FACTORS INVOLVED IN THE RE-EMERGENCE OF MALARIA IN BORDERLINE OF IRAN, ARMENIA, AZERBAIJAN AND TURKEY

H Vatandoost[1], H Ashraf[1], SH Salari Lak[2], R Entezar Mahdi[2], MR Abai[1] and M Nazari[1]

[1]School of Public Health and Institute of Health Research, Tehran University of Medical Sciences, Tehran, Iran;
[2]Urumieh University of Medical Scienecs, Iran

Abstract. The borderline of Iran with Azerbaijan, Armenia and Turkey had been considered a malaria free region. However, in 1991, after the independence of the southern countries of the former Soviet Union, a new threat of malaria importation emerged from those countries into Iran, which was affected by serious epidemics of *Plasmodium vivax* malaria. Various factors can affect malaria resurgence in this region, such as socioeconomic conditions, especially the displacement of massive populations from war-stricken zones in the Republic of Azerbaijan. Accordingly, in some parts of West-Azerbaijan, East-Azerbaijan, Ardebile and Gilan provinces of Iran, several malaria foci were observed. Construction of dams, people traveling from neighboring countries to Iran, urbanization, irrigation projects, lack of malaria vector control, shortage of drug supplies are also major factors in malaria outbreaks in the region. An investigation was carried out on the bionomics of the main malaria vectors in the region. The result showed that *Anopheles sacharovi* plays an important role in malaria transmission and *An. maculipennis* and *An. superpictus* can be secondary vectors. Larvae were found in slow flowing water and channels with water plants. They were more abundant in June. The parity rate of blood-fed females was high in May. *An. sacharovi* is active from May to October with two peaks of activity, which occur in August and October. The population of this species is higher in animal shelters with a zoophicity of 95%. About 90% of bites took place in the second half of the night. A CDC light trap can also catch this species. Susceptibility testing using the WHO-recommended diagnostic doses of insecticides, revealed that this species is resistant to DDT and dieldrin, but susceptible to malathion, fenitrothion, propoxur, bendiocarb, lambdacyhalothrin, permethrin, cyfluthrin, etofenprox and deltamethrin.

INTRODUCTION

Malaria is endemic in southern parts of Iran. According to recent information, a total of 18,000 cases was reported from these areas. In the northern part of Iran, only small numbers of imported cases of malaria have been reported. Although West-Azerbaijan Province in the Maku area, which has been considered a malaria-free region (Edrissian *et al*, 1985a), in 1991, after the independence of the southern countries of the former Soviet Union, and the occurrence of the Nagorno-Karabakh civil war, a new threat of malaria importation recently emerged from the Republic of Azerbaijan, to the north-west of Iran, which was affected by a serious epidemics of *Plasmodium vivax* malaria. Various factors can affect malaria resurgence in this region, such as socioeconomic conditions, especially displacement of massive populations from war-stricken zones in the Republic of Azerbaijan. Cases have been reported in refugee camps since 1993, while probably more cases outside the camps have gone unreported. By 1996, about one million refugees and displaced persons were living in refugee camps and other makeshift dwellings in malaria-endemic areas in the marshy lowland regions of the interior and along the southern border near Iran (Velibekov, 2000). Accordingly, in some parts of West-Azerbaijan, East-Azerbaijan, Ardebile and Gilan provinces of Iran, several malaria foci were observed. Construction of dams, people traveling from neighboring countries to Iran, urbanization, irrigation projects, lack of malaria vector control, shortage of drug supplies are also major factors in malaria outbreaks in the north of the Zagros Mountain Range. The geographical range distribution included the southern former USSR, Yugoslavia, Austria, Italy, Greece, Cyprus, Turkey, Iraq, Syria, Lebanon, Jordan and Palestine (Knight and Stone, 1977).

Anopheles sacharovi is one of the malaria vectors in Iran, and has a more localized distribution in central, northwest, west, southwestern and Fars province (Manouchehri, 1974). According to the investigations of Daw in 1958 (Daw, 1958), Nasiri-amini during 1964-1967 (Nasiri-amini, 1967), Saebi through 1983-1985 (Saebi, 1985), the *Anopheles* fauna of the province includes *Anopheles sacharovi* (Favre, 1903), *An. maculipennis melanoon* (Hackett, 1934), *An. maculipennis* (Meigen, 1818) and *An. superpictus*

Correspondence: Dr H Vatandoost, School of Public Health and Institute of Health Research, Tehran University of Medical Sciences, PO Box 6446-14155, Tehran, Iran.
E-mail:hvatandoost@yahoo.com

(Grassi, 1899). Although little is known about the bionomics and vector potential of the anophelines of the province, *An. sacharovi* is the only one considered to be a principal vector of malaria (Macan, 1950). *An. sacharovi* is also the most important vector of malaria in Turkey, Iraq, Armenia and the Eastern Mediterranean region (Al-Tikrity, 1964; Caglar and Alten, 2000; Romi *et al*, 2002), especially in the most receptive Aras areas along the lowland Aras River basin, which is at an altitude of 150 m. The villages on both sides of the border are within flying range of *Anopheles sacharovi*. This species is widespread and abundant in the foothills and the escarpment (Zahar, 1990). It also transmits malaria in the Shat-al-Arab river area (Etherington and Sellick, 1946). Along these locations, among 185 specimens of *An. sacharovi* were dissected during November 1943 giving a sporozoite rate of 3.8% (7/185 dissected). During October 1943, one sporozoite-positive specimen was found among 353 female *An. sacharovi* dissected in the Kermanshah Valley, western Iran and the sporozoite rate for November 1943 was 0.415% (2/486 dissected). Both sporozoite-positive specimens were found in January 1943 (WHO, 1963), also in Turkey *An. sacharovi* overwinter as adults, when it is found in a large number of buildings. In general, human dwellings are efficiently heated during the winter, and are too hot and dry for mosquito survival. By contrast, stables and other animal shelters are cold during the day when empty, but at night, when they are full of animals, are warm and humid enough tn‡ permit mosquitos to feed, and thus afford optimum conditions for survival through the winter. Therefore, *An. sacharovi* was assumed to be the most efficient malaria vector based on epidemiological evidence, and the above results confirmed that *An. sacharovi* can transmit some malaria, even throughout the winter.

West-Azerbaijan Province has experienced several epidemics of malaria. The most noteworthy of these have been in the years 1976, 1977, 1978, 1990 and 1995, resulting in 293, 186, 210, 144 and 149 total human cases, respectively. A malaria outbreak in 1999, in some parts of the province bordering Armenia, Naxcivan and Turkey, resulted in 189 human cases in the Maku and Poldasht counties. This outbreak prompted disease surveillance coupled with field research aimed at determining which *Anopheles* species occur in this region, and also the behavioral and ecological characteristics of the local populations of principal and potential vector mosquitos, including their breeding habitats, feeding contact with man, relative abundance, and to elucidate the current susceptibility status of *Anopheles sacharovi* to different insecticides.

MATERIALS AND METHODS

Study area

West-Azerbaijan Province (surface area 37,598 km^2) is located in north-western Iran (between 35º, 47´-39º, 47´N latitude, and 44º, 14´-47º, 19´E longitude). The province is bounded by Nacxivan Autonomous Republic (with Armenia proper) in the north, borderline of 135 km, by Turkey and Iraq in the west, (686 km), by Kurdestan Province in the south and by East-Azerbaijan and Zanjan Provinces in the east. In August 1999, localities with recent autochthonous malaria cases were visited to determine the types of dwellings, their accessibility and their proximity to potential mosquito breeding sites. Based on preliminary surveys, six villages with relatively high numbers of anophelines were selected for continued sampling. The investigation was conducted monthly between August through October 2000 and May through July 2001 in four villages (Kholkholeh, Zilakeh-ye-olya, Dimgeshlag, Gareh-Gowyun) in Northern Chipasar Rural District (44º,44´E longitude, 39º, 40´N latitude) 80-84 km from the city of Maku, and in two villages (Pezik, Zanganeh) in the western Chipasar Rural District (44º57´E longitude, 39º35´N latitude) 16-35 km from the city of Poldasht. Poldasht is situated at an altitude of 795m in the plain area alongside the banks of the Aras River. In August and January, the maximum and minimum annual average monthly temperatures were 35ºC and -10ºC, respectively. The minimum mean monthly relative humidity was 27% (August) and the maximum was 86% (April-May). The total local population is 9,700 people. Maku is situated at an altitude of 1,410 m in a mountainous area. In 2000, the maximum and minimum mean monthly temperatures were 37.5ºC and -16ºC in August and February, respectively. The average total annual precipitation is 390 mm. The minimum mean monthly relative humidity was 35% (August) and the maximum was 73% (April-May). The total population is 33,406 people. The climate of the study area is characterized by a hot summer (July-September), and moderate winter season (January-March). The main occupation of the people in selected villages is husbandry of domesticated animals, business and agriculture.

Larval collection and breeing place characteristics

Searches for anopheline larvae were made in perennial and transient man-made or natural bodies of water using standard dippers (500ml) from breeding places close to adult collection sites. Larvae were calculated on the basis of the mean monthly number of larvae collected per 10 dips. Third and fourth instar larvae were immediately preserved for later identification. The larvae were counted during the period May to October. Water was sampled for chemical analysis from breeding places of *An. sacharovi* and *An. maculipennis s.s.* in two villages (Kholkholeh and Zanganeh) of Maku and Poldasht counties. Samples were sent to the Water and Waste Water Laboratory, Urumieh University of Medical Sciences for chemical analysis. Vegetation and insect breeding places were also studied and identified.

Adult collection

Pyrethrum Space Spray Catches (PSSC). PSSC were conducted monthly in eight fixed shelters (four human and four animal) located in different parts of the study villages by standard methods (WHO, 1963), using 0.2% pyrethrum spray. The density was calculated as the number of mosquitos per shelter. *Anopheles* were sampled intra-domiciliarly before 0900 hr. *Anopheles* mosquitos were sexed and identified using taxonomic keys (Shahgudian, 1960) and sorted according to abdominal condition (WHO, 1975). Females were classified as being unfed, blood-fed, gravid or semi-gravid. Only females of *An. sacharovi* were examined for parity rate because this species is responsible for maintaining malaria transmission in neighboring countries and provinces near this focus, and also becouse the remaining species were abundant only during limited periods of the year. The parous rates of blood-fed and unfed females were compared by computing a z statistic. These examinations were made in the laboratory of the Poldasht Health Center.

Human and animal bait collections. In Kholkholeh Village, biting collection was conducted outdoors because all people and animals spent the night in the open and slept outdoors during most of the study period. Outdoor collection was done from 21 00 hr to 06 00 hr for one night in August 2001. Catches were made by 2 collectors equipped with torches and aspirators. Human-biting anopheles were captured from the exposed legs, feet, arms and head of one subject seated in the yard of a house (WHO, 1975).

Parallel outdoor animal-bait catches were made in front of shelters, using a pair of collectors similarly equipped. A cow tethered outside its shelter, about 15m away from the human bait and 200m from larval habitats, was used as animal bait. A night biting catch consisted of 12 episodes of 50 minutes of uninterrupted catching. Each episode began on the hour, and all mosquitos were collected and stored in a plastic cup. Each plastic cup collected nightly was labeled by hour and station. During the 10-minute hourly rest period, weather conditions were taken on the site. Human-bait and animal-bait teams exchanged positions on the hour to avoid collector bias. Mosquitos obtained by hourly man-bait and animal-bait collections were held in cages until they were identified and counted. These collections were used to determine the periodicity of the biting activity, man-biting density, the relative abundance of *Anopheles sacharovi* and host preference intensity.

CDC light trap collection. CDC light traps were used to sample mosquitos at night at 6 sites. The traps were suspended from the thatched roofs of three animal shelters; the ceilings of two human dwellings, and one at a cave near the study area.

Blood meal identification

The stomach contents of the identified semi-gravid and engorged female anophelines were squashed onto circles of Whatman No.1 filter paper for the determination of host-blood source. They were sent with the necessary information to the Department of Parasitology in the Pasteur Institute of Iran for enzyme linked immunosorbent assay (ELISA) testing (Edrissian *et al*, 1985b).

Mosquito dissection

To determine the age composition and epidemiological potential of the population of *An.sacharovi,* the parity of each female was recorded using the method of Detinova (1962).

Insecticide susceptibility test

In the study area, engorged female *An.sacharovi* were collected from indoor resting sites using mouth aspirators. A significant proportion of this species resting in human dwellings had fed upon the occupants. In order to reduce the mortality of the adult mosquitos, they were kept in holding tubes covered with a wet towel, and then transferred to the laboratory for insecticide testing.

Tests on adults were carried out according to the method of the WHO (1981, 1988). In each test, at least 100 mosquitos, representing 4-5 individual replicates of 20-25 adults, were tested. To reduce variability in the replicates, engorged females were used. The exposure tubes were held in a vertical position during the tests. The exposure time for each insecticide, except for fenitrothion, was 1 hour. For fenitrothion, a 2-hour exposure time was used. The mortality rate was scored after a 24-hour recovery period. Insecticide exposure took place in a room with a temperature of 27±2°C and the holding tubes were held in a room condition of 27±2°C and relative humidity 55-60%. The following insecticide impregnated papers were supplied by WHO: DDT 4%, dieldrin 0.4%, malathion 5%, fenitrothion 1%, bendiocarb 0.1%, propoxure 0.1%, lambdacyhalothrin 0.05%, deltamethrin 0.05%, permethrin 0.75%, cyfluthrin 0.15%, etofenprox 0.5%. For the control of organochlorine insecticides, organophosphate, carbamate and pyrethroid insecticides, mineral oil, olive oil and silicon oil impregnated papers were used, respectively.

RESULTS

Larval composition and breeding places

Of the total number of larvae samplings conducted at the breeding sites, four species of *Anopheles* were collected during the study rounds, including *An. sacharovi* and *An. maculipennis* (95.6%). *An. hyrcanus* (4.2%) and *An. superpictus* (0.2%). At the breeding sites, immature stages were present in clear samples along the edges of slow flowing rear-ends of water supply channels, and roadside ditches. Perennial, temporal and transient pools were the most important larval habitats. Larvae were also found in sun-exposed freshwater stream pools with clear, shallow, stagnant water containing abundant filamentous green algae and aquatic vegetation. No larvae were observed in stagnent water, marshes and ponds, but in the wet season, particularly in May, larvae were found in seepage areas, meadows, and in waterlogged grassy and reedy lands resulting from overflowing spring and stream waters. Physicochemical measurements are shown in Table 1. Larvae were most commonly associated with water plants, such as *Sorgum* spp (Family: Graminaeae), *Typha* spp (Family: Typhaceae), *Lemna* spp (Family: Lemnaceae) and most commonly aquatic insects were from the families Coenagrionidae (Order: Odonata), and Notonectidae (Order: Hemiptera). It should be noted that systematic keys for the exact identification of genus and species were not available. Larvae of *An. sacharovi* and *An. maculipennis* were found in small numbers (6.3/10 dips) by the end of May. The maximum larval density (28/10 dips) was in late June. They became most abundant near the study villages during July (15.3/10 dips). The density subsequently increased in August (25/10 dips), declined in September (8.5/10 dips), and increased in October (23/10 dips). No larvae sampling was done near the study villages from late October to the next April because of high rainfall and cold.

Abundance, trophic status, and parity

Of the 3,498 mosquitos collected during the study, the majority were *An. sacharovi* (88%). The other species present were *An. superpictus* (0.12%), *An. hyrcanus* (3.48%), and *An. maculipennis* (8.4%). The proportion of blood-fed female *An. sacharovi* in the collection period markedly increased in the latter part of the study. The majority of these blood-fed females were nulliparous with immature ovaries (primary follicles at Christopher's stage or I-II), indicating teneral or diapusing mosquitos.

The parity rate of the blood-fed females declined from 0.79% at the beginning of the study to 0.17% at its conclusion. The parity rates of the blood-fed females captured in the study villages were found to be high in May but low in October. The nulliparous females were not collected from November to April in the study area because of cold weather and high rainfall. The day-time population density of *An. sacharovi* indicated a gradual increase, beginning in May and reaching a maximum level in June, then followed by a gradual decrease until July. However, it was found that the population did not disappear entirely. The number of mosquitos was the highest during August, but the population decreased further in September when the natural larval habitats were arid, and increased again in October. Results showed that *An. sacharovi* was active from May to October, with two peaks of activity

Table1
Physicochemical measurements of water of breeding places of mosquiro larvae.

Villages	Dissolved oxygen mg/l	pH	Turbidity	Salinity %	F	Cl	Ca	Mg	COD	Conductivity μSIMENS
Kholkhole	4.2	8.0	4.5	0.5 (0.05Mg/l)	0.78 1.06	62 560	57 29	62 36	23 132	944 4,040
Zanganeh	0.8	8.1	200	2.2 (0.22Mg/l)						

in August and October. However, *An. maculipennis s. s.* started to appear in early May and disappeared in late June. It was found that the population did not clear entirely and was present as a sprinkling from August to October. The maximum density of *An. maculipennis* was in dwellings and shelters of villages in Poldasht and Maku counties. They were more prevalent in May, when relative humidity was high. Two other species, *An. superpictus* and *An. hyrcanus*, were present in study villages from August through October, but at low densities. The density of *An. sacharovi* was consistently higher in animal shelters, as compared to human dwellings, and more prevalent than the density of *An. maculipennis s. s.*

Biting behavior and biting cycle

Catches began at about 30 minutes prior to sunset and ended at 30 minutes after sunrise. The results showed that out of 105 *An. sacharovi* females collected, 68.6% were caught on cow bait, and 31.4% were caught on human bait. *An. sacharovi* human biting started at 20 00 hr and continued until 07 00 hr, with a peak between 04 00 hr and 06 00 hr. About 90% of the bites took place in the second half of the night (12 00-06 00 hr) and only 10% in the three hours after sunset. The biting pattern on the cow was more or less the same as the human, but the number of bites on the cow was much greater than the number of bites received by the human. *An. sacharovi* cow biting started at 20 00 hr and continued until 07 00 hr, with two peaks, first in (01 00-02 00 hr) and second in (05 00-06 00 hr) in which maximum biting was observed in the second half of night. It seems that adult mosquitos were active after dusk and before dawn. The average biting rate was 6.6 bites per person per night.

CDC light trap catches

Using the CDC light traps, only 13 female adult *Anopheles* were collected during the study periods. They included *An. hyrcanus* (69.2%), *An. maculipennis s.s.* (7.7%), *An. sacharovi* (23.1%). Other species that were obtained included *Culex* spp. All female *Anopheles* were dissected and the result revealed that only one *An. sacharovi* was blood-fed and the remaining were unfed.

Host preference pattern

In ELISA testing of 100 blood meals of *An.sacharovi*, the proportion giving a positive reaction with alkaline phosphatase anti-human conjugate was 5%, indicating the high zoophilic habit of this species.

Susceptibility status

The mortality rates of *An. sacharovi* after 1-hour exposure, followed by 24-hour recovery period are given in Table 2. By applying WHO criteria (98-100% mortality indicates susceptibility, 80-97% mortality requires confirmation of resistance with other methods, and <80% mortality suggests resistance), it was found that field samples were resistant to DDT, tolerant to dieldrin, and from the results it can be concluded that they were susceptible to malathion, fenitrothion, bendiocarb, propoxur, lambdacyhalothrin, deltamethrin, permethrin , cyfluthrin, and etofenprox.

Table 2
Mortality of *Anopheles sacharovi* adult females to different insecticides in the study area.

Insecticides	Number of mosquitos tested	Percentage knock-down after 1-hour exposure	Mortality ± standard error	Exposure time (hr)
DDT 4%	101	25	39±5	1
Dieldrin 0.4%	100	82	90±3	1
Malathion 5%	100	99	100	1
Fenitrothion 1%	100	100	100	2
Bendiocarb 0.1%	100	86	96±2	1
Propoxur 0.1%	100	95	100	1
Lambdacyhalothrin 0.1%	100	95	98±2	1
Deltamethrin 0.05%	100	95	100	1
Permethrin 0.75%	100	95	98±2	1
Cyfluthrin 0.15%	100	98	100	1
Etofenprox 0.5%	100	90	96±2	1
Control	100	0	1	1

DISCUSSION

The study of the bionomics of mosquitos in the borderline areas of Iran, Armenia, Azerbaijan and Turkey revealed that the main prevalent malaria vectors may be *An. sacharovi* and *An. maculipennis*. The remaining include *An. superpictus* and *An. hyrcanus*, which may play a role as secondary vectors of malaria in the region. Larvae of *An. sacharovi* and *An. maculipennis* were collected from diffrent breeding places with various plants and aquatic insects. The highest larval collection occurred in June. In this month and season, different breeding places are made by running water and rainfall. In our study, the salinity of the breeding places ranged between 0.05-0.22 mg/l. Yaghoobi-Ershadi *et al* (2001) found that a water salinity up to 414 mg/l is appropriate for *An. sacharovi* in other parts of Iran. In the villages of Bogorodica, formerly Yugoslavia, the salinity in a breeding place of *An. sacharovi* was reported as 74 mg/l (Lepes and Vitanovic, 1962). In central Asia, high salinity is better tolerated by the larvae of *An. sacharovi* than any other member of the *An. maculipennis* complex. Its larvae can develop in water having salinities up to 200 mg/l (Artemiev, 1980). Gokberk (1961) reported that breeding places of larvae of *An. sacharovi* and *An. maculipennis* vary in different areas of Turkey.

Our results showed that *An. sacharovi* was active from May to October, with two peaks of activity in August and October. However, *An. maculipennis s. s.* started to appear in early May and disappeared in late June. According to Yaghoobi-Ershadi (2001), *An. sacharovi* was active from June to late November with a peak of activity in early August on the Plain of Parsabad, and from late June to late November with one peak of activity in late August in the mountainous area of Germi. In our study, *An. sacharovi* was active for 7 months of the year. Our finding revealed that *An. maculipennis* appears from the beginning of the year and lasts until the end of June, but after June-July the more prevalent species is *An. sacharovi*, so that, in some villages, more than 95% of the species composition was *An. sacharovi*. It seems that humidity and temperature are the main factors for the seasonal activities of the speceis. CDC light traps are able to attract *An. hyrcanus* and less *An. sacharovi*. Our finding showed that *An. sacharovi* is a mainly zoophilic species with 5% anthropophilicity index.

In 1935, precipitin tests of *An. sacharovi* blood meals in Greece demonstrated positive human reactions from 61.5% of specimens taken from human dwellings and from 7.5% of those from animal shelters. Studies in Greece, in 1973, reported positive human reactions in 38.5% of mosquitos from human dwellings and 1.1% from animal shelters. In 1966, human-positive reactions of *An. sacharovi* taken from a variety of shelters were 5.6% in Greece and 30.5% in Syria. Values of 26.4% from human dwellings and 9.4% from animal shelters were obtained in Iran in 1985.

These findings suggest that the feeding preferences of *An. sacharovi* vary from year to year and place to place. No comparative figures from Turkey were found, so this study was undertaken to provide comparative data. The feeding habits of *An. sacharovi* in Adana Province, Turkey, were investigated by use of the gel diffusion technique. Mosquitos were collected from various villages of Cukurova, Turkey, and from feeding rooms especially prepared for these experiments. A human, cow, sheep, chicken, horse, and donkey were used as hosts in these rooms. The results showed that *An. sacharovi* is a zoophilic species. The females preferred donkey when human, cow, sheep, chicken, and horse were equally available. Their preference changed to horse, cow, and sheep in the absence of donkey. The host preference index (HPI) was always smaller than the one for humans in habitats offering a choice of hosts. The human blood index was high only in human dwellings. In other habitats, the numbers of mosquitos feeding on animals were higher than on humans.

Although the human blood index was low, *An. sacharovi* is the principal vector of human malaria in Turkey, partly because a significant proportion of those resting in human dwellings have fed upon the occupants, and partly because of the uneven distribution of human and animal hosts (Kasap *et al*, 2000).

From the results of susceptibility tests it can be concluded that *An. sacharovi* exhibits resistance to DDT and is tolerant to dieldrin. In 1957, for the first time, *An. sacharovi* were tested against DDT 4% in Fars Province. The results showed that this species was susceptible to DDT 4%. In 1957, DDT was used for malaria vector control in the region. In 1959, the mortality of *An. sacharovi* to DDT 4% decreased to 35-40%, indicating resistance in this population. Due to DDT resistance, dieldrin was replaced for vector control in 1961. Subsequently, after 2 years of dieldrin application, resistance to this insecticide was reported (Zahar, 1990). The first report of *An. sacharovi* resistance to DDT occurred in 1959, from Kazeroon, and subsequently from Izeh and Meshkinshahr (Manouchehri *et al*, 1974). Several studies have been done on the ecology of malaria vectors in Iran. In 1967, malathion was used in the region and until 1973 there was no report of malathion resistance in this population. In 1998, Ghavami (1999) showed that the *An. sacharovi* collected from Kazerun region are susceptible to DDT. It is often asserted that insecticide resistance in a mosquito population is gradually lost and reversion to susceptibility occurs after withdrawal of insecticide pressure (Abedi and Brown, 1960; Pal, 1974; Brown, 1986). Yaghoobi-Ershadi *et al*, in 2001, showed that this species in Parsabad and Germi counties in Ardebil Province of Iran is resistant to DDT and dieldrin. Kasap *et al*, in 2000, reported resistance to 12 insecticides by specimens of *An. sacharovi*, both in the laboratory and in those collected in the malarious areas located in the southern part of Turkey. In Adana, Adiyaman and Antalya, *An. sacharovi* was susceptible only to malathion and pirimiphos-methyl. In our study, pirimiphos-methyl was not used. In other parts of Turkey this species was susceptible to dieldrin, fenitrothion, lambdacyhalothrin, cyfluthrin, etofenprox, malathion and pirimiphos-methyl. It should be noted that both vertical and horizontal positions were used during the exposure times. Hemingway (1985) reported that DDT resistance in *An. sacharovi* was scattered in the population in 1984, despite the replacement of DDT by malathion for malaria control 13 years earlier. It was also reported that populations of this species in Cukurova had an altered acetyl cholinesterase resistance mechanism, conferring broad-spectrum resistance against organophosphates and carbamates. Specimens of *An. sacharovi* collected in the field in 1989-1990 were still resistant to DDT, organophosphate and carbamates, although at lower frequencies than in 1984 (Hemingway, 1992). In addition to the acetyl cholinesterase resistance mechanism, there is evidence of an increased level of glutathione S-transferase in some of the *An. sacharovi* population tested. This is known to be correlated with DDT resistance in other anophelines.

In Iraq, Manouchehri *et al* (1980) reported that *An. sacharovi* was resistant to DDT, but susceptible to malathion. In this study, DDT resistance and dieldrin tolerance was investigated. Selection for resistance in malaria vectors can result from the agricultural application of insecticides (Georghiou, 1972; Brown, 1983; Lines, 1988). According to the department of agriculture in provinces of Iran, 9 types of organophosphates, and 3 types of pyrethroid insecticides are distributed among farmers for agricultural pest control, due to the extensive use of permethrin for agricultural pest control and the occurrence of cross-resistance between permethrin and DDT in malaria vectors (WHO, 1986). From our results, it can be assumed that DDT resistance in the *An. sacharovi* population may be due to cross-resistance to permethrin. Study of the mechanisms of DDT and permethrin resistance is highly recommended. From our results it can be concluded that, except for the organochlorine insecticides, other groups of insecticides may be recommended for malaria vector control. In our studies, *An. sacharovi* partly fed on animals. Sponging cattle with deltamethrin has recently been shown to be effective in killing zoophilic anophelines (Hewith and Rowland, 1999). The results can provide a clue for the control of malaria vectors in the region.

ACKNOWLEDGEMENTS

The authors would like to express their appreciation to the health centers of West Azerbaijan Province and the staff of the Department of Medical Entomology, School of Public Health and Institute of Health Research, Tehran University, for their close collaboration and help.

REFERENCES

Abedi ZH, Brown AW. Development and reversion of DDT-resistance in *Aedes aegypti*. *Can J Cytol* 1960;2:252-61.

Al-Tikrity AB. The geographical distribution of *Anopheles* species and vectors of malaria in Iraq. *Bull End Dis (Baghdad)* 1964;6:91-117.

Artemiev MM. *Anopheles* mosquito main malaria vectors in USSR. In: International scientific project on ecologically safe method for control of malaria and its vectors. Moscow, USSR: The United Nations Environment Programme (UNEP) 1980;2:45-71.

Brown AW. Insecticide resistance as a factor in the integrated control of Culicidae. In: Laired M, Miles JW, eds. Integrated mosquito control methodologies. Vol I. New York: Academic Press: 1983:161-235.

Brown AWA. Insecticides resistance in mosquitoes: a pragmatic review. *J Mosq Control Assoc* 1986;2:123-40.

Caglar SS, Alten B. Malaria situation and its vectors in Turkey. Ankara: Proceedings of the 13th European Sove Meeting. 2000:275pp.

Daw J. Scientific studies in regard to malaria report, Parasitology and Malariology Institute. Ramsar, Iran: 5th Medical Congress. 1958:350 pp.

Detinova TS. Age grouping methods in Diptera of medical importance with special references to some vectors of malaria. *WHO Monogr Ser* 1962;47:216.

Etherington D, Sellick G. Notes on the bionomics of *Anopheles sacharovi* in Persia and Iraq. *Bull Entomol Res* 1946;37:191-5.

Edrissian GH, Ghorbani H, Afshar M. IFA serological surveys of malaria in north, northwest and south-west parts of Iran. *Bull Soc Pathol Exot* 1985a; 78:349-59.

Edrissian GH, Manouchehri AV, Hafizi A. Application of an enzyme-linked immunosorbent assay (ELISA) for determination of human blood index in anopheline mosquitoes collected in Iran, *J Am Mosq Cotnrol Assoc* 1985b;3:349-52.

Georghiou GP. Studies on resistance to carbamates and organophosphorus insecticides in *Anopheles albimanus*. *Am J Trop Med Hyg* 1972;21:797-806.

Ghavami S. Diagnostic dose of pyrethroids against *An.stephensi* and susceptibility status of *An.stephensi* and *An.sacharovi* to malathion, fenitrothion and DDT in Fars province, Iran. Tehran: School of Public Health, Tehran University of Medical Sciences. 1999. MSc dissertation.

Gokberk, C. *Anopheles sacharovi* (Favor 1903) in Turkey. *Mosq News* 1961;2:101-2.

Hemingway J. The biochemistry of insecticide resistance in *Anopheles sacharovi*: comparative studies with a range of insecticide susceptible and resistance *Anopheles* and *Culex* species. *Pestic Biochem Physiol* 1985;24:68-76.

Hemingway J. Insecticide resistance gene frequencies of *Anopheles sacharovi* populations of Cukurova plain, Adana province, Turkey. *Med Vet Entomol* 1992;6:342-8.

Hewith S, Rowland M. Control of zoophilic malaria vectors by applying pyrethroid insecticides to cattle. *Trop Med Int Health J* 1999;4:481-6.

Kasap H, Kasap M, Aleptekin D, Luleyap U, Herath PRJ. Insecticide resistance in *Anopheles sacharovi* favor in southern Turkey. *Bull WHO* 2000;78:687-92.

Knight KL, Stone A. A catalogue of the mosquitoes of the world. Vol VI. Washington DC: Thomas Say Foundation 1977:1-611.

Lepes TR, Vitanovic R. The appearance of *Anopheles sacharovi* in the Republic of Macedonia, Yogoslavia. *Bull WHO* 1962;26:126-8.

Lines JD. Do agricultural insecticides select for insecticide resistance in mosquitoes? A look at the evidence. *Parasitol Today* 1988;4:S17-S20.

Macan TT. The anopheline mosquitoes of Iraq and North Persia , 1950. In: Lee S *et al*, eds. Anopheles and malaria in the Near East. London School of Hygiene and Tropical Medicine, Memoir No.7. London HK: Lewis and Co, 1950:113-29.

Manouchehri AV, Zaini A, Javadian E, Saebi E. Resistance of *Anopheles sacharovi* favor to DDT in Iran. *Iranian J Public Health* 1974;4:204-11.

Manouchehri AV, Shalil AK, Al-Saaidi SH, Al-Okailv AK. Status of resistance of *Anopheles* mosquito in Iraq. *Mosq News* 1980;35:278-80.

Pal R. The present status of insecticide resistance in *Anopheles* mosquitoes. *J Trop Med Hyg* 1974;5:28-41.

Nasiri-amini, M. The anopheles fauna of West Azerbaijan province. Tehran: Faculty of Veterinary Medicine, Tehran University. 1967. PhD dissertation.

Romi R, Boccolini B, Hovanesyan L, Grigorian G, Di luca M, Sabatinelli G. *Anopheles sacharovi* (Diptera: Culicidae): a reemerging malaria vector in the Ararat Valley of Armenia. *J Med Entomol* 2002;39:446-50.

Saebi MA. Morphological identification and distribution of larvae anopheles fauna of Iran. Tehran: School of Public Health, Tehran University of Medical Sciences, 1985. PhD dissertation.

Shahgudian ER. A key to the anophelines of Iran. *Acta Med Iranica III* 1960;3:38-48.

Velibekov AS. Malaria in Azerbaijan. *Med Parazitol (Mosk)* 2000;2:16-7.

World Health Organization. Practical entomology in malaria eradication. Part I. Field and laboratory techniques. *WHO/PA/62*, 1963:8-11.

World Health Organization. Manual on practical entomology in malaria, part II. Geneva: WHO, offset publication 1975:13.

World Health Organization. Instruction for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organophosphate and carbamate insecticides, diagnostic test. *WHO VBC* 1981;81: 806.

World Health Organization. Resistance of vectors and reservoirs of disease to pesticides. *WHO Tech Rep Ser* 1986;737.

World Health Organization. Test procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces. Geneva: World Health Organization. *WHO CPC MAL/98*, 1988:12.

Yaghoobi-Ershadi MR, Namazi J, Piazak N. Bionomics of *Anopheles sacharovi* in Ardebil province, northwestern Iran during a larval control program. *Acta Tropica* 2001;78:207-15.

Zahar AR. Vector bionomics in the epidemiology and control of malaria Part 2. The WHO European region and the WHO Eastern Mediterranean region. *WHO VBC* 1990;90.3:1-166.