Original Article The Fauna and Ecology of Mosquitoes (Diptera: Culicidae) in Western Iran

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Abstract

Background: The aim of this study was to obtain new data which would be valuable to develop programs for future planning of mosquito controls in in western Iran.

Methods: Larvae and adult collections were carried out from different habitats using standard dipping and animal baited trap methods during May, June and July 2012 in two provinces (Kurdistan and Kermanshah) in the west of Iran. Characteristics of breeding places were studied based on the habitat type (River edge, Ground pool), water conditions (clear or turbid, stagnant or running), vegetation (With or without vegetation), water temperature, sunlight exposure (full or partial sunlight) and so on.

Results: Overall, 4081 third- and fourth-instars larvae and 2013 Adult were collected. Five genera and eleven species of the family Culicidae were identified. Mosquitoes collected in larval and adult stages including, *Anopheles maculipennis* s.l. (4.1%, 4.42%), *An. superpictus* (1.35%, 1.39%), *An. turkhudi* (1.75%, 1.68%), *Aedes vexans* (2.5%, 8.78%), *Culex hortensis* (1.59%, 1.04%), *Cx. mimeticus* (5%, 2.38%), *Cx. pipiens* (16.5%, 8.15%), *Cx. theileri* (10%, 46.4%), *Culiseta longiareolata* (24%, 4.27%), and *Cs. subochrea* (24%, 4.27%) and *Ochlerotatus caspius* s.l. (9.1%, 21.46%). *Culiseta longiareolata* and *Cs. subochrea* found predominant species in larval collection, whereas *Culex theileri* was dominant in Adult collection. *Anopheles turkhudi* is reported for the first time in Kermanshah Province. **Conclusion:** Due to the geographical location of the two provinces, extensively studies with emphasis on mosquito ecology, to having comprehensive and up to date information is essential.

Keywords: Culicidae, Larval habitat, Fauna, Iran

Introduction

The family Culicidae (Diptera) comprises at least 3531 species representing 112 genera divided into two subfamilies, Culicinae and Anophelinae (Harbach 2013). According to the newest checklist of Iranian mosquitoes, 64 species representing seven genera occur in the country (Azari-Hamidian 2007). Oshaghi et al. (2008) recently identified *Anopheles superpictus* Grassi as a complex of three genotypes (X, Y, and Z). Naddaf et al. (2010, 2012) reported *An. fluviatilis* James species U from Fars Province. Besides, Mehravaran et al. (2011) recorded the species U in southeastern Iran. Mosquito-borne diseases such as malaria, West Nile, as well as dirofilariasis are a major public health threat in Iran (Naficy and Saidi 1970, Azari-Hamidian et al. 2007, Ahmadnejad et al. 2011). Malaria cases in Kurdistan Province, western Iran, increased from 1994 to 1996 (543, 633, and 236, respectively), however, they dramatically decreased after that. There were no cases of malaria in 2009, and just 2 and 3 cases in 2010 and 2011, respectively. All were imported from outside of the Province (Banafshi et al. 2013).

There are only three recent studies on mosquitoes in Kurdistan Province, including

the faunistic investigation of anophelines by Vahabi et al. (2001) and culicines in Sanandaj County by Moosa-Kazemi et al. (2010). Banafshi et al. (2013) studied fauna and ecology of mosquito larvae in the Province. By now, 6 genera and 18 species of mosquitoes have been recorded in Kurdistan Province (Macan 1950, Lotfi 1973, Zaim 1987, Vahabi 2001, Moosa Kazemi et al. 2010, Banafshi et al. 2013). Seven species of Anopheles have been found in the province (Macan 1950, Vahabi 2001) including An. algeriensis Theobald, An. claviger (Meigen), An. maculipennis s.l., An. marteri Senevet and Prunnelle, An. sacharovi Favre, An. sergentii (Theobald), and An. superpictus Grassi. Of the Maculipennis group, An. maculipennis s.l. and An. sacharovi have been recorded in the Province based on morphological characters. Zaim (1987) mentioned 6 species of Culex Linnaeus and 2 species of *Culiseta* genus in the province: *Culex* hortensis Ficalbi, Cx. mimeticus Noe, Cx. perexiguus Theobald, Cx. theileri Theobald, Cx. pipiens Linnaeus, Cx. territans Walker, Culiseta longiareolata (Macquart), and Cs. subochrea (Edwards). Moosa-Kazemi et al. (2010) recently reported Aedes vexans (Meigen) and Ochlerotatus caspius (Pallas) s.l. in Kurdistan Province for the first time. Zaim and Cranston (1986) noted that a female specimen from Koolan of Marivan, Kurdistan Province, which had been previously identified by Lotfi (1973) as Cx. impudicus Ficalbi, was possibly a new species of *Coquillettidia* Dyar.

There is little and scattered information about the fauna of Culicidae in Kermanshah, including at least 4 genera and 22 species (Macan 1950, Manouchehri et al. 1976, Zaim 1987, Harbach 1988). Eleven species of Anopheles have been reported in the Province including An. algeriensis, An. claviger, An. dthali Patton, An. fluviatilis, An. marteri, An. maculipennis s.l., An. pulcherrimus Theobald, An. turkhudi Liston, An. superpictus, An. sacharovi, An. stephensi Liston (Macan 1950, Manouchehri et al. 1976, unpublished data from Institute of Malariology, Tehran University of Medical Sciences).

Certainly, Kermanshah Province has not been recognized as an endemic region for Malaria at least in 4 past decades, but some sporadic cases were reported in several regions (Vejdani 2012).

This paper presents the results of recent surveys of Culicidae mosquitoes from samples taken from a range of larval breeding sites in which they occur in nature, and adult mosquitoes captured in residential and sparsely populated areas by animal bait traps collection in the two provinces. The aim was to obtain new data which would be valuable to develop programs for future planning of mosquito control in this area of western Iran.

Materials and Methods

To better understand the Culicidae fauna of Kurdistan and Kermanshah Provinces and gather information on their distribution and the kinds of larval breeding sites in which they occur in nature, a descriptive cross-sectional study was performed from May, June and July 2012 in western Iran. This study took place in three randomly selected rural villages in Kermanshah Province (34°31' 76" N 47°08'69" E) and three villages in Kurdistan Province (35°.31'13''N 46°99' 60"E). The district is bounded by the Iraq in the west, Hamadan and Lorestan Provinces in the east. Ilam Province in the south, and western Azerbaijan and Zanjan Provinces in the north (Fig. 1). In 2011 the maximum and minimum mean monthly temperatures in Kurdistan were 30 °C and -1 °C in July and February, respectively. The total annual rainfall was 800 mm, the minimum of 3.9 mm in April and maximum of 126.2 mm in November. The mean annual relative humidity was 39%. In the same time the maximum and minimum mean monthly temperatures in Kermanshah were 28.7 °C and 6.9 °C in August and December, respectively. The total annual rainfall was 406.4 mm, the minimum of 0.1mm in July and maximum of 155.7 mm in November. The mean annual relative humidity was 41%. The main economic activity of the people is agriculture and husbandry of cow and goats. Three villages in the different topographical areas, including: Hassan-Abad-e-Sofla, Hassan-Abad-e-Olia. and Hossain-Abad in Kurdistan Province and Cham-e-Seved Sadegh, Kaleh Ghandi and Saleh Abad in Kermanshah Province were selected. To study the mosquito fauna, Sampling was carried out using dipping method for collecting larvae and animal baited traps using suction tubes for adult mosquitoes. Female mosquitoes were collected primarily through the use of suitable bait (Cow) by suction tube, between 20.00 and 03.00 h monthly in fixed animal shelter randomly on each village. Before catches, all cows were fixed by tight were closed the shelter. The collected mosquitoes were transferred to cups container with moist cotton. All collected mosquitoes were pinned and then identified using the keys of Azari-Hamidian and Harbach (2009). Mosquito name abbreviations are cited based on Reinert (2009).

Larval habitats present within 500 m radius of each village were identified, and sampled for mosquito larvae two per month. Three randomly selected breeding places located within a 500 m radius of the study site were also included and formed the sampling unit for mosquito larvae. All larvae were collected from natural habitats, generally feeding with seepage water, such as swamps, seepages, streams, river banks, drying river beds, pools, and grasslands. Up to 20 dipper samples were taken at intervals along the edge of each larval breeding place using a standard mosquito dipper (350 ml) depending on the size of the habitat.

The larvae for each habitat were placed separately in whirl packs and transported to the laboratory where they were sorted by

genera and instars counted and recorded. All third and fourth instars of mosquitoes were preserved in lactophenol solution and later identified morphologically (Shahgudian 1960, Azari-Hamidian and Harbach 2009). In order to determine the association of mosquito larvae, samples for each habitat type were pooled together in each site irrespective of date of collection. Physical characteristics of the larval habitats including distance to the nearest homestead, habitat stability, water depth, turbidity, presence of other aquatic invertebrates, and presence of floating, emergent, and submerged vegetation were recorded. Water depth was measured using a metal ruler. The habitat was recorded as unstable if it did not contain water during the next weekly sampling visit. The Culicinae larvae along with the description of their breeding sites were sent to the School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.

Results

A total of 2013 adult and 4081 larvae of mosquitoes were collected, in which 5 genera and 11 species were recognized including, Aedes vexans, Ochlerotatus caspius s.l., Anopheles maculipennis s.l. An. superpictus, An. turkhudi, Culex hortensis, Cx. pipiens, Cx. mimeticus, Cx. theileri, CS. longiareolata, and Cs. subochrea. Results of adult and larvae mosquitoes collected presented in Table 1 and 2. These species were collected from the plain, slope and mountainous areas of the Kurdistan and Kermanshah Provinces on 6 six selected villages. An. turkhudi is recorded for the first time in Kermanshah Province. Culiseta longiarolata collected only in the adult stage in Hossain Abad of Kurdistan Province.

The number and location of the mosquito larvae at the ecological station of this area is shown in Table 1. In the larval collection, 3913 Culicidae larvae were collected, both *CS. longiareolata* (25.9%), and *Cs. subochrea* (25.9%) predominated, followed by *Cx. pipiens* (17.56%), *Cx. theileri* (10.68%), *Ochlerotatus caspius* s.l. (8.35%), *Cx. mimeticus* (5.26%), *Aedes vexans* (3.066%), *An. turkhudi* (1.84%), *An. superpictus* (1.43%), and *Cx. hortensis* (1.66%) (Table 1).

Number and prevalence of mosquitoes catches by animal bait trap through the night, are shown in Table 2. Overall, *Culex theileri* was found predominant species (46.4 %), followed by *Ochlerotatus caspius* s.l. (21.46%), *Aedes vexans* (8.78%), *Cx. pipiens* (8.14%), *An. maculipennis* s.l. (4.42%), *CS. subochrea* (4.27%), *Cx. mimeticus* (2.38%), *An. turkhudi* (1.68%), *An. superpictus* (1.39%), *Cx. hortensis* (1.04%), and *Cs. longiareolata* (0.04%).

Two species including *Culex theileri*, and *Oc. caspius* s.l., with 46.4% and 21.46% respectively formed about 68% of the total adult collections. The other species were less frequent and in total accounting for 32% of the collections (Table 2).

Culex theileri was the most frequent mosquitoes collected in this area, with total of 934, and 418 specimens, by animal bait trap and larval collection respectively. Feeding activity of Oc. caspius s.l. was increased in the first third of the night and decrease slowly in the second and the end of the third night whereas blood feeding activity of CS. subochrea was increased in the second and end third of night and then decreased in first third of the night. Culex theileri was active thought the night and others mosquitoes species such as Cx. pipiens, Cx. hortensis, Cx. mimeticus, and Ae. vexans were active in the second third of the night. Blood feeding activity of An. superpictus and An. turkhudi was increased in the second and end third of night and then decreased in first third of the night.

Two species including *Culex theileri*, and *Oc. caspius* s.l. accounted for 82.02% of the adult collection after the sun set in the animal bait trap collection whereas in the se-

cond third of the night these species with *Cx. pipiense* were predominant and accounted for 76.22% of the adult collection. In the end of night *Culex theileri*, *Oc. caspius* s.l., and *Ae. vexans* were predominant and about 85.36% of the total mosquito catches.

The larval habitat characteristics and occurrence percentages of different mosquito larvae and their association are shown in Table 3 and Table 4. Anopheles maculipennis s.l. was collected from 45 larval breeding sites. This species was found mostly in transient, stagnant, clear water in natural habitats without vegetation, partial sunlight. Natural habitats without vegetation transient, stagnant, clear water in, partial sunlight were found for the species of An. superpictus. Anopheles turkhudi were collected from 4 larval breeding sites. This species was found most in transient, stagnant, clear water in natural habitats without vegetation, partial sunlight. Natural habitats without vegetation, permanent, slow ranning, turbid water in, full sunlight were found for the species of Ae. vexans. Culiseta longiareolata was collected from 14 larval breeding sites. This species was found most in transient, stagnant, clear water in natural habitats without vegetation, partial sunlight. Natural habitats without vegetation, permanent, slow running, turbid water in, full sunlight were found for the species of Cs. subochrea.

Species	Ku	rdistan sel villages	ected	Kerman	n	%		
	Hassan- Hassan- Hos Abad-e- Abad-e- A		Hossain- Abad	Cham- e-Seyed	Kaleh Ghandi	Saleh Abad	_	
	Sofla,	Olia,		Sadegh,				
An. maculipennis	9	12	14	19	101	13	168	4.1
s.l.								
An. superpictus	-	10	14	19	-	13	56	1.35
An. turkhudi	-	-	-	18	51	3	72	1.75
Ae. vexans	-	9	-	98	4	9	120	2.5
Cx hortensis	-	12	14	19	20	-	65	1.59
Cx. mimeticus	8	12	114	-	19	53	206	5
Cx. pipiens	12	100	119	156	229	69	685	16.5
Cx. theileri	114	48	124	26	101	5	418	10
Cs. longiareolata	221	146	231	58	223	103	982	24
Cs. subochrea	-	265	-	230	198	289	982	24
Oc. caspius s.l.	89	12	98	19	101	8	327	9.1
Total	453	626	728	662	1047	565	4081	100

 Table 1. Composition and localities of the larvae of mosquitoes collected in the West of Iran, May–July 2012



Fig. 1. Map of Iran indicating the location of the study area in Kurdistan and Kermanshah Provinces situated in western Iran

1= Hossain-Abad	2= Hassan-Abad-e-Olia	3=Hassan-Abad-e- Sofla
4= Cham-e- Seyed Sadegh	5=Kaleh Ghandi	6= Saleh Abad

Species	K	urdiatan sel	ected	Kerm	n	%			
		villages			_				
	Hassan- Abad-e- Sofla,	Hassan- Hassan- Hossain Abad-e- Abad-e- Abad Sofla, Olia,		Cham-e- Kaleh Seyed Ghan Sadegh,		Saleh Abad			
An. maculipennis s.l.	3	14	18	6	39	9	89	4.42	
An. superpictus	2	6	5	8	2	5	28	1.39	
An. turkhudi	-	-	-	18	13	3	34	1.68	
Ae. vexans	-	13	26	98	20	19	176	8.78	
Cx hortensis	-	3	5	2	6	5	21	1.04	
Cx. mimeticus	8	10	9	-	9	12	48	2.38	
Cx. pipiens	26	26	19	67	19	7	164	8.147	
Cx. theileri	201	262	199	133	89	50	934	46.4	
Cs. longiareolata	-	-	1	-	-	-	1	0.04	
Cs. subochrea	-	7	-	29	31	19	86	4.27	
Oc. caspius s.l.	89	17	98	19	101	108	432	21.46	
Total	329	358	380	380	329	237	2013	100	

 Table 2. Number and prevalence of adult culicidae mosquitoes catches by animal baited trap in the West of Iran, May–July 2012

 Table 3. The larval habitat characteristics and occurrence percentages of different mosquito larvae in the West of Iran, May–July 2012

Larval breeding site: characteristics and habitats	An. maculipennis s.l. (%)	An. superpictus (%)	Ân. turkhudi (%)	Ae. vexans (%)	Cx hortensis (%)	Cx. mimeticus (%)	Cx. pipiens (%)	Cx. theileri (%)	Cs. longiareolata (%)	Cs .subochrea (%)	Oc. caspius s.l. (%)
A) Habitat type											
Permanent	40.5	89.6	76.4	99.4	54.6	99	74.5	58.6	99	100	100
Transient	59.5	10.4	23.6	0.6	45.4	1	25.5	41.4	1	0	0
B) Water conditions											
Stagnant water	95.5	95.7	95.2	0.6	99.8	40.5	63.4	92.6	98	95.6	99
Slow-running water	4.5	4.3	4.8	99.4	0.2	59.5	36.6	7.4	2	4.4	1
C) Water situation											
Clear	99.5	84.2	81.7	0.8	95.5	98	98	95.5	97	87.6	98
Turbid	0.5	15.8	18.3	99.2	4.5	2	2	4.5	3	12.4	2
D) Vegetation situation											
With vegetation	39.4	44.8	39.6	0.9	3.5	81.5	67.2	43.6	100	99	100
Without vegetation	51.6	56.2	59.4	99.1	96.7	18.5	22.8	56.4	0	1	0
E) Sunlight exposure											
Full sunlight	6.4	6.3	3.9	94.5	69	65.5	64.6	8.6	1	1	0
Partial sunlight	93.6	93.7	96.1	5.5	31	34.5	33.4	81.4	99	99	100
F) Habitat Type											
Natural	82.4	99	65	89	71.3	98	73	60.5	89	99	100
Artificial	17.6	1	35	11	28.7	2	27	39.5	11	1	0

Larval breeding site: characteristics and habitats	Total Occasions	An. maculipennis s.l.(%)	An. superpictus (%)	An. turkhudi (%)	Ae. vexans(%)	Cx hortensis (%)	Cx. mimeticus (%)	Cx. pipiens (%)	Cx. theileri (%)	Cs. longiareolata (%)	Cs. subochrea (%)	Oc. caspius s.l. (%)
An. maculipennis s.l. (%)	45	*	4	4	0	3	8	14	8	2	1	2
An. superpictus (%)	6	4	*	1	1	2	2	1	2	1	1	4
An. turkhudi (%)	13	4	1	*	0	0	1	1	0	1	2	1
Ae. vexans (%)	7	0	0	0	*	1	2	1	0	1	1	2
Cx hortensis (%)	12	3	2	0	1	*	1	2	2	3	1	0
Cx. mimeticus (%)	22	8	2	1	2	1	*	5	1	1	1	2
Cx. pipiens (%)	32	14	1	1	1	2	5	*	4	2	1	2
Cx. theileri (%)	23	8	2	0	0	2	1	4	*	1	0	0
Cs. Longiareolata (%)	14	2	1	1	1	3	1	2	1	*	0	3
Cs. Subochrea (%)	6	1	1	2	2	1	1	1	0	0	*	1
Oc. caspius s.l. (%)	18	2	4	1	1	0	2	2	0	3	1	*

Table 4. The association of different mosquito larvae with other species in West of Iran, May–July 2012

Discussion

By now 7 species of Anopheles have been recorded in Kurdistan Province and 11 species in Kermanshah Province (Macan 1950, Vahabi 2001, Manouchehri et al. 1976, Moosa-Kazemi et al 2010, Banafshi et al 2013). In addition two species including An. stephensi and An. pulcherrimus has been reported in Kermanshah Province in past decades (Manouchehri et al. 1976). Three Anopheles species were collected in this investigation, including An. maculipennis s.l., An. superpictus, and An. turkhudi. The species of An. algeriensis, An. marteri, An. sacharovi, and An. sergentii were not found. In the previous study, Vahabi (2001) found only one adult An. marteri in the Province, and An. algeriensis and An. sergentii were not common species. He also did not distinguish the Maculipennis Group specimens in larval stage and identified the adults of An.maculipennis s.l. and An. sacharovi using morphological characters. An. maculipennis reported as the complex species and molecular study was carried out to determine the species complex in the northern areas of the country (Sedaghat et al. 2003). Anopheles turkudi is reported in Kermanshah Province for the first time, this species found in 15 Provinces in central plateau, western, southwestern, eastern, southeastern, and southern Iran (Saghafipour et al. 2012). However, the occurrence of *An.* turkhudi in the Islam Abad-e-Gharb, Kermanshah Province was mentioned only in the unpublished documents of the School of Public Health and the Institute of Public Health Research (Saebi 1987, unpublished data).

In our study only *An. maculipennis* s.l of the Maculipennis Group was identified using the mean number of seta 2 branches of the fourth- and fifth-abdominal segments of larvae, which is 36.8 in *An. sacharovi* whereas it is 16.5 in *An. maculipennis* s.l. (Azari-Hamidian and Harbach 2009). In our study, also *An. superpictus* found 1.35% and 1.39% of larval and adult collection respectively. Vahabi (2001) also reported *Anopheles superpictus* (49.8%) and the *An. maculipennis*

group (40.5%) as the most abundant anopheline species in Kurdistan Province. In parallel, Mousakazemi et al. (2000) reported the An. maculipennis s.l. (including 2.08% of the whole samples) and An. superpictus (0.16%) from rice fields in Lenian and Mobarakeh areas (Isfahan Province, central Iran). Abai et al. (2007) found An. maculipennis s.l. one of the most abundant species in East Azerbaijan Province, northwestern Iran. Azari-Hamidian et al. (2009) found the species of An. maculipennis s.l. (19.0%) in Ardebil Province, northwestern Iran. Out of 11 species of culicine mosquitoes, which had been recorded previously in Kurdistan Province (Zaim. 1987, Vahabi 2001, Moosa-Kazemi et al. 2010, Banafshi et al. 2013) 8 species were collected in this investigation. Culex theileri, Oc. caspius s.l., and Ae. vexans were the most prevalent species respectively, and collected in almost all of the sampling methods (Table 1 and 2).

In this study, two species of the tribe Aedini including, *Oc. caspius* s.l. and *Ae. vexans* were collected in larvae and adult stages. There is no information about the *Oc. caspius* sibling species (A or B) in the country (Azari-Hamidian 2007).

The species of CS. longiarolata was one of the most frequent culicine mosquitoes collected in this area, with total of 982 specimens, by larval collection. This species was the fewest culicine mosquitoes collected at adult collection. Culex pipiens can be separated from Cx. torrentium, and Cx. vagans by Harbach's Key (1988) using seta 1-III-V, seta 1-M, seta 1-X, seta 1-C, and some other characters. Based on this key, in this survey only Cx. pipiens was identified. Results of this survey were almost as the same of findings by previous study in Kurdistan Province (Zaim 1987, Vahabi 2001, Moosa-Kazemi et al. 2010, Banafshi et al. 2013). Ochlerotatus caspius s.l. and Ae. vexans was reported as the potential vectors of human and domesticated animal pathogens (Horsfall 1955,

Harbach 1988). The present study confirms the occurrence of these species. The species of Cx. theileri was one of the culicine mosquitoes collected in the Kermanshah, with total of 934, and 418 specimens, by adult and larval collection respectively. This mosquito is the more prevalent species at higher altitudes and in rural areas of Isfahan (Mousa-Kazemi et al. 2000), East Azerbaijan (Abai et al. 2007), Ardebil (Azari-Hamidian et al. 2009) and West Azerbaijan Provinces (Khoshdel-Nezamiha et al. 2014). Azari-Hamidian et al. (2009) reported Cx. theileri as 27.1% in Ardebil Province, northwestern Iran, more than any other culicidae species, our finding also showed the most prevalent species in western Iran. In eastern Turkey, Cx. theileri was the most prevalent *Culex* species (Aldemir et al. 2009, Alkan and Aldemir 2010). In agreement with previous investigations (Mousa-Kazemi et al. 2000, Ghavami and Ladonni 2005, Abai et al. 2007, Azari-Hamidian et al. 2009, Khoshdel-Nezamiha et al. 2014) this species is most abundant mosquito in the northwestern Iran. Observations made during the present study agree with those of Simsek (2004) who noted that Cx. theileri in Turkey prefers large natural larval habitats that are generally present in rural areas.

Conclusion

In our research, some potential vectors in medical and veterinary importance identified such as *Aedes. vexans, An. maculipennis* s.l., *An. superpictus, Cx. pipiens, Cx. theileri*, and *Oc. caspius* s.l. On the other hand, malaria and West Nile have been reported in this area shows the importance of mosquito control programmes (Ahmadnejad et al. 2011, Vejdani 2012). The ecology of other mosquitoes in Iran needs to be studied extensively.

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