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## Impact of Environmental Factors on the Distribution of Sandflies in Different Localities of Moulay Yaâcoub Province, North Central Morocco

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### ABSTRACT

Leishmaniasis is a parasitic disease with a wide range of clinical manifestations. Sandflies are the only known vector of this disease. This study was conducted to investigate the relationship between the spatial distribution of sandflies and environmental factors in Moulay Yaâcoub Province, Morocco. Sandfly distribution in the study area was assessed using four factors: altitude, soil type, vegetation, and environmental hygiene. The Sandfly collection was carried out from April to October 2014, during the active period of the sandflies. Adhesive traps and miniature light traps were used in the capture. The Geographic Information System (GIS) was employed to evaluate the distribution of sandflies based on environmental factors. Principal Component Analysis (PCA) was performed with the XLSTAT software. The results of the study showed that a total of 3,287 sandfly specimens from the *Phlebotomus* and *Sergentomyia* genera were collected. The genus *Phlebotomus* accounted for 94.3% of the sandflies sampled, while the genus *Sergentomyia* represented 5.7%. The dominant species of sandflies in the total samples was *P. papatasi*, which accounted for 31.45%, followed by *P. longicuspis* (26.1%), *P. perniciosus* (19.35%), and *P. sergenti* (17.40%). *Sergentomyia falax* represented only 3.85%, closely followed by *S. antennata* (0.85%), *S. minuta* (0.76%), and *S. dreyfussi* (0.24%). The findings of this study have demonstrated that the nature of the environment can favour the abundance of one species over another, depending on the species' tolerance to the four environmental factors studied.

**Keywords:** Environment, Leishmaniasis, Morocco, *Phlebotomus* species, Sandflies, *Sergentomyia* species.

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### Introduction

Sandflies are the only known vector of leishmaniasis, a parasitic disease that has a wide range of clinical manifestations, with a global prevalence of 12 million human cases, an annual incidence of 1.5 to 2 million new cases, and 350 million people at risk.<sup>1</sup> They are very sensitive to weather and climatic variations.<sup>2-4</sup> Sandflies are inextricably linked to the state of the environment.<sup>5</sup> This sensitivity to environmental components strongly influences their distribution and abundance on global, national, regional, or even local levels and determines the location of leishmaniasis forms. Because there are no vaccines available before the emergence of new outbreaks, control programs are the only application that can alleviate or even eliminate this infection. Vector control is recommended,

but its success is dependent on the results of the entomological investigation. This work exploited our entomological survey on sandflies conducted in 2014 at Moulay Yaâcoub province, which showed that sandflies are unevenly distributed and that abundance and species richness vary from one locality to another, and to understand and explain the spatial distribution of species of sandflies in the Moulay Yaâcoub Province and study the impact of certain factors that seem to affect this distribution. This study is based on the analysis of four environmental factors and their relationship with the abundance and distribution of sandflies species in the study area. These factors are: altitude, soil type, vegetation, and environmental hygiene. This study was aimed at investigating the effect of environmental factors on the spatial distribution of sandflies in different areas of Moulay Yaâcoub Province in North Central Morocco.

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### Materials and Methods

#### Study zone

The study was conducted in the province of Moulay Yacoub, which was established in 2003. Moulay Yacoub Province is part of the Fez-Meknes region and consists of eleven localities, one municipality (Moulay Yaâcoub), and ten rural towns, which include: Ain Chkef, Mikkès, Sebaa Rouadi, Sebt Loudaya, Ain Bou Ali, Laajajra,

Louadaine, Oulad Mimoun, Sidi Daoud and Ain Kansara. It is located in north-central Morocco, where the climate is continental, with hot and dry summers and cold and humid winters. The average annual rainfall ranges from 150 to 300 mm; while the average monthly temperature is 9°C in January and 40°C in July (Meteorology Center of North Branch). The population of this province is estimated at 174,049 inhabitants according to the 2014 census and is essentially rural. The rural population accounts for nearly 85.5% of the provincial population.

#### Collection and identification of sandflies

The collection of sandflies was carried out during the period of activity of sandflies from April to October 2014.<sup>2</sup> The capture was done using adhesive traps (A4 paper coated with castor oil),<sup>7</sup> and miniature light traps from the Center for Disease Control.<sup>8</sup> Traps were set up at dusk and recovered the next day at dawn, once a month. After collection, the captured specimens were removed from the traps using a brush dipped in alcohol, and preserved in ethanol at 95%.<sup>9</sup> Sandflies caught in light traps were refrigerated for about 30 minutes before being removed. The specimens were thinned by Marc-André solution (acetic acid chloral hydrate) and were mounted in Canadian balsam for identification.<sup>10</sup> The identification was made by the identification key of Moroccan sandflies based on the examination of the morphology of the male's genital organs, spermatid, and throat for identifying the females.<sup>11</sup>

#### Development of thematic maps

Thematic maps were created using the ASTER GDEM data,<sup>12</sup> which was used to create the digital module terrain (DMT) and the vegetation cards. The soil data were obtained from the Moroccan soil database, which was accessible via the website of National Institute of Agricultural Research (NIAR).<sup>13</sup> Sandfly abundance has been investigated and determined in previous sandfly allocation studies conducted in all localities of the Moulay Yaâcoub Province. The analysis of the environmental situation of localities was carried out using detailed data from the locality's monographs and on-site visits conducted during the study period. The topographic coordinates of the sites were identified by Global Positioning System (GPS) coordinates and were converted to Lambert. The following software was used to process images and maps for the elaboration of thematic maps: 2.8 QGIS, GRASS GIS, and GIS Post. Geographic Information System (GIS) was used to determine the distribution of sandflies based on environmental factors that appear to predict the presence or absence, abundance or rarity of sandflies, and species type.

#### Statistical analysis

The data were analyzed using the Principal Component Analysis (PCA) method to investigate the existence of correlations between environmental factors and species distribution and abundance, as well

as sites of similarity. The PCA was performed with the XLSTAT software (2016.02.28.451 version).

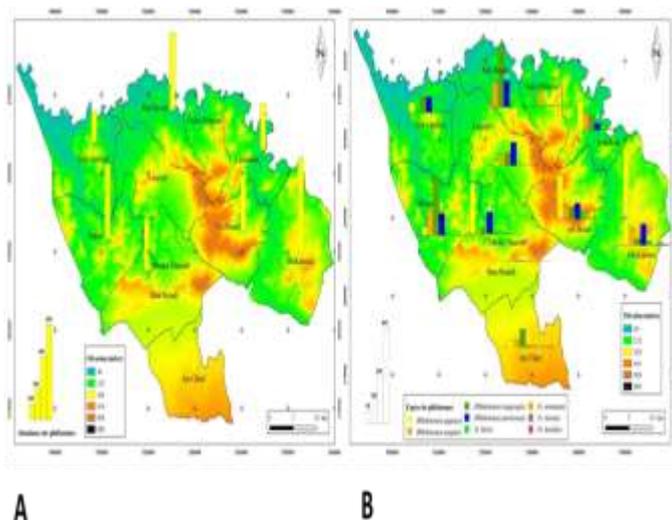
## Results and Discussion

A total of 3,287 sandfly specimens were collected using two types of traps: adhesive (A4 paper coated with castor oil),<sup>7</sup> and light traps.<sup>8</sup> The genus *Phlebotomus* represented 94.3% of the sampled sandflies, while the genus *Sergentomyia* represented 5.7%. Of the total samples, the dominant species of sandflies was *P. papatasi*, which constituted 31.45%. This was closely followed by *P. longicuspis* (26.1%), *P. perniciosus* (19.35%), and *P. sergenti* (17.40%). *Sergentomyia falax* represented only 3.85%, closely followed by *S. antennata* (0.85%), *S. minuta* (0.76%), and finally *S. dreyfussi* (0.24%). The difference between the collected species is statistically significant ( $\chi^2$ -2929, 85,  $p < 0.001$ ). The abundance of species collected in each locality is shown in Table 1. To visualize the results of the entomological study on thematic maps relating to each of the environmental factors studied (elevation, soil type, dominant vegetation, and environmental health), the two results, overlaying total sandfly abundance and relative abundance of each species, were combined. This was done to determine sandfly species preferences concerning the variables being studied (Figures 1-4). The results obtained allowed for the prediction of the proliferation of certain species of leishmaniasis vectors and, as a result, the identification of risk areas. Table 2 shows the results of combining the entomological survey and thematic map results.

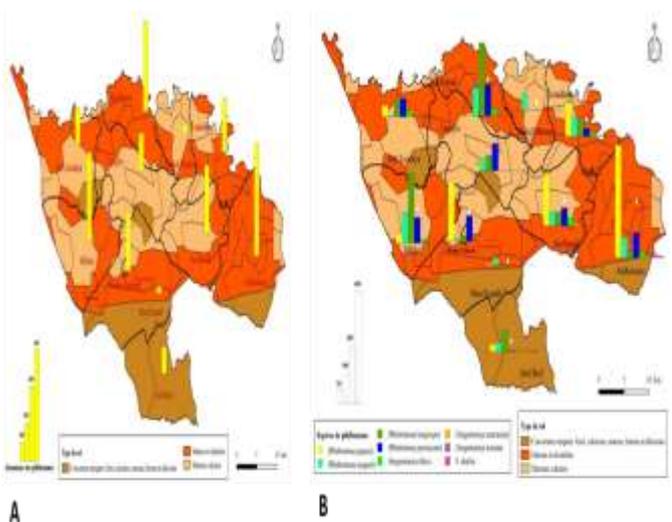
From Table 2, the preferences of the species compared to factors presented in Tables 3-6 were summarized. The preferences of each species in terms of the altitude of the study area are presented in Table 3. Also, Table 4 indicates the preferences of each identified species in terms of the nature of the soil in their environment. The abundance of the identified species is different according to the nature of the vegetation in the environment. Table 5 depicts the preferences of each species concerning the nature of vegetation. It was also observed that the hygiene of the environment influenced the sandfly species; the preferences of each species regarding the cleanliness of the environment are shown in Table 6. Principal component analysis was performed to obtain an overview of the similarities and differences between species and to study the relationship between the studied environmental factors. Four PCAs were calculated according to the nature of the data obtained. PCA 1 (altitude factor), PCA 2 (natural factor of the soil), PCA 3 (vegetation factor), and PCA 4 (hygiene environmental factor). The PCA 1 results reveal that the first three components can be used to explain the variability of the data. However, most of the information is explained by the first two factorial axes. In the factorial plan F1xF2, the values of the two components F1 and F2, as well as their contribution to the total inertia, are displayed in Table 7 of the neat values.

**Table 1:** Relative abundance of the species of sandflies collected in each locality

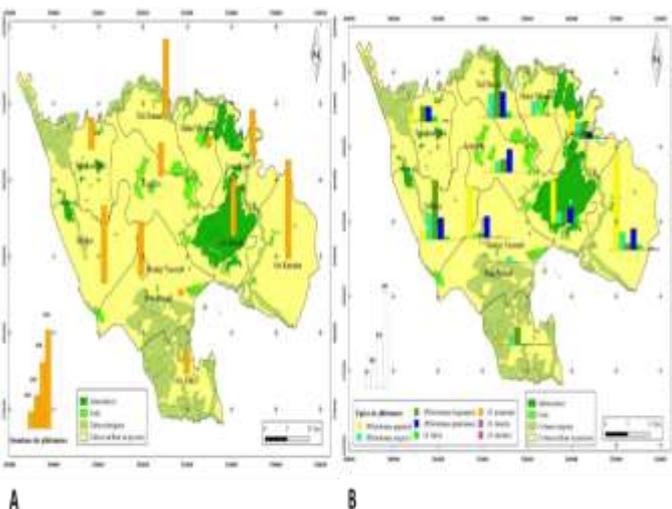
Locality	Genus <i>Phlebotomus</i>				Genus <i>Sergentomyia</i>				Total
	<i>P. papatasi</i>	<i>P. sergenti</i>	<i>P. longicuspis</i>	<i>P. perniciosus</i>	<i>S. falax</i>	<i>S. antennata</i>	<i>S. minuta</i>	<i>S. dreyfussi</i>	
Ain kansara	63.62	11.53	4.32	14.1	3.2	0.64	1.76	0.8	624
Ain Chkef	17.33	21.33	49.34	2	3.33	2.67	2	2	150
Sbaâ Rouadi	15.4	66.66	12.82	5.12	0	0	0	0	39
Ouled Mimoun	3.33	93.34	0	3.33	0	0	0	0	60
Centre Moulay Yaâcoub	62.01	3.26	5.64	26.4	0.6	1.79	0.3	0	337
Louadain	39.33	23	21	9.67	4.67	1.67	0.66	0	300
Ain Bouali	47.47	13.33	12.8	16.8	7.2	2.4	0	0	375
Sidi Daoud	1.6	20	51.6	21.8	5	0	0	0	500
Laâjajra	7.14	21.44	25.71	45.71	0	0	0	0	210
Mikkès	7.14	22.04	50	18.58	2.24	0	0	0	490
Sebt Loudaya	19.8	1.48	32.17	31.69	10.9	0	3.96	0	202



**Figure 1 :** Abundance of sandflies with altitude classes of MNT. A: Total abundance; B: Relative abundance



**Figure 2:** Abundance of sandflies depending on the nature of the soil. A: Total abundance; B: Relative abundance



**Figure 3:** Abundance of sandflies according to the dominant vegetation. A: Total abundance; B: Relative abundance.

The two axes used to describe the correlations between variables related to spatial structures account for 86.21% of the total information, with axis 1 accounting for 62.96% and axis 2 accounting for 23.25% (Figure 5A). Sandflies were collected in all localities of the province and at all altitudes. The total abundance varies from one locality to another. The highest abundance of this species was found in the commune of Ain Kansara (Table 1 and Figure 1A).

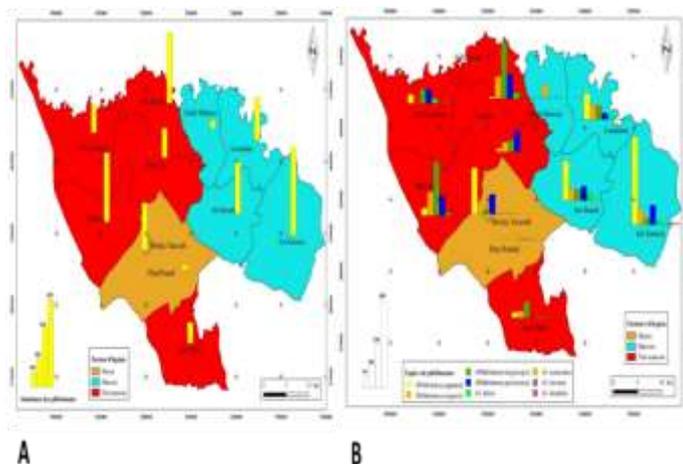
The abundance of species was different according to the altitude classes; the highest (30.42%) was calculated between 301 and 405 m. The species *P. papatasi*, *P. sergenti*, and *S. dreyfussi* were collected at an altitude of 301 to 600 m. *Phlebotomus longicuspis*, *P. perniciosus*, *S. falax*, *S. antennata*, and *S. minuta* were abundant at an altitude lower than 0 to 300 m (Tables 2-3 and Figure 1B). The first two factors account for 86.21% of the total variability of the data. The correlation circle (Figure 5B) indicates that *P. perniciosus*, *S. falax*, *S. antennata*, and *S. minuta* correlate positively with the F1 axis (0.75). *Phlebotomus longicuspis papatasi* also had a weak and positive correlation with F1. *Phlebotomus sergenti* and *S. dreyfussi* have a negative correlation with the first group. Also, *P. longicuspis* has a negative correlation with F1 but a positive correlation with F2.

The factorial map database observations (Figure 5C) show that the first group correlates with a low level of 0-150 m. (A1). *Phlebotomus papatasi* positively correlates with F1 but it exists in the negative part of F2, thus it correlates with an altitude of 301 to 450 m (A3). The figure indicates that *P. sergenti* and *S. dreyfussi* correlate with altitudes ranging from 451 to 600 m (A4), whereas *P. longicuspis* correlates with altitudes ranging from 151 to 300 m.

*Phlebotomus papatasi* is known for its wide distribution in the Mediterranean basin.<sup>14</sup> It is widely distributed in Morocco.<sup>15</sup> *Phlebotomus papatasi* was collected at all sites but it was more abundant in sites, located at an altitude of 301 to 450 m. The species was most abundant in altitudes ranging between 400 and 600 m in 2002, according to Works in the High Atlas,<sup>4</sup> and it was absent between 1,200 and 1,400 m. As a result, it is classified as a plain species, with decreasing abundance as altitude increases. This species was collected by Kahime et al.,<sup>16</sup> up to 1,800 m, with a peak at 800-1,000 m. This species is well adapted to arid conditions,<sup>3,15,17</sup> and its density increases with aridity.<sup>18</sup> This explains the prevalence of zoonotic cutaneous leishmaniasis in Morocco's Saharan and pre-Saharan zones, given that this species has been confirmed as a proven vector of leishmania major, the agent of zoonotic cutaneous leishmaniasis.<sup>19</sup> The PCA 2 (nature of soil component) results show that the first two factorial axes explain all of the information. Table 8 of the own values highlights the values of the two components F1 and F2 and their contribution to the total inertia in the factorial plain F1xF2. The two axes describe the correlations between variables related to spatial structures and hold alone at 100% of the total information, with 61.84% for axis 1 and 38.16% for axis 2 (Figure 6A). Sandflies were collected from different soil types with a very high total abundance (60.0%) on marl limestone soil (Table 2 and Figure 2A). The abundance of species was different according to the type of soil. *Phlebotomus papatasi* and *S. falax* were collected in very high abundance in limestone marl soil, and *P. perniciosus* was also abundantly found in marl soil.

*Phlebotomus sergenti*, *P. longicuspis*, *S. dreyfussi*, *S. falax*, *S. antennata*, and *S. minuta* have a high relative abundance in soil with a neogene nature composed of sandstone, limestone, marl, loam, and silt as presented in Table 4 and Figure 2B. The first two factors explain 100% of the total variability of the database. The correlation circle (Figure 6B) shows that *S. antennata*, *S. minuta*, *P. longicuspis*, and *S. dreyfussi* correlate with the F1 axis (0.5). *Phlebotomus sergenti* also positively correlates with F1 (0.75). *Phlebotomus papatasi* and *S. falax* negatively correlate with the F1 axis, but they correlate positively with the F2 axis, while *P. perniciosus* negatively correlates with the F1 axis. The database factorial map (Figure 6C) reveals that the first group of *S. antennata*, *S. minuta*, *P. longicuspis*, *S. dreyfussi* and *P. sergenti* correlates with neogene soil (S3).

*Phlebotomus papatasi* and *S. falax* exist in the negative side of F1 and the positive part of F2 and therefore have similarities with the type of limestone marl soil (S2). *Phlebotomus perniciosus* negatively correlates with F1 and F2 axes and prefers S1 soil with marl types. It was observed that the highest abundance of *P. papatasi* was found in calcareous marl soils.



**Figure 4:** Abundance of sandflies according to environmental health. A: Total abundance; B: Relative abundance

The province of Moulay Yaâcoub is at the beginning of the Rif Mountains, which are characterized by marl. The PCA results showed a strong correlation (0.75) of this species with the marled limestone soil. Kahime et al.,<sup>15</sup> found that it was surrounded more by sandy loam soil. The analysis of the PCA 3 (type of vegetation component) results shows that the first three components can be taken into account in explaining the variability of the data. However, most of the information is explained by the first two factorial axes. In the factorial design F1xF2, the values of the two components F1 and F2 and their contribution to the total inertia are shown in Table 9 of the neat values.

The two axes are taken into consideration to describe the correlations between variables related to spatial structures. They account for 85.75% of the total information, with 48.70 and 37.05%, respectively for axes 1 and 2 (Figure 7A).

The highest abundance of sandflies was found in the town of Ain Kanassra, a town whose canopy is dominated by crops and forest land. The correlation circle (Figure 7B) shows that *P. papatasi* has a strong positive correlation (close to 1) with the F1 axis. *Sergentomyia antennata* also correlates positively (> 0.5) with the same axis. Meanwhile, *S. falax* shows a very weak positive correlation with F1 and a negative correlation with F2. *Phlebotomus perniciosus* correlates very well with the axis F2 (close to 1) and very weakly with F1. The other species all have a strong negative correlation with F1. A positive but weak correlation with F2 was observed for *P. sergenti* and *P. longicuspis*; while a low negative correlation was also observed for *S. dreyfussi* and *S. minuta*. According to the database's factorial map of observations (Figure 7C), the first group of species found on the positive side of F1 and the negative side of F2 can be distinguished, which is dominated by arboriculture, based mainly on olives and followed by almonds and citrus. The second group, *S. dreyfussi*, and *S. minuta*, correlates with V1, which is mainly composed of cereal and leguminous crops as well as trees. The third group of *P. sergenti* and *P. longicuspis* exists on the positive side of F2 and the negative side of F1; thus, they correlate with V3, which has mostly crops and forest trees as vegetation. *Phlebotomus perniciosus* is located in the positive part of F1 and F2. As a result, it shares many similarities with V4, which consists of a covered plant that has been severely degraded due to the nature of the soil. The study of the vegetation effect on species distribution revealed that *P. papatasi* has a strong correlation with arboriculture-dominated sites, which are primarily composed of olive trees.

**Table 2:** Relative abundance of each species of sandflies collected in 2014 according to the altitude class, soil, vegetation and environmental health.

Different parameters	code	Species of sandflies							
		<i>PPa</i>	<i>PS</i>	<i>PL</i>	<i>PPer</i>	<i>SF</i>	<i>SA</i>	<i>SM</i>	<i>SD</i>
<b>Altitude</b>									
0-150	A1	33.63	7.4	22.49	24.24	9.06	1.2	1.98	0
151-300	A2	23.23	22.52	35.5	14.13	3.45	0.84	0.33	0
301-450	A3	47.01	27.16	7.6	15.2	1.28	0.81	0.68	0.26
451-600	A4	7.35	39.03	31.66	18.21	2.09	0.66	0.5	0.5
<b>Soil</b>									
Marl	S1	20.19	35.005	18.59	22.575	2.56	0.585	0.495	0
Marl- limestone	S2	35.15	12.62	24.32	20.63	5.12	1.17	0.99	0
Sandstone, limestone, marl, loam and silt	S3	16.36	43,99	31.09	3.56	1.66	1.34	1	1
<b>Vegetation</b>									
Cultivation + arboriculture	V1	17.51	29.82	31.45	12.94	4.74	0.89	1.99	0.66
Arboriculture	V2	43.4	18.16	16.91	13.25	5.91	2.03	0.34	0
Cultivation+ Forest	V3	18.93	36.58	20.4	21.23	2.05	0.17	0.44	0.2
Degraded vegetation cover	V4	34.57	12.65	27.82	22.49	1.42	0.9	0.15	0
<b>Hygiene</b>									
Medium	H1	38.7	34.97	9.23	15.76	0.3	0.89	0.15	0
Bad	H2	38.44	35.3	9.53	10.98	3.77	1.18	0.6	0.2
Very bad	H3	10.6	17.26	41.76	23.96	4.3	0.53	1.19	0.4

PPa: *Phlebotomus papatasi*; PS: *P. sergenti*; PL: *P. longicuspis*; PPer: *P. perniciosus*; SF: *Sergentomyia fallax*; SA: *S. antennata*; SM: *S. minuta*; SD: *S. dreyfussi*

**Table 3:** Preferences of each species of the sandflies compared to the altitude

Species	Altitude (m)	
	0-300	301-600
Genus <i>Phlebotomus</i>	<i>P. perniciosus</i>	<i>P. papatasi</i>
	<i>P. longicuspis</i>	<i>P. sergenti</i>
Genus <i>Sergentomyia</i>	<i>S. antennata</i>	<i>S. dreifussi</i>
	<i>S. minuta</i>	
	<i>S. falax</i>	

**Table 4:** Preferences of each species of the sandflies compared to the nature of the soil.

Species	Soil texture		
	Marl	Marl- limestone	Sandstone, limestone, marl, loam and silt
<i>P. perniciosus</i>	<i>P. papatasi</i>		<i>P. longicuspis</i>
		<i>S. falax</i>	<i>P. sergenti</i>
<i>P. papatasi</i>			<i>S. antennata</i>
			<i>S. minuta</i>
			<i>S. dreifussi</i>

**Table 5:** Preferences of each species of sandflies compared to the dominant vegetation species

Species	Vegetation type			
	Cultivation + arboriculture	Arboriculture	Cultivation + Forest	Degraded vegetation cover
<i>P. longicuspis</i>		<i>P. papatasi</i>		<i>P. perniciosus</i>
		<i>S. fallax</i>		
<i>P. papatasi</i>		<i>S. antennata</i>		

**Table 6:** Preferences of each species of the sandflies compared to the hygiene type

Species	Hygiene type		
	Medium	Bad	Very bad
<i>P. papatasi</i>	<i>P. papatasi</i>		<i>P. perniciosus</i>
	<i>P. sergenti</i>	<i>P. sergenti</i>	<i>P. longicuspis</i>
<i>P. sergenti</i>		<i>S. antennata</i>	<i>S. falax</i>
			<i>S. minuta</i>
			<i>S. dreifussi</i>

**Table 7:** Initial values

	F1	F2	F3
Initial values	5.037	1.860	1.103
(%) of variance	62.958	23.252	13.790
(%) cumulative	62.958	86.210	100.000

**Table 8:** Initial values

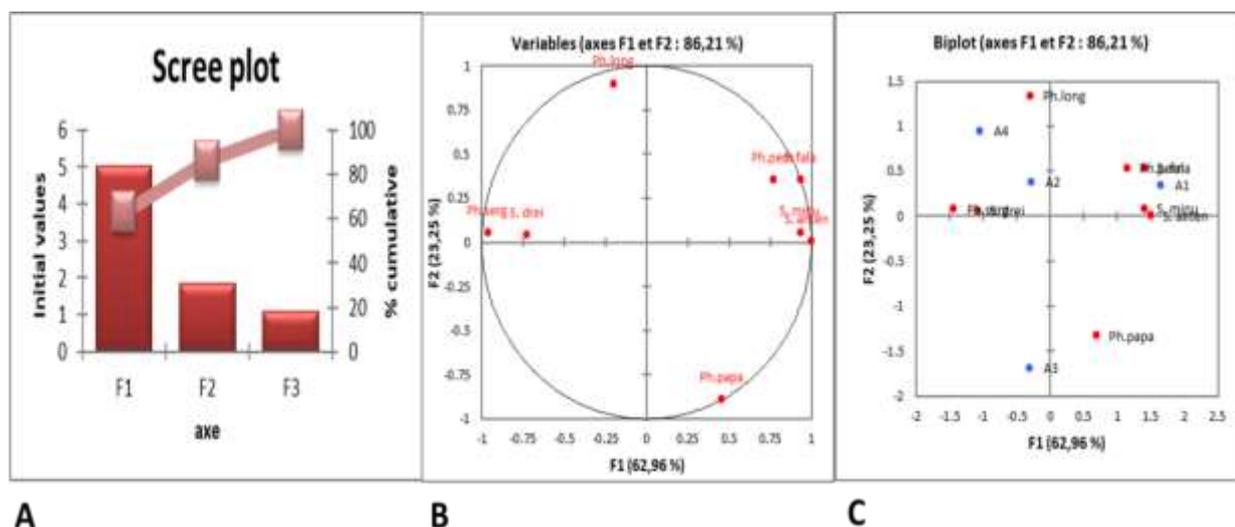
	F1	F2
Initial values	4.947	3.053
(%) of variance	61.839	38.161
(%) cumulative	61.839	100.000

This result explains the dominance of this species throughout the Mediterranean basin. In terms of environmental health, *P. papatasi* thrives in a less-than-ideal environment, which is primarily defined by the presence of manure (very strong correlation). Manure is a very favorable environment for the development and proliferation of sandflies.<sup>20</sup>

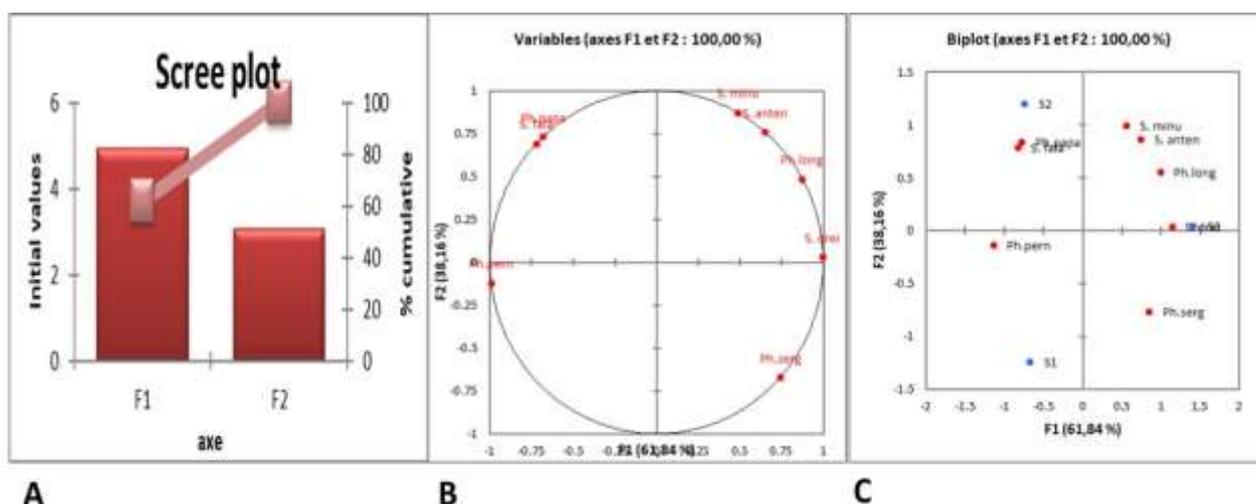
The analysis of the PCA 4 results shows that the first two factorial axes explain all of the information. In the factorial design F1xF2, the values of the two components F1 and F2, and their contribution to the total inertia are shown in Table 10 of the eigenvalues. As displayed in Figure 8A, the two axes are considered to describe the correlations between variables related to spatial structures; they hold alone for 100% of the total information with 83.20 and 16.80% for axes 1 and 2, respectively. During terrain visits and outings to collect sandflies, it was discovered that some areas were cleaner than others, but the majority suffered from health and environmental issues. As a result of this situation, each locality was assigned a hygiene index ranging from 1 to 3. The determination of the index assigned to each locality was based on two main elements: the presence of manure in abundance and the presence of wild dumps. Three classes have been defined, an index equal to 1 for nearly clean towns; an index equal to 2 for localities with poor conditions, and an index equal to 3 for towns that suffer from very bad hygiene. Environmental health greatly contributes to the proliferation of sandflies, especially in rural regions where manure constitutes a very favorable environment, rich in organic materials necessary for the development of eggs and larvae of sandflies. Sandflies were collected in all localities with a different abundance determined by the hygiene indices (Table 2 and Figure 4A). The abundance of each species also differs from one locality to another according to the hygienic indices, as shown in Table 6 and Figure 4B.

*Phlebotomus papatasi*, *P. sergenti*, and *S. antennata* show peaks in an environment in a state of medium to bad hygienic conditions. Other species have their maximum abundance in very bad hygienic conditions. The correlation map (Figure 8B) reveals that *P. papatasi* and *P. sergenti* have a very strong positive correlation (= 1) with the F1 axis. *Sergentomyia antennata* also has a strong positive correlation with the same axis (>0.75). However, *P. longicuspis* highly correlates negatively (= -1) with the F1 axis. *Phlebotomus perniciosus* negatively correlates with both axes, while *S. falax*, *S. minuta*, and *S. dreifussi* correlate positively with the F2 axis. The factorial map of the observations database (Figure 8C) depicts that *P. papatasi*, *P. sergenti*, and *S. antennata* are correlated with H2. *Phlebotomus longicuspis* and *P. perniciosus* exist in the negative part of F1 and F2, hence they correlate with H3. *Sergentomyia falax*, *S. minuta*, and *S. dreifussi* are on the positive side of F2 and the negative side of F1, which are aligned with H3 and therefore correlate with it.

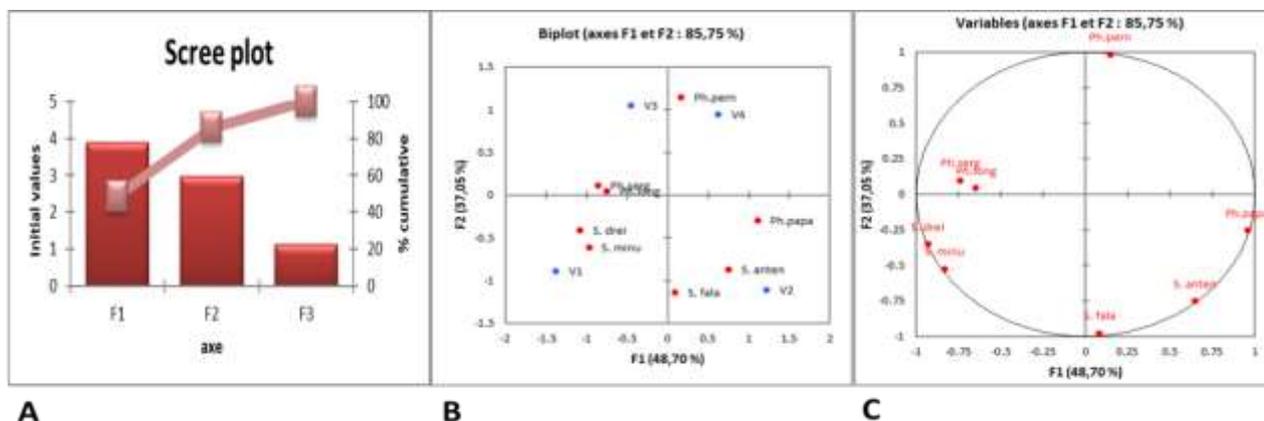
*Phlebotomus sergenti* is the second most abundant species in Morocco. Several studies have shown the dominance of this species in Morocco, especially in this region.<sup>21, 22, 23</sup> It is very prevalent in the country,<sup>22</sup> but it is more widespread in arid and sub-humid areas.<sup>25</sup> Yahia et al.,<sup>26</sup> demonstrated that it is a species with significant genetic diversity. This species was most abundant in the current study at altitudes higher than *P. papatasi* (451 to 600 m). *Phlebotomus sergenti* is abundant in rural and urban habitats with semi-arid bioclimatic space at altitudes of up to 1,400 m in the 2000s.<sup>3,4,26-28</sup> It is thought to be a species that prefers neogene soil made up of sand, limestone, marl, loam, and silt. *Phlebotomus sergenti* is also dominant in areas dominated by crops and forest trees. Similar to *P. papatasi*, it was abundantly collected in sites with more or less poor sanitary conditions due to the abundance of manure.



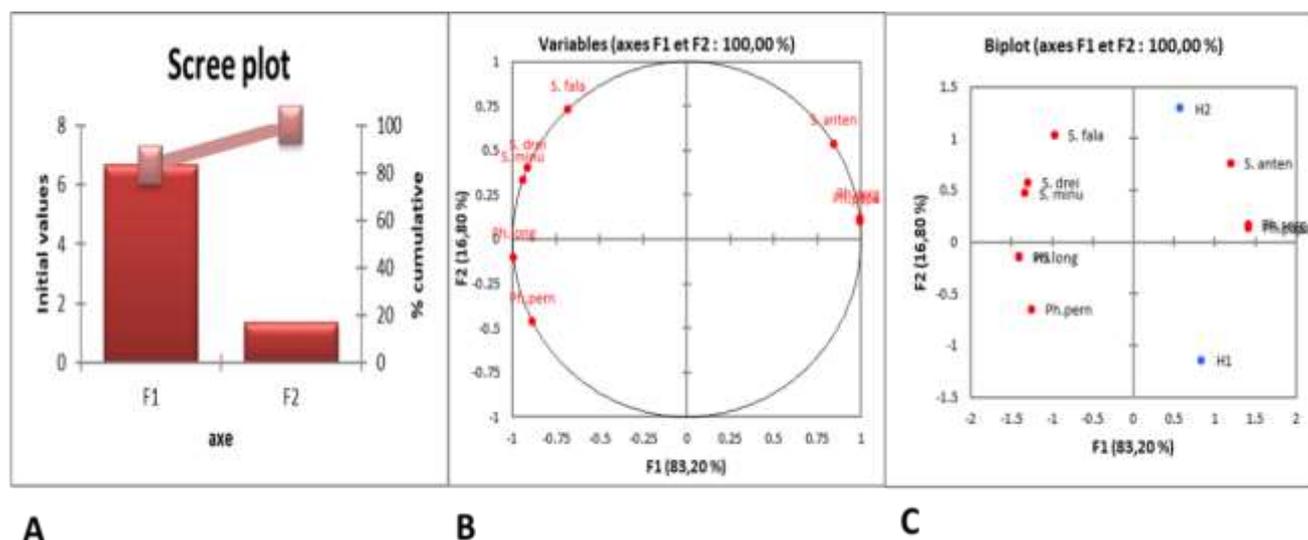
**Figure 5:** Graphic approach to the principal component analysis (PCA) according to the factorial plan (F1x2) of PCA 1. A: Distribution of inertia between axes; B: Correlation map between the variables and factors; C: Factorial observation map coordinates. *Phlebotomus papa*: *P. papatasi*; *P. serg*: *P. sergentii*; *P. long*: *P. longicuspis*; *P. per*: *P. perniciosus*; *S. fala*: *Sergentomyia falax*; *S. anten*: *S. antennata*; *S. minu*: *S. minuta*; *S. drey*: *S. dreyfussi*



**Figure 6:** Graphic approach to the principal component analysis (PCA) according to the factorial plan (F1x2) of the PCA 2. A: Distribution of inertia between axes; B: Correlation map between the variables and factors; C: Factorial observation map coordinates. *Phlebotomus papa*: *P. papatasi*; *P. serg*: *P. sergentii*; *P. long*: *P. longicuspis*; *P. per*: *P. perniciosus*; *Sergentomyia fala*: *S. falax*; *S. anten*: *S. antennata*; *S. minu*: *S. minuta*; *S. drey*: *S. dreyfussi*



**Figure 7:** Graphic approach to the principal component analysis (PCA) according to the factorial plan (F1x2) of the PCA 3. A: Distribution of inertia between axes; B: Correlation map between the variables and factors; C: Factorial observation map coordinates. *Phlebotomus papa*: *P. papatasi*; *P. serg*: *P. sergentii*; *P. long*: *P. longicuspis*; *P. per*: *P. perniciosus*; *Sergentomyia fala*: *S. falax*; *S. anten*: *S. antennata*; *S. minu*: *S. minuta*; *S. drey*: *S. dreyfussi*



**Figure 8:** Graphic approach to the principal component analysis (PCA) by the factorial plan (F1x F2) of the PCA 4. A: Distribution of inertia between axes; B: Correlation map between the variables and factors; C: Factorial observation map coordinates.

*Phlebotomus papa* : *P. papatasi*; *P. serg*: *P. sergenti*; *P. long*: *P. longicuspis*; *P. per*: *P. perniciosus*; *Sergentomyia fala*: *S. fallax*; *S. anten* : *S. antennata*; *S. minu*: *S. minuta*; *S. drey* : *S. dreyfussi*

**Table 9:** Initial Values

	F1	F2	F3
Initial values	3.896	2.964	1.140
(%) of variance	48.702	37.052	14.246
(%) cumulative	48.702	85.754	100.000

**Table 10:** Initial values

	F1	F2
Initial values	6.656	1.344
(%) of variance	83.197	16.803
(%) cumulative	83.197	100

According to the findings of Kahim et al.,<sup>16</sup> *P. sergenti* was collected at an altitude that ranged from 800 to 1,000 m above sea level, in all bioclimatic zones, particularly in semi-arid climates (cold and fresh in winter), with a texture of sandy-clay soil. In terms of soil texture, the characterization of *P. sergenti* breeding habitats in the city of Marrakech revealed a preference for sandy soil.<sup>29</sup> *Phlebotomus longicuspis* and *P. perniciosus* were collected in the lower altitudes of 151 to 300 m and from 0 to 150 m, respectively. Guernaoui et al.,<sup>4</sup> collected *P. longicuspis* at all altitudes, but it was most abundant between 600 and 799 m. *Phlebotomus perniciosus* was absent only at elevations of 400-599 m, but it was more abundant at elevations of 1,000-1,199 m. These species have different preferences depending on the type of soil. *Phlebotomus longicuspis* correlates significantly with neogene soil, while *P. perniciosus* abounds on marled soil. According to the vegetation, the first abounds with vegetation essentially based on crops and forest trees. The second, on the other hand, is most abundant in areas with sparse plant cover and almost bare land. Both species have their peaks in a much-polluted environment characterized by the presence of both manure and domestic waste in uncontrolled landfills. Kahim et al.<sup>16</sup> discovered the complex *P. perniciosus* at an altitude of 800 to 1,000 m with the texture of loamy sand soil, particularly in the Saharan climate. In general, *P. longicuspis* and *P. perniciosus* were collected in a significant abundance. They are usually considered among the most popular species in Morocco,<sup>24</sup> *Phlebotomus longicuspis* and *P. perniciosus* are two vectors suspected of carrying *Leishmania infantum* in the Mediterranean.<sup>30</sup> The species of the genus *S.* were poorly represented, with only 9.36% of the total collection. *Sergentomyia minuta* and *S. fallax* are found in

three North African countries.<sup>24,31</sup> Four types of species were collected, most of which have similarities with the environmental factors being studied. *Sergentomyia fallax*, *S. minuta*, and *S. antennata* were collected at lower elevations of 0 to 150 m with a high correlation (close to 1), while *S. dreyfussi* was collected at 451-600 m altitude. *Sergentomyia dreyfussi*, *S. minuta*, and *S. antennata* are most abundant in a neogene-rich environment, while *S. fallax* prefers limestone-marled soil. *Sergentomyia fallax* and *S. antennata* prefer dense, arboriculture-based vegetation. *Sergentomyia minuta* and *S. dreyfussi* correlate with vegetation composed of crops and tree crops. *Sergentomyia antennata* was more prevalent in poor areas with an abundance of manure, whereas three species abound in environments with very poor conditions where manure and mismanaged household waste coexist.

According to Berdjane et al.,<sup>32</sup> the genus *Sergentomyia* may be implicated in the transmission of leishmaniasis in some cases. In the Marrakech region, Boussaa et al.<sup>33</sup> classified the species *Sergentomyia* into rural cash and ubiquitous cash (*S. minuta* and *S. fallax*) that were collected in urban and rural areas. Kahim et al.,<sup>16</sup> collected *S. minuta* and *S. fallax* in a semi-arid climate at elevations ranging from 800 to 1,000 m to 2,000 m. According to the same authors, *S. fallax* is most common in sandy loam soil, while *S. minuta* prefers purely sandy soil. Guernaoui et al.,<sup>4</sup> collected these species as well, primarily at 800-1,000 m, with the exception of *S. minuta*, which can survive at 1,200 m.

## Conclusion

The findings of this study revealed that the environmental factors studied have an impact on the distribution of sandfly species in the province of Moulay Yaâcoub. Each species has preferences based on the existing environmental conditions. Altitude, soil, vegetation, and environmental health may all play a role in the abundance of one or more species, thereby determining risk zones. Despite the abundance of sandflies and certain vector species, the leishmaniasis risk can exist only if all leishmaniasis factors, namely the parasite, the tank, and the sandfly vector, are combined. Population and environmental health are risk factors to be studied.

## 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.

**References**

- WHO. Control of leishmaniasis. Technical Report Series, Report of a meeting of the WHO Expert Committee on the Control of Leishmaniasis, Geneva, 22–26 March 2010. [Online] Available from: [http://apps.who.int/iris/bitstream/10665/44412/1/WHO\\_TRS\\_949\\_eng.pdf](http://apps.who.int/iris/bitstream/10665/44412/1/WHO_TRS_949_eng.pdf).
- Lahouiti K, El Ouali Lalami A, Maniar S, Bekhti K. Seasonal fluctuations of phlebotomines sand fly populations (Diptera: Psychodidae) in the Moulay Yaâcoub province, Centre Morocco: effect of ecological factors. *Afr J Envi Sc Tech*. 2013; 7(11):1028–1036.
- Boussaa S, Guernaoui S, Pesson B, Boumezzough A. Seasonal fluctuations of phlebotomine sand fly populations Diptera: Psychodidae) in the urban area of Marrakech, Morocco. *Act Trop*. 2005; 95(2):86-91.
- Guernaoui S, Boumezzough A, Laamrani A. Altitudinal structuring of sandflies (Diptera: Psychodidae) in the High-Atlas Mountains (Morocco) and its relation to the risk of leishmaniasis transmission. *Acta Trop*. 2006; 97(3):346–351
- Ashford RW. The leishmaniasis as emerging and reemerging zoonoses. *Int J Parasitol*. 2000; 30(12-13):1269-1281.
- Lahouiti K, Bekhti K, Fadil M, A. El Ouali Lalami. Entomological Investigations In Moulay Yacoub, Leishmaniasis Focus In The Center Of Morocco. *AJPCR*. 2016; 9(6):1-5.
- Rioux JA, Golvan YJ, Croset H, Houin R, Juminer B, Bain O, and Tour S. Ecology of leishmaniasis in Southern France 1. *Phlebotomus*. Sampling – ethology. *Ann Parasitol Hum Comp*. 1967; 42(6):561-603.
- Sudia WD and Chamberland RW. Battery operated light trap, an improved model. *Mosq News* 1962. 1988;4(4):536-538
- Leger N and Depaquit J. Sandflies and their role in the transmission of leishmaniasis. *Rev Française Lab* 2001; 338:41-48.
- Floch H and Abonnenc E. Sandflies diptera of guyana and French antilles. *Faune Union Fr* 1957; 14:1-207.
- Anon. Activities Guide. Directorate of Epidemiology and Fight Against Diseases. Parasitic Diseases Service. Morocco: Ministry of Health; 1997.
- Aster GDEM, [Online] Available from: <http://www.gdem.aster.ersdac.or.jp/>
- National Institute for Agricultural Research (INRA environment) [Online] Available from: <http://www.inra.org.ma>
- Dedet JP and Belazzoug S. Leishmaniasis in North Africa. Elsevier Science Publishers B.V. (Biomedical Division). 1985
- Rioux JA, Akalay O, Perieres J, Dereure J, Mahjour J, Le Houérou H.N. An eco-epidemiological evaluation of the risk of leishmaniasis in the Atlantic Sahara of Morocco. The predictive value of the relationship between sandflies and climate [Phlebotomus, Sergentomyia, Leishmania. *Ecol Mediterr*. 1997; 23 (3-4):73-92.
- Kahime K, Boussaa S, El Mzabi A, Boumezzough A. Spatial relations among environmental factors and phlebotomine sand fly populations (Diptera: Psychodidae) in central and southern Morocco. *J Vec Eco*. 2015; 40 (2):342-354.
- Rioux JA, Rispaill P, Lanotte G, Lepart J. Phlebotomine-bioclimatic relationships in leishmaniasis ecology. Epidemiological corollaries. The example of Morocco. *Bull Soc Bot Fr*. 1984; 131(2-4):549-557.
- Croset H, Rioux JA, Léger N, Houin R, Cadi Soussi M, Benmansour N, Maistre M. Sampling methods for sandfly populations in the Mediterranean region. In: Rioux, J.A. (Ed.), *Ecology of leishmaniasis*. CNRS, Paris. 1974. 139-151p.
- Rioux JA, Guilvard E, Dereure J, Lanotte G, Denial M, Pralong F. « Natural infestation of *Phlebotomus papatasi* (Scopoli, 1786) by *Leishmania major* MON-25. About 28 strains isolated in an outbreak in southern Morocco», In: *Leishmania. Taxonomy and Phylogeny. Eco-epidemiological applications*. Int Coll CNRS/INSERM. IMEEE, Montpellier, France. 1986: 471-480p.
- WHO. Urbanization: an increasing risk factor for leishmaniasis. *Weekly Epidemiological Record* 2002; 77(44):365-372.
- Faraj C, Adlaoui E, Ouahabi S, El Kohli M, El Rhazi M, Lakraa L, Ameur B. Distribution and bionomic of sandflies in five ecologically different cutaneous leishmaniasis foci in Morocco. *ISRN Epidemiol*. 2013; 1-8.
- El Miri H, Rhajaoui M, Himmi O, Ouahabi S, Benhoussa A, Faraj C. Entomological study of five foci of cutaneous leishmaniasis in the province of Sidi Kacem in northern Morocco, *Annals of the Entomological Society of France; (N.S.)*. *Int J Ent*. 2013; 1-6.
- Talbi FZ, El Ouali Lalami A, Janati Idrissi A, Sebti F, Faraj C. Leishmaniasis in Central Morocco: Seasonal Fluctuations of Phlebotomine Sand Fly in Aichoun Locality, from Sefrou Province; Hindawi Publishing Corporation; *Pathol Res Int*. 2015; 1-4.
- Bailly-Chaumara H, Abonnec E, Pastre J. Contribution to the study of the sandflies of Morocco (Diptera: Psychodidae). Faunistic and ecological data. ORSTOM notebooks, series of Ent Méd and Para IX. 1971; 4:431-460.
- Rioux JA. Eco-epidemiology of leishmaniasis in Morocco: review of 30 years of cooperation. *D.E.L.M. Epidemiol Bull*. 1999; 37:2-10.
- Yahia H, Ready PD, Hamdani A, Testa JM, Guessous IN. Regional genetic differentiation of *Phlebotomus sergenti* in three Moroccan foci of cutaneous leishmaniasis caused by *Leishmania tropica*. *Parasite*. 2004; 11:189-199.
- Guernaoui S and Boumezzough A. Habitat preferences of phlebotomine sandflies (Diptera: Psychodidae) in southwestern Morocco. *J Med Entomol*. 2009; 46:1187-1194.
- Talbi FZ, Sbai A, El Omari H, Najy M, El Ouali Lalami A. Spatial Relation Among Incidence of Leishmaniasis and Altitude Factor of Different Communes of Sefrou Province: Contribution of Geographic Information Systems. [Online] Available from: [https://doi.org/10.1007/978-3-030-37629-1\\_87](https://doi.org/10.1007/978-3-030-37629-1_87).
- Boussaa S and Boumezzough A. Identification and characterization of sandfly breeding sites (Diptera: Psychodidae) in Marrakech (Maroc). *Entomol Faunist* 2014; 67:93-101.
- Killick Kendrick R. Phlebotomine vectors of leishmaniasis: a review. *Med Vet Ent*. 1990; 4:1624.
- Dedet JP, Rioux JP, Rispaill P, Lanotte G, Lepart J. Phlebotomine-bioclimatic relationships in leishmaniasis ecology Epidemiological corollaries. The example of Morocco, *Bulletin of the Botanical Society of France*. *Act Bot*. 1984; 131:2-4.
- Berdjane-Brouk Z, Koné AK, Djimé AA, Charrel RN, Ravel C, Delaunay P, del Giudice P, Diarra AZ, Doumbo S, Goita S, Thera MA, Depaquit J, Marty P, Doumbo OK, Izri A. First detection of *Leishmania major* DNA in *Sergentomyia* (Spelaeomyia) darlingi from cutaneous leishmaniasis foci in Mali. *PLoS ONE*. 2012; 7:1-5.
- Boussaa S, Boumezzough A, Sibold B, Alves-Pires C, Morillas Marquez F, Glasser N, and Pesson B. Phlebotomine sandflies (Diptera: Psychodidae) of the genus *Sergentomyia* in Marrakech region, Morocco. *Parasitol Res*. 2009; 104(5):1027-1033.