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Research Article

Entomological Survey and Impact of Climatic Factors on the Dynamics of Sandflies in Central Morocco

Hajar El Omari,[1] **Abdelkader Chahlaoui**,[1] **Fatima Zahra Talbi**,[2,3] **Amina Chlouchi**,[4] **Fouad El-Akhal**,[5] **Khadija Lahouiti**,[6] **Amal Taroq**,[7] **Meryem Mrani Alaoui**,[8] **and Abdelhakim El Ouali Lalami**[9]

[1]*Natural Resources Management and Development Team, Laboratory of Health and Environment, Faculty of Sciences, Moulay Ismail University, Meknes, Morocco*
[2]*Hassan First University of Settat, Faculty of Sciences and Technologies, Laboratory of Biochemistry, Neurosciences, Natural Resources and Environment, P. O. Box 577, Settat 26000, Morocco*
[3]*Sidi Mohamed Ben Abdellah University, Faculty of Sciences Dhar El Mahraz, Laboratory of Biotechnology, Conservation and Valorization of Naturals Resources (LBCVNR), Fez 30000, Morocco*
[4]*Laboratoire Chimie Organique Catalyse et Environnement, Ibn Tofail University, Kenitra, Morocco*
[5]*Higher Institute of Nursing Professions and Health Techniques of Tetouan (Annex Al Hoceima), Regional Health Directorate, Mohammed V Hospital, Al Haceima 32000, Morocco*
[6]*Laboratory of Microbial Biotechnology, Department of Biology, Faculty of Sciences and Technology, University Sidi Mohamed Ben Abdellah, BP 2202, Road of Immouzer, Fez, Morocco*
[7]*Laboratory of Natural Substances, Pharmacology Environment, Modilesation, Health and Quality of Life, Faculty of Sciences Dhar Mahraz, University Sidi Mohamed Ben Abdellah, Fez, Morocco*
[8]*Laboratory of Biochemistry, Environment and Agrifood, URAC36, Faculty of Sciences and Technologies, Hassan II University Casablanca, BP 146, 20650 Mohammedia, Morocco*
[9]*Higher Institute of Nursing Professions and Health Techniques Fez, Regional Health Directorate, El Ghassani Hospital, Fez, Morocco*

Correspondence should be addressed to Hajar El Omari; elomari.hajar.gie@gmail.com

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Sandflies are small insects belonging to the order Diptera, which make up the Phlebotominae subfamily within the Psychodidae family. In the fight against leishmaniasis in the prefecture of Meknes, Morocco, a study of the phlebotomist population was carried out during the period of activity of sandflies while studying the link between the abundance of these insects and climatic factors, namely, temperature, moisture, rainfall, and wind speed. A total of 958 phlebotomus were captured, 73% of which belong to *Phlebotomus sergenti*, a vector of *Leishmania* species causing human cutaneous leishmaniasis. This study also showed the existence of two months of risk in July and September with a positive correlation between temperature and abundance of sandflies and a negative correlation between abundance of sandflies and rainfall and moisture. Indeed, it is necessary to strengthen the means to fight this disease during periods of activity and to use medium-term climatological forecasts to develop an alert system for leishmaniasis.

1. Introduction

Sandflies are small, light-colored hematophagous insects [1, 2] that are vectors of protozoa causing clinical parasitic disease with a global prevalence estimated at 12 million human cases, an annual incidence of 1.5 to 2 million new cases, and 350 million people at risk [3]. Phlebotomus are considered exclusive vectors of visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL) [4], which are endemic in Morocco [5, 6]. In Morocco, the average annual incidence rate was 0.4 cases of VL per 100,000 inhabitants between 1990 and 2014 [7].

However, for cutaneous leishmaniasis, more than 3,700 cases are reported annually, with two peaks in 2010 and 2018 with, respectively, 8,707 and 9,700 cases [8]. Cutaneous leishmaniasis is widely distributed in three nosogeographic entities across Morocco: zoonotic cutaneous leishmaniasis (ZCL; caused by *L. major*); localized in arid regions along the northern edge of the Sahara desert and transmitted by *Ph. papatasi* (Scopoli), anthroponotic cutaneous leishmaniasis (ACL; caused by *L. tropica*); localized in the semiarid regions of central and southwestern Morocco and transmitted by *Ph. sergenti* Parrot [9] and LC caused by *L. infantum* in the northern regions of the country [10, 11] whose vector is *Ph. ariasi*.

Phlebotomus have a nocturnal and twilight activity that varies according to the temperature [12–14]. Some species of adult sandflies require closely related ecological niches in human and animal domestic dwellings that provide them with optimal shelter. Different biotic and abiotic factors can affect the seasonality of sandflies [15–17]. The habitat of sandflies is conditioned by three needs: a source of blood for adult females, food for larvae, and a suitable habitat. In general, sandflies are insects that are very sensitive to meteorological and climatic variations [18, 19].

Epidemiological interest in sandflies is due to the vector role of certain species in the transmission of infectious agents that cause human and animal diseases [20, 21]. These species can be the cause of allergic reactions and nuisances because of their perpetual pitting, but they are more dangerous in the transmission of first-line pathogens such as parasites of Leishmania. They also transmit the sandfly fever virus. Although a number of entomological studies have been carried out in Morocco [22–24], very few studies have used the seasonal fluctuation of sandflies with climatic factors to study the effect of climatic factors on the distribution of sandflies [25].

It is with this in mind that this study aims to carry out an inventory of sandflies in the prefecture of Meknes and study the impact of certain climatic factors on their seasonal fluctuation.

2. Materials and Methods

2.1. Study Area. The prefecture of Meknes is located in the Fes-Meknes region extending over an area of approximately 1786 square kilometers. The study stations represent the prospected environment in which the sites or the biotopes favorable to the development of sandflies are located (laying and/or resting environment). The study stations are chosen according to their environmental factors and epidemiological histories (Figure 1).

The study stations are mainly barns, but there are also human habitations and caves (Table 1). The different biotopes were chosen according to their proximity to the population, their epidemiological history, and the level of risk [25]. Indeed, the regions most affected by this disease in our study area are the two rural communes of Dkhissa and Walili (S2 and S1) and the urban sectors of Ras Aghil, Sidi Amer, and Ouislane (S3 S4 S5), with a high incidence in 2012 of the order of 65.1; 59.1; 23.64; and 9.49, respectively. As far as station S6 is concerned, it contains caves and spaces used for grazing, which is why we chose it despite the high incidence that was recorded at 6.61.

2.2. Data Processing

2.2.1. Sampling of Sandflies. To achieve our goals, we used adhesive traps. This is a nonselective technique suitable for the qualitative and quantitative inventory of sandflies in the Mediterranean region [26, 27]. Impregnated with castor oil, the adhesive traps were placed either in cones or inserted in the wall. The study stations represent environments conducive to the development of sandflies. They are essentially barns made of human dwellings and caves. Six different biotopes distributed in the municipality of Meknes (Table 1) were chosen according to environmental factors and especially the epidemiological history of the localities [28].

Forty-eight traps were placed in the evening (eight traps for each site), and they were retrieved the next morning to avoid deterioration of the sandflies. Two monthly trapping sessions of sandflies were conducted (separated by 15 days) using the adhesive traps during the activity period of sandflies which ran from April to November 2017.

2.2.2. Identification of Sandflies. The captured insects are placed for about 48 hours at 95% alcohol to completely solubilize the oil and then kept in plastic tubes containing 70% ethyl alcohol and bearing the date and name of the station. The captured specimens are subjected to a separation of the sexes which is essentially based on the genital organ: the genital organs are elongated in males and simple in females (internal genital organs). The male genital frame, spermathecae, and the oral frame of the female vary in terms of morphology and are used in the identification and classification of species. Differentiation between males of *Ph. perniciosus* and *Ph. longicuspis* has been made by examining both the shape of the copulatory valves and the number of coxite hairs. The identification of species was performed under the microscope using the determination key established by the Ministry of Health guide [29].

2.2.3. Recording Climatic Factors. To determine the relationship between climatic factors (including temperature, moisture, rainfall, and wind speed) and vector dynamics, meteorological data were obtained from the Agropolis meteorological station in Meknes.

2.3. Statistical Analysis of the Data. To determine the relationship between climatic factors and sandfly dynamics, we used Pearson's correlation coefficient which gives us information on the existence of a linear relation between the two quantities considered:

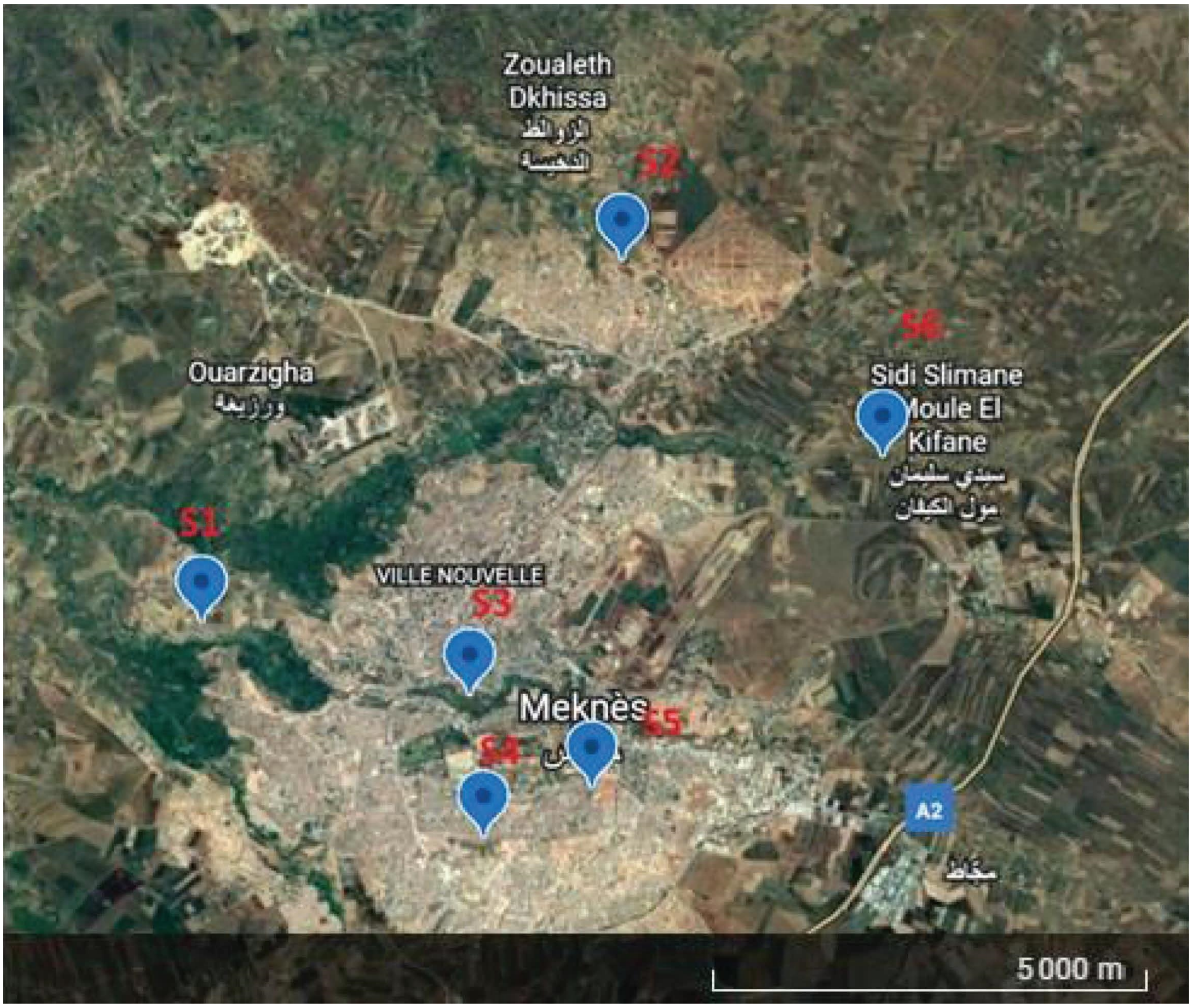

Figure 1: Study stations.

Table 1: Description of study stations.

Stations	Geographical coordinates	Description
S1	33,8936347, −5,5635208	Anarchic neighbourhood that contains barns and stables (dozens of sheep, less than ten cows and horses)
S2	33,9144661, −5,4754414	Barns not far from the population (dozens of sheep, less than ten cows)
S3	33,8468291, −5,5361417	Human dwellings containing barns (dozens of sheep, less than ten cows)
S4	33,8872592, −5,5349912	Human dwellings containing barns (dozens of sheep, less than ten cows)
S5	33,8516269, −5,4813507	Human dwellings containing uncontrolled discharge
S6	33,8681401, −5,4693706	Caves close to land used for grazing

$$r = \frac{\sum (X - \overline{X}).(Y - \overline{Y})}{\sqrt{\sum (X - \overline{X})^2} \times \sqrt{\sum (Y - \overline{Y})^2}}. \quad (1)$$

To study the relationship between the number of sandflies and the stations, we used the t-student. For the coefficient of t-student to be significant, the p value must be less than 0.05 (p value < 0.05). The calculations of t-student and the p value are performed using IBM SPSS statistical software.

3. Results

3.1. Inventory of Phlebotomian Fauna. The results of the entomological study show the existence of two genera: the genus Phlebotomus representing 96% of the total genera and the genus Sergentomyia representing 4%. A total of 5 species have been identified (Table 2). The most abundant species is *Ph. sergenti* (73%), followed by *Ph. perniciosus* (8%), *Ph. longicuspis* (8%), *Ph. papatasi* (6%), and *S. minuta* with 4%.

Table 2: Number and relative abundance of species.

Genus	Subgenus	Species	Total	Relative abundance (%)
Phlebotomus	*Larroussius*	*Ph. perniciosus*	78	8
		Ph. longicuspis	82	8
	Paraphlebotomus	*Ph. sergenti*	696	73
	Phlebotomus	*Ph. Papatasi*	61	6
Sergentomyia	*Sergentomyia*	*S. minuta*	41	4
		Total	958	100

The distribution of sandflies according to localities is represented in the following table (Table 3). According to the distribution of the species in different localities (Table 3), we can note that the species: *P. papatasi, P. sergenti, P. perniciosus, P. longicuspis, and S. minuta* are qualified as constants (or common species) because they were found in all stations.

The statistical analysis shows that there is no correlation between the number of sandflies and the study station ($p > 0.05$).

3.2. The Dynamic Evolution of Phlebotomian Fauna. The evolution of the relative abundance of the harvested species (Figure 2) shows that the most abundant evolutions were recorded during the two months of July and September being, respectively, 22% and 24%. This period coincides with the dry period of the year.

3.3. Impact of Climatic Factors. In comparison with climatic factors, we find that during periods of maximum activity, the average temperature is, respectively, between 22 and 20°C. In June and September, when the greatest number of sandflies were collected, the average temperature was 27 degrees Celsius and 22.4 degrees Celsius, respectively, while the rainfall rate was low (0 mm; 7 mm). On the other hand, the minimum number of sandflies was sampled in April and November with an average temperature of 14.2 degrees Celsius and 12.7 degrees Celsius and a maximum relative moisture exceeding 62%. We also note that the rainy months are the months when the relative abundance of sandflies is low (Figure 3).

The study of the correlation between temperature, rainfall, and the abundance of phlebotomian species shows the existence of a very positive correlation between temperature and the different phlebotomian species ($r = 0.81$) with a confidence interval of 95%. However, this correlation is negative between rainfall and the abundance of this fauna ($r = -0.80$) (Figure 4).

On the other hand, statistical analysis shows a negative correlation between moisture and sandfly abundance ($r = -0.76$) and a low correlation with wind speed ($r = 0.17$) with a confidence interval of 95%.

4. Discussion

The entomological study shows that during the study year, the phlebotomine fauna of our study area was made up of two genera, Phlebotomus and Sergentomyia, distributed among the four subgenera Phlebotomus, Paraphlebotomus, Larroussius, and Sergentomyia:

(i) In the Paraphlebotomus genus, which is the most abundant one, we reported only one species, *Ph.*

Table 3: Number of sandflies at the studied stations.

	S1	S2	S3	S4	S5	S6
Species	Nb	Nb	Nb	Nb	Nb	Nb
P. sergenti	128	110	112	112	124	110
P. papatasi	12	3	9	12	16	9
P. perniciosus	12	7	8	24	18	9
P. longicuspis	11	12	8	22	20	9
S. minuta	4	5	6	10	9	7
Total	167	137	143	180	187	144

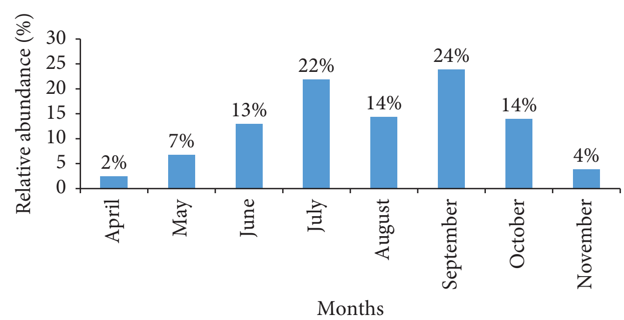

Figure 2: Temporal evolution of the relative abundance of sandflies.

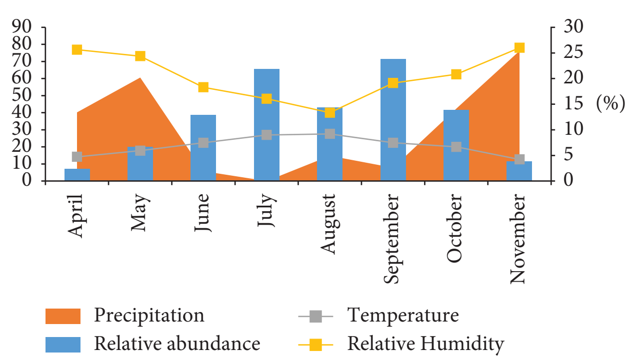

Figure 3: Representation of the relative abundance of sandflies and variation of the monthly temperature, relative humidity, and precipitation in Meknes prefecture.

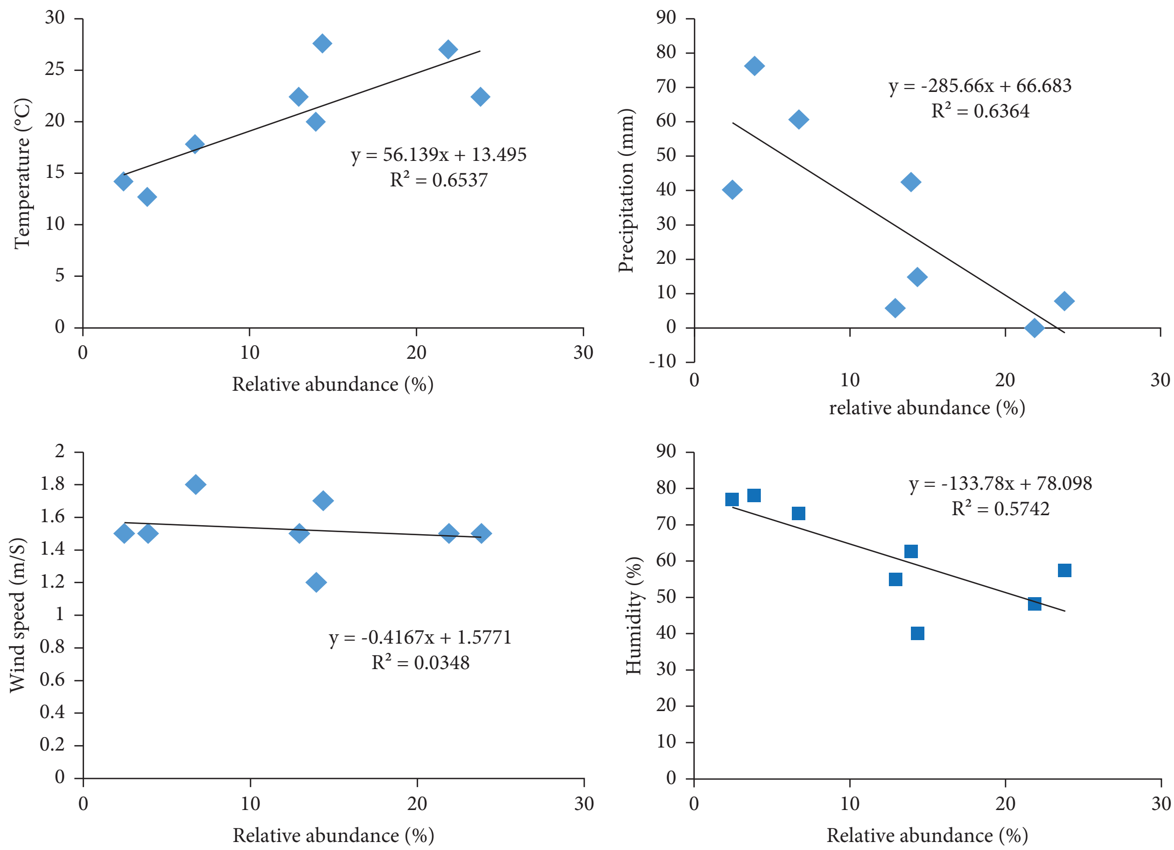

Figure 4: There is a correlation between the abundance of sandflies and climatic factors.

sergenti (73%), which is the only vector of *Leishmania tropica* in several countries and in Morocco in particular [30–33].

(ii) In the genus Larroussius, we identified two species *Ph.perniciosus* and *Ph.longicuspis* with 16% of the total population. These two species are vectors of *L. infantum*, the causative agent of visceral leishmaniasis [34–37].

(iii) In the Paraphlebotomus genus, we have identified only one species, *Ph. papatasi*, an agent of zoonotic CL confirmed as a vector of *L. major* in Mediterranean countries and in particular in arid and Saharan areas [38, 39]. This species is considered to be a part of the species that are well adapted to the domestic (predomestic) environment in the Saharan arid zones, while it is rare in the humid zones [40].

(iv) The Sergentomyia genus has been reported in a single species, *Sergentomyia minuta,* which feeds mainly on reptiles. This genus has no epidemiological significance [41].

The five species captured in our survey have also been found in other regions of Morocco [18, 22, 23].

The study of the spatiotemporal variation of the captured species shows that all the species found are common in the chosen stations: this could be explained by the fact that the chosen stations are all not far from the population at risk. Indeed, the stations are located less than 100 m from the population for stations S1 and S6 and less than 1 km from the other stations.

The number of the captured species is variable from one month to another, which proves that the variations in climatic factors influence the fluctuations of sandflies.

The study of the relationship between the abundance of sandflies and climatic factors therefore makes it possible to have prior knowledge of the risk periods. The evolution of the relative abundance of the harvested species reveals two risk periods, namely, the months of July (22%) and September (24%), which coincide with the dry period of the year. The results of the study could be explained by the meteorological disturbances in the region, especially the temperature and the rainfall. The statistical study also showed the positive correlation between these specimens and the variation in temperature, as well as the negative correlation with rainfall. Indeed, temperature favors the multiplication of these insects, increasing their activities and frequencies, which reinforce the vectorial capacity of sandflies and the transmission of leishmaniasis. However, rainfall inhibits their proliferation. Sandflies are most active during the dry season [42–44]. The two peaks of activity were noted in June and September. During these months, the rainfall rate is low (0 mm; 7 mm), and the average temperature is reaching its maximum (27 degrees Celsius; 22.4

degrees Celsius). These results are consistent with those of Lahouiti et al. [24] and Talbi et al. [45], who showed that sandfly activity coincides with the dry period of the year when rainfall is low and temperatures are high. In addition, some authors have confirmed the correlation between environmental factors (rainfall and temperature) and sandfly abundance [46, 47].

The optimum moisture or sandfly proliferation is between 57% and 62%, so density decreases when moisture exceeds 62%. These results are consistent with other research that has shown that temperature and moisture are the main parameters of the biological evolution of the disease [24]. In addition, the work of Wittmann et al. has shown that increased temperatures increase the proliferation of vectors and also foster their vectorial capacity [44]. This result is consistent with the results obtained in Benslimane, where relative moisture (30% to 70%) constitutes the climatic zone that is favorable to the development of sandflies [46]. Similarly, the work of Galvez et al. [48], Desjeux [49], and Senghor et al. [50] has shown that the period of activity of sandfly species is conditioned by climate change. A recent study on the effects of climate factors upon the frequency distribution of local sandflies in Iraq shows that climate can affect the activity, spread, and distribution of sandflies [51].

These results are in agreement with those obtained in Morocco by Guessous-Idrissi et al. in the province of Taza and with those obtained by El Miri in 2013 [52, 53], particularly in the Fez-Meknes region, where temperature, rainfall, and moisture are confirmed as the main factors in the spread of the disease [28, 45].

Since there is currently no vaccine or preventive medication for leishmaniasis, in order to fight against this vector, it is necessary to encourage the separation between human and animal habitats, to improve human habitats, to treat animal waste which allows to get rid of the sandflies, and also to fight the reservoirs [24].

It is also important to note that the control of leishmaniasis in Morocco is essentially based on the detection and treatment of cases of anthroponotic leishmaniasis and on the fight against reservoirs (rodents) for zoonotic leishmaniasis. Vector control is becoming increasingly important in national disease control strategies [26]. It is mainly based on promoting the improvement of hygiene conditions. Insecticide spraying campaigns have occasionally been recorded for some epidemics, but the impact has not been assessed. In fact, the success of a pest control campaign cannot be judged without a good knowledge of vector bioecology.

This article discusses the different abundances of leishmaniasis vectors as a function of climatic conditions based on the results of entomological monitoring and climatic data from our study area, which could help in predicting when disease transmission is going to be high. Those data will therefore be of great use in planning prevention and control measures by adopting preventive strategies during the high-risk months such as awareness-raising and training campaigns for the population concerning the risk months and the existing means of prevention such as the spraying of insecticides in the biotopes conducive to the multiplication of vectors.

5. Conclusions

The study of the inventory and the dynamics of sandflies in the region of Meknes shows the existence of five leishmaniasis vector species with two peaks, the first of which was observed in July and the second in September.

This study reveals two periods of increased risk of transmission of leishmaniasis, characterized by great abundance. Our study also shows that climatic factors affect the abundance of sandflies, hence the need to strengthen control through participatory strategies involving all actors, including the general population.

It is indeed necessary to strengthen the surveillance activities of leishmaniasis in time and space by taking into account the epidemiological history of the disease in the affected areas and also the risk factors, including climatic factors, and by strengthening the means of control during the high-risk months.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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