Journal of Medical Entomology, 59(3), 2022, 801–809
https://doi.org/10.1093/jme/tjac009
Advance Access Publication Date: 14 February 2022
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Mosquito Repellent Potential of *Carpesium abrotanoides* Essential Oil and Its Main Components Against a Dengue Vector, *Aedes aegypti* (Diptera: Culicidae)

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Subject Editor: Roberto Pereira

Received 18 July 2021; Editorial decision 4 January 2022

Abstract

Disease vectoring mosquitoes are a serious threat to humans. However, till today only few mosquito repellents have been identified. The current study was conducted to evaluate the repellent potential of *Carpesium abrotanoides* essential oil against *Aedes aegypti* females by human bait technique. Essential oil was extracted by steam distillation process while the identification of chemical constituents was carried out by gas chromatography–mass spectrometry. Time span repellent bioassays of *C. abrotanoides* essential oil in comparison to DEET were performed at three different doses (33 μg/cm^2, 165 μg/cm^2, and 330 μg/cm^2) under laboratory conditions. Highest repellency periods for essential oil and DEET were observed at the tested dose of 330 μg/cm^2 with 315 min and 720 min, respectively. Lowest repellency period of 45 min for essential oil and 105 min for DEET was recorded at the tested dose of 33 μg/cm^2. Major constituents caryophyllene (24.3%) and *trans*-nerolidol (12.0%) of *C. abrotanoides* essential oil were also evaluated as repellents at three different doses (330 μg/cm^2, 165 μg/cm^2, and 33 μg/cm^2) against *Ae. aegypti*. Surprisingly, *trans*-nerolidol completely inhibited *Ae. aegypti* landings for 45 min when tested at 330 μg/cm^2. However, caryophyllene did not completely inhibit *Ae. aegypti* landing even after immediate application at the tested dose of 330 μg/cm^2. At the tested dose of 330 μg/cm^2, the mixture (*trans*-nerolidol + caryophyllene) completely inhibited *Ae. aegypti* landing for 60 min indicating the synergistic effect of caryophyllene. Hence, *C. abrotanoides* as well as its major constituent, especially *trans*-nerolidol, have potential to formulate as mosquito repellent comparable of DEET.

Key words: essential oil, *Carpesium abrotanoides*, mosquito, *Aedes aegypti*, repellency

Females of each mosquito species depend on vertebrates for bloodmeal in order to lay eggs (Harrison et al. 2021). In the process of blood feeding, numerous species have the potential to transmit extremely harmful pathogens of several diseases such as dengue fever, malaria, encephalitis, filariasis, etc. (Chen et al. 2013). The members of genus *Aedes*, especially *Aedes aegypti* (L.) is trending as the most threatening pest for human around the globe (Jansen and Beebe 2010). For instance, *Ae. aegypti* mosquitoes have a potential to carry and transmit four serotypes of dengue (DENV-1, DENV-2, DENV-3, and DENV-4) in humans, and as a consequence thousands of humans die annually (WHO 2009, Emran et al. 2018, Shirin et al. 2019).

Dengue virus is a single positive-stranded RNA virus from the family Flaviviridae and a casual organism responsible for break bone fever, dengue hemorrhagic fever, and dengue shock syndrome in humans (Byard 2016). About 129 countries having 3.9 billion people are at risk of dengue infection while the incidence rate of dengue has raised up to 8 folds (i.e., from 505,430 cases in 2000, to over 2.4 million in 2010, and 4.2 million cases in 2019) over the last two decades (WHO 2020). Frequent movement of visitors from dengue virus infected regions to dengue free countries is considered as basic reservoir playing key role in rapid increase of dengue incidences (Lo et al. 2013).

A new emerging challenge that is also directly linked with genus *Aedes* is zika virus. This is another single positive-stranded RNA virus that also belongs to the family Flaviviridae, just like dengue virus (Calvez et al. 2018). In most of the cases, zika virus does not

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evolve any symptom, but in case of major zika virus outbreak in French Polynesia, 8,200 cases of microcephaly and congenital malformations in infants, preterm births, and miscarriages were reported out of 268,000 human beings in 2013 (Rabaan et al. 2017). The most efficient, precise, and promising way to minimize the rate of dengue and zika incidence is to manage *Ae. aegypti* population.

In order to manage *Ae. aegypti* population, different insecticides such as organochlorines (DDT), organophosphate (malathion and pirimiphos methyl), carbamate (propoxur and bendicarb), and pyrethroids (permethrin, deltamethrin, and lambda cyhalothrin) were utilized (Mazzarri and Georghiou 1995). Repeated application of such insecticides made *Ae. aegypti* population able to gain resistance against DDT, malathion, bendiocarb, and permethrin (Arslan et al. 2016). Besides insecticides usage to control mosquito population, personal protection was considered a suitable approach to prevent mosquito bites (Gupta and Rutledge 1994). For this purpose, synthetic repellents such as DEET had been utilized to maintain the distance between human and mosquitoes, but later on its hazardous impact on humans i.e., rashes, swelling, eye irritation, brain swelling in children, anaphylactic shock, and low blood pressure have also been reported (Patel et al. 2012). By keeping in view human health at priority basis, there is a need to find the natural alternates such as plant essential oils as substitutes for synthetic repellents. Numerous plants belonging to the families Zingiberaceae, Lamiaceae, Lauraceae, Rutaceae, Apiaceae, and Poaceae have been investigated and described as promising natural insect repellents (Prajapati et al. 2005, Choochote et al. 2007, Park et al. 2010, Kumar et al. 2011, Sathantriphop et al. 2015).

Twenty-one plant species belonging to genus *Carpesium* (Asterales: Asteraceae) are distributed worldwide. Most of them are perennial while few of them are annual. Being a medicinal plant, various *Carpesium* species are used for the treatment of diseases in various countries. Almost 143 compounds were isolated and identified from *Carpesium* plants which include secondary metabolites, i.e., monoterpenes, diterpenes, sesquiterpenes, and phenolic compounds (Zhang et al. 2015). Secondary metabolites of genus *Carpesium* are reported as main source of various bioactivities such as antiplasmodial, antifungal, antitumor, antiinflammatory, antioxidant, and antibacterial (Rodriguez et al. 1976, Lee et al. 2002, Wang et al. 2006, Gao et al. 2007, Zhang et al. 2015, Hu et al. 2018).

Carpesium abrotanoides (L.) is a perennial herb and commonly distributed in Europe and Eastern Asia, mainly in Japan, Korea, and China (Mayur et al. 2010). The aerial parts of *C. abrotanoides* have been utilized as insecticides as well as in the treatment of wounds (Wang et al. 2009, Mayur et al. 2010). Moreover, *C. abrotanoides* have been used to cure bronchitis, stomach ulcer, boils, and tonsillitis (Mayur et al. 2010). Results of previous studies proved that *C. abrotanoides* is a source of numerous bioactive compounds but its repellent potential against any insect has not been explored yet. In the current study, the essential oil extracted from *C. abrotanoides* was evaluated as repellent against *Ae. aegypti* as well as the chemical composition of oil was analysed to identify the behaviourally active chemical constituents, which can be used to develop the commercial mosquito repellent.

Materials and Methods

Plants Collection and Maintenance

Fresh aerial parts of wild-grown plants of *C. abrotanoides* (Asteraceae) were collected from the Abbottabad (34° 07′ 26.5″ N, 73° 20′ 04.5″ E), Pakistan. Plant was identified by plant taxonomists at the Department of Environmental Sciences, COMSATS University Islamabad, Abbottabad Campus, Abbottabad, Pakistan. The collected fresh plant material was kept in a freezer at −20°C until used in the essential oil extraction process.

Essential Oil Extraction

To extract the essential oil from the fresh aerial part of collected plant, steam distillation process was carried out (Azeem et al. 2019). Pruning shear was utilized to cut the plant material into pieces. Approximately, 2 kg of plant material was filled in a stainless-steel vessel having 2 litres of water in a way that plant material did not directly contact the water. An electric hot plate was utilized to heat up the stainless-steel vessel. Produced steam forced the plant material to release volatile compounds. Condenser was installed on the upper side of the vessel to cool the steam. The distillate (plant volatiles and water) collected in a separating funnel for at least 4 h. The collected distillate was subjected to the liquid–liquid extraction by utilizing (70 ml × 3) HPLC grade *n*-hexane. The pooled extracted hexane layers dried with the addition of anhydrous magnesium sulphate and were filtered. With the help of a rotary evaporator at 25°C solvent was evaporated. Essential oil obtained was weighed and the percentage yield of extracted essential oil was calculated by following formula

$$\text{Percentage yield} = \frac{\text{Mass of essential oil}}{\text{Total mass of plant material}} \times 100$$

Extracted essential oil was stored in glass vial at −20°C for bioassay and chemical analysis.

Chemical Analysis of Plant Essential Oil

To analyse the essential oil sample, gas chromatography equipped with mass-spectrometry (Hewlett Packard GC–MS) system was used where the stationary phase of the GC column possessed 5% diphenyl, 95% dimethylpolysiloxane. The GC injector was isothermally set at 235°C while the GC oven temperature was programmed with the initial temperature of 40°C for the period of 2 min. After 2 min, temperature increased up to 240°C at the rate of 4°C/min, and afterwards remained constant at 240°C for 8 min. With the constant flow of 1 ml/min, pure Helium gas was utilized as mobile phase. Diluted solution of essential oil injected with the volume of 1 µl in the splitless mode for 30 s. As far as the parameters for mass spectrometer are concerned, the electron ionization was employed at 70 eV, ion source temperature of the MS was constantly set at 180°C with the filament off time for 5 min while the mass spectra scan range was 30–400 amu. To calculate the percentage composition of each compound in an essential oil the total ion chromatogram peak areas were utilized. Identification of the separated compounds was done by comparing their mass spectra with NIST-2008 MS library. The retention indexes of separated compounds were determined relative to the retention times of a series of *n*-alkanes (C9-C24) analyzed at the same GC–MS parameters utilized for the essential oil. Retention indexes of separated compounds were compared with the published data to determine the elution order and identification of compounds. Finally, wherever possible, the identification of a compound was verified by injecting standard compound purchased from Sigma–Aldrich at the same parameters which were adopted for essential oils analysis (Azeem et al. 2019).

Rearing of *Ae. aegypti*

Ae. aegypti population obtained from the Health Department Multan (Dengue Control Unit, Railway Hospital, Multan) in the larval stage was maintained under controlled conditions in the

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laboratory at Department of Entomology, Faculty of Agricultural Sciences & Technology, Bahauddin Zakariya University Multan, Pakistan by keeping in view the published methods (Das et al. 2007, Akram et al. 2011). The obtained population shifted to a plastic container containing 1 litre of distilled water under the controlled conditions (temperature 25 ± 2°C, 65 ± 5% RH, and a photoperiod of 12:12 (L:D) h. Larval population up to 4th instar was fed on fish diet containing (28% crude protein, 3% crude fat, 4% crude fibre, and 10% moisture) once in a day until pupation occurred. Pupae were collected in a plastic cup containing distilled water. The pupae cup was shifted to the adult cage (30 × 30 × 30 cm) for adult emergence (Nararak et al. 2019). After emergence, adults were fed on 10% sucrose solution. Females were fed with pigeon blood for egg laying after 4–5 d. The blood-feeding was carried out within the certain time period (12:00 pm–4:00 pm). Egg laying medium (butter paper and distilled water) was provided in an adult cage. After egg laying, the eggs were shifted to a plastic container containing 1 litre of water for hatching (Azeem et al. 2019). *Ae. aegypti* were reared for three generations in the above-mentioned controlled conditions to minimize possible maternal effects and to achieve homogeneous population before the start of experiment.

Repellent Bioassay

Repellent potential of essential oil against *Ae. aegypti* females was evaluated by using the human bait technique prior to the scotophase period. The 1%, 5%, and 10% solutions of *C. abrotanoides* essential oil, caryophyllene, and *trans*-nerolidol were prepared with ethanol to test their repellent potential against the females of *Ae. aegypti* whereas 1%, 5%, and 10% DEET (*N,N′*-diethyl-3-methylbenzamide) solution in ethanol was used as a positive control. Moreover, to check the synergistic effect, the mixture of *trans*-nerolidol and caryophyllene was tested at 10% concentration against the females of *Ae. aegypti*. Twenty laboratory reared blood-starved mated females of 3–4 d old with one day sugar starved were placed in the experimental cage (30 × 30 × 30 cm). Before each test, the hand of the human subject washed with scent free soap and allowed to dry for 10 min. The subject (author of this manuscript) wore gloves on hands that cover the entire hand and arm except for a circular area of 30 cm^2 on the dorsal side of the hands. An aliquot of 100 µl solution of test substance or negative control (ethanol solvent) was applied on the exposed area of the hand and solvent was allowed to evaporate for 3 min prior to the experiment. Both test substance treated and only solvent treated (control) hand were placed alternatively in the same cage each for a period of 5 min. Numbers of mosquito landings were counted on negative control or sample-treated hand. However, the subject was allowed to shake off his hand immediately after the landing of female to avoid being bitten. The experiment was repeated randomly five times for both the test sample and negative control. For each replication a new group of 20 females was used. Repellency bioassay method was adopted from previous reports with slight modification (Gillij et al. 2008, Govindarajan 2011, El-Sheikh et al. 2016) while the percentage of repellency was calculated by the formula

$$\text{Percentage Repellency} = [(\text{Ta} - \text{Tb})/\text{Ta}] \times 100$$

Where;

Ta is the number of mosquitoes on the untreated skin
Tb is the number of mosquitoes on the treated skin

Time Span Repellent Bioassay

Time span bioassay was performed in the same way as the repellency bioassay described above, except exposing the same treated hand to the females of *Ae. aegypti* for the period of 5 min after each 15 min time period until the number of landings on control and treatment became equal. The time span bioassays were conducted by using 1% (33 µg/cm^2), 5% (165 µg/cm^2), and 10% (330 µg/cm^2) concentrations of test samples while the mixture of caryophyllene and *trans*-nerolidol was tested at only 10% (330 µg/cm^2) concentration.

Statistical Analysis

Two sample *t*-test for independent samples was adopted for time span bioassays for the comparison of test samples and DEET where α was set at 0.05. To compare the different doses of caryophyllene and *trans*-nerolidol, data was subjected to ANOVA followed by a post-hoc test, Tukey HSD, for multiple comparisons while the significant level was set at $P < 0.05$. All statistical analyses were done using the statistical software Statistix 8.1.

Results

Essential Oil Yield and Chemical Composition

The fresh aerial parts of *C. abrotanoides* contained 0.04% of essential oil. The GC–MS analysis of essential oil revealed the presence of 39 compounds. The major compounds included 24% caryophyllene, 12% *trans*-nerolidol, 10.63% geranyl isobutyrate, 8.83% δ-cadinene, and 4.67% β-eudesmene (Table 1).

Repellent Potential of Essential Oil and DEET at the Tested Dose of 33 µg/cm²

Immediately after application, the essential oil of *C. abrotanoides* exhibited significant repellency similar to DEET ($P = 0.5812$) against *Ae. aegypti*. However, after 15 min the repellency of *C. abrotanoides* treated hand significantly reduced ($P = 0.0030$) as compared to DEET. The repellency of *C. abrotanoides* at 30 min ($P < 0.0001$) and 45 min ($P < 0.0001$) was shown to some extent but significantly less than DEET. Zero repellency was observed in case of *C. abrotanoides* at 60 min, however, DEET showed repellency till 105 min (Fig. 1).

Repellent Potential of Essential Oil and DEET at the Tested Dose of 165 µg/cm²

The essential oil of *C. abrotanoides* exhibited repellency similar to DEET against *Ae. aegypti* at 0 and 15 min ($P = 1$ and $P = 0.8899$). However, the repellency exhibited by *C. abrotanoides* significantly reduced ($P = 0.0013$) as compared to DEET at 30 min. At 45 min repellency exhibited by *C. abrotanoides* ($P < 0.0001$) remained approximately half of the DEET. From 60 min ($P < 0.0001$) to 90 min ($P > 0.0001$) *C. abrotanoides* showed repellency to some extent but significantly less than DEET. The repellency of *C. abrotanoides* reached zero after 90 min and no repellency was observed at 105 min or later time intervals. However, DEET was showing repellency till 390 min (Fig. 2).

Repellent Potential of Essential Oil and DEET at the Tested Dose of 330 µg/cm²

The essential oil of *C. abrotanoides* showed repellency similar to DEET from 0 min till 210 min post-treatment ($P > 0.05$) against *Ae. aegypti*. The repellency exhibited by *C. abrotanoides* continuously decreased at each interval from 225 min ($P = 0.0011$) till 315 min ($P > 0.0001$) and the essential oil repellency reached its minimum level which was significantly lower than DEET. After 315 min repellency of *C. abrotanoides* was observed to be zero, however, DEET was showing repellency till 720 min (Fig. 3).

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Table 1. Chemical composition of essential oil (*Carpesium abrotanoides*)

RI	Identified compounds	% Composition	Identified through[a]
847	*trans*-3-Hexen-1-ol	0.63	MS, RI
861	*n*-Hexanol	0.85	MS, RI
975	1-Octen-3-ol	0.87	MS, RI
982	3-Octanone	0.08	MS, RI, Std
1003	*cis*-3-Hexenyl acetate	0.33	MS, RI, Std
1026	Limonene	0.11	MS, RI, Std
1039	Benzeneacetaldehyde	0.34	MS, RI
1089	2,6-Dimethyl-7-octen-2-ol	0.24	MS, RI
1096	Linalool	0.66	MS, RI, Std
1101	Nonanal	0.18	MS, RI
1134	Benzyl nitrile	1.45	MS, RI
1152	Nerol oxide	0.33	MS, RI
1165	Lavandulol	0.07	MS, RI
1175	4-Terpineol	0.14	MS, RI, Std
1188	α-Terpineol	0.40	MS, RI, Std
1202	Decanal	0.32	MS, RI
1213	6-Allyl-2-methylphenol	0.31	MS
1226	*cis*-Geraniol	0.47	MS, RI
1241	Carvone	0.27	MS, RI, Std
1364	Piperitenone oxide	0.11	MS, RI
1369	Cyclosativene	0.48	MS, RI
1377	Copaene	2.02	MS, RI
1392	*trans*-β-Elemene	0.29	MS, RI
1396	*cis*-Jasmone	0.08	MS, RI
1423	Caryophyllene	24.30	MS, RI, Std
1456	α-Caryophyllene	3.50	MS, RI, Std
1488	Geranyl isobutyrate	10.63	MS, RI
1489	β-Eudesmene	4.67	MS, RI
1498	α-Selinene	0.27	MS, RI
1501	α-Muurolene	0.44	MS, RI, Std
1512	*trans*-lachnophyllum ester	3.87	MS, RI
1525	δ-Cadinene	8.83	MS, RI
1562	*trans*-nerolidol	12.03	MS, RI, Std
1574	Neryl 3-methylbutyrate	4.67	MS, RI
1581	Linalyl 3-methylbutanoate	1.67	MS
1587	Caryophyllene oxide	3.89	MS, RI
1644	τ-Muurolol	0.79	MS, RI
1689	4-(2,2-Dimethyl-6-methylenecyclohexylidene)-3-methylbutan-2-one	1.32	MS
2029	Propanoic acid, 2-methyl-, 2-[3[(acetyloxy)methyl]oxiranyl]-5-methylphenyl ester	2.35	MS
	Total Identified	**94.23**	

[a]Compounds were identified by matching with MS = mass spectra and RI = retention indexes of the NIST and Wiley MS libraries as well as of synthetic standards (Std) of the available compounds.

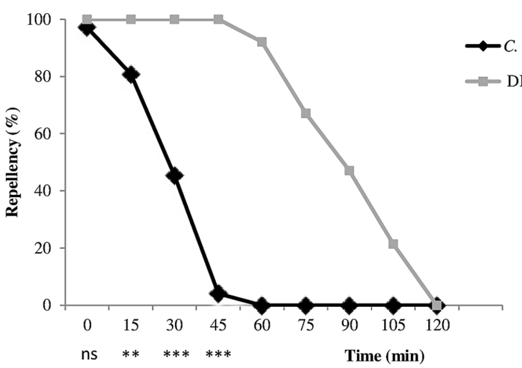

Fig. 1. Repellence to *Ae. aegypti* female adults exhibited by *C. abrotanoides* essential oil and DEET at the tested dose of 33 µg/cm² while α was set at 0.05; **, *** represent $P \leq 0.01$, $P \leq 0.001$, respectively.

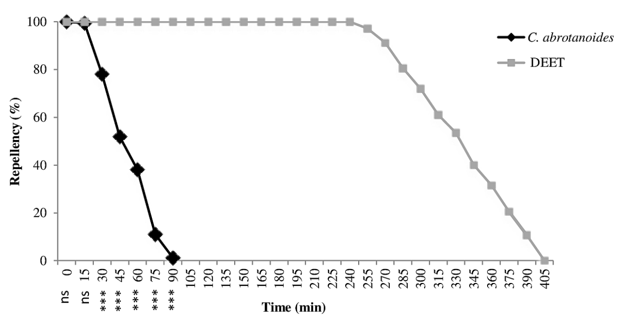

Fig. 2. Repellence to *Ae. aegypti* female adults exhibited by *C. abrotanoides* essential oil and DEET at the tested dose of 165 µg/cm² while α was set at 0.05; *** represents $P \leq 0.001$.

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Repellent Potential of Caryophyllene at Three Different Doses (330 µg/cm², 165 µg/cm², and 33 µg/cm²)

Caryophyllene was evaluated as repellent at three different doses 330 µg/cm², 165 µg/cm², and 33 µg/cm² against *Ae. aegypti*. Results showed that at the 0-min time interval more than 55% repellency was exhibited by caryophyllene at the tested dose of 330 µg/cm² while at 165 µg/cm² showed more than 35 % repellency. More than 20% repellency was observed at the tested dose of 33 µg/cm². In case of 15 min time interval, repellency was reduced below 40% at the tested dose of 330 µg/cm² while at 165 µg/cm² it showed approximately 20% repellency and in case of 33 µg/cm² repellency was reduced to zero. At 30 min time interval, repellency at 165 µg/cm² reduced to zero, where at 330 µg/cm², 20% repellency was observed. However, at 45 min time interval, zero repellency was observed at the tested dose of 330 µg/cm² (Fig. 4).

Repellent Potential of *trans*-Nerolidol at Different Concentrations (330 ug/cm², 165 ug/cm², and 33 ug/cm²)

According to the results both doses of *trans*-nerolidol (330 µg/cm², 165 µg/cm²) showed 100% repellency after application at 0 min time interval, while the significant difference was observed at the tested dose of 33 µg/cm² with less than 80% repellency. At 15 min time interval, significant difference was observed among all three doses (330 µg/cm², 165 µg/cm², and 33 µg/cm²) where repellency at the tested dose of 330 µg/cm² was the same (100%) as in the 0 min time interval. At 30 min time interval, *trans*-nerolidol at the tested dose of 330 µg/cm² exhibited 100% repellency, on the other hand, 165 µg/cm² showed approximately 70% repellency, while zero repellence was observed at the tested dose of 33 µg/cm². For 45 min time interval, the repellency at the tested dose of 330 µg/cm² remained 100%, which is equal to the previous time intervals, while 40% repellency was observed at the dose of 165 µg/cm². Decrease in the repellency from 100% to 90% was observed at the tested dose of 330 µg/cm², while less than 20% repellence was exhibited by 165 µg/cm² at 60 min time interval (Fig. 5).

Repellent Potential of Mixture (trans-Nerolidol+Caryophyllene) and DEET at the Tested Dose of 330 µg/cm²

The mixture of *trans*-nerolidol and caryophyllene at the tested dose of 330 µg/cm² exhibited repellency similar to DEET from 0 min to 60 min ($P = 1$), while from 75 min ($P = 0.0287$) repellency of mixture (*trans*-nerolidol+caryophyllene) started differing from the DEET significantly. More than 70% repellency of mixture (*trans*-nerolidol+caryophyllene) was observed at 90 min ($P = 0.0008$). Above 50% repellency of mixture (*trans*-nerolidol+caryophyllene) was recorded at 105 min ($P < 0.0001$), which significantly differ from the DEET repellency. The repellency of mixture (*trans*-nerolidol+caryophyllene) continuously decreased at each interval from 120 min ($P < 0.0001$) to 165 min ($P < 0.0001$) and reached its minimum level which was significantly lesser than DEET. After 165 min, repellency of mixture (*trans*-nerolidol+caryophyllene) was recorded as zero, however DEET was showing repellency till 720 min (Fig. 6).

Discussion

Plant essential oils are important source to discover novel insect repellents, especially to get protection from blood-sucking insect pests of medical importance such as mosquitoes and bed bugs. The current study was conducted to evaluate the repellent potential of essential oil of an herb, *C. abrotanoides*, against *Ae. aegypti* adult females as well as to identify the chemical constituents of this essential oil. Immediately after application, *C. abrotanoides* exhibited repellency

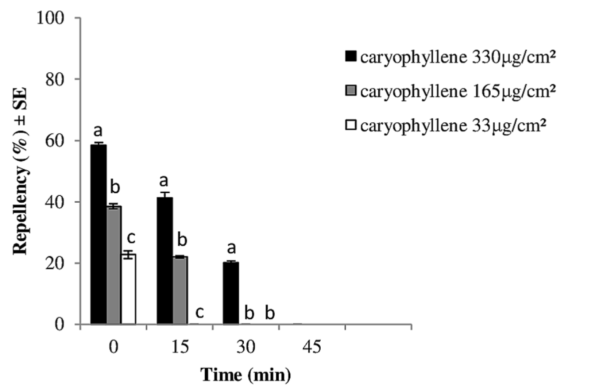

Fig. 4. Repellence to *Ae. aegypti* female adults exhibited by caryophyllene at three different doses (330 µg/cm², 165 µg/cm², and 33 µg/cm²) while bars with different letters for each time interval are significantly different ($P \leq 0.05$) from each other.

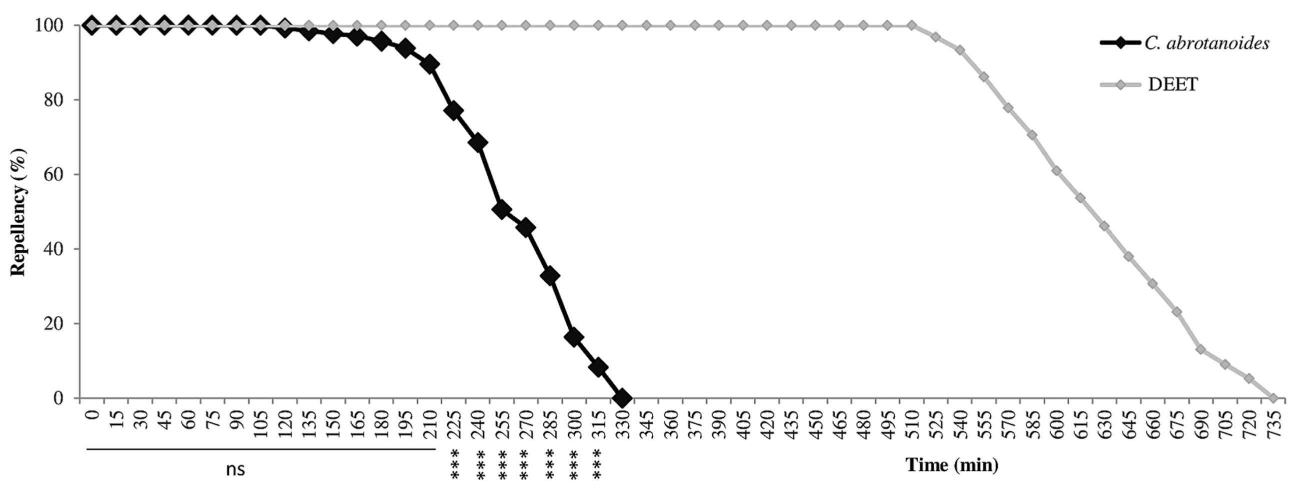

Fig. 3. Repellence to *Ae. aegypti* female adults exhibited by *C. abrotanoides* essential oil and DEET at tested dose of 330 µg/cm² while α was set at 0.05; *** represents $P \leq 0.001$.

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significantly similar to DEET at all the tested doses (330 μg/cm², 165 μg/cm², and 33 μg/cm²). Repellency period exhibited by essential oil of *C. abrotanoides* was dose dependent, i.e., repellency period was increased with increase in dose of essential oil, indicating the potential of *C. abrotanoides*. In case of time span bioassays, the maximum repellency periods for both *C. abrotanoides* and DEET were observed at the tested dose of 330 μg/cm² with 315 min and 720 min, respectively. However, at the tested dose of 165 μg/cm², repellency duration decreased to 90 min for *C. abrotanoides* and 390 min for DEET. The repellency period of 45 min for *C. abrotanoides* and 105 min for DEET were observed at the tested dose of 33 μg/cm². Previously, *Mentha spicata* (L.) (Lamiales: Lamiaceae) essential oil has been reported with complete inhibition of female *Ae. aegypti* landing on human hands for a period of more than 45 min which was similar to the synthetic repellent (DEET) where the maximum protection duration observed for *M. spicata* was 75 min at the tested dose of 33 μg/cm² (Azeem et al. 2019). According to our investigation, essential oil of *C. abrotanoides* inhibited landing of *Ae. aegypti* females for the period of 45 min which is less than *M. spicata* at the same tested dose. There is published data describing the 50% solution of *Myrtus communis* (L.) (Myrtales: Myrtaceae) and *Calendula officinalis* (L.) (Asterales : Asteraceae) essential oils as effective repellent with complete inhibition of *Anopheles stephensi* (Liston) (Diptera: Culicidae) landing for the period of 2.15 and 4.36 h, while the 25% solution of DEET showed complete inhibition for the period of 6.23 h (Tavassoli et al. 2011). In another comparative study, *Ocimum sanctum* (L.) (Lamiales: Lamiaceae), *Plectranthus amboinicus* (Lour.) (Lamiales: Lamiaceae), and *Mentha piperita* (L.) (Lamiales: Lamiaceae) proved equally effective as repellent when compared to DEET, with complete inhibition of *Ae. aegypti* landing for the period of 6 h at 20% solution (Mathew 2017). Complete protection from *Ae. aegypti* also exhibited by *Mentha arvensis* (L.) (Lamiales: Lamiaceae) was reported at three different concentrations (25%, 50%, and 100%) with protection duration of 45 min, 90 min, and 165 min, respectively (Manh and Tuyet 2020). Present investigation clearly supported the essential oil of *C. abrotanoides* as most effective repellent with protection duration of 315 min as compared to the plant essential oils mentioned in the previous studies, even at the lower tested dose of 330 μg/cm² (10%) against *Ae. aegypti* (Mathew 2017). Essential oils obtained from Verbenaceae plants proved effective in the field as repellent and insecticide against mosquitoes (Gleiser et al. 2011). Petroleum ether extract of *Vitex agnus-castus* (L.) (Lamiales: Lamiaceae) leaves protected for the period of 8 h at the dose of 2 mg/cm² against different mosquito species in the field (Karunamoorthi et al. 2008a,b). Another field experiment conducted in Kenya, also support plant essential oils as effective alternates of synthetic repellent, where 10% *Hyptis suaveolens* (L.) (Lamiales: Lamiaceae) essential oil and 30% DEET concentration showed significantly similar protection from mosquito bites for the period of 6 h post-application (Abagli and Alavo 2011). A study from Thailand also reported *Zanthoxylum piperitum* (L.) (Sapindales: Rutaceae), *Anethum graveolens* (L.) (Apiales: Apiaceae), and *Kaempferia galangal* (L.) (Zingiberales: Zingiberaceae) essential oils exerted complete protection against *Ae. aegypti* for the period of 1, 0.5, and 0.25 h while the essential oil of *Z. piperitum* with 10% vanillin exerted repellency for 2.5 h (Choochote et al. 2007). In another research, four *Nepeta* species (Lamiales: Lamiaceae) and hybrids were tested at two doses 10 μg/cm² and 100 μg/cm² as repellents against *Ae. aegypti*. *Nepeta* species and hybrids exhibited lesser impact at 10 μg/cm², however, similar at 100 μg/cm² when compared with DEET at 25 nmol/cm² (Ali et al. 2016). Despite the use of different reasons behind the variation in the repellency exhibited by reported plant species, with different chemical compositions, and tested against different mosquito species using different solvents and doses of plant essential oils, it can be said, in the light of previous and current research work, that plant essential oils have the

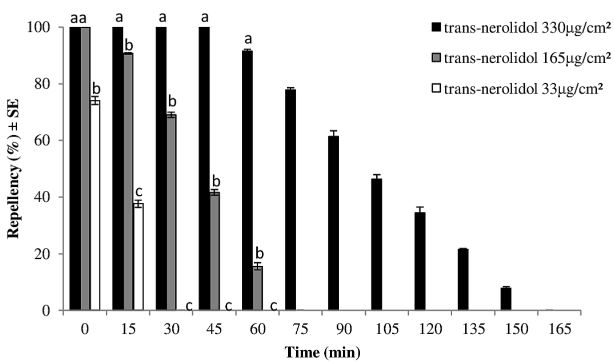

Fig. 5. Repellence to *Ae. aegypti* female adults exhibited by *trans*-nerolidol at three different doses (330 μg/cm², 165 μg/cm², and 33 μg/cm²) while bars with different letters for each time interval are significantly different ($P \leq 0.05$) from each other. Bars without letters from 75 min to 150 min time interval describe the effectiveness of *trans*-nerolidol at the tested dose of 330 μg/cm² for remaining time span.

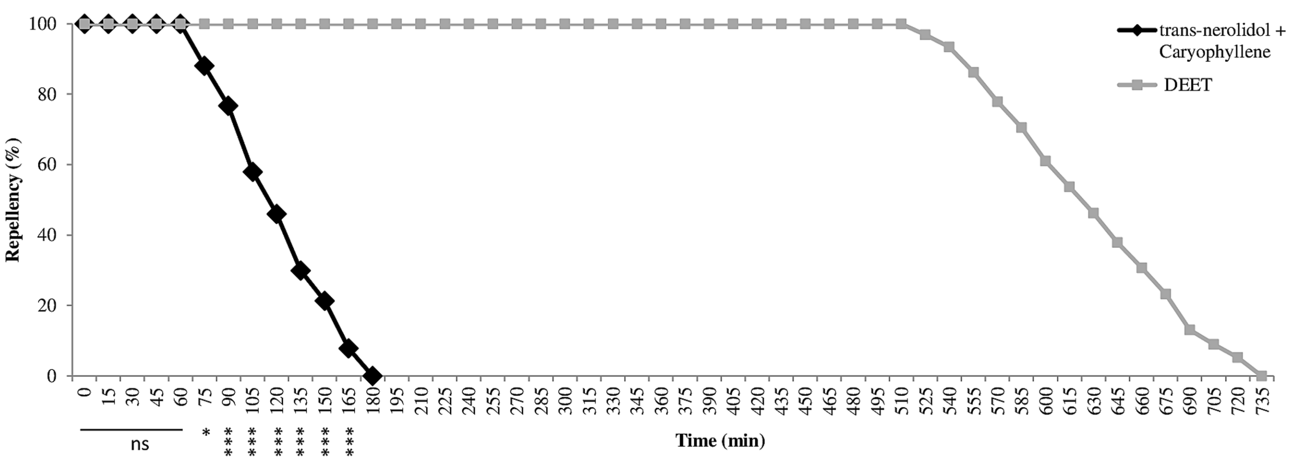

Fig. 6. Repellence to *Ae. aegypti* female adults exhibited by mixture (*trans*-nerolidol + caryophyllene and DEET at the tested dose of 330 μg/cm² while α was set at 0.05; *, *** represent $P \leq 0.05$, $P \leq 0.001$, respectively.

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potential to replace commercial repellents to overcome the human health hazards caused by the synthetic repellents.

Bioactivity of any plant essential oil is thought to be due to its major constituents (Salehi et al. 2019, Hassan et al. 2021). *Rosmarinus officinalis* (L.) (Lamiales: Lamiaceae) showed 100% repellence with major constituent's camphor (33.6%), verbenone (25%), and linalool (4.1%). However, pulegone (51.2%), menthone (30.7%), and limonene (10.1%) were identified as the main constituents of *Minthostachys mollis* (Kunth.) (Lamiales: Lamiaceae), this plant caused complete inhibition of *Ae. aegypti* landing. In same the study, *Eucalyptus saligna* (Smith) (Myrtales: Myrtaceae), with major constituent 1, 8-cineole (93.2%), and *Aloysia citriodora* (Palau) (Lamiales: Verbenaceae), with geranial (22.7%), neral (19.4%), and α-thujone (14.2%) as its major constituents, exerted complete protection against *Ae. aegypti* (Gillij et al. 2008).

Previously, carpabrotalactone A and B, 11(13)-dehydroivaxillin, eriolin,2α,5α-dihydroxy-11αH-eudesma-4(15)-en-12,8β-olide,(5α)-5-hydroxyasperilin,1α,4α-dihydroxy-guaia-11(13)-ene-12,8α-olide,4α,5α-dihydroxy-guaia-11(13)-en-12,8α lactone, 9β-hydroxy-1βH, 11αH-guaia-4,10(14)-dien-12,8α-olide, 9β-hydroxy-1 βH, 11βH-guaia-4,10(14)-dien-12,8 αolide and carpesiumaleimides A–C were identified from *C. abrotanoides* while the chemical analysis of current investigation differs from the previous studies (Wang et al. 2009, Wu et al. 2016, He et al. 2020). In the current investigation, chemical analysis highlighted caryophyllene (24.30%) and *trans*-nerolidol (12.03%) as the main constituents of *C. abrotanoides*. As far as the bioactivities of *C. abrotanoides* is concerned except repellency, *C. abrotanoides* proved effective against cancer, viruses (influenza), and tumor cells (Wang et al. 2015a, Wang et al. 2018, Chai et al. 2019, Wang et al. 2019, He et al. 2020, Qian et al. 2020). Moreover, insecticidal activities of *C. abrotanoides* against *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), *Bradysia odoriphaga* (Yang and Zhang) (Diptera: Sciaridae), *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), *Pieris rapae* (L.) (Lepidoptera: Pieridae), and *P. xylostella* were also reported (Hongming et al. 1999, Feng et al. 2012, Xuejiao et al. 2012, Wu et al. 2016). All these bioactivities totally depend on the presence of major and minor constituents in *C. abrotanoides*.

Each chemical constituent possesses specific ecological signals to affect insect behaviour, i.e., caryophyllene has been reported as an attractant for *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), while *Collops vittatus* (say) (Coleoptera: Melyridae) attracted by caryophyllene alcohol (Flint et al. 1979, 1981). *Peristenus spretus* (Chen and Van Achterberg) (Hymenoptera: Braconidae), *Aphidius gifuensis* (Ashmead) (Hymenoptera: Braconidae), and *Anagrus nilaparvatae* (Pang et Wang) (Hymenoptera: Mymaridae) were attracted towards the plants which are rich source of (*E*)-β-caryophyllene (Zhang et al. 2020). As far as the repellent activity of (E)-β-caryophyllene is concerned, this compound has the potential to repel *Diaphorina citri* (Kuwayama) (Hemiptera: Liviidae), *Apolygus lucorum* (Meyer-Dür) (Hemiptera: Miridae), *Aphis gossypii* (Glover) (Homoptera: Aphididae), and *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). However, the caryophyllene has not been reported as repellent yet against *Ae. aegypti* (Wang et al. 2015b, Alquézar et al. 2017, Zhang et al. 2020). *Trans*-nerolidol was found from various plant species, i.e., *Celastrus angulatus* (Maxim) (Celastrales: Celastraceae), *Momordica charantia* (L.) (Cucurbitales: Cucurbitaceae), *Eugenia haberi* (Barrie) (Myrtales: Myrtaceae), *Eugenia monteverdensis* (Barrie) (Myrtales: Myrtaceae), *Eugenia zuchowskiae* (Barrie) (Myrtales: Myrtaceae), *Eugenia cartagensis* (O. Berg) (Myrtales: Myrtaceae), *Piper hispidum* (Sw.) (Piperales: Piperaceae) (Cole et al. 2007, Braca et al. 2008, Benitez et al. 2009, Li et al. 2021). Previously, *trans*-nerolidol was reported as an effective larvicide while it showed lowest repellency against *Ae. aegypti,* although the tested dose of *trans*-nerolidol as repellent was not clearly mentioned (Ali et al. 2013). In current study, caryophyllene and *trans*-nerolidol were evaluated singly at three different doses 330 μg/cm², 165 μg/cm², and 33 μg/cm² while the mixture of both constituents examined only at 330 μg/cm² against *Ae. aegypti* landings. Surprisingly, *trans*-nerolidol proved effective and completely inhibited *Ae. aegypti* landings for a period of 45 min at 330 μg/cm². At 60 min post-treatment, effectiveness of *trans*-nerolidol started decreasing and reached zero at 165 min post-treatment. On the other hand, caryophyllene did not show complete inhibition to *Ae. aegypti* landing even at the tested dose of 330 μg/cm² at 0 min posttreatment. In case of mixture (*trans*-nerolidol+caryophyllene) at the tested dose of 330 μg/cm², complete inhibition of *Ae. aegypti* landing increased to 60 min while the total protection time increased by the mixture (*trans*-nerolidol+caryophyllene) for the period of 165 min as compared to the *trans*-nerolidol alone at the same tested dose.

Conclusion

According to the results, we conclude that the essential oil of *C. abrotanoides*, its major constituents, especially *trans*-nerolidol as well as the mixture of caryophyllene and *trans*-nerolidol, have potential as a mosquito repellent comparable of DEET and can be used to develop safer commercial products to repel mosquitoes.

Acknowledgment

We are thankful to Dr. A Waheed from BZU for the guidance in statistical analyses. This research was funded by International Foundation for Science (IFS), Sweden under grant number I-1-F-6041-1.

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