

Bionomic studies of the Mosquitoes (Diptera: Culicidae) in Kermanshah Province, Western IranSeyed Hassan Moosa-Kazemi ^{1*}, Faranak Firoozfar ^{2,3}^{*}¹Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran² Department of public health, North Khorasan University of Medical Sciences, Bojnurd, Iran³Vector-borne Diseases Research Center, North Khorasan University of Medical Sciences, Bojnurd, IranE-mail: moosakazemi@sina.tums.ac.ir

Abstract: Ecological data are important in the vector control management of mosquitoes specially in tropical area. The aim of this study was to design of programs for future planning of mosquito control. This investigation was carried out to study the mosquito fauna and ecology in Kermanshah Provinces, western Iran, during May–June 2014. Mosquitoes were collected using the standard dipping and night mosquitoes landing catch methods. Five genera and 10 species of the subfamilies, Culicinae and Anophelinae (Diptera: Culicidae) were collected, and morphologically identified including; *Aedes vexans* Meigen, *Anopheles turkhudi* Liston, *An. superpictus* Grassi, *Culex hortensis* Ficalbi, *Cx. pipiens* Linnaeus, *Cx. mimeticus* Noe, *Cx. theileri* Theobald, *Culiseta longiareolata* Macquart, and *Cs. subochrea* Edwards and *Ochlerotatus caspius s.l.* Pallas. In this study the *Cx. theileri* was predominant and comprised 10.68%, and 48.54% of larvae and adult collection, respectively. It is noteworthy that *Aedes vexans* and *Ochlerotatus caspius s.l.* had been recorded in Kermanshah, western Iran, high lighting the deficient knowledge of the fauna and distribution of *Aedinae* and *Ochelotinae* mosquitoes in this area.

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Key words: Culicidae; Mosquito; Fauna; Ecology; Kermanshah; Iran.

Introduction

Mosquito-borne diseases such as malaria, West Nile and Sindbis viruses, Japanese encephalitis, Rift Valley fever as well as *Dirofilaria immitis* (dog heart worm) and *D. repens* (dirofilariasis) are a major public health in tropical areas of the world, Asian countries, including Iran (Naficy & Saidi 1970, Saidi et al. 1976, Moosa-Kazemi et al. 2006, 4 Azari-Hamidian 2007).

Malaria transmission in west of Iran occurs in summer, most of the malaria cases were observed from border line with Iraq (Beklemishev & Gontaeva 1943). By now, 64 species, three sub species and seven genera of mosquitoes have been reported in Iran (Azari-Hamidian 2007). The old report about the culicidae in this area mentioned by Ghaffari in 1954. The species of *An maculipennis*, *An sacharovi*, *An superpictus* has been reported (Ghaffari 1954). *Anopheles maculipennis* and *An superpictus* was reported by Shaghudian in 1960 subsequently. *Anopheles dthali* was reported by Manuchehri et al 1972. *Culex*. (Cux.) *mimeticus*, *Cx.* (Cux.) *perexiguus*, *Cx.* (Cux.) *pipiens*, *Cx.* (Cux.) *theileri*, *Cx.* (Cux.) *tritaeniorhynchus*, *Cx.* (Mai.) *hortensis*, *Cs.* (All.) *longiareolata*, *Uranotaenia* (Pfc.) *unguiculata* was reported by Zaim et al 1987. *Anopheles superpictus* was reported by Edrissian et al 1985. *Anopheles claviger*, *An. superpictus*, *An marteri*, *An turkhudi* was reported by Saebi 1987. The species of *Aedes vexans*, *Ochlerotatus caspius s. l.* *Anopheles maculipennis s. l.*

An. superpictus, *Cx. hortensis*, *Cx. pipiens*, *Cx. mimeticus*, *Cx. theileri* *Culiseta longiareolata*, *Cs. subochrea* had been reported in western Iran (Moosa-Kazemi et al. 2015).

Six genera of *Anopheles*, *Culex*, *Culiseta*, *Uranotaenia*, *Aedes* and *Ochlerotatus* and 18 species of the subfamilies Culicinae and Anophelinae have been reported in Kermanshah Provinces as follow: *Anopheles claviger* Meigen, *An dthali* patton, *An. maculipennis* Meigen s. l., *An. marteri sogdianus* Keshishian, *An. sacharovi* Favre, *An. superpictus* Grassi, *An. turkhudi* Liston, *Culex hortensis* Ficalbi, *Cx. perexiguus* Theobald, *Cx. pipiens* Linnaeus, *Cx. mimeticus* Noe, *Cx. torrentium* Martini, *Cx. theileri* Theobald, *Cx. tritaeniorhynchus* Giles,, *Culiseta longiareolata* Macquart, and *Cs. subochrea* Edwards and *Uranotaenia unguiculata* Edwards(Zaim 1987, Saebi 1987).

Two genera of *Aedes*, and *Ochlerotatus*, have found in Kermanshah previously (Moosa-Kazemi et al. 2015), although were mentioned in the checklist of Iranian Culicidae previously(Saebi 1987). There is only one recent study on the mosquitoes in the county in relation to westrn Iran(Moosa-Kazemi et al. 2015). There are no more references on the culicidae mosquitoes in this part of Iran. This paper presents the first report the results of recent surveys of culicidae mosquitoes from samples taken from a range of breeding sites in which they occur in nature, and adult

culicidae mosquitoes captured in residential and sparsely populated areas by Animal bait trap collection. The aim was to obtain new data which would be valuable to develop programs for future planning of mosquito control in western Iran.

Materials & Methods

To better understand the Culicidae fauna of Kermanshah Provinces and gather information on their distribution and the kinds of larval breeding sites in which they occur in nature, a field trial study was performed from May, June and July 2014 in the western Iran.

This study took place in four randomly selected rural villages in Kermanshah district (34°23'N, 47°03'E). The district is bounded by the Iraq in the west, Hamedan provinces in the east, Lorestan & Illam Province in the south, and Kurdistan province in the north. In 2014 the maximum and minimum mean monthly temperatures in Kermanshah 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 0.1 mm 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. On the basis of available epidemiological data from the Kurdistan and Kermanshah Health Centers, there are no villages were short-listed and entomological surveys were carried out. On this basis, four villages in Kermanshah were selected. To study the mosquito fauna, sampling was carried out by dipping method for collecting larvae and night catch from animal baited trap (ANH) for adult mosquitoes. The specimens were collected from three villages in the different topographical areas. Including; Cham-e- Seyed Sadegh, Kaleh Ghandi and Saleh Abad in Kermanshah Province. Night catch mosquitoes collection were conducted between 20.00 and 03.00 h monthly in fixed animal shelter randomly on each villages. Before catches, all cows were fixed by tight were closed the shelter. Night catch was occurred by suction tube. After catches the mosquitoes by suction tube, the mosquitoes were transferred to cups container, was kept closed and then collected with forceps and placed in Petri-dishes lined with moist cotton. Attempts were also made to catch mosquitoes landing on animals under bed net using mouth suction tube.

All collected mosquitoes were pinned and then identified using the keys of Shaghdian (Shaghdian

1960). Zaim and Cranston (Zaim 1987), Harbach (Harbach 1988), and Darsie and Samanidou-Voyadjoglou (Darsie & Samanidou-Voyadjoglou 1997), Azari-Hamidian (Azari-Hamidian & Harbach 2009). Mosquito name abbreviations are cited based on Reinert (Reinert 2001).

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 genus and instars counted and recorded. All third and fourth instars of mosquitoes were preserved in 100% lactophenol solution and later identified morphologically to species (Zaim 1987, Shahgudian 1960). 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 larvae were preserved in lactophenol and the microscopic slides of the preserved larvae were prepared using Berlese medium. The culicinae larvae along with the description of their breeding sites were sent to the School of Public Health, Tehran University of Medical Sciences, where the author identified using the keys mentioned above.

Results

The number of culicinae mosquito species at the Ecological Station of this area is shown in Table I. A total of 1924 adult and 3913 larvae collected, in which 5 genera and 10 species and were recognized including; *Aedes vexans*, *Ochlerotatus caspius* s. l. (indicate new occurrence records for this area), *Anopheles superpictus*, *An. turkhudi*, *Culex hortensis*, *Cx. pipiens*, *Cx. mimeticus*, *Cx. theileri*, *Culiseta longiareolata*, and *Cs. subochrea*. A total of 1924 adult mosquitoes were collected by Night catch from cow bait and bait net trap in living quarters and stables

(Table 1). Overall, *Cx. theileri* was predominant (48.54%), followed by *Oc. caspius* s. l. (22.45%), *Ae. vexans* (9.14 %), *Cx. pipiens* (8.52 %), *Cs.* (4.46%), *Cx. mimeticus* (2.49%), *An. turkhudi* (1.76%), *An. superpictus*(1.45%), *Cx. hortensis* (1.09%), and *Cs. longiareolata* (0.05%). In the larval collection, of 3913 culicidae larvae collected, both *Cs. longiareolata* (25.9%), and *Cs. subochrea* predominant (25.9%), followed by *Cx. pipiens* (17.56%), *Cx. theileri* (10.68 %), *Oc. caspius* s. l.(8.35%), *Cx. mimeticus* (5.26%), *Ae. vexans* (3.066%), *An. turkhudi* (1.84%), *An. superpictus* (1.43%), and *Cx. hortensis* (1.66%) (Table 1).

Two species including *Cx. theileri*, and *Oc. caspius* s. l., accounted for 71% of the adult collection respectively. The other species less frequent rare, each accounting for 29% of the total (Table 1).

Culex theileri, was the most frequent mosquitoes collected in this area, with total of 934, and 418 specimens, by Night catch and larval collection respectively. *Ae. vexans*, and *Oc. caspius* s. l. are recorded from ochlerotinae and aedinae species in this area. Number and prevalence of mosquitoes catches by animal bait trap through the night, are shown in (Table 2). 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 culicinae mosquitoes species such as *Cx. pipiense*, *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 *Cx. 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 second third of the night these species with *Cx. pipiens* were predominant and accounted for 76.22% of the adult collection. In the end of night, *Cx. theileri*, *Oc. caspius* s. l., and *Ae. vexans* were predominant and about 85.36% of the total mosquitoes catches.

In the present study, larval breeding place of some mosquito species were diverse. *An. turkhudi* larvae were collected only in permanent larval habitats (Table 3). *An. Superpictus* was found in the larval habitats without vegetation, whereas *Cs. longiareolata* and *Cs. subochrea* were collected from habitats with vegetation (Table 3). Most larval habitats were found with substrate of mud and sand bottom and fewer larvae were collected in rocks and cement substrates.

Also, total number of samples of *Anopheles* species was collected in fresh water (Table 3).

Discussion

Five genera and ten species of mosquitoes were identified, including the *Ae. vexans* and *Oc. caspius* s. l. in this area.

All 8 species of culicinae mosquitoes, which had been recorded previously in this area not found during the study including; *Anopheles maculipennis*, *An sacharovi*, *An dthali*, *An claviger*, *An marteri*, *Culex perexiguus*, *tritaeniorhynchus*, *Uranotaenia unguiculata*. *Cx. perexiguus*, and *Cx. tritaeniorhynchus* have not found during of this investigation. However, *Ae. vexance* and *Oc. caspius* s. l., were reported before^[6], although many references counted *Ae. vexans* as the more common species of the other parts of Iran(Reinert 2001). *Cx. 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 were recorded including; *Oc. caspius* s. l. and *Ae. vexans*. These species were differentiated from each other in the larval stage (Darsie 1997). 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 mosquitoes collected in this area, with total of 982 specimens, by larval collection. This species was the lowest mosquitoes collected at adult collection. *Cx. pipiens* larvae can be separated from *Cx. torrentium*, and *Cx. vagans* by Harbach's key using seta 1-III-V, seta 1-M, seta 1-X, seta 1-C, and some other characters(Harbach 1988, Dehghan et al. 2010). According to morphological characters in this survey only *Cx. pipiens* was identified.

Results of this survey is almost the same of finding by previous study in western Iran (Zaim 1987, Moosa Kazemia et al. 2010, Moosa-Kazemi et al. 2015). It should be mention that in this study collected other mosquitoes, which are potential vectors of human and domesticated animal pathogens, such as *Oc. caspius* s. l. and *Ae. vexans* (Harbach 1988, Horsfall 1955).

The species of *Cx. theileri* was one of the mosquitoes collected at the Kermanshah, with total of 934, and 418 specimens, by adult and larval collection respectively. This species is the more prevalent species at higher altitudes and in rural areas of Isfahan (Mousakazemi et al. 2000), and East Azerbaijan Provinces (Abai et al. 2007), Observations made during the present study agree with those of Simsek(Simsek 2004)who noted that *Cx. theileri* in Turkey prefers large natural larval habitats that are generally present in rural areas. Among the other mosquitoes of

the County that their ecology need to be studied extensively.

Manuchehri et al. 1972 was reported the occurrences of *Anopheles superpictus* as a malaria vector in Iran. Salivary gland infection was reported as ranging from 0.65 to 4.6% (Manuchehri et al. 1972). This species association with *An. maculipennis* was considered as the malaria vector during the outbreak of the diseases which had occurred in Iraq, Azerbaijan, Armenia, and Turkey and borderline areas of the countries in 1990. However, after the independence of the southern republics of the former Soviet Union, Iran was threatened by imported malaria cases (Oshaghi et al. 2011). In this present study, *An. superpictus* was collected in natural habitats. The characteristics of breeding place of this species were mainly in permanent water without vegetation, clear water, semi-sunlight and shaded habitats with muddy substrate. Zolotarev (1945), Dow (1953) and Nikookar et al. (2014) have reported the occurrence of this species in Mazandaran Province. *Anopheles superpictus* larvae was found in permanent, stagnant, with muddy substrate, transparent water, semi-shady, natural with vegetation habitats in Neka county, northern Iran (Nikookar et al. 2014). Moreover, Azari-Hamidian et al. (2011) have stated its presence in stagnant, transient, muddy substrate, full sunlight water with vegetation in natural habitats in Guilan Province, northern Iran.

Further support for our results comes from some previous studies carried out in western Iran (Moosa-Kazemi et al. 2015, Macan 1950), Zarrin-Shahr and Mobarakeh areas of Isfahan Province, center of Iran (Mousakazemi et al. 2000), Ardabil Province, northwestern Iran (Yaghoobi-Ershadi et al. 2001), Rasht County of Guilan Province, northern Iran (Azari-Hamidian et al. 2002) and in Iranshahr,

southeastern part of the country (Ghanbari et al. 2005). Three genotypes named X, Y, and Z within *An. superpictus* during the molecular study were reported in Iran by Oshaghi et al. (2008). By now, there are no reports about the genotypes of this species in Kermanshah Province. However, it needs to be studied in the future.

Conflict of interest:

The authors declare no conflict of interest

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Table 1 Number and prevalence of adult and larvae culicidae mosquitoes collected by Night catch and larval collections in the western Iran, May-July 2014

Species	Larvae		Adult	
	No	%	No	%
<i>Ae. vexans</i>	120	3.06	176	9.15
<i>An. superpictus</i>	56	1.42	28	1.45
<i>An. turkhudi</i>	72	1.84	34	1.76
<i>Cx. hortensis</i>	65	1.66	21	1.1
<i>Cx. pipiens</i>	685	17.55	164	8.53
<i>Cx. mimeticus</i>	206	5.26	48	2.5
<i>Cx. theileri</i>	418	10.68	934	48.55
<i>Cs. longiareolata</i>	982	25.09	1	0.05
<i>Cs. subochrea</i>	982	25.09	86	4.46
<i>Oc. caspius s.l.</i>	327	8.35	432	22.45
Total	3913	100	1924	100

Table 2 Number and prevalence of mosquitoes catches by animal bait trap through the night in the western Iran, May-July 2014

Species	First Round		Second Round		Third Round	
	No	%	No	%	No	%
<i>Ae. vexans</i>	41	23.4	88	50	47	26.6
<i>An. superpictus</i>	7	25	12	42.85	9	32.14
<i>An. turkhudi</i>	4	11.76	18	52.94	12	35.29
<i>Cx. hortensis</i>	6	25	14	68	1	7
<i>Cx. pipiens</i>	29	17.6	134	82	1	0.4
<i>Cx. mimeticus</i>	6	14	25	51	17	35
<i>Cx. theileri</i>	292	31	361	38.8	281	30.2
<i>Cs. longiareolata</i>	1	100	-	-	-	-
<i>Cs. subochrea</i>	23	26	35	41	28	33
<i>Oc. caspius s.l.</i>	242	56	121	28	69	16
Total	651	-	808	-	465	-

Table 3 Larval habitat characteristics of mosquitoes collected in Kermanshah County, 2014

Larval habitat	<i>A. vexans</i>	<i>An. turkhufti</i>	<i>An. superpictus</i>	<i>Cx. theileri</i>	<i>Cx. hortensis</i>	<i>Cx. mineticus</i>	<i>Cx. pipiens</i>	<i>Cs. longiareolata</i>	<i>Cs. subochrea</i>	<i>Oc. caspius</i>
Habitat										
Permanent	93.7	100	91.8	72	64.3	35.1	91.8	75.2	23	91
Temporary	6.3	0	8.2	28	33.7	64.9	8.2	24.8	76	9
Slow-running water	84	8	54.5	61	1.3	11	63.5	32	45	61
Stagnant water	16	92	45.5	39	98.7	89	36.5	68	55	39
Vegetation										
With	95.3	0	0	55	31	56	69.7	100	100	59
Without	4.7	100	100	45	69	44	29.3	0	0	41
Substrate										
Mud	14	100	80	45	65	73	31.3	69	65	96
Sand	86	0	20	35	25	27	47.7	21	35	2
Rock and Cement	0	0	0	20	10	0	21	10	0	2
Water Situation										
Turbid	0	0	0	64	49	61	79	65	78	83
Clear	100	100	100	36	51	39	21	35	22	17
Sunlight situation										
Full sunlight	94.5	0	0	56	61	59	61	89	24	62
Partial sunlight	5.5	65	65	44	39	41	39	11	76	38
Shaded	0	35	35	0	0	0	0	0	0	0
Habitat Type										
Natural	53.8	100	55.9	87	71	89	100	65	82	36
Artificial	46.2	0	44.1	13	29	11	0	35	18	64

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