Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Indication of pyrethroid resistance in the main malaria vector, *Anopheles stephensi* from Iran

Hassan Vatandoost*, Ahmad Ali Hanafi-Bojd

Department of Medical Entomology and Vector Control, School of Public Health & National Institue of Health Research, Tehran University of Medical Sciences, P.O. Box: 6446-14155, Tehran, Iran

ARTICLE INFO

Article history:
Received 21 January 2012
Received in revised form 24 February 2012
Accepted 15 March 2012
Available online 20 September 2012

Keywords:
Anophels stephensi
Malaria
Pesticides
Resisatnce

ABSTRACT

Objective: To investiagte insecticide resistance in target species for better insecticide resistance managemnet in malaria control programs. **Methods:** The status of insecticide resistance to different imagicides in *Anopheles stephensi* (*An. stephensi*) including DDT 4%, lambdacyhalothrin 0.50%, deltamethrin 0.05%, permethrin 0.75%, cyfluthrin 0.15% and etofenprox 0.50% was performed according to WHO standard method. **Results:** The mortality rate to lambdacyhalothrin, permethrin, cyfluthrin, deltamethrin, etofenprox and DDT was (88.0±3.2), (92.0±2.7), (52.0±5.0), (96.0±2.2), (90.0±3.0) and (41.0±5.7) percent, respectively at diagnostic dose for one hour exposure time followed by 24 h recovery period. **Conclusions:** These results showed first indication of pyrethroid resistance in *An. stephensi* in a malarious area, from southern Iran. There is widespread, multiple resistances in the country in *An. stephensi* to organochlorine and some report of tolerance to organophosphate insecticides and recently to pyrethroids. However, results of this paper will provide a clue for monitoring and mapping of insecticide resistance in the main malaria vector for implementation of any vector control.

1. Introduction

Malaria is still a major endemic disease in foci located in south and southeast of Iran. According to the report of Minsitry of Health and Medical Education of the country the annual malaria cases have been reported from 66 075 to 3 000 during 1995-2010, indicating the sharp decline of disease It is unstable with two seasonal peaks mainly in spring and autumn. These areas include the provinces of Sistan & Baluchistan, Hormozgan and Kerman. In this part of the country six Anopheline mosquitoes including *Anopheles culicifacies (An. culicifacies) s.l.*, *Anopheles stephensi (An. stephensi)*, *Anopheles dthali (An. dthali)*, *Anopheles fluviatilis (An. fluviatilis) s.l.*, *Anopheles superpictus (An. superpictus)*, and *Anopheles pulcherrimus (An. pulcherrimus)* are known to be the malaria vectors, while *Anopheles sahacrovi (An. sahacrovi)* and *Anopheles maculipennis (An. maculipennis)* s.l. are considered as malaria vectors in northern part of the country[1-11]. There are also occasionally outbreak of malaria in northwest of Iran. The country is now launching the elimination of malaria.

Different studies have been conducted during more than 80 years on malaria and its vectors. So far 33 species from 2 subspecies (*Anopheles* and *Cellia*), siblings, genotype and type forms are recorded in the country, 18 out of them are in complexes or groups that introduced as malaria vectors. Different studies have introduced *An. stephensi* (type, intermediate and mysorensis froms), *An. superpictus* (X, Y, Z genotypes), *An. culicifacies* (sibling A,B), *An. fluviatilis* (sibling T,U), *An. maculipennis* group (*An. atroparvus*, *An. maculipennis*, *An. melanoon*, *An. messeae*, *An. persiensis*, *An. sacharovi*) and *An. dthali* as confirmed malaria vectors in the country. Also A*n. pulcherrimus* is detected serologically positive to malaria parasite(s) in Baluchistan malaria foci, southeastern of the country. Figure 1 shows the distribution of *An. stephensi* in the country.

Seasonal activity of Anopheline mosquitoes varies in different area due to environmental condition. It shows one peak in northwest especially in summer, however, there are two peaks of activity in coastal warm and humid region

*Corresponding author: Prof. H. Vatandoost, Department of Medical Entomology and Vector Control, School of Public Health & National Institue of Health Research, Tehran University of Medical Sciences. P.O. Box: 6446-14155, Tehran, Iran.
E-mail: hvatandoost1@yahoo.com; vatando@tums.ac.ir

in the southern part of Iran with oriental epidemiological characteristics.

Plasmodium vivax (P. vivax) is dominant agent of malaria in Iran, while *Plasmodium falciparum (P. falciparum)* is reported and infects about 15% of patients.

There are different methods including biological, chemical and environmental management for malaria vector control. Chemical control had been the main method for combating against adult stage of malaria vector since eradication era. Insecticide application for adult mosquito control started with organochlorines (DDT, dieldrin and BHC) during the 1960′s, followed by organophosphates (malathion and pirimiphos-methyl) for 2 decades from 1966 and continued with the carbamate, propoxur during 1977-1990, and then with pyrethroids (lambdacyhalothrin/deltamethrin). Temephos, chlorpyriphos-methyl and pirimiphos-methyl was used for larviciding from 1970 to 1992. Resistance of *An. stephensi* to DDT, dieldrin and malathion was reported for the first time in 1957, 1960 and 1976, respectively. Resistance of *An. stephensi*, especially its adult stage against DDT, dieldrin and malathion have been widely reported in Middle east and Indian subcontinent regions[12].

Malaria control in the country is now based on use of deltamethrin (5% WP) as an adulticide as 25 mg/m^2 for indoor residual spraying (IRS) and *Bacillus thuringiensis* as a larvicide. Recently Long Lasting Impregnated Net of Olyset is recommended for southern part. Due to simultanous use of pyrethroids either in IRS and LLINs against target mosquitoes, and the lack of no rotation sttategy with other classes of insecticides, it is expected to evolve resistance gene of tolerant and resistance in the opulation of Anopheline vectors. Therefore the aim of this study was to determine the current status of susceptibility to insecticides in *An. stephensi*, the main malaria vecotr of coastal southern part of Iran.

2. Materials and methods

2.1. Study area

This study was carried out in Chabahar sea port (25°25′ N, 60°45′ E) Sistan and Baluchistan province, Iran. The larvae were collected from breeding places and then maintained in the insectary. All the tests were carried out on this species during spring 2011.

2.2. Mosquito sampling, rearing and identification

Collected larvae were reared under insectary conditions at 25-29 ℃, 12:12 (light:dark) h photo-period and 50%-70% relative humidity, and were fed with 10% aqueous sucrose solution.

The insectary is using for International Diploma Course of Malaria Programe and Planning which designated as WHO collborating centre. The field strain was reared and F1 progeny of 2-3-day-old sugar fed females were used for the tests. Mosquitoes were identified morphologically in the field by use of a standardized key for medically important anophelines of Iran.

2.3. Adult susceptibility test

Tests on adults were carried out according to the method of WHO[12]. At each test at least 100 mosquitoes representing 4-5 individual replicates of 20-25 adults were tested. To reduce variability in the replicates, sugar fed females were used. The exposure tubes were held in a vertical position during the tests. The exposure time for each insecticide was 1 h. The mortality rate was scored after a 24 h recovery period. Insecticide exposure took place in a room with a temperature of (27±2) ℃ and holding tubes were held in a room condition of (27±2) ℃ and relative humidity of 55%-60%. Error bars for each mortality were calculated based on statistical method at α =5%

2.4. Insecticides impregnated papers

The following insecticides impregnated papers were supplied by WHO: DDT 4%, lambdacyhalothrin 0.05%, deltamethrin 0.05%, permethrin 0.75%, cyfluthrin 0.15% and etofenprox 0.5%. For the control of organochlorine insecticides and pyrethroids insecticides the, mineral oil, and silicon oil impregnated papers were used, respectively.

2.5. Statistical analysis

The bioassay result was corrected using the Abbott formula when the control mortality was between 5% and 20%[13]. In some circumstance we ploted regression line based for calculation of LT_{50} and its 95% confidence intervals.

2.6. Criteria for susceptibility

The bioassay results were summarized in three resistance classes as defined by WHO[12]: (1) susceptible when mortality was 98% or higher, (2) possible resistant so called tolerant, when mortality was between 97% and 80%, and (3) resistant when the mortality was lower than 80%.

3. Results

The mortality of *An.stephensi* to lambdacyhalothrin, permethrin, cyfluthrin, deltamethrin, etofenprox and DDT was (88.0±3.2), (92.0±2.7), (52.0±5.0), (96.0±2.2), (90.0±3.0) and (41.0±5.7) percent, respectively (Table 1). Based on WHO criteria, it should be concluded that, this species is exhibiting resistance to DDT and some pyrethdoids. Table 2 shows the LT_{50} of *An. stephensi* to different insecticides.

Table 1

Mortality of *An. stephensi* to different insecticides at diagnostic dose for one hour exposure time and 24 h recovery period, southeastern Iran, 2011.

Insecticide	No. tested	No. Dead	Mortality (%)	Abbotts correction ±EB
Lambdacyhalothrin 0.05%	103	91	88.4	88.0±3.2
Permethrin 0.75%	99	92	92.3	92.0±2.7
Cyfluthrin 0.15%	100	55	55.0	52.0±5.0
Deltamethrin 0.05%	77	74	96.0	96.0±2.2
Etofenprox 0.50%	100	91	91.0	90.0±3.0
DDT 4.00%	75	34	45.0	41.0±5.7
Control	179	13	7.0	–

Tabale 2

Probit regresion line parameters of different insecticdes against *An.stephensi*, southeastern Iran, 2011.

Insecticides	A	B ± SE	LT_{50} ,95% *CI*	$\chi^2(df)$	*P*	Y=aX +b
Lambdacyhalothrin	−3.281	1.305 ±0.326	72.992 327.110 1101.824	11.215(3)	<0.05	Y = −3.281 + 1.305 X
Permethrin	−4.112	1.577 ± 0.152	314.375 404.947 507.061	6.093(4)	>0.05	Y = −4.112 + 1.577 X
Cyfluthrin	−4.682	1.863 ± 0.480	– 326.099 –	6.173(2)	<0.05	Y = −4.682 + 1.863 X
Deltamethrin	−3.529	1.468± 0.161	183.455 253.752 331.758	5.302(3)	>0.05	Y = −3.529 + 1.468 X
Etofenprox	−2.763	1.214 ± 0.157	120.613 188.678 263.165	7.780(3)	>0.05	Y = −2.763 + 1.214 X
DDT	−2.567	0.708 ± 0.144	2 126.921 4 208.983 16 430.847	5.613(3)	>0.05	Y = −2.567 + 0.708 X

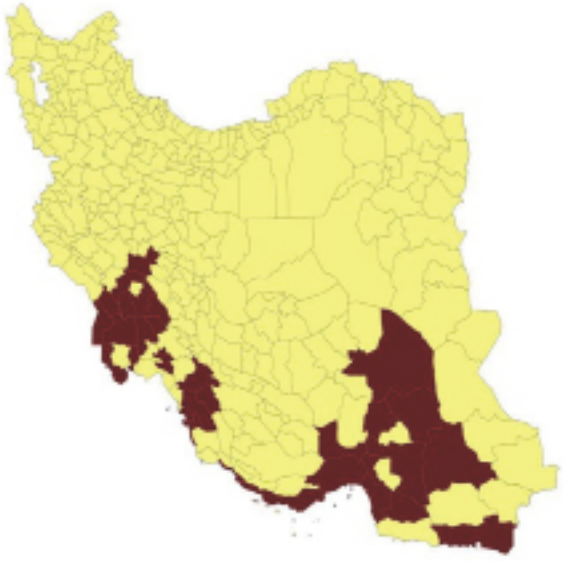

Figure 1. Distribution of *An. stephensi* in Iran.

4. Discussion

An. stephensi is found in Afghanistan, Bahrain, Bangladesh, China, Egypt, India, Iran, Iraq, Myanmar (Burma), Nepal, Oman, Pakistan, Saudi Arabia and Thailand. Previous investigations have shown that it is the most prevalent anopheline species in the malarious area of southern Iran[11]. This species is considered to be endophagous and endophilic. It is the main vector responsible for transmission of malaria to human in Persian Gulf area. Sporozoite rates of samples from the southern parts of Iran were reported to be between 0.2% and 1.8%. *An. stephensi* is one of the main malaria vectors in Hormozgan province[9].

In Iran, insecticide resistance monitoring of *An. stephensi* has been carried out regularly, and its resistance to DDT, dieldrin and malathion was first reported in 1957, 1960 and 1976, respectively. Recent studies have shown that *An. stephensi* from Bandar Abbas region is resistant to DDT and dieldrin[14-15]. Studies in other countries showed that

An. stephensi is resistant to DDT in Afghanistan, India, Pakistan, Iraq, Oman, United Arab Emirates and Saudi Arabia. In Iran, after appearance of malathion resistance in *An. stephensi*, propoxur was substituted in 1978 and it was used for 13 years. In recent years, pyrethroids have been used for residual spraying in malaria control programmes. From 1990 till 2009, pyrethroid resistance has been monitored in Iran showing that almost all tested *An. stephensi* were susceptible[17-20]. The results of new study conducted in Jiroft district, Kerman province, showed that this species is tolerant to DDT and dieldrin with mortality rates of 91.3% and 90% respectively; but susceptible to pyrethroid insecticides. *An. stephensi* larvae in Hormozgan province showed susceptibility to malathion, chlorpyrifos and temephos, but resistance to fenitrothion (73% mortality in WHO susceptibility tests)[21-22]. In Kahnooj district, south of Kerman province, it found to be resistant to DDT and dieldrin and susceptible to malathion, fenitrothion, propoxur, lambdacyhalothrin, permethrin, cyfluthrin, deltamethrin and etofenprox. That study showed larvae of *An. stephensi*, exhibited 100% mortality for temephos and malathion, but (44.00±4.32)% for discriminative dose of fenitrothion[17]. In a study it was shown that *An. stephesi* is susceptible to all types of insecticides in Kazerun in 1999 (Unpublished data). However different strains of Bandar Abbas, Iranshahr and Kazerun revealed resistant to DDT, dieldrin and fipronil and susceptible to lambdacyhalothrin, permethrin, cyfluthrin, and deltamethrin[15]. A new reoprt from Bashagard area, which is located in malarious area of Iran, revealed that diagnostic dose of insecticides exhibited resistance of *An. stephensi* to DDT and tolerance in *An. stephensi* to deltamethrin and bendiocarb. That study also reported (85.0±1.8)% mortality of *An. stephensi* larvae against diagnostic dose of fenthion[10].

Because *An. stephensi* is the main malaria vector in the country and its rearing is easy, it used for different biological assays such as larvicides, irritability tests, olfaction studies, bioassay tests for bednets and indoor residual spraying, biological tests for plant extraction and repellents[23-34].

Biochemical and molecular assays is recommended for understanding the mechanisms of pyrethroid resistance in this vector.

The waste use of pyrethroids for malaria vector control has increased in the past decade. They are used for insecticide treated net and indoor residual spraying. The major malaria vectors of the world have developed resistance to pyrehtoids and the resistance genes are spreading rapidly throughout world[35]. There are several reports on mechanism of pyreyhoid resistance in malaria vectors. However there are some control strategy for combating agianst resistance such as rotation, mixture, using biological control and Integrtaed Vector Management (IVM). The control strategy of the country is moving into the malaria elimination era, so that it is vital that the implications of insecticide resistance are fully understood. Monitoring and mapping of insecticide resistance in the country is an essential tool for implementation of malaria elimination.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Acknowledgments

The authors would like appreciate very much for all supports of School of Public Health & National Institute of Health research, Tehran University of Medical Sciences, World Health Organization and postgraduate students for helping for conduction of study. Special thanks to Mr. AbdulRasool Mojahedi, Mr. Abbas Pakari, Mr. Hassan Javdan and Mr. Alireza Sanei Dehkordi for their kind assitance.

References

[1] Abai MR, Mehravaran A, Vatandoost H, Oshaghi MA, Javadian E, Mashayekhi M, et al. Comparative performance of imagicides on *Anopheles stephensi*, main malaria vector in a malarious area, southern Iran. *J Vector Borne Dis* 2008; **45:** 307-312.

[2] Oshaghi MA, Vatandoost H, Goruhi A, Abai MR, Madjidpour A, Arshai S, et al. Anopheline species composition in borderline of Iran-Azerbaijan. *Acta Trop* 2011; **119(1):** 44-49.

[3] Vatandoost H, Emami SN, Oshaghi MA, Abai MR. Ecology of malaria vector *Anopheles culicifacies* in a malarious area of Sistan va Baluchestan province, south-east Islamic Republic of Iran. *Eastern Mediterr Health J* 2011; **17(5):** 439-445

[4] Oshaghi MA, Yaghoobi F, Vatandoost H, Abai MR, Akbarzadeh K. *Anopheles stephensi* biological forms, geographical distribution, and malaria transmission in malarious regions in Iran. *Pak J Biol Sci* 2006; **9:** 294-298.

[5] Oshaghi MA, Yaaghoobi F, Abaei MR. Pattern of mitochondrial DNA variation between and within *Anopheles stephensi* (Diptera: Culicidae) biological forms suggests extensive gene flow. *Acta Trop* 2006; **99:** 226-233.

[6] Chavshin AR, Oshaghi MA, Vatandoost H, Pourmand MR, Raeisi A, Enayati AA, et al. Identification of bacterial microflora in the midgut of the larvae and adult of wild caught *Anopheles stephensi*: A step toward finding suitable paratransgenesis candidates. *Acta Trop* 2012; **121(2):**129-134.

[7] Sedaghat MM, Linton YM, Oshaghi MA, Vatandoost H, Harbach RE. The *Anopheles maculipennis* complex (Diptera: Culicidae) in Iran: molecular characterisation and recognition of a new species. *Bull Entomol Res* 2003; **93:** 527-535.

[8] Vatandoost H, Abai MR. Irritability of *Anopheles sacharovi* to different insecticides in a malaria- prone area, Iran. *Asian Pac J Trop Med* 2012; **5(2):** 113-116.

[9] Vatandoost H, Oshaghi MA, Abaie MR, Shahi M, Yaghoobi F, Baghaii M, et al. Bionomics of *Anopheles stephensi* Liston in the malarious area of Hormozgan province, southern Iran. *Acta Trop*

2006; **97:** 196-205.

[10]Hanafi-Bojd AA, Vatandoost H, Oshaghi MA, Haghdoost AA, Shahi M, Sedaghat MM, et al. Entomological and epidemiological attributes for malaria transmission and implementation of vector control in southern Iran. *Acta Trop* 2012; **121:** 85-92.

[11]Hanafi-Bojd AA, Azari-Hamidian S, Vatandoost H, Charrahy Z. Spatio-temporal distribution of malaria vectors (Diptera: Culicidae) across different climatic zones of Iran. *Asian Pac J Trop Med* 2011; **6:** 498-504.

[12]World Health Organization. *Entomology and vector control. Training module on malaria control. Guide for participants*. World Health Organization: Global Malaria Programme; 2002, p. 204.

[13]Abbott WS. A method for computing the effectiveness of an insecticide. *J Econ Entomol* 1925; **18:** 265-267.

[14]Davari B, Vatandoost H, Oshaghi MA, Ladonni H, Enayati AA, Shaeghi M, et al. Selection of *Anopheles stephensi* with DDT and dieldrin and cross-resistance spectrum to pyrethroids and fipronil. *Pest Biochem Physiol* 2007; **89:** 97-103.

[15]Davari B, Vatandoost H, Ladonni H, Shaeghi M, Oshaghi MA, Basseri HR, et al. Comparative efficacy of different imagicides against different strains of *Anopheles stephensi* in the malarious areas of Iran, 2004-2005. *Pak J Biol Sci* 2006; **9**: 885-892.

[16]Tavassoli M, Shayeghi M, Abai MR, Vatandoost H, Khoobdel M, Salari M, et al. Repellency effects of essential oils of myrtle (*Myrtus communis* L.), marigold (*Calendula officinalis* L.) compared with DEET against *Anopheles stephensi* on human volunteers. *Iran J Arthropod-Borne Dis* 2011; **5(2):** 10-22.

[17]Vatandoost H, Mashayekhi M, Abaie MR, Aflatoonian MR, Hanafi-Bojd AA, Sharifi I. Monitoring of insecticides resistance in main malaria vectors in a malarious area of Kahnooj district, Kerman province, southeastern Iran. *J Vec Borne Di*s 2005; **42:** 100-108.

[18]Vatandoost H, Borhani N. Susceptibility level and irritability of synthetic pyrethroids against main malaria vectors in the endemic areas of Iran. *Acta Med Iran* 2004; **42:** 247-255.

[19]Mehravaran A, Vatandoost H, Oshaghi MA, Abai MR, Edalat H, Javadian E, et al. Ecology of *Anopheles stephensi* in a Malarious area, Southeast of Iran. *Acta Medica Iranica* 2012; **50(1):** 61-65.

[20]Vatandoost H, Ramin E, Rassi Y, Abai MR. Stability and wash resistance of local made mosquito bednets and detergents treated with pyrethroids against *Anopheles stephensi. Iran J Arthropod-Borne Dis* 2009; **3:** 19-28.

[21]Vatandoost H, Hanafi-Bojd AA. Current resistant status of *Anopheles stephensi* Liston to different larvicides in Hormozgan province, southeastern Iran. *Pak J Biol Sci* 2005; **8**: 1568-1570.

[22]Vatandoost H, Shahi M, 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 Public Health* 2004; **35:** 22-25.

[23]Sedaghat MM, Sanei-Dehkordi A, Abai MR, Khanavi M, Mohtarami F, Salim Abadi Y, et al. Larvicidal activity of essential oils of apiaceae plant. *Iran J Arthropod-Borne Dis* 2011; **5(2):** 51-59.

[24]Vatandoost H, Khazani A, Rafinejad J, Khoobdel M, Kebriai-Zadeh A, Abai MR. Comparative efficacy of Neem and dimethyl phthalate (DMP) against malaria vector, *Anopheles stephensi* (Diptera: Culicidae). *Asian Pac J Trop Med* 2008; **1:** 1-6.

[25]Vatandoost H, Hanafi-Bojd AA. Laboratory evaluation of 3 repellents against *Anopheles stephensi* in the Islamic Republic of Iran. *East Mediterr Health J* 2008; **14:** 260-267.

[26]Vatandoost H, Ramin E, Rassi Y, Abai MR. Stability and wash resistance of local made mosquito bednets and detergents treated with pyrethroids against *Anopheles stephensi. Iran J Arthropod-Borne Dis* 2009; **3:** 19-28.

[27]Rafinejad J, Vatandoost H, Nikpoor F, Abai MR, Shaeghi M, Duchen S, et al. Effect of washing on the bioefficacy of insecticide-treated nets (ITNs) and long-lasting insecticidal nets (LLINs) against main malaria vector *Anopheles stephensi* by three bioassay methods. *J Vector Borne Dis* 2006; **45:** 143-150.

[28]Vatandoost H, Dehakia M, Djavadia E, Abai MR, Duchson S. Comparative study on the efficacy of lambdacyhalothrin and bifenthrin on torn nets against the malaria vector, *Anopheles stephensi* as assessed by tunnel test method. *J Vector Borne Dis* 2006; **43:** 133-135.

[29]Vatandoost H, Abai MR, Abbasi M, Shaeghi M, Abtahi M, Rafie F. Designing of a laboratory model for evaluation of the residual effects of deltamethrin (K-othrine WP 5%) on different surfaces against malaria vector, *Anopheles stephensi* (Diptera: Culicidae). *J Vector Borne Dis* 2009; **46**: 261-267.

[30]Khanavi M, Bagheri Toulabi P, Abai MR, Sadat N, Hadjiakhoondi F, Hadjiakhoondi A, et al. Larvicidal activity of marine algae, *Sargassum swartzii* and *Chondria dasyphylla*, against malaria vector, *Anopheles stephensi*. *J Vector Borne Dis* 2011; **48:** 241-244.

[31]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). *Pharmacogn Res* 2011; **3:** 135-139.

[32]Shahi M, Hanafi-Bojd AA, Iranshahi M, Vatandoost H, Hanafi-Bojd MY. Larvicidal efficacy of latex and extract of *Calotropis procera* (Gentianales: Asclepiadaceae) against *Culex quinquefasciatus* and *Anopheles stephensi* (Diptera: Culicidae). *J Vector Borne Dis* 2010; **47:** 185-188.

[33]Omrani SM, Vatandoost H, Oshaghi MA, Shokri F, Guerin PM, Yaghoobi-Ershadi MR, et al. Fabrication of an olfactometer for mosquito behavioural studies. *J Vector Borne Dis* 2010; **47:** 17-25.

[34]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. Iran J Pharm Sci* 2006; **2:** 97-102.

[35]Himeidan YE, Abdel Hamid MM, Jones CM, Ranson H. Extensive permethrin and DDT resistance in *Anopheles arabiensis* from eastern and central Sudan. *Parasit Vectors* 2011; **4:** 154.