

## Original Article

# Susceptibility of Culicidae Mosquitoes to Some Insecticides Recommended by WHO in a Malaria Endemic Area of Southeastern Iran

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## Abstract

**Background:** According to the national strategy plan on monitoring of insecticides resistance, this study was carried out to determine the base line susceptibility of the Culicidae mosquitoes to the WHO-recommended insecticides in an endemic focus of malaria in southeastern Iran.

**Methods:** Larval collection was carried out by dipping method and adult collection occurred by suction tube from January to December 2010. The susceptibility test was assessed to DDT 4 %, malathion 5 %, propoxur 0.1 %, deltamethrin 0.05 %, lambda-cyhalothrin 0.05 %, and cyfluthrin 0.15 % at different interval times (discriminative dose) followed by 24 h recovery period. The LT<sub>50</sub> and LT<sub>90</sub> values were calculated for plotting the regression line using Microsoft office Excel software ver. 2007.

**Results:** *Anopheles stephensi* was quite resistant to DDT and showed susceptible or tolerant to other insecticides. The LT<sub>50</sub> and LT<sub>90</sub> values to DDT in this species were 29.07, and 98.26 minutes, respectively. *Anopheles culicifacies* and *Anopheles dthali* were found susceptible or tolerant to insecticides. *Culex pipiens* was found resistance to DDT, propoxur, lambda-cyhalothrin and cyfluthrin whereas observed susceptible to malathion and tolerant to deltamethrin. *Ochlerotatus caspius sl.* was resistant to DDT, whereas found susceptible to other insecticides. *Culisita longiareolata* was susceptible to deltamethrin, whereas tolerant to other insecticides. The LT<sub>50</sub> and LT<sub>90</sub> values of *Cs. longiareolata* to DDT were 17.82, and 51.26 minutes.

**Conclusion:** We suggested the same study in different parts of the country for monitoring and evaluation of control measures.

**Keywords:** Culicidae, Malaria, Susceptibility test, Southeastern Iran

## Introduction

Arthropod borne diseases are very important in the world. Malaria occurs often in poor, tropical and subtropical areas and still took an estimated 627 000 lives in 2012, mostly those of children under five years of age in Africa. In fact 1300 young lives lost to malaria every day (WHO 2013).

The Culicidae mosquitoes are responsible for transmission of the worm parasites of heart dogs such as *Dirofilaria immitis*, *Dirofilaria repense*, *Wuchereria bancrofti*, some Arboviral diseases such as Japanese Encephalitis, Rift valley fever, Western equine encephalitis and

Eastern equine encephalitis, Tahyna, Sagiya, Trivittatus, Lymphocytic Choriomanangitis, West Nile virus, St. Louis encephalitis, California encephalitis. About 70 *Anopheles* species in the world are able to transmit malaria and of these 40 species have been identified as the major vectors (Azari-Hamidian and Harbach 2009).

By now, 64 species of Culicidae mosquitoes due to 3 sub genera and 7 genera are reported from Iran (Azari-Hamidian and Harbach 2009). *Anopheles stephensi* is the main malaria vector in Iran. By now, seven

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species of *Anopheles* reported as the malaria vectors in the country including: *An. fluviatilis* s.l., *An. culicifacies* s.l., *An. sacharovi*, *An. maculipennis* s.l., *An. superpictus*, *An. stephensi* and *An. dthali* (Faghah 1969, Vatandoost et al. 2004c, Vatandoost and Borhani 2004a, Vatandoost and Moinvaziri 2004b, Vatandoost et al. 2005b, Sedaghat and Harback 2005, Hanafi-Bojad et al. 2006, Oshaghi et al. 2006, Vatandoost et al. 2006a, 2006b, 2007, 2008, 2009a, 2009b, Moosa-Kazemi et al. 2009, 2010, Omrani et al. 2010). In addition, Zaim et al. reported the *An. pulcherrimus* as secondary vectors of malaria in the South East of Iran (Zaim et al. 1993). Oocyst of *Plasmodium* found at the first time in *An. multicolor*, while not found in salivary glands (Eshghy 1978). Avian malaria reported in Iran by Ghaffari (Ghaffari 1955).

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 pyrethroids, three organophosphates and two carbamates (Pluess et al. 2010). DDT resistance in the adult of *An. stephensi* was reported in Iran in 1957, subsequently to dieldrin in 1960, and then to malathion in 1976 (Eshghy 1978, Manouchehri et al. 1974). Propoxur was used after reports about to malathion tolerance of *An. stephensi* in 1978 (Eshghy 1978). Release of larvivorous fish, 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 (Vatandoost and Hanafi Bojd 2005a, Moosa-Kazemi et al. 2007).

In spite of more than 50 years malaria-control programming, 42%–60% of the total malaria cases reported from Sistan and Baluchistan Province. Out of 112 malaria cases representing 79 males and 33 females have been reported in Chabahar County, southeastern Iran in 2010. Out of 38 cases were

from Bangladesh, Pakistan and Afghanistan and the rest were the local Aborigines (MOH and ME 2010). Chabahar is commercial and industrial free zone as well as important due to agricultural and husbandry in Sistan and Baluchistan Province in southeastern Iran and also the border line of Pakistan.

There are scatter data about susceptibility level of malaria vectors in Iran, the susceptibility level of Culicinae mosquitoes in southeastern Country had not been reported. Therefore, this study was carried out to determine susceptibility level of mosquitoes to monitoring and evaluation of insecticides resistance. This information could provide an essential clue for judicious use of insecticides and will be very useful to health authorities for future planning of vector control in this endemic malarious area.

## Materials and Methods

### Study area

The study was carried out in Chabahar City, Sistan and Baluchistan Province, southeastern Iran (25°17'GN, 60°37'GE) (Fig. 1). 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 36 °C and 16.5 °C, respectively (Meteorological Organization of Iran, 2010). Average annual rainfall and humidity was 90mm and 79 %, respectively. Kajoo and Bishmont rivers are the largest rivers located in this area. Chabahar County with 24, 729 square kilometers (19.3%) comprised 3 cities, 5 districts and 11 rural districts. The population of Chabahar reported 211,081 including 143,535 rural (68%) and 67,546 urban (32%). Naval Base near the 22 Bahman Squar (25°18'GN, 60°37'GE) in Chabahar city selected as fix station including, 30 households, and 114 population. Baluchi-Adam village (25°47'GN, 61°16'GE) with 71 households, and

379 populations, and Bahoo-kalat village (25°43'GN, 61°25'GE), 198 households and 1064 population selected randomly as fixed stations. Sampling methods such as larval collection, Hand catch was carried out during January to December 2010 (WHO 1975, WHO 1975). These studies were conducted once every 15 days and collected mosquitoes were identified by specific systematic keys (Shahgudian 1960, Zaim and Cranston 1986).

### Larval collection and rearing

In each fixed and variable station larvae was collected from January to December 2010. Larval mosquitoes 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 sucrose 10% in bottom of the cage. The adults were kept in the temperature (22–24 °C), relative humidity (60–70%) and 12L-12D photoperiod (WHO 1975a, 1975b).

### Hand collection

Culicidae mosquitoes were collected from the villages that spraying was not performed before, between 06.30 and 09.30 am. Sampling was carried out in each human dwelling, cattle and goat sheds for 15 min using suction tube and torch (WHO 1975b). The mosquitoes were inserted in the cage as dimensions of 40×40 cm and sent to the laboratory. Total of 200 to 250 mosquitoes were entered in each cage and covered with wet towel. The sucrose 5 % solution was placed inside the cage. The mosquitoes were kept in standard condition (25 °C, 75% H).

### Insecticide impregnated papers

Impregnated papers with DDT 4 %, malathion 5 %, propoxur 0.1 %, deltamethrin

0.05 %, lambda-cyhalothrin 0.05 %, cyfluthrin 0.15 %, and control papers were supplied by World Health Organization.

### Adult susceptibility test

The adult susceptibility test kit including holding and exposure tubes, slides, copper and silver rings and related covers were washed with detergent, and then washed three times with tap water to be free of detergents and insecticides. After drying of the pipe holders, standard paper impregnated with insecticides fixed inside the tubes marked with red dot and copper ring. For holding tube used paper control (no insecticide) tube with a green sign and silver pipe fixed to the wall (WHO 2006). 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 Culicidae mosquitoes was assessed to the insecticides impregnated papers. The mosquitoes were exposed to different insecticides by different interval times and 24 hours recovery period. Smoking and use of pesticides during the test was strictly prohibited.

### WHO criteria for susceptibility test

The following criteria have been used for interpretation and classification of results, based on WHO recommendations (African Network on Vector Resistance 2005).

A. Susceptibility test carried out with less than 20 mosquitoes per test have not been considered

B. At least 80 mosquitoes used per test, three resistance classes as defined. Resistant when the mortality was lower than 80 %, susceptible when mortality was 98 % or higher and possible resistant or tolerant when mortality was between 97 % and 80 %.

C. Three resistance classes as defined when twenty to 79 mosquitoes used per test, sus-

ceptible when mortality was 98 % or higher, resistance suspected, when mortality was between 95 % and 97 %, and resistant when the mortality was lower than 95 %.

### 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 (Shahgudian 1960, Zaim and Cranston 1986, Azari-Hamidian and Harbach 2009).

### 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 % (Abbott 1925, WHO 2006).

Data were analyzed by probit analysis (Finney 1971, 1978). Regression lines of the species were measured through the  $\chi^2$  test. The  $LT_{50}$  and  $LT_{90}$  were calculated for plotting the regression line using Microsoft Excel software ver. 2007.

## Results

Totally, nine species were collected including: *An. stephensi*, *An. dthali*, *An. culicifacies*, *An. fluviatilis*, *Cx. pipiens*, *Cx. quinquefasciatus*, *Cx. theileri*, *Cs. longiareolata*, *Oc. caspius* s.l (Fig. 2). In adult collection *An. stephensi* was dominant species 34.76 % allocated mosquitoes collected. *Anopheles dthali* and *An. culicifacies* were followed 15 %, 12.92 %, respectively. *Culiseta longiareolata* had the lowest density with 1.09 %. *An. culicifacies*,

*An. stephensi*, *Cx. pipiens*, *Cx. theileri* were collected in all months. In larval collection, *An. stephensi*, with 1495 specimens (28.9%) was predominant followed by *Cx. pipiens* 753 (14.1%), *An. culicifacies* 12.8 %, *Cx. quinquefasciatus* 6.3 % in the same month. It should be noted that *An. fluviatilis* larva was collected in May and December.

Mortality of Culicidae mosquitoes exposed to DDT and other insecticides has shown in tables 1 and 2.  $LT_{50}$  and  $LT_{90}$  values of *An. stephensi* to DDT 4% were 29.70 and 98.26 minutes, respectively. This species was quite resistant to DDT while susceptible to deltamethrin and tolerant to other insecticides (Fig. 3). It is concluded that *An. culicifacies* is tolerant to DDT, malathion, and propoxure whereas susceptible to deltamethrin, cyfluthrin and lambda-cyhalothrin (Table 2). The  $LT_{50}$  and  $LT_{90}$  values of this species to DDT 4 % were 18.12 and 46.42 minutes (Table 1, Fig. 3). *Anopheles dthali* was tolerant to DDT and propoxur whereas susceptible to deltamethrin, lambda-cyhalotrin, cyfluthrin and malathion (Table 2, Fig. 3). The  $LT_{50}$  and  $LT_{90}$  values to DDT 4 % were 17.86 and 48.42 minutes (Table 1).

It is concluded that *Cx. pipiens* is quite resistance to DDT, propoxur, lambda-cyhalothrin, and cyfluthrin whereas susceptible to Malathion (Table 2, Fig. 3). In our study *Oc. caspius* found to be resistant to DDT whereas susceptible to other insecticides (Table 2, Fig. 3). The  $LT_{50}$  and  $LT_{90}$  values to DDT 4 % were 44.68, and 164.01 minutes (Table 1). *Culiseta longiareolata* observed tolerant to DDT, propoxur, cyfuthrin, lambda-cyhalothrin whereas susceptible to deltamethrin (Table 2, Fig. 3). The  $LT_{50}$  and  $LT_{90}$  values to DDT 4 % were 17.82 and 51.26 minutes (Table 1).

**Table 1.** Regression line parameters of various species exposed to DDT 4% in Chabahar County, Sistan and Baluchistan Province, southeastern Iran, 2010

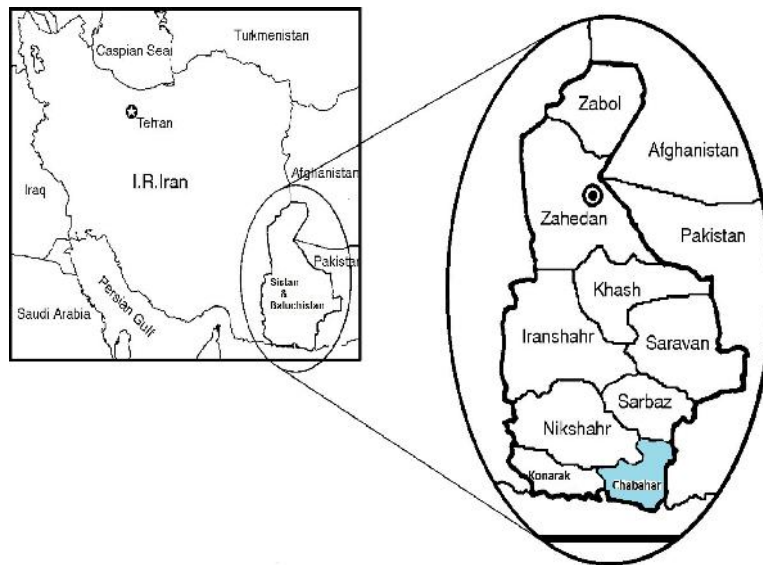
Species	*A	**B±SE	***LT <sub>50</sub> 95% C.I. (minute)	****LT <sub>90</sub> 95% C.I. (minute)	X <sup>2</sup> (df)	P value	Y=A+ BX
<i>An. stephensi</i>	-8.0186	2.4666±1.655	29.70	98.26	98.062(2)	<0.05	Y= -8.0186+ 2.4666 X
<i>An. culicifacies</i>	-9.5283	3.1379±3.222	18.12	46.42	265.565(2)	<0.05	Y= -9.5283+ 3.1379 X
<i>An. dthali</i>	-8.9653	2.9588±3.713	17.86	48.42	397.042(2)	<0.05	Y= -8.9653+ 2.9588 X
<i>Cx. pipiens</i>	-7.3266	2.2321±1.042	31.93	119.72	59.087(2)	<0.05	Y= -7.3266+ 2.2321 X
<i>Oc. caspius</i>	-8.2580	2.3891±3.687	44.68	164.01	409.134(2)	<0.05	Y= -8.2580+ 2.3891 X
<i>Cs. longiareolata</i>	-8.4636	2.7939±1.086	17.82	51.26	41.551(2)	<0.05	Y= -8.4636+ 2.7939 X

\*Intercept.

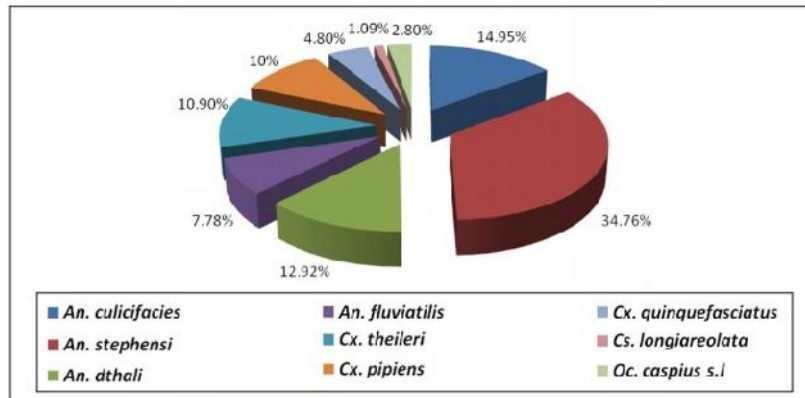
\*\*B± SE= slope and its standard error.

\*\*\*LT<sub>50</sub>, 95 % CI= lethal time causing 50 % mortality and its 95 % confidence interval.

\*\*\*\*LT<sub>90</sub>, 95 % CI= lethal time causing 90 % mortality and its 95 % confidence interval.



**Fig. 1.** Location of study area in southeastern Iran, 2010



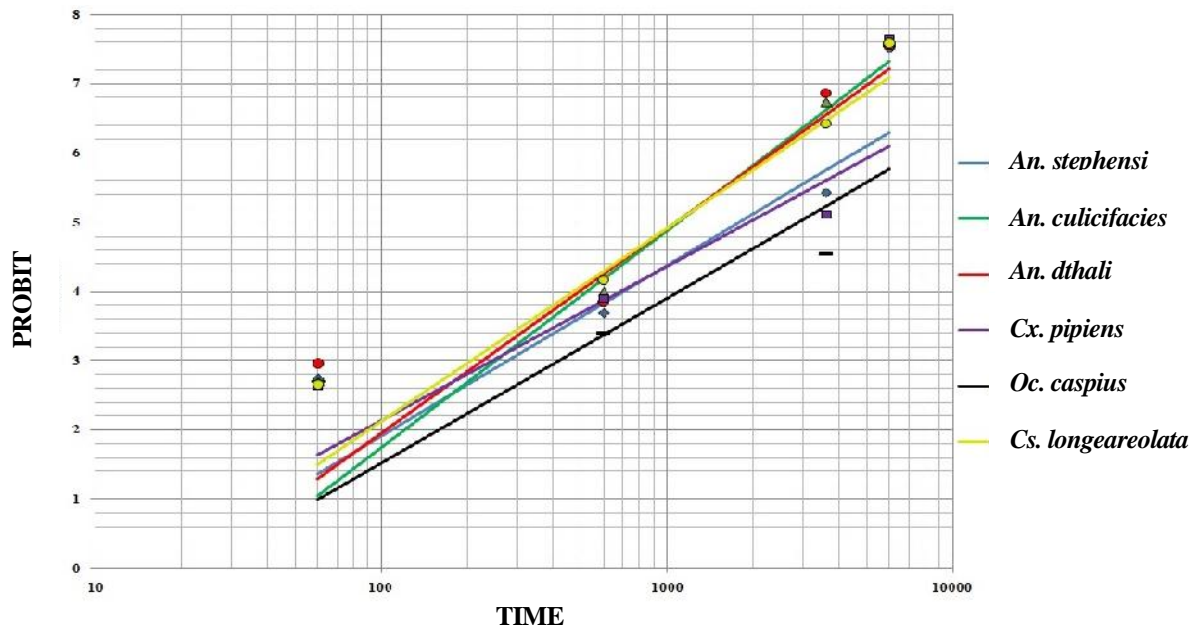
**Fig. 2.** The species composition of adult Culicidae mosquitoes collected by different sampling methods in Chabahar County, Sistan and Baluchistan Province, southeastern Iran, 2010

**Table 2.** Susceptibility level of various Culicidae mosquitoes exposed to some insecticides in Chabahar County, Sistan and Baluchistan Province, Southeastern Iran, 2010

Species	Insecticide	*MR±ER	**Resistance status
<i>An. culicifacies</i>	DDT 4%	96±2	T
	Malathion 5%	95±2	T
	Propoxur 0.1%	97±2	T
	Deltamethrin 0.05%	100	S
	Cyfluthrin 0.15 %	98±1	S
	Lambda-cyhalothrin 0.05%	99±1	S
<i>An. dthali</i>	DDT 4%	97±2	T
	Malathion 5%	98±1	S
	Propoxur 0.1%	97±2	T
	Deltamethrin 0.05%	100	S
	Cyfluthrin 0.15 %	98±1	S
	Lambda-cyhalothrin 0.05 %	99±1	S
<i>An. stephensi</i>	DDT 4%	67±5	R
	Malathion 5%	90±3	T
	Propoxur 0.1%	95±2	T
	Deltamethrin 0.05%	100	S
	Cyfluthrin 0.15 %	96±2	T
	Lambda-cyhalothrin 0.05 %	96±2	T
<i>Cx. pipiens</i>	DDT 4%	54±5	R
	Malathion 5%	100	S
	Propoxur 0.1%	20±4	R
	Deltamethrin 0.05%	93±2	T
	Cyfluthrin 0.15 %	72±4	R
	Lambda-cyhalothrin 0.05 %	72±5	R
<i>Cs. longiareolata</i>	DDT 4%	92±3	T
	Malathion 5%	-	-
	Propoxur 0.1%	92±3	T
	Deltamethrin 0.05%	100	S
	Cyfluthrin 0.15 %	88±3	T
	Lambda-cyhalothrin 0.05 %	96±2	T
<i>Oc. caspius</i>	DDT 4%	33±5	R
	Malathion 5%	100	S
	Propoxur 0.1%	100	S
	Deltamethrin 0.05%	100	S
	Cyfluthrin 0.15 %	100	S
	Lambda-cyhalothrin 0.05 %	100	S

\*Mortality rate ±Error Bar

\*\*R Resistance, S Susceptible, T Tolerance



**Fig. 3.** Regression line of adult Culicidae mosquitoes exposed to DDT 4 % in Chabahar County, Sistan and Baluchistan Province 2010

## Discussion

In our study, 4 genera and 9 species of mosquito larvae and adults were identified based on morphological characters. Culicidae species were belongs to the genus of *Anopheles*, *Culex*, *Culisita* and *Ochleratatus*. The Species of *Cx. quinquefasciatus* and *Cs. longiareolata* 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. (2004c) 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 (Vatandoost et al. 2006b).

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 (Mofidi et al. 1958, 1960, Manuchehri et al. 1976a, 1976b) and subsequently to dieldrin in 19609 (Mofidi and saminmi 1960) and

matlathion in 1976 (Eshghy 1978). Following the emergence of resistance of *Anopheles stephensi* to DDT, other organophosphours, 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 1985–88. The results revealed that this species was resistant to DDT and it is quite susceptible to Dieldrin (Manouchehri et al. 1976). In a similar study in south of Iran, the results showed that this species was resistant to DDT (Eshghy and Janbakhsh 1976). Prior of this study, Vatandoost et al. (2004) reported three biological forms of *An. stephensi* was susceptible to Bendiocarb, Propoxur, Malathion, Fenitrothion, Deltamethrin, Permethrin, Cyfluthrin and Lambda-cyhalothrin, resistance to DDT and tolerant to Dieldrin in Hormozgan Province, south of Iran. The same study was carried out on *An. stephensi* of Bandar Abbas strain, showed a relatively high adult resistance of

4.66-fold (LT<sub>50</sub>= 108min) to 4 % DDT (Davari et al. 2007). In our study, the LT<sub>50</sub> values of DDT 4.0 % against this species were 29.7 minutes, a manifestation of DDT resistance. Some investigations indicated *An. stephensi*, the main malaria vector, resistant to pyrethroid insecticides in southeastern Iran (Vatandoost and Hanafi-Bojd 2012).

*Anopheles culicifacies* comprised of five sibling species of A, B, C, D and E in North and South of India reported as A and B, in north east found D associated sympatric with A and B whereas in central area reported all of sibling species (Goswami et al. 2006). Polytene chromosome examination has been reported only available method that able to differentiate four members of this complex in areas where species E is not prevalent (Goswami et al. 2006). Species A has been reported in Oman and Iran (Zaim and Javaherian 1991). This species is known as vector of malaria in East Afghanistan (Zahar 1990). In our study, *An. culicifacies* was tolerant to DDT, malathion and propoxur while susceptible to other insecticides. In contrast, DDT resistance reported for the first time after residual spraying in Iran by Vatandoost et al. (2011). They reported susceptibility of *An. culicifacies* to pyrethroid and irritability to fenitrothion, cyfluthrin, and permethrin while high extremely resistance to DDT. There are several reports revealing DDT resistance of *An. culicifacies* in Afghanistan, and also resistance to dieldrin, malathion and DDT in India (Goswami et al. 2006).

In our study, *An. dthali* found tolerant to DDT and propoxur while susceptible to Deltamethrin, Lambda-cyhalothrin, Cyfluthrin and Malathion. In contrast, there are also some reports emphasizing to susceptibility levels of this species in different countries (WHO 1992, Hanafi-bojd et al. 2007). In spite, the existence of many reports about to susceptibility of *An. dthali* to insecticides, resistance reported to malathion, chloropyrifos,

bromophos, carbamate in Egypt and temephos in Jordan (WHO 1992).

In our research, *Cx. pipiens* larvae with 1073 (51.14%) collection, was found one of the most predominant species. In adult collection, *Cx. pipiens* catches 12.14 % and *Cx. quinquefasciatus* found as 4.82 % of total collection

Gjullin and Peters (1952) cited decrease of susceptibility level of *Cx. pipiens* complex to DDT and organophosphorus insecticide. First report related to resistance of the *Cx. quinquefasciatus* to organophosphorus insecticide was cited by Isaak in 1961 (Isaak 1961). Increasing in the resistance level of *Cx. pipiens* to organophosphorus insecticides reported by Toma et al. (2011). In our study, *Cx. pipiens* was quite resistance to DDT, propoxur, lambda-cyhalothrin, and cyfluthrin, whereas tolerant to deltamethrin and susceptible to malathion. In parallel, there are many reports in relation to some organophosphorus and pyrethroid insecticides resistance in Tunisia (Ben Cheikh et al. 1998), Cuba (Bisset et al. 1991), Burkina Faso (Chandre et al. 1998), Saudi Arabia (Amin and Hemingway 1998) and China (Jinfu 1999).

Another study showed, pyrethroid insecticides had tolerance of *Cx. pipiens pipiens* var *molestus* in North America (McAbee et al. 2003). In contrast, DDT resistance of *Cx. pipiens* complex reported in southern Tehran (Nazari and Janbakhsh 2000). LT<sub>50</sub> value for propoxur and malathion calculated as 51, and 31 minutes respectively (Nazari and Janbakhsh 2000). The species reported quit susceptible to dieldrin, propoxur and malathion (Nazari and Janbakhsh 2000). In our study, *Cx. pipiens* was highly resistance to DDT, propoxur, cyfluthrin and lambda-cyhalothrin as evidenced by the discriminating concentrations of times. The results may be related to the present of the species complex, application of IRS and distribution of LLINs in southern Iran.



In the present study, *Oc. caspius* found to be quite resistance to DDT whereas susceptible to other insecticide. The  $LT_{50}$  and  $LT_{90}$  values to DDT 4 % were 47.68 and 164.01 minutes. In addition, our study showed *Cs. longiaerolata* quite resistance to DDT and susceptible to other insecticides. The  $LT_{50}$  and  $LT_{90}$  values to DDT 4 % were 17.82 and 50.26 minutes. Based on the literature, no reports were available on the susceptibility levels of *Oc. caspius* and *Cs. longiaerolata* to WHO recommended insecticides in the world.

## Conclusion

Iran is in the malaria elimination stage (WHO 2011). By now, IRS in human dwellings and animal shelters, space-spraying, personal protection through distribution of LLINs and curtains (ICNs), repellents measures used to control of malaria vectors in Iran. In addition, some biological and chemical agents against larval and adult stages of Culicidae mosquitoes had been evaluated in the laboratory. Results obtained from susceptibility tests of the malaria vectors on DDT revealed that only *An. stephensi* was highly resistance to DDT in Chabahar area, precautionary measures should be taken in future vector control operations. Moreover, the status of resistance in other locations in malaria endemic area should be investigated. Since the country relies on deltamethrin for IRS operation, tolerant populations of *Cx. pipiens* implies careful consideration and regular monitoring of susceptibility level of mosquitoes in the future.

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