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CHEMICAL COMPOSITION, ADULTICIDAL AND REPELLENT ACTIVITY OF ESSENTIAL OILS FROM *Mentha longifolia* L. AND *Lavandula dentata* L. AGAINST *Culex pipiens* L.

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ABSTRACT

The essential oils from *Mentha longifolia* L., and *Lavandula dentata* L. were evaluated for their insecticidal and repellent activity against adult females of *Culex pipiens* L. The two oils showed LC_{50} values of 0.215 and 0.217 µl / l, respectively. *Lavandula dentata* oil showed higher repellent activity than *Mentha longifolia* oil against adults of *Culex pipiens*; paraffin oil significantly prolonged the time of protection for the two oils. The longest time of protection was 165 min for *L dentata* oil, at 1 µl/cm^2, when the tested oils were applied in paraffin oil. The principle compounds in the two oils were pulegone, l-menthone, 1-8-cineole, eucarvone and borneol for (*M. longifolia*); and camphor, fenchone, linalool and fenchol for (*L. dentata*). The two oils were effective as repellent substances when compared with commercial materials. Further studies are needed to isolate the most effective mosquito control agents from these oils.

Keywords: essential oils, *Culex pipiens*, adulticidal activity, *Mentha longifolia*, repllents, *Lavandula dentata*

INTRODUCTION

Mosquitoes are serious pests; they annoy man and animals and transmit many fatal diseases (Service, 2004; Smith *et al.,* 1993; Sardelis, 2003; Peng et al, 2004; Morales *et al.,* 2006). Conventional pesticides are heavily used to combat mosquitoes, but these pesticides have many disadvantages as: development of resistance, residue problems and bad effects on environment and human beings (WHO, 1992; Briassoulis, 2001). Therefore, a need is found to replace these hazardous pesticides with safe ones. Plant-derived pesticides are being tried as optional alternatives. While a number of botanicals, especially essential oils, were reported to be effective as mosquito repellents (Sritabutra and Soonwera, 2013; Phasomkusolsil and Soonwera, 2011; Gillij *et al.,* 2008; Prajapati *et al.,* 2005), many exhibited larvicidal activity (Govindarajan *et al.,* 2013; Manimaran *et al.,* 2012; Ansari *et al.,* 2000; Cetin *et al.,* 2004; Cetin and yanikoglu, 2006; Michaelakis *et al.,* 2007). Few studies were carried out on adulticidal properties of essential oils against mosquitoes (Pal *et al*, 2011; Kang et.al. 2009; Dolan, *et. al*., 2007; Yang *et al.,* 2005). At least, five-seven years are required to introduce a new commercial insecticide into the market; therefore, it is important to discover the pesticidal properties of local plant species to be used as soon as the onset of insect resistance to conventional pesticides. The two medicinal plants, *Mentha longifolia* L., and *Lavandula dentata* L. were reported to have insecticidal and/or antimicrobial properties in many places (Odeyemi *et al.,* 2008; Imelouane *et al.,* 2009; Koliopoulos *et al.,* 2010; Hafedh *et al.,* 2010; Hussain *et al.,* 2010; Baka, 2010). Therefore, this work was undertaken to investigate the constituents of *Mentha longifolia*, and *Lavandula dentata* oils and evaluate their insecticidal and repellent activity against *Culex pipiens* adults.

MATERIALS AND METHODS

The tested Plants:

The samples of *L. dentata* were collected from Asir region, Saudi Arabia, while samples of *M. longifolia* were purchased from a local supermarket. The tested Plants were identified by the Botany Department, king Saud University.

Extraction of essential oils

Semi-dried leaves and flowers of *L. dentata* and fresh leaves of *M. longifolia* were steam distillated. Essential oils were extracted from steam distillates with diethyl ether; extracts were dried over anhydrous sodium sulfate and solvent was evaporated under vacuum, using Rotavapor-R-215 rotary evaporator. Oils were kept in deep freezer, in glass vials with Teflon screw caps.

GC-MS analysis

The components of the tested oils were identified by Agilant Technologies 6890 N Network system, equipped with Agilant 5973 mass selective detector. Agilant DB-35 MS capillary column was used with the following oven temperature program: 40^{o}C initial temperature, 2 ^{o}C /min ramp to 200 ^{o}C, held for 5 min. Carrier gas (helium) flow rate was 1 ml/min, injection of oil sample (1µl dissolved in methanol) was carried out at 200 ^{o}c. Electron ionization mode with a transfer line temperature at 280 ^{o}C was used. A wiley 7 data base was employed to identify the compounds.

Insecticidal activity

Insects tested

Adult females of *Culex pipiens* were obtained from a susceptible strain reared in the insectary of Plant Protection Department, King Saud University. The colony was kept at 24 ± 2 ^{o}C, with a photoperiod of 12: 12 h (light:dark). Larvae were fed on animal feed and adults were fed on 10% sucrose.

Adulticidal trials

The fumigant toxicity of the tested oils against female *C*. pipiens was performed in airtight conical flasks according to our method (Al-Sarrar *et al.,* 2014), with minor modifications. The tested oil was pipetted into a 3 ml glass vial. The opening of the treated vial was covered with a cloth netting to prevent contact of mosquitoes with the oil inside the vial. Twenty adult females (5-10-days-old) were introduced into the conical flask with the aid of an aspirator. The treated vial was hanged inside the conical flask with a string. The conical flask was sealed with its quick fit led. To prevent any leakage of oil vapor outside the flask, the led was wrapped with parafilm.

Dead insects were counted 24 h after treatment. Overturned insects without any movement were considered dead. Three oil-free flasks were used as control with same number of insects. Three replicates were used for each concentration.

Repellency test

The abdomen of a pigeon (4×5 cm^2 area) was shaved to expose the skin to the blood starved female mosquitoes. Test oil was applied to skin as pure oil or diluted with ethanol or paraffin oil with the aid of a micropipette at 0.5 and 1 $\mu l/cm^2$; the dose was evenly distributed on the treated area. Controls with and without ethanol or paraffin were used and three replicates were used for each treatment. Treatments were placed against wire netted cages (45× 45×45 cm^3), each containing more than 250 blood starved female mosquitoes. The time when 2 or more mosquitoes started to bite and feed on the treated area was considered as the protection time, according to Tawatsin *et al*. (2006).

Statistical analysis

Median lethal (LC_{50}) values, 95 % fiducial limits and slopes were estimated according to Finney (1971). Repellency results were analyzed by the Costat 2 statistical program, employing the Student- Newman-Keuls Test to compare among means of treatments.

RESULTS AND DISCUSSION

The Chemical composition of the tested oils

Adulticidal trials

Results of adulticidal activity are shown in Table 2. The two oils showed similar LC_{50} values of 0.215 and 0.217 µl/ l, respectively. At higher concentrations, *M. longifolia* oil was more toxic than *L. dentata* oil, where LC_{95} values were 0.309 and 0.695 µl/ l, respectively. Essential oil of *Plectranthus incanus* Link showed LC_{50} value of 19.6 µl/ l against *Culex fatigans*, the main compounds in this oil were fenchone, piperitone oxide and piperitenone oxide, where the LC_{50} value of the commercial citronella oil was 31 µl/ l (Pal *et al.,* 2011); fenchone was one of the main compounds in *L. dentata* oil used in the present study. Yang et al. (2005) reported LC_{50} value of 254 µl/ l for *Mentha piperita* oil against *Culex pipiens quinquefasciatus* adults. Wintergreen oil showed knockdown activity against *C. pipiens pallens* adults at 12 µL/L and cinnamon oil exhibited fumigation toxicity against *C. pipiens pallens* adults with the LC_{50} value of 0.31 µL/L (Wei-Bin *et al.,* 2013)

Table 1: Chemical Composition of tested essential oils

Constituent	R_t	*L. dentata*	*M. longifolia*
(-)-Borneol	27.03		1.72
(+)-Fenchol	22.55	1.74	
(+)-Sabinene	11.70		0.42
1,8-Cineole	16.20		7.35
Camphor	27.55	60.39	
Carvacrol	37.20		
Carvacrol methyl ether	31.78		
Caryophyllene Oxide	53.80		
Cis-Isopulegone	29.51		1.47
dl-Limonene	14.94	0.41	0.30
Eucarvone	42.73		2.68
Fenchone	22.34	28.05	
Gamma-Terpinene	13.44		0.55
Germacrene	45.39		0.20
Linalool	21.72	1.80	
Linalool oxide	19.23	0.47	
L-Menthone	26.87		6.62
Myrtenal	31.16	1.04	
Myrtenol	29.30	0.62	
NI	25.48	1.19	
NI	28.10	0.74	
NI	51.21		0.13
NI	57.12		0.52
NI	81.25		0.23
Para-cymene	16.06		
Para-Cymene-8-ol	30.30	0.27	
p-Cymenene	21.49		
Piperitone	35.53		0.15
Pulegone	34.69		74.95
Trans-caryophyllene	40.53		0.61
Trans-linalool oxide	20.88	0.49	
trans-p-Menthan-3-one	27.68		0.63
trans-β-Ocimene	15.81		
Verbenone	32.78	0.30	
α-Caryophyllene	43.37		0.17
α-Pinene	8.38		0.19
α-terpinene	47.03	0.37	
α-terpinolene	19.09		
β-Bisabolene	46.21		
β-Myrcene	12.74		
β-Pinene	11.8	0.89	0.13
β-Selinene	45.64	0.36	

R_t = retention time

Table 2: Probit analysis for adulticidal activity of test oils against *C pipiens* females

Oil	Oils Toxicity		
	LC_{50}* (95% FL)	**LC_{95} (95% FL)**	**Slope ± SE**
M. longifolia	0.215 (0.204- 0.225)	0.309 (0.292-0.334)	10.44 ± 0.97
L. dentata	0.217 (0.193- 0.239)	0.695 (0.565- 0.95)	3.25 ± 0.34

***LC_{50} = µl/L**

Repellency results

The results of repellent activity of *L. dentata* and *M. longifolia* oils are presented in Table 3. The longest protection time against mosquito biting (165 min) was achieved by *L. dentata* oil at 1 µl/cm^2 when the oil was applied in paraffin oil, while the same dose applied as pure oil or dissolved in ethanol gave 85 min protection time, that is, paraffin oil prolonged the protection time by two fold and there was a significant difference between the two treatments. *M. longifolia* oil, at 1 µl/cm^2, applied in paraffin oil, gave protection against mosquito attack for only 64.67 min, which means that *L. dentata* oil, at the same dose, is 3 times more effective than *M. longifolia* oil. Moreover, *L. dentata* oil, at 1 µl/cm^2, without paraffin oil, was more effective than *M. longifolia* oil dissolved in paraffin oil at the same dose, and gave protection against mosquito biting for 85 min, while *M. longifolia* oil protection time was 65 min only with a significant difference between the two treatments. The protection time of the two oils, dissolved in paraffin oil, at 0.5 µl /cm^2, decreased to be 44.67 and 36 min, respectively. *L. dentata* oil was significantly more effective than *M. longifolia* oil. At the same dose, without paraffin oil, the protection time for the two oils declined to 32.67 and 12 min, respectively. *Zingiber officinals* volatile oil, at 4 mg/cm^2, showed repellent activity for 120 min against adult *Culex quinquefasciatus* (Pushpanathan, *et al.,* 2008). At 0.005 mg/cm^2, essential oils of clove and juniper berry gave good repellency against female *Culex pipiens pallens* (Kang *et al.,* 2009). *Lavandula gibsoni* essential oil, at 2 mg/cm^2, gave 100% protection against *Aedes aegypti* adults for more than 7 h (Kulkarni *et al.,* 2013). Kumar et al. (2011) reported that the protection time of *Mentha pipertia* essential oil against bits of *Aedes aegypti* was 150 min when the oil was applied at 4 µl /cm^2. Peng *et al*. (2009) reported that essential oil from *Zanthoxlum beecheyamum* var. alatum provided protection for 6.54 h against *Culex pipens quinquefasciatus* at 1.5 mg/cm^2. *Cymbopogon citratus* oil gave protection for 84 min against adults of *Culex uinquefasciatus* at 0.21 mg/cm^2 (Phasomkusolsil *et al.,* (2011).

Table 3: Protection time (min) of test oils against bits of *C pipiens* adults

Treatment	Protection time (min) Mean ± SD*
L. dentata oil in paraffin (1µl/cm^2)	165 ± 5^a
L. dentata oil (1µl/cm^2)	85 ± 5^b
M. longifolia oil in paraffin (1µl/cm^2)	64.67 ± 5^c
L. dentata oil in paraffin (0.5µl/cm^2)	44.67 ± 1.6^d
M. longifolia oil (1µl/cm^2)	43.67 ± 1.2^d
longifolia oil in paraffin (0.5µl/cm^2)	36 ± 2.65^e
L. dentata oil (0.5µl/cm^2)	32.67 ± 2.5^e
M. longifolia oil (0.5µl/cm^2)	12 ± 2^f
Controls	1± 0.0 ^{g}

***Means with the same letters are not significantly different at the 0.05 level.**

CONCLUSION

The results of the present study showed that the essential oils of tested plant species have insecticidal and repellent activity against adults of *C pipiens*. The oil of *L. dentata*, diluted with paraffin oil, could be used as protection agent against biting of *C pipiens* adults to protect people against disease transmission. Essential oils have many advantages over conventional pesticides; they have pleasant smell, they are blend of many compounds which render them difficult for insect to develop resistance, and they do not cause residue problems in the environment because they are volatile. Further studies are needed to isolate the most effective compounds from these oils. The results indicated that plants are potential safe alternatives to hazardous conventional pesticides, especially for the control of the public health pests.

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التركيب الكيميائى والنشاط الابادى والطارد للزيوت الأساسية المعزولة من نباتى الحبك والخزامى ضد الطور البالغ من بعوض الكيولكس بيبينز.

على سعيد آلـ -سرار
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تم تقييم النشاط الابادى والطارد للزيوت الأساسية المعزولة من نباتى الحبك والخزامى ضد الاناث البالغة من بعوض الكيولكس بيبينز. كان التركيز القاتل لــ 50% من الاناث البالغة هو 215و. و217و. ميكرولتر/ لتر لكلا الزيتين على التوالى. أظهر زيت نبات الخزامى نشاطاً أعلى من زيت نبات الحبك كمادة طاردة. أطال زيت البرافين وقت الحماية لكلا الزيتين وكان أطول وقت للحماية هو 165 دقيقة لزيت الخزامى عند تطبيقه بتركيز 1 ميكرولتر / سم مربع فى زيت البرافين. كانت المركبات الأساسية فى زيت الحبك هى بوليجون، 1- منثون، 1و8- سنيول، يوكارفون، بورنيول- بينما كانت المركبات الأساسية فى زيت نبات الخزامى هى كامفور، فنكون، لينالول، وفنكول. كان كلا من الزيتين فعالاً كمادة طاردة مقارنة بالزيوت التجارية. هناك حاجة لمزيد من الدراسة لعزل أكثر المركبات فعالية من هذة الزيوت.

الكلمات الدالة: كيولكس بيبينز، حبك، خزامى، مواد طاردة، النشاط الابادى ضد البالغات.