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Publication Date (Web): December 13, 2011 | doi: 10.1021/bk-2011-1090.ch004

Chapter 4

Catnip Essential Oil and Its Nepetalactone Isomers as Repellents for Mosquitoes

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The effect of catnip *Nepeta cataria* essential oil and two isomers of nepetalactone, the major components, on the distribution of *Aedes aegypti* (yellow fever mosquito) mosquitoes in a static-air olfactometer response was examined to determine their activity as spatial repellents. A glass cylinder was used as a choice-test chamber. The catnip (*Nepeta cataria*) essential oil, as well as the *E,Z*- and *Z,E*-isomers of nepetalactone were significantly repellent after application of one ml of 1% and 0.1% solution to filter paper (conc. of 157 and 15.7 μg/cm^2). Diethyl-*m*-toluamide (DEET) a positive control was significantly repellent at 157 μg/cm^2 in this assay. Both nepetalactone isomers and the catnip essential oil had excellent spatial repellency while DEET only exhibited spatial repellency at higher concentrations. The bioassay allowed for definition of and delineation between spatial and contact repellency.

Keywords: *Aedes aegypti*; repellent; *Nepeta cataria*; nepetalactone; spatial repellent

Introduction

For several decades, diethyl-m-toluamide (DEET) has been the most widely used insect repellent available. First synthesized in the early 1950s, DEET is usually regarded as safe, but up to 50% of the applied dose of DEET may be absorbed into the skin within six hours, and toxic effects have occasionally been documented in the literature, e.g. (*1*), (*2*), (*3*). In the United States, citronella is a popular botanical ingredient in insect repellent formulations. Candles and incense

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containing oil of citronella are also sold as insect repellents. It was reported (*4*) that citronella candles or incense were ineffective for reducing the biting pressure of mosquitoes. There is a strong desire among consumers for alternative choices for insect repellents, including high interest in natural alternatives.

The mint catnip *Nepeta cataria* L. is a common plant that frequently grows as a weed in many parts of the United States. Folklore had considered it an insect repellent for decades. Its oil was shown to keep ants from scavenging on a dead insect (*5*). Later it was shown to reduce the amount of time spent by German cockroaches *Blattella germanica* (L.) on the treated side of a choice-test arena (*6*, *7*). The essential oil of catnip has been shown to consist primarily of nepetalactone (70 to 98%), which is present primarily as two isomers, *Z,E*- and *E,Z*-nepetalactone (Figure 1) (*8*). Peterson (*7*) reports a separation process for these two isomers. The current study examines the effect of this essential oil and the individual isomers of nepetalactone on the distribution of yellow fever mosquitoes *Aedes aegypti* L. in a choice-test chamber. We used a 9 x 60-cm static-air glass repellency chamber to compare the effects of the catnip essential oil, the two pure individual isomers of nepetalactone at two concentrations (157 and 15.7 μg/cm^2) on the mosquitoes, and DEET at three concentrations (1572, 786, and 157 μg/cm^2).

Materials and Methods

Insects. A colony of *Aedes aegypti* was established in the summer of 1999 from wild mosquitoes collected in Costa Rica. The colony was blood-fed on a sedated rabbit. Eggs from mosquitoes were dried and stored in an incubator until needed. Eggs were placed in deoxygenated water and two to three drops of ground fish food were added to the water to feed the larvae. Pupae were removed from the larval pans as they appeared and were placed into mesh-covered paper cups. Adults were removed as they emerged by using an aspirator. The adults were separated by sex, and the females were retained for the repellency tests. The mosquitoes were allowed to feed on a cotton ball soaked with 10% (0.3 M) sucrose solution for four days before testing. The cotton ball was removed about 24 hr before the test was run.

Catnip Essential Oil and Nepetalactone isomers. Catnip plants were collected in the wild in Ames, Iowa. The catnip essential oil was obtained by steam distillation as described in Peterson (*9*). The nepetalactone from the catnip essential oil was identified by high performance liquid chromatography (HPLC) to consist of 85% *Z,E*-nepetalactone and 15% *E,Z*-nepetalactone. *Z,E*-nepetalactone was purchased as "catnip oil" from Kong Pet Products, Golden, CO. The HPLC method used an Hewlett-Packard 1100 HPLC with a Pirkle Covalent Phenylglycine Hi-chrom preparative column (25 cm x 10 mm I.D., 5μm S5NH Modified Shereosorb) and a mobile phase of 9:1 hexane:ethyl acetate at 2.5 ml/min flow rate, and detection with a Spectroflow 757 variable-wavelength UV-detector at 254 nm. Analysis of this oil determined that it consisted of 97.5% *Z,E*-nepetalactone, 0.8% *E,Z*-nepetalactone and 1.7% of an unknown. *E,Z*-Nepetalactone was purified from the catnip essential oil by preparative

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thin-layer chromatography as previously described (*10*). Diethyl-*m*-toluamide (DEET, 97%) was purchased from Aldrich Chemical Co., Milwaukee, WI.

Bioassay. A static-air choice-test apparatus consisted of a 9 x 60-cm section of glass tubing with a 2-cm hole drilled at the midpoint along the length for central introduction of the insects. One ml of test solution (oil, isomer, or DEET dissolved in acetone) was pipetted on a 9-cm filter paper and allowed to dry for five minutes. Another filter paper was treated with 1 ml certified acetone and allowed to dry for five minutes. The filter papers were placed inside the lids of 9-cm plastic petri dishes, and the lids were placed over the ends of the glass tube. The position of the treated side, to the right or to the left, was selected by using a random-number table. Twenty unmated adult female mosquitoes (four-day post emergence) were starved for 24 hours prior to the test, then anesthetized with carbon dioxide, and then introduced into the tube by using an aspirator. The insects were allowed to disperse through the tube for 15 minutes and the number of insects in each side (30 cm) were counted. A preliminary test was run with DEET at 1,572 and 786 $\mu g/cm^2$ to confirm if the assay was capable of detecting repellency. The catnip essential oil (CNEO) and two pure isomers were tested at two treatment concentrations, a high concentration and a low concentration, consisting of 157 $\mu g/cm^2$ and 15.7 $\mu g/cm^2$, respectively, which were prepared by pipetting one ml of 1% or 0.1% solution of repellent. Acetone controls (application of 1 ml of acetone, the carrier solvent) were conducted for each concentration. Five replications were conducted. The repellency observed is considered to be "spatial" repellency, since the vapors of the treatment are responsible for the movement of the mosquitoes to positions further away from the treated filter paper. Percentage repellency was calculated by subtracting the number of insects present on the treated side from the number on the untreated side, then dividing by the total number of insect in the chamber and the multiplying by 100 to convert the result to a percentage. The number of insects on each side of the tube was compared by using a paired t-test to determine if a treatment significantly altered insect distribution. Analysis of variance (ANOVA) was calculated by hand to determine significance due to treatments.

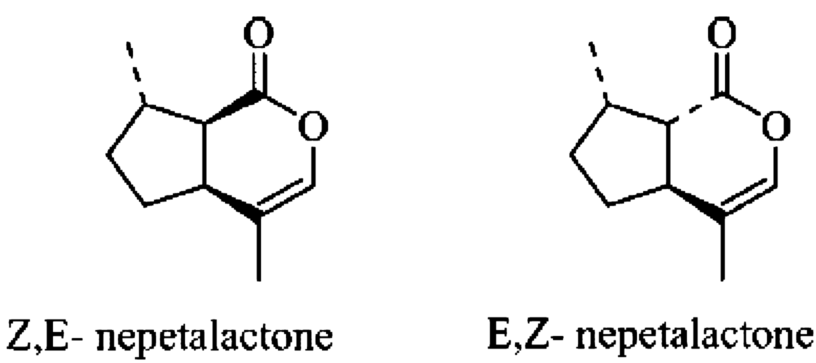

Figure 1. Z,E and E,Z racemic nepetalactone isomers in catnip.

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Results

The results with 1,572 and 786 µg/cm² DEET show that the bioassay is capable of detecting spatial repellency for *A. aegypti* (Table 1). These levels were produced from 1 ml of 10% and 5% DEET, respectively; commercial DEET products have a range of concentrations of the active ingredient, from 7% to 50% and higher. Table 1 shows that at the relatively high concentrations (1,572 and 786 µg/cm², DEET was effective as a spatial repellent.

Table 1. Spatial repellency of a high concentration and a low concentration of catnip essential oil (CNEO), the isomes of nepetalactone, and positive control treatments to *Aedes aegypti*

Treatment	*% Repellency*[a]	*SEM*	*t-value*
High conc. (157 µg/cm²)			
CNEO	58.7	9.46	5.57*
Z,E isomer	49.6	14.8	3.62*
E,Z isomer	56.2	14.0	4.02*
DEET	10.0	7.41	1.38
Control	0.22	10.5	0.0
Low conc. (15.7 µg/cm²)			
CNEO	53.3	11.9	4.12*
Z,E isomer	45.6	12.4	3.67*
E,Z isomer	38.8	5.3	7.38*
DEET	9.7	6.76	1.4
Control	17.4	11.4	1.53
Positive controls			
1,572 µg/cm² DEET	78.9	5.43	13.0*
786 µg/cm² DEET	85.3	3.94	9.90*
Control	-4.82	6.20	0.77

* Indicates that treatment was significantly different from a random distribution by paired t-test ($\alpha = 0.05$). [a] Percentage repellency = [(# of insects on untreated side - # insects on treated side)/total] x 100.

At 157 μg/cm2 the catnip essential oil (CNEO) and each isomer were all significantly (spatially) repellent, while the DEET did not show spatial repellency. At the lower concentration, from the application of 1 ml of 0.1% solution, the CNEO and the individual isomers were significantly repellent and the DEET was not. When DEET was evaluated, the distribution of the mosquitoes in the repellency chamber was not statistically different from the distribution for the "Control" treatment, in which no chemicals were applied other than the solvent.

Significant differences due to treatment were found for the high-concentration test (F = 5.20, df = 4,20), as well as the low-concentration test (F = 3.48, df = 4,20). These represent significance at the 99% and 95% levels, respectively. Significant differences in insect distribution were found by the paired t-test for CNEO, and for the *Z,E*- and *E,Z*-isomers. Significant effects on the distribution of the mosquitoes were not observed for DEET at either concentration, although is must be noted that the assay system was designed for testing spatial repellency. DEET is a very effective contact repellent; it is a more effective contact repellent than the monoterpenoids from catnip that were tested here.

Discussion

Thomas Eisner demonstrated that a drop of catnip essential oil repelled insects (*5*). Our first report on quantifying the repellency utilized a choice arena, with treated and untreated sides, with individual male German cockroaches (*6*). We first reported on the mosquito-repellent activity of the essential oil of catnip and of the two principal isomers of nepetalactone in 2001 (*9–11*). Quwenling and citronella are other monoterpenoid-based products that were marketed as mosquito repellents.

Essential oil of lemon eucalyptus was shown to be repellent, and its mostly active ingredient, p-menthane-3,8-diol, is also used as a natural, monoterpenoid insect repellent.

It was hoped that examination of the catnip essential oil and nepetalactone isomers would provide leads for development of mosquito-repellent products that may be safer and more accepted by the consumer than DEET. Other laboratories have also evaluated the oil of catnip and compared it to DEET. A triple-cage olfactometer was used to demonstrate that catnip oil was a better spatial repellent but poorer contact repellent, compared to DEET (*12*), while another study found it to be as effective as DEET in the K & D module *in vitro* assay, but not as good in bite-prevention on human subjects (*13*). Catnip essential oil was also demonstrated to be repellent to subterranean termites (*14*). Another study employed a slow-release formulation and showed excellent spatial repellency against stable flies (*15*). Two U.S. patents were issued (*16, 17*), and most recently DuPont has evaluated, patented and registered a synthetic dihydonepetalactone, which is a close analog of the natural monoterpenoid (*18*). Clearly the nepetalactone molecule has spurred new interest and efforts in development of natural, alternative repellents. A recent review of insect repellents has been published (*19*).

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The simple static-air repellency chamber assay has provided a rapid and reliable tool for initial screening of potential repellent compounds, especially volatile ones that demonstrate spatial repellency. This assay is useful for comparison of spatial repellency activity of many essential oils and individual terpenoids. It does not involve any attractant or host, so the value is limited by that aspect, but it has proven to be useful for quantifying spatial, as well as contact, repellency; comparisons of the monoterpenoids in catnip essential oil with the sesquiterpenoids in osage orange essential oil showed that the monoterpenoids were more effective spatial repellents (in particular at early time points) and that the sesquiterpenoids were more effective contact repellents, although they slowly developed some spatial repellency as well (*20*), (*21*). A study of the ratio of *Z,E* to *E,Z* isomers produced by the catnip plants in Iowa revealed that the time of season clearly affected the ratio of the isomers (*22*). Blends of the catnip monoterpenoids with sesquiterpenoids yielded a repellent that demonstrated both early spatial repellency and long-lasting contact repellency (*23*).

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Acknowledgments

We thank professors Lyric Bartholomay and Wayne Rowley of the Medical Entomology Laboratory at Iowa State University. The undergraduate laboratory assistants also assisted ably in the research: Leah Nemetz, Leah Jones, Gretchen Schultz, Sara Erickson, Mary Beth Penisten and Jaime Kutcher and the graduate student Jennifer Remmers. This is a journal paper of the Iowa Agriculture and Home Economics Experiment Station, Iowa State University, Ames, Iowa, project number 5075.

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