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Behavioral response of *Aedes aegypti* (Diptera: Culicidae) to essential oils of *Cymbopogon nardus* (L.) *Eucalyptus camaldulensis* (Dehnh) and their blend in Y-maze olfactometer

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Background: *Aedes aegypti* vectors several important arboviruses including dengue and yellow fever. This vector mosquito is controlled mainly by using synthetic insecticides and repellents. Overusing these insecticides causes mosquito resistance, harms the environment, and affects human health. This report reevaluates the repellent activities of *Cymbopogon nardus*, *Eucalyptus camaldulensis* essential oils (EOs), and their mixtures against laboratory-reared adult *Ae. aegypti*.

Methods: The chemical composition of *C. nardus*, *E. camaldulensis* EOs, and their 1:1 combination was identified by gas chromatography-mass spectrometry (GC-MS). We evaluated the repellent activities of these oils against *Ae. aegypti* using a Y-maze olfactometer. The preference index (PI) was evaluated and compared with the binary data obtained from the olfactometer assay with samples that did not contain EOs (control) using an Exact Binomial test ($\alpha = 0.05$)

Results: Several monoterpenes and sesquiterpene compounds were found in EOs and their mixture. The EOs of *E. camaldulensis* and the mixture of the two oils showed a repellent activity of 50%, whereas *C. nardus* was less active and attracted mosquitoes at 1 ppm.

Conclusion: We show that EOs from *C. nardus* and *E. camaldulensis* contain compounds that repel *Ae. aegypti*. Future studies will identify specific compounds with the highest repellent activities and use them to formulate in the future a potent repellent against *Ae. aegypti* for human protection.

KEYWORDS

essential oil, repellent, *Aedes aegypti*, mixture, GC-MS

1 Introduction

Dengue, chikungunya, and zika viruses are transmitted by *Aedes* mosquitoes. Among these, dengue virus (DENV) is a significant global health concern, affecting millions of people annually (1, 2). Historically, dengue fever was more prevalent in South America than in other parts of the world (3). Over the past two decades, the reported cases of dengue have increased many folds, and the World Health Organization (WHO) reports a more than eight folds increase from 505,430 cases in 2000 to over 2.4 million in 2010 and 5.2 million cases in 2019 (4). Following a slight decline in cases between 2020 and 2022 mainly due to lower reporting during the COVID-19 pandemic and a global upsurge in dengue cases was observed in 2023 (5). Outbreaks have been reported in 15 of the 47 surveyed African countries, including Benin, Burkina Faso, Cape Verde, Chad, Côte d'Ivoire, Ethiopia, Ghana, Guinea, Mali, Mauritius, Niger, Nigeria, São Tomé and Principe, Senegal, and Togo (5). Burkina Faso was the most affected country in the sub-Saharan Africa region in 2023 with a cumulative total of 154,867 suspected cases and 709 recorded deaths (6). This situation is explained by the presence of discarded car tires in the cities and the water-based handwashing stations introduced into public places to reduce or prevent transmission of the SARS-CoV-2 virus (7).

The primary vector responsible for transmitting DENV is female *Aedes aegypti*, that predominantly inhabits urban areas and breeds in human-made containers. *Ae. aegypti* exhibits diurnal biting behavior, with peak activity observed during early mornings and evenings before sunset (4, 8). Current strategies for disease control involve the use of insecticides to reduce mosquito populations and the application of chemical repellents to protect humans from mosquito bites (9, 10). The widespread use of synthetic insecticides, however, has led to a surge in insecticide resistance among mosquito populations (11–14) contributing to environmental toxicity that adversely impacts biodiversity and human health (15, 16). It has been shown that *Aedes* resistant to DDT and pyrethroid are less sensitive to the effects of repellents such as N, N-diethyl-3-methylbenzamide (DEET), ethyl 3-[acetyl (butyl)amino] propanoate (IR3535), and 2-undecanone (17). Similarly, conventional topical repellents, such as DEET, IR3535, and picaridin are used to protect against mosquito bites (18, 19). However, using repellents such as DEET has raised significant health concerns including allergic reactions and skin irritation, and brain disease such as encephalopathy, especially for children (20, 21). Additionally, DEET has limitations, including high production costs, plasticizing effects on polymers, limited efficacy against specific insect species, and nontarget effects (22–24). Keeping in mind the problems associated with chemical insecticides, synthetic repellents, and diseases spread by *Ae. aegypti*, there is a dire need to find natural chemical ingredient to develop new plant-based mosquito repellents.

Plant-derived oils and extracts are safe and eco-friendly alternatives. Several plant essential oils (EOs) are known to have insecticidal, repellent, and growth-reducing properties (25–29). EOs from the leaves of *Lantana camara* L., *Hyptis suaveolens* Poit., *Hyptis spicigera* Lam, and *Ocimum canum* Sims show strong oviposition deterrence, excito-repellent and blood-feeding inhibitory activities against *An. coluzzii* and *An. gambiae* (28). Various aromatic plant EOs, such as *Zanthoxylum piperitum*, *Anethum graveolens*, *Kaempferia galanga* (30), *Lippia alba*, *L. origanoides*, *Eucalyptus citriodora*, *Cymbopogon citratus*, *Cymbopogon flexuosus*, *Citrus sinensis*, *Cananga odorata*, *Swinglea glutinosa*, and *Tagetes lucida* (31), are known for their repellent effects against *Ae. aegypti*. Several EOs mixtures exhibit synergistic effects, eliciting stronger repellency when compared with single EOs. For instance, a mixture of *Litsea cubeba* and *Litsea salicifolia* oils are highly repellent and is comparable to DEET against *Ae. aegypti*, than each EO (32). This synergistic effect results from the interaction between specific EO compounds, such as monoterpenes and sesquiterpenes, which deter mosquitoes from landing on treated surfaces (33).

In this study we report the repellent activities of two EOs from local plant species: *Eucalyptus camaldulensis* and *Cymbopogon nardus*. The chemical compositions of these EOs were determined using gas chromatography-mass spectrometry and their repellent activity was quantified against female *Ae. aegypti* mosquitoes using an olfactometer. The repellent potential of these EOs, will contribute to the development of safe and natural mosquito repellents.

2 Materials and methods

2.1 Plant sample collection and essential oil extraction

Plant leaves (Figure 1) were sampled at the "Institut de Recherche en Sciences Appliquées et Technologies" (IRSAT) in Ouagadougou, Burkina Faso. The leaves are the parts of the plant that contain the most essential oils. The taxonomic identification of the collected samples was carried out at the 'Laboratoire de Biologie et d'Ecologie Végétales' (Université joseph KI-ZERBO, Burkina Faso). Essential oils (EOs) were extracted from *Eucalyptus camaldulensis* (EC) and *Cymbopogon nardus* (CN) by hydro distillation at IRSAT. The EOs were distilled, dried over anhydrous sodium sulfate, and stored at 4°C.

2.2 Gas chromatography coupled with mass spectrometry (GC-MS) analysis of essential oils

Gas chromatography-mass spectrometry (GC-MS) analysis identified and quantitated the major and minor constituents of *C. nardus*, *E. camaldulensis* EOs alone, and their 1:1 combination. An aliquot (20 µL) was extracted from each EO sample using a micropipette, diluted at 1/5000 in hexane, and placed into a vial with an insert (VWR, Radnor, PA), allowing it to be injected into a GC-MS (Trace 1310, Thermo Fisher Scientific) equipped with a 30 m column (IDD 0.25 mm, #36096-1420, Thermo Fisher Scientific). Five microliters of the prepared samples were loaded into the GC-MS using an autosampler (TriPlus RSH, Thermo Fisher Scientific). Helium was used as the carrier gas at a constant flow of 1 cc/min1 cc/min. The oven temperature was set at 45°C, held for 4 minutes, followed by a heating gradient ramping to 230°C (10°C/min), and the 230°C temperature was maintained isothermally for 6 minutes (total run: 28.5 min.). Each sample was analyzed in triplicates. Chromatographic peaks were integrated using the Chromeleon software MS quantitative processing method (Thermo Fisher Scientific), and chemical compounds were identified using the online NIST library. Major peaks found with consistently high abundances across multiple samples for each EO were then recorded, and compared with all Eos.

2.3 Dilution of essential oils

The EOs were diluted in mineral oil, and the tested concentrations were obtained from a stock solution of 10,000 ppm (0.5 ml of oil diluted in 49.5 ml of ethanol or 0.25 ml in 24.75 ml of ethanol). A stock solution (10,000 ppm) of the two oil combinations (1:1) was prepared by mixing 0.25 ml of each EO with 49.5 ml of mineral oil from a stock solution (10,000 ppm) aliquots of 1, 10, and 100 µl were removed and added to 10 ml of mineral oil to obtain 1, 10, and 100 ppm dilutions, respectively [Sigma Life Science (M8410)].

2.4 Mosquito rearing

Aedes aegypti (Rockefeller strain: MR-734, MR4, ATCC®, Manassas, VA, USA) mosquitoes were reared at Virginia Tech (Blacksburg, VA, USA) in an incubator at 26°C and 60% relative humidity under a 12:12 h (light: dark) cycle. Eggs were hatched in deionized water, and larvae were fed with fish food (Hikari Tropic First Bites, Petco, San Diego, CA, USA). After emergence, adult females were fed on 10% sucrose solution for five days. On day

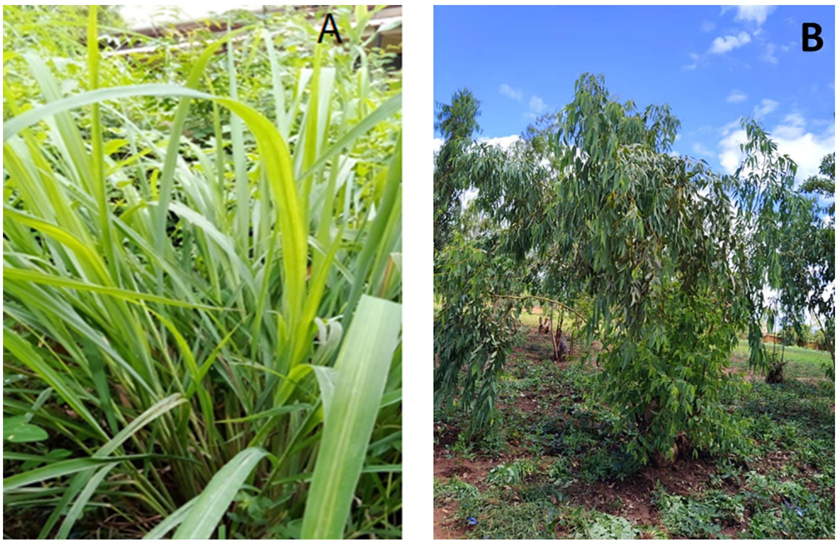

FIGURE 1
Plants used for the study: **(A)** *Cymbpogon nardus*; **(B)** *Eucalyptus camaldulensis*.

six, the sucrose solution was withdrawn for 24 hours, and female mosquitoes seeking response were determined using an olfactometer.

2.5 Seeking and deterrence behavioral response in the presence of EOs

A Y-maze olfactometer [as described in (34)] was used to quantify the seeking or deterrence response of mosquitoes in the presence of single and mixed EOs at concentrations of 1, 10, and 100 ppm. The olfactometer, constructed using custom-cut acrylic sheets, comprises two choice arms connected to an entry arm via a central chamber (100 cm long, 10 cm internal diameter, with choice arms positioned at an angle of 120°). Two fans, mounted at the end of the choice arms, generated a constant airflow (Rosewill, Los Angeles, CA, USA, air speed ~30 cm.s-1), with incoming air filtered through a series of activated charcoal filters and honeycomb mesh (10 cm long) to create contaminant-free laminar airflow. Charcoal-filtered air (~3 cm.s-1) passed through scintillation vials containing either the test (EOs) or control odor (mineral oil) and was delivered as olfactory stimuli at the distal end of the olfactometer's choice arms through divided circuits of polytetrafluoroethylene (PTFE) tubing (McMaster-Carr, Elmhurst, IL, USA). The position of the test and control olfactory stimuli were randomized between the two choice arms across experimental trials to eliminate bias (35). The behavior of individual mosquitoes in the olfactometer was observed for 5 minutes. Additionally, statistical analysis of mosquitoes' behavioral preference toward two clean air currents (i.e., mineral oil-laden air delivered in both choice arms of the olfactometer) confirmed the absence of directional bias in the assay (n = 16; Binomial Exact test: P = 0.59) (35). For each EO concentration, assays evaluating the behavioral preference of individual females were spread across two to three experimental sessions to address reproducibility and block biased effects. For every EO concentration tested, 24-28 female mosquitoes (released individually into the olfactometer) that showed attraction or deterrence during the assay were included in the analysis of behavioral preference. The sample size that we used is sufficient to achieve a statistical power > 0.8. All assays were conducted in a well-ventilated room at a constant temperature (26 ± 1.5°C) and relative humidity (50–60%). DEET (98.11%) served as a positive control to demonstrate deterrence.

2.6 Statistical analysis

The olfactory preference of mosquitoes in the Y-maze olfactometer assay was quantified as a preference index (P). The preference index was calculated using the formula: $P = (O - C)/(O + C)$, where O denotes the number of mosquitoes that preferred the odor arm, and C denotes the number of mosquitoes that preferred the control arm. The standard error for the preference index was computed using the formula $\sqrt{[q(1-q)]/[O+C]}$, where q denotes the proportion of active mosquitoes. The binary data quantified in the olfactometer assay were compared to chance using the Binomial Exact test (α = 0.05). The percentage repellency of the EOs and the proportion of active and decision-making mosquitoes were compared across treatments in the olfactometer assay using a Generalized Linear Model assuming binomially distributed errors (36). *Post hoc* comparisons reported in this study were performed using the Tukey's HSD method and corrected for pairwise multiple comparisons. All analyses were performed using R software (version 4.2.2).

3 Results

3.1 Chemical composition and yield of essential oils

Thirty-seven compounds with concentrations higher than 0.1% were identified in *C. nardus* (Table 1). The main constituents of this oil were citronellal (28.41%), geranial (16.03%), cis-geraniol (10.30%), and elemol (7.39%). The remaining compounds were less than 5% in this oil. In *E. camaldulensis*, 25 compounds were identified (Table 1), with the major compounds being eucalyptol (48.59%), p-cymene (23.86%), γ-Terpinene (6.83%), and α-Phellandrene (5.13%). The blend (1:1) of *C. nardus* and *E. camaldulensis* contained a reduced number of compounds (21), with the primary constituents being eucalyptol (36.61%), citronellal (29.67%), nerol (9.5%), α-Pinene (7.19%), and p-Cymene (6.51%). Some compounds in the mixture were less abundant compared with the individual oil samples. Nerol, γ-Elemen, and β-Elemen were present in the mixture despite their initial absence in the individual oils.

3.2 Mosquito olfactive response to essential oils

In the Y-maze olfactometer, when given the choice between two clean air currents (negative control), the mosquitoes showed random orientation between the two arms, indicating no bias in the experimental setup (n=44, P > 0.05). At a 1 ppm concentration (Figure 2A), the EO from *E. camaldulensis* (EC) demonstrated significant repellent activity both alone (n=27, P = 0.026) and in combination with the EO from *C. nardus* (CN) (n=70, P = 0.021). The EO from CN at a 1 ppm concentration showed low levels of attraction that were not statistically significant (n=25, P > 0.05). At a 10 ppm concentration, the EOs from both EC and CN did not exhibit repellent activity (n=25 per EO, P > 0.05). The combination of EOs from EC and CN at a 10 ppm concentration did not show repellent activity (n=26, P > 0.05). At a 100 ppm concentration, the EO from EC showed the strongest repellent activity (n=25, P = 0.007) (Figure 2A). Additionally, the EOs from EC and CN, in combination at a 100 ppm concentration, displayed significant repellent activity (n=27, P = 0.026). However, when used alone at a 100 ppm concentration, the EO from CN did not demonstrate significant repellent activity (n=27, P > 0.05).

TABLE 1 Essential oil chemical composition and yields.

R. time	Compounds	*C. nardus (%)*	*E. camaldulensis (%)*	*C. nardus + E. camaldulensis (%)*
12.182	β-Thujene	–	0.21	–
12.216	α-Pinene	0.13	–	7.19
12.624	Camphene	–	0.23	–
13.087	β-Pinene	–	0.61	–
13.305	β-Myrcene	0.17	0.70	–
13.638	α-Phellandrene	1.68	5.13	0.76
13.985	p-Cymene	1.74	23.86	6.51
14.039	Ethylhexanol	0.18	–	–
14.097	D-Limonene	4.31	–	0.53
14.148	Eucalyptol	2.57	48.59	36.61
14.505	Melonal	0.13	–	–
14.624	γ-Terpinene	0.23	6.83	0.73
15.114	α-Terpinolene	0.18	–	–
15.339	Linalool	2.09	0.13	0.23
15.587	isovalerate	–	0.77	–
16.124	(-)-trans-Pinocarveol	–	1.11	–
16.294	Citronellal	28.41	–	29.67
16.481	(+)-isodihydrocarveol	0.48	–	–
16.638	Pinocarvone	–	0.16	–
16.815	Terpinen-4-ol	0.20	2.12	0.24
17.073	α-Terpineol	–	2.45	-0.25
17.097	α-Terpinyl propionate	0.16	–	–
17.233	Decanal	0.13	–	–
17.437	Citronellol	0.20	–	3.09
17.682	Neral	0.20	–	–
17.828	Geraniol	16.03	–	–
17.964	cis-Geraniol	10.30	–	–
17.971	Nerol	–	–	9.50
18.046	p-Menth-6-en-2-one.	–	0.22	–
18.1	α-Citral	0.99		–
18.117	Piperitone	–	0.23	–
19.389	γ-Elemene	–	–	0.50
19.485	Eugenol	1.05	–	–
19.723	Geranyl acetate	3.05	–	–
19.821	Nerol acetate	–	–	0.68
20.015	Elemene	2.12	–	–
20.162	β-Elemene	–	–	0.27
20.563	Caryophyllene	0.19	0.29	–

(Continued)

TABLE 1 Continued

R. time	Compounds	*C. nardus* (%)	*E. camaldulensis* (%)	*C. nardus* + *E. camaldulensis* (%)
20.726	Aromandendrene	1.08	1.57	–
20.947	β-Longipinene	1.05	1.09	–
21.362	Germacrene D	1.49	–	–
21.485	Aromandendrene	2.29	–	–
21.985	δ-Cadinene	–	–	0.26
22.138	Elemol	7.39	–	1.12
22.365	β-Elemol	0.57	0.15	–
22.505	Thunbergol	4.40	–	0.66
22.794	(+)-Spathulenol	–	0.95	–
22.814	(-)-Globulol	0.55	1.09	0.14
22.93	Caryophyllene oxide	–	–	0.15
23.413	.tau.-Cadinol	0.71	–	–
23.474	(-)-Spathulenol	–	0.38	–
23.491	.tau.-Muurolol	0.86	–	–
23.559	α-Cadinol	1.92	–	0.20
23.573	β-Acorenol	–	0.24	–
26.841	m-Camphorene	0.17	0.25	–
	Total	99.41	99.38	99.29

R. time, retention time; Compounds representing less than 0.1% in the same row in all essential oils were not listed.

The activity levels of mosquitoes in the olfactometer assay (i.e., the proportions of mosquitoes initiating flight to move out of the release arm in the olfactometer) were consistently over 50% (Figure 2B). When presented with no odorant stimulus, i.e., negative control, 93.4% of the mosquitoes were active in the assay (n=57). In comparison, the EOs from CN and EC, both in isolation and in combination at 1 ppm concentration, elicited statistically comparable levels of flight initiation and activity (CN: 96.6%, n=56; EC: 98.2%, n=55, CN+EC: 98.9%, n=89; $P > 0.05$ for all comparisons). At 10 ppm concentration, the activity levels of mosquitoes exposed to the EO from CN were significantly lower when compared to the negative control (CN: 62.9%, n=44; $P = 0.012$). The EO from EC, in isolation and in combination with the EO from CN at 10 ppm concentration, elicited activity levels statistically comparable to the negative control (EC: 86.8%, n=66, CN+EC: 98.5%, n=64; $P > 0.05$ for all comparisons) (Figure 2B). The EOs, at 100 ppm concentration, did not affect the activity levels of mosquitoes in the olfactometer assay (CN: 83.5%, n=66; EC: 96.8%, n=60, CN+EC: 100%, n=48; $P > 0.05$ for all comparisons).

The analysis of the proportion of decision-making mosquitoes (i.e., the proportions of active mosquitoes that made a choice between control and test odors) revealed that 77.2% of the active mosquitoes, in the absence of an odorant stimulus i.e., negative control, made a choice in the olfactometer assay (n=44) (Figure 2C). The proportion of decision-making mosquitoes in the olfactometer assay across the EOs, both in isolation and in combination, as well as across the three concentrations, although slightly lower compared to the negative control, were statistically comparable.

4 Discussion

GC-MS analyses revealed several compounds in the extracted plants' essential oils (EOs) and their mixtures. Previous studies have shown variations in the chemical composition of EOs of *E. camaldulensis* (37, 38) and *C. nardus* (39, 40), exhibiting different chemotypes. For example, in the repellent study by Azeem et al., *E. camaldulensis* major compounds were limonene, trans-β-ocimene, and germacrene D, while in the larvicidal study by Manh et al. (38), the major compounds were 1,8-cineol, α-pinene, and citronellyl acetate. Citronellal and geraniol are consistently major compounds in *C. nardus* (41, 42). The composition of *E. camaldulensis* is typically varied, containing compounds such as 1,8 cineole, γ-terpinene, α-Phellandrene, and p-Cymene (43, 44). The use of 12 EOs of the *Eucalyptus* genus against *Ae. aegypti* highlighted major compounds like 1,8-cineole, α-pinene, α-phellandrene, β-phellandrene, γ-terpinene, 4-terpineol, α-terpineol, p-cymene, and spathulenol (45). In our study, eucalyptol and p-cymene appear to be specific to *E. camaldulensis* oil. The high concentration of eucalyptol in *E. camaldulensis* (48.59%) might be more influential in repellent activity compared to the lesser concentrations of other compounds.

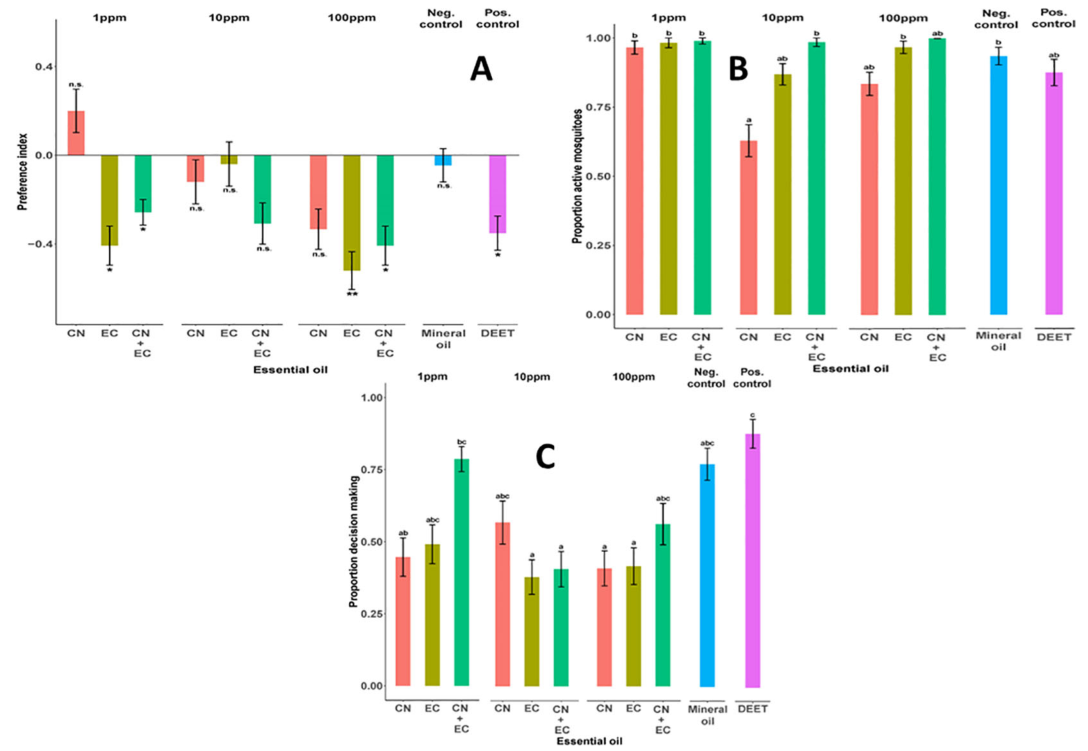

FIGURE 2
Olfactory responses of *Ae. aegypti* adult female mosquitoes: **(A)** Mosquito olfactory preference represented as a preference index. Asterisks denote responses that were significantly different from chance (Binomial Exact test, p < 0.05), **(B)** Proportion of active mosquitoes during bioassay, **(C)** Proportion of mosquitoes making an active choice between the two choice arms of the olfactometer; CN, *Cymbopogon nardus*; EC, *Eucalyptus camaldulensis*. The different lowercase letters show Statistical Significance of essential oil effect. Graphs with the same letters are not statistically different.

Compounds such as α-Pinene, α-Terpinolene, Citronellal, α-Terpinyl propionate, Decanal, Geraniol, cis-Geraniol, α-Citral, Eugenol, Geranyl acetate, Germacrene D, Aromandendrene, Elemol, Thunbergol, α-Cadinol known to have mosquito repellent properties (46–53) are present in *C. nardus* and absent in *E. camaldulensis*. Previous studies in Burkina Faso showed the presence of only 1,8-cineole in large quantities in *E. camadulensis* while *C. nardus* mainly contained geraniol, citronelal, elemol and nerol, compounds known for their antifungal and insecticidal properties against *Ae. aegypti*. This justifies the repellent effect of and *E. camaldulensis* in this study. In the combination of oils, only four compounds (α-Pinene, Citronellal, Elemol, and α-Cadinol) recognized as good repellents were detected, explaining the weak repellent activity of the EO combination. Eucalyptol, p-cymene, and α-phellandrene, known for their repellent properties (54, 55), were found in large quantities in the oil of *E. camaldulensis* and could justify its strong repulsion against *Ae. aegypti*.

In the combination of the two oils, we detected fewer compounds than in each individual EO. Muturi et al., who studied the EOs of *Origanum vulgare*, *Syzygium aromaticum*, and *Leptospermum scoparium* against *Ae. aegypti*, reported similar findings (56). This could be attributed to the dilution of minor constituents into undetectable levels in the oil mixtures. Additionally, similar to other research on EOs combinations, we identified new compounds such as Nerol, γ-Elemen, and β-Elemen (29, 56). These new compounds may be linked to a different distribution based on solubility and mixing, which likely altered the composition of the original EOs (56). The variations in the composition of EOs between the two plants can be attributed to various factors, such as the age of the plant, the plant parts used in oil extraction, the extraction method, the seasonal sampling period, and environmental and climatic conditions (57). Moreover, ethanol which was used as a solvent may have an effect as well. It might be interesting to explore why some compounds, like nerol or γ-Elemen, appear in the blend but not in the individual oils.

Based on the results of the olfactometer test, the EOs repelled female *Ae. aegypti* at 1ppm and 100 ppm concentrations for EC. However, *C. nardus* at 1 ppm attracted mosquitoes. The activity was dose-dependent for EC and the EC + CN mixture at 1 and 100ppm concentrations. These results were similar to those obtained by Uniyal et al. (26) which showed the repulsive effect of 23 EOs against *Ae. aegypti* by olfactometry (26). The EOs of *C. nardus* were reported to act in a dose-dependent repellent activity against *Ae. aegypti*, especially when mixed with citral and myrcene (40). In previous studies, several EOs showed repellent properties against *Ae. aegypti* either through formulations (58–61) or through combinations of EOs (56, 62, 63) or by using simple oils (56, 64). *E. camaldulensis* showed weak repellent activity significantly different from the response to the EO at 1 ppm. Of the three types of oils used, *E. camaldulensis* was most active, followed by the CN + EC mixtures at 1 and 100ppm and *C. nardus* at 100 ppm however it was weakly attractive at 1 ppm. While the response at 10 ppm does not exhibit a straightforward dose-dependent trend, the increase in the preference index at 100 ppm highlights the complex, non-linear nature of mosquito behavior in response to essential oils (EOs). We speculate that this pattern can be explained by the dynamics of olfactory receptor adaptation, where intermediate concentrations like 10 ppm may temporarily saturate or desensitize receptors, leading to a plateau (65, 66). However, at higher concentrations such as 100 ppm, these effects can be overcome, potentially due to the activation of additional sensory pathways or cumulative receptor effects, resulting in a renewed and stronger behavioral response (67, 68). The non-linear dose-response relationships observed in insect behavior, including that of mosquitoes, are well-documented and suggest that behavioral responses can vary widely with concentration (69, 70). This underscores the importance of considering the intricate mechanisms at play in mosquito olfactory systems, reinforcing the validity of our findings despite the lack of a simple linear relationship (71, 72). Finally, the observed mosquito behavior at 10ppm leads us to further questions on mechanisms underlying the dose-dependent responses to repellents which could be addressed in future studies.The EOs contain citronellal, 1,8-cineole, carvacrol, geraniol, caryophyllene, p-cymene, pinene that were reported to act as repellents (73), similar to some of the EOs compositions in this study and are known to be mosquito repellents. In recent study, researchers show that the performance of the citronellal is comparable (95% protection for ≤3.5 h) with those of the most widespread synthetic repellents DEET and Picaridin, tested at a four-fold higher doses against *Aedes albopictus* and *An. gambiae* (74). Eucalyptol, the major component in the oil of *E. camaldulensis* in combination with CN + EC mixture, is an effective repellent. In some studies where pure compounds were tested, eucalyptol was more repellent than the other compounds against *Rhodnius prolixus* Ståhl and *Ae. aegypti* (73, 75). This mixture did not show any synergy between the two oils and this would be due to antagonism between certain compounds of the two plants, which would reduce the repellent potential of the mixture. Cases of antagonisms are reported when evaluating the repellent effects of oils against ticks and mosquitoes (73). These results using eucalyptus oils make it possible to use the eucalyptus leaves, in tropical zones, which are often discarded after the tree trunks are used in the construction of buildings. At low doses, *C. nardus* seems to attract mosquitoes, probably due to some of the volatile compounds in this oil mixture that would attract mosquitoes and therefore could be used in mosquito traps. Several compounds have been reported to attract mosquitoes like limonene, menthone, and hexanal, however, they are weak attractants as compared with 3-methyl indole (76). Screening of the individual compounds identified in *C. nardus* EOs could in the future identify repellent compounds and those that are attractants.

5 Conclusion

The EOs tested in this study contain several compounds that are potential repellents *of Ae. aegypti*. The EO of *E. camaldulensis* and strongly repelled *Ae. aegypti* than the EOs of *C. nardus*, which lost potency at high concentrations and exhibited weak attraction at 1 ppm. The mixture of *C. nardus* and *E. camaldulensis* EOs had a little repellent effect compared to *E. camaldulensis* oil. Future characterizations of these compounds could be used for vector control applications.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions

DW: Conceptualization, Data curation, Funding acquisition, Methodology, Writing – original draft. KC: Data curation, Formal analysis, Methodology, Software, Visualization, Writing – review & editing. FU: Investigation, Writing – review & editing. DB: Supervision, Visualization, Writing – review & editing. IS: Supervision, Writing – review & editing. CV: Supervision, Validation, Writing – review & editing. AB: Writing – review & editing. SA: Supervision, Writing – review & editing. CL: Conceptualization, Supervision, Validation, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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