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Contact and Fumigant Toxicity of a Botanical-Based Feeding Deterrent of the Stable Fly, *Stomoxys calcitrans* (Diptera: Muscidae)

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ABSTRACT: The stable fly, *Stomoxys calcitrans* (L.), has been considered one of the most serious biting flies of confined and pastured livestock. The economic losses caused by the stable fly to the cattle industry in the United States exceed $2 billion annually. Current practices for managing stable flies using insecticides provide only marginal control. Insecticide resistance has also been recently reported in stable flies. The present study reports the use of plant-based insecticides, for example, essential oils, as alternatives for managing this fly pest. The toxicity of several plant essential oils and selected ingredient compounds was evaluated by contact and fumigant toxicity bioassays. Catnip oil (20 mg dosage) showed the highest toxicity against stable flies, the shortest knock-down time (∼7 min), and the quickest lethal time (∼19 min). Toxicity levels similar to catnip oil were found among three insect repellent compounds (*N*,*N*-diethyl-3-methylbenzamide, 2-methylpiperidinyl-3-cyclohexene-1-carboxamide, (1*S*,2′*S*)-2-methylpiperidinyl-3-cyclohexene-1-carboxamide). No differences in knock-down and lethal times were found among the catnip oil and its two active ingredient compounds. Similar stable fly mortality was observed using a 20 mg dose of catnip oil in a modified K&D system and a fumigant jar. When catnip oil was topically applied to stable flies, the least lethal dose was 12.5 μg/fly, and a 50 μg/fly dose resulted in 100% mortality. The blood-feeding behavior of stable flies was also negatively affected by the topical application of catnip oil, and the effect was dose-dependent. This study demonstrated that catnip oil has both contact and fumigant toxicity against the stable fly and thus has the potential as an alternative for stable fly control.

KEYWORDS: biting fly, *Nepeta cataria*, essential oil, toxicity, fumigant, topical treatment, blood feeding

INTRODUCTION

The stable fly, *Stomoxys calcitrans* (L.), is a serious biting fly that mainly feeds on bovids and equines in livestock barns and stables.[1] Recently, it has been reported that stable flies also cause significant economic losses to pastured livestock[2] (Taylor, unpublished data). Their painful attacks on grazing hosts are one of main causes leading to reproductive failure and reduction of weight gain and milk production, with estimated annual economic losses up to $2 billion for the cattle industry in the United States alone.[3−5] Furthermore, stable flies have been reported to transmit a wide variety of pathogens that are primary disease agents leading to cattle mortality.[1,6,7]

Insecticides and cultural sanitation are primary methods for stable fly control in confined and pasture settings. However, the direct application of insecticides provides only marginal control.[8,9] Furthermore, stable fly insecticide resistance has previously been detected, although it has been largely associated with organochlorines and organophosphates.[8,10] Resistance to permethrin, one of the currently used insecticides, has also been reported recently in several field populations of the stable fly in Florida.[11]

Plant derivatives have been used as botanical-based insecticides and repellents against arthropods for over two centuries.[12,13] Plant essential oils have been suggested as alternative materials for insect control because they are biodegradable and generally nontoxic or less harmful to nontarget organisms.[14,15] Zhu et al.[16,17] reported that catnip (*Nepeta cataria* L.) essential oil acts as an effective antifeedant/repellent against several filth fly species (including stable flies) and as a mosquito larvicide in laboratory assays. They also demonstrated that catnip oil is a relatively safe repellent with an extremely low toxicity in rabbits and rats. The use of botanical-based insecticides could be an important approach for reducing the impact of the stable fly on livestock without development of insecticide resistance.[18,19] Because bioactive chemicals often act at multiple and novel target sites, the potential for developing resistance is significantly reduced.[13,20−22]

The objectives of this study were (1) to assess the potential of three plant essential oils, previously reported as mosquito larvicides, for use against adult stable flies; (2) to compare the toxicity of the active ingredient compounds of catnip oil against stable flies; and (3) to evaluate the toxicological effects of fumigant activity and topical applications of the most effective catnip essential oils on stable flies.

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MATERIALS AND METHODS

Insects. Stable fly colonies have been maintained at the U.S. Department of Agriculture, Agricultural Research Service, Agroecosystem Management Research Unit (Lincoln, NE) and Knipling−Bushland U.S. Livestock Insects Research Laboratory (Kerrville, Texas) for over 3 years. The flies were maintained at 25−27 °C with variable humidity (50−60% relative humidity) and a 12 light/12 dark photoperiod. Adults were fed citrated bovine blood (3.7 g of sodium citrate/L) from a blood-soaked absorbent pad (Stayfree, McNeil-PPC Inc., Skillman, NJ) placed on top of the screened cage.

Catnip Essential Oil and Other Chemicals. Catnip essential oil, *N. cataria* L. (Lamiaceae), was purchased from Bramble Berry Inc. (Bellingham, WA). Its chemical composition included 90% *ZE*- and *EZ*-nepetalactone and 10% caryophyllene, which were determined previously by gas chromatography−mass spectrometry (GC-MS) analysis.[16] The two nepetalactones were accumulated and purified (>95%) from the purchased catnip essential oil following the methods described by Peterson.[23] Caryophyllene (>98%) was purchased from Sigma-Aldrich (St. Louis, MO). Two additional essential oils were also tested. Sandalwood oil (*Santalum album*) and amyris oil (*Amyris balsamifera* L.) were purchased from Olympia Laboratories Inc. (Scottsdale, AZ) and Sigma-Aldrich Inc. (St. Louis, MO), respectively.

Comparisons of feeding repellency were also made with other known arthropod repellents. *N*,*N*-Diethyl-3-methylbenzamide (DEET) was purchased from Morflex Inc. (Greensboro, NC) with >98% purity. 2-Methylpiperidinyl-3-cyclohexene-1-carboxamide (AI3-37220) was also purchased from Morflex, Inc., as a mixture of four diastereoisomers. Optically pure diastereoisomer (1*S*,2′*S*)-2-methylpiperidinyl-3-cyclohexene-1-carboxamide (SS-220) was purchased from Sai Dru Syn Laboratories Ltd., Hyderabad, India (95% stereoisomeric and >99% chemical purity).

Feeding Deterrency and Toxicity Bioassay. The laboratory feeding deterrency and toxic effect tests were first conducted in a device consisting of six small boxes (4 × 5 × 5 cm) aligned in a row and similar to the in vitro Klun and Debboun module (renamed as K&D boxes in the present study because it has been modified for the stable fly test) described by Klun et al.,[24] but modified for stable fly testing.[17] Newly emerged adult stable flies were supplied with 10% sugar water on the first day. The sugar water was then removed, and flies were fed bovine blood one or two times. Adults (2−3 days old) were starved for 48 h prior to each test. Twenty milligrams of each plant essential oil, synthetic constituent compounds of catnip oil, and other repellent chemicals was first weighed using a precision electric balance (Mettler-Toledo, Oakland, CA). The weighed material was dissolved in 200 μL of hexane (Burdick & Jackson High-Purity Solvent, Muskegon, MI) and then evenly applied to the outer layer of a feminine hygiene pad (4 × 5 cm). After the solvent had evaporated in approximately 2−3 min, the impregnated layer was placed on top of a blood-soaked sanitary pad in the reservoir well. Starved stable flies of mixed sexes were transferred into each of the six testing boxes (average of three to five flies in each box) and allowed to walk on and feed through the impregnated layer. During the experiments, toxic effects (including time to knock-down and lethal time) were recorded. Knock-down was defined as flies lying on the floor of the box (unable to fly and abdomen up), whereas lethal time was defined as flies not moving after being touched with a thin wooden stick. The surviving stable flies were anesthetized with CO_2, and all tested flies' feeding status was checked by squashing their abdomens to determine the presence of blood. Flies in this toxicity bioassay were exposed to randomized treatments (essential oil candidates, catnip ingredient compounds, and other tested repellent compounds) until at least six replicates were completed (new groups of flies were used for all replicated experiments).

To evaluate the lethal concentration (LC) of catnip oil against stable flies, an additional test was carried out using various dosages (0.02, 0.2, 2, 6.35, and 20 mg) described above. Hexane was used as the control. The experiment was repeated at least five times.

Topical Treatment Bioassay. A second mortality test was performed to determine the toxicity of catnip oil to stable fly adults via topical application. Four-day-old stable flies of mixed sexes were used. A solution containing approximately 100 μg of catnip oil/μL of hexane was prepared. Serial (50%) dilutions were then made to produce nine test concentrations of catnip oil: 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, and 0.39 μg/μL. Approximately 800 stable flies of mixed sexes were anesthetized briefly with CO_2, placed on a cold table, and sorted into three replicates of 20 flies per treatment dose. A repeating dispenser (Hamilton model PB600-1, Reno, NV) was used to topically apply 0.5 μL of test solution to the dorsal surface of the thorax of each fly. This resulted in treatment doses ranging from 0.19 to 50 μg/fly. Acetone (0.5 μL/fly) alone was used as the control. Each set of treated flies was placed in a Petri dish with a 9 mm circular Whatman no. 1 filter paper (Whatman Ltd., Maidstone, U.K.) on the bottom. Flies were allowed to recover at a room temperature (23−25 °C), and live and dead flies were counted at 2, 4, 6, and 24 h after treatment. Flies not moving after the application were considered to be dead.

Fumigant Jar (Vapor Phase) Toxicity Bioassay. The fumigant toxicity of catnip oil against adult stable flies was investigated using a 1 L glass jar sealed with a lid. Differing amounts of catnip oil (0.02, 0.2, 2, 6.35, and 20 mg) dissolved in 100 μL of hexane were impregnated in strips (1 × 5 cm) of Whatman no. 1 filter paper. Hexane was used as the control. Each catnip-impregnated paper strip was fixed on a metal-wire hook attached to the bottom of the lid and placed inside the glass jar. Ten adult stable flies (4−5 days old) were transferred into a 4 cm diameter × 12 cm high metal wire cage, which was then placed inside a treatment jar. The wire cage was used to prevent contact between the catnip-impregnated filter paper and the stable flies. In all cases, filter papers were allowed to evaporate for 2−3 min (to dry) before being placed in the jars. The numbers of knocked-down stable flies were recorded every 3 min for 4 h and then after 24 h. Flies lying on the bottom of the wire cage and unable to walk or fly were considered to be knocked-down. Flies showing no movement were recorded as dead. A total of six replications were performed for each bioassay.

Sublethal Effects of Catnip Oil on Blood Feeding. In toxicity bioassays conducted in the modified K&D boxes, we observed some test flies successfully engorging blood through the catnip-impregnated layer, but these flies later died. In an additional bioassay accessing the sublethal effect of catnip oil on adult blood feeding, a group of approximately 800 adult stable flies (3 days old) was placed in a cage following a blood meal. The flies were then starved for 24 h prior to the feeding bioassay. On the basis of the results from the topical toxicity bioassay, only four concentrations (0.39, 0.78, 1.56, and 3.13 μg/μL) of catnip oil were selected to evaluate the sublethal effects of catnip oil on blood feeding. To each fly was applied only half of the above four concentrations. The control group included flies that were treated with acetone only. Each treatment had five replicates of 20 flies. The topical treatment of flies was performed as previously described. To determine the prefeeding fly weight, flies were weighed in groups of 20 flies in 20 mL glass vials. Flies in each group were released into one small fly cage (3.8 × 3.8 × 3.8 cm) with screens on the top and bottom. A small piece (4 × 3 cm) of blood-soaked sanitary pad was placed on top of each fly cage, and flies were allowed to feed for 75 min. At the end of this feeding period, flies that remained at the bottom of the cage without any physical movement were recorded as inactive. The small fly cages were then placed on the cold table, and flies in each cage were transferred to the labeled glass vials for weighing. Blood ingestion was measured by the weight gain in each group of flies and was expressed as milligrams of blood per fly.

Statistical Analysis. The observed mean knock-down and survival times (minutes) of stable flies in toxicity tests (modified K&D boxes) were compared using multiway ANOVA followed by the Scheffe test

Table 1. Observed Knock-down and Lethal Times of Stable Flies Responding to Tested Repellent Candidate Materials in Laboratory Repellency Assays[a]

candidate repellent (tested at 20 mg dosage)	knock-down time, min (mean ± SE)	survival time, min (mean ± SE)	*N*
I. Plant Essential Oils			
catnip	7.33 ± 0.93 a	19.17 ± 2.93 a	28
sandalwood	73.27 ± 5.78 b	87.15 ± 5.65 b	22
amyris	62.86 ± 9.89 b	93.71 ± 7.07 b	20
II. Catnip Ingredient Compounds			
catnip	6.45 ± 0.89 a	14.47 ± 0.88 a	24
ZE-nepetalactone	6.95 ± 0.95 a	15.60 ± 1.49 a	11
EZ-nepetalactone	8.03 ± 0.46 a	17.68 ± 2.83 a	10
caryophyllene	44.91 ± 7.15 b	55.98 ± 5.32 b	20
III. Repellent Candidates			
catnip	5.63 ± 0.42 a	15.14 ± 1.80 a	32
DEET	64.39 ± 8.52 c	89.83 ± 11.21 b	16
SS-220	36.42 ± 4.72 b	154.05 ± 16.12 c	19
AI3-37220	51.11 ± 10.24 bc	127.20 ± 16.85 b	12
control	0.00 ± 0.00	0.00 ± 0.00 a	122

[a] Means followed by different letters in the same test are significantly different at $P < 0.05$. DEET, *N*,*N*-diethyl-3-methylbenzamide; SS-220, (1*S*,2′*S*)-2-methylpiperidinyl-3-cyclohexene-1-carboxamide; AI3-37220, 2-methylpiperidinyl-3-cyclohexene-1-carboxamide.

(PASW Statistics 18, SPSS Inc.). Percentage data of mortalities observed among treatments of the modified K&D boxes and fumigant jar tests were transformed using square root ($x + 1$). The significances of differences between individual means (feeding percent and mortalities) were determined by Student's *t* test. A POLO PC program was used for probit analysis of concentration− and dose−mortality data.[25,26] The toxicity was considered to be significantly different when 95% confidence limit levels of the LC values failed to overlap. The mean weight gains of the five treatment groups in the blood-feeding test were compared using the GLM procedure (SAS Institute, Cary, NC).

RESULTS

Comparisons of Knock-down and Lethal Time in Modified K&D Boxes. All three tested plant essential oils at 20 mg in the modified K&D boxes knocked stable flies down in <74 min (Table 1). Only 7 min was needed before flies were knocked down by catnip oil. The knock-down time by catnip oil was significantly shorter than those observed from amyris and sandalwood oil between 60 and 75 min. Of the major compositional compounds of catnip oil, the two nepetalactones had similar knock-down times as catnip oil. However, significantly longer time was required for caryophyllene, a minor compound, to knock down stable flies. Over 6-fold longer times were required for SS-220, AI3-37220, and DEET to knock down stable flies relative to catnip oil. In comparison to catnip oil, the lethal time of stable flies after exposure to caryophyllene, the other plant oils, and repellents was 4 times longer. The mean lethal time of stable flies exposed to catnip oil was approximately 16 min, whereas those for the remainder of chemicals were 56−154 min. No knocked-down or dead flies were observed in the control treatments.

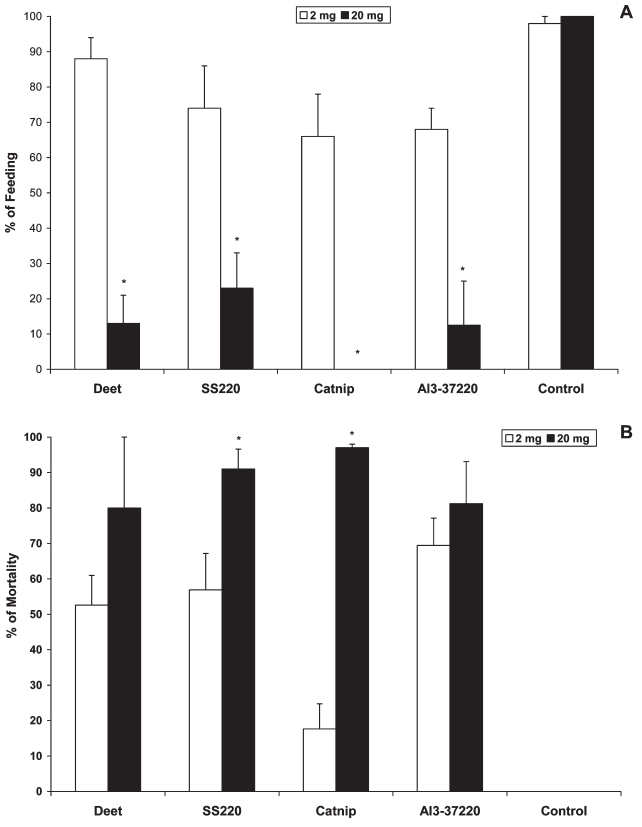

Figure 1. Feeding deterrence of catnip oil at two dosages to adult stable flies (A, $N = 30$) and percent mortality observed in the modified K&D boxes (B, $N = 28$). An asterisk on the top of a bars indicates significant difference between the two dosages tested ($P < 0.05$, Student *t* test).

Effects of Catnip Oil Dosages on Feeding and Mortality. When treated with a 2 mg dose of catnip oil or other tested repellents in the modified K&D boxes, 70−88% of the starved stable flies were found to successfully engorge blood (Figure 1A). However, significant decreases in blood feeding were observed when the dose rate of tested chemicals was increased to 20 mg, with <20% of test flies blood-fed. The order of feeding deterrence was catnip oil > AI3-37220 > DEET > SS-220. Over 95% of control stable flies were observed to engorge blood successfully. Significantly higher mortalities of adult stable flies were also observed when flies were treated with a 20 mg dosage for all four chemicals (Figure 1B). Highest mortality occurred with catnip oil at a 20 mg dosage followed by SS-220, AI3-37220, and DEET. Interestingly, significant reductions in mortality were observed from stable flies when tested with a 2 mg dosage of catnip oil and SS-220, compared to those of the 20 mg dosage. No significant decreases in mortality were detected from the 2 mg DEET and AI3-37220. All control stable flies survived.

Comparisons of Toxicity Using Two Bioassays. We also compared the mortality of stable flies when treated with five different doses of catnip oil using the modified K&D boxes and fumigant jars. No significant differences in mortality were found between the two devices, except at the two lower dosages (0.02 and 0.2 mg) after 24 h (Figure 2). However, significantly longer knock-down and lethal times were required for catnip oil at a dose of 20 mg. Average knock-down times in the fumigant jar and modified K&D boxes were 15.4 ± 2.5 and 6.4 ± 0.5 min, respectively. The lethal time of flies after knock-down in the

K&D boxes was 16.3 ± 1.4 min compared to 82.1 ± 6.1 min in the fumigant jar.

Contact and Vapor Phase Toxicity. The toxicity of catnip oil to adult stable flies was evaluated by comparing the LC_{50-99} values using the modified K&D boxes and fumigant jar bioassays. On the basis of up to 24 h of observation LC values were not significantly different between the two devices (Table 2). Topical applications of catnip oil had no significant toxic effect on adult stable flies at doses <12.5 μg/fly (Figure 3A). Higher doses (12.5, 25, and 50 μg/fly) were toxic to flies, and 100% mortality was observed at 50 μg/fly. The catnip dose−mortality responses at different times post-treatment are summarized in Table 3, and the probit line of 24 h post-treatment is shown in Figure 3B.

Effects of Catnip Oil on Feeding. The effect of catnip oil on stable fly blood feeding was determined by measuring mean weight gains of flies treated with different doses of catnip oil. The amount (mg) of blood ingested during the test period (75 min) in catnip oil-treated flies (0.78−3.13 μg/fly) was significantly lower than that of the control group (Figure 4A). There was a significant negative correlation between the mean fly weight gain (due to feeding) and the applied catnip oil doses ($Y = 5.18 - 0.77X$; $r^2 = 0.96$; $P < 0.01$). Topical treatment of stable flies with catnip oil at 3.13 μg/fly affected the stable fly behavior significantly, with >35% of flies found to be inactive at the bottom of the feeding cage. However, no significant effects on fly behavior were observed when lower doses (0.39−1.56 μg/fly) were tested ($P = 0.053$).

DISCUSSION

Historically, catnip has been used as a folk remedy to repel insects, and indeed, Eisner[27] reported it repelled at least 13 families of insects. Recently, the essential oil of catnip has been shown to be an effective alternative insect repellent against several urban pests including mosquitoes, cockroaches, and flies, in addition to those earlier reported against several agricultural pests on crops.[17,23,28−30] Catnip oil has also been reported as an effective larvicide against three mosquito species.[16]

Using the modified K&D boxes, we have shown that a 20 mg dose of catnip oil can effectively discourage stable fly blood feeding, but the deterrence dissipates at a lower dose rate (2 mg). The same trend is also found for other biting insect repellents and monoterpene-rich sandalwood and amyris oils. From trials using the modified K&D boxes we observed significant mortality (>98%) of stable flies treated with catnip oil. This mortality could be caused by either direct contact with catnip oil or exposure to

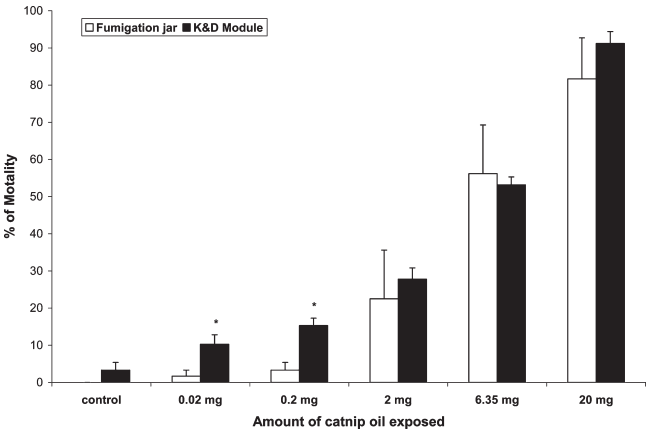

Figure 2. Percent mortality observed from stable flies exposed to 20 mg of catnip oil in the modified K&D boxes and fumigant jar ($N = 60$). An asterisk on the top of a bars indicates significant difference between the two devices tested ($P < 0.05$, Student t test).

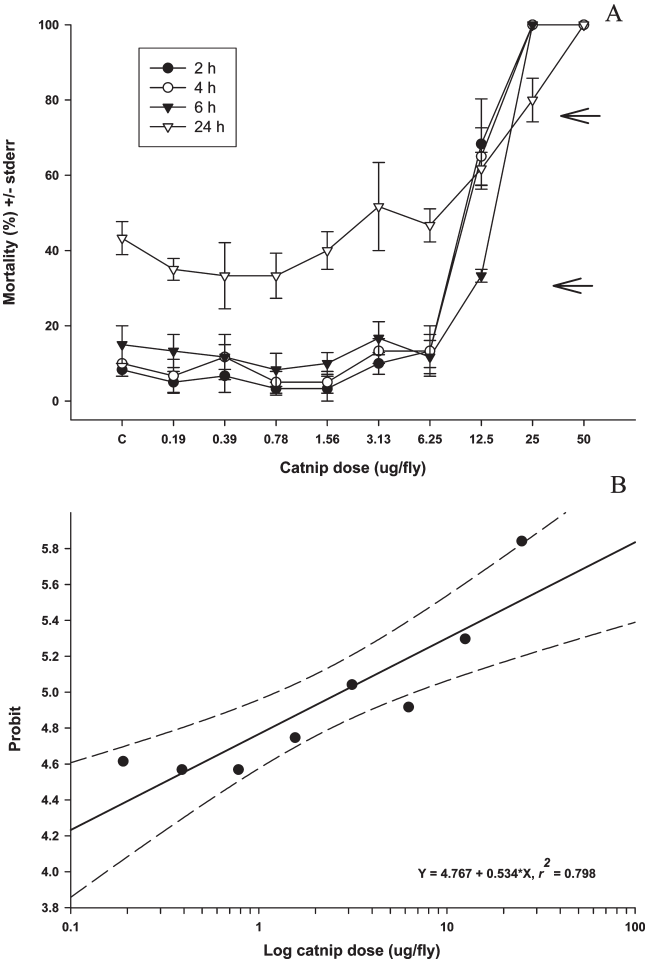

Figure 3. (A) Dose−mortality responses of catnip oil applied topically to adult stable flies. Mortalities were determined at 2, 4, 6, and 12 h post-treatment. Recovery (arrows) of flies was noted at 6 h post-treatment in flies treated with 12.5 μg of catnip/fly (32%) and at 24 h post-treatment in flies treated with 25 μg of catnip/fly (20%). (B) Probit line of dose−mortality response at 24 h post-treatment.

Table 2. Toxicity of Catnip against Stable Flies, *Stomoxys calcitrans*, Using Contact and Fumigant Toxicity Bioassays during a 24 h Exposure

	N^a	X^2	slope (±SE)	LC_{50}, mg/cm^3 (95% cl[b])	LC_{90}, mg/cm^3 (95% cl)	LC_{99}, mg/cm^3 (95% cl)
K&D module	60	14.7	0.12 ± 0.01	7.7 (6.3−9.5)	18.15 (15.2−22.6)	26.7 (22.2−33.6)
fumigant jar	60	108.7	0.09 ± 0.01	10.7 (7.2−16.6)	23.90 (17.6−40.1)	34.69 (25.2−60.2)

[a] Number of stable flies tested. [b] Confidence limit.

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Table 3. Contact Toxicity of Catnip Oil against Stable Flies, *Stomoxys calcitrans*, by Topical Applications of Various Concentrations during Periods of 2, 4, 6, and 24 h Exposures

exposure, h	N^a	X^2	slope (±SE)	LC_{50}, μg/fly[b] (95% cl[c])	LC_{90}, μg/fly (95% cl)	LC_{99}, μg/fly (95% cl)
2	600	28.1	6.7 ± 1.2	10.6 (9.1−11.9)	16.4 (14.3−21.2)	23.5 (18.9−37.0)
4	600	19.5	7.6 ± 2.2	11.3 (8.9−12.2)	16.6 (14.7−24.3)	22.8 (18.2−50.5)
6	600	16.4	n/a[d]	13.2	14.7	16.0
24	600	28.6	3.8 ± 1.0	16.4 (8.1−21.2)	35.9 (27.8−74.2)	68.0 (43.0−354)

[a] Number of stable flies tested. [b] Lethal dose. [c] Confidence limit. [d] n/a, not available.

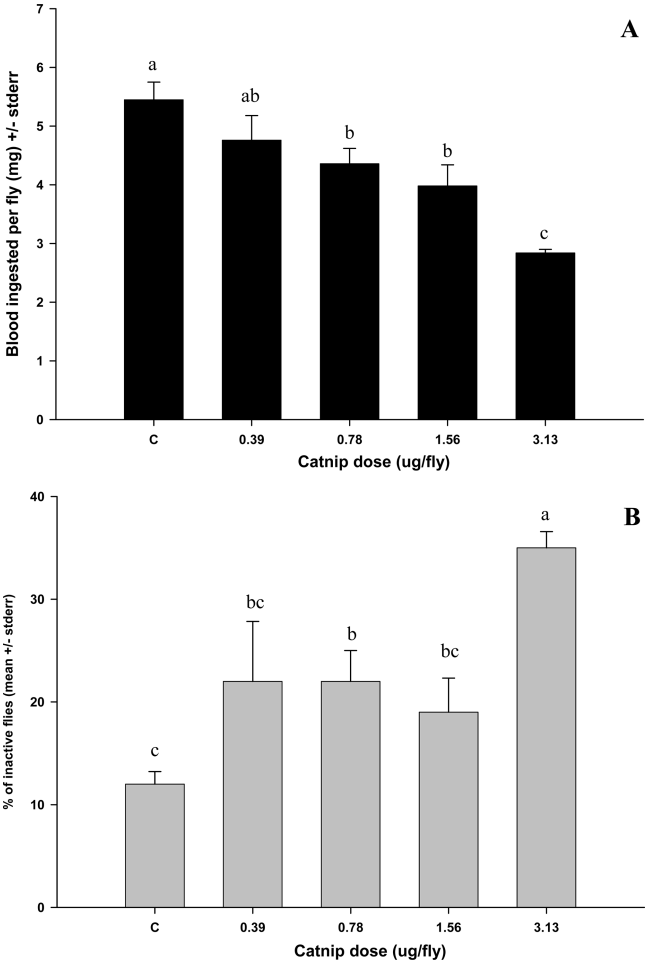

Figure 4. (A) Effect of sublethal doses of catnip oil on blood feeding of the stable flies. Blood ingested by flies in different treatment groups was determined by weight gain of flies during the feeding period. Means with different letters are statistically different (F = 11.08; df = 4, 20; $P < 0.001$). (B) Proportion of inactive flies during feeding in each of the treatment groups. Means with different letters are statistically different (F = 5.67; df = 4, 20; $P < 0.005$).

toxic vapor. The contact toxicity test revealed that the half-lethal dose of catnip oil against adult stable flies was around 11 μg. This is the first report of toxicity of catnip oil to adult flies, although previous studies have shown larvicidal activity to mosquito larvae and lower toxicity of catnip oil to small rodents.[16,17] Knock-down and mortality of stable flies treated with catnip oil are much faster than with sandalwood and amyris oils and other repellent compounds. However, at a lower dosage (2 mg), lower mortality is found from catnip oil than from other repellents including DEET and AI3-37200. This suggests that catnip oil's main constituents, such as nepetalactone, may have a different mode of action, which interferes with the fly's octopamine or GABA receptors as reported for other insects.[31,32] Further neurophysiological and biochemical experiments are needed to elucidate the mode of action for catnip oil.

It has been hypothesized that the insecticidal mode of action of essential oils may be attributable to the vapor phase of the active ingredients.[33−35] From our observations in the modified K&D boxes stable flies tend to fly away from treated surfaces but were subsequently knocked-down due to the toxic vapor. The time between fly knock-down and death was approximately 10 min. The fumigation jar test prevented direct contact between stable flies and the catnip oil, demonstrating the oil's fumigant activity. However, the lethal time required in the fumigation jar was 5 times longer than that observed in the modified K&D boxes. This can partly be explained by direct contact between flies and catnip oil on the treated surface in the K&D boxes or the relatively lower vapor concentrations presented in the fumigation jar (which is ∼10 times bigger than the K&D box in volume).

The mortality of stable flies in topical treatment experiments may also reflect both contact and fumigant toxicity of catnip oil. Catnip oil treatments at lower doses (0.19−6.25 μg/fly) caused similar mortality to the control treatment (acetone) even at 24 h post-treatment. This is not a typical dose response to an insecticide. Mortality (65−68%) was observed for the higher dose (12.5 μg/fly) at 2 and 4 h; however, mortality dropped to 33% after 6 h with the observation of a recovery of approximately 50% of the "dead" flies (see arrows in Figure 3A). Similarly, all flies topically treated with 25 μg/fly were also recorded as dead at 2, 4, and 6 h post-treatment, but only 20% of these "dead" flies had recovered by 24 h post-treatment (Figure 3A). Although the mode of action of catnip oil toxicity remains unclear, it is dose-dependent. The lower recovery at higher doses of catnip oil suggests that it may work more as a fumigant than as a contact insecticide. Therefore, it is possible that a toxic catnip oil vapor accumulated in the atmosphere sufficient to kill stable flies with an application of catnip oil-based products at a relatively higher dose.

Lack of difference in stable fly mortality at different exposure times suggests catnip oil acts as a fast knock-down agent with limited residual toxicity. This is probably due to the well-known "volatile" nature of primary active ingredient compounds from most plant-based insecticides that display low residual toxicities.[36] However, a lack of residual toxicity and environmental persistence in plant essential oil would be beneficial if rapid and permanent knock-down is obtained.[37]

The present and earlier studies using the modified K&D boxes has been proven as a reliable tool for accessing the efficiency in feeding repellency, but caution should be taken when using such a device for toxicity tests. Differences in observed mortalities

resulted from toxicity tests conducted in the modified K&D boxes and the fumigation jar, especially at the lower concentrations tested (0.02 and 0.2 mg). Whereas the current work confirms that catnip oil is toxic to adult stable flies, it is interesting to note that topical applications had a significant sublethal effect on blood feeding at doses as low as 1 μg/fly (Figure 4A). This feeding reduction may result from the reduced activity of the flies (Figure 4B) rather than from repellency.

The toxic activity of catnip oil is manifested both by vapor exposure and by contact. This is similar to the action of other plant essential oils used as insecticides.[37] The rapid knock-down effects of catnip oil against stable flies suggest that it could be used as a stand-alone treatment. However, for longer term control a combination of catnip oil with other control strategies should be considered. In addition to the previously reported repellent activity of catnip oil, this study documented its toxicity to stable flies. We believe that the further development of combining slower acting and longer term alternative management options involving plant essential oils could provide new insights to integrated stable fly management.

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