Original Article

Assessing the Susceptibility Status of Mosquitoes (Diptera: Culicidae) in a Dirofilariasis Focus, Northwestern Iran

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Abstract

Background: Mosquitoes are considered as the vectors of dirofilariasis and some vector borne disease in Iran. The objective of this study was to determine the susceptibility level of the vectors to various insecticides recommended by WHO for any control measures in an endemic area in northwestern Iran.
Methods: Mosquito larval and adult collections were carried out using different methods provided by WHO including dipping and hand catch techniques. The susceptibility level was assessed to DDT 4%, malathion 5%, propoxur 0.1%, deltamethrin 0.05% and lambda-cyhalothrin 0.05%.
Results: Totally, 749 adults and 5060 larvae of Culicidae mosquitoes were collected comprising seven species of adult and larvae, including: *Anopheles claviger*, *An. maculipennis*, *An. sacharovi*, *Culex hortensis*, *Cx. pipiens*, *Cx. theileri* and *Culiseta longiaerolata*. Frequency of larvae and adults of *An. maculipennis* was very low, so susceptibility tests on this species did not performed. Results showed that *Cx. theileri*, *Cs. longiaerolata* and *Cx. pipiens* were resistant to DDT 4%, lambda-cyhalothrin 0.05%, and propoxur 0.1% whereas found tolerant to deltamethrin 0.05% and malathion 5%. The LT_{50} and LT_{90} values for five insecticides were calculated.
Conclusion: We suggest the same study in different parts of the world to obtain the data due to bionomic and susceptibility status of dirofilariasis vectors. This information will help the health authorities for monitoring and evaluation of control measures.

Keywords: Culicidae, Vector, *Dirofilaria immitis*, Susceptibility test**,** Iran

Introduction

Mosquitoes (Diptera: Culicidae) are the most important groups of arthropods in medical and veterinary. They act as vectors of several diseases such as malaria, yellow fever, dengue, filariasis, setariasis and encephalitis, causing serious health problems to humans (Service 2003, Almeida et al. 2008).

The Culicidae mosquitoes are vector of dogs heartworm parasites, *Dirofilaria immitis*, *Di. repens*, *Wuchereria bancrofti*, some arboviral diseases such as Japanese Encephalitis, Rift valley fever, Western equine encephalitis and Eastern equine encephalitis, Tahyna, Sagiyama, Trivitatus, Lymphocytic Choriomanangitis, West Nile virus, St Louis encephalitis, California encephalitis (Muller 2002, Mullen and Durden 2009).

Dirofilariasis is a metazoonotic disease that is transmitted by certain species of mosquitoes. *Dirofilaria immitis* could be found in carnivores especially dogs and reported from various countries including Turkey (Atas et al. 1997), Japan (Hatsushika et al. 1997), Brazil (Reifur et al. 2004), Canada (Slocombe and Villeneure 1993) and other countries (Chen and Liang 1993, Rosa et al. 2002).

Dirofilaria immitis is commonly causes canine heartworm disease in many countries

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(Genchi et al. 2005). Human dirofilariasis has been shown as pulmonary or subcutaneous infection, but in Korea one case of hepatic dirofilariasis has been reported by *Di. immitis* agent (Choi et al. 2002). Dirofilariasis produced by *Di. immitis* parasites have been reported from all tropical, subtropical and moderate areas and the highest prevalence is in North America, South America, Australia, Italy and Japan (Venco et al. 2004). Dirofilariasis is a worldwide distributed disease, but the most endemic areas are those with moderate, tropical and subtropical climates, where mosquito populations are high and stable. Other regions with cold climates or with hot summers and with rivers, lakes and widely irrigated lands are also suitable for the development of the disease. Endemic occurrence of *Di. immitis* has been reported in the USA, Canada, South America, Africa, Australia, Asia and Europe (Yildirim et al. 2006).

Dirofilaria immitis was first reported from a dog in Iran in 1969 (Sadighian 1969). Then infection to heartworm was reported from different areas of Iran including Ardebil (Bokai et al. 1998), Shiraz (Jafari et al. 1996), Tehran (Meshgi and Eslami 2001), Tabriz (Meshgi et al. 2002) Tonekabon (Ranjbar-Bahadori et al. 2005), and Mashhad (Razmi 1999). Iran reported one of the disease foci in the world (WHO/FAO/OIE 1984). Zoonotic dirofilariasis has been reported in 11 provinces of Iran (Azari-Hamidian et al. 2007). By now, *Di. immitis* has been isolated from peripheral blood in a cat in Tabriz (Ashrafi et al. 2001). Azari-hamidian et al. (2007) reported *Di. immitis* in dogs, jackals, foxes, wolves and cats, and *Di. repens* in dogs and jackals in different areas of the country. The natural infection has been reported about 26.7% on stray dogs in Tonekabon (former Shahsavar), Mazandaran Province (Sadighian 1969). In addition, contamination of 0.95 and 36.8 % has been reported in the East and West Azerbaijan, Ardebil and Tehran, Fars and Khuzestan, Mazandaran, Golestan and East Azerbaijan Provinces (Sadighian 1969, Abai 1990, Zarriffard 1993, Jafari et al. 1996, Jamali and Hashemzade Farhang 1996, Bokaie et al. 1998, Farahnak et al. 1998, Razm Arayi et al. 2000, Ariamanesh 2000, Mashgi and Eslami 2001, Sadjjadi et al. 2004, Fallah et al. 2005, Ranjbar-Bahadori and Eslami 2005). Infection of subcutaneous dirofilariasis has been reported in the Anzali Port and Lahijan, Karaj and Ahvaz County (Siavashi and Massoud 1995, Athari 2003, Maraghi et al. 2006, Radmanesh et al. 2006, (Khanmohammadi et al. 2011).

By now, human and animal dirofilariasis cases have been recorded in 11 of the 31 provinces of Iran. Azari–Hamidian et al. (2009) reported the fourteen human cases of infection, including eight subcutaneous and three ocular cases of *Di. repens*, a testicular hydrocele case of *Di. immitis* and two pulmonary cases probably attributable to *Di. immitis.*

Two species of *Dirofilaria* include *Di. immitis* (canine heartworm) and *Di. repens* have been reported in Iran. Microfilarial worms of *Di. immitis* as 258±7µm, and for infected larvae ranged 0.75–1.3mm (Anderson 2000). The length of adult calculated as 250–300×1 mm (Muller 2002). Female mosquitoes serve as an intermediate host by sucking blood from dog which circulating *Di. immitis* microfilaria (Montoya et al. 1998). The diagnosis of canine heartworm infection is based upon the detection of *Di. immitis* circulating in blood or upon the detection of serum antibodies by serologic methods (Peribanez et al. 2001). *Wolbachia* (Rickettsiales: Rickettsiaceae) found in filarial nematodes such as *Dirofilaria* spp., have an important role in the filarial infection pathogenesis (Bazzocchi et al. 2000). The immune response of dog against *Wolbachia*in, considered as a diagnostic method for canine heartworm diseases (Tiawsirisup et al. 2010).

Some of mosquito species are able to transmit infected of third-stage larvae of

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Dirofilaria parasite (Aranda et al. 1998). Many of *Di. immitis* vectors reported in the world (Grieve et al. 1983). The different species of the genera of *Aedes*, *Ochlerotatus*, *Psorophora*, *Anopheles*, and *Culex* found susceptible to *Di. immitis* in USA (Mullen et al. 2009). *Anopheles maculipennis* and *Cx. theileri* reported naturally infected with infective larvae of *Di. immitis* and *Setaria labiatopapillosa* in Iran (Azari-Hamidian et al. 2009).

In Iran, the insecticides residual spraying against malaria vectors was carried out during 1950–1968. Following the resistance of the vectors to DDT (1957), dieldrin (1960) and malathion (1976), carbamate and pyrethroid insecticides were used (Zaim 1987, Moosa-Kazemi et al. 2007). Some enzymes such as monooxygenases, esterases and glutathione S-transferases have been considered as a reason for resistance to Permethrin insecticides. The efficacy of these insecticides will be increased by using some synergists like piperonyl butoxide (PBO) that inhibits monooxygenases ortribufos (DEF) that inhibits esterases (Enayati et al. 2003).

Prior of this study, there are no reports on the susceptibility of mosquitoes to insecticides in Ahar County, East Azarbaijan Province. The aim of this study was to determine the susceptibility level of dirofialriasis vectors to insecticides in the area.

Materials and Methods

Study area

East Azarbaijan Province is located in the northwest of the country, bordering Armenia and the Republic of Azarbaijan. The investigation was carried out during the summer of 2011 in the rural district of Ahar County (38˚45´N, 47˚11´E). The population of Ahar County has been reported 147.781 and 34.067 families. Agriculture, horticulture, livestock and making hand crafts making carpet and needlework are introduced as the main jobs. Cold and moderate weather provides the suitable condition for agricultural and husbandry activities. Three fixed Afil, (38˚37´N, 47˚31´E), Noghdose (38˚39´N, 47˚36´E), Bohol (38˚36´N, 47˚35´E) and six variable villages selected randomly (Fig. 1).

Mosquito collection

Hand collection: In each fixed and variable places, mosquitoes were collected from indoor places by the using suction tubes. Adult mosquitoes were collected from the villages, 05.00 to 08.00 AM by using suction tube. Then the adult mosquitoes released in the cage and covered with wet towel and mosquitoes feed by sucrose 5 % solution (Silver 2008).

Larval collection: In each fixed station the mosquitoes were collected from June to August 2011. The related data such as water temperature, larval type, larval number and the date of sampling were recorded. The larvae were collected from the villages, 08.00 to 11.00 AM by using standard dipper (350 ml) and eye dropper. The larvae were transferred into a closed container, sent to the laboratory and placed within a few cups into cages to obtain F_1 generation. The samples were reared (Silver 2008).

Adult susceptibility test

The mosquitoes were transferred into the exposure tubes at different logarithmic exposure time, and mortality was then calculated after a 24 h recovery period (25 ˚C, 75%RH). The mortalities between 5.0 to 20.0 % were corrected by Abbott's correction formula. All the tests were accepted when the mortality was less than 5% and ignored when the mortality was more than 20% in the control group (Abbott 1925, Silver 2008, WHO 1998). The susceptibility level of the species was considered in three classes as susceptible, tolerant and resistant based on WHO criteria (WHO 2002). The mortality more than

98 % was considered as susceptible, less than 80 % noted as resistant and between 97 to 98 % defined as tolerant.

Insecticides impregnated papers

Impregnated papers, DDT 4 %, malathion 5 %, deltamethrin 0.05 %, propoxur 0.1 % and lamda-cyhalothrin 0.05 % were supplied by WHO.

Statistical analysis

The LT_{50} was considered as lethal time causing 50 % mortality and its 95 % confidence interval and LT_{90} mentioned as lethal time causing 90 % mortality and its 9 5% confidence interval. The LT_{50} and LT_{90} values of *Cx. theileri*, *Cx. pipiens* and *Cs. longiaerolata* and probit regression line parameters were calculated to Finney's test (Finney 1971). Plotting the regression line was calculated through the χ2 test using Microsoft Excel ver. 2007.

Identification of mosquito using morphological characteristics

The samples were mounted and identified by systematic keys (Shahgudian 1960, Zaim and Cranston 1986, Azari-Hamidian and Harbach 2009).

Results

Totally, 749 adults and 5060 larvae of Culicidae mosquitoes were collected including seven species, *An. maculip*ennis sl, *An. sacharovi*, *An. claviger*, *Cx. pipiens*, *Cx. hortensis*, *Cx. theileri* and *Cs. longiaerolata*. *Culex theileri* was found to be dominant species as 42.8 %, 33.6 % allocated in adult and larval stages, respectively. *Cx. pipiens* was followed by 29.9 %, 25.3 % in adult and larvae respectively. *An. maculipennis* sl was found 6.9 % in larvae collection. *An. claviger* was collected as low density (4.7%), *An. sacharovi*, *Cx. hortensis* and *Cs. longiaerolata* were also collected in larval habitats. The maximum and minimum temperature in larval habitats was 29 ˚C and 22 ˚C, respectively.

An. maculipennis sl was collected very low, so susceptibility tests on this vector did not perform. The LT_{50} and LT_{90}values for five insecticides are shown in Table 1, Figs. 2–6. The mortality rates of field collected *Cs. longiareolata*, *Cx. theileri* and *Cx. pipiens* to DDT 4 % at diagnostic exposure time were 18 %, 15 %, 23 %, respectively.

Considering the criteria of resistance of the species described by WHO (Table 2), it should be mentioned that the species of *Cx. pipiens*, *Cx. theileri* and *Cs. longiaerolata* were resistant to DDT 4 %, Lambda-cyhalothrin 0.05 %, and propoxur 0.1 %. These mosquitoes were found tolerant to deltamethrin 0.05 % and malathion 5 %. Our results based on probit regression line showed that *Cs. longiaerolata* was more susceptible than *Cx. theileri* and *Cx. pipiens* when exposed to deltamethrin 0.05 % and malathion 5 %. In addition, *Cs. longiaerolata* was found more susceptible to *Cx. pipiens* and *Cx. theileri* after exposed to lambda-cyhalotherin 0.05 %, propoxur 0.1 % and DDT 4 %.

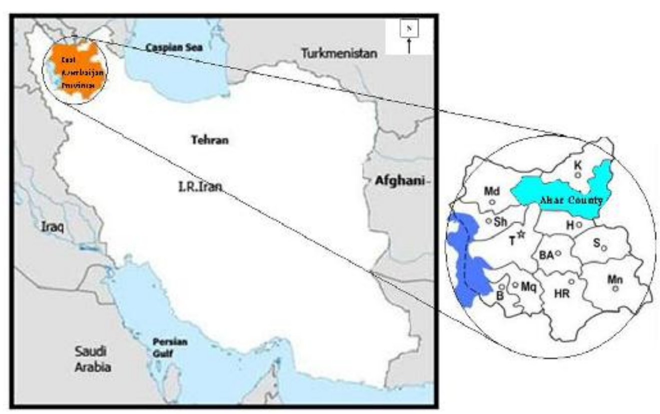

Fig. 1. Map of the study area, East Azerbaijan Province, northwestern Iran

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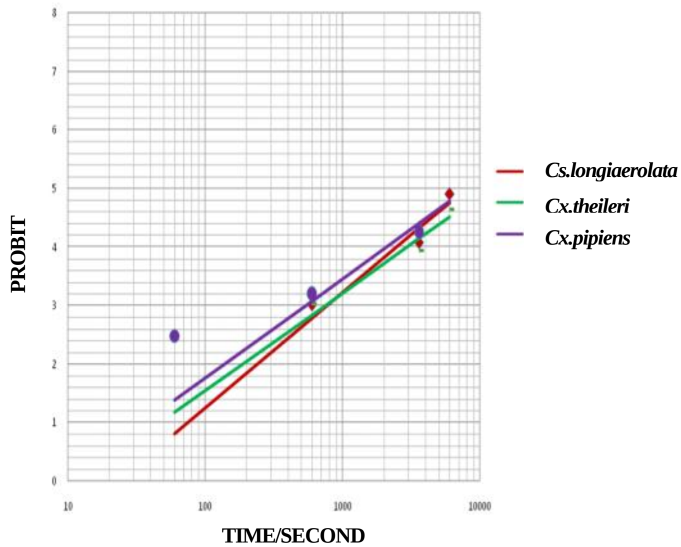

Fig. 2. Probit regression line of Culicinae mosquitoes to DDT 4 % in Ahar County, East Azarbaijan Province, 2011

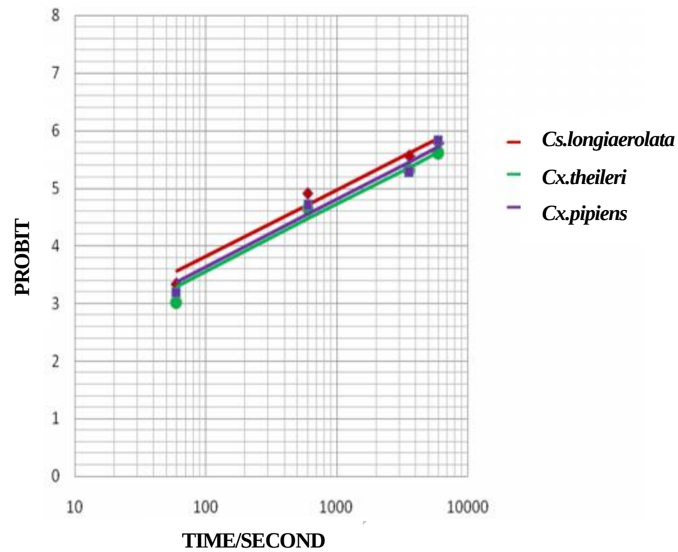

Fig. 4. Probit regression line of Culicinae mosquitoes to Lambda-cyhalothrin 0.05 % in Ahar County, East Azarbaijan Province, 2011

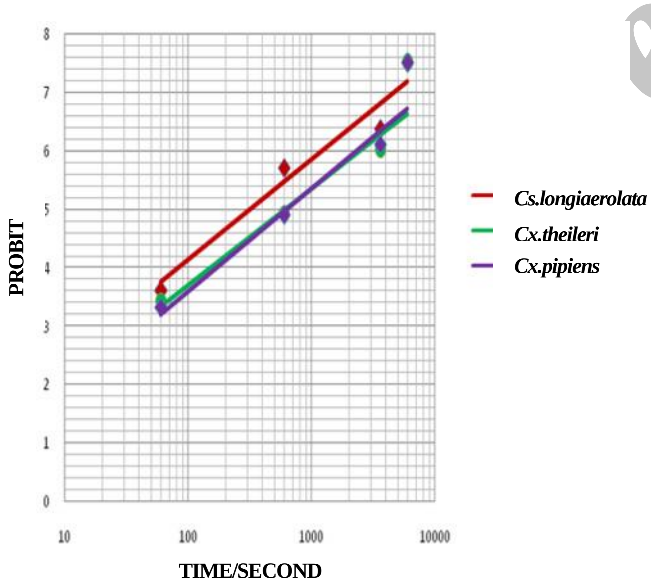

Fig. 3. Probit regression line of Culicinae mosquitoes to Deltamethrin 0.05 % in Ahar County, East Azarbaijan Province, 2011

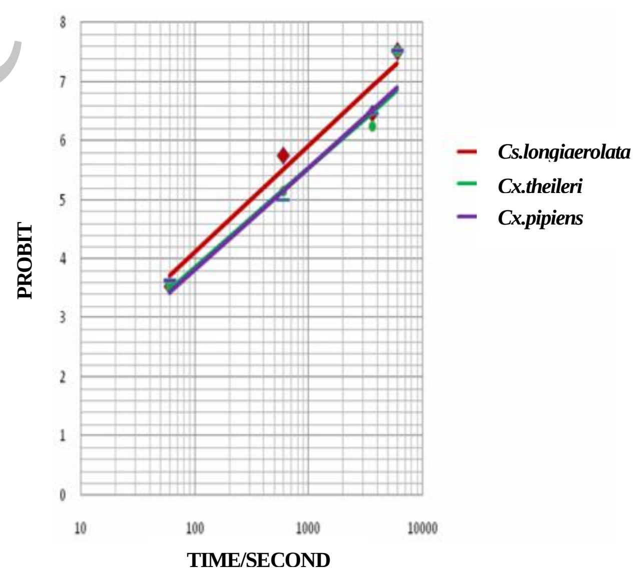

Fig. 5. Probit regression line of Culicinae mosquitoes to Malathion 5 % in Ahar County, East Azarbaijan Province, 2011

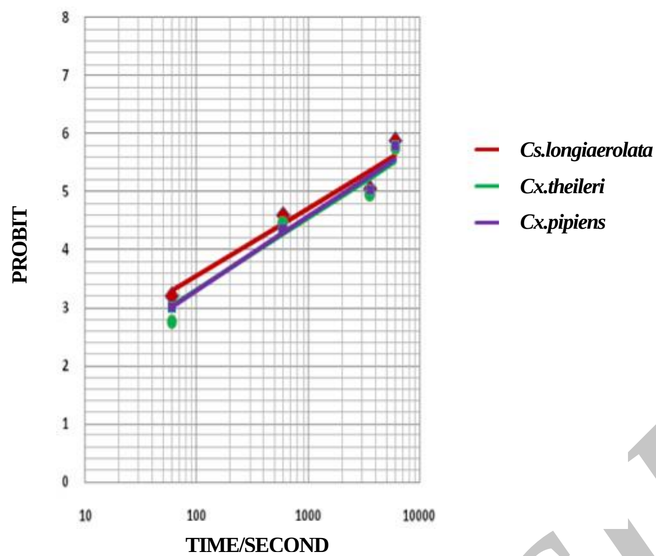

Fig. 6. Probit regression line of Culicinae mosquitoes to Propoxur 0.1 % in Ahar County, East Azarbaijan Province, 2011

Table 1. Probit regression line parameters of insecticides against mosquitoes in Ahar County, East Azarbaijan Province, 2011

Insecticides	species	*A	**B±SE	***LT_{50} 95%C.I (Minute)	****LT_{90} 95%C.I (Minute)	X^2 (df)	Y= A+BX	*P*-value
DDT 4%	*Cs. longiaerolata*	-7.6975	1.9745±0.381	131.94	588.13	4.119(2)	Y= -7.6975+1.9745 X	<0.05
	Cx. theileri	-6.7965	1.6702±0.363	195.48	1144.01	2.624(2)	Y= -6.7965+1.6702 X	<0.05
	Cx. pipiens	-6.6372	1.6985±0.305	134.75	765.75	1.778(2)	Y= -6.6372+1.6985X	<0.05
Deltamethrin 0.05%	*Cs. longiaerolata*	-4.2737	1.7124±0.148	5.21	29.24	9.996(2)	Y = -4.2737+1.7124 X	<0.05
	Cx. theileri	-4.691	1.6581±0.141	10.179	60.350	7.516(2)	Y = -4.6191+1.6581 X	<0.05
	Cx. pipiens	-4.9528	1.7710±0.152	10.432	55.211	5.921(2)	Y = -4.9528+1.7710 X	<0.05
Lambda-cyhalothrin 0.05%	*Cs. longiaerolata*	-3.4762	1.1496±0.114	17.60	229.26	3.329(2)	Y= -3.4762+1.1496 X	<0.05
	Cx. theileri	-3.7667	1.1630±0.123	28.87	365.2	2.621(2)	Y= -3.7667+1.1630 X	<0.05
	Cx. pipiens	-3.7260	1.1772±0.121	24.37	298.77	4.048(2)	Y= -3.7260+1.1772 X	<0.05
Malathion 5%	*Cs. longiaerolata*	-4.4964	1.8031±0.157	5.19	26.69	9.939(2)	Y= -4.4964+1.8031 X	<0.05
	Cx. theileri	-4.5115	1.6842±0.142	7.95	45.87	4.676(2)	Y= -4.5115+1.6842 X	<0.05
	Cx. pipiens	-4.6712	1.7413±0.148	8.025	43.70	5.028(2)	Y= -4.6712+1.7413 X	<0.05
Propoxur 0.1%	*Cs. longiaerolata*	-3.7575	1.1588±0.123	29.12	371.76	8.809(2)	Y= -3.7575+1.1588 X	<0.05
	Cx. theileri	-4.1764	1.2443±0.137	37.86	405.64	9.727(2)	Y= -4.1764+1.2443 X	<0.05
	Cx. pipiens	-4.2249	1.2666±0.137	36.105	371.07	5.825(2)	Y= -4.2249+1.2666 X	<0.05

*A= intercept.
**B±SE= slope and its standard error.
***LT50, 95 % CI= lethal time causing 50 % mortality and its 95 % confidence interval.
****LT90, 95 % CI= lethal time causing 90 % mortality and its 95 % confidence interval.

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Table 2. Susceptibility status of culicinae species to insecticides in Ahar County, East Azarbaijan Province, 2011

Species	Insecticide	MR±EB*	Resistance status**
Cs. longiaerolata	DDT 4 %	18±4	R
	Deltamethrine 0.05 %	87±4	T
	Lambda-cyhalothrine 0.05	73±5	R
	Malathion 5 %	90±3	T
	Propoxur 0.1 %	55±5	R
Cx. theileri	DDT 4 %	15±4	R
	Deltamethrine 0.05 %	88±4	T
	Lambda-cyhalothrine 0.05	65±5	R
	Malathion 5 %	93±3	T
	Propoxur 0.1 %	50±6	R
Cx. pipiens	DDT 4 %	23±5	R
	Deltamethrine 0.05 %	91±3	T
	Lambda-cyhalothrine 0.05	63±5	R
	Malathion 5 %	97.5±2	T
	Propoxur 0.1 %	53±6	R

*Mortality Rate±Error Bar
**R: Resistance, T tolerance

Discussion

In this study, a total of 3 genera and 7 species of mosquito larvae and adults were collected in Ahar County. Culicidae species belonged to the genus of *Anopheles*, *Culex* and *Culiseta*.

The most predominant species was *Cx. theileri* as 42.8% of adult and 33.6% larvae collection. This species is reported as one of the most predominant species in Ardebil (Azari-Hamidian et al. 2009), Isfahan (Moosa-Kazemi et al. 2000) and Guilan Province (Lotfi 1970, Azari-Hamidian et al. 2000, Azari-Hamidian et al. 2001, Azari-Hamidian 2007). This species cited as predominant in Zanjan (Ghavami and Ladoni 2005), East Azarbaijan (Abai et al. 2007), and Southeastern Iran (Moosa-Kazemi et al. 2009). Simsek (2004) noted the dominant species in Ankara, Turkey (Simsek 2004). Zoophilic index on goat, dog, cattle, and human reported as 58.3%, 50%, 44.4% and 13.6%, respectively. *Culex theileri* found 1.49% of the total collection in Neka County (Nikookar et al. 2010). The seasonal activity of larvae started at the end of May reach to peak in the end of June in Isfahan Province. Seasonal activity of adults began in the end of May reach to peak in mid of July (Mousakazemi 1995). The maximum blood feeding activity reported as 22.30 PM–00.30 AM, 23% of feeding observed in the second third of the night while 19% found in the first third of the night (Moosa Kazemi e al. 2010).

For the first time *Di. imimitis* was isolated from *Cx. theileri* with 10% infection in Meshkin Shahr (Azari-Hamidian et al. 2009). Nazari and Janbakhsh (2000) noted the resistance of this species to DDT, tolerant to propoxur and dieldrin in southern Tehran.

In our study, LT_{50} value of *Cx. theileri* for DDT, deltamethrin, lambda-cyhalothrin, malathion and propoxur was calculated as 195.48, 10.179, 28.87, 7.95 and 37.86 minutes respectively. LT_{90} value found as 1144.01, 60.35, 365.2, 45.87 and 405.64 minutes for DDT, deltamethrin, lambda-cyhalothrin, malathion and propoxur respectively. In contrast, LT_{50} value of this species for propoxur and malathion reported as 31, and 22 minutes respectively (Nazari and Janbakhsh 2000). At present, seems to, this species was resistance to DDT, lambda-cyhalothrin and propoxur whereas tolerant to deltamethrin and malathion.

In our study, *Cx. pipiens* was dominant species comprised as 29.9% of adult and 25.3% of larvae. Minimum and maximum temperature in the larval habitat found as 22 ˚C, 29 ˚C respectively. The larvae collected in the larval habitat as a range of altitude 834–1382 meters. Based on morphological characteristic *Cx. pipiens* with *Cx. vagans* and *Cx. torentium* has mentioned in one group by Zaim and Cranston (1986). Lotfi (1970) reported the *Cx. torentium* from Fars Province based on the siphon index and size of siphonal setae (Lotfi 1970). *Culex pipiens* complex have a great abundance in north and north west of the country (Lotfi 1970, Azari-Hamidian et al. 2000, Azari-Hamidian et al. 2001) East Azarbaijan (Abai et al. 2007), Zanjan (Ghavami and Ladoni 2005). This species reported the dominant species in Isfahan Province. Larvae were collected from rice fields (Mousakazemi 1995). The larval activity in Isfahan began in late May reached peak in late June and then declined. The seasonal activity of adult started in the end of June and reached peak in mid-July and declined in end of August. The attractiveness of this species to light trap had been reported more than to human bait collection (Mousakazemi 1995).

Azari-Hamidian et al. (2001) reported the blood feeding of this species on cattle in north of Iran. In contrast Mousakazemi (1995) reported the tendency of blood feeding on human in central Iran (Mousakazemi 1995). It seems that the limitation of animal host in the urban area of Lenjan County, leads to attractive of this species to light trap. This species was reported one of the most predominant species in Mazandaran Province in North of Iran (Nikookar et al. 2010). The adaptation of *Cx. pipiens* to variety of breeding places with different degrees of pollution near the human residence reported by Ghavami and Ladoni (2005) and Dehghan et al. (2010).

Culex pipiens and *Cx. quinquefaciatus* larvae were detectable based on Siphon/ Saddle index and seta 1 on abdominal segment III-IV (Harbach 1985, Azari-Hamidian and Harbach 2009, Dehghan et al. 2010). Seta 1 on abdominal segment III-IV in *Cx. pipiens* is usually single while this character in *Cx. quinquefaciatus* is usually double. *Culex pipiens* form *molestus* can be separated from *Cx. pipiens pipiens* mainly by ecological and physiological characters. *Culex pipiens* form *molestus* this found in open spaces and the accumulated water. The adults are able to laying the first batch of eggs without a blood-sucking (Autogenes). They are mating in small spaces and they hibernate in the winter (Stenogamus). The blood feeding of *Cx. pipiens* form *molestus* reported from mammals more than other animals.

The main different characteristic between the adult of *Cx. quinquefasciatus* and *Cx. pipiens pipiens* was reported as the ratio of DV/D in male genitalia (Sundaramans 1949). The distance of tip of ventral arm to dorsal arm of phalosoma considered as DV and D mentioned the distance of tip of dorsal arms of phalosoma (Barr and Kabtman 1951, Mattingly et al. 1951). In addition some characters are important including: the number of setae on the side in the ninth tergit hairs on maxillary pulp of the adult male, and DV/D (Vinogradova 2000). The most reliable character for identifying larval stages of *Cx. torentium* and *Cx. pipiens* reported as siphon/saddle index and Seta 1 on abdominal segment III-IV. In *Cx. torentium* reported with four branches (Harbach 1985, Azari-Hamidian and Harbach 2009, Dehghan et al. 2010).

Culex pipiens includs *pipiens* and *molestus* forms. *Culex pipiens pipiens* is considered as

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unatogenous laying eggs on open breeding places, and pass the winter with diapose while *Cx. pipiens* form molestus is considered as autogenous, stenogamus, and pass the winter without diapose. *Culex pipiens pipiens* blood feeds on birds than human whereas *Cx. pipiens* form *molestus* feed on human. In addition blood feeding of *Cx. pipiens* form molestus is reported from birds, rodents and guinea pigs. *Culex pipiens pipiens*, *Cx. quinquefasciatus* and *Cx. pipiens* form *molestus* as the most common and most widespread in the world. *Culex pipiens* form *molestus* habitat found in temperate climates, whereas *Cx. quinquefasciatus* has been distributed in most parts of the world and considered as cosmotropical (Vinogradova 2000).

Gjullin and Peters (1952) reported the tolerant of *Cx. pipiens* complex to DDT and organophosphoros insecticidae. The first reports related to resistance of the *Cx. quinquefasciatus* to organophosphoros insecticidae was reported by Isaak (Isaak 1961). In addition, the increase of the resistance to organophosphoros insecticides reported by Toma et al. (2011). Many studies indicated the tolerance to organophosphoros from Tunisia (Ben Cheikh et al. 1998), Cuba (Bisset et al. 1991, Rodriguez et al. 1993), Burkina Faso (Chandre et al. 1998), Saudi Arabia (Amin and Hemingway 1998), China (Jinfu 1999) and North America (McAbee et al. 2003).

In Iran, indoor residual spraying was carried out during 1950–1968 against malaria vector. Following the resistance of *An. stephensi*, the main malaria vector to DDT, other insecticides such as dieldrin, malathion, propoxur, lambda-cyhalothrin and recently deltamethrin used. The resistance of *Cx. pipiens* complex to DDT reported in southern Tehran. LT_{50} for propoxur and malathion calculated as 51, and 31 minutes respectively. The species was quit susceptible to propoxur and malathion (Nazari and Janbakhsh 2000). Khyami-Horani et al. (1995) reported two standard strains, *Bacillus thuringiensis*, and *Ba. sphaericus* had killing effects as 0.0006 to 0.006 ppm and 0.003 to 0.06 ppm respectively against *Cx. pipiens molestus* larvae.

In our study, LT_{50} value of *Cx. pipiens* for DDT, deltamethrin, lambda-cyhalothrin, malathion and propoxur calculated as 134.75, 10.43, 24.37, 8.025 and 36.105 minutes respectively. LT_{90} value found as 765.75, 55.211, 298.77, 43.70 and 371.07 minutes for DDT, deltamethrin, lambda-cyhalothrin, malathion and propoxur respectively. LT_{50} value of this species in our study was lower than the previous study cited by Nazari and Janbakhsh (2000).

In our study, *Cs. longiaerolata* observed resistance to DDT, propoxur, lambda-cyhalotrin and tolerant to malathion and deltamethrin. LT_{50} value found as 131.94, 5.21, 17.60, 5.19 and 29.12 minutes for DDT, deltamethrin, lambda-cyhalothrin, malathion and propoxur respectively. LT_{90} value of this species for DDT, deltamethrin, lambda-cyhalothrin, malathion and propoxur calculated as 588.13, 29.24, 229.26, 26.69 and 371.76 minutes respectively.

Some reports emphasizing to susceptibility of *Cs. longiareolata* larvae to *Ba. sphaericus* and *Ba. thuringiensis* (Katbeh Bader et al. 1999). The range of mortality found as a 0.003–0.004 ppm. The mortality of larvae in temperature of 28±1 ˚C found more than 20±1 ˚C (Katbeh Bader et al. 1999). Bouaziz et al. (2011) reported the LC_{50} values of *Novaluron* (IGR) against *Cs. longiareolata* as 0.51 and 0.91 μg/l respectively. The LC_{90} values were reported as 2.32–4.30 μg/l (species was collected to 41.1 % in larval collection (Bouaziz et al. 2011).

Conclusion

Cross-resistance of *Cs. longiareolata*, *Cx. pipiens* and *Cx. theileri* found after exposed to DDT and lambda-cyhalothrin. Also, we recommend the same procedure in field trial

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in different parts of Iran such as West Azerbaijan, Ardebil, Tehran, Fars, Khuzestan, Mazandaran and Golestan Provinces to obtain the unique conclusion about criteria for susceptibility status. The results could be useful for controlling of vector-borne diseases and *Dirofolaria immitis* in the future.

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