



*Research Paper*

**ECOLOGY OF MALARIA VECTOR, *Anopheles maculipennis* IN A RESURGENT AREA, BORDERLINE OF IRAN AND REPUBLIC OF AZERBAIJAN**

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**Abstract**

Malaria is an important vector borne diseases worldwide and Iran . A field trial study was carried out on the ecology of *Anopheles maculipennis*. Different methods of adult collection including pyrethrum space spray , human and animal bait collections were used. Monitoring of susceptibility tests against different insecticides followed by WHO guideline. Pyrethrum space spray collection captured the highest number of *An.maculipennis* female (93%) as compared to human and animal bait collections (7%). This species was active throughout the night with two peaks of blood meal. Age determination by ovary dissection indicated long longevity and high potency of transmission. From different methods of adult collection and ovary dissection it should be concluded that at least 5.6% of the population could reach to the dangerous age, in relation to malaria transmission. The gonotrophic cycle, survival rate, life expectancy of this species was 4, 0.82, 5 days; respectively. Vectorial capacity was measured as 0.028. The mortality of *An. maculipennis* larvae exposed to WHO-recommended diagnostic doses of fenitrothion 0.125 mg/l, temphos 0.25 mg/l, malathion 3.125 mg/l and chloropyrifos 0.025 mg/l showed that larvae of this species exhibits susceptible to malathin and fenitrothion while resistant to the next two larvicides.

Key words: Ecology, *Anopheles maculipennis*, Gillan, Iran.

**INTRODUCTION**

Malaria is prevalent in about 100 countries and more than 2400 million of population of the world are at risk of the disease. The annual worldwide incidence is estimated 300-400 million and the deaths from malaria, mostly among children under the age of 5 years, are

about 1.1 to 2.7 million each year. The most morbidity and mortality occur in Africa, south of Sahara [1,2] .

In East Mediterranean region 95% of cases are from Afghanistan, Somalia, Sudan and Yemen (WHO, 2000) [2] . At the present in Iran, malaria mostly occurs in the Southeast parts of the country in Hormozgan, Sistan & Baluchistan and south of Kerman (Kahnoudj area), during the years 1991 to 1999, the annual malaria cases varies from 96,340 in 1991 to 22,854 in 1999. The rather high percentage (33% in 1999) of the annual malaria cases in Iran occurs among foreigners, mostly Afghan refugees [3] .

Over 50 years ago it was realized that *A. maculipennis* a common mosquito associated with malaria in Europe, later, careful morphological, biological and hybridization experiments showed that this species was composed of six sibling species, such as *An. maculipennis*, *An. masseae*, *An. atroparvus*, *An. labranchia*, *An. melanon* and *An. sacharovi*. Morphology of species complex or sibling species indistinguishable but reproductively isolated. Azari-Hamidian (2007) [4] and Hanafi-Bojd et al. (2011) [5] mentioned that the *An. maculipennis* Group includes seven species in Iran.

*An. maculipennis* complex has a wide distribution in the world continental Europe, South West Asia to Persian Gulf and Siberia (*Anopheles maculipennis*), Northern palearctic region (*An. messeae*), Holland, largely littoral of Europe (*An. atroparvus*). Italy, Spain, Sardinia, Corsica, Balkans, Caucasian region (*An. melanoon*). Italy, Spain, Corsica, Sardinia, Sicily, Morocco, Algeria, Tunisia (*An. labranchiae*). Italy, Sardinia, Corsica, Greece, Syria, Iraq, Phalestine, Iran, Austria, Cyprus, Yugoslavia, Turkey. Lebanon, Jordan (*An. sacharovi*).

In Iran, occurs mainly in Caspian littoral, north and south slopes of Alborz mountains, Isfahan, Kermanshahan, Azerbaijan, Gillian and Mazandaran, Kordestan, Hamedan, Fars, Khorasan, Semnan, Tehran and Markazi provinces [6] . Slow moving streams, ditches, rice field, pools, marshes, and other types of waters with or without vegetation have been reported as the breeding site. *An. maculipennis* larvae . However, rice fields, marshes, and clean stagnant and slow running water; with or without regulation also have bent reported as breeding places of this species [7] .

Adults of this species were found in human dwellings and animal shelter . This species rest door and outdoor shelters and actively bite man and animal and has been potential as a vector in Europe, incriminated as a vector of malaria in Turkey and Syria, morocco, Algeria

and Tunisia [7]. This species is mostly exophagic and endophagic also Zoophilic [6]. Early-morning pyrethrum space spray catch of indoor-resting mosquitoes in animal and human shelters also human and animal bait collection considered to be the best sampling methods for studying the population of this species *An. maculipennis* has been reported to resistance with DDT and some of organophosphate, carbamate and pyrethroid compounds in Bulgaria, Greece, Romania, Turkey, Romania, Algeria, Morocco and Tunisia [8].

*An. maculipennis* and *Anopheles hyrcanus* are the most common anopheline species in the Gillian area. *An. superpicuts*, *An. claviger*, *An. plumbeus* the other known *Anopheles* in Iran, are also present in this area but in very small numbers [7]. However, *An. maculipennis*, identified as complex species such as *An. maculipennis* typicus Meigen 1818, *An. melanoon melanoon*, *An. melanoon subalpinus* and *An. messeus*. *Anopheles maculipennis* typicus was reported as common anopheles, regarded as the main vector. Distribution of *An. maculipennis* is shown in Fig. 1.

A new species (*Anopheles persiensis*) has since been described for the first time from Iran [9]. The *Anopheles maculipennis* complex formally comprised 12 Palearctic members including *Anopheles atroparvus*, *Anopheles beklemishevi*, *Anopheles labranchiae*, *Anopheles maculipennis*, *Anopheles martinus*, *Anopheles melanoon*, *Anopheles messeae*, *Anopheles sacharovi*, *Anopheles persiensis*, *An. daciae*, *An. lewisi* and *An. artemievi* [10]. Molecular analysis of the species complex indicated the presence of *Anopheles sacharovi* 984 (67.6%) and *A. maculipennis* 137 (9.4%) in northern region of Iran [11]. This species recently reported from Serbia [12]. *Anopheles maculipennis* has been distributed in 20 provinces of Iran [13].

Out of 24 members of this complex species, seven species including *Anopheles atroparvus*, *Anopheles labranchiae*, *An. maculipennis*, *Anopheles messeae*, *Anopheles melanoon*, *An. sacharovi* and *Anopheles persiensis* were reported from Iran [14].

## MATERIAL AND METHODS

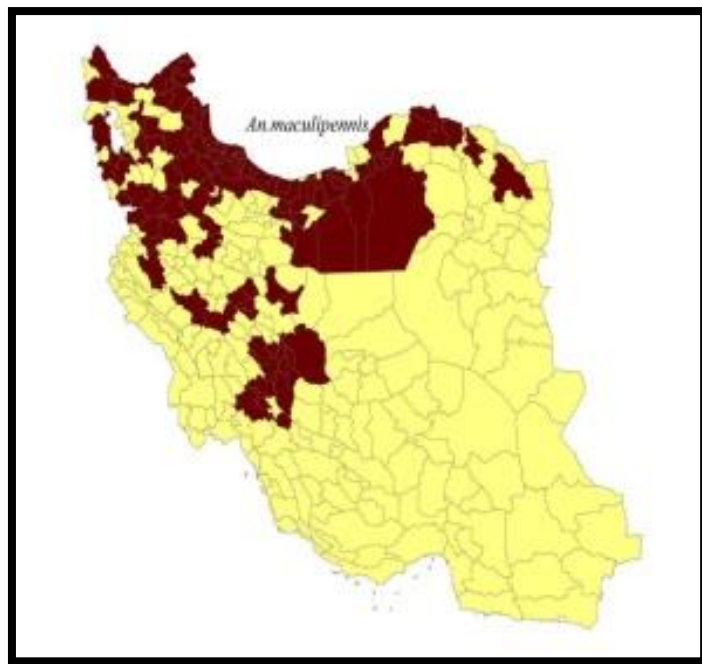
This investigation was carried out in Astará area, Gillian province between 2000-2002 over a 12 month period. Astará area with a total population of about 67584 in a malarious area located in 48° 51' N longitude, 38° 26' E latitude on the northern parts of Gillian province, boarder line with Azerbaijan republic, Ardabil province also Caspian sea, where the annual incidence of malaria average about 0.5 per thousand population. Three villages

with a total population 2956 were selected randomly for this study. The average maximum and minimum temperature in summer was 32.4<sup>0</sup> C and 24.2<sup>0</sup> C and in winter 23.2<sup>0</sup> C and 10.4<sup>0</sup> C respectively. This area are rice and vegetable also lemon and orange growing region irrigated by Astarachi and Taleseyfi rivers, irrigation canals, which the major sources of mosquito breeding places (Fig.2) .

The average yearly rainfall is about 320 mm. Anitmalaria measures such as residual spraying was started by applying DDT 75% with a rate of 2gr a.i /m<sup>2</sup> from 1950 to 1954, and was stopped following the decrease of malaria cases. An entomological evaluation was carried out in three villages, space spray collection was made in eight fixed shelters, four animal and four human shelters in each village. Collection was carried out in a 15 days cycle in the early morning [8].

Night biting collections using man and animal baits in three villages were started at the beginning of the seasonal activity of vectors and continued for more than two months, two cow and two men were considered as baits. Collection was made from 6.00m until 5.00 am in fortnightly intervals [8] .The anopheline species were collected and classified according to the blood digestion stage (empty, freshly fed, half-gravid, and gravid). Female mosquitoes were dissected and scored as nulliparus and parous on the basis of the tracheal skin of their ovaries and scored as number of dilatation or sac of their ovaries [15,16]. Human biting rate habit, expectation of life and infective life of vector also Victoria capacity, gonotrophic and sporogony cycle has been detected by Macdonald's formula [17].

The susceptibility test of anopheles larvae were carried out using of *An. maculipennis* larvae at the 3rd or 4th stage and dipping method from 07.00 till 10.00 AM during May and June and they were transported with care into the laboratory . Emulsions of diagnostic dose of 3.125 mg/l malathion., 0.125 mg/l fenitrothion, 0.25 mg/l temephos, 0.025 mg/l chloropyrifhos were used for treated samples and ethanol for control. All the insecticides were supplied by WHO. Mortality rates were determined 24 hours after exposure. Mortality of larvae were corrected by Abbott's formula and the results subjected to Epi-info computer program, using students-t-test.



**Fig.1. Distribution of *An. maculipennis* in Iran**



**Fig.2. Map of Study area in Iran**

## RESULTS

Because of the importance of malaria in that region antimalarial measures such as indoor residual spraying with lambda-cyhalothrin. The peak of *Plasmodium vivax* is in July-August. The standard WHO test on malathion, fenitrothion, chlorpyrifos and temephos against *Anopheles maculipennis* larvae conducted in Astar area. The assessment of effectiveness of larvicides was conducted on the basis of WHO headlines. Mortality of Anopheline larvae exposed to these compounds presented in table 1. There were 100% mortality in *An.*

*maculipennis* larvae exposed to diagnostic doses of malathion, fenitrothion whereas 53% and 77% mortality were observed with temephos and chlorpyrifos, respectively. Results of the abdominal condition and parous rate of *An. maculipennis* female captured by pyrethrum space spray catches method is presented in table 2. Species composition of anopheles catches by this method consists *An. maculipennis* 92% and *An. hyrcanus* 8%. A total of 903 *An. maculipennis* were examined, followed by unfed (23.5%), freshly fed (54%), semigravid (12.1%) and Gravid (10.4%). The parity of *A. maculipennis* was (31.1%). Parous rate and parity condition of *An. maculipennis* females captured by night biting collection and hand catch are presented in table 3. This species was active throughout the night with 2 peaks of blood meal, 21.00-22.00 PM and 01.00-02.00 AM. Six dilation detected after dissection of ovary in hand catch collection which indicate long longevity and high potency of transmission. Parity condition, probability of survival, expectation of life and expectation of infective life of *An. maculipennis* in Astara area are presented in Table 6. Duration period between bloods fed and laying egg by Anopheles female known gonotrophic cycle and calculated by Blunk formula. In this study gonotrophic cycle calculated 3.4 days. The period is necessary for the development of the plasmodium in the vector were 12.5, 16 and 16 days for *P. vivax*, *P. falciparum* and *P. malariae* respectively, the direct estimation of the physiological age of *An. maculipennis* calculated by intricate dissection methods that showed that number of dilation in the ovaries. Each dilatation in an ovarie usually taken to represent one successful oviposition. The proportion of parous and, the parity rate determined. The probability of survival through 1 day is equivalent to the square root of the proportion of gravid female. This criteria for total catch, hand catch and night biting collection methods were 0.75, 0.84 and 0.90 days, respectively. The expectation of infective life of vector can be defined as their mean number of days of life in the infective condition and total catch method for *P.vivax*, *P. falciparum* and *P.malariae* calculated 0.1, 0.04 and 0.04 day respectively. This criteria for *P.vivax*, *P.falciparum* and *P.malariae* by night collection method was 2.5, 1.8, 1.8 days, whereas by hand collection method the figures was 0.6, 0.4 and 0.4 respectively (table 4).

**Table 1 - Mortality of *Anopheles maculipennis* larvae exposed to different diagnostic concentrations of larvicides in Astara area, Gilan province**

larvicides	replicates	No. mosquito tested	No. mosquito dead	Mortality rate
Malathion 3.125 mg/l	4	100	100	100
Fenitrothion 0.125 mg/l	4	100	100	100
Temephos 0.25 mg/l	4	100	53	53
Chloropyifos 0.025 mg/l	6	150	115	77

**Table 2- Abdominal conditions and parous rate of *Anopheles maculipennis* female captured by Pyrethrum space spray catches method at Astara, Gilan**

Density per shelter	No. of mosquito collected	Abdominal condition				No. of mosquito dissected	N	P	P%
		U	F.F	S.G	G				
112.8	903	U	F.F	S.G	G	135	93	42	31
		215	490	104	94				

U= Unfed , F= freshly fed, SG= Semi-Gravid, G= gravid, T= Total catches, N= Nulliparus  
P= Parus

**Table 3- Parous rates and condition of *Anopheles maculipennis* females captured by different collection methods at Astara, Gilan**

Collection methods	No. of mosquitoes dissected	Parity condition							
		N	1P	2P	3P	4P	5P	6P	Sac
Hand catch	132	65	40	16	5	1	1	1	3
Human bait collection	29	9	13	3	2	0	0	0	2
Animal bait collection	28	10	11	4	3	0	0	0	0
Total	189	84	64	23	10	1	1	1	5

N=Nulliparus, P=Parus

**Table 4- Parity condition, probability of survival, expectation of life and expectation of infective life of *Anopheles maculipennis* in Astara**

Collection methods	No. of mosquito catches	No. of mosquito dissected	Parity condition		Probability of survival	Expectation of life	Expectation of infective life of vector		
			N	P			<i>P. vivax</i>	<i>P. falciparum</i>	<i>P. malariae</i>
Total catch	903	135	93	42	0.75	3.15	0.1	0.04	0.04
Hand collection	132	132	65	67	0.84	5.7	0.6	0.4	0.4
Night collection	60	58	19	39	0.90	9.5	2.5	1.8	1.8

## DISCUSSION

Malaria continues to be a major public health problem in Southeast of Iran. Despite the considerable research and control efforts devoted to malaria, it is still the most prevalent and forms a public health stand point. In Gillan province, malaria control was started with residual spraying by DDT from 1950 and was stopped following the decreased malaria cases in 1945. Following the emergence Azerbaijan and Armenia republics in borderline of Gillan province and Astara area, Annual parasite incidence (API) has been increased. This criteria was measured as 0.047 cases per thousand population in 1995 and increased as 1.27 in 1998. There are new foci such as Astara, Pars-Abad, Kaleybar, Maco, in relation to malaria epidemic. The rater high percentage (80% in 1999) of the annual malaria cases in Astar area occurs among foreigners, monthly from other provinces or country. Species composition of *An. maculipennis* and *hyrcanus* catches by larval collection was 28%, 72% whereas by pyrethrum space spray catches and night biting collection was 93%, 7% and 6%, 94% respectively. *An. maculipennis*, the main vector of malaria in this area was endophagic and endophilic as shown by the freshly fed/gravid and semigravid proportion of 1/0.41 obtained in Pyrethrum pace spray collection. This behavior has been mentioned [3]. Results of pyrethrum space spray and hand collections from two different habitats of this species, in relation to latitude indicated occurrence of two sibling species. *An. maculipenni* typicus was presented in high latitude habitats, there were different between eggs shape, parity and number dilation. From 1095 females *Anopheles* collected by different techniques in that study, 257 (23%) was *An. maculipennis*, it was found that at least 5.6% of the population could reach to the dangerous age in order to transmit the malaria parasites. From total of females *Anopheles* collected by animal and human biting



collection *An. hyrcanus* (94%) was the most common Anopheline species with 2 peak of feeding activity in 24.00-01.00 and 02.00-03.00 followed by *An. maculipennis* (6%) with 2 peak in 21.00-22.00 and 01.00-02.00 respectively. Age determination by ovary dissection revealed 6 dilation, which indicate long longevity and high potency of transmission.

The mortality of *An. maculipennis* larvae exposed to different WHO-recommended diagnostic dose of fenitrothion, temephos, malation and chloropyrifos showed that larvae of this species exhibit susceptible to these larvicides specially pyrethroid compound . The use of organophosphate compound for control of agricultural pests decreased susceptibility level of this species. Adults susceptibility test using diagnostic dose of different insecticides showed that this species exhibits resistance to DDT and dieldrin and bendiocarb, tolerance to permethrin and deltamethrin, whereas susceptible to malathion and lambda-cyhalothrin [18]. Resistance to DDT also reported by Eshgy et al,(1980) [3] . In a study varied out by Chavshin et al ( 2015) [19] they reported that *An. maculipennis* is tolerant to permethrin, deltamethrin and dielderin, but displayed resistance against propoxur, bendiocarb and malathion. Organophosphate and carbamate resistance in *An. maculipennis* group was reported [20].

## CONCLUSION

Malaria is a public health problem in the country [21] . There are several reports on resistant status of malaria vectors including *An. stephensi* [22-24] , *An. maculipennis* [19] , *An. sacharovi* [25], *An. culicifacies* [26-28], *An. fluviatilis* [29]. Monitoring and mapping of insecticide resistance in *An. maculepennis* should be carried out regularly.

**Conflict of interest:** All authors that there is no conflict of interest.

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