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# Nocturnal activity of Phlebotomus species (Diptera: Psychodidae) in a visceral leishmaniasis endemic area of northwest Ethiopia

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**Abstract**
*Phlebotomus orientalis* is the most likely vector of *Leishmania donovani* causing visceral leishmaniasis in northwest Ethiopia. Understanding of sand fly night activities is very essential to design appropriate sand fly control methods in order to reduce *Leishmania* infection. The aim of this study was to determine the nocturnal activity of *Phlebotomus* species. This study was conducted for six consecutive months from January to June 2013 in Adebay village where visceral leishmaniasis is endemic. Sandflies were collected using CDC Light traps changed at one hour interval, in periphery village (peridomestic area) and farm field. The traps were activated from 18:00 to 7:00 hours. Overall, 5,902 sandflies were collected. Eight *Phlebotomus* species representing four subgenera were indentified: *Phlebotomus* (*Larroussius) orientalis, P.*(*Phlebotmus*) *papatasi, P.*(*Phlebotmus*) *bergeroti, P.* (*Phlebotomus*)*duboscqi, P.* (*Paraphlebotomus*) *alexanderi, P. (Anaphlebotomus) rodhani* and other two *Parvidens* species(*P. lesleyae* and *P. heischi*). Among eight species of sand flies collected *P.orientalis* were the most predominant species followed by *P. papatasi* and *P. lesleyae*. The result indicated that female and male *P.orientalis* showed similar activity pattern (19:00-05:00hrs). Both sexes were active throughout the night (19:00-06:00 hours), reaching a peak between 01:00 and 03:00hrs (mean density of 16.46 females/trap/hour/night; and 33.83 males/trap/hour/night). Male *P. papatasi* were the dominant having two peaks, an early smaller peak between 21:00-22:00 hrs and a larger second one around midnight (24:00-02:00 hrs). Females displayed similar activity patterns, with an early peak at 21:00-22:00 and a late smaller peak at 02:00-03:00 hours. Hourly collections of *P.orientalis* and *P. papatasi,* the corresponding temperatures revealed no significant correlations. In conclusion, *P.orientalis* and *P. papatasi* remained active throughout the night. High risk of VL transmissions is likely concentrated during the peak hours and local inhabitants could reduce the risk of infection by using appropriate personal protective measures such as repellents and bed nets.
**Keywords:** visceral leishmaniasis, *Phlebotomus* species, nocturnal activity, northwest Ethiopia

## 1. Introduction

Visceral leishmaniasis (VL) is caused by *Leishmania donovani–L.infantum* complex (WHO, 2010). Most infections are asymptomatic, although some victims ultimately develop clinical VL. Malnutrition and immune suppression, such as HIV co-infection, frequently result in clinical disease (WHO, 2010). In Ethiopia, VL is mostly endemic in the lowland areas but has recently spread into the highlands, particularly in northwest Ethiopia (Libo-Kemkem and Fogera districts) (Alvar *et al*., 2007). This disease is transmitted by the bite of phlebotomine sand flies (Diptera: Psychodidae).

 

Adult phlebotomine sand flies are nocturnal or crepuscular (Lane, 1993; Killick-Kendrick, 1999), but sometimes they can bite during the day if disturbed or in cloudy weather in forests (Lane, 1993). Sand flies are weak fliers and move in short hops; they travel up to 2.2 km over a period of a few days in open habitats (Lane, 1993). In a single night a sand fly can move several hundred metres in their search for food, mates, as well as resting and breeding sites (Lane, 1993; Galati *et al*., 2009). Sand flies tend to fly close to the ground, probably to avoid being swept away by wind gusts (Faiman *et al.* 2011). During the day they rest in microhabitats, where they are protected from extreme environmental conditions (Foster, 1972; Quate 1964, Ashford, 1974). Movement and biting activities of sand flies are governed by light intensity and influenced by climatic and environmental factors (Quate 1964, Gibson and Torr, 1999). Different species of phlebotomine sand flies have distinct peaks of blood feeding activity, which can influence their vectorial capacity (Reza and Mansour, 2006; Rivas *et al*., 2008; Kasap *et al*., 2009).

Several studies have been carried out on the nocturnal activities of *Leishmania* vectors in the Old World (*Phlebotomus martini*, *P. celiae*, *P. tobbi*, *P. perfiliewi*, *P. papatasi* and *P. sergenti* (Gebre-Michael and Lane, 1996; Reza and Mansour, 2006; Rivas *et al*.,2008; Kasap *et al*., 2009) and in the New World (*Lutzomyia intermedia*, *Lu. whitmaniand Lu. longipalpis)*(Souza *et al*. 2005). Their activity peaks time interval was as follows: *P. tobbi* and *P. perfiliewi* peaked at 04:00-06:00 hrs (Kasap *et al*., 2009) and the peak activity of adult *P. papatasi* and *P. sergenti* was at 20:00h (Reza and Mansour, 2006). According to Gebre-Michael and Lane (1996) both *P. martini* and *P. celiae* showed peak activity between 20:00 and 22:00hrs around termite hills and in human dwelling compounds. The New World sand flies, *Lu. whitmani* collected in the forest is more active in the middle of the night, whereas *Lu. intermedia* collected in the peridomestic area shows a peak of activity in the early evening (Souza *et al*., 2005) and *Lu. longipalpis* showed crepuscular activity (Rivas *et al*., 2008). According to Gonzalez *et al.*(1999) *Lu. panamensis*, beginning and ending also in the crepuscular periods of dusk and dawn, but with maximum activity in the 02:00-03:00hrs period.

VL is prevalent in northwest Ethiopia particularly in Kafta Humera district where *P.orientalis* is the most abundant and probable vector of *L .donovani* (Gebre-Michael et al., 2010). This disease mainly affects seasonal migrant labourers, military personnel, and residents of rural farming communities. Studies in Sudan demonstrated that *P. orientalis* bites humans and is able to transmit *L. donovani* (Quate, 1964, Hoogstraal and Henyenman, 1969; Elnaiem *et al*., 1997; WHO 2010). In Humera and other lowland areas, due to hot weather conditions, people tend to sleep outside and frequently work in the fields during night time. Hence, they are highly exposed to biting *P.orientalis* (Gebre-Michael *et al*., 2010). To date, there have not been any, studies on activities and nocturnal periodicity of *P.orientalis* and other *Phlebotomus* spp. close to human dwellings in the Kafta-Humera region.

Studies on sand fly nocturnal activity patterns especially near human dwellings are essential to understand the more risky hours for contracting infection. It may also be useful to know the best possible time to collect sandflies for epidemiological purposes at village level. The current study was undertaken to improve our understanding of the nocturnal activity of *P.orientalis* and other *Phlebotomus* species domestic and peridomestic environment in one of the villages in Kafta-Humera district, northwest Ethiopia.

## 1.1. Materials and methods

### 1.1.1. Study area

This study was carried out from the period of January to June 2013 in Adebay village in Kafta Humrea district in the Western Tigray region, northwest Ethiopia, which borders Eritrea to the north and Sudan to the west. Agriculture comprise sesame, cotton and sorghum are the major economic activity in the area. It seems the area is more conducive for the availability of the vector and reservoir host. Cracked Vertisol and forests (dominated by *Balanites* spp.) are more abundant as compared to other areas in the district [Moncaz et al 2014]. The villages are located at altitude ranges of 500m-650meters a.s.l. and along coordinates 14° 11.47'N and longitudes of 36°46.07'E. Temperature reaches an average of $42^0$C between April and June and falls to between 25 and $35^0$C during the months between June and February. The average annual rainfall is 400-650mm; with July and August receiving the highest amounts and rain is absent between October and April.

### 1.1.2. Sand fly collections

The study of the nocturnal activity of sand flies was carried out in Adebay village where recent VL cases had been reported and treated at Kahsay Abera Hospital (Yared *et al*., 2014). The study was conducted for a period of six months (January-June 2013). Sand flies were collected bimonthly in the periphery of the village and in farm fields adjacent to the village. Two CDC light traps per sampling night per habitat were placed about 70 meters apart. The traps functioned from 18:00 to 7:00 hours i.e. 13h collections/night. A total of 26 collection cages were prepared for the two CDC light traps in the periphery of village and a similar number for collection in the farm fields. The collection cages were removed at the end of every hour and replaced by another set following the protocol of (El-Badry *et al*., 2009). Temperature and relative humidity measured every hour using Data loggers.

 

### 1.1.3. Mounting and Identification of sandflies

The trapped sandflies were separated according to the hour and place of collection and brought to the laboratory, anaesthetized with chloroform and sorted out *Phlebotomus* and *Sergentomyia* under a dissecting microscope. The head and abdominal tip of each sand fly were removed and mounted on slides in a drop of Hoyer's medium for species identification. Sand flies were identified to species level based upon cibarial and pharyngeal armature as well as spermatheca of females and external genitalia of males using morphological keys (Quate, 1964; Lewis, 1982).

### 1.1.4. Statistical analysis

The total number of phlebotomine sand flies captured in the different study sites was compared using independent t test. Numbers captured at different time periods were expressed as number of sand flies per trap per hour of each sand fly species. The differences between the 1-h intervals were compared using non parametric one way ANOVA. Sand fly numbers were log-transformed [log (n + 1)] to normalize the distribution. The correlation coefficient (r) was used to estimate relationships between hourly temperature and relative humidity and the overall density per trap per hour per night *P. orientalis* and *P. papatasi*. The analysis was performed using SPSS 20.0 software (SPSS Inc, Chicago, IL, USA).

## 1.2. Results

Overall, 5,902 phlebotomine sand flies were collected from the village periphery and farm fields in Adeby village. Of these *P. orientalis* was the most dominant species (93.53%) followed by *P. papatasi*(4.17%) and *P. lesleyae* (1.52%). Other species included: *P. bergeroti* (0.51%), *P. duboscqi* (0.2%), *P. alexanderi* (0.03%), *P. heischi* (0.02%) and *P. rodhaini* (0.02%) (Table1). The abundance of *P.orientalis* showed no significant deference between the periphery of the village and farm field ($P>0.05$) whereas *P. papatasi* was significantly more abundant in the periphery of the village than in the cultivated fields ($P<0.05$). There were significant differences between the abundance of adult males and females for both *P.orientalis* (1.85:1 males: females) and *P. papatasi* (1.96:1) ($P<0.05$)(Table 1).

### 1.2.1. Nocturnal activity

Figure 1 depicts the nocturnal activity of *P. orientlais* plotting the mean numbers per hour throughout the six month period. There were significance differences in the numbers of both sexes of *P.orientalis* in both habitats during different times of the night (Female: Kruskal Wallis Test =71.586, df=12, P=0.000; male: Kruskal Wallis Test=67.903, df=12, P=0.000). The result indicated that female and male *P.orientalis* showed similar activity pattern (19:00-05:00hrs). No sand flies were caught before sunset or after sunrise. Both sexes were active throughout the night (19:00 -06:00 hours), reaching a peak between 01:00 and 03:00 (mean density of 16.46 females/trap/hour/night; and 33.83 males/trap/hour/night at about 22-23.11$^{\circ}$C hourly average temperature and 36%-38% relative humidity. The activity decreased sharply after 03:00 hours and stopped completely at 06:00. Overall, males were more abundant than females till 03:00 hours after which they sharply decreased and were at par with the females. Hourly collections of *P.orientalis,* the corresponding temperatures (Female: r=-0.136, P=0.089; male: r=-015, P=0.061) and relative humidity (Female: r=0.082, P=0.312; male: r=-0.025, P=0.755) revealed no significant correlations.

### 1.2.2. Monthly variations of the nocturnal activity of *P.orientalis* in different habitats

The activity patterns of *P. orientalis* in the different months of the experiment were not identical (Figure 2). However, the periodicities in activity of *P. orientalis* in periphery of village and farm varied considerably, not showing any pattern of activity with the progress of the dry season from January to June.

The density of *P.orientalis* was more abundant in farms fields than in periphery of villages. However, the peak of activity in the periphery of seemed to be progressively pushed back with the progress of the dry season from January to June (Fig. 2). Thus, it can be seen that in January *P.orientalis* were reached a peak between 20:00 and 22:00 hrs; in February, peaked at 00:00-01:00hrs. The peak time of *P. orientalis* was at 01:00-02:00hrs in March and April. In May, *P.orientalis* had one major peak of equal abundance at 00:00-01:00hrs. In June, *P. orientalis* had three peaks of activities between midnight and morning hours, the biggest at 02:00-03:00 hrs.

In the farm field, the activity pattern of *P. orientalis* was similar in January and April with different densities. It was reached peak at 02:00-03:00hrs. Similarly, *P.orientalis* also showed similar activity pattern in February and March and reached a peak at 22:00-23:00hrs. In May, *P. orientalis* had two peaks, a smaller one in the early evening (20:00-23:00hrs) and a bigger one after midnight (24:00- 04:00hrs). In June, *P. orientalis* had two peaks (an early one at 21:00-23:00 hrs and second one around mid night of 24:00:02:00 hrs.

### 1.2.3. Nocturnal activity of *P. papatasi*

Figure 3 shows the nocturnal activity of *P. papatasi* based on the mean catch of during hourly collections throughout the six month period. The pattern was similar to *P. orientalis*. Males were the dominant having two peaks, an early smaller peak between 21:00-22:00 hrs and a larger second one around midnight (24:00-02:00 hrs). Females displayed similar activity patterns, with an early peak at 21:00-22:00 and a late smaller peak at 02:00-

 

03:00 hours. The activities of both sexes ceased after 04:00 hrs. No correlation was found between activity of *P. papatasi* and temperature (female: r=-0.09, P=0. 431, male: -0.097, P=0.396) or relative humidity (female: r=-0.196, P=0.085; male: r=-0.156, P=0.172). Both sexes were active at temperatures ranging between 31-20°C and relative humidity of 29%-45%.

### 1.2.4. Monthly variations of the nocturnal activity of *P. papatasi* in different habitats

Figure 4 shows the monthly nocturnal activity patterns of *P. papatasi* in the periphery of village and farm field. Different patterns of nocturnal activities of *P. papatasi* were observed in each month in the village periphery but not active in the farm, with the progress of the dry season. In periphery of village, peak nocturnal activity was at 21:00-22:00hrs in January, 23:00-00:00hrs in February, 01:00-03:00hrs in March. In April, *P. papatasi* showed two peak nocturnal activities at 21:00-22:00 and 00:00-01hrs. Peak nocturnal activity was at 00:00-01:00hrs in May and in June, *P. papatasi* was active at time interval 00:00-05:00hrs and reached peak at 04:00-05:00hrs.

## 1.3. Discussion

We found eight species of *Phlebotomus*, of which *P. orientalis* was the dominant followed by *P. papatasi*. Overall, the abundance of *P.orientalis* didn't differ significantly between the periphery of village and cultivated fields despite the presence of domestic animals and humans in the villages.

Historically, nocturnal activity of sand flies or mosquitoes had been routinely monitored using landing/biting collections on humans. However, these approaches are now regarded as unethical and banned in Ethiopia despite their usefulness. As an alternative, we carried out the investigation using CDC light traps by replacing the collecting bags with new ones every hour of the night. We assume that the nocturnal activity patterns of *P. orientalis* and *P. papatasi* determined by light trap catches, would correspond with human landing catches.

Although *P. orientalis* was active throughout the night, both the males and females exhibited two peaks of activity, a smaller one, just before midnight and a major one, just after midnight with males predominating during these times. Very little information is available on nocturnal activity of *P.orientalis* in Ethiopia. In Arbaya, highlands in northern Ethiopia, *P. orientalis* females bit in large numbers soon after sunset and reduced their activity about four hours after sunset when the temperature fell below 16°C (Ashford, 1974). Similarly, in the Rift Valley (Eastern Ethiopia), almost all *P. orientalis* bit before mid-night and most biting took place between 20:00-22:00 hours (Kebede *et al.,* 2010). These reports were both based on brief experimental man-landing catches. Recently the same pattern of nocturnal activity in warmer months has been seen in the same species in the forest extra domestic area in northwest Ethiopia (Lemma *et al*. 2014). In Sudan, Quate (1964) reported that in the Upper Nile region, the biting activity of *P. orientalis* was between 18:30 and 20:30 and sometimes, the biting occurred up to 21:30 or 22:00 during suitable environment. In Western Upper Nile areas, *P. orientalis* was found biting throughout the night and in Dinder national Park in eastern Sudan, the hourly light traps and human-landing collections of *P. orientalis* continued until late in the night (Elnaiem, 2011). On the other hand, biting activities of *P. martini* and *P. celiae*, vectors of VL in southern Ethiopia were shown to peak between 20:00 and 23:00 hours and subsided afterwards (Gebre-Michael and Lane, 1996). In Israel, three species (*P. tobbi*, *P. arabicus* and *P. sergenti*) all exhibited peaks of activity soon after sun set and subsided after midnight (Kravchenko *et al.*, 2004). Elsewhere, the biting activity of *P. argentipes* peaked between 22:00 and 02:00 hours (Dinesh *et al*., 2001). *P. argentipes* in Sri Lanka, displayed peaks of activity from 01:00 onwards (Lane *et al*., 1990). Thus, like *P. orientalis*, *P. argentipes* appears to display distinct activity patterns in different parts of its distribution, perhaps due to variations of weather conditions or season or collection method.

We found different nocturnal activity pattern of *P.orientalis* in different months. This variation could be due to environmental factors affecting the activity of *P.orientalis* during collection time (Quate, 1964; Ashford, 1974, Elnaiem, 2011). Sand flies cannot fly in windy weather, so maybe gusty winds at night cause their variation. Very few sand flies were collected during windy nights whereas high number of sand flies was caught on very calm night (Quate, 1964; Ashford, 1974; Coleman *et al*., 2007). Our result showed that no effect of hourly temperature and relative humidity on the hourly density of *P. orientalis* per trap per night. Similarly, Quate (1964) also found a biting activity that could not be correlated with temperature, wind velocity, or direction. However, we observed no female and male *P. orientalis* was collected below 18°C temperature. Similarly, Ashford (1974) observed that the activity of *P. orientalis* was reduced when temperature fell below 16°C during the night in Arabya. In the present study, it was also observed that *P. orientalis* started its activity early in the evening and continued until late 05:00hr in May and June in the farm. In this area, the daily labourers and farmers are also engaged working in the agricultural fields during the night time to avoid the extreme heat during the day, especially during May and June. Hence, they would be exposed to the bite of *P. orientalis* and other sand flies that can transmit *Leishmania.*

In agreement with several studies (Morsy *et al*., 1993; EI-Badry *et al*. 2008; Fahmy *et al*., 2009) *P. papatasi* was highly prevalent in the periphery of villages (peridmoestic habitat). Over all, both female and male *P. papatasi* exhibited similar nocturnal activity pattern and they were active between 19:00 and 05:00hrs. Female

 

*P. papatasi* reached its maximum at 21:00-22:00hrs whereas male *P. papatasi* showed bimodal activity at 21:00-22:00hrs and 00:00-01:00hrs. Similar studies carried out on *P. papatasi* in different countries have shown different results. Thus, the nocturnal peak activity of *P. papatasi* was around midnight in Turkey (23:00 to 00:01 hours)(Kassiri *et al*., 2013), in Morocco, the nocturnal activity of *P. papatasi* was between 20:00 and 22:00 hours (Guernaoui *et al*., 2006), and in Egypt, *P. papatasi* exhibited peaked after midnight (24:00 – 02:00)(Fahmy *et al*., 2009). Furthermore, *P. papatasi* was active from 20:00 to 04:00hrs in Iraq (Coleman *et al*., 2007), whereas in the Palestine West Bank, human-biting activity of *P.papatasi* and other sand flies occurred through the night with peaks between 24:00 and 03:00 hours (Sawalha *et al*., 2003). In Saudi Arabia, *P. papatasi* increased biting activity at 20:00-22:00hrs with lower temperature and high humidity (EI-Badry *et al*. 2008). The present finding found that no effect of hourly relative humidity on the nocturnal activity of *P. papatasi*. In contrast, *P. papatasi* showed a significant negative correlation with the hourly relative humidity ($r=-0.9$, $p=0.01$) in Morocco (Guernaoui *et al*., 2006). This variation occurs may be the method of collection and time of collection.

The large number of male *P.orientalis* and *P.papatasi* collected in the present study were probably that they were attracted to the females for mating. Similar observations were also made in *P. argentipes* in India and Sri Lana (Lane *et al*., 1990; Dinesh *et al*., 2001) and in three other species in Israel (*P. tobbi*, *P. arabicus* and *P. sergenti*) (Kravchenko, *et al*., 2004). However, these observations were in contrast with the finding of EI-Badry *et al*. (2008), where female *P. papatasi* were found to be higher than the males in Saudi Arabia, showing there might be other factors (e.g. method of collection) involved in biased sex ratios, even though the ratio is expected to be 1:1 in nature.

The findings of the present study have important practical implications for the community so that they would take appropriate measures to avoid the risky hours of the night (e.g use of repellents, ITNs or avoiding outdoor activities).

In conclusion, *P.orientalis* and *P. papatasi* remained active throughout the night. High risk of VL transmissions is likely concentrated during the peak hours and local inhabitants could reduce the risk of infection by using appropriate personal protective measures such as repellents and bed nets. Further study should investigate the activity of *P. orientalis* in relation to the sleep habits of farmers and migrant labourers during the night time.

**Competing interest**

The authors declare that they have no competing interests.

**Author contributions**

Conceived and designed the study: SY, TGM Performed data collection: SY Analyzed the data and interpretation the results: SY, MB AH, TGM, AW. Wrote the paper: SY, MB, AH, TGM, AW. All authors read and approved the final manuscript.

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Table 1 Phlebotomine sand flies collected by CDC light traps from January to June 2013

| | Village periphery | | | Farm field | | | |
|---|---|---|---|---|---|---|---|
| Species | Female N(%) | Male N(%) | Total N(%) | Female N(%) | Male N(%) | Total N(%) | Overall total N(%) |
| *P.orientalis* | 773(13.10) | 1,472(24.94) | 2,245(38.04) | 1,163(19.71) | 2,112(35.78) | 3,275(55.49) | 5,520(93.53) |
| *P.papatasi* | 83(1.41) | 153(2.59) | 236(4.00) | 0(0.00) | 10(0.17) | 10(0.17) | 246(4.17) |
| *P. bergeroti* | 7(0.12) | 19(0.32) | 26(0.44) | 1((.02) | 3(0.05) | 4(0.07) | 30(0.51) |
| *P. duboscqi* | 4(0.07) | 7(0.12) | 11(0.19) | 0(0.00) | 1(0.02) | 1(0.02) | 12(0.20) |
| *P.lesleyae* | 17(0.29) | 36(0.61) | 53(0.90) | 16(0.27) | 21(0.36) | 37(0.63) | 90(1.52) |
| *P. heischi* | 0(0.00) | 0(0.00) | 0(0.00) | 1(0.02) | 0(0.00) | 1(0.02) | 1(0.02) |
| *P. alexanderi* | 0(0.00) | 0(0.00) | 0(0.00) | 2(0.03) | 0(0.00) | 2(0.03) | 2(0.03) |
| *P. rodhaini* | 0(0.00) | 0(0.00) | 0(0.00) | 1(0.02) | 0(0.00) | 1(0.02) | 1(0.02) |
| Total | 884(14.98) | 1,687(28.58) | 2,571(43.56) | 1,184(20.06) | 2,147(36.38) | 3,331(56.44) | 5,902(100) |

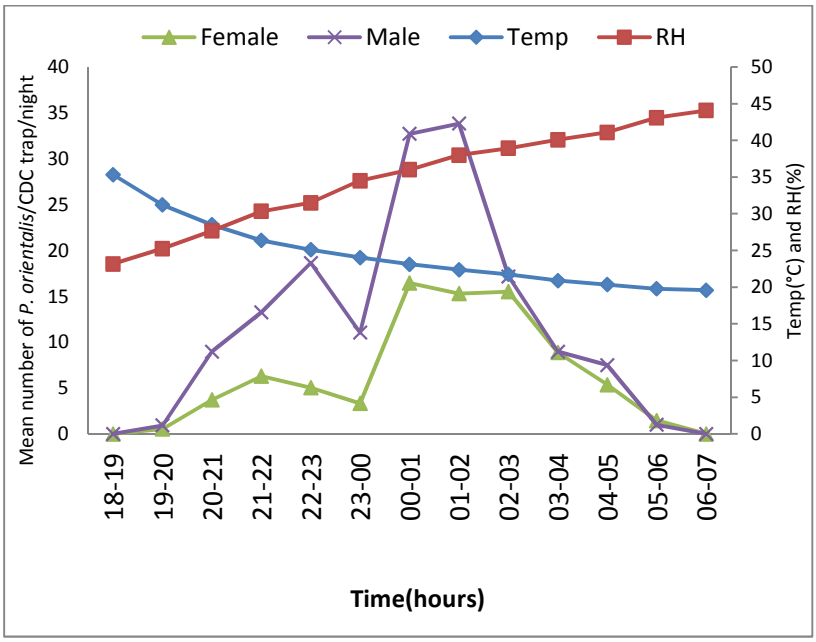


Figure 1: Nocturnal activity of *P. orientalis* in Adeby village, Kafta Humera and variation in hourly mean temperatures and relative humidity from January to June 2013.

 

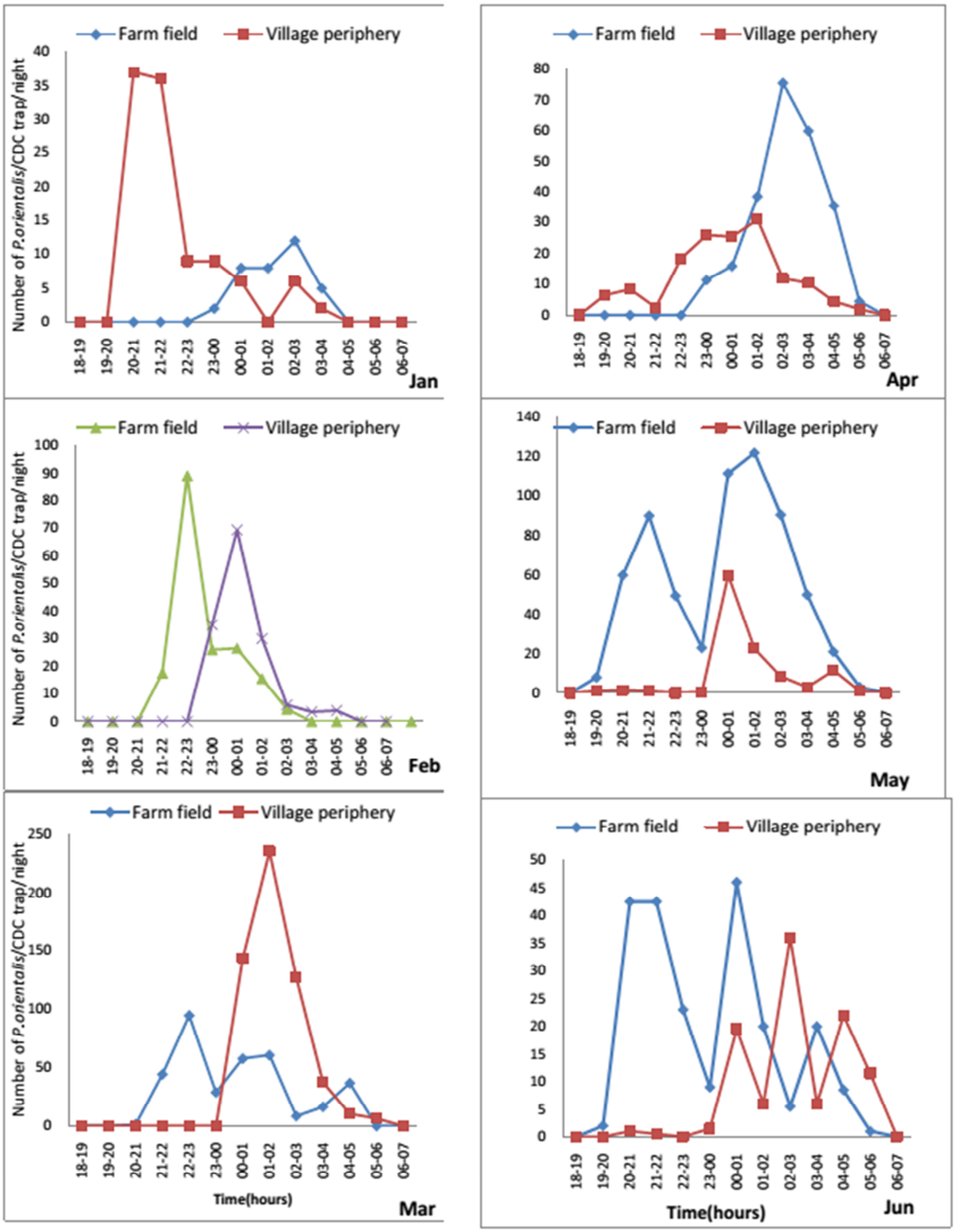


Figure 2: Nocturnal activity patterns of *P.orientalis* in the periphery of villages and farm field from January to June 2013

 

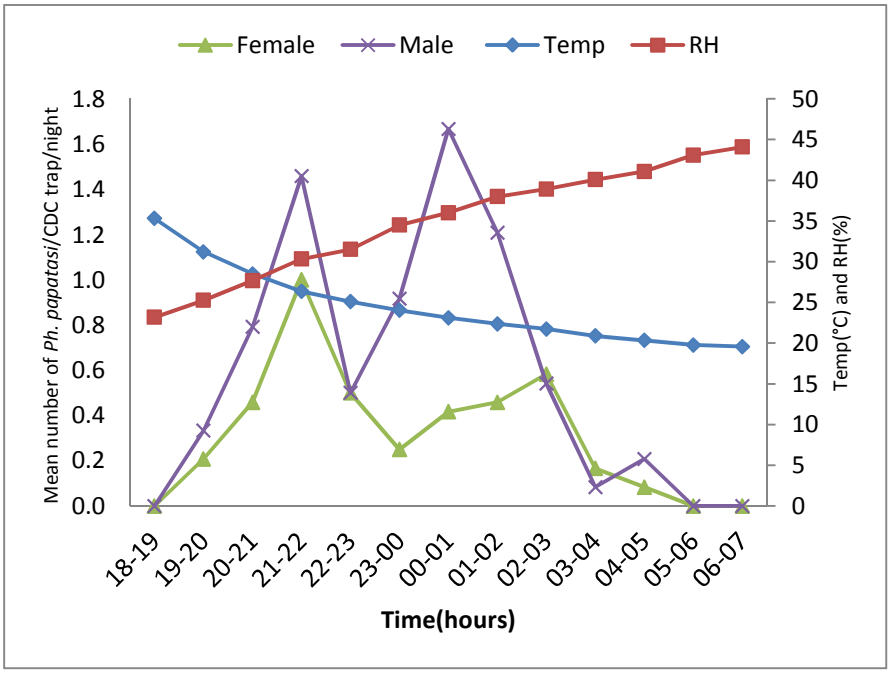


Figure 3 Nocturnal activities of *P. papatasi* and variation in temperature and relative humidity from January to June 2013 in Adebay village

 

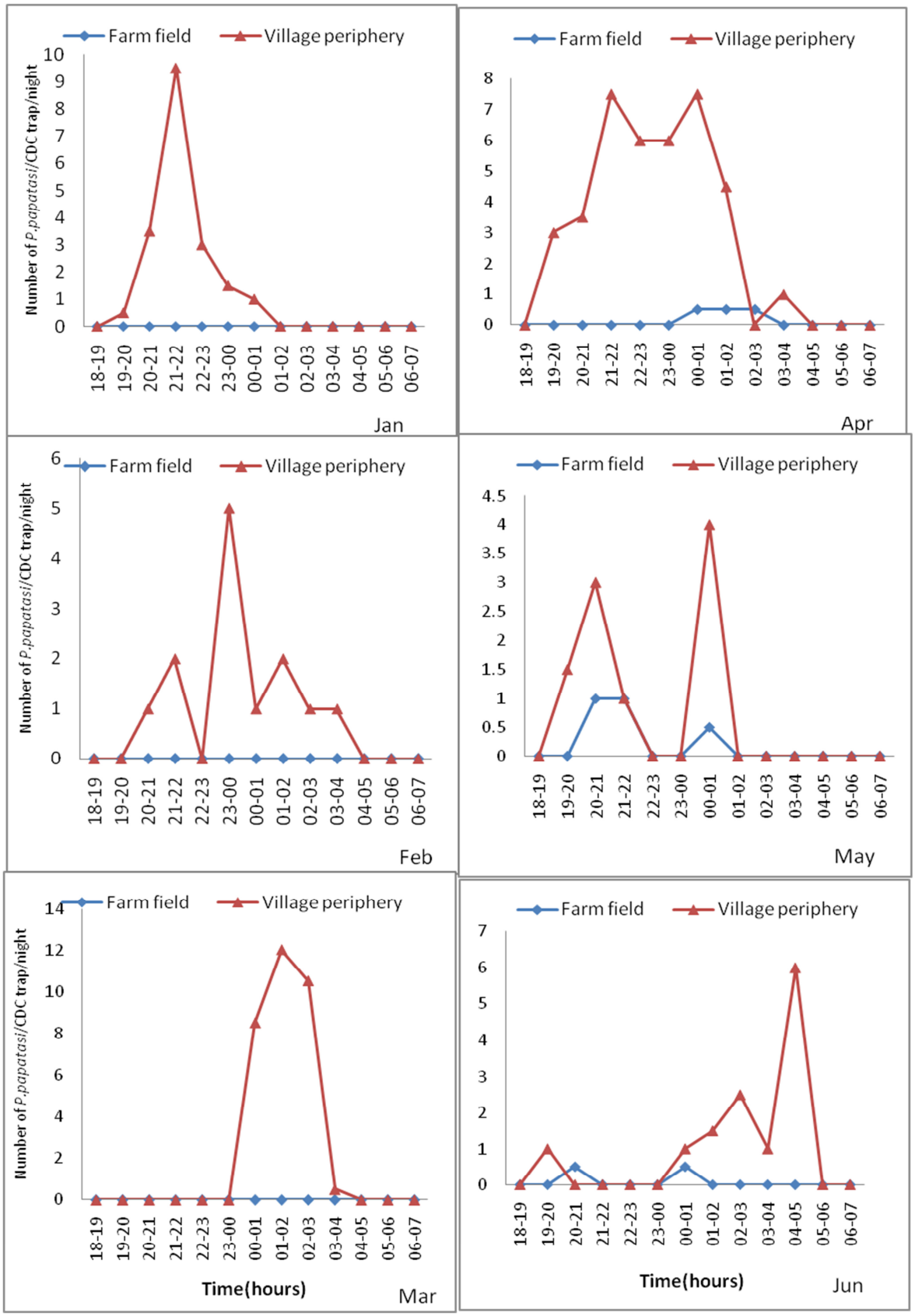


Figure 4 Nocturnal activity of *P.papatasi* from January to June 2013 in the periphery of Adebay village.

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