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A study of phototropism of sand flies species (Diptera: Psychodidae) gathered in a pastoral area in Central Morocco

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An inventory of a year was achieved using two traps techniques (light and sticky trap) in Oulad Aid (center of Morocco), a focus of cutaneous leishmaniasis (CL), to understand better the phototropism of circulating species of the sandflies dominance according to their physiological states (total number of sandflies collected are to be given). The most abundant species were respectively *Phlebotomus papatasi* (50.52%), *Phlebotomus sergenti* (24.7%), *Phlebotomus perniciosus* (9.69%), *Phlebotomus longicuspis* (5.54%), *Sergentomyia falax* (5.22%), *Sergentomyia minuta* (3.64%), *Sergentomyia antenatal* (0.4%) and *Sergentomyia dreyfussi* (0.2 %). With sticky traps, *P. papatasi* constituted 59% of collected samples against 23% for *P. sergenti*, 12% for *P. perniciosus* and 6% for *P. longicuspis*. However with light traps, phototropism of *P. sergenti* was high and represented 56% of collected samples against 36% for *P. papatasi* and only 4% for *P. perniciosus* and *P. longicuspis*. The trap technique influenced the dominance of females collected according to their physiological state. Non engorged females were dominant (77.73%) when sticky traps were used but gorged and gravid females were, respectively dominant (32.62 and 21.98%) when light traps were used. Statistical analysis showed that significantly more number of sand flies were obtained using sticky traps, compared with that of light traps. Sticky traps were found to be effective, as the sand flies entering the dwellings either for blood meal or mating got trapped, while light traps yielded relatively lesser number of sand flies species. Perhaps the sand flies which are influenced by the light were attracted towards the light traps. This information may be necessary for designing intervention measure and evaluation of the impact on sand fly prevalence in a CL focus area.

Key words: Sand flies, Psychodidae, phototropism, leishmania, Morocco.

INTRODUCTION

Leishmaniasis are endemic in Morocco and remain a great health problem which represents the second most common vector-borne disease in the Mediterranean region.

On a large scale, worldwide, the leishmaniasis infects between 1.5 and 2 million people each year (World Health Organization, 2007). It is estimated that leishmaniasis

disease has an undesirable morbid effect corresponding to 2.34 million years of life (World Health Organization, 2010). In Morocco, the vectors involved in the transmission of the disease are: *Phlebotomus (larroussius) ariasi* Tonnoir 1921 (Rioux et al., 1984a; Rhajaoui, 2011) and *Phlebotomus (Larroussius) perniciosus* Newstead 1911 (Guernaoui et al., 2005). These species are found throughout the Rif mountains and lowlands of pre-Rif, where visceral leishmaniasis due to *Leishmania (Leishmania) infantum* is abundant (Nejjar et al., 1998); *Phlebotomus (larroussius) longicuspis* Nitzulescu 1930 is considered as the vector of sporadic cutaneous leishmaniasis due to *L. (Leishmania) infantum* in the semi-arid belt (Dereure et al., 1991) and even in the arid belt (Dereure et al., 1986).

Phlebotomus (Paraphlebotomus) sergenti Parrot 1917 was found infested by *Leishmania (Leishmania) tropica* (Ajaoud et al., 2013), responsible for the dry skin which is very recurrent in the semi-arid belt in Western slope of the Atlas from the province of Taza to Essaouira on the Atlantic coast (Guilvard et al., 1991); *Phlebotomus (Phlebotomus) papatasi* Scopoli 1786 is dominant in the zoonotic cutaneous leishmaniasis focus due to *Leishmania (Leishmania) major* localized in the arid south of the Atlas mountains that extend from the province of Jrada east to Tata province west (Rioux et al., 1986a; Boussaa, 2008; Guernaoui, 2011).

Morocco is also jointly involved in leishmaniasis control programme which is based on the detection and monitoring of vectors' density. Moulay Yacoub, located about 10 km from the city of Fez, is monitored for skin focus (Fellah et al., 2007). For implementing effective vector control strategy to suppress the leishmania infection, the investigations were carried out on the seasonal abundance and behavior of sandflies, mainly the females, depending on the nature of the technique used in the capture in the province of Moulay Yacoub, which is a known focus of cutaneous leishmaniasis (Lahouiti et al., 2013).

MATERIALS AND METHODS

Studied area

Douar Ouled Aid is located (34° 05' N, 4° 45' W, 345 m MSL) about 35 km to the N- N-east of the city of Fez. It is part of the province of Moulay Yacoub known as a new "microfocus" of human cutaneous leishmaniasis due to *L. tropica* (Fellah et al., 2007). The area is rural and is located on one of South Rif hills and has about 709 inhabitants; the majority of houses are built with clay and straw. Regions depend on rainfed agriculture and breeding livestock

which are considered the only activity for the population of the region. Vegetation is dominated by *Olea europaea*, *Zizyphus lotus*, *Eucalyptus sp*, *Cylindropuntia imbricata* and *Opuntia sp*.

Sandfly collection

Light traps "C. D. C. Miniature Light Trap" (Mulhern, 1942; Sudia and Chamberlain, 1988) was used for sandfly collection both indoor and outdoor (Figure 1). This device is composed of a motor assuring the functioning of a small fan to maintain a continuous low intensity suction, an ampoule of 0.3 A, with a transparent plastic cylinder inside which the fan-motor-lamp is located, a cage covered with a very fine mesh cloth piece and a very flat metal cover that covers the whole device and protects the camera from rain and projections. Insects attracted by the lights will be drawn and collected in the collection bottle. Light traps are placed from 6 pm to 8 am. They are placed inside the house and stables at an average height of 2 m. Sticky traps made of sheets of white A4 paper (21 x 29.7 cm) smeared with castor oil (Rioux et al., 1967) were placed upright in the walls of human dwellings (Figure 2) and stables entries (Figure 3). The oiled sheets are held by a thread (Figure 2) or maintained by a rigid support (Figure 3).

The sticky traps placed from 6 pm to 8 am

The traps are placed in many habitats, intra-domestic (Figures 1 and 2) and peri-domestic (Figure 3) in the cow's, donkey's, horse's and sheep's stables. Period of capture lasted all night, traps were placed at the site in the previous evening (6 pm) and collected in the subsequent morning (8 am). Sand flies obtained in the collection, using light and sticky traps, were transferred in a vial containing 70% alcohol, using fine brush and again preserved in 95% absolute alcohol. The samples were then cleaned with a Marc-André solution before being mounted in Canada Baum (Floch and Abonnec, 1957). Taxonomic identification is based on different morphological criteria described by Abonnec (1972) and Niang et al. (2000), such as cibarium and pharynx in both male and female, spermathecae in female and the armature of genitalia in male.

Statistical analyses

Results were statistically performed by the software R using the Pearson's chi-squared test comparing percentages. For the entire test, the significance level was 0.05.

RESULTS

The results shown in this paper included data from the entomological survey undertaken in Douar Ouled Aid during a year between April, 2011 and March, 2012 to show the particular phototropism of sand fly species and of females according to their physiological states. Data were subjected for statistical analysis, using a software

R and the Pearson's chi-square test for comparing percentage. For the entire test, the significance level is 0.05.

A total of 3,064 sand flies were obtained during the study, of which 87.8% (N = 2690) were obtained from sticky traps and the remaining (12.2%; N = 374) from light traps (Table 1). The number of sand flies captured using sticky traps was greater than the number collected using light traps. The difference was statistically significant between the two traps ($\chi^2 = 1750, 606, p$ -value $< 2.2e^{-16}$). Of the total sand flies caught, the sand flies of the genus *Phlebotomus* were predominant in the sticky trap collections (86.9%), compared to that of light trap collections (13.1%). Similarly, sand flies of the *Sergentomyia* collected using sticky traps were 95.9% and light traps were 4.1%, indicating a statistically significant difference between the efficacy of the traps in sand fly collection ($\kappa^2 = 18.9182, p$ -value = 1.364×10^{-05}).

In both types of trap collection, male sand flies were predominant (Table 2) that is, 82% male in sticky trap collections and 61.7% male in light trap collections. However, *Sergentomyia fallax* species were the only females captured using both types of traps. On the contrary, *Sergentomyia antennata* males were abundant in the collections. The difference was statistically significant between the two traps ($\kappa^2 = 2.7778, p$ -value = 0.09558).

Species composition

Among the total collections (N = 3064), *P. papatasi* was represented at 50.5%. The other species of sand flies obtained in order of abundance are as follows; *P. sergenti* (24.7%), *P. perniciosus* (9.7%) and *P. longicuspis* (5.5%). The difference was statistically significant between these species ($\kappa^2 = 52, p$ -value = 2.995×10^{-11}). Phlebotomine's collection was carried out twice a month during one year. The traps were placed from 6 pm to 8 am. The morpho-anatomical identification of species was based on the identification of key sand flies (Abonnec, 1972; Niang et al., 2000). The result shows the number of males (M) and females (F) for each species and for each type of trap.

By examining the distribution of species according to the type of trap, we have noticed that with sticky traps, *P. papatasi* was dominant at 59% ($\kappa^2 = 706.2147, p$ -value $< 2.2 \times 10^{-16}$) followed by *P. sergenti* (23%) ($\chi^2 = 308443, p$ -value $< 2.2 \times 10^{-16}$), *P. perniciosus* (12%) ($\chi^2 = 216.5634, p$ -value $< 2.2 \times 10^{-16}$) and *P. longicuspis* (6%) ($\chi^2 = 70.2229, p$ -value $< 2.2 \times 10^{-16}$) (Figure 4). However, with light traps (Figure 5), *P. sergenti* was collected at 56% ($\chi^2 = 15.3725, p$ -value = 8.826×10^{-05}) followed by *P. papatasi* at 36% and *P. perniciosus* and *P. longicuspis* at 4%.

The physiological states of the females are given in Table 3. The sticky trap and light traps yielded about 70.44% of

Table 1. Distribution and abundance of sand flies inventoried for each trap.

Species	Sticky traps	Light traps
<i>P. papatasi</i>	1416	132
<i>P. sergenti</i>	553	204
<i>P. perniciosus</i>	284	13
<i>P. longicuspis</i>	157	13
<i>S. falax</i>	157	3
<i>S. minuta</i>	106	6
<i>S. antennata</i>	14	1
<i>S. dreyfussi</i>	3	2
Total	2690	374

Table 2. Distribution and abundance of sand flies inventoried for each trap according to their sex.

Species	Sticky traps	Light traps
<i>P. papatasi</i>	1208 M	72 M
	208 F	60 F
<i>P. sergenti</i>	483 M	130 M
	70 F	74 F
<i>P. perniciosus</i>	266 M	12 M
	18 F	1 F
<i>P. longicuspis</i>	131 M	10 M
	26 F	3 F
<i>S. falax</i>	0 M	0 M
	157 F	3 F
<i>S. minuta</i>	103M	6 M
	3F	0 F
<i>S. antennata</i>	14 M	1 M
	0 F	0 F
<i>S. dreyfussi</i>	2 M	0 M
	1 F	1 F
Total	2690	373

non engorged females, 19.64% for engorged females and 9.90% for gravid females. The difference is statistically significant between the three physiological states of females ($\chi^2 = 8.2439, p$ -value = 0.01621). However, with each trap, this percentage changes, with sticky traps at 77.73% of harvested females which are non engorged,

Table 3. Abundance of female *Phlebotomus* species inventoried in each trap according to their physiological state.

Species	Sticky traps	Light traps
<i>P. papatasi</i>	125 FN	34 FN
	71 GS	18 GS
	12 G	8 G
<i>P. sergenti</i>	57 FN	24FN
	2 GS	27 GS
	11 G	20 G
<i>P. perniciosus</i>	15 FN	1 FN
	0 GS	0 GS
	3 G	1 G
<i>P. longicuspis</i>	23 FN	1 FN
	3 GS	1 GS
	2 G	1 G
<i>S. falax</i>	153 FN	3 FN
	1 GS	0 GS
	3 G	0 G
<i>S. minuta</i>	3 FN	0 FN
	0 GS	0 GS
	0 G	0 G
<i>S. antenata</i>	0 FN	0 FN
	0 GS	0 GS
	0 G	0 G
<i>S. dreyfussi</i>	1 FN	1 FN
	0 GS	0 FGS
	0 G	1 G

Non engorged (FN), engorged females without (GS), gravid (G), normal females (NF).

15.87% of the females which are engorged and 6.39% which are pregnant. With light traps, 45.39% of collected females are non engorged, 32.62% are engorged and 21.98% are pregnant. The difference between the three physiological states females with these types of traps is statistically significant ($\chi^2 = 90.0431$, p -value $< 2.2 \times 10^{-16}$).

P. sergenti and *P. papatasi* were the two common species in this study; we have investigated the female's distribution by types of traps according to their physiological states. The results (Figure 6) shows that 6% of attracted *P. papatasi* specimens are pregnant females,

34% are females engorged with blood and 60% are neither pregnant females nor engorged. These percentages were almost the same with light traps (Figure 7). However *P. sergenti* females had a clear trap preference depending on their physiological conditions; with sticky traps (Figure 8), 81% of females were normal, 16% of females were engorged and 3% were pregnant but with light traps (Figure 9), 38% of females were gorged with blood, 28% were pregnant and 34% were normal females.

Phlebotomine's collection was carried out twice a month during one year. The traps were placed from 18 pm to 8 am. The morpho-anatomical identification of species was based on the sand flies key identification (Abonnec, 1972; Niang et al., 2000). The result shows the distribution of *P. papatasi* females collected by light trap according to their physiological states: non engorged (FN), engorged females without (GS) and gravid (G).

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DISCUSSION

The results reported in this paper shows for the first time the distribution of sand fly species and the physiological state of females according to the techniques of traps used for the collection in this semi-arid rural area of Douar Oulad Aid characterized as an anthropic niche of *L. tropica* (Rhajaoui et al., 2004; Fellah et al., 2007). The density of sand flies captured by sticky traps (87.8%) is higher than the density of sand flies collected by the light



Figure 1. Light traps (A and B) inside houses.



Figure 2. Sticky traps inside the houses.

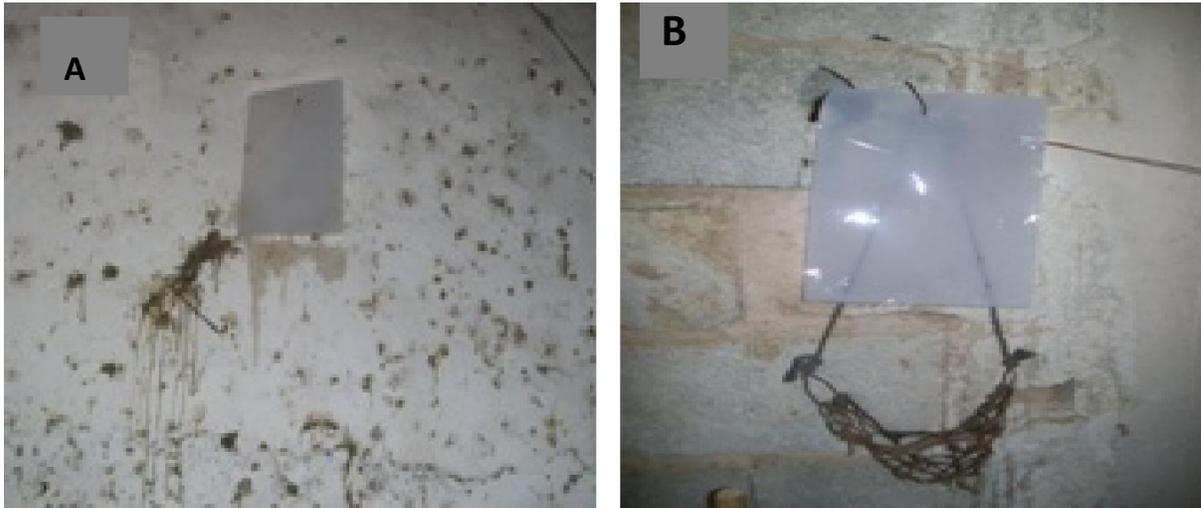


Figure 3. Sticky traps (A and B) inside the stables.

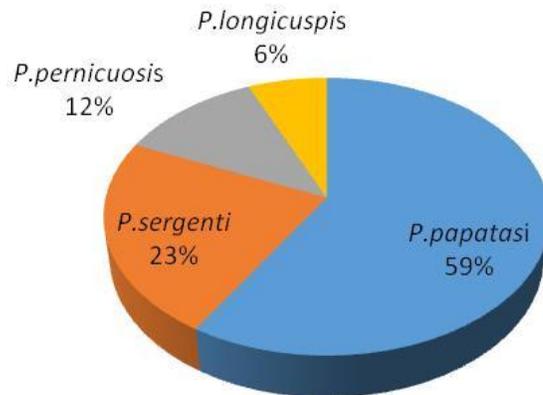


Figure 4. Abundance of species of *Phlebotomus* genus collected by sticky traps (ST).

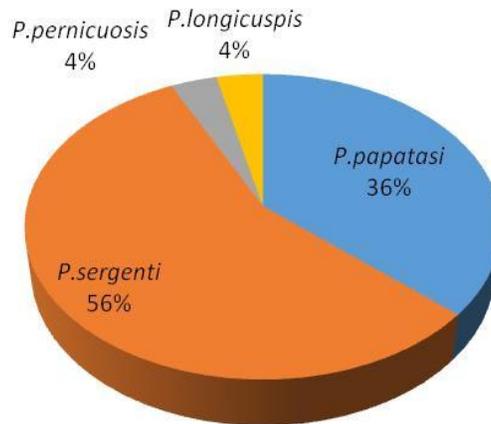


Figure 5. Abundance of species of *Phlebotomus* genus collected by light traps (LT).

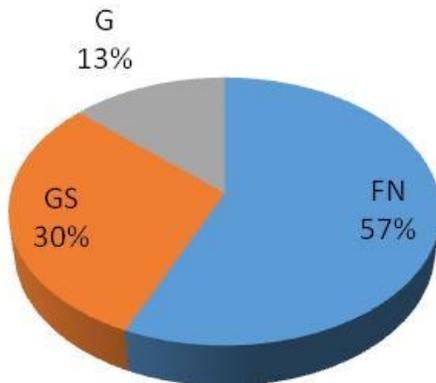


Figure 6. Abundance of female *P. papatasi* collected by light traps (LT) according to their physiological states FN (normal females), GS (engorged females) and G (gravid females).

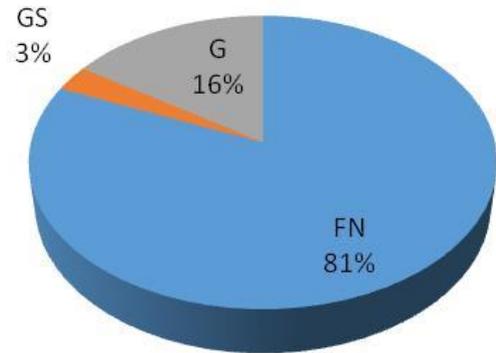


Figure 8. *P. sergenti* females' abundance by sticky traps (ST) according to their physiological states FN (normal females), GS (females engorged) and G (pregnant females).

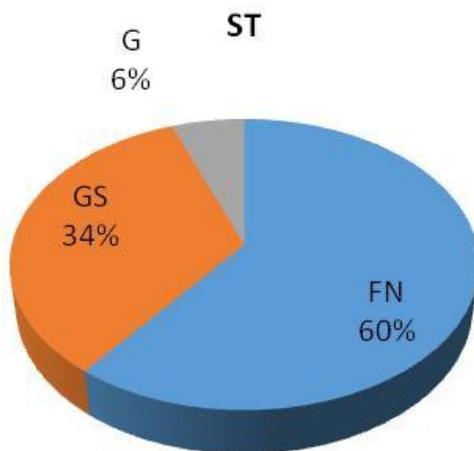


Figure 7. Abundance of female *P. papatasi* by sticky traps (ST) according to their physiological states FN (normal females), GS (females engorged), G (gravid females).

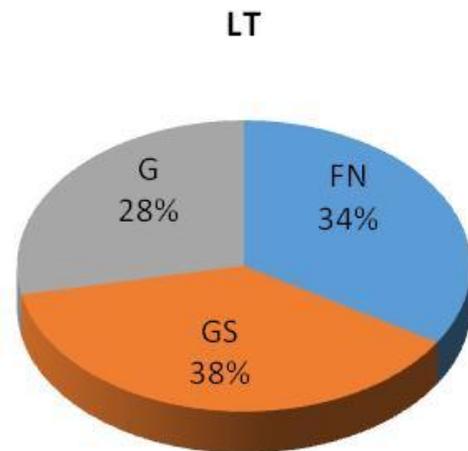


Figure 9. *P. sergenti* females abundance by light traps (LT) according to their physiological states FN (non engorged), GS (engorged females), G (pregnant females).

traps (12.2%). With both traps, *Phlebotomus spp* was collected at 89.46% while *Sergentomyia* was collected at 10.54%. *Phlebotomus spp* is the most abundant, they have been also reported by several authors (Bailly-Choumara et al., 1971; Rioux et al., 1984; Dereure et al., 1991; Raspail et al., 2002; Guernaoui et al., 2000; Boussaa, 2008; Faraj et al., 2013) as the only vectors implicated in the transmission of leishmaniasis in Morocco. Sticky traps captured more of both species of the *Phlebotomus* (86.9%) and *Sergentomyia* genus (94.5%). *Sergentomyia* species (*S. falax*, *S. minuta*, *S. antenata*, *S. dreyfussi*) phototropisms seems undeveloped (5.5%) since its species are not attracted by light

traps. These results confirm the effectiveness of the use of oiled paper as a good sampling and monitoring of sand flies populations technique (Boukraa et al., 2010). The sticky traps have the advantages of not being repellents, being very viscous; they are also soluble in alcohol which facilitates insects' retrieval. However sand flies collected using sticky traps are damaged and the taxonomic study by morpho-anatomical identification can be difficult.

For the entire collection and within the *Phlebotomus* genus, *P. papatasi*, *P. sergenti*, *P. perniciosus* and *P. longicuspis* are the circulating species in the heart of central Morocco, with *P. papatasi* dominating at 56.38%,

followed by *P. sergenti* dominating at 27.3%. This result confirms the work of Lahouiti (2013), who reported the dominance of *P. papatasi* in this province and those of Rioux et al. (1984) which reported the widespread that the dominance of a species of sand fly in an outbreak of leishmaniasis depends on used trap technique. It also shows that phototropism of *P. sergenti* species is higher compared to *P. papatasi*, *P. perniciosus* and *P. longicuspis* that are captured only at 4%, with light traps contrary to 12% when sticky traps is used. Blood sucking females are the leishmaniasis vectors, the relationship between sand fly-leishmania is close and the study of the distribution by type of trap of females according to their physiological state may be interesting to the extent in which we want to study the vector's infestation or the sand flies trophic power. Our study showed that the sample is composed of 70.44% of normal females (neither gravid nor engorged), with 19.64% of engorged females and 9.90% of pregnant females. However, the abundance of females according to their physiological state in each of the types of traps showed that sticky traps attracts more normal females (77.73%) than engorged females (15.87%) and pregnant females (6.39 %), while light traps attracted 32.62% of engorged females and 21.98% of pregnant females. This original result shows the effectiveness of the use of the technique of light traps in the collection in order to have more females engorged with blood which are necessary to perform more specific studies. The same study is applied to the two most abundant species in this semi-arid outbreak which are *P. papatasi* and *P. sergenti*. The physiological state of *P. papatasi* females is not affected by light, the percentage of distribution of normal females, females engorged and gravid females is, respectively 57 to 60, 30 to 34 and 6 to 13% for sticky and light traps which is probably explainable by the lack of infectious activity of this species in this location whose vector is *P. sergenti*. The latter species is confirmed as being the only vector of *L. tropica* MON 101 by the works of Rhajaoui (2004) and Fellah et al. (2007) in this area and demonstrated infested by *L. tropica* by Ajouad et al. (2013). We have shown dominance and a high phototropism for the species of *P. sergenti*; females also have a net density difference according to the used traps and to their physiological states. Indeed, females engorged and gravid females are attracted to light traps, respectively 38% (GS) and 28% (G), while the sticky traps set at 81% that of normal females and only 3% are females engorged with blood which likely confirms the infectious activity of *P. sergenti* in this focus.

Conclusion

We have shown that the calculated dominance in an out-

break of leishmaniasis depends on the trap chosen. We have also shown that in the locality of Oulad Aid (central Morocco), *P. papatasi* may be dominant if we use sticky traps and *P. sergenti* may be dominant if we use light traps. We have also shown that the sticky traps are effective and therefore, monitoring the population during surveillance sticky traps could be used. While the light traps though yielded a relative lower number of sand flies species, these traps are used in identification, which would also be used to know the species abundance and distribution.

Conflict of Interest

The authors declare that they have no conflict of interests.

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