Original Article

Resistance Status of *Anopheles maculipennis* and *Anopheles superpictus* to the Conventional Insecticides in Northeastern Caspian Littoral, Iran

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(Received 22 Mar 2020; accepted 23 May 2021)

Abstract

Background: Malaria resurgence has occurred in the northern half parts of Iran. The resurgence of malaria in the prone area could arise from various factors, e.g. wide use of pesticides in the agriculture sector and factors such as habitual patterns of movement of local people from problematic southeastern foci in Iran toward the Caspian Littoral. There are no new data on the resistance status of main malaria vectors in the Caspian Littoral, and this study was aimed at renewal data on conventional insecticides.

Methods: The field strain of adult *Anopheles superpictus* and *Anopheles maculipennis* were collected using the hand catch method and transferred to the laboratory. The susceptibility tests were carried out against DDT 4%, Malathion 5%, Permethrin 0.75%, Deltamethrin 0.05%, and Lambda-cyhalothrin 0.05%, followed by the WHO's procedure.

Results: The primary malaria vector in Caspian Littoral is *An. maculipennis*, revealed to be still resistant to DDT and mortality rate, LT_{50} and LT_{90} of female mosquitoes were 75.0%, 54.2, minutes and 111.3 minutes. The under 'verification required' status of *An. maculipennis* was also revealed to Lambda-cyhalothrin based on recent WHO's criteria. The malaria vector *An. superpictus* is also considered the second malaria vectors in the west parts of the studied area, which showed to be susceptible to all insecticides tested.

Conclusion: DDT resistance is persisted in *An. maculipennis* despite stopping residual spraying with DDT since 1978 in the Caspian Littoral, but the occurrence of pyrethroid under 'verification required' status is a progressive threat to the possible development of cross-resistance in the future.

Keywords: *Anopheles maculipennis*; *Anopheles superpictus*; Insecticide resistance; Malaria; Caspian Littoral

Introduction

Malaria is one of the most important vector-borne diseases globally, especially in developing countries, and Iran is located in the Eastern Mediterranean Region with lower malaria endemicity. The country's southeastern parts, including the provinces of Sistan- Baluchistan, Hormozgan, and southern Kerman are characterized by "refractory malaria". Later on up to the year 1944, malaria epidemiology was studied by some Iranian and overseas investigators, and it was found the hypo-endemic situation at some littoral parts of the Caspian Sea in North of Iran (1). In the past years, studies were carried out in the Golestan Province from 1949 to 1957 and, the spleen index was measured in 21 villages. The classical malariometric measure causing splenic enlargement rate was estimated at 52.1% in the Bandar-e-

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Published Online: June 30, 2021

Gaz and 32.5% in the Gorgan area during 1949–1959. However, the annual parasite index was reported as 5.7% in the Gorgan area, northern Iran. The prevalence of malaria was stated as 100 per 10,000 populations in 1949 to 8 per 10000 populations in 1959, and the mortality decreased from 40% to 2%. Malaria cases were recorded 164 in the Gorgan, 50 in the Bandar-e-Shah, 57 in the Komish-Tapeh, 52 in the Gonbad-e-Kavous, 44 in the Haji-Lar, and 103 in the Gaz among the age group 2–12 years old in 1935. The percentage of malaria parasites was 50.0% *Plasmodium malariae*, 46.1% *Plasmodium vivax*, and 3.9% *Plasmodium falciparum* (2). Seven Anopheline mosquitoes, including *Anopheles stephensi*, *Anopheles culicifacies*, *Anopheles fluviatilis*, *An. superpictus*, *Anopheles sacharovi*, *An. maculipennis* complex and *Anopheles dthali* are involved in the transmission of malaria in Iran (3-5). A total of three species of malaria vectors was reported in North of Iran, and *An. maculipennis* was introduced as the primary vector (3) and *An. superpictus* as a secondary vector (6-7) and *Anopheles sacharovi* are also considered a malaria vector in the northwestern parts of Iran (8). Malaria was considered a significant health problem from 1941 to 1948, so that no other disease has caused such irreparable financial and human losses in the country. The disease has become more common in the populated areas of the Caspian territory, due to the presence of a favorite climate for the development of *Anopheles* mosquitoes. Control strategies were using the residual spraying of indoor places with DDT, larval control with oil derivation at different habitats, and treating the patients with quinine. Malaria eradication program (MEP) was started in 1957 in Iran and from 1957–1971 caused interruption of transmission in the North of Iran (9). Due to prone condition of study area, and favorite climate for malaria vectors, routine movement of local people from the southeastern parts to northeastern of the Caspian area as well as the wide application of pesticides in the agricultural sector, this study aimed to determine the susceptibility level of two *Anopheles* species to the conventional insecticides in the Kalaleh district, northeastern of the Caspian territory.

Materials and Methods

Study area

The study was conducted in the Kalaleh district (37° 22' N, 55° 29' E), Golestan Province, from April to October 2016. This province was split off from Mazandaran Province in 1998. The province is bounded by the Caspian Sea and the Mazandaran Province in the west, the Semnan Province in the south, the North Khorasan Province in the East, and a borderline with Turkmenistan in the North (Fig. 1). This study was carried out in three fixed villages and five randomly selected one in Kalaleh District. Most parts of the Golestan Province are plain, and more than 2/3 of the plains have arid and semiarid climates, and 1/3 of the others have a temperate climate. The district area is 1985km^2 with 117660 population located in the northeast parts of the Golestan Province. The main agricultural products are alfalfa, rice, watermelon, and cotton. Maximum and minimum temperatures were recorded as 40.8 and -0.2 °C, respectively, and the mean annual relative humidity was recorded as 74.0 %. The total annual rainfall was 772mm, the minimum precipitation in August and maximum in February. The sampling of mosquitoes carried out in 3 villages of Kalaleh District, including Aziz-Abad (37°32'45"N, 61°41'52"E), Gharanki-Jangal (37°34'31"N, 61°46'43"E) and Gorgandoz (37°31'24"N, 61°43'10"E) with the mean elevation of 65 meters above sea level.

Mosquito collection

The sampling plan for collecting of adult mosquitoes was carried out from April to February 2016. The fresh-fed mosquitoes were dominant compared to unfed and gravid physiologic conditions, so only the fresh-fed mosquitoes were used in order to the homogeneity of test

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Published Online: June 30, 2021

data. The indoor-resting mosquitoes were collected by mouth aspirator before sunrise, transferred into the wooden cages, and transported in a cool condition to the laboratory in the Health Center of Kalaleh District, Golestan Province, Northeast of Iran.

Susceptibility Test

Susceptibility levels of field-collected mosquitoes to insecticides were determined by exposing freshly fed females to the diagnostic doses of insecticide-impregnated papers supplied by WHO, i.e. DDT 4%, Malathion 5%, Permethrin 0.75%, Deltamethrin 0.05%, and Lambda-cyhalothrin 0.05%. The exposure time for all the insecticides tested was 60min, followed by a 24h recovery period. To calculate the LT_{50} for DDT, the logarithmic exposure times ranged 15, 30, 60, and 120 minutes, followed by 24h holding period were carried on. Each logarithmic exposure time was replicated four times using 25 female field-caught mosquitoes. The susceptibility exposure tubes were held in the vertical position during testings with pyrethroids, organochlorine and organophosphate insecticides (12). The recovery period of exposed mosquitoes was kept in a room with a temperature of 25±2 °C. Simultaneously, the control group also was exposed 60 minutes to untreated papers. After exposure, the mosquitoes spent the recovery period at 25±2 °C and 70–80% relative humidity with access to soaked cotton pads in 10% sucrose solution for 24h until scoring the mortality. If control mortality was within 5–20%, test mortality was corrected by Abbott's formula. The mortality rate was ranked as the susceptible, under 'verification required' status, and resistant, based on WHO's criteria e.g., 98–100%, 90–97%, and below 90%, respectively (10-11).

Results

The dominant species were, *An. superpictus* and *An. maculipennis* that tested for resistance/susceptibility level to DDT, malathion, deltamethrin, permethrin, and lambda-cyhalothrin (Table 1). The response of 100 mosquitoes of *An. maculipennis* to DDT 4.0% for 1h, followed by a 24h recovery period resulted in the survival of 25 mosquitoes, and the mortality was 75.0%. The regression parameters of DDT time-response, including intercept (a), slope ± standard error (b±SE), heterogeneity of mortality data with the degree of freedom (χ^2(df)), LT_{50} ±95% confidence interval (CI), and LT_{90} ± 95% CI were calculated (Table 2). The LT_{50} and LT_{90} values for *An. maculipennis* were 54.2 and 111.3 minutes, respectively. The regression line and the equation was shown in Fig. 2. The susceptibility level of *An. superpictus* to the tested insecticides is summarized in Table 1, which showed complete susceptible to all tested insecticides.

Table 1. Susceptibility levels of dominant species of *Anopheles* at the diagnostic doses to different insecticides using WHO-recommended method (10), northeastern parts of the Caspian Littoral, Iran

Insecticide	*Anopheles superpictus*				*Anopheles maculipennis*			
	Total mosquito tested	No. dead	Mortality rate (%)	Resistance status*	Total mosquito tested	No. dead	Mortality rate (%)	Resistance status*
DDT 4%	100	99	99.0	S	100	75	75.0	R
Malathion 5%	100	100	100.0	S	100	100	100.0	S
Deltamethrin 0.05%	100	98	98.0	S	100	98	98.0	S
Permethrin 0.75%	100	100	100.0	S	100	100	100.0	S
Lambda-cyhalothrin 0.05%	100	100	100.0	S	100	96	95.5	V
Control	100	0	0.0	-	100	0	0.0	-

*S=Susceptible; V=under 'verification required' status; R=Resistant

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Table 2. Regression analysis of bioassay data of *Anopheles maculipennis* exposed to DDT 4.0% using WHO-recommended method, northeastern parts of the Caspian Littoral, Iran

a	b±SE	LT_{50} (min)±95%CL	LT_{90} (min)±95%CL	χ^2 (heterogeneity)	χ^2 Table (df)	p
-7.1132	4.1014±0.337	49.4392 **54.2415** 59.6185	97.2503 **111.3806** 132.7855	24.685 *	5.991 (2)	0.05

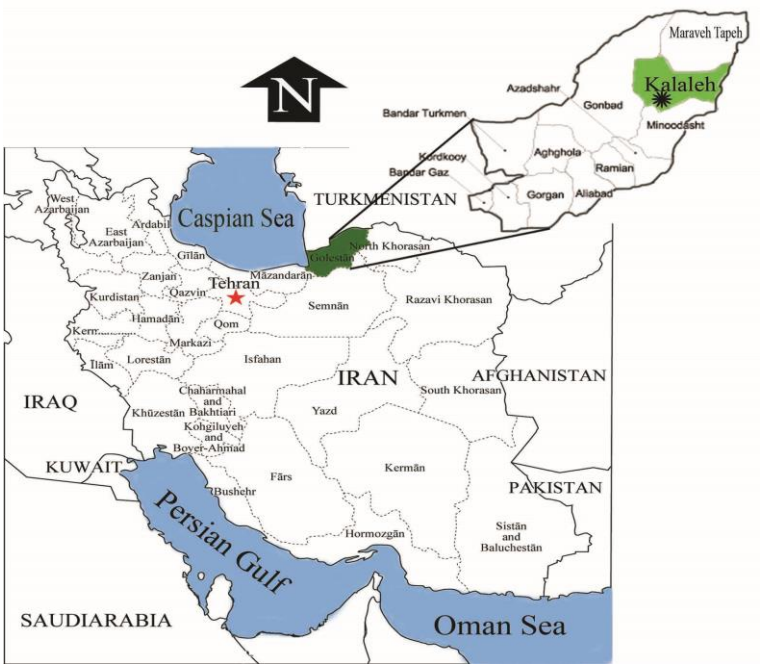

Fig. 1. Map of study area showing Kalaleh District, Golestan Province, northeast of Iran where two main species of Anopheline were collected

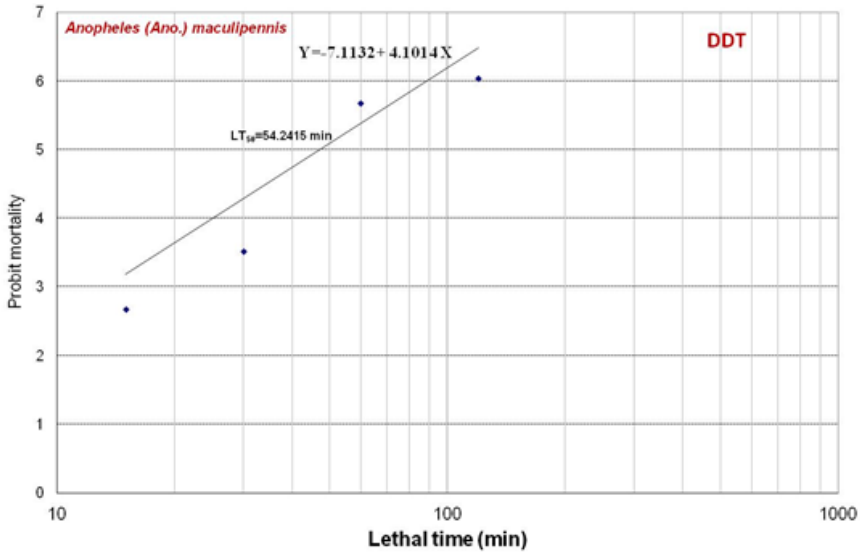

Fig. 2. Regression parameters estimating the lethal time of *Anopheles maculipennis* exposed to DDT 4.0%, northeastern parts of the Caspian Littoral, Iran

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Discussion

Currently, malaria is regarded as an infectious disease causes financial losses and workforce health. It is still concerned with health authorizes at the Sistan and Baluchistan, Hormozgan, and southern Kerman provinces (12). With attention to development achieved during five decades of vector control programs and the reduction of prevalence, malaria elimination is in the joint approach of the Iranian Ministry of Health and the World Health Organization (12). Due to the risk of malaria reemergence in northeastern parts of the Caspian Littoral, which is caused by numerous climatic, environmental, and social factors, determining of susceptibility level of *Anopheles* vectors was noticed. The Maculipennis complex comprised 12 Palearctic members that distributed in different provinces including West Azarbaijan, East Azarbaijan, Ardabil, Guilan, Mazandaran, Golestan, Isfahan, Fars, Kohgiluyeh and Boyer-Ahmad, Kermanshah, Kurdistan, Zanjan, Tehran and Khuzistan but the exact distribution of each member of Maculipennis complex as well as its bioecology is not clear (3). The Resistance ratio (RR) which calculated by dividing the LC_{50} of the resistant population by the LC_{50} of the susceptible strain had been calculated for *An. maculipennis* in different localities of Iran during 1970–1977, e.g., central parts (Isfahan Province, RR= 64.2 min), the northwestern parts adjusting to borderlines of Republic Azarbaijan and Armenia (Ardabil Province, RR= 57.5min, the East Azarbaijan Province, RR= 58.4min), Caspian littoral (Guilan Province, RR= 77.1min; Mazandaran Province, RR= 58.1min; Golestan Province, RR= 63.7min) and northeastern parts (Razavi Khorasan, RR= 74.6min) (13-14). During the malaria resurgence at the Caspian littoral in 2008, it was shown that *An. maculipennis* (strain Astara, Guilan Province, Caspian littoral) exhibited low resistance (84.0%) to DDT, whereas susceptible to Malathion, Lambda-cyhalothrin, and Deltamethrin during 1998–1999 (15). A similar study conducted at different villages of the Mazandaran Province, in the Caspian plateau during 1988–1989 and the susceptibility level of *An. maculipennis* was determined against DDT 4% after 60min of exposure time using WHO's method. The results showed the resistance of *An. maculipennis* to DDT ranged 72.5–94.4%, which followed 93.9% mortality after 120min exposure. The latter species was susceptible to Dieldrin 4% and Malathion 5.0% but surprisingly showed under 'verification required' status to Deltamethrin 0.025 % with a mean of mortality of 96.5% (16). Also, the susceptibility level of *An. maculipennis* to DDT 4.0% was also determined in the Guilan Province, in the west of the Caspian littoral during 1987 with the mortality rate of 87.5–91.7%, 90.5–94.3%, and 96.1–97.1% after 120, 150, and 180min exposure time which indicated a high resistance level of *An. maculipennis* to DDT 4% in Guilan Province (16). Another study conducted on the susceptibility level of *Anopheles messeae* against DDT 4% using the WHO's method at 60min in Sari, Amol, and Tonekabon districts, Mazandaran Province, Caspian littoral during 1989–1990. The results also revealed a high resistance level of *An. messeae* to DDT ranged 8.9–61.2%, with a mean of 40.1%. The latter species was reported as susceptible to Dieldrin 4% and Malathion 5.0% (17). During a recent trial in the northwestern part of Iran, it was indicated that *An. maculipennis* (strain West Azarbaijan, the borderline of Turkey) displayed high resistance (50.0%) against Malathion and under 'verification required' status to Permethrin and Deltamethrin (18). In neighboring countries of Iran, susceptibility tests on *An. maculipennis* were carried out since 1974 in Turkey, revealing resistance to organophosphate insecticides (19). The resistance of *Anopheles artemievi*, one member of the Maculipennis complex, was established to DDT (26.7%) at different parts of Uzbekistan. The variations in susceptibility level of

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Published Online: June 30, 2021

An. maculipennis were shown related to seasonal change and mosquito collection months (20-21). The resistance of five strains of *An. maculipennis* was confirmed to DDT, Malathion, Permethrin, and Deltamethrin in Turkey (20, 22). During this study, it was revealed that *An. superpictus* still remained susceptible to all tested insecticides from different groups. A similar situation of the susceptibility of *An. superpictus* was shown in different parts of Iran during 1971–74 (23) and then in Ilam Province, west of Iran during 2000 (24), whereas a record of resistance (56.0%) of *An. superpictus* was recently recorded in the Sistan and Baluchistan Province, southeastern Iran (25). In the piedmont and mountainous districts of Uzbekistan, *An. superpictus* was also highly sensitive to the insecticides, while the diapausing female *An. superpictus* mosquitoes in the population were found to be resistant to DDT (82.8%) and highly resistant to Malathion (43.8%) (26). More than a half-century has passed since the newer investigations revealed the *An. superpictus* still remained susceptible to DDT, Malathion, and pyrethroids (27). In Tajikistan, *An. superpictus* was proved to be exophile and completely susceptible to the Malathion, but with a low DDT resistance (28). A different pattern of susceptibility was shown a low resistance (85.0%) to the Deltamethrin, but susceptible to the DDT, Malathion, and Permethrin between the field population of *An. superpictus* collected from the Badakhshan Province, Afghanistan (29). The adult *An. superpictus* that collected from the Jordan in the Middle East showed a transit susceptibility (96.0%) to the Deltamethrin, whereas completely susceptible to the Lambda-cyhalothrin (30). The study's results and comparison of the past and present data in different countries indicated a serious alert status for pesticide management both in health and agriculture arthropod control.

Conclusion

Susceptibility level of *An. maculipennis* to DDT remained with the least change in the eastern part of Caspian littoral despite withdrawal of indoor spraying with DDT since 1971, but under 'verification required' status to pyrethroids could be considered a threat to the possible development of resistance in the future. The results of the tests on Malathion, Deltamethrin, Permethrin revealed susceptibility to both *An. maculipennis* and *An. superpictus* to these insecticides.

Acknowledgments

This study was supported by the Research Deputy of Golestan University of Medical Sciences (GUMS) under research code 35/678. The authors would like to appreciate the kind collaboration of GUMS Deputy and the staff of the Health Center of Kalaleh District. The authors declare that there is no conflict of interest.

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Published Online: June 30, 2021