Journal of the American Mosquito Control Association, 34(4):272–280, 2018

REPELLENT ACTIVITY OF CARROT SEED ESSENTIAL OIL AND ITS PURE COMPOUND, CAROTOL, AGAINST MOSQUITOES

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ABSTRACT. In our natural products screening program for mosquitoes, carrot seed essential oil showed high repellency. The gas chromatography (GC)/flame ionization detector and GC/mass spectrometry analysis of the essential oil revealed the presence of 47 compounds. Carotol was more than 75% w/w, followed by muurolene (4.86%), (*Z*)-β-farnesene (2.9%), and diepicedrene (1.1%). Systematic bioassay-guided fractionation of the essential oil was performed to identify active repellent compounds. In both Klun and Debboun (K&D) and Ali and Khan (A&K) bioassays, carotol showed biting deterrent activity similar to *N*,*N*-Diethyl-3-methylbenzamide (deet) and carrot seed essential oil against both *Aedes aegypti* and *Anopheles quadrimaculatus*, while in in vivo cloth patch bioassay, the minimum effective dose (MED) of deet was lower (12.5 μg/cm^2) than the essential oil and carotol (25 μg/cm^2) against *Ae. aegypti*. In the A&K bioassay, the MED values were similar, whereas the values of the mixtures of deet with essential oil and carotol was lower ($6.25 + 6.25 = 12.5$ μg/cm^2) than their individual treatments (25 μg/cm^2). In direct skin application bioassay, both the essential oil and carotol provided good repellency. The mixtures of deet and essential oil or carotol significantly increased the residual activity, indicating synergism. Mosquito repellency of the essential oil and carotol is reported for the 1st time. These data indicate the potential of these natural products to be developed as commercial repellents.

KEY WORDS *Aedes aegypti*, *Aedes albopictus*, *Anopheles quadrimaculatus*, biting deterrent, repellent, carrot seed essential oil, carotol

INTRODUCTION

Mosquitoes are important in global public health because they transmit many disease pathogens. *Aedes aegypti* (L.) and *Ae. albopictus* (Skuse) are considered the primary and secondary vectors of Zika virus, respectively, as well as other arboviruses (Ali et al. 2017a). *Anopheles* spp. transmit malaria (Sanders et al. 1996, Meslin 1997) and *Culex quinquefasciatus* Say transmits West Nile virus (Godsey et al. 2005). The use of synthetic insecticides in mosquito control has proved to be one of the major components for prevention and reduction of mosquito-borne disease incidence (Bhatt et al. 2015).

Insect repellents play an important role in the reduction of disease incidence by preventing infected mosquitoes from biting humans (Leal 2006). Moreover, repellents have always been used against host-seeking mosquitoes as they provide immediate, localized, personal protection. The most widely used repellent, *N*,*N*-Diethyl-3-methylbenzamide (deet), has been in use for >60 years and is the standard to which all repellents are measured in the marketplace (Frances 2007). The discovery of novel repellents against mosquito vectors from nontoxic plant sources that are biodegradable and safe for humans and the environment is continued by several researchers (Wedge et al. 2006, Tabanca et al. 2013, Cantrell et al. 2016).

Carrot (*Daucus carota sativus* L.) is a biennial root vegetable that belongs to the family Apiaceae. In addition to its use as a vegetable, carrot seeds have medicinal properties and are used in many ways to cure certain medical conditions in humans, while their essential oils have multiple uses including the treatment of skin conditions (Sumit et al. 2012). Carotol is the major compound present in carrot seed essential oils (Ozcan and Chalchat 2007). In this study we report the chemical composition of carrot seed essential oil and document its repellent activity as well as its major component, carotol, against adult *Ae. aegypti*, *Ae. albopictus*, and *Anopheles quadrimaculatus* Say.

MATERIALS AND METHODS

Pure therapeutic essential oils of carrot were purchased from Edens Garden (1322 Calle Avanzado, San Clemente, CA). Deet was purchased from Sigma-Aldrich (St. Louis, MO). Thin-layer chromatography (TLC) plates were purchased from Merck (Darmstadt, Germany) and silica gel 60 for column chromatography (CC) were obtained from Silicycle (Quebec, Canada).

Spectroscopic analysis of carrot oil and carotol

Proton nuclear magnetic resonance (NMR) and carbon-13 NMR spectra of carrot essential oil were measured using a 400-MHz Bruker NMR spectrometer (Brucker, Billerica, MA). In addition, gas chromatography/flame ionization detector (GC/FID) and gas chromatography/mass spectrometry (GC/MS) analyses were performed on this material with a Varian CP-3380 (Varian, Walnut Creek, CA) and Thermo Finnegan Trace, respectively. Both instru-

ments were equipped with autosamplers. The temperature programming, column dimensions, detector types, and other experimental parameters were followed after Wanas et al. (2016). Column chromatographic separations were performed on silica gel 60 (0.04–0.063 mm; Silicycle) for the column. Thin-layer chromatography was carried out on precoated silica gel TLC plates 60 F254 (0.2 mm; Merck), using *n*-hexane, ethylacetate (EtOAc) (95:5) as the TLC developing system.

Bioassay-guided fractionation, using 700 mg of carrot essential oil, was performed on silica gel 60 (0.04–0.063 mm; Silicycle) eluted with EtOAc with increasing polarity to 5%. In addition, TLC was carried out on precoated silica 60 F254 gel plates (0.2 mm), using EtOAc (95:5) as the TLC developing system. Carrot seed essential oil (6.0 g) was subjected to silica gel CC, and 7 fractions were collected, concentrated under vacuum, and screened for biting deterrent activity. The active fraction was rechromatographed on silica gel CC and isocratically eluted with 2% EtOAc/hexane to yield 3.51 g of an active compound identified as carotol.

Insects

Adults of *Ae. aegypti*, *Ae. albopictus*, and *An. quadrimaculatus* used in these studies were from the laboratory colonies maintained at the Mosquito and Fly Research Unit at the Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, Gainesville, FL. For biting deterrence and repellent bioassays, the larvae hatching from the eggs were reared to adults in the laboratory maintained at 27 ± 2°C and 60 ± 10% RH with a photoperiod regimen of 12 h light and 12 h dark. Adult females 8–18 days old were used in these bioassays.

General methodology

Both in vitro and in vivo bioassays were conducted to determine the repellent activity of carrot seed essential oil, its fractions, carotol, and deet. In in vitro bioassays, a feeding solution consisting of citrate phosphate dextrose adenine-1 (CPDA-1) and adenosine triphosphate (ATP) was used instead of blood as described by Ali et al. (2012). Details of the feeding solution (CPDA-1 + ATP and green fluorescent tracer dye), experimental procedures, and data collection were followed after Ali et al. (2012, 2015). These bioassay systems are based on the concept that mosquitoes are attracted to warm temperatures. The temperature of feeding solution in the reservoirs was maintained at 37°C by continuously passing the warm water through the reservoir using a circulatory bath. All the treatments were freshly prepared in molecular biology–grade 100% ethanol (Fisher Scientific Chemical Co., Fairlawn, NJ) at the time of bioassay. In in vitro Ali and Khan (A&K) bioassay, a series of dosages were tested to achieve ≤1% biting as the minimum effective dose (MED). Two hundred (±5%) female mosquitoes were transferred into 30 × 30 × 30-cm test cages using an aspirator (John W. Hock Company, Gainesville, FL). To ensure proper landing and biting, 3–4 cages were used at a time and only 1 treatment replication of individual samples was completed in a single cage. A total of 5–10 replicates were completed in each bioassay.

For in vivo bioassays, 500 (±5%) female mosquitoes were transferred into 45 × 45 × 45-cm test cages using an aspirator. The test started when the arm or hand with treated muslin cloth or direct skin application was inserted into the mosquito cage. After 1 min of exposure, the hand was gently shaken and the number of biting females (feeding females do not fly) was recorded. Any treatment with ≤5 females biting during 1 min of exposure was considered as passed, whereas a treatment with >5 bites out of 500 mosquitoes was considered as failed. A next lower or higher serial dose was tested to reach the MED. Residual repellent activity was determined by exposing the females to the treated muslin cloth or treated skin at an interval of 30 min. Solvent control treatment was tested first to observe the response of the mosquitoes. The test was started only if >20 females successfully landed in 20 sec in solvent control. The caged mosquitoes were tested against ethanol control after every 5 successive exposures to determine the response of the mosquitoes. A protocol approved by the University of Mississippi Human Use Institutional Review Board (IRB protocol no. 15-070) was followed in both the in vivo bioassays.

In vitro Klun and Debboun biting deterrent bioassay

Bioassays were conducted using a 6-celled in vitro Klun and Debboun (K&D) module bioassay system developed by Klun et al. (2005) for quantitative evaluation of biting deterrence. Briefly, the assay system consists of a 6-well reservoir with each of the 3 × 4-cm wells containing 6 ml of feeding solution. The reservoirs were covered with a layer of collagen membrane (Devro, Sandy Run, SC). The test compounds were applied to 6 4 × 5-cm marked areas of organdy cloth (G Street Fabrics, Rockville, MD) and positioned over the collagen-covered feeding solution. The K&D module containing 5 female mosquitoes per cell was positioned over treated organdy and the trap doors were opened to expose the females to the treatment. The number of mosquitoes biting through the treated organdy in each cell was recorded after a 3-min exposure. Sets of 5 replications each with 5 females per treatment were conducted on 2–3 different days, using a newly treated organdy and a new batch of mosquitoes in each replication. Proportion not biting (PNB) was calculated using the following formula:

$$\text{PNB} = 1 - \left(\frac{\text{Total number of females biting}}{\text{Total number of females}}\right).$$

In vitro A&K repellent bioassay

Bioassays were conducted using the A&K bioassay system developed by Ali et al. (2017b) for quantitative evaluation of repellency against mosquitoes. Briefly, the bioassay system consists of a 30 × 30 × 30-cm collapsible aluminum cage having 1 panel of clear transparent acrylic sheet with 120 × 35-mm slit through which the blood box containing a removable feeding device was attached. The top of the blood box had a sliding door used to expose female mosquitoes to treatment during the bioassay. Rectangular areas of either 3 × 4 cm or 4 × 7.5 cm were marked on the collagen sheet that matched the measurement of the rectangular liquid reservoirs. Treated collagen was secured on the feeding reservoir containing the feeding solution using a thin layer of grease. The feeding device was then pushed inside the blood box and the sliding door was opened to expose the insects to the treatment. The number of females biting through the treated collagen during a 1-min exposure was recorded.

In vivo cloth patch bioassay

Cloth patch bioassays were conducted by using an in vivo bioassay system described by Katritzky et al. (2010) and Ali et al. (2017a). A piece of muslin cloth measuring 8 × 13 cm with a 4 × 7.5-cm marked area in its center was used. Approximately 2.5 × 7-cm pieces of cardboard were stapled onto the sides of the muslin cloth to secure the treated cloth on the plastic sleeve by using an adhesive tape. Each volunteer's hand was covered with a soft-embossed long-cuff poly glove and a powder-free latex glove (Diamond Grip; Microflex Corporation, Reno, NV) followed by a knee-high stocking (L'eggs Everyday Knee Highs; Hanes, Winston-Salem, NC). A polyvinyl sleeve with 4 × 7.5-cm opening cut halfway between the wrist and the elbow was placed around the arm with Velcro strips. The treatment was applied to the marked area by using a pipette, and the treated muslin cloth was secured on the plastic sleeve. The arm was inserted into the cage, and data were recorded after 1 min of exposure. This opening permitted attractive odors from the skin surface to emanate out and attract mosquitoes.

In vivo direct skin application bioassay

Direct skin application bioassays were conducted using a powder-free latex glove (Diamond Grip; Microflex Corporation). An opening of 3 × 4 cm was cut through the glove to fit on the dorsal surface of the hand. After wearing the glove, a wristband was used to prevent the biting on the hand near the border of the glove. Marked skin surface was treated with the test compound in a volume of 50 µl of ethanol. The treated hand was inserted into the cage and data on biting were recorded after 1 min of exposure.

Statistical analysis

Data on the PNB were analyzed using SAS Proc ANOVA (SAS Institute, Inc., Cary, NC), and means were separated using Ryan–Einot–Gabriel–Welsch multiple range test. Means and standard errors of MED values were calculated using SAS Proc Means (SAS Institute, Inc., Cary, NC) or Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA).

RESULTS

In the initial screening, the essential oil with PNB value of 0.84 provided biting deterrent activity similar to deet (PNB = 0.85) in K&D bioassays. Therefore, we selected carrot seed essential oil for further investigation.

The GC/FID and GC/MS analysis of carrot seed essential oil revealed the presence of 47 compounds, mainly mono- and sesquiterpenes (Table 1). Carotol was >75% (w/w), followed by muurolene (4.86%), (*Z*)-β-farnesene (2.9%), and diepicedrene (1.1%). Of the 7 fractions of carrot seed oil (6.0 g) obtained through silica gel CC, fraction 3 appeared to be the most active fraction. The GC/FID and GC/MS analysis indicated that fraction 3 contained >95% of carotol (Fig. 1), which was isolated as a colorless oil. The identity of carotol was confirmed by NMR analysis and by comparing its spectroscopic data to those reported by Jasicka-Misiak et al. (2004).

In vitro K&D biting bioassay

Data on the biting deterrent activity of the carrot seed essential oil, fractions, and carotol against different species of mosquitoes are given in Table 2. Carrot seed essential oil at 10 µg/cm^2 showed a biting deterrent activity similar to deet at 4.8 µg/cm^2. The results indicated that the biting deterrent activity of fraction 3 with a PNB value of 0.72 was similar to that of deet. Carotol, which was the major compound in this fraction, resulted in a PNB value of 0.9 at 10 µg/cm^2 and was similar to deet (PNB = 0.85) at 4.8 µg/cm^2. However, the biting deterrent activity of carotol at 5 µg/cm^2, with PNB value of 0.66, was slightly lower than deet. Carrot seed essential oil and carotol were also tested against *An. quadrimaculatus*. Results from carrot seed essential oil and carotol with PNB values of 0.84 and 0.78 at 10 µg/cm^2 showed activity similar to deet. The biting deterrence of carotol at 5.6 µg/cm^2, with PNB value of 0.72, was also similar to deet at 4.8 µg/cm^2.

In vitro A&K repellency bioassay

In A&K bioassay with treated surface area of 30 cm^2, MED of deet, carrot essential oil, and carotol against *Ae. aegypti* was 25 µg/cm^2 (Table 3). The

Table 1. Major components of carrot seed essential oil from gas chromatography analysis.

Component	Area (%)	Rt (min)	*m/z*	Class
α-pinene	0.82	7.8	136	Monoterpene
2(10)-pinene	0.46	9.4	136	Monoterpene
β-pinene	0.40	9.8	136	Monoterpene
Limonene	0.75	11.4	136	Monoterpene
Anisole	0.37	23.4	148	Monoterpene
Muurolene	4.86	27.5	204	Sesquiterpene
Cryophyllene	0.5	29.4	204	Sesquiterpene
(*z*)-β-farnesene	2.9	33.2	204	Sesquiterpene
Diepicedrene	1.1	35.6	222	Sesquiterpene
Carotol	75.6	37.56	222	Sesquiterpene
Daucol	2.0	38.9	238	Sesquiterpene
Total area	89.47			

mixture of deet with either the essential oil or carotol showed MED value of 12.5 μg/cm^2 (6.25 + 6.25 = 12.5 μg/cm^2), which is half the dose of individual compounds. Amounts of the individual components in the mixture were one-fourth of the MED value of individual compounds, which indicated a synergistic activity. In residual repellent activity bioassay, the essential oil and carotol gave activity similar to deet at 46.9 μg/cm^2 up to 120 min posttreatment (Table 4). At 23.4 μg/cm^2, deet showed full activity up to 120 min, whereas the carrot seed essential oil and the carotol crossed the MED limit after 60 min posttreatment. At 11.7 μg/cm^2, deet was within range of MED value up to 30 min posttreatment, whereas the essential oil and carotol failed at this dose.

In vivo cloth patch repellent bioassay

Data on the repellent activity of carrot seed essential oil and carotol are given in Table 5. The MED value of carrot seed essential oil and carotol was higher (25 μg/cm^2) than deet (12.5 μg/cm^2) against *Ae. aegypti*. The MED value of deet and carotol was 12.5 μg/cm^2 against *Ae. albopictus*, whereas the MED value of carotol was lower than *Ae. aegypti* (25 μg/cm^2). The MED value of deet and carotol (6.25 μg/cm^2) against *An. quadrimaculatus* was lower than *Ae. aegypti* (deet = 12.5 and carotol = 25 μg/cm^2, respectively) or *Ae. albopictus* (MED = 12.5 μg/cm^2).

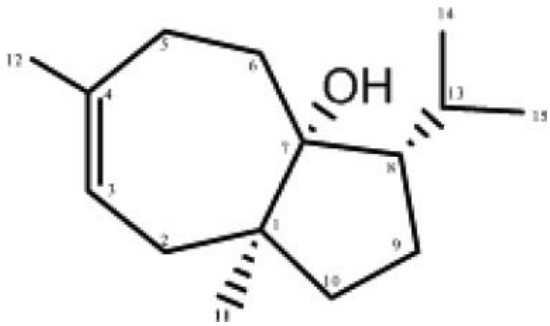

Fig. 1. Chemical structure of carotol.

In cloth patch bioassay carrot seed essential oil and carotol showed residual repellency similar to deet up to 120 min posttreatment at the dosages of 50 and 25 μg/cm^2 (Table 6) against *Ae. aegypti*, whereas only deet showed activity at 12.5 μg/cm^2.

Table 2. Biting deterrent activity of deet (*N*,*N*-Diethyl-3-methylbenzamide), carrot seed essential oil, and carotol against different species of mosquitoes in Klun and Debboun bioassay.

Treatment	*N*[1]	PNB ± SEM[2]
Aedes aegypti	75	0.85 ± 0.04 A
Experiment no. 1		
Deet 4.8 μg/cm^2		
Carrot seed essential oil 10 μg/cm^2	75	0.84 ± 0.05 A
Ethanol	75	0.36 ± 0.06 B
Experiment no. 2 (Fractions [F])	25	0.84 ±0.04 AB
Deet 4.8 μg/cm^2		
Carrot seed essential oil 10 μg/cm^2	25	0.88 ± 0.05 A
F-1 10 μg/cm^2	25	0.64 ± 0.04 BC
F-2 10 μg/cm^2	25	0.60 ± 0.06 BC
F-3 10 μg/cm^2	25	0.72 ± 0.05 ABC
F-4 10 μg/cm^2	25	0.52 ± 0.08 CD
F-5 10 μg/cm^2	25	0.48 ± 0.05 CD
F-6 10 μg/cm^2	25	0.52 ± 0.05 CD
F-7 10 μg/cm^2	25	0.60 ± 0.06 BC
Ethanol	25	0.32 ± 0.05 D
Experiment no. 3	75	0.85 ± 0.04 A
Deet 4.8 μg/cm^2		
Carotol 10 μg/cm^2	75	0.90 ± 0.03 A
Carotol 5 μg/cm^2	75	0.66 ± 0.03 B
Ethanol	75	0.29 ± 0.05 C
Anopheles quadrimaculatus		
Deet 4.8 μg/cm^2	50	0.84 ± 0.03 A
Carrot seed oil 10 μg/cm^2	50	0.84 ± 0.04 A
Carotol 10 μg/cm^2	50	0.78 ± 0.04 A
Carotol 5.6 μg/cm^2	50	0.72 ± 0.04 A
Ethanol	50	0.18± 0.02 B

[1] *N*, number of females tested.
[2] PNB, mean proportion of females not biting; SEM, standard error of the mean. Means within a column in an experiment not followed by the same letter are significantly different (Ryan–Einot–Gabriel–Welsch multiple range test $P \leq 0.05$).

Table 3. Repellant activity of deet (*N*,*N*-Diethyl-3-methylbenzamide) and combinations with carrot oil (C) and carotol (F) against *Aedes aegypti* in an in vitro Ali and Khan (A&K) bioassay. Values show the percentage (mean ± SEM) of females biting out of 200 in the cage.[1]

		Dose (μg/cm^2)			
Product[2]	*N*[3]	50	25	12.5	6.25
Compound					
Deet	15	0	0.53 ± 0.08	>1	>1
C	15	0	0.23 ± 0.08	>1	>1
F	15	0	0.43 ± 0.08	>1	>1
		25 + 25 = 50	12.5 + 12.5 = 25	6.25 + 6.25 = 12.5	3.12 + 3.13 = 6.25
Mixture					
Deet + C	15	0	0.13 ± 0.06	0.57 ± 0.07	>1
Deet + F	15	0	0.1 ± 0.05	0.63 ± 0.08	>1

[1] Minimum effective dose is ≤1% biting, which was 2 females out of 200 in the cage.
[2] The data are from A&K bioassay using 30-cm^2 treated surface area.
[3] *N*, number of replications.

In vivo direct skin application bioassay

In direct skin application bioassay, both the essential oil and carotol at 8%, 12.5%, 25%, and 50% application rates were active and reached to the MED values at 1.5, 1.5, 2, and 2.5 h postapplication, respectively (Table 7).

Data on the repellent activity of mixtures of deet with carrot seed essential oil and carotol against *Ae. aegypti* are shown in Table 8. These mixtures of deet with the essential oil and carotol showed very promising results. All the mixtures were active, showing a substantial increase in residual repellent activity at lower dosages when compared with various treatments used alone. Deet at 1% rate of application crossed the MED level at 1 h postapplication and with the addition of 1% carrot essential oil or carotol residual activity increased by 100% against *Ae. aegypti*. There was a 33% increase in residual activity when the activity of 1 + 1 = 2% mixtures was compared with 2% of deet alone. Addition of 2% essential oil or carotol in 2% deet increased the residual repellent activity by 100% (1.5 h), whereas this increase was 25% when the mixture was compared with the MED value of 4% deet. Mixture of 4% of the essential oil or carotol with 4% deet increased residual activity by 175% (3.5 h) when compared with the MED value of 4% deet, whereas the residual repellent activity of this mixture was similar to 8% application rate of deet. Mixture of 8.3% of the essential oil or carotol with 4.2% deet increased the residual activity by 225–250% (4.5–5 h), 30–40% (1.5–2 h), and 0%, when compared with 4%, 8%, and 12.5% application rate of deet, respectively.

DISCUSSION

Many researchers have reported the composition of carrot oil. Ozcan and Chalchat (2007) reported

Table 4. Residual repellent activity of deet (*N*,*N*-Diethyl-3-methylbenzamide), carrot seed essential oil, and carotol against *Aedes aegypti* females at different dosages in an in vitro Ali and Khan (A&K) bioassay. Values show the percentage (mean ± SEM) of females biting out of 200 in the cage.[1]

		Time after treatment (min)				
Compound by dose	*N*[2]	0	30	60	90	120
46.9 μg/cm^2						
Deet	15	0	0	0	0	0
Carotol	15	0	0	0	0.03 ± 0.03	0.03 ± 0.03
Carrot seed essential oil	15	0	0.07 ± 0.05	0.13 ± 0.06	0.33 ± 0.06	0.8 ± 0.1
23.4 μg/cm^2						
Deet	15	0	0.0 ± 0.0	0.0 ± 0.0	0.03 ± 0.03	0.43 ± 0.07
Carotol	15	0	0.4 ± 0.05	0.57 ± 0.08	>1	>1
Carrot seed essential oil	15	0.03 ± 0.03	0.16 ± 0.06	0.43 ± 0.07	>1	>1
11.7 μg/cm^2						
Deet	15	0.26 ± 0.07	0.53 ± 0.08	>1	>1	>1
Carotol	15	0.1 ± 0.05	>1	>1	>1	>1
Carrot seed essential oil	15	0.87 ± 0.05	>1	>1	>1	>1

[1] Minimum effective dose (MED) is ≤1% biting, which was 2 females out of 200 in the cage. Data are from A&K bioassay using 12-cm^2 treated surface area. Residual repellent activity based on MED is 46.9 μg/cm^2 in all treatment up to 120 min, whereas at 23.4 μg/cm^2 carotol and carrot seed essential oil crossed MED levels after 60 min posttreatment. Ethanol was regularly tested at the beginning and after every 5 replications as solvent control. The bioassays were continued only if the ethanol treatment failed (feeding ≥ 1%).
[2] *N*, number of replications.

Table 5. Repellent activity of deet (*N*,*N*-Diethyl-3-methylbenzamide), carrot seed essential oil, and carotol against 3 different species of mosquitoes in an in vivo "cloth patch" bioassay. Values show the percentage (mean ± SEM) of females biting out of 500 in the cage.[1]

Compound	N[2]	Dose (μg/cm^2)				
		50	25	12.5	6.25	3.125
Aedes aegypti						
Deet	25	0.02 ± 0.01	0.14 ± 0.03	0.5 ± 0.04	>1	—
Carotol	25	0.13 ± 0.03	0.53 ± 0.04	>1	—	—
Carrot seed oil	25	0.24 ± 0.03	0.57 ± 0.03	>1	—	—
Aedes albopictus						
Deet	5	—	0	0.23 ± 0.05	>1	—
Carotol	5	—	0.16 ± 0.06	0.65 ± 0.05	>1	—
Anopheles quadrimaculatus						
Deet	5	—	0	0.08 ± 0.05	0.32 ± 0.12	>1
Carotol	5	—	0	0.12 ± 0.08	0.36 ± 0.12	>1

[1] Minimum effective dose (MED) is ≤1% biting, which was 5 females out of 500. The MED in deet was 12.5 μg/cm^2 whereas comparable MED of carotol and the carrot seed essential oil was 25 μg/cm^2. Data are based on 25 replications for 50 and 25 μg/cm^2 treatments, respectively. Ethanol was used as solvent control, which showed heavy landing and biting in this bioassay (feeding ≥ 1%).
[2] *N*, number of replications.

Table 6. Residual repellent activity of deet (*N*,*N*-Diethyl-3-methylbenzamide), carrot seed essential oil, and carotol against *Aedes aegypti* females in an in vivo "cloth patch" assay. Values show the percentage (mean ± SEM) of females biting out of 500 in the cage.[1]

Compound	Dose (μg/cm^2)	N[2]	Time after treatment (min)				
			0	30	60	90	120
Deet	50	5	0.04 ± 0.04	0.08 ± 0.05	0.16 ± 0.04	0.28 ± 0.05	0.28 ± 0.05
Carotol	50	5	0.2 ± 0.06	0.44 ± 0.04	0.64 ± 0.04	0.68 ± 0.08	0.8 ± 0.06
Carrot seed oil	50	5	0.2 ± 0.06	0.28 ± 0.05	0.44 ± 0.09	0.6 ± 0.06	0.72 ± 0.05
Deet	25	5	0.08 ± 0.05	0.16 ± 0.04	0.36 ± 0.04	0.48 ± 0.05	0.48 ± 0.05
Carotol	25	5	0.4 ± 0.06	0.44 ± 0.04	0.56 ± 0.07	0.72 ± 0.05	0.76 ± 0.04
Carrot seed oil	25	5	0.52 ± 0.05	0.6 ± 0.0	0.64 ± 0.04	0.72 ± 0.05	0.92 ± 0.5
Deet	12.5	5	0.52 ± 0.1	>1	>1	>1	>1
Carotol	12.5	5	>1				
Carrot seed oil	12.5	5	>1				

[1] Minimum effective dose (MED) is ≤1% biting, which was 5 females out of 500 in the cage. Residual repellency based on MED is 50 and 25 μg/cm^2 in all treatments up to 120 min, whereas deet was also active at 0 min at 12.5 μg/cm^2. Ethanol was regularly tested at the beginning and after every 5 replications as solvent control. The bioassays were continued only if the ethanol treatment failed (feeding ≥ 1%).
[2] *N*, number of replications.

Table 7. Residual activity of carrot oil (C) and carotol (F) in direct skin application bioassay against *Aedes aegypti*. Values show the percentage (mean ± SEM) of females biting out of 500 in the cage.[1]

Time (h)	Dose (% age)							
	8		12.5		25		50	
	C	F	C	F	C	F	C	F
0	0	0	0	0.13 ± 0.07	0	0	0	0
0.5	0.13 ± 0.13	0.4 ± 0.12	0	0.07 ± 0.07	0	0	0	0
1	0.6 ± 0.0	0.6 ± 0.12	0.6 ± 0.28	0.27 ± 0.26	0.27 ± 0.13	0.67 ± 0.07	0	0.2 ± 0.12
1.5	>1	>1	>1	>1	0.93 ± 0.07	>1	0.33 ± 0.07	0.47 ± 0.13
2	>1	>1	>1	>1	>1	>1	0.73 ± 0.18	0.73 ± 0.27
2.5	>1	>1	>1	>1	>1	>1	>1	>1

[1] Minimum effective dose is ≤1% biting, which was 5 females out of 500 in the cage. The data are the means of 3 replications done on different days. Ethanol was regularly tested at the beginning and after every 5 replications as solvent control. The bioassays were continued only if the ethanol treatment failed (feeding ≥ 1%).

Table 8. Residual activity of deet (*N*,*N*-Diethyl-3-methylbenzamide) and combinations with carrot essential oil (C) and carotol (F) against *Aedes aegypti* in direct skin application bioassay. Values show the percentage (mean ± SEM) of females biting out of 500 in the cage.[1]

Time (h)	Dose (% age)											
	1.0	(1 + 1 = 2)		2.0	(2 + 2 = 4)		4.0	(4 + 4 = 8)		8.0	(4.2 + 8.3 = 12.5)		12.5
	Deet	Deet + C	Deet + F	Deet	Deet + C	Deet + F	Deet	Deet + C	Deet + F	Deet	Deet + C	Deet + F	Deet
0	0.3 ± 0.2	0	0	0	0	0	0	0	0	0	0	0	0
0.5	0.33 ± 0.07	0	0	0.07 ± 0.07	0	0	0	0	0	0	0	0	0
1	>1	0.27 ± 0.26	0.4 ± 0.12	0.27 ± 0.13	0.2 ± 0.0	0	0	0	0	0	0	0	0
1.5	>1	0.53 ± 0.29	>1	>1	0.33 ± 0.13	0.13 ± 0.13	0.13 ± 0.13	0	0	0	0	0	0
2	>1	>1	>1	>1	0.53 ± 0.18	0.33 ± 0.13	0.73 ± 0.18	0	0	0	0	0	0
2.5	>1	>1	>1	>1	0.6 ± 0.12	0.4 ± 0.0	>1	0	0	0	0.07 ± 0.07	0.07 ± 0.07	0
3	>1	>1	>1	>1	>1	>1	>1	0	0	0	0.07 ± 0.07	0.13 ± 0.07	0
3.5	>1	>1	>1	>1	>1	>1	>1	0.07 ± 0.07	0.27 ± 0.07	0.13 ± 0.13	0.07 ± 0.07	0.27 ± 0.07	0
4	>1	>1	>1	>1	>1	>1	>1	0.2 ± 0.12	0.4 ± 0.12	0.0 ± 0.0	0.07 ± 0.07	0.33 ± 0.07	0
4.5	>1	>1	>1	>1	>1	>1	>1	0.27 ± 0.18	0.53 ± 0.07	0.4 ± 0.23	0.13 ± 0.07	0.33 ± 0.13	0
5	>1	>1	>1	>1	>1	>1	>1	0.47 ± 0.13	>1	0.73 ± 0.3	0.2 ± 0.12	0.47 ± 0.07	0
5.5	>1	>1	>1	>1	>1	>1	>1	0.73 ± 0.18	>1	>1	0.27 ± 0.07	0.73 ± 0.18	0
6	>1	>1	>1	>1	>1	>1	>1	>1	>1	>1	0.33 ± 0.07	0.87 ± 0.13	0.13 ± 0.07
6.5	>1	>1	>1	>1	>1	>1	>1	>1	>1	>1	0.53 ± 0.13	0.93 ± 0.07	0.27 ± 0.27
7	>1	>1	>1	>1	>1	>1	>1	>1	>1	>1	0.6 ± 0.2	>1	0.53 ± 0.24
7.5	>1	>1	>1	>1	>1	>1	>1	>1	>1	>1	>1	>1	>1

[1] Minimum effective dose is ≤1% biting, which was 5 females out of 500 in the cage. The data are the means of 3 replications done on different days. Ethanol was regularly tested at the beginning and after every 5 replications as solvent control. The bioassays were continued only if the ethanol treatment failed (feeding ≥ 1%) in this bioassay.

carotol (66.8%), daucene (7.84%), (*Z*,*Z*)-α-faenesene (5.86%), germacrene D (2.34%), trans-α-bergamotene (2.41%), and β-selenene (2.2%) as the major contents of the carrot seed essential oil, while the seed oil contained carotol (30.6%), daucol (12.6%), and copaenol (0.62%) as main constituents. Jasicka-Misiak et al. (2004) reported 38.6% carotol in carrot seed oil purchased from Augustus Oils Ltd. London, with β-caryophyllene (10.66%), caryophyllene oxide (4.34%), α-pinene (3.94%), and farnesene (3.35%) as the other major contents. Mazzoni et al. (1999) reported carotol as the major content (73.1%, 69.7%, and 36.1%) in 3 commercial carrot seed essential oil samples. The published data suggested that the quantity of the major constituents of the carrot seed essential oil can vary. This variation could have been due to the variety, plant parts used, harvest timings, geographical location as affected by the climatic factors, genetic origin, and the way the samples were prepared (Merghache et al. 2008).

The K&D bioassay is widely used as a tool to test the biting deterrence of compounds against mosquitoes where deet is used as a positive control. As such, many studies have reported data on deet whereas biting deterrent activity of the carrot seed essential oil and carotol is reported for the 1st time in the present study. In most of the published studies, the PNB value of deet is >0.8. Data on the biting deterrent activity of deet in this study were similar to the published literature (Klun et al. 2005, Ali et al. 2012).

The A&K bioassay is a newly developed bioassay and data on deet are limited, whereas repellent activity of carrot seed essential oil and carotol against mosquitoes has not been reported in the literature. Data from this study corroborate the findings of Ali et al. (2017a), who reported an MED value of 18.7 μg/cm^2 in a similar experimental setup.

In vivo cloth patch bioassay is widely used to test repellency against mosquitoes where deet is used as a positive control. As such, many studies have reported the repellency of deet against different species of mosquitoes, whereas data on repellent activity of carrot seed essential oil and carotol are reported for the 1st time. The MED reported in the literature ranges from 6 to 23 μg/cm^2 as reported by Ali et al. (2017a). Data from the cloth patch bioassay on deet (MED = 12.5 μg/cm^2) in this study corroborates the findings of Ali et al. (2017a) and Katritzky et al. (2010), reporting MED values of 11.1 and 9 μg/cm^2 for *Ae. aegypti*, respectively.

Residual activity of deet (7 h) at 12.5% application rates of deet in direct skin application bioassay corroborates the findings of Logan et al. (2010), who reported 100% protection for 6 h against *Ae. aegypti* in arm in cage repellency trials. Witting-Bissinger et al. (2008) reported >96% protection for 6 h at a dose of 15%. Repellent activity of carrot seed essential oil and carotol is reported for the 1st time. In this study, the residual activity of carrot seed essential oil and carotol lasted 1 h at a dose of 8% (Table 7) and deet at 4% lasted for 2 h (Table 8). Residual activity of the mixture of these natural products with deet at 8.3 + 4.2%, respectively, synergistically increased from 1 h to 6.5 h.

The carrot seed essential oil and carotol showed excellent biting deterrent activity in all the bioassays. The data on the mixtures of deet with the carrot seed essential oil and carotol indicated a substantial increase in residual repellency compared with the essential oil, carotol, or deet alone. Data on repellency of these natural products or their mixtures with deet in direct skin application are very promising, indicating good potential of these natural products to be developed as effective repellents. This is the 1st report on the biting deterrent and repellent activity of carrot seed essential oil and carotol against mosquitoes. Synergy of these natural products in mixtures is a unique characteristic that makes them strong candidates for development as commercial repellents against mosquitoes alone or in blends. Carrot seed essential oil is on the US Food and Drug Administration's Generally Recognized as Safe list and is used commercially in cosmetic formulations. A major commercial advantage of carrot seed essential oil is that it is safe for skin use, biodegradable, and commercially available. Carotol, which is the major part (>75%) of the carrot seed essential oil, can be economically extracted. Further studies will be needed to test these natural products under field conditions.

ACKNOWLEDGMENTS

This study was supported in part by USDA/ARS grant No. 58-6066-6-043. We thank James J. Becnel and Dan Kline, Mosquito and Fly Research Unit, Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, Gainesville, for supplying mosquito eggs. We thank N. Tabanca, C. Cantrell, and S. Duke, USDA-ARS, for their valuable suggestions during the course of this study. We thank J. F. Parcher, NCNPR, The University of Mississippi for his critical review of the manuscript.

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