Original Article

Insecticide Resistance Status of Malaria Vectors in a Malarious Area, Southeast of Iran

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Abstract

Background: Malaria continues to be the main vector-borne disease in Iran. The endemic foci of malaria are in Sistan and Baluchistan Province, the borderline of Iran and Pakistan. By the year 2020 the program of the country is malaria elimination. The main vector control is using insecticide as Indoor Residual Spraying. The aim of the study was to evaluate the susceptibility of main malaria vectors to different insecticides recommended by WHO.
Methods: All the insecticides papers supported by WHO and evaluation of insecticide resistance of *Anopheles stephensi*, *Anopheles culicifacies*, *Anopheles superpictus* to different chemical groups of imagicides including DDT 4%, malathion 5%, propoxur 01.%, lambdacyhalothrin 0.05%, deltamethrin 0.025% and permethrin 0.75% were followed by the WHO guideline.
Results: Results of the susceptibility test against different insecticides revealed that *An. stephensi* and *An. culicifacies* are resistant to DDT and susceptible to other insecticides. *An. superpictus* is susceptible to all groups of pesticides.
Conclusion: Knowledge on insecticide resistance in target species is a basic requirement to guide insecticide use in malaria control programmes in local and global scales.

Keywords: *Anopheles stephensi; Anopheles culicifacies; Anopheles superpictus*; Resistant; Pesticides

Introduction

Malaria is the main vector borne diseases worldwide. According to the recent record of the World Health Organization, a total of 228 million cases have been reported in 2018 mainly in the African region (1). According to the report of the Ministry of Health of Iran, less than 89 locally-transmitted cases have been reported in 2017. The aim of country is to eliminate the disease by 2025 (2). The campaign against malaria vectors started with organochlorines (DDT, dieldrin and BHC) during the 1960's, followed by organophosphates (malathion and pirimiphos-methyl) for 2 decades from 1966 and continued with the carbamate, propoxur during 1977–1990, and then with pyrethroids including lambdacyhalothrin and Deltamethrin. Temephos, Reldan and pirimiphos-methyl was used for larviciding. The last checklist of Iranian mosquitoes

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shows 31 *Anophele*s species including sibling, biological forms and genotypes, 17 out of them are reported as malaria vector transmission. These vectors are considered as sibling, genotype and type forms. *Anopheles stephensi*, *An. culicifacies*, *An. fluviatilis*, *An. dthali* are the main vector species of south-eastern foci, while *An. sacharovi* and *An. maculipennis* are included in malaria transmission in northwest focus. *Anopheles superpictus* has wide distribution in all malaria foci of the country (Fig. 1). *Anopheles stephensi* is reported from the Indian subcontinent It is also distributed across the Middle East and South Asia region, existing in countries such as: Afghanistan, Bahrain, Bangladesh, China, Egypt, India, Iran, Iraq, Oman, Pakistan, Saudi Arabia, and Thailand (3, 4). It is also reported from Djibouti and Ethiopia (5, 6). *Anopheles culicifacies* reported from Afghanistan, Bahrain, Cambodia, China, Eritrea, Ethiopia, India, Iran, Iraq, Laos, Myanmar (Burma), Nepal, Oman, Pakistan, Sri Lanka, Thailand, Vietnam, Yemen. It has five sibling species as A, B, C, D, and E. *Anopheles superpictus* is a main malaria vector in Palearctic region, Middle Eastern countries, northern Africa, India, Afghanistan, Pakistan, central and southern Europe, and Russia (7). Insecticide resistance is the selection of a heritable trait in an insect population that results in an insect-control product no longer performing as intended. Establishing the baseline of insecticide resistance and conducting a comprehensive situation analysis is the starting point for tracking resistance. This will require collecting available background data and, if necessary, conducting additional tests on vector susceptibility and on resistance mechanisms. Interpretation of the data must take into account the resistance situation in neighbouring countries as well as previous experience elsewhere with the same type of resistance mechanisms. Countries should design a monitoring plan that includes data on vector distribution and relevant vector attributes for transmission and control. Investigation on susceptibility/resistance to currently used insecticides, and on the quality of vector control interventions. Experience suggests that if nothing is done, resistance will stabilize in the vector population and reversal will be difficult or even impossible, so that, some of the most effective insecticides will no longer be usable.

Materials and Methods

Study area

The study was carried out in Sarbaz City, Sistan and Baluchistan Province, borderline of Iran and Pakistan (Fig. 2).

Mosquito collection

Mosquitoes were collected in the breeding places by a dipper equipment. The larvea were ternsferred to the insectary to become adults.

Mosquito identification

All the adutls females were identified using valuable key identification (8).

Insecticide papers resource

All the insecticides papers were supported by WHO.

Insecticide susceptibility tests

Aduls suceptibiity tests were carried out accordig to the WHO guideline. Susceptible when mortality is 98% or higher, possible resistant when mortality is between 97 and 90%, and resistant when the mortality is lower than 90%. An excel sheet was created for insecticide resistance based on the applied insecticide at diagnostic dosage recommended by WHO.

Statistical analysis

The mortality quantities of 50% and 90% of imagicides (LT_{50} and LT_{90}) and the level of confidence of 95%, the equation of the regression line were estimated using a regression probit analysis as described by Finney (1971) (9). When the mortality of the control group is less than 5%, then the data of biometric tests have not been corrected, but if the mortality of the control group is between 5% and 20%, they have to

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be corrected. The percentage mortality was calculated using Abbot's formula (1925) (10).

Results

Results of exposure of *An. stephensi* to different logarimic time of pesticides is shown in Table 1. Probit regression line is shown in Fig. 3. The results of the susceptibility test at diagnosctic dose are shown in Fig. 4. The order of LT_{50} value is DDT> Propoxur>Malathion >Bendiocarb>deltamethrin. Mortality at dignostic dose considering mortality less than 90% revealed that *An. stephensi* is resistant to DDT and suscpetible to other insecticides. Results of exposure of *An.*

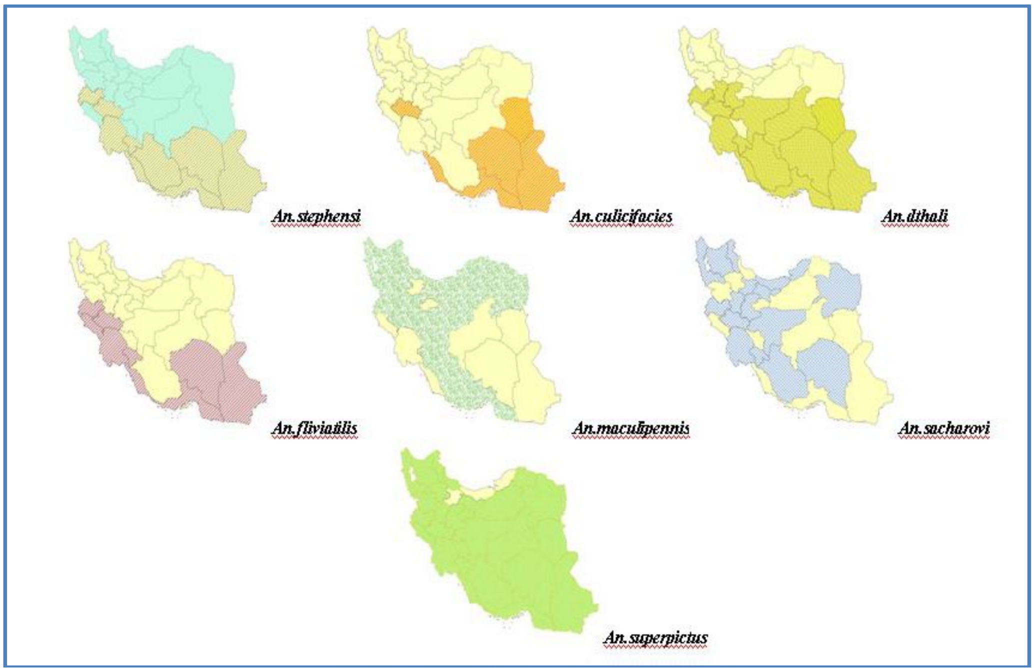

Fig. 1. Spatial distribution of malaria vectors in Iran

Table 1. Parameters of probit regression lines of *Anopheles stephensi* exposed to different insecticides

Insecticide	A	B±SE	LT_{50}	LT_{90}	χ^2 (df)	P	Y=A+BX
DDT	-10.22	2.94 ±0.309	2942.2500	8008.1993	5.747 (2)	>0.05	Y=-10.2229+2.9472X
Malathion	-9.58	3.1 ±0.287	1109.7414	2833.6861	1.174 (2)	>0.05	Y=-9.5860+3.1479X
Propoxur	-11.31	3.67 ±0.324	1195.6603	2668.2999	2.307 (2)	<0.05	Y=-11.3137+3.6761X
Deltamethrin	-10.85	3.66 ±0.313	909.8464	2033.9448	0.634 (2)	<0.05	Y=-10.8544+3.6683X
Bendiocarb	-13.87	4.6 ±0.410	977.1575	1845.5122	0.401 (2)	<0.05	Y=-13.8762+4.6409X

A= (interceptor), B±SE (Slope± standard error), LT_{50} (lethal time cause 50% mortality according to seconds), LT_{90} (lethal time cause 90% mortality according to seconds)

Table 2. Parameters of probit regression lines of *Anopheles culicifacies* exposed to different insecticides

Insecticide	A	B±SE	LT_{50}	LT_{90}	χ^2 (df)	P	Y=A+BX
DDT	-13.42	4.3 ±0.362	1339.7000	2664.5821	0.902 (2)	>0.05	Y=-13.4202+4.2917X
Malathion	-11.05	3.6 ±0.325	1099.0019	2475.2129	0.002 (2)	>0.05	Y=-11.0527+3.6346X
Propoxur	-12.15	3.9 ±0.336	1327.2653	2832.0720	1.419 (2)	>0.05	Y=-12.1599+3.8937X
Deltamethrin	-11.81	3.9 ±0.333	1005.5845	2128.8572	1.912 (2)	>0.05	Y=-11.8132+3.9346X
Bendiocarb	-12.19	4.0 ±0.358	1034.0933	2145.1592	1.635 (2)	>0.05	Y=-12.1914+4.0442X

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Table 3. Parameters of probit regression lines of *Anopheles superpictus* exposed to different insecticides

Insecticide	A	B±SE	LT_{50}	LT_{90}	χ^2 (df)	P	Y=A+BX
DDT	-11.87	3.3±0.874	3709.0662	9007.5451	7.837 (2)	<0.05	Y=-11.8709+3.3259 X
Malathion	-10.50	3.4±0.444	1059.0387	2476.6908	3.980 (2)	>0.05	Y=-10.5072+3.4735X
Propoxur	-13.30	4.2±0.489	1279.4752	2548.2910	3.360 (2)	>0.05	Y=-13.3080+4.2832X
Deltamethrin	-11.88	4.0±0.371	868.1769	1801.2125	2.307 (2)	>0.05	Y=-11.8821+4.0435X
Lambdacyhalothrin	-11.58	3.9±0.380	851.7902	1796.5675	4.225 (2)	>0.05	Y=-11.5871+3.9542X
Permethrin	-12.49	3.9±0.726	1424.9688	3000.9704	8.819 (2)	<0.05	Y=-12.4960+3.9622X

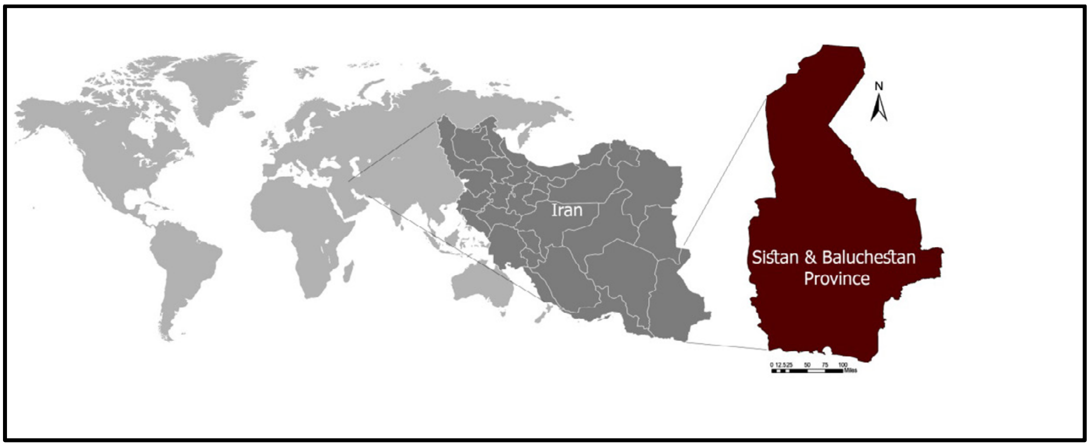

Fig. 2. Map of study area, Sistan and Baluchistan Province, Iran

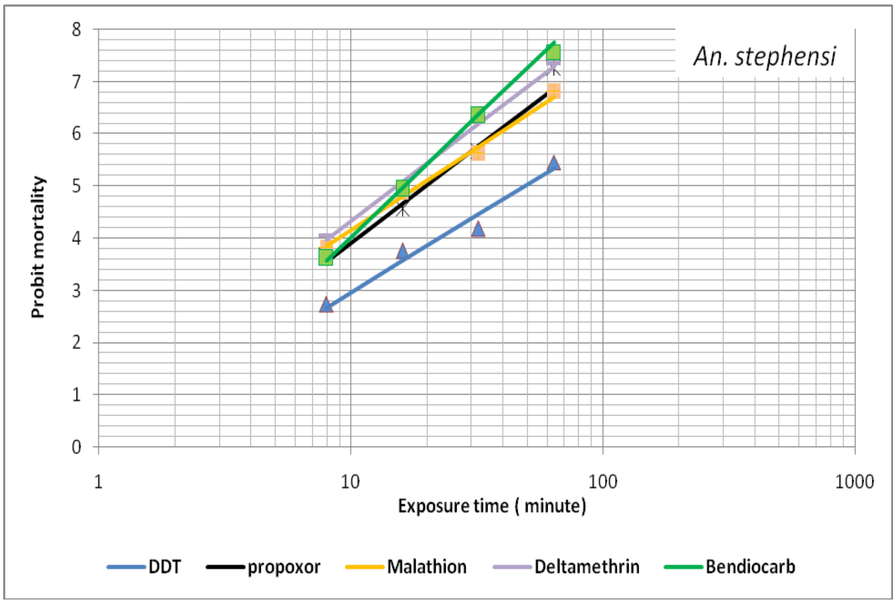

Fig. 3. Probit regression line of *Anopheles stephensi* exposed to different insecticides

culicifacies to different logarimic time of pesticides are shown in Table 2. Probit regression line is shown in Fig. 5. The results of susceptibility test at diagnosctic dose are shown in Fig. 6. The order of LT_{50} value is DDT> Propoxur>Malathion >Bendiocarb>deltamethrin. Mortality at dignostic dose considering mortality less than 90% revealed that *An. culicifacies* is suscpetible to all insecticides. Results of

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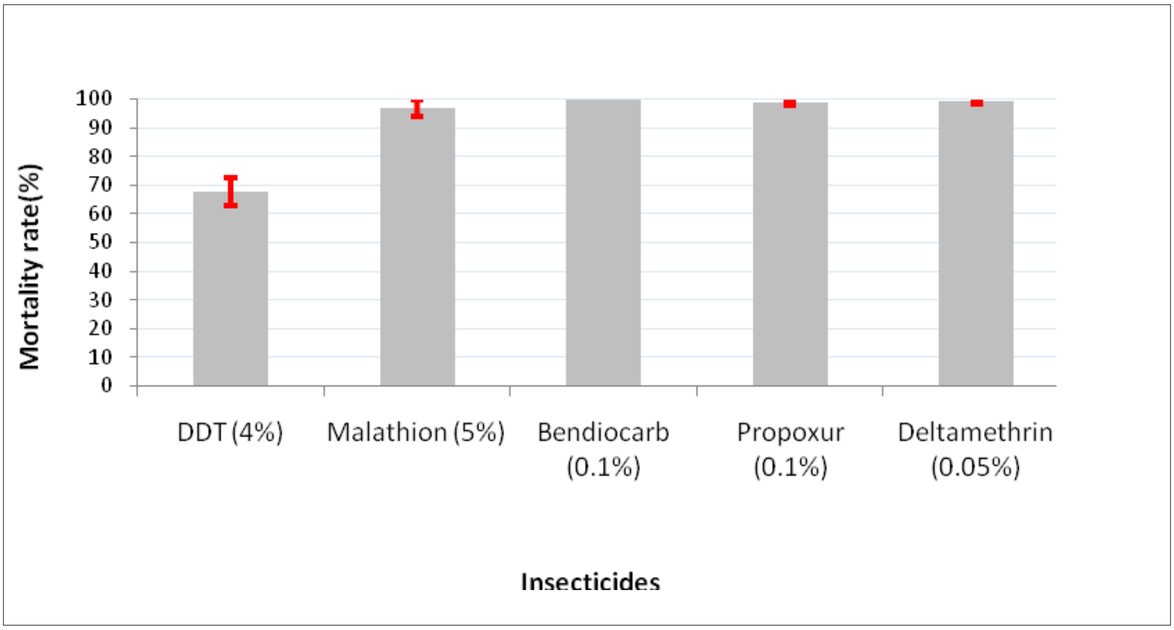

Fig. 4. Resistance of *Anopheles stephensi* to different insecticides at diagnostic doses

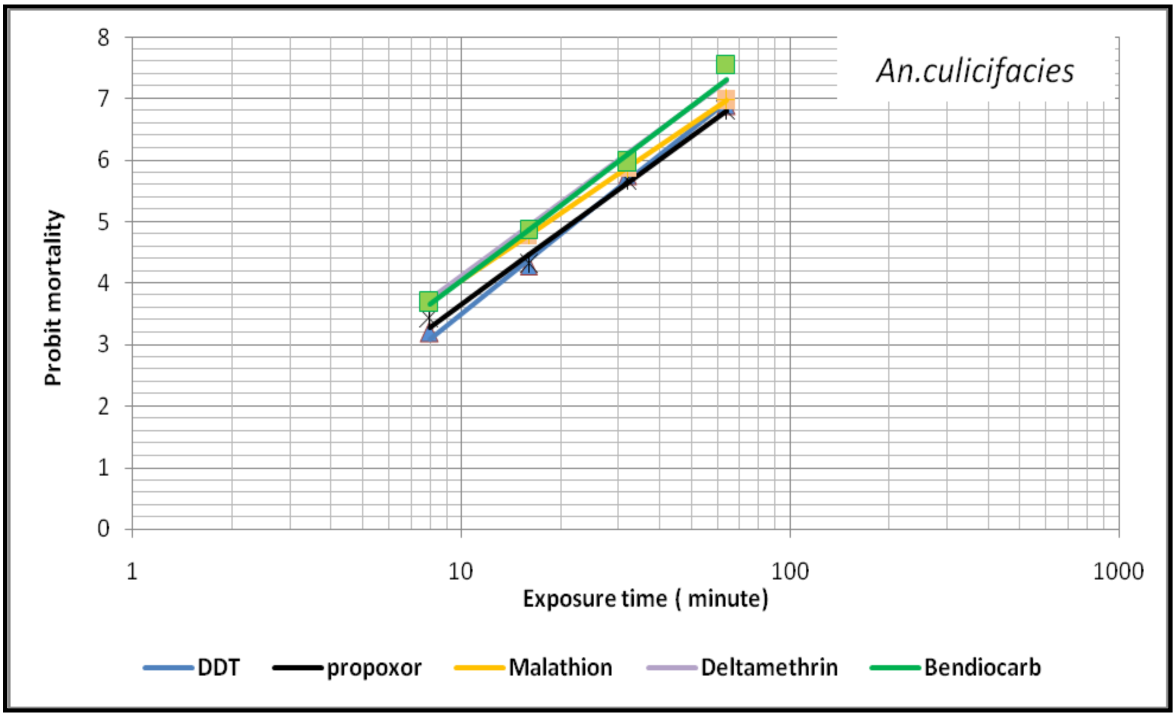

Fig. 5. Probit regression line of *Anopheles culicifacies* exposed to different insecticides

exposure of *An. superpictus* to different logarimic time of pesticides are shown in Table 3. The results of susceptibility test at diagnosctic dose are shown in Fig. 7. The order of LT_{50} value is DDT> Propoxur >Malathion> Permetrhin> Deltamethrin> lamabdacyhalothrin. Mortality at dignostic dose considering mortality less than 90% revealed that *An. superpictus* is resistsnt to DDT and suscpetible to all insecticides.

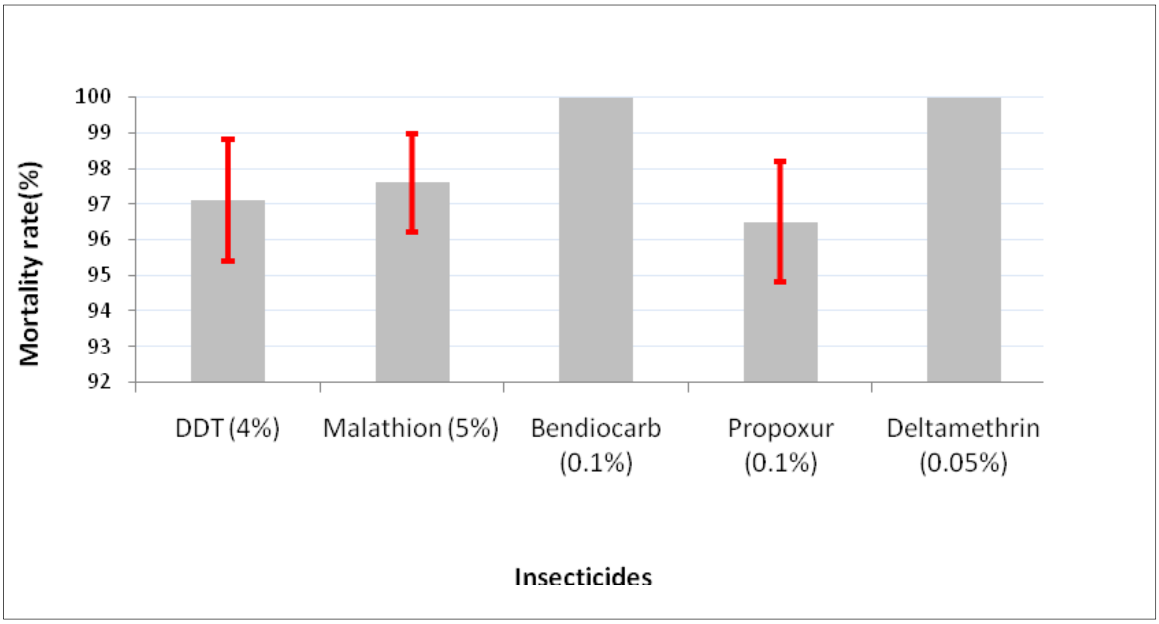

Fig. 6. Resistance of *Anopheles culicifacies* to different insecticides at diagnostic doses

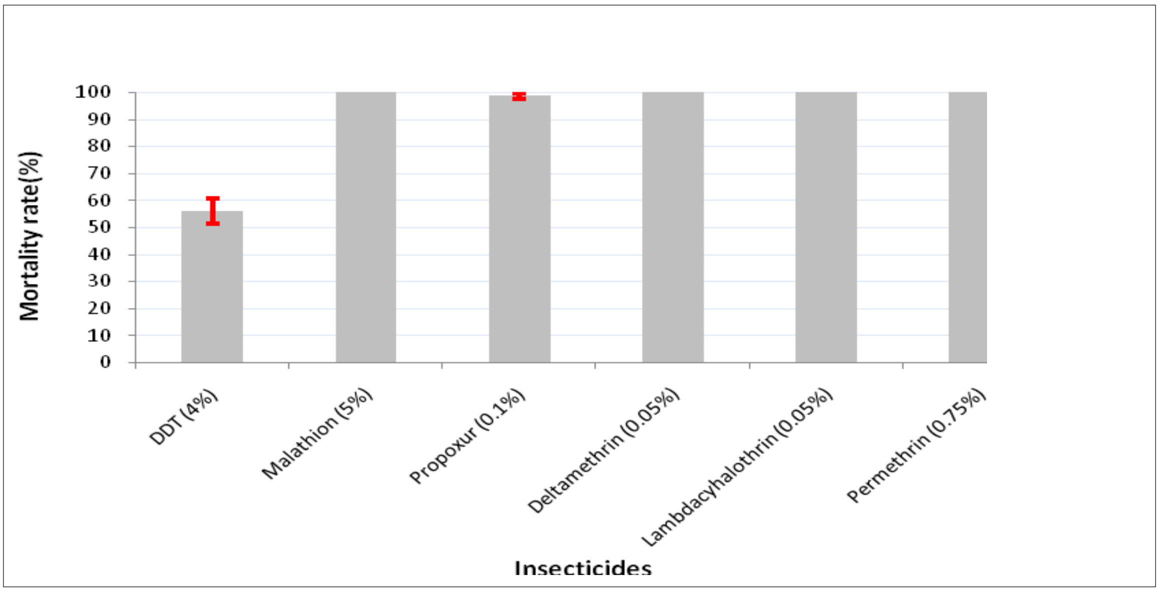

Fig. 7. Resistance of *Anopheles superpictus* to different insecticides at diagnostic dose

Discussion

Results of susceptibility tests against different WHO recommended insecticides, including DDT, Malathion, Propoxur, Bendiocarb, Deltamethrin, Lambdacyhalothrin and Permethrin against *An. stephensi*, *An. culicifacies* and *An. superpictus* revealed that only *An. superpictus* is susceptible to all insecticides. However, *An. stephensi* and *An. culicifacies* showed resistant to DDT. There are several reports on resistant status of malaria vectors including *An. stephensi* (11-17). *Anopheles stephensi* showed resistance to lambdacyhalothrin, deltamethrin, permethrin, and bendiocarb in Bandar Abbas County, southern Iran (17). *Anopheles stephensi* samples were resistant bendiocarb, propoxur, deltamethrin, permethrin, DDT, malathion and pirimiphos-methyl in Somali region (18). Resistant to DDT, malathion, bendiocarb, permethrin and deltamethrin was reported in *An. stephensi* and *An. culicifacies* from Afghanistan. Resistant to only deltamethrin and bendiocarb was observed in *An. superpictus* (19). There are also report of resistant to pyrethroids in Afghanistan (20). *Anopheles superpictus* populations were confirmed resistant to DDT, malathion and propoxur and susceptible to pyrethroid insecticides in different parts of Turkey (21). *Anopheles culicifacies* was resistant to organochlorine insecticides and tolerant to carbamates insecticides and susceptible to other insecticides (22). Insecticide resistance in *An. culicifacies* was reported (23-24). In order to suggest pyrethrpids for malaria vector control addition studies is necessary to find the mechanisms of resistant to DDT and cross-resistant to pyrethroids. According to the results of Gorouhi et al. (2018) (25), metabolic mechanisms play a crucial role in the development of DDT and cyfluthrin resistance in *An. stephensi*. Global results showed a wide variety of susceptibility/resistance status of malaria vectors to these chemicals according to the location, historical context of pesticide used, genetic background of vectors, age and abdominal conditions of adults, use of pesticides for agricultural pest control may play important role in the susceptibility status of these species to different insecticides (26-32).

Conclusion

The results of this study are providing a guideline for the country to manage their vector control activities against insecticide resistance of malaria vectors and provide novel approaches.

Acknowledgments

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