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Biodiversity of mosquitoes (Diptera: Culicidae), vectors of important arboviral diseases at different altitudes in the central part of Iran

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Abstract: Risk assessment and vector control programs rely on a comprehensive knowledge of mosquito diversity in different areas. The present study aims to evaluate mosquito biodiversity in different areas of Sirjan County, Kerman Province, as one of the most important economic zones of Iran. Totally 4538 mosquitoes representing 19 species and four genera were collected and identified. Results showed that *Culiseta longiareolata* and *Culex quinquefacsiatus* were constant, *Culex destricola, Culex pusillus, Culex pipiens and Culex laticinctus* were common and the other captured species were accidental species in Sirjan County. In this study, *Aedes vittatus* was recorded for the first time in Kerman Province. The highest rate of richness was in Balvard (2034 meters above sea level) and the highest rate of evenness and Shannon-Wiener indices belong to Zeid Abad (1718 m.a.s.l.) districts. The lowest similarity rate was between Balvard (2034 m.a.s.l.) and Pariz (2322 m.a.s.l.), and the highest was between Homashahr (1984 m.a.s.l.) and Khajo-Shahr districts (1723 m.a.s.l.). In this study, an agricultural area showed the highest; conversely, the area neighboring a copper mine showed the lowest rate of biodiversity. Biotic and abiotic factors and human activities can affect mosquito biodiversity; therefore, mosquito surveillance and control programs should consider these factors.

Key words: Richness, evenness, Shannon-Wiener index, Culicidae, Sirjan, Kerman

1. Introduction

Mosquitoes (Diptera: Culicidae) transmit some medically and veterinary important pathogens and parasites to humans and animals worldwide. About 750,000 deaths occur from mosquito-borne diseases annually and so they are the deadliest arthropods in the world (Mobin et al., 2022; Nebbak et al., 2022).

Human and avian malaria (protozoal), avian pox, bovine ephemeral fever, dengue fever, Rift Valley fever, Sindbis fever, West Nile fever (arboviral), anthrax, tularemia (bacterial), and *Deraiophoronema evansi* infection, dirofilariasis, lymphatic filariasis, setariasis (helminthic diseases) have been reported in Iran (Azari-Hamidian et al., 2019). Up till now, two subfamilies of Culicidae including Anopheline and Culicinae have been known. Currently, 113 genera and 3563 species of mosquitoes have been reported in the world, of which 11 genera and 69 species have been recorded in Iran (Azari-Hamidian et al., 2019).

Some mosquito-borne diseases including malaria, avian pox, *Deraiophoronema evansi* infection, and dirofilariasis have been reported in Kerman Province (Akhtardanesh et al., 2011; Reza et al., 2013; Sazmand et al., 2013; Azari-Hamidian et al., 2019). All of the mosquito ecological studies have been done in southern parts of Kerman Province because these areas are malaria foci. Up to now, four genera (*Anopheles*, *Culex*, *Culiseta*, and *Uranotaenia*) and 19 species of mosquito have been reported in Kerman Province and generally, the species composition of anopheline mosquitoes is better documented than for culicines in this province (Baseri et al., 2005; Mehravaran et al., 2011; Edalat et al., 2020). Unfortunately, there is no information about the diversity and geographical distribution of mosquitoes in the northern parts of this region, especially in Sirjan County, as an important economic and tourist area in Iran that many travelers come to visit annually.

Diversity and abundance of mosquito increase in suitable environmental conditions, and so favorable conditions, enhanced mosquito-borne diseases (Attaullah et al., 2021).

There are three levels of diversity including alpha (α), beta (β), and gamma (γ). Alpha diversity can be assessed at a local level, while beta and gamma diversity can be

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assessed between habitats and across regional scales, respectively (Whittaker, 1972).

Studies on mosquito biodiversity indices such as species richness, species evenness, Shannon-Wiener, degree of presence or occurrence, and Jaccard's similarity indices are essential to develop vector surveillance and control strategies in different areas (Jahanifard et al., 2014; Zeroual et al., 2016). In the present study, alpha and beta diversity indices were studied to determine the geographical distribution, frequency, and species diversity of mosquitoes. Entomological surveys were restricted to breeding places of mosquitoes in different landscapes of Sirjan County because breeding places are important determinants of adult distribution and frequency of mosquitoes (Piyaratne et al., 2005; Mwangangi et al., 2012).

2. Materials and methods

2.1. Study area

Sirjan, with 13552.811 km^2, is located in the northwest of Kerman Province at 29°21′0″N, 55°32′0″E. Elevation is about 1738 meters above sea level (m.a.s.l.), and the mean annual rainfall is 144 mm; therefore, it is an arid area with hot summer and cold winter. It also has various geographical regions such as mountainous and plain areas. The human population of Sirjan County is about 300,000 inhabitants. The most economic activities are agriculture and mining. Sirjan County is located on the main road connecting the south to the north of Iran.

This county shares borders with Hormozgan and Fars provinces, and also Share-Babak and Rafsanjan counties.

2.2. Sampling methods and identification

Larval sampling was done every two weeks from March to November 2019 in different parts of Sirjan County, including Balvard (2034 m.a.s.l), Pariz (2322 m.a.s.l.), Zeid Abad (1718 m.a.s.l.), Homashahr (1984 m.a.s.l.), and Khajo-Shahr districts (1723 m.a.s.l.) (Figure 1). A standard metal dipper (350 mL capacity) was used for collecting mosquito larvae. The mosquito breeding sites consist of stagnant and nonstagnant water such as river banks, seepage, dry river bed, grasslands, irrigation channel, pools, etc. In each sampling, 10 dips were taken from each breeding place each sampling time. Collected larvae were preserved in glass vials filled with lactophenol and taken to the Sirjan School of Medical Sciences laboratory for species identification. Fourth-instar larvae were identified using valid morphological identification keys (Azari-Hamidian and Harbach, 2009). In the present study, molecular methods for species identification were not used.

2.3. Data analysis

Alpha diversity indices

Species richness (S) is the number of species represented in an ecological community (Spellerberg and Fedor, 2003).

Shannon-Wiener index $H = -\sum_{i=1}^{s} Pi * lnPi$ indicates species biodiversity in an ecological area (Magurran, 2021).

Evenness $E = \frac{H}{ln\, S}$ describes the relative abundance of the different species in a region (Magurran, 2021).

Degree of presence $C = \frac{n}{N*100}$ is the number of places where a specific species is collected divided by the total number of the study areas. This index is divided into several categories, which are constant species, common species, accidental species, and very accidental species that are found in 50% or more, 25%–49%, 12.5%–24%, and less than 12% of the study areas, respectively (Neffar et al., 2016).

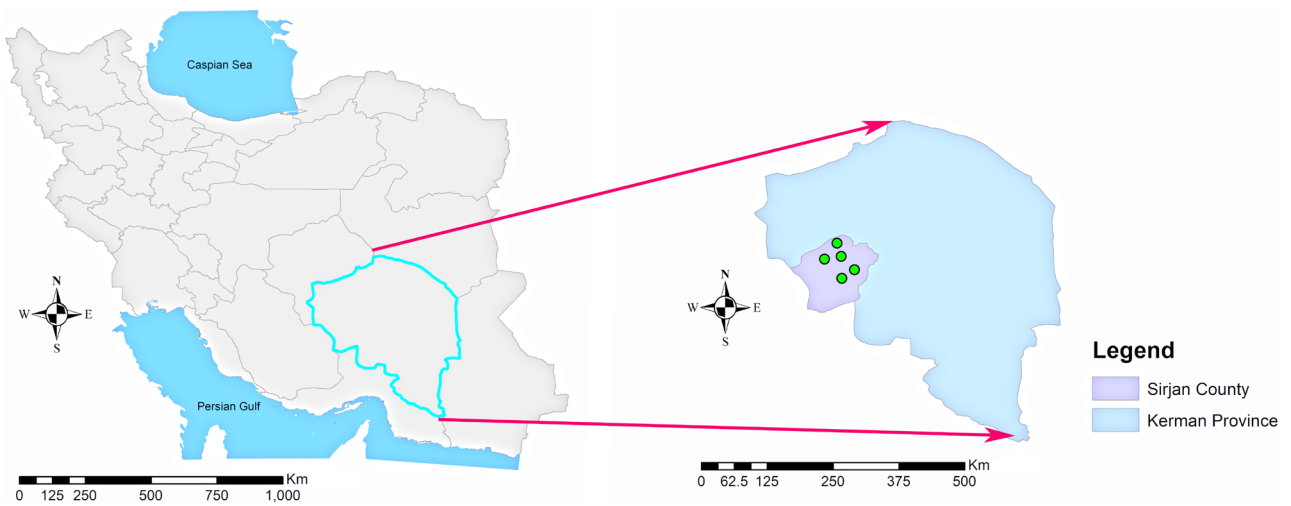

Figure 1. Sampling locations in Sirjan County, Kerman Province, Iran.

Beta diversity indices

Jaccard's similarity index: $j = \frac{a}{a+b+c}$.

This index measures the degree of community similarity and dissimilarity between two different areas (Hao et al., 2019).

3. Results

Overall, 4538 specimens of mosquito larvae belonging to four genera and 19 species were collected from stagnant and nonstagnant water in different (mountainous and plain) areas of Sirjan County, including Balvard (21.75%), Pariz (8.70%), Zeid Abad (8.9%), Homashahr (40.4%), and Khajo-Shahr (20.3%) districts (Table 1). Balvard and Pariz are mountainous and the other mentioned regions are plain. The captured species in Balvard are *Cu. longiareolata, Cx. quinquefasciatus, Cx. desetricola, Ae. vittatus, Cx. pusillus, Cx. sitiens, Oc. leucomelas, Cx. pseudovishnui, Oc. detritus,* and *Cu. subochrea*. The species captured in Pariz are *Cu. longiareolata* and *Cx. laticinctus* (Table 2). The species collected in Zeid Abad are *Cu. longiareolata, Cx. quinquefasciatus, Cx. territans, Cx. pusillus, Cx. tritaeniorhynchus, and Oc. flavescens* (Table 3).

The five species captured in Homashahr include *Cu. longiareolata, Cx. quinquefasciatus, Cx. pipiens, Cx. theileri, and Cx. arabieeni* (Table 3). The species collected in Khajo-Shahr are *Cu. longiareolata, Cx. quinquefasciatus, Cx. desetricola, Cx. hortensis, Cx. impudicus, Cx. pipiens,* and *Cx. laticinctus* (Table 4). Generally, the most frequent species was *Cu. longiareolata* (67.84%) followed by *Cx. quinquefasciatus* (20.14 %), and *Cx. territans* (4.03%), and the highest rate of their frequency was observed in summer.

The highest and the lowest rates of richness were observed in Balvard (R = 10) and Pariz (R = 2), respectively.

The highest evenness and Shannon-Wiener indices rates were observed in Zeid Abad (H = 1.337, E = 0.746), and the lowest rate was in Pariz (H = 0.056, E = 0.080).

The similarity index between different areas was generally low; however, the highest level (Sj = 0.2) was between Homashahr and Khajo-Shahr, and the lowest level was between Balvard and Pariz (Sj= 0.0769).

Table 1. Mosquitoes species composition in studied areas, Sirjan County, Iran.

Species	Balvard N (P)	Pariz N (P)	Zeid Abad N (P)	Homashahr N (P)	Khajo-Shahr N (P)	Total
Culiseta longiareolata	832 (84.30%)	391 (98.99%)	53 (13.18%)	1775 (96.78%)	28 (3.04%)	3079
Culex quinquefasciatus	12 (1.22%)	0	95 (23016%)	48 (2.62%)	759 (82.50%)	914
Culex deserticola	4 (0.41%)	0	0	0	16 (1.74%)	20
Culex territans	0	0	183 (45.52%)	0	0	183
Culex hortensis	0	0	0	0	31 (3.37%)	31
Culex impudicus	0	0	0	0	71 (7.72%)	71
Aedes vittatus	90 (9.12%)	0	0	0	0	90
Culex pusillus	24 (2.43%)	0	63 (15.67%)	0	0	87
Culex pipiens	0	0	0	4 (0.22%)	11 (1.20%)	15
Culex laticinctus	0	4 (1.01%)	0	0	4 (0.43%)	8
Culex sitiens	8 (0.81%)	0	0	0	0	8
Ochlerotatus leucomelas	8 (0.81%)	0	0	0	0	8
Culex pseudovishnui	4 (0.41%)	0	0	0	0	4
Culex tritaeniorhynchus	0	0	4 (1%)	0	0	4
Ochlerotatus detritus	3 (0.30%)	0	0	0	0	3
Ochlerotatus flavescens	0	0	4 (1%)	0	0	4
Culiseta subochrea	2 (0.20%)	0	0	0	0	2
Culex theileri	0	0	0	4 (0.22%)	0	4
Culex arbieeni	0	0	0	3 (0.16%)	0	3
Total	987 (100%)	395 (100%)	402 (100%)	1834 (100%)	920 (100%)	4538

N: number; P: percentage.

Table 2. Alpha diversity indices of mosquitoes (Family: Culicidae) in Balvard and Pariz districts, Sirjan County, Iran.

Region	Mosquito species	Frequency	Percent	Proportion (Pi)	Log (pi)	$Pilog_epi$	Richness	Shannon index	Evenness
Balvard	*Culiseta longiareolata*	832	84.295	0.842	–0.171	–0. 143	**10**	**0.649**	**0.281**
	Culex quinquefasciatus	12	1.215	0.012	–4.422	–0.053			
	Culex deserticola	4	0.405	0.004	–5.521	–0.022			
	Aedes vittatus	90	9.11	0.091	–2.396	–0.215			
	Culex pusillus	24	2.431	0.024	–3.729	–0.089			
	Culex sitiens	8	0.810	0.008	–4.828	–0.038			
	Ochlerotatus leucomelas	8	0.810	0.008	–4.828	–0.038			
	Culex pseudovishnui	4	0.405	0.004	–5.521	–0.022			
	Ochlerotatus detritus	3	0.303	0.003	–5.809	–0.017			
	Culiseta subochrea	2	0.202	0.002	–6.21	–0.012			
	Total	987							
Pariz	*Culiseta longiareolata*	391	98.987	0.989	–0.011	–0.010	**2**	**0.056**	**0.080**
	Culex laticinctus	4	1.012	0.010	–4.605	–0.046			
	Total	395							

Table 3. Alpha diversity indices of mosquitoes (Family: Culicidae) in Zeid Abad and Homashahr districts, Sirjan County, Iran.

Region	**Mosquito species**	**Frequency**	**Percent**	**Proportion (Pi)**	**Log_epi**	**$Pilog_epi$**	**Richness**	**Shannon index**	**Evenness**
Zeid Abad	*Culiseta longiareolata*	53	13.184	0.131	–2.032	–0.266	**6**	**1.337**	**0.746**
	Culex quinquefasciatus	95	23.631	0.236	–1.443	–0.340			
	Culex territans	183	45.522	0.455	–0.787	–0.358			
	Culex pusillus	63	15.671	0.156	–1.857	–0.289			
	Culex tritaeniorhynchus	4	0.995	0.009	–4.710	–0.042			
	Ochlerotatus flavescens	4	0.995	0.009	–4.710	–0.042			
	Total	402							
Homashahr	*Culiseta longiareolata*	1775	96.782	0.967	–0.360	–0.348	**5**	**0.472**	**0.293**
	Culex quinquefasciatus	48	2.617	0.026	–3.649	–0.094			
	Culex pipiens	4	0.218	0.002	–6.214	–0.012			
	Culex theileri	4	0.218	0.002	–6.214	–0.012			
	Culex arbieeni	3	0.163	0.001	–6.907	– 0.006			
	Total	1834							

Calculation of the degree of presence showed that *Cu. longiareolata* and *Cx. quinquefacsiatus* are constant species, *Cx. destricola, Culex pusillus, Cx. pipiens and Cx. Laticinctus* are common species, and the other captured species are accidental. There is no record of very accidental species in this region.

4. Discussion

Sirjan is an important economic zone and the most densely populated county in the northwest of the Kerman Province. Risk assessment and vector control programs rely on a comprehensive knowledge of mosquito diversity in different areas (WHO, 1982; WHO, 1995; Hazratian et al., 2019). The mosquito diversity in our study area has not been previously described; therefore, the present study aimed to determine alpha and beta diversity indices and species distribution in different parts of Sirjan County. The species of mosquitoes collected in this study represented 28% of the mosquito fauna found in Iran, which is 69 species. Among the collected species,

Table 4. Alpha diversity indices of mosquitoes (Family: Culicidae) in Khajo-Shahr district, Sirjan County, Iran.

Region	Mosquito species	Frequency	Percent	Proportion (Pi)	$Log_e pi$	$Pilog_e pi$	Richness	Shannon index	Evenness
Khajo-Shahr	*Culiseta longiareolata*	28	3.043	0.030	–3.506	–0.105	**7**	**0.732**	**0.376**
	Culex quinquefasciatus	759	82.5	0.825	–0.216	–0.178			
	Culex deserticola	16	1.739	0.017	–4.074	–0.069			
	Culex hortensis	31	3.369	0.033	–3.411	–0.112			
	Culex impudicus	71	7.717	0.077	–2.563	–0.197			
	Culex pipiens	11	1.195	0.011	–4.509	–0.049			
	Culex laticinctus	4	0.434	0.004	–5.521	–0.022			
	Total	920							

some medically and veterinary important vectors have not been recorded previously in this province.

Cu. longiareolata, as the vector of avian influenza, West Nile virus and an intermediate host of avian plasmodium (Hazratian et al., 2019; Bozorg-Omid et al., 2020; Soltanbeiglu et al., 2022), was the most abundant species in this site, representing about 68% of the specimens. This species was recorded in different parts of Iran and in agreement with our investigation, it is the most abundant species in Kurdistan, Kermanshah, Sistan and Baluchistan, East and West Azerbaijan provinces (Moosa-Kazemi et al., 2015; Ghahvechi Khaligh et al., 2020).

Cx. quinquefasciatus is the second most abundant species captured in this study (20.1%). It transmits some pathogens such as Sindbis virus and *Dirofila immitis* and is reported from most parts of Iran, including Kerman, Fars, Bushehr, Hormozgan, Tehran, Khuzestan, Sistan and Baluchistan provinces (Saidi et al., 1976; Vatandoost et al., 2004; Soltani et al., 2017). This species is a constant species, captured from the most breeding places in our study areas. The other medically and veterinary important species captured in the present study are *Cx. tritaeniorhynchus,* the vector of Japanese encephalitis virus, Rift Valley fever virus, Sindbis virus, West Nile virus, *Wuchereria bancrofti*, *Dirofilaria immitis*, chikungunya virus, and dengue virus (Azari-Hamidian et al., 2005; Moosa-Kazemi et al., 2005; Azari-Hamidian and Harbach, 2009; Chinikar et al., 2010).

Cx. theileri is the vector of *Dirofilaria spp.* and Rift Valley fever virus (Chinikar et al., 2010). *Cx. pipiens* transmits important pathogens such as *Wucheria bancrofti*, West Nile virus, Japanese encephalitis virus, Eastern Equine encephalitis virus, Rift Valley fever virus, and Saint Louis encephalitis virus (Kramer and Ebel, 2003; Kilpatrick et al., 2008; Farajollahi et al., 2011).

In this study, *Ae. vittatus* was reported for the first time from Kerman Province, although it was recorded in other parts of Iran such as Hormozgan and Khuzestan provinces previously (Nasirian et al., 2014; Doosti et al., 2016). This species is the vector of yellow fever, dengue fever, and chikungunya viruses in some parts of the world (Angel and Joshi, 2008; Vazeille et al., 2008; Ngoagouni et al., 2012).

The diversity, distribution, and abundance of mosquitoes are affected by some factors including climatic conditions, urbanization, and local ecological condition (Montagner et al., 2017; Biteye et al., 2018; Chaverri et al., 2018).

In a study conducted in different parts of China species richness was between 6 and 8, and Gini-Simpson index was between 0.07 and 0.42 (Li et al., 2020). There was a significant variation in mosquito density and species richness in different parts of India (Vanlalruia et al., 2014). Similar to these two surveys, biodiversity indices were different in various parts of our study areas.

Generally, biodiversity indices have been largely neglected in Iran. In a survey conducted in different parts of East Azerbaijan Province, located in the northwest of Iran, alpha diversity indices including species richness, evenness, and Shannon-Wiener are in the ranges of 6–11, 0.55–0.727, and 1.01–1.07, respectively. In another study in this region, five species including *An. maculipennis*, *Cx. pipiens*, *Cx. theileri*, *Cs. longiareolata*,and *Ae. caspius* were reported and species richness was equal to five (Naseri-Karimi et al., 2015; Hosseinpour et al., 2019; Paksa et al., 2019).

Seven species were reported in the West Azerbaijan Province with *Cx. pipiens* and *Cx. mimeticus* being the dominant species. *Cx. pipiens* was collected from 68.2% out of the 22 collection sites. *Cx. theileri* was found in 59.1% of collection sites. *Cx. mimeticus*, *Cs. Longiareolata*, and *Cx. modestus* were found in 4.5% of sites (Amini et al., 2020). In the present study, *Cx. pipiens* was common species and *Cs. longiareolata* was constant species. However, the ecological condition is different

between these two areas and this influences mosquito distribution and dispersal.

In a study conducted in Zanjan Province, four species of mosquitoes including *An. maculipennis*, *Cx. pipiens*, *Cx. theileri*, and *Cs. longiareolata* were reported with species richness values equal to four, but no calculations on the diversity indices were performed (Ghavami and Ladoni, 2005).

In the northern part of Iran, the species richness, evenness, and Shannon-Wiener indices were equal to 4–13, 0.229–0.444, and 0.471–1.268, respectively (Nikookar et al., 2015).

In various areas in the south of Iran, the Shannon-Wiener index ranged from 1.50 to 1.64 (Khoobdel et al., 2019). In another study in the south of Iran, the Shannon-Wiener index was 0.38–1.7 (Azari-Hamidian and Harbach, 2009).

Abovementioned studies showed that diversity indices are different in various areas. Therefore, it is not unexpected that these indices are different in various ecological zones of Sirjan County.

In Kerman Province, ecological studies of mosquitoes have focused on southern parts of the province, and also the species composition of anopheline mosquitoes is better documented than for culicines because these areas are malaria foci. Up till now, six species including *An. fluviatilis*, *An. stephensi*, *An. culicifacies*, *An. superpictus*, *An. dthali*, and *An. turkhudi* have been reported from southern parts of Kerman Province (Mehravaran et al., 2011; Edalat et al., 2020). None of the mentioned above Anopheles mosquitoes were caught in our study area because the ecological conditions differ between the south and north parts of Kerman Province. Generally, biodiversity indices have been largely neglected in this province.

The results of the present study showed that Zeid Abad and Khajo-Shahr districts with the lowest elevations have the highest value of the Shannon-Wiener index. This index is influenced by richness and evenness; although Zeid Abad and Khajo-Shahr's richness was lower than Balvard's, the community in these places was evener than Balvard.

In a study conducted in Kosovo, there was a negative correlation between mosquito community size and increasing altitude (Muja-Bajraktari et al., 2019). But in our study, the highest (40.41%) and the lowest (8.70%) ranges of community size were observed in areas with 1984 m.a.s.l. and 2322 m.a.s.l., respectively. It seems that this is due to the difference in the study areas that affect the ecological conditions.

According to Eisen et al.(Eisen, et al. 2008), species richness was highest in plain habitats than in mountainous areas, but this does not coincide with our findings (Eisen et al., 2008). Other factors, such as human activities, seem to affect alpha diversity indices because Pariz with the highest elevation has the lowest richness, evenness and Shannon-Wiener indices. Pariz is neighboring the Sarcheshmeh mineral zone as one of the most prominent copper mines in the world, and it seems that these circumstances influence the diversity indices. The highest level of Shannon-Wiener and evenness indices was observed in the Zeid Abad district (1718 m.a.s.l.) (Table 3). This area is the most populated district in Sirjan County and also one of the most important agricultural zones; therefore, there are many suitable artificial and natural breeding places, plants and animal hosts that can increase mosquito biodiversity.

It seems there is no study on the mosquito similarity index in Iran. Generally, the similarity index was low among different regions of the study area.

Calculating Jaccard's similarity index between different regions of the study areas (Table 5) showed that the lowest rate of similarity was between Balvard (2034 m.a.s.l.) and Pariz (2322 m.a.s.l.) and the highest was between Homashahr (1984 m.a.s.l.) and Khajo-Shahr (1723 m.a.s.l.) The Jaccard's similarity index is affected by species richness and common species between the two regions. The highest and the lowest species richness were observed in Balvard and Pariz, respectively, and common species between the two areas were equal to one. Generally, man-made changes and human intervention in Balvard are lesser than in Pariz, and it influences the species richness in these areas. Although the number of

Table 5. Jaccard's similarity index between different areas of Sirjan County, Iran.

Places	Balvard	Pariz	Zeid Abad	Homashahr	Khajo-Shahr
	10*	2*	6*	5*	7*
Balvard	-	1	3	2	3
Pariz	0.0769	-	1	1	2
Zeid Abad	0.1578	0.1111	-	2	2
Homashahr	0.1176	0.1250	0.1538	-	3
Khajo-Shahr	0.1500	0.1818	j	0.2000	-

common species between Balvard and Khajo-Shahr, and Khajo-Shahr and Homashahr are the same, the species richness in Balvard is the highest and this issue has caused that similarity index between Khajo-Shahr and Homashahr to be higher than the previous two.

In this study, the physicochemical factors of mosquito breeding places were not investigated. And also species identification was done according to morphological characters and molecular methods were not used.

5. Conclusion

Based on the results of the present study, the alpha and beta diversity indices of mosquitoes are different in various ecological zones of Sirjan County. Therefore, different intervention strategies are required in various areas for cost-effective vector control programs.

Conflict of interest

The authors have no competing interests to declare that are relevant to the content of this article.

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Contribution of authors

All authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by Saideh Yousefi, Azim Paksa and Sara Rahimi. The first draft of the manuscript and the revisions were written by Mozaffar Vahedi and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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