# Resistance status of main malaria vector, *Anopheles stephensi* Liston (Diptera: Culicidae) to insecticides in a malaria Endemic Area, Southern Iran

Madineh Abbasi[1], Ahmad Ali Hanafi−Bojd[1✉], Mohammad Reza Yaghoobi−Ershadi[1], Hassan Vatandoost[1,2], Mohammad Ali Oshaghi[1], Teimour Hazratian[3], Mohammad Mehdi Sedaghat[1], Sajjad Fekri[4], Reza Safari[4], Abdol Rasoul Mojahedi[4], Yousef Salari[4]

[1]Department of Medical Entomology & Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

[2]Department of Environmental Chemical Pollutants and Pesticides, Institute for Environmental Research, Tehran University of Medical Sciences, Tehran, Iran

[3]Department of Parasitology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

[3]Department of Diseases Control, Hormozgan University of Medical Sciences, Bandar Abbas, Iran

## ARTICLE INFO

## ABSTRACT

**Objective:** To evaluate the susceptibility of *Anopheles stephensi* (*An. stephensi*) Liston, the main malaria vector in southern Iran, to WHO recommended insecticides. **Methods:** Larvae of *An. stephensi* were collected from three different larval habitats in both urban and rural area of Bandar Abbas city and one rural area in Rudan county southern Iran. WHO standard method was used for evaluation of adult and larval mosquito susceptibility. Bendiocarb, permethrin, lambda-cyhalothrin, deltamethrin as insecticide and temephos and chlorpyriphos as larvicide were used at the diagnostic dosages recommended by WHO. **Results:** Findings of this study showed all larval populations of *An. stephensi* were completely susceptible to temephos and candidate for resistance to chlorpyriphos. Adult mosquitoes in rural areas of Bandar Abbas city were resistant to pyrethroid and carbamate insecticides. **Conclusion:** Comparison of the results of this survey with previous studies indicates that the resistance to pyrethroids and carbamates in this malaria endemic region is increasing. Wide use of pesticides in agriculture is certainly effective in increasing resistance. The inter-sectoral coordination and collaboration in health and agriculture seem to be necessary to manage insecticide resistance in malaria vectors.

## 1. Introduction

Malaria is considered one of the most important infectious blood diseases in tropical and subtropical developing countries around the world. In 2016, an estimated 216 million cases of malaria occurred worldwide, of which 445 000 has resulted in deaths[1]. Malaria elimination is the common goal of World Health Organization (WHO) and the health system in Iran. Elimination of malaria is defined as the reduction to zero of the incidence of locally acquired infection from human malaria parasites in a defined geographical area as a result of planned attempts[2]. Following a decline in malaria cases in recent years, the malaria elimination program, technically supported by WHO, has initiated since 2009 in Iran[3].

Due to successful implementation of malaria control plan, a reduction trend from 1 847 to 81 cases between 2010 and 2016 was found in indigenous malaria positive cases in Iran[1].

Long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS) and larviciding are the most powerful and most broadly applied interventions against malaria vectors. These methods work by reducing human-vector contact and decreasing the lifespan of female mosquitoes so that they do not survive long enough to transmit the parasite[4,5]. Other personal protection method is to wear impregnated clothes for military and other field workers in the malaria endemic areas[6]. In the short time after the 1940s, when insecticides were widely used for the first time in the world, insecticide resistance, which has unintended side effects in controlling malaria vectors, has been raised. Identifying and preventing the development and spread of insecticide resistance in malaria endemic countries are very important and the program for monitoring and managing insecticide resistance is one of the national strategies of these countries. The emergence and expansion of resistance over the last two decades has referred to a high dependence on only one group of insecticides (pyrethroids) for both public health and agricultural purposes.

In order to maintain significant gains in malaria control and the success of malaria elimination program, it is critical to prevent further development of resistance and to maintain the effectiveness of vector control interventions[7]. Since 2010, resistance to at least one class of insecticides has been reported in at least one malaria vector species in 60 of the 96 malaria-endemic countries that conducted monitoring; also, 49 countries reported resistance to at least two classes of insecticides[7].

There are seven *Anopheles* mosquitoes as the malaria vectors in Iran, *i.e. Anopheles stephensi* (*An. stephensi*), *Anopheles culicifacies s.l.*, *Anopheles superpictus s.l.*, *Anopheles fluviatilis s.l.*, *Anopheles maculipennis* complex including *Anopheles sacharovi* and *Anopheles dthali*[8,9]. Despite the implementation of the malaria control program over the years, malaria is still a major health problem in the southern regions of Iran. Most local malaria transmission in Iran occurs in Sistan va Baluchestan, Hormozgan and Kerman provinces[2,10].

There is a long history of using insecticides and resistance to them in malaria vectors of Iran. Earlier studies showed resistance of *An. stephensi* to DDT[11], dieldrin[12], malathion[13], lambda-cyhalothrin[14] and deltamethrin[15,16]; *Anopheles culicifacies s.l.* to DDT[17], *Anopheles dthali* to DDT[18], and *Anopheles maculipennis* complex to DDT, propoxur, bendiocarb and malathion[19] in Iran. In recent years, pyrethroids have been used for residual spraying and long-lasting bed net treatment in national malaria control program.

The distribution of *An. stephensi* is from China, Thailand, Bangladesh, India, Pakistan, Afghanistan, Iran, Iraq, Oman, Myanmar (Burma), Nepal, Bahrain, Saudi Arabia and Egypt[8]. According to the earlier studies, *An. stephensi* is the dominant anopheline species in coastal areas of southern Iran[8]. This species is the main and the most important malaria vector in Hormozgan province, especially in plane areas[20].

One of widely used methods for identifying resistance to insecticides in mosquitoes is a simple response-to-exposure test developed by world health organization[21]. This test will differentiate between susceptible and resistant populations of mosquitoes and intended to be used as a field and laboratory surveillance tool[21]. The aim of this study was to evaluate the current susceptibility of *An. stephensi* to common chemical insecticides in Southern Iran, for implementing in vector control program.

## 2. Materials and methods

### 2.1. Study area

This survey was conducted on the samples of *An. stephensi* collected from Bandar Abbas and Rudan counties, Hormozgan province, Southern Iran.

Bandar Abbas (with an average altitude of 9 m above sea level and geographical coordinates: $27^{o}11'11.4''N$; $56^{o}16'50.9''E$), the capital city of Hormozgan province, is located on the southern coast of Iran, north of the Persian Gulf (Figure 1). This city has strategic location in the Strait of Hormoz and is the main site of the naval base of Iran. According to the Koppen BWh climate classification, the area has hot desert climate (tropical), maximum temperature at summer can reach 49 ℃ in summer and the minimum temperature may drop to 5 ℃ in winter. Annual precipitation is about 170 mm and average relative humidity is 65%.

Rudan County is composed of topographic and mountainous features. The plain and smooth sections cover most of the central and southern regions. The lands of this area are often flat and between 150 and 700 meters above sea level. The mountainous area has significant heights in the north, west and east. It has a very hot and dry climate, so the average of annual temperature shows the highest degree and lowest relative humidity percentage in Hormozgan province. The average annual precipitation in this county is 250 mm.
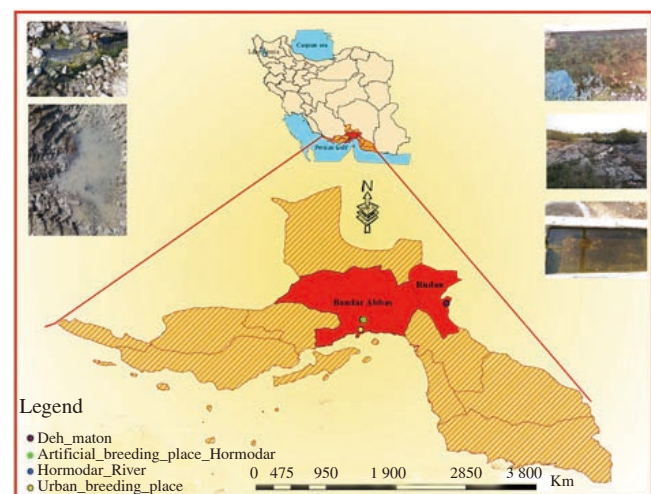


**Figure 1.** Geographical position of the study area and collection sites in south of Iran.

## 2.2. Sampling

This study was carried out from February until the end of May 2018 with aims of finding the best larval habitats for *An. stephensi*, sampling and conducting the susceptibility tests. Geographic coordinates of the larvae and adult mosquito collection sites were recorded by using a GPS device. Larval stage of *An. stephensi* was collected from different larval habitats in the urban area of Bandar Abbas city (27°12'18.71"N; 56°17'32.11"E), Hormoodar village (27°19'14.72"N; 56°19'14.80"E) in Bandar Abbas County, and Deh Matoon village (27°30'43.05"N; 57°17'50.61"E) in Rudan County, by using standard dipping method. Physical characteristics of the breeding sites were registered in the relevant forms and chemicals elements were tested in water quality laboratory, Hormozgan University of Medical Sciences. The collected larvae were transferred to the insectary for rearing to adults. Hormoodar population successfully reared and eggs float ridges for detection of *An. stephensi* biotype was studied[22]. A number of specimens from each breeding site were mounted and identified using morphological key[23]. Breeding sites with *An. stephensi* were considered for further sampling and tests.

## 2.3. Adult susceptibility tests

According to the guideline developed by WHO[21], diagnostic dose of insecticides was used against female adult and larvae of *An. stephensi* mosquitoes. A total of four replicates were used as exposure (20-25 mosquitos per test, totally 100 specimens) for each insecticide and two replicates as control (totally 50 specimens). Test kits and insecticide-impregnated papers were purchased from the WHO collaborative center in University Sains Malaysia (USM), Penang, Malaysia. Bendiocarb 0.1% (Batch No.159, Expiry date: March 2019), permethrin 0.75% (Batch No. 428, Expiry date: August 2018), lambda-cyhalothrin 0.05% (Batch No. 262, Expiry date: July 2018) and deltamethrin 0.05% (Batch No. 527, Expiry date: August 2018) were used against adult mosquitoes. The control test mosquitoes were exposed to papers impregnated only with the appropriate carrier oil for pyrethroids (Batch No. 262, Expiry date: August 2018) and carbamates (Batch No. 181, Expiry date: July 2020); that was without insecticide.

Tests were performed on the $F_1$ progeny of wild-caught adult females of Hormoodar population, 3–5 days old fed with sugar. The mosquitoes were exposed to different insecticides by 60-minutes exposure time and 24 hours recovery period. A pad of a cotton wool soaked in 10% sugar solution was provided as feeding source of mosquitoes during the recovery period. Tests were carried out in an insectary maintained at (27±2)℃ temperature and (75±10)% relative humidity, 14:10 light: dark[21]. Mortality rate for each test was estimated at the end of tests.

## 2.4. Larval susceptibility tests

Two larvicides, *i.e.* temephos (0.25 mg/L) and chlorpyriphos (0.025 mg/L) were used at the diagnostic dose provided by WHO[24]. Collected larvae from breeding places were transferred to insectary of Bandar Abbas Research Station. After 24 h recovery time, late $3^{rd}$ or early $4^{th}$ instars were used for the tests by WHO test procedure for mosquito larvae[25] and mortality rate was recorded after 24 h exposure time. Totally 100 specimens were used per each test and 50 specimens per each control. Tests were carried out in an insectary maintained at (27±2)℃ temperature and (75±10)% relative humidity.

## 2.5. Data analysis

If the results of control mortality were less than 5%, the tests were considered to be correct, but in the cases of between 5%–20%, it is necessary to correct by Abbotts' formula and for more than 20% the tests were discarded and repeated by new specimens. Based on the latest guideline of WHO, mortality of the test between 98%–100% was considered as susceptible, 90%–97% as candidate of resistance that should be confirmed using specific methods, and less than 90% was considered as resistant[21].

## 3. Results

The physical condition and chemical elements of the larval habitats of *An. stephensi* during the study period is shown in Tables 1 and 2. The biotype of *An. stephensi* that was reared from Hormoodar was intermediate because the number of eggs ridges was 15.44±0.82.

**Table 1**

Physical characters of *Anopheles stephensi* breeding places, Bandar Abbas County, Southern Iran, 2018.

| Larval habitat characters | Hormoodar natural habitat | Hormoodar artificial habitat | Urban habitat |
|---|---|---|---|
| Habitat type | River | Cement pool | Leakage |
| Substrate | Sandy | Cement | Muddy |
| Sunlight | + | - | + |
| Algae | + | + | + |
| Vegetation | + | - | + |
| Permanency | Permanent | Temporary | Temporary |

**Table 2**

Chemical elements of *Anopheles stephensi* breeding places, Hormoodar village, Bandar Abbas County, Southern Iran, 2018.

| Chemical elements | March | April | May | Mean ± SD |
|---|---|---|---|---|
| Turbidity (NTU) | 0.67 | 0.74 | 0.72 | 0.71±0.03 |
| Conductivity (μS/cm) | 2173 | 3120 | 2950 | 2 747.60±504.80 |
| pH | 6.9 | 6.9 | 6.8 | 6.86±0.05 |
| $CL^-$(mg/L) | 292 | 496 | 461 | 416.30±109.00 |
| $Ca^{++}$(mg/L) | 69.3 | 224 | 164.4 | 152.50±78.00 |
| $Mg^{++}$(mg/L) | 211.2 | 132.5 | 199.2 | 180.90±42.40 |
| $CaCO_3$(mg/L) | 69.6 | 130.4 | 72 | 90.60±34.40 |
| $CaCO_3$(mg/L) | 56.8 | 1112 | 134.8 | 434.50±587.90 |
| $HCO_3$(mg/L) | 818 | 159.1 | 798 | 591.70±374.70 |

As shown in Table 3, our findings showed resistance to both pyrethroid and carbamate insecticides with mortality rates of

**Table 3**

Response of *Anopheles stephensi* collected from Southern Iran to different insecticides/larvicides, 2018.

| Population | Insecticide/Larvicide | Exposure time (h) | Total (No.) | Mortality (%) | | Resistant status |
|---|---|---|---|---|---|---|
| | | | | Mean±SD | Control | |
| Hormoodar | Bendiocarb 0.1% | 1 | 223 | 86.10±3.61 | 0.00±0.00 | R |
| | Permethrin 0.75% | 1 | 220 | 82.27±5.20 | 0.00±0.00 | R |
| | Lambda-cyhalothrin 0.05% | 1 | 102 | 84.31±2.62 | 0.00±0.00 | R |
| | Deltamethrin 0.05% | 1 | 116 | 89.66±2.34 | 3.03±0.00 | R |
| | Temephos (0.25 mg/L) | 24 | 100 | 100.00±0.00 | 0.00±0.00 | S |
| | Chlorpyriphos (0.025 mg/L) | 24 | 101 | 97.92±0.99 | 0.00±0.00 | CR |
| Bandar Abbas City | Temephos (0.25 mg/L) | 24 | 100 | 100.00±0.00 | 2.13±0.00 | S |
| | Chlorpyriphos (0.025 mg/L) | 24 | 99 | 96.67±0.70 | 2.13±0.00 | CR |
| Deh Matoon | Temephos (0.25 mg/L) | 24 | 106 | 100.00±0.00 | 0.00±0.00 | S |
| | Chlorpyriphos (0.025 mg/L) | 24 | 106 | 96.22±1.40 | 0.00±0.00 | CR |

R= Resistance, S= Susceptible, CR= Candidate of resistance.

(84.31±2.62)%, (89.66±2.34)%, (82.27±5.20)% and (86.10±3.61)% to lambda-cyhalothrin, deltamethrin, permethrin and bendiocarb, respectively. Indications of resistance to chlorpyriphos were observed with mortality rates of (96.22±1.40)%, (96.67±0.70)% and (97.92±0.99)% in Deh Matoon, Bandar Abbas city, and Hormoodar populations. Findings of this study showed three populations (Hormoodar, Deh Matoon, and Bandar Abbas city) of *An. stephensi* from Bandar Abbas and Rudan Counties, Southern Iran, were completely susceptible to temephos and candidate of resistance to chlorpyriphos in the diagnostic dose recommended by WHO. *An. stephensi* showed resistance to both pyrethroid and carbamate insecticide groups that used in this study (lambda-cyhalothrin, deltamethrin, permethrin, and bendiocarb) in Hormoodar population.

## 4. Discussion

*An. stephensi* is the main vector responsible for transmission of the pathogen that causes human malaria, *i.e. Plasmodium vivax* and *Plasmodium falciparum*, in the northern plates of Persian Gulf[24]. It considered being endophagous and endophilic, so it is exposed to the insecticides used in malaria vector control program. With the prolonged and continuous use of insecticides, the selection of insecticide resistant individuals occurs, increasing populations of mosquitoes more tolerant and less sensitive to the product and, therefore, promoting the reduction of the effectiveness of insecticides[20].

Among the insecticide used in this study, only temephos induced 100% mortality. Earlier studies showed *An. stephensi* was susceptible to this larvicide in south of Iran[26,27] and resistant in southwest[28]. A recent study showed that the altered enzymes lead to temephos resistance in *An. stephensi*[29]. There is a report of resistance to

temephos from *An. stephensi* in the Al-Dhahira region from Oman country that located in the Middle East[30].

First clues of pyrethroid resistance was found at 2011 from Iran[15] and then confirmed in another study conducted in Hormozgan province[27]. The results of a new study at 2015 conducted in Chabahar district, Sistan va Baluchestan province, showed that *An. stephensi* was resistant to DDT, tolerant to malathion, propoxur, cyfluthrin, and lambda-cyhalothrin and susceptible to deltamethrin[18]. Another study in this district determined tolerance to deltamethrin and permethrin and resistance to lambda-cyhalothrin[16]. Two earlier studies in Jask district, east of our study area in Hormozgan province, showed *An. stephensi* was susceptible to pyrethroids that we used in our study, although it was resistant to DDT and dieldrin[14,31]. A recent study in Hormoodar area showed *An. stephensi* was candidate of resistance to deltamethrin with 91% mortality rate[32]. In the Punjab province, Pakistan, *An. stephensi* was susceptible to permethrin and deltamethrin, while confirmed or potential resistance to cypermethrin, $\lambda$-cyhalothrin, and cyfluthrin was observed from all the study sites[33]. According to a report published in 2016, *An. stephensi* was resistant against all tested insecticides in Afghanistan[34].

Regarding carbamates, which are using in rotation with pyrethroids in the national malaria vector control program in Iran, first indication of potential resistance in *An. stephensi* was reported from the southeast of Iran, with 95% mortality[27]. Although next study conducted in the area[35] resulted to 99.3% mortality, a study conducted in 2015 in 10 sentinel sites in southern Iran (Ministry of Health, Unpublished data) confirmed resistance to bendiocarb (86.41% mortality) in Kerman province and potential resistance to this insecticide in Sistan va Baluchestan province. In a cross-sectional study at 2010, *An. stephensi* (Minab population) was susceptible to bendiocarb and deltamethrin (100% mortality rate);

however, it was candidate of resistance to DDT and fenitrothion with mortality rates of 97% and 95%, respectively[35]. Slight resistance of *An. stephensi* to bendiocarb reported from Afghanistan[35]. Among other main malaria vectors, *Anopheles gambiae* is reported to be resistant to bendiocarb in West African[36].

Inquire about the history of insecticide use in the agricultural sector from the farmers in the study area showed organophosphate and pyrethroid insecticides (especially deltamethrin) have an extensive use in agriculture and horticulture. Cross resistance maybe the reason for carbamate resistance in the study areas, where organophosphates have an extensive use. Obviously, the excessive use of this insecticide can also contribute to the resistance of other insects living in the area. Also, the waste use of pyrethroids insecticide in treated net and indoor residual spraying for malaria vector control has increased in the past decade.

Selection of resistant individuals to pyrethroids was observed in major malaria vectors of the world, and the resistance genes are spreading rapidly throughout the world[37]. So, monitoring and mapping of insecticide resistance is an essential tool for implementation of malaria elimination in the areas under coverage of program. Although enzymes have role in the resistant of *An. stephensi* to DDT and pyrethroids[38] in Iran, it is found that piperonyl butoxide can suppress resistance to pyrethroid[32]. Therefore, it seems using synergists can be suggested as a new tool for prevention of pyrethroids resistance. However, there are a number of control strategies for combating against resistance such as rotation, mixture, using biological control and integrated vector management. These methods should be considered in vector control programs in future.

It can be concluded that resistance to pyrethroids in the main malaria vector of southern Iran is increasing. Extensive use of pyrethroids in agriculture is certainly effective in creating this event. The intersectoral coordination and collaboration in health and agriculture seem to be necessary to manage the resistance. Larviciding by temephos and *Bacillus thuringiensis* may be considered as the main method for control of *An. stephensi* in urban areas of Bandar Abbas, while it can help reduce the mosquito density in rural areas.

## Conflicting interest statement

Authors declare that they have no conflict of ineterst.

## Acknowledgements

## Foundation project

## References

[1] World Health Organization. *Malaria report. WHO* 2017. Geneva: WHO; 2017.

[2] World Health Organization. *Malaria elimination, Guide for participants. WHO 2016*; Geneva: WHO; 2016.

[3] Hemami MR, Sari AA, Raeisi A, Vatandoost H, Majdzadeh R. Malaria elimination in Iran, importance and challenges. *Int J Prev Med* 2013; **4**(1):88.

[4] World Health Organization. A *framework for malaria elimination. WHO* 2017; Geneva: WHO; 2017.

[5] United States Agency for International Development. Integrated vector management programs for malaria vector control. Version 2017. USA: USAID; 2017, p. 5.

[6] Khoobdel M, Shayeghi M, Vatandoost H, Rassi Y, Abaei MR, Ladonni H, et al. Field evaluation of permethrin-treated military uniforms against *Anopheles stephensi* and 4 species of *Culex* (Diptera: Culicidae) in Iran. *J Entomol* 2006; **3**(2): 108-118.

[7] World Health Organization. Insecticide resistance. Geneva: WHO; 2017. [Online] Available from: http://www.who.int/malaria/areas/vector_control/insecticide_resistance/en/ [Accessed on 17th November 2017].

[8] Salahi-Moghaddam A, Khoshdel A, Dalaei H, Pakdad K, Nutifafa GG, Sedaghat MM. Spatial changes in the distribution of malaria vectors during the past 5 decades in Iran. *Acta Trop* 2017; **166**: 45-53.

[9] Saghafipour A, Abai MR, Farzinnia B, Nafar R, Ladonni H, Azari-Hamidian S. Mosquito (Diptera: culicidae) fauna of Qom Province, Iran. *J Arthropod–Borne Dis* 2012; **6**(1): 54-61.

[10]Sharifi-Sarasiabi K, Haghighi A, Kazemi B, Taghipour N, Mojarad EN, Gachkar L. Molecular surveillance of *Plasmodium vivax* and *Plasmodium falciparum* DHFR mutations in isolates from Southern Iran. *Rev Inst Med Trop Sao Paulo* 2016; **58**:5.

[11]Mofidi Ch, Samimi B, Eshghi N, Ghiassudin M. Further studies of anopheline susceptibility to insecticide in Iran: result of Bosvine and Nash methods. *Inst Iran Parasit Malariol Publ Tehran* 1958; **585**:7.

[12]Mofidi, Ch., Samimi, B. Resistance of *Anopheles stephensi* to dieldrin, Iran. *Inst Iran Parasit Malariol Publ Tehran* 1960; **650**: 3–4.

[13]Manouchehri AV, Zaini A, Yazdanpanah H. Selection for resistance to malathion in *Anopheles stephensi* mysorensis. *Mosq News* 1975; **35**: 278–280.

[14]Zare M, Soleimani-Ahmadi M, Davoodi SH, Sanei-Dehkordi AR. Insecticide susceptibility of *Anopheles stephensi* to DDT and current insecticides in an elimination area in Iran, *Parasite Vectors* 2016; **9**: 571.

[15]Vatandoost H, Hanafi-Bojd AA. Indication of pyrethroid resistance in the main malaria vector, *Anopheles stephensi* from Iran. *Asian Pac J Trop Med* 2012; **5**(9): 722-726.

[16]Gorouhi MA, Vatandoost H, Oshaghi MA, Raeisi A, Enayati AA, Mirhendi H, et al. Current susceptibility status of *Anopheles stephensi* (Diptera: Culicidae) to different imagicides in a malarious area, Southeastern of Iran. *J Arthropod–Borne Dis* 2016; **10**(4): 493–500.

[17]Vatandoost H, Hanafi-Bojd AA, Raeisi A, Abai MR, Nikpour F. Ecology, monitoring and mapping of insecticide resistance of malaria vector, *Anopheles culicifacies* (Diptera: Culicidae) to different imagicides in Iran. *Asian Pac J Trop Dis* 2017; **7**(1): 53–56.

[18]Fathian M, Vatandoost H, Moosa- Kazemi SH, Raeisi A, Yaghoobi-Ershadi MR, Oshaghi MA, et al. Susceptibility of culicidae mosquitoes to some insecticides recommended by WHO in a malaria endemic area of Southeastern Iran. *J Arthropod–Borne Dis* 2015; **9**(1): 22-34.

[19]Chavshin AR, Dabiri F, Vatandoost H, Mohammadi Bavani M. Susceptibility of *Anopheles maculipennis* to different classes of insecticides in West Azarbaijan Province, Northwestern Iran. *Asian Pac J Trop Biomed* 2015; **5**(5): 403-406.

[20]Vatandoost H, Oshaghi MA, Abaie MR, Shahi M, Yaaghoobi F, Baghaii M, et al. Bionomics of *Anopheles stephensi* Liston in the malarious area of Hormozgan Province, southern Iran. *Acta Trop* 2006; **97**(2): 196-203.

[21]WHO. *Test procedures for insecticide resistance monitoring in malaria vector mosquitoes*. 2nd ed. Geneva: WHO; 2016, p.55.

[22]Subbarao SK, Vasantha K, Adak T, Sharma VP, Curtis CF. Egg-float ridge number in *Anopheles stephensi*: ecological variation and genetic analysis. *Med Vet Entomol* 1987; **1**(3): 265-271.

[23]Azari-Hamidian S, Harbach RE. Keys to the adult females and fourth instar larvae of the mosquitoes of Iran (Diptera: Culicidae). *Zootaxa* 2009; **2078**: 1– 33.

[24]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.

[25]World Health Organization. *Guidelines for laboratory and field testing of mosquito larvicides*. Geneva: WHO; 2005.

[26]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 Public Health* 2004; **35**: 22–25.

[27]Hanafi-Bojd AA, Vatandoost H, Oshaghi MA, Charrahy Z, Haghdoost A, Zamani G, et al. Spatial analysis and mapping of malaria risk in an endemic area, south of Iran: a GIS based decision making for planning of control. *Acta Trop* 2012; **122**(1): 132-137.

[28]Abai MR, Hanafi-Bojd AA, and Vatandoost H. Laboratory evaluation of temephos against *Anopheles stephensi* and *Culex pipiens* Larvae in Iran. *J Arthropod–Borne Dis* 2016; **10**(4): 510–518.

[29]Soltani A, Vatandoost H, Oshaghi MA, Maleki Ravasan N, Enayati AA, Asgarian F. Resistance mechanisms of *Anopheles stephensi* (Diptera: Culicidae) to Temephos. *J Arthropod–Borne Dis* 2015; **9**(1): 71–83.

[30]Anderasen MH. Emerging resistance to temephos in *Anopheles stephensi* in the Al-Dhahira region of Oman. *Mission Report WHO* 2006; 1–13.

[31]Yeryan M, Basseri HR, Hanafi-Bojd AA, Raeisi A, Edalat H, Safari R. Bio-ecology of malaria vectors in an endemic area, Southeast of Iran. *Asian Pac J Trop Med* 2016; **9**(1): 32–38.

[32]Nikpour F, Vatandoost H, Hanafi-Bojd AA, Raeisi A, Ranjbar M, Enayati AA, et al. Evaluation of deltamethrin in combination of piperonyl butoxide (PBO) against pyrethroid resistant, malaria vector, *Anopheles stephensi* in IRS implementation: An experimental semi-filed trial in Iran. *J Arthropod–Borne Dis* 2017; **11**(4): 469–469.

[33]Ali Khan HA, Akram W, Lee S. Resistance to selected pyrethroid insecticides in the malaria mosquito, *Anopheles stephensi* (Diptera: Culicidae), From Punjab, Pakistan. *J Med Entomol* 2018; **55**(3): 735-738.

[34]Ahmad M, Buhler C, Pignatelli P, Ranson H, Nahzat SM, Naseem M, et al. Status of insecticide resistance in high-risk malaria provinces in Afghanistan. *Malar J* 2016; **15**: 98.

[35]Azizi K, Soltani A, Poodat A, Khodadadi M, Yaran M, Hasanvand B. Susceptibility of *Anopheles stephensi* against five current chemical insecticides. *Hormozgan Med J* 2011; **14**(4): 305-311.

[36]Aikpon R, Agossa F, Osse R, Oussou O, Aizoun N, Oke-Agbo F, Akogbeto M. Bendiocarb resistance in *Anopheles gambiae* s.l. populations from Atacora department in Benin, West Africa: a threat for malaria vector control. *Parasit Vectors* 2013; **26**(6): 192.

[37]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.

[38]Gorouhi M.A. *Investigating of biochemical and molecular resistance mechanisms in Anopheles stephensi to DDT and some pyrethroid insecticides*. PhD thesis. Tehran University of Medical Sciences; 2016.