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CLIMATIC FACTORS INFLUENCING THE ABUNDANCE OF *PHLEBOTOMUS PAPTASI* (SCOPOLI) (DIPTERA: PSYCHODIDAE) IN THE NILE DELTA

By

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Abstract

Ecological studies on sand flies were carried out in two villages of the Nile Delta Governorates namely; Kafr Tahla (Qalyubiya) and el Quantara el Beida (Kafr el-Sheikh) from September 2003 to August 2005. Sand flies were collected weekly from around houses, stables and near rodent burrows using sticky paper traps. A total of 9529 sand flies were collected from the two villages, all of which were identified as *Phlebotomus papatasi*. Sand fly activity started from April to December with a bimodal annual pattern. The sex ratio of collected sand flies was significantly male biased. Data analyses revealed that sand fly densities were strongly correlated to temperature but not to relative humidity or wind velocity. Variation in the densities of *P. papatasi* in both villages did not show a significant effect due to lunar phases. However, sand fly activity was highly positively correlated to fraction illumination.

Key words: Sand flies-*Phlebotomus papatasi*-seasonal variation-climate-Lunar phases-Egypt.

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Introduction

Phlebotomine sandflies are the sole vectors of leishmaniasis besides their role as vectors of several viral and bacterial diseases of humans and other animals (Lane,

1993). Cutaneous leishmaniasis affects people in 88 countries around the world, with 1-1.5 million new cases reported annually (Desjeux, 2001). In Egypt, zoonotic cutaneous leishmaniasis (ZCL) due to *Leishmania major* is endemic in

Sinai (Mansour *et al.*, 1987) and in the Nile Delta (Morsy, 1983). The vector has been identified as *Phlebotomus papatasi* (Fryauff *et al.*, 1993) and different rodent species were considered potential reservoir hosts (Morsy *et al.*, 1982; El Hossary *et al.*, 2000).

The distribution and incidence of infectious diseases particularly those transmitted by vectors, are affected by environmental factors. Cyclical variations in population densities of insect vectors reflect their survival strategies in response to climatic variations (Ximenes *et al.*, 2006). There have been studies on the influence of physical factors on the seasonality of phlebotomine sand flies in Egypt (e.g. El Naggar *et al.*, 2006; El Hossary *et al.*, 2000; El Said *et al.*, 1985) and other countries in the region (Boussaa *et al.*, 2005; Ibrahim and Abdoon, 2005; Wasserberg *et al.*, 2003; Janini *et al.*, 1995). However, findings varied depending on the species type and the different geographical areas.

Based on existing knowledge on the biology of sand flies, one might expect these insects to be affected by the lunar cycle. Few studies have demonstrated an influence of the phases of the moon on phlebotomine behavior (Souza *et al.*, 2005; Santos-De Marco *et al.*, 2002).

Here we present results of a phlebotomine survey in two villages of the Nile Delta, from September 2003 to August, 2005 with the aim to determine the activity periods of

sand flies and to identify the climatic factors that influence them. Also, the influence of the lunar phases and fraction illumination of the moon was one of the factors investigated in the two villages. Being able to predict the times of peak population density and activity will facilitate design of more timely and effective control strategies.

Materials and Methods

The present investigation was carried out in two villages namely, Kafr Tahla in Qalyubiya Governorate and el Quantara el Beida in Kafr el Sheikh Governorate. Kafr Tahla village is located in the southern parts of the Delta and lies adjacent to Damietta Nile Branch. It represents a typical Egyptian village where latrine dumps are found at convenient sites outside houses as there were no sewage or garbage disposal systems. Animal rooms or stables are usually found inside houses. El Quantara el Beida village is located in the northern parts of the Nile Delta; about three km North of Kafr el Sheikh town. It is an urbanized densely populated area provided with the sanitation system. Animal pens were not set up inside houses but adjacent to them.

Adult sand flies were collected weekly from sunset to sunrise from September 2003 to August 2005 using sticky paper traps (A4 blank paper sheets coated with castor oil from both sides). Traps were held by 35 cm long stick and placed 15-

20 cm above the ground. In each village, an average of 20 traps was placed, in the same places, around houses and stables and near rodent burrows in the close surroundings.

The specimens caught were preserved in 70% ethanol. They were cleared in chloral hydrate and phenol, 1:1 v/v then mounted in Puri's medium (Smart *et al.*, 1965). The identification was made by examining the morphology of male genitalia, female spermathecae and pharynges using the key of Lane (1986). Sand fly densities were calculated as the number of flies recovered per sticky paper trap.

Air temperature, relative humidity, and wind speed were measured at the two villages each time the traps were installed or checked using digital humidity & temperature meter and turbo meter (Cole Parmer U.S.A.) respectively.

The influence of the lunar cycle on the activity of sand flies was assessed at the two villages. Sand flies were collected outdoors using sticky paper traps for 96 nights during September 2003 to August 2005 on one night of each of the four lunar phases of each month. Sticky traps were set at sunset and recovered at sunrise. The total number of flies caught at each lunar phase was recorded.

The moon phases were defined according to Theodore (1983): (1) Crescent: the moon appears to be partly but less than one-half illuminated by direct sunlight, where the

fraction illumination of the moon's disk, that is illuminated is increasing; (2) Full moon: the moon's illuminated side is facing the earth, where the moon appears to be completely illuminated by direct sunlight; (3) $\frac{3}{4}$ moon: the moon appears to be more than one-half but not fully illuminated by direct sunlight, and (4) New moon: the moon's illuminated side is facing the earth, where the moon is not visible. The data concerning the fraction illumination of the moon during the four lunar phases was obtained from the nautical almanac for the year 2006 (Defense Department, Naval Observatory). The moon age and its fraction illumination for each phase are presented in Table (1).

Statistical analysis: Data were analyzed using SPSS for Windows, release 17.0 (SPSS Inc., Chicago, IL, USA). Differences between the numbers of sand flies collected from the two villages were assessed using the Mann-Whitney *U* test as they were significantly not normally distributed ($D(48)=0.189$; $P<0.001$). Multiple regression analysis was used to analyze the relationship between the number of sand flies collected and weather conditions. Sex ratio was analyzed using Chi-square test for goodness of fit. The non-parametric Kruskal Wallis test was used to compare the number of flies collected during different lunar phases because data showed skewed distribution.

Spearman's rank correlation coefficient was used to investigate the relation between sand fly activity and fraction illumination during different lunar phases. Data were presented as mean \pm standard deviations. A *P*-value of ≤ 0.05 was considered indicative of statistical significance.

Results

In total, 9529 sand flies were collected from the two villages using sticky paper traps. All the collected flies were identified as *Phlebotomus papatasi*. A total of 6247 sand flies was collected from Kafr Tahla village which is significantly higher than the total number of flies (3282) collected from el Quantara el Beida ($U = 838.5$; $z = -2.298$; $P = 0.02$).

In Kafr Tahla village, *P. papatasi* could be collected outdoors from April till end of December and no sand flies were found from January to March. Sand flies peaked in June & September where densities reached 4.55-10.60 fly/trap respectively.

For the two peaks, the monthly mean temperature was 28°C and 25°C; RH% was 61% and 59% and wind speed was 0.67 m/s and 0.28 m/s respectively. Peaks of both sexes were recorded within same temperature range (Fig 1).

According to the number of sand flies collected, the mean sex ratio (Male: total) was (0.70 \pm 0.10). Chi square test for goodness of fit revealed that the sex ratio outdoors is significantly male biased ($X^2=16.0$;

df=1; $P=0.00001$). Multiple regression analysis showed that the model was significant (Adjusted $r^2 = 0.77$; df = 3, 20; $F = 26.94$; $P = 0.0001$). According to the developed model, only temperature could be used as a predictor variable for *P. papatasi* ($\beta=0.92$; $P=0.00001$) (Table 2).

In el Quantara el Beida, a total of 2192 males and 1090 females were collected using sticky paper traps. Data (Fig. 2) showed that *P. papatasi* activity started in April and ended in January. No sand flies were collected during months of February and March. High numbers of *P. papatasi* were collected in May and September with no distinct peak during summer time. Fly densities ranged between 3.20 fly/trap in June 2004 to 2.86 fly/trap in October 2004, when the monthly mean temperature was 24°C and 27°C; RH% was 74% and 89%, and wind speed was 0.31 m/s and 0.21 m/s respectively.

Highest sand fly densities were recorded in September 2003 where densities of flies reached 5.47 fly/trap and the monthly mean temperature was 27°C; RH% was 75% and wind speed was 0.93 m/s. Very low densities were observed during December 2003 and January 2004, where it reached 0.43 and 0.14 fly/trap respectively. According to the number of sand flies collected, the mean sex ratio (Male: total) outdoors was (0.72 \pm 0.11). Chi square test of goodness of fit revealed that sex ratio outdoors is significantly

male biased ($X^2=19.36$; $df = 1$; $P=0.0001$). The multiple regression analysis revealed that the regression model was significant (Adjusted $r^2 = 0.71$; $df = 3, 20$; $F = 19.96$; $P = 0.00001$). As indicated by the developed model, only temperature could be used as a predictor variable for *P. papatasi* densities ($\beta = 0.90$; $P = 0.00001$) (Table 2).

To assess potential lunar influence on sand fly population densities, flies were collected for a period of two years on one night of each of the four lunar phases of each month. Totals of 6247 and 3282 *P. papatasi* were caught in Kafr Tahla and el Quantara el Beida, respectively. Data (Fig. 3) revealed that the activity of sand flies reached maximum during full moon in Kafr Tahla and

el Quantara el Beida (111.50 ± 75.23 & 59.17 ± 37.14 , respectively) where fraction illumination of the moon was maximum; while it was minimal during new moon (59.55 ± 49.16 and 37.44 ± 25.52 , respectively) where fraction illumination was also minimal (Table 1).

Statistical analysis of data using Kruskal-Wallis test revealed that lunar phase had no significant influence on sand fly activity in Kafr Tahla and el Beida villages ($H=6.14$; $df=3$; $P=0.11$ and $H=4.917$; $df=3$; $P=0.18$, respectively). But, sand fly activity was highly positively correlated to fraction illumination during different lunar phases both in Kafr Tahla ($r=0.79$) and el Quantara el Beida ($r=0.94$).

Table 1: Number *P. papatasi* (males and females) collected during different lunar phases in Kafr Tahla, Qualubya Governorate and el Quantara el Beida, Kafr el Sheikh Governorate.

Village	Moon Phase	Fraction Illumination	Number of Flies collected
Kafr Tahla	Crescent	0.07	1508
	Full Moon	1	2007
	¾ Moon	0.73	1541
	New Moon	0.03	1191
el Quantara el Beida	Crescent	0.13	708
	Full Moon	1	1006
	¾ Moon	0.62	894
	New Moon	0.01	674

Table 2: Significant predictor variables in the multiple regression model for *P. papatasi* in the Nile Delta.

Village	Coefficient	B	S.E	β	Significance
Kafr Tahla	Constant	-277.15	321.260		0.40
	Relative humidity (RH%)	-6.57	4.58	-0.15	0.17
	Temperature (°C)	39.36	4.74	0.92	0.00001
	Wind velocity (m/s)	199.92	143.187	0.16	0.18
el Quantara el Beida	Constant	-214.28	172.70		0.23
	Relative humidity (RH%)	-1.48	2.22	-0.83	0.51
	Temperature (°C)	22.50	3.37	0.90	0.00001
	Wind velocity (m/s)	-1.190	56.60	-0.003	0.98

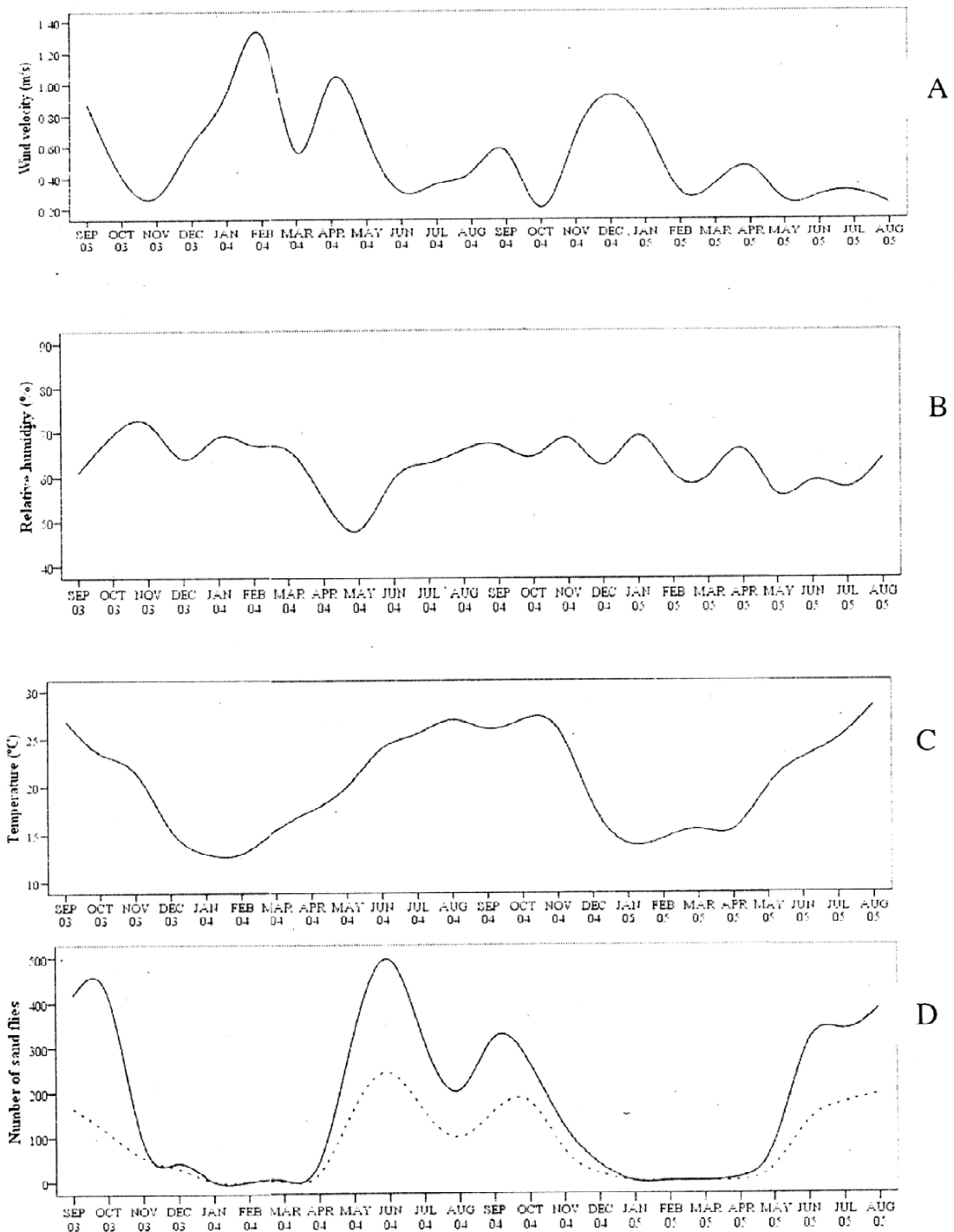


Fig. 1: Annual variation in population density of *Phlebotomus papatasi* relative to variation in climatic factors in Kafra Tahla, Qualubia Governorate. (A) Wind velocity. (B) Relative humidity. (C) Temperature. (D) Number of sand flies (dashed line, females; solid line, males).

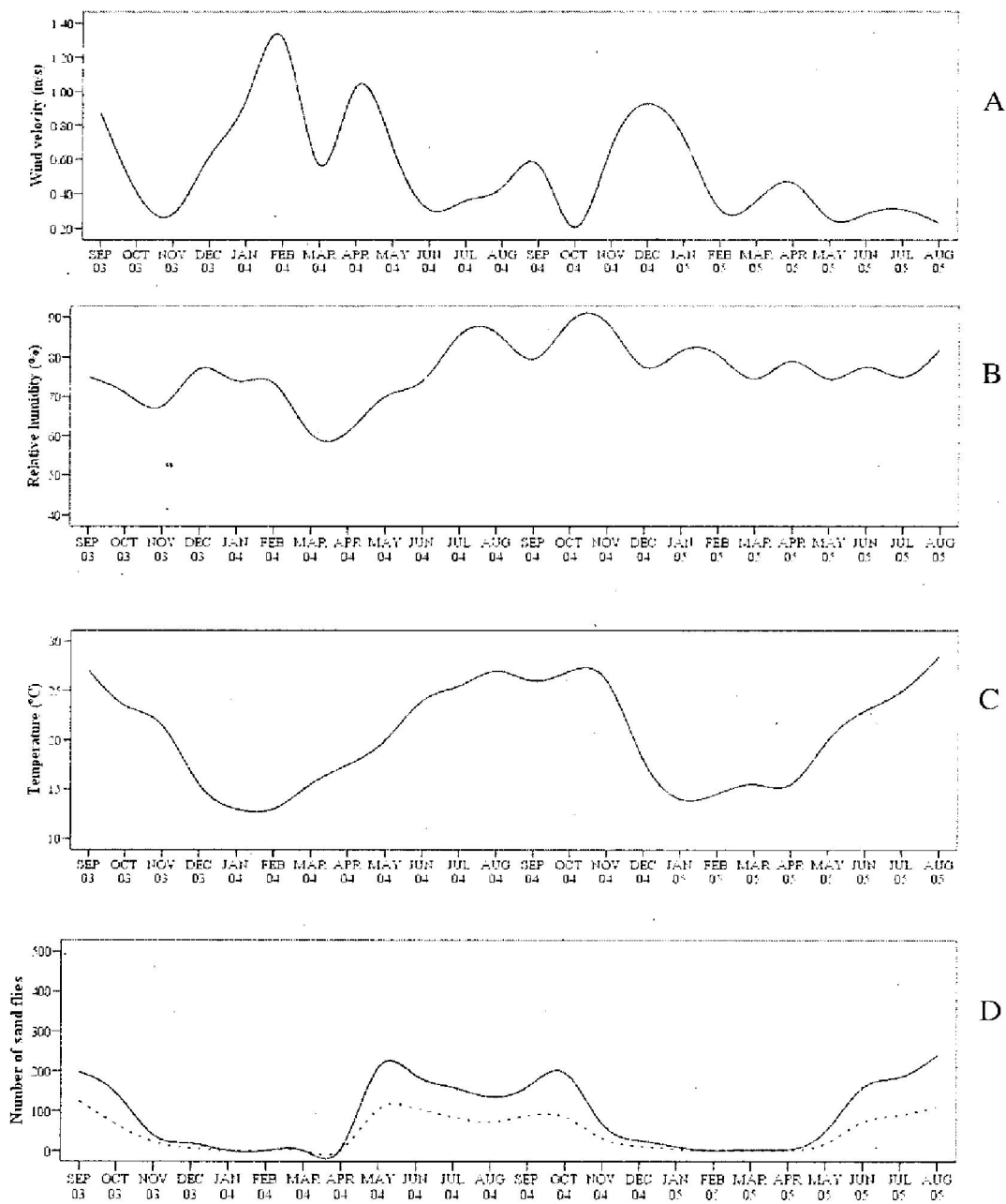


Fig. 2: Annual variation in population density of *Phlebotomus papatasi* relative to variation in climatic factors in el Quantara el Beida, Kafr el Sheikh Governorate. (A) Wind velocity. (B) Relative humidity. (C) Temperature. (D) Number of sand flies (dashed line, females; solid line, males).

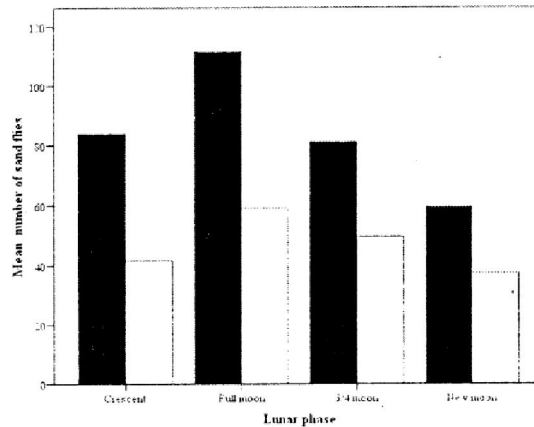


Fig. 3: *Phlebotomus papatasi* collected during lunar phases in Kafr Tahla, Qualubya Governorate (■) and in el Quantara el Beida, Kafr el Sheikh Governorate (□)

Discussion

Sand fly species composition was studied in two villages representing two of the Nile Delta Governorates namely; Kafr Tahla, (Qalyubiya Governorate) and el Quantara el Beida, (Kafr el Sheikh Governorate). This study aimed in part to update the faunal list of sand flies in Egypt. Sand fly collections were made using sticky paper traps. Sticky paper trap is the most efficient method for collection when studying seasonal abundance and species composition of sand flies (WHO, 1990). Only *P. papatasi* was collected from all sites in the two villages as the ecological conditions and topographic features of these areas were similar. *P. papatasi* was previously recorded in other domestic areas of the Nile Delta (Morsy *et al.*, 1990).

During this two years study, sand fly densities were higher in Kafr Tahla than in el Quantara el Beida village. Kafr Tahla is a typical

Egyptian village, where mud houses provide sand flies with much humid, darker and less ventilation microhabitats. However, the period of sand fly activity was longer in el Quantara el Beida. This could be the result of the difference in temperature between the two villages being more favorable for longer survival of *P. papatasi* in el Quantara el Beida. The results of this study proved that males of *P. papatasi* outnumbered females. The animal hosts attract sand fly males by the odor and carbon dioxide, and the males in turn attract the females by emitting pheromones and compete for mating privileges (Ward *et al.*, 1993). This might explain why the sex ratio in collections is skewed in favor of males, because they remain near the host longer than females. The female reproductive strategy seems to be to take a blood meal, mate and then fly off to shelter. Similar findings were previously reported for *P. papatasi* in Sinai Egypt (Hassan *et*

al., 1999); in Jordan Valley (Yuval and Schlein, 1991) and for *Lutzomyia longipalpis* in northern Brazil (Ximenes *et al.*, 2006). However, it is possible that some variations may occur due to many factors such as geographic location, the method and time of collection, climate and the species.

Sand flies appeared to have a bimodal peak of activity for both sexes, one in June and the other in September. After the second peak sand flies could be found in low numbers till late December and early January and disappears during February and March. These are the coldest months of the year and sand flies pass over such unfavorable conditions as dormant immature stages. One of the most important factors determining the distribution of any insect species is temperature. For sand flies, temperature is known to affect the survival and speed of development of the different stages in the life cycle (Elnaiem *et al.*, 1998). Multiple regression analysis showed that the seasonal changes in sand fly densities in Kafr Tahla and el Quantara el Beida were closely related to the changes in temperature. The importance of temperature as determinant of sand fly distribution was studied by Cross and Hyams (1996) and predicted that raising the ambient temperature by 1-5°C would result in the expansion of the geographical distribution of *P. papatasi* and increase disease transmission. Recorded values of wind

velocity and relative humidity were higher in el Quantara el Beida than in Kafr Tahla; however, such climatic factors did not significantly affect sand fly densities in both villages. This could be attributed to unnoticeable changes in humidity and wind velocity during summer months where sand fly activity reached maximum. In the laboratory, high relative humidity (75-80%) is an important determinant for the successful colonization of sand fly; it promotes adult emergence and oviposition (Kassem, 1998). In the field, the ambient relative humidity in the sand fly microhabitat is sufficiently high to maintain soil moisture at a level that supports survival of immature stages. The abundance of sand flies is influenced by wind velocity (Ximenes *et al.*, 2006). The increased abundance of the New-World *Lu. longipalpis* was related to a reduction in wind velocity. When considered as secondary climatic factor, wind velocity can modify temperature and precipitation by transporting heat and humidity as well as affecting evaporation rate, which could affect the development of immature insects.

The views expressed in the available literature are rather contradictory regarding the question of whether the changing light of the moon influences the catch of insects. Despite several decades of research, the influence of the moon light trapping has to this day remained one of the most complex and least known

problems. From the point of view of clarifying the relationship between the light of the moon and light-trap effectiveness, studies examining the moonlight-related activity of insects by use of other methods are of great significance.

This is the first long-term study of the influence of the lunar cycle on the activity of *P. papatasi* using the sticky paper trap method. Based on the number of *P. papatasi* collected from Kafr Tahla and el Quantara el Beida, the activity of flies reached maximum during the full moon (highest catch) and was significantly positively correlated to the fraction illumination. The most likely explanation is that the moon light helps sand flies to navigate and to find its host (Mellor and Hamilton, 2003). This result agrees with that obtained by Souza *et al.* (2005) who found positive correlation between moon light intensity and the number of New World sand flies (*Lu. intermedia* and *Lu. whitmani*) collected while feeding (using aspirator). The authors explained their findings as did others, on the basis of in darker nights (new moon) the light of CDC light traps is more effective in attracting sand flies, while in the moon light the decreased catch is due to increased competition between the light from the trap and from a full moon (Bowden 1973). Nevertheless, it would seem that lunar phase intensity is a factor that should be taken into consideration when planning or analyzing data on

sand fly captures by different methods. Considering that an increase in CL follows an increase in *P. papatasi* population density and that there is a positive correlation between sand fly density and certain climatic variations, careful monitoring of both climatic variables and sand fly populations should allow prediction of leishmaniasis outbreaks.

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