Entomology, Ornithology & Herpetology: Current Research

Gorbani et al., Entomol Ornithol Herpetol 2018, 7:2
DOI: 10.4172/2161-0983.1000212

Research Article Open Access

Ecological and Climatic Factors Affecting on the Abundance and Vector Capacity of the Main Vector of Visceral Leishmaniasis (*Ph. Kandlakii*) in North-Western of Iran

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Received date: May 10, 2018; **Accepted date:** May 28, 2018; **Published date:** June 04, 2018

Abstract

Sand flies are vectors of leishmaniasis, which spread all over the world. One of the most important vectors of visceral leishmaniasis in Iran is *Ph. kandlakii*, which has been known as the main vector in northwestern Iran. Understanding habits and ecology of this vector can help us abundantly to control and prevent visceral leishmaniasis outbreak. This study has been conducted in Meshkin Shahr which is one of the main foci endemic in Northwest of Iran and in this study 4 villages (2 villages of polluted area and 2 villages of unpolluted area) were selected for examining and collected sand flies and comparing ecological factors. Totally 1100 sand flies were collected in two areas which 700 of them were from polluted area and 400 sand flies were from unpolluted area. Out of this population, 19 sand fly (4.75%) of *Ph. kandlakii* were in east area (unpolluted) and 29 sand fly (4.14%) of *Ph. kandlakii* were in west area (polluted). in polluted area (west), frequency of *Ph. kandlaky* is more than east unpolluted areas which causes the advent of more diseases in west areas.

Keywords: Visceral leishmaniasis; *Ph. kandlakii*; Meshkin shahr; Ardebil

Introduction

Diseases transmitted by vectors in humans and animals in the world are increasing rapidly and this includes one third of all infectious diseases [1]. Leishmaniasis is a parasitic disease; this disease is appeared in different types such as cutaneous leishmaniasis, mucocutaneous, diffuse and visceral leishmaniasis. The vectors of the disease are sand flies polluted with paradise which is transmitted to human body by the bite of the sand fly [2]. At least 20 different species of parasites of *Leishmania* genus can be transmitted by sand flies. Approximately 1.5 cutaneous Leishmaniasis and 500000 visceral Leishmaniasis occur over the world yearly. After Malaria, this disease is considered as the second health problem in the world [2]. Currently 12 million people in the world are polluted with the paradise and 350 million people are exposed to leishmaniasis. Most of Cutaneous leishmaniasis is observed in Afghanistan, Saudi Arabia, Iran, North and South America, and 90% of leishmaniasis has been seen in Middle East [3]. Vectors of leishmaniasis are bloodsucking sand flies that two important genus phlebotomus in the Old World and lutzomyia in the New World are vectors and more than 700 species of sand flies have been identified so far that about 30 species can transmitted *Leishmania* to human being [4-6]. Visceral leishmaniasis is the severe and hard form of this group of diseases [7]. 0.2-0.4 million cases were from this kind of disease and it has over 40,000 deaths per year [8]. Visceral leishmaniasis (VL) in Iran is Mediterranean and caused by *Leishmania infantum* [9], dogs and canines were reservoirs and every year 100-300 cases of human of this disease are reported in Iran [10,11]. VL in Iran is more in children and according to the examination more than 89 per cent of patients in endemic areas are consisted of less than 5 years old [12]. According to studies of researchers, 5 species of sand flies are proven vectors of visceral leishmaniasis in Iran: Phlebotomus perfiliewi, *Ph. kandelakii*, *Ph. major, Ph. Alexandri* and *Ph. tobbi* [13-18]. Regarding the presence of proven vectors and proper reservoirs in Ardebil has made this Province as a high-risk endemic area. Currently 25-50 per cent of visceral leishmaniasis cases of the state occur in Ardebil Province and therefore it is one of the most important endemic foci of disease in Iran [19,20]. According to conducted studies, more than 80 per cent of visceral leishmaniasis occurs in Meshkin Shahr in western part and 20 per cent of it occurs in eastern area [12]. Therefore most of *Ph. kandelakii* sand fly cases have been collected from villages of west areas [21]. So, a full review should be done about indicators of habitat of sand flies in both eastern and western areas of Meshkin Shahr in order to compare the type of soil, plant, temperature and humidity with together.

Material and Methods

This study has been conducted in Meshkin Shahr which is one of the main endemic foci in Iran and in this study four villages (two villages from polluted area and two villages from unpolluted area) were selected for study and collection. The polluted villages were selected from western area (Qortappeh and Niaz soee) and unpolluted villages were selected from eastern area (Kangarloo and Dadeh Bigloo) (Figure 1). In these areas abundance and variety of sand flies and some climatic factors such as temperature, humidity, rainfall, soil type and vegetation and places and different habitats were done from May 2013 to December 2013.

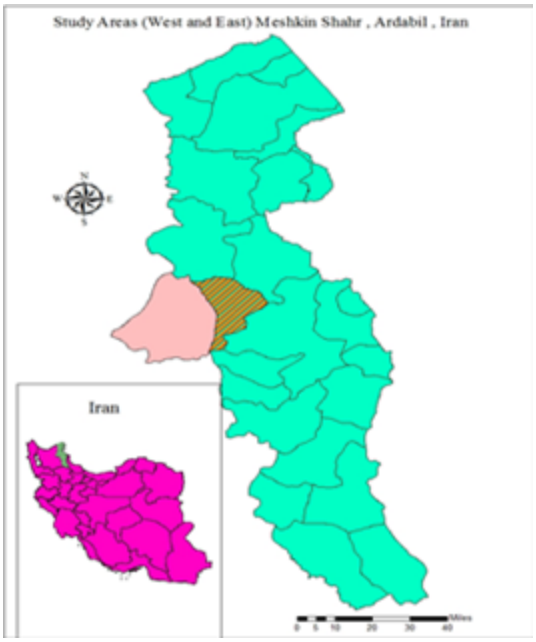


Figure 1: The study area, Meshkin Shahr, Ardabil province, Iran (2013).

For studying sand flies, every 15 days for 8 months 60 sticky paper traps were used for each village-30 traps for indoors such as bedroom, barn, aviary, haystack and storage and 30 traps for outdoors such as yard, nests, fox burrows, crevices of rocks, stone walls, ruined places and riverbank. In the range of selected biotopes, climatic parameters were measured by daily digital hydrometer. For studying the type of soil, it was collected in a one-meter chip in standard containers and transferred to the lab. The collection sand flies were permanently mounted by pouri solution and then they were identified by using standard identification keys (Rashti and Nadim 1992) and (Nadim and Javadian 1976) and (Theodor 1958), (Perfelio 1968) and (Louis 1982).

Results

East region

East area of Kangarloo village has semi-arid with mild intensity and Dadeh Beigloo village has semi-arid with moderate intensity. The average of ground water level in east region in the village was determined as 8.5 meter and 23.02 meter out of the village. The number of cloudy days, sunny days and rainy days in Meshkin Shahr in second half of May till end of December were 74, 125 and 175 days respectively. In terms of vegetation in east region, *Poa buibosa* has the most per cent of cover and *Thymus kotseyanus* has the lowest percentage of cover and composition (Table 1).

West region (high risk)			**East region (Low Risk)**		
Species of plants	**Composition%**	**Coverage %**	**Species of plants**	**Composition%**	**Coverage%**
poa buibosa	7	12/28	poa buibosa	9	17/31
Artemsiai sibry	12	21/05	Artemsiai sibry	6	11/55
salsola rigida	3	5/26	salsola rigida	5	9/61
Erodium sp	6	10/54	Erodium sp	5	9/61
Medicage sp	5	8/77	Medicage sp	4	7/69
Astragalus sp	2	3/51	Astragalus sp	4	7/69
Stipa barbata	4	7/02	Hordeum morinum	3	5/77
Bromus tectorum	1	1/75	Stipa barbata	3	5/77
A Ropjron.sp	5	8/77	Bromus tectorum	3	5/77
Centaurea virjata	4	7/02	Cynodon dactylon	2	3/85
Secale careale	3	5/26	Bromus danthonia	2	3/85
Noea macronata	2	3/51	Bromus tomentellus	2	3/85
Atraphaxis spinosa	2	3/51	Noea muceronata	1	1/92
Sanguisorba minor	1	1/75	Atriplex sp	1	1/92
-	-	-	Alyssum sp	1	1/92
-	-	-	Thymus kotseyanus	1	1/92

Table 1: Composition and percentage of vegetation cover in west and east regions of Meshkin Shahr.

Sand fly analyses

Totally 400 sand flies were collected that out of them 19 sand flies (4.75%) were *Ph. kandelakii* collected from indoors and outdoors by sticky paper trap. 8 sand flies were collected from indoors which include 15.78% from barn, from aviary 15.78% and from living room 10.5% and from outdoors totally 11 *Ph. kandelakii* were collected which include 10.5% from yard, 10.5% from gap mountain and 36.94% from fox den (Table 2). Seasonal activity of this species in indoors starts from second half of June and continues to second half of July and seasonal activity in outdoors starts from second half of July and continues till first half of September.

Soil sample analysis

The results of analysis of soil sample in outdoor (fox den) show that soil texture in the range of 30-100 cm depth profiles, Silty clay loam type and composition of silt, clay and sand are 54-46, 14-10 and 44-32 per cent respectively. The percentage of organic materials in soil samples has been 0.87-1.83 and salinity rate has been 1.2-12.8 Ds per square meter. The acidity of the examined soil was 7.25-7.8 and the rate of Total Neutralizing Value (TNV), Calcium (Ca), Magnesium (Mg) and Nitrogen (N) have been achieved in the range of 10.63-27.21 per cent, 6-48.8 and 9.2-98.4 mil gram per litre and 0.1-0.2 per cent respectively. The results of soil samples excavated profiles in near indoor biotopes in which *Ph. kandelakii* was collected that soil texture has been in the range of 30-100 cm depth profiles and the composition of silt, clay and sand have been 48-54, 10-810 and 40-44 per cent respectively (Figure 2). The percentage of organic materials in soil samples has been 1.03-1.54 and salinity rate has been 2-11.2 Ds per square meter. The acidity of examined soil was 7.75-8.05 and the rate of TVN, Ca, Mg and N has been attained in the range of 14.34-38.98 per cent, 7.2-32.8 and 12-66.4 mil g per litre and 0.1 per cent (Figures 3 and 4). The range of air changes in outdoors in biotope of fox den for *Ph. kandelakii* during season of activity and at the time of emergence was minimum 14.4 and maximum 25 degrees and for the stage of disappearance minimum 21.3 and maximum 32.5 degree. In outdoors, the range of humidity for emergence and disappearance were recorded as 44-99 per cent and 10-51 per cent respectively (Figure 5). The range of temperature in indoor was at the time of emergence minimum 20.5 and maximum 32.4 and for the step of disappearance it was minimum 23.3 and maximum 32.2 degree. The relative humidity in the places at the time of emergence was 35.4-81.4 and for disappearance was 20-62.26 per cent (Figure 4).

Area of study	Total		outdoor						Indoor					
			Fox kennels		Rock crevices		Yards		Human boriding		Hen nests		stable	
	%	N	%	N	%	N	%	N	%	N	%	N	%	N
East (Low Risk)	100	19	36/94	7	10/5	2	10/5	2	10/5	2	15/8	3	15/8	3
West (high risk)	100	29	0	0	24/14	7	48/3	14	Oct-34	3	Mar-44	1	13/78	4

Table 2: The number of *Ph. kandelakii* collected from different locations in the study area (2013).

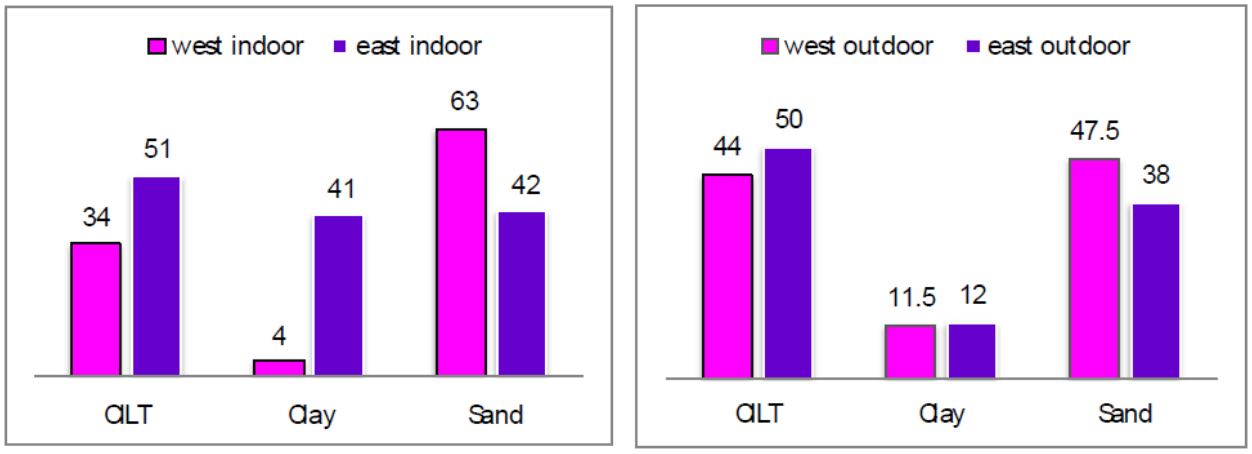
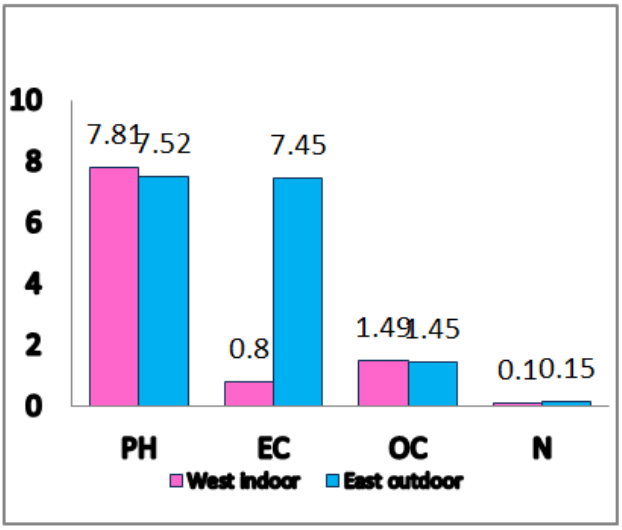


Figure 2: Synthetic of soil texture in outdoor and indoor places in both East and West area Meshkin shahr (2013).



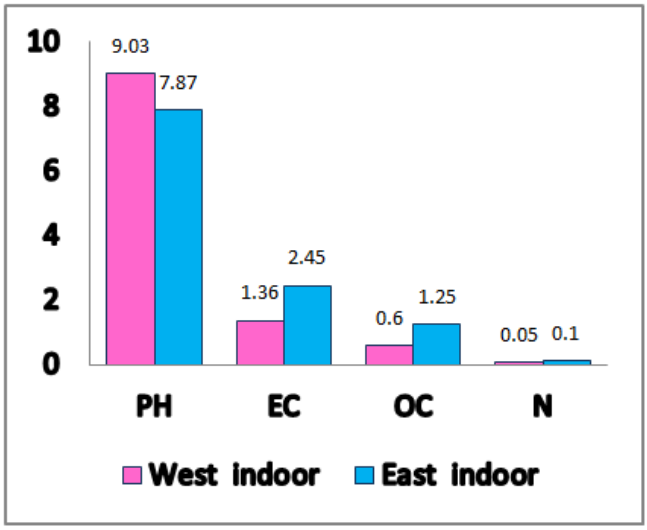
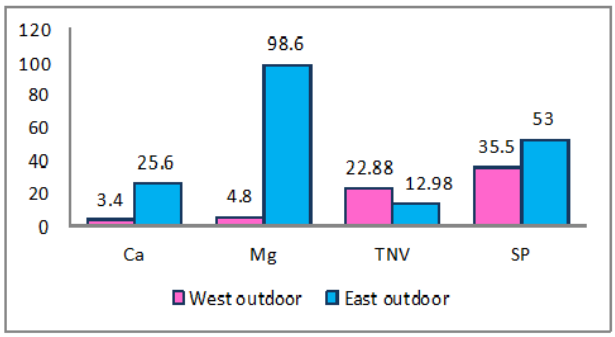


Figure 3: Compare soil organic matter in both East and West areas (Outdoor and Indoor), Meshgin shahr, Ardabil, Iran (2013) Potential of hydrogen (PH), Electrical Conductivity (EC), Organic Carbon (OC), Nitrogen (N).



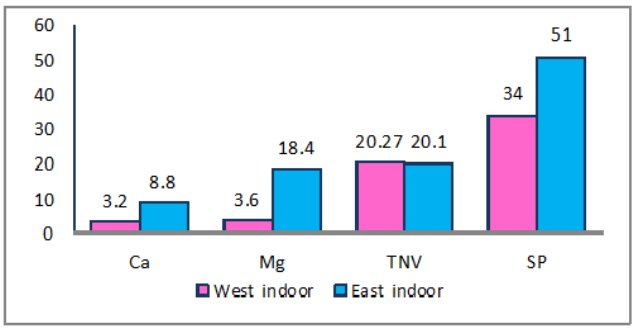


Figure 4a: Compare soil organic matter in both East and West areas (Outdoor and Indoor), Meshgin shahr, Ardabil, Iran (2013) Calcium (Ca), Magnesium (Mg), Total Neutralizing Value (TNV), Saturation Percentage (SP).

Western region

In west area, Qort Tappeh and Niaz were selected. The climate of both villages was semi-arid with moderate intensity. The average of underground water level in west area was determined in the village as 18 meters and out of the village as 22.12 meters. The number of cloudy, sunny and rainy days in west area of Meshkin Shahr from the second half of June to the end of December was 60, 138 and 140 days respectively. In terms of vegetation, in west area *Poa buibosa* species has the most per cent of covering and composition and *Sanguisorba* minor species has the least per cent (Table 1).

Sand fly analyses

Totally 700 sand flies were collected that out of it 29 sand flies (4.14%) were *Ph. kandelakii* collected by sticky paper trap from outdoor and indoor places. 8 sand flies (42.1%) of indoors include 4 (15.78%) were netted from barn, 1 (15.78%) from aviary and 3 from living room (10.5%) and from outdoors totally 11 *Ph. kandelakii* (57.9%) include 14 numbers were collected from yard (10.5%) and 7 numbers from Rock crevices (Table 2).

Seasonal activity of this species in indoors starts from the second half of June and continues to the first half of September and seasonal activity in outdoors starts from the second half of June and continues to the second half of October. The results of soil sample analysis in outdoors show that soil texture in the range of 30-100 cm soil depth profiles has been from Loamy to sandy loam type and the combination of silt, clay and sand has been 36-52, 11-12 and 36-58.6 per cent respectively. The percentage of organic materials in soil samples has been 0.87-1.58 and the salinity rate has been 0.5-10.5 Ds per square meter. The acidity of examined soil was 0.87-1.58 and the rate of TNV, Ca, Mg and N has been attained in the range of 14.34-38.35 per cent, 2.8-14 ml g per litre and 0.1 per cent (Figures 3 and 4).

Soil sample analysis

The results of soil sample profile in indoors in which *Ph. kandelakii* were netted that soil texture in 30-100 cm soil depth profiles was sandy

loam type and the combination of silt, clay and sand has been 32-36, 4 and 62-64 per cent respectively. The percentage of organic materials in soil samples has been 0.26-0.97 and salinity rate has been 0.5-0.55 Ds per square meter. The acidity of examined soil was 8.05-8.1 and the rate of TNV, Ca, Mg and N has been attained as 0.42-1.69 per cent, 2.4-48 and 3.2-9.6 ml g per litre and 0-0.1 per cent (Figures 3 and 4a).

The range of temperature changes in outdoors in biotope *Ph. kandelakii* during season of activity and at the time of appearance was minimum 22.1 and maximum 33 degrees and for the step of disappearance was minimum 24.5 and maximum 34.9 c. In outdoors, the humidity range for the emergence and disappearance were recorded as 50-62 per cent and 10-60 per cent (Figure 5). The temperature ranges in indoors at the time of emergence was minimum 22.3 and maximum 31.2 and for the step of disappearance was min 18.9 and max 29.1 degrees. The relative humidity in indoors at the time of appearance was 29.3-70.1 and for disappearance was 32.3-86.7 per cent (Figure 4b).

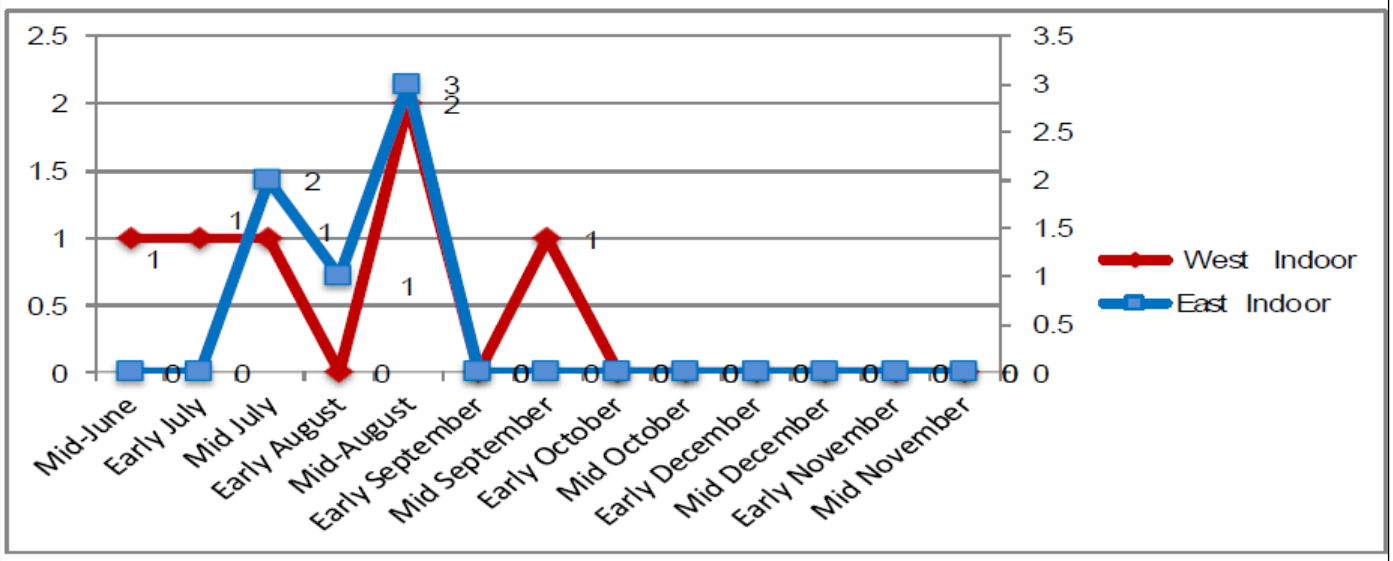


Figure 4b: Monthly activity of *Ph. kandelakii* in indoors with separation of east and west in Meshkin Shahr.

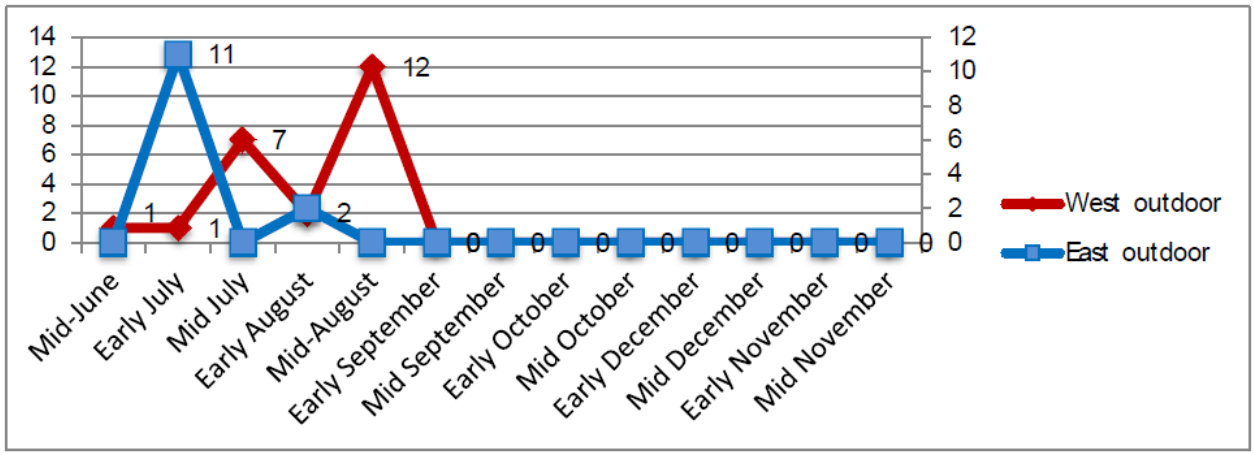


Figure 5: Monthly activity of *Ph. kandelakii* in outdoors with separation of east and west in Meshkin Shahr.

Discussion

The east studied area is in weak semi-arid climate and the west region which has many diseases is placed in moderate semi-arid climate that regarding the results, the collection of *Ph. kandelakii* sand flies done more in west than east and this is according to the previous reports [16,22]. In terms of vegetation covering east region had less plant density than west region but the species of most plants was the same and it was Poa buibosa. The number of plant species was more in east than west. In the study of Moncaz in Ethiopia the dominant vegetation in the region was Sorgham due to the presence of sand fly and the effect of vegetation density has been stressed on the emergence

process of sand flies [23]. The start of seasonal activity of *Ph. kandelakii* in east region was the second half of June but in west region was the first half of July that is 15 days after which this is due to the difference of temperature and climate. The end of seasonal activity during the year in east was till the end of September and in west was till the first half of October. Totally the activity range of *Ph. kandelakii* in east and west regions was observed as maximum 6 months from June to November. In the study in west-north of Ethiopia, the emergence of *Ph. Papatasi* was evaluated in early February and Ph. argenetipes after February till June [24]. The analysis of soil texture in both regions and indoors and outdoors show that in both regions soil was of silt, clay and loam types that its composition was determined with analysis, the clay rate in west in indoors and outdoors was less than east and the sand rate in east was more and this is one of the most essential differences in soil texture which is the main place of laying *Ph. kandelakii*. However in Moncaz Ph. seregenti has been found more in sandy, loam and clay [25]. In terms of acidity of soil, the examined results show that the soil acidity in east area is 6 times more than its east area and the rate of Ca and Mg in east areas is 3 times more than its west area which is one of the effective factors in habitat of this species. In Suhakar study, the rate of aluminium, magnesium and calcium in endemic regions of sand flies was stated as an effective factor [26]. The relative humidity of soil is another effective factor in the growth of sand flies that according to the estimation of west regions, the relative humidity of soil was more than east regions; therefore, the frequency of *Ph. kandelakii* in west was also more than east areas and consequently with high frequency of this species which is the main carrier of North-West Iran the incidence rate of VL (disease) in west region was also more than in east region. The rate of raining and humidity in west is higher but in east humidity and rain is less; therefore, the frequency of sand fly in west is more than east. In areas that the temperature is more than vectors vital activity threshold, precipitation events can provide activity field and proliferation of the fly with temperature adjustment, creation of vegetation cover and increasing relative humidity of air and soil and it causes fly migration to this favourable area. There is a significant correlation between frequency of sand fly and soil temperature in 10 cm depth ($r=0.522$, $p=0.009$, $df=20$) and 30 cm depth ($r=0.530$, $p=0.008$, $df=20$). Another study in 2012 year have also conducted a research called Examination of the map of environmental risks of *Leishmania* in France so that they have examined the rate of outbreak in these two regions with different climates from the view of temperature and high relative humidity, temperature and low relative humidity and finally they have drawn the distribution map of disease outbreak in the two intended regions [27]. Also, Bavia showed that high vegetation covers and heavy rainfall cause increasing the number of sand fly and finally it will increase the disease outbreak [28]. In Europe examined the relationship between climate, sand fly and Leishmaniasis by using Multivariate statistics and he showed that heating the earth is effective in prediction of increasing outbreak of visceral leishmaniasis and decreasing the rate of phlebotomine pernicisus [29]. But in 2012 Gonzalez have examined the relationship between climate change and Leishmaniasis outbreak in North America.

The research results show that climate change in North America and the temperature rise have provided a natural environment and proper habitat for carrier of the disease [30]. Regarding Sink studies who considers the 30 per cent relative humidity as the vital threshold of carrier fly, sand fly can have a better growth and proliferation in Meshkin Shahr but regarding the range of relative humidity intended of Sink which is 31-85 per cent, we can conclude that yearly relative humidity cannot influence significantly on the frequency of sand fly in the studied region [31].

Conclusion

According to this study, the rate of humidity and proper rainfall and favourable temperature and vegetation cover are the effective factors in growth and maintenance of *Ph. kandelakii* sand flies in west of Meshkin Shahr. The high frequency of vectors in the West region causes to increase the emergence rate of visceral leishmaniasis in this region.

Acknowledgement

I appreciate the cooperation of all colleagues and nice people of Meshkin Shahr who contributed us in conducting the research. This article is a part of research project with codes 20504-27-04-91 and Sponsored by the Tehran University of Medical Science.

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