ORIGINAL ARTICLE

Bionomics of Mosquitoes (Diptera: Culicidae) to Design a Comprehensive Control Program in Important Tourism Centers of Fars Province, Southern Iran

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Abstract

Background: The general purpose of this study was to investigate the species diversity and characteristics of the larval habitats of culicids in important tourism centers of Fars province to design a comprehensive program to control them.

Methods: In a cross-sectional study conducted from April to the end of September 2021, the species diversity of mosquito larvae and the characteristics of larval habitats were investigated. Mosquito larvae were collected using the dipping method and the characteristics of larval habitats such as permanent or temporary habitat, water running, intensity of sunlight, vegetation, habitat floor, turbidity, natural or artificial were recorded according to the hydro-ecological characteristics.

Results: Totally, 8825 specimens were collected and identified from 9 selected locations in Marvdasht city and 6 selected locations in Maharloo wetland. Collected species were *Culex pipiens* (39.77%), *Culex sinaiticus* (3.80%), *Culex quinquefasciatus* (23.68%), *Culiseta longiareolata* (17.52%), *Uranotaenia unguiculata* (9.02%), and *Anopheles stephensi* (6.21%). Most specimens (79%) were collected from natural habitats. There is a significant difference between the number of collected mosquitoes with the permanent habitat, stagnant water, partial sunlight, vegetation, turbidity, and natural habitat ($P<0.0001$). The dominant species were *Cx. pipiens* (39.77%) and *Cx. quinquefasciatus* (23.68%).

Conclusion: These species are potentially involved in the transmission of many pathogens to humans and domestic animals and should be extensively studied. The results of this study suggest that environmental factors play an important role in larvae's habitat preference.

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Introduction

Vector-borne diseases account for around 17% of the estimated global burden of communicable diseases and lead to the death of more than 700000 people annually.[1] The most important vector-borne diseases are malaria, dengue fever, lymphatic filariasis, chikungunya, onchocerciasis, Chagas disease, leishmaniasis, zika virus disease, yellow fever, and Japanese encephalitis. Almost 64% of the mentioned diseases are transmitted

by mosquitoes, so mosquitoes are considered the world's deadliest arthropods.[2, 3]

So far, some important mosquito-borne diseases such as arboviruses (avian pox, bovine ephemeral fever, dengue fever, Rift Valley fever, Sindbis fever, and West Nile fever), bacterial infections (anthrax and tularemia), helminthic parasites or infections (*Deraiophoronema evansi* infection, dirofilariasis, and seteriasis), and protozoal infections (human malaria, avian malaria) have been reported from Iran.[4]

8 cases of these diseases, including avian pox, bovine ephemeral fever,[5] West Nile virus,[6] Deraiophoronema evansi,[7] dirofilariasis,[3] and human and avian malaria,[9, 10] are found in Fars Province. Female mosquitoes lay eggs in various breeding places. Knowledge about mosquito breeding places is important for investigating the mosquito ecology and biology. Seven genera and more than 98 species of mosquitoes (Diptera: Culicidae) are found in southwestern Asia. Up till now, 11 genera and 69 species of mosquito have been recorded in Iran, of which 5 genera and 17 species have been reported in Fars province.[11, 12]

Fars province, due to its special geographical location and the existence of suitable climate, changes in the ecosystem of urban and rural areas, as well as climate change over the past 20 years, the spread of suburbanization around cities, deforestation, the spread of irrigated agriculture, and rice planting have suitable breeding places for mosquitoes to reproduce. Therefore, due to the existence of suitable larval habitats and some mosquito-borne diseases in this area, Fars is one of the potentially high-risk areas for mosquito borne diseases such as human malaria, Dirofilariasis, and West Nile Fever, and other Arboviruses.[6] Fars province is one of the most important historical and economic regions in Iran. Thousands of tourists and businessmen travel to this area annually.[13] Therefore, due to the high potential of the region in the transmission of vector-borne diseases, it is important to study the species diversity and characteristics of larval habitats of mosquitoes in this province.

Given that the species diversity of mosquitoes in Marvdasht city has not been studied coherently and considering the existence of several paddy fields in this city that have created favorable conditions for the reproduction of mosquitoes, it seems necessary to research this issue.

Also, the Maharloo wetland area, as one of the tourist attractions of the province, annually receives the largest number of migratory birds entering Fars Province; due to the possibility of transferring important Arboviruses in terms of pathogenicity for humans and lack of studies on the diversity of mosquito in this region, it seems necessary to conduct a study in this regard.[14]

This study aimed to investigate the species diversity and characteristics of the larval habitats of culicids in important tourism centers of Fars province and design a comprehensive program to control them. The results of this study provide critical important information for the Department of Infectious Diseases at the Ministry of Health to prevent the diseases transmitted by mosquitoes and plan to control vectors, especially in the event of an epidemic disease.
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Methods

Study Area

Fars Province (29.62° N and 52.53° E), with about 133 thousand square kilometers, is located in the south of Iran. Marvdasht is one of the northern cities of Fars province, with an altitude of 1620 meters above the sea level and an area of 4649 square kilometers. The annual rainfall of this city is between 450 to 600 mm. This city is considered a tourist area due to the location of Persepolis. It is also considered one of the habitats of mosquitoes due to the existence of numerous paddy fields and farms.

Maharloo wetland area with geographical coordinates of 29° 20′27، 5 ″ North 52° 49′14، 3 ″ East is located near Maharloo Lake (Salt Lake) at 27.0 km (16.8 mi) southeast of Shiraz and is located on the southern slopes of the high mountains of the Zagros. It has a temperate and mountainous climate (Figure 1).

Sample and Data Collection

Mosquito larvae were collected from 9 selected locations of Marvdasht city (Central part, Kamfiruz, Doroodzan) and 6 selected locations of Maharloo wetland (Maharloo village, Kuhenjan village) from various breeding sites such as riverbanks, riverbeds, river banks, rainwater harvesting sites, swamps, grasslands, paddy fields, farms, irrigation canals, wells, and pipette. In each area, there were one fixed and three variable locations (different topographic areas). Larvae were collected for 15-20 minutes in selected locations during the spring, summer, and autumn seasons. Physical and biological characteristics of larval habitats such as habitat status (permanent or temporary, standing or running), habitat type (natural or artificial), vegetation (with or without vegetation), sunlight status (full or partial or shady sunlight), and water status (clear or turbid) were recorded. The water turbidity was determined using a portable turbidimeter (Nephelometer, model: HACH-2100Q), which is reported with nephelometric turbidity units (NTU). According to the standards, any degree of turbidity read by the nephelometer that is above 5 units is considered turbid.

Figure 1: Location of the study area and sampling sites in Fars Province, southern Iran; 1. Marvdasht County, 2. Maharloo area (Courtesy of Google map)

Table 1: Frequency (number and percentage) of mosquito larvae in selected areas of Fars province, Iran, from April to November 2021

Districts	Cx. pipiens n (%)	Cx. quinquefasciatus n (%)	Cx. sinaiticus n (%)	Cs. longiareolata n (%)	An. stephensi n (%)	Ur. unguiculata n (%)	Total n (%)
Central	1082 (39.39)	685 (24.94)	0 (0)	780 (28.39)	200 (7.28)	0	2747 (100)
Kamfiruz	844 (47.23)	453 (25.35)	72 (4.03)	235 (13.15)	183 (10.24)	0	1787 (100)
Doroodzan	548 (41.83)	395 (30.15)	0	202 (15.42)	165 (12.60)	0	1310 (100)
Maharloo	560 (34.63)	316 (19.54)	164 (10.14)	173 (10.70)	0	404 (24.50)	1617 (100)
kuhenjan	476 (34.90)	241 (17.67)	99 (7.26)	156 (11.44)	0	392 (28.74)	1364 (100)
Total	3510(39.77)	2090 (23.68)	335 (3.80)	1546 (17.52)	548 (6.21)	796 (9.02)	8825 (100)

Diagnosis and Sampling

Identification of the specimens was done according to Shahgudian morphological identification key.[15] The name of species, characteristics of breeding sites, date of collection, and mean of temperature and relative humidity were recorded in predesign tables.

Statistical Analysis

Data analysis was done using the Chi-square test and Fisher's exact test. This test is used to check the relationship between the mosquito type and habitat type, water running, intensity of sunlight, vegetation, habitat floor, turbidity, and habitat kind.

Results

Totally, 8825 mosquito larvae including *Cx. pipiens, Cx. quinquefasciatus*, *Cx. sinaiticus*, *Cs. longiareolata*, *Uranotaenia*. *unguiculata* and, *Anopheles stephensi* were caught (Table 1).

There is a statistically significant difference between the type of mosquito and whether their habitat is permanent or temporary, water running (stagnant water), intensity of sunlight (partial sunlight), vegetation, turbidity, and habitat floor (sandy) (P=0.0001). Also, there is a significant difference between the type of mosquito and habitat type (natural) (P=0.03). The distribution and characteristics of larval habitats of the identified species are as follows:

Cx. Pipiens

Of the 8825 specimens, 3510 (39.77%) were *Cx. pipiens*. Among the larval habitats containing this species, 81% were permanent and 19% temporary, 67% were paddy fields and 33% were water storage ponds, and 96% were stagnant waters and 4% were low-flow waters. Habitats with partial sunlight, vegetation, and sandy and almost muddy floors were the highest percentage of larval nests of this species; it should be noted that 79% of the larvae of this species were in natural habitats and 21% were found in artificial habitats (Table 2).

Cx. Quinqufasciatus

The number of larvae of this species was 2090 (23.68%). The larval breeding sites of this species were 78% permanent and 22% temporary, 86% paddy fields and 14% water storage ponds, and 92% stagnant waters and 8% low-flow water. Habitats with partial sunlight, vegetation, and sandy and almost muddy floors formed the highest percentage of larval nests of this species. It should be noted that 76% of the larvae of this species were in natural habitats and 24% in artificial habitats (Table 2).

Cx. Sinaiticus

Out of 8825 caught specimens, 335 (3.80%) belonged to this species. The larval habitats of this species were 87% permanent and 13% temporary, 26%

Table 2: Characteristics of larval habitat and percentage of Culicid larvae, selected areas of Fars province, Southern Iran

Breeding site-characteristics and habitats	Variables	Culex pipiens (%)	Culex. qinqufasciatuss (%)	Culex. sinaiticus (%)	Culiseta longiareolata (%)	Anopheles stephensi (%)	Uranotaenia unguiculata (%)	P value*
Habitat type	Permanent	81	78	87	76	79	97	0.0001
	Temporary	19	22	13	24	21	3	
Water running	low-flow	4	8	6	12	34	0	
	Stagnant water	96	92	94	88	66	100	0.0001
Intensity of sunlight	Full sunlight	40	38	62	77	2	4	
	Partial sunlight	52	58	38	16	79	27	0.0001
	Shaded	8	4	0	7	19	69	
Vegetation	With vegetation	73	65	36	89	36	98	0.0001
	Without vegetation	27	35	64	11	64	2	
Habitat floor	Mud	38	27	29	32	0	99	
	Sand	42	66	58	62	43	1	0.0001
	Gravel	20	7	13	6	57	0	
Turbidity	Clear	11	9	22	31	56	0	0.0001
	Turbid	89	91	78	69	44	100	
Habitat Kind	Natural	79	76	58	63	62	100	
	Artificial	21	24	42	37	38	0	0.03

*P value for chi-square and fisher exact test

paddy fields and 74% water storage ponds, and 94% stagnant waters and 6% low-flow waters. Habitats with partial sunlight, vegetated and non-vegetated habitats, and sandy and almost muddy habitats constituted the highest percentage of habitats of this species. It should be noted that 58% of the larvae of this species were in natural habitats and 42% were found in artificial habitats (Table 2).

Cs. Longiareolata

Totally 1546 (17.52%) specimens of *Cs. longiareolata* were collected. The larval habitats of this species were 76% permanent and 24% temporary, 33% paddy and 67% water storage pool, and 88% stagnant water and 12% low-flow waters. Habitats with full sunlight, vegetation, and sandy and almost muddy floors had the highest percentage of larval nests of this species. It should be noted that 63% of the larvae of this species were in natural habitats and 37% in artificial habitats (Table 2).

An. Stephensi

548 (6.21%) specimens were *An. stephensi*. The larval habitats of this species were 79% permanent and 21% temporary, 46% paddy fields and 54% water storage ponds, and 66% stagnant waters and 34% low-flow waters. Habitats with low sunlight, vegetation, with pebble and sandy soils, and mostly in clean water constituted the highest percentage of larval habitats of this species. It is noteworthy that 62% of the larvae of this species were found in natural habitats and 38% in artificial habitats (Table 2).

Ur. Unguiculata

The number of larvae was 796 (9.02%). The larval habitats of this species were 97% permanent, 3% temporary, and 100% stagnant. Shaded larval habitats, containing vegetation, with mud and mud floor, constituted the highest percentage of larval habitats of this species. It is noteworthy that 100% of the larvae of this species were found in natural habitats (Table 2).

Discussion and Conclusion

Totally 8825 specimens belonging to 4 genera and 6 species were collected. The result showed that *Cx. pipiens* (39.77%), *Cx. quinqufasciatus* (23.68%), and *Cs. longiareolata* (17.52%) had the highest geographical distribution in the areas and *Cx. pipiens* larvae have been found in a variety of natural and man-made habitats, including water storage ponds, paddy fields, water storage containers, and natural swamps. This great variety of breeding places can explain why this species is one of the most abundant species of mosquitoes in Iran.

The noteworthy point in this study was that *Uranotaenia unguiculata* was the only shade-loving species reported in this research. Also, in this study, *Anopheles stephensi* and *Culex sinaiticus* two species were mostly found in habitats without vegetation. Similar observations in different parts of Iran confirm that *Cx. pipiens* was one of the dominant species and was present in natural and permanent habitats.[16-19]

The permanency of mosquito larval breeding sites was recognized as the main reason related to the presence of *Cx. pipiens* complex larvae (39.77%). Relatively permanent water bodies such as water bodies along river fringes, ponds and puddles, and water bodies with no predators should be paid more attention for future *Cx. pipiens* larvae abatement campaigns in Lhasa, China.[20] According to other studies, members of the *Cx. pipiens* complex readily breed in storm sewer catch basins, clean and polluted ground pools,

ditches, animal waste lagoons, effluent from sewage treatment plants, and other sites that are slightly to very eutrophic or polluted with organic wastes. Generally, *Cx. quinquefasciatus* is associated with more eutrophic water than *Cx. pipiens*.[21-23] Fillinger et al. found that semi-permanent and permanent habitats were suitable for the proliferation of Culicines and *Anopheles gambiae* sensu lato. Fort Ternan et al. found that permanent habitats were more preferred by the Culicines and Anopheline mosquitoes.[24]

In a study conducted in Tarhuna Region, Libya, *Cx. pipiens* and *Cx. quinquefasciatus*, we found that only semi-permanent habitats with the presence of vegetation and shade, and also water turbidity and velocity had affected mosquito breeding.[25] Also, in a study conducted in the central part of Iran, *Cu. longiareolata* and *Cx. quinquefacsiatus* were constant species and *Cx. pipiens* was found in stagnant and flowing water.[26] In two studies conducted in the northern and northeastern regions of Iran, 14 species of the genus *Anopheles*, *Culex*, *Aedes* and, *Culiseta* were reported. *Cx. pipiens* was dominant species.[27, 28] According to Zaim et al., the genus *Culiseta* is distributed in all regions of Iran and lives in different types of breeding places. There are different species of *Culiseta* in other parts of the world and Iran.[29] *Cs. longiareolata* and *Cs. subochrea* have been reported from Kurdistan and Kermanshah provinces in western Iran that were the dominant species with high abundance in larval breeding places.[30]

Cs. longiareolata was reported from the province of West Azerbaijan in northwestern Iran.[31] In a study conducted in Neka city, Mazandaran Province, two species of *Cs. longiareolata* and *Cs. annulata* were reported from larval breeding places, and the abundance of *Cs. longiareolata* was more than *Cs. annulata*.[27] Nejati et al. reported *Cs. longiareolata* in Chabahar city of Sistan and Baluchestan Province.[32] In a study, *Cs. longiareolata* and *Cs. subochrea* were caught from breeding places (natural larval habitats, temporary larval habitats, stagnant water) in Hamedan, Qom, and northeastern provinces of Iran, respectively.[19, 33, 34] Dehghan et al. showed that in the central part of Iran, many factors are involved in the life and growth of mosquito larvae, including fauna and flora, temperature and water flow rate, light and shade, chemical composition, turbidity, and water pollution.[35]

The presence of aquatic plant density can have a positive or negative effect on spawning and abundance of Culicid larvae.[36] Different species of mosquitoes need different biological and environmental conditions to grow. Some species lay eggs in permanent water habitats, others prefer temporary aquatic habitats for reproduction, some need freshwater, and some need salty water. Others prefer high temperatures and others like low temperatures. Some of them prefer running water and some stagnant water and mud to lay eggs. Also, for many species to reproduce, the habitat must be sunny or shady.[37]

The status of aquatic habitat and the presence of sand, sludge, and other sediments, the amount of water salt play a role in determining the existing species. In a study in Golestan Province, most of the captured larvae were related to ponds, agricultural streams, wetlands, and rice fields.[28] In Mazandaran province, wetlands and discarded tires are the main habitats of larvae for *Cx. pipiens*.[27] In Isfahan, the above species is mostly found in rice fields and natural habitats. The larval habitats of this species have mostly been stagnant water and swamps, irrigation canals, and barrels full of water.[29, 37] In Yazd city, *Cs. logiareolata* and *Cx. pipiens* were found in larval habitats contaminated with organic matter and industrial waste.[38] In a study conducted in Kurdistan Province, *Ochlerotatus* was found in wet forest areas.[39]

Little is known about the ecology of Culicids larvae in Fars Province. In a study conducted in the west of this region, three genera and six species of Culicidae including *An. superpictus*, *An. d'thali*, *Cx. sinaiticus*, *Cx theileri*, *Cx. mimeticus*, and Cu. Longiareolata were collected and *Cx. theileri* was the most dominant species.[40] Keshavarzi et al. recognized five genera and 17 species of mosquito in southern parts of Fars Province including *An. dthali, An. fluviatilis, An. stephensi, An. superpictus, Cx. quinquefasciatus, Cx. mimeticus, Cx. perexiguus, Cx. pipiens, Culex tritaeniorhynchus, Cx. theileri, Cx. tritaeniorhynchus, Cx. sinaiticus, Cx. torrentium, Cx. modestus, Oc. caspius, Cs. Logiareolata, and Ae. Vexans;*in this study, *Cx. pipiens* (27.3%), *Cx. theileri* (15.9%) and Cx. quinquefasciatus (9.4%) were the most abundant species.[14] Due to its large size, Fars Province has a wide variety of climates and so different species of mosquitos are found in different parts of this province. However, based on our study, *Cx. pipiens* and *Cx. quinquefasciatus* were among the dominant species in Southern parts. Considering that some mosquito-borne diseases such as avian pox,[39] bovine ephemeral fever,[4] West Nile virus[5], *Deraiophoronema evansi*,[7] dirofilariasis,[3] and human and avian malaria[8, 9] are found in Fars Province, so studying the ecology of mosquitoes is essential in different parts of this Province.

The results of these studies help the health system managers to reduce disease vectors as much as possible by using environmental improvement methods. To control mosquitoes, the conditions for their reproduction should be limited. Among these methods, the following can be mentioned: eliminating containers, tires, etc. in which water can be saved and mosquitoes can lay their eggs and eliminating their breeding by changing the environment to make

it unfavorable for mosquitoes to breed, sub-soil drainage.

Authors' Contribution

Masoumeh Amin: Conceptualization (equal); funding acquisition (lead); investigation (equal); methodology (equal); project administration (equal); resources (lead); supervision (equal); validation (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal). Mohsen Kalantari: Conceptualization (lead); data curation (equal); formal analysis (equal); investigation (lead); methodology (equal); project administration (equal); resources (equal); validation (equal); visualization (equal); writing-original draft (head); writing-review and editing (lead). Saideh Yousefi: investigation(equal); writing-review and editing (equal); data curation(equal). Mozaffar Vahedi: investigation(equal); writing-review and editing (equal); data curation(equal). Sorna Dabaghmanesh: samples Collection, investigation(equal); data curation(equal). Hamid Reza Ghorbani: samples Collection, investigation(equal). Marzieh Shahriari Namadi: samples Collection, investigation(equal); data curation(equal). Parisa Amin: writing-review and editing.

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