Original Article

Ecology of Malaria Vectors in an Endemic Area, Southeast of Iran

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Abstract

Background: Malaria has long been regarded as one of the most important public health issues in Iran. Although the country is now in the elimination phase, some endemic foci of malaria are still present in the southeastern areas of the country. In some endemic foci, there are no data on the malaria vectors. To fill this gap, the present study was designed to provide basic entomological data on malaria vectors in the southeastern areas of Iran.

Methods: Adult and larval stages of *Anopheles* mosquitoes were collected by using different catch methods. Resistance of the main malaria vector in the study area to selected insecticides was evaluated using diagnostic doses advised by the World Health Organization in 2013–2014.

Results: A total of 3288 larvae and 1055 adult *Anopheles* mosquitoes were collected, and identified as: *Anopheles stephensi* (32.1%), *Anopheles culicifacies* s.l. (23.4%), *Anopheles dthali* (23.2%), *Anopheles superpictus* s.l. (12.7%), and *Anopheles fluviatilis* s.l. (8.6%). *Anopheles stephensi* was the most predominant mosquito species collected indoors at the study area, with two peaks of activity in May and November. This species was found to be resistant to DDT 4%, tolerant to malathion 5% and susceptible to other tested insecticides.

Conclusion: All the five malaria vectors endemic to the south of Iran were collected and identified in the study area. Our findings on the ecology and resting/feeding habitats of these malaria vectors provide information useful for planning vector control program in this malarious area.

Keywords: Malaria; *Anopheles*; Malaria vectors; Bio-ecology; Iran

Introduction

Malaria has long been regarded as one of the most important public health issues in Iran. The disease caused irreparable financial and fatality losses in the country, which made initiation of elimination necessary. The most important endemic foci in the country are Sistan and Baluchistan, Hormozgan and Kerman Provinces, which in total, account for 96% of all cases. At present, Iran is in the process of eliminating malaria, and under this condition, even low number of reported cases is very important (1).

Adequate understanding of the association between the behavioral characteristics of the disease vectors and their ecology is important in the planning and determination of strategies to fight against the disease. *Anopheles* species has been considered an important part in malaria transmission cycle after its role in the transmission of the disease was discovered. The ability of *Anopheles* mosquitoes to transmit *Plasmodium* infections is attributed to the physiology and biochemistry of their bodies, which are different according to species characteristics. Other factors such as frequency of blood feeding, longevity, ecological and environmental conditions are important in this respect (2). According to the last checklist of mosquitoes of Iran, there are 30 *Anopheles* species (3). Kerman Province has a long history of ma-

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laria, and even though the disease has been well controlled in other endemic areas of the country, malaria is still considered a major health problem in the province. Previous studies have identified nine *Anopheles* species in the province, and among them, *Anopheles fluviatilis* s.l., *Anopheles dthali*, *Anopheles culicifacies* s.l., *Anopheles stephensi* and *Anopheles superpictus* s.l. have been reported as vectors of malaria (4-6).

Residual spraying and use of insecticide-treated bed nets have recently been implemented in Qaleh-Ganj County in Kerman Province. Due to the higher potential of malaria transmission by *Anopheles* vectors, it was necessary to carry out studies on the fauna and ecology of *Anopheles* species in the area and their susceptibility to some of the conventional pesticides. The aim of this study was to collect information about the fauna and bioecology of *Anopheles* mosquitoes in the area, and to determine their susceptibility to some selected insecticides. Our study intended to provide data that can be useful for future vector control programs in the area.

Materials and Methods

Study area

Kerman Province is located in the southeast of the central plateau of Iran (Fig. 1). The province has a mean annual rainfall of 152.9mm, and according to the current national Counties distribution, the province is composed of 16 counties. Qaleh-Ganj is a county located in the south of the province (27.5277° N, 57.8651° E), with a population of about 70,000 people, 17% of the people live in urban and 83% in rural areas. According to the Koppen-Geiger climate classification, Qale-Ganj County is classified under hot desert climate (BWh); however, in recent years due to reduced rainfall, drought has gripped the area. The maximum and minimum recorded temperatures in the district are 52 and 2 °C, respectively. Monthly maximum and minimum relative humidity in the district is 70% and 35%, respectively, and rainfall in the area is between 0–125mm (Statistical Yearbook of Kerman Province, 2015).

Entomological survey

In this study, sample collection was carried out in three villages including: Shah-Kahan (27.519126°N, 57868245°E), Marz (27.537 180°N, 57.852005°E), and Rameshk (27.521 183°N, 57.857334°E), during 2015. Study sites were chosen according to the WHO standard techniques (7). Mosquitoes were collected using the simple sampling method, before the activity season of the vectors. Specimen collection was carried out over a period of 12 months using total catch, pit shelter, light trap, window trap and dipping methods (7) and by using night catch method during the month of May in 2015.

Total catch method was used to determine species richness of each location and monthly changes in species population and diversity, and to determine the physiological status of endophagic and endophilic species. Artificial pit shelters were created for the collection of outdoor adult mosquitoes. A pit was drilled in each study location (village). Cavities with a dimension of 30×30cm were dug in the walls of the pit shelters about 0.5 meters from the floor of the shelter. These attractive cavities served as resting-sites for mosquitoes entering the pit. Mosquitoes were aspirated from the pit shelters using an aspirator, before sunrise (6–9am) in each day of collection. Another method used for mosquito sampling in our study was the light trap method. During the study, a CDC light trap was used, before sunset and until sunrise in the next day, to collect mosquitoes in Ramsehk Village.

To study the feeding and resting behaviors of adult mosquitoes, window exit traps were installed on the outside of window frames in selected locations. Collected females were identified at species level and their abdominal condition was recorded. Night collection method using human and animal (cow) baits was conducted to identify the host preference of *Anoph-*

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eles mosquitoes in the study area. In this method, mosquitoes were collected as soon as they landed on the host using torch and aspirator, from sunset to sunrise. To determine the peak of host seeking and blood feeding activity, samples collected in each hour were kept in individual cups covered by a fine net and labeled based on the place and time of capture.

After adult specimen collection, the mosquito species were mounted on entomological pins and were identified with a morphological key (8). Female mosquitoes collected by the different methods were classified according to their abdominal condition as: gravid (G), semigravid (SG), unfed (U) and/or freshly blood-fed (F) (7).

In the present study, anopheline mosquito larvae were collected using the dipping method (7). Physical characteristics of the larval habitats were recorded during larval collection. Collected larval specimens were preserved in lacto-phenol for least 24h prior to specimen preparation for microscopic identification. The specimens were mounted on glass slides using Chloral-Gum mounting media and were covered with coverslips. The glass slides were then placed in an incubator at 37 °C to dry before observing under a microscope. Identification key was used to identify the mounted samples based on morphological characteristics (8).

Susceptibility test studies

Susceptibility tests were performed using insecticide-impregnated papers on 2 to 3 day old dominant *Anopheles* species fed with 5% sugar. Mosquitoes were exposed to insecticide-impregnated papers at diagnostic doses, as described by the World Health Organization pesticide scheme guidelines, for one hour, and mortality after 24-hour recovery period was recorded. The tests were carried out at a temperature between 22–26 °C and relative humidity of 60%. In the present study, DDT 4%, Malathion 5%, Propoxur 0.1% and Deltamethrin 0.05% were used for susceptibility testing. Mortality rate of 98–100% was considered as susceptible, 90–97.99% as tolerant, and < 90% as resistant (9). For each insecticide, four replicates of 25 2 to 3day old sugarfed female mosquitoes were tested for susceptibility, whilst two replicates were used as controls. In this study: if control mortality was less than five percent, the results of tests were considered acceptable; if control mortality was between 5–20%, the test results were corrected using Abbotts' formula; and if control mortality was > 20%, the tests were repeated (9).

Results

Mosquitoes Collection

A total of 3288 larvae and 1055 adult *Anopheles* mosquitoes were collected and identified as *An. stephensi* (32.1%), *An. culicifacies* s.l. (23.4%), *An. dthali* (23.2%), *An. superpictus* s.l. (12.7%) and *An. fluviatilis* s.l. (8.6%). Results are described, according to the method of collection, as follows:

Total catch

A total of 541 *Anopheles* species were collected indoors using this method and *An. stephensi* (47.31%) was the most predominant species. The abundance of other species collected by this method is as follows: 114 (21.07%) belonged to *An. culicifacies* s.l., 123 (22.73%) belonged to *An. dthali*, 7 (1.32%) belonged to *An. fluviatilis* s.l., and 40 (7.57%) belonged to *An. superpictus* s.l. (Fig. 2). *Anopheles stephensi* was collected throughout the sampling period except in January and February.

Pit shelter

A total of 137 *Anopheles* species were collected from shelter pits, and *An. fluviatilis* s.l. was the most predominant species (35.76%). *Anopheles superpictus* s.l. was also identified by this method (Fig. 3).

Light trap

Three *Anopheles* species were collected by this method, and *An. superpictus* s.l. was the most numerically dominant. Other species collected by this method include *An. culicifacies* s.l. and *An. stephensi* (Table 1).

Window trap

A total of 57 female *Anopheles* mosquitoes representing four species were trapped by window traps. *Anopheles dthali* (33.3%), *An. culicifacies* s.l. (31.6%), *An. stephensi* (26.3%), and *An. superpictus* s.l. (8.8%) were collected and identified using this method.

Night catch using human/animal baits

Table 2 shows the abundance of adult mosquitoes collected by night catch method using human and animal baits during May 2015. Using human baits, the highest collection occurred in the first third of the night, and the species were identified as *An. superpictus* s.l. and *An. fluviatilis* s.l. *An. culicifacies* s.l. and *An. fluviatilis* s.l. were also collected using animal baits, with the highest collection also occurring at the first third of the night. Blood feeding peak of *An. fluviatilis* s.l. and *An. culicifacies* s.l. were 8–9pm and 10–11pm, respectively.

Abdominal status of collected female mosquitoes

Abdominal condition of the female *Anopheles* mosquitoes collected by three different methods was examined. We classified the females as unfed (U), blood fed (F), and gravid/ semigravid (G/SG), according to their abdominal condition (Tables 3–5).

Total catch

The number of gravid and semi gravid *An. stephensi* collected by this method was higher than the number of unfed and blood-fed *An. stephensi*. Unlike *An. stephensi*, the number of gravid and semi gravid *An. culicifacies*, *An. fluviatilis* s.l., *An. superpictus* s.l. and *An. dthali* was lower than the number of unfed and blooded ones (Table 3).

Pit shelter

The number of gravid and semi gravid *An. culicifacies* s.l. species was higher than the number of unfed and blood-fed *An. culicifacies* s.l. In contrast, the number of gravid and semi gravid *An. stephensi*, *An. fluviatilis* s.l., *An. superpictus* s.l. and *An. dthali* was lower than that of unfed and blood fed ones (Table 4).

Window trap

The number of gravid and semi gravid *An. culicifacies* s.l. and *An. stephensi* was higher than the number of unfed and blood-fed ones; in contrast, the number of gravid and semi gravid *An. fluviatilis* s.l., *An. superpictus* s.l. and *An. dthali* was lower than the number of unfed and blood-fed species (Table 5).

Larval collection

Anopheles stephensi (31.59%) and *An. fluviatilis* s.l. (7.29%) were respectively the most abundant and least abundant larval species collected in the study area. The results of species abundance per 10 dips have been summarized in Fig. 4. Characteristics of larval habitats in the study area have been detailed in Table 6.

Susceptibility tests

Mortality rate of *An. stephensi* against DDT 4%, malathion, propoxur, and deltamethrin was 25%, 97%, 99% and 98%, respectively. Mortality in the control against all insecticides tested were zero except for malathion and propoxur. *Anopheles stephensi* was quite resistant to DDT, but tolerant to Malathion and sensitive to the remaining insecticides.

Table 1. The number of adult mosquitoes collected by light trap in Rameshk Cllage, Qaleh-Ganj County, Kerman Province of Iran, 2015

Species	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Total**	**%**
An. stephensi	0	2	0	0	0	0	0	0	3	1	0	0	6	27.27
An. culicifacies	0	0	0	0	0	0	0	0	0	2	2	0	4	18.18
An. superpictus	0	2	3	1	0	0	0	2	0	1	3	0	12	54.55

Table 2. The abundance of mosquitoes collected on human/animal baits by night catch method, Qaleh-Ganj County, Kerman Province of Iran, May 2013

Bait	**Species**	**Catch Time (Night)**			**Total**
		1st third	2nd third	3rd third	
Human	*An. stephensi*	7	2	1	10
	An. culicifacies	5	2	2	9
	An. superpictus	9	5	4	18
	An. dthali	6	3	1	10
	An. fluviatilis	11	6	2	19
	Total	38	18	10	66
Animal	*An. stephensi*	4	3	2	9
	An. culicifacies.	15	3	4	22
	An. superpictus	9	5	4	18
	An. dthali	12	2	1	15
	An. fluviatilis	19	6	4	29
	Total	59	19	15	93

Table 3. Abdominal status of collected female mosquitoes by total catch, in the study area, Qaleh-Ganj County, Kerman Province of Iran, 2015

Time	**Species/Abdominal Status**																			
	An. stephensi				***An. culicifacies***				***An. superpictus*.**				***An. dthali***				***An. fluviatilis***			
	U	**F**	**G+SG**	**Total**	**U**	**F**	**G+SG**	**Total**	**U**	**F**	**G+SG**	**Total**	**U**	**F**	**G+SG**	**Total**	**U**	**F**	**G+SG**	**Total**
Mar	6	15	28	49	3	9	7	19	1	3	2	6	6	14	12	32	0	0	0	0
Apr	7	17	44	68	2	14	25	41	2	5	7	14	0	21	15	36	0	3	2	5
May	5	22	14	41	1	8	6	15	0	2	5	7	3	6	2	11	0	2	0	2
Jun	2	6	3	11	0	2	2	4	0	3	0	3	0	2	1	3	0	0	0	0
Jul	0	2	3	5	0	2	0	2	0	1	0	1	1	2	0	3	0	0	0	0
Aug	0	0	2	2	0	0	1	1	0	0	0	0	0	0	1	1	0	0	0	0
Sep	2	2	5	9	1	1	2	4	0	1	2	3	1	4	4	9	0	0	0	0
Oct	9	15	24	48	2	5	8	15	0	0	0	0	3	5	4	12	0	0	0	0
Nov	2	7	5	14	2	6	2	10	0	3	1	4	2	7	3	12	0	0	0	0
Dec	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jan	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Feb	3	3	3	9	0	2	1	3	0	2	1	3	0	3	1	4	0	0	0	0
Total	36	89	131	256	11	49	54	114	3	20	18	41	16	64	43	123	0	5	2	7

Table 4. Abdominal states of collected female mosquitoes by shelter pit, in the study area, Qaleh-Ganj County, Kerman Province of Iran, 2015

Time	Species/Abdominal Status																			
	An. stephensi				*An. culicifacies*.				*An. superpictus*				*An. dthali*				*An. fluviatilis*			
	U	F	G+SG	Total	U	F	G+SG	Total	U	F	G+SG	Total	U	F	G+SG	Total	U	F	G+SG	Total
Mar	0	3	1	4	0	1	1	2	1	1	3	5	0	0	0	0	1	4	2	7
Apr	1	2	2	5	0	1	2	3	1	4	4	9	0	2	1	3	2	7	3	12
May	0	1	1	2	0	1	2	3	1	2	1	4	1	2	0	3	0	3	3	6
Jun	0	1	2	3	0	1	0	1	1	1	0	2	1	0	1	2	1	1	1	3
Jul	0	1	0	1	0	2	1	3	0	0	2	2	0	1	0	1	0	0	0	0
Aug	1	1	0	2	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Sep	1	1	1	3	0	0	1	1	0	2	1	3	0	2	1	3	0	2	1	3
Oct	0	3	2	5	0	0	0	0	0	0	1	1	0	0	1	1	1	2	3	6
Nov	2	0	1	3	0	1	1	2	0	0	0	0	0	0	0	0	2	2	2	6
Dec	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2
Jan	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Feb	0	2	0	2	0	1	0	1	0	0	0	0	0	1	1	2	0	2	2	4
Total	5	15	10	30	0	8	9	17	4	10	12	26	2	8	5	15	7	24	18	49

Table 5. Abdominal status of collected female mosquitoes by outdoor window trap, in the study area, Qaleh-Ganj County, Kerman Province of Iran, 2015

Time	Species/Abdominal Status															
	An. stephensi				*An. culicifacies*				*An. superpictus*				*An. dthali*			
	U	F	G+SG	Total	U	F	G+SG	Total	U	F	G+SG	Total	U	F	G+SG	Total
Mar	0	0	2	2	0	0	1	1	0	0	0	0	0	1	1	2
Apr	0	0	1	1	0	1	2	3	0	0	1	1	0	0	2	2
May	0	0	1	1	0	0	1	1	0	0	0	0	0	0	1	1
Jun	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jul	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
Aug	0	1	1	2	0	0	0	0	0	0	0	0	0	0	0	0
Sep	0	0	0	0	0	1	1	2	0	0	0	0	0	0	0	0
Oct	0	1	1	2	0	1	3	4	1	1	0	2	0	2	1	3
Nov	1	1	2	4	0	1	1	2	0	0	0	0	0	3	1	4
Dec	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jan	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	3
Feb	0	1	2	3	1	1	2	4	0	1	1	2	0	3	1	4
Total	1	4	10	15	1	5	12	18	1	2	2	5	0	11	8	19

Table 6. Characteristics of larval habitats in the study area, Qaleh-Ganj County, Kerman Province of Iran, 2015

	Categories	*An. stephensi*	*An. culicifacies*	*An. superpictus*	*An. dthali*	*An. fluviatilis*
Habitat Situation	Constant	64	75	67	64	72
	Temporary	36	25	33	36	28
	Running Water	100	100	100	100	100
	Resident Water	0	0	0	0	0
Vegetation Situation	Without Vegetation	64	75	67	64	72
	With Vegetation	36	25	33	36	28

Type	Muddy	36.4	25	33	36	28
	Sand	27.6	38	23	26	30
	Stone or Cement	36	37	46	38	42
Water Quality	Turbid	64	75	67	64	72
	Fresh	36	25	33	36	28
Sunlight Situation	Sunny	27.5	38	23	26	30
	Semi-Shade	72.5	62	77	74	70
	Shade	0	0	0	0	0
Habitat	Natural	64	75	67	64	72
	Artificial	36	25	33	36	28
Temperature (ºC)	Mean	29.5	28	26.5	31.5	22
pH	Mean	7.6	7.6	7.6	7.6	7.6

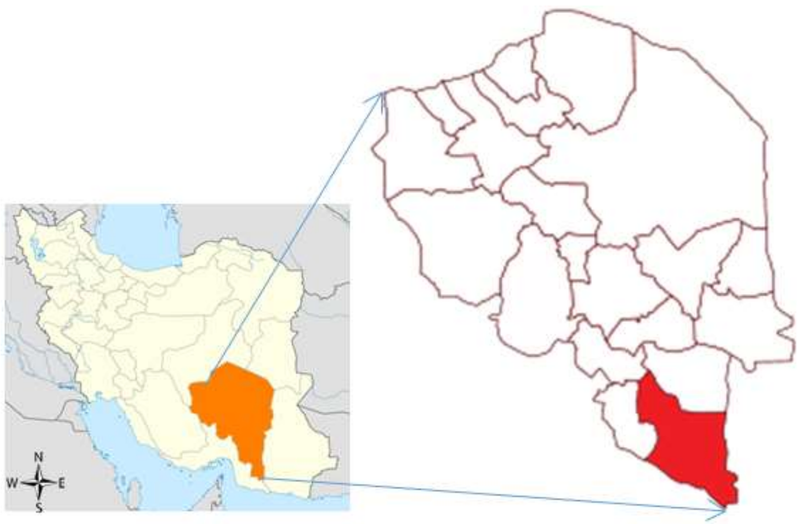

Fig. 1. Study area in Kerman Province of Iran

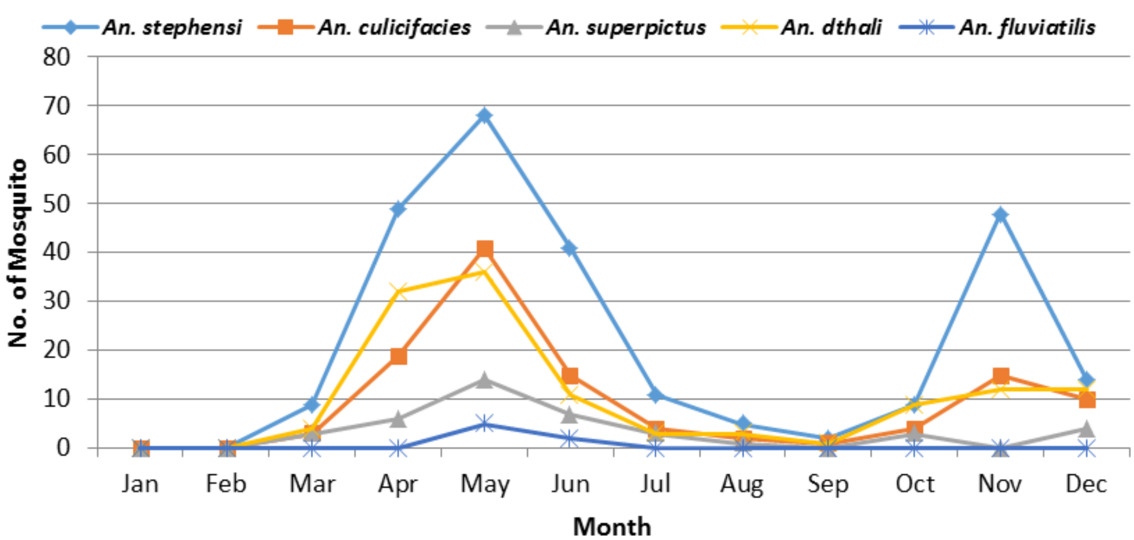

Fig. 2. The abundance of mosquitoes collected from indoors by total catch, in the study area, Qaleh-Ganj County, Kerman Province of Iran, 2015

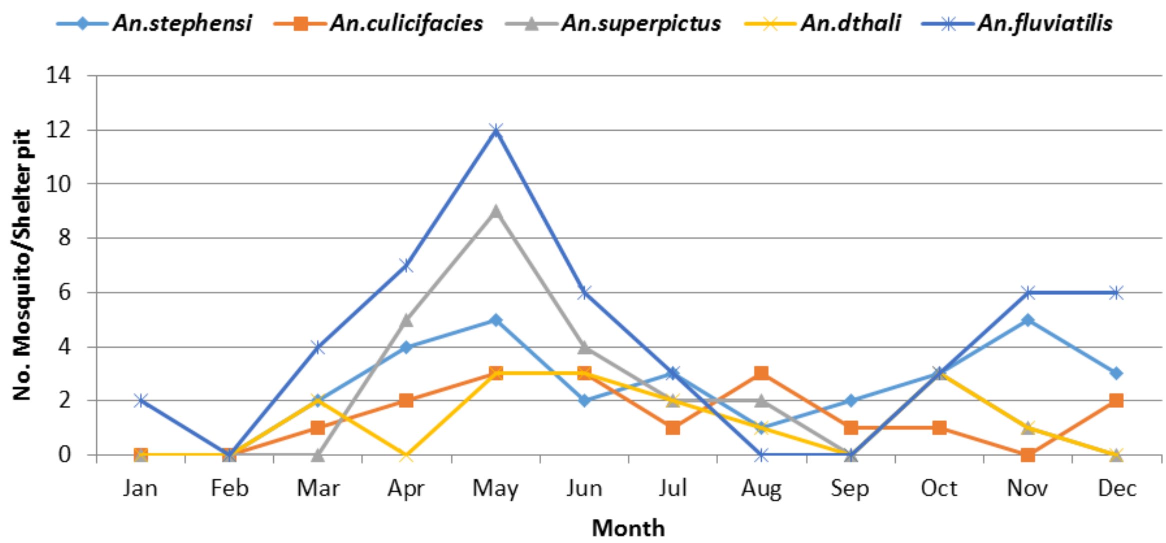

Fig. 3. The abundance of mosquitoes collected by shelter pit method in the study area, Qaleh-Ganj County, Kerman Province of Iran, 2015

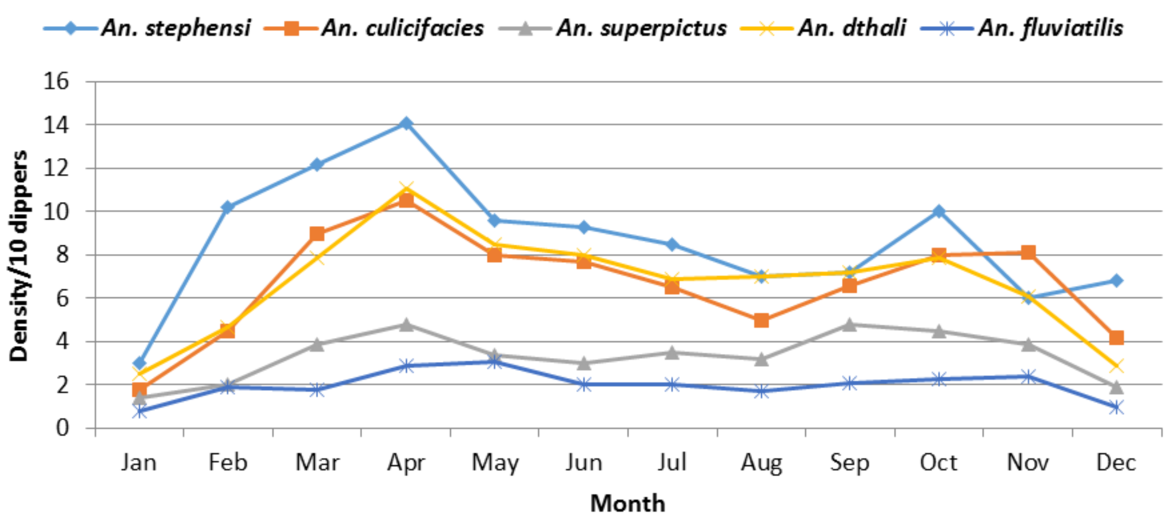

Fig. 4. Larval abundance per 10 dips in the study area, Qaleh-Ganj County, Kerman Province of Iran, 2015

Discussion

Anopheles stephensi

Among the 541 *Anopheles* species collected indoors by the total catch method, *An. stephensi* was the most predominant species. It was highly abundant in May. In contrast to our study, *An. stephensi* was sampled throughout the year in another study in southeastern Iran, with the peak of activity occurring in February and September–October (10). In another study, the peak of *An. stephensi* activity was found in May and November in Bandar Abbas in southern Iran (11). However, a recent study conducted in Jask County, south of our study area, reported two peaks of activity for *An. stephensi* in March–April and October (12). The peak of activity of *An. stephensi* is directly affected by weather variables especially temperature, which varies between different study areas.

The density of *Anopheles stephensi* collected in shelter pits was low. A similar result was reported in the southern Iran (10). With the night catch method, the highest number of *An. stephensi* mosquitoes was collected in the first third of the night between 9–10pm. In a study conducted at Khesht area in Fars Province in Iran, similar results were obtained (13). It should be mentioned that this method was only performed during the month of May. We recommend this method to be used at least in all months of the malaria transmission season in future studies in the study area. In this study, the number of gravid and semi gravid *An. stephensi* collected by shelter pit method was lower than the number of unfed and blood-fed ones, but in total catch method, the gravid and semi gravid ratio was more than 1. This shows that *An. stephensi* has a higher endophilic tendency. In a similar study conducted in Sistan and Baluchistan and Hormozgan Provinces of Iran, *An. stephensi* was found to be the most numerically dominant among the sampled mosquito species. Moreover, the number of gravid and semi gravid *An. stephensi* collected in the study, both in shelter pits and indoors, was higher than the number of unfed and blood-fed species. The investigators indicated that *An. stephensi* is more endophilic compared with the other species sampled in the study areas; with G+SG/F+UF ratio lower than one both outdoors and indoors (18)

Anopheles culicifacies s.l.

In our study, the number of gravid and semi gravid *An. culicifacies* s.l. collected by total catch method was lower than unfed and blood-fed ones, but in the pit shelters and outdoor window trap methods, the gravid and semi gravid ratio was more than 1. Moreover, the high number of unfed mosquitoes compared with gravid and blood-fed mosquitoes collected by indoor window trap method shows a high endophagic tendency of this species. In contrast to our findings, in a study conducted in Sistan and Baluchistan, the number of gravid and semi gravid *An. culicifacies* s.l. collected indoors was lower than the number of unfed and blood-fed mosquitoes. The researchers also reported the same abdominal state findings for *An. culicifacies* s.l. mosquitoes collected from pit shelters (14). Another study conducted in Sistan and Baluchistan indicated that this *Anopheles* species is more endophilic (15, 16). Consistent with our study, based on ventral aspect ratio (G+SG/U+F), another study stated that *An. culicifacies* s.l. prefers both indoors and outdoors as their resting places (10). Similar results have also been reported in different regions of India (17).

Although this species had two peaks of activity during March–April and October–November, a study conducted in Sistan and Baluchistan in Iran, larvae of *An. culicifacies* s.l. were more abundant in April to December in rice fields with palm trees (18). In terms of breeding sites, this species was mostly collected from sites which have turbid water, semi-shade and have no vegetation. The mean temperature and pH of the study area were 28 ^{o}C and 7.6, respectively. An earlier study conducted in the south of our study area reported clear, no vegetation, sunny, and natural breeding sites with average temperature and pH of 25–30 ^{o}C and 7.14–8.90, respectively as climate preferences of this species (19).

Anopheles dthali

This species was most abundant in May, June and October during the study period. A previous study conducted in a relatively warmer area in Iran reported that the peak of activity of this species occurs in April and September–October, which is one month earlier than observed in our study (20). It seems that weather conditions play crucial role in the period of activity of this species, making it necessary to take into consideration the weather condition of the area before planning any vector control measures. The highest abundance of this species, using night catch method with human and animal baits, occurred in the first third of the night between 8–9pm. We thus recommend that people be encouraged to use bed nets or avoid outdoors at these times.

Larvae of *An. dthali* were mostly found in stagnant, turbid waters, natural breeding sites in river banks which have no vegetation, and semi-shade. In agreement with our results, this *Anopheles* species was sampled from breeding sites without vegetation (19), but unlike our findings, it was mostly collected from clear and sunny sites. Although a previous study reported that breeding places with temperature ranging between 13 ºC and 28 ºC and pH between 6.9 and 8.0 are preferred by this species (21), the mean temperature and pH of our study sites were 31.5% and 7.6, respectively. Another study also reported that about 50% of *An. dthali* larvae were collected from breeding sites with temperature ranging between 25.1–30 ºC and pH between 7.14 and 8.20 (19).

Anopheles fluviatilis s.l.

This *Anopheles* species is also considered as a secondary vector of malaria in most of its' distribution areas in Iran (22). In some studies, it was captured in outdoor habitats and on animal baits (23). We found *An. fluviatilis* s.l. was the most predominant species (35.76%) caught by pit shelter method, confirms its exophilic habit. It was also collected by night catch method using human and animal baits in May and November, mostly in the first third of the night between 9–10pm. The number of unfed and blood-fed *An. fluviatilis* s.l. was higher than the number of gravid and semi gravid *An. fluviatilis* s.l. captured by total catch method, which shows high exophilic tendency of this species.

Breeding sites for this species in our study area were natural water bodies, stagnant, turbid waters, and semi-arid areas without vegetation. Mean temperature and pH of the study sites were 22 ºC and 7.6, respectively. This species usually breeds in fresh, slow flowing or even stagnant waters (22).

Anopheles superpictus s.l.

The highest collection of this species occurred in the first third of the night between 8 to 9pm. In our study, the number of unfed and blood-fed *An. superpictus* s.l. was lower than the number of gravid and semi gravid *An. superpictus* s.l. captured by total catch method, which shows high exophilic tendency of this species. Contrary to our results, a study conducted in south west of Iran, reported endophilic habit of this species in agreement with earlier studies (24).

Studies on the larval habitats of *An. superpictus* s.l. in Iran revealed that this species is frequently abundant in river banks, both in natural breeding sites and artificial habitats created by human activities like mining pools (22). We collected this species from natural breeding sites, stagnant, turbid waters and semi-arid areas with no vegetation. Mean temperature and pH of the collection sites of this species were 26.5 ºC and 7.6, respectively. An earlier study reported clear, sandy bed, full sunlight and natural water bodies as the main breeding sites for *An. superpictus* s.l. (19).

Insecticide resistance

Our results show that *Anopheles stephensi* is quite resistant to DDT, tolerant to malathion and susceptible to deltamethrin and Propoxur. This result is in accordance with that of a study conducted in Chabahar (25), in which *An. stephensi* was found to be resistant to DDT but tolerant to malathion. Resistance of this species to DDT in Iran has been reported in the last two decades (11, 13, 26-30). Fortunately, in our study, this species was found to be susceptible to both deltamethrin and propoxur insecticides. Thus, these insecticides can be used in indoor residual spraying for vector control against this main endophilic malaria vector. Furthermore, it is recommended that more susceptibility tests be conducted on other species with high endophilic and endophagic tendencies, especially *An. culicifacies* s.l. and *An. dthali*, in the area.

Conclusion

In conclusion, five malaria vectors are active in the study area. Regular entomological studies are recommended to monitor their monthly activity and susceptibility status to insecticides. The peak of host seeking activity of the indoor mosquitoes occur in the first 3rd of the night, therefore, community-based training programs should be designed and implemented to enforce proper use of mosquito nets and personal protective measures against mosquitoes.

Country has a long history of work on malaria and publication of several papers on different aspects of malaria including insecticide resistance monitoring, sibling species, molecular study, new record, novel methods for vector control, faunestic study, use of plants for larval control, using bednets and long lasting impregnated nets, morphological studies, malaria epidemiology, ecology of malaria vectors, biodiversity, community participation, vector control, repellent evaluation, anthropophilic index of malaria vectors, training is designated as malaria training center by WHO. There are several reports on different aspects of malaria vectors recently (31-118).

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