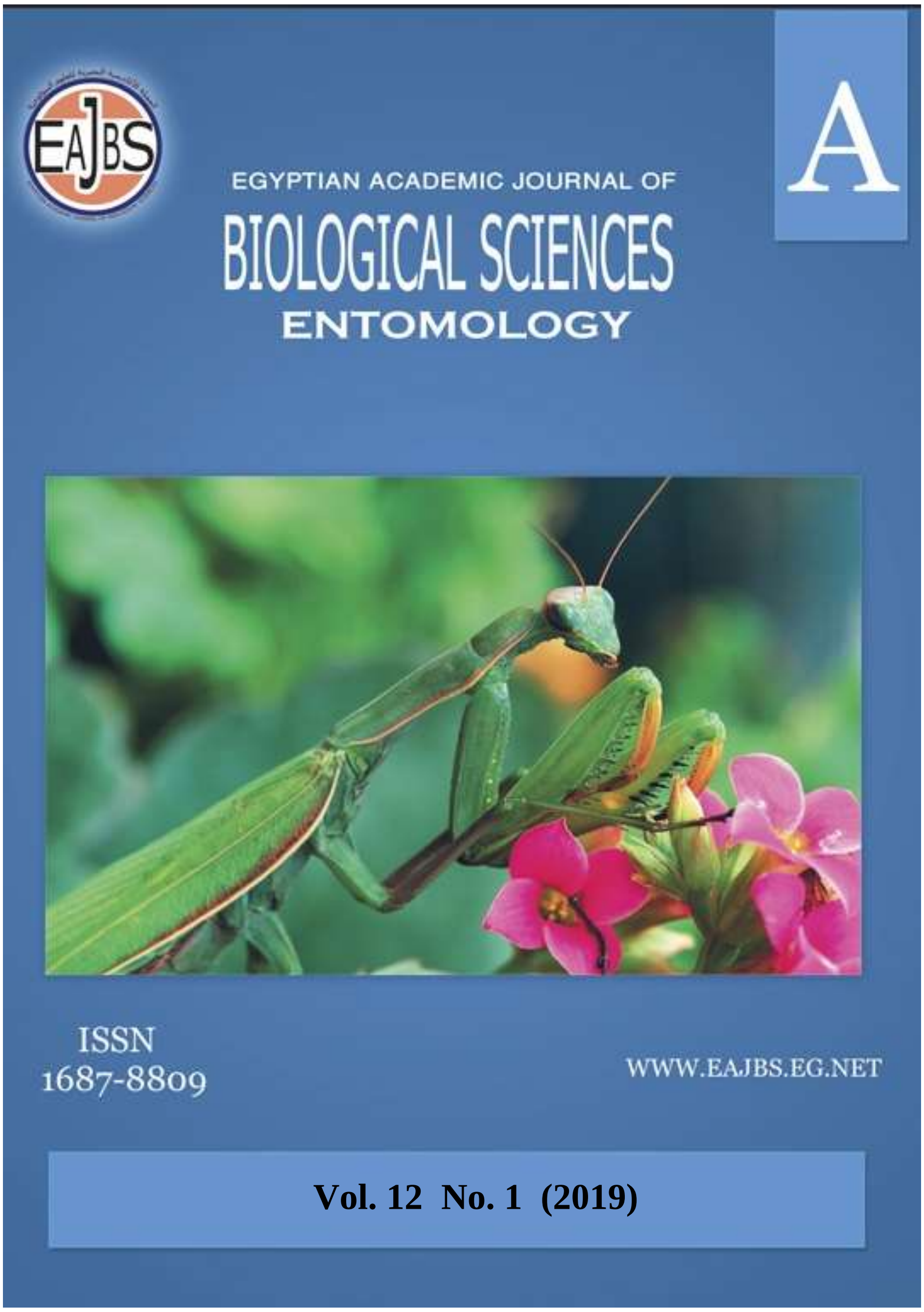

Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences, Department of Entomology, Faculty of Sciences Ain Shams University.
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www.eajbs.eg.net

Citation: *Egypt. Acad. J. Biolog. Sci. (A. Entomology) Vol. 12(1) pp: 131- 144 (2019)*

Egypt. Acad. J. Biolog. Sci., 12(1):131– 144 (2019)

Egyptian Academic Journal of Biological Sciences
A. Entomology

ISSN 1687- 8809
http://eajbsa.journals.ekb.eg/

Ecological and Behavioral Studies of Mosquito Vectors in Monufia, Nile Delta, Egypt.

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ARTICLE INFO
Article History
Received:13/1/2019
Accepted:6/2/2019

Keywords:
Culicine mosquitoes-indoor and outdoor collection set - Seasonal variation-resting behavior-Monufia Governorate-Egypt- parity.

ABSTRACT

Continuous monitoring of vector species composition, abundance, dynamics, biting and resting habits is the base to determine the local vector species that affect disease transmission patterns and consequently the choice of cost-effective vector control options. For targeting that, adult mosquito surveys were conducted in Monufia as a representative of the Egyptian governorates in Nile-Delta (Spring 2016– Winter 2018). Totally, 4 species were reported in the selected villages: *Culex (Culex) pipiens* Linnaeus, *Cx.* (*Cx.) perexiguus* Theobald, *Cx. (Cx.) antennatus* Becker and *Ochlerotatus* (*Ochlerotatus*) *caspius* Pallas. *Culex pipiens* was the predominant species in all study regions. For each species, the following were examined: the relation of adult indoor and outdoor density with temperature and relative humidity as well as its indoor and outdoor resting density and its relation with temperature and relative humidity, determination of the endophagic and exophagic indices for mosquito biting reference and the seasonal changes in parity status.

INTRODUCTION

Mosquitoes are widespread insects in the world with about 3000 species grouped in 39 genera and 135 subgenera (Clements, 1992 and Reinerti, 2001). In Egypt, *Culex* mosquitoes have been implicated in the transmission of filariasis and various viral diseases included Rift Valley Fever and West Nile Fever (Darwish and Hoogstraal, 1981). Prevalence of the vector species, its high density and seasonal distribution influence the rate of disease transmission in time and space (Das *et al.* 2004). Samples of resting mosquitoes are also required to enable assessment of host feeding patterns through blood meal analysis. Mosquito resting behavior can be divided into two categories, an endophilic and an exophilic behavior (resting indoor or outdoor, respectively) (Pates and Curtis, 2005 and WHO, 2013). The host-seeking behavior is an innate behavior of the female mosquitoes to secure a blood meal which is essential for the completion of the gonotrophic cycle and oviposition, to continue their species propagation. Biting activity is one of the important aspects of the biology of vectors to which disease transmission is depended. The intensity of disease transmission depends upon the high biting density, anthropophily and high survival rates of a vector (Kaul and Wattal, 1968). Blood ingestion in the female mosquito is needed for the ovarian development, one complete around of the ovarian development is called the gonotrophic cycle. The potential for transmission comes from the female mosquitoes that survive a minimum of at least two gonotrophic cycles (Shriram *et al*., 2005). Age determination could be used to evaluate control measures success against the vector population. The parity status of an adult female mosquito indicates whether the female has oviposited during her lifetime or not (Service, 2012). This information is particularly useful at the early stage, to avoid sitting camps in high-risk areas, and later for selecting potential additional prevention measures.

The present work aimed to study the ecology and behavior of adult mosquitoes in Monufia governorate, Nile Delta, Egypt via conduction of indoor and outdoor adult mosquito survey, study the resting behavior (indoor and outdoor) using light and window traps, detection of seasonal changes in indoor and outdoor resting mosquito density, determination of the endophagic and exophagic (feed indoor or outdoor, respectively) indices for mosquito biting reference and study the seasonal changes in parity status.

MATERIALS AND METHODS

Study Area:

The present study was carried out seasonally during two years from Spring 2016 to Winter 2018 (4 visits annually during April, July, October and January). The study was conducted in five villages follow Berket El-Sabie district, Monufia governorate in Nile Delta, El-Ganzor (30° 40.490'N and 31° 1.667'E), El-Roda (30° 39.763'N and 31°4.418'E), El-Shaheed fekry (30° 40.897'N and 31° 4.246'E), Mit Om-Saleh (30° 38.863'N and 31° 2.527'E) and Shintina Al-Hagar (30°38.661''N and 31° 3.173'E) villages. The distance between each village ranged from about 2 to 4 kilometer.

Sampling of Adult Mosquitoes and Identification:

Outdoor Collection:

Mosquito collection was carried out from sunset to sunrise from five sites in each of the five villages, using dry cell battery-operated CDC light traps which were hung on small trees approximately 1.5-2.0 m of the ground near mosquito breeding sites and outside of the houses according to **Chen *et al.*, (2011) and WHO, (2013).** The trapped mosquitoes were collected and knocked down by freezing, then the females were identified morphologically using **Harbach (1985)** key and classified according to their feeding stage as unfed, fed, half gravid and gravid females.

Indoor Collection:

A list of households at five localities in the five study villages was used as a sampling frame. Again dry cell battery-operated CDC light traps were hung near the bed of the occupants who slept under untreated nets, about 1.5 m from the floor and 50 cm from the bed net between 6:00 pm and 6:00 am **(Lines *et al.*, 1991)**. Also, females were identified morphologically to species and grouped according to their feeding stage as previously mentioned. Human biting rate (BR) was determined using the following formula:

BR = f/w where **F**= total number of freshly fed mosquitoes of the particular species and **W**= total number of human occupants in houses.

Outdoor Resting Mosquito Collection:

Outdoor resting mosquitoes were collected using endowindow traps which were fitted into the window of the sleeping room according to **WHO, 2013**. The collected mosquito females were identified to species and grouped according to their feeding stage.

Indoor Resting Mosquito Collection:

Indoor resting mosquito density was detected by collecting the resting mosquitoes with exowindows trap between 07:00am and 09:00 am according to **WHO, 2013**. Then females were identified morphologically to species. The indoor resting mosquito density (IRD) was determined using the following formula:

IRD = No. of females of a particular species/ No. of houses inspected

Determination of Parity Ratios:

All fresh fed and unfed female mosquitoes that were collected from the different sites were frozen at (-18^{O}C) to arrest ovarian development until they were identified and used to establish the physiological age of females. The used method to determine parity in this study was described by Detinova (1962). After the females dissection, the ovaries were examined using a compound microscope. The parity rate was determined using the following formula:

Parity rate = (No. of parous female / total no. of female dissected) X 100

Statistical Analyses:

Statistical analyses were performed using IBM© SPSS© Statistics version 20 (SPSS Inc., an IBM Company, 1New Orchard Road, Armonk, New York 10504-1722, USA). Standard Deviations and Means were conducted and compared between five villages for each species and each collected set using One-way ANOVA test. The relationship between each mosquito species density and each environmental factor was calculated using partial correlation. Indoor means density of each species was compared with outdoor and indoor resting with outdoor resting using T-test. Chi-square test was used to compare the parity of mosquitoes at different seasons.

RESULTS

The Sampling of Adult Mosquitoes (outdoor and indoor survey):

During the period of the study, 3482 mosquito females were collected from the five villages, out of them 38.9% (1355) were caught outdoors from all five sites. After identification all collected mosquito females were found to be belonging to four species, *Culex pipiens* 55.1% (747), *Culex antennatus* 26.1% (354), *Culex perexiguus* 13.6% (184) and *Ochlerotatus caspius* 5.2% (70). In figure (1) the result showed that, there was no significant difference in the mean density of *Cx. pipiens, Cx. antennatus* and *Cx. perexiguus* among five villages (P>0.05). However, the mean density of *Ochlerotatus caspius* was differed significantly (P>0.05) since it was higher in El-Ganzor (1.6 ±3.5) and El-Roda (0.19±.73) villages than the other three villages. The seasonal abundance of *Cx. pipiens* was higher in Spring and Autumn than in the other two seasons of the year and its density no significant (P>0.05) increases as temperature and relative humidity elevated (Fig. 1a). *Culex antennatus* and *Cx perexiguus* largest collection were occurred in Spring (Fig. 1b, 1c). *Ochlerotatus caspius* was predominant in Spring season and collected in small number in Summer (Fig. 1d). However, *Cx. antennatus* density showed no significant decreases as temperature and relative humidity increased (P>0.05). *Culex perexiguus* density showed no significant decreases as temperature increased (P>0.05) and significantly decreases as relative humidity decreased (P<0.05). *Ochlerotatus caspius* density increases as temperature increased and relative humidity significantly increased (P<0.05). For all species, unfed females comprised a higher proportion of outdoor collections than fed females (Fig. 1).

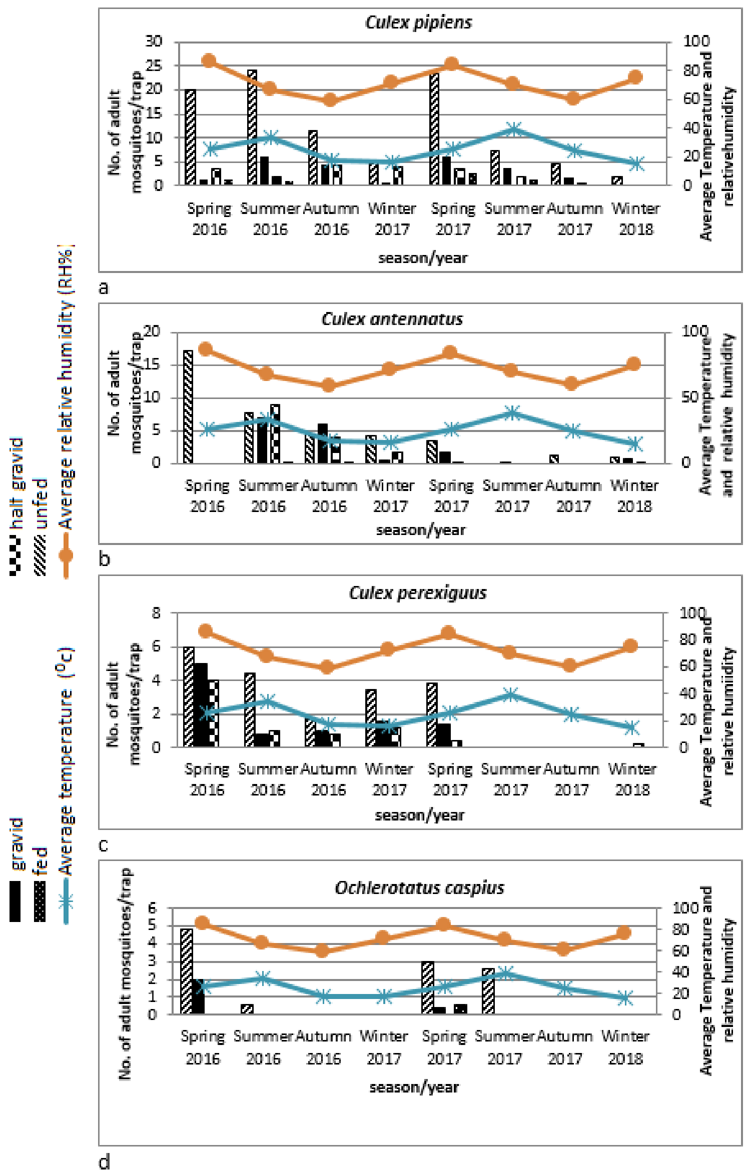

Fig. 1. Outdoor collected mosquito species throughout the four seasons in two years; plotted with temperature and relative humidity.

The indoor collection resulted in trapping 38.5% (1341) mosquito females, *Cx. pipiens* represented 58.٨% (788) of the total catch (1341), *Cx. antennatus* 21.5% (289), *Cx. perexiguus* 18.٦% (249) and *Och. caspius* 1.1% (15). The results were plotted in figure (2). There was no significant difference in the mean density of *Cx. pipiens* and *Cx. antennatus* among study villages (P>0.05). In contrast, the mean density of *Cx. perexiguus* was significantly differed in abundance (P<0.05) as it was significantly higher in two villages, El-Ganzor (1.6 ±2.5) and El-Roda (3.2±6.1) than the other three villages. There was a significant difference in mean density of *Ochlerotatus caspius* since it was significantly higher in two villages, El-Ganzor (1.6 ±3.5) and El-Roda (0.2±0.9) than the other three villages.

Culex species were caught throughout the year while, *Ochlerotatus caspius* was predominant in the Spring season and its density increase as temperature decreased and relative humidity increased while, its highest biting rate was observed during Spring (0.3) (Fig. 2d). *Culex pipiens* and *Cx perexiguus* were collected in

Spring and Autumn more than in the other seasons of the year (Fig 2a, 2c). On the other hand, *Cx. pipiens* showed higher biting rate during Spring and Autumn and its density decreases as temperature and relative humidity increased (Fig. 2a).The biting rate of *Cx perexiguus* was higher during Summer (0.3) and its density decreases as temperature increased and relative humidity decreased (Fig. 2c). *Culex antennatus* density increases as temperature increased and relative humidity decreased, while the largest collection occurred in the Summer and Autumn and its highest biting rate was during Spring (0.9) (Fig. 2b). Gravid, half gravid and unfed mosquito comprised the high percentage of indoor collections (Fig. 2). Variation in the indoor and outdoor density of *Cx. antennatus* wasn't significant and higher than that of *Cx. pipiens* and *Cx. perexiguus* (P>0.05). There was significant variation in *Och. caspius* density between indoor and outdoor (P<0.05) as its mean was higher indoor (1.8±.2) than outdoor (0.3±.1).

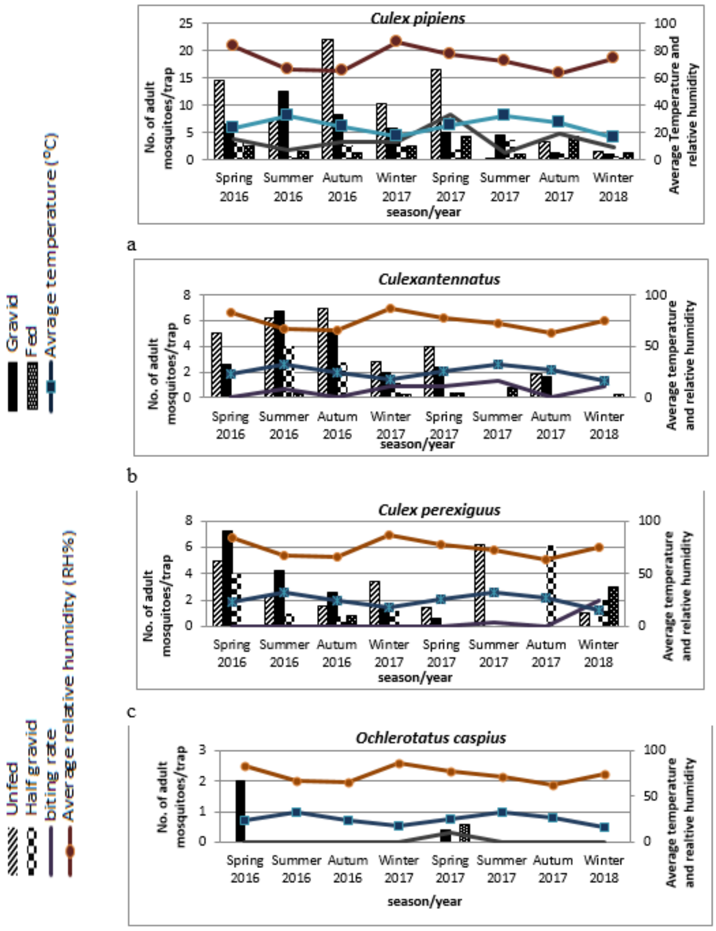

Fig.2. Indoor collected mosquito species throughout the four seasons in two years; plotted with temperature and relative humidity and biting rate.

Outdoor and Indoor Resting Mosquitoes:

During the period of the study, 8.52% (297) of mosquito female species were resting outdoor. Among these females, *Cx. pipiens* 55.2% (164), *Cx. antennatus* 20.5% (61) and *Cx. perexiguus* 24.3% (72) represented of these numbers while *Ochlerotatus caspius* did not collect from any of the used traps. Figure (3) illustrated that, there was no significant difference in the mean density of *Cx. pipiens* and *Cx. antennatus* at the different villages (P>0.05). In construct, the mean density of *Cx.*

perexiguus was significantly differed as it was higher in three villages, Ganzor (0.4 ±.3), El-Roda (0.4±0.2) and Shintina (0.4±0.1) than the other two villages, El-Shaheed (0.2±0.1) and Mit Om Saleh (0.3±0.1). For instance, most species were collected in Spring season and the smallest numbers were collected in Winter (Fig. 3). The result showed that, there was no significant increase in *Cx. pipiens* and *Cx. antennatus* density as temperature and relative humidity increased (*P*>0.05). However, *Cx perexiguus* density was no significant decreases as temperature and relative humidity increased (*P*>0.05). Almost the dominant *Cx. pipiens* were collected as gravid (65.2%) and only a few individuals as fed (1.8%) (Fig. 3a). There were no *Cx. antennatus* fed females located at the traps, while about 44.4% of the collected females were gravid (Fig. 3b). However, *Cx. perexiguus* were collected in highest number as half gravid 42.6% and also there were no any fed females (Fig. 3c).

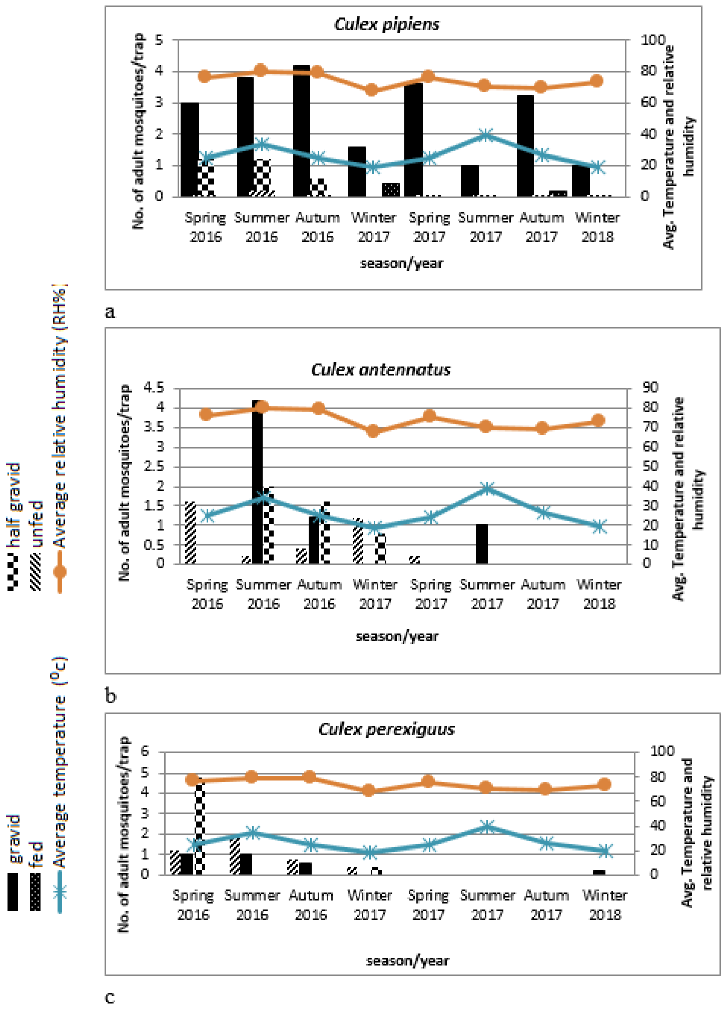

Fig.3. Adult mosquito species collected resting outdoors throughout the four seasons in two years; plotted with temperature and relative humidity

During the study, 14.1% (489) of female mosquitoes were collected as indoor-resting ones. Among these females *Cx. pipiens* (55.4%) 271, *Cx. antennatus* (28.6%) 140 and *Cx. perexiguus* (16%) 78 represented of these numbers while, *Ochlerotatus caspius* did not found in any one of the used traps. Figure (4) illustrated that, *Cx. pipiens* were collected resting indoors in four seasons (with a peak in Autumn and

Spring season) (Fig. 4a) meanwhile, the *Cx. antennatus* were collected during Autumn and Summer seasons only (Fig. 4b).The mean density of *Cx. pipiens* between five villages was differed significantly (*P*<0.05) as this mean was higher in two villages, El-Ganzor (3.1 ±0.5) and El-Roda (2.4±0.3) than the other villages and lowest in Shintina (0.6 ±0.2), However. *Cx. antennatus* did not differ significantly the mean density of *Cx. perexiguus* differ significantly (*P*<0.05) as it was higher in El-Roda (0.9 ±0.3) village and lowest (0.1 ±.07) in Mit Om Saleh and Shintina. Gravid (43.2%) and fed (23.6%) *Culex pipiens* females comprised a higher proportion of indoor resting collections than other species and the lowest number of indoor resting unfed females (10.7%) (Fig.4a). In the case of *Cx. perexiguus* and *Cx. antennatus* the largest number was collected as gravid and half gravid with (47.4 %-27%) and (59.3% - 22.1%) respectively (Fig. 4b, 4c). The result showed that, there was no significant decrease in *Cx. pipiens* and *Cx perexiguus* density as temperature increased and relative humidity decreased (*P*>0.05). However, *Cx. antennatus* density wasn't significant increase as temperature increased and relative humidity decreased (*P*>0.05). There was no significant variation of *Cx. pipiens* density that resting indoor and outdoor (*P*>0.05), while this variation was significant and observed between indoor resting and outdoor resting populations of *Cx. antennatus* and *Cx. perexiguus* (*P*<0.05).

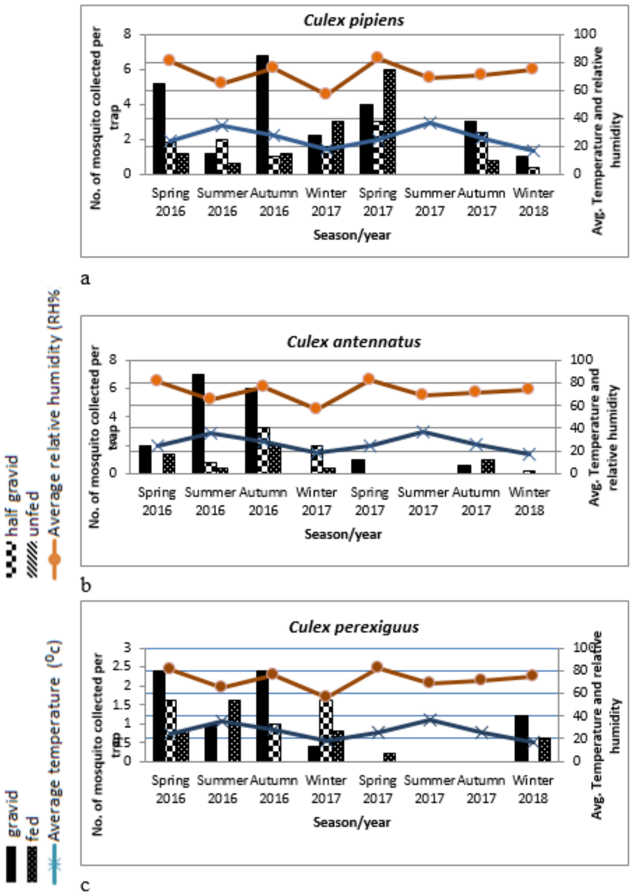

Fig.4. Adult mosquito species collected resting indoors throughout the four seasons in two years; plotted with temperature and relative humidity.

Mosquito Females Parity Ratio:

A total of 1355 female mosquitoes were caught from outdoor during the study out of this, 65.01% (881) were dissected. Ovarian dissections were conducted on 59.6% (525) *Cx. pipiens*, 22.1% (195) *Cx. antennatus*, 12% (106) *Cx. perexiguus* and 6.3% (55) *Ochlerotatus caspius*. However, a total of 1341 mosquitoes were caught from indoor out of this, (43.8%) 588 were dissected. Ovarian dissections were conducted on (66.5%) 391 *Cx. pipiens*, (23.1%) 136 *Cx. antennatus* and (10.4%) 61 *Cx. perexiguus*. Table (1) showed that, parity rates of *Cx. pipiens* females collected from outdoor and indoor were highest during Spring and Summer (Table 1a and 1b) and it was varied significantly (P<0.05) as the mean of indoor *Cx. pipiens* parity (66.3±7.6) was higher than outdoor (32.1±3.6). Overall, 297 adult female mosquitoes were collected resting outdoor and 26.9% (80) were dissected. During the study ovarian dissections were conducted on 51.2% (41) *Cx. pipiens*, 22.5% (18) *Cx. antennatus* and 26.3% (21) *Cx. perexiguus*. The results showed that, the total number of mosquitoes was caught resting indoors 489, out of this, 7.77% (38) were dissected. Ovarian dissections were conducted on 78.9% (30) *Cx. pipiens* and 21.1% (8) *Cx. antennatus*. Table (2) showed that, parity rate was highest among *Cx. pipiens* females collected resting outdoor in Spring 2016 and 2017 and among females collected resting indoor during Autumn 2016 and Summer 2017 **(Table 2a and 2b)** and the mean of *Cx. pipiens* parity that resting indoor (39.5±13.6) higher than outdoor (16.8±6.1). In the case of *Cx. antennatus* collected outdoor and indoor the parity rate was highest during Autumn 2017 and Spring 2017 and during Spring 2016 and 2017 respectively (Table 1a and 1b). The parity rate of *Cx. antennatus* that resting outdoor and indoor was highest during Spring 2016 and Winter 2017 and during Autumn 2016 and 2017 respectively (Table 2a and 2b). However *Cx. perexiguus* females were collected outdoor with a low number in Winter but its parity rates were the highest at Winter and for that collected indoor it was high during spring (Table 1a and 1b). However ovarian dissections were conducted on *Ochlerotatus caspius* females collected outdoor only, its parity rates were highest during Spring 2016 (79.2%) and Summer 2017(84.6%) than the other seasons (Table 1a). The result showed that, for *Cx. pipiens* species that collected outdoor parity rate didn't significantly increase as temperature decrease and relative humidity increased (P>0.05). However, *Cx. antennatus* and *Cx. perexiguus* parity rate wasn't significantly increased as temperature decreased and relative humidity increase (P>0.05). For species that resting outdoor parity rate wasn't significantly increased as temperature and relative humidity increased (P>0.05). During the study the differences between season and parity rate of *Cx. pipiens* and *Cx. antennatus* females collected resting indoors wasn't significant (P>0.05).

Table(1). The total seasonally numbers of dissected female mosquitos that collected from outdoor and indoor, number of parous females, parity rate and environmental factors

a

	Outdoor collected mosquito species								Environmental factors	
	Cx. pipiens		*Cx. antennatus*		*Cx. perexiguus*		*Och. caspius*			
Season/year	D.	P. (PR%)	D.	P. (PR%)	D.	P. (PR%)	D.	P. (PR%)	Av. T°c	Av RH%
Spring 2016	131	57 (43.5)	86	28 (32.5)	30	7 (23.3)	24	19 (79.1)	25	85.6
Summer 2016	120	29 (24.1)	39	13 (33.3)	22	6 (27.2)	3	2 (66.6)	34	67
Autumn 2016	57	16 (28)	23	5 (21.7)	9	3 (33.3)	0	0(0)	17	59
Winter 2017	28	12 (42.8)	21	6 (28.5)	17	7 (41.1)	0	0(0)	16	71.6
Spring 2017	118	54 (45.7)	18	8 (44.4)	19	6 (31.5)	15	7 (46.6)	26	84
Summer 2017	37	9 (24.3)	0	0 (0)	0	0 (0)	13	11 (84.6)	39	70
Autumn 2017	24	8 (29.1)	3	2 (66.6)	0	0 (0)	0	0 (0)	25	60
Winter 2018	10	2 (20)	5	3 (60)	9	6 (66)	0	0 (0)	22	75

b

	Indoor collected mosquito species						Environmental factors	
	Cx. pipiens		*Cx. antennatus*		*Cx. perexiguus*			
Season/year	D.	P. (PR%)	D.	P. (PR%)	D.	P. (PR%)	Av. T°c	Av. RH%
Spring 2016	74	57 (77)	25	18 (72)	17	11 (64.7)	24	83.4
Summer2016	37	29 (78.3)	33	23 (69.6)	12	5 (41.6)	32	66.6
Autumn 2016	11 1	86 (77.4)	35	25 (71.4)	8	5 (62.5)	24	65.4
Winter 2017	52	36 (69.2)	14	6 (42.8)	12	7 (58.3)	17	86.8
Spring 2017	83	77 (92.7)	20	11 (55)	7	5 (71.4)	25	77.4
Summer 2017	10	3(30)	7	2 (28.5)	0	0(0)	32	72
Autumn 2017	16	11 (68.7)	2	0 (0)	0	0 (0)	27	63
Winter 2018	8	3 (37.5)	0	0 (0)	5	2 (40)	16	74.4

D. Dissected **P.** Parous female **PR**. Parity rate

Table (2).The total seasonally numbers of dissected female mosquitos that collected as rested from indoor and outdoor, number of parous females, parity rate and environmental factors

a

	Outdoor resting collected mosquito species						Environmental factors	
	Cx. pipiens		*Cx. antennatus*		*Cx. perexiguus*			
Season/year	D.	P. (PR%)	D	P. (PR%)	D.	P. (PR%)	Av. T°c	Av. RH%
Spring 2016	5	1 (20)	8	2 (25)	6	1 (16.6)	25	76.4
Summer 2016	18	3 (16.6)	1	0 (0)	9	3 (33.3)	34	79.6
Autumn 2016	3	0 (0)	2	0 (0)	4	0 (0)	24	79.2
Winter 2017	0	0 (0)	6	1 (16.6)	2	0 (0)	18	67.8
Spring 2017	7	2 (28.5)	1	0 (0)	0	0 (0)	24	75.6
Summer 2017	1	0 (0)	0	0 (0)	0	0 (0)	39	70
Autumn 2017	5	1 (20)	0	0 (0)	0	0 (0)	26	69
Winter 2018	2	1 (50)	0	0 (0)	0	0 (0)	19	73

b

	Indoor resting collected mosquito species				Environmental factors	
	Cx. pipiens		*Cx. antennatus*			
Season/year	D.	P. (PR%)	D.	P. (PR%)	Av. T°c	Av. RH%
Spring 2016	5*	4 (80)	0	0 (0)	24	81
Summer 2016	0	0 (0)	0	0 (0)	35	65
Autumn 2016	10	6 (60)	4	2 (50)	28	76
Winter 2017	1	0 (0)	0	0 (0)	18	56.8
Spring 2017	6	2 (33.3)	0	0 (0)	25	82.2
Summer 2017	1	1 (100)	0	0 (0)	37	70
Autumn 2017	7	3 (42.8)	4	1 (25)	26	71
Winter 2018	0	0 (0)	0	0 (0)	17	74.3

D. Dissected **P.** Parous female **PR**. Parity rate

DISCUSSION

Entomological monitoring of vectors provides information on the characteristics of disease transmission in an area as well as the behavior and habitats of the specific vector species. These information are essential components of disease control programs, operational activities and research. For targeting that, a preliminary survey was carried out to provide ecological and behavioral information about, the identity of specific mosquito species, seasonally prevalence, it's feeding and parity status. During the present study, only four adult species were collected in the study areas in Monufia governorate, *Culex (Culex) pipiens*, *Culex (Culex) antennatus*, *Culex (Culex) perexiguus* and *Ochlerotatus (Ochlerotatus) caspius*. However, a previous conducted study in Monufia governorate reported six culicine mosquito species *Culex pipiens*, *Culex antennatus*, *Culex perexiguus, Ochlerotatus caspius*, *Ochlerotatus detritus* and *Culiseta* as these two species were collected as larvae only in that study (Abdel-Hamide *et al*., 2011). *Culex pipiens* was the most abundant species in the majority indoor and outdoor adult collection sets of the present study in agreement with the previous observations by El-Said and Kenawy (1983), Mostafa *et al.*, (2002) and Zayed *et al.*, (2015). This species is the primary vector of many viruses and may complicate the risk of RVFV transmission during RVF outbreaks in the Nile Delta. The same conclusion was noticed by Zayed *et al.*, (2015)

The relationship between climatic factors and mosquito seasonal abundance is very important to determine parasite activity levels and disease risk (Bashar and Tuno, 2014). The present work indicated that, population densities vary strongly with temperature since *Cx. pipiens* and *Ochlerotatus caspius* density increased as outdoor temperature increased and indoor temperature decreased, while *Cx. antennatus* density increased as outdoor temperature decreased and indoor temperature increased. On the other hand, *Cx. perexiguus* density increased as outdoor and indoor temperature decreased. These variations between indoor and outdoor population densities may be due to variation between outdoor and indoor temperature which strongly depend on factors such as season, location/altitude, the nature of the building structure, its surroundings, the number of occupants and whether people burn wood indoors additionally, even within a single house there is likely to exist a gradient of temperature microhabitats (Paaijman and Thomas, 2011). Adult mosquitoes showed higher incidence during Spring and Autumn seasons when the temperature was moderate or suitable (22-28°c), while the abundance decreased during Summer when the temperature rose (35^{O}c - 39^{O}c) which may kill and affected the lifespan of the mosquito (Pratt and Moor, 1993).In the present study, population density decreased also during Winter as low temperature probably prolonged the development period from egg to adult as well as it could be inducing mortality of larval and adult stages as suggested by (Kaul and Wattal, 1968). Also, heavy rain during Winter season inhibits mosquito oviposition and movement (Chave, 2010).

The obtained results indicated also that, population densities vary with relative humidity as temperature since *Cx. pipiens* density increased as outdoor relative humidity increased and indoor relative humidity decreased. On the other hand, the density of *Cx. antennatus* decreased as indoor and outdoor relative humidity increased. These observations disagree with findings of Abdel-Hamide *et al.* (2009) and Zayed *et al.* (2015) who reported that, adult mosquito density increases as relative humidity increased. In the present study, *Cx. perexiguus,* and *Oc. caspius* density increase with the increased of outdoor and indoor relative humidity which was explained by Dow *et al.* (1970) and Chaves *et al.* (2010) who found that, relative humidity influences mosquito activity patterns and the dynamics of oviposition. The biting rate of the four collected species was high during Spring which may be due to their delayed flight activities (e.g. host searching) until more favorable temperature conditions occur and considerably decrease their activities at an unfavorable temperature **(Lebl *et al*., 2013).** The lowest number of the fed status of the three *Culex* species collected from outdoor sites may suggest a more endophagic and endophilic behaviour of these species (Kaul and Wattal, 1968).

Mosquito species differ in their resting behavior and they may prefer resting indoor (endophilic) or outdoor (exophilic) (Pates and Curtis, 2005). In the present study, the three *Culex* species rested indoor more than rested outdoor throughout the year, which indicates the endophilic nature of the *Culex* species which also were reported by Gowda and Vijayan (1992). Blanford *et al.* (2009**)** stated that, mosquito individuals select resting locations where microclimate is favourable for optimum physiological activities**.** The abdominal condition of day time resting females provides additional evidence of their resting behavior. Among the indoor resting females of *Culex*, the proportion of gravid ones was higher than feds, indicating that a large proportion rest indoors before completion of their gonotrophic cycle which also confirm that, the populations of *Culex* species in the study areas tend to be more endophilic for resting as recorded before by **Gowda and Vijayan (1992)** who collected fed and semi-gravid more than unfed *Cx. quinquefasciatus* females from indoor resting sites**.**

Age determination of the insect vectors is important to estimate rates of population increase (Lehane, 1985; Milby and Reisen, 1989). Several methods have been suggested to determine the age of insects but the technique introduced by Detinova (1945) still the easer and accurate method to differentiate between parous and nulliparous females. The potential for transmission diseases comes from the female mosquitoes that survive for at least two gonotrophic cycles (Marc and Briegel, 1994). The study indicated that, there is seasonal variation in parity rate evident as it was highest among *Cx. pipiens* females collected from outdoor during Spring 2016 and 2017, while *Cx. antennatus* the highest parity rate occurred at Autumn 2017 and Spring 2017. This increase in parity ratio may be due to suitable climatic conditions such as rainfall and temperature, as well as higher relative humidity, which largely influences mosquito biology as suggested by Sang *et al*. (2010); Lutomiah *et al*. (2013) and Arum *et al.* (2015). In the case of *Ochlerotatus caspius* females, the highest parity rate was recorded in Spring 2016 and Summer 2017 which disagree with Nathan (1981). During the present study the lowest parity ratios were recorded during Winter season, which means an increase in the nulliparous proportion of the populations, observed during the study period is probably due to high number of breeding sites that formed by rain full as estimated by De Meillon and Khan (1967); Graham and Bradley (1972). Also in Summer, the females showed low parity rate which may be due to higher ambient temperatures that lead to faster blood meal digestion and thus shorter gonotrophic cycle duration and higher biting frequency by the mosquitoes as estimated by Mala *et al.* (2014). This is also may be due to dry weather conditions resulting in reabsorption of follicles by mosquitoes and retaining eggs or entering dormancy as a way of avoiding dry conditions as Medlock *et al.* (2006) illustrated.

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