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Eupatorium capillifolium Essential Oil: Chemical Composition, Antifungal Activity, and Insecticidal Activity

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Natural plant extracts often contain compounds that are useful in pest management applications. The essential oil of *Eupatorium capillifolium* (dog-fennel) was investigated for antifungal and insecticidal activities. Essential oil obtained by hydrodistillation of aerial parts was analyzed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC/MS). The major components were determined to be thymol methyl ether (=methyl thymol) (36.3%), 2,5-dimethoxy-*p*-cymene (20.8%) and myrcene (15.7%). Antifungal activity of the essential oil was weak against the plant pathogens *Colletotrichum acutatum*, *C. fragariae*, and *C. gloeosporioides* in direct bioautography assay. The *E. capillifolium* oil showed promising repellent activity against the yellow fever mosquito *Aedes aegypti*, whereas the oil exhibited moderate activity against the mosquito's first instar larvae in a high throughput bioassay. Topical applications of the oil showed no activity against the blood-feeding female adults of *A. aegypti*. *Eupatorium capillifolium* essential oil showed a linear dose response between adult lace bug (*Stephanitis pyrioides*) mortality and increasing oil concentration in an adulticidal activity bioassay. The dog-fennel oil was more potent than the conventional insecticide malathion. In conclusion, these combined results showed *Eupatorium capillifolium* oil is a promising novel source of a biological insecticide with multiple modes of action.

Keywords: *Eupatorium capillifolium*, strawberry anthracnose, plant pathogens, bioautography, thymol methyl ether, mosquito control, *Aedes aegypti*, *Stephanitis pyrioides*.

Pyrethroids have been historically used to control a wide range of insect pests in agriculture and public health situations [1]. Public health pest control attempts to balance the amount and use of chemicals to minimize residues found in foods, in the environment, and at the same time reduce levels of pests responsible for transmission of vector-borne diseases. However, frequent use of pyrethroids or other pesticides such as diazinon, malathion, or carbaryl, can lead to the development of insecticide resistance, and potentially result in negative environmental effects and human health problems [2,3]. Therefore, there is continued need to develop natural insecticides as alternatives to synthetic pesticides for control of a variety of insects and insect-vectored diseases. In recent years, research into essential oils and their constituents as pest control agents has increased because these compounds have the desirable property of being biodegradable [3]. Additionally, it is unlikely that these natural products will leave toxic residues for long periods of time [3,4]. Continuing with our effort to find potent natural based alternatives to conventional pesticides, we extended our studies to investigate the essential oil of *Eupatorium capillifolium* (Lam.) Small, and to study its biological activity for pest management applications.

Eupatorium capillifolium (dog-fennel) is an herbaceous perennial in the family Asteraceae. *Eupatorium*

capillifolium is native to North America and found primarily in the southeastern United States [5,6]. The genus *Eupatorium* L. includes approximately 42 species distributed in eastern temperate North America, Europe, and eastern Asia [6]. Other species found in the southeastern United States include *E. hyssopifolium* L., *E. perfoliatum* L., *E. rotundifolium* L., and *E. serotinum* Michx. [7]. While some 800 species have previously been classified under *Eupatorium*, most have now been reclassified under other genera [6]. *E. capillifolium* is characterized by its fine-textured leaves, narrow leaf segments, and very distinctive odor. It is considered one of the 10 most problematic weeds in southeastern U.S. pastures and may be eaten by cattle if other forage is not available. The leaves contain low levels of the toxin tremetol, which can cause dehydration in cattle [8]. The garden cultivar 'Elegant Feather' is a selected, sterile form of *E. capillifolium* [9].

Pharmacological and phytochemical investigations of *Eupatorium* species (including many species now reclassified into other closely related genera) have been carried out by several researchers [10-18]. Sesquiterpene lactones, flavonoids, phenolic and acetylenic compounds, triterpenes and alkaloids with cytotoxic, antitumoral, antimicrobial, antioxidant and anti-inflammatory activities have been reported [10]. *Eupatorium* species are noted to have anti-inflammatory properties in Argentinean folk medicine. The antinociceptive effect of infusions of *E. laevigatum*, *E. arnottianum* and *E. subhastatum* was investigated by Clavin *et al*. and the results showed analgesic activity that supported their use in folk medicine [10]. Three South American *Eupatorium* species, *E. buniifolium*, *E. articulatum* and *E. glutinosum* were described as inhibitors of herpes viruses [11]. *Eupatorium purpureum* (gravel root and joe pye weed), *E. cannabinum* (hemp agrimony, avicenna) and *E. perfoliatum* (boneset) have a long history of use in traditional medicine and their infusions were used for treatment of fever, cold, flu, arthritic and rheumatic pains by native Americans [12]. *Eupatorium purpureum* has mostly been used as a diuretic and to treat urinary tract infections [12]. More recently, the crude ethanol extract of *E. purpureum* was further investigated for its significant anti-inflammatory activity [13]. Cistifolin and four benzofurans were isolated and subjected to bioassay guided-fractionation using *in vitro* monocyte–endothelial and monocyte–fibronectin adhesions bioassays. Only cistifolin showed strong activity and this result supports its potential use for treatment of inflammation [13]. There have been few studies published on the use of *Eupatorium* species in pest management. The potential molluscicidal activities of aqueous extracts of *E. adenophorum* were recently reported against *Oncomelania hupensis*, the intermediate host snail of *Schistosoma japonicum* [14]. The chloroform extract from the leaves of *E. quadrangulare* showed significant repellency activity against the leafcutter ant (*Atta cephalotes*) [15]. Bioassay-guided fractionation of this extract resulted in isolation of five sesquiterpene lactones and two of them (secoeudesmanolide and alloalantolactone) were determined to be the most active repellent compounds [15]. Yankanchi and Patil reported that the ethanol extract of *E. odoratum* demonstrated more impact on cotton bollworm (*Helicoverpa armigera*) than had the extracts of other plant species [16]. The essential oil of *E. buniifolium* was evaluated against *Varroa* mite (*Varroa destructor*) and showed no attractive or repellent effect on this parasitic mite of honey bees [17]. Macedo *et al*. reported that the ethanol extract of the aerial parts of *E. amphidictyum*, *E. bupleurifolium*, *E. capilare*, *E. halimifolium*, *E. kleniodes*, *E. laevigatum* and *E. squaliudum* showed no activity against *Aedes fluviatilis* fourth instar larvae [18].

Table 1: The Composition of the Essential Oil of *E. capillifolium*.

RRI	Compound	%
1032	α-Pinene	0.3
1076	Camphene	0.4
1118	β-Pinene	0.3
1151	δ-4-Carene	1.1
1174	Myrcene	15.7
1176	α-Phellandrene	6.5
1203	Limonene	0.4
1218	β-Phellandrene	0.4
1246	(*Z*)-β-Ocimene	tr
1266	(*E*)-β-Ocimene	2.4
1280	*p*-Cymene	7.7
1290	Terpinolene	0.1
1571	*trans-p*-Menth-2-en-1-ol	0.1
1591	Bornyl acetate	0.3
1604	Thymol methyl ether (=*methyl thymol*	36.3
1614	Carvacrol methyl ether (=*methyl carvacrol*)	0.5
1617	Lavandulyl acetate	0.3
1686	Lavandulol	0.1
1687	α-Humulene	0.1
1726	Germacrene D	1.2
1823	*p*-Mentha-1(7),5-dien-2-ol	0.7
1878	2,5-Dimethoxy-*p*-cymene	20.8
1993	2,6-Dimethoxy-*p*-cymene	tr
2037	Salvial-4(14)-en-1-one	0.1
2185	γ-Eudesmol	0.1
2257	β-Eudesmol	0.1
	Total	96.0

RRI Relative retention indices calculated against *n*-alkanes
Relative percentages (%) calculated from GC-Flame Ionization Detector (FID). tr Trace (< 0.1 %)

The chemical composition, antifungal and insecticidal activities of essential oil obtained by hydrodistillation of *E. capillifolium* aerial parts were investigated in this study. The yellow essential oil with a strong aromatic odor was obtained from *E. capillifolium* with a yield of 0.4% v/w, dry weight basis. This essential oil was analyzed both by GC and GC-MS. Compounds were characterized and reported along with their relative percentages listed in Table 1. Twenty-six identifiable compounds represented 96.0% of the total oil. The main

constituents were found to be thymol methyl ether (=methyl thymol) (36.3%), 2,5-dimethoxy-*p*-cymene (20.8%) and myrcene (15.7%). The chemical compositions of *E. capillifolium* from two different geographic localities were previously studied [19,20]. The oil from the aerial parts of *E. capillifolium* grown in Cuba contained cymene (23.7%), selin-11-en-4α-ol (12.3%), methyl thymol (8.9%) and β-bisabolene (8.24%) as the major constituents [19]. Another study from Mexico reported that the major constituent of the oil from *E. capillifolium* was limonene (65%) [20]. In our study, we found limonene to represent only 0.4% of the essential oil (Table 1).

In a program aimed at discovering natural fungicides as alternatives to conventional synthetic agrochemicals, *E. capillifolium* essential oil was evaluated for antifungal activity using direct bioautography assays against three *Colletotrichum* species. Anthracnose diseases of strawberry (*Fragaria* × *ananassa*) is caused by the fungal pathogens *Colletotrichum acutatum*, *C. fragariae* and *C. gloeosporioides* [21]. Evaluation of *E. capillifolium* essential oil in direct bioautography assay at 80 μg/spot against three *Colletotrichum* species demonstrated clear zones (2.9 ± 0.14 mm) of fungal growth inhibition. This was weak activity in the bioautographical screens compared with growth inhibition provided by the fungicide captan (clear zones of 17 ± 1.53 mm). Captan is a well known multi-site inhibitor fungicide with no systemic activity and is used as a protectant fungicide in commercial strawberry production to prevent anthracnose of fruits and plants caused by *Colletotrichum* species [21]. Due to less activity with the *E. capillifolium* oil, the oil was not subjected to further bioassay-guided fractionation.

Mosquitoes are important vectors of diseases of humans and livestock, as well as being nuisance pests [22]. In the search for environmentally safe and effective ways of controlling mosquitoes, *E. capillifolium* essential oil was evaluated for repellent, larvicidal and adulticidal activity against *Aedes aegypti* (L.). Personal protection is one approach to preventing mosquito bites [22]. The preliminary evaluation of feeding deterrent effects using this oil was done with three human volunteers using the "cloth patch assay" with 0.200 mg/cm^2 of the oil dissolved onto the cloth [23]. The result of this assay indicated that the oil was repellent, implicating that one or more components of the oil were producing this observed repellency against adult *A. aegypti* mosquitoes. From past studies of this nature it is known that the standard repellent DEET is normally efficacious down to levels of 0.0005-0.047 mg/cm^2; however, because oils are comprised of many individual compounds of which many may not be effective, any natural oil exhibiting repellency at < 0.375 mg/cm^2 is considered noteworthy. This promising result will lead to further study of the individual compounds within this oil. The major constituent of *E. capillifolium* oil, thymol methyl ether (TME), has also been tested for the "Minimum Effective Dosage" (MED) at which it provides repellency [23,24]. The TME was repellent down to an average concentration (± SE) of 0.258 ± 0.117 mg/cm^2. Since this average concentration for repellency was a higher concentration than that of the oil, additional components within the oil are being assayed for repellent activity since they are likely to be more potent repellents.

An effective integrated mosquito management strategy relies heavily on the control of the larval and/or adult stages. *Eupatorium capillifolium* oil and its major pure compound TME were subjected to high throughput larval bioassay and adult toxicity against *A. aegypti*. *Eupatorium capillifolium* oil was able to kill 100% and 80% of first instar larvae of *A. aegypti*, respectively, at the concentrations of 62.5 and 31.25 ppm. The TME was then evaluated in larval assay and it exhibited 80% and 60% mortality at the concentrations of 62.5 and 31.25 ppm, respectively. Based on the moderate activity found in the larval assays, the whole essential oil and TME were not considered suitable for further testing. There was no adult mortality observed for these compounds at the screening rate of 3.125 ppm per insect. The results described here for *E. capillifolium* oil and TME determined that these samples appear to have limited potential for mosquito control.

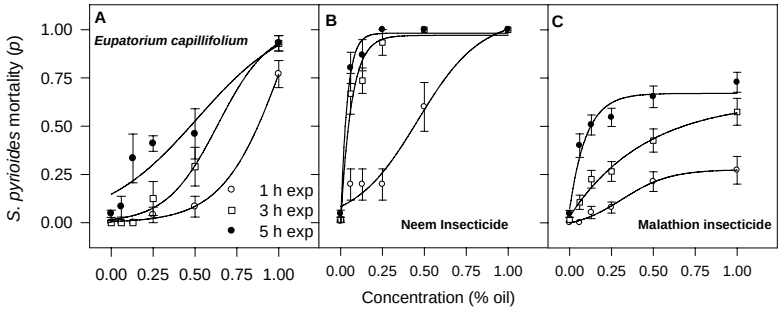
The azalea lace bug (*Stephanitis pyrioides* (Scott)), a major insect pest of azalea plants in nurseries and landscapes, was used as test subject in insecticidal bioassays. Oil of *E. capillifolium* was unusual among other essential oils tested [25]. Mortality was low initially; however by hours 3, 4 and 5, mortality increased to about 95% (Fig. 1). Many of more potent essential oil compounds such as Neem oil can inflict mortality that resembles data in Figure 1b. Although *Eupatorium* oil had a slow knockdown and ranked third in potency (Table 2, LC_{50} = 5800 ppm), it did kill greater than 95% of adult bugs at 1% concentration after 3h exposure. This was nearly as many bugs that were killed by 100% neem oil and greater than the percentage bugs killed by 75% malathion (Figure 1a – 1c). The major constituent of *E. capillifolium* oil, TME, was relatively slow to kill adult lace bugs as well.

However, no bugs survived after 5 h of exposure to 1% TME (Figure 1d). These findings indicate that TME appears to be the leading active component of *E. capillifolium* oil, but its dilution by other more benign components may have reduced its potency.

Table 2: Insecticidal bioassays for *Stephanitis pyrioides* with summary of probit analyses for *E. capillifolium* oil and two commercial insecticides (Malathion, Neem) topically applied to adult azalea lace bugs, *S. pyrioides*.

Sample	Insect toxicity[b] ranking for lace bug mortality			Average LC_{50} for adult *S.pyrioides* after 1, 3 and 5 h exposure (ppm oil)		
Treatment		n	Slope (±SEM)	(95% confidence limits)	Chi^2	*P*
Eupatorium capillifolium oil	3	10	-0.71 (0.12)	5800 (5200 – 6400)	35.82	< 0.0001
Neem insecticide[a]	1	10	1.61 (0.15)	1000 (200 – 1600)	108.93	< 0.0001
Malathion insecticide[a]	2	25	0.00 (0.00)	3300 (2400 – 4700)	---[a]	---[a]
log_econcentration	.	695	0.93 (0.06)	. . .	262.58	< 0.0001
exposure time	.	695	0.44 (0.04)	. . .	135.87	< 0.0001

[a] Malathion and Neem used as positive baseline controls for testing the relative biological activity (H_o: slope = 0) of the other materials.
[b] Insect toxicity rankings based on pairwise probit comparisons ($P < 0.05$, 1: active, 3: least active)



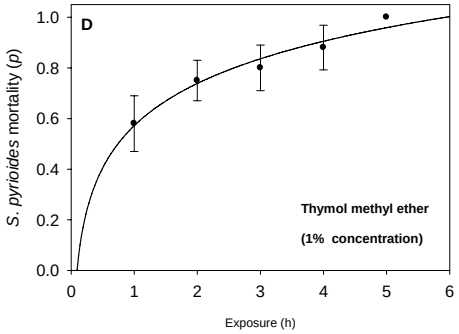


Figure 1: Figures 1a – 1c show mortality (0.00 = 0% mortality; 1.00 = 100% mortality) for adult azalea lace bugs, *Stephanitis pyrioides*, after being exposed to six concentrations [0ppm (0%), 600ppm (0.06%), 1300ppm (0.13%), 2500ppm (0.25%), 5000ppm (0.50%), 10,000 ppm (1.00%)] of oils from *E. capillifolium*, neem, and malathion for 1 h (○), 3 h (□), and 5 h (●). Figure 1d shows test results for the activity of the lead component of *E. capillifolium* oil, thymol methyl ether (TME), at a concentration of 1%.

In conclusion, no work had been conducted in search of antifungal and insecticidal activity of *E. capillifolium* oil. The principle compound, thymol methyl ether, was for the first time evaluated as mosquito repellent, larvicidal and adulticidal topical assays against *A. aegypti* and for adulticidal topical activity against azalea lace bugs, *S. pyrioides*. *E. capillifolium* essential oil could make an excellent slower acting insecticide, but perhaps its greatest strength would be for use as an insect repellent.

Experimental

General: Thymol methyl ether (>98%, Aldrich-Sigma, St., Louis, MO) and fungicide technical grade standards benomyl, cyprodinil, azoxystrobin, and captan (Chem Service, Inc. West Chester, PA) were purchased from commercial sources.

Samples: *Eupatorium capillifolium* was collected from plants growing as weeds in containerized ornamentals at the USDA-ARS, Southern Horticultural Laboratory, Poplarville, Mississippi, USA. A voucher specimen (EUCAA1055) was deposited in the repository of National Center for Natural Product Research, University of Mississippi, University, Mississippi, USA.

Isolation of the essential oils: Air dried aerial parts of *Eupatorium capillifolium* were water distilled for 3 h using a Clevenger-type apparatus to produce an essential oil at a yield of 0.4%.

GC/FID and GC/MS conditions: Chemical composition of *E. capillifolium* oil was analyzed by capillary GC and GC/MS using an Agilent GC/MSD system. The same column and analysis conditions were used for both GC and GC/MS.

The GC/MS analysis was carried out with an Agilent 5975 GC/MSD system. A Hewlet Packard-Innowax FSC column (60 m x 0.25 mm, 0.25 μm film thickness) was used with helium as carrier gas (0.8 mL/min). The GC oven temperature was kept at 60°C for 10 min and increased up to 220°C at a rate of 4°C/min, then held constant at 220°C for 10 min with a final programmed ramp up to 240°C at a rate of 1°C/min, and held a second time at 240°C for 20 min. Split ratio was adjusted at 40:1. The injector temperature was at 250°C. The mass spectrometer was operated with an electron energy of 70 eV. Mass spectra were acquired with the instrument set to scan from *m/z* 35 to 450 at a scan rate of s^{-1}. The GC analysis was carried out using an Agilent 6890N GC system. FID detector temperature was 300°C. In order to obtain the same elution order with GC/MS, simultaneous injection was done by using the same column and appropriate operational conditions.

Identification of the essential oil components was carried out by comparison of their relative retention times with those of authentic samples or by comparing their relative retention index (RRI) to series of *n*-alkanes. Computer matching identified compounds using as references (Wiley and MassFinder 3.1) [26,27], an in-house "Başer Library of Essential Oil Constituents" composed of genuine compounds and components of known oils, and MS literature data [28-30] was also used for the identification. The relative concentrations of the separated compounds based on percentage were computed from FID chromatograms.

Direct bioautography assay: A number of bioautography techniques were used as primary screening systems to detect antifungal activity [31]. Matrix, one-dimensional protocols on silica gel TLC plates along with *Colletotrichum* spp. as the test organisms were used to identify the antifungal activity according to published methods [31]. Matrix bioautography was used to screen large numbers of crude extract at 80 μg/spot. One-dimensional thin-layer chromatography (1D TLC) was subsequently used to purify and identify the number of antifungal agents in extracts. Each plate was subsequently sprayed with a spore suspension (10^5 spores/mL) of the fungus of interest and incubated in a moisture chamber for four days at 26°C with a 12 h photoperiod. Clear zones of fungal growth inhibition on the TLC plate indicated the presence of antifungal constituents in each extract. Fungal growth inhibition was evaluated 4–5 days after treatment by measuring zone diameters. Antifungal metabolites were readily located on the plates by visually observing clear zones where the active compounds inhibited fungal growth. Fungicide technical grade standards benomyl, cyprodinil, azoxystrobin, and captan were used as controls at 2 mM in 2μL of EtOH.

Mosquito repellent assay: A 10 mg sample of *Eupatorium capillifolium* oil was dissolved in 1 mL of acetone and applied to 50 cm^2 muslin cloth, resulting in a surface concentration of 0.200 mg/cm^2. The individual compound that comprised most of the oil (thymol methyl ether) was tested by application of a suitable amount to cloth to produce successive serial dilutions of 1.500, 0.750, and 0.375 mg/cm^2. Each concentration was tested to determine the point where the repellent failed for each of the volunteers in the study; this concentration was averaged and reported. The test was conducted by having each volunteer affix the treated cloth onto a plastic sleeve to cover a 32 cm^2 window previously cut into the sleeve. Each of the volunteers wore this sleeve/cloth assembly above a nylon stocking that covered the arm with the hand of each volunteer protected by a glove. [23]. The arm with the sleeve/cloth assembly was inserted into a cage where approximately 500 female *A. aegypti* mosquitoes (age 7 days) had been preselected as host-seeking using a draw box [32]. Failure of the repellent treatment is predetermined to be 1% bite through, i.e. the volunteer receives 5 bites through the cloth over the sleeve window in the 1 minute assay. All human volunteers in this study provided informed consent to participate in this study as part of a protocol (2005-636) approved by the University of Florida Human Use Institutional Review Board (IRB-01).

Mosquito larvicidal assay: All essential oils/extracts or pure compounds were diluted in dimethyl sulfoxide (DMSO) and serial dilutions were performed for each test compound (six concentration between 8 and 500 ppm). Larvae assays were performed in 24-well plates using 5 first instar-larvae in each well. Each well contained 950 μL of water, 40 μL of larvae food solution, and 10 μL of DMSO (control) or 10 μL of serially diluted test compound. Mortality data were recorded twenty-four hours post-exposure [33].

Mosquito adult topical assay: To determine the toxicity of each chemical against female *A. aegypti*, the compound was serially diluted in acetone and topically applied to individual mosquitoes. Prior to topical application, 5 to 7 day-old females were briefly anaesthetized for 30 seconds with carbon dioxide and

placed on a 4°C chill table. A droplet of 0.5 µL of chemical solution was applied to the dorsal thorax using a 700 series syringe and a PB600 repeating dispenser. A screening dose of 3.125 ppm per female was used on 25–30 females. Tests were replicated three times. If no mortality was found at this dose, further testing was not warranted. Control treatments with 0.5 µL of acetone alone gave mortality with less than 10%. After treatment, mosquitoes were kept in plastic cups and supplied with 10% sucrose solution for 24 h before mortality was recorded. Temperature and humidity were maintained at 26°C and 80% RH, respectively [34].

Adulticidal activity against azalea lace bug: Adult azalea lace bugs *Stephanitis pyrioides* were chosen as test organisms. They were easily cultured in sealed plastic containers when provided with bouquets of azalea hosts (*Rhododendron* sp.) kept in plant growth chambers (Percival Scientific, Perry, IA). Climate in the chambers was uniform: 27°C, 65% RH, and a photoperiod of 14:10 L:D. Emulsions of malathion and neem served as positive controls, which enabled comparison of *E. capillifolium* oil as a promising source of a biological insecticide. Test solutions were prepared at an initial concentration of 100 mg mL^{-1} (10%) by adding 90% DMSO as a nontoxic solvent and emulsifying agent and formed an emulsion of 10 mg mL^{-1} or 1% oil by adding 99% deionized water. One percent oil concentration was used based on commercial botanical insecticides such as Neem (Azadiractin), Ecotrol (*Rosmarinus officinalis*) and Requiem (*Chenopodium ambroisoides*). One percent oil has also proven to be a consistently effective concentration that most essential oil bioassays require to kill most if not all minute insect pests such as aphids, midges and plant bugs [25,35,36]. This appropriate baseline control or blank for these trials was a 10% aqueous solution of DMSO, a non-toxic emulsifier. According to a randomized complete block design (RCBD), 20µl of each oil emulsion and baseline solution (DMSO) were pipetted into plastic wells of a standard 96-well microtiter plate. At the bottom of each well was an absorbent disc of Whatman no. 2 filter paper, which prevented bugs from drowning in residual fluid and served as a model of a host leaf where an insecticide would be aerially applied. For each test, 75 adult *S. pyrioides* were uniformly distributed from their holding vial to 25 wells treated with oil and DMSO. Adult *S. pyrioides* observed under a dissecting microscope at the top of every hour for 5 h at 21°C, to see if any had died. Between these inspections, treated bugs were kept at 23°C in a separate growth chamber. The RCBD design and probit analyses were similar to those used in previous bioassays [25,35,36]. Probit analysis also calculated the LC_{50} and mortality rates of bugs exposed to six concentrations of the test compounds: 0%, (0 ppm) 0.06% (600 ppm), 0.13% (1,300 ppm), 0.25% (2,500 ppm), 0.50% (5,000 ppm) and 1.00 % (10,000 ppm). Thymol methyl ether (TME) was also evaluated at a concentration of 1% against adult *S. pyrioides* and killed lace bugs over a 5 h period. TME is a leading compound that constituted 36% of *E. capillifolium* oil (Table 2).

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References

[1] Carter SW. (**1989**) A review of the use of synthetic pyrethroids in public health and vector pest control. *Journal of Pest Science*, ***27***, 361-374.

[2] Kolaczinski JH, Curtis CF. (**2004**) Chronic illness as a result of low-level exposure to synthetic pyrethroid insecticides. *Food and Chemical Toxicology*, ***42***, 697–706.

[3] Isman MB. (**2000**) Plant essential oils for pest and disease management. *Crop Protection*, ***19***, 603-608.

[4] Silva WJ, Doria GAA, Maia RT, Nunes RS, Carvalho GA, Blank AF, Alves PB, Marcal RM, Cavalcanti SCH. (**2008**) Effects of essential oils on *Aedes aegypti* larvae: Alternatives to environmentally safe insecticides. *Bioresource Technology*, ***99***, 3251–3255.

[5] Schmidt GJ, Schilling EE. (**2000**) Phylogeny and biogeography of *Eupatorium* (Asteraceae: Eupatorieae) based on nuclear ITS sequence data. *American Journal of Botany*, ***87***, 716-726.

[6] King RM, Robinson H. (**1987**) The genera of Eupatorieae (Asteraceae). *Monographs in systematic botany*, *Missouri Botanical Garden*, ***22***, 1-581.

[7] Timme SL. (**2007**) Wildflowers of Mississippi. *University Press of Mississippi*, Jackson, Mississippi, 74-76.

[8] Sellers BA, Ferrell JA, MacDonald GE, Kline WN. (**2009**) Dogfennel (*Eupatorium capillifolium*) size at application affects herbicide efficacy. *Weed Technology*, ***23***, 247-250.

[9] http://www.provenwinners.com/plants/detail.cfm?photoID=7958 [May 24, 2010].

[10] Clavin ML, Gorzalczany S, Mino J, Carina Kadarian C, Martino V, Ferraro G, Acevedo C. (**2000**) Antinociceptive effect of some Argentine medicinal species of *Eupatorium*. *Phytotherapy Research*, ***14***, 275-277.

[11] Abad MJ, Bermejo P, S. Sanchez Palomino S, Chiriboga X, Carrasco L. (**1999**) Antiviral activity of some South American medicinal plants. *Phytotherapy Research*, ***13***, 142-146.

[12] Chevallier A. (**1996**) The encyclopedia of medicinal plants. DK Publishing Inc., New York, 206-207.

[13] Habtemariam S. (**2001**) Antiinflammatory activity of the antirheumatic herbal drug, gravel root (*Eupatorium purpureum*): Further biological activities and constituents. *Phytotherapy Research*, ***15***, 687-690.

[14] Zou FC, Duan G, Xie YJ, Zhou Y, Dong GD, Lin RQ, Zhu XQ. (**2009**) Molluscicidal activity of the plant *Eupatorium adenophorum* against *Oncomelania hupensis*, the intermediate host snail of *Schistosoma japonicum*. *Annals of Tropical Medicine and Parasitology*, ***103***, 549-553.

[15] Okunade AL, Wiemer DF. (**1985**) Ant-repellent sesquiterpene lactones from *Eupatorium quadrangularae*. *Phytochemistry*, ***24***, 1199-1201.

[16] Yankanchi SR, Patil SR. (**2009**) Field efficacy of plant extracts on larval populations of *Plutella xylostella* L. and *Helicoverpa armigera* Hub. and their impact on cabbage infestation. *Journal of Biopesticides*, ***2***, 32-36.

[17] Ruffinengo S, Eguara M, Floris I. Faverin C, Bailac P, Ponzi M. (**2005**) LD_{50} and repellent effects of essential oils from Argentinean wild plant species on *Varroa destructor*. *Journal of Economic Entomology*, ***98***, 651-655.

[18] Macedo ME, Consoli RAGB, Grandi TSM, Anjos AMG dos, Oliveira AB de, Mendes NM, Queiroz RO, Zani CL. (**1997**) Screening Asteraceae (Composite) plant extracts for larvicidal activity against *Aedes fluviatilis* (Diptera: Culicidae). *Memórias do Instituto Oswaldo Cruz*, ***92***, 565-570.

[19] Pino JA, Rosado A, Fuentes V. (**1998**) Essential oil of *Eupatorium capillifolium* (Lam.) Small from Cuba. *Journal of Essential Oil Research*, ***10***, 79-80.

[20] Dominguez, XA, Gomez ME, Gomez PA, Villarreal AN, Rombold C. (**1971**) Physical data on the essential oils of five Composite plants. *Planta Medica*, ***19***, 52-54.

[21] Wang X, Wedge DE, Tabanca N, Johnson RD, Cutler SJ. Pace P, Smith BJ, Zhou L. (**2008**) Development of A miniaturized 24-well strawberry leaf disk bioassay for evaluating natural fungicides. *Natural Product Communications*, ***3***, 1079-1084.

[22] Hoel D, Pridgeon, JW, Bernier, UR, Chauhan, K, Meepagala, K, Cantrell, C. (**2010**) Departments of Defense and Agriculture team up to develop new insecticides for mosquito control. *Wingbeats*, ***21***, 19–34.

[23] Katritzky AR, Wang Z, Slavov S, Tsikolia M, Dobchev D, Akhmedov NG, Hall CD, Bernier UR, Clark GG, Linthicum KJ. (**2008**) Synthesis and bioassay of novel mosquito repellents predicted from chemical structure. *Proceedings of the National Academy of Sciences*, ***105***, 7359-7364.

[24] Bernier UR, Furman KD, Kline DL, Allan SA, Barnard DR. (**2005**) Comparison of contact and spatial repellency of catnip oil and *N*,*N*-Diethyl-3-methylbenzamide (Deet) against mosquitoes**.** *Journal of Medical Entomology***,** ***42***, 306-311.

[25] Sampson BJ, Tabanca N, Kirimer N, Demirci B, Baser KHC, Khan IA, Spiers JM, Wedge DE. (**2005**) Insecticidal activity of 23 essential oils and their major compounds against adult *Aphis pseudobrassicae* (Davis) (Aphididae: Homoptera). *Pest Management Science*, ***61***, 1122-1128.

[26] Koenig WA, Joulain D, Hochmuth DH. (**2004**) *Terpenoids and related constituents of essential oils*. MassFinder 3. Ed. Hochmuth D.H. Convenient and Rapid Analysis of GCMS, incorporating, Hamburg, Germany.

[27] McLafferty FW, Stauffer DB. (**1989**) *The Wiley/NBS registry of mass spectral data*. J. Wiley and Sons, New York.

[28] Joulain D, Koenig WA. (**1998**) *The atlas of spectra data of sesquiterpene hydrocarbons*. E.B.Verlag, Hamburg.

[29] ESO 2000. (**1999**) *The complete database of essential oils*. Boelens Aroma Chemical Information Service, The Netherlands.

[30] Jennings WG, Shibamoto T. (**1980**) *Quantitative analysis of flavor and fragrance volatiles by glass capillary GC*. Academic Press, New York.

[31] Tabanca N, Wedge DE, Wang X, Demirci B, Baser KHC, Zhou L, Cutler SJ. (**2008**) Chemical composition and antifungal activity of *Angelica sinensis* essential oil against three *Colletotrichum* species. *Natural Product Communications*, ***3***, 1073-1078.

[32] Posey KH, Schreck CE. (**1981**) An airflow apparatus for selecting female mosquitoes for use in repellent and attraction studies. *Mosquito News*, ***41***, 566-568.

[33] Pridgeon, JW, Becnel JJ, Clark GG, Linthicum KJ. (**2009**) A high throughput screening method to identify potential pesticides for mosquito control. *Journal* of *Medical Entomology*, ***46***, 335-341.

[34] Pridgeon JW, Pereira RM, Becnel JJ, Allan SA, Clark GG, Linthicum KJ. (**2008**) Susceptibility of *Aedes aegypti*, *Culex quinquefasciatus* Say, and *Anopheles quadrimaculatus* Say to 19 pesticides with different modes of action. *Journal of Medical Entomology*, ***45***, 82-87.

[35] Sampson, BJ, McLaughlin JL, Wedge DE. (**2003**) Evaluating the extract of the Paw Paw tree as a botanical insecticide. *Arthropod Management Tests*, ***28***, L5.

[36] Wedge DE, Tabanca N, Sampson BJ, Werle C, Demirci B, Baser KHC, Nan P, Duan P, Liu Z. (**2009**) Antifungal and insecticidal activity from two *Juniperus* essential oils. *Natural Product Communications*, ***4***, 123-127.