Original Research

Abundance, composition, peak active-period and vertical flight range of sand flies (Diptera: Psychodidae), the vector of leishmaniasis in Kurunegala district

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Abstract

Background: Leishmaniasis is identified as a neglected tropical disease in the world and considered a notifiable disease in Sri Lanka. It has shown an upward trend in Kurunegala district since 2008. Studies on vector bionomics, although important for implementing effective vector control, are still limited in Sri Lanka.

Objective: To describe the abundance, composition, peak active-period and vertical flight range of sand flies in the Kurunegala district

Methods: A descriptive study was conducted in two selected disease-endemic areas in Rideegama and Ibbagamuwa Medical Officer of Health areas in Kurunegala district during February-March, 2019. sand flies were collected using different standard sampling techniques (CDC Light-traps, Sticky-traps and Cattle-Baited-Net-Traps and by hand collections). The identification of sand flies was done up to Genus level. Sticky traps were set up at different heights on walls of houses to determine the vertical flight range of sand flies. The hourly sand fly collections were used to determine the active peak times. The abundance was calculated as per trap density and hourly collections were monitored to identify peak active-period of sand flies.

Results: The total number of sand flies collected during the study period was 773. The light- traps were found to be more productive than other collection methods. The numbers of sand flies belonging to geni Phlebotomus and Sergentomyia were 525 and 248 respectively. The average vertical flight range of flies was less than two feet from the ground level. A higher percentage of sand flies was collected from outdoors (n = 74%) than indoors (n = 26%). The highest active period of sand flies was between 18.00 hr and 22.00 hr of the day, although it was slightly different in Phlebotomus (from 18.00 hr to 20.00 hr) species and Sergentomyia species (from 18.00.00 hr to 22.00 hr).

Conclusions and Recommendations: Sand flies belonging to the genus Phlebotomus and Sergentomyia were present in the area. The peak aggregation time was at night starting at 18.00 hrs in the day for both genera. The vertical flight range of sand fly was approximately less than two feet from the ground level.

Key Words: Sand flies, composition, peak aggregation time, flight range

Background

Leishmaniasis is a disease affecting about 89 countries and is endemic to Asia, Africa, parts of North and South America and the Mediterranean. Globally it has been suggested that 12 to 15 million are infected. About 350 million worldwide are at risk of acquiring the disease which has made it one of the seven most important tropical diseases according to the World Health Organization (WHO). According to the statistics of WHO, around one million new cases are reported every year. WHO reported that poverty and unplanned urbanisation, deforestation, migration from non-endemic to endemic settlements have contributed to the epidemic of leishmaniasis [1]. Sand flies are considered as the vector of the Leishmania parasite which causes public health problems in many parts of the world. The dipteran flies transmit the Leishmania parasites, *Leishmania donovani* a protozoan parasite that causes leishmaniasis. The disease has several different forms including three main forms, namely, visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), and muco-cutaneous leishmaniasis (MCL), where VL is considered to be the most virulent form of the disease.

In Sri Lanka first notified cutaneous leishmaniasis (CL) was in 1992. [2] By the year 2002, 65 cases, mainly from the northern dry zone were reported. From 2008, cutaneous leishmaniasis was included in the list of notifiable diseases in Sri Lanka. *Leishmania donovani* zymodeme MON-37 was identified as the causative agent of the CL in Sri Lanka [2]. Phlebotomus argentipes Annandale and Brunetti (Diptera: Psychodidae) are the major vectors of VL (kala-azar) in India [3]. The predominant sand fly species in Sri Lanka is also *Phlebotomus argentipes* [4].

In 2018, there was a considerable increase of leishmaniasis cases reported from Kurunegala district compared to the last three years (Weakly Return of Communicable Disease Data from Epidemiology Unit, Kurunegala). This is four folds higher than the average number of cases reported in 2017 and 2016 in the district. More than 25 cases were reported from the Rideegama, Ibbagamuwa, Giribawa. Alawwa, Polpitigama and Pannal MOH areas. The most affected area with the highest number of reported cases was Rideegama MOH area. Even though certain measures have been taken to control the vector and the disease condition, the efforts have failed mainly due to the lack of proper understanding of the disease and vector. Sufficient information, especially on vector behaviour, host preference, peak biting time, breeding sites, resting preference, oviposition are unavailable to implement effective control measures timely.

There has been relatively little published research on the taxonomy, geographical distribution, behavioural habitats, feeding and breeding preferences as well as insecticide susceptibility patterns of sand flies in Sri Lanka. Without sufficient understanding of these aspects, the vector control strategies may not effective to the expectation. Due to the prevailing disease conditions in the country, it is a timely need to identify and adopt correct preventive strategies to control this disease condition in Sri Lanka. These strategies need to be developed in an evidence-based manner. Therefore, comprehensive studies on vector species composition and bionomics will help to minimize the gaps in our understanding of the biology and ecology of this vector to implement proper control strategies.

Therefore, this study aimed to describe the composition, distribution, active seasonal fluctuation and bionomics of sand fly species in selected localities of Kurunegala district. This will help developing an effective vector control programme to minimize the possible leishmaniasis disease outbreak situation in the district.

Objectives

To determine the composition, abundance, peak active time and the vertical flight range of sand flies in selected localities in Kurunegala district

Methods

This descriptive study was conducted in two disease-endemic areas from February 25th, 2019 to March 31st, 2019. The selected localities were Ogodapola and Omaragolla in Kurunegala district in the lowland plain. Ogodapola area was located in Rideegama MOH area (N 7.55'67.56", E 80.47'55.3") and Omaragolla area was located in Ibbagamuwa MOH area (N 7.38'39.12", E 80.30',34.2"). Ogodapola area was known as a rural area and Omaragolla was a semi-urban area. Wild plants and bushes were abundant in gardens making the environment cool and well-nourished organic debris in the soil. Many

physical characteristics of the houses and surrounding environment support the biology and ecology of sand flies in these areas.

Sand flies were collected using CDC light traps, sticky traps, cattle-baited net traps, and hand collection using aspirators. CDC light traps were set in houses where positive cases had been reported. In each sampling site, two CDC light traps were set: one inside and the other outside the house for two consecutive nights for one sampling effort. Samplings were done for twelve hours from 18.00 hrs to 6.00 hrs and data were reported hourly. Since traps were negative for sand flies from midnight, the sampling was continued for six hours between 18.00 hrs to 12.00 hrs. A total of 20 CDC light traps were set during the experimental period for eleven nights in two selected localities.

Sticky traps prepared using transparent sheets coated with olive oil were placed in selected places, inside the house and surrounding environment including animal housing as well as close to the vegetation. These traps were set up in the sampling site at 18.00 hrs and they were collected for data reporting after twelve hours. For monitoring the occupied height level from the ground level and to understand the flying pattern of sand flies, these sticky traps were placed in 30 selected houses each in Omaragolla and Ogodapola areas. These traps were attached to the interior walls of houses at one foot, two feet, three feet from the ground level. Sand flies were also collected using a WHO-recommended cattle baited trap. Cattle-baited traps were set between 18.00 hrs–6.00 hrs within 10 m of houses where positive cases of leishmaniasis had been reported. Hand collection was performed using mouth aspirators indoor and outdoor.

Collected Sand flies were separated into labelled cups, stored in 70% v/v ethanol and morphological identification was done using available keys as specified by Lewis [5]. Temperature and Relative Humidity (RH) data were obtained from the Meteorology Department, Colombo, Sri Lanka. The findings were presented as numbers/frequencies and percentages.

Data were entered into Microsoft Excel and was analysed by Minitab version 16 for statistical analysis. Descriptive statistics were calculated including percentage, mean and standard deviation to describe the univariate analysis. One-way ANOVA was used to test the differences in the number of sand flies across the different levels of independent variables such as per trap density, the height of ground levels. Pearson's correlation analysis was applied to determine the relationship between climatic parameters and the number of sand fly in two selected study sites. The level of significance was taken as 0.05.

Results

A total of 773 sand flies were collected which belong to two genera (Phlebotomus and Sergentomyia) during the experimental period and 57% (n = 417) of them were males while 43% (n = 316) of them were females. Sixty-nine per cent of the total population of sand flies were found to be exophilic. Of those exophilic flies, 61% were males.

The collected sand flies were belonging to the genus Phlebotomus (n = 199) and genus Sergentomyia (n = 132). Table 02 shows the relative abundance of two sand fly genera by the locality. According to the data, Phlebotomus was the predominant genus identified in Rideegama, Oogodapola area during the study period. There was no significant statistical difference between the abundance of Sergentomyia spp and Phlebotomus spp in study areas ($p = 0.119$, $t = 1.23$). The recorded average temperatures and relative humidities were 34°C and 59% in February and 38°C, 47% in March 2019. Figure 01 shows the behaviour of meteorological parameters and the number of sand flies. According to the correlation analysis, there is no statistically significant relationship between the meteorological parameters and the number of sand flies in two study sites during the study period.

When used the cattle baited traps, the total per trap density collection of sand flies was 41. The results showed that no significant difference was obtained between females (50.16%) and males (49.84%) in cattle-baited traps during the study period. It was observed that locality wise more females were attracted to cattle-baited traps in Ormaragolla area (n = 40, 86.95%) in Ibbagamuwa MOH division compared to the Oogodapola (n = 76, 50.16%) in Rideegama area. A total of 253 sand flies were collected using CDC light traps and 183 (72.33%) were found to be females. Males were 27.67% of the total. The number of sand flies collected using different types of traps is presented in Table 01. The CDC light traps and cattle baited trap methods were most productive compared to the other

two methods ($p = 0.02$, $F = 38.8$). Approximately 253 (12.65 per trap density) of sand flies were collected using CDC light traps.

Three rows of sticky traps were set up at three different heights (one Foot, two Feet and three Feet) on the indoor ($n = 54$) and outdoor walls ($n = 54$) of selected houses to determine the flight range of sand flies. There were 18 sticky traps set for in one house overnight. Out of 100 such sticky traps, four traps were positive for sand flies (Male = 4, female = 7). However, all these positive traps had been placed at the level of two feet from the ground indoors. These results suggest that these flies frequently rest close to the ground level.

The hourly collections of sand flies showed that Phlebotomus spp have two active peaks between 17.00 hrs–20.00 hrs and 2.00 hrs–4.00 hrs (Fig 02), while it was found to be between 18.00 hrs–23.00 hrs for Sergentomyia spp. However, these two genera are predominant between 18.00 hrs–23.00 hrs. Hourly collection of sand flies in indoor samples (Fig 03) indicated that Phlebotomus spp are highly active between 18.00 hrs–21.00 hrs while Sergentomyia spp are active between 20.00 hrs–23.00 hrs and 2.00 hrs–3.00 hrs. An outdoor sampling of these flies showed (Fig 04) that Phlebotomus spp are active between 18.00 hrs–24.00 hrs, while Sergentomyia spp are active between 20.00 hrs–23.00 hrs and 2.00 hrs–3.00 hrs.

Discussion

A study carried out to analyse epidemiological data showed that almost 90% of the leishmaniasis cases reported between 2009 and 2016 were from Anuradhapura, Hambantota, Polonnaruwa, Kurunegala, and Matara as endemic districts [4]. This research is the first preliminary study that has been carried out to determine the composition, abundance, flight range and peak active times of sand fly species in an endemic focus of leishmaniasis in Kurunegala District. According to our findings, Phlebotomus is the most abundant genus compared to Sergentomyia. Previous findings [6,7] have shown that sand flies belong to the genera of Phlebotomus and Sergentomyia are present in Kurunegala. According to past literature, the suspected vector of leishmaniasis in Sri Lanka belongs to genera Phlebotomus [8]. The findings of the present study suggest that these flies are frequent close to ground level.

The highest activity of sand flies was recorded during the time period of 18.00–22.00 hr (84%) which is different from a previous study conducted in Sri Lanka where the peak aggregation of sand flies was observed during the hours of 20:00 to 23:00 hr [8]. In a study conducted in Italy, the peak activity of sand flies was found to be during 20:00–2.00 hr [9]. Some studies suggested that the peak activity of sand flies shifted from early evening (20:00–22:00) to late-night (22:00–00:00) due to seasonal changes [10]. Understanding monthly variations and activities of sand flies at different time intervals is important to determine the period of highest risk of leishmania transmission as well as to implement proper monitoring and efficient control strategies. However, we identified the time and the areas to be considered for the application of insecticides. Information obtained from this work conducted for the first time in Kurunegala district will facilitate the development of effective control programmes.

A study about the role of climatic factors in the seasonal distribution of sand flies in arid areas of India had revealed that the majority of species preferred comparatively high temperature and relatively low humid conditions [3]. The preliminary data of this study are not sufficient to make strong conclusions regarding this aspect. Therefore, further studies are needed for a proper understanding of the preferred environmental conditions, weather patterns as well as activity patterns of the reported sand flies.

Leishmaniasis vector control methods should be implemented after thorough investigations on vector, for instance, exact vector species, behaviour, breeding habits and places, resting places, distribution and other bionomic characteristics such as population fluctuations. In Sri Lanka, very few studies have been conducted on these aspects, hence, minimal evidence is there to support control programmes. The present study provides baseline information on the time and height levels to facilitate the implementation of chemical control methods.

Conclusions and recommendations

Sand flies belonging to the genus Phlebotomus and Sergentomyia were present in the area. The peak

aggregation time was at night starting at 18.00 hrs in the day for both Genera. The vertical flight range of sand fly was approximately less than two feet from the ground level. The findings of the present study will be useful for leishmaniasis vector control methods since this is the first study of this nature conducted in this study setting.

Author declaration

Author contributions: All authors contributed to the conceptualization and design of the study. JMMKH and MRSSB designed the study. JMMKH, MRSSB, PGA, RPS and MACMM carried out the experiment and JMMKH, MRSSB, PGA, RPS supervised research team activities. PMRBIP and AMPCL contributed to the implementation of research. JMMKH wrote the manuscript with the help of MRSSB and PGA.

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Ethics approval: Ethics approval was obtained from the Ethics Review Committee of the Provincial Department of Health Services of the North Western Province.

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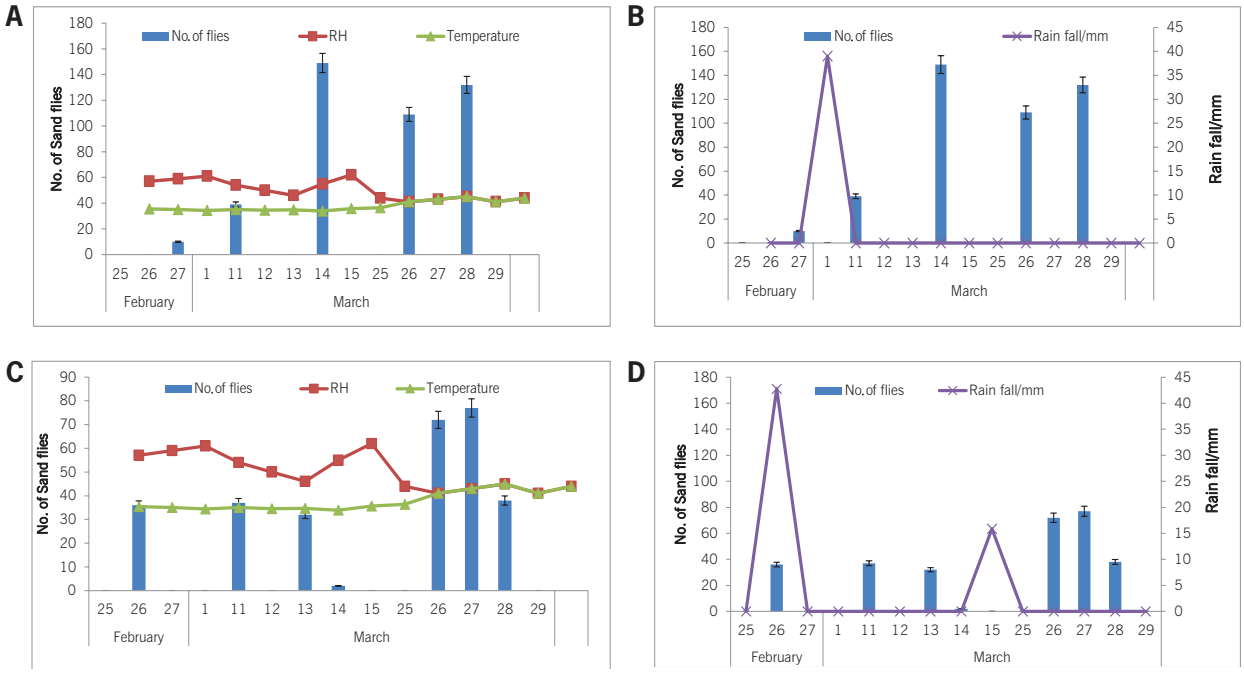
Table 01: Summary of the sand fly collection by different methods during the study period

Sampling Techniques	Total No. of Sampling Units	Total No. of collected sand flies	Per trap density
Light trap	20	253	12.65
Cattle baited net trap	9	369	41
Sticky trap	100	11	0.11
Hand collection	11 (Hours)	61	5.54 (per Hour)

Table 02: Relative abundance of Phlebotomus spp and Sergentomyia spp at each locality during the study period

Locality	Gender	Month	Sergentomyia	Relative abundance%	Phlebotomus	Relative abundance%
Ibbagamuwa	Female	Feb	11	8.3	16	8.0
		March	20	15.2	27	13.6
	Male	Feb	8	6.1	3	1.5
		March	55	41.7	22	11.5
Rideegama	Female	Feb	5	3.8	3	1.5
		March	9	6.8	15	7.5
	Male	Feb	0	0.0	51	25.7
		March	24	18.1	62	31.1
Total			132	100.0	199	100.0

Relative abundance: nx/N * 100, nx: number of individuals belonging to species x, N: total number of sampled individuals.



RH: Relative Humidity

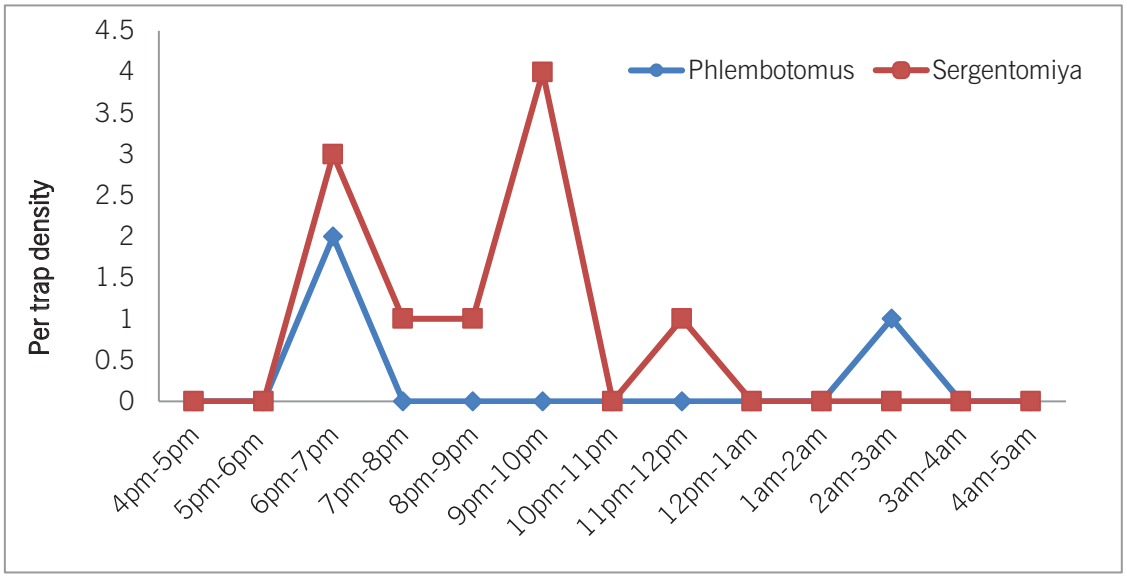
A–Effects of RH and temperature on no. of flies in Ogodapola locality

B–Effects of rainfall on no. of flies in Ogodapola locality

C–Effects of RH and temperature on no. of flies in Omaragolla locality

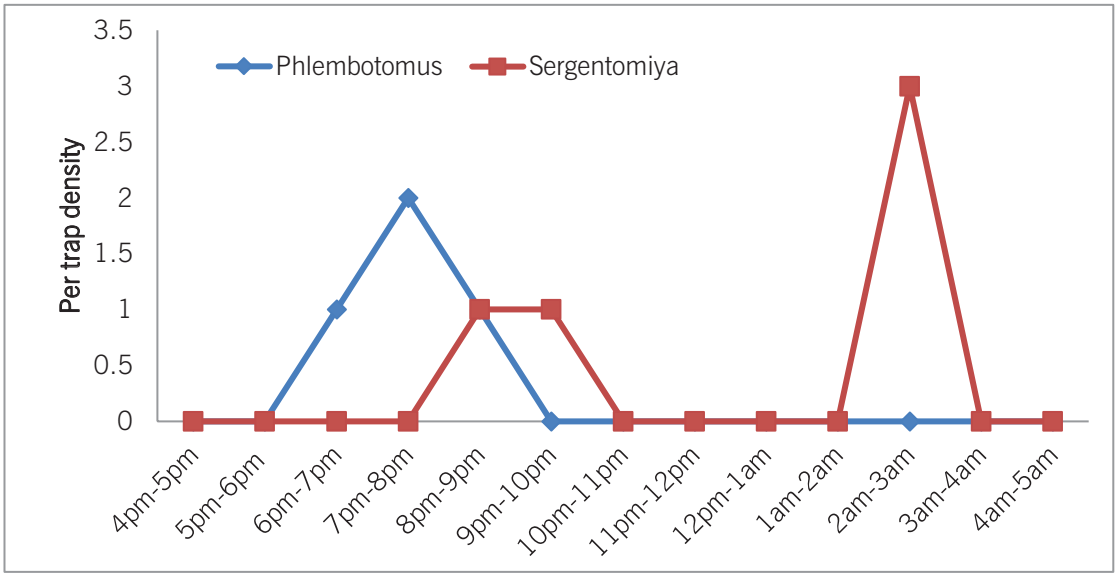
D–Effects of rainfall on no. of flies in Omaragolla locality

Figure 01: Effect of meteorological factors on the number of sand flies in the two selected localities



Per trap density–No. of flies per light trap

Figure 02: Two hourly activities of sand flies



Per trap density–No. of flies per light trap

Figure 03: Hourly activity patterns of sand flies–Indoor collection

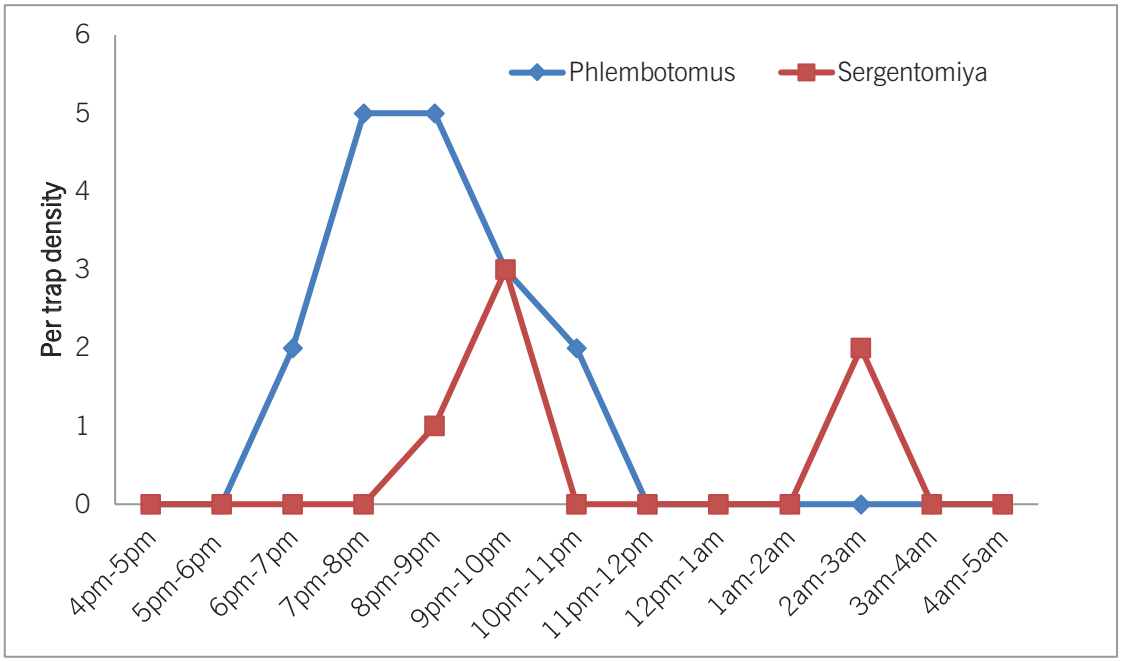


Figure 04: Hourly activity patterns sand flies–Outdoor collection