



Repellent activity and toxicity of the essential oil of *Calypttranthes concinna* DC. using the model insect *Drosophila melanogaster*

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Abstract

Insects, belonging to the phylum Arthropoda, play essential roles in ecosystems, such as pollination, pest control, and decomposition. However, some species are vectors of diseases, which makes the development of control methods crucial. The use of synthetic insecticides, although effective, causes serious environmental and health problems. As an alternative, plant essential oils, which are biodegradable and can have repellent and toxic effects on insects, have gained prominence. This study focuses on the species *Calypttranthes concinna* DC., belonging to the Myrtaceae family, whose repellent and toxicological properties were evaluated using *Drosophila melanogaster* Meigan (1830) as an experimental model. The botanical material of *Calypttranthes concinna* was collected from the Private Natural Heritage Reserve in Palmeira-PR, and the essential oil was extracted from the dried leaves by hydrodistillation. The oil, with a yield of 0.26%, was stored for later analysis. The species *Drosophila melanogaster* was used as a model for toxicity and repellent activity tests. The tests involved fumigation of flies with different doses of oil, evaluation of damage to locomotor capacity and repellency test using filter paper. Data were analyzed statistically using two-way ANOVA. *Calypttranthes concinna* essential oil demonstrated low toxicity to *Drosophila melanogaster*, with 100% survival at all concentrations tested over 48 h and deaths that were not statistically significant compared to the water control. Impairment of locomotor capacity was observed only at the highest concentrations (40 µg/mL) after 3 h, and at intermediate concentrations (20 µg/mL) after 6 h, but these effects were equal to the control after 12 h. The repellency test revealed that the essential oil has a significant efficacy, especially at higher concentrations (39 µg/cm²), comparable to the commercial repellent DEET, indicating that *C. concinna* essential oil is a promising alternative to synthetic repellents. *Calypttranthes concinna* essential oil has demonstrated potential as a natural repellent, with low toxicity and efficacy comparable to commercial DEET. Compared to studies with other essential oils and similar compounds, *C. concinna* oil has shown consistent results, suggesting that it may be an effective and environmentally friendly alternative to synthetic repellents. The presence of terpenes in the essential oil may be related to its repellent effect. These results support the use of *C. concinna* essential oil as a promising natural repellent.

Keywords Myrtaceae · Alternative models · Repellency · Terpenes

1 Introduction

Insects are beings belonging to the phylum Arthropoda, which have a body divided into three segments: head, thorax, abdomen and three pairs of legs (Yaqoob 2023). Over the course of evolution, these organisms have developed the ability to adapt to the most diverse terrestrial ecosystems (Schowalter 2022). It is estimated that there are around 5 million species of terrestrial insects (Stork 2018), and this

number could increase even further with sophisticated molecular analysis techniques (Wagner et al. 2021).

Most of these species are beneficial as they contribute to the balance of communities. This is accomplished by providing essential ecosystem services, such as important mutualistic associations (Elizalde et al. 2020), plant pollination, seed dispersal, pest control, decomposition of organic matter and renewal of soil nutrients (Samways et al. 2021), food production such as honey production, as well as being used as sources of protein for food security (Belluco et al. 2023).

However, some species have been scrutinised by the public health sector, as they can act as vectors of disease

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transmission (Socha et al. 2022). Examples include members of the order *Diptera*, such as flies, mosquitoes, midges, and blackflies, which are involved in the transmission of viruses, protozoa, bacteria, trematodes, cestodes, nematodes, among others (Ombugadu et al. 2018; Rizwan et al. 2023).

The primary method for controlling insects in Brazil has been the use of synthetic insecticides. This method has generated major environmental problems, including poisoning of humans and animals, extinction of non-target species, resistance to insecticides, and an increase in disease transmission (Bezerra et al. 2016; Carvalho et al. 2017).

Medicinal plants are sources of numerous secondary metabolites, which are frequently reported in studies with diverse clinical applications, such as the suppression or regulation of microRNAs associated with certain types of cancer, either as complex mixtures of compounds, such as green tea, or in their isolated forms (Şahin et al. 2023). Isolated flavonoids, for instance, have been reported to exhibit antitumor effects, either alone or in combination with clinical agents, thereby reducing adverse effects (Fernandez et al. 2021). The use of plant-derived secondary metabolites has also been associated with a reduction of harmful or adverse effects on the human body caused by synthetic agents, as highlighted in the study by Alcorta et al. (2024), which reported gastrointestinal protection through the use of nutraceuticals during chemotherapy treatment.

Thus, botanical sources have been proposed as an alternative for controlling insect pests and repelling mosquitoes, especially vectors responsible for the transmission of arboviruses (Silva Sá et al. 2023). In this aspect, essential oils are considered environmentally friendly because they are biodegradable and have a broad spectrum due to different chemical constituents (Falleh et al. 2020), in addition to interfering in essential metabolic, biochemical, and physiological functions of insects (Garrido-Miranda et al. 2022).

Plant Essential Oils (EO) are commonly used by the population as mosquito repellents and related research has demonstrated their effectiveness either through the direct action of an isolated component, through the mixture of its components, or through synergy with other oils or synthetic compound (Mishra et al. 2023).

The species *Calyptanthes concinna* DC. is a tree species belonging to the Myrtaceae family, popularly known by the names camboim-ferro, guamirim-facho and guamirim. This species can reach up to 5 m in height, has a tortuous trunk and grayish bark (Marchiori and Brum 1997). The EO derived from its leaves has antioxidant (Antonelo et al. 2022), antifungal and allelopathic (Fernandes et al. 2024) activities already described in the literature.

A previous study on *C. concinna* identified the chemical composition and antibacterial activity (Costa et al. 2020). This investigation will be the first to evaluate the plant's toxicity and repellent activity.

In this research, the model insect *Drosophila melanogaster* was used as a result of the immense scientific research related to human health, its easy maintenance and manipulation in laboratories, and its use as a toxicity and repellence model (Neto et al. 2021; Wang et al. 2021; Bezerra et al. 2022; Cook and Parks 2022; Yoon and Tak 2022).

This study is aimed at reporting the repellency activity and toxicity of *C. concinna* by evaluating the damage to the locomotor activity of the model insect *D. melanogaster* (Meigen 1830).

2 Materials and methods

Collection of botanical material The collection of plant material, specifically the leaves of *Calyptanthes concinna*, was carried out at the Private Natural Heritage Reserve (RPPN), Butugara, in the municipality of Palmeira-PR, southern Brazil (25° 20.884' S and 049° 47.258' W). The collection and transportation of this plant material was carried out under license issued by the Environmental Institute of Paraná under no. 284/11. An exsiccate was prepared and deposited in the Herbarium of the Faculdades Integradas Espírita, being registered in the collection under no. HFIE 8.820.

Essential oil extraction The extraction of the essential oil of *Calyptanthes concinna* was performed by the hydrodistillation method in a Clevenger apparatus for a period of 2.5 h, using 50 g of dried leaves in 1 L of distilled water, with 3 repetitions (Wasicky 1963). The leaves were dried using an electric dryer model FANEM—Mod. 320 SE with air circulation at 40 °C for 24 h.

To determine the moisture content of fresh leaves, 20 g samples were collected in triplicate at the time of extraction and dried in an electric dryer, model FANEM—Mod. 320 SE, with air circulation at 65 °C until reaching a constant weight. After extraction, the samples were collected with a precision pipette and stored in a freezer, where they remained until analysis. To determine the essential oil content on a dry basis, the total mass of essential oil produced was measured in relation to the amount of dry mass of botanical material used in the extraction. The yield of *C. concinna* essential oil was 0.26% of the mass of the dry leaf sample.

***Drosophila* stock and culture** The species *Drosophila melanogaster* (Harwich strain) was used as an alternative model for toxicity testing and evaluation of repellent activity. It was obtained from the National Species Stock Center, Bowling Green, OH. The flies were reared in 2.5 × 6.5 cm² bottles containing 5 mL of standard medium (83% corn mass, 4%

sugar, 4% lyophilized milk, 4% soybean meal, 4% wheat bran, and 1% salt). After cooling, 1 mL of a solution containing *Saccharomyces cerevisiae* was placed in the growth vessels. The flies were kept at a temperature of 25 °C and a relative humidity of 60%.

Toxicity test The exposure of flies to the EO was performed by a fumigation protocol as described: 20 adult flies (male and female) were placed in 330 cm³ vials containing filter paper located at the bottom, soaked with 1% sucrose in distilled water. A polyethylene terephthalate (PET) counter-cap was inserted into the screw cap of the vial, to which a filter paper was fixed on the inside of the cap for application of different doses of the essential oil. The vials received the following treatments: 1% sucrose (control) and 1, 5, 10 µL of EO, resulting in final concentrations of 10, 20, and 40 µg/mL, respectively. Fly survival readings were performed at 3, 6, 9, 12, 24, 36, and 48 h. The results were presented as the percentage (%) of live flies (mean ± SD) obtained from three independent replicates.

Assessment of damage to locomotor capacity The determination of damage to locomotor capacity was performed through the negative geotaxis test described by Coulom and Birman (2004). Each group of live flies exposed to the essential oil were led to the bottom of the container, and after 5 s the number of flies that reached the surface 4 cm from the height of the container was recorded. The tests were repeated twice for each experiment at 1-min intervals.

Repellency test The repellent activity of *C. concinna* essential oil was evaluated using the area preference method of Zhang and collaborators (2011) with modifications, against *D. melanogaster* adults. The oil was diluted in distilled water at three concentrations: 6000, 3000 and 100 µg.mL⁻¹ respectively. Filter paper (14 cm in diameter) was cut into two equal parts. One part was uniformly moistened with 500 µL of the test solution, whose concentrations varied in the plate area 39, 19.5 and 0.65 µg.cm² respectively, the other part

was moistened with 500 µL of distilled water as a control of the experiment. Each paper was fixed to the bottom of the 14 cm diameter plate side by side. For each test, 20 flies were placed in the center of the filter paper and the plate was closed with the lid. Each concentration was performed with 3 replicates. The number of flies presented on the control filter paper (Nc) and on the test parts (Nt) were recorded after 30 min, 1, 2 and 4 h, respectively. A commercial repellent (DEET 10%) was used as a positive control. The repellency percentage (PR) value will be calculated as follows: $PR (\%) = [(Nc - Nt)/(Nc + Nt)] \times 100$.

Statistical analysis The results obtained in the tests were analyzed using the arithmetic mean as central data ± standard deviation of the mean. The two-way ANOVA test with Bonferroni post hoc test was used ($p < 0.05$ considered as significance).

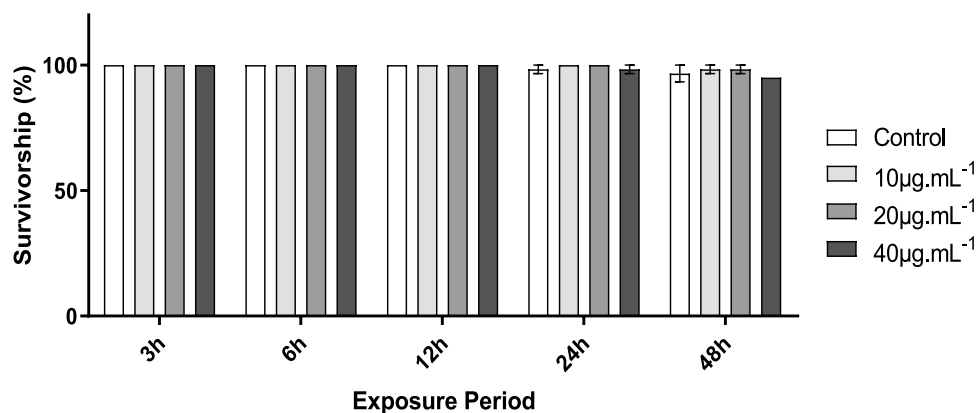
3 Results

Toxicity test The exposure of flies to *C. concinna* essential oil showed 100% survival in all concentrations tested over a period of up to 48 h (Fig. 1), demonstrating that the essential oil under study has low toxicity against *D. melanogaster*.

After 24 h, fly deaths occurred at the highest concentration used and after 48 h, at all concentrations, including the control, however the number of deaths in the presence of deaths in the presence of the essential oil was not statistically different from the aqueous control, indicating that these deaths did not occur due to exposure to the oil.

Assessment of damage to locomotor capacity When evaluating the damage to locomotor capacity caused to *D. melanogaster*, we observed that only at the highest concentration (40 µg.mL⁻¹) did the flies present damage to locomotor capacity at the first measurement time, with 3 h of exposure, as demonstrated in Fig. 2. After 6 h of exposure, the concentration of 20 µg.mL⁻¹ also began to cause damage to the

Fig. 1 Toxicity of *C. concinna* essential oil against *D. melanogaster*, ns = no significance; $p < 0.05$



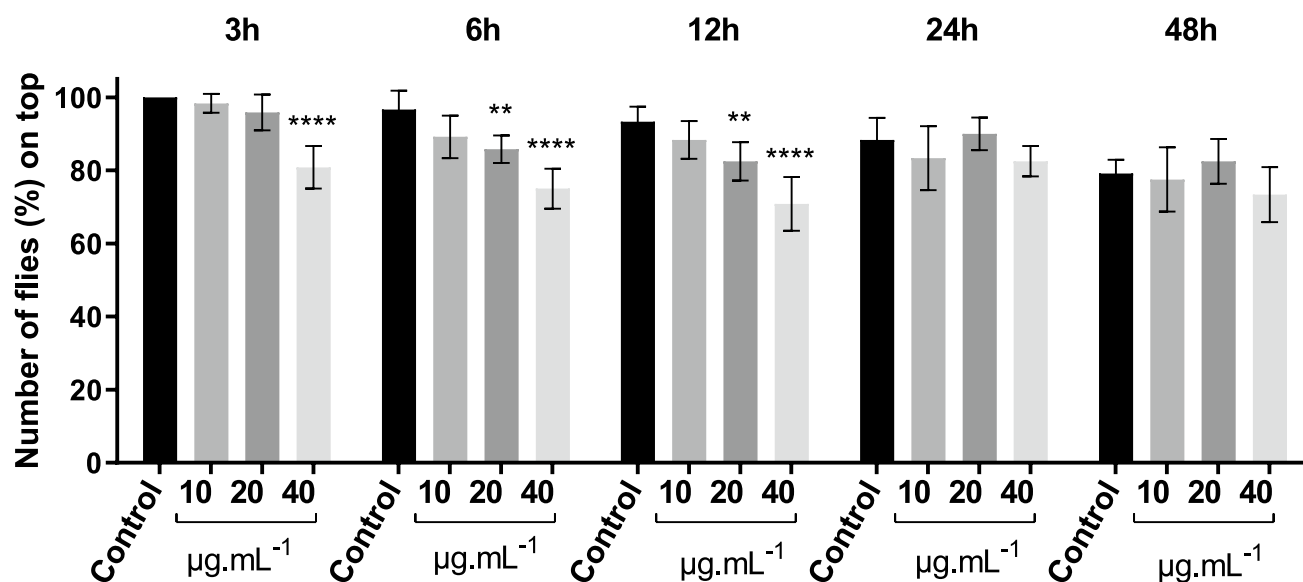


Fig. 2 Damage caused to locomotor capacity in *D. melanogaster* exposed to *C. concinna* essential oil, ns=no significance; $p < 0.05$

locomotor apparatus of the flies, together with the concentration of 40 $\mu\text{g.mL}^{-1}$.

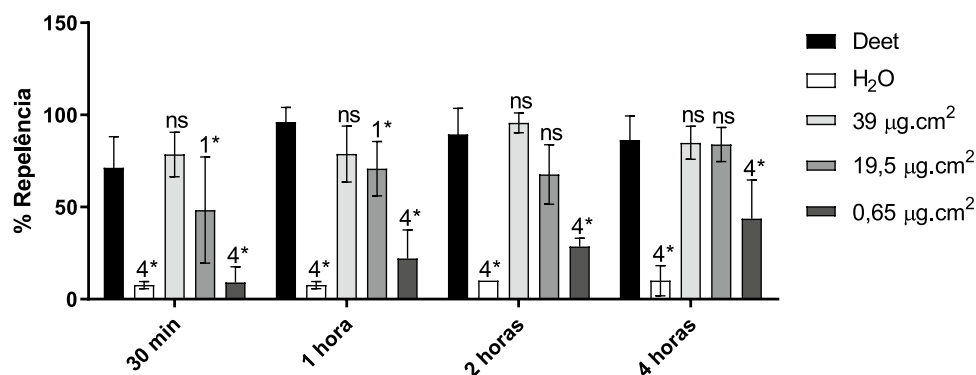
The damage caused to the locomotor apparatus of the flies differed from the aqueous control until the evaluation carried out 12 h after the start of exposure. From this moment on, the damage became statistically equivalent in all vials, for all concentrations. This can be explained by the beginning of the deaths of the flies after 24 h, as demonstrated in Fig. 1. At the lowest concentration (10 $\mu\text{g.mL}^{-1}$) there was no damage to the locomotor apparatus of the flies that differed this concentration from the control at any of the evaluation times, showing it to be a safe concentration,

Repellency test It is worth noting that the repellency test checks the distribution of flies throughout the petri dish. A uniform distribution means that the compound does not interfere with the behavior of the insects, approaching the distribution of the aqueous control. A less uniform dispersion, tending to move away from the substance and behaving

similarly to the distribution behavior in the DEET control, means repellency. The data found in the repellency test are described in Fig. 3.

When evaluating the repellent activity of *C. concinna* essential oil against *D. melanogaster*, we observed that the highest concentration, 39 $\mu\text{g.cm}^2$, presented an effect similar to that of the commercial product throughout the evaluation period. The intermediate concentration of 19.5 $\mu\text{g.cm}^2$ presented a lower level of repellency when compared to DEET, but superior to the water control. The lowest concentration of 0.65 $\mu\text{g.cm}^2$ presented an effect comparable to that of water in the first three evaluation points and subsequently an intermediate level of repellency after 4 h of exposure. This indicates the durability of the action of the essential oil over time. This fact also explains the increase in the level of repellency in the concentrations of 19.5 $\mu\text{g.cm}^2$.

Fig. 3 Repellency of *C. concinna* essential oil against exposed *D. melanogaster*, ns=no significance; $p < 0.05$



4 Discussion

Biological control is a crucial challenge. Insects play undesirable roles in the transmission of diseases and damage to agriculture (Collinge et al. 2022). This is further compounded by the negative effects caused by synthetic insecticides on both humans and animals and the environment (Pandit et al. 2022).

The use of essential oils (EOs) as insect repellents is a promising alternative to synthetic agents, offering protection against pests in a natural way. The effectiveness of EOs depends on the appropriate choice of oil to repel specific insects. However, safety issues still need to be resolved through research on the safety and efficacy of their use (Marsin et al. 2020).

Litsea cubeba (Lour.) Pers. essential oil, rich in terpene compounds, showed a concentration- and time-dependent effect on fly *Drosophila suzukii* (Matsumura 1931) mortality. At the highest concentrations tested, there was 100% death in the first 24 h, while at the lowest concentration of 20 $\mu\text{L.L}^{-1}$, there was only 10% mortality over 72 h (Gao et al. 2024).

Nanoemulsions of *Ayapana triplinervis* (M.Vahl) R.M.King & H.Rob. essential oils demonstrated effective action in the mortality of *A. aegypti* L. adults at the highest concentration (150 $\mu\text{g.mL}^{-1}$), reaching 100% mortality. At the lowest concentration (50 $\mu\text{g.mL}^{-1}$) of nanoemulsions, there was no death of the mosquito (Rodrigues et al. 2022).

In both studies presented, the concentrations tested were quite high when compared to those used in the *C. concinna* test. Comparing with the second study, the maximum concentration used in this test (40 $\mu\text{g.mL}^{-1}$) is lower than the lowest concentration of the nanoemulsions (50 $\mu\text{g.mL}^{-1}$), meaning that the findings are consistent, since there was no statistical difference between the survival of the flies at the various concentrations used as a function of time for *C. concinna*, nor for the lowest concentration of the nanoemulsions.

Studies with essential oils of *Eugenia uniflora* L. (Cunha et al. 2015) and *Psidium guajava* L. (Pinho et al. 2014), whose compositions contain terpenes, also found in the EO of *C. concinna*, showed damage to the locomotor system of flies at the two highest concentrations (30 and 23 $\mu\text{g.mL}^{-1}$) from the first observation point of the tests (3 and 6 h, respectively). After this first period, there was damage at all other observation points for all concentrations tested. Damage to the locomotor system was associated with increased mortality over time.

Unlike these studies, the EO of *C. concinna* did not show an increase in damage to the locomotor system associated with the time factor, nor an increase in mortality. This indicates that the mixtures of the different

compounds in each essential oil analyzed impacted the different results.

In another study with *Eugenia uniflora* essential oil, a mechanism of action of the essential oil on the mortality of *Drosophila melanogaster* was explored. It was found that an action on the insect's mitochondria considerably reduced its bioenergetic potential. This effect was associated with the presence of sesquiterpene, monoterpene and phenylpropanoid compounds (Carvalho et al. 2017).

The reduction of the bioenergetic potential may also be the probable mechanism of action of *C. concinna* essential oil, however, with a lesser effect due to the difference in the mixture of compounds in the essential oils. This fact reinforces the low toxicity of *C. concinna* essential oil against *D. melanogaster*, indicating that its use as a repellent may be quite promising.

The data suggest that *C. concinna* essential oil, at higher concentrations, has a repellent effect equivalent to the commercial repellent DEET.

Essential oils (EOs) and their bioactive compounds exert direct repellent activity when they penetrate the cuticles and respiratory system of insects, acting to inhibit acetylcholinesterase (AChE) which is crucial in the transmission of nerve impulses. In addition, they also cause indirect intoxication by blocking important receptors and altering antioxidant defense systems. In this way, contact with these substances repels insects (Chaudhari et al. 2021).

This repellent effect may be related to the presence of some compounds from the terpene class, which interfere with the respiratory system of insects, altering their behavior and, in this way, forming a protective barrier (Sikhan et al. 2023).

In a previous study with the essential oil of *C. concinna*, Costa and collaborators (2020) identified the following compounds as the major ones present: Elimicin, alpha-cadinol, Globulol, cis-beta-guaiene, alpha-pinene, beta-pinene.

Silva and collaborators (2019) described in their research the composition of the essential oil of the species *Myrciaria delicatula* (DC.) O.Berg, *Campomanesia xantocarpha* (Mart.) O.Berg, *Campomanesia aurea* O. Berg, *Calypttranthes clusiifolia* O. Berg, *Myrcia splendens* (Sw.) DC., *Eugenia osoriana* Mattos & D.Legrand, *Myrciaria tenella* (DC.) O.Berg, *Myrceugenia reitzii* D. Legrand., *Calypttranthes concinna* and *Myrcia arborensis* O. Berg, all from the Myrtaceae family. They showed that the chemical composition of the samples presented mono and sesquiterpenes, with sesquiterpenes in higher percentages.

Several plants from the Myrtaceae family have essential oils with repellent properties already described in the literature.

Study with *Psidium galapageium* Hooker essential oil rich in monoterpenes and sesquiterpenes, as well as its fractions, were tested against *Anopheles arabiensis* (Patton

1905) and *Philornis downsi* (Dodge and Aitken 1968), showing high repellency potential when compared with a negative control (Martina et al. 2022).

Essential oils derived from the plants *Eucalyptus rudis* Endl., *Eucalyptus crebra* F. Muell. and *Melaleuca genistifolia* Sm. were tested for repellent activity against larvae and adults of *Tribolium castaneum* Herbst. Monoterpenes and sesquiterpenes were found in the composition of the oils, which showed repellent activity against adults and larvae in varying degrees and dependent on time (Ain et al. 2024).

In fumigation tests against *Rhyzopertha dominica* Fabricius, essential oil of *Eucalyptus resinifera* Sm. demonstrated high action against the adult insect after 48 h of exposure, showing greater activity than the isolated compounds of its mixture. The effect of the essential oil of *Eucalyptus resinifera* was more potent than the commercial insecticide used as a positive control (Filomeno et al. 2020).

Another plant from the Myrtaceae family, *Temu cruckshanksii* (Hook. & Arn.) O.Berg, had its essential oil tested against the horn fly *Haematobia irritans* (Linnaeus 1758), with satisfactory repellency results, making it a suitable and environmentally friendly candidate for use in fly repellency (Espinoza et al. 2021). The repellent potential of *C. concinna* essential oil found in this article is corroborated with repellency data previously described in the literature on plants of the same family.

The essential oil of *C. concinna* exhibits low toxicity toward *Drosophila melanogaster*, demonstrating safety at the lowest concentrations tested. Although transient impairment of locomotor activity was observed at higher concentrations, these effects subsided within 12 h, reaching levels comparable to the control group. In addition, the oil showed a pronounced repellent effect, particularly at higher concentrations, comparable to that of commercial DEET. Overall, these findings highlight *C. concinna* essential oil as a promising, eco-friendly alternative to synthetic repellents, providing an effective and sustainable approach to pest management. Furthermore, considering its broad bioactive profile and low toxicity, *C. concinna* emerges as a potential source of bioactive compounds with valuable therapeutic and pharmacological applications.

Author contributions MdSC, TSF and MASO—Conceptualization; NJSA, SPS, ZSS and NSM—Investigation; HSS and AMRT—Software; FABC and ICPN—Resources; JCA-P and HDMC—Supervision; HDMC—Project Administration.

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Data availability All data will be available after request to the corresponding author.

Declarations

Conflict of interest The authors declared no conflict of interest.

Ethical approval Not applicable.

Consent of publication Not applicable.

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