

Repellence properties of catnip essential oil against the mosquito species *Aedes aegypti*

Charles Batume

Uganda Virus Research Institute (UVRI)

Ivan Mugeni Mulongo

University of California Davis

Richard Ludlow

Cardiff University

John Ssebaale

CEMPOP Uganda Limited

Peter Randerson

Cardiff University

John A. Pickett

Cardiff University

Ivan M. Mukisa

Makerere University

Simon Scofield (✉ ScofieldS@cardiff.ac.uk)

Cardiff University

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Abstract

The mosquito species *Aedes aegypti* is known to act as a vector in the transmission of various diseases, including dengue fever and yellow fever. The use of insect repellents is one of precautionary measures used to mitigate the risk of these diseases in humans by reducing mosquito biting. Nepetalactone, a potent natural insect repellent primarily found in catnip (*Nepeta cataria*) essential oil, has emerged as a promising candidate for mosquito repellence. Here, we evaluated the potential of catnip essential oil (>95% nepetalactone) for use as a mosquito repellent. Using a Y-tube olfactometer and human hands as an attractant, we analysed the effectiveness of catnip oil at repelling the mosquito species *Aedes aegypti*. We tested a range of dilutions of catnip essential oil and found that concentrations as low as 2% were effective at repelling >70% of mosquitoes for between one and four hours after repellent application. These findings suggest that nepetalactone could be used as a natural and highly effective alternative to synthetic mosquito repellents, thereby offering enhanced protection against vector-borne diseases.

Introduction

Insect-borne diseases pose a significant threat to human health, with mosquitoes acting as vectors of pathogens causing diseases such as malaria, dengue fever, filariasis, and West Nile virus¹. Protecting against mosquito bites has traditionally involved limiting outdoor activities during peak mosquito activity, wearing protective clothing, and using insect repellents. While effective control measures, such as insecticide-treated nets and indoor residual sprays, have been implemented for indoor-biting mosquitoes in sub-Saharan countries such as Uganda, outdoor exposure to mosquitoes during work hours remains a challenge, particularly for *Aedes aegypti*, *Anopheles gambiae sensu stricto*, and *Culex quinquefasciatus*. Therefore, there is a need for an effective, user-friendly and affordable solution to prevent mosquito bites in these scenarios.

The most commonly used and effective mosquito repellent is the synthetic compound DEET (*N,N*-diethyl-3-methylbenzamide). However, concerns have been raised about the potential adverse effects of DEET, particularly in pregnant and lactating women, as well as in young children²⁻⁴. Despite these concerns, the Centers for Disease Control and Prevention (CDC) still recommend DEET for vector protection. While studies have shown that DEET can inhibit human acetylcholinesterase and modulate various receptors and ion channels, conflicting reports argue against significant adverse health effects. Additionally, DEET is rapidly cleared from the body, unless ingested or used for prolonged periods. Nonetheless, the cost of DEET remains a barrier for many individuals living in mosquito-prone regions like sub-Saharan Africa.

Exploring ethnobotanical resources has led to the identification of plant oils with insect-repellent properties, such as citronella, geraniol from lemongrass oil, and neem oil⁵. However, these natural alternatives have limitations, including shorter duration of repellence compared to DEET. Nevertheless, preliminary studies have indicated that nepetalactone, an iridoid monoterpene found in catnip (*Nepeta cataria*) essential oil, exhibits comparable effectiveness to DEET as a mosquito repellent⁶⁻⁹. Moreover, catnip leaves have been used in various applications, including tea, meat tenderizer, and folk remedies for

fevers, colds, cramps, and migraines, suggesting that is safe for humans¹⁰. Safety assessments, such as acute oral, dermal, and inhalation toxicity tests, have deemed *N. cataria* essential oil safe for human use, as confirmed by the United States Environmental Protection Agency¹¹.

Clinical data have demonstrated that a 15% concentration of nepetalactone in lotion provides more than 6 hours of protection against black fly and mosquito bites⁶. Furthermore, Suschke *et al.*, 2007 reported that catnip oil did not cause irritation when applied to human skin at a concentration of 25%¹². Building upon these findings, this report evaluates the repellent properties of essential oil from a *Nepeta cataria* chemotype grown in Uganda which contains the 4a*S*,7*S*,7a*R* isomer of nepetalactone at a concentration of >95%, as determined by gas chromatography coupled mass spectrometry (GC-MS). This study explores the effectiveness of catnip oil concentrations ranging from 0% to 100%, using a Y-tube olfactometer to assay repellence. By investigating the repellent properties of essential oils derived from catnip, specifically against *Aedes aegypti* mosquitoes, this study aims to provide valuable insights into the development of effective and accessible mosquito repellents for populations in mosquito-prone regions. The affordability of locally sourced nepetalactone makes it a promising alternative or supplement to chemical-based repellents like DEET.

Results

Validation of Y-tube olfactometer function

Prior to testing the effectiveness of catnip oil as a mosquito repellent, we first evaluated our Y-tube olfactometer (designed according to WHO guidance¹³; Supplementary Fig. S1) for use in repellence assay experiments (Fig. 1; Supplementary Table S1). We first established whether the *Aedes aegypti* mosquitoes displayed a preference for a particular branch of the olfactometer, irrespective of the use of attractants or repellents. Twenty mosquitoes were released from the holding port into the apparatus and after 30 seconds, the number of mosquitoes in each branch port was recorded. A total of six replicates were performed. The distribution of mosquitoes in the olfactometer showed that an average of 13% travelled to the end of each collecting branch, while the remaining mosquitoes were distributed in the base leg or holding port (Fig. 1A; Supplementary Table S1). These findings indicate a random, uniform and repeatable distribution pattern of mosquitoes in both olfactometer branches. Thus, the Y-tube olfactometer is suitable for conducting unbiased repellence assays.

We next tested our apparatus to confirm that the presence of a human hand in one of the branches acted as an attractant to mosquitoes. In line with this, we found that an average of 57% of mosquitoes were attracted to the branch containing the human hand while 15% were found in the empty control branch and 28% remained in the holding port or base leg of the olfactometer (Fig.1B; Supplementary Table S1).

We then performed a series of experiments to evaluate the performance of the mosquito repellent *N,N*-diethyl-3-methylbenzamide (DEET) using our olfactometer. We first placed an open vial containing 100µl of 20% DEET in one branch and left the other branch empty. An average of 85% of mosquitoes remained

in the holding tube/ base leg, while 14% were found in the empty branch and <1% were found in the branch containing DEET (Fig. 1C; Supplementary Table S1). In the next experiment we placed a human hand in one branch as an attractant and a vial containing 100µl of 20% DEET in the other branch. We found that 49% of mosquitoes were attracted to the human hand, while 51% remained in the holding tube/ base leg. No mosquitoes were found in the branch containing DEET (Fig. 1D; Supplementary Table S1). Finally, we placed a human hand in one branch and a human hand with a vial containing 100µl of 20% DEET in front of it in the other branch. An average of 62% of mosquitoes were found in the branch containing the human hand, while 33% remained in the holding tube/ base leg and 5% were found in the branch containing the human hand + 20% DEET (Fig. 1E; Supplementary Table S1). These data confirm that human hands act as an attractant to *Aedes aegypti* mosquitoes and that DEET functions as an effective repellent using our Y-tube olfactometer and experimental methodology.

Testing efficacy of commercially available repellents with the Y-tube olfactometer

We tested our experimental setup and testing procedure with commercially available insect repellents *Peaceful Sleep* (15% DEET) and *Odomos*, which contains 12% *N,N*-diethylbenzamide, a compound closely related to DEET, that has been shown to be effective in repelling *Anopheles stephensi* and *Aedes aegypti* mosquitoes¹⁴. We found that *Peaceful Sleep*-treated human hands attracted an average of 14% of responding *Aedes aegypti* mosquitoes (ie. only those mosquitoes that moved to either branch and did not remain in the base leg/ holding port) while the untreated control hand attracted an average of 86% of mosquitoes (Fig.2; Supplementary Table S2; Supplementary Fig. S2). Likewise, *Odomos*-treated human hands attracted an average of 14% of mosquitoes while the untreated control hand attracted an average of 86% mosquitoes (Fig.2; Supplementary Table S2; Supplementary Fig. S2). These data confirm that our experimental design and apparatus can replicate the known mosquito repellence quality of DEET and *N,N*-diethylbenzamide, establishing that it is suitable for analysing the repellence qualities of other compounds.

Testing efficacy of catnip oil in repelling Aedes aegypti

We next investigated the effectiveness of different concentrations of catnip essential oil in repelling *Aedes aegypti* mosquitoes. We used essential oil obtained by hydro-distillation of leaves, stems and flowers of a variety of *Nepeta cataria* designated as Chemotype A, which has previously been shown to contain the 4a*S*,7*S*,7a*R* isomer of nepetalactone⁷. In line with this, our GC-MS analysis showed that the oil contained ~97.5% nepetalactone, ~1.5% caryophyllene and <1% caryophyllene oxide (Table 1).

We assessed the efficacy of a series of catnip oil dilutions ranging from 100% catnip oil with no diluent (olive oil), to 0% catnip oil with 100% diluent. A human hand was placed at the control branch of the Y-tube olfactometer, while in the other branch a 100 µl aliquot of catnip oil or diluent was placed in a vial cap in front of the human hand. We found that catnip oil at concentrations ranging from 100% to just 2% provided significant repellence of mosquitoes, with the percentage of responding mosquitoes in the control branch ranging from 74% to 90%, and the percentage of mosquitoes in the branch containing

repellent ranging from 26% to 10% (Fig.3A; Supplementary Tables S3 and S4; Supplementary Fig. S3). Catnip oil diluted to 1%, or diluent alone, did not show any significant repellence properties. These data demonstrate the catnip oil is effective in repelling *Aedes aegypti* mosquitoes at a broad range of concentrations, with 2% representing the lower limit of efficacy.

Assessing the duration of mosquito repellence using 2% catnip oil

Having established that 2% catnip oil provided substantial *Aedes aegypti* mosquito repellence, we next investigated the duration of repellence after the initial application to human skin. We tested a 2% dilution of catnip oil, again using olive oil as the diluent, and the effect of 2% catnip oil in a standard hand lotion formulation (see Materials and Methods for ingredients). We performed the repellence assay over a 5-hour period and found that 2% catnip oil diluted in olive oil gave significant repellence for up to one hour after application to skin, with 85% of responding mosquitoes found in the branch containing the human hand and 15% of mosquitoes found in the branch containing the human hand and 2% catnip oil one hour after application (Fig.3B; Supplementary Table S5 and S6; Supplementary Fig. S3). Some repellence was observed at later timepoints, with ~60% of responding mosquitoes found in the branch containing the human hand and ~40% of mosquitoes found in the branch containing the human hand and 2% catnip oil, but these results were not statistically significant. For 2% catnip oil added to a hand lotion, we observed significant repellence for up to 4 hours after application to the skin, with 82% of responding mosquitoes found in the branch containing the human hand and 18% in the branch containing the human hand and 2% catnip lotion 1 hour after application, and 61% and 39% respectively after 4 hours (Fig.3C; Supplementary Table S7 and S8; Supplementary Fig. S3). Hence, 2% catnip oil has significant repellence properties for variable amounts of time after the initial application, depending on the diluent used.

Discussion

In this study we investigated the use of catnip essential oil derived from the herbaceous plant *Nepeta cataria* (a member of the Lamiaceae or mint family), as a repellent of the mosquito species *Aedes aegypti*, which acts as a vector for several infectious human diseases in sub-Saharan Africa and elsewhere. The active component in catnip oil, the iridoid monoterpene nepetalactone, has been shown previously to have potent repellence properties on several other hematophagous arthropod species⁶⁻⁹. Here, we used a Y-tube olfactometer and used WHO approved experimental protocols to assess mosquito repellence effectiveness of catnip oil comprising >95% nepetalactone.

Our initial experiments validated our experimental design and apparatus, as we detected no inherent bias in the mosquito preference for a particular branch of the olfactometer, and we were able to demonstrate effective mosquito attraction using human hands and repellence using 20% DEET. We also showed that the application of two different commercial brands of insect repellent, one containing 15% DEET (*Peaceful Sleep*) and one containing 12% of the closely related compound *N,N*-diethylbenzamide (*Odomos*), acted to effectively repel mosquitoes using our experimental system, with ~86% of

mosquitoes repelled. These data show that our repellence assay can recapitulate the known repellence properties of DEET and *N,N*-diethylbenzamide.

Previous studies have shown the nepetalactone can actively reduce mosquito landing events using 37°C heat packs⁹. A further study demonstrated reduced landing events on human subjects using range of concentrations of hydrogenated nepetalactone⁶. Our study builds upon these findings by directly assaying mosquito repellence using the Y-tube olfactometer to provide a binary choice for landing and by using human hands as the attractant. We performed mosquito repellence assays using a range of different concentrations of catnip oil, diluted as appropriate in the olive oil diluent. We showed that concentrations as low as just 2% catnip oil provided effective repellence similar to that observed with higher concentrations of up to 100% catnip oil, and to the degree of repellence observed with 15% DEET. At concentration of 1% and 0% catnip oil, no repellence was observed, indicating that 2% is the lower limit for repellence, and that the olive oil diluent itself possesses no repellence properties.

We showed that 2% catnip oil provides statistically significant repellence for a period of up to 1 hours when diluted in olive oil and applied as an aliquot in a vial cap, and for up to 4 hours when mixed with an unscented lotion that can be applied directly to human skin. The use of just 2% catnip oil is far more cost-effective than using higher concentrations, and reduces the apparent strength of the odour as perceived by humans. This implies that, for people living in sub-Saharan Africa, 2% catnip oil can provide protection for the period spent undertaking chores in the evening, when many species of mosquito are most active. However, *Aedes aegypti* has also been found to bite during day, and therefore application of the repellent containing 2% catnip oil several times per day can be potentially as effective as the 15% catnip oil concentration tested by Spero *et al*, (2008) ⁶.

Conclusions

This study highlights the efficacy of catnip oil as a natural insect repellent, specifically for repelling *Aedes aegypti* mosquitoes. The results demonstrate that even at a low concentration of 2%, catnip oil provides significant protection for up to 4 hours, making it a viable option for inclusion in consumer products like lotions, and may represent an effective and cheaper alternative to repellents such as DEET. The catnip plant is easily cultivated in much of sub-Saharan Africa, especially in Uganda, and a number of indigenous catnip varieties exist which could be analysed and potentially used as a nepetalactone feedstock. Further research is warranted to validate these findings in different settings, against other species of mosquitoes and other biting arthropods, and to explore potential applications in the field for mosquito-borne disease prevention.

Materials and Methods

Rearing and feeding of Aedes aegypti mosquitoes

The mosquito repellence aspect of this project was carried out at Makerere University College of Food Science technology, Nutrition and Bioengineering, Kampala, Uganda. The colony of *Aedes aegypti* was established by obtaining eggs and larvae from ovitraps set in the field (grassland) around Makerere University and rearing was conducted at the insectary at the Makerere University College of Food technology, Nutrition and Bioengineering. Traps containing eggs were brought back to the insectary facility and placed in distilled water to hatch. Larvae were reared in water at 27 °C under a 12-hour day/night light cycle. After egg hatching, larvae were separated from the unhatched eggs and placed into fresh water. A small amount (about 10 mg) of tetramine fish food purchased from Tetra (Blacksburg, VA) was added to feed the larvae twice daily. Pupae were separated from larvae and placed into a (30x30x30) cm rearing cage where the pupae were allowed to mature into adults. These were given 5 days to mate. Mature females were visually identified, then separated out of the population by aspirating them into a separate rearing cage where they were given a 10% sucrose solution as an energy source. The method of rearing mosquitoes in the insectary followed well established protocols^{15,16}. De-fibrinated bovine/ovine blood was used to feed the adult mosquitoes. Blood was stored at 4°C and placed in warm water before feeding the mosquitoes. Mature females were maintained 27+/- 1 °C with 72-80% relative humidity in rearing cages until experimentation. Female mosquitoes that were nulliparous and 5–8 days post-emergence were used in the repellence experiments. Insectary hygiene, safety and security measures were implemented according to appropriate guidelines^{15,16}.

Purification and analysis of catnip essential oil

Nepeta cataria 'Chemotype A' seeds were sourced from CN Seeds (Cambridgeshire, UK). Essential oil from this *N. cataria* chemotype (Chemotype A) has previously been shown to contain >91% 4a*S*,7*S*,7a*R* nepetalactone and has substantial insect repellent quality that has been demonstrated to work effectively on several species of mosquito, ticks and mites⁷. Plants were grown in 4L pots, in a potting mix consisting of 4 parts sieved compost to 1 part grit and 1 part John Innes No. 3. They were grown under heated glasshouse conditions and harvested at the point of floral opening. Plants were dried in a drying oven at 25°C before being distilled. Catnip essential oil was obtained by hydro-distillation of dried mature aerial *Nepeta cataria* tissue using a Clevenger apparatus and a 5L round bottomed flask. 100g of dried *N. cataria*, 2.5L of water and 2ml of hexane was boiled for 5 hours and the oil distillate was collected and analysed by GC-MS. All plant research was conducted in compliance with international and UK guidelines. No endangered species were used in this research.

Essential oil samples were diluted by a factor of 100 in hexane (>99%, Sigma-Aldrich). Samples were injected into a Thermo Trace 1300 gas chromatograph fitted with a Thermo TG-5MS column (30m x 0.25mm x 0.25µm) and detected using a Thermo ISQ LT mass spectrometer. The injection port was operated at 200°C, into which 1 µL of the sample was injected and loaded onto the column at a 1:5 split ratio, with the column being operated at 1 mL min⁻¹ He carrier gas. The GC oven was run with a ramped temperature profile; initial temperature 50°C for 2 min, ramp at 5°C min⁻¹ to 230°C and held for 12 min.

The mass spectrometer was operated with a transfer line temperature of 250°C, an ion source temperature of 230°C and a mass scan range of 35-350, with a 2 min solvent delay.

GC-MS data were processed using Chromeleon (Version 7.2 SR4; Thermo Scientific, USA) and the deconvolution and integration of signal peaks occurred in AMDIS (NIST, 2014), using a custom retention-indexed mass spectral library as previously described¹⁷. MS spectra were compared to the NIST 2014 library¹⁸. Compounds scoring >80 % in both forward and reverse fit which also had a retention index match of ± 15 were included in the custom library as putatively identified. VOC data are presented as a percentage of the total signal for each sample.

Repellence testing procedure

The effectiveness of catnip oil and the branded repellents in mosquito repellence was tested using a Y-tube olfactometer based on that described by Geier and Boeckh (1999)¹⁹ in accordance with WHO protocols¹³ (Supplementary Fig. S1). Preference for the subject's treated/untreated hands, positioned at the ends of the Y-tube branches, was measured²⁰. The positioning of test compounds (left or right branch) was varied to eliminate possible biases derived from the apparatus itself. Commercially available brands of mosquito repellents were used to validate our testing apparatus and protocol. These were *Peaceful Sleep* (15% DEET) and *Odomos* (12% w/v *N,N*-diethylbenzamide).

Mosquitoes were starved for 3-4 hours prior to performing each experiment. The olfactometer was cleaned with 70% ethanol before and between experiments. Repellence assays were performed between 8:00 am and 6:00 pm with presence of natural light. Dilutions of catnip oil were prepared using a micropipette at a range of concentrations ranging from 0% to 100% diluted as appropriate using olive oil as the diluent and are hereafter termed 'repellent' while the control olive oil is termed 'diluent only'. The lotion for assessing the duration of repellence contained 2% catnip oil, petrolatum, dimethicone, glycerol, sunflower oil, olive oil, shea butter, methyl paraben, propyl paraben, cetyl alcohol and stearyl alcohol. A 100 μ l sample of repellent or control mixtures was applied to a 9mm vial cap and placed in front of the human subject's hand to assay repellence. For repellence assays, 20 adult female *Aedes aegypti* mosquitoes were placed in the central holding port of the olfactometer and allowed to acclimatize for 5 minutes after the airflow fan was engaged at a rate of 0.4 m/s. 100 μ l of repellent mixture was placed in a 9mm vial cap in one olfactometer branch port and 100 μ l of control mixture in front of the other, and human hands were placed at each port of the olfactometer. The placement of repellent and control treatments was fully randomized. The holding port door was opened for 30 seconds allowing the mosquitoes to fly along the base leg and into the two branches. The trapping port doors were closed and the number of mosquitoes in each trapping port were counted, along with those that remained in the holding port or base leg. Mosquitoes that were physically damaged, incapable of flying or walking are discounted. Trapping ports and the holding port were removed and cleaned between replicates, and airflow was engaged for 5 minutes before the next replicate. The percentage attracted to the treatment port was calculated by dividing the number of mosquitoes trapped in the treatment port by the total number of mosquitoes in the test. The mean percentage attraction was calculated from the responses of

a minimum of three replicates of each treatment. Data were analysed using Microsoft Excel. Statistical significance ($p \leq 0.01$) was determined using an unpaired 2-tailed *t*-test assuming equal variance in the data.

Declarations

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Author contributions

CB designed and conducted the experiments, analysed data and wrote the first draft of the manuscript. IMM conducted experiments. RL extracted and analysed the catnip oil. JS and PR coordinated the research between Uganda and the UK. JAP provided the *N. cataria* germplasm, provided funding and critically reviewed the data and the manuscript. IM supervised and led the work in Uganda and coordinated the study. SS led the overall project as PI, provided funding, analysed data and prepared the final version of the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that there are no competing interests.

Data Availability statement

The raw data generated as part of this study are available from the corresponding author upon reasonable request.

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Table 1

Table 1 is available in the Supplementary Files section.

Figures

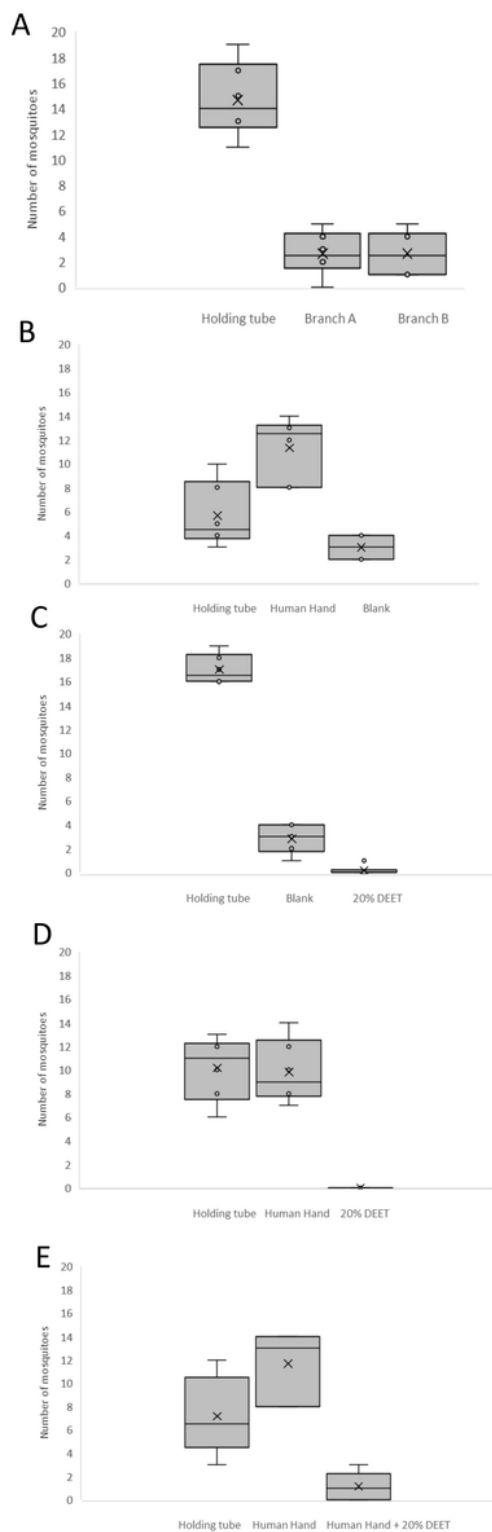


Figure 1

Validation of Y-tube olfactometer function. Box and whisker plots show mean number of mosquitoes per treatment and associated standard deviations. (A) Olfactometer bias experiment with no attractants or repellents. (B) Attraction assay using a human hand and empty olfactometer branch (blank). (C) Repellence assay using 20% DEET in one branch and an empty second branch (blank). (D) Attraction/repellence assay using human hand in one branch and 20% DEET in the other branch. (E) Attraction/

repellence assay using human hand in one branch and human hand + 20% DEET in the other branch. All experiments were performed with six replicates of 20 mosquitoes per replicate. Branches for different treatments were alternated for experiments shown in B-E. *t*-tests were performed as described in Materials and Methods and are shown in supplementary data.

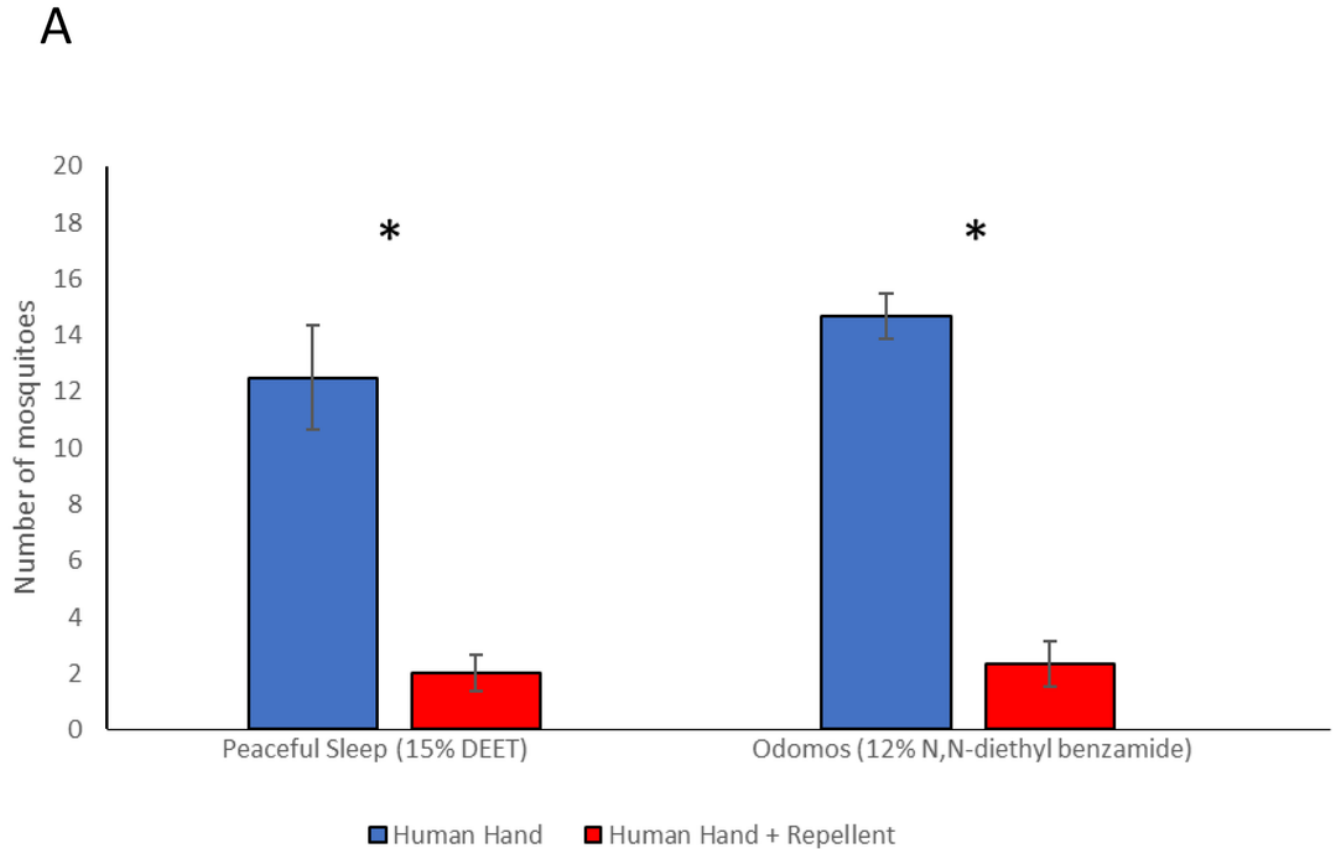


Figure 2

Repellence efficacy of commercially available mosquito repellents *Peaceful Sleep* (15% DEET) and *Odomos* (12% *N,N*-diethyl benzamide) using the Y-tube olfactometer. 18-20 mosquitoes were used for each replicate. A total of 6 replicates were used for each repellent. Error bars indicate standard deviations. *t*-tests were performed as described in the Materials and Methods. Asterisks indicate statistical significance ($p \leq 0.01$).

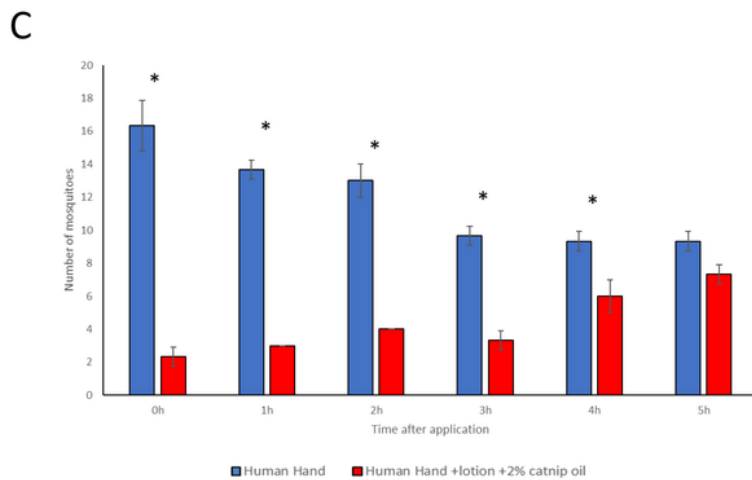
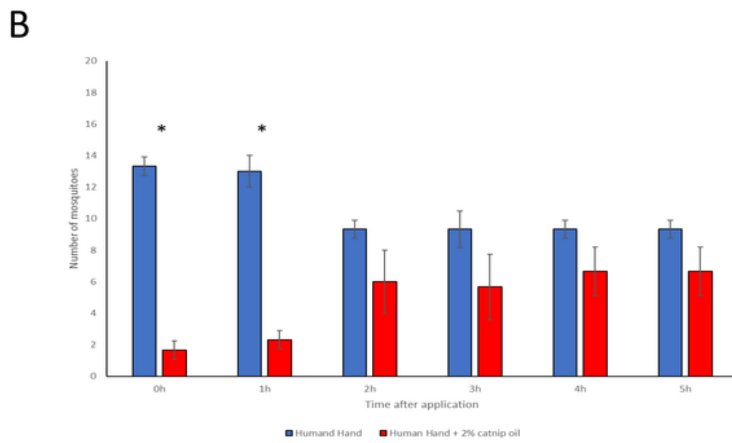
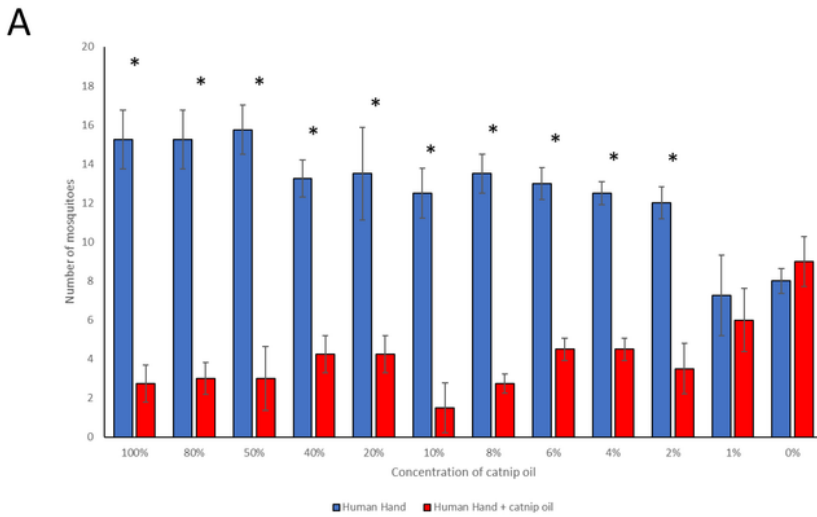


Figure 3

Evaluation of catnip oil as a mosquito repellent. (A) Number of mosquitoes attracted to a human hand (no catnip oil, blue bars) or human hand with catnip oil (red bars) applied at differing concentrations, diluted to the appropriate concentration in olive oil. 100% catnip oil had no olive oil diluent, 0% catnip oil was olive oil only. 20 mosquitoes were used for each replicate and 4 replicates were performed for each concentration. (B) Residual activity of 2% catnip oil diluted in olive oil over a 5-hour time-course post-

application. 20 mosquitoes were used for each replicate and 3 replicates were performed for each concentration. (C) Residual activity of hand lotion supplemented with 2% catnip oil diluted hand lotion over a 5-hour time-course post-application. 20 mosquitoes were used for each replicate and 3 replicates were performed for each concentration. Error bars represent standard deviations. Asterisks indicate statistical significance using a *t*-test with a *p*-value of ≤ 0.01 .

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryData.pdf](#)
- [Table1.pdf](#)