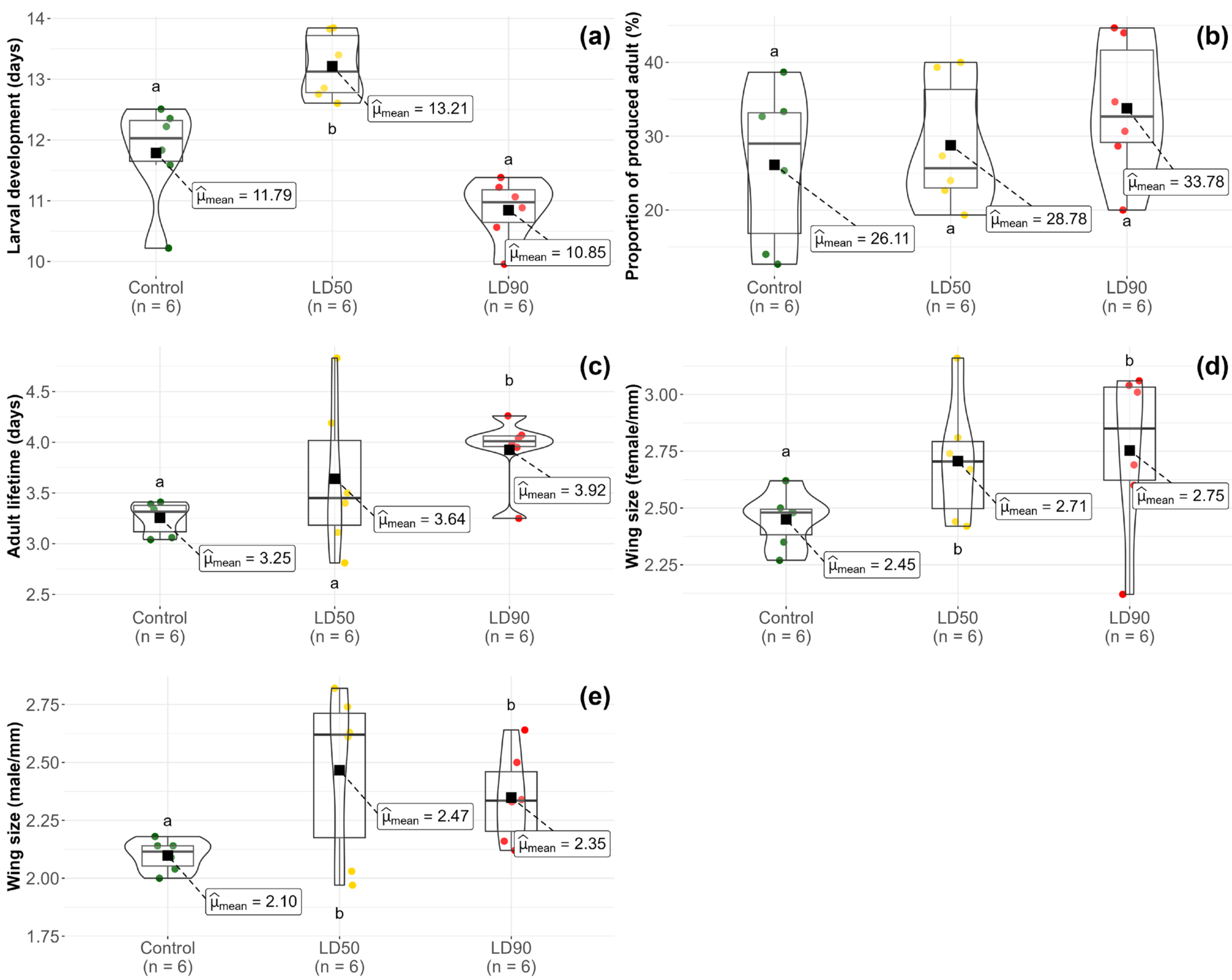

Figure 2 – Responses of *Aedes aegypti* for (a) larval development time in days, (b) adult produced in proportion, (c) adult lifetime in days, (d) female wing size in mm and (e) male wing size in mm among control, LD50 and LD90 *Eucalyptus benthamii* essential oil treatments in a laboratory test. The violin boxes represent quartiles; the black squares in the horizontal represent the mean; the horizontal-bold line represents the median; and the vertical line represents the upper and lower limits. Distinct under/superscript letters represent statistically different groups.

Consequently, the presence of these carcasses may have contributed to the survival and enhanced the development of the remaining larvae, potentially explaining the significant difference in larval mortality between the LD90 treatment and the LD50 and control groups.

We attributed the extended larval development time observed in the LD50 treatment to reduced food availability per larva due to higher larval density compared to the control, as larval mortality in the control group intensified between the 2nd and 4th weeks. However, despite the initial mortality in LD50 reducing larval density and alleviating intraspecific competition, the per capita food availability may not have been sufficient to sustain larval development at the same rate observed in the LD90 treatment. This could explain why larval development in LD50 was slower than in LD90, where a greater reduction in larval density at the beginning of the experiment provided the remaining larvae with more abundant resources. Our findings align with those of Beserra et al. (2009) and Alto and Lord (2016), who reported that reduced larval populations are associated with faster development. In the LD90 treatment, an acceleration in larval development was observed, consistent with these previous studies. These results suggest that while increased mortality alleviates competition, the extent of mortality and the resulting per capita food availability are key determinants of larval development rates (Walker et al., 2021; Marsaro et al., 2023; Cozzer et al., 2024).

Although our microcosm experiments showed a biological trend suggestive of higher adult emergence in the LD90 treatment compared to the control, the statistical analysis did not reveal a significant difference among treatments. The heightened larval mortality at 18.5 ppm (LD90) of the essential oil, paired with increased wing size in both females and males exposed to LD50 and LD90 compared to the control is an important result. These results hint at an overcompensatory response to higher concentrations of *E. benthamii* essential oil. Density-dependent processes strongly influence mosquito larval populations under stress from interventions aimed at reducing or altering population density (Alto and Lord, 2016; Cozzer et al., 2025). Additional mortality during early life stages may relieve intraspecific competition among the surviving larvae. Consequently, even if the total number of adults does not increase, the reduced larval density can lead to larger adults, a paradoxical outcome consistent with the overcompensatory concept (Abrams and Matsuda, 2005; Abrams, 2009). New experiments can be conducted to assess whether there is an increase in population size. One way to do this is to allow exposed adult mosquitoes to mate within the treatments and quantify the adult offspring, maintaining the conditions of the larval development environment.

Larger adult mosquitoes, both male and female, were observed in the *E. benthamii* essential oil treatments compared to the control. This finding is concerning, as mosquito size is typically positively correlated with longevity, reproductive capacity, virus transmission efficiency, and fecundity (Bellamy et al., 2024). Alto and Lord (2016) found a strong positive relationship between mosquito size and the number of eggs laid by *A. aegypti* females, comparing those that developed from larvae exposed to *B. thuringiensis* var. *israelensis* (Bti) with those that did not. Similarly, *A. aegypti* larvae exposed to the chemical insecticide Malathion also produced larger adult females (Muturi et al., 2011). The results further indicate that adult mosquitoes emerging from larvae treated with essential oil at LD90 concentration lived longer than those from untreated larvae. However, a similar experiment with *A. aegypti* larvae exposed to Bti did not find differences in adult longevity between treatments and controls (Alto and Lord, 2016). It is plausible that mosquitoes in the LD90 treatment lived longer due to the alleviation of competition caused due to the initial decline in population density at the start of the experiment. This reduction provided the remaining larvae with increased per capita access to available resources. Additionally, the larvae may have benefited from the extra resources provided by the carcasses of conspecifics that died early in the experiment. As observed by Marsaro et al. (2023), these larvae developed into larger adults than those in the control group, as evidenced by the increased wing sizes observed in both females and males. Larger adult mosquitoes typically possess greater nutritional reserves, which can contribute to increased longevity (Bellamy et al., 2024; Cozzer et al., 2025). This relationship between body size and longevity has been documented in various studies, highlighting the ecological and epidemiological implications of larger mosquito sizes (Bellamy et al., 2024; Cozzer et al., 2025).

In general, any external source of mortality (e.g., plant extracts, BTI, predators, or chemical control) can produce unintended, counterintuitive effects on population dynamics (Borges et al., 2023; Cozzer et al., 2023). Nonetheless, compensatory and/or overcompensatory mortality have seldom been considered in mosquito control strategies. Ecologically, larger mosquito females tend to live longer and have greater flight capacity, are better equipped to locate and bite multiple hosts, consequently leaving larger offspring (Bellamy et al., 2024). Epidemiologically, larger females tend to have a higher capacity for virus replication due to their greater body tissue mass, providing more resources for viral replication (Aldridge et al., 2024; Hall et al., 2025). Living longer also allows for more blood feeding events, increasing the chances of arbovirus transmission (Bellamy et al., 2024), same expected with increased offspring (Cozzer et al., 2023; Bellamy et al., 2024). Additionally, mosquito larvae are frequently exposed to sublethal doses of larvicides, especially when biotic factors such as heavy rainfall or warm temperatures dilute the larvicide concentration in treated containers (Williams et al., 2014). This can lead to sublethal exposures that fail to control the population effectively (Marsaro et al., 2023) and can accelerate the selection process for resistant strains (Keumeni et al., 2025), reinforcing the importance of reevaluating the use of larvicides in integrated pest management.

Conclusion

By demonstrating that *Eucalyptus benthamii* essential oil, when applied at sublethal concentrations, can trigger density-dependent responses that alter the development of *Aedes aegypti* larvae and the adult's fitness, the main goal behind this research was achieved. Rather than functioning solely as a stressor, the induced early larval mortality alleviated intraspecific competition and ultimately enhanced the performance of surviving individuals. Responses such as greater body size and increased adult longevity are characterized as overcompensatory and indicate that the essential oil may paradoxically strengthen mosquito populations instead of suppressing them. The implications of this finding are substantial: control measures that produce only partial mortality may unintentionally generate adults with higher reproductive potential, longer lifespan, and increased capacity for pathogen transmission. Such unintended ecological and epidemiological outcomes challenge the assumption that natural larvicidal agents are inherently benign or uniformly effective. Therefore, the results highlight the need for vector management strategies that can incorporate density-dependent mechanisms and compensatory responses into their design and assessment. Future research should expand to multigenerational and population-level evaluations to determine whether these trait-level enhancements really translate into increased vector abundance and transmission risk.

Authors' Contributions

Gallon, C.: conceptualization; data curation; formal analysis; investigation; methodology; writing – original draft. **Cozzer, G. D.:** data curation; investigation; methodology; writing – original draft. **Pessetti, R. C.**: writing – original draft. **Godoy, B. S.**: writing – review & editing. **Rosa, C. S.**: writing – review & editing. **Silva, I. M.**: writing – review & editing. **Rezende, R. S.:** formal analysis; writing – review & editing. **Oliveira, J. V.**: writing – review & editing. **Dal Magro, J.**: conceptualization; funding acquisition; project administration; writing – review & editing. **Albeny-Simões, D.**: conceptualization; formal analysis; funding acquisition; methodology; project administration; supervision; writing – review & editing.

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