

## Review Article

# Monitoring and Mapping of Insecticide Resistance in Medically Important Mosquitoes (Diptera: Culicidae) in Iran (2000–2020): A Review

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## Abstract

**Background:** Mosquitos due to their role in the transmission of different pathogens to humans are considered as an important group in the phylum Arthropoda. According to the WHO and FAO guideline different groups of insecticide applied for controlling pests in both the agricultural and public health sectors.

**Methods:** All the data published about resistant status of the mosquitoes *Anopheles*, *Culex*, *Aedes* and *Culiseta* species were searched on PubMed, Elsevier, Web of Science, Magiran and google scholar. The objectives of this study was to review the trend of resistance to insecticides during 2000–2020 in medically important mosquitoes in Iran. The criteria for resistant are followed according to WHO guideline.

**Results:** The Results showed that there are widespread, multiple resistances in the country to different organochlorine, organophosphates, carbamate and pyrethroids insecticides in the mosquitoes.

**Conclusion:** The effect of pesticide residues on the environment could be a cause for selection pressure on mosquitos and lead to insecticides resistance to them. Insecticides resistance is main challenge of the vector control program. Also result will provide a guideline for control of the mosquito-borne diseases in the country as well as the world.

**Keywords:** Resistance; Insecticide; Mosquito; Iran

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## Introduction

Malaria is one of the most important parasitic diseases transmitted by the genus *Anopheles* to human (1-3). In Iran malaria has been present for a long time ago (4, 5) and mainly mentioned as a big public health challenge in Sistan and Baluchistan, Hormozgan and Ker-

man Provinces in the south-eastern part of Iran also featured as refractory malaria (6). The favorable results obtained in reducing of malaria transmissions by application of DDT for controlling of mosquito in a hyperendemic area in Iran for the first time in 1947 and then

spraying with DDT in combination with other vectors control interventions continued in the most malaria-endemic areas of the country until 1956, finally for first time in 1957 resistance to DDT was recorded in *Anopheles stephensi* as vector of malaria in Iran (4, 5). In Iran there are some species of malaria vectors (Fig. 1) including: *An. stephensi*, *An. dthali*, *An. culicifacies* s.l., *An. fluviatilis* s.l., *An. superpictus* s.l., *An. sacharovi*, *An. maculipennis* s.l. (3, 5). *Culex pipiens pipiens* and *Culex quinquefasciatus* as complex members of *Cx. pipiens* are competent vectors for some filarial and arboviral disease and also *Dirofilaria immitis* moreover West Nile and Sindbis viruses have been detected from mentioned species in Iran (1, 7-9). *Culex pipiens* habitat mainly is sewage system and there are different reagents and also residues of insecticides belong to several group of pesticides which have been previously used in agriculture and public health sectors (9, 10). For the first time resistance to insecticides was shown in 1975 in *Cx. pipiens pipiens* about DDT in the northern part of Iran (9, 11). Fig. 2 shows the distribution of *Cx. pipiens* and *Cx. quinquefasciatus* in Iran. After 20 years later from the first report of resistance to DDT in *An. stephensi*, this report was the second alarm related to resistance in medically important mosquitos in Iran. The presence of West Nile virus also has been reported in *Aedes caspius* or *Ochlerotatus caspius* from Iranian wetlands during recent years (12). Even though to date there is no report about the detection of pathogens among *Culiseta longiareolata* in Iran but, *Ae. caspius* (13) and *Cs. longiareolata* (14) currently showed their resistance to DDT. Figs. 3 and 4 shows the distribution of *Ae. caspius* and *Cs. longiareolata* in Iran. Continuation of the previous report related to the appearance of insecticide resistance about DDT in *An. stephensi* and *Cx. pipiens* as vectors of important diseases in Iran. According to the latest studies only during 2012 to 2014 approximately 14,000 tons of pesticides consist of herbicides, insecticides,

acaricides, and fungicides were used for agricultural pests (15). Due to the effect of pesticide residues on the environment it could be a cause for selection pressure on mosquitos which their breeding places are water and finally lead to their resistance to different group of insecticides indirectly (9, 10, 16). In the public health sector also different groups of insecticide applied for controlling *Anopheles* mosquitos in malarious areas of Iran such as DDT and Dieldrin belong to organochlorine compounds, Malathion and Pirimiphos-methyl (organophosphates), Propoxur (carbamates), Deltamethrin and Lambda-cyhalothrin belong to pyrethroids compounds (4, 5). Previously for testing the insecticide susceptibility level among adult of mosquitos all bioassay studies have been performed referring to the test procedures of World Health Organization (WHO) recommended for insecticide resistance monitoring in mosquitos that suggested for each concentrations, six replicate samples of 20–25 adult female mosquitos per tube (2 replicates as control) should be expose for one hour at diagnostic dose of each insecticide and the number of mortality determined 24 hours after recovery period, finally the mortality results divided in three categories include: 98–100% mortality indicates susceptibility, 80–97% mortality considered as tolerance and also requires confirmation of resistance with other methods and if mortality was less than 80% in tested samples, mosquitos considered as resistant to insecticide (17). Previous guideline was revised recently and considering the current WHO categories for susceptibility level, the following criteria have been used for interpretation of results related to mortality rate: higher than 98% was considered as susceptible, mortality between 90% to 97% considered as resistance candidate and more investigation is needed for the confirmation of resistance and finally mortality less than 90% demonstrated resistance (18). Here there are examples regarding resistance to DDT lonely in medically important mosquitos and its trend from the beginning of the resistance to insecticides,

ticides. In the present paper, we aimed to review the trend of resistance to insecticides during 2000–2020 in medically important mosquitoes and also the distribution of resistant specimen plotted using Arc-GIS10.2 software (Redlands, CA) In Iran.

## Materials and Methods

All the data published about resistant status of mosquitoes *Anopheles*, *Culex*, *Aedes* and *Culiseta* species were searched during 2000–2020 on PubMed, Elsevier, Web of Science, Magiran and google scholar.

## Results

### Review of resistance to insecticides in *Anopheles* mosquitoes as malaria vectors

According to recent studies, seven species of *Anopheles* mosquitoes (Fig. 1) introduced as malaria vectors in Iran including: *An. stephensi*, *An. culicifacies* s.l., *An. fluviatilis* s.l., *An. superpictus* s.l., *An. dthali*, *An. sacharovi* and *An. maculipennis* s.l. while *An. pulcherrimus* is considered as a suspect vector moreover five of these vectors can be found in the southeast of the country, where the majority of malaria cases is reported also *An. stephensi* considered to be the main malaria vector in the same area (3, 19). The resistant status of *Anopheles* mosquitoes to organochlorine compounds in Iran (Table 1 and Figs. 5–7) indicated that approximately all malaria vectors in Iran were resistant to the DDT and Dieldrin which have been used previously for control of mosquitoes (6, 13, 20–37). After the first report about resistance to DDT in *An. stephensi* in 1957 other malaria vectors gradually, showed their resistance to organochlorine compounds in Iran up to now. But about *An. sacharovi* and *An. maculipennis* s.l. as main malaria vectors in the northern part of the country, all studies which have been performed on the susceptibility level of these species to organochlorine compounds

showed tolerance to dieldrin (21, 23, 30, 33). Similar to this finding about Dieldrin, tolerance to DDT in *An. dthali* in south eastern part of Iran also has been shown (13). Although in some districts in southern parts of the country in Jiroft District in Kerman Province *An. stephensi* was tolerant to DDT and dieldrin (27). Moreover, in Bashagard District in Hormozgan Province tolerant to dieldrin in *An. stephensi* as well as tolerant to DDT in *An. culicifacies* s.l. also have been shown (28, 29).

The susceptibility level of *Anopheles* mosquitoes about pyrethroids compounds (Table 2 and Fig. 6) showed that, to date among malaria vectors in Iran, just in *An. stephensi* resistant to Cyfluthrin, and Lambda-cyhalothrin has been shown in Chabahar District in southeastern part of the country (31, 34, 35). Although tolerant to others pyrethroids insecticides such as deltamethrin, permethrin and etofenprox also have been reported in *An. stephensi* in this area (31, 34). Tolerant to deltamethrin also shown in *An. stephensi*, *An. culicifacies* s.l. and *An. dthali* in Bashagard District and *An. stephensi* in Jask District in Hormozgan Province (28, 35) and also in *An. culicifacies* s.l. in Chabahar District in Sistan and Baluchistan Province (36). Similar to mentioned finding about tolerant to pyrethroids insecticides in *Anopheles* mosquitoes, tolerant to deltamethrin and permethrin also has been shown in *An. maculipennis* in the northern part of Iran (33).

The susceptibility level of *Anopheles* mosquitoes to organophosphates compounds (Table 3 and Fig. 7) indicated that *An. maculipennis* s.l. in West Azarbaijan Province in the Northwestern part of the country has become resistant to Malathion (33) but about other *Anopheles* mosquitoes tolerant to Malathion in southern part also in *An. dthali* in Hormozgan Province (28) and also in *An. stephensi* and *An. culicifacies* s.l. in Sistan and Baluchistan Province were shown (13).

The status of *Anopheles* mosquito resistant to carbamates compound's (Table 4 and Figs. 6, 7) indicated that among all malaria vectors

around the country resistance to carbamates insecticides such as propoxur and bendiocarb is shown in *An. maculipennis* s.l. in North-western part and resistance to bendiocarb in *An. stephensi* is in the southern part of the country (33, 37). Although in *An. stephensi*, *An. culicifacies* s.l. and *An. dthali* tolerant to propoxur in some parts of the country has been reported (13, 22, 28). By reviewing the resistance status of *Anopheles* mosquitos during 2000 to 2020 it is concluded that approximately all *Anopheles* mosquitos found resistance to organochlorine insecticides in Iran and although about other classes of insecticides there are a few reports for resistance about some malaria vectors to one or more insecticides, but considering the modification of the guideline for susceptibility test in mosquitos From 2013 which demonstrated resistance in mosquitos with mortality less than 90%, susceptible species in previous studies can be categories as resistant to insecticides (36).

### Review of resistance to insecticides in *Culex* mosquitos

The susceptibility level of *Culex* mosquitos against organochlorine compounds in Iran (Table 5 and Figs. 8–10) revealed that *Cx. pipiens*, *Cx. quinquefasciatus* and *Cx. theileri* in most area of the country showed their highly level of insecticide resistance to DDT and deildrin (9, 13, 14, 16, 38, 39, 41, 42). Although, *Cx. quinquefasciatus* in a study in Southeast area showed its 90% mortality rate to DDT and according to the WHO considered as tolerant or candidate for resistance (40), similar *Anopheles* species, resistance to DDT in *Culex* mosquitos around the country have been developed during recent years after the first report for resistance to DDT in the northern part of Iran about *Cx. pipiens pipiens* in 1975 (9, 11).

The status of *Culex* mosquitoes resistant to pyrethroids compound's (Table 6 and Figs. 8–10) showed that they are almost resistant to most insecticides belong to pyrethroids (9, 13, 14, 39, 41-43). For example, in the north of

Iran, *Cx. pipiens* in Sari District was highly resistance to all tested pyrethroids insecticides including Cyfluthrin, Lambda-cyhalothrin, Deltamethrin, Etofenprox and Permethrin (41).

Resistance to organophosphates compounds in *Culex* mosquitos (Table 7 and Figs. 8, 9) indicated that members of *Cx. pipiens* complex were found resistant to Malathion and Fenitrothion (40, 41) although in Ahar District in East Azarbaijan tolerance to malathion in *Cx. pipiens* and *Cx. theileri* have been reported (14).

The susceptibility level of *Culex* mosquitos to carbamates compounds (Table 8 and Figs. 8–10) revealed that *Cx. quinquefasciatus*, *Cx. theileri* and *Cx. pipiens* were found to be resistant to all tested insecticides belong to carbamates compounds (14, 16, 40, 41).

Comparing the resistance status of *Culex* mosquitos with *Anopheles* mosquitos during the past 20 years ago revealed that *Culex* species almost were found resistant to most insecticides belong to pyrethroids and also about other classes of insecticides the number of species which showed resistance to insecticides are more than *Anopheles* mosquitos.

### Review of resistance to insecticides in *Aedes caspius*

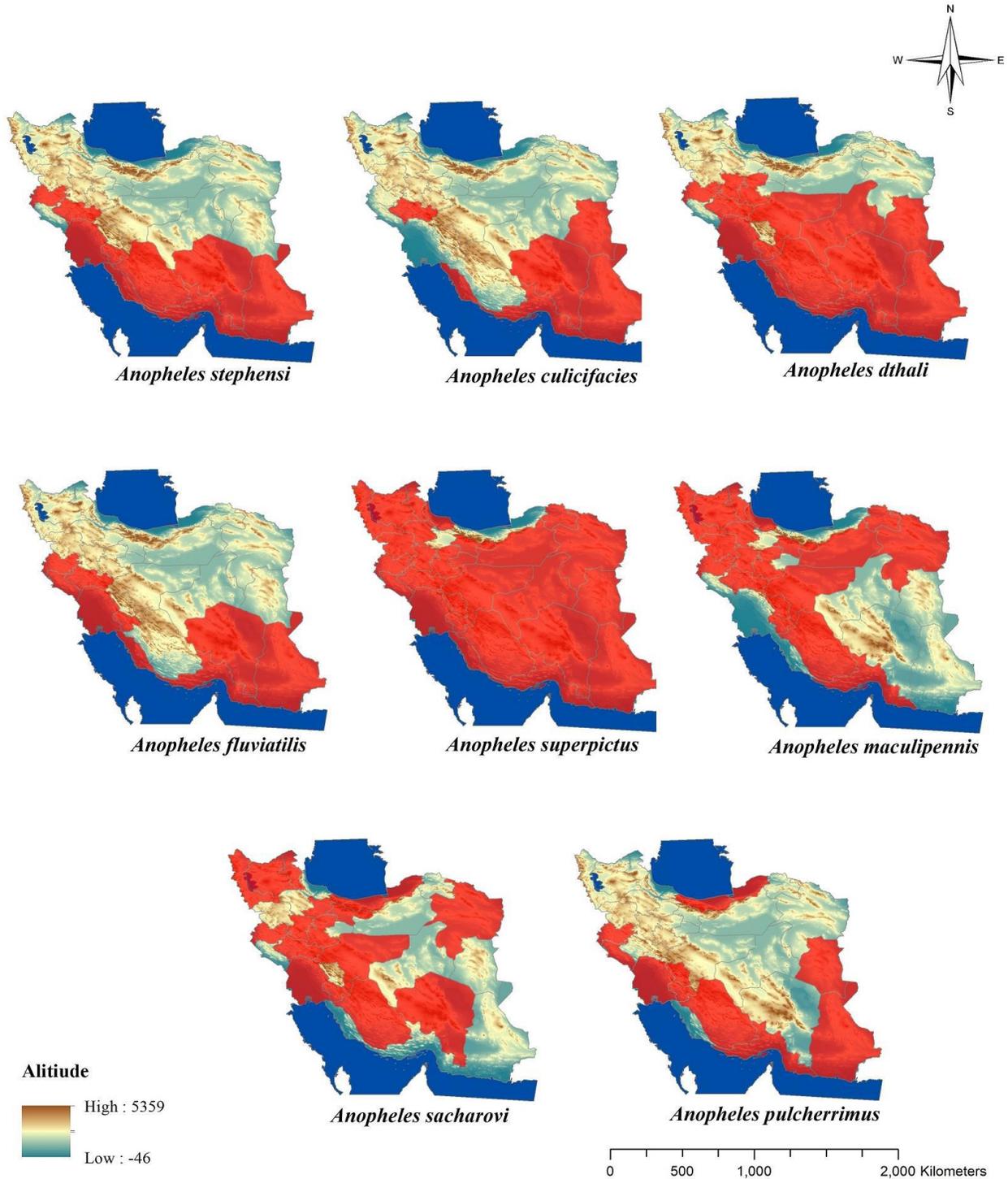
The resistance status of *Ae. caspius* to different groups of insecticides in Iran (Table 9 and Fig. 11) showed that *Ae. caspius* is resistant to DDT, Bendiocarb, Malathion, Permethrin and Lambdacyhalothrin (13, 44). Actually this species was found to be resistant to all classes of insecticides although in some part this species was susceptible to deltamethrin (44).

### Review of resistance to insecticides in *Culiseta longiareolata*

The susceptibility level of *Cs. longiareolata* to different groups of insecticides (Table 10 and Fig. 12) indicated that this species similar *Ae. caspius* was resistant to all classes of insecticides (14, 45). There are a few studies about the susceptibility level of *Cs. longiareolata* and *Ae. caspius* in Iran, but both of them were found to be resistant to all groups of in-

secticides. In the following resistance to insecticides in medically important mosquitos in Iran resistance to insecticides in mentioned

species also can be considered as a problem in the vector control program.



**Fig. 1.** Distribution of malaria vectors in Iran

**Table 1.** List of *Anopheles* mosquitos resistant to organochlorine compounds in Iran using the WHO insecticide susceptibility tests (2000–2020)

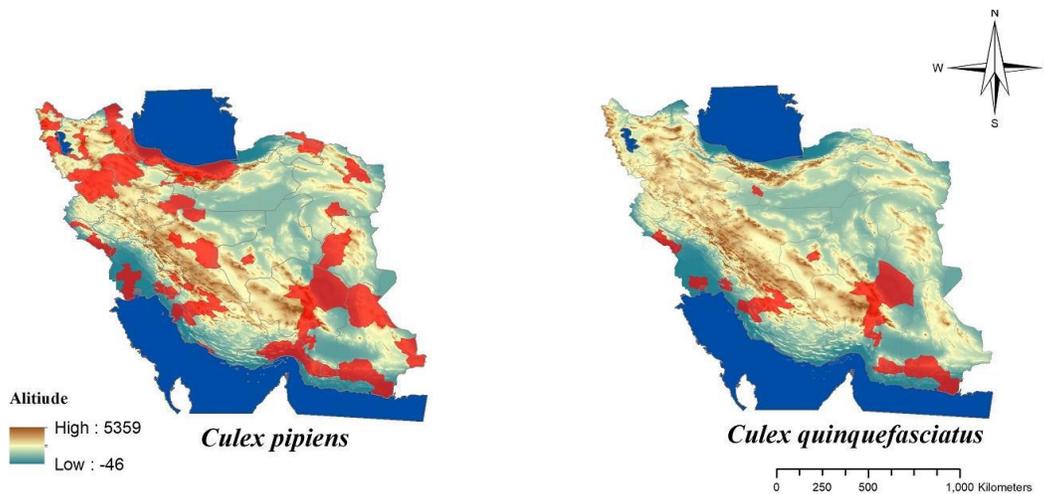
Species	Insecticides	Location (Province-District)	References
<i>An. pulcherrimus</i>	Dieldrin	Sistan and Baluchistan-Ghasreghand	20
<i>An. sacharovi</i>	DDT	West Azerbaijan-Poldasht	21
<i>An. stephensi</i>	DDT	Sistan and Baluchistan-Iranshahr	22
<i>An. Sacharovi</i>	DDT	East Azerbaijan-Kalibar	23,30
<i>An. stephensi</i>	DDT, Dieldrin	Kerman-Kahnooj	24
<i>An. stephensi</i>	DDT	Hormozgan-Siahoo, Geno and Bandar Abbas Hormozgan-Bandar Abbas	25
<i>An. stephensi</i>	DDT, Dieldrin	Sistan and Baluchistan-Iranshahr Fars-Kazeroon	26
<i>An. maculipennis</i> s.l.	DDT, Dieldrin	Gilan-Astara	6
<i>An. stephensi</i>	DDT	Hormozgan-Bashagard	28,29
<i>An. stephensi</i>	DDT	Sistan and Baluchistan-Chabahar	13, 31, 34, 37
<i>An. superpictus</i> s.l.	DDT	Sistan and Baluchistan-Sarbaz	32
<i>An. stephensi</i>	DDT	Hormozgan-Jask	35
<i>An. culicifacies</i> s.l.	DDT, Dieldrin	Sistan and Baluchistan-Chabahar	36

**Table 2.** List of *Anopheles* mosquitos resistant to pyrethroids compounds in Iran using WHO insecticide susceptibility tests (2000–2020)

Species	Insecticides	Location (Province-District)	References
<i>An. stephensi</i>	cyfluthrin, lmbda-cyhalothrin	Sistan and Baluchistan-Chabahar	31, 34
<i>An.stephensi</i>	lmbdacyhalothrin	Hormozgan-Jask	35

**Table 3.** List of *Anopheles* mosquitos resistant to organophosphates compound’s in Iran using WHO insecticide susceptibility tests (2000–2020)

Species	Insecticides	Location (Province-District)	References
<i>An. maculipennis</i> s.l.	Malathion	West Azerbaijan	33



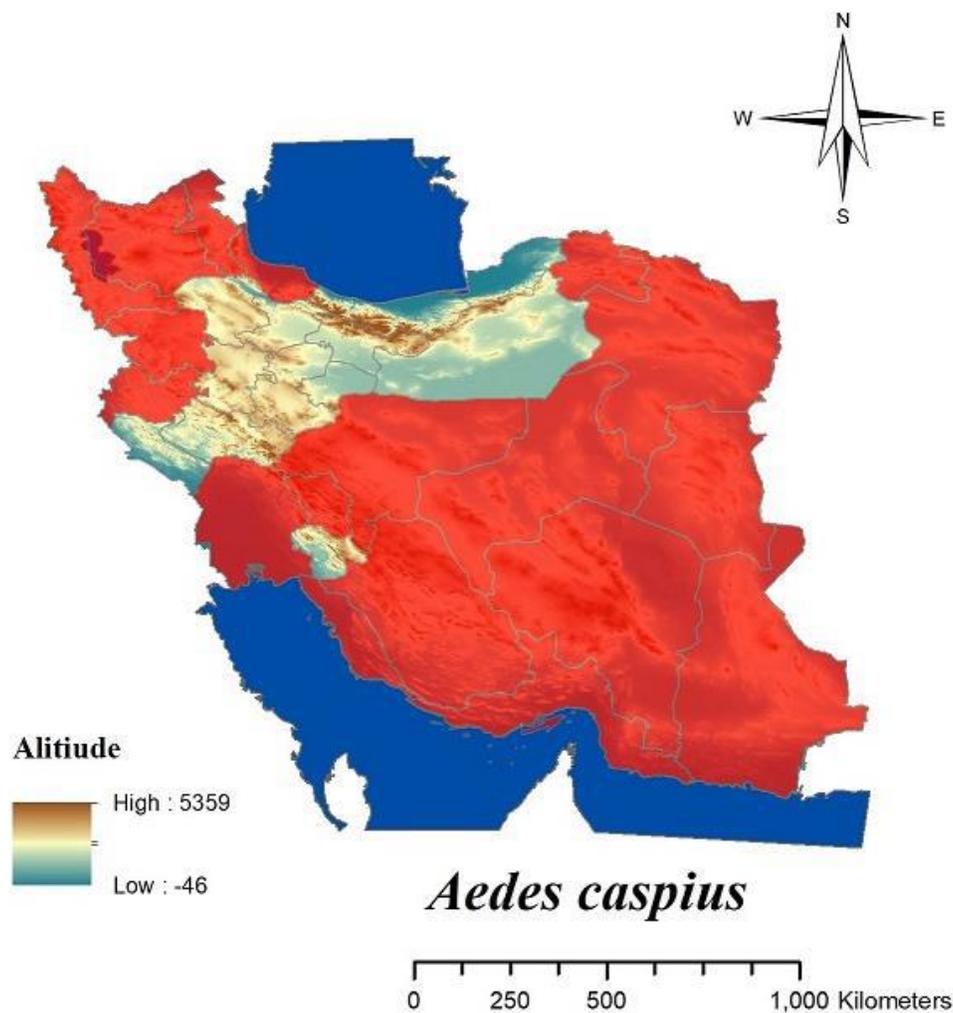
**Fig. 2.** Distribution of *Culex pipiens* and *Culex quiquefasciatus* in Iran

**Table 4.** List of *Anopheles* mosquitos resistant to Carbamates compound's in Iran using WHO insecticide susceptibility tests (2000–2020)

Species	Insecticides	Location (Province-District)	References
<i>An. maculipennis</i> s.l.	Propoxur and Bendiocarb	West Azerbaijan	33
<i>An. stephensi</i>	Bendiocarb	Sistan and Baluchistan-Chabahar	37

**Table 5.** List of *Culex* mosquitos resistant to organochlorine compounds in Iran using WHO insecticide susceptibility tests (2000–2020)

Species	Insecticides	Location (Province-District)	References
<i>Cx. theileri, Cx. pipiens</i>	DDT	Tehran-Tehran	38
<i>Cx. quinquefasciatus</i>	DDT	Tehran-Varamin	16
<i>Cx. pipiens</i>	DDT	Sistan and Baluchistan-Chabahar	13
<i>Cx. theileri and Cx. pipiens</i>	DDT	East Azarbaijan -Ahar	14
<i>Cx. pipiens</i>	DDT	West Azerbaijan -Urmia	39
<i>Cx. pipiens</i>	DDT	Tehran-Tehran	9
<i>Cx. pipiens</i>	DDT, Dieldrin	Mazandaran-Sari	41
<i>Cx. quinquefasciatus</i>	DDT	Hormozgan-Suru	42



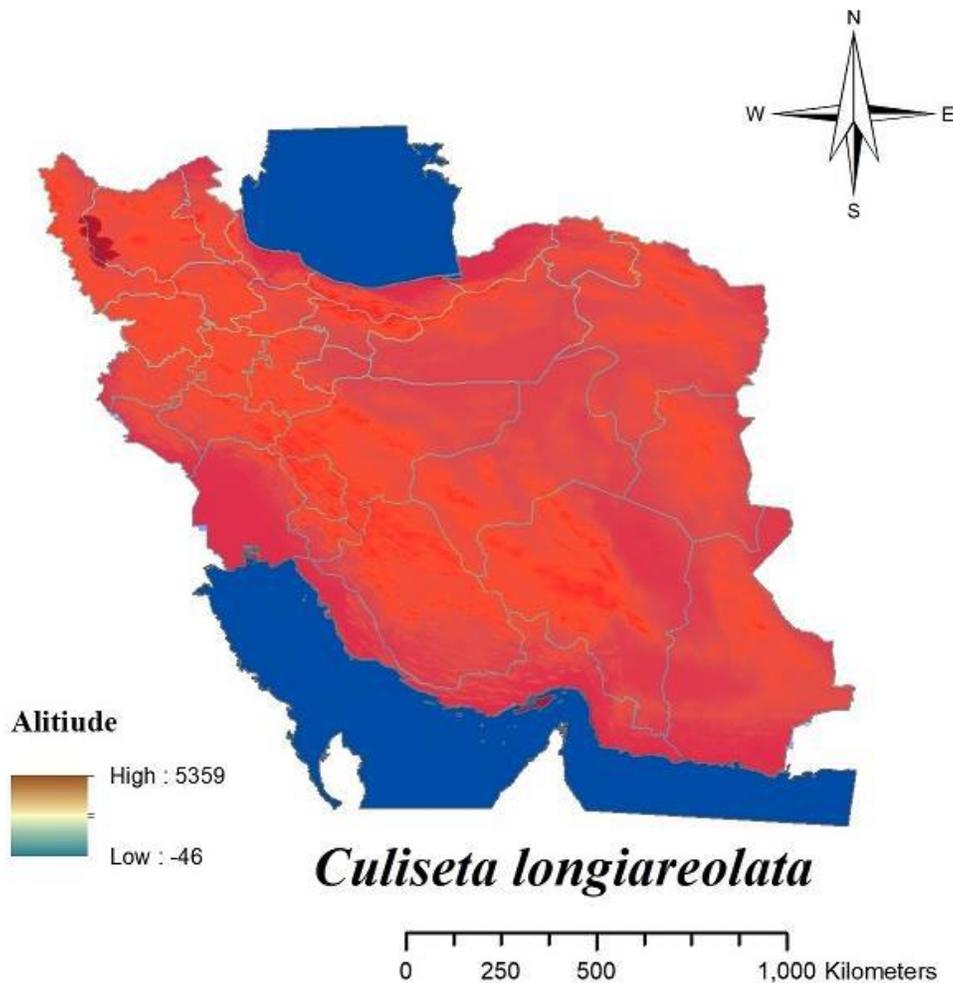
**Fig. 3.** Distribution of *Aedes caspius* in Iran

**Table 6.** List of *Culex* mosquitos resistant to pyrethroids compound's in Iran using WHO insecticide susceptibility tests (2000–2020)

Species	Insecticides	Location (Province-District)	References
<i>Cx. pipiens</i>	Cyfluthrin and Lambdacyhalothrin	Sistan and Baluchistan-Chabahar	13
<i>Cx. theileri, Cx. pipiens</i>	Lambdacyhalothrin	East Azarbaijan-Ahar	14
<i>Cx. pipiens</i>	Deltamethrin	West Azerbaijan-Urmia	39
<i>Cx. pipiens</i>	Cyfluthrin, Lambdacyhalothrin, Deltamethrin	Tehran-Tehran	9
<i>Cx. pipiens</i>	Cyfluthrin, Lambdacyhalothrin, Deltamethrin, Etofenprox, Permethrin	Mazandaran-Sari	41
<i>Cx. quinquefasciatus</i>	Deltamethrin	Hormozgan-Suru	42
<i>Cx. pipiens complex</i>	Deltamethrin	Tehran-Gharchak	43

**Table 7.** List of *Culex* mosquitos resistant to organophosphates compound's in Iran using WHO insecticide susceptibility tests (2000–2020)

Species	Insecticides	Location (Province-District)	References
<i>Cx. quinquefasciatus</i>	Malathion	Kerman-Rafsanjan	40
<i>Cx. pipiens</i>	Malathion, Fenitrothion	Mazandaran-Sari	41



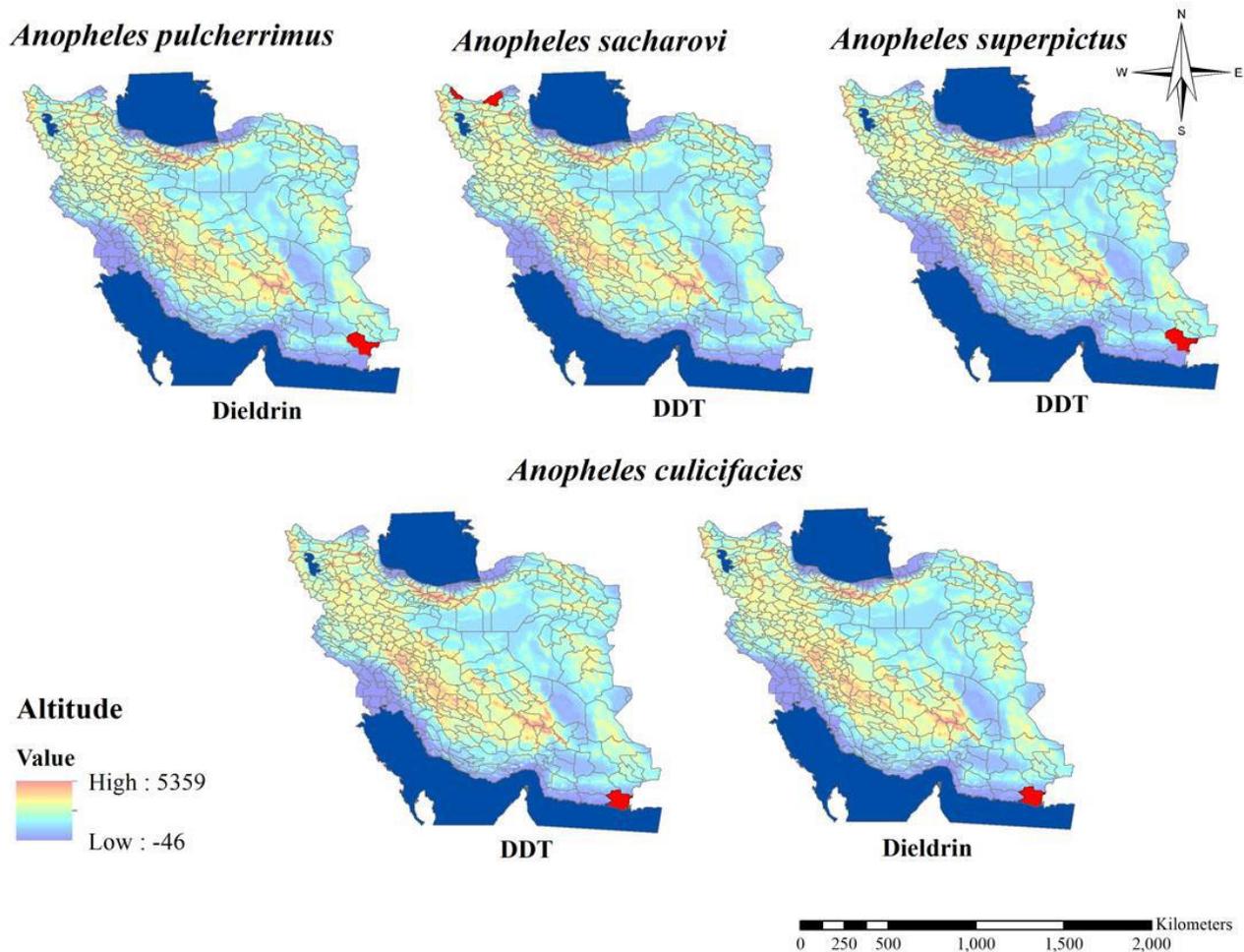
**Fig. 4.** Distribution of *Culiseta longiareolata* in Iran

**Table 8.** List of *Culex* mosquitos resistant to carbamates compounds in Iran using WHO insecticide tests (2000–2020)

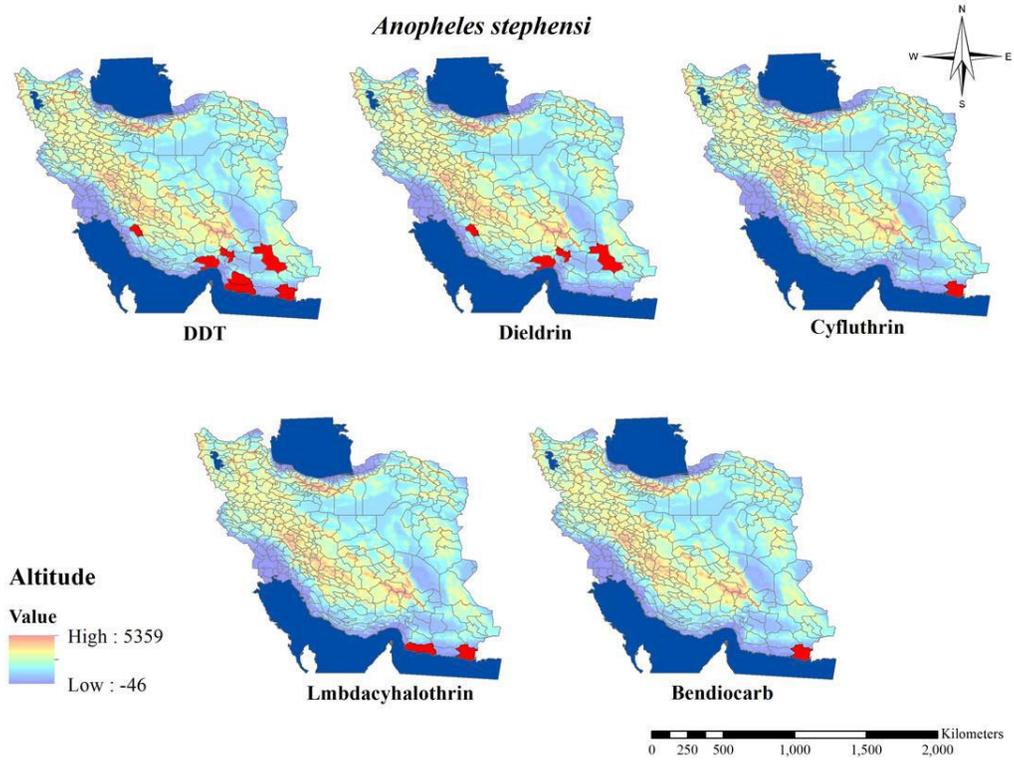
Species	Insecticides	Location (Province-District)	References
<i>Cx. theileri</i> and <i>Cx. pipiens</i>	Propoxur	East Azarbaijan-Ahar	14
<i>Cx. quinquefasciatus</i>	Bendiocarb	Tehran-Varamin	16
<i>Cx. quinquefasciatus</i>	Bendiocarb	Kerman-Rafsanjan	40
<i>Cx. pipiens</i>	Bendiocarb	Mazandaran-Sari	41

**Table 9.** Resistance status of *Aedes caspius* to different groups of insecticides in Iran using WHO insecticide susceptibility tests (2000–2020)

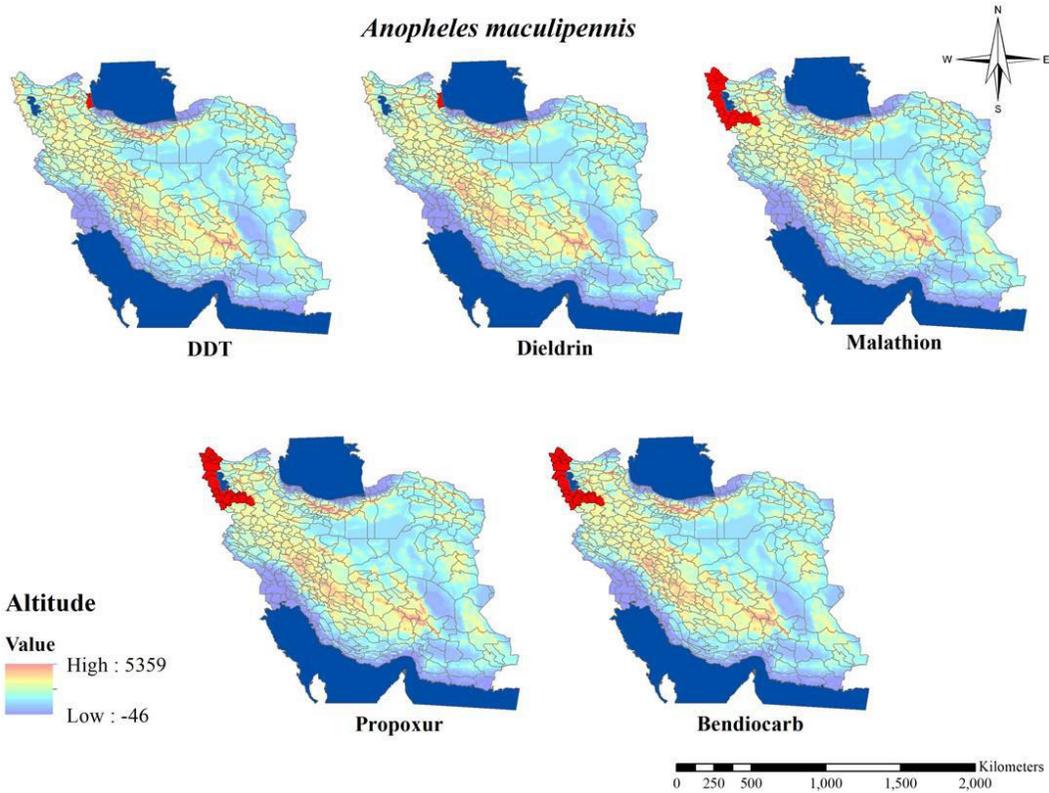
Species	Insecticides	Location (Province-District)	References
<i>Ae. caspius</i>	DDT	Sistan and Baluchistan-Chabahar	13
		Hormozgan-Siahoo	44
	Malathion	Hormozgan-Siahoo	44
	Bendiocarb		
Permethrin, lambdacyhalothrin			



**Fig. 5.** Distribution of *Anopheles* mosquitos with resistance to organochlorine compounds in Iran (2000–2020)



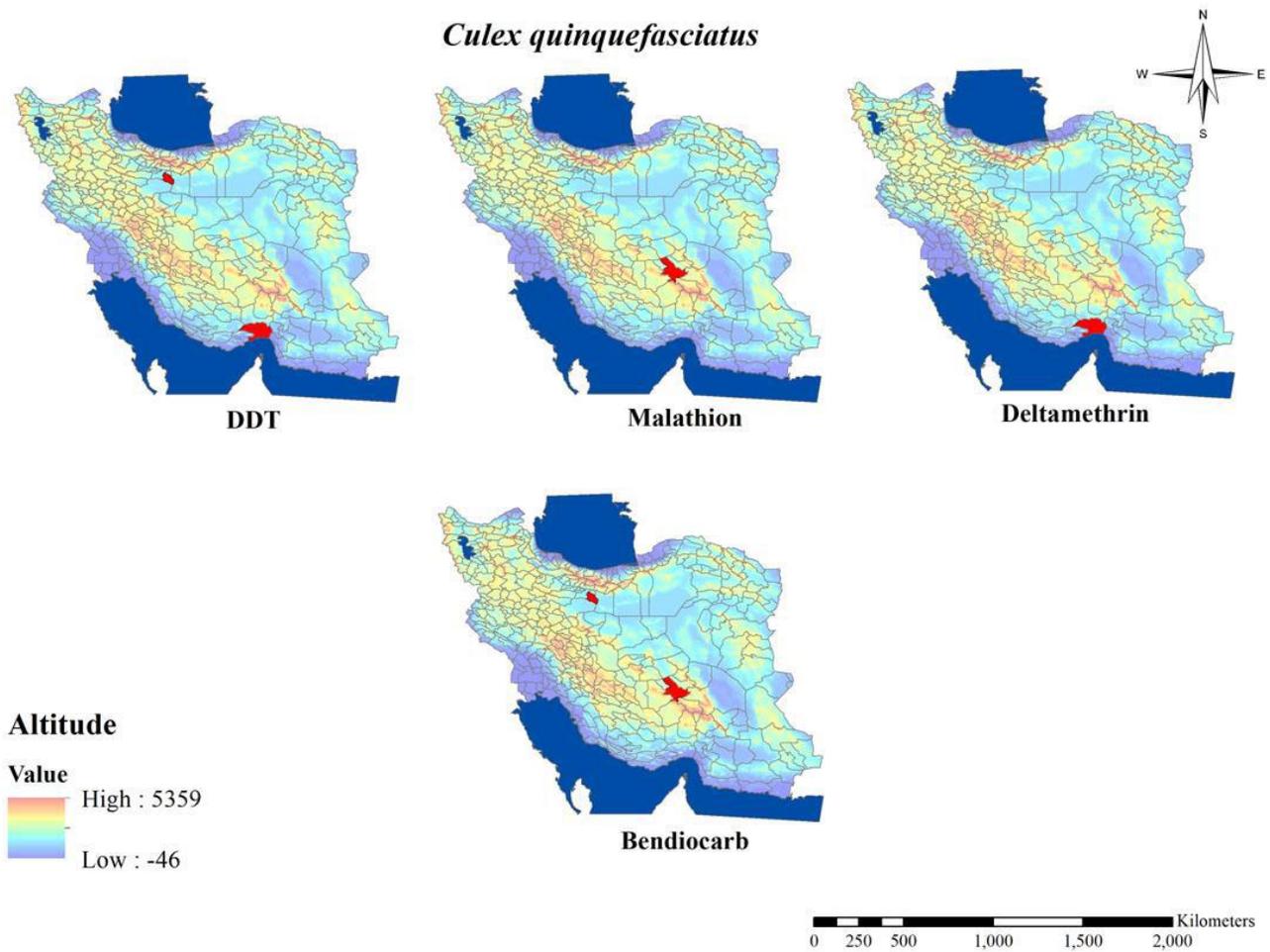
**Fig. 6.** Distribution of *Anopheles stephensi* with resistance to different insecticides in Iran (2000–2020)



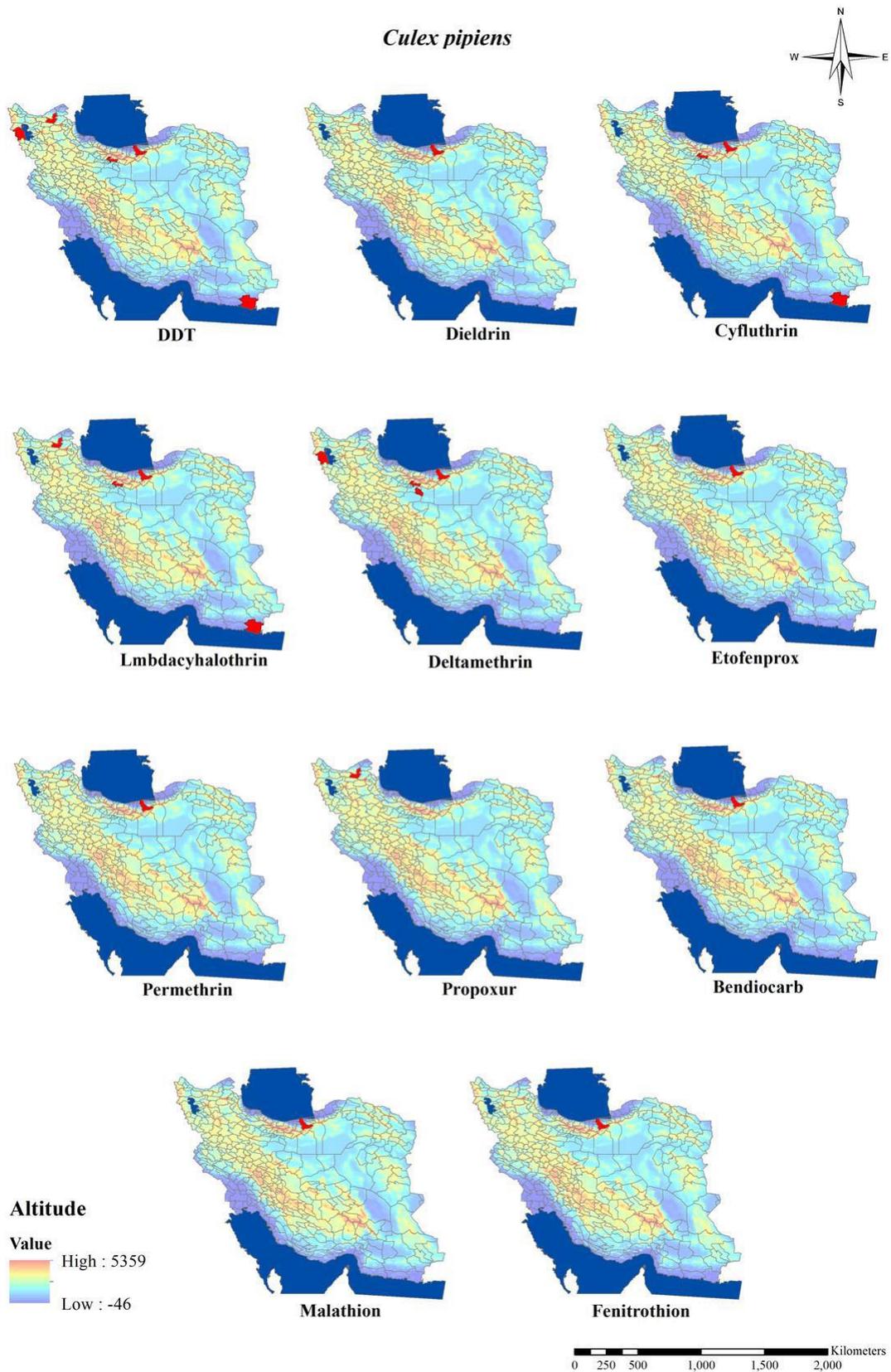
**Fig. 7.** Distribution of *Anopheles maculipennis* with resistance to different insecticides in Iran (2000–2020)

**Table 10.** Resistance status of *Culiseta longiareolata* to different groups of insecticides in Iran using WHO insecticide susceptibility tests (2000–2020)

Species	Insecticides	Location (Province-District)	References
<i>Cs. longiareolata</i>	DDT	East Azarbaijan-Ahar	14
	Propoxur	East Azarbaijan-Marand	45
		East Azarbaijan-Ahar	14
	Lambdacyhalothrine	East Azarbaijan-Marand	45
	Deltamethrin	East Azarbaijan-Ahar	14
Fenitrothion	East Azarbaijan-Marand	45	



**Fig. 8.** Distribution of *Culex quinquefasciatus* with resistance to different insecticides in Iran (2000–2020)



**Fig. 9.** Distribution of *Culex pipiens* with resistance to different insecticides in Iran (2000–2020)

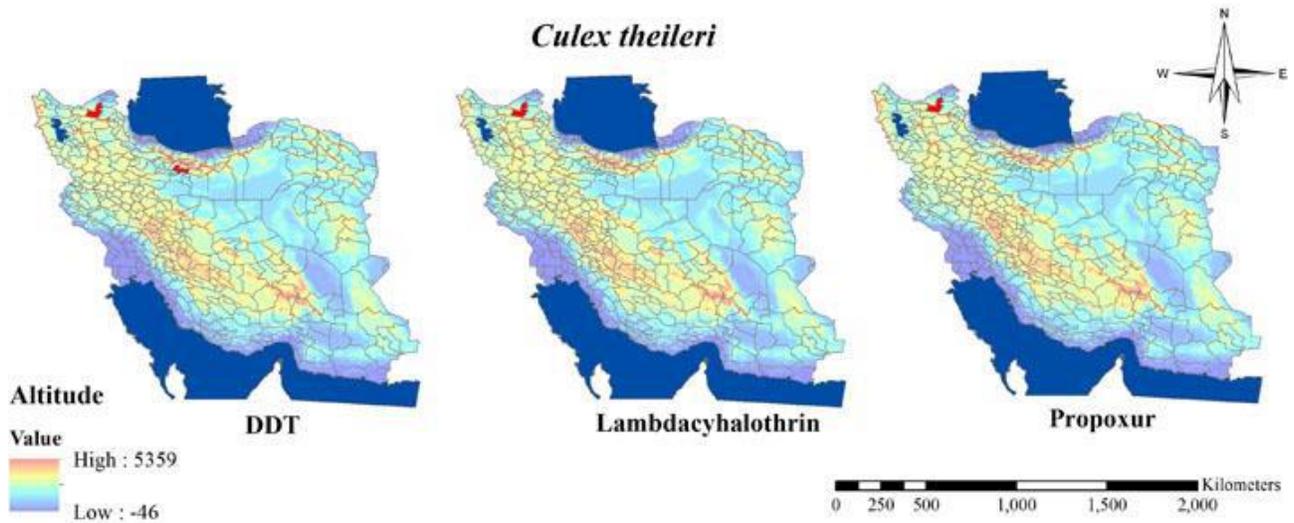


Fig. 10. Distribution of *Culex theileri* with resistance to different insecticides in Iran (2000–2020)

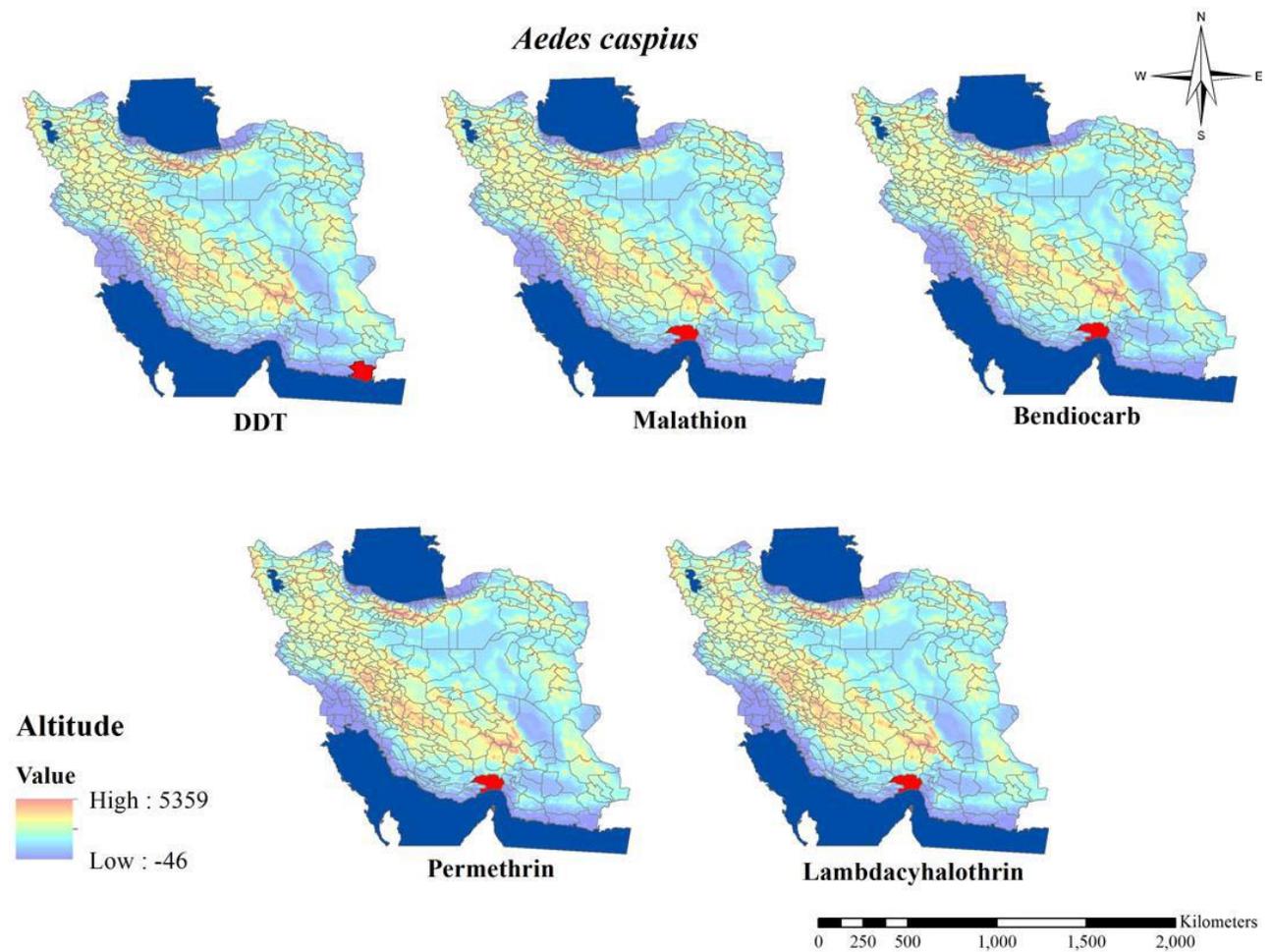
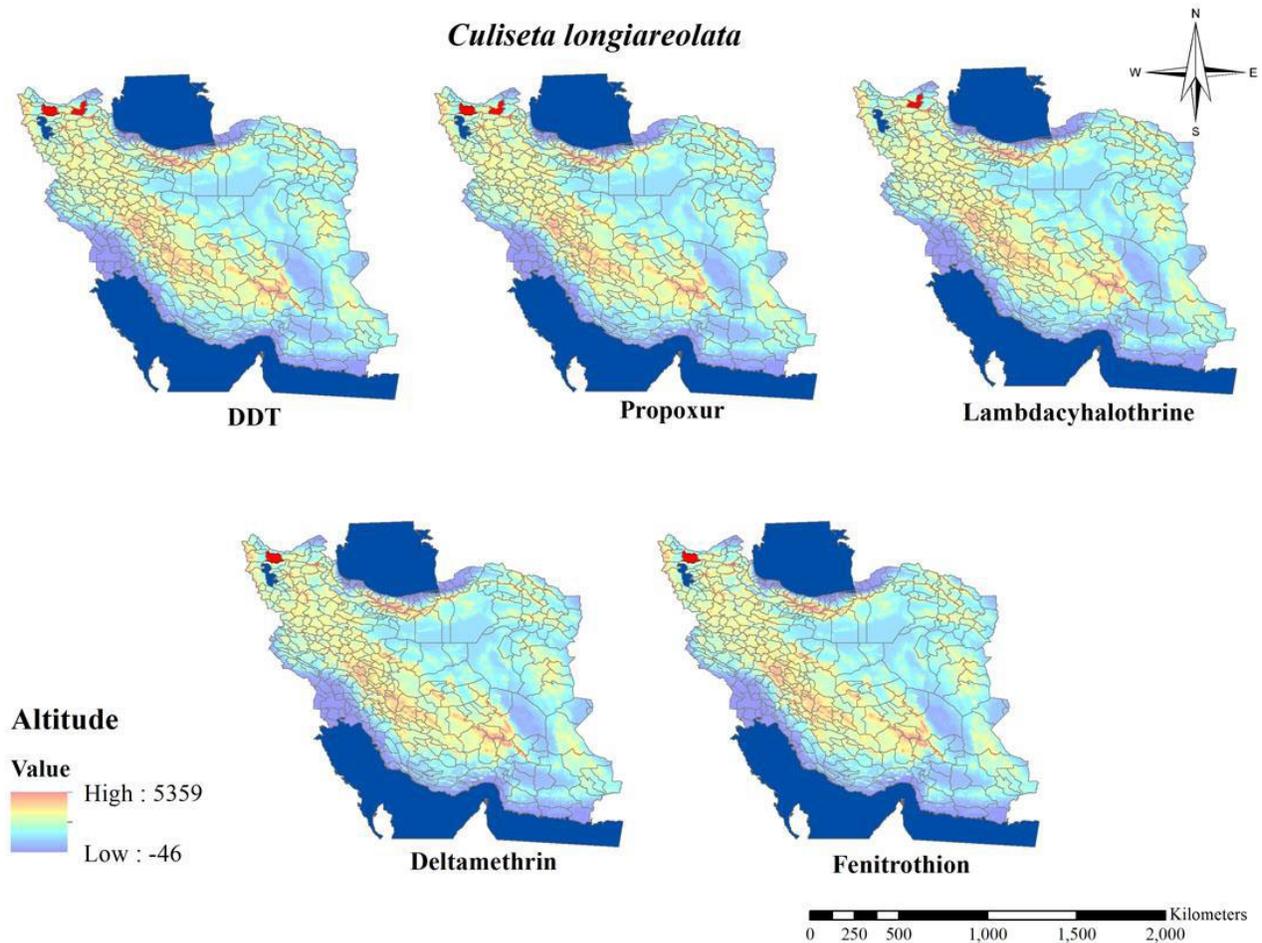


Fig. 11. Distribution of *Aedes caspius* with resistance to different insecticides in Iran (2000–2020)



**Fig. 12.** Distribution of *Culiseta longiareolata* with resistance to different insecticides in Iran (2000–2020)

## Discussion

The trend of insecticides resistance in medically important mosquitos in Iran revealed that among specimens of *Anopheles* mosquitoes as malaria vectors, approximately all of them have been found resistant to one or more insecticides and also most malaria vectors are resistant to the organochlorine compounds in Iran. It cannot conclude certainly that all the malaria vectors are resistant to insecticide because there are no definite report about resistance to insecticides in *An. fluviatilis* s.l. and *An. dthali* although there are some reports about tolerant in *An. dthali* to some insecticides. In Iran, *An. stephensi* as the main malaria vector has been found resistant to most classes of insecticides during recent years and this resistance may be

caused by others intervention for controlling of malaria vectors such as Indoor Residual Spraying (IRS) and Insecticide Treated Nets (ITNs) or usage of insecticide in the agriculture sector (34). Resistance to all classes of insecticides also reported in other countries in *An. stephensi* for example in Afghanistan, *An. stephensi* has been found resistant to DDT, malathion, bendiocarb, deltamethrin, and permethrin and in Ethiopia, it was highly resistant to deltamethrin, permethrin pirimiphos-methyl, malathion, DDT, propoxur, and bendiocarb (46, 47). Similar finding of *Anopheles* mosquitos, *Culex* specimens also were resistant to several insecticides belong to each class moreover *Cx. pipiens* in the north of Iran, in Mazandaran Province was high

resistance to all tested insecticides of all major classes (41) around the world some studies also reported a high level of resistance in members of *Cx. pipiens* complex to many groups of insecticides (48-50). Based on the literature, there were no reports available on monitoring the susceptibility level of *Ae. caspius* and *Cs. longiaerolata* to insecticides which recommended by WHO around the world. Recent studies about baseline susceptibility of mentioned species in Iran revealed that both of these mentioned species were found to be resistant to all classes of insecticides in the study areas of *Ae. caspius* and *Cs. longiaerolata* (13, 14, 44, 45). During recent years the development of resistance to insecticides in mosquitos as vectors of important diseases in Iran were increased. So that almost all medially important mosquitos were found to be resistant to all different classes of insecticides. The use of alternative insecticide which is made from natural products and some biological agents can be appropriate method for vectors control programs (45, 51, 52). Secondary metabolites of plants such as essential oils are candidates for the discovery of new compounds against vector mosquitos. Insecticide-based plants have the advantage of exhibiting novel modes of action against mosquito vectors that could lessen the risk of resistance (45, 52-61). Moreover, for controlling of mosquito populations, *Wolbachia* as an intracellular organism that infect different groups of arthropods, also introduced as a bioagent due to its environmentally friendly feature (62, 63).

## Conclusion

Relevant studies about resistance to insecticides during the quarter of a century about medially important mosquitos in Iran indicated that the development of resistance to all classes of insecticides in mosquitos is happening gradually, so alternative and efficient interven-

tion methods should be used to preventing the development of resistance to insecticides in mosquitos.

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