Original Article

Current Susceptibility Status of *Anopheles stephensi* (Diptera: Culicidae) to Different Imagicides in a Malarious Area, Southeastern of Iran

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Abstract
Background: *Anopheles* mosquitoes are an important group of arthropods due to their role in transmission of malaria. The present study was conducted for determination of susceptibility status of *Anopheles stephensi* to different imagicides collected from malarious area in Chabahar city, Iran.
Methods: In the present study seven insecticides including: DDT 4%, lambdacyhalothrin 0.05%, deltamethrin 0.05%, permethrin 0.75%, cyfluthrin 0.15% and etofenprox 0.5% were tested based on WHO method. Regression line was plotted for each insecticide using mortality of different exposure times. Bioassay data were analyzed using Probit software and the lethal time for 50% and 90% mortality (LT_{50} and LT_{90}) values were calculated.
Results: The susceptibility levels of field strain of *An. stephensi* to the discriminative dose of different imagicides were determined 100, 98, 96, 89, 82 and 62% for etofenprox, permethrin, deltamethrin, lambdacyhalothrin, cyfluthrin and DDT, respectively. Our finding indicated that *An. stephensi* is resistant to DDT, lambdacyhalothrin and cyfluthrin, and susceptible to etofenprox and permethrin and candidate of resistant to deltamethrin based on WHO criteria.
Conclusion: Our findings indicated that *An. stephensi* is resistant to DDT and some pyrethroid insecticides which can be developed due to application of insecticides in health and agriculture. These results can provide a clue for future chemical control program in the study area.

Keywords: Susceptibility test, *Anopheles stephensi*, Chabahar, Pyrethroid resistance

Introduction

Mosquitoes as a big group of arthropods play an important role in transmission of many diseases to human such as malaria, filariasis, yellow fever, dengue fever (Horsfall 1955, Tabachnick 1991, Service 2003, Azari-Hamidian 2011). Some species of *Anopheles* mosquitoes are vectors of malaria in different parts of the world. For example, *Anopheles stephensi* Liston (Diptera: Culicidae) is the main malaria vector in Eastern Mediterranean region and south of Asia continent (Zahar 1974,Vatandoost et al. 2006). In Iran there are some species of malaria vectors including: *An. stephensi*, *An. dthali*, *An. culicifacies*, *An. fluviatilis*, *An. superpictus* s.l., *An. sacharovi*, *An. maculipennis* Complex (Naddaf et al. 2003, Azari-Hamidian 2011, Mehravaran et al. 2011, Oshaghi et al. 2011).

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Before initiating of national malaria control program in 1957, malaria cases were reported from most parts of Iran, Since then, due to implementing of many continuous interventions, malaria confined to south eastern parts of the country including Sistan va Baluchestan, Hormozgan and southern parts of Kerman Provinces (Edrissian 2006, Vatandoost et al. 2011). For controlling of malaria, vector control is one the most important approach which focuses on chemical control of mosquitoes. Up to now different group of insecticides including: organochlorines (DDT, dieldrin and BHC), organophosphates (pirimiphos-methyl, malathion), carbamates (propoxur) and pyrethroids (lambdacyhalothrin, delthamethrin) in different forms of application such as Indoor Residual Spraying (IRS), Insecticide Treated Nets (ITN_S) for adult stage and some organophosphates for larviciding were used in malariaous area of Iran (Salim Abadi et al. 2010, Hanafi-Bojd et al. 2012, Vatandoost and Hanafi-Bojd 2012). Resistance of *Anopheles* spp to DDT and pyrethroid insecticides were reported from different countries around the world like China, Turkey, India, some countries of Africa and Latin America (Kasap et al. 2000, Hargreaves et al. 2003, Syafruddin et al. 2010, Lol et al. 2013, Soltani et al. 2013, Chang et al. 2014). In Iran many researches have evaluated susceptibility status of malaria vectors against different insecticides (Vatandoost and Hanafi-Bojd 2005, Hanafi-Bojd et al. 2006, Vatandoost et al. 2006, Vatandoost et al. 2011, Vatandoost and Hanafi-Bojd 2012). Approximately in all previous conducted studies on *An. stephensi* in Iran, resistance to DDT and susceptibility to pyrethroids have been reported, but in 2012 first indication of resistance to pyrethroid compounds was reported from south eastern parts of the country (Vatandoost and Hanafi-Bojd 2012). Resistances to DDT, mainly in the adult stage of *An. stephensi*, have been widely distributed in Middle-East and Indian subcontinent causing operational problems for control programs (WHO 1985, WHO 1992). This study aims to monitor susceptibility status of main malaria vector, *An. stephensi*, to some insecticides in Chabahar City, Sistan va Baluchestan Province, Iran.

Materials and Methods

Study area

This study was performed in Chabahar seaport (25^{o} $25^{/}$ N, 60^{o} $45^{/}$ E), Sistan va Baluchestan Province of Iran during April to June 2013 (Fig. 1).

Mosquito sampling and rearing

Collected larvae from the study area were transferred to the insectary for rearing under standard conditions (Temperature= 25–29^{o} C, photo-period=12:12 Hours (light: Dark) and Humidity=50–70%). Emerged adult mosquitoes were fed with 10% aqueous sucrose solution.

Adult susceptibility test

Adult susceptibility tests were carried out according to the current World Health Organization method (WHO 2013). For each insecticide mortality rate in various times also were calculated and then regression line to each insecticide plotted using Microsoft Excel (version. 2013).

Insecticide impregnated papers

The following insecticides impregnated papers were supplied according to WHO Test procedure including: DDT 4%, lambdacyhalothrin 0.05%, deltamethrin 0.05%, permethrin 0.75%, cyfluthrin 0.15% and etofenprox 0.5%. Mineral oil, and silicon oil impregnated papers were used for organochlorine insecticides and pyrethroids as control, respectively (WHO 1981, WHO 2013).

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Statistical analysis

Results were analyzed by using of Probit program (Finney 1971). In case of mortality, when the control mortality was between 5% to 20% it was corrected by Abbott's formula (Abbott 1925). Error bars for each mortality were calculated based on statistical method at α=5%. The lethal Time for 50% and 90% mortality (LT_{50} and LT_{90}) values and their 95% confidence interval also Probit regression line parameters were determined with Finney method and then the regression line of all Insecticides were plotted using Microsoft Excel (version. 2013).

Results

The results of susceptibility test for each insecticides are shown in tables 1,2. Mortality rate and lethal Time for 50% mortality (LT_{50}) of different insecticides were calculated. Our finding indicated that Etofenprox, Deltamethrin, Lambdacyhalothrin, Permethrin, Cyfluthrin and DDT have the lowest to highest LT_{50} value respectively (Fig. 2).

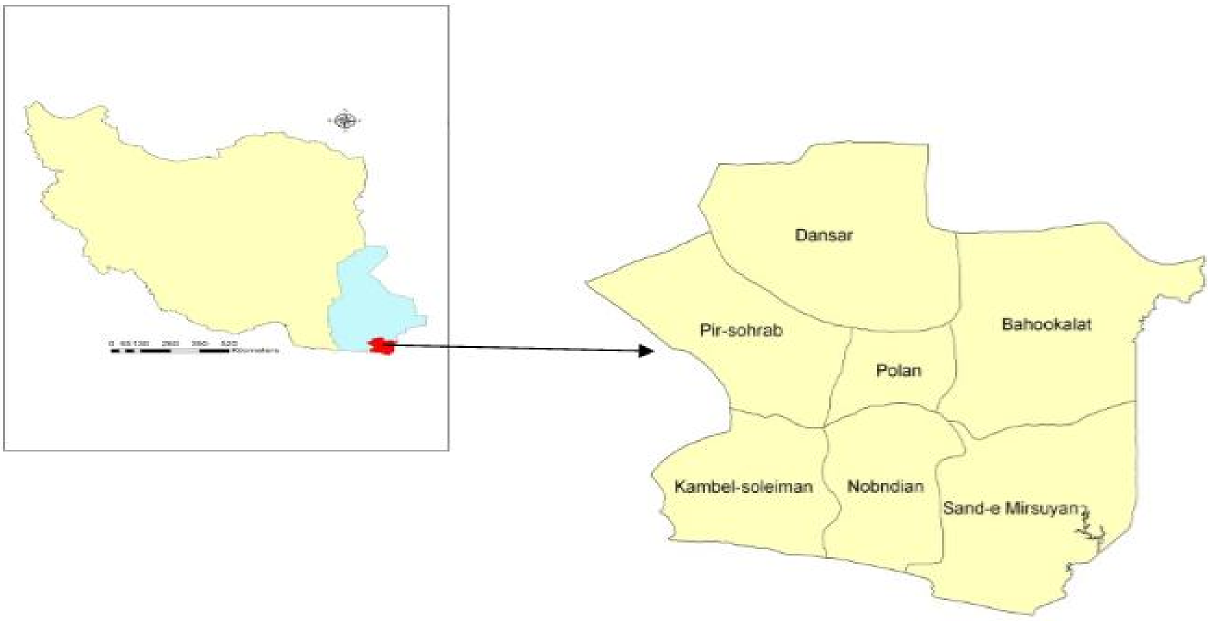

Fig. 1. The map of Chabahar City representing rural districts, Sistan and Baluchistan Province (Study area), Iran

Table 1. Probit regression line parameters of *Anopheles stephensi* exposed to different insecticides

Insecticide	A	B±SE	LT_{50}, 95% C.I. (Second)	LT_{90}, 95% C.I. (Second)	X^2(df)	P value
			75	626		
Etofenprox 0.05%	-2.68	1.33± 0.14	104	957	5.11 (3)	>0.05
			138	1749		
			277	984		
Permethrin 0.75%	-5.61	2.22±0.21	335	1266	2.89 (2)	>0.05
			401	1775		
			656	5121		
Cyfluthrin0.15%	-3.79	1.3±0.12	812	7805	1.97(4)	>0.05
			1010	14160		

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Table 1. Continued…

Lambdacyhalothrin 0.05%	-3.25	1.36±0.12	185	1414	0.04(2)	>0.05
			246	2146		
			324	3791		
Deltamethrin 0.05%	-2.69	1.22±0.15	101	1194	0.95(2)	>0.05
			159	1785		
			221	3277		
DDT 4%	-4.47	2.5±0.33	2820	7560	6.99(3)	>0.05
			3240	10200		
			3840	16560		

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.

Table 2. Mortality rate and susceptibility status of *Anopheles stephensi* exposed to different insecticides Chabahar, southeastern Iran, 2013

Insecticide	MR±EB*	Resistance status**
Deltamethrin	96±3.8	RC
Lmbdacyhalothrin	89±2.8	R
Cyfluthrin	82±3.5	R
Permethrin	98±1	S
Etofenprox	100	S
DDT	62±4.8	R
control		-

*Mortality Rate±Error Bar

**R Resistance, RC Resistant Candidate, T Tolerance, S Susceptible

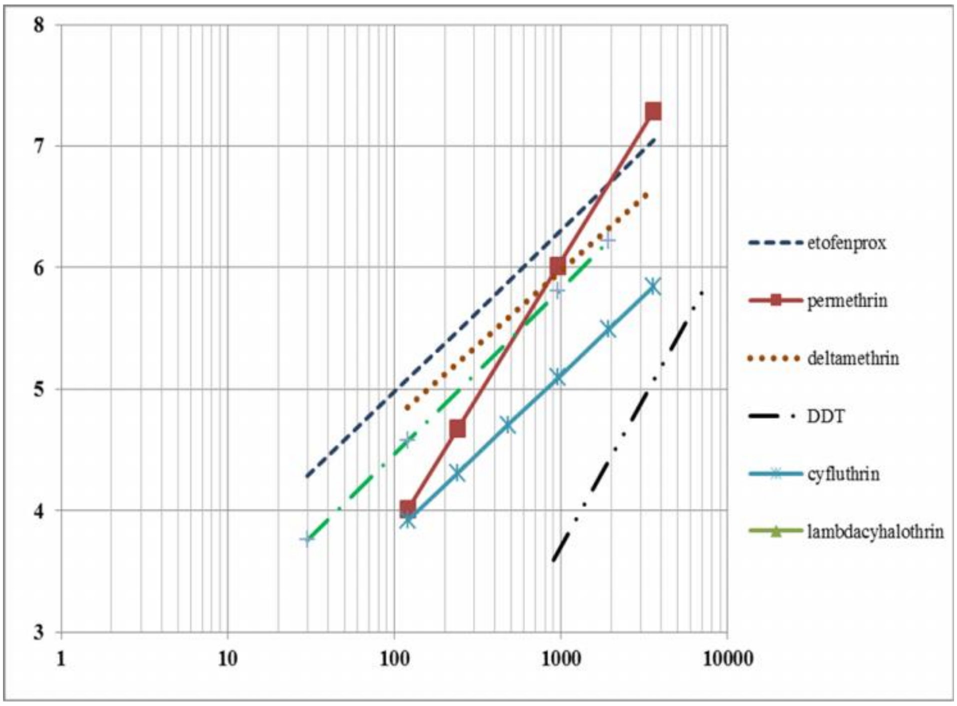

Fig. 2. Regression lines of *Anopheles stephensi* exposed to different insecticides (field population), 2013

Discussion

In the current study seven insecticides including: DDT 4%, lambdacyhalothrin 0.05%, deltamethrin 0.05%, permethrin 0.75%, cyfluthrin 0.15% and etofenprox 0.5% were used to determine susceptibility status of *An. stephensi* collected from Chabahar City. Based on WHO criteria that suggested (98–100% mortality indicates susceptibility, 90–97% mortality indicates resistance candidate (more investigation is needed) and less than 90% mortality suggests resistance (WHO 2013). Results indicated that species is resistant to DDT, cyfluthrin and Lambda-cyhalothrin, However, susceptible to permethrin and Etophenprox. The indication of resistant to deltamethrin at the early stages of evolution has also be documented. Our findings reveal that *An. stephensi* is resistant to DDT which is in line with previous researches results that have been performed in our study area (Vatandoost and Hanafi-Bojd 2012, Fathian et al. 2015). Majority of susceptibility tests which performed during the past decade in different malarious area revealed resistance to DDT in southern part of Iran (Borhani 2004, Vatandoost et al. 2005, Vatandoost et al. 2006) as well as in the most distribution area of *An. stephensi* in the world (Rathor et al. 1980, Thavaselvam et al. 1993, Tikar et al. 2011, Chang et al. 2014, Singh et al. 2014). Furthermore, there are many resistance reports to DDT in other species of *Anopheles* mosquitoes from different part of the world (Hemingway and Ranson 2000, Hemingway et al. 2002, Zahirnia et al. 2002, Lak et al. 2002, Balkew et al. 2006 , Raghavendra et al. 2010, Tikar et al. 2011, Vatandoost et al. 2011, Nardini et al. 2013, Wang et al. 2013). In the present study resistance to cyfluthrin and lambda-cyhalothrin were indicated and these findings are in line with previous research results that have been conducted in the same area (Vatandoost and Hanafi-Bojd 2012).

On the other hand our finding about cyfluthrin susceptibility status is not in concordance with another research that has been performed previously in the same area by Fathian et al. (2015) that showed this species is susceptible to cyfluthrin. (Fathian et al. 2015). It may be due to different sampling localities. Resistance of *An. stephensi* to pyrethroid compounds were reported from its different distribution regions, for instance in the study was performed by Rathor et al. (2013) in Punjab Province of Pakistan, resistance to three commonly used pyrethroids, permethrin, lambda-cyhalothrin, and deltamethrin were indicated from the majority of test localities (Rathor et al. 2013). In the present study *An. stephensi* was susceptible to Etophenprox and Permethrin that these findings are in parallel with other previous conducted researches results (Vatandoost et al. 2005). In the current study deltametthrin was indicated as resistant candidate so that more investigation is needed. Molecular and biochemical assays for this species as a main malaria vector must be conducted for accurate evaluating of resistance status of pyrethroid insecticides specially those commonly used in malaria control program.

Conclusion

In the present study *An. stephensi* was found resistant to DDT and some pyrethroid insecticides. This enhanced resistance status may be due to previous chemical control programs against malaria vectors, such as IRS/ITNs or insecticide application in agriculture. However, more investigation for determination of resistance mechanisms is necessary. Furthermore regular monitoring of resistance status by standard bioassay tests and other complementary methods especially in active foci of malaria transmission is suggested.

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