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Ecology, monitoring and mapping of insecticide resistance of malaria vector, *Anopheles culicifacies* (Diptera: Culicidae) to different imagicides in Iran

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ABSTRACT

Objective: To work on bioecology and to monitor and map the insecticide resistance of malaria vector, *Anopheles culicifacies* (*An. culicifacies*) (Diptera: Culicidae) in Iran.
Methods: Mosquitoes were collected from different breeding places in Sistan and Baluchistan Province and then reread at insectary. F1 generation was used for susceptibility tests. All the impregnated papers were provided by World Health Organization (WHO) and tests were carried out according to WHO guideline.
Results: Results of adult susceptibility tests against female *An. culicifecies* revealed that this species was resistant to dichloro-diphenyl-trichloroethane, dieldrin, tolerated to bendiocarb, propoxur and deltamethrin and susceptible to other imagicides recommended by WHO. *An. culicifecies* was resistant to organochlorine insecticides and tolerant to organophosphae, carbamate and pyrethroids.
Conclusions: Results of the ecology and susceptibility status of malaria vectors will help authorities to make decision for vector control. More biomedical assays was required to found the mechanisms of insecticide resistance.

1. Introduction

Malaria is one of the most important communicable diseases transmitted by anopheline mosquitoes (Diptera: Culicidae) to humans. In 2013, there were 97 countries and territories with ongoing malaria transmission and 7 countries in the prevention of reintroduction phase, making a total of 104 countries and territories in which malaria is presently considered endemic. World Health Organization (WHO) estimated that 207 million cases of malaria occurred globally in 2012, resulting in 627000 deaths[1]. Malaria is one of the important infectious diseases in Iran with an average of about 15000 annual cases in the last decade, while total recorded cases have dropped to less than 500 in 2013. More than 80% of malaria cases in Iran are reported from three provinces of Sistan and Baluchistan, Hormozgan, and Kerman in southern and southeastern areas. The most routes of malaria cases transmit from Afghanistan and Pakistan to southern and southeastern areas of the country. Over the last 20 years, there has been a dramatic reduction of the malaria burden in Islamic Republic of Iran. While in 1991, nearly 100000 cases were reported and in 2014, only 246 autochthonous cases were reported. In 2009, Islamic Republic of Iran set some objectives of time-bound elimination for its malaria program. There has been excellent progress, but the continued risk of importation of malaria cases from Pakistan poses a huge challenge, politically, socially, operationally and technically, to malaria elimination in Iran. The situation in the next decade will be absolute elimination or a few small short-lived foci emerge from time to time as a result of importation. The latest number of autochthonous cases in the whole country is 42 including 23 local malaria patients, 7 relapsed cases, 12 imported from the other districts by end of July 2016.

Country has a long history of work on malaria including monitoring insecticide resistance. So far, 31 species from 2 subspecies (*Anopheles* and *Cellia*), of which siblings, genotype and type forms are recorded in the country and 17 out of them related to 7 species are in complexes or groups that are introduced as malaria vectors. *Anopheles culicifacies* (*An. culicifacies*) with two siblings (A and B) in Iran, is confirmed as the malaria vector in southeastern part of the country. Also there are several scientific works on different aspects of malaria including sibling species, molecular

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study, new record, novel methods for vector control, faunistic study, use of plants for larval control, use of bed nets and long lasting impregnated nets, morphological studies, malaria epidemiology[2-10].

Different studies have been conducted during more than 90 years on malaria and its vectors in Iran. The last checklist of Iranian mosquitoes shows 31 *Anopheles* species including sibling, biological forms and genotypes and 17 out of them are reported to be included in malaria transmission. These vectors are considered as sibling, genotype and type forms. *Anopheles stephensi*, *An. culicifacies*, *Anopheles fluviatilis* and *Anopheles dthali* are the main vector species of south-eastern foci, while *Anopheles sacharovi* and *Anopheles maculipennis* are included in malaria transmission in northwest foci, and *Anopheles superpictus* has wide distribution in all malaria foci of the country. Seasonal activity of *Anopheline* mosquitoes varies in different areas due to environmental condition. It shows one peak in northwest especially in summer, however, there are two peaks of activity in coastal warm and humid region in the southern part of Iran with oriental epidemiological characteristics[11-15].

Campaign against malaria vectors was started from 1952 by spraying dichloro-diphenyl-trichloroethane (DDT) and then replaced by dieldrin, malathion, propoxur, lambda-cyhalothrin 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 conducted by *Bacillus thuringiensis* as larvicide. The status of insecticide resistance of *An. culicifacies* is different with other imagicides including 4% DDT, 0.4% diledrin, 5% malathion, 1% fenitrothion, 0.1% bendiocarb, 0.1% propoxur, 0.1% and 0.05% lambda-cyhalothrin, 0.025% and 0.05% deltamethrin, 0.25% and 0.75% permethrin, 0.15% cyfluthrin and 0.5% etofenprox. Knowledge on insecticide resistance of target species is a basic requirement to guide insecticide use in malaria control programs in local and global scales. The main criteria for susceptibility status, which are recommended by WHO, are considered. The results showed that *An. culicifacies* is resistant to DDT and dieldrin and tolerant to some tested insecticides.

The main governmental use of insecticide in the health sector is their application for adult mosquito control. The campaign against malaria vectors started with organochlorines (DDT, dieldrin and benzene hexachloride) 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 lambda-cyhalothrin and deltamethrin. Temephos, reldan and pirimiphos-methyl were used for larviciding[15-20].

2. Materials and methods

2.1. Larval collection activities

Field visits were carried out to collect *Anopheles* larvae. Collected larvae were transferred to the insectary, to rear larvae into F1 generation for subsequent tests.

2.2. WHO susceptibility test kit

The WHO susceptibility tests kits were provided by Ministry of Health and Medical Education, Iran. Tests were conducted by WHO insecticide-impregnated papers at the appropriate discriminating concentrations.

Insecticides and concentrations were presented as 4% DDT, 0.04% dieldrin, 1% fenitrothion, 5% malathion, 0.1% bendiocarb, 0.05% deltamethrin, 0.75% permethrin and 0.05% lambda-cyhalothrin.

2.3. Test method

For testing each insecticide, a minimum of 150 female mosquitoes 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). 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 and the WHO recommendation for interpretation of susceptibility test results were considered as follows: susceptible, if mortality was in the range of 98%–100%; resistant, if mortality of test sample was less than 80% and suggestive of resistance, verification required, if the observed mortality is between 80% and 97%.

3. Results

Figure 1 shows the distribution of *An. culicifacies* in Iran. *An. culicifacies* mosquitoes was susceptible against 4% DDT, 0.4% dieldrin, 5% malathion, 1% fenitrothion, 0.1% bendiocarb, 0.1% propoxur, 0.05% and 0.1% lambda-cyhalothrin, 0.025% and 0.05% deltamethrin, 0.15% cyfluthrin and 0.5% etofenprox. According to the criteria of WHO, this species was resistant to organochlorine insecticides and tolerant to carbamates insecticides and susceptible to other imagicides.

From 2013, the guideline for susceptibility test was modified, 90% mortality indicating the resistance, so that the results of previous years indicated the susceptible strains resistant to insecticides.

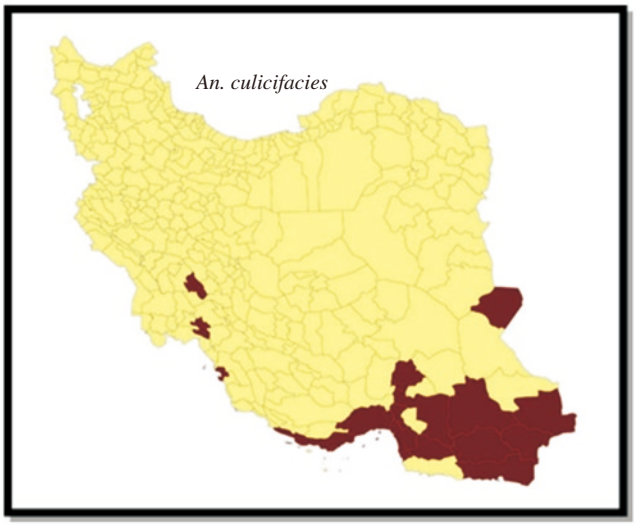

Figure 1. Distribution map of *An. culicifacies* in Iran (brown color).

4. Discussion

There are several works on different aspects of malaria vectors including ecology of malaria vectors, biodiversity, community participation, vector control, repellent evaluation and anthropophilic index of malaria vectors and training is designated as malaria training center by WHO[21-30]. *An. culicifacies* as malaria vector is widely distributed in the Asia and Indian subcontinent. Although it is predominantly zoophilic in many areas, it is abundant even altitudes up to 3000 m and is an important vector of malaria throughout its distribution. *An. culicifacies* occurs mainly in Sistan and Baluchistan Province, Kerman Province and Hormozgan Province. This species was implicated as a vector of malaria in Iran during an epidemic. Among the secondary vectors, *An. culicifacies* is considered as a potential vector, since its role in malaria transmission has been reported from Sistan and Baluchistan Province in southeast of Iran, bordering Afghanistan and Pakistan. This species is largely responsible for the epidemic of malaria in this province. There are important differences in the bionomics of *An. culicifacies* and other species in different regions, including differences in seasonal abundance, diurnal activity and man-biting behavior[31-40].

Behavioral resistance appears more rapidly in endophilic species than exophilic one. *An. culicifacies* has more exophilic habit. This species is resistant to DDT, diledrin, tolerant to bendiocarb, propoxur and deltamethrin and susceptible to other WHO-recommended imagicides.

There is widespread resistance to organochlorines in the country and widespread tolerant to organophosphate, carbamates and pyrethroids. The results could be a clue for controlling malaria vector in the country.

Conflict of interest statement

We declare that we have no conflict of interest.

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