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RESEARCH ARTICLE

MONITORING AND MAPPING OF INSECTICIDE RESISTANCE ON MALARIA VECTOR, *ANOPHELES FLUVIATILIS* (DIPTERA: CULICIDAE) IN A MALARIOUS AREA OF IRAN

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ABSTRACT

Malaria continues to be a main vector-borne public health problem in Iran. The endemic foci of malaria are mainly located in south-eastern part of the country. Iran is now launching the elimination of malaria. Susceptibility of malaria vectors to insecticide is essential. Mosquitoes were collected from different breeding places in Hormozghan province and reread at insectary and F1 generation was used for susceptibility tests. All the impregnated paper provide by WHO and tests was carried out according to WHO guideline. Results of adult susceptibility tests against females of *An.fluviatilis* revealed that, this species is susceptible to all WHO-recommended imagicides. Results of the evaluating will help for decision making of authorities for vector control.

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INTRODUCTION

There are some important arthropod-borne diseases in Iran including: Malaria , Cutaneous leishmaniasis, Visceral leishmaniasis, Crimean-Congo hemorrhagic fever, Tick relapsing fever, Furthermore scorpions are one of the risk factors for life in some parts, while other arthropod-related diseases such as myiasis exist more or less across the country. Some probable Arthropod-borne disease in the future may be: Q-fever, Papatasi fever, Tularemia, Rift valley fever, Dengue fever, Yellow fever, West Nile viruses, Lactrodictism (spider bite), Plague, scabies, Nuisance insects of horseflies and Culicidae mosquitoes, Cockroach-borne diseases; damages by fire ants, blister beetles and bee stings. Also there is potential of diseases transmitted by black flies, rove beetles, lice and bedbugs. Estimating the burden of diseases according to the future climate change will provide a clue for decision makers for appropriate disease control. Malaria is one of the most important communicable diseases transmitted by anopheline mosquitoes (Diptera: Culicidae) to humans.

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In 2013, there are 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. Based on WHO estimate, 207 million cases of malaria occurred globally in 2012 resulted to 627 000 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 has dropped to less than 500 locally transmitted cases in 2013. More than 80% of malaria cases in Iran are reported from three provinces of Sistan and Baluchistan, Hormozghan, and Kerman in southern and southeastern areas of the country. The most routes of malaria cases are immigration from Afghanistan and Pakistan to southern and southeastern areas of the country (Ministry of Health, annual reports). Over the last 20 years there has been a dramatic reduction of the malaria burden in I.R. Iran. While in 1991, nearly 100,000 cases were reported, in 2014, only 246 autochthonous cases were reported. All observations indicate that the data reflect the real situation and that the overwhelming majority of cases, which occur, are included in the national system, although there is room for improvement in the surveillance system. The spectacular progress can be

ascribed to effective implementation of appropriate curative and preventive control interventions through a strong health care infrastructure. Social and economic development allowing better housing, use of air-conditioning etc. has also played a role. Locally transmitted cases are now concentrated in the south-eastern part of the country, which are affected by extensive population movement across the border with Pakistan, where malaria control faces serious difficulties. In 2009, I.R. Iran set time-bound elimination objectives for its malaria program. There has been excellent progress since, 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 one where 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 insecticide resistance monitoring, So far 31 species from 2 subspecies (*Anopheles* and *Cellia*), siblings, genotype and type forms are recorded in the country, 17 out of them related to 7 species, are in complexes or groups that introduced as malaria vectors. *An. culicifacies* with two siblings: A and B in Iran, is confirmed as the malaria vector in southeastern part of the country (2-12), sibling species, molecular study, new record (13-20), novel methods for vector control (21-26), faunestic study (27, 28), use of plants for larval control (29-41) using bednets and long lasting impregnated nets (42-48), morphological studies (49-51), malaria epidemiology (52-55), ecology of malaria vectors (56-64), biodiversity (65, 66), community participation (45, 54), vector control (67), repellent evaluation (68), anthropophilic index of malaria vectors (65, 69), training (70) is designated as malaria training center by WHO. There are several reports on different aspects of malaria vectors recently (71-96).

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, 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, 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, and *An. superpictus* has wide distribution in all malaria foci of the country. Seasonal activity of *Anopheline* mosquitoes varies in different area 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. 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. Knowledge on insecticide resistance in 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, were considered. Agriculture in Iran remains highly sensitive to climate developments; the country's most important crops are wheat, rice and other grains, sugar beet, fruits, nuts, cotton, and tobacco, which require the use of insecticides. So far different groups of insecticides are using for crops protection in the country. 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 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.

MATERIALS AND METHODS

Larval collection activities

Field visits were carried out to collect *Anopheles* larvae from). Collected larvae were transferred to the insectary of Bandar Abbas Research Station, School of Public Health, to rear larvae into F1 generation for subsequent tests.

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: DDT 4%; Dieldrin 0.04%; Fenitrothion 1%; Malathion 5%; Prppoxur 0.1%, Bendiocarb 0.1%; Deltamethrin 0.05%; Permethrin 0.75%; Lambda-cyhalothrin 0.05%, Cyfluthrin 0.15%, Etofenprox 0.5%.

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. 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: The WHO recommendation for interpretation of susceptibility test results were considered as follows:Susceptible - if mortality is in the range of 98 – 100%; Resistant - if mortality of test sample is less than 80%; Suggestive of resistance, verification required – if the observed mortality is between 80% and 97%.

RESULTS AND DISCUSSION

Anopheles fluviatilis is distributed on the southern slopes of the Zagros chain, from southwest of Kermanshah to Baluchistan in the southeastern part of Iran (97-98).

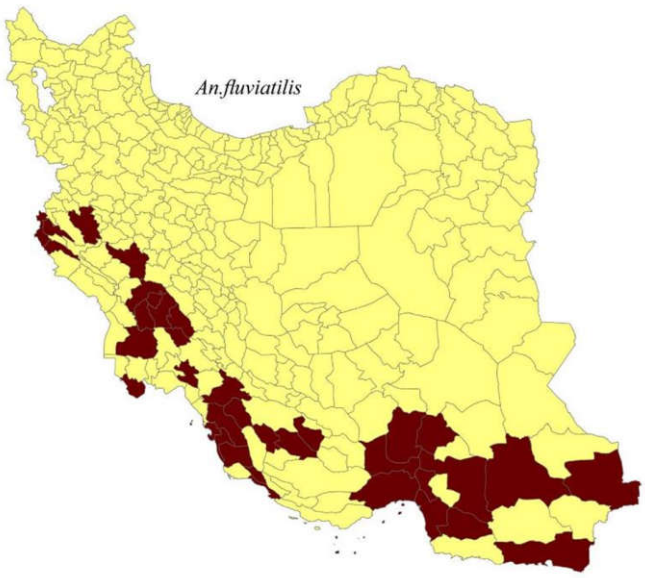

Fig. 1. Distribution map of *Anopheles fluviatilis* in Iran

Table 1. Status of insecticide resistance in *An.fluvitilis*

Insecticide	Resistant status
DDT 4%	S
Dieldrin 0.4-4%	S
Malathion 5%	S
Fenitrothion 1%	S
Propoxur 0.1%	S
Bendiocarb 0.1%	S
Permethrin 0.25-0.75%	S
Lambdacyhalothrin 0.025-0.1%	S
Deltamethrin 0.025-0.05%	S
Cyfluthrin 0.15%	S
Etofenprox 0.5%	S

S = Susceptible, T= Tolerant, R = Resistant

It is considered a secondary vector of malaria in most of its distribution area, and found at altitude from 50 meter to 1100 meter. Naddaf *et al*. (2003) (19) had captured this species from outdoor habitats and on animal baits. The low prevalence of *An. fluviatilis* might be attributed to the specific conditions required for breeding places (Fig1). It is reported the presence of species T in Iran based on rDNA ITS2 sequence. *Anopheles fluviatilis s.l.* is generally considered to be a major vector in southern areas of Iran, and a secondary vector in the southeastern corner of the country (99-100). Studies in other countries showed that *An. fluviatilis* is resistant to DDT in Afghanistan, India, Nepal and Pakistan. Also in Pakistan and Saudi Arabia it is reported resistant to dieldrin (101). A study on insecticides resistance in India showed that *An. fluviatilis* has developed resistance to HCH and is susceptible to DDT (102). There is no indication of resistance/tolerance in this species in Iran.

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