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Original Research Article

Influence of Basic Environmental Factors on Seasonal Variation and Distribution of Sand Flies at Ben Slimane Sites in Fez City, Morocco

Fatima Zahra Talbi[1]*, Abdelilah Merabti[2], Fouad El-Akhal[3], Abdellatif Alami[4], Hajar El Omari[5], Mohamed Najy[6], Rachid Amaiach[2], Meryem Mrani Alaoui[7], Amal Taroq[8], Fatiha El Khayyat[6], Moussa Benboubker[9], Said Hilali[1], Abdelkarim Taam[10], Abdelhakim El Ouali Lalami[2]

[1]*Hassan First University of Settat, Faculty of Sciences and Technologies, Laboratory of Biochemistry,Neurosciences, Natural Resources and Environment, P.O. Box 577, 26000 Settat, Morocco*
[2]*Higher Institute of Nursing Professions and Health Techniques of Fez, Regional Health Directorate Fez-Meknes, EL Ghassani Hospital, 30000 Fez, Morocco*
[3]*HigherInstitute of Nursing Professions and Health Techniques of Tetouan (annex Al Hoceima), Regional Health Directorate, Mohammed V Hospital, Al Hoceima, 32000, Morocco*
[4]*Laboratory of Applied Organic Chemistry, Faculty of Science and Technology, Sidi Mohamed Ben Abdellah University, Fez, Morocco*
[5]*Ministry of National Education, Early Education, and Athletics, Regional Academy of Education and Formation of Fez-Meknes, Qualifying Secondary School Teacher, Moulay Ismail High School, Meknes, Morocco*
[6]*Natural Resources and Sustainable Development Laboratory, Faculty of Sciences, Ibn Tofail University, BP133, 14000, Kenitra, Morocco*
[7]*Laboratory of Biochemistry, Environment and Agrifood, URAC36, Faculty of Sciences and Technologies, Hassan II University Casablanca, BP 146, 20650, Mohammedia, Morocco*
[8]*Laboratory of Natural Substances, Pharmacology, Environment, Modilesation, Health and Quality of Life, Faculty of Sciences Dhar Mahraz, University Sidi Mohamed Ben Abdellah, Fez, Morocco*
[9]*Laboratory of Human Pathology, Biomedicine and Environment, Faculty of Medicine and Pharmacy, Sidi Mohammed Ben Abdellah University, Fez, Morocco*
[10]*Laboratory of Engineering Sciences, National School of Applied Sciences, Ibn Tofail University, Morocco*

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ABSTRACT

Cases of cutaneous leishmaniasis are reported yearly in Fez, Morocco, although the inventory of its responsible mechanical vectors remains undocumented. The present study was aimed at investigating the impact of environmental conditions on the seasonal variation and dispersion of sand flies in Ben Slimane sites in Fez City, Morocco. Samplings were conducted every fortnight from March to September 2009 in five selected sites in Ben Slimane, Morocco. The collected sand fly samples were preserved and identified. Also, monthly temperature and relative humidity data were obtained from each site. All the data were analyzed statistically. A total of 13,081 sand flies of the *Phlebotomus* genus namely,*P. perniciosus* (52.58%), *P. sergenti* (30.14%), and *P. longicuspis* (17.29%) were recovered. The seasonal activity of the total captured sand flies reached a bimodal evolution for the three species collected. Its peak was in June (43.47%). *Phlebotomus perniciosus* was found to be the most abundant with two peaks, the first was in June (30.43%) and the second was in August (22.42%). Sand fly activity temperatures ranged between 23 and 30°C in Ben Slimane sites, with relative humidity ranging from 30 to 70%. The interaction between numerous climatic factors indicated that the period of activity and abundance of sand flies are substantially influenced by the study site's climatic conditions. Sand flies become more abundant as the temperature rises. The findings will help better understand the dynamics of leishmaniasis transmission in Fez and will contribute to the design of a surveillance strategy.

***Keywords*::** Fez, Ben Slimane, Monitoring, Morocco, Sand fly, *Phlebotomus*, Temperature.

Introduction

Sand flies are minute dipterans in the family Psychodidae that transmit the causative agents of leishmaniasis.[1] Worldwide, leishmaniasis affects 88 countries, of which 66 are in Africa, Asia, and Europe, while the other 22 are on the American continent.[2]

*Corresponding author. E mail: fatimazahra.talbi@uhp.ac.ma; eloualilalami@yahoo.fr
Tel: +212671543020

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About 12 million people are affected by the disease, with about two million new cases reported yearly, three-quarters of which are cutaneous and one-quarter visceral infections.[3,4] Morocco is an endemic country for cutaneous leishmaniasis with alarming epidemiology. According to the data on parasitic diseases of the Directorate of Epidemiology and Disease Control under the Ministry of Health, 23,008 cases were reported between 1997 and 2007.[5] The causative parasite is transmitted by sand flies, especially those in the genus *Phlebotomus*. Zoonotic cutaneous leishmaniasis is caused by *Leishmania major* and transmitted by *Phlebotomus papatasi*.[6] Anthroponotic cutaneous leishmaniasis (ACL), caused by *L. tropica* is transmitted by *P. sergenti*.[6] Also, cutaneous and visceral leishmaniasis can be caused by *L. infantum* found in domestic dogs and humans and is transmitted by *Phlebotomus* species of subgenus *Larrossius*.[7]
In Morocco, there are 22 species of sand flies, 13 of which are *Phlebotomus* and 9 of which are Sergentomyia.[8] Five of these species are known to transmit parasites, whereas three, namely *Phlebotomus ariasi*, *Phlebotomus perniciosus*, and *Phlebotomus longicuspus* are

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responsible for visceral leishmaniasis including the pathogenic parasite of *L. infantum*, which is prevalent in northern Morocco.[9]*Phlebotomus papatasi* has been implicated for cutaneous leishmaniasis with the major parasite *Leishmania*, as observed in the south and southeast of the Atlas Mountain ranges. The *P. sergenti* is the vector of cutaneous leishmaniasis caused by the tropical *Leishmania* parasite in the central region of the country.[10] In retrospect, numerous cases of cutaneous leishmaniasis were documented in the city of Fez in central Morocco between 2000 and 2008 (unpublished data), with the Ben Slimane Health Center being considered as the most affected location, with an occurrence of 39.4% of 59 cases. Despite efforts to suppress the disease from 2002 to 2004, the incidence of the disease remained high from 2005 to 2008.[11] This observed pattern prompted health officials to evaluate the extent of the outbreak in 2009, with a focus on Fez, a city in central Morocco, by questioning key factors influencing the proliferation of sand flies.

This study was conducted to investigate the influence of environmental factors on the seasonal variation and distribution of sand flies in Ben Slimane sites located in Fez City, Morocco.

Materials and Methods

Study sites

The study sites were in Fez, a city in Morocco renowned for its sand fly population. The survey was conducted at five different locations in the city (Figure 1). The sites are prospective high-risk areas with known epidemiological records.[11] Also, there is the existence of caves, rodent burrows, and unmanaged waste sites, all of which are potential biotopes for leishmaniasis vectors and reservoirs. Table 1 contains further information on all of the sites.

Figure 1: Types of studied sand fly sites at Ben Slimane, Fez City, Morocco.

Figure 2: Installation of sticky traps.

Sampling, preservation, and identification of sand fly samples

Samplings were conducted every fortnight from March to September 2009, using sticky traps. Each site was sampled for two nights per month with 80 sticky traps made with paper placed at fixed interior stations for 12 h from dusk to dawn. The sticky traps were made from white sheets (A4 size; Figure 2) that were saturated with purified castor oil,[12] and placed in the cavities (entry terriers, caves, soil cracks, holes, rocks, and crevices of trees). The combined morning sand fly counts forthe period yielded a density estimate, and the mean number of sand flies per-paper-per night, was counted and collated.

Captured sand flies were removed from the oil-impregnated paper using a fine brush dipped largely in 75% alcohol and placed in properly labeled collection tubes (per station).The collected specimens were identified using an identification key.[5] The females were differentiated from the males using their spermathecae, ciborium, and posterior pharynx, as described by the Regional Diagnostic Laboratory of Epidemiological and Environmental Health in Fez, Morocco.

Collection of data on climatic conditions

Temperature and relative humidity data were collected month-to-month from each site using a thermo-hygrometer and a Brannan-type trap.

Statistical analysis

Researchers can determine the relative abundance of sand flies by evaluating the degrees of dependence between the various parameters studied. Statistical analysis was conducted using the correlation coefficients determined by statistical calculations (XLSTAT Trial version). Thus, by using the principal component analysis (PCA), correlations were established between the studied parameters taken, two by two. This may be termed a data mining method because it allows for the easy extraction of information from large sets of data, which enables the generation of binary correlation diagrams for interpretation.

Results and Discussion

Distribution of sand flies in the studied sites

Entomological investigations were carried out in five stations of Ben Slimane in Fez City. In this survey, a total of 13,081 individual insects were collected, belonging to 23 species in the genus *Phlebotomus* (Table 2). Recalling that the site is constituted by an uncontrolled waste of all kinds: stables, caves, and one permanent larval site rich in *Culicidae* species also.[13] The existence of a large number of sand flies can be explained by the bad hygienic conditions in the analyzed areas. Morocco is an endemic country, with cutaneous leishmaniasis foci situated in the arid and semi-arid areas of the southern and eastern Atlas Mountains, specifically from the province of Tata in the south to the province of Jrada in the east via Zagora, Ouarzazate, Errachidia, and Figuig. Dry cutaneous leishmaniasis outbreaks occur throughout the central region of the country, including Essaouira, Azilal, Taza, Zouagha Moulay Yacoub, and Chichaoua. Visceral leishmaniasis is observed as sporadic cases in the rural mountainous areas of the north of the country.[14]These were reported to be the most abundant of the 23 species found in Morocco.[5,15]In the western Mediterranean Sea, the species *P. perniciosus, P. sergenti*, and *P. longicuspis* can be found. The geographic distribution extends from the northern edge of the Sahara to the southern Iberian Peninsula.[16]

Table 1: Characteristics of sand fly study sites

Station	Altitude	Latitude	Longitude	Type of biotope	Type of pollution	Surface (m^2)
S1	417 m	34° 04’ 20,66’’N	4° 59’ 06,24’’W	Stable	Domestic wastes and animals	70
S2	405 m	34° 04’ 17,93’’ N	4° 58’ 57,76’’W	Human habitation	Domestic wastes	90
S3	402 m	34° 04’ 19,55’’ N	4°58’ 58,31’’W	Stable	Domestic wastes	30
S4	415 m	34° 04’ 23,74’’ N	4°59’ 11,67’’W	Stable	Domestic wastes and animals	45
S5	401 m	34° 04’ 03,24’’ N	4°58’ 58,31’’W	Stable	Domestic wastes and animals	500

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Phlebotomus longicuspis is a ubiquitous sand fly in Morocco. It has been found in 50 scattered stations at different geographical and bioclimatic levels. In contrast, Gaud and Laurent,[17] reported that the two sand fly species, *P. longicuspis* and *P. perniciosus* were found to be associated in Rabat, but only at low density. The results of Bailly-Choumara et al.,[18] showed that *P. longicuspis* was distributed in the different bioclimatic stages (humid, semi-arid, arid, and Saharan) and all geographical regions (the Rif, plains and hills, North Atlantic, Central Plateau, Middle Atlantic plains and plateaus, Middle Atlas, High Atlas, Anti-Atlas, Eastern Morocco, South Atlantic, and Southern Interior).In a study of sand flies conducted in 2006 in the Moroccan Atlas, Guernaoui et al.,[19] showed that *P. longicuspis* preferentially lives between 600 and 800 m altitude. It was observed that the altitude recorded in the Ben Slimane outbreak was between 401 and 417 m. The Health Epidemiology Department (Regional Health Observatory) of the Regional Health Directorate in Fez reported nine cases of VL in 2008 (unpublished data). This is consistent with the results of the present study of the presence of the vector *P. longicuspis* responsible for the transmission of this disease. *Phlebotomus sergenti* is the proven vector of *Leishmania tropica* in Morocco. This species is found throughout the country,[18] but it is most common in arid and semi-arid areas,[20] and it has a high genetic diversity. Similar to other countries in the Mediterranean basin, this species persists from June, which corresponds to the humidity of the dry season, to September, with a considerable peak of activity. These results are in agreement with those obtained by Guessous- Idrissi et al.,[21] in the province of Taza, east of the province of Sidi Kacem, and with those obtained by El Miri in 2013.[22] Other studies along the same lines can be found in the provinces of Sefrou and Meknes, respectively, by Talbi et al.,[23] and El Omari et al.[24]*Phlebotomus perniciosus* is an abundant species in the whole Mediterranean basin. In North Africa, it is mostly localized in the northernmost part, although it has also been reported in the central Sahara, where the climate is sub-humid to semi-arid.[18,25]

Sand fly seasonal variation in Ben Slimane sites

Figure 3 depicts the seasonal fluctuation of different species captured at different sites of Ben Slimane. From May to September 2009, the observed species of the sand fly were *P. perniciosus* (52.58%), *P. sergenti* (30.14%), and *P. longicuspis* (17.29%). The seasonal activity reaches a bimodal evolution for the three species collected. Its peak from June to September 2009 was 4.34% in June, but it rose to 43.47% in August and 26.08% in September. *Phlebotomus perniciosus* was found to be the most abundant with two peaks, the first was in June (30.43%) and the second was in August (22.42%). *Phlebotomus* sergenti, which ranked second in abundance, had the first peak in June (13.04%) but the second peak in September (4.05%). In May (4.34%) and July (12.95%), *P. longicuspis* was present with a diphasic evolution. These results are in agreement with those obtained by El Aasri in Sidi Yahya in 2013,[26] and Talbi in Sefrou province.[25] Outside this period, the density of *P. perniciosus* is low and eventually zero under the effect of climatic factors. The presence of this species is important from an epidemiological point of view, as it has been implicated in the transmission of VL.

Effect of climatic factors on the sand fly distribution

The variation of different parameters (temperature and humidity) that affect the geographical distribution of sand fly insects during the study period is shown in Figures 4 and 5. Studies of the relationship between different climatic factors have shown that the period of activity and abundance of sand flies are strongly determined by the climatic conditions (relative humidity and temperature) in the present study site. Sand flies were abundant in the Ben Slimane area when the temperature was between 23 and 30°C, but they disappeared when the temperature was below 23°C (Figure 4). Their populations peaked when relative humidity reached 65% (Figure 5). Sand fly abundance is influenced by climatic conditions, as peaks were reported in June, July, and August for all three species when these two criteria are combined. According to studies carried out in the central region of Morocco,[27-30] climatic factors such as temperature (between 16 and 30°C), humidity (56 to 82%), and poor hygiene (existence of caves, rodent burrows, and uncontrolled dumps) contribute to sand fly population proliferation.

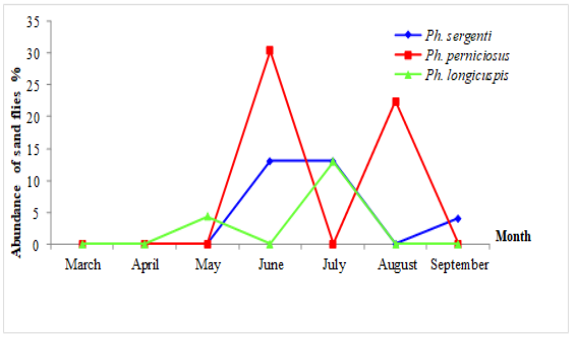

Figure 3: Seasonal variation of sand flies collected in Aichoun by sticky paper traps.

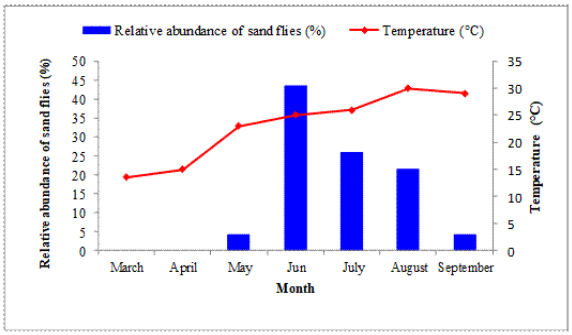

Figure 4: Abundance of sand fly fauna and variation of the monthly temperature in the study area from March to September 2009.

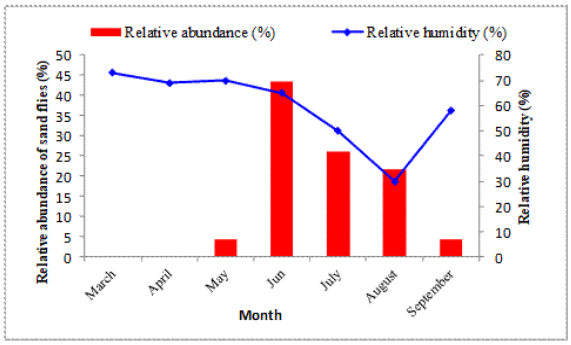

Figure 5: Abundance of sand fly fauna and variation of the monthly relative humidity in the study area from March to September 2009.

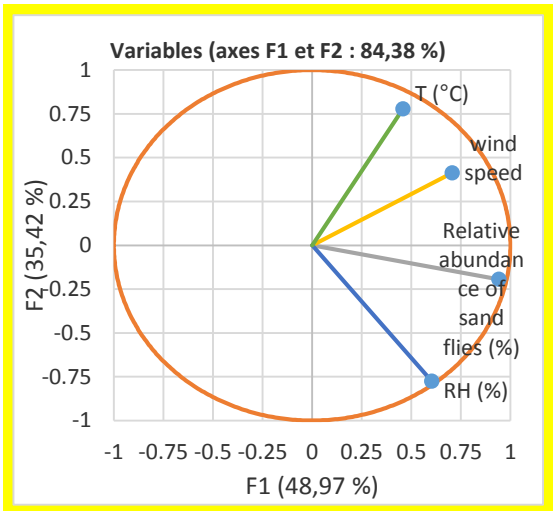

Figure 6: Distribution of variables on the factorial plane (F1 and F2).

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This result is consistent with that obtained in Ben Slimane, while the recorded temperature (between 23 and 30°C) and relative humidity (30 to 70%) constitute the climatic zone favorable to the development of sand flies. The results agree with those obtained in the same region of Fez Meknes,[24,31,] especially in the Province of Sefrou.[23,29,30]It was confirmed that the temperature parameter is strongly correlated with the distribution of the seven species of sand flies captured in the Aichoun locality. On the other hand, relative humidity and precipitation do not have a positive effect on the existence and abundance of these species; rather, the correlation is strongly negative.[23,29,30] The impact of climatic factors on the abundance of this fauna was researched on another biotope in Meknes prefecture, revealing a strong positive correlation between temperature and the different species of phlebotomine, and a negative correlation between humidity and phlebotomine activity was also confirmed.[24]

Correlations between the studied parameters

According to Table 3, the majority of correlation coefficients are positive, indicating a direct and significant relationship between the above variables in the study area. Values in bold indicate the correlation coefficients of variables that are highly correlated with each other. This is true for all correlation matrices. There is a strong and positive correlation between relative sand fly abundance (%) and relative humidity (%) with r = 0.69, and a medium correlation between relative sand fly abundance (%) and wind speed with r = 0.68. On the factorial axes F1-F2 (Figure 6), which absorb 84.38 % of the total variance, half of the variables are positively correlated to the F1 axis (48.97 %), the F2 axis is characterized by the RH % and the temperature (T) (well positively correlated). These two variables are differentiated from the others. Figure 7 depicts the distribution of the factorial plane of variables and individuals (F1, F2), which shows that on-axis F2 is distinguished by the months (September and August), while on-axis F1, passing from left to right, the months' June and July are controlled by the relative abundance of sand flies, indicating that as the temperature rises, the relative abundance of sand flies rises as well.

Given that there is presently no vaccine or preventative medicine for leishmaniasis, the fight against this disease is divided into two aspects. The first is based on the control of wild or domestic reservoirs of the parasite. When humans are the reservoir, control consists of giving a diagnosis and a treatment to the patients and making them aware of the means of protection. In the second aspect, an awareness that the fight against the vector requires a good mastery of the biology and ecology of the vector and the circulating species to guarantee the effectiveness of any action. However, such controls will be ineffective and inefficient unless entomological surveillance of sand flies is established and extended to other areas of Fez. It is also necessary to raise awareness among the population and local authorities about the potential of leishmaniasis and the role of hygiene in the fight against this disease.

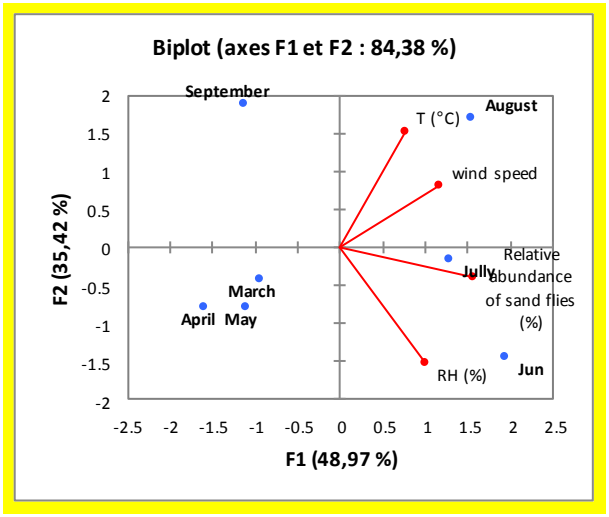

Figure 7: Distribution of variables and individuals at the factorial level

Table 2: Composition of sand fly species

Subgenus	Species	M	F	Ratio sex	Total	Relative abundance
Larroussisus	*P. longicuspis*	1800	461	3,9	2261	17,29
	P. perniciosus	4650	2227	2,08	6877	52,58
Paraphlebotomus	*P. sergenti*	2863	1080	2,65	3943	30,14

Table 3: Correlation matrix

Variable	RA (%)	Ws	RH (%)	T (°C)
Relative abundance of sand flies (%)	1	0.468	0.693	0.337
Wind speed	0.468	1	0.077	0.414
RH (%)	0.693	0.077	1	-0.283
T (°C)	0.337	0.414	-0.283	1

RA: Relative abundance; Ws: Wind speed; RH: Relative humidity (%); T: Temperature

Conclusion

The findings of this study reveal the presence of three species of the genus *Phlebotomus: P. perniciosus*, *P. sergenti*, and *P. longucuspis* in the study site. *Phlebotomus perniciosus* was the most abundant species (52.58%), followed by *P. sergenti* (30.14%), and *P. longucuspis* (17.29%). Prospection of the sites investigated in the Ben Slimane outbreaks also revealed that sand fly development conditions are most prevalent in May and June, which corresponds to the start of the vectorial activity period, as reported in the literature. The major recommendation of this entomological study is the establishment of a monitoring system (design of data sheets of outbreaks and a survey calendar) for sand fly entomology in both time and location. This is a critical control measure in the fight against disease vectors, particularly the sand flies that transmit leishmaniasis.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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