Original Article

Efficacy of Different Sampling Methods of Sand Flies (Diptera: Psychodidae) in Endemic Focus of Cutaneous Leishmaniasis in Kashan District, Isfahan Province, Iran

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Abstract

Background: The aim of the study was to evaluate and compare the efficiency and practicality of seven trapping methods for adult phlebotominae sand flies. The results of this investigation provide information to determine the species composition and nocturnal activity pattern of different sand fly species.

Methods: The study was carried out in both plain region (about 5km far from northeast) and mountainous region (about 40km far from southwest of Kashan City). Seven traps were selected as sampling methods and sand flies were collected during 5 interval times starting July to September 2011 and from 8:00PM to 6:00AM in outdoors habitats. The traps include: sticky traps (4 papers for 2 hours), Disney trap, Malaise, CDC and CO_2 light traps, Shannon traps (black and white nets) and animal-baited trap.

Results: A total of 1445 sand flies belonging to 15 species of *Phlebotomus* spp. and five of *Sergentomyia* spp. were collected. Females and males comprised 44.91% and 55.09% of catches, respectively. Of the collected specimens, *Se. sintoni* was found to be the most prevalent (37.86%) species, while *Ph. papatasi*, accounted for 31.76% of the sand flies.

Conclusion: Disney trap and sticky traps exhibited the most productivity than other traps. In addition, in terms of the efficiency of sampling method, these two trapping methods appeared to be the most productive for both estimating the number of sand flies and the species composition in the study area.

Keywords: Psychodidae, Sand flies, Nocturnal activity, Trapping, Sampling methods, Iran

Introduction

Phlebotomine sandflies (Diptera: Psychodidae) are widespread in the tropics and subtropics, and they transmit *Leishmania*, protozoan parasites which cause visceral leishmaniasis (VL) and various forms of cutaneous leishmaniasis (CL) infecting more than 350 million people in more than 80 countries. They have a wide distribution, though mainly in the tropics and subtropics (Lane 1993). Approximately 700 species of phlebotomine sand flies distributed among 6 genera but only two of them, *Phlebotomus* in the old world and *Lutzomyia* in the new world are introduced medically importance (Lewis 1982, Lane 1987, Lane 1993, Sharma and Singh 2008), and only 10% of them act as the disease vector. Further, about 30 species of these are important from public health standpoint (WHO 1990, Desjeux 2000, Sharma and Singh 2008). The blood-feeding females of phlebotomine sand flies (Diptera: Psychodidae, Phlebotomine) include natural vectors of protozoa of the genus *Leishmania* (Kinetoplastida: Trypanosomatidae), which are the parasitic causative agents of mammalian leishmaniasis (Killick-Kendrick 1990).

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Cutaneous leishmaniasis are reported from more than 50% of the 31 provinces in Iran (Yaghoobi-Ershadi et al. 1999, Rassi et al. 2004, Rassi et al. 2006, Rassi et al. 2007, Rassi et al 2007, Rassi et al. 2011).

In the Middle East, phlebotomine sand fly populations occasionally occur in great numbers, resulting in biting pressure of 1,000 bites per person per night (Coleman et al. 2006). An extraordinarily high number compared with CDC light traps. Although the impact on adult sand fly biting activity was not determined (Blow et al. 2007), these preliminary results suggested that commercial mosquito traps might be good candidates for inclusion into integrated sand fly control or suppression programs in desert settings (Hoel et al. 2000).

In this study, sampling of sand flies were carried with Disney trap, Malaise, Sticky traps, CDC light trap, CO_2 Light trap, Shannon traps and Animal-baited trap. The purpose of the current study was to assess which of these traps could catch a larger number or show the most diversity of sand flies in the interval times. Some of them are currently used in routine sand fly surveillance programs (Alexander 2000). Previous studies aiming at determining the species composition and distribution patterns of sand flies in Kashan revealed eleven *Phlebotomus spp.* and six *Sergentomyia spp*.

Some ideas for precise sampling in order to determine species composition, nocturnal activity and biodiversity of different sand fly was species in CL focus at different topographic condition e.g. mountain/plain. Furthermore the effects of some environmental factors such as temperature, relative humidity and height above sea level were studied in relation to fluctuation considered in pattern of nocturnal activity as well as sand fly biodiversity.

Materials and Methods

Study area

The study was carried out in both plain region about 5km far from northeast and mountainous region about 40 km far from southwest of Kashan District, Isfahan Province, in central Iran (Fig. 1). The geographical coordinates is 51º 29' 54.6" E and 33º 58' 52.9" N for plain region and 51º 13' 54.6" E and 33º 58' 52.9" N mountainous region with at an altitude of 951m and 1823m respectively. The climate is hot and dry in summer and cold during the winter. The maximum and minimum temperatures were 38 °C and 19 °C in plain region and were 45 °C and 15 °C in mountainous region during the study period.

Sand fly sampling

Seven traps were employed for sampling of sand flies. The sampling was repeated three times during the peak activity of sand flies in both plain and mountainous regions. Turning of sand flies sampling were 5 times per night starting 8:00PM and ending 6:00 AM at outdoors using sticky traps (4 papers for 2 hours, totally 20 papers per day), Disney trap, Malaise, CDC light trap, CO_2 Light trap (0.5kg dry ice), Shannon traps (both black and white nets) and animal-baited trap (Fig. 2). The study was extended from July to September 2011. The traps were set up at least 15m from each other. Sticky traps were changed every two hours and as the traps were changed, the new ones were replaced in the same location. Other collection methods were also performed over the same 10 hours period using mouth aspirator to collect the sand flies. All sand flies at these traps were captured during 10–15min in every two hours. Collected sand flies for sticky traps (20×30cm papers coated with castor oil) and Disney trap were removed from sticky papers using entomological needles or fine brushes, preserved in 70% ethanol, and kept in microtubes before identification. At other traps, sand flies were also collected at interval of 2 hours by aspirator starting 8:00 PM ending 6:00 AM. The sand

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flies were anesthetized with chloroform and transferred to microtube containing 70% ethanol. Then specimens transported to laboratory research center for mounting and identification.

Mounting and Morphological identification

After recording the sampling data and locations, some sand fly specimens which collected with sticky traps were washed in acetone and rest of them transferred to 70% ethanol. At appropriate time, the sand flies were mounted in Puri's medium. All the identifications were carried out based on their genus and then species. The identification keys of Theodor and Mesghali 1964, Lewis 1982, Rassi et al. 2006 and Absavaran et al. 2009 were used. The permanent mounted, labelled and identified microscope slides were deposited in Medical Entomology and Zoology Museums, School of Public Health, TUMS under code No. GC22ST5-92.

Recording climatic conditions

To determine the relationship between temperature and relative humidity (RH) and the abundance or activity of sand flies in both study regions (plain and mountain) associated with traps some meteorological data were recorded. The temperature and relative humidity were recorded every two hours during the night.

Statistical Analysis

Data were analyzed using SPSS 20. In order to ensure normality and homogeneity of variances before subjecting to statistical analysis, data on the number of flies collected were square-root transformed. The significance difference of effect of the traps and time was analyzed in relation to sand flies species caught in two regions using univariate analysis of variance (ANOVA) and Chi-square tests. Total sand fly species caught constituted the dependent variable, while sex and traps and time were chosen as the fixed factor. Basic correlation matrices were used to determine the existence of a correlation between the abundance of the sand flies, average hourly temperature and relative humidity in the study area. Some of graphs were prepared using Microsoft Excel 2010.

Results

A total of 1445 sand flies belonging to 15 species of the genus *Phlebotomus* and 5 of the genus *Sergentomyia* were collected. Females and males comprised 44.91% and 55.09% of catches during the study period respectively. Of the collected total, *S. sintoni* was found to be the most prevalent (37.86 %) species while *Ph. papatasi* the proven vector of *L. major*, *L. turanica* and *L. gerbili* in Isfahan accounted for 31.76% of the sand flies that were identified. Other species were *S.*(*Ser.*) *dentata* (0.3%), *S.*(*Ser.*) *antennata* (0.2%), *S.*(*Ran.*) *pawlowskyi* (0.3%), *S.*(*Par.*) *palestinensis* (0.1%), *Ph.*(*Syn.*) *ansarii* (0.1%), *Ph.*(*Para.*) *kazeruni* (1.7%), *Ph.*(*Par.*) *caucasicus group* (0.4%), *Ph.*(*Par.*) *alexandri* (1.1%), *Ph.*(*Lar.*) *wenyoni* (0.6%), *Ph.*(*Lar.*) *tobbi* (1%), *Ph.*(*Lar.*) *major* (3.5%), *Ph.*(*Lar.*) *keshishiani* (0.2%), *Ph.*(*Lar.*) *kandelaki* (0.1%), *Ph.*(*Adl.*) *longiductus* (0.1%), *Ph.*(*Adl.*) *halepensis* (0.1%), *Ph.*(*Adl.*) *chinensis group* (0.1%) and *Ph.*(*Adl.*) *brevis* (0.3%).

During the study period, the population of sand flies was found to be the lowest in CDC light trap and CO_2 Light trap. Population size was raised with two highest peaks in Disney trap and sticky trap at 22:00–24:00 PM.

The average monthly temperature and relative humidity values ranged between 21.83–28.33 °C and 24.83–36.33% respectively. During the night, when the maximum number of sand flies (29.25%) was collected at 22:00–24:00 PM, the average temperature and RH were found to be 26.92 °C and 26.5%, respectively, while the minimum number of sand flies was sampled at 02:00–04:00 AM (11.62 %) with an average temperature of 23.08 °C and 33.5% relative humidity (Fig. 3).

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The number of sand flies collected using different types of traps during the study period is presented in Table 1. Almost all sand fly species were commonly caught with Disney trap and sticky traps. Approximately 37.3% of the total collection was made up using Disney trap. 24.2% and 10.5% of sand flies were collected using sticky traps and Shannon trap (black net), respectively. Collections using animal baited trap and Shannon trap (white net) constituted 8.5% and 7.3% of the total collection. Neither CO_2 Light trap (5.0%) nor CDC light trap (3.3%) and Malaise trap (0.1%) showed a high efficiency during the study. A significant interaction was revealed between the collection methods and the number of individuals collected using of univariate analysis of variance (ANOVA) ($P< 0.05$), and there was significant interaction between the trapping methods and the proportion of males and females collected ($P< 0.05$). When significant effects of traps were established ($P< 0.05$), differences among traps were exposed that CO_2 and standard CDC light traps, Shannon trap (black and white net) and animal baited trap displayed some similar efficiency, whereas sticky papers and Disney trap differed from the others ($P< 0.05$).

Results with respect to the nocturnal activity indicated that even though the number sand flies declined rapidly between 02:00 and 04:00h. There was no significant difference between hourly pattern either in the species prevalence or in the activity of the species in different traps (Fig. 4).

Fig. 1. Kashan District, Isfahan Province, central Iran

Fig. 2. Different methods used for sand fly sampling

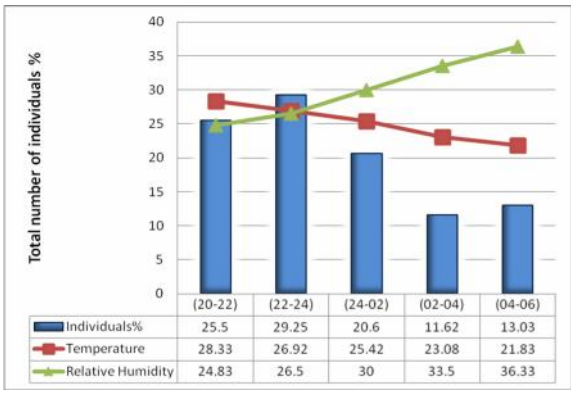

Fig. 3. Correlation of two environmental conditions with nocturnal activity of sand flies

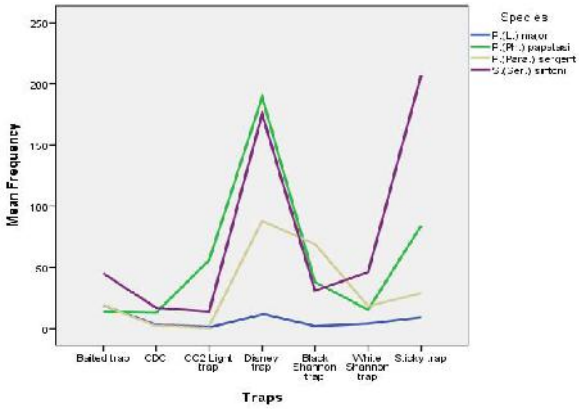

Fig. 4. Correlation between collection methods and density of abundance sand flies species in the study area in Kashan City

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Table 1. Prevalent sand flies collected by different traps, Kashan, Isfahan Province, 2011

Species	Traps							Total
	Baited trap	CDC	CO2 Light trap	Disney trap	Black Shannon trap	White Shannon trap	Sticky trap	
Ph.(Lar.) major	19	3	0	12	2	4	9	49
Ph.(Phl.) papatasi	14	18	56	208	38	22	86	442
Ph.(Par.) sergenti	21	4	2	114	73	22	36	272
S.(Ser.) sintoni	45	17	14	179	31	46	207	539
Total	99	42	72	513	144	94	338	1302

Discussion

This study is the first detailed research in terms of species composition, density and nocturnal activity of sand flies using different methods of capturing in an endemic focus of cutaneous leishmaniasis in Kashan district, Isfahan Province, Iran. Trapping over the summer season during the peak of sand flies activity revealed that *S. sintoni* (37.9 %), *P. papatasi* (31.8%) and *P. sergenti* (20.1%) are the most abundant and prevalent species in these regions respectively (Doroudgar et al. 1999). Both the previous and recent findings showed that *P. papatasi* the main vector of *L. major*, *L. turanica* and *L. gerbili* in Isfahan Province, *P. sergenti* the proven vector of *L. tropica* and *L. gerbili* in this region, also *S. sintoni* were represented with high populations in the study areas (Seyedi Rashti and Nadim 1992, Doroudgar et al. 1999, Yaghoobi-Ershadi 2012).

Although sand fly abundance was not strongly and significantly influenced by the variations in average monthly temperature and relative humidity during the six months of survey in Cukurova Plain, south Anatolia, Turkey, the maximum number of sand flies was recorded in the hottest and driest seasons between June and September when the average temperature was comparatively high, the relative humidity was low and the rainy days ranged between 1.5 to 3.5 days. (Kasap et al. 2009).

The role of climatic factors on the seasonal distribution of sand flies in the arid areas of India exhibited that the majority of species preferred comparatively higher temperatures and low RH%, a prerequisite for survival in arid and semi-arid conditions (Singh 1990).

Our study results are concordance with the other survey in relation to effects of temperature and relative humidity on abundance of sand flies. It seems that special range of both high temperature and low relative humidity caused the high abundance of sand fly species in our study regions. The results showed that before midnight (between 22:00–24:00 PM) sand flies were very active.

Although CDC and CO_2 light traps are used extensively in the field studies of sand flies (Alexander 2000), in Cukurova Plain in Turkey sticky traps have no known attractiveness and have generally been used for determining species composition of an area as they randomly sample the species where they are set (Kasap et al. 2009), whereas our findings showed that Disney trap and sticky traps have been attractiveness more than other traps. Our findings in terms of the efficiency of the sampling methods, showed these two trapping methods appeared to be the most productive for both estimating the number of sand flies and the species composition in the study area, in agreement with some previous studies. It seems that the sticky traps provide more realistic results than CDC light traps that could attract additional phototropic sand flies. The effective range of

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CDC light traps was less than 5m (Wheeler et al. 1996). Study in northern Italy showed that when compared to sticky traps, CO_2 traps were more effective in collecting sand flies and addition of a light source improved the catches (Veronesi et al. 2007).

The nocturnal activity patterns of sand flies have been reported for Old World species (Roberts 1994, Guernaoui et al. 2006, Coleman et al. 2007). According to our results, sand flies activity was not significantly different over time even though total counts decreased between 04.00–06.00h during late summer. No significant difference was found in the species prevalence throughout the nights. It seems to be the most important factor affecting sand fly nocturnal activity in similar studies was low humidity, followed by low wind velocity and high temperature (Roberts 1994, Guernaoui et al. 2006, Kasap et al. 2009).

Conclusion

Disney trap and sticky traps exhibited the most productivity than other traps. Therefore, more detailed studies with respect to the seasonal variations and the effects of abiotic conditions, other than temperature and relative humidity, such as cloud cover, wind velocity and lunar cycle on the nocturnal activity of sand flies may improve knowledge of the behavior of sand flies for important epidemiologically surveys.

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