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Species Structure and Distribution of Sand Flies (Diptera: Psychodidae) in Different Habitats of Soba and Tutti Island Areas, Khartoum State, Sudan (October 2016 - September 2017)

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Abstract

Presently, there are over 927 known species or subspecies of sand flies, of which approximately 500 species occur in the New World. This study aimed to collect data and gain knowledge on species structure and distribution of sand fly vectors in Soba and Totti Areas, Khartoum State. This study was conducted in Soba and Tutti Island during (October 2016 - September 2017). Sand flies were collected from the study area mainly using the collection method; sticky paper traps (The STs was used to collect sand flies from indoor, outdoor, domestic and forest sites in the area). The STs made of A4 white papers coated by castor oil on both sites to collect sand flies between 6 pm - 6 am. At outdoor sites, the STs were fixed using wood stick at 5 - 10 cm above the ground level. The collected sand fly samples were labeled and identified according to the standard keys. From a total of 223 sand flies collected, a number of 41 (18.4%) were identified as *Phlebotomus papatasi*, 64 (28.7%) as *Sergentomyia claudia*, 96 (43%) as *Sergentomyia antanata* and 22 (9.9%) were identified as *Sergentomyia chowzai* in Soba area. Also, from 59 sand flies collected a number of 16 (27.1%) were identified as *Phlebotomus papatasi*, 4 (6.8%) as *Sergentomyia claudia*, 19 (32.2%) as *S antanata* and 20 (33.9%) were identified as *Sergentomyia chowzai* in Tutti island area. The mean total of sandflies collected in Soba area outdoor was 3.1, indoor (4.4), forest (1.2) and domestic (3.8). While in Tutti island the mean total of sand flies collected at forest were 1.5 and at domestic were 2.7. Soba and Tutti island areas were infested with sand fly species include: *Phlebotomus papatasi, Sergentomyia claudia*, *Sergentomyia antanata* and *Sergentomyia chowzai*. Understanding the requirement of sand flies is very crucial for their breeding habitats in order to control sand flies by treating specific areas, rather than using area-wide applications of pesticides. Knowledge on sandflies habitats and ecological distribution is prerequisite as to specific locations, logic control methods should be implemented, and treatment has to be applied.

Keywords: *Sandflies; Soba; Tutti Island; Khartoum*

Introduction

Phlebotomine sand flies (Diptera: Psychodidae) are of considerable public health importance because of their ability to transmit several viral, bacterial, and protozoan disease-causing organisms to humans and other animals [1]. Of these diseases, leishmaniasis is the most important one, especially the visceral form because it is often fatal if untreated. Leishmaniasis is considered as a major public health problem, 88th in the world causing morbidity and mortality [2]. The disease also causes serious economic loss and impedes socioeconomic development in many countries [2].

Leishmaniasis is an endemic disease spread over a wide geographical area in Sudan [3,4]. Cutaneous leishmaniasis (CL) occurs in a fluctuating pattern in the country mainly in the west, central and northern parts of Sudan [3]. Whereas, visceral leishmaniasis (VL) is endemic in Savannah areas extending from the Sudanese-Ethiopian border in the east to the banks of the White Nile in the west, and from Kassala in the East towards Blue Nile State to the south with scatter foci (sandflies habitats) in Nuba Mountain and Darfur [4].

In Sudan, studies on sand flies revealed that the sand fly *Phlebotomus orientalis* and *P. papatasi*, are the principal vectors of VL and CL respectively [2,5-7]. *P. orientalis* the vector of *Leishmania donovani* has been found to prefer habitats of woodlands dominated by *Acacia seyal* and *Balanites aegyptiaca* which grow on black cracking clay soil [5]. However, it has been collected from villages [11,14] which interfere such habitats. Currently, this species was collected from villages in White Nile and Khartoum states in a semi-desert region [8,9]. In contrast, *P. papatasi* the vector of *L. major*, has a wide range of distribution including many parts of the arid areas of Sudan [3,8]. Moreover, the two sand fly species, showed seasonal variation in their numbers [10,11], which disappear during the rainy season (July-October) and reach its peak during the dry season (April-May) [10,11]. Leishmaniasis (both VL and CL) remain one of the most serious public health problem in many regions in Sudan. Visceral leishmaniasis affect population in rural productive agricultural areas like White Nile and Gedarif states whereas, the CL affect most of the arid region in the country including, Darfur, Kordofan, White Nile, Khartoum, River Nile, Northern and Kassala states. The control of leishmaniasis in affected areas mainly depends on the case-management (diagnosis and treatment). Although, insecticide impregnated bed nets (ITNs) has recently been used for control of VL transmission in Gedarif area. Insecticide impregnated bed nets vector control interventions targeting sand flies remain controversial. In most regions in Sudan, sand flies control is mainly a collateral benefit of mosquitoes control programs. The lack of sand flies specific control interventions most probably due to little information known about the biology and ecology of the sand fly vectors of *Leishmania* parasites of both VL and CL. In order to develop or improve current control programs, information about species composition, spatio-temporal distribution and abundance of sand fly vectors in leishmaniasis-affected regions in Sudan is a prerequisite. Therefore, the current study was carried out for one year in Khartoum state to determine some aspects on ecology of sand flies.

Aim of the Study

This study aimed to study species structure and distribution of sand flies (Diptera: Psychodidae) in different habitats of Soba and Tutti island areas, Khartoum state, Sudan (October 2016 - September 2017).

Materials and Methods

Study design

This study was carried out as cross-sectional entomological surveys at different locations in study area. Sand flies were collected monthly during October2016 - September 2017 from different locations in Khartoum State. The sand flies collection sites were selected to represent all the habitats prevailing in the State. Sticky paper traps (STs) were used to collect sand flies from each location. Moreover, detailed descriptions of the habitats related to sand fly species were recorded. Then sand flies will be collected STs. Sand flies were collected from outdoor, indoor, domestic and forest sites using sticky paper traps in Soba area while forest and domestic collection were only used in Tutti island area. In each site, 60 STs/night were used to collect sand flies for three consecutive nights every month. Moreover, in each study site, sand flies were collected from possible resting places (i.e. rooms, animal sheds and cracks on the ground close the Blue Nile). The captured sand flies from each site every night/month was preserved in 70% ethanol, and then identified and recorded.

Study area

The present study was carried out in Khartoum State, Sudan from October 2016 to September 2017. Khartoum State is one of the eighteen states although it is smallest state by area (22.142 km^2) it is the most populous (5.274.321 in 2008 census). It contains three largest city's Omdurman, Bahri and Khartoum cities, which is the capital of the state as well as the national capital of Sudan (Map 1).

Map 1: *Map of Khartoum State, showing the study sites (Source: https://www.google.com/search).*

The State locate in the east north in the central Sudan at the confluence of the White Nile and Blue Nile, where the two rivers unite to form the River Nile. The State lies between longitude 31.5 to 34 eastern and latitude 15 to 16 northern. It's surrounded by River Nile state in the north-east in the north-west by Northern States, in the east and southeast by the States of Kassala, Gedaref, Gezira and the west by North Kordofan. The landscape is a vast plain sloping gently towards the river. Moreover, the ground on either side of the river is rocky more sand than silt is brought down by seasonal floodwaters.

Study sites selection

The two sites Soba and Tutti island area (Map 2), were selected for the study of sand fly species composition because of their location besides Blue Nile River where preferred habitats for sand fly expected to be found in addition to suitability of climate to the species survival.

Map 2: *Map of Khartoum State, showing Tutti Island the study sites 1 (Source: https://www.google.com/search).*

Soba area

It is located between Latitude/longitude: 15°30′24″N32°38′37″E with decimal coordinates: 15.5069 32.6437 and Altitude: 386 m. The area is near the high road traffic Medani-Khartoum. The most features of the area were agriculture beside the bank of Blue Nile, and Brick factories. There were many institutional there such as Soba Teaching Hospital and College of forest, University of Sudan in addition to Veterinary Center for researches.

Described sand fly habitat

Tutti Island is an island in Sudan where the White Nile and Blue Nile merge to form the main Nile. It is surrounded by Khartoum (the capital of Sudan), Omdurman (the largest city in Sudan), and Bahri, a large industrial center). Despite this, Tutti island include only one small village (founded in the late 15th century), with grassland being the main makeup of the island. In the past the only approach to Tutti island was via several ferries that cross the river every so often, but now the Tutti island Bridge, a modern suspension bridge, has been completed and can be used instead. island mainly their works is agricultural activities, where Khartoum gets most of its supply of fruits and vegetables and therefore, many farms situated all around the island, many of them still using manual methods of farming. There of acres of green fields and lime groves. Its eight square kilometers of fertile land are covered with citrus orchards, vegetable farms, gorse hedgerows and narrow muddy lanes where donkeys and rickshaws are the main source of transport.

Data collection

Sampling techniques

Sand flies were collected from the study area mainly using sticky paper traps (STs). The STs were used to collect sand flies from indoor, outdoor, domestic and forest sites in the study area. The STs made of Xerox A4 white papers (15 × 21) coated by castor oil on both sites was used to collect sand flies between 6 pm - 6 am. The STs were fixed using wood stick at 5 - 10 cm above the ground level and collected next morning.

Preservation, mounting and identification of sand flies

Captured sand flies were immersed in detergent solution (one drop of baby shampoo in 30 ml of water) and sorted out from other insects. For those collected by STs were washed carefully to remove the oil and for initial clearance of specimens. The captured sand flies was then be transferred and kept in 70% ethanol in 30 ml glass vials and well-labeled. The labels included the trap number, the site (location), habitat type and date of collection. The ethanol-preserved sand flies in the vials were kept in room temperature for subsequent species identification. The preserved sand flies were mounted on glass slides on Berlese's medium for species identification. Each individual sand fly was placed on a drop of Berlese's medium on a clean glass slide, then under a dissecting microscope the head of sand fly will be separated and placed (on its dorsal side) near the rest of the body. Then a cover slip was applied gently on the mounted specimen. The mounted specimens were left at least for 12 hours to permit clearing of chitin us layer of the insect body. The mounted sand flies were examined under the microscope at 40× (magnification). Structural features used for species identification, were spermatheca of females, external genitalia of males, pharynx and cibarium structure for both sexes. Identification was done using sand flies proper identification keys constructed by Kirk and Lewis [12] and Quate [10].

Entomological studies

Seasonal variation in numbers of sand fly species in the study area

To determine the fluctuation of number of sand fly species in different insects was collected from different location and habitats during October 2016 - Sept. 2017 by two aforementioned trapping methods. For this purpose, 20 - 30 STs was used in each site to collect sand flies for three consecutive nights every month. Captured sand flies by each trap from each site were preserved in 70% ethanol, mounted, identified and recorded.

Abundance and geographical distribution of sand flies in the study area

Sand flies were collected from different location (sentinels) in the state set for entomological surveillance. Moreover, each sentinel site was divided into habitats representing those prevailing in the area. In each sentinel site, sand flies was collected from each habitats by STs. Sand flies collected by each type of trap from each habitat in the selected sites were preserved in 70% ethanol, mounted, identified and recorded.

Description of sand flies habitats

In this study, the selected habitats in each location for sand flies collection in the area was fully described using a well-design format. The form was designed to record information on the soil type and texture, vegetation cover, animals, household description and population activities. Moreover, data of mean monthly temperature, rainfall and humidity was collected from Meteorological Department, Khartoum State.

Data analysis

The data obtained from this study was entered in a computer and analyzed using SPSS software ver. 20. Data from different experiments in this study was analyzed by suitable statistical tests. Descriptive test was used to analyze data of sand fly species. ANOVA was used to compare abundance of sand flies between months, locations and habitats. In addition, chi-square test was used to determine the sand fly vector habitat preference.

Results

Study findings

In this study, two-genus and four species sand fly was recorded; one species *Phlebotomus* and three species *Sergentomyia* sand flies. These species namely were *P. papatasi*. *S. claudia*, *S. antanata* and *S. schwetzi.*

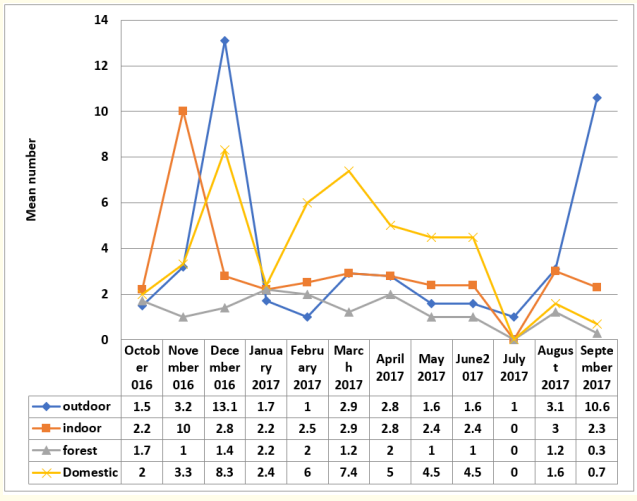
Collection of sticky traps from different habitats in soba and Tutti island areas

Table 1 and figure 1 shows the mean number of sand fly collected from different habitats in Soba area during the period from October 2016 to September 2017.

Month	Location	Total sand fly collected
		Mean ± SE
October 2016	Outdoor	1.5 ± 0.5
	Indoor	2.2 ± 0.5
	Forest	1.7 ± 0.5
	Domestic	2.0 ± 0.5
	Total	1.9 ± 0.5
November 2016	Outdoor	3.2 ± 0.9
	Indoor	10.0 ± 2.9
	Forest	1.0 ± 1.0
	Domestic	3.3 ± 1.1
	Total	4.4 ± 1.1
December 2016	Outdoor	13.1 ± 2.1
	Indoor	2.8 ± 0.5
	Forest	1.4 ± 0.4
	Domestic	8.3 ± 0.5
	Total	6.4 ± 0.2
January 2017	Outdoor	1.7 ± 0.6
	Indoor	2.2 ± 0.2
	Forest	0.5 ± 0.2
	Domestic	2.4 ± 0.5

	Total	1.7 ± 0.2
February 2017	Outdoor	1.0 ± 0.0
	Indoor	2.5 ± 0.4
	Forest	2.0 ± 0.4
	Domestic	6.0 ± 0.5
	Total	2.9 ± 0.7
March 2017	Outdoor	2.9 ± 0.7
	Indoor	2.9 ± 0.1
	Forest	1.2 ± 0.2
	Domestic	7.4 ± 0.7
	Total	3.6 ± 0.1
April 2017	Outdoor	2.8 ± 0.6
	Indoor	2.8 ± 0.1
	Forest	2.0 ± 0.5
	Domestic	5.0 ± 0.4
	Total	3.2 ± 0.7
May 2017	Outdoor	1.6 ± 0.4
	Indoor	2.4 ± 0.8
	Forest	1.0 ± 0.0
	Domestic	4.5 ± 0.3
	Total	2.4 ± 0.5
June 2017	Outdoor	1.6 ± .4
	Indoor	2.4 ± .8
	Forest	1.0 ± .00
	Domestic	4.5 ± .3
	Total	2.4 ± .5
July 2017	Outdoor	1.0 ± .5
	Indoor	.00 ± .00
	Forest	.00 ± .00
	Domestic	.00 ± .00
	Total	.3 ± .2
August 2017	Outdoor	3.1 ± .6
	Indoor	3.0 ± 1.4
	Forest	1.2 ± .2
	Domestic	1.6 ± .4
	Total	2.2 ± .4
September 2017	Outdoor	10.6 ± 0.6
	Indoor	2.3 ± 0.1
	Forest	.3 ± .3
	Domestic	.7 ± .2
	Total	3.5 ± .9

Table 1: *Mean number of sand fly collected from different habitats in soba area during October 2016-Setember 2017.*



	October 016	November 016	December 016	January 2017	February 2017	March 2017	April 2017	May 2017	June2017	July 2017	August 2017	September 2017
outdoor	1.5	3.2	13.1	1.7	1	2.9	2.8	1.6	1.6	1	3.1	10.6
indoor	2.2	10	2.8	2.2	2.5	2.9	2.8	2.4	2.4	0	3	2.3
forest	1.7	1	1.4	2.2	2	1.2	2	1	1	0	1.2	0.3
Domestic	2	3.3	8.3	2.4	6	7.4	5	4.5	4.5	0	1.6	0.7

Figure 1: *Mean sand fly collected from different habitats in Soba area during October 2016 - September 2017.*

Collection from outdoor habitats

High collection of outdoor was obtained in December 2016 with mean of (13.1) followed by September 2017 with mean of (10.6).

Collection from indoor habitats

Regarding indoor collection of sand fly the high mean was found in November 2016 with mean of (10) followed by March 2017 with mean of (2.9).

Collection from forest habitats

In forest collection the high captured was observed in January 2017 (2.2) followed by February and April 2017 with mean of (2.0).

Collection from domestic habitats:

The high domestic collection was found during December 2016 with mean of 8.3 followed by March 2017 (7.4).

Table 2 and figure 2 shows that the mean total of sand fly collected during the study period in outdoor collection was (3.1), (4.4) in indoor collection, (1.2) in forest collection and (3.8) in domestic collection. However, high collection was observed in forest collection (4.4) followed by domestic collection (3.8).

Area	Location	Total Sand Fly Collected
		Mean ± SE
Soba	Outdoor	3.1 ± .4
	Indoor	4.4 ± .8
	Forest	1.2 ± .1
	Domestic	3.8 ± .7

Table 2: *Mean total of sand fly collected from different habitats in soba area.*

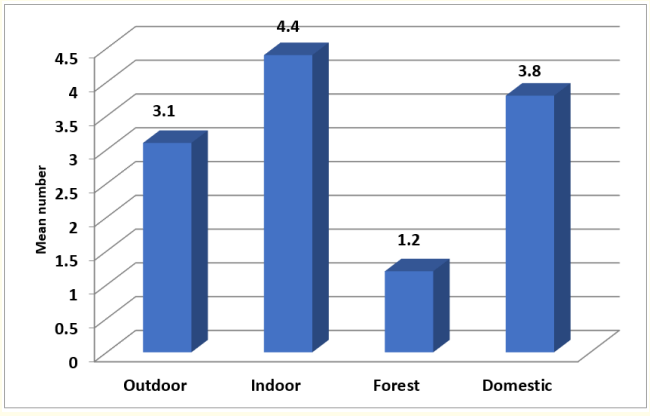


Figure 2: *Mean total of sand fly collected from different habitats in Soba area.*

Table 3 explains that there were no significance differences between sand fly collected in different months of the study period in Soba and Tutti island area, $p > 0.05$.

Month	Location	Total sand fly collected
		Mean ± SE
October 2016	Forest	.00 ± .00
	Domestic	2.0 ± .1
	Total	1.0 ± .8
November 2016	Forest	3.0 ± .00
	Domestic	1.7 ± .6
	Total	2.4 ± .5
December 2016	Forest	3.5 ± .5
	Domestic	3.0 ± .00
	Total	3.3 ± .4
January 2017	Forest	3.0 ± .5
	Domestic	6.1 ± .8
	Total	4.6 ± .7
February 2017	Forest	0.0 ± 0.0
	Domestic	0.3 ± .3
	Total	0.2 ± .3
March 2017	Forest	1.5 ± .1
	Domestic	2.2 ± .9
	Total	1.9 ± .7
April 2017	Forest	0.3 ± .3
	Domestic	1.7 ± .8

	Total	1.0 ± .5
May 2017	Forest	2.0 ± .00
	Domestic	2.0 ± .00
	Total	2.0 ± .00
June 2017	Forest	1.5 ± 1.5
	Domestic	.00 ± .00
	Total	0.8 ± .1
July 2017	Forest	.00 ± .00
	Domestic	.00 ± .00
	Total	.00 ± .00
August 2017	Forest	1.0 ± .5
	Domestic	.00 ± .00
	Total	0.5 ± .4
September 2017	Forest	1.3 ± .3
	Domestic	2.0 ± .1
	Total	1.7 ± .5

Table 3: *Mean of sand fly collected from different habitats in tutti island area during October 2016-Setember 2017.*

Table 4 and figure 3 demonstrates that, the high mean of forest collection was recorded in December 2016 (3.5) followed by November 2016 (3.0). While the high mean of sand fly collected in domestic was reported in January 2017 (6.1) and March 2017 (2.2).

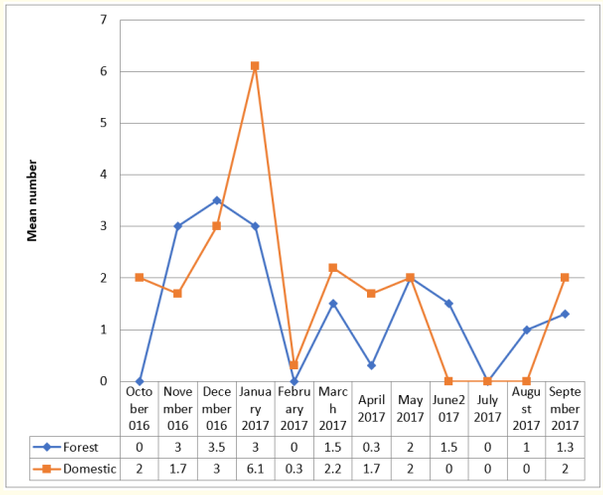


Figure 3: *Mean of sand fly collected from different habitats in Tutti island area during October 2016 - September 2017.*

Collection from forest and domestic habitats in Tutti island area

The mean total of sand fly collected during the study period in island area was found to be (2.2) and the mean total of forest collection was (1.5) while the mean total for domestic collection was (2.7) as shown in table 4.

Location	Mean ± SE
Forest	1.5 ± .3
Domestic	2.7 ± .7
Total	2.2 ± .4

Table 4: *Mean total of sand fly collected from different habitats in tutti island area.*

Table 5 indicates the mean of male and female sand fly collected from different habitats in Soba area during October 2016 - September 2017.The high mean of male sand fly was collected during December 2016 and November 2016 with mean of (3.6) and (3.1), respectively. However, the high mean of female of sand fly collected was observed during December 2016 (3.9) followed by September 2017 (2.2).

Month	Mean ± SE	
	Male	Female
October 2016	1.2 ± 0.3	0.7 ± 0.3
November 2016	3.1 ± 0.7	1.8 ± 0.5
December 2016	3.5 ± 0.9	3.9 ± 1.3
January 2017	1.4 ± 0.3	0.3 ± 0.1
February 2017	1.7 ± 0.5	1.3 ± 0.3
March 2017	2.5 ± 0.7	1.1 ± 0.3
April 2017	2.0 ± 0.6	1.0 ± 0.2
May 2017	0.9 ± 0.2	0.9 ± 0.2
June2017	1.4 ± 0.4	1.2 ± 0.3
July 2017	0.3 ± 0.1	0.0 ± 0.0
August 2017	1.6 ± 0.3	0.8 ± 0.3
September 2017	2.0 ± 0.7	2.2 ± 0.5
Total	1.9 ± 0.2	1.4 ± 0.2

Table 5: *Mean number of male and female sand fly collected from different habitats in soba area during October 2016-September 2017.*

Table 6 shows the means of male and female sand fly collected from different habitats in Tutti island area during October 2016 - September 2017. The high mean number of male sand fly collected was captured January 2017 (3.3) followed by December 2016 (2.0). While the high mean of female caught was found during January 2017 (1.7) followed by November 2016 (1.5).

Month	Mean ± SE	
	Male	Female
October 2016	1.0 ± 0.7	0.5 ± 0.2
November 2016	1.2 ± 0.6	1.5 ± 0.9
December 016	2.0 ± 0.1	1.3 ± 0.6
January 2017	3.3 ± 1.2	1.7 ± 0.6
February 2017	0.0 ± 0.0	0.3 ± 0.3
March 2017	0.9 ± 0.3	1.0 ± 0.6
April 2017	0.3 ± 0.1	0.8 ± 0.4
May 2017	1.0 ± 0.1	1.0 ± 0.1

June2017	0.3 ± 0.3	0.3 ± 0.1
July 2017	0.0 ± 0.0	0.0 ± 0.0
August 2017	0.4 ± 0.2	0.2 ± 0.1
September2017	0. 6 ± 0.2	1.1 ± 0.3
Total	1.2 ± 0.3	1.9 ± 0.1

Table 6: *Mean number of male and female sand fly collected from different habitats in tutti island area during October 2016-September 2017.*

Table 7 shows that there was no significant difference between male collected in study areas, $p > 0.05$ and also no significance difference was found between female sand fly collected in study areas, $p > 0.05$.

Month	Mean ± SE			
	Unfed	fed	Half gravid	Gravid
October 2016	0.5 ± 0.3	.00 ± .00	0.1 ± 0.1	0.4 ± 0.2
November 2016	1.4 ± 0.3	.00 ± .00	0.00 ± 0.00	0.2 ± 0.1
December 2016	3.7 ± 1.2	.00 ± .00	0.04 ± 0.04	0.2 ± 0.1
January 2017	0.2 ± 0.1	.00 ± .00	0.00 ± 0.00	0.1 ± 0.1
February 2017	1.1 ± 0.9	.00 ± .00	0.00 ± 0.00	0.2 ± 0.1
March 2017	0.9 ± 0.3	.00 ± .00	0.00 ± 0.00	0.2 ± 0.1
April 2017	0.8 ± 0.2	.00 ± .00	0.00 ± 0.00	0.3 ± 0.1
May 2017	0.7 ± 0.2	.00 ± .00	0.00 ± 0.00	0.3 ± 0.1
June 2017	0.5 ± 0.2	.05 ± .05	0.1 ± 0.01	0.4 ± 0.1
July 2017	0.0 ± 0.0	.00 ± .00	0.00 ± 0.00	0.00 ± 0.00
August 2017	0.7 ± 0.3	.04 ± .01	0.04 ± 0.01	0.1 ± 0.1
September 2017	2.0 ± 1.1	.00 ± .00	0.05 ± 0.01	0.1 ± 0.01
Total	1.1 ± .1	.01 ± .01	0.02 ± 0.001	0.2 ± 0.03

Table 7: *Mean number of female abdominal appearance during different months of the study period in soba area.*

Sella stage classification

Table 8 explains the mean of female abdominal appearance during different months of the study period in soba area. The high mean of unfed sand fly was observed during December 2016 (3.7) while the high mean fed sand fly was recorded in June 2017 (0.05) while high half gravid was shown in October 2016 (0.1) and June 2017 (0.1). However, the high mean of gravid female was reported in April and May 2017 respectively (0.4) for each.

Month	Mean ± SE			
	Unfed	fed	Half gravid	Gravid
October 2016	.5 ± .2	.00 ± .00	.00 ± .00	.00 ± .00
November 2016	1.5 ± .9	.00 ± .00	.00 ± .00	.00 ± .00
December 2016	1.3 ± .7	.00 ± .00	.00 ± .00	.00 ± .00
January 2017	.8 ± .3	.00 ± .00	.00 ± .00	.8 ± .4

February 2017	.3 ± .3	.00 ± .00	.00 ± .00	.00 ± .00
March 2017	1.0 ± .5	.00 ± .00	.00 ± .00	.00 ± .00
April 2017	.5 ± .2	.00 ± .00	.00 ± .00	.3 ± .1
May 2017	.5 ± .1	.00 ± .00	.00 ± .00	.5 ± .1
June 2017	. 7 ± .1	.00 ± .00	.00 ± .00	.00 ± .00
July 2017	.00 ± .00	.00 ± .00	.00 ± .00	.00 ± .00
August 2017	.2 ± .1	.00 ± .00	.00 ± .00	.00 ± .00
September 2017	.3 ± .1	.3 ± .1	.00 ± .00	.6 ± .4
Total	.7 ± .1	.03 ± .02	.00 ± .00	.3 ± .1

Table 8: *Distribution of female abdominal appearance during different months of the study period in tutti island area.*

Table 9 explains the mean of female abdominal appearance during different months of the study period in Tutti island area. The high mean of unfed sand fly was observed during January 2017 (0.8) while the high mean fed sand fly was recorded in September 2017 (0.3). However, the high mean of gravid female was reported in January 2017 (0.8).

Month	Species identified				Total	P-value
	P. papatasi	*S. claudia*	*S. antanata*	*S. chowzai*		
October 2016	1(2.4)	4(6.2)	6 (6.2)	2 (9.1%)	13 (6.1%)	0.000*
November 2016	5 (12.2%)	7 (10.9%)	9 (9.4%)	4 (18.2%)	25 (11.4%)	
December 2016	5 (12.2%)	8 (12.5%)	8 (8.3%)	1(4.5%)	22(9%)	
January 2017	2 (4.9%)	7(10.9%)	7 (7.3%)	1(4.5%)	17(7.8%)	
February 2017	6 (14.6%)	7 (10.9%)	9 (9.4%)	0 (0.0%)	22 (9%)	
March 2017	7 (17.1%)	6 (9.4%)	12 (12.5%)	4 (18.2%)	29 (11.8%)	
April 2017	4 (9.8%)	1 (1.6%)	11 (11.5%)	2 (9.1%)	18 (7.8%)	
May 2017	3 (7.3%)	7 (10.9%)	7 (7.3%)	1 (4.5%)	18 (7.3%)	
June 2017	2 (4.9%)	6 (9.4%)	9 (9.4%)	1 (4.5%)	18 (7.3%)	
July 2017	0 (0.0%)	1 (1.6%)	1 (1%)	0(0.0%)	2 (4.5%)	
August 2017	3 (7.3%)	8 (12.5%)	9 (9.4%)	3 (13.6%)	23 (9.4%)	
September 2017	3 (7.3%)	2 (3.1%)	8(8.3%)	3 (13.6%)	16 (7.5%)	
Total	41 (100%)	64 (100%)	96 (100%)	22 (100%)	223 (100%)	

Table 9: *Distribution of sand fly species identified during different months of the study period in soba area χ^2 = 95.576, df=44; *p-Value significant at less than 0.05 levels.*

Seasonal variations of sand fly species in different areas:

Table 10 and figure 4 indicate the distribution of species composition in summer, winter and rainy season in Soba area. There was highly significance differences between species composition during different season (p = 0.003).

Month	*P. papatasi*	*S. claudia*	*S. antanata*	*S. chowzai*	Total	P-value
October 2016	1 (6.3%)	1 (25%)	1 (5%)	1 (5%)	4 (6.5%)	0.522
November 2016	0 (0.0%)	1 (25%)	2 (10%)	1 (5.3%)	4 (6.5%)	
December 2016	0 (0.0%)	0 (0.0%)	1 (5%)	2 (10.5%)	3 (4.8%)	
January 2017	0 (0.0%)	1 (25%)	6 (30%)	5 (26.3%)	12 (21%)	
February 2017	2 (12.5%)	0 (0.0%)	1 (5%)	0 (0.0%)	3 (4.8%)	
March 2017	3 (18.8%)	0 (0.0%)	2 (10%)	3 (15.8%)	8 (14.5%)	
April 2017	3 (18.8%)	0 (0.0%)	1 (5%)	3 (15.8%)	7 (11.3%)	
May 2017	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (10.5%)	2 (3.2%)	
June2017	2 (12.5%)	0 (0.0%)	0 (0.0%)	1 (5%)	3 (3.2%)	
July 2017	2 (12.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (3.2%)	
August 2017	3 (18.8%)	0 (0.0%)	2 (10%)	0 (0.0%)	5 (8.1%)	
September 2017	0 (0.0%)	1 (25%)	3 (15%)	2 (10.5%)	6 (11.3%)	
Total	16 (100%)	4 (100%)	19 (100%)	20 (100%)	59 (100%)	

Table 10: *Distribution of sand fly species identified during different months of the study period in tutti island Area χ^2 = 112.160, df = 44; *p-value significant at less than 0.05 levels.*

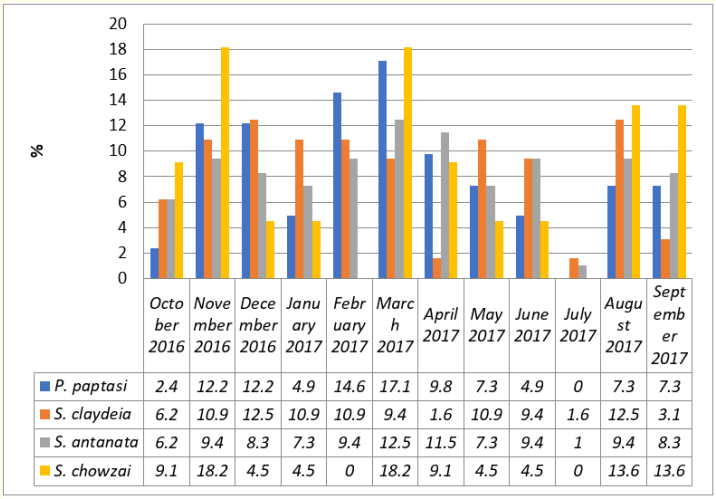


Figure 4: *Distribution of sand fly species identified during different months of the study period in Soba area.*

The high prevalence of *S. antanata* significantly was sharply increased during summer season (49.1%) compared to 40.8% in winter and 41.9% in rainy season. *P. papatasi* was significantly increased in rainy season (18.6%) while *S. chowzai* significantly showed less prevalent during different season.

Table 10 and figure 5 shows the seasonal variations of species composition in different seasons in Tutti island area. The high occurrence of *P. papatasi* and *S. antanata* were not significantly (p = .481) appeared in summer (rainy season; (50.0%) for *P. papatasi* and for *S.*

antanata increased in summer (62.7%). In addition, the high occurrence of *S. claudia* was not significantly observed in summer (25.0%) and rainy season (16.7%). *S. chowzai* not significantly showed high occurrences in winter (46.1%).

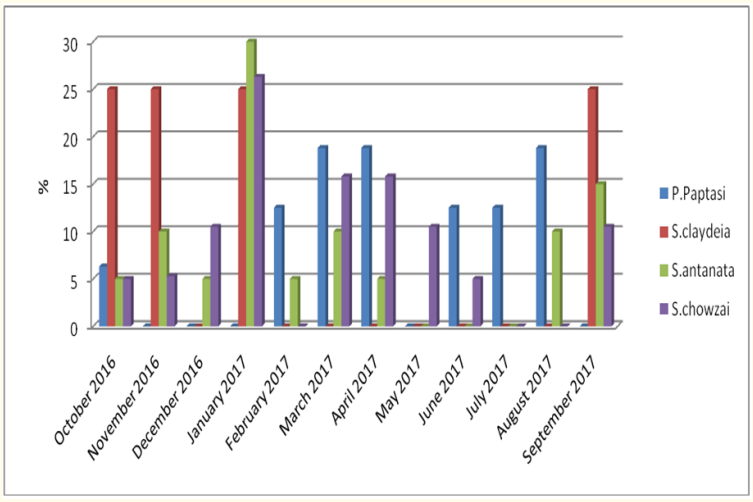
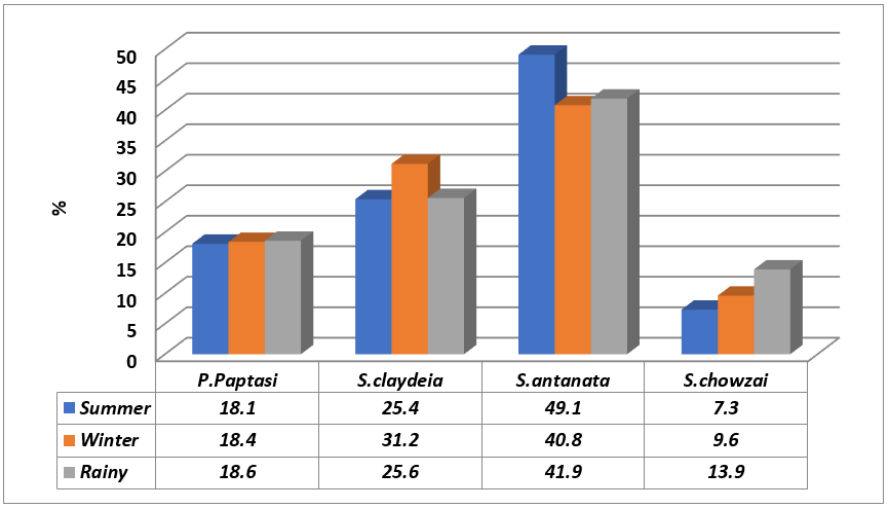


Figure 5: *Distribution of sand fly species identified during different months of the study period in Tutti area.*

Species composition identification

Table 11 and figure 6 indicates the distribution of sand fly species identified during different months of the study period in Soba area.



	P.Paptasi	S.claydeia	S.antanata	S.chowzai
Summer	18.1	25.4	49.1	7.3
Winter	18.4	31.2	40.8	9.6
Rainy	18.6	25.6	41.9	13.9

Figure 6: *Seasonal variations of sand fly species composition in different seasons in Soba area.*

Area	Species composition	Season				P-value
		Summer	Winter	Rainy	Total	
Soba	*P. Papatasi*	10 (18.1%)	23 (18.4%)	8 (18.6%)	41 (18.4%)	.003*
	S. claudia	14 (25.4%)	39 (31.2%)	11 (25.6%)	64 (28.7%)	
	S. antanata	27 (49.1%)	51 (40.8%)	18 (41.9%)	96 (39.0%)	
	S. chowzai	4 (7.3%)	12 (9.6%)	6 (13.9%)	22 (9.9%)	
	Total	55 (100%)	125 (100%)	43 (100%)	223 (100%)	

Table 11: *Seasonal variations of sand fly species composition in different seasons in soba area.*

**p-value significant at less than 0.05 levels.*

From 213 sand flies collected, a number of 41 (19.2%) were identified as *P. papatasi*, 64 (30.0%) were *S. claudia*, 96 (45.1%) were *S. antanata* and 22 (10.3%) were identified as *S. chowzai*. High appearance of *P. papatasi* was observed in March 2017 (17.1%), while high distribution of *S. claudia* was also found in December 2016 (12.5%) and August 2017 (12.5%). However, a high percentage of *S. antanata* was recorded during March 2017 (12.5%) and high appearance of *S. chowzai* was seen during November 2016 (18.2%) and March 2017 (18.2%). However, *P. papatasi, S. claudia, S. antanata* and *S. chowzai* were significantly present during the months of the study period in Wad alagali area, $p < 0.05$. Table 12 and figure 7 indicates the distribution of sand fly species identified during different months of the study period in island area. From a total of 59 sand flies collected a number of 16 (27.1%) were identified as *P. papatasi*, 4 (6.8%) were *S. claudia*, 19 (32.2%) were *S. antanata* and 20 (33.9%) were identified as *S. chowzai*. High appearance of *P. papatasi* was observed in August 2017 and March 2017 (18.8%), while high distribution of *S. claudia* was also found in October 2016, November 2016 and September 2017 (18.8%). However, a high percentage of *S. antanata* was recorded during January 2017 (30.0%) and high appearance of *S. chowzai* was seen during January 2017 (26.3%). However, *P. papatasi*, *S. claudia, S. antanata* and *S. chowzai* were not significantly present during the months of the study period in island area, $p > 0.05$.

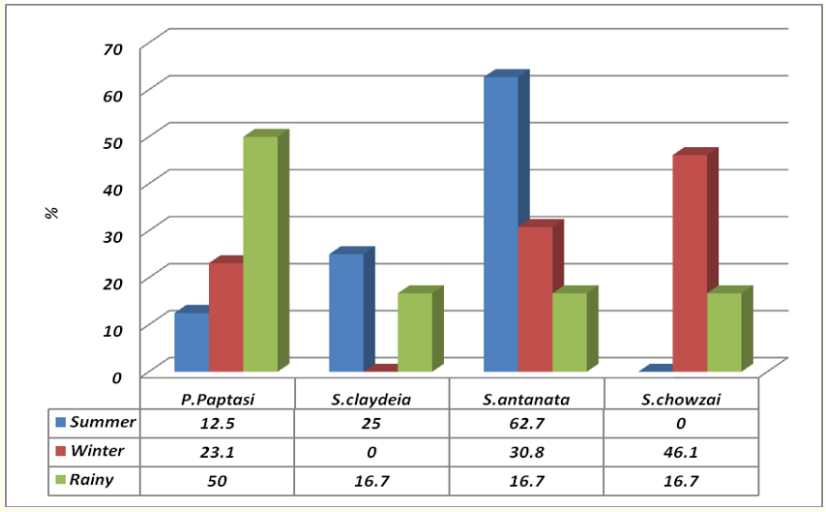


Figure 7: *Seasonal variations of sand fly species composition in different seasons in Tutti island area.*

Are	Species composition	Season				P-value
		Summer	Winter	Rainy	Total	
Tuti	*P. Papatasi*	1 (12.5%)	9 (23.1%)	6 (50.0%)	16 (27.1%)	.481
	S. claudia	2 (25.0%)	0 (0.0%)	2 (16.7%)	4 (6.8%)	
	S. antanata	5 (62.75%)	12 (30.8%)	2 (16.7%)	19 (32.2%)	
	S. chowzai	0 (0.0%)	18 (46.1%)	2 (16.7%)	20 (33.9%)	
	Total	8 (100%)	39 (100%)	12 (100%)	59 (100%)	

Table 12: *Seasonal variations of sand fly species composition in different seasons in tutti island area.*

**p-value significant at less than 0.05 levels.*

Discussion and Conclusion

The current study showed that the high collection of outdoor was obtained in December 2016 followed by September in Soba area. Regarding indoor collection of sand fly, the high collection was found in November 2016 followed by March 2017. In forest, collection the high captured was observed in January 2017 followed by February and April 2017. However, the high domestic collection was found during December 2016 followed by March 2017. The findings demonstrate that sand fly species was captured from different habitats in different months; this may be because of availing of ideal environmental factors. Similar studies proved collection through sticky traps from exophilic (resting outdoor) behavior and it is in agreement with those of earlier studies in Sudan [13,14] and neighboring districts in northern Ethiopia [15]. One study identified the sandfly species and their behavior in forest and anthropic environments in Paraná State, Brazil (Silva., *et al.* 2008) was agreed with the study finding. Habitats of these flies can be in different areas, including rain forests, desert, and rural, urban, sylvatic and domestic areas in Asia, Africa, Europe and South America [16,17]. Singh and Ipe [18] identified the distributional patterns of sand flies in India.

There were no significant differences between sand fly collected in different months of the study period in Soba and Tutti island areas, $p > 0.05$. This difference may be due to variation in climatic factors as rainfall, winds and temperature may be the most important factors effecting the distribution of sand fly species. The study indicated that high mean of male sand fly was collected during December 2016 and November 2016, respectively and high mean of female of sand fly collected was observed during December 2016 (3.9) followed by September 2017 in soba area. High mean number of male sand fly was collected captured in January 2017 followed by December 2016 and the high mean of female caught was found during January 2017 followed by November 2016. The male to female ratio was found 1.3:1.0 in soba and 1:1.6 in Tuti island area. The study indicated high number of male caught in Soba this maybe because the sticky traps were caught near the breeding habitats.

The sex ratio in the present study was similar to other reports [19,20]. They consider this finding as a strategy used by the species that is caused by the production of male pheromones and as a consequence females are attracted and other males are recruited to feeding and mating sites [19,20]. At first, the males seem to be attracted by the odor of wild host animals and then the pheromone produced by the first male may act as an additional attractant for male and female sand flies [20].

In soba area the high mean of unfed sand fly was observed during December 2016 while the high mean fed sand fly was recorded in June 2017, high half gravid was shown in October 2016 and June 2017. However, the high mean of gravid female was reported in April and May 2017, respectively.

The mean of female abdominal appearance (Sella stage) during different months of the study period in Tutti island area showed that the high mean of unfed sand fly was observed during January 2017 while the high mean fed sand fly was recorded in September 2017.

However, the high mean of gravid female was reported in January 2017. The finding indicated that high abundance of sand fly with different sella stage, which indicated availability of climatic factors and reservoirs. Similar study proved that females present at the sites were nullipars, resting after a blood meal, and gravid females, searching for protection from possible predation and environmental pressure as rain and wind. The absence of wind, the humidity, the type of soil and the decomposing organic matter found in armadillo shelters seem to contribute to the presence of the five species captured at these sites. The odor of organic matter attracts gravid females and in addition, food extracts and rabbit feces combined with oviposition pheromones elicit a positive response from gravid *Lu. longipalpis* females [21].

The study showed that there were main four sand fly species predominant in Soba and Tutti island areas includes; *P. papatasi*, *S. claudia*, *S. antanata* and *S. chowzai*. Surveys of the Phlebotomine fauna in a focus of zoonotic cutaneous leishmaniasis (ZCL) in the Al-Hassa oasis, Eastern Province, Saudi Arabia, revealed only one species of *Phlebotomus* (*P. papatasi*) and three of *Sergentomyia* (*S. antanata*, *claudia* and *S. fallax*) [22].

P. papatasi is the vector of *L. major* that causes zoonotic CL in humans in many countries in Africa and Asia [1]. It can be found locally all around southern Europe but there are no studies of its vectorial role in Greece [23]. Given that *P. papatasi* has an established presence in Crete and mainland Greece [24] as well as in Cyprus [25]. Other consistent study showed the relative abundances of sand fly species in the highland and lowland areas in Libo-Kemkem, *S. schewtzi*, *S. clydei* and *P. papatasi*. Of all sand fly species in the lowland area, *S. clydei* was the most abundant species accounting for 27.6% of the total collection and followed by *S. antanata* (10.3%) and *S. schewtzi* (7.3%) [26]. Also, the sandfly population in the vegetation was composed of *Sergentomyia bedfordi* (61%), *S. antennatus* (31%), *S. ingrami* (3%), *S. schwetzi* (2%), and *S. africanus* (1%). *Phlebotomus* species rarely occurred in the study area. Our study revealed that there were highly significant differences between species composition during different season. This may be attributed to variations in climatic condition during summer, winter and rainy season. The finding in line with Jeanneth., *et al*. [27] who stated that sand fly abundance was positively correlated with temperature and RH and negatively correlated with rainfall. The number of captured sandflies was observed to decrease as the temperature decreased [27].

In summary, the study showed that Soba and island areas were infested with sand fly species, which include: *P. papatasi, S. claudia, S. antanata* and *S. chowzai* and fly caught by sticky traps from all different habitats (outdoor, indoor, forest, domestic), the preferred habitats for sand fly species detected were domestic habitats followed by both indoor and forest and there were significant differences between species composition in summer, winter and rainy season. Hence, the *Sergentomyia* sand flies were more prevalent in the study area than *Phlebotomus* sand flies. However, *P. papatasi, S. claudia,* and *S. antanata* were seasonal species. While the medically important species prevalent in the area were *P. papatasi*. Investigation on the infection rates of Leishmania parasites in sandfly vectors by dissecting large numbers of Sandfly sampled over a longer time and covering different seasons of the year is needed to determine the transmission season of the disease in the area. Also further investigation is required to elucidate the seasonal abundance and distribution of the vector, as well as the transmission season of VL in both habitats so that appropriate control strategies for the vector can be designed.

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