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Full Length Research Paper

Seasonal fluctuations of phlebotomines sand fly populations (Diptera: Psychodidae) in the Moulay Yacoub province, centre Morocco: Effect of ecological factors

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An entomological survey of phlebotomine sand flies was conducted in the Moulay Yacoub province, central Morocco. An anthropic niche (Ouled Aid) and a wild niche (Zliligh) were selected. Sand flies were collected twice a month between April 2011 and March 2012, using sticky traps and CDC light traps. 3675 specimens were collected (78.3% males/21.7% females) which composed of eight species divided into two genera: *Phlebotomus* (90.34%), wide *Phlebotomus papatasi* (49.78%), *Phlebotomus sergenti* (27.17%), *Phlebotomus longicuspis* (13.69%), *Phlebotomus pernicuosis* (9.36%) and *Sergentomyia* (9.66%) composed of *Sergentomyia fallax* (52.36%), *Sergentomyia minuta* (34.26%) *Sergentomyia antennata* (11.16%) and *Sergentomyia dreyfussi* (2.22%). *P. papatasi* is dominant in the anthropic niche and *P. longicuspis* is dominant in the wild niche. The population dynamics showed a bimodal pattern with a first peak in June for both studied stations and a second one in September for Ouled Aid and in August for Zliligh. A significant positive correlation between the density and the temperature(r = 0.64, r = 0.66) and a significant negative correlation with humidity (r = -0.64, r = -0.62) are shown. Density (119 specimens/m^2/night) of sand flies showed that Moulay Yacoub's province presents a risk for the inhabitants in the vicinity of Fez. This situation requires continuous monitoring to prevent and reduce the leishmania risk.

Key words: Sand flies, leishmaniasis, seasonality; risk period, Moulay Yacoub, Morocco.

INTRODUCTION

The phlebotomine sand flies (Diptera Psychodidae) are the vectors of leishmaniasis diseases caused by the *Leishmania* protozoaires parasites. 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, as is the case in many Mediterranean countries, the leishmaniasis is endemic in some regions and presents a real health problem. Indeed, in 2010, 8846 cases have been reported among which 8707 were

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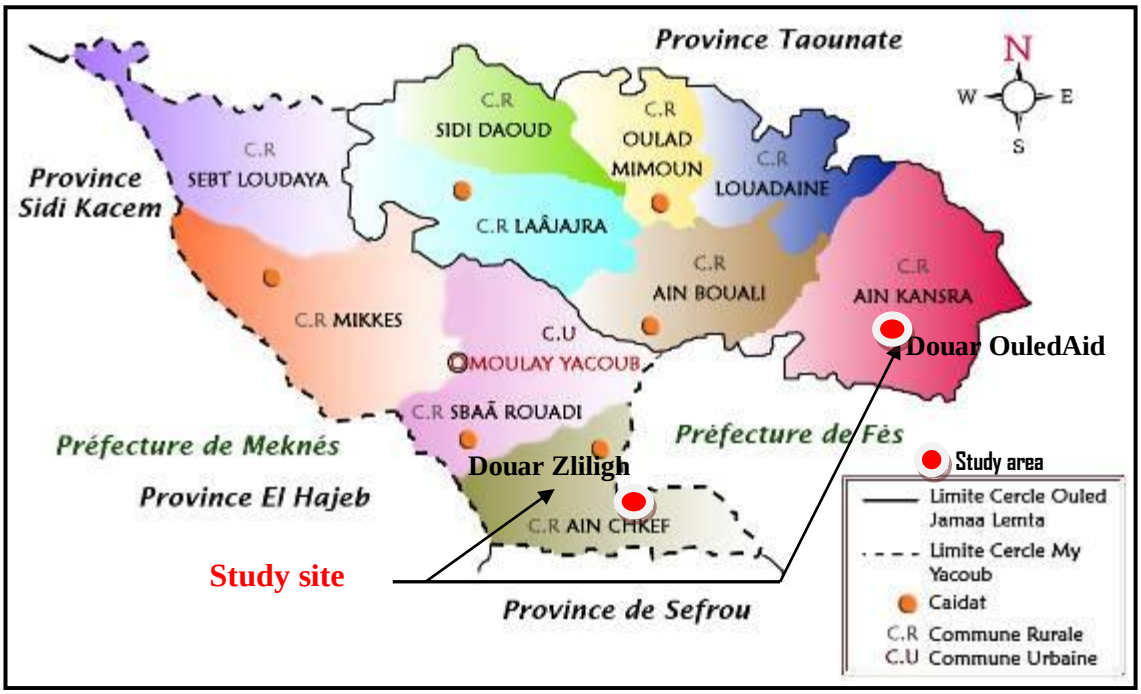

Map 1. The province of Moulay Yacoub showing the study area, Douar Ouled Aid and Douar Zliligh (Source: Province of Moulay Yacoub DTU).

cutaneous leishmaniasis and 139 cases were visceral leishmaniasis (Moroccan Ministry of Health, 2010).

Twenty two (22) species of phlebotomines have been reported; 13 species of *Phlebotomus* genus and nine species of *Sergentomyia* genus (Anonymous, 1997) of which five species have been known to transmit the disease: *Phlebotomus ariasi, Phlebotomus perniciosus* and *Phlebotomus longicuspus* are known to produce the visceral leishmaniasis and are spread mainly in northern regions (Dereure et al.,1986; Rioux et al., 1986a), *Phlebotomus papatasi* is responsible for the humid form seen in the South and Southeast of the Atlas chain of mountains, while *Phlebotomus sergenti* is the vector for the dry skin disease leishmaniasis seen in the center of the country (Rhajaoui, 2009; Adler and Ber, 1941; Rioux et al., 1986b).

During the year 2001, an outbreak of *Leishmania tropica* was declared in the province of Moulay Yacoub at the outskirts of Fez city. The epidemic aggressions have infected a large number of persons. More than 1600 cases were reported (Fellah et al., 2007; Rhajaoui, 2011).

Since the last five years, 138 cases of leishmaniasis have been recorded in the province of Moulay Yacoub of which 79 cases were visceral leishmaniasis and 59 were cutaneous leishmaniasis (Prefectural Services for Environment's Hygiene, a Delegation of Ministry of Health - Province of MoulayYacoub).

The province of Moulay Yacoub is characterized by its rural aspect and by its semi-dry climate, two factors prone to the development of phlebotomies that could present a risk of contamination for the surrounding areas with more important populations. This province is at the outskirts of Fez which has a population of more than one million inhabitants.

Our goal is to study the dynamics of sandflies by specifying the periods when their density is high; so the risk of transmission of leishmaniasis is increased. We are studied abiotic and environmental factors that could influence the distribution of species of sand flies and their biological development. All this work is in order to carry out a successful anti-vector campaign.

MATERIALS AND METHODS

The entomologic surveys was conducted in the province of Moulay Yacoub, neighboring Fez city in the center of the country. This area undergoes a continental semi dry climate, hot in summer and very cold in winter. The yearly average precipitations range between 150 and 300 mm. The winds are dry and cold in winter and hot in summer. The average temperature is between 9 and 40°C from January to July (Direction Meteorology North- East, Morocco). For this study, we have chosen two stations (Map 1): Douar Ouled Aid (O.Aid), an anthropic niche (34°05'N, 4°45'W; 345 m) at about 35 km north-eastern of Fez city and DouarZliligh (Zgh), a wild niche (33°57° N; 5°05W; 500 m) about 20 km west of Fez Prefecture.

The collection of phlebotomies was done twice in a months during the year starting from April 2011 to March 2012. The capture was performed with a sticky material (paper A4 covered with resin oil) (Rioux et al., 1967) and with phosphorescent traps of CDC type (Sudia and Chamberland, 1962). The traps were put in many biotopes:

Table 1. Number of specimens according to the genus in the two stations.

Station	Traps type	Genre Phlebotomus (number of specimens)		Genre Sergentomyia (number of specimens)		Total
		Male	Female	Male	Female	
Ouled Aid	Sticky traps	2088	322	119	165	2694
	CDC light Traps	224	134	7	5	370
Zliligh	Sticky traps	411	137	39	24	611
Total		2723	593	165	194	3675

1. Intra-domestic and around neighboring areas (stables for cows, donkeys, horses, sheep) (Ouled Aid Station). Two kinds of traps were used here.
2. The wild regions far from human dwellings (the caverns of former pits) (station Zliligh). Only sticky traps were used here.

The traps were placed in the evening around 18.00 h and taken back the next day morning around 8.00 h. In the collections with sticky material, the captured specimens were removed with a brush soaked with alcohol and reserved in ethanol 95% (Leger and Depaquit, 2001). The captured insects with shining traps were cooled in a fridge for about half an hour before being taken off.

The specimens were then cleaned with the Marc-Andre solution (chloral hydrate/acetic acid) before being mounted in the Canada Balsam medium for identification (Floch and Abonnenc, 1957). The characterization was undertaken following the identification key of Moroccan phlebotomies which consists on the morphological examination of sexual organs of males and sperm and females pharynx (Anonymous, 1997).

In this paper three parameters have been studied: Temperature and humidity: The atmospheric temperatures and humidity were recorded (°C) at the time of building and taking away the traps with a thermometer and a hygrometer (Fisherbrand); The precipitations data of this study period were obtained at the Hydraulic Agency of Sebou Basin(HASB). Data analysis is based on three characteristics of the sand fly fauna:

Density:

For sticky traps,

D = ph/S/night

S = 2 x L x I x P

For CDC light traps

D = ph/P

Ph = number of sand flies

S = surface trapped

P = number of traps

L and I are the length and width (in meters) of the used papers.

Abundance: The relative number of individuals from the same gender as compared to the whole harvest of phlebotomies in percent.

Correlation index:

$$r = \frac{\sum (X - \overline{X}).(Y - \overline{Y})}{\sqrt{\sum (X - \overline{X})^2} \times \sqrt{\sum (Y - \overline{Y})^2}}$$

r = 0 no correlation

-0,5 < r < 0,5 non significant correlation

-0,5 > r > 0,5 significant correlation

r = 1 perfect correlation

RESULTS

During the trapping period, a total of 3675 sand flies were caught (Table 1). The sand flies were composed of *Phlebotomus* (90.34%) and *Sergentomyia* (9.66%) *genus*. The anatomic analysis of *Phlebotomus* species show a higher number of males (81.7 %) while the *Sergentomyia* species show a higher female number (53.8%) (Table1). *Phlebotomus* species caught (Table 2) were *Phlebotomus papatasi* (49.78%), *P. sergenti* (27.17%), *P. longicuspis* (13.69%) and *P. pernicuosis* (9.36%); *Sergentomyia* species are *Sergentomyia fallax* (52.36%), *Sergentomyia minuta* (34.26%) *Sergentomyia antennata* (11.16%) and *Sergentomyia dreyfussi* (2.22%).

Phlebotomine's collection was carried out twice a month for one year. The traps were placed from 18 pm to 8 am. The specimens were reserved in ethanol 95%, cleared up with the Marc-Andre solution, mounted in the Canadian Baum. The identification key of Moroccan phlebotomies is applied. Results represent the number of genus captured by sexes, study stations and traps type.

Table 1 shows that sticky traps appear to be more efficient because it have trapped 2694 specimens whereas The CDC light traps caught only 370 specimens. Taking into consideration, the data and results obtained with the sticky technique we noted that the number of captured sand flies in the region of Ouled Aid is very high and represents 81.51% of the total number of sand flies captured while the number is lower at Zliligh where it was not higher than 18.49%.

The evolution density of the captured fauna through the year has shown that sand flies infesting the region of Ouled Aid (Figure 1a) appear during the month of April and continue spreading till the month of November; while in the Zliligh station, sandflies appear starting from late May until November. The activity reaches its pick at Ouled Aid (Figure 1a) from June till October to 60.4

Table 2. Number of species caught in the two stations using two types of traps.

Station	Types of trap	Total number of species	*P. papatazi*		*P. sergenti*		*P. longicuspis*		*P. pernicuosis*		*S. fallax*		*S. minuta*		*S. antennata*		*S. dreyfussi*	
			M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Ouled Aid	Stiky traps	2694	1208	208	483	70	131	26	266	18	0	161	103	3	14	0	2	1
	CDC light traps	370	72	60	130	70	10	3	12	1	0	3	6	0	1	0	0	2
Zlilig	Stiky traps	611	77	26	110	38	217	67	7	6	0	24	11	0	25	0	3	0

specimens/m^2/night during the month of June but go higher during the trimester, August-September-October with 119 specimens/ m^2/night in September.

At Zliligh (Figure 1b) the period of activity of the sand flies starts half-June till the end of September with a first pick in June with 22.79 specimens/ m^2/night and a second lasting for August-September higher in August with 36.01 specimens/m^2/night.

Calculation of life-table statistics

The data analysis shows a significant positive correlation between density and temperature (r = 0.64) at Ouled Aid and (r = 0.62) at Zliligh (Figure 2a and Figure 3a). A significant negative correlation has been found between the phlebotomies density and the ambient humidity (r = - 0.64) at Ouled Aid and (r = - 0.66) at Zliligh (Figure 2b and Figure 3b). No significant correlation has been found in either of the two stations between density and rain (Figure 2c and Figure 3c).

At Ouled Aid, most phlebotomies were captured when temperature varied between 31 and 35°C while the maximum number of specimens found in Zliligh was recorded at 26 to 30°C (Figure 4).
In both study sites, the highest number of phlebotomies was found when the humidity scale changed from 30 to 40%.

Proportion between male and female (Figure 5) is different following temperature variations and the studied spot. This seems to have more impact at Ouled Aid as compared to Zliligh station when temperature goes between 26 and 30°C and diminishes when temperature exceeds 35°C. At Zliligh, this result records no significant change. This survey has also shown that females seem to be more sensitive to temperature changes (r = 0.7) than males (r = 0.62).

An entomological survey of phlebotomine sand flies (Diptera: Psychodidae) was conducted in the MoulayYacoub province, central Morocco. Two stations were selected, the first is an anthropic niche, Ouled Aid and the second is a wild niche, Zliligh. Sand flies were collected twice in a month between April 2011 and March 2012, using sticky traps and CDC light traps. 3675 specimens were collected with 78.3% males and 21.7% females. The results of the morphotaxonomic study of collected sand flies in different stations in the study area show the presence of eight species of sand flies divided into two genuses.

DISCUSSION

The present paper reports on an entomological survey of sand fly populations conducted in two different stations (anthropic and wild) of Moulay Yacoub area (Morocco), a focus on cutaneous leishmaniasis (Rhajaoui et al., 2004; Fellah et al., 2007) during a twelve month period. The results of the composition of sand flies species circulating, the ecological effects (temperature, humidity, precipitatios) and environmental factors in the dynamic of sand flies was shown.

The analysis of this study is based on the results of the sticky traps since the use of glowing traps for the capture of sand flies proved to be less efficiency than the sticky papers (Table 1); this result confirms the work of Faiman et al. (2011). The sand flies captured were much more abundant in the anthroponotic sites of Ouled Aid with 119 specimens/m^2/night as a maximal density as compared to the wildness of Zliligh site where their maximum density does not exceed 36.01 specimens/m^2/night (Figure 1a and b) this result confirms the work of Guernaoui et al. (2005), Izri et al. (2006) and Dujardin (2006) which show that anthropic sites modified by rural human activities are favorable for the development of sand flies.

The activity of phlebotomine fauna appears half year from April to November which corresponds to the dry season when there are no rainfalls but when the weather gets hot and dry. The data analysis has shown a bimodal pattern for both stations. The fauna had reached its pick in June then in September in Ouled Aid station (Figure 1a), and in June and August in Zliligh station (Figure 1b). Those season variations should be related to many different environmental factors, especially meteorologically. In countries where dry seasons last a long rigorous time (tropical zones), the phlebotomies are rare in contrast with regions

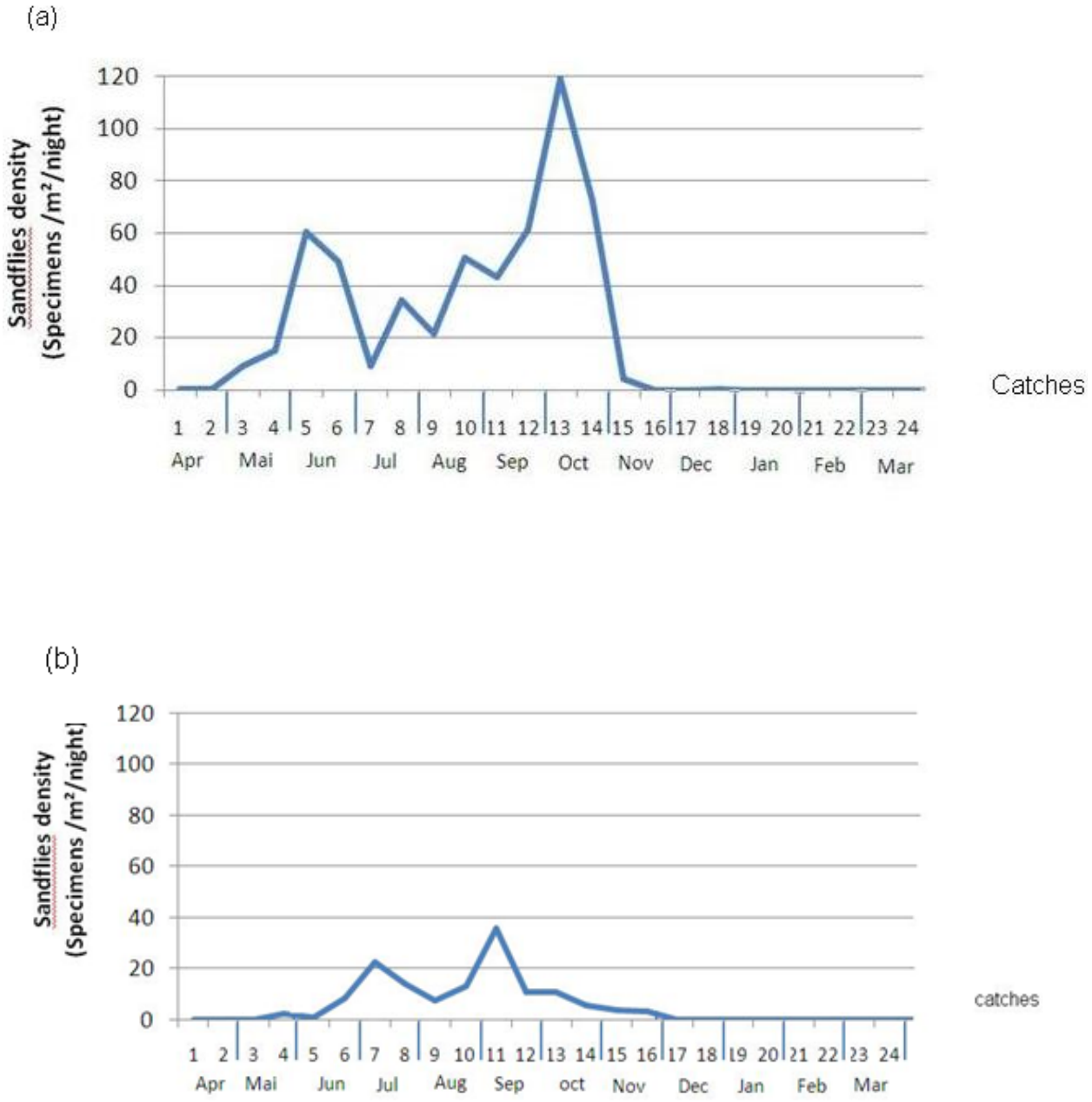

Figure 1. Evolution of the sand flies density (sticky traps) from April 2011 to March 2012 in Ouled Aid (a) and Zliligh (b). Phlebotomine's collection was carried out twice a month during one year. The traps were placed from 18 pm to 8 am. The specimens were reserved in ethanol 95%.The results show the density of sandflies captured each month for each study station.

where humidity and weather stay unchanged the whole year; infestation risks are then high (Abonnenc, 1972; Niang AA University of Cheikh Anta Diop, Sénégal, thesis 1992; Ba et al., 1998), but the dynamic and the activity period could be different even under the same climate. At Chichaoua, south of Morocco, the activity period is short and spreads from June to November in an arid climate (Guernaoui et al., 2005), while in Marrakech, the sand flies was active the whole year under the same climate (Boussaa et al., 2005). These results could be explained by the adaptation of the population to the climate. The length and activity period and the phlebotomies density are highly conditioned by climatic changes (Galvez et al., 2010).

Correlation studies have shown that the activity period and the phlebotomies density are highly determined by the climatic conditions of the site. Temperature and humidity are the main factors for the nature and proliferation

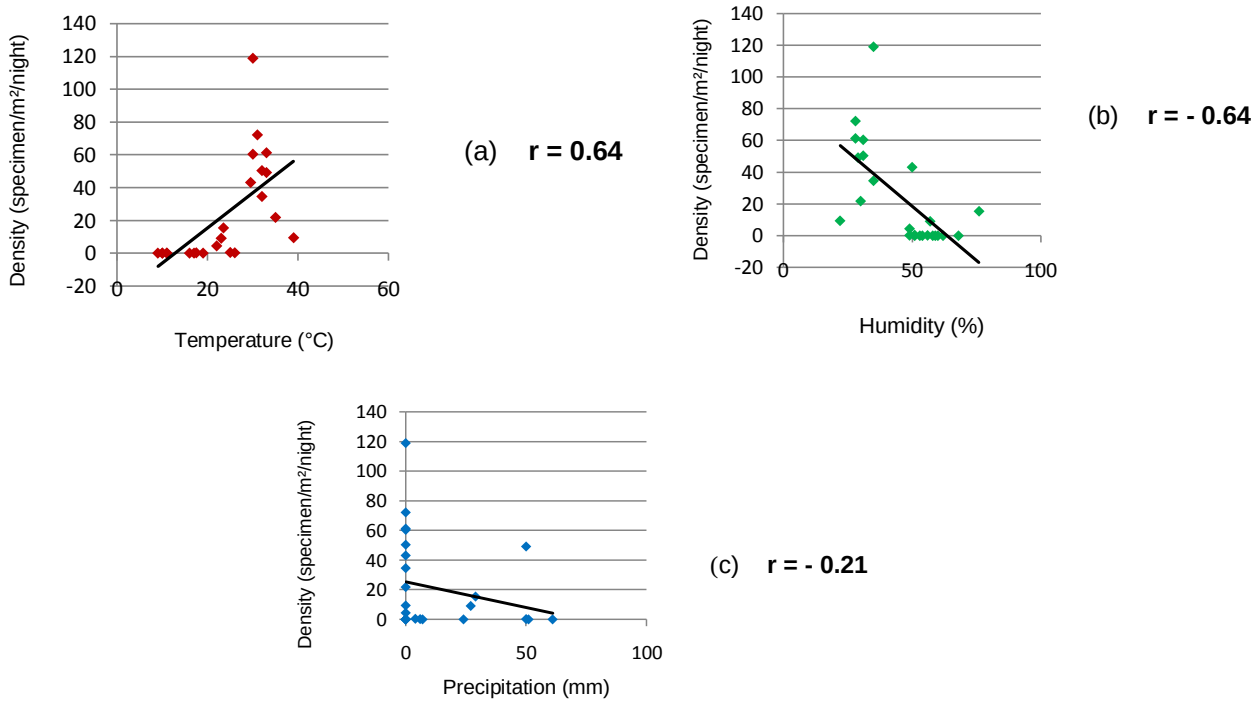

Figure 2. Correlation between the density of sandflies, in Ouled Aid, and temperature (a), humidity (b) and precipitation (c). Temperature (°C) and humidity (%) are measured at the time of laying and retrieving traps in both study stations. Precipitation is obtained from the Agency hydraulics Sebou (AHBS) basin. The results show the correlation between the density of sandflies, in Ouled Aid.

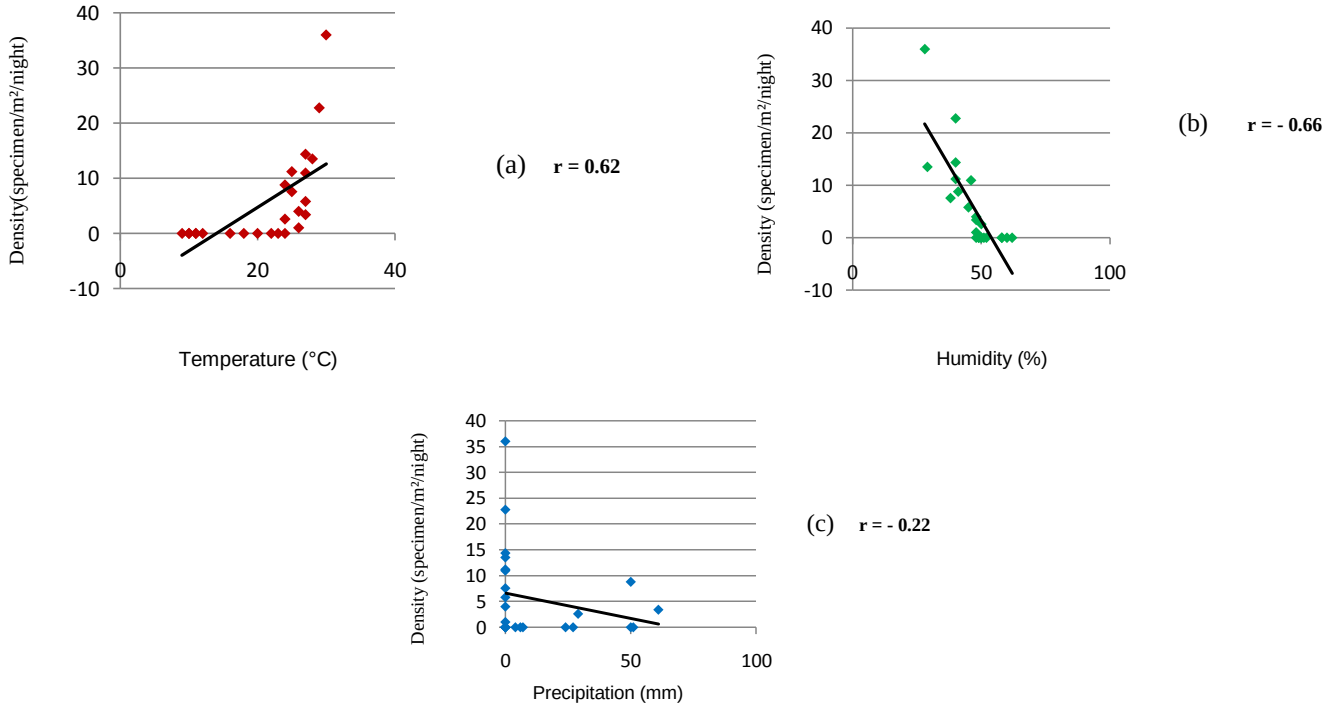

Figure 3. Correlation between the density of sandflies, in Zlilig, and temperature (a), humidity (b) and precipitation (c). Temperature (°C) and humidity (%) are measured at the time of laying and retrieving of traps in in Zlilig. Precipitation is obtained from the Agency hydraulics Sebou (AHBS) basin. The results show the correlation between the density of sand flies, in Zlilig.

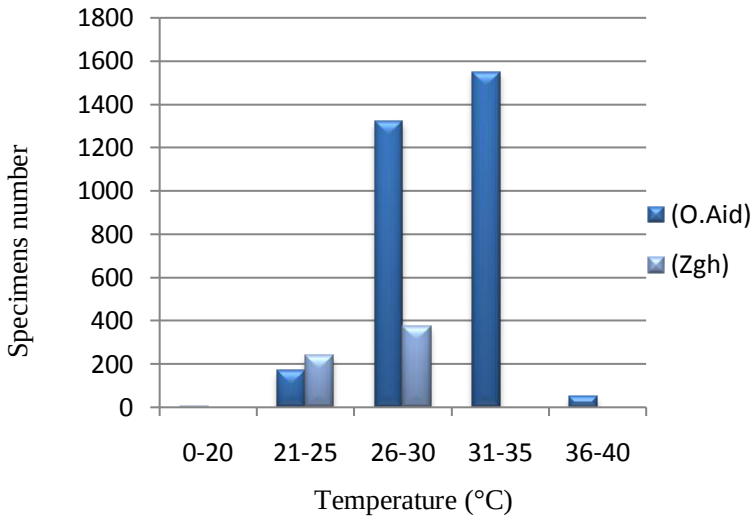

Figure 4. Number of specimens in the different temperature ranges in the two sites. The temperature is measured for every capture. Different ranges of temperature are formed and the specimens are counted for every range.

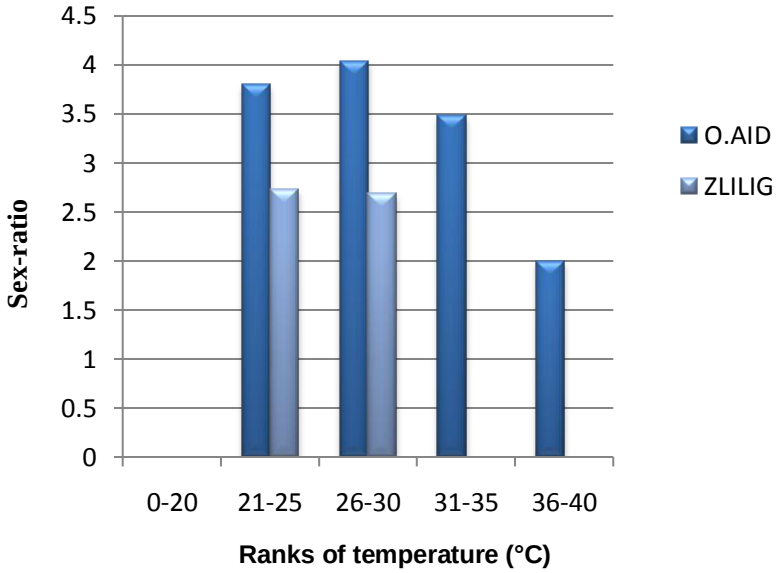

Figure 5. The sex-ratio in the different temperature ranges in the two sites. The temperature was measured for every capture. Different ranges of temperature are seen. The specimens are counted for every range of temperature and the sex-ratio is calculated.

of the disease.

Sand fly density in Ouled Aid station reaches its maximum when the temperature is between 30 and 35°C. Sand flies population vanishes when the temperature exceeds 35°C. In Zliligh site, the maximum density is obtained at a temperature between 21 and 25°C (Figure 4). The phlebotomies sex-ratio shows a net predominance of the male population in both sites (Figure 5). It seems thus that temperature affects greatly the development of the insects. Females are known to be more sensitive (r = 0.7) than males (r = 0.62).

A significant negative correlation (r = -0.64 (Ouled Aid) ; r = -0,66 (Zlilig)) was found between the density of sand flies and humidity. Density peaked in Ouled Aid when the humidity was between 30 and 40%, and decreased when the humidity exceeded 40% from November to March. In

Figure 6. Animal droppings (a, b, c).and junk (d).

Zlilig, the optimum humidity for the proliferation of sand flies hover around 30%. The density difference in the two stations could be explained by the presence of different species each having its specific survival climatic conditions (Table 2).

The dominance of *P. papatasi at* Ouled Aid (vector of *Leishmania major* (Rioux et al., 1986c), by 49.7% of *Phlebotomus* genus (45% in total) confirms its wide distribution in the region as well as its adaptation to different environments and tolerance to the anthropic environment and high temperature (Cross et al., 1996).

Ouled Aid station reflects Moulay Yacoub region's rural area, most of its inhabitants live in shanty towns and Douars, the houses are built with argil and straw. Animal droppings (Figure 6a, b and c) and junk are accumulated in open areas without any control (Figure 6d). This offers well adapted focal outbreaks for the proliferation of the insects larvae, rodents and dogs reservoirs of *Leishmania*.

The role of clay houses and manure in the proliferation of sandflies has been reported by Guessous et al. (1997) Razmjou et al. (2009) and Zougaghi et al. (2011).

The dominance of *P. longicuspis* (vector of *L. infantum) at* Zlilig station can be explained by the effect of the studied habitat (a cave) characterized by a moderate temperature and humidity. *P. longicuspis* is a vector of *L. infantum* which explains the case of visceral leishmaniasis in this site declared in 2001 (Prefecture's Services for Environment Hygiene, a Delegation of Ministry of Health - Province of Moulay Yacoub).

Conclusion

This study has allowed us to determine the phlebotomine sand flies activity periods in the province of Moulay Yacoub, to understand the existing relationship between their density and meteorological factors in that region. The results can contribute to the planning of preventive measures against leishmaniasis and determine the intervention periods to fight responsible vectors.

The high sand flies density shows that the study zone presents a leishmaniasis foyer which means that there is a high risk for Moulay Yacoub and its immediate vicinities like Fez.

The period presenting a higher risk of proliferation starts from April to November, the hot season ends with the downpours and diminution of the temperature. Moroccan climate changes, the extension of the rural area changes and non hygienic houses sharpen the risks. This situation demands a nonstop surveillance in terms of time and space to prevent the leishmaniasis risks.

The destruction of vectors' habitats, the improvement of human habitats, the treatment of scraps, animal droppings, dung and the separation between animal and human dwellings could be an efficient way to get rid of sand flies and consequently diminish the risks of contamination.

Teaching local population through campaigns of Information-Education-Communication is the best way to achieve these goals.

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