

Research Paper

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Corresponding author:

Renan de Souza Rezende;

Email: renanrezende30@gmail.com

Less is more: partial larvicidal efficacy of plant leachate leads to larger *Aedes aegypti* mosquitoes

Isabella Bodanese Marsaro, Gilberto Dinis Cozzer ,

Emanuel Rampanelli Cararo , Raquel De Brito , William Gabriel Borges ,

Jacir Dal Magro , Cássia Alves Lima-Rezende  and

Renan de Souza Rezende 

Communitarian University of Chapecó Region – UNOCHAPECÓ, Servidão Anjo da Guarda, 295-D, Efapi, 89809-000 Chapecó, Santa Catarina, Brazil

Abstract

Major efforts to control the population of *Aedes aegypti* mosquitoes involve the use of synthetic insecticides, which can be harmful to the environment. Most plant compounds are eco-friendly and some of them have biocontrol potential, whereas a fraction of these compounds is released into the environment through the leaf-leaching process. We evaluated the effects of secondary compounds from *Ateleia glazioviana* and *Eucalyptus grandis* senescent leaf leachates on *Ae. aegypti* larval mortality, adult emergence time, and wing size using a microcosm approach. The microcosms consisted of 10 larvae kept in water (control) and under four treatments with leachates from a combination of plant species and leaching time (7 or 14 days). Chemical analyses of the leachates showed the presence of carboxaldehyde and Heptatriocotanol, which have antimicrobial properties, potentially reducing the food available for larvae. β -Sitosterol, Stigmasterol, α -Amyrin, and Lupeol are compounds with inhibitory, neurotoxic, and larvicidal effects. Both plant species' leachates increased larval mortality and decreased emergence time due to the presence of compounds toxic to the larvae. Larger organisms emerged in treatments with 7-days leachates, likely due to the high concentration of dissolved organic matter in the leachates. The higher mortality in 7-days leachates may also increase the organic matter from co-specific decomposition, improving adult size. Therefore, if the mosquito population is not locally extinct, compounds present in leaf leachates may act as a resource enhancing larvae growth, potentially increasing survivors' fitness. In conclusion, biocontrol attempts using urban green spaces may have unexpected outcomes, such as resulting in larger pest organisms.

Introduction

Aedes (Stegomyia) aegypti (Linnaeus, 1762) (Diptera: Culicidae) mosquito is one of the main vectors of arboviruses (e.g., dengue, chikungunya, zika, and yellow fever) to humans (Feitosa *et al.*, 2015; Zara *et al.*, 2016). Therefore, the control of mosquito populations poses a significant public health challenge (Lima-Camara, 2016; Cozzer *et al.*, 2022). Currently, the main tool for controlling the population of *Ae. aegypti* is the use of chemical insecticides (Quimbayo *et al.*, 2014), which poses several risks to human health, non-target species, and the environment, including soil, water, and air contamination (Fraga *et al.*, 2016; Silva *et al.*, 2021). The use of chemical pesticides can also contribute to the emergence of resistant organisms, as tolerance to lethal doses may be a heritable trait (Poletti and Omotto, 2003). Additionally, chemical pesticides are not selective and can be lethal to both target and non-target organisms, such as pollinators and biological control agents (Pimentel and Burgess, 2014). Given these concerns, natural insecticides have emerged as an alternative to chemical insecticides (George *et al.*, 2014).

Biological insecticides can help reduce the environmental impact, as they are potentially less toxic to non-target organisms and the environment compared to chemical pesticides (Caneppele *et al.*, 2010). However, the toxicity of biological insecticides tends to decrease over time, especially in aquatic ecosystems (Rezende *et al.*, 2019, 2021). There is also the possibility that these compounds could become a food resource for resistant organisms (Gallo and Sarachine, 2009). As a result, these insecticides may alter the competition dynamics in populations regulated by density-dependent processes (Bellamy and Alto, 2018; Cozzer *et al.*, 2021). In *Ae. aegypti* populations, density-dependent processes are regulated by indirect competition for food resources (Legros *et al.*, 2011). Thus, the density-dependent effect, regulated by food resources, may determine the dynamics of the *Ae. aegypti* larval stage (Bellamy and Alto,

2018). Therefore, understanding the larvicidal effect of natural compounds on target organisms and their impact on density-dependent processes is key to successful biological control (Legros *et al.*, 2011; Rezende *et al.*, 2015).

Secondary plant metabolites are currently the main compounds used as biological insecticides (Ukoroiye and Otoyor, 2020; Barros *et al.*, 2022). These compounds can be obtained through different methods: extraction of essential oils (Barros *et al.*, 2022), leaf leaching (Rezende *et al.*, 2021), plant exudates (Hanley *et al.*, 2007), or even during leaf decomposition (Rezende *et al.*, 2023). The extraction of secondary plant metabolites takes into consideration both plant properties and the chemical characteristics of the target molecules (Soares *et al.*, 2015). The efficacy of plant compounds as biological insecticides varies among different animal species (Clemensen, 2018). Therefore, a comprehensive understanding of the interactions between biotic and abiotic components within ecosystems is crucial (Rezende *et al.*, 2019, 2023).

Ateleia glazioviana Baill (Fabaceae), commonly known in Brazil as Timbó, is a native tree found in the southern region of the Neotropics in areas near wetlands and known for its anti-herbivory potential with allelopathic potential (Alievi *et al.*, 2021). *Eucalyptus grandis* W. Hill ex Maiden (Myrtaceae), commonly known in Brazil as Eucalyptus, is an exotic tree in the Neotropics (Liu *et al.*, 2008) with allelopathic potential, inhibiting the germination of several species (Goetze and Thomé, 2004). Both *A. glazioviana* and *E. grandis*, are frequently used in urban green areas to maintain a clean understory due to their allelopathic potential, thus facilitating the recreational use of these areas. These species have been tested for their larvicidal effects tested essential oils or wood leachate (Liu *et al.*, 2008, Gallon *et al.*, 2020). However, their effects on the population of mosquitoes that survived the treatments have not been tested. Finally, little is known about the larvicidal efficacy of leaves leachate from these plant species (Saito and Luchini, 1998; Rezende *et al.*, 2019), which corresponds to the predominant form of secondary plant metabolites input in the environment (Rezende *et al.*, 2021, 2023), with potential use in biological control programs in urban areas.

In this context, our aim was to evaluate the effects of secondary metabolites from *A. glazioviana* (Timbó) and *E. grandis* (Eucalyptus) leachates as potential biological insecticides against *Ae. aegypti*. Therefore, this study can contribute to the understanding of how plant species selection for urban green space planning and management can be used in the biocontrol of mosquito vectors, specifically *Ae. aegypti*. The main premises of our study are (i) that plants produce secondary metabolites with protective functions (e.g., anti-herbivore), and these traits persist in senescing leaves (Rezende *et al.*, 2019, 2021); (ii) that secondary metabolites are gradually released through leaf leaching over time (Rezende *et al.*, 2019; Müller *et al.*, 2022); (iii) that secondary metabolites increase larval mortality (Gallon *et al.*, 2020; Barros *et al.*, 2022); and (iv) that reduced larval density increases resource availability (Rezende *et al.*, 2015; Cozzer *et al.*, 2021, 2022). Based on these premises, our hypothesis is that the high toxicity of secondary metabolites present in leaf leachate, will increase larval mortality, but also enhance the fitness of the survivors. Our predictions are that (i) the use of leaf leachate will result in high larval mortality in *Ae. aegypti*, and (ii) over time, the reduction in population density will enable survivors to attain a large body size, which serves as a proxy for mosquito fitness.

Materials and methods

Experimental room and microcosms

To obtain leaf leachates, leaves of *A. glazioviana* and *E. grandis* were collected from different locations in Chapecó, SC – Brazil. The collected leaves were transported to the laboratory, air-dried, and stored in the dark until further use. For each plant species, 100 g (OHAUS scale model ARD110) of dried leaves were leached in 8L of distilled water for 7 and 14 days (fig. 1) at 27°C (±2°C) and humidity of 70 to 80% (Beleza *et al.*, 2019).

Ae. aegypti eggs were obtained from the insectary at the Ecological Entomology Laboratory (LABENT-Eco; nº 001/CEUA/2022) at the Communitarian University of Chapecó Region (Unochapecó). Oviposition strips were placed in the experimental room of the laboratory and immersed in a Becker containing 1 l of tap water for 24 hours to allow the eggs to hatch (Cozzer *et al.*, 2022). The larvae were then counted and transferred to the microcosms. The microcosm experiment was conducted at a temperature of 27°C (± 2°C), a humidity of 70 to 80%, and a 12:12 hours light:dark cycle (Cozzer *et al.*, 2022).

Microcosms consisted of entomological traps used to capture emerging adults, each filled with 250 ml mineral water (hereinafter referred to as the control) or leaf leachate (hereinafter referred to as treatment; Cozzer *et al.*, 2022). All microcosms received 10 first-instar larvae (0.04 larva/ml). A total of four treatments, in addition to the control, were tested with four replicates each, resulting in a total of 20 microcosms. Treatments were named based on plant species and leaching time as follows: Ag7 - *A. glazioviana* 7 days leachate, Ag14 - *A. glazioviana* 14 days leachate, Eg7 - *E. grandis* 7 days leachate, and Eg14 - *E. grandis* 14 days leachate. All treatments and the control were fed with 0.012 g of Spirulina Alcon (fig. 1) (Cozzer *et al.*, 2022).

Experimental procedures

The time of adult emergence was recorded, and the adults were captured using entomological traps. Subsequently, the mosquitoes were sexed, and their left-wing length was measured in ventral view for allometric purposes (Hidalgo *et al.*, 2015). First, individuals were photographed using a Zeiss Stemi 305 binocular stereoscopic microscope, and then the images were used to obtain linear wing sizes in ImageJ v.1.51. All measurements, except for mortality, were taken from each individual. Mortality of *Ae. aegypti* was quantified as the percentage of larval mortality in the microcosm, calculated as:

$$\text{Mortality} = (N_{\text{larvae}} - N_{\text{adults}}) / N_{\text{larvae}} \times 100,$$

where N_{larvae} is the number of larvae at the start of the experiment and N_{adults} is the number of emerged mosquitoes at the end of the experiment.

GC-MS analysis of leaves leachates

The chemical composition of the bioactive fraction in the *A. glazioviana* and *E. grandis* leaf leachates at the start of the experiment was determined using Gas Chromatography and Mass Spectrometry (GC-MS) with the headspace injection method. For each leachate, nine milliliters were transferred to a 15 ml HS vial, and then 1 ml of methanol was added. The GC-MS analyses were performed using the HS-trap method with an Agilent 7890B gas chromatograph coupled to a 5977A quadrupole mass

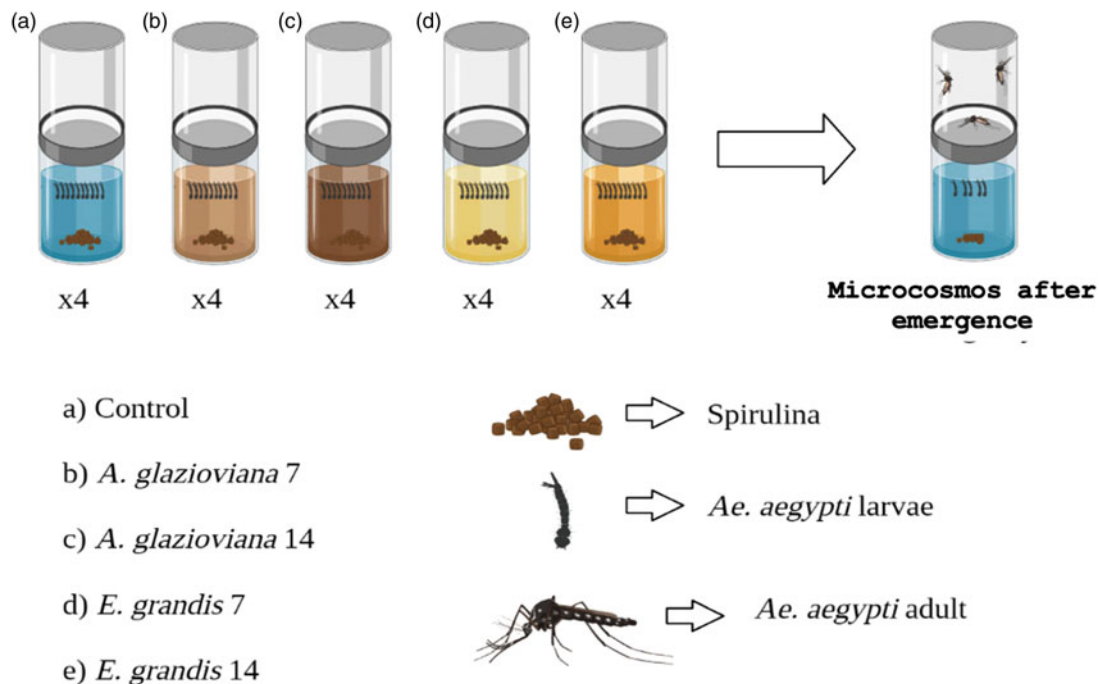


Figure 1. Experimental design to test the effects of *Ateleia glazioviana* and *Eucalyptus grandis* leaf leachates on *A. aegypti* survival.

spectrometer (Agilent Technologies, Palo Alto, CA, USA). As the HS sampler, a CTC-PAL sample injector from Agilent Technologies was used. The temperature of the HS-Trap oven was set to 70°C for 10 min. Helium was used as the carrier gas with a column head pressure of 150 kPa. The GC temperature program started at 40°C (held for 2 min), then increased to 200°C at a rate of 5°C/min, and further increased to 300°C at a rate of 40°C/min (held for 3 min). The MS transfer line temperature was set to 250°C and the ion sources temperature was set to 230°C. An electron ionization system was used for GC-MS detection with an ionization energy of 70 eV and a mass range of m/z 40–400. The analytes were detected in time windows and identified using the NIST-MS library search. Leachate compounds were identified by comparing their mass spectra with those in the National Institute of Standards and Technology (NIST 5.01) libraries provided by the computer controlling the GC-MS system (Agilent P/N G1033A).

Statistical analysis

Larval mortality, emergence time, and adult size (for both females and males separately) were evaluated as response variables among treatments (Ag7, Ag14, Eg7, and Eg14), as well as the control, using One Way Generalized Linear Models (GLM). For all four GLMs, a Gaussian error distribution was used (link = identity, test = F; Crawley, 2007). Contrast analyses were also conducted to assess the differences between the response variables in an orthogonal *post-hoc* test among different treatments using the ‘vegan’ package. In contrast analyses, the response variable was ranked from lowest to highest mean and tested pairwise. Subsequently, the model was gradually simplified by sequentially adding treatment values that did not affect the model and testing with the next variable in the sequence [for more details, see chapter 9 in (Crawley, 2007)]. All GLMs were adjusted to correct for cases of under or overdispersion (Crawley, 2007).

The effect size was calculated by comparing treatments to the control using a method analogous to the response ratio commonly used in meta-analysis (Koricheva *et al.*, 2013). The ratios were calculated for larval mortality and adult size (separately for female and male) between each treatment and the control of their respective sample battery. The ratio was calculated for each replicate. Then, to provide a consistent estimate of the magnitude of change from the null value, the values of larval mortality and adult size (separately for female and male) were log-transformed. Subsequently, 95% confidence intervals were obtained with 1000 bootstrap replicates (Davison and Hinkley, 1997) and used to test whether the magnitude and direction for each treatment were different from the control using the BCa method, as implemented in the ‘boot’ function and package (Canty and Ripley, 2016). All analyses were performed using the R statistical program version 3.6.2 (R Development Core Team, 2014).

Results

Chemistry of the leaf leachate extract

The chemical analysis of *A. glazioviana* and *E. grandis* leachates showed the presence of at least ten compounds, with five of them exclusively found on *E. grandis* leachates and one compound exclusive to *A. glazioviana*. β -Sitosterol was the main compound found in *E. grandis* 7 days leachate (74.76%), whereas significant amounts of α -Amyrin (41.95%) and Lupeol (26.71%) were found in *E. grandis* 14 days leachate (table 1). Stigmasterol was primarily found in *A. glazioviana* 7 days leachate (70.63%) and was also present in *E. grandis* 7 days leachate (9.61%). 1-Heptatriocotanol (37.53%) and 2-[4-methyl-6-(26,6-trimethylcyclohex-1-enyl)hexa-13,5-trienyl]cyclohex-1-en-1-carboxaldehyde (59.3%) were the main compounds found in *A. glazioviana* 14 days leachate. Although 1-Heptatriocotanol was also present in *E. grandis* 14 days leachate, it was found in a lower amount (9.12%).

Table 1. Chemical compounds of *Eucalyptus grandis* and *Ateleia glazioviana* leaf leachates

| Compound | <i>E. grandis</i> | | <i>A. glazioviana</i> | |
|---|------------------------------------|---------|-----------------------|---------|
| | Area of each compound (%) by GC/MS | | | |
| | 7 days | 14 days | 7 days | 14 days |
| β -Sitosterol | 74.76 | – | – | – |
| Hexacosane | 9.95 | – | 2.45 | – |
| Stigmasterol | 9.61 | – | 70.63 | – |
| Undecane | 1.14 | – | – | – |
| α -Amyrin | – | 41.95 | – | – |
| Lupeol | – | 26.71 | – | – |
| 1-Heptatricotanol | – | 9.12 | – | 37.53 |
| Eucalyptol | – | 5.96 | – | 1.2 |
| D-Limonene | 0.73 | 0.88 | – | – |
| 2-[4-methyl-6-(26,6-trimethylcyclohex-1-enyl)hexa-13,5-trienyl]cyclohex-1-en-1-carboxaldehyde | – | – | 2.5 | 59.3 |

Larval mortality, emergence time, and adult size

Leachate treatments had a significant effect on *Ae. aegypti* larval mortality (fig. 2a; table 2a). Mean larval mortality was lower in the control ($20 \pm 7\%$; hereinafter, all values are expressed as mean \pm SD) and the treatment with *E. grandis* 14 days leachate ($30 \pm 17\%$), with no significant differences between these two (fig. 2a; table 2a). In contrast, the mortality rates of *Ae. aegypti* were increased by *E. grandis* 7 days leachate ($63 \pm 25\%$) and both *A. glazioviana* leachates compared to the control (fig. 2a; table 2b). Additionally, there were no significant differences in larval mortality between the 7-day ($74 \pm 14\%$) and 14-day ($79 \pm 19\%$) leaching times for *A. glazioviana* leachates. On the other hand, the effect size (treatments vs. control) was significant for all treatments, resulting in an increased *Ae. aegypti* mortality compared to the control (mean increase: Ag7 = 56%, Eg7 = 46%, Ag14 = 58%, and Eg14 = 18%; Fig. 3a).

Leachate treatments had a significant effect on adult *Ae. aegypti* emergence time (fig. 2b; table 2b). Compared to the control (25 ± 0 days), the emergence times of *Ae. aegypti* were shorter in *A. glazioviana* 7 days leachate treatment (21.78 ± 0.6 days), *A. glazioviana* 14 days leachate treatment (10 ± 2 days), and *E. grandis* 7 days leachate treatment (21 ± 5 days) (fig. 2b; table 2b). In contrast, the emergence time of *Ae. aegypti* did not differ between the *E. grandis* 14 days leachate treatment (25 ± 0 days) and the control (fig. 2b; table 2b).

Leachate treatments had a significant effect on both female and male wing sizes (fig. 2d; table 2d). In the 7 days leachate treatments, both males (Ag7 = 2.534 ± 0.276 mm; Eg7 = 2.44 ± 0.132 mm) and females (Ag7 = 3.305 ± 0.243 mm; Eg7 = 3.346 ± 0.193 mm) had larger wing sizes compared to the control (males = 2.238 ± 0.204 mm; females = 2.871 ± 0.296 mm) and 14 days leachate treatments (males: Ag14 = 2.073 ± 0.199 ; Eg14 = 2.402 ± 0.216 ; females: Ag14 = 3.275 ± 0.554 ; Eg14 = 3.040 ± 0.485). When assessing the effect size, we found that females in the 7 days leachate treatments were, on average, 14% larger compared to the control, while males in the 7 days leachate treatments were 7% larger. Adult sizes did not differ from the control in both *A. glazioviana* and *E. grandis* 14 days leachate treatments (fig. 3b).

Discussion

Larval mortality

The compounds present in *A. glazioviana* and *E. grandis* leachates are potentially toxic to *Ae. aegypti* larvae. Despite the scarcity of studies, some of these compounds are known for their larvicidal effects (Gade *et al.*, 2017). Specifically, Stigmasterol, the main compound found in *A. glazioviana* 7 days leachate, has been reported to cause *Ae. aegypti* larval mortality by inhibiting acetylcholinesterase activity (Gade *et al.*, 2017). Additionally, the presence of 2-[4-methyl-6-(26,6-trimethylcyclohex-1-enyl)hexa-13,5-trienyl]cyclohex-1-en-1-carboxaldehyde, which is mostly a terpenoid (Erharuyi *et al.*, 2017), can enhance cellular uptake, thereby amplifying the effect of Stigmasterol (Simas *et al.*, 2004), resulting in elevated larval mortality even at lower concentrations of Stigmasterol. On the other hand, compounds found in *A. glazioviana* leachates have been found to block cellular uptake (Singer and Ramsay, 1994) and inhibit mitochondrial respiration in *Ae. aegypti*, leading to larval mortality (Mascaro *et al.*, 1998).

Regarding 1-Heptatricotanol, there is no study evidencing its larvicidal activity on *Ae. aegypti*. However, it is known that 1-Heptatricotanol is an alcoholic compound that exhibits antimicrobial activities (Erharuyi *et al.*, 2017). Thus, considering the high amounts of this compound in *A. glazioviana* leachate, further studies could focus on determining its larvicidal effects on *Ae. aegypti* by decreasing microbial biomass, one of the main larvae food resources. Similarly, Eucalyptol has been shown to inhibit bacterial proliferation in microcosms (Mączka *et al.*, 2021; Saleemi *et al.*, 2022). Although a low amount of this compound was found in *A. glazioviana* leachate, we cannot rule out its potential to diminish microbial biomass, leading to a decrease in food resources for *Ae. aegypti* larvae and potentially increasing larval mortality due to starvation (Mączka *et al.*, 2021; Saleemi *et al.*, 2022). Additionally, Eucalyptol has also been shown to have anti-herbivory and larvicidal properties (Ebadollahi, 2013).

E. grandis leachates contain the same compounds discussed for *A. glazioviana*, except for carboxaldehyde. β -Sitosterol and Hexacosane were the major compounds found in *E. grandis* 7 days leachate, and α -Amyrin and Lupeol were the major compounds in 14 days leachate. The larvicidal property of

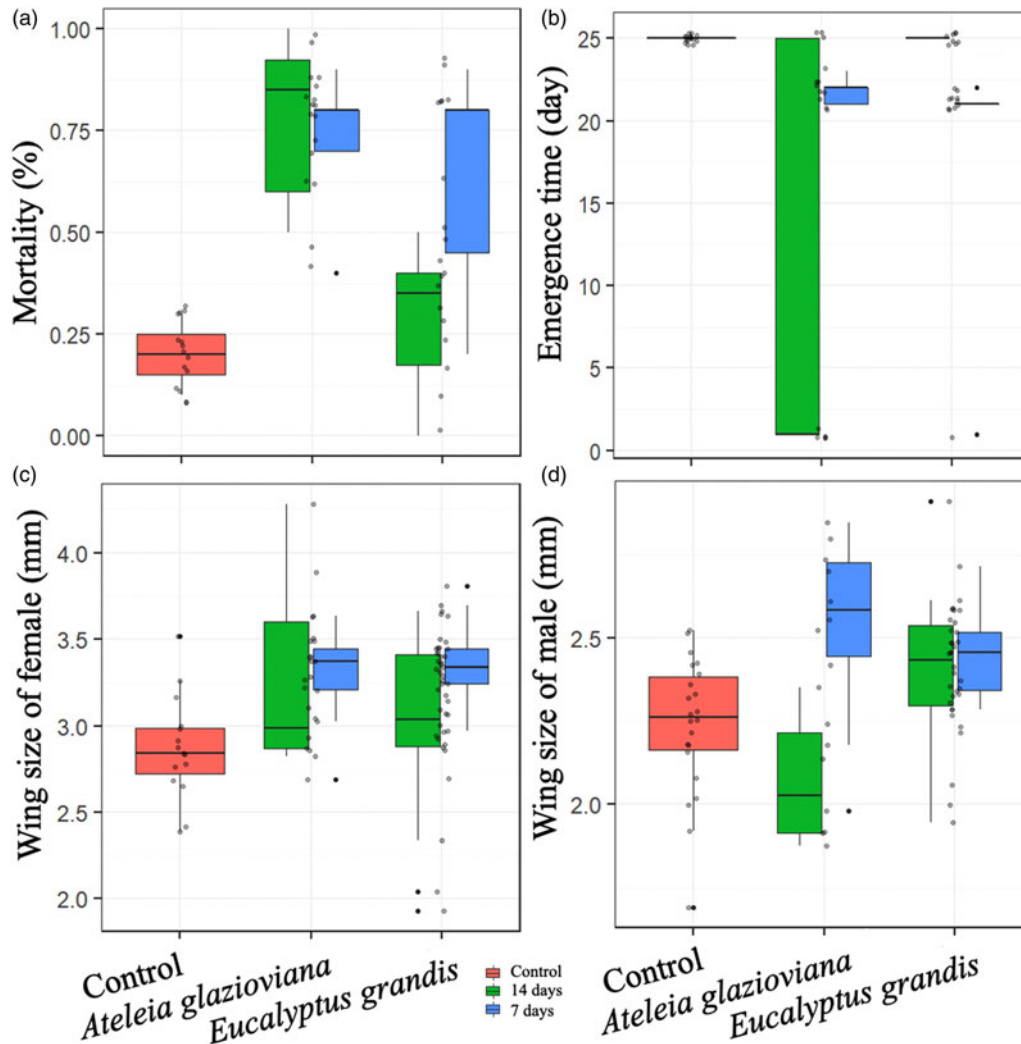


Figure 2. Responses in larval mortality (a), emergence time (b) and adult size (female - c - and male - d, separately) to different treatments (control, *Ateleia glazioviana* 7 days and 14 days leachates and *Eucalyptus grandis* 7 days and 14 days leachates). The boxes represent the quartiles, the average is indicated by a horizontal black bar, the vertical lines represent the upper and lower limits, open circles represent the sampling units, and closed circles represent the extreme values (outliers).

Table 2. We employed Generalized Linear Models (GLMs) to investigate the impact of plant species and leaching time (*A. glazioviana* 7 days leachate 'Ag7', *A. glazioviana* 14 days leachate 'Ag14', *E. grandis* 7 days leachate 'Eg7', *E. grandis* 14 days leachate 'Eg14', and control) on larval mortality (a), emergence time (b), and adult size (separately for females - c - and males - d -)

| GLMs | Df | Dev. Resid. | Df Resid. | Dev. | F | Pr(>F) | Contrast analyses |
|---------------------------|----|-------------|-----------|-------|------|--------|-----------------------------------|
| a) Null | 50 | 4.5 | | | | | |
| Mortality | 4 | 3.3 | 46 | 1.2 | 32.2 | <0.001 | Control = Eg14 < Eg7 < Ag7 = Ag14 |
| b) Null | 50 | 2826.0 | | | | | |
| Emergence time | 4 | 1996.6 | 46 | 829.5 | 27.7 | <0.001 | Ag14 = Eg7 = Ag7 < Eg14 = Control |
| c) Null | 80 | 12.5 | | | | | |
| Wing size (female) | 4 | 2.7 | 76 | 9.8 | 5.3 | <0.001 | Control = Ag14 = Eg14 < Eg7 = Ag7 |
| d) Null | 72 | 4.2 | | | | | |
| Wing size (male) | 4 | 1.2 | 68 | 3.0 | 7.0 | <0.001 | Ag14 = Control = Eg14 < Eg7 = Ag7 |

Additionally, orthogonal contrast analyses were conducted to assess the differences among the treatments. The results, including degrees of freedom (DF), residual deviance (Dev), F-statistic, and p-values (Pr > F), provide insights into the effects of the experimental variables

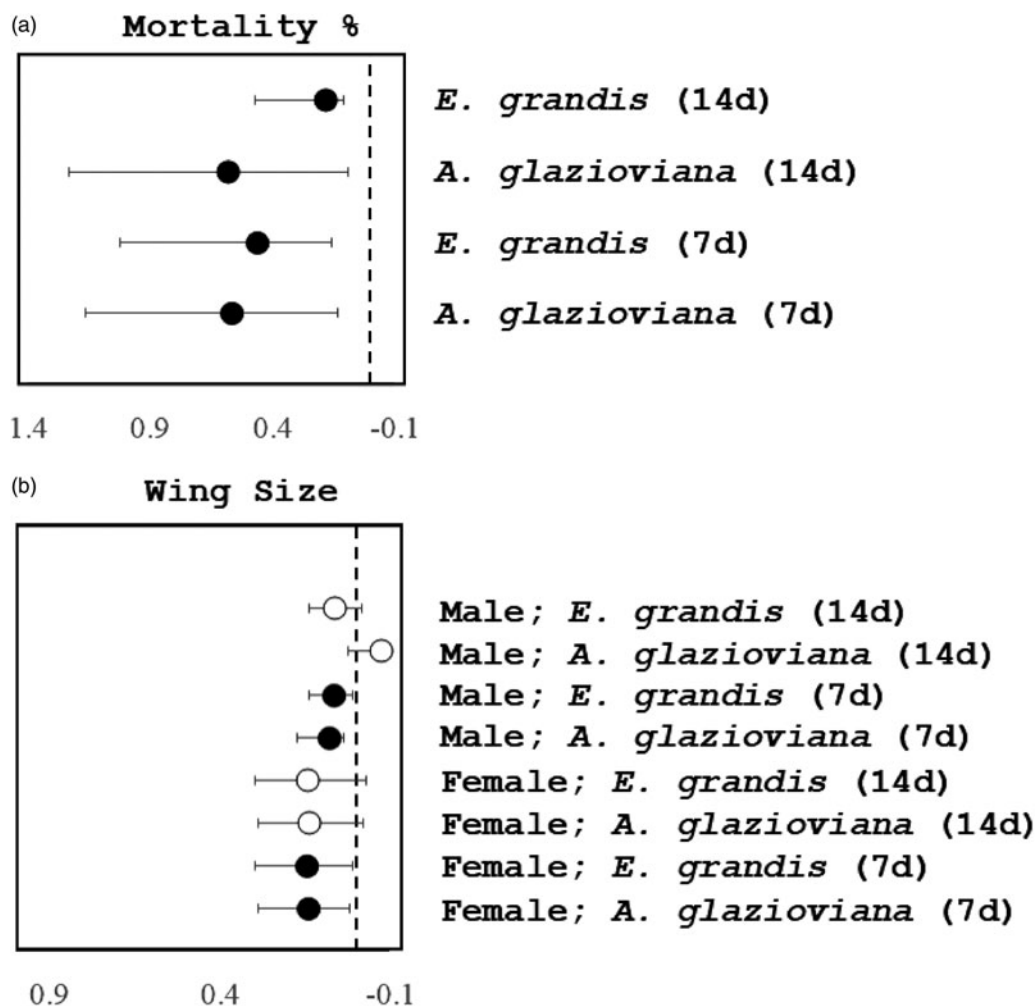


Figure 3. Size and direction of the effect of plant species and leaching time on larval mortality (a) and adult size (b) expressed by the logarithmic relationships between the different treatments (*Ateleia glazioviana* 7 days and 14 days leachates and *Eucalyptus grandis* 7 days and 14 days leachates) and their respective controls. Circles represent the means, and the lines depict the 95% confidence intervals. Black circles indicate statistically significant results, with intervals that reject the null hypothesis (indicated by not touching the dashed line at the 0 effect size). Open circles represent statistically non-significant results, with intervals that do not reject the null hypothesis (indicated by touching the dashed line at the 0 effect size).

β -Sitosterol is associated with its neurotoxicity in organisms (Viana, 2018). Similarly, Hexacosan acts by inhibiting acetylcholinesterase activity in *Ae. aegypti*, disrupting normal cholinergic neuronal transmission in the mosquito (Gade *et al.*, 2017). Additionally, previous studies have demonstrated the larvicidal potential of α -Amyrin and Lupeol on culicid larval mortality (Kuppusamy *et al.*, 2009). Larval mortality increases with increasing concentrations of α -Amyrin (Kuppusamy *et al.*, 2009), and Lupeol shows strong larvicidal effects on insects (Nobsathian *et al.*, 2018). Therefore, the greater toxicity of *A. glazioviana* can be attributed to the high concentrations of Stigmasterol and 1-Heptatriacotanol, whereas larval mortality in *E. grandis* treatments may be linked to the wider range of compounds with potential larvicidal effects, as *E. grandis* leachates had lower concentrations of Stigmasterol and 1-Heptatriacotanol.

Emergence time

A. glazioviana (at 7 and 14 days) and *E. grandis* (at 7 days) treatments reduced emergence time compared to the control and *E. grandis* at 14 days. We infer that this is an effect of increased

toxicity in the treatments. Toxic compounds found in *A. glazioviana* and *E. grandis* leachates caused the mortality of individuals (Gava *et al.*, 2001, 2021), leading to an increase in the amount of organic matter in the system due to the decomposition of co-specific individuals (Fader and Juliano, 2014). The organic matter input derived from decomposed larvae enriches the system and, coupled with reduced competition, shortens the adult emergence time of the surviving larvae, accelerating their exit from the hostile system caused by leachate toxicity (Erharuyi *et al.*, 2017). This result may also support a density-dependent regulatory mechanism in the immature stages of *Ae. aegypti* (Legros *et al.*, 2011; Cozzer *et al.*, 2021). The overcompensatory density-dependence process in *Ae. aegypti* larvae can raise serious concerns for the implementation of control methods, as it may increase the fitness of natural populations (Legros *et al.*, 2011).

Nevertheless, some compounds also may quickly deteriorate or decompose after leaching (Rezende *et al.*, 2019; Cozzer *et al.*, 2021), or even be consumed by microorganisms (Rezende *et al.*, 2021) or invertebrates (Sena *et al.*, 2020; Rezende *et al.*, 2023). Certain compounds found in *E. grandis* leachates can exhibit inhibitory activity (β -Sitosterol and Hexacosane) when present

at high concentrations, but at low concentrations, they can act as a stimulant (Della Bruna *et al.*, 1989; Subramaniam *et al.*, 2014). High concentrations of β -Sitosterol and Hexacosane were found in *E. grandis* 7 days leachate, whereas these compounds were not detected in *E. grandis* 14 days leachate, possibly explaining the high larval mortality at 7 days leachate and a non-significant larval mortality in the 14 days leachate treatment. This result suggests that a specific leaf litter, even with toxic potential, may benefit the larval development of *Ae. aegypti* during a certain stage of the leaf decomposition process. This result can also raise concerns, as it indicates that even toxic leaf litter can increase the fitness of natural populations of *Ae. aegypti* during a specific period.

Adult size

Females and males of *Ae. aegypti* were larger in the 7 days leached treatments compared to the control, regardless of the plant species. This result is attributed to the input of dissolved organic matter from the leachates, which increases resource availability and positively impacts the size of these organisms (Denno *et al.*, 1995). Additionally, the organic matter input from the death of co-specific individuals may have contributed to the increased resource availability for the survivors (Yee *et al.*, 2007; Fader and Juliano, 2014). Our results showed that mosquitoes in 7 days leachate treatments were larger compared to those in 14 days leachates. This difference can be explained by the loss of leached organic matter and secondary metabolites due to microbial decomposition over the leaching process (Marangoni *et al.*, 2013). Thus, mosquitoes exposed to leachates obtained from shorter leaching times incorporated more biomass compared to those exposed to leachates obtained from longer leaching times.

E. grandis 7 days leachate showed significant amounts of β -Sitosterol and Hexacosane. Phytosterols such as β -Sitosterol exhibit estrogenic effects (Ju *et al.*, 2004), whereas Hexacosane has been found to be part of insects' sex pheromone (Wattanchaiyingcharoen *et al.*, 2020). On the other hand, *E. grandis* 14 days leachate contained high levels of the triterpene α -Amyrin and the sterol Lupeol. The triterpene α -Amyrin, an oleanolic acid, possesses growth-regulating properties in insects (Kuppusamy *et al.*, 2009), whilst the sterol Lupeol exhibits larvicidal effects against certain insect species (Nobsathian *et al.*, 2018). Although the combination of α -Amyrin and Lupeol did not impact the mortality or emergence time of *Ae. aegypti*, it may have inhibited the growth of surviving individuals.

The effect size of 7 days leachate on female wing size was twice that observed in males, regardless of the plant species. This finding may be attributed to the shorter development period among males compared to females, a phenomenon known as protandry (Kleckner *et al.*, 1995), and previously described in *Ae. aegypti*. In this regard, the shorter emergence times of males may lead to smaller body sizes due to the intense competition resulting from the high larval density at the beginning of the experiment, which hampers their access to food resources. On the other hand, the reduced competition among females due to the early emergence of males enables females to attain larger sizes. Therefore, by accelerating emergence period of males, shorter leaching periods promote the emergence of even larger females.

Conclusion

Our study was conducted based on a microcosm experiment, and although it represents a simplification of natural systems, it

provides important insights into the effects of plant species leachates, which occur in the study region, on the mosquito populations. We found that plant leachates had significant effects on larval mortality, emergence time, and wing sizes. These results are possibly associated with the presence of secondary metabolites that possess antimicrobial properties, thereby decreasing the availability of food for *Ae. aegypti* larvae.

Additionally, the presence of compounds with inhibitory, neurotoxic, and larvicidal effects on *Ae. aegypti* altered larval mortality and emergence time, consequently affecting the size of adult mosquitoes in comparison to the control treatment. The emergence time was reduced by *A. glazioviana* and *E. grandis*, possibly due to the input of dissolved organic matter present in the leachates and the toxicity of the leachates. The toxicity resulted in increased larval mortality and provided an additional resource input through the decomposition of dead larvae, thus alleviating density-dependent competition.

Furthermore, the abundance of resources also led to larger adults in treatments with shorter leaching times, potentially benefiting the fitness of surviving individuals, as larger adults tend to have more energy available for reproduction. This suggests that if the population is not eradicated, the plant compounds can serve as a strong significant resource for the growth of *Ae. aegypti* larvae, potentially increasing the fitness of the survivors. In conclusion, careful consideration should be given to plant selection in urban green space planning and management, particularly in the context of *Ae. aegypti* biocontrol, as it may lead to unexpected outcomes, such as the enlargement of pest organisms.

Data

Not applicable. All data is available on paper.

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Ethical standards. The experimental procedures were conducted following ethical guidelines for invertebrate use in research. The authors have no conflicts of interest to disclose.

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