PHCOG RES. ORIGINAL ARTICLE

Chemical composition and larvicidal activity of essential oil of *Cupressus arizonica* E.L. Greene against malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae)

Mohammad Mehdi Sedaghat, Alireza Sanei Dehkordi, Mahnaz Khanavi[1], Mohammad Reza Abai, Fatemeh Mohtarami, Hassan Vatandoost

Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, [1]Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

Submitted: 19-01-2011 Revised: 25-03-2011 Published: 08-06-2011

ABSTRACT

Background: Using botanical insecticides as an alternative biocontrol technique for vector control is considered by some scientists. **Materials and Methods:** Chemical composition of the essential oil was analyzed using gas chromatography–mass spectrometry (GC–MS). In addition, the mosquito larvicidal activity of leaf essential oil of *Cupressus arizonica* was investigated against fourth instar larvae of laboratory-reared *An. stephensi* according to the method of the World Health Organization. **Results:** Of 46 constituents in the oil, limonene (14.44%), umbellulone (13.25%) and α-pinene (11%) were determined as the main constituents. *Cupressus arizonica* volatile oil showed significant larvicidal activity against *An. stephensi* with LC_{50} and LC_{90} values 79.30 ppm and 238.89 ppm respectively. Clear dose-response relationships were established with the highest dose of 160 ppm essential oil with almost 100% mortality. **Discussion:** The results from this study revealed that *C. arizonica* essential oil could be considered as a natural larvicide against *An. stephensi*. However, the field evaluation of the formulation is necessary.

Key words: *Anopheles stephensi*, botanical insecticide, *Cupressus arizonica*, essential oil, Iran, vector control

Access this article online
Website: www.phcogres.com
DOI: 10.4103/0974-8490.81962
Quick Response Code:

INTRODUCTION

Anopheles mosquitoes are bloodsucking insects that are responsible for the transmission of malaria, filariasis and arboviruses; on the other hand they are important as nuisance mosquitoes in particular areas while they may not be vectors of any disease.[1] Human malaria is considered as the most important disease among the vector-borne diseases. Malaria still remains an endemic disease in the southeastern corner of the country.[2,3] There are more than 25 currently recognized Anopheles species out of which seven of them have important roles in malaria transmission. Among these species, *Anopheles stephensi* is considered as a primary vector of malaria in the southern parts of the country.[2,4-9]

Address for correspondence:
Dr. Hassan Vatandoost, PO Box: 14155-6446 Tehran, Iran.
E-mail: hvatandoost1@yahoo.com

There are several methods for malaria control, most of which have been applied in Iran. Synthetic insecticides which are generally used have side-effects on human and animal health and also the environment. The side-effects of synthetic organophosphorus compounds on fish and other organisms in the environment are being increasingly reported.[10,11] A lot of attention is being paid to natural products in vector control as they are environmentally safe, degradable and target-specific. Recent studies have demonstrated the insecticidal properties of plant essential oils and their efficacies against larvae of different species of mosquito.[4,12-20] The repellent effect of the extract and essential oil of plants has been investigated on some mosquito species in Iran.[21-23]

The purpose of this paper is to investigate the composition and larvicidal activity of the leaf essential oil of *Cupressus arizonica* E.L. Greene. This species is a medium-sized evergreen tree with a conic to ovoid-conic crown known

as a native plant in the southwestern United States.[24,25] It was introduced to Iran in 1954 and has been commonly cultivated in many parts of the country.[26] The biological effect of *C. arizonica* have been investigated and its effects as a potential mosquito repellent have been reported earlier.[27,28] Previous studies indicated that α-pinene, limonene and umbellulone were obtained as major components from the oil of *C. arizonica* leaves in Iran,[29] Italy,[30] USA,[31] Algeria,[32] France,[33] Tunisia[27] and Argentina.[34]

This study was conducted to find out the efficacy of the leaf essential oil of *C. arizonica* against fourth instar larvae of *An. stephensi* under laboratory conditions and determine the chemical composition of the essential oil.

MATERIALS AND METHODS

Plant materials

A fresh leaf from an eight-year-old *C. arizonica* tree was collected in May 2009 from Tehran, Iran (51° 23'E, 35° 43'N, elevation: 1329 Meter). The plant was identified and authenticated and the voucher specimen was deposited at the Herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

Essential oil isolation

Fresh leaves (200 g) of *C. arizonica* were subjected to hydrodistillation using a modified clevenger-type apparatus for 3 h, dried over anhydrous sodium sulphate and transferred into amber-colored vials at 5°C for further work. Essential oil was stored in an airtight container prior to analysis by gas chromatography–mass spectrometry (GC and GC–MS).

Gas chromatography-mass spectroscopy

Analytical gas chromatography was carried out using an HP gas chromatograph. The separation was achieved by use of a HP_1 (Fused silica) capillary column (30 m × 0.25 mm; film thickness 0.25 μm); split ratio, 1:25, and using a flame ionization detector. The GC settings were programmed as follows: initial oven temperature was held at 40°C for 1 min, rising to 250°C at 5°C /min. The injector temperature was maintained at 250°C. The detector temperature was at 230°C. Helium was used as the carrier gas at a flow rate of 1 ml/min.

GC-MS was performed on Agilent Technology 5973 mass selective detector connected with a HP 6890 gas chromatograph. The separation was achieved by use of a HP_1MS (Fused silica) capillary column (30 m × 0.25 mm; film thickness 0.25 μm); split ratio, 1:25, and using a flame ionization detector. The MS operated at 70 eV ionization energy. Quantitative data were obtained from the electronic integration of the Flame Ionization Detector (FID) peak areas.

Determination of oil composition

Identification of the oil components was based on retention indices which were calculated by using retention times of *n*-alkanes that were injected after the oil at the same chromatographic conditions. The components of the oils were identified by comparison of their mass spectra and retention indices with Wiley library and those published in the literature.[27,35] The percentage of each component is presented in Table 1.

Mosquito rearing

The fourth-instar larvae of *An. stephensi* Bandar-Abass strain was obtained from the Department of Medical Entomology, Tehran University Medical Sciences. The mosquito colony was maintained continuously at 27°C with 12:12 light and dark photoperiod in 80 ± 10% relative humidity. Larvae of *An. stephensi* were continuously available for the mosquito larvicidal experiments.

Bioassays and larval mortality

Bioassays were performed according to the standard method recommended by the World Health Organization (WHO).[36] The fourth-instar larvae of *An. stephensi*, collected from the maintained laboratory stock served as the test organisms in this study. Methanol 99.0% was used as co-solvent and then different concentrations of the essential oil in distillated water were prepared. A 400-ml glass beaker was used for each experiment or control. Controls included batches of mosquitoes from the colony exposed to water and the solvent alone.

The larvae were exposed to the concentrations of 10, 20, 40, 80 and 160 ppm of essential oil extract in distilled water for 24 h. In the control beakers only 1 ml of solvent was dissolved into the water. Mortality was counted after 24 h recovery period. The percentage of mortality was reported from the average for the five replicates taken.

Statistical analysis

LC_{50} (lethal concentration to cause 50% mortality in the population) and LC_{90} (lethal concentration to cause 90% mortality in the population) were determined by the use of regression line employed by Finney.[37] In case of mortality in control beakers, it was corrected by Abbott's correction.[38] Differences between means were considered significant at $P \leq 0.05$.[39]

RESULTS

Yields and chemical constituents of essential oil

The hydrodistillation of the *C. arizonica* leave gave oil in

Table 1: Chemical constituents of leaf essential oil from *Cupressus arizonica*

Compound	RI[a]	Concentration %
Tricyclene	893	0.20
α-Thujene	898	0.58
α-Pinene	907	11.00
Camphene	921	0.31
Verbenene	934	0.11
Sabinene	941	4.04
β-Pinene	946	0.25
β-Myrcene	960	1.95
α-Phellandrene	970	0.20
δ-2-Carene	972	0.85
α-Terpinene	985	1.54
p-Cymene	992	1.61
Limonene	996	14.44
(E)-β-Ocimene	1019	0.04
γ-Terpinene	1026	2.15
cis-Sabinene hydrate	1042	0.27
Terpinolene	1057	1.65
trans-Sabinene hydrate	1065	0.20
Linalool	1067	0.92
1-terpineol	1102	0.68
Camphor	1115	1.68
Umbellulone	1137	13.25
Terpinen-4-ol	1150	7.29
ρ-Cymen-8-ol	1155	0.42
α-Terpineol	1159	0.71
cis-Piperitol	1169	0.35
Thymol, methyl ether	1204	0.28
Citronellyl formate	1242	1.14
Phellandral	1249	0.12
Bornyl acetate	1264	0.52
Thymol	1265	0.81
trans- carvyl acetate	1320	0.14
α-Terpinyl acetate	1325	1.76
α-Cedrene	1378	0.17
β-funbrene	1382	2.64
trans-Caryophyllene	1387	0.15
cis-Muurola-4(14),5-diene	1457	7.36
epi-Zonarene	1467	2.90
trans-Calamenene	1495	2.89
δ-Cadinene	1498	0.73
α-Calacorene	1515	0.33
Caryophyllene oxide	1557	0.59
Cedrol	1574	1.18
β-Acorenol	1608	2.56
α-Cadinol	1621	1.33
cis-14-nor-muurol-5-en-4-one	1655	3.04
Total		97.33
Terpenoids		
Monoterpene hydrocarbons		40.72
Oxygenated monoterpenes		30.54
Sesquiterpene hydrocarbons		17.17
Oxygenated sesquiterpenes		8.7
Non-terpenoids		0.2

[a]RI = Retention Index on HP-5 in reference to *n*-alkanes injected after the oil at the same chromatographic conditions

0.8% (w/w) yield on fresh weight material. The essential oil was yellow with a distinct sharp odor.

Table 1 shows constituents of the oil. Forty-six constituents in the leaf essential oil of *C. arizonica* were identified corresponding to 97.33% of the total oil. The main constituents in the *C. arizonica* leaf essential oil were limonene (14.44%), umbellulone (13.25%) and α-pinene (11%) respectively. The results revealed that monoterpene hydrocarbons in the leaf oil were predominant (40.72%), whereas oxygenated sesquiterpenes were present in low amounts.

Mosquito larvicidal activity of essential oil

The larvicidal potency of different concentrations of leaf essential oil of *C. arizonica* against *An. stephensi* is given in Table 2. Among the five concentrations tested, the dosage of 160 ppm was found to be the most toxic with 100% larval mortality. *Cupressus arizonica* leaf essential oil showed toxicity against the larvae. There was no mortality in the control groups. The LC_{50} and LC_{90} values against *An. stephensi* larvae were 79.30 ppm and 238.89 ppm respectively.

DISCUSSION

The efficacy of several plant essential oils and extracts as natural larvicides has been reported.[13,14,16-20,40] In this study, major constituents and bioactivity of leaf essential oil of *C. arizonica* were considered. Limonene (14.44%), umbellulone (13.25%) and α-pinene (11%), *cis*-muurola-4(14),5-diene (7.36%) and terpinen-4-ol (7.29%) were known as the main compounds of the oil.

In previous studies, various constituents of the oil of *C. arizonica* were reported.[27,29-34] Although umbellulone is the most abundant constituent in the oils obtained from all mentioned studies, the percentage of the compound was varied, based on the origins of the plant. While it has been reported as 45.1% from *C. arizonica* cultivated in Italy, 37.3% in Algeria, 18.4% in Tunisia, 16.5% in Argentine and 5.4% in Texas, it was recorded as 13.25% in this study which is close to the result obtained from Argentinean samples. Although the oil obtained in this study has a moderate amount of limonene (14.44%) and known as the most abundant constituent, it was recorded 5.8% in Tunisia, 8.5% in Argentina, 8.7% in France and 14% in Texas.

The amount of limonene in this study is similar to the US samples. In the previous researches, α-pinene has been reported from the Texas (7.6%), Algeria (10.5%), Tunisia (20.0%) and Argentina samples (22.9%). In a similar study, it was discovered as the main constituent of the leaf oil (19.2%) in Isfahan, Iran, which is similar to the results of our study.[27,29-34]

According to the results of the larvicidal tests, the essential oil *C. arizonica* was effective against *An. stephensi* with LC_{50} and LC_{90} values of 79.30 and 238.89 ppm, respectively. The

Table 2: Probit regression line parameters of *Anopheles stephensi* to leaf essential oil extraction of *Cupressus arizonica* at different interval concentrations

Specimen	A	B ± SE	LC_{50}, 95% C.I.	LC_{90}, 95% C.I.	X^2 (df)	*P* value
Leaf essential oil	-5.083	2. 676 ± 0.677	34.37 **79.30** 199.30	125.05 **238.89** 290.37	27.74 (3)	<0.05

bioactivity of different extracts from the plants has been investigated on *An. stephensi* larvae. There is a report about the efficacy of the methanolic extract of *Tagetes minuta* L. on the *An. stephensi* in which, the LC_{50} and LC_{90} values were obtained as 2.5 mg/l and 11.0 mg/l, respectively.[17] The larvicidal activity of *Tagetes patula* oil has been reported on the larvae of *An. stephensi* with the LC_{50} and LC_{90} values of 12.08 mg/l and 57.62 mg/l, respectively.[41] Vatandoost and Moein-Vaziri reported LC_{50} and LC_{90} values of about 0.35 mg/l and 1.81 mg/l respectively of neem tree (*Azadirachta indica*) extract against *An. stephensi* larvae.[40] Our previous studies demonstrated the efficacy of *Eucalyptus camaldulensis* oil against *An. stephensi* larvae with the LC_{50} and LC_{90} values of 89.85 mg/l and 397.75 mg/l respectively.[20] The comparison of the efficacy of essential oils of *C. arizonica* and *E. camaldulensis* against *An. stephensi* larvae revealed that the oil of *C. arizonica* is more potent than *E. camaldulensis*. It seems that the presence of a high amount of limonene and α-pinene as larvicidal compounds in *C. arizonica* essential oil, could demonstrate its efficacy against *An. stephensi* larvae.[42]

The usage of plant essential oils as larvicides in vector control can be considered as a substitute method to reduce the side-effects of synthetic insecticides on the environment. Extensive efforts have been made to find new plants and their oils as natural and eco-friendly larvicides. In this way, our finding suggests that the leaf essential oil of *C. arizonica* has a potential larvicide effect. It is essential to conduct more research in this area in order to find good candidates and isolate the effective compounds.

ACKNOWLEDGMENTS

This work was supported by a grants from the Centre of Environmental Health Research, Tehran University of Medical Sciences, Tehran, Iran, Project No.88-01-46-8661.

REFERENCES

1. Service MW. A Guide to Medical Entomology. London: MacMillan; 1980. p. 226.
2. Sedaghat MM, Linton YM, Oshaghi MA, Vatandoost H, Harbach RE. The *Anopheles maculipennis* complex (Diptera: Culicidae) in Iran: Molecular characterization and recognition of a new species. Bull Entomol Res 2003a;93:527-35.
3. Sedaghat MM, Linton YM, Nicolescu G, Smith L, Koliopoulos G, Zounos AK, *et al*. Morphological and molecular characterization of *Anopheles* (*Anopheles*) *sacharovi* Favre: A primary vector of malaria in the Middle East. Syst Entomol 2003b;28:241-56.
4. Vatandoost H, Shahi H, Abai MR, Hanafi-Bojd AA, Oshaghi MA, Zamani G. Larval habitats of main malaria vectors in Hormozgan province and their susceptibility to different larvicides. Southeast Asian J Trop Med Pub Health 2004;35:22-5.
5. Vatandoost H, Oshaghi M, Abaie MR, Shahi M, Yaghoobi F, Baghai M, *et al*. Bionomics of *Anopheles stephensi* Liston in the malarious area of Hormozgan province, southern Iran. Acta Trop 2006;97:196-205.
6. Oshaghi MA, Sedaghat MM, Vatandoost H. Molecular characterization of the *Anopheles maculipennis* complex in the Islamic Republic of Iran. East Mediterr Health J 2003a;9:659-66.
7. Naddaf SR, Oshaghi MA, Vatandoost H, Asmar M. Molecular characterization of the *Anopheles fluviatilis* species complex in Iran. East Mediterr Health J 2003;9:257-65.
8. Doosti S, Vatandoost H, Oshaghi MA, Hosseini M, Sedaghat MM. Applying Morphometric Variation of Seta 2 (Antepalmate Hair) among the Larvae of the Members of the Maculipennis Subgroup (Diptera: Culicidae) in Iran. Iranian J Arthropod Borne Dis 2007;1:28-37.
9. Sedaghat MM, Harbach RE. An annotated checklist of the *Anopheles* mosquitoes (Diptera: Culicidae) in Iran. J Vect Ecol 2005;30:272-6.
10. Mittal PK, Adak T, Sharma VP. Acute toxicity of certain organochlorine, organophosphorus, synthetic pyrethroid and microbial insecticides to the mosquito fish *Gambusia affinis* (Baird and Girard). Indian J Malariol 1991;28:167-70.
11. NICC-Comunicação e Saúde. Available from: http://www.cpqam.fiocruz.br/nicc/comunic/jc200101.htm. [cited in 2003].
12. Saxena SC, Sumithra L. Laboratory evaluation of leaf extract of new plant to suppress the population of malaria vector *Anopheles stephensi* Liston (Diptera:Culicidae). Curr Sci 1985;54:201-2.
13. Kumar A, Dutta GP. Indigenous plant oils as larvicidal agent against *Anopheles stephensi* mosquitoes. Curr Sci 1987;56:959-60.
14. Markouk M, Bekkouche K, Larhsini M, Bousaid M, Lazrek HB, Jana M. Evaluation of some Moroccan medicinal plant extracts for larvicidal activity. J Ethanopharm 2000;73:293-7.
15. Hadjiakhoondi A, Aghel N, Zamanizadeh N, Vatandoost H. Chemical and Biological study of *Mentha spicata* L. essential oil from Iran. Daru 2000;8:19-21.
16. Hadjiakhoondi A, Vatandoost H, Jamshidi A, Bagherj AE. Chemical constituents and efficacy of *Cymbopogon olivieri* (Boiss) bar essential oil against malaria vector, *Anopheles stephensi*. Daru 2003;11:125-8.
17. Hadjiakhoondi A, Vatandoost H, Khanavi M, Abaee MR. Biochemical investigation of different extracts and larvicidal activity of *Tagetes minuta* L on *Anopheles stephensi* larvae. Iranian J Pharma Sci 2005;1:81-4.
18. Hadjiakhoondi A, Sadeghipour-Roodsari HR, Vatandoost H, Khanavi M, Abaee MR, Vosoughi M, *et al*. Fatty acid composition and toxicity of *Melia azedarach* L. fruits against malaria vector *Anopheles stephensi*. Iranian J Pharma Sci 2006;2:97-102.

19. Tare V, Deshpande S, Sharma RN. Susceptibility of two different strains of *Aedes aegypti* (Diptera: Culicidae) to plant oils. J Econ Entomol 2004;97:1734-6.
20. Sedaghat MM, Sanei AR, Khanavi M, Abai MR, Hadjiakhoondi A, Mohtarami F, *et al*. Phytochemistry and larvicidal activity of *Eucalyptus camaldulensis* against malaria vector, *Anopheles stephensi*. Asian Pac J Trop Med 2010;3:841-5.
21. Oshaghi MA, Ghalandari R, Vatandoost H, Shayeghi M, Kamali-nejad M, Tourabi-Khaledi H, *et al*. Repellent effect of extracts and essential oil of Citrus limon (Rutaceae) and *Melissa officinalis* (Labiatae) against main malaria vector, *Anopheles stephens*i (Diptera: Culicidae) in Iran. Iranian J Public Hlth 2003b;32:47-52.
22. Vatandoost H, Khazani A, KebriainZadeh A, Rafinejad J, Khoobdel M, Abai MR, *et al*. Comparative efficacy of Neem and dimethyl phthalate (DMP) against malaria vector, *Anopheles stephensi* (Diptera: Culicidae). Asian Pac J Trop Med 2008;1:1-6.
23. Yaghoobi-Ershadi MR, Akhavan AA, Jahanifard E, Vatandoost H, Amin G, Moosavi L, *et al*. Repellecy effect of Myrtle essential oil and DEET against *Phlebotomus papatasi* Scopoli, the main vector of zoonotic cutaneous leishmaniasis under labo ratory conditions. Iranian J Public Hlth 2006;35:713.
24. Elmore FH, Janish JR. Shrubs and trees of the southwest uplands. Tucson, AZ, USA: Southwest Parks and Monuments Association; 1976.
25. Farjon A. World Checklist and Bibliography of Conifers. 2nd ed. England, UK: The Bath Press; 2001.
26. Sabeti H. Native and Exotic Tree and Shrubs of Iran. Tehran: Tehran Univ Press; 1966. p. 430 (in persian).
27. Cheraif I, Jannet HB, Hammami M, Khouja ML, Mighri Z. Chemical composition and antimicrobial activity of essential oils of *Cupressus arizonica* Greene. Biochem Syst Ecol 2007;35:813-20.
28. Akob CA, Ewete FK. Laboratory Evaluation of Bioactivity of Ethanolic Extracts of Plants Used for Protection of Stored Maize against *Sitophilus zeamais* Motschulsky in Cameroon. Afr Entomol 2009;17:90-4.
29. Afsharypuor S, Tavakoli P. Essential oil constituents of leaves and fruits of *Cupressus arizonica* Greene. J Essent Oil Res 2005;17:225-6.
30. Flamini G, Cioni PL, Morelli I, Bighelli A, Castola V, Casanova J. GC/MS and 13C NMR integrated analysis of the essential oils from leaves, branches and female cones of *Cupressus arizonica* from Italy. J Essent Oil Res 2003;15:302-4.
31. Adams RP, Zanoni, TA, Lara A, Barrero AF, Cool LG. Comparisons among *Cupressus arizonica* Greene, C. benthamii Endl., C. lindleyi Klotz. ex Endl. and C. lusitanica Mill. using leaf essential oils and DNA fingerprinting. J Essent Oil Res 1997;9:303-9.
32. Chanegriha N, Baaliouamer A, Meklati BY, Charetien JR, Keravis G. GC and GC/MS leaf oil analysis of four Algerian cypress species. J Essent Oil Res 1997;9:555-9.
33. Pierre-Leandri C, Fernandez X, Lizzani-Cuvelier L, Loiseau M, Fellous R, Garnero J. Chemical composition of cypress essential oils: Volatile constituents of leaf oils from seven cultivated Cupressus species. J Essent Oil Res 2003;15:242-7.
34. Malizia RA, Acardell D, Molli JS, Gonzalez S, Guerra PE, Grau RJ. Volatile constituents of leaf oils from the cupressaceae family part I. *Cupressus macrocarpa* Hartw., *C. arizonica* Greene and C. torulosa Don species growing in Argentina. J Essent Oil Res 2000;12:59-63.
35. Adams RP. Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. 4th Ed. Carol Stream, Illinois: Allured Publishing Corporation; 2007.
36. WHO. Instructions for determining susceptibility or resistance of mosquito larvae to insecticides. Geneva: WHO/VBC;1981. p. 807.
37. Finney DJ. Probit Analysis, 3rd ed. London, UK: Cambridge University Press; 1971.
38. Abbot WS. A method of computing the effectiveness of an insecticide. J Eco Entomol 1925;18:265-7.
39. Cary NC. Saxena SC, Sumithra L. Laboratory evaluations of leaf extract of new plant to suppress the population of malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae): SAS Institute. The SAS System for Windows, release 8.1. Curr Sci 2001;54:201-2.
40. Vatandoost H, Moein-Vaziri V. Larvicidal activity of Neem extract (*Azadirachta indica*) against mosquito larvae in Iran. East Mediterr Health J 2004;10:1-9.
41. Dharmagadda VS, Naik SN, Mittal PK, Vasudevan P. Larvicidal activity of *Tagetes patula* essential oil against three mosquito species. Bioresource Technol 2005;96:1235-40.
42. Cheng SS, Huang CG, Ying-Ju C, Jane-Jane Y, Wei-June C, Shang-Tzen C. Chemical compositions and larvicidal activities of leaf essential oils from two Eucalyptus species. Bioresource Technol 2009;100:452-6.

Cite this article as: Sedaghat MM, Dehkordi AS, Khanavi M, Abai MR, Mohtarami F, Vatandoost H. Chemical composition and larvicidal activity of essential oil of *Cupressus arizonica* E.L. Greene against malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae). Phcog Res 2011;3:135-9.

Source of Support: Centre of Environmental Health Research, Tehran University of Medical Sciences, Tehran, Iran, Project No.88-01-46-8661., **Conflict of Interest:** None declared.