ORIGINAL ARTICLE

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Rec. Nat. Prod. 10:2 (2016) 137-147

records of natural products

Chemical Composition and Biological Activity of Essential Oils from Four *Nepeta* Species and Hybrids against *Aedes aegypti* (L.) (Diptera: Culicidae)

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(Received April 02, 2014; Revised April 07, 2015; Accepted April 08, 2015)

Abstract: Essential oils of four ornamental species and hybrids of *Nepeta*: *N. racemosa* Lam. hybrid 'Select', *N. sibirica* L., *N. subsessilis* Maxim, and *N.* ×*faassenii* Bergmans ex Stearn 'Dropmore were studied for their chemical composition, larvicidal and biting deterrent activity. Water-distilled essential oils from aerial parts of *Nepeta* species were analyzed by gas chromatography (GC-FID) and gas chromatography mass spectrometry (GC-MS). *Nepeta racemosa* hybrid 'Select' and *N.* ×*faassenii* 'Dropmore' essential oils were rich in 1,8-cineole whereas *N. sibirica* and *N. subsessilis* essential oils mainly consisted of sesquiterpenes: (*Z*)-β-farnesene, β-bisabolene, δ-cadinene or β-caryophyllene, and caryophyllene oxide. Many *Nepeta* species essential oils are reported to be rich in nepetalactone isomers, but essential oils from these species contained either very low or no nepetalactone content. In biting deterrent bioassays, essential oils of these *Nepeta* species and hybrids at 100 μg/cm^2 showed activity similar to DEET at 25 nmol/cm^2 against *Aedes aegypti*, whereas this activity at the concentration of 10 μg/cm^2 was lower than DEET. All the essential oils showed weak larvicidal activity and mortalities were observed only at highest dose of 125 ppm against *Ae. aegypti*.

Keywords: *Nepeta racemosa*; *Nepeta sibirica*; *Nepeta subsessilis*; *Nepeta faassenii*; essential oil; biting deterrent; larvicide; mosquito; catmint.

1. Introduction

The genus *Nepeta* L. (Lamiaceae) contains more than 250 species endemic to temperate regions of central and southern Europe, the Near East, and central and southern Asia. [1, 2]. The best-known species of the genus, *N. cataria* L. (catnip), is known for its unusual behavioral effects on cats [3].

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The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 08/01/2015 EISSN:1307-6167

Freshly harvested flowering shoots of catnip yield nepetalactone and other aromatic compounds [4]. Plants of *N. cataria* are grown as a garden herb and for use in herbal preparations. This species is reported to be used as folk remedy for cancer, toothache, colds, anemia, headache, diarrhea, indigestion, tuberculosis, and other various ailments [5, 6]. Nepetalactone, a major compound isolated from catnip, has been reported to be effective as an insect repellent [7].
Nepeta species are commonly used in traditional medicine in Tibet, Russia, Mongolia, and other eastern and central Asian countries against infectious diseases as a mouthwash to treat laryngitis, to soothe nervous irritations, for treatment of eczema, and as an antidepressant, antiseptic, and anti-inflammatory. *Nepeta* species have reportedly been used by some cultures in veterinary practices, especially as pest repellents, in equine and pet breeding, for fur producing animals, and in deer production areas of southwestern Siberia [8]. *Nepeta meyeri* essential oil showed inhibition of germination of the seeds of *Amaranthus retroflexus*, *Chenopodium album*, *Cirsium arvense* and *Sinapsis arvensis* [9]. Approximately 20 species of *Nepeta*, along with many selected cultivars, are grown as landscape ornamentals and are favored for their profusion of flowers, long season of bloom, and attractive foliage [2].

Essential oils of some species of *Nepeta* have been reported to have insecticidal activity, but information on the activity of essential oils of *N. racemosa* hybrid ‘Select’, *N. sibirica*, *N. subsessilis* and *N. ×faassenii* ‘Dropmore’ is not available. Four ornamental species and hybrids of *Nepeta: N. racemosa* Lam. hybrid ‘Select’, *N. sibirica* L., *N. subsessilis* Maxim, and the horticultural hybrid *N. ×faassenii* Bergmans ex Stearn Dropmore were investigated in this study for chemical composition, larvicidal and biting deterrent activity of these essential oils against the yellow fever mosquito, *Ae. aegypti*.

2. Materials and Methods

2.1. Plant material

Plants of *N. racemosa* hybrid 'Select', *N. sibirica*, and *N. subsessilis* used in this study were propagated from seeds obtained from Jelitto Perennial Seeds (Louisville, KY, USA). Plants of *N. ×faassenii* 'Dropmore' were propagated by cuttings obtained from Yoder Brothers, Inc. (Lancaster, PA, USA). Plants of *N. ×faassenii*, *N. racemosa* hybrid 'Select', and *N. sibirica* were grown in a sandy loam soil and plants of *N. subsessilis* were grown in 15-gallon containers in a pine bark-based substrate under 50% shade at the South Mississippi Branch Experiment Station (SMBES) in Poplarville, MS, USA (30°50'26"N, 89°32'46"W; USDA hardiness zone 8b). Voucher specimens #36 (*N. racemosa* hybrid 'Select'), #84 (*N. sibirica*), #76 (*N. subsessilis*), and #52 (*N. ×faassenii* 'Dropmore') were deposited at the SMBES for future reference. Aerial parts were harvested from one-year-old plants of *N. racemosa* 'Select', *N. sibirica*, and *N. ×faassenii* 'Dropmore' in June 2009 and from one-year-old plants of *N. subsessilis* in June 2010 and air-dried for three weeks inside an air-conditioned building (25°C max.). Dried plant material was packed loosely into cardboard boxes to avoid crushing and stored in the same building until shipment to the National Center for Natural Products Research, The University of Mississippi, University, MS, USA in September 2010.

2.2. Essential oils

Aerial parts of *N. racemosa* hybrid ‘Select’, *N. sibirica*, *N. ×faassenii* ‘Dropmore’ and *N. subsessilis* were separately subjected to hydrodistillation for 3 h using a Clevenger-type apparatus to produce the oils. Light yellow oils were obtained from the *Nepeta* essential oils with 0.07; 0.08; 0.08 and 0.04% (v/w) yields, respectively.

2.3. GC and GC-MS Analysis for Essential Oils

Nepeta essential oils were analyzed by gas chromatography (GC) with a flame ionization detector (FID) and gas chromatography–mass spectrometry (GC-MS) using an Agilent GC-mass selective detector (MSD) system. The GC-MS analyses were done with an Agilent 5975 GC-MSD

system. An Innowax fused silica capillary (FSC) column (60 m × 0.25 mm, 0.25 μm film thickness) was used with helium as the carrier gas (0.8 mL/min). Oven temperature was kept at 60ºC for 10 min, then programmed to 220ºC at a rate of 4ºC/min, then maintained constant at 220ºC for 10 min, and finally programmed to 240ºC at a rate of 1ºC/min. Injector temperature was set at 250ºC. Split flow was adjusted at 50:1. Mass spectra were recorded at 70 eV with the mass range *m/z* 35 to 450.

GC analyses were performed using an Agilent 6890N GC system. FID detector temperature was set to 300 ºC and the same operational conditions were applied to a duplicate of the same column used in GC-MS analyses. Simultaneous auto injection was done to obtain equivalent retention times. Relative percentages of the separated compounds were calculated from integration of the peak areas in the GC-FID chromatograms (Table 1).

Individual components were identified by computer matching with commercial mass spectral libraries (Wiley GC/MS Library, MassFinder 3 Library) and in-house "Baser Library of Essential Oil Constituents", which includes over 3200 authentic compounds with Mass Spectra and retention data from pure standard compounds and components of known oils as well as MS literature data, were also used for the identification [10- 13]. These identifications were accomplished by comparison of retention times with authentic samples or by comparison of their relative retention index (RRI) to a series of *n*-alkanes [14].

2.4. Mosquitoes

Aedes aegypti larvae and adults used in these studies were from a laboratory colony maintained at the Mosquito and Fly Research Unit at the Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, Gainesville, Florida. For biting deterrence bioassays, pupae were maintained in the laboratory at 27 ± 2°C and 60 ± 10% RH, and 8-18-d-old adult females were used. For larval bioassays, the eggs were hatched and the larvae were maintained at a temperature of 27 ± 2˚C and 60 ± 10 % RH with a photoperiod regimen of 12:12 h (L: D).

2.5. Mosquito Biting Bioassays

Experiments were conducted by using a six-celled *in vitro* Klun and Debboun (K&D) module bioassay system developed by Klun *et al.* [15] for quantitative evaluation of biting deterrent properties of candidate compounds. Briefly the assay system consists of a six-well reservoir with each of the 3 × 4 cm wells containing 6 mL of blood. As described by Ali *et al.* [16], a feeding solution consisting of CPDA-1 and ATP was used instead of blood. Green fluorescent tracer dye (www.blacklightworld.com) was used to determine feeding by the females. Essential oils from aerial parts of *N. racemosa* hybrid 'Select', *N. sibirica, N. subsessilis* and *N.* ×*faassenii* 'Dropmore' were tested in this study. Samples were applied at concentrations of 10 and 100 μg/cm^2, pure compounds were tested at 25 nmol/cm^2, and DEET (97%, *N, N*-diethyl-*meta*-toluamide) (Sigma Aldrich, St. Louis, MO) at 25 nmol/cm^2 was used as positive control. All treatments were freshly prepared in molecular biology grade 100% ethanol (Fisher Scientific Chemical Co. Fairlawn, NJ) at the time of bioassay.

Temperature of the solution in the reservoirs was maintained at 37°C by continuously passing warm water through the reservoir using a circulatory bath. Reservoirs were covered with a layer of collagen membrane (Devro, Sandy Run, SC). Test compounds were randomly applied to six 4 × 5 cm areas of organdy cloth and positioned over the membrane-covered CPDA-1+ATP solution with a Teflon separator placed between the treated cloth and the six-celled module to prevent the contamination of the module. A six-celled K&D module containing five female mosquitoes per cell was positioned over the cloth treatments covering the six CPDA-1 + ATP solution membrane wells, and trap doors were opened to expose the treatments to these females. The number of mosquitoes biting through cloth treatments in each cell was recorded after a 3-min exposure and mosquitoes were prodded back into the cells to check the actual feeding. Mosquitoes were squashed and the presence or absence of green fluorescent tracer dye in the gut was used as an indicator of feeding. A replicate consisted of six treatments: four test materials, DEET (a standard biting deterrent) and ethanol-treated

organdy as solvent control applied randomly. Two sets of 5 replications each with 5 females per treatment were conducted on 2 different days using a newly treated organdy and a new batch of females in each replication. Treatments were replicated 10 times.

2.6. Larval Bioassays

Bioassays were conducted to test essential oils of *N. racemosa* hybrid 'Select', *N. sibirica*, *N. subsessilis*, and *N. ×faassenii* 'Dropmore' for their larvicidal activity against *Ae. aegypti* by using the bioassay system described by Pridgeon *et al.* [17]. Five 1-d-old *Ae. aegypti* larvae were added in a droplet of water to each well of 24-well plates (BD Labware, Franklin Lakes, NJ) by use of a disposable 22.5 cm Pasteur pipette. Fifty microliters of larval diet (2% slurry of 3:2 beef liver powder (Now Foods, Bloomingdale, Illinois) and Brewer's yeast (Lewis Laboratories Ltd., Westport, CT) was added to each well by using a Finnpipette stepper (Thermo Fisher, Vantaa, Finland). All chemicals tested were diluted in dimethyl sulfoxide (DMSO). Eleven microliters of the test chemical was added to the labeled wells, while 11 µL of DMSO was added to control treatments. After the treatment application, the plates were swirled in clockwise and counterclockwise motions and front and back and side to side five times to ensure even mixing of the chemicals. Larval mortality was recorded 24-h post treatment. Larvae that showed no movement in the well after manual disturbance of the water were recorded as dead. Three dosages, 125, 62.5 and 31.25 ppm, were used in the screening bioassay to determine the larvicidal activity and each treatment was replicated twice.

2.7. Statistical Analyses

Proportion not biting (PNB) was calculated using the procedure described by Ali *et al.* [16]. As the K&D module bioassay system can handle only 4 treatments along with negative and positive controls, in order to make direct comparisons among more than four test compounds and to compensate for variation in overall response among replicates, biting deterrent activity was quantified as Biting Deterrence Index (BDI) [15]. The BDI's were calculated using the following formula:

$$\left[BDI_{i,j,k}\right] = \left[\frac{PNB_{i,j,k} - PNB_{c,j,k}}{PNB_{d,j,k} - PNB_{c,j,k}}\right]$$

where $PNB_{i,j,k}$ denotes the proportion of females not biting when exposed to test compound *i* for replication *j* and day *k* (*i*=1-4, *j*=1-5, *k*=1-2), $PNB_{c,j,k}$ denotes the proportion of females not biting the solvent control "*c*" for replication *j* and day *k* (*j*=1-5, *k*=1-2) and $PNB_{d,j,k}$ denotes the proportion of females not biting in response to DEET "*d"*(positive control) for replication *j* and day *k* (*j*=1-5, *k*=1-2). This formula makes an adjustment for inter-day variation in response and incorporates information from the solvent control as well as the positive control.

A BDI value of 0 indicates an effect similar to ethanol, while a value significantly greater than 0 indicates biting deterrent effect relative to ethanol. BDI values not significantly different from 1 are statistically similar to DEET. BDI values were analyzed using the ANOVA procedure [single factor: test compound (fixed)] of SAS (version 9.2; SAS Institute, Cary, NC), and means were separated using the Ryan-Einot-Gabriel-Welsch Multiple Range Test. To determine whether confidence intervals include the values of 0 or 1 for treatments, Scheffe's multiple comparison procedure with the CLM option was used. Biting deterrent activity was compared among treatments based on non-overlapping 95% CI [18].

Table 1. Composition of the essential oils of four *Nepeta* species and hybrids.

RRI	Compound	*Nepeta*				Identification
		***racemosa* hybrid 'Select' (%)[a]**	***sibirica* (%)[a]**	**×*faassenii* 'Dropmore' (%)[a]**	***subsessilis* (%)[a]**	
1032	α-Pinene	0.4	-	0.1	5.5	RRI, MS
1035	α-Thujene	-	-	-	0.6	RRI, MS
1051	2,5-Dimethyltetrahydrofuran	-	-	0.2	-	MS
1076	Camphene	-	-	-	-	RRI, MS
1118	β-Pinene	1.1	-	1.6	1.1	RRI, MS
1132	Sabinene	-	-	-	0.7	RRI, MS
1174	Myrcene	0.1	-	-	0.2	RRIMS
1188	α-Terpinene	-	-	-	0.2	RRI, MS
1203	Limonene	0.2	-	0.5	0.8	RRI, MS
1213	1,8-Cineole	51.2	0.2	42.1	2.1	RRI, MS
1225	3-Methylcyclopentanone	0.5	-	-	-	MS
1225	(*Z*)-3-Hexenal	-	-	-	0.3	MS
1246	(*Z*)-β-Ocimene	0.4	-	-	0.4	MS
1255	γ-Terpinene	-	-	-	0.8	RRI, MS
1265	3-Octanone	0.9	-	1.1	3.8	RRI, MS
1266	(*E*)-β-Ocimene	-	-	-	0.2	MS
1280	*p*-Cymene	0.7	-	0.5	3.5	RRI, MS
1290	Terpinolene	-	-	-	0.2	RRI, MS
1337	3-Methyl cyclohexanone	0.2	-	-	-	MS
1386	Octenyl acetate	-	-	-	0.1	MS
1393	3-Octanol	0.2	0.5	0.9	1.3	RRI, MS
1391	(*Z*)-3-Hexenol	0.3	-	-	0.3	MS
1435	1-Cyclohexenyl methylketone	2.4	-	0.9	0.1	MS
1450	*trans*-Linalool oxide (*Furanoid*)	0.3	-	0.2	0.2	MS
1452	1-Octen-3-ol	1.3	0.2	0.4	2.4	RRI, MS
1452	α,*p*-Dimethylstyrene	-	-	-	0.2	MS
1466	α-Cubebene	-	0.1	-	0.1	RRI, MS
1466	4,4-Dimethyl-2-cyclohexen-1-one	0.7	-	0.3	-	MS
1478	*cis*-Linalool oxide (*Furanoid*)	-	-	-	0.2	MS
1479	Furfural	-	-	-	0.2	MS
1493	α-Ylangene	-	-	-	<0.1	MS
1494	(Z)-3-hexenyl isovalerate	-	-	-	0.2	MS
1497	α-Copaene	0.9	0.2	0.3	0.1	RRI, MS
1503	Unknown I	1.4	-	1.0	-	
1505	Dihydroedulane II	-	1.1	-	-	MS
1528	α-Bourbonene	-	-	0.2	-	MS
1535	β-Bourbonene	0.7	1.1	2.6	0.8	MS
1541	Benzaldehyde	-	-	0.2	0.7	RRI, MS
1553	Linalool	1.7	1.0	2.4	2.6	RRI, MS
1586	Pinocarvone	-	-	0.5	-	RRI, MS
1589	β-Ylangene	-	0.2	0.6	0.4	MS
1597	β-Copaene	0.2	-	-	-	MS
1611	Terpinen-4-ol	0.6	-	0.6	2.8	RRI, MS
1612	β-Caryophyllene	0.2	3.8	0.3	6.2	RRI, MS
1638	β-Cyclocitral	-	-	-	0.7	MS
1648	Myrtenal	-	-	0.5	0.4	MS

1662	Pulegone	1.2	-	1.2	-	RRI, MS
1668	(*Z*)-β-Farnesene	0.3	9.4	-	-	MS
1670	*trans*-Pinocarveol	0.3	-	1.0	-	RRI, MS
1678	Unknown II	3.0	1.5	2.9	1.6	
1682	δ-Terpineol	1.5	-	-	-	MS
1687	α-Humulene	-	1.5	-	3.3	RRI, MS
1704	γ-Muurolene	1.6	2.7	1.1	2.2	MS
1706	α-Terpineol	1.5	0.5	-	0.9	RRI, MS
1726	α-Zingiberene	-	3.6	-	-	MS
1726	Germacrene D	0.3	2.1	0.9	0.7	RRI, MS
1740	α-Muurolene	1.0	-	0.5	1.4	MS
1741	β-Bisabolene	-	6.8	-	-	RRI, MS
1744	α-Selinene	-	-	-	0.2	MS
1751	Carvone	-	-	0.4	-	RRI, MS
1758	(*E*,*E*)-α-Farnesene	-	2.0		0.2	MS
1773	δ-Cadinene	1.6	5.5	0.7	3.4	MS
1776	γ-Cadinene	0.5	1.3	0.3	1.0	MS
1783	β-Sesquiphellandrene	0.2	2.0	-	-	MS
1786	*ar*-Curcumene	0.5	1.3	0.3	-	MS
1798	Methyl salicylate	0.2	-	-	0.6	RRI, MS
1804	Myrtenol	-	-	0.4	-	RRI, MS
1807	Perilla aldehyde	-	-	-	0.2	RRI, MS
1807	α-Cadinene	-	0.3	-	0.5	MS
1838	(*E*)-β-Damascenone	-	0.4	0.6	0.1	MS
1845	*trans*-Carveol	-	-	0.2	0.4	RRI, MS
1849	Calamenene	0.8	0.4	-	0.6	MS
1857	Geraniol	-	-	0.2	0.3	RRI, MS
1864	*p*-Cymen-8-ol	-	-	0.2	0.7	RRI, MS
1866	Methyl hydrocinnamate	0.6	-	-	-	MS
1868	(*E*)-Geranyl acetone	-	0.1	0.1	0.1	MS
1904	Ethyl-3-phenyl propionate	0.2	-	-	-	MS
1941	α-Calacorene	0.3	0.4	-	-	MS
1945	1,5-Epoxy-salvial(4)14-ene	-	-	0.6	-	MS
1958	(*E*)-β-Ionone	0.5	0.5	0.4	-	MS
1996	Unknown III	-	1.1	-	1.7	
2008	Caryophyllene oxide	0.7	4.4	3.4	5.0	RRI, MS
2016	4aα, 7α, 7aα-Nepetalactone	-	2.6	-	1.1	MS
2037	Salvial-4(14)-en-1-one	-	-	1.9	-	MS
2046	Norbourbonone	-	-	0.7	-	MS
2069	4aα, 7α, 7aβ-Nepetalactone	-	1.7	-	2.8	MS
2071	Humulene epoxide-II	-	1.3	0.4	2.0	MS
2080	Cubenol	-	0.6	-	-	MS
2080	1,10-*diepi*-Cubenol	-	0.5	-	-	MS
2088	4aβ, 7α, 7aβ-nepetalactone	0.6	-	-	-	MS
2112	Unknown IV	4.2	-	-	-	
2113	Cumin alcohol	-	-	0.3	-	RRI, MS
2130	Salviadienol	-	-	0.4	-	MS
2131	Hexahydrofarnesyl acetone	0.5	1.3	0.4	0.7	MS
2144	Spathulenol	2.6	0.9	0.5	-	MS
2150	Unknown V	-	-	1.4	-	
2179	3,4-Dimethyl-5-pentylidene-2(5H)-furanone	-	0.8	0.3	-	MS
2179	Nor-Copaonone	-	-	0.6	-	MS

2080	Junenol (=Eudesm-4(15)-en-6-ol)	-	-	0.4	-	MS
2186	Eugenol	1.0	3.5	2.1	3.6	RRI, MS
2187	T-Cadinol	-	1.5	-	0.8	RRI, MS
2198	Thymol	1.5	3.7	0.3	0.7	RRI, MS
2200	3,4-dimetil-5-pentyl-5H-furan-2-one	-	0.7	0.3	-	MS
2209	T-Muurolol	-	1.0	-	0.1	MS
2218	4-Vinyl guaiacol	-	1.3	-	-	MS
2232	α-Bisabolol	-	-	-	1.2	RRI, MS
2239	Carvacrol	0.2	0.1	0.4	-	RRI, MS
2255	α-Cadinol	-	2.2	-	1.6	RRI, MS
2278	Torilenol	-	-	0.4	-	MS
2279	Unknown VI	-	-	1.8	-	
2289	4-Oxo-α-Ylangene	-	-	0.3	-	MS
2324	Caryophylla-2(12),6(13)-dien-5α-ol (=*Caryophylladienol II*)	-	-	0.4	-	MS
2369	Eudesma-4(15),7-dien-4β-ol	-	1.3	-	-	MS
2389	Caryophylla-2(12),6-dien-5α-ol (=*Caryophyllenol I*)	-	-	0.5	-	MS
2392	Caryophylla-2(12),6-dien-5β-ol (=*Caryophyllenol II*)	-	-	0.6	-	MS
2456	Unknown VII	-	3.8	-	1.2	
2500	Pentacosane	-	-	0.3	-	RRI, MS
2607	14-Hydroxy-δ-cadinene	-	-	0.2	-	MS
2622	Phytol	-	1.5	0.1	1.9	MS
2628	Unknown VIII	-	2.6	-	1.2	
2700	Heptacosane	-	-	0.4	-	RRI, MS
2900	Nonacosane	-	-	0.6	-	RRI, MS
2931	Hexadecanoic acid	-	2.0	-	1.2	RRI, MS
	Total identified	**85.4**	**82.1**	**82.3**	**79.9**	
	Total	**94.0**	**91.1**	**89.4**	**85.6**	

RRI Relative retention indices calculated against *n*-alkanes

[a] % calculated from FID data

Identification method: RRI, identification based on the relative retention indices of authentic compounds on the HP Innowax column; MS, identified on the basis of computer matching of the mass spectra with those of the Wiley and MassFinder libraries and comparison with literature data

Unknown I: EIMS, 70 eV, *m/z* (rel. int.): 112(7.0), 97(59.2), 83(20.1), 69(100), 55(47.8), 43(68.9).
Unknown II: EIMS, 70 eV, *m/z* (rel. int.): 204(0.1), 174(0.6), 159(1.9), 138(59.2), 123(100), 109(18.1), 95(37.3), 81(47.9), 67(34.5), 55(17.7), 41(26.9).
Unknown III: EIMS, 70 eV, *m/z* (rel. int.): 192(3.5), 174(6.2), 159(100), 147(19.0), 119(49.5), 105(49.3), 91(50.1), 79(22.4), 43(43.3).
Unknown IV: EIMS, 70 eV, *m/z* (rel. int.): 141(1.6), 127(7.5), 101(62.6), 96(30.7), 87(100), 81(13.9), 69(15.9), 55(18.5).
Unknown VI: EIMS, 70 eV, $C_{15}H_{26}O$, *m/z* (rel. int.): 222$[M]^+$(21.1), 165(23.4), 151(100), 133(42.7), 123(35.7), 111(44.4), 95(65.7), 81(64.0), 67(21.3), 55(22.0).
Unknown VII: EIMS, 70 eV, $C_{20}H_{30}$, *m/z* (rel. int.): 270$[M]^+$(52.7), 255(100), 227(10.1), 185(4.4), 148(14.2), 133(17.8), 119(23.7), 105(17.1), 91(19).
Unknown VIII: EIMS, 70 eV, *m/z* (rel. int.): 268$[M]^+$(10.0), 253(100), 238(1.5), 199(12.7), 159(4.8), 141(4.3), 129(5.1), 117(4.9), 91(4.5).

3. Results and Discussion

The water-distilled essential oils from aerial parts of the four *Nepeta* species and hybrids were characterized by GC-FID and GC-MS. The compounds identified from the essential oils along with their relative percentages are listed in Table 1. A total of 47, 48, 64, and 67 compounds were identified from the essential oils of *N. racemosa* hybrid 'Select', *N. sibirica*, *N. ×faassenii* 'Dropmore' and *N. subsessilis*, respectively, which represented 85.4, 82.1, 82.3, and 79.9% of the oils. Using commercial library (Wiley GC/MS Library, MassFinder 3 Library) and in-house "Baser Library of Essential Oil Constituents" database contained no information on unknowns with RRIs 1503, 1678, 1996, 2112 and 2150, therefore we could not identify these compounds. Mass spectrum of unknown compounds is given as dip note in Table 1. 1,8-Cineole was the major compound in *N. racemosa* hybrid 'Select' (51.2%) and *N. ×faassenii* 'Dropmore' (42.1%) oils. *Nepeta sibirica* essential oil mainly consisted of sesquiterpene hydrocarbons such as (*Z*)-β-farnesene (9.4%), β-bisabolene (6.8%) and δ-cadinene (5.5%). *Nepeta subsessilis* essential oil contained β-caryophyllene (6.2%), α-pinene (5.5%) and caryophyllene oxide (5.0%). Although previous reports indicate that *Nepeta racemosa* essential oil consists mainly of nepetalactones and 4aα, 7α, 7aα-nepetalactone [19-21, 1, 22], the essential oil samples tested in this study were rich in 1,8-cineole (51%) and only one of the nepetalactone isomers (4aβ, 7α, 7aβ-nepetalactoone) was found in minor amount (0.6%). This variation could be attributed to *N. racemosa* hybrid 'Select' being of hybrid origin and not the pure species. However, Daryasari *et al.* [23] studied *N. racemosa* essential oils from samples collected from a wild source in western Iran, extracted through microwave-assisted hydrodistillation technique, and found 1,8-cineole (37%) and nepetalactone (2.3%) as major compounds. Letchamo *et al.* [8] reported a high percentage of neoepinepetalactone (78.8%) in samples of *N. sibirica* essential oil collected from its natural habitat in Siberian Altai and Tsuruoka *et al*. [24] found 4aα, 7α, 7aα-nepetalactone as a principal compound of *N. sibirica* essential oil collected from Mongolia. However, the samples investigated in the present study were rich in sesquiterpene hydrocarbons and percentages of 4aα, 7α, 7aα-nepetalactone (2.6%) and 4aα, 7α, 7aβ-nepetalactone (1.7%) were low. De Pooter *et al.* [25] studied the essential oils of *N. ×faassenii* and *N. sibirica*. Their *N. ×faassenii* sample was a commercial botanical garden and *N. sibirica* was obtained from the botanical garden of Munich and grown under greenhouse conditions. Both essential oils were mainly rich in nepetalactone isomers. *Nepeta subsessilis* essential oil is reported here for the first time which contained 4aα, 7α, 7aα-nepetalactone (1.1%) and 4aα, 7α, 7aβ-nepetalactone (2.8%) in small percentages. In this study, *N. subsessilis* essential oil was obtained from the plant material of horticultural origin rather than native sources; this species need to be collected from natural sources for the comparison of essential oil compositions. There is no study published on natural *N. subsessilis* essential oil. It seems that more work on *N. subsessilis*. Baser *et al.* [26] divided the chemical composition of *Nepeta* essential oils into two main groups: nepetalactone-containing and nepetalactone-less. Most *Nepeta* species contain nepetalactones as the main constituents, whereas monoterpenes and sesquiterpenes are present in smaller quantities in the essential oils. Results from this study indicated a very low percentage or complete absence of nepetalactone in samples of *Nepeta* essential oils tested. It can be concluded from these data that *N. racemosa* hybrid 'Select', *N. ×faassenii* and *N. sibirica* feature a mixed parentage and their essential oil composition support this conclusion. Previous research suggests the range of terpenoids present in various samples of essential oils from *Nepeta* species depends upon the cultivated or natural sources. *Nepeta* species are distinguished by the production of nepetalactones which are repellent or beneficial for insects. Other than nepetalactones, *Nepeta* essential oils contain other natural compounds such as monoterpenes (1,8-cineole, linalool) or sesquiterpenes (caryophyllene oxide, germacrene D, spathulenol) [26]. The current knowledge of composition of essential oils make it indicates that there is strong need to study the essential oils of various *Nepeta* cultivars from different sources to determine the effects of sample source and chemotypes on the composition of the essential oils.

The *in vitro* K&D system used in this study specifically quantified the mosquito biting deterrent properties of *N. racemosa* hybrid 'Select', *N. sibirica*, *N. ×faassenii* 'Dropmore' and *N. subsessilis*

essential oils. In biting deterrent bioassays, essential oil of *Nepeta* species at 10 µg/cm^2 showed activity significantly lower than DEET at 25 nmol/cm^2 (Figure 1). Biting deterrent activity significantly increased with increase in test concentrations from 10 to 100 µg/cm^2. Based on 95% CI, all four essential oils at 100 µg/cm^2 showed activity similar to DEET. Essential oils of some species of *Nepeta* especially *N. cataria* (catnip) are well studied for insecticidal activities [27-28]. It has been reported that topical application of catnip essential oil can effectively prevent biting by various mosquito species, and there is an evidence of spatial repellency [28- 30]. Major contents of catnip oil, 4aα, 7α, 7aα-nepetalactone and 4aα, 7α, 7aβ-nepetalactone, were either present in small percentage or totally absent in these current essential oils we studied. Chauhan *et al*. [27] showed high activity of nepetalactones from catnip oil against *Ae. aegypti* in *in vitro* K & D bioassays. However, efficacy of these compounds in *in vivo* bioassay was significantly lower than DEET. Caryophyllenes (β-caryophyllene and caryophyllene oxides) are the other group of compounds which are present in catnip oil as well as in these four essential oils. β-Caryophyllene has shown moderate activity [31] and the increase in activity at 100 µg/cm^2 may be because of increase in levels of the compounds present in smaller quantity to the level at which activity matches that of DEET. This may very well be the result of combinations of other sesquiterpenes present in these oils.

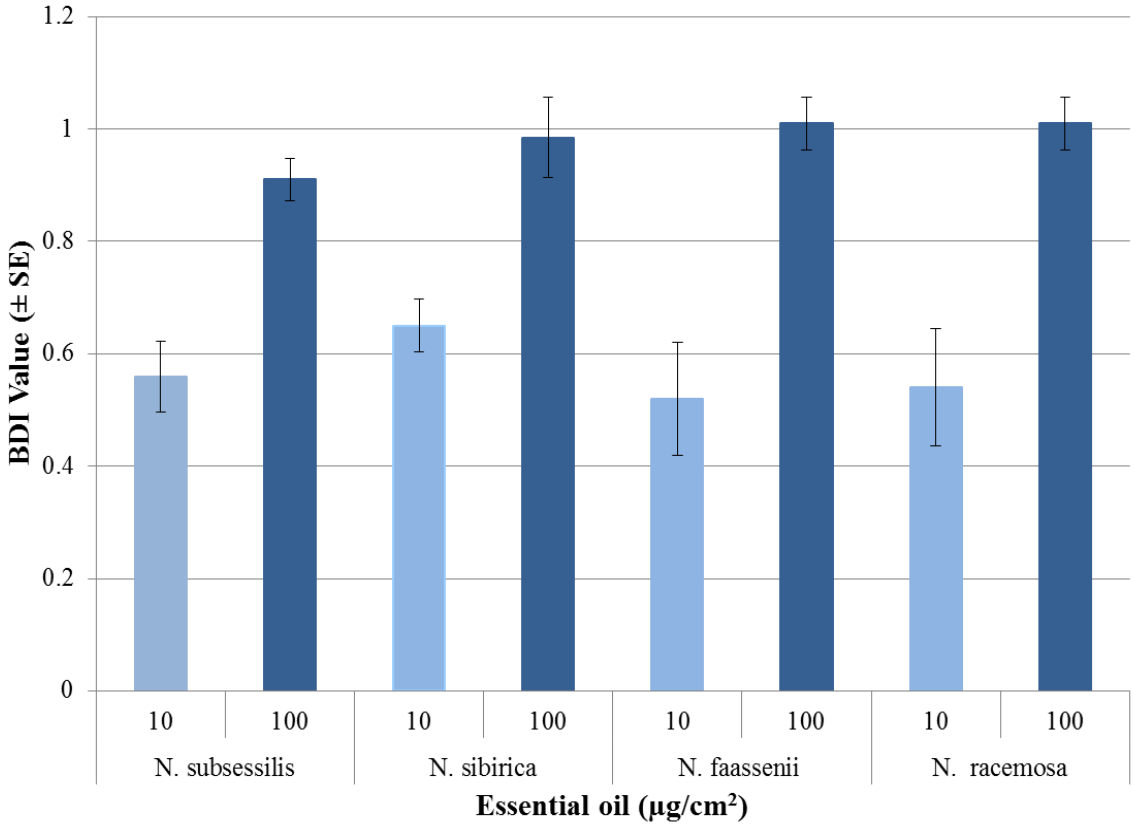

Figure 1. Biting deterrence index (BDI) of the essential oils extracted from samples of aerial parts of *N. subsessilis, N. sibirica, N. ×faassenii* 'Dropmore', and *N. racemosa* hybrid 'Select' against female *Ae. aegypti*. Ethanol was the solvent control and DEET at 25 nmol/cm^2 was used as positive control.

In larvicidal screening bioassays, toxicity was low in all the essential oils tested. Only 90, 30, 28, and 30% mortality was observed in *N. racemosa* hybrid 'Select', *N. sibirica*, *N. subsessilis* and *N. ×faassenii* 'Dropmore' essential oils, respectively, at the highest dose of 125 ppm. Zhu *et al.* [29] reported larvicidal activity of *N. cataria* (catnip) essential oil with LC_{50} values of 70, 298 and 122 ppm

against *Ae. aegypti, Ae. albopictus* and *Culex pipens pallens* larvae, respectively. However, ethanol extracts of *N. cataria* roots and leaves did not show any larvicidal activity at highest concentration of 125 ppm (A. Ali personal communication). Since the chemical composition of essential oils of the *Nepeta* species tested differ from catnip oil, the results cannot be directly compared.

4. Conclusions

Nepeta racemosa hybrid 'Select' and *N.* ×*faassenii* 'Dropmore' essential oils are rich source of monoterpene-1,8-cineole, whereas *N. sibirica* and *N. subsessilis* essential oils mainly contained sesquiterpenes. Essential oils of *N. racemosa* hybrid 'Select', *N. sibirica*, *N. subsessilis*, and *N.* ×*faassenii* 'Dropmore' have a high biting deterrent activity against *Ae. aegypti*. This is the first report on biting deterrent activity of these oils against mosquitoes. These results indicate that sesquiterpenes present in these oils should be further studied individually to explore their activity. Since the chemical composition of *Nepeta* species essential oils varies, there is a need to investigate the composition of *Nepeta* species, especially from wild populations. Due to the insufficient oil yields, the unknown compounds could not be isolated. These compounds may be important and should be isolated to study detailed biological activity of these essential oils. Further research can lead to finding horticultural traits or genes that can improve the concentration of more desirable compounds and combination of compounds to improve the efficacy of these oils as biting deterrents against insects.

Acknowledgments

This study was supported in part by USDA/ARS grant No. 56-6402-1-612, Deployed War-Fighter Protection Research Program Grant funded by the U.S. Department of Defense through the Armed Forces Pest Management Board, and a Special Research Initiative grant from the Mississippi Agricultural and Forestry Experiment Station. We thank Dr. James J. Becnel, USDA-ARS, Gainesville, FL for supplying mosquito eggs.

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