Original Article

Evaluation of Susceptibility of Aedes caspius (Diptera: Culicidae) to Insecticides in a potent arboviral-prone Area, Southern Iran

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Abstract

Background: Southern part of the country is a high risk for mosquito transmitted Arboviruses. This study was carried out to determine the base line susceptibility of the Aedini mosquitoes to the WHO-recommended insecticide.

Methods: Larval collection was carried out by dipping method and adult collection occurred by suction tube from January to December 2017. The adult susceptibility test was assessed to Bendiocarb 0.1%, DDT 4%. Deltamethrin 0.05%, Lambda-cyhalothrin 0.05%, Malathion 5% and, Permethrin 0.75% at different interval times as well as at discriminative dose recommended by WHO. The larval susceptibility test was occurred using Temephos and Bacillus thuringiensis serotype H-14, at different concentrations. The LT₅₀, LT₉₀ and LC₅₀, LC₉₀ values were calculated for plotting the regression line using Microsoft office Excel software ver. 2007.

Results: Aedes caspius was quite resistant to DDT, Malathion, Bendiocarb and showed susceptible or tolerant to other insecticides. The LT5₀ and LT9₀ values to DDT in this species were 157.896, and 301.006 minutes, respectively. The LC5₀ and LC9₀ values of Ae. caspius to Temephos were 0.000068, and 0.000130 ppm, the figures for B. thuringiensis was 111.62 and 210.2 ppm, respectively.

Conclusion: A routine and continuous study for monitoring and evaluation of different species of Aedes to insecticides is recommend at different parts of country for decision making.

Keywords: Aedes caspius; Susceptibility; Iran

Introduction

Arthropod borne diseases are very important in the world. The tribe Aedini (Family Culicidae) contains approximately one-quarter of the known species of mosquitoes, including vectors of deadly or debilitating disease agents. This tribe contains the genus Aedes, which is one of the three most familiar genera of mosquitoes (1). The Aedini mosquitoes are responsible for transmission of the Barmah Forest, Batai, Babanki, Bouboui, Bunyanwera, Chikungunya, Cache, Valley, Dengue, Eastern Equine Encephalitis, Edge Hill, Everglades, Getah, GanGan, HighlandJ, Illinois, James Canyon, Kedougou, La Crosse, Lebombo, Murray Valley River, Nyando, Nagari, Oriboca, Orungo, Pongola, Ross River, Rift Valley Fever, Semiliki Forest, Sindbis, St Louis, Encephalitis; Spondweri, Tahyna, Tensaw, Trivittatus, Uganda S, Venezuelan Equine Encephalitis, West Nile, WSLV, Wesselbron, Wyeomyia, Yellow Fever, and Zika (1). The number of Dengue cases reported annually by WHO ranged from 0.4 to 1.3 million in the decade 1996–2005 (2). As an infectious disease, the number of death cases varies substantially from year to year (3). At the present, Culicidae includes; 2 sub families, 11 tribes, 113 genera and 3526 species

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(4). The Iranian mosquitoes includes 69 species, that 7 or 11 genera depending on the classification used for aedines (5-6). Recent epidemics of mosquito-borne viral infections in countries neighboring Iran i.e. dengue, chikungunya and West Nile infections in Pakistan, dengue and Rift Valley fever in Saudi Arabia, and West Nile infection in Iraq have placed this country at a serious risk for mosquito-borne diseases (7-9). *Aedes caspius* (Pallas) is the vector of Tahina and West Nile Viruses (7-8, 10). At the present seven *Anopheles* species reported as the malaria vectors in the country including: *An. flaviatilis* l, *An. culicifacies* l, *An. sacharovi*, *An. maculipennis* l, *An. superpictus*, *An. stephensi* and *An. dthali* (11). In addition, Zaim et al. reported the *An. pulcherrimus* secondary vectors of malaria in the South East of Iran (12). Oocyte of *Plasmodium* found at the first time in *An. multicolor*, while not found in salivary glands (13). Avian malaria reported in Iran by Ghaffari (14). Spraying with residual insecticide (IRS) considered an important mosquito control measure. Twelve insecticides recommended by WHO for IRS currently, which belong to four chemical groups including one organochlorine, six pyrethrins, three organophosphates and two carbamates (15-16). DDT resistance in the adult of *Aedes aegypti*, *Ae. albopictus* and susceptibility to Temephos, *Bacillus thuringiensis* and metabolic resistance of the current species to deltamethrin and DDT have been reported in Africa (17). Resistance of *Ae. aegypti* larvae to Temephos has been reported in Asia (18-19). In addition, larval resistance of *Aedes albopictus* to Temephos have been reported in Malaysia (20), Thailand (21). Adult susceptibility test on *Ae. aegypti* against some pyrethroids has been reported in various research study (21-24). In spite of some reports due to resistance of *An. stephensi* against DDT, Dieldrin and Malathion in Iran (13, 25-31). Mechanism of resistance of *An. stephensi* against temephos has been reported by (32-33). By now there are no evidence of resistance of *Ae. vexans* and *Ae. caspius* in Iran. Release of larvivorous fish and microbrial agent using the *Bacillus thuringiensis*, and larviciding by chlorpyrifos-methyl are the main larval control measures and pyrethroid as new insecticides are being used as IRS and LLINs in Iran (34-35). In spite of more than 50 years’ malaria control programming more than 60% of the total malaria cases reported from Southern Iran. Malaria is one of the most important communicable diseases transmitted by anopheline mosquitoes (Diptera: Culicidae) to humans. In 2013, there are 97 countries and territories with ongoing malaria transmission, and 7 countries in the prevention of reintroduction phase, making a total of 104 countries and territories in which malaria is presently considered endemic. Based on WHO estimate, 207 million cases of malaria occurred globally in 2012 resulted to 627 000 deaths (2). Malaria is one of the important infectious diseases in Iran with an average of about 15000 annual cases in the last decade, while total recorded cases has dropped to less than 500 locally transmitted cases in 2013. More than 80% of malaria cases in Iran are reported from three provinces of Sistan and Baluchistan, Hormozgan and Kerman in southern and southeastern areas of the country. The most routes of malaria cases are immigration from Afghanistan and Pakistan to southern and southeastern areas of the country (36). Over the last 20 years there has been a dramatic reduction of the malaria burden in Iran. While in 1991, nearly 100,000 cases were reported, less than 100 locally transmitted cases in 2017 (Ministry of Health, annual reports unpublished data). All observations indicate that the data reflect the real situation and that the overwhelming majority of cases, which occur, are included in the national system, although there is room for improvement in the surveillance system. The spectacular progress can be ascribed to effective implementation of appro-
appropriate curative and preventive control interventions through a strong health care infrastructure. Social and economic development allowing better housing, use of air-conditioning etc. has also played a role. Locally transmitted cases are now concentrated in the southeastern part of the country, which are affected by extensive population movement across the border with Pakistan, where malaria control faces serious difficulties. In 2009, Iran set time-bound elimination objectives for its malaria program. There has been excellent progress since, but the continued risk of importation of malaria cases from Pakistan poses a huge challenge, politically, socially, operationally and technically, to malaria elimination in Iran. The situation in the next decade will be absolute elimination or one where a few small short-lived foci emerge from time to time as a result of importation. The latest number of autochthonous cases in the whole country is 42 including 23 local malaria patients, 7 relapsed cases, 12 imported from the other districts by end of July 2016 (Ministry of Health, annual reports unpublished data). *Aedes albopictus* and *Ae. aegypti* has been recently reported in Algeria, Lebanon, Palestine, Syria, and Turkey (37). *Aedes albopictus* has been identified along the southeastern Iran (6) and Mediterranean coast of Europe for decades along with local transmission of DENV and chikungunya since 2007 (38). Near the Pakistan border, serologic evidence suggests possible DENV transmission in Iran (39-41), in Afghanistan (42), though local transmission has not been confirmed to our knowledge (41). The presence of *Aedes* or DENV transmission in these areas should not be ruled out (41). Qeshm and Kish are commercial and industrial free zones in Hormozghan Province. This area also is important due to agricultural and husbandry in southern Iran in the border line of Persian Gulf and Oman sea. The study area is endemic to malaria, however in recent years, the nuisance’s aedini species have been increased. There are no data about susceptibility level of Aedini vectors in Iran, so, the susceptibility level of Aedeini mosquitoes has been studied during this research. The results could provide an essential clue for judicious use of insecticides and will be very useful to health authorities for future planning of vector control.

**Material and Methods**

**Study area**

The study was carried out in Hormozgan (27°11’18”N 56°16’36”E/27.1884°N 56.276 8°E), Province, southern Iran. The people engaged to agriculture, horticulture, livestock, fishing sailing, and hand crafts including needlework, making carpet and musical instruments. The absolute maximum and average of temperature was reported 52 °C and 26.5 °C in Hormozghan Province, respectively. Average annual rainfall and humidity was 140.28mm and 79%, respectively. The absolute maximum and average of temperature in Isfahan was reported 40.6 °C and 17.1 °C. In this area average annual rainfall and humidity was 63.5 mm and 22%, respectively (43) (Fig. 1).

Hormozghan province with 70,697km² (27,296 sq. mi) square kilometers comprised of 21 counties (or districts), 69 municipalities, 13 major cities and 2,046 villages. In 2011 a little more than 1.5 million people resided in Hormozghan Province. Daregaz village (27° 49’GN, 56°17’GE) with 268 households, and 926 populations, and Kovea village (27°44 ’GN, 56°22’GE), 38 households and 112 populations, Talsooro village (27°46’GN, 56°23 ’GE), 92 households and 309 populations, as fixed stations and Zakin village (27°49’GN, 56°16’GE), 158 households 571 population selected randomly as variable stations.

**Sampling methods**

Sampling methods such as larval collection, hand catch was carried out during January to December 2017 (44). These studies
were conducted once every 30 days and collected mosquitoes were identified by specific systematic keys (5, 45).

**Larval collection and rearing**

In each fixed and variable station larvae was collected from January to December 2017. Mosquitoes larvae were picked up from the water using a dropper, pipette or fine net and inserted into the bulb. The related data such as water temperature, larval type, number and date sampling was recorded. Larvae and pupae in holding container filled with water were transferred to the laboratory for rearing. Mosquito larvae feed by dry fish food. Adult mosquitoes live quite well on bowl of water and covered with wet towel. In larval collection, An. stephensi was dominant species 34.76% in the same month. It should be noted that Aedes caspius larvae was collected in May and December.

**Insecticide impregnated papers**

Impregnated papers with DDT 4%, malathion 0.08%, bendiocarb 0.1%, deltamethrin 0.05%, lambda-cyhalothrin 0.03%, permethrin 0.25%, and control papers were supplied by World Health Organization.

**Larvicides solutions.**

Five concentration of Temephos as (0.00015, 0.000031, 0.000062, 0.000125, 0.000250ppm) and four concentrations of Bti as (4, 36, 296, 2368ppm) were immersed in 249mL of tap water separately and larval test was applied based of WHO criteria guideline 2016 (2).

**Adult susceptibility test**

The adult susceptibility test was carried out according WHO guideline (2). Each time 4–5 mosquito collected and insert to holding tube overall 20–25 mosquito were kept into holding tube. The susceptibility tests performed on their standard condition (22–26 °C, 60% H). The susceptibility of the wild strain of Aedini mosquitoes was assessed to the insecticides impregnated papers. The mosquitoes were exposed to different insecticides by different interval times and 24 hours’ recovery period.

**WHO criteria for susceptibility test**

Based on WHO recommendations (2), the following criteria have been used for interpretation and classification; Mortality in the range 98–100% indicates susceptibility. A mortality of less than 98% is suggestive of the existence of resistance and further investigation is needed. The observed mortality (corrected if necessary) is between 90% and 97%, the presence of resistant genes in the vec-
tor population must be confirmed. The confirmation of resistance may be obtained by performing additional bioassay tests with the same insecticide on the same population or on the progeny of any surviving mosquitoes (reared under insectary conditions) and/or by conducting molecular assays for known resistance mechanisms. If at least two additional tests consistently show mortality below 98%, then resistance is confirmed. If mortality is less than 90%, confirmation of the existence of resistant genes in the test population with additional bioassays may not be necessary, as long as a minimum of 100 mosquitoes of each species was tested. However, further investigation of the mechanisms and distribution of resistance should be undertaken. When resistance is confirmed, pre-emptive action must be taken to manage insecticide resistance and to ensure that the effectiveness of insecticides used for malaria vector control (2).

Identification of mosquitoes using morphological Characteristics

The mosquitoes after the test were mounted and identified by specific systematic keys. The samples were recorded in the special forms by and the appropriate time of deaths associated with history of collection, relative humidity and temperature (5, 45).

Statistical analysis

Results were considered reliable if the control mortality was less than 5% and rejected if more than 20%. Results were corrected by Abbott’s formula when mortality rates of control group were between 5 to 20% (47-48). Data were analyzed by probit analysis (49). Regression lines of the species were measured through the $\chi^2$ test. The $LT_{50}$ and $LT_{90}$ values were calculated for plotting the regression line using Microsoft Excel software ver. 2013.

Results

Adult bioassay

Adult bioassays using various insecticides showed that $LT_{50}$ and $LT_{90}$ values for DDT 4% against *Ae. caspius* were ranged from 157.896–301.006 minutes for the BAND strain. Bioassay test for other insecticides against is shown in Table 1, Fig. 2.

Larval bioassay

Larval bioassays using Temephos showed that $LC_{50}$ and $LC_{90}$ for *Ae. caspius* ranged from 0.000068–0.000130mg/l for the BAND strain (susceptible reference strain) to 111.62–210.2 mg/L for the B. T (Table 2, Figs. 3, 4).

Mortality of *Aedes caspius* exposed to DDT and other insecticides has shown in Tables 1 and 2. $LT_{50}$ and $LT_{90}$ values of this species to DDT 4% were 157.89 and 301.006 minutes, respectively. This species was quite resistant to DDT and other insecticides except deltamethrin (Fig. 2).

It is concluded that *An. caspius* is resistant to DDT, malathion, and bendiocarb, permethrin, lambdacyhalothrin whereas susceptible to deltamethrin, (Table 1). The $LT_{50}$ and $LT_{90}$ values of this species to DDT 4% were 157.896 and 301.006 minutes (Table 1, Fig. 2).

Mortality of *Aedes caspius* larvae exposed to temephos and *Bti* has shown in (Table 2 and Figs. 3, 4). $LC_{50}$ and $LC_{90}$ values of this species to temephos were 0.000068 and 0.000130ppm, respectively. $LC_{50}$ and $LC_{90}$ values of this species to *Bti* were 111.62 and 210.2ppm, respectively.
Table 1. Regression line parameters of *Aedes caspium* adult stage exposed to some insecticides recommended by WHO in a arboviral-prone Area Southern Iran, 2017

| Insecticide | A       | B±SE     | LT<sub>50</sub> 95% C.I (minute) | LT<sub>90</sub> 95% C.I (minute) | P-Value | book | Y = BX+A |
|-------------|---------|----------|-------------------------------|-------------------------------|---------|------|----------|
| DDT4%       | 1.1511  | 0.0167±0.196 | 157.896                       | 301.006                       | P>0.05  | 5.99 | y= 0.0167x+1.1511 |
| Malathion 5%| 1.2944  | 0.0081±0.190 | 160.229                       | 304.435                       | P>0.05  | 5.99 | y= 0.0081x+1.2944 |
| Bendiocarb 0.1% | 1.6845 | 0.0135±0.087 | 42.124                        | 80.0356                       | P>0.05  | 5.99 | y= 0.0135x+1.6845 |
| Deltamethrin 0.1% | 1.7745 | 0.0141±0.077 | 48.735                        | 92.5965                       | P>0.05  | 5.99 | y= 0.0141x+1.7745 |
| Lambda-cyhalothrin 0.05% | 1.8494 | 0.0132±0.166 | 46.129                        | 87.6451                       | P>0.05  | 5.99 | y= 0.0132x+1.8494 |
| Permethrin 0.75% | 1.5955 | 0.0156±0.196 | 29.652                        | 56.3388                       | P<0.05  | 5.99 | y= 0.0156x+1.5955 |

Table 2. Regression line parameters of *Aedes caspium* larval stage exposed to Some Larvicides Recommended by WHO in arboviral-prone Area Southern Iran, 2017

| Larvicide     | A         | B±SE     | LC<sub>50</sub> 95% C.I | LC<sub>90</sub> 95% C.I | P-Value | book | Y = BX+A |
|---------------|-----------|----------|--------------------------|--------------------------|---------|------|----------|
| Temephos      | 6.8275    | 3322.2±0.385 | 0.0000068                | 0.000130                  | P>0.05  | 7.81 | y = 3322.2x+ 6.8275 |
| B.thuringiensis | 1.7839    | 0.0004±0.256 | 111.62 | 210.2 | 173.914 (2)  | P<0.05  | 7.81 | y= 0.0004x+1.7839 |

Fig. 1. Map of study area, Hormozghan province, Iran
Fig. 2. Regression line of *Aedes caspius* Adult stage exposed to Some Insecticides Recommended by WHO in arboviral-prone Area Southern Iran, 2015

Fig. 3. Regression line of *Aedes caspius* larval stage exposed to Temephos Larvicide Recommended by WHO in a potent Dengue Endemic Area of Central and Southern Iran, 2015

Fig. 4. Regression line of *Aedes caspius* Larval stage exposed to *B. thuringiensis* Larvicides Recommended by WHO in arboviral-prone Area Southern Iran, 2015
Discussion

In our study, 4 genera and 10 species of mosquito larvae and adults were identified based on morphological characters. Culicidae species were belongs to the genus of Anopheles, Culex, Culisita and Aedes. The species of Ae. caspius and Ae. vexans found by larval collection. The most predominant species was An. stephensi with 34.76% of adult and 29.36% of larvae collection. Vatandoost et al. (2004b) (50), reported three biological forms of this species including type, intermediate and mysorensis in southern Iran. Type and intermediate forms cited as vector in urban areas whereas, mysorensis form as vector in rural area (51). In Iran, indoor residual spraying (IRS) with DDT was carried out for malaria control during 1950–1968. In this species, resistance to DDT was first recognized in 1958 malathion in 1976 (13). Following the emergence of resistance of An. stephensi to DDT, other organophosphorus, carbamate and pyrethroid insecticides were used. The susceptibility level of An. stephensi to DDT and Dieldrin was studied at various parts of Iran bordered in Persian Gulf and Oman Sea during 1985–2016. The situation of Dengue fever and dengue hemorrhagic fever has been changed in imported to indigenous cases in Iran and probable Aedes albopictus is responsible for these endemic diseases due to unplanned urbanization (6). In southern Iran, the climatic conditions are suitable for mosquito’s life cycle. The changes in temperature, humidity and wide range of water grades may have a significant effect on the population growth and also vector control programmers (52). Potent dengue vector in Iran has exophilic behavior, so, the efficacy of larvicing materials is very important to vector control programs. Temephos and Bti were evaluated in Lab scale against Ae. caspius larvae in the current study. In this research work, different concentrations of Bti were prepared as done by previous workers (53-54). Bacillus turingiensis is safe and effective biocontrol agent used widely to control of mosquitoes for the recent years (55-58). The experiment was conducted in tape water. Abdalmagid et al. (2012) (53) checked the efficacy of Bti dunks in field water and studied the physio-chemical properties of water. They concluded that these properties have no impact on the efficacy of Bti (P> 0.05). Mulla (1990) (59) studied that it was difficult to handle 1st instar larvae because of high mortality rate during handling. Due to this reason we used 3rd and 4th instar larvae for our experiments. In the present study we found low mortality rate in case of Bti. In agreement with this study, Rodrigues et al. (1999) (60) reported the low mortality of Ae. aegypti post treatment by Bti and 24h. Recovery periods. Ramathilaga et al. (2012) (61) studied the impact of Bti against 3rd instar larvae of Ae. aegypti as was recorded in the present study against 3rd and 4th instar larvae of Ae. caspius. In the present study, 40% and 78% mortality was recorded for 592 and 1184ppm of Bti respectively after 24h in tape water while Ramathilaga et al. (2012) (61) recorded (16%) mortality at the 1mg concentration of Bti for 24h treatment in tap water. Haung et al. (1993) (62) recorded 52.1, 69.5 and 78.2% mortality after 12, 24 and 48h respectively in 0.10ppm against Ae. aegypti larvae while 97.1, 97.1 and 97.1% mortality after 12, 24 and 48h in 0.20ppm. Gbehou et al. (2010) (63) compared the efficacy of Bti on Aedes, Culex and Anophleles species and observed 40, 80 and 100% mortality after 2, 4 and 6h against Aedes species. Many other factors such as species, genera susceptibility, feeding behavior of larvae, instar susceptibility to bioicides, suspended organic matter, water temperature, larval density, and water depth influence the efficacy of Bti against mosquitoes (Boisvert 2005) (64). Some of these factors like organic, inorganic, muddy, food and floating particles decreased the efficacy of Bti due to
adsorption of Bti onto suspended particles followed by a slow sedimentation (65-66). In the present study, we found higher concentration of Bti is enquired for 100% mortality rate. In parallel, Ohana et al. 1987 (67), Mulla 1990 (59) reported more concentration need to control of Ae. aegypti larvae due to Bti a few toxic suspended crystals particles ingested by larvae. In this research study, different concentrations of Temephos were prepared as done by previous workers (68). This larcicide is safe and effective agent used widely to control of mosquitoes for the recent years. Kemabonta and Nwankwo 2013 (68) checked the efficacy of Temephos in field water with comparison to spinozad. They concluded that these properties have good impact on the 3rd and 4th Ae. aegypti larvae (P > 0.05). The LC$_{50}$ values for wild Aedes caspius larvae were 0.000068mg/l and 0.000130mg/l, while the LC$_{50}$ values for the laboratory bred and wild Aedes aegypti larvae were 7.418g/l and 8.150 g/l respectively (68). In the present study, 100% mortality was recorded at 0.000250mg/L of temephos respectively after 24h in tape water while Kemabonta and Nwankwo (2013) (68) recorded (100%) mortality at the 30g/L concentration of temephos for 24h treatment in tap water. Many other factors such as species, genera susceptibility, feeding behavior of larvae, instar susceptibility to biocides, suspended organic matter, water temperature, larval density, and water depth influence the efficacy of Bti against mosquitoes (64). Some of these Many other factors like organic, inorganic, muddy, food and floating particles decreased the efficacy of Temephos. In addition, many factors effects of efficacy of Bti due to adsorption of Bti onto suspended particles followed by a slow sedimentation (65-66).

In the present study, we found higher concentration of Bti will be needed for 100% mortality rate. In parallel, Ohana et al. 1987 (67), Mulla 1990 (59) were reported more concentration need to control of Ae. aegypti larvae due to Bti a few toxic suspended crystals particles ingested by larvae.

The interruption in the efficacy of Bti was found to be caused by bacterial adsorption to soil particles, but the inactivation could be inverted by washing the mud away (44). Due to these reasons, the mean value of LC$_{50}$ was higher against Ae. caspius larva in comparison to temephos. The mean LC$_{50}$ values of Bti and Temephos were 111.62ppm and 0.000068ppm after 24h for tape water respectively. The results of the present study revealed the higher mortality post treatment by Temephos in tape water because temephos is considered as contact larciding in comparison to Bti as digestive effects and it is free of any particles due to suspended particles. Based on the literature, no reports were available on the susceptibility levels of Aedes caspius.

**Conclusion**

Iran is near the Dengue endemic area, Aedes albopictus was reported for the first time in southeastern Iran in 2014. By now, IRS in human dwelling sand animal shelters, space-spraying, personal protection through distribution of LLINs and curtains (ICNs), repellents measures used to control of vectors in Iran. In addition, some biological and chemical agents against larval and adult stages of mosquitoes had been evaluated in the laboratory. Results obtained from susceptibility tests of Aedes caspius on some WHO recommended insecticides revealed that highly resistance to them in southern Iran. Precautionary measures should be taken in future vector control operations. Moreover, the status of resistance in other locations in this area should be investigated. Since the country relies on deltamethrin for IRS operation, tolerant populations of Aedini species implies careful consideration and regular monitoring of susceptibility level of mosquitoes in the future.
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References

1. Wilkerson RC, Lintom YM, Fonseca DM, Schultz TR, Price DC, Strickam DA (2015) Making mosquito taxonomy useful: a stable classification of tribe Aedini that balances utility with current knowledge of evolutionary relationships. PLoS One. 10(7): e0133602.
2. WHO (2016) Test procedures for insecticide resistance monitoring in malaria vector mosquitoes.1. Insecticide resistance. 2. Malaria-prevention and control. 3. Insecticides. 4. Insectcontrol. 1. World Health Organization. ISBN 978 92 4 150515 4 (NLM classification: WA 240) 2016. WHO Press, Avenue Appia, Geneva, Switzerland
3. Suaya JA, Shepard DS, Beatty ME (2007) Dengue burden of disease and costs of illness. Working paper 3.2 in: Report of the Scientific Working Group meeting on Dengue, Geneva, 1–5 October. Geneva, World Health Organization, Special Programmed for Research and Training in Tropical Diseases. pp. 35–49.
4. Harbach R (2007) The Culicidae (Diptera): a review of taxonomy, classification and phylogeny. Zootaxa. 1668: 591–638.
5. Azari-Hamidian S, Norouzi B, Harbach RE (2019) A detailed review of the mosquitoes (Diptera: Culicidae) of Iran and their medical and veterinary importance). Acta Trop. 194: 106–122.
6. Doosti S, Yaghoobi-Ershadi MR, Schaffner F, Moosa-Kazemi SH, Akbarzadeh K, Gooya MM, Vatandoost H, Shirzadi MR, Mostafavi E (2016) Mosquito surveillance and the first record of the invasive mosquito species Aedes (Stegomyia) albopictus (Skuse) (Diptera: Culicidae) in southern Iran. Iran J Public Health. 45 (8): 1064–1073.
7. Ali-Khan HA, Akram W, Shehzad K, Shaalan EA (2011) First report of filed evolved resistance to agrochemical in dengue mosquito, Aedes albopictus (Diptera: Culicidae), from Pakistan. Parasit Vectors. 4: 146–152.
8. Rasheed SB, Butlin RK, Boots MA (2013) review of dengue as an emerging disease in Pakistan Public Health. 127(1): 11–17.
9. Afzal MF, Naqvi SQ, Sultan MA, Hanif A (2015) Chikungunya fever among children presenting with nonspecific febrile illness during an epidemic of dengue fever in Lahore, Pakistan. Merit Res J Med Sci. 3(3): 069–073.
10. Ergunay K, Gunay F, Oter K, Kasap OE, Orstein S, Akkutay AZ, Erdem H, Ozkul A, Alten B (2013) Arboviral surveillance of field-collected mosquitoes reveals circulation of West Nile Virus Lineage 1 strains in Eastern Thrace, Turkey. Vector Borne Zoonot Dis. 13: 744–752.
11. Faghih MA (1969) Malaria and malaria eradication. Tehran University Press. p. 726.
12. Zaim M, Zahirnia AH, Manouchehri AV (1993) Survival rates of Anopheles culicifacies: 1 and Anopheles pulcherrimus in sprayed and unsprayed villages' in Ghassre Ghand District, Baluchistan, Iran. J Am Mosq Control Assoc. 9: 421–425
13. Eshghy N (1978) Tolerance of Anopheles stephensi to Malathion in the province of Fars, southern Iran. Mosq News. 38 (4): 580–583.
14. Ghaffari AN (1955) The classification of Culicidae (Diptera: Nematocera): the study of Culex linneaeus in Iran. Tehran University, School of Medicine, Iran, p. 189.

15. Pluess B, Tanser FC, Lengeler C, Sharp BL (2010) Indoor residual spraying for preventing malaria. Cochrane Database Syst Rev. 4: 49.

16. Hanafi-Bojd AA, Vatandoost H, Oshaghi MA, Haghdost AA, Shahi M, Sedaghat MM, Abedi F, Yeryan M, Pakari A (2012) Entomological and epidemiological attributes for malaria transmission and implementation of vector control in southern Iran. Acta Trop. 121(2): 85–92.

17. Kamgang B, Marcombe S, Chandre F, Nchoutpouen E, Nwane P, Etang J, Corbel V, Paupy C (2011) Insecticide susceptibility of Aedes aegypti and Aedes albopictus in Central Africa. Parasit Vectors. 15(4): 79.

18. Lee HL, Lime W (1989) A reevaluation of the susceptibility of field collected Aedes (Stegomyia) aegypti (Linnaeus) larvae to temephos in Malaysia. Mosquito-Borne Dis Bull. 6: 91–95.

19. Chen CD, Nazni WA, Lee HL, Sofian-Azirun M (2005) Susceptibility of Aedes aegypti and Aedes albopictus to temephos in four study sites in Kuala Lumpur City center and Selangor State, Malaysia. Trop Biomed. 22(2): 207–216.

20. Nazni WA, Kamaludin MY, Lee HL, Rogayah TAR, Sa'diyah I (2000) Oxidase activity in relation to insecticides resistance in vectors of public health importance. Trop Biomed. 17: 69–79.

21. Ponlawat A, Scott JG, Harrington LC (2005) Insecticide susceptibility of Aedes aegypti and Aedes albopictus across Thailand. J Med Entomol. 42(5): 821–885.

22. Mouchet J, Cordellier V, Germain M, Carnevale P, Barathe J, Sannier C (1972) Ré-sistance aux insecticides d' Aedes aegypti et Culex pipiens fatigues en Afrique centrale. WHO/VBC/72/381, P, Geneva, Switzerland, World Health Organization.

23. Hemingway J, Ranson H (2000) Insecticide resistance in insect vectors of human disease. Annu Rev Entomol. 45(1): 371–391.

24. Ranson H, Burhan J, Lumjuan N, Black WC (2010) Insecticide resistance in dengue vectors. Trop IKAnet J. 1(1): 379–386.

25. Vatandoost H, Shahi H, Abai MR, Hanafi-Bojd AA, Oshaghi MA, Zamani G (2004a) Larval habitats of main malaria vectors in Hormozgan Province and their susceptibility to different larvicides. Southeast Asian J Trop Med Public Health. 35(2): 22–25.

26. Vatandoost H, Abai MR, Abbasi M, Shaeghi M, Abtahi M, Rafie F (2009) 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. 46(4): 261–267.

27. Vatandoost H, Abai MR (2012) Irritability of malaria vector, Anopheles sacha-rovi to different insecticides in a malaria-prone area. Asian Pac J Trop Med. 5(2): 113–116.

28. Vatandoost H, Zahirnia AH (2010) Responsiveness of Anopheles maculipennis to different imagicides during resurgent malaria. Asian Pacific J Trop Med. 3: 360–363.

29. Vatandoost H, Hanafi-Bojd AA (2012) Indication of pyrethroid resistance in the main malaria vector, Anopheles stephen-si from Iran. Asian Pac J Trop Med. 5 (9): 722–726.

30. Vatandoost H, Sanei Dehkordi A, Sadeghi SM, Davari B, Karimian F, Abai MR, Sedaghat MM (2012) Identification of chemical constituents and larvicidal activity of Kelussia odoratissima Mozaf-
farian essential oil against two mosqui-
to vectors Anopheles stephensi and Cu-
lex pipiens (Diptera: Culicidae). Exp Par-
asitol. 132(4): 470–474.
31. Fathian M, Vatandoost H, Moosa-Kazemi
SH, Raeesi A, Yaghoobi-Ershadi MR,
Oshaghi MA, Sedaghat MM (2015) Sus-
ceptibility of culicidae mosquitoes to
some insecticides recommended by
WHO in a malaria endemic area of south-
eastern Iran. J Arthropod Borne Dis. 9
(1): 22–34.
32. Soltani A, Vatandoost H, Oshaghi MA,
Enayati AA, Raeesi A, Eshraghian MR,
Soltan-Dallal MM, Hanafi-Bojd AA,
Abai MR, Rafi F (2013) Baseline sus-
tceptibility of different geographical
strains of Anopheles stephensi (Diptera:
Culicidae) to Temephos in malarious ar-
eas of Iran. J Arthropod Borne Dis. 7
(1): 56–60.
33. Soltani A, Vatandoost H, Oshaghi MA,
Ravasan NM, Enayati AA, Asgarian F
(2015) Mechanisms of Temephos re-
sistant in Anopheles stephensi. J Ar-
thropod Borne Dis. 9(1): 71–83.
34. Vatandoost H, Hanafi-Bojd AA (2005)
Current resistant status of Anopheles
stephensi Liston to different larvicides
in Hormozgan Province, southeastern
Iran. Pakistan J Bio Sci. 8: 1568–1570.
35. Moosa-Kazemi SH, Vatandoost H, Rae-
isi A, Akbarzadeh K (2007) Deltame-
thrin impregnated bed nets in a malaria
control program in Chabahar, South-
east Baluchistan, Iran. Iran J Arthro-
pod–Borne Dis. 1: 43–51.
36. Ministry of Health (MOH) (2010) Med-
cal Education (ME) of Iran Annual re-
port of malaria control department.
Tehran: CDC, Iran, pp. 50–65.
37. Garabedian GA, Matossian RM, Musalli
MN (1971) Serologic evidence of ar-
bovirus infection in Lebanon. Le Jour-
nal medical libanais. The Lebanese
Med J. 24(4): 339–350.

38. European Centre for Disease Prevention
and Control. Guidelines for the surveil-
ance of invasive mosquitoes in Europe
2015. Available at: ecdc.europa.eu/en/.../TER-Mosquito-
surveillance-guidelines.pdf
39. Aghaie A, Aaskov J, Chinikar S, Niedrig
M, Banazadeh S, Mohammadpour HK
(2014) Frequency of dengue virus in-
festation in blood donors in Sistan and
Baluchistan province in Iran. Transfus
APher Sci. 50(1): 59–62.
40. Saidi S (1971) Survey of antibodies to
arboviruses in human population of
Iran. P. Med J. 2(3): 485–490.
41. Chinikar S, Ghiasi SM, Shah-Hosseini
N, Mostafavi E, Moradi M, Khak-
ifarouz S (2013) Preliminary study of
dengue virus infection in Iran. Travel
Med Infect Dis. 11(3): 166–169.
42. Elyan DS, Moustafa L, Noormal B, Ja-
cobs JS, Aziz MA, Hassan KS, Wasfy
MO, Monestersky JH, Oyofo BA
(2014) Serological evidence of Fla-
viruses infection among acute febrile
illness patients in Afghanistan. J Infect
Dev Ctries. 8: 1176–1180.
43. Meterological organization of Iran.
Available at: http://theiranproject.com/blog/tag/iran-
2015
44. WHO (2004) A review of entomological
sampling methods and indicators for
dengue vectors focks infectious disease
UNICEF/UNDP/WORLD
BANK/WHO. Special Programme for
Research and Training in Tropical
Diseases (TDR). p. 40.
45. Zaim M, Cranston PS (1986) Checklist
and keys to the Culiciniae of Iran (Dip-
tera: Culicidae). Mosq Syst. 18: 233–
245.
46. Vosshall (2014) Laboratory Mosquito
rearing standard operating procedures
last revised: December 12, 2014 La-
boratory of neurogenetics and behav-
ior, The Rockefeller University, 1230 York Avenue, Box 63, New York, NY 10065, USA.
47. Abbott WS (1925) A method of computing the effectiveness of an insecticide. J Econ Entomol. 8: 265–267.
48. WHO (2006) Pesticides and their application. For the control of vectors and pests of public health importance. Volume WHO/CDS/NTD/WHOPES/GCDPP/2 006.1.WHO Press, Avenue Appia, Geneva, Switzerland.
49. Finney DJ (1971) Probit analysis (3rd ed): Cambridge University Press, Cambridge, UK. 333: 3.
50. Vatandoost H, Shahi H, Abai MR, Hanafi-Bojd AA, Oshaghi MA, Zamani G (2004b) Larval habitats of main malaria vectors in Hormozgan Province and their susceptibility to different larvicides. Southeast Asian J Trop Med Pubic Health. 35: 22–25.
51. Vatandoost H, Oshaghi MA, Abaie MR, Shahi M, Yaaghoobi F, Baghaii M, Hanafi-Bojd AA, Zamani G, Townsend H (2006) Bionomics of Anopheles stephensi Liston in the malarious area of Hormozgan Province, southern Iran, 2002. Acta Trop. 97(2): 196–203.
52. Tun-Lin W, Burkot TR, Kay BH (2000) Effects of temperature and larval diet on development rates and survival of the dengue vector Aedes aegypti in north Queensland Australia. Med Vet Entomo-l. 14: 31–37.
53. Abdalmagid MA, Elkhaliifa SM, Ismail AB, Ismail SM, Abdalrahman AH, Jamal AE, Brair M, Elnaeim IH (2012) The mosquito dunk (Bacillus thuringiensis israelensis) as a control agent against mosquito larvae in Khartoum State, Sudan. Sudan J Public Health. 7: 51–55.
54. Jahan N, Shahid A (2012) Evaluation of resistance against Bacillus thuringiensis israelensis WDG in dengue vector from Lahore, Pakistan. Pak J Zool. 44(4): 945–949.
55. Lacey LA, Siegel JP (2000) Safety and ecotoxicology of entomopathogenic bacteria. In: Charles JF, Dele`cluse A, Nielsen- LeRoux C (ed) Entomopathogenic bacteria: from laboratory to field application. Kluwer Academic Publishers, Dordrecht, pp. 253–273.
56. Mittal PK (2003) Bio larvicides in vector control: Challenges and prospects. J Vector Borne Dis. 40: 20–32.
57. Junwei Z, Xiaopeng Z, Yanna TL, Ting L, Kuen Q, Yuhua H, Suqin X, Brad T (2006) Adult repellency and larvicidal activity of five plant essential oils against mosquitoes. J Am Mosq Control Assoc. 3: 515–522.
58. Lacey LA (2007) Bacillus thuringiensis israelensis and Bacillus sphaericus for mosquito control. J Am Mosq Control Assoc. 23: 133–163.
59. Mulla MS (1990) Activity, field efficacy and use of Bacillus thuringiensis israelensis against mosquitoes. In: Bar-jac H, Sutherland DJ (ed) Bacterial control of mosquitoes and black flies: Biochemistry, genetics and applications of Bacillus thuringiensis israelensis and Bacillus sphaericus. Rutgers University Press, New Brunswick, New Jersey, pp. 134–160.
60. Rodrigues IB, Tadei WP, Dias JS (1999) Larvicidal activity of Bacillus sphaericus 2362 against Anopheles nuneztovara-ci, Anopheles darling and Anopheles braziliensis (Diptera: culicidae). Rev Inst Med Trop S Paulo. 41(2): 101–105.
61. Ramathilaga A, Murugesan AG, Sathesh C (2012) Prabu Bio larvicidal activity of Peani bacillus macerans and Bacillus subtilis isolated from the dead lar-vae against Aedes aegypti-Vector for Chikungunya. Proc Int Acad Ecol Environ Sci. 2: 90–95.
62. Haung RN, LO IP, Ho C, Haung JS, Hsu
63. Gbehou NA, Christophe HS, Yilian L (2010) Effect of Bacillus thuringiensis var. israelensis (H-14) on Culex, Aedes and Anopheles larvae (Cotonou; Benin). Stem Cell. 1: 60–67.

64. Boisvert M (2005) Utilization of Bacillus thuringiensis var. israelensis (Bti)-based formulations for the biological control of mosquitoes in Canada. In: 6th Pacific Rim Conference on the biotechnology of Bacillus thuringiensis and its environmental impact, Victoria BC, pp. 87–93.

65. Margalit J, Bobroglo H (1984) The effect of organic materials and solids in water on the persistence of Bacillus thuringiensis var. israelensis Serotype H-14. Zeitschrift für Angewandte Entomologie. Available at: https://doi.org/10.1111/j.1439-0418.1984.tb03785.x.

66. Margalit JC, Pascar-gluzman H, Bobroglo Z, Barak, Lahkim- tsror L (1985) Biocontrol of mosquitoes in Israel. In: Laird M, Miles JW (ed) Integrated mosquito control methodologies. San-93 Diego, Academic Press. pp. 361–374.

67. Ohana B, Margalit J, Barak Z (1987) Fate of Bacillus thuringiensis subsp. israelensis under Simulated Field Conditions. Appl Environ Microbiol. 53(4): 828–831.

68. Kemabonta KA, Nwankwo A (2013) larvicidal effectiveness of spinosad and temephos on Anopheles gambiae and Aedes aegypti. Int J Sci Nat. 4(2): 214–222.

69. World Health Organization. (2009) Dengue guidelines for diagnosis, treatment, prevention and control: new edition. Available at: World Health Organization. https://apps.who.int/iris/handle/10665/44188 . p. 147.