Original Article

High Insecticides Resistance in *Culex pipiens* (Diptera: Culicidae) from Tehran, Capital of Iran

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Abstract

Background: During recent years transmission of *Dirofilaria immitis* (dog heart worm) by *Culex pipiens* and West Nile virus have been reported from Iran. The present study was preformed for evaluating the susceptibility status of *Cx. pipiens* collected from capital city of Tehran, Iran**.**

Methods: Four Insecticides including: DDT 4%, Lambdacyhalothrin 0.05%, Deltamethrin 0.05% and Cyfluthrin 0.15 % according to WHO standard methods were used for evaluating the susceptibility status of *Cx. pipiens* from Tehran moreover For comparison susceptibility status a Laboratory strain also was used. Bioassay data were analyzed using Probit program. The lethal time for 50% and 90% mortality (LT_{50} and LT_{90}) values were calculated from regression line.

Results: The susceptibility status of lab strain of *Cx. pipiens* revealed that it is susceptible to Lambdacyhalothrin, Deltamethrin, Cyfluthrin and resistant to DDT. Moreover cyfluthrin with LT_{50}=36 seconds and DDT with LT_{50}=3005 seconds had the least and most LT_{50}s. Field population was resistance to all tested insecticides and DDT yielded no mortality.

Conclusion: Highly resistance level against all WHO recommended imagicides were detected in field populations. We suggest more biochemical and molecular investigations to detect resistance mechanisms in the field population for further decision of vector control.

Keywords: Susceptibility status, *Culex pipiens*, Tehran

Introduction

Mosquitoes known as the main groups of arthropods in medical and public health due to their role in transmission of Malaria, filariasis, several types of encephalitis and also many arboviral diseases (Horsfall 1955, Service 2003, Mullen 2009). In the Culicidae Family, *Culex* genus and specifically *Cx. pipiens* complex members including: *Cx. pipiens pipiens*, *Cx. quinquefasciatus*, *Cx. p. pallens*, *Cx. pipiens* form molestus act as efficient vectors for Sindbis virus, West Nile virus, Equine encephalitis, St Louis, Oro-pouche, Rift Valley fever,moreover *Cx. pipiens* transmit *Plasmodium relictum* that

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causing bird malaria. Its distribution is wide so that this species approximately present in all continent of the world (Mitchell et al. 1980, Vinogradova 2000, Smith and Fonseca 2004, Savage et al. 2007, Mullen 2009, Strickman and Fonseca 2012). In Iran during recent years transmitting of *Dirofilaria immitis* (dog heart worm), West Nile and Sindbis viruses by *Culex* mosquitoes have been reported (Naficy and Saidi 1970, Azari-Hamidian et al. 2007, Azari-Hamidian et al. 2009). *Culex* Genus habituated capital city of Tehran in sewage system of the houses, where there are different insecticides which have been used for controlling of household and agricultural pests such as: permehrin, bioallethrin, dursban, pirimiphos-methyl, malathion, chlorpyrifos-methyl, allethrin, propoxur. I addition different reagents also exist in the sweage systems. we postulated that resistance to insecticides is as a result of continous exposure to Different group of insecticides which may indirectly cause selection pressure on the susceptibility of mosquitoes mainly breed in wastewater habitats (Horsfall 1955, Lotfi 1976, Golestani 1976, Lines 1988, Vatandoost et al. 2004, Calhoun et al. 2007). Here upon the evaluation of susceptibility level of mosquitos for monitoring of resistance to insecticides is necessary and for this approach in Iran in recent years the most studies about evaluating susceptibility level of mosquitos to common insecticides have been on *Anopheles* mosquitos (Enayati et al. 2003, Vatandoost and Borhani 2004, Vatandoost and Hanafi-Bojd 2005, Vatandoost et al. 2005, Hanafi-Bojd et al. 2006, Shahi et al. 2006, Abai et al. 2008, Hanafi-Bojd et al. 2010, Oshaghi et al. 2011, Hanafi-Bojd et al. 2012, Soltani et al. 2013) and there are just a limited study about susceptibility status of *Culex* genus in Iran for example in the performed studies in Caspian sea coast about susceptibility level of *Cx. pipiens* complex results indicated that this species is resistant to DDT and susceptible to Dieldrin (Lotfi et al. 1975). Nazari and Janbakhsh in 2000 reported that *Cx. pipiens* in the southern area of Tehran is resistance to DDT (Nazari and Janbakhsh 2000). In 2004 the susceptibility level of laboratory and field collected strains of *Cx. Quinquefasciatusin* a medically important member belong to *Cx. pipiens* complex to different insecticides evaluated, DDT resistant was observed in both laboratory and field collected strains (Vatandoost et al. 2004). According to the most performed study around the world it seems that this species approximately is resistant to many insecticides or have multiple insecticide resistances (Davidson 1964, Mukhopadhyay et al. 1993, Ben Cheikh et al. 1998, Bisset et al. 1999, Martinez-Torres et al. 1999, Corbel et al. 2007, Tantely et al. 2010, Toma et al. 2011, Jones et al. 2012, Pocquet et al. 2013). The present study was preformed for evaluating susceptibility status of *Cx. pipiens* of Tehran City. The result of this study can be useful for future chemical control programs in the study area.

Materials and Methods

Study area

This study was conducted in Tehran city (35° 41′ 46″ N, 51° 25′ 23″ E), Tehran Province, Iran. Tehran is the capital of Iran and also is Iran's largest city (Fig. 1).

Mosquito strains and adult susceptibility test

In this study four Insecticides including: DDT 4%, Lambdacyhalothrin 0.05%, Deltamethrin 0.05%, Cyfluthrin 0.15% were used for evaluating susceptibility status of *Cx. pipiens*. Moreover For comparison susceptibility status a Laboratory strain also was used. All tested species were reared in the insectary of School of Public Health, Tehran University of Medical Sciences under the standard condition.

Data analysis

Bioassay data were analyzed with Probit

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program (Finney 1971). For correction mortality, when control mortality is greater than 5% but less than 20%, then the observed mortality was corrected using Abbott's formula (Abbott 1965). By method of Finney the lethal Time for 50% and 90% mortality (LT_{50} and LT_{90}) values and their 95% confidence interval and Probit regression line parameters were determined for both strains (Lab and field population). The regression line belong to each Insecticides after different exposure times were plotted using Microsoft Excel (ver. 2013).

Results

The result of susceptibility test for lab and filed strains of *Cx. pipiens* has been summarized in Table 1 and 2. Lab strain exhibited different LT50 valuses to different insecticides. Cyfluthrin with LT_{50}=36Sec, Lambdacyhalothrin (LT_{50}=79), Deltamethrin ($LT_{50=}$ 326) and DDT ($LT_{50=}$3005) had the lowest to highest lethal time (Table 1 and 3). Although this result for field population indicated that DDT exhibited no mortality. Cyfluthrin with LT_{50}=27minutes, Lambdacyhalothrin (LT_{50}= 111 minutes) and Deltamethrin ($LT_{50=}$ 182 minutes) had lowest to highest LT50 (Table 2 and 3). The result also showed that among these insecticides, Lab strain is susceptible to Lambdacyhalothrin, deltamethrin, cyfluthrin and resistance to DDT according to WHO criteria that suggested (98-100% mortality indicates susceptibility, 90-97% mortality indicates resistance candidate (more investigation is needed or requires confirmation of resistance with other methods) and <90% mortality suggests resistance) (WHO 2013). And also cyfluthrin with LT50=45_{Sec} and DDT with LT50=3005 were the most and least effect (Table 1,3 and Fig. 2,3).

Fig.1. The map of Iran and location of Tehran city

Table 1. Probit regression line parameters of lab strain of *Culex pipiens* exposed to different insecticides

Insecticides	A	B ± SE	LT_{50} , 95% C.I. (Second)	LT_{90}, 95% C.I. (Second)	X^2 (df)	P value
DDT 4%	-9.21	2.64± 0.31	2647 3005 3455	7061 9156 13701	3.56(3)	>0.05
Lambdacyhalothrin 0.05%	-2.50	1.31±0.18	43 79 120	510 752 1302	2.41(2)	>0.05
Deltamethrin 0.05%	-4.17	1.6± 0.16	255 326 406	1443 1937 2872	5.85 (4)	>0.05
Cyfluthrin 0.15%	-1.84	1.17±0.18	14 36 64	308 448 725	3.60(4)	>0.05

A= y-intercept, B= the slope of the line, SE= Standard error, CI= confidence interval, x^2= heterogeneity about the regression line, df= degree of freedom, P> 0.05 = represent no heterogeneity in the population of tested mosquitos.

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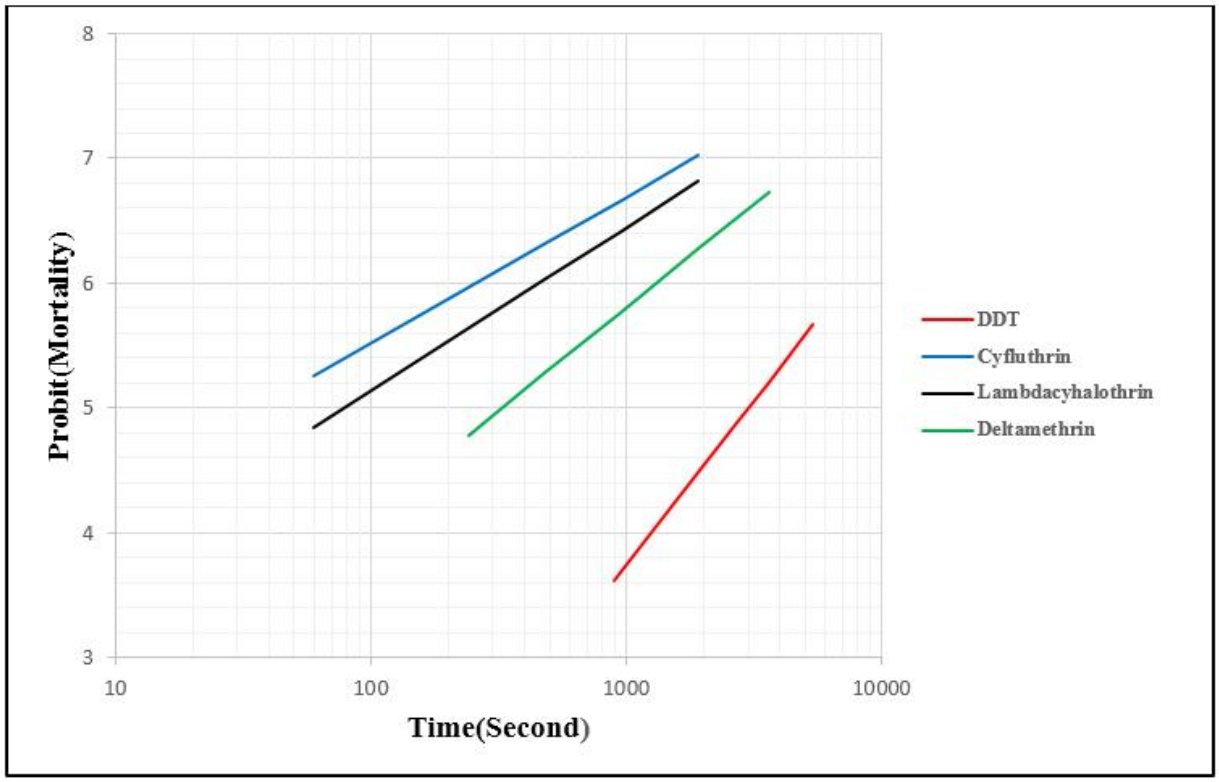

Fig. 2. Regression lines for lab strain of *Culex pipiens* exposed to different insecticides

Table 2. Probit regression line parameters of field population of *Culex pipiens* exposed to different insecticides

Insecticides	A	B ± SE	LT_{50} , 95% C.I. (Minute)	LT_{90}, 95% C.I. (Minute)	X^2 (df)	P value
Lambdacyhalothrin 0.05%	-7.58	3.71±0.36	99 111 122	214 245 293	5.34(2)	>0.05
Deltamethrin 0.05%	-4.36	1.9± 0.35	152 182 234	511 838 2322	0.37 (2)	>0.05
Cyfluthrin 0.15%	-2.29	1.59±0.23	21 27 33	117 172 336	3.47(2)	>0.05
DDT 4%*	-	-	-	-	-	-

*No mortality after 24 hours exposure

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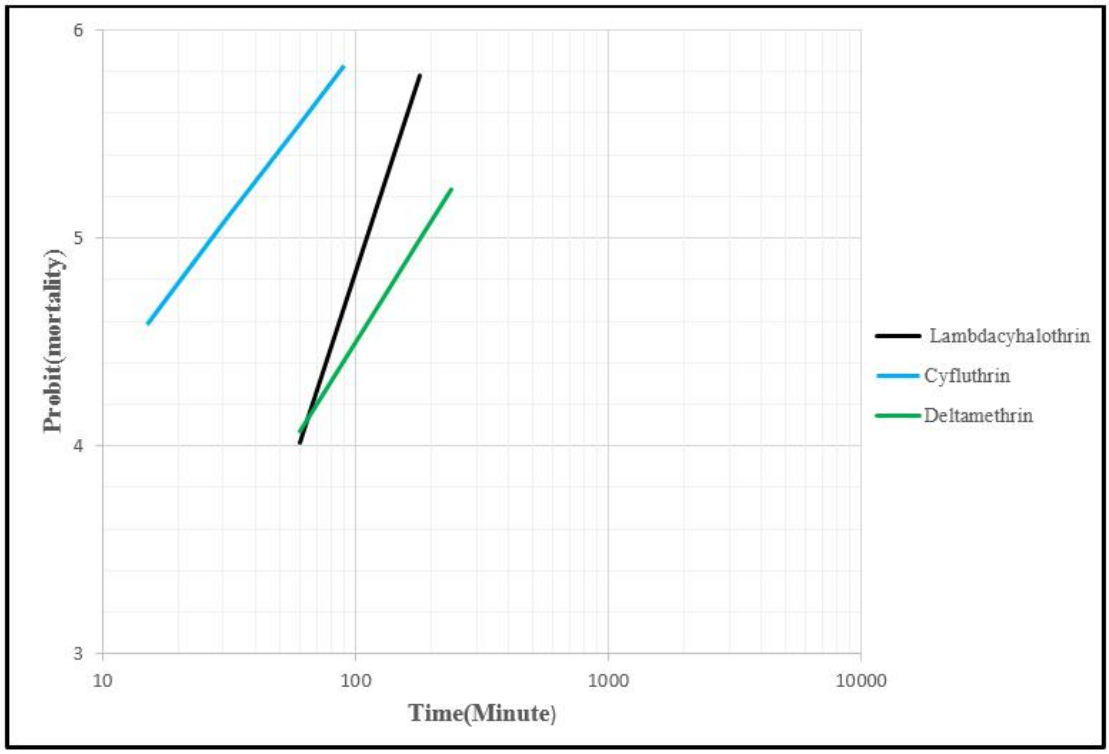

Fig. 3. Regression lines for field population of *Culex pipiens* exposed to different insecticides

Table 3. Mortality rate and susceptibility status of *Culex pipiens* (Lab and field population) exposed to different insecticides at one hour exposure and 24 hours recovery period

Insecticides	MR±EB*		Resistance status**	
	Lab strain	Field population	Lab strain	Field population
Lambdacyhalothrin 0.05%	100	20±**2**	S	R
Deltamethrin 0.05%	98±**1**	18±**3**	S	R
Cyfluthrin 0.15%	100	66±**3**	S	R
DDT 4%	55±**3**	0	R	R

*Mortality Rate±Error Bar
**R Resistance, S Susceptible

Discussion

In the present study four Insecticides including: DDT 4%, Lambdacyhalothrin 0.05%, Deltamethrin 0.05% and Cyfluthrin 0.15% were used for evaluatin the susceptibility status of *Cx.pipiens* According to WHO criteria lab starin was resistant to DDT and Filed strain exhibited resistant to all insecticides used. In the both strains a highly level of resistance to DDT were determined and also the LT_{50} for DDT >Deltamethrin > Lambdacyhalothrin >Cyfluthrin respectively. In the two previous studies conducted in Tehran, resistant to DDT have been reported in *Cx. pipiens* and *Cx. quinquefasciatusin* and these results was in parallel to our finding (Nazari and Janbakhsh 2000, Vatandoost et

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al. 2004). It seems that *Cx. pipiens* in the most part of the world has been resistant to DDT (Mukhopadhyay et al. 1993, Nazni et al. 2005, Corbel et al. 2007, Sarkar et al. 2009, Jones et al. 2012, Pocquet et al. 2013). In the study conducted by Nazni et al. (2005) mosquitoes from two field sites in Kuala Lumpur exhibited resistance to DDT with no mortality 24h after exposure, and this result was same to our result about field population (Nazni et al. 2005). In another study, high frequencies of resistance to DDT in *Cx. quinquefasciatus* from Benin have been reported and mortality rate ranged from 5 to 54% (Corbel et al. 2007). In northeastern India *Cx. quinquefasciatus* which caught from 7 different field sites of study area this species was resistance to DDT in all sites and its mortality rate varied from 11.9 to 50.0% (Sarkar et al. 2009). Result of susceptibility test in northwest and southeastern part of Iran indicated that this species is highly resistant to DDT (Ataie et al. 2015, Fathian et al. 2015). In the current study, Field population also was resistance to Lambdacyhalothrin, Deltamethrin and Cyfluthrin and in some same studies this result also reported, for example resistance to Lambdacyhalothrin and Cyfluthrin reported in the Southeastern part of Iran and resistance to Lambdacyhalothrin in Northwestern part of Iran (Ataie et al. 2015, Fathian et al. 2015). In *Cx. quinquefasciatus* from Wete on Pemba Island in Zanzibar resistance to Deltamethrin and Lambdacyhalothrin also have been reported (Jones et al. 2012). In Thailand, *Cx. quinquefasciatu*s belong to The Baan Suan strain was highly resistant to Deltamethrin even its mortality was very lower than our results (Sathantriphop et al. 2006). Resistance to Deltamethrin also reported by Chen et al. (2010), so that all the six surveyed *Cx. pipiens pallens* populations strains were resistance to Deltamethrin and also their mortality Ranged from 20.2% to 78.6% (Chen et al. 2010). In some studies resistance to the others group of insecticides like organophosphates and Carbamate also have been reported (Bisset et al. 1999, Corbel et al. 2007, Tantely et al. 2010, Toma et al. 2011, Ataie et al. 2015 Fathian et al. 2015). for example in the both study that performed in Northwestern and Southern part of Iran, *Cx. pipiens* showed resistance to Propoxur (Ataie et al. 2015, Fathian et al. 2015). Tolerance to Deltamethrin (with mortality=86%) in Culex populations from Kilimani, Unguja Island in Zanzibar also reported by Jones et al. (2012). Although this populations was susceptible to Lam-bdacyhalothrin. Moreover in this study *Culex* from the nearby site of Tibirinzi in Pemba was relatively susceptible to Deltamethrin and also Lambdacyhalothrin (Jones et al. 2012). In the same previously study that performed by Vatandoost et al in 2004, field population of *Cx. Quinquefasciatus* which collected from Sewage System of Tehran after evaluating their susceptibly status to insecticides result showed that this species is susceptible to Cyfluthrin and also have tolerance to Lambdacyhalothrin and Deltamethrin. Routine use of pesticides in Household and agricultural pest control might have developed this Enhanced Tolerance to insecticide in the wastewater mosquito, *Cx. Quinquefasciatus* (Vatandoost et al. 2004).

Conclusion

In the present study, in the population whitch collected from field highly resistance to all insecticides exhibited it might be due to pollution of wastewater with chemical substances Findings of this research could provide a clue for logical operations of future chemical control program. Next step of this research will focus on the biochemical and molecular investigation.

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