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RESEARCH ARTICLE

Repellent efficacy of *Coleus amboinicus* Lour. (Lamiales: Lamiaceae) essential oil against *Aedes aegypti* L. (Diptera: Culicidae)

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ABSTRACT

The increasing prevalence of mosquito-borne diseases such as dengue fever necessitates the development of effective and safe repellents. This study evaluated the repellent efficacy of *Coleus amboinicus* essential oil (CAEO) against *Aedes aegypti*, a primary vector of dengue in Malaysia. Essential oil was extracted via hydrodistillation and analyzed using GC-MS, revealing 17 compounds, with carbamic acid (30.75%) as the major constituent, followed by caryophyllene (6.52%) and ρ-cymene (6.40%). Repellency assays were conducted using human volunteers at concentrations of 2.5%, 5.0%, 10.0%, and 20.0% CAEO and DEET. Results indicated that CAEO exhibited a concentration-dependent repellent effect comparable to DEET, with 20% CAEO offering 95.83% repellency. Complete protection time evaluation showed CAEO provided 100% protection for 30 minutes and >80% efficacy for up to 3.5 hours, while DEET offered complete protection for 4 hours. The findings highlight CAEO's potential as a natural repellent alternative, although variability in composition and efficacy underscores the need for further research, including field trials and formulation enhancements to improve longevity and effectiveness.

Keywords: *Coleus amboinicus*; essential oil; *Aedes aegypti*; repellent; mosquito-borne disease.

INTRODUCTION

In Malaysia, the most common mosquito-borne diseases are dengue fever, chikungunya, and malaria, with dengue fever being the most prevalent and a major public health concern (NHMS, 2020). *Aedes* mosquitoes, particularly *Ae. aegypti* and *Ae. albopictus*, are the main vectors responsible for transmitting the dengue virus (CDC, 2023; NHMS, 2020).

To reduce the risk of mosquito bites and the transmission of mosquito-borne diseases, proactive preventive measures are essential. These include using insect repellents, wearing long sleeves and pants, and utilizing bed nets, particularly during peak mosquito activity periods (Jones *et al*., 2021).

While synthetic mosquito repellents are effective in preventing bites and reducing the risk of diseases such as malaria and dengue, they also have certain drawbacks. A key concern is the presence of potentially harmful chemicals, such as DEET, picaridin, and permethrin, which may cause skin irritation, allergic reactions, or other adverse effects in some individuals (Aldridge, 1990; Heng *et al*., 2017; McHenry & Lacuesta, 2014). Additionally, prolonged and excessive use of synthetic repellents raises environmental concerns, as these chemicals can be released into the air and water (Roy *et al*., 2017). Furthermore, the growing resistance of mosquitoes to synthetic repellents poses a challenge, diminishing their long-term effectiveness (Yang *et al*., 2019).

In recent years, natural product-based repellents have gained increasing attention. Plant-based repellents have been found to be as effective as synthetic alternatives, though they offer shorter protection durations and require more frequent reapplication (Maia & Moore, 2011).

Coleus amboinicus, commonly known as Indian borage or country borage, has significant potential as a natural mosquito repellent (Mathalaimuthu *et al*., 2017). This aromatic plant is rich in essential oils, particularly carvacrol and thymol, which are known for their insect-repelling properties (Wadikar & Patki, 2016). Studies suggest that the volatile compounds in *C. amboinicus* exhibit mosquito-repelling effects, making it a promising candidate for botanical-based mosquito repellents (Govindaraju *et al*., 2016; Mathalaimuthu *et al*., 2017).

Given that most studies on *Coleus amboinicus* essential oil (CAEO) originate from India, investigating its activity against *Aedes aegypti* in Malaysia is necessary, considering potential differences in environmental factors and vector susceptibility. This preliminary study aims to investigate the optimal concentration and its effectiveness of CAEO against *Ae. aegypti*.

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MATERIALS AND METHODS

Source of plant

A total of 5 kg of dried *C. amboinicus* leaves, sourced from locally grown plants in Malaysia, were purchased from HERBagus Trading Sdn. Bhd. (PG0186524-U). A sample of the fresh leaves and stems provided by the company was sent to the Herbarium of Universiti Kebangsaan Malaysia for identification and authentication. The plant was confirmed as *Coleus amboinicus* Lour. (syn. *Plectranthus amboinicus* (Lour.) Spreng) from the family Lamiaceae (syn. Labiatae) and assigned the voucher number ID096/2023. The fresh leaves were then oven-dried at 55°C for 10 to 12 hours before being coarsely ground (Babu *et al*., 2018). To preserve the dried leaves, the relative humidity was consistently maintained below 10%, to prevent any fungal or bacterial infections on the leaves.

Extraction of essential oil

The essential oil was extracted according to Lalthazuali & Mathew (2017) using a Clevenger-type laboratory-scale hydro distillation apparatus. A total of 500 g of leaves were transferred into a distillation flask and fully immersed in distilled water. The leaves were boiled at 100°C for 6–7 hours. The water and oil vapour mixture were condensed by indirect cooling with cold water. From the condenser, the distillate flowed into a separator, where the oil separated automatically from the distillate water. The final product of the hydrodistillation process was a liquid containing water and essential oil. The essential oil was dried using anhydrous sodium sulphate to separate the water. After the drying process, the essential oil was weighed. The volatile compound was transferred to a glass vial, covered with aluminium foil, and stored at 4°C.

Gas chromatography mass spectrometry analysis

The gas chromatograph mass spectrometer (GC/MS) analysis was performed using an Agilent 7890A GC system coupled to an Agilent 5975C inert MSD with a triple-axis detector. The MSD ChemStation was used to find all the peaks in the raw GC chromatogram. A library search was carried out for all the peaks using the NIST/EPA/NIH version 2.0.

Mosquito colony

Aedes aegypti was colonised in the insectarium of Universiti Kebangsaan Malaysia, Kuala Lumpur. The *Ae. aegypti* eggs on filter paper were immersed in containers with dechlorinated tap water to hatch the eggs. The hatched larvae were reared with dried beef liver powder and allowed to grow into adults. The mosquitoes were maintained in an insectarium under controlled conditions of 24-26°C and relative humidity 60-80% in a 12h:12h photoperiod room. A 10% sugar solution with vitamin B complex was supplied as a food source for adult mosquitoes. The laboratory strains of *Ae. aegypti* used in this study have been colonized for >65 generations with no history of exposure to insecticides. Adult female of *Ae. aegypti*, aged between 3 and 6 days and in a pre-oviposition state, were used to evaluate the efficacy of the repellent.

Evaluation of effective dose

This study was conducted and modified from the method described by Buescher *et al*. (1982). Five volunteers were participated in this study and provided informed consent before participation. On each volunteer's forearm, a circle of 29mm diameter was drawn using a marker pen to align with Buescher's plastic cage.

Four serial dilutions of the essential oil with concentration of 2.5%, 5.0%, 10.0% and 20.0% were prepared using 70% ethanol as the solvent. Similarly, 70% ethanol served as the negative control. The diluted essential oil solutions and the control were randomly applied to the designated circles, with a consistent dosage of 25 µl per application.

Laboratory-bred, overnight starved of 3-6 days old female adults *Ae*. *aegypti* were used in the tests. A number of 15 female adults were collected into the plastic cage. Then, the cage containing female adults was secured over the treated areas on the arm, matching the cut-outs of its floor using Velcro® tape. The cover beneath the cage was slide out to expose the mosquitoes to the treated arm. The landing of each mosquito at every treated side was recorded throughout 90 seconds of exposure. The test was conducted in four replicates. The mean percentage of repellency was calculated. The same procedure was repeated using DEET as a positive control at concentrations of 2.5%, 5.0%, 10.0%, and 20.0%. The test on DEET and CAEO was performed on different days with different batches of mosquitoes. The percentage of repellency was calculated following the formula by Jantan & Zaki (1998):

$$\text{Repellency (\%)} = 100 - \frac{\text{No. of landing on treated circle}}{\text{No. of landing on control circle}} \times 100$$

Complete protection time evaluation

The World Health Organization (WHO) guidelines for efficacy testing of mosquito repellents for human skin was used and modified to determine the complete protection time of the repellent (WHO, 2009). This experiment involved three volunteers of whom provided informed consent and agreed to adhere to this study procedures. A total of 600 overnight starved aged 3-6 days old female mosquitoes were used. Using an aspirator, the mosquitoes were transferred into two cages, each containing 100 mosquitoes, assigned to a single volunteer (Lalthazuali & Mathew, 2017). The cage measuring 35cm x 35cm x 35cm with a cloth sleeve with a circumference of 15cm was used in this experiment.

Initially, the mosquitoes' inclination to land and/or probe was evaluated by introducing an untreated (treated with 70% alcohol) arm into a cage, counting 10 landings or probing within 30 seconds for acclimatisation. This process was repeated with the other arm in the second cage.

After the pre-treatment trial, a 32 cm^2 square was drawn on the volunteer's forearm. The right arm was treated with 100 µl of 20% CAEO, applied evenly onto the designated area, and allowed to dry for 15 minutes. The left arm was treated with 70% ethanol as a negative control and allowed to dry. To limit the landing of the mosquitoes to the designated area, the volunteers wore a thick rubber glove with an exposed 32 cm^2 cut-out that matched the treated area. Both arms were then introduced into the cage for 3 minutes, one after another. After 3 minutes of exposure, the arms were taken out, and at intervals of 30 minutes, the arms were introduced into the cage for another 3 minutes. This procedure was conducted for 4 hours. The assessment time points were 0, 30, 60, 90, 120, 150, 180, 210 and 240 minutes. The same procedures were repeated using 20% of DEET as a positive control. The test was conducted in triplicate, spanning different days and involving different batches of mosquitoes. The effectiveness of the CAEO was assessed through the calculation of the percentage of repellency as shown below (Schreck, 1977):

$$\text{Repellency (\%)} = \frac{MU - MT}{MU} \times 100$$

Where MU = number of mosquitoes on the untreated arm and MT = number of mosquitoes on the treated arm.

Statistical analysis

Results obtained from the experiment were analysed using SPSS software version 26 with a 95% confidence level. The mixed ANOVA test was used to assess the mean difference of DEET and CAEO on the percentage of repellency across time.

Table 1. Chemical composition of *C. amboinicus* essential oil (CAEO)

N	Compound[a]	Molecular formula	Rt[b]	Area percentage*
1.	Carbamic acid	$C_{12}H_{16}NO_2$	15.8275	30.75
2.	Caryophyllene	$C_{15}H_{24}$	18.4014	6.52
3.	ρ-Cymene	$C_{10}H_{14}$	8.7744	6.40
4.	Caryophyllene oxide	$C_{15}H_{24}O$	21.7513	4.83
5.	Humulene	$C_{15}H_{24}$	19.2594	4.64
6.	γ-Terpinene	$C_{10}H_{16}$	9.6072	4.45
7.	α-Bergamotene	$C_{15}H_{24}$	18.6979	4.42
8.	Terpinen-4-ol	$C_{10}H_{18}O$	13.5942	3.89
9.	α-Humulene epoxide II	$C_{15}H_{24}O$	22.1929	1.79
10.	Caryophylla-4(12),8(13)-dien-5.beta.-ol	$C_{15}H_{24}O$	22.7102	1.44
11.	4-Hydroxy-3-methylacetophenone	$C_9H_{10}O_2$	16.9	1.36
12.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	28.5898	1.34
13.	(+)-4-Carene	$C_{10}H_{16}$	8.4337	1.29
14.	Caryophyllene oxide	$C_{15}H_{24}O$	23.36	1.14
15.	Hexadecanoic acid, trimethylsilyl ester	$C_{19}H_{40}O_2Si$	29.4794	1.08
16.	Adamantane	$C_{10}H_{16}$	27.4984	1.03
17.	Thymol	$C_{10}H_{14}O$	29.7128	0.15

[a]Compounds listed in order of their composition percentage.
[b]Retention time (minutes).
*Only the two first decimal places are presented.

Ethic approval

This research had obtained ethical approval from the Research Ethics Committee, The National University of Malaysia (RECUKM) with the ethics reference number of JEP-2023-682.

RESULTS

Chemical composition

A total of 0.12% essential oil was yielded from 5 kg of *C. amboinicus* leaves, which is equivalent to 6.03 g of goldish essential oil in 6.7 ml. The detailed chemical constituents of CAEO analysed by GC–MS are presented in Table 1, where components below 1.0% are omitted (except for Thymol). Seventeen compounds representing 76.52% of the total oil content were identified and quantified in CAEO, indicating the complex nature of its chemical composition. The three major components were carbamic acid (30.75%), caryophyllene (6.52%) and ρ-cymene (6.40%), followed by caryophyllene oxide (4.83%), humulene (4.64%), γ-terpinene (4.45%) and α-bergamotene (4.42%).

Evaluation of effective dose

The repellency percentages of DEET and CAEO increased consistently with rising concentrations, reflecting a concentration-dependent trend. Notably, CAEO demonstrated a better repellency effect at higher concentrations compared to DEET. However, there was no significant difference ($p > 0.05$), indicating that the CAEO has a relatively comparable repellency effect with DEET.

Table 2. Percentage of repellency against various concentrations of DEET and CAEO

Concentration (%)	Percentage of Repellency (± SD)		t-test
	DEET	CAEO	
2.5	48.63 ± 14.29	23.33 ± 22.54	p = 0.176
5.0	80.37 ± 2.79	52.78 ± 25.45	p = 0.135
10.0	82.41 ± 6.99	83.33 ± 14.43	p = 0.926
20.0	92.96 ± 6.12	95.83 ± 7.22	p = 0.627

Protection time

A concentration of 20% of CAEO was selected for the test, in order to compare with 20% DEET as a positive control, as recommended by WHO (2009). In addition, both 20% of DEET and CAEO exhibited >90% repellency activity in the previous test.

This study revealed that DEET exhibited complete repellency over 4 hours duration. On the other hand, CAEO only exhibited complete repellency up to 30 minutes and was effective up to 3.5 hours (with percentage repellency >80%). Repellency percentages are presented as mean ± standard error of the mean (SEM) (Figure 1). The SEM reflects the variability across replicates at each time point.

A mixed ANOVA showed no significant interaction between repellent type (DEET vs. CAEO) and time ($p = 0.116$, $p>0.05$), indicating that the pattern of repellency decline over time was comparable for both repellents. A significant main effect of time ($p = 0.009$, $p<0.01$) was observed, driven mainly by a decrease in CAEO's repellency across the measured intervals. Additionally, the main effect of repellent type was significant ($p = 0.015$, $p<0.05$), demonstrating that overall, DEET maintained higher repellency than CAEO throughout the experiment.

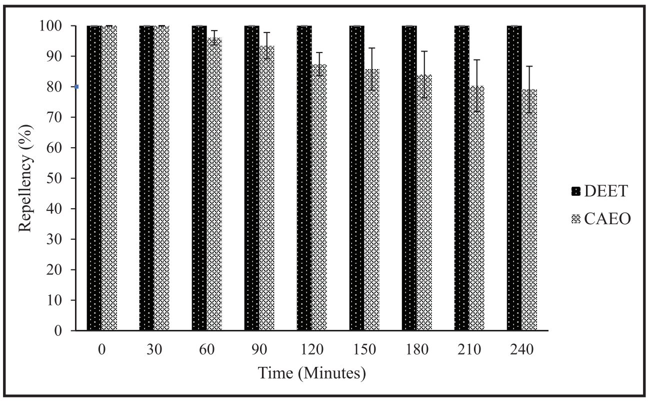

Figure 1. The percentage of repellency effect against time of DEET and CAEO treated arm. Dotted line indicating good repellency effect (>80%). Error bars represent the standard error of the mean (SEM).

DISCUSSION

Coleus amboinicus, a perennial herb renowned for its medicinal properties, has gained attention for its potential as a natural insect repellent. With the increasing concern over vector-borne diseases, the exploration of alternative repellents has become essential. Native to Southeast Asia, *C. amboinicus* also known as *Plectranthus amboinicus*, *Coleus aromaticus* and *Plectranthus aromaticus*, has a long history of traditional use across various cultures. Its essential oil is a complex mixture of bioactive compounds, with carvacrol and thymol identified as key contributors to its repellent properties (Govindarajan *et al*., 2013).

This study revealed carbamic acid as the major compound (30.75%) found in CAEO. This is in line with a study conducted in Indonesia, which identified carbamic acid (11.73%) as the primary compound in *C. amboinicus* essential oil (Suryowati *et al*., 2015). The carbamic acid substitution rules the pesticide activity. The N-methyl esters of carbamic acid are insecticides, while other derivatives of carbamic acid are herbicides and fungicides (Heinzen & Cesio, 2024).

Additionally, this study detected γ-terpinene, p-cymene, and humulene in CAEO, which aligned with previous research, though with variations in their relative concentrations (Govindarajan *et al*., 2013; Jimmy, 2021; Joshi *et al*., 2011; Leesombun *et al*., 2022; Selvakumar *et al*., 2012; Wadikar & Patki, 2016). Earlier research highlighted thymol (50.7%–57.4%) and carvacrol (13.5%–50.7%) as major constituents, along with notable amounts of γ-terpinene (5.6%–13.1%), p-cymene (5.2%–8.7%), γ-caryophyllene (8.7%–13.1%), and patchoulane (8.7%–13.1%) (Govindarajan *et al*., 2013; Jimmy, 2021; Joshi *et al*., 2011; Leesombun *et al*., 2022; Selvakumar *et al*., 2012; Wadikar & Patki, 2016). Carvacrol and thymol are widely recognized as the primary mosquito-repelling agents in *C. amboinicus* (Govindarajan *et al*., 2013; Govindaraju *et al*., 2016). However, low thymol and no carvacrol were detected in this study. Variability in the composition of essential oils is influenced by factors such as plant maturity, seasonal fluctuations, geographic origin, genetic diversity, and the specific plant parts used for extraction (Ślusarczyk *et al*., 2021).

Essential oil extraction was performed using hydrodistillation to preserve key repellent compounds, yielding 0.12% oil from dried *C. amboinicus* leaves. Comparatively, previous studies reported yields of 0.08%, 0.24%, 0.89% and 1.0% (v/w) from fresh leaves (Leesombun *et al*., 2022; Leesombun *et al*., 2023; Mangathayaru *et al*., 2005; Selvakumar *et al*., 2012). The solubility of CAEO has been well documented, with high solubility observed in 80% ethanol and diethyl ether (Wadikar & Patki, 2016). However, this study found good solubility in 70% ethanol. Variability in oil yield and composition can be attributed to factors such as geographical cultivation conditions, plant parts used and extraction methods (Leesombun *et al*., 2022).

Geographical differences such as altitude, soil type, climate, and seasonal variations can significantly affect the plant's metabolism and the biosynthesis of secondary metabolites, including essential oils (Soni *et al*., 2015). Additionally, different parts of the plant, such as leaves, stems, flowers, or roots, may contain varying concentrations and profiles of volatile compounds, which in turn impact both the quantity and quality of the oil extracted (Mazza & Cottrell, 1999). Furthermore, the choice of extraction method, such as hydrodistillation, steam distillation, solvent extraction, or supercritical CO_2 extraction, can also influence not only the yield but also the chemical profile of the oil, as some methods may degrade or fail to extract certain thermolabile or less volatile constituents (Stratakos & Koidis, 2016). Therefore, inconsistencies in essential oil yield and composition reported across different studies are often the result of these interacting biological and technical factors.

The study also examined the duration of protection offered by CAEO. Volunteers exposed to 20% CAEO experienced complete protection for 30 minutes and at least 80% protection for up to 3.5 hours. However, this result differed from the findings of Lalthazuali & Mathew (2017), who observed no *Ae. aegypti* landings within the first 6 hours following application of 20% CAEO. Despite using the same concentration and formulation, these discrepancies may be attributed to differences in *Ae. aegypti* behavior between India and Malaysia. Studies have shown that mosquito biting behavior can vary geographically due to environmental and ecological factors (Chen *et al*., 2014; Saifur *et al*., 2012; Zahid *et al*., 2023). Genetic differences among *Ae. aegypti* populations across regions may also contribute to variations in repellent efficacy (Sumitha *et al*., 2023). Geographic factors influence mosquito genetic variation, affecting their sensory responses. For instance, differences in chemoreceptor genes can alter mosquitoes' ability to detect and react to specific repellent compounds. In addition, the chemical composition of the extract will also exhibit different repellent efficiency.

Previous studies demonstrated that *C. amboinicus* extracts can provide substantial repellency against *Ae. aegypti* mosquitoes. Mathalaimuthu *et al*. (2017) reported 100% repellency for at least 320 minutes (5 hours 20 minutes) with 2.5 mg/cm² of methanol extract. Baranitharan & Dhanasekaran (2014) found that diethyl ether extracts of *C. amboinicus* at 3.0 mg/cm² provided 100% repellency for 120 minutes (2 hours), while 2.0 mg/cm² and 1.0 mg/cm² offered protection for 80 and 40 minutes, respectively. These findings suggest that solvent selection can influence the chemical composition of the oil or extract, subsequently affecting its repellent efficacy. Research has shown that methanol, diethyl ether, and ethyl acetate extracts of *C. amboinicus* demonstrate high repellent and larvicidal effectiveness even at minimal concentrations (Baranitharan & Dhanasekaran, 2014; Deepak *et al*., 2020; Mathalaimuthu *et al*., 2017).

When comparing the repellent efficacy of DEET and CAEO, it was evident that DEET demonstrated superior and longer-lasting protection against mosquito bites. This outcome was anticipated, as synthetic repellents like DEET are specifically formulated for prolonged activity and have lower volatility, allowing them to remain on the skin surface for extended periods (Qiu *et al*., 1998). In contrast, essential oil-based repellents, including CAEO, tend to provide shorter protection times. This is primarily due to the high volatility of their active components, which causes them to evaporate more rapidly upon exposure to air and body heat. As a result, the concentration of repellent compounds on the skin diminishes quickly, leading to reduced effectiveness over time. According to Yoon *et al*. (2015), this rapid evaporation is a major limitation of natural repellents, necessitating frequent reapplication to maintain efficacy. Therefore, while essential oils may offer a more natural and potentially safer alternative, their limited persistence poses a challenge for sustained protection in field conditions.

Throughout the study, no adverse effects were reported by volunteers. However, research on the toxicity of *C. amboinicus* extracts remains limited. Existing studies on bioactivity and toxicity have been conducted on a small number of compounds under in vivo conditions using animal models (Arumugam *et al*., 2016; Asiimwe *et al*., 2014; Pillai *et al*., 2010). Sub-acute toxicity assessments involving albino mice indicated that the highest dose (2000 mg/kg) of crude extract and methanol extracts at 200 and 400 mg/kg did not result in mortality, adverse effects, or significant changes in hematological or biochemical parameters. Histological examinations of vital organs revealed no morphological abnormalities (Pillai *et al*., 2010).

The promising results of this study open new avenues for further research. One potential direction is the integration of essential oils with nanoparticles, which could enhance the stability and sustained release of active compounds, potentially extending the duration of repellent effects (Abrantes *et al*., 2021). Combining essential oils with nanotechnology by encapsulating them in nanoemulsions or nanostructured lipid carriers can slow volatilization and enable controlled release, thereby improving longevity and efficacy of repellency compared to conventional formulations (Kechagia *et al*., 2024). Additionally, synergistic studies combining CAEO with other essential oils could broaden its spectrum of protection against a wider range of insect species (Jantan *et al*., 2003). Understanding how CAEO interacts with other essential oils will help determine whether the effects are additive or synergistic.

To further validate laboratory findings, additional studies should assess the real-world applicability and safety of CAEO. Field trials are essential to evaluate the repellent's effectiveness under natural conditions, considering factors such as environmental variations, diverse insect species and human activity. Furthermore, extending the duration of repellency assessments to 8 – 12 hours would provide critical insights into the long-term protective effects of the essential oil, especially in settings where individuals are exposed to mosquito vectors for extended periods.

CONCLUSION

This study demonstrated that *Coleus amboinicus* essential oil (CAEO) exhibits concentration-dependent repellency against *Aedes aegypti*, with comparable effectiveness to DEET at 20% concentration, achieving over 80% protection for up to 3.5 hours. Although DEET provided longer complete protection, these findings highlight the potential of CAEO as a natural and environmentally friendly alternative to synthetic repellents. As DEET continues to serve as the gold standard, the rising interest in botanicals reflects global efforts to adopt more sustainable public health strategies. However, further research is essential to improve the formulation, stability, and longevity of essential oil-based repellents in order to match the efficacy of conventional products. Continued exploration in this area is critical to diversifying vector control tools and reducing reliance on synthetic chemicals, especially in addressing the growing need for eco-friendly solutions to combat mosquito-borne diseases such as dengue.

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