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Original Article

The Effects of Different Lunar Phases on Sand Flies (Diptera: Psychodidae) Biodiversity in an Important Focus of Leishmaniasis, Southeast Iran

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Abstract
Background: Anthroponotic cutaneous leishmaniasis (ACL) caused by *Leishmania tropica* is endemic in most parts of Iran; however, its vector ecology has not been extensively studied. This study investigates the effects of lunar phases and climatic factors on the biodiversity and frequency of sand flies in various biotopes, with and without artificial light.
Methods: Sand flies were collected using sticky paper traps in four lunar phases (new moon, first quarter, full moon, and last quarter). Alpha and beta diversity indices were calculated using the relevant formulas. Poisson regression analysis was used to study the relationship between lunar phases and climatic factors with the sand fly frequency.
Results: In places with artificial light, the highest richness was observed in the new moon and the first quarter (S= 7), and the highest species evenness and Shannon-Wiener indices were observed in the last quarter (E= 0.464, H= 0.832) phases. But in areas without artificial light, the maximum value of richness was in the first quarter and full moon (S= 9), and the highest value of evenness and Shannon–Wiener was in the first quarter (E= 0.748, H= 1.645). Non-parametric tests revealed that among climatic factors, only wind speed exhibited a significant correlation with sand fly frequency.
Conclusion: The highest diversity in biotopes with and without artificial light was observed in the last quarter and first quarter, respectively, and also, wind speed influenced the frequency and diversity of sand flies in different habitats, so in sand fly surveillance, it is crucial to consider these important factors.

Keywords: Anthroponotic cutaneous leishmaniasis; Ecology; Phlebotomine sand flies; Diversity

Introduction

Leishmaniasis, classified as a neglected tropical disease, holds considerable public health importance worldwide (1). There are three types of leishmaniasis, each with different clinical manifestations. These include visceral leishmaniasis (VL), the most severe form that can be fatal without treatment; cutaneous leishmaniasis (2), the most common form typically resulting in skin ulcers; and mucocutaneous leishmaniasis (MCL), which affects the mouth, nose, and throat (3).

Approximately 95% of CL cases occur in the Americas, the Mediterranean basin, the Middle East, and Central Asia. It is estimated that between 600,000 to 1 million new cases arise each year globally; however, only about 200,000 are formally reported to the World Health Organization (WHO) (3). The majority of new cases of CL (2) occur in countries such as Iran, Iraq, Afghanistan, Pakistan, Syria, Tunisia, Libya, Brazil, Colombia, Peru, and Algeria, accounting for approximately 87% of annual cases (4, 5).

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In Iran specifically, approximately 15,000 cases of leishmaniasis are reported each year, with 99% being CL (6). Kerman University of Medical Sciences documented 4,993 confirmed cases of CL from 2014 to 2020 (7). Bam County, located in the southeast of Kerman Province, had the highest infection rate at 63.6%, followed by Kerman County at 24.7%, with other districts showing lower rates of infection. In Kerman Province, *Leishmania tropica* has been identified as the primary causative agent, accounting for 95.5% of CL cases, while *Leishmania major* contributed to 4.5% of the total cases. Bam, Kerman, and the southern counties of Kerman Province are classified as purely anthroponotic cutaneous leishmaniasis (ACL) areas (8). Bam County, located in Kerman Province, is one of the most important foci of ACL in southeastern Iran (9, 10).

Phlebotomine sand flies (Diptera: Psychodidae), the primary vectors of various types of leishmaniasis, are nocturnally and crepuscularly active insects and their activity is influenced by temperature, relative humidity (RH), wind speed, and lunar phases (11–14). There are 44 species of sand fly (26 from the *Phlebotomus* genus and 18 from the *Sergentomyia* genus) in Iran, of which 9 species, including 3 *Phlebotomus* (*Ph. sergenti*, *Ph. papatasi*, and *Ph. longiductus*) and 6 *Sergentomyia* (*Se. baghdadis*, *Se. dentata*, *Se. antennata*, *Se. sintoni*, *Se. powlowski*, and *Se. theodori*) are recorded in Bam County (5, 15).

To control the sand flies population, knowledge about their ecology is essential. The biodiversity indices of sand flies represent crucial aspects of their ecology (16). There are three levels of diversity, including alpha, beta, and gamma. Alpha diversity represents the species diversity in an ecosystem. Beta diversity quantifies the species diversity between two ecosystems, and gamma diversity, on the other hand, denotes the overall biodiversity in a vast geographical region (17, 18).

Species diversity refers to the quantity and proportional distribution of species present in a given biological community or ecosystem. Within species diversity, species richness and evenness play crucial roles. Species richness signifies the number of species existing in a particular area, while species evenness describes the equitable distribution of each species (17, 19).

According to the biology of sand flies, lunar phases are expected to influence their activities (20, 21). The phases of the Moon exhibit a pattern of rotation and illumination that varies throughout the lunar cycle, regardless of whether viewed from the northern or southern hemisphere. There are four primary lunar phases, including new moon, first quarter, full moon, and last quarter, each occurring at specific angles relative to the Sun. These phases are followed by intermediate phases, such as crescent or gibbous, which last around 7.38 days on average. The waxing phases signify the Moon's thickening shape towards a full moon, while waning phases indicate a thinning shape. The duration between a full moon and a new moon, or vice versa, ranges from about 13 days 22+1⁄2 hours to 15 days 14+1⁄2 hours. The positioning of the moon in the sky relative to the meridian and the ecliptic varies throughout the year, with different lunar phases appearing highest or lowest during solstices and equinoxes (22–24).

It seems that no study has been done on the effects of moonlight on the sand flies biodiversity indices in Iran, so the present study assessed the influence of different lunar phases on the species richness, evenness and Shannon–Wiener, and similarity indices of sand flies. The study focused on domestic and peri-domestic biotopes (with artificial light) as well as agricultural and sylvatic biotopes (without artificial light), and also the effects of the temperature, RH, wind speed, and moonlight on the frequency of sand flies in the southeast of Iran.

Materials and Methods

Study area

Dehbakri District (29°03'13.89"N, 57°54' 37.75"E) is a new focus of ACL located in the southwest of Bam County (Fig. 1). The mean annual RH, temperature, and rainfall are 45.5%, 14.1°C, and 325 mm, respectively (25). This mountainous area, 2052 meters above sea level, is located between Bam and Jiroft Counties, which experience high temperatures during the summer; therefore, many people from these two populated regions visit Dehbakri District, which is an infected focus of ACL, for summer vacations.

Sand fly sampling

In the study area, four sampling biotops were selected, encompassing domestic and peri-domestic habitats (with artificial light) as well as agricultural and sylvatic environments (without artificial light). Sand flies were collected during six months from mid-May to mid-November in 2018. Sampling was conducted at different lunar phases, including the new moon, first quarter, full moon, and last quarter. Each month involved four sampling sessions during distinct lunar phases at the four different biotopes mentioned earlier. This resulted in a total of 24 sampling events across the four biotopes.

Specimens were captured using sticky paper traps (A4 size, white colored, impregnated with castor oil), with 40 traps deployed at each biotope for each nighttime sampling session. During each sampling event, RH, temperature, and wind speed were also recorded. The collected sand flies were individually preserved in small vials with 75% ethanol. Data regarding lunar phases and other environmental variables were carefully recorded in dedicated tables. For species identification, sand flies were mounted on microscopic slides with Puri's medium. Following the separation of the head and two terminal abdominal segments, morphological keys (13) were utilized for species identification.

Biodiversity analyses

Alpha diversity indices

Richness (S) is the number of species captured in a place or at a particular time (new moon, first quarter, last quarter, and full moon) (26).

The following formulas were used to calculate Shannon-Wiener (H´) and evenness (E) (19):

$$H' = -\sum p_i \times \ln P_i$$

Pi= ratio of a species to the total number of captured specimens

$$E = \frac{H}{\ln S}$$

H= Shannon–Wiener
S= Richness

Beta diversity indices

Jaccard's similarity index is used to check the similarity between two places or two lunar phases (12).

$$Sj = \frac{a}{a + b + c}$$

a= The number of species found in both lunar phases
b= Number of species in the first lunar phase
c= Number of species in the second lunar phase

Statistical analysis

The effects of qualitative variables, such as biotope type (domestic and peri-domestic biotopes with artificial light, and agricultural and sylvatic biotopes without artificial light) and lunar phases (new moon, first quarter, full moon, and last quarter) on *Ph. sergenti* frequency were analysed using the Kruskal-Wallis and Mann-Whitney tests. The impact of meteorological variables, including RH, temperature, and wind speed, was assessed using Spearman correlation analysis. All analyses were performed using SPSS version 27.

Results

Composition of the sand fly species

In total, 6227 sand fly specimens were captured, with 60% found in sylvatic and agricultural biotopes (without artificial light) and the remaining 40% in domestic and peri-domestic environments (with artificial light). Eleven species belonging to two genera, *Phlebotomus* (*Ph. sergenti*, *Ph. papatasi*, *Ph. alexandri*, and *Ph. kazeruni*) and *Sergentomyia* (*Se. baghdadis*, *Se. dentata*, *Se. squamipleuris*, *Se. sintoni*, *Se. theodori*, *Se. antennata*, and *Se. pawlowsky*) were captured. In domestic and peri-domestic biotopes, 9 species were collected, including 4 species of *Phlebotomus* and 5 species of *Sergentomyia*, and in sylvatic and agricultural biotopes, 10 species, including 3 species of *Phlebotomus* and 7 species of *Sergentomyia*, were captured. *Phlebotomus sergenti* was the most abundant species with 69% of collected specimens, followed by *Sergentomyia baghdadis*, which accounted for 19% (Table 1).

Moreover, the dominant species, *Ph. sergenti*, and the second most abundant species, *Se. baghdadis*, were present under both light conditions and various biotope types. Among the different lunar phases, the abundance of total sand flies caught was 31%, 24.4%, 24% and 20.6% during the full moon, new moon, last quarter, and first quarter, respectively. In all lunar phases except the first quarter, the abundance of sand flies was greater in biotopes without artificial light compared to those with artificial light. The three dominant species in all lunar phases are *Ph. sergenti*, *Se. baghdadis*, and *Se. dentata* (Table 1).

Biodiversity analyses

Alpha diversity indices

In biotopes with artificial light, the highest species richness was recorded during the new moon and first quarter phases, while the last quarter phase exhibited the highest evenness and Shannon-Wiener indices. In contrast, biotopes without artificial light showed the highest species richness during the first quarter and full moon phases, with the first quarter phase having the highest evenness and Shannon-Wiener indices (Table 2).

Beta diversity indices

In environments with artificial light, the highest similarity (Sj= 0.333) was observed between the full moon and last quarter phases, while the lowest (Sj= 0.263) was found between the first quarter and new moon phases. Conversely, in natural biotopes such as sylvatic and agricultural areas without artificial light, the highest similarity index (Sj= 0.316) was noted between the new moon and last quarter phases, with the lowest (Sj= 0.286) occurring between the last quarter and first quarter, as well as between the full moon and last quarter phases (Table 3).

When comparing environments with and without artificial light, the lowest similarity (Sj= 0.263) was found between the new moon phases. However, the highest similarity (Sj= 0.333) was observed between the last quarter phases in both types of environments and also last quarter full moon phases. Additionally, there was a notable similarity between the full moon phase in areas with artificial light and the last quarter phase in locations without artificial light (Table 3).

The meteorological data

The temperature, RH, and wind speed for different lunar phases and biotops during the study period are shown in Fig. 2. Over the 6-month study period, the maximum and minimum values recorded were: temperature 29 °C and 13 °C, RH 60% and 39%, and wind speed 4.12 m/s and 2.14 m/s.

Statistical analyses

Table 4 presents the detailed outcomes of the statistical analyses conducted to examine the influence of biotopes and lunar phases on the abundance of *Ph. sergenti* populations. The non-parametric tests, including the Mann-Whitney U test and Kruskal-Wallis, revealed no significant differences in sand fly frequency across various biotopes or lunar phases. Correlation analyses using Pearson's R indicated no significant associations between sand fly frequency and RH, and temperature. Among the meteorological variables examined, only wind speed exhibited a significant negative correlation with sand fly frequency in agricultural and sylvatic areas (R= -0.44, P-value= 0.02). Regarding lunar phases, only temperature exhibited a significant positive correlation with sand fly frequency during the full moon phase (R= 0.63, P-value= 0.02). However, no statistically significant differences were found regarding the abundance of *Ph. sergenti*, and the strongest correlations with meteorological parameters were weak, suggesting that environmental variables and lunar phases may have a limited direct impact on the distribution patterns of this species within the studied area (Table 4).

Fig. 1. Geographical location of sand fly collection site, Bam County, Kerman Province, Iran, 2018

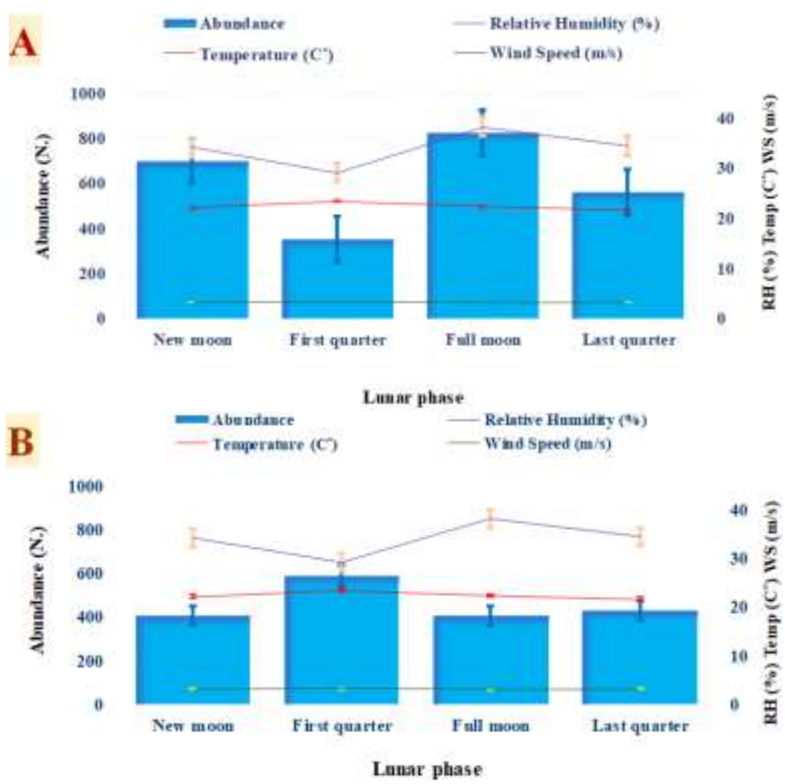

Fig. 2. Temporal variation in temperature, relative humidity (RH), wind speed, and sand fly frequency rate across four lunar phases during the six-month sand fly activity period (mid-May–mid-October 2018), in Bam County, Kerman Province, southeast Iran. (A) agricultural and sylvatic and (B) domestic and peri-domestic biotopes. Bars represent the mean ± SEM (standard error of the mean) for each lunar phase calculated across sampling sites within each biotope

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Table 1. Frequency of collected sand flies in different lunar phases and biotopes, Bam County, Kerman Province, 2018

Biotopes / Species	Lunar phases												Total		
	New moon			First quarter			Full moon			Last quarter					
	*D/PD (N.) (%)	** A/S (N.) (%)	Total (N.) (%)	D/PD (N.) (%)	A/S (N.) (%)	Total (N.) (%)	D/PD (N.) (%)	A/S (N.) (%)	Total (N.) (%)	D/PD (N.) (%)	A/S (N.) (%)	Total (N.) (%)	D/PD (N.) (%)	A/S (N.) (%)	Total (N.) (%)
Phlebotomus sergenti	408 (77.56)	702 (70)	1110 (73)	592 (72)	355 (76.5)	947 (73.8)	409 (74.7)	827 (60)	1236 (64)	432 (72)	563 (62.5)	995 (66)	1841 (73.9)	2447 (65.5)	4288 (68.9)
Phlebotomus papatasi	16 (3)	9 (1)	25 (1.6)	12 (1.5)	9 (1.9)	21 (1.6)	14 (2.55)	20 (1)	34 (1.7)	30 (5)	17 (2)	47 (3.1)	72 (2.8)	55 (1.4)	127 (2)
Phlebotomus alexandri	2 (0.3)	0 (0)	2 (0.1)	0 (0)	2 (0.43)	2 (0. 002)	0 (0)	1 (0.07)	1 (0.05)	0 (0)	0 (0)	0 (0)	2 (0.1)	3 (0.08)	5 (0.08)
Phlebotomus kazeruni	0 (0)	0 (0)	0 (0)	1 (0.02)	0 (0)	1 (0.001)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0)	0 (0)	1 (0)
Sergentomyia baghdadis	88 (16.8)	165 (17)	253 (16.6)	184 (22.5)	70 (15)	254 (19.8)	92 (16.8)	286 (20.8)	378 (19.6)	118 (19.7)	196 (21.7)	314 (21)	482 (19.3)	717 (19.2)	1199 (19.3)
Sergentomyia dentata	9 (1.7)	102 (10.2)	111 (7.2)	22 (2.5)	12 (2.6)	34 (2.7)	25 (4.6)	121 (8.8)	146 (7.6)	15 (2.5)	120 (13.3)	135 (9)	71 (2.8)	355 (9.5)	426 (6.8)
Sergentomyia squamipleuris	1 (0.19)	0 (0)	1 (0.05)	0 (0)	1 (0.02)	1 (0.002)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0)	1 (0)	2 (0)
Sergentomyia sintoni	0 (0)	3 (0.3)	3 (0.1)	5 (0.6)	11 (2.3)	16 (1.2)	5 (1)	76 (5.53)	81 (4.2)	4 (0.66)	3 (0.3)	7 (0.5)	14 (0.6)	93 (2.5)	107 (1.7)
Sergentomyia theodori	2 (0.38)	14 (1.4)	16 (1.05)	3 (0.07)	3 (0.006)	6 (0.05)	2 (0.3)	29 (2.1)	31 (1.6)	1 (0.16)	2 (0.2)	3 (0.2)	8 (0.3)	48 (1.3)	56 (0.9)
Sergentomyia pawlowsky	0 (0)	1 (0.1)	1 (0.05)	0 (0)	1 (0.02)	1 (0.001)	0 (0)	1 (0.07)	1 (0.07)	0 (0)	0 (0)	0 (0)	0 (0)	3 (0.1)	3 (0)
Sergentomyia antennata	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	13 (1)	13 (0.70)	0 (0)	0 (0)	0 (0)	0 (0)	13 (0.3)	13 (0.2)
Total	526 (100)	996 (100)	1522 (100)	819 (100)	464 (100)	1283 (100)	547 (100)	1374 (100)	1921 (100)	600 (100)	901 (100)	1501 (100)	2492 (100)	3735 (100)	6227 (100)

* **D/PD:** Domestic and pre-domestic biotopes with artificial light
** **A/S:** Agriculture and sylvatic biotopes without artificial light

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Table 2. Alpha diversity indices in different lunar phases and biotopes, Bam County, Kerman Province, 2018

Lunar phase	Biotope					
	Domestic and peri-domestic			Agriculture and sylvatic		
	Richness	Evenness	Shannon-Wiener	Richness	Evenness	Shannon- Wiener
New moon	7	0.364	0.708	7	0.462	0.899
First quarter	7	0.395	0.769	9	0.748	1.645
Full moon	6	0.450	0.806	9	0.539	1.185
Last quarter	6	0.464	0.832	6	0.554	0.993

Table 3. The Jaccard similarity index between different lunar phases in places with and without artificial light, Bam County, Kerman Province, 2018

Lunar phase/ Artificial light		With artificial light				Without artificial light			
		New moon	First quarter	Full moon	Last quarter	New moon	First quarter	Full moon	Last quarter
With artificial light	New moon	-	5	5	5	5	7	6	5
	First quarter	0.263	-	6	6	6	6	6	6
	Full moon	0.278	0.316	-	6	6	6	6	6
	Last quarter	0.278	0.316	0.333	-	6	6	6	6
Without artificial light	New moon	0.263	0.300	0.316	0.316	-	7	7	6
	First quarter	0.304	0.272	0.286	0.286	0.304	-	8	6
	Full moon	0.272	0.272	0.286	0.286	0.304	0.308	-	6
	Last quarter	0.278	0.316	0.333	0.333	0.316	0.286	0.286	-

The top and right panel shows the number of common species in lunar phases compared pairwise.

Table 4. Statistical analysis of the effects of lunar phases and climate conditions on sand fly frequency, Bam County, Kerman Province, 2018

Data	Test	Variable	Sample Size (No.)	Test Statistic	P-value
Total	Mann-Whitney U	Biotopes	24 per group	U= 268.50	0.687
	Kruskal-Wallis	Lunar phase	12 per group	H= 0.807	0.848
	Spearman's Rank-Order correlations	Relative humidity (%)	48 per group	R= 0.122	0.407
		Temperature (°C)	48 per group	R= 0.26	0.07
		Wind speed (m/s)	48 per group	R= - 0.36	0.02*
Domestic and Peri-domestic	Spearman's Rank-Order correlations	Relative humidity (%)	24 per group	R= 0.2	0.33
		Temperature (°C)	24 per group	R= 0.3	0.07
		Wind speed (m/s)	24 per group	R= - 0.19	0.35

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Table 4. Continued ...

Agricultural and Sylvatic	**Spearman's Rank-Order correlations**	**Relative humidity (%)**	24 per group	R= 0.24	0.24
		Temperature (°C)	24 per group	R= 0.14	0.44
		Wind speed (m/s)	24 per group	R= - 0.44	0.02*
Domestic and Peri-domestic	**Kruskal-Wallis**	**Lunar phase**	6 per group	H = 2.5	0.47
Agricultural and Sylvatic	**Kruskal-Wallis**	**Lunar phase**	6 per group	H= 0.1	0.99
New moon	**Spearman's Rank-Order correlations**	**Relative humidity (%)**	12 per group	R= 0.7	0.81
		Temperature (°C)	12 per group	R= 0.28	0.37
		Wind speed (m/s)	12 per group	R= - 0.5	0.08
First quarter	**Spearman's Rank-Order correlations**	**Relative humidity (%)**	12 per group	R= 0.19	0.53
		Temperature (°C)	12 per group	R= 0.001	1
		Wind speed (m/s)	12 per group	R= - 0.34	0.37
Full moon	**Spearman's Rank-Order correlations**	**Relative humidity (%)**	12 per group	R= 0.46	0.12
		Temperature (°C)	12 per group	R= 0.63	0.02*
		Wind speed (m/s)	12 per group	R= 0.33	0.29
Last quarter	**Spearman's Rank-Order correlations**	**Relative humidity (%)**	12 per group	R= 0.01	0.96
		Temperature (°C)	12 per group	R= 0.22	0.47
		Wind speed (m/s)	12 per group	R= - 0.54	0.06

Discussion

Cutaneous leishmaniasis continues to be a significant public health concern worldwide, especially in tropical and sub-tropical regions (27). Understanding the ecology of leishmaniasis vectors is crucial for surveillance studies and control programs (16, 28, 29). Biodiversity indices of sand flies play a critical role in understanding their ecology (30). They are active insects that are primarily active during the night and at dawn or dusk. Their activity is affected by a range of environmental factors, including the lunar phases (31-33).

In the present study, we first examined the effects of different lunar phases (new moon, first quarter, full moon, and last quarter) on sand fly biodiversity indices in different biotopes (with and without artificial light), and then the role of different environmental factors on their frequency was investigated.

Although the overall species richness in this study was 11 (S= 11), in agricultural and sylvatic biotopes with no artificial light, species richness was highest during the first quarter and full moon phases (S= 9). Also, in biotopes with artificial light, the highest species richness was observed in the first quarter and new moon phases (S= 7).

A study conducted by Aghasi et al. before the devastating 2003 earthquake in Bam County, a total of 5 species (2 species of *Phlebotomus* and 3 species of *Sergentomyia* genera) were captured. But in the present study, species richness was more than double (34). Perhaps one of the most reasons for this difference

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is the severe environmental changes that occurs after the Bam County earthquake, which provide suitable environment for establishment and reproduction of new species of sand flies and, in addition to this factor the role of global warming in the dispersion and establishment of sand fly species should also be considered.

Yousefi et al. collected a total of nine sand fly species from mountainous and plain areas of Bam County (10). They collected sand flies bimonthly without considering lunar phases, but we collected sand flies weekly in various lunar phases. However, more frequent sampling increases the probability of capturing rare sand fly species.

The Jaccard similarity index is influenced by species richness (S) and the number of common species between two lunar phases (a). In biotopes with artificial light, the greatest Jaccard similarity index was observed between the full moon and last quarter phases, where all six captured species were common in both phases (S= 6, a= 6). The lowest similarity index was between the first quarter and new moon phases, where only five species were shared between the two lunar phases (S= 7, a= 5).

In agricultural and sylvatic biotopes, without artificial light, the highest species richness was observed in the first quarter and full moon phases (S = 9), while species evenness and the Shannon-Wiener index varied across different lunar phases. The highest Shannon-Wiener index was recorded in the first quarter phase due to the combination of high richness and evenness in this phase.

In biotopes without artificial light, the highest similarity index was found between the new moon (S= 7) and last quarter (S= 9) phases, with seven common species shared between these two phases (a= 7). The lowest similarity index was between the last quarter (S= 6) and first quarter (S= 9), as well as between the last quarter and full moon phases (S= 9), with six common species shared between each pair (a= 6). Generally, a higher number of common species leads to a higher similarity index.

The comparison of different lunar phases in biotopes with and without artificial light revealed that the highest similarity index was observed between the last quarter phases in both types of biotopes, as well as between the last quarter phase in areas without artificial light and the full moon phase in areas with artificial light. In these areas, the species richness and number of common species were both six (S= 6, a= 6). The lowest similarity index was found between the new moon phases in areas with and without artificial light, with species richness (S) and common species (a) being 7 and 5, respectively. Based on these findings, it appears that the impact of moonlight on sand fly activity is more significant than the effect of artificial lights, as the similarity index between similar phases in different biotopes is higher than that of dissimilar phases.

Environmental factors such as temperature, relative humidity, wind speed, and moonlight effectively affect phlebotomine sand flies activity and frequency and, subsequently, the occurrence of leishmaniasis (31–33). In the present study, there was a significant negative correlation between wind speed ($P< 0.05$) and sand fly frequency. Thies et al. indicated that there was no significant correlation between sand fly frequency with relative humidity, temperature, and wind speed (35).

In this study, unlike studies conducted in Brazil, Morocco, and Iran, no significant relationship was found between the frequency of sand flies with temperature and relative humidity (36–38).

Sand flies, being nocturnal insects, have been the subject of studies investigating their frequency concerning various lunar phases in different areas. However, no such study had been conducted in Iran until our research. Our study revealed that there was no positive correlation between sand fly frequency and various lunar phases.

A study conducted in Ethiopia using the CDC light trap method found that the abundance of sand flies during the new and full moon phases was higher compared to the first and last quarter phases, although this difference was not statistically significant (20). Similarly, studies in Brazil and Colombia using aspirator and CDC light trap methods reported a higher abundance of phlebotomine sand flies during the full moon phase compared to other phases (32, 38). However, Santos-De Marco et al. observed reduced sand fly activity during the crescent and full moon phases in their study, where the new moon and waning phases did not significantly affect sand fly abundance; they employed Shannon and Falcao light traps (21). In contrast, studies by Kassem et al. found no significant difference in sand fly frequency across different lunar phases (39). Consistent with these findings, studies in Brazil and Kenya also supported this result (21, 40). Studies mentioned above did not compare the frequency of sand flies in different habitats (with and without artificial light), and they also utilized varying capture methods. Nonetheless, it appears that the moonlight patterns in different lunar phases have a non-significant impact on the frequency of sand flies across all habitats.

Although a recent study showed that there was no significant relationship between various lunar phases and the frequency of sand flies, it should be noted that the biodiversity indices varied among different lunar phases. Therefore, in surveillance studies for leishmaniasis vectors, it is important not only to sample from various locations but also to sample in different lunar phases.

Conclusions

This research showed that the highest biodiversity was observed in the last and first quarter phases in biotopes with and without artificial light, respectively, and also wind speed influenced the frequency and biodiversity of sand flies in different biotopes, so it is essential to consider these issues in biodiversity research and surveillance programs.

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Ethical consideration

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The Tehran University of Medical Sciences ethics board permitted the study procedures with the ethical approval number: IR.TUMS.SPH. REC.1396.3601.

Conflicts of interest statement

The authors declare that there is no conflict of interest.

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