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Resistant Status of Main Malaria Vector, Anopheles Stephensi Liston (Diptera: Culicidae) to WHO Recommended Insecticides in Bandar Abbas, The Remain Transmission Area in Southern Iran

Vatandoost H[1*], Nikpoor F[1], Sanei Dehkordi AR[2], Soleimani-Ahmadi M[2], Madani A[2], Mojahedi AR[3], Yarian M[3] and Raeisi A[1]

[1]*Department of Environmental Chemical Pollutants and Pesticides, Institute for Environmental Research, Tehran University of Medical Sciences, Tehran, Iran*

[2]*Research Institute for Health, Hormozgan University of Medical Sciences, Bandar Abbas, Iran*

[3]*Department of Diseases Control, Hormozgan University of Medical Sciences, Bandar Abbas, Iran*

*** Corresponding Author**

Vatandoost H, Department of Environmental Chemical Pollutants and Pesticides, Institute for Environmental Research, Tehran University of Medical Sciences, Tehran, Iran, E-mail: hvatandoost1@yahoo.com; vatando@tums.ac.ir

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Abstract

Malaria continues to be main vector-borne dieses in Iran. The remain transmission area in Southern Iran is Bandar Abbas . By year 2020 the program of country is malaria elimination. The main vector control is using insecticide as Indoor Residual Spraying. The aim of study was to evaluate the susceptibility of main malaria vector *An. stephensi* to different insecticides recommended by WHO. All the insecticides papers supported by WHO and evaluation of insecticide resistance of *Anopheles stephensi*, to different chemical groups of imagicides including DDT 4%,Dieldrin 0.4%, Malathion 5%, Deltamethrin 0.05%, Deltamethrin 0.25%, Permethrin 0.75% , Lambda-cyhalothrin 0.05%, Bendiocarb 0.5%,Bendiocarb 0.1%, PBO 4% +Deltamethrin 0.05% were followed by WHO guideline. Results of susceptibility test against different insecticides revealed that *An.stephensi* is candidate of resistance to DDT and resistant to Deltamethrin 0.05%, Bendiocarb 0.5%, Bendiocarb 0.1% and susceptible to other insecticides. 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;* Resistant; Pesticides; Iran

Introduction

Malaria and other mosquito-borne disease are the major problems worldwide. Malaria presents a major health problem globally. It is estimated that globally 243 million cases of malaria led to 863,000 deaths in 2008. In most countries where malaria is endemic, the disease disproportionately affects poor and disadvantaged people, who have limited access to health facilities and can barely afford the recommended treatment. In 2019, an estimated 229 million cases of malaria occurred worldwide, resulting in 409,000 deaths [1]. According the latest information of WHO in 2021 (Figure 1) the indigenous cases in different parts of the world increased [2].

Anopheles stephensi is found across the Indian subcontinent, extending from the Arabian Peninsula, through Iran and Iraq, across to Bangladesh, southern China, Myanmar and Thailand (Figure 2) In southern part of the Iran, five anopheline mosquitoes, *Anopheles stephensi, An. dthali, An. fluviatilis, An. superpictus* and *An. culicifacies* (Diptera: Culicidae) are known to be malaria vectors. The distribution of *An.stephensi* is presented in Figure 3

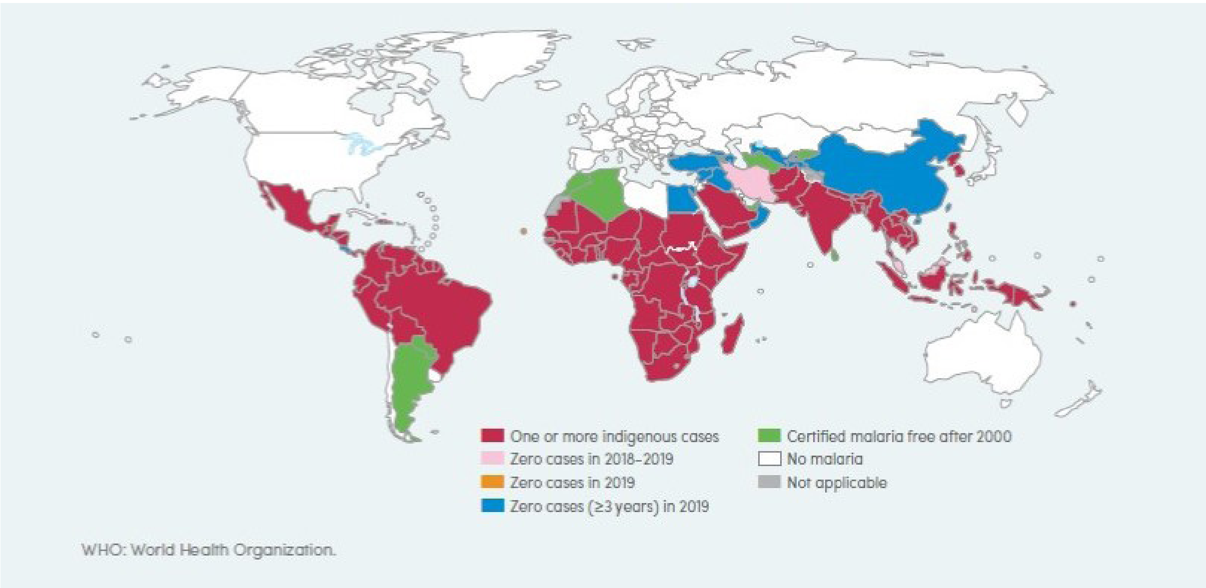
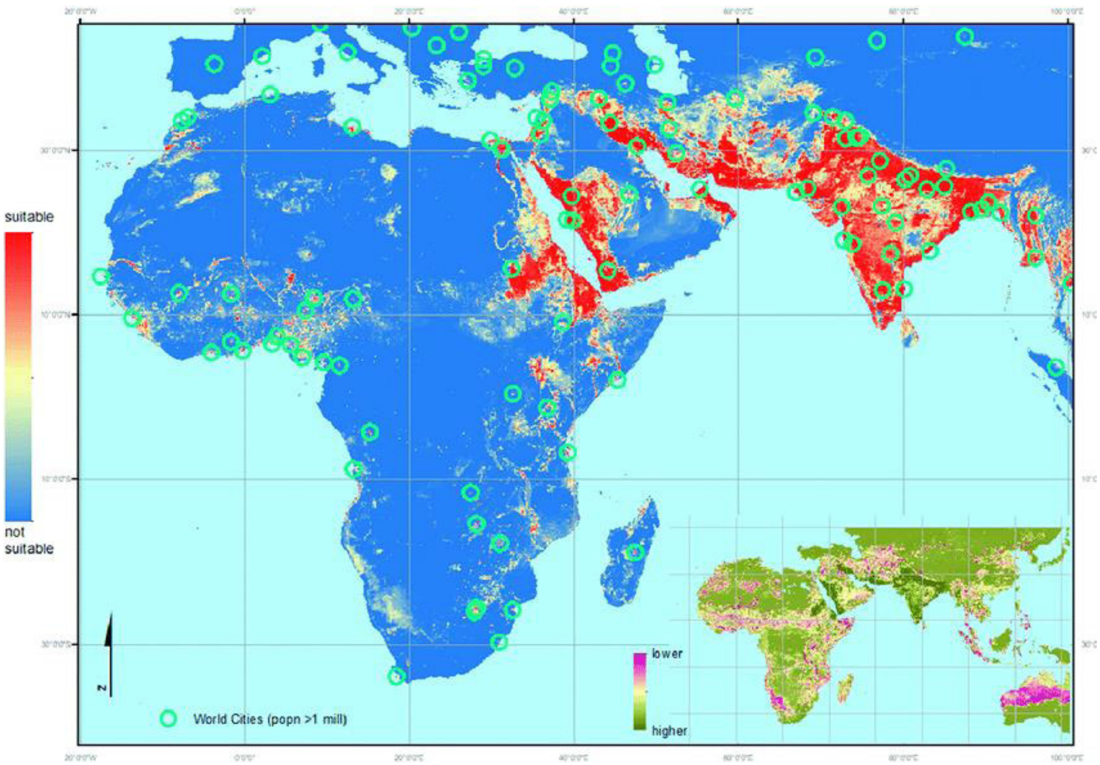


Figure 1: Global malaria distribution



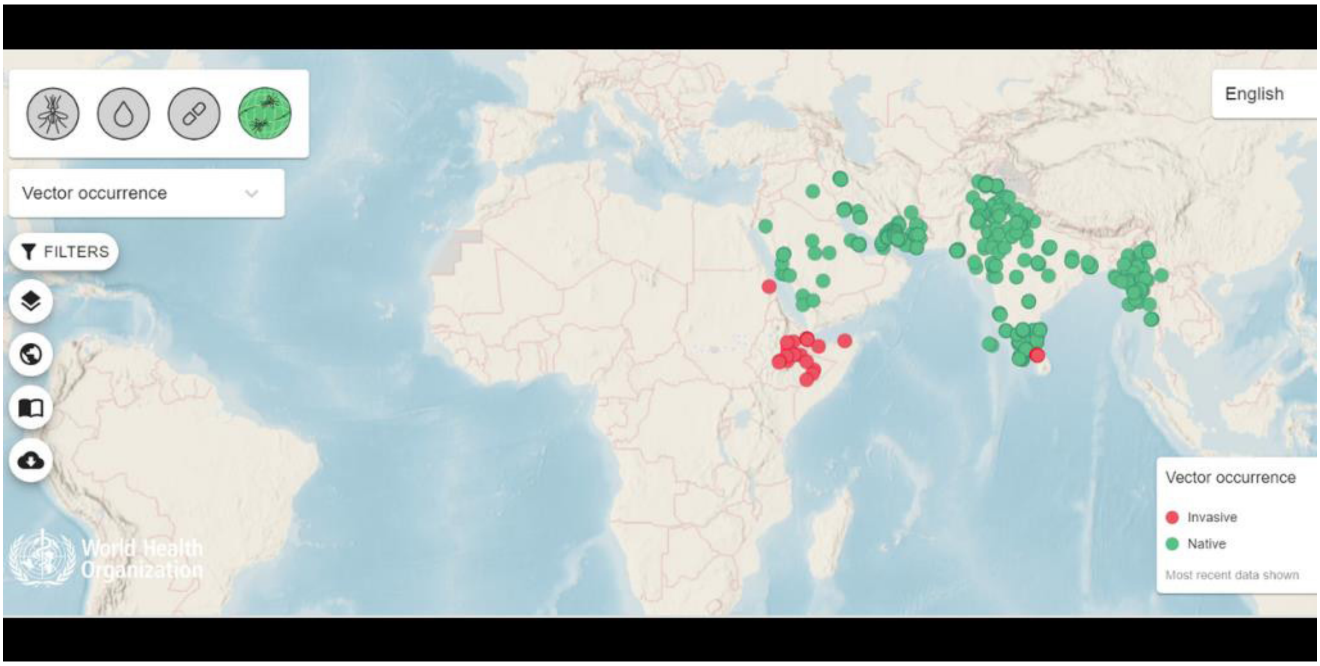


Figure 2: Distribution *of An.stephensi* worldwide

Figure 3: Distribution of *An.stephensi* in Iran

WHO recommended insecticides for indoor residual spraying against malaria vectors are: DDT ,Malathion, Fenitrothion, Pirimiphos-methyl, Bendiocar , Propoxur, Alpha-cypermethrin ,Bifenthrin, Cyfluthrin, Deltamethrin, Etofenprox, Lambda-cyhalothrin, Clothianidin [3].

Similarly main malaria cases in the country has been reported from southern and southeastern areas. The most routes of malaria cases are immigration from neighboring countries to southern and southeastern areas of the country (WHO, 2017) [4]. The main important vector control in the country are using insecticides as indoor reisula sprying, impregnate bednets and larviciding by applying *Bacillus thuringiensis* in the breeding places.

Currently there are proven and effective tools to fight against malaria including vector control measures [5]. A total of 228 million cases of malaria occurred worldwide in 2018. Most malaria cases (93%) were in African Region. *Plasmodium falciparum* is the most prevalent malaria parasite [6]. Iran is one of the malaria-endemic countries in the world, especially in southern provinces. The total number recorded cases have dropped to less than 89 locally-transmitted cases in 2017. Iran started a malaria elimination programme with a goal to achieve this target by 2025. There has been excellent progress since, but the continued risk of importation of malaria cases from Pakistan and Afghanistan. Main malaria vectors are *Anopheles stephensi*, *An.culicifacies*, *An.dthali*, *An.fluviatilis*, *An. superpictus An.maculipennis* and *An. sacharovi* [7]. Distribution of malaria vectors is shown in Figure 4.

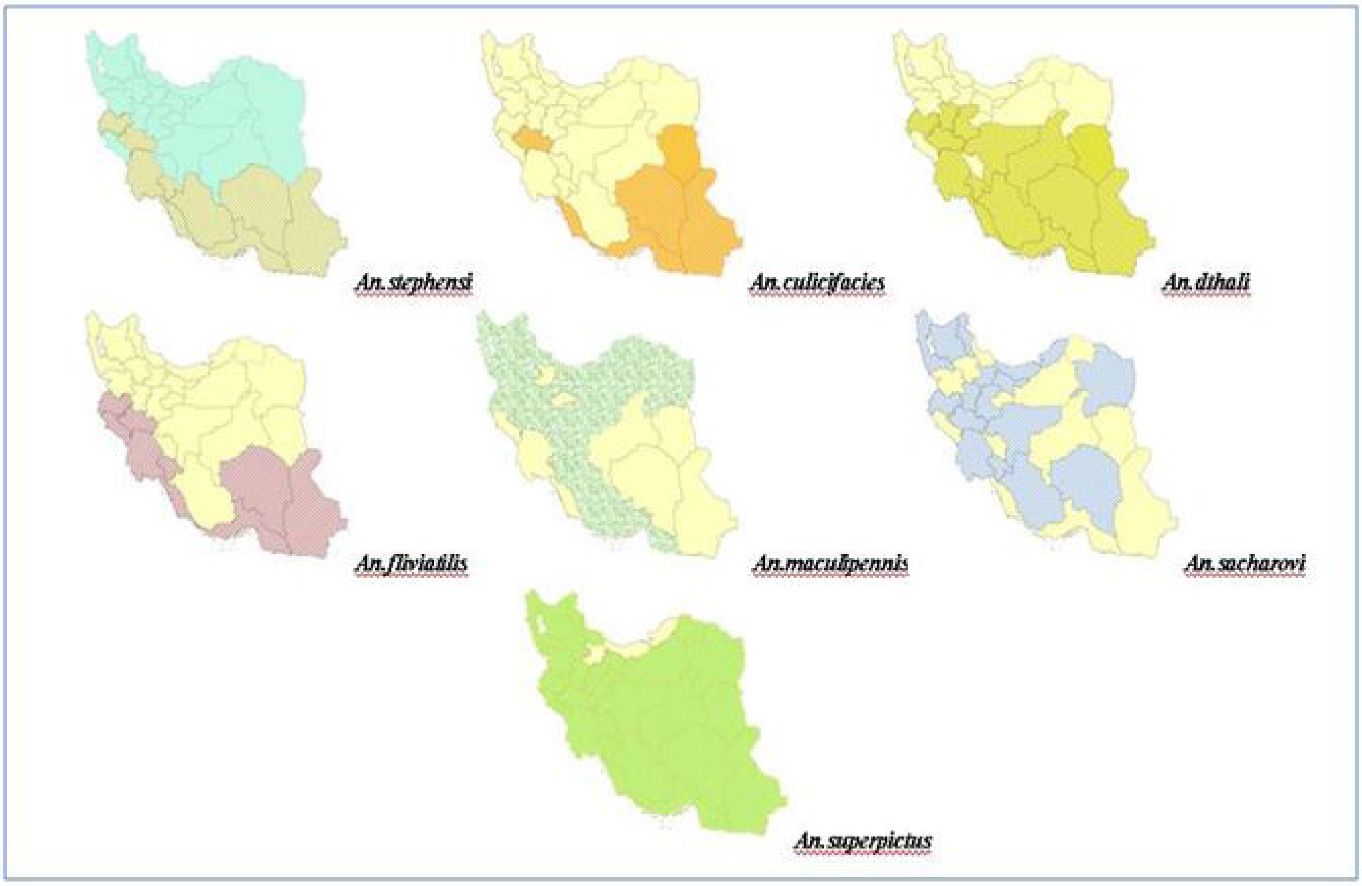


Figure 4: Spatial distribution of malaria vectors in Iran

Campaign against malaria vectors was started from 1952 by DDT spraying and then replaced by dieldrin, Malathion, propoxur, lambdacyhalothrin and deltamethrin, respectively. The chemical control of vectors now is restricted to endemic malarious areas of south-eastern part of the country with Deltamethrin and residual spraying and long lasting permethrin impregnated nets (Olyset) for personal protection, while biological control is conducting by *Bacillus thuringiensis* as larvicide.

Material and Methods

Study area

Malarious area of Bandar Abbas southern Iran was selected for experiments (Figure 5) . This is a port city and capital of Hormozgān Province on the southern coast of Iran, on the Persian Gulf. The city occupies a strategic position on the narrow Strait of Hormuz (just across from Musandam Governorate, Oman), and it is the location of the main base of the Iranian

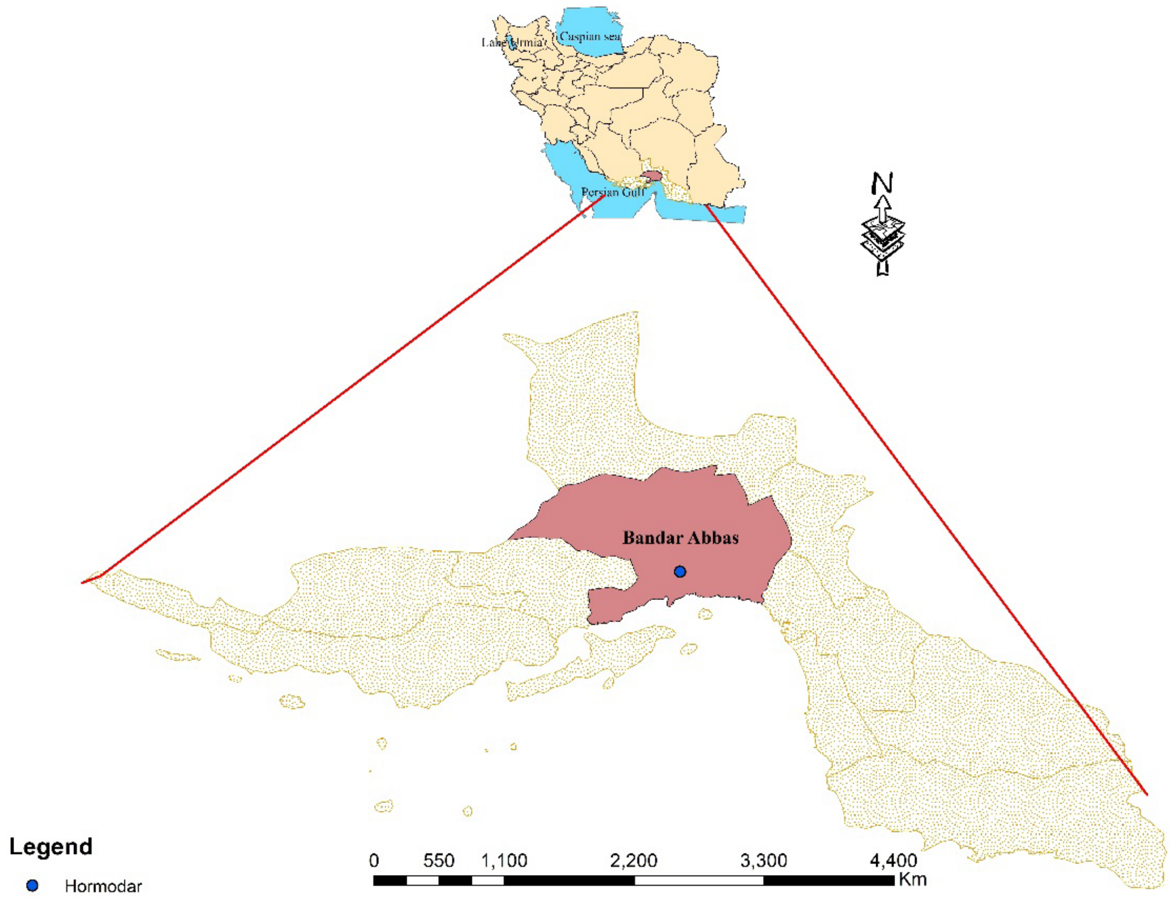


Figure 5: Geographical position of the study area and collection sites in south of Iran

Navy. Bandar Abbas is also the capital and largest city of Bandar Abbas County. At the 2016 census, its population was 526,648.

Preparation of tests kit and impregnated papers

Test kits and insecticide-impregnated papers were purchased from the WHO collaborative center in University Sains Malaysia (USM), Penang, Malaysia. They are: DDT 4%, Dieldrin 0.4%, Malathion 5%, Deltamethrin 0.05%, Deltamethrin 0.25%, Permethrin 0.75% , Lambda-cyhalothrin 0.05%, Bendiocarb 0.5%, PBO 4% +Deltamethrin were used against adult mosquitoes.

Mosquito species tested

Anopheles stephensi larvae were collected from Hormoodar village (27°19'14.72"N, 56°19'14.80"E), is located on the southern coast of Iran, north of the Persian Gulf (Figure 5), during Aug-Sep 2020 and were transferred to the insectary of Bandar Abbas School of Public Health. The larvae were reared into F1 generation for subsequent tests. The F1 offspring of adult females caught in the wild from the Hormoodar population were tested for between 3 and 5 days and given sugar. Mosquitoes were exposed to various insecticides with an exposure time of 60 minutes and a recovery period of 24 hours. A cotton swab soaked in 10% sugar solution was provided as a food source for the mosquitoes during the recovery period. The tests were performed in insectium maintained at a temperature of 27 ± 2 ° C and a relative humidity of 75 ± 10%, light 14:10: dark (8). The mortality rate for each test was estimated at the end of the tests

Test Method

For testing each insecticide a minimum of 150 female mosquitoes of a given species were used, so that 100 of which were exposed to the insecticide being tested at the diagnostic concentration (in four replicates of 25 mosquitoes) and the remaining were served as controls (two replicates of 25 mosquitoes). The mortality of the test sample was calculated by summing the number of dead mosquitoes across all exposure replicates and expressing as a percentage of the total number of exposed mosquitoes:

$$\text{Observed mortality} = \frac{\text{Total number of dead mosquitoes}}{\text{Total sample size}} \text{X } 100$$

A similar calculation was made for the control mortality. If the control mortality was above 20%, the tests were discarded. When control mortality was between 5-20%, then the observed mortality of exposed mosquitoes were corrected using Abbots formula, as follows:

$$\frac{\%\ \text{observed mortality} - \%\ \text{control mortality}}{100 - \%\ \text{control mortality}} \text{X } 100$$

The current WHO recommendation for interpretation of susceptibility test results were considered as follows (8):

- Susceptible - if mortality is in the range of 98 – 100%;
- Confirmed resistance - if mortality of test sample is less than 90%;
- Possible resistance, verification required – if the observed mortality is between 90% and 97%.

Statistical analysis

Data obtained from different replicates were collected for each surface. The mortality rate under 80% is considered as threshold level. Tests with control mortality rate between 5 and 20%, were corrected using Abbott's formula.

Results

Results of susceptibility test on different impregnated insecticide paper presented as follows (Table 1):

Results revealed that *An.stephensi* is candidate of resistance to DDT and resistant to Deltamethrin 0.05%, Bendiocarb 0.5%, Bendiocarb 0.1% and susceptible to other insecticides.

The results of susceptibility tests on *An. stephensi* against impregnated paper DDT 4%,Dieldrin 0.4%, Malathion 5%, Deltamethrin 0.05%, Deltamethrin 0.25%, Permethrin 0.75% , Lambda-cyhalothrin 0.05%, Bendiocarb 0.5%,Bendiocarb 0.1%, PBO 4% +Deltamethrin 0.05% indicated candidate of resistance to this insecticide according to the new WHO criteria (8)

An. stephensi is the main vector responsible for transmission of the pathogen that causes human malaria, *i.e. Plasmodium vivax* and *Plasmodium falciparum*, in the northern plates of Persian Gulf. It considered being endophagous and endophilic, so it is exposed to the insecticides used in malaria vector control program

Insecticide Impregnated paper	**Trial**		**Control**		**Resistant status**
	No. of Mosquitos	Trial	No. of Mosquitos	Control	
Malathion 5%	100	99%	50	0%	S
DDT 4%	100	97%	50	0%	CR
Dieldrin 0.4%	100	99%	50	0%	S
Deltamethrin 0.05%	200	95%	50	0%	R
Deltamethrin 0.25%	100	100%	50	0%	S
Permethrin 0.75%	100	100%	50	0%	S
Lambdacyhalothrin 0.05	100	100%	50	0%	S
Bendiocarb 0.1%	100	95%	50	2%	R
Bendiocarb 0.5%	100	96%	50	0%	R
Deltamethrin 0.05% after exposed PBO 4%	100	100%	50	0%	S

*R= Resistance, **S= Susceptible, ***CR= Candidate of resistance

Table 1: Response of *Anopheles stephensi* to different insecticides collected from Southern Iran, 2020

Discussions and Conclusion

An. stephensi is reported from 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 [9,10]. It is also reported from Djibouti and Ethiopia. [11,12]. First clues of pyrethroid resistance was found at 2011 from Iran and then confirmed in another study conducted in Hormozgan province. The results of a new study at 2015 conducted in Chabahar district, Sistan va Baluchestan province, showed that An. stephensi was resistant to DDT, tolerant to malathion, propoxur, cyfluthrin, and lambda-cyhalothrin and susceptible to deltamethrin. Another study in this district determined tolerance to deltamethrin and permethrin and resistance to lambdacyhalothrin. Two earlier studies in Jask district, east of our study area in Hormozgan province, showed *An. stephensi* was susceptible to pyrethroids that we used in our study, although it was resistant to DDT and dieldrin. A recent study in Hormoodar area showed *An. stephensi* was candidate of resistance to deltamethrin with 91% mortality rate. In the Punjab province, Pakistan, *An. stephensi* was susceptible to permethrin and deltamethrin, while confirmed or potential resistance to cypermethrin, λ-cyhalothrin, and cyfluthrin was observed from all the study sites. According to a report published in 2016, *An. stephensi* was resistant against all tested insecticides in Afghanistan. Regarding carbamates, which are using in rotation with pyrethroids in the national malaria vector control program in Iran, first indication of potential resistance in *An. stephensi* was reported from the southeast of Iran, with 95% mortality. Although next study conducted in the area resulted to 99.3% mortality, a study conducted in 2015 in 10 sentinel sites in southern Iran (Ministry of Health, Unpublished data) confirmed resistance to bendiocarb (86.41% mortality) in Kerman province and potential resistance to this insecticide in Sistan va Baluchestan province. In a crosssectional study at 2010, *An. stephensi* (Minab population) was susceptible to bendiocarb and deltamethrin (100% mortality rate) [13-25]. There are several reports on resistant status of malaria vectors including *An.stephensi* [26-30]. *An. stephensi* showed resistance to lambdacyhalothrin, deltamethrin, permethrin, and bendiocarb in Bandar Abbas province, southern Iran . *An. stephensi* samples were resistant bendiocarb , propoxur, deltamethrin , permethrin, DDT , malathion and pirimiphos-methyl in Somali region [31]. The results of this study is providing a guideline for country to manage their vector control activities against insecticide resistance of malaria vectors and provide novel approaches.

Ethics approval and consent to participate

Not applicable

Consent for publication

Applicable

Availability of data and material

applicable

Competing interests

Not applicable

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Authors' contributions

all authors are involved

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