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# On the Biology and Thermal Developmental Requirements of the Cotton Mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) in Egypt

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# ABSTRACT

This study evaluated the thermal requirements for development of the cotton mealybug Phenacoccus solenopsis depending on different biological parameters on Okra leaves Abelmoschus esculentusat under two constant temperatures (20°C and 30°C). The effect of temperature on eggs was ineffective since it hatched shortly to 1<sup>st</sup> nymphal instars after deposition. While the tested temperature caused significant effects on nymphal durations, pupation rate (pre-male stage), females emergence %, pre-oviposition, longevity, post-oviposition periods and fecundity in females (egg deposition, ovisacs numbers and hatchability %). The thermal constant and developmental zero were calculated to be 7.29°C and 79.9 DDs for eggs, 11.67°C and 272.9 DDs for nymphal stages, 11.06°C and 46.4 DDs for males and then 3.31°C and 554.1 DDs for females, respectively. The duration of the life cycle was  $65.6 \pm 10.36$  days at 20°C; this was shortened to  $35.51 \pm 1.12$  days at 30°C. The thermal requirements to complete the insect development for one generation was 8.2°C for the developmental zero and 774.1 DDs for the thermal constant. Based on the thermal requirements values, the average life cycle duration from January to December 2016 was 61.78 days and the number of annual generations was 7.143 when the average annual temperature was  $23.29^{\circ}$ C.

Keywords: Biology, Insects, Cotton Mealybugs, Phenacoccus solenopsis, Thermal requirements

# INTRODUCTION

The cotton mealybug, *Phenacoccus solenopsis* [1] [Hemiptera: Pseudococcidae] is a polyphagous insect pest. It was originally reported on ornamentals and fruit crops in New Mexico [1] and then spread in different regions of the world. In Texas, USA in 1989 it was reported as a pest of cotton [2]. Recently, it was recorded in other countries such as Ecuador [3], Chile [4], Argentina [5], Brazil [6], Pakistan [7], Nigeria [8], India [9], China [10], Italy [11] and Iraq [12].

In Egypt, *P.solenopsis* was first recorded on weeds [13] and subsequently as a new insect pest on tomato plants in Qalyoubia governorate [14]. In Alexandria governorate [15], recorded it on two ornamental plants. While, Beshr et al. [16] recorded this insect species on different host plants. For the first time, *P. solenopsis* was recorded as a new insect pest attacking cotton plants (Gossypium barbadense var. Giza 86) in Kafr El-Sheikh governorate [17].

Damage of this insect to host crops is due to sucking of twigs, leaves and fruits. This insect excretes honey dew resulting in sooty mould growth, which hinders photosynthesis [18]. At high infestation rate, the infested host plants become stunted, weak and produce only a few small cotton bolls. The leaves appear distorted, turn yellow and eventually drop off causing severe damage to the yield [6,19,20]. Its damage increase in the hot regions due to increasing of the insect population, because of the presence of positive correlation among the mealybugs population with temperature [21].

The temperature represents an important environmental factor that affects development, survival and abundance of mealybugs [22,23]. Zalom et al. [24] reported that the thermal units provide a valuable tool for insect pest control, in forecasting infestations monitoring and timing of insecticide applications. Accumulated thermal units have been used to predict the seasonal development and emergence of various insects [25,26].

This study aims to evaluate the thermal requirements for complete development of *P. solenopsis* depending on assessment of its life history parameters including developmental durations, survival and reproduction on leaves of okra *Abelmoschus esculentus* under two constant temperatures (20 and 30°C). The investigations can help in predicting annual generations and expected times of female frequency in the field under current and expected future climate changes by using the relationship between the accumulated thermal heat units expressed as degree days and its population fluctuation in the experimental area.

# MATERIALS AND METHODS

A field survey was carried out during June, 2016 in different cultivations in Sharkia governorate, Egypt. The collected samples of mealybugs were transferred to the laboratory and these were identified as *Phenacoccus solenopsis* Tinsley by Prof. Dr. Saber Faheim, Plant Protection Institute, the Ministry of Egyptian Agriculture.

#### Colony establishment

Culture of *P.solenopsis*, was maintained under laboratory conditions. Potato sprouts and okra fruits were used for rearing the mealybugs in rectangular plastic boxes ( $50 \times 30 \times 30$  cm) provided with sawdust and covered with nylon net. Specimens of the collected mealybugs have been released in the rearing boxes and in a short time they settled on okra fruits and potato sprouts for feeding by sucking the plant juice. The incubation period of eggs was very short and after hatching, the neonate crawlers (1<sup>st</sup> nymphal instar) emerged out and then transferred to glass jars (4 L) provided with okra fruits and leaves. The crawlers were very fast-moving but after 24-48 h. They settled on the host (near to the veins) and started in piercing it for sucking the juice for nutrition and development until they became adults. The female nymphs moulted three times while the males four times. The presence of exuviae indicated the successful development from one instar to another. Finally, the moulted adults were transferred to rearing plastic boxes for starting the next generation. Adult females live for a long period and feed on plant juice till oviposition, while the male life span was very short and it has no feeding mouthparts.

## Biological studies

The biological studies of *P. solenopsis* were conducted according to Nikam, Vennila and Sreedevi [27-29]. Different biological parameters of *P. solenopsis* were estimated on okra leaves under two constant temperatures (20 and 30°C). In each experiment, ten mated females were taken from the laboratory culture then transferred individually on okra in plastic cups, all cups were maintained at the tested temperature. These females were observed daily for ovisacs; the ovisac was excreted and formed below the posterior end of the abdomen. All ovisacs have been removed carefully and examined using stereomicroscope for egg count and transferred to new Petri dishes provided with okra leaves and then divided into five replicates and returned to the set temperature. The average number of the incubated eggs, incubation period and % of hatching were calculated.

After hatching, the neonate crawlers (1<sup>st</sup> nymphal instar) are active but became less mobile when they settled on the feeding site. The five incubated Petri dishes were examined daily for the shed exuvia. The successful moulting was confirmed by the presence of exuivum on the leaf or on the posterior end of molted nymphs. The colour, shape and size of each nymphal instar (1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup>) were critically observed. The developmental time of each instar was recorded depending on observation of exuvia and increasing of the size. The female nymphs moulted three times and the males four times. Winged adult males emerged from the pre-male stage (pupa) which was loosely woven, silky, filamentous capsule-like structures and then the developmental durations of pupae, males, females and the cumulative duration of the life cycle were computed. After adult emergence, twenty females were reared separately  $(1 \bigcirc :1 \circ)$  in plastic cups on okra leaves under the tested temperatures to study the pre-oviposition, longevity and post-oviposition periods. Since the females laid their eggs in cottony sacs located at posterior end of its abdomen, the ovisacs were collected during the oviposition period to count the number of ovisacs and eggs in each ovisac for calculating the fecundity.

#### The thermal requirements and annual generations

An experiment was conducted to calculate both of the expected date for female appearance and the annual generations of *P. solenopsis* under field conditions. Degree-days were calculated from daily temperatures recorded by the Central Laboratory for Agriculture Climate, Agriculture Research Centre during 2016.

The previous biological parameters of *P. solenopsis* were estimated to determine the thermal requirements (Thermal constant & Developmental zero) for the developmental stages (eggs, nymphs, males and females) and the biological life cycle. For this purpose, the formula of Bodenheimer [30] and Jasic [31] has been applied. The formula is as follows:

y= n (t - x)

Where, y is the total effective temperatures required for development of each stage (heat units) or the so called thermal constant; x is the thermal threshold or zero of development and below which development of any stage was inactivated; n is the average duration of the stages at the temperature (t). Accordingly, the values of the thermal threshold and thermal constant for each stage of the insect can be determined. The calculation of thermal requirements of the pest helps in prediction of its occurrence.

## **RESULTS AND DISCUSSION**

## **Biological studies**

Different biological parameters of *P. solenopsis* were estimated under two constant temperatures (20 and 30°C). The data presented in Table 1 showed the duration of the life cycle of *P. solenopsis* (which is successfully completed from crawlers to adults at the tested temperatures). At low temperature (20°C), the developmental time occurred at a much slower rate for each stage, so the development of the life cycle lasted a long time. In contrast, the life cycle was fast at high temperature (30°C) because the duration of each stage was short.

	Biological aspects		Rearing temperature	s at RH 65%
			20 ± 1 °C	30 ± 1 °C
Egg stage	No. of incubated eggs		$117.6 \pm 18.26$	111.00 ±
	Incubation periods / h		$7.70 \pm 1.22$	$4.31 \pm 0.41$
	No. of hatched eggs (1 <sup>st</sup> nymphal inst	ar)	$104.00 \pm 18.89$	103.60 ± 7.23
	% of hatching		$88.44 \pm 5.98$	93.33 ± 1.63
Nymphal sage	Duration of 1 <sup>st</sup> instar/day		$10.20\pm0.31$	$4.40 \pm 0.22$
	Duration of 2 <sup>nd</sup> instar/day		$10.24 \pm 1.69$	5.50 ± 0.43
	Duration of 3 <sup>rd</sup> instar/day		$12.32 \pm 1.20$	4.99 ± 0.28
	Total duration of nymphal stage / day	1	$32.76\pm2.29$	$14.89\pm0.5$
Male	No. of pre-male stage (pupae)		$11.20 \pm 4.12$	7.10 ± 2.83
	% of pupation		$10.77 \pm 2.31$	$6.86 \pm 2.53$
	Pupal duration/day		$7.60\pm0.61$	$4.54 \pm 0.35$
	No. of emerged male		$7.40 \pm 2.94$	6.60 ± 2.65
	% of male emergence		$66.07 \pm 19.01$	$92.96 \pm 8.89$
	Longevity of male/day		$5.19\pm0.49$	$2.45 \pm 0.31$
Female	No. of moulted female		$47.80 \pm 9.37$	$62.60\pm6.71$
	% of female moulting		$45.96 \pm 5.17$	$60.42 \pm 9.18$
	Pre-Oviposition period/day	$12.48\pm0.69$	$4.85\pm0.78$	
	Longevity of female/day		$32.84 \pm 2.07$	20.62 ± 1.11
	Post-Oviposition/day		$5.07 \pm 2.86$	$2.46\pm0.93$
	No. of ovisacs		$3.92\pm0.95$	3.58 ± 0.64
	(Range)		(2-5)	(2-4)
		1 <sup>st</sup> Ovisac	$12.92 \pm 1.14$	$5.16\pm0.77$
	Female age at production of ovisac		(10-14)	(4-6)
	(Range)	2 <sup>nd</sup> Ovisac	$18.15\pm2.95$	$10.10 \pm 0.78$
		and a s	(17-20)	(9-11)
		3 <sup>rd</sup> Ovisac	$23.50 \pm 2.04$	15.75 ± 2.11
		th c :	(20-22)	(14-18)
		4 <sup>th</sup> Ovisac	$28.11 \pm 1.99$ (27-29)	19.33 ± 0.65 (18-20)
		5 <sup>th</sup> Ovisac	$31.00 \pm 0.00$	Not secreted
		0 0 11540	-31	The secreted
	Egg deposition / female		$191.08 \pm 31.34$	239.92 ±
				32.65
	Hatchability		$125.15 \pm 29.63$	228.69 ± 31.78
	% of hatchability in the second generati	ion	$65.32 \pm 8.71$	$95.31\pm2.07$
	Total life cycle of female (days)		$65.60 \pm 10.36$	35.51 ± 1.12

Table 1: Effect of temperature on biological aspects of *Phenacoccus solenopsis* at two different constant temperatures

## Egg stage

Females of *P. solenopsis* deposited and protected their eggs in an ovisac. The ovisac was located below the posterior end of the abdomen and composed from thin and soft cottony filaments, creamy yellow in colour and like cotton candy in shape. The eggs were light creamy yellow in colour, elongate in shape with tapering ends like rice. The average number of the incubated eggs at 20 and 30°C for starting the biology studies was  $117.6 \pm 18.26$  and  $111.0 \pm 7.72$  eggs, respectively. The incubation period was very short; it was  $7.7 \pm 1.22$  h at 20°C then decreased significantly to  $4.31 \pm 0.41$  h. As the temperature increased to 30°C. Also, the hatching rate was  $93.33 \pm 1.63\%$  at 30°C then decreased to  $88.44 \pm 5.98\%$  at 20°C.

## Nymphal stage

The insect passes through three nymphal instars. Just after hatching, neonate crawlers (1<sup>st</sup> nymphal instar) emerged from eggs. They were very small, soft creamy in colour with no wax coating on their bodies. This instar is very active up to 24 h of hatching but became less mobile after settling on the feeding site. After first moulting, the second instar nymphs emerged and appeared to be very slow in motion with a significant increase in size and simple change in colour. Two black strips on the thorax and on the abdomen and light white waxy filaments on the dorsal side after about one day of first moulting were observed. The exuvium of the instar was observed near the posterior end of the abdomen. After second moulting, the third instar emerged with light green in colour after moulting immediately. Two black strips were observed on the thorax and the abdomen with increase in size compared with the previous instars. White waxy layer appeared after 2 days of moulting.

Data in Table 1 indicated that temperature significantly influenced the nymphal development. The developmental period of 1<sup>st</sup> instar of *P. solenopsis* was shorter than 2<sup>nd</sup> and 3<sup>rd</sup> instars. The mean developmental periods of 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> in stars were 10.2  $\pm$  0.31, 10.24  $\pm$  1.69 and 12.32  $\pm$  1.2 days, respectively at 20°C. Increase of temperature to 30°C shortened significantly the nymphal durations being 4.4  $\pm$  0.22, 5.5  $\pm$  0.43 and 4.99  $\pm$  0.28 days, respectively. The total nymphal duration was 32.76  $\pm$  2.29 days at 20°C and significantly decreased to 14.89  $\pm$  0.5 days with increase in temperature to 30°C.

## Male

Male nymphs moulted four times, it passes through three nymphal intars and pupae prior to reaching the adulthood. Pupae were small, cylindrical and enclosed in white cottony cocoon. Adult males emerged from the pupae. Males appeared to be thin and elongate in shape, grey in color and characterized by the presence of a single pair of fore transparent wings. Two long waxy filaments were present at the abdominal end of the body.

Data in Table 1 indicated that, production of pupae (pre-male stage) was lower than female production under the tested temperatures. The average number of recorded pupae was  $11.2 \pm 4.12$  pupae (10.77%) and  $7.1 \pm 2.83$  pupae (6.86%), while number of recorded females increased to  $47.8 \pm 9.37$  females (45.96%) and  $62.6 \pm 6.71$  females (60.42%) at 20 and 30°C, respectively. The developmental durations of pupae and male longevity decreased significantly from  $7.6 \pm 0.61$  to  $4.54 \pm 0.35$  days for pupa and from  $5.19 \pm 0.49$  to  $2.45 \pm 0.31$  days for the male with increase in temperature from 20 to  $30^{\circ}$ C, respectively.

#### Female

Female nymphs moulted three times. Females undergo three immature nymphal instars only prior to reach the adulthood. Female adults of *P. solenopsis* were wingless, wide and oblong in shape and dark brown in colour immediately after emergence and then light white waxy filaments appeared after an hour of moulting. The waxy secretions increased within 2 days to coat the body with two black strips on the dorsal side of body region. Results in Table 1 on pre-oviposition, longevity and post-oviposition periods of females revealed that these parameters were temperature dependent with significant decreasing trend and they were longest at 20°C and shortest at 30°C. The pre-oviposition periods lasted  $12.48 \pm 0.69$  then decreased to  $4.85 \pm 0.78$  days. The longevity of females were  $32.84 \pm 2.07$  then decreased to  $20.62 \pm 1.11$  days. While the post-oviposition period lasted  $5.07 \pm 2.86$  then decreased to  $2.46 \pm 0.93$  days when the temperature changed from 20 to 30°C. Total number of ovisacs and eggs laid by a single female during its life period varied depending on the temperature. Observations indicate the first record for the number of ovisacs and their timing. The ovisacs produced by a single female ranged from 2-5 with an average of  $3.92 \pm 0.95$  ovisacs at 20°C. The first sac was produced at  $12.92 \pm 1.14$  days post-female emergence, while the second, third, fourth and fifth ovisacs were produced at  $18.15 \pm 2.95$ ,  $23.5 \pm 2.04$ ,  $28.11 \pm 1.99$  and  $31 \pm 0.0$  days post-female emergence. While at 30°C, the ovisacs produced by single female ranged from 2-4 with an average of  $3.58 \pm 0.64$  ovisacs. The first sac produced at an age of  $5.16 \pm 0.77$ , while the last was produced at an age of  $19.33 \pm 0.65$  days post-adult emergence. The highest egg production was observed at  $30^{\circ}$ C ( $239.92 \pm 32.65$  eggs/female) and 95.31% hatchability compared with  $191.08 \pm 31.34$  eggs/female and the hatchability was 65.32% at  $20^{\circ}$ C.

Finally, the period required for complete development of the life cycle of *P. solenopsis* (from newly hatched crawlers to adult female stage) was  $65.6 \pm 10.36$  days at 20°C then decreased to  $35.51 \pm 1.12$  days at 30°C.

#### The thermal requirements and annual generations

The biological parameters of *P. solenopsis* were estimated to determine the thermal requirements (Thermal constant & Developmental zero) for the insect developmental stages (eggs, nymphs, males and females) and the biological life cycle. For this purpose, the following formula has been applied

y = n (t - x)

In the egg stage, the incubation period of the newly deposited eggs, showed to be 7.7  $\pm$  1.22 and 4.31  $\pm$  0.41 h at constant temperature of 20 and 30°C, respectively.

The zero of development (x) and the thermal constant (y) for the egg stage can be calculated as follows:

Since, y=n (t-x) where, n=Incubation period; t=Average temperature.

At an average temperature of 20°C; Thermal constant (y)=(7.7 h)×[20–x]

Also, at an average temperature 30°C;

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Thermal constant (y)=(4.31 hrs)×[30–x]
i.e. y =154.0 – 7.70 x .....at 20 °C \rightarrow (1)
y =129.3 – 4.31 x ....at 30 °C \rightarrow (2)
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When equation 1=equation 2
154.0-7.70x = 129.3-4.31x
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So, 24.7=3.39x i.e. Developmental zero (x) = $7.29^{\circ}$ C.

By adding value of (x) the value of the thermal constant (y) were calculated as 97.9 So, y = 97.9 Degree Days (DDs) for the egg stage.

The thermal constant and developmental zero for the nymphal stage, male, female and total life cycle were calculated as explained above and the values are reported in Table 2.

Table 2: The thermal	requirements for	development of I	Phenacoccus solenopsis

	Duratio	ons at	Thermal requirements		
Biological stages	20 ± 1 °C	30 ± 1 °C	Thermal threshold (Developmental zero. x)	Thermal constant (y)	
Egg stage	7.70 ± 1.22 (h)	$4.31 \pm 0.41$ (h)	7.29°C	79.90 DDs	
Nymphal stage	$32.76 \pm 2.29$ (days)	$14.89 \pm 0.50$ (days)	11.67℃	272.90 DDs	
Male	$5.19\pm0.49~(days)$	$2.45 \pm 0.31$ (days)	11.06°C	46.40 DDs	
Female	32.84 ± 2.07 (days)	20.62 ± 1.11 (days)	3.31°C	554.10 DDs	
Total life cycle	65.60 ± 10.36 (days)	35.51 ± 1.12 (days)	8.20°C	774.10 DDs	

### The expected frequencies of annual generations of Phenacoccus solenopsis in Sharkia governorate, Egypt

Studies on the life cycle of *P. solenopsis* showed that the heat required for completing the life cycle per day and then per month in Sharkia governorate in 2016 were calculated and reported in Table 3.

Months	No. of days (n)	Average temp. °C (t)	D. Zero (x)	Thermal constant (y)	Heat units (Degree days)		Duration	Rate of generation development			
					t - x	n (t-x)		(G)			
January	31	14.2			6	186	126.91	0.24			
February	29	14.6	8.2		6.4	185.6	117.29	0.24			
March	31	17.7		8.2 774		9.5	294.5	80.64	0.38		
April	30	24.7				16.5	495	46.92	0.639		
May	31	25.9					17.7	548.7	43.98	0.709	
June	30	28.8			774 1	20.6	618	37.4	0.798		
July	31	30.1			8.2	8.2	774.1	21.9	678.9	35.35	0.877
August	31	30.4					22.2	688.2	34.87	0.89	
September	30	28.5					20.3	609	37.76	0.787	
October	31	26.6				18.4	570.4	41.62	0.737		
November	30	22.9			14.7	441	52.66	0.57			
December	31	15.1			6.9	213.9	86.01	0.276			
Total	266	279.5		•		•	741.41	7.143			
Average	- 366	23.29					61.78	0.595			

Table 3: Calculated heat units required for complete development of Phenacoccus solenopsis in Sharkia governorate during 2016

Data in Table 3 indicate that when the developmental zero (x) was 8.2°C and thermal constant (y) was 774.1 DDs, the life cycle duration was 126.91 days in January 2016 when the average temperature was 14.2°C; this decreased gradually in the following months where the minimum values of life cycle duration were recorded as 34.87 and 35.35 days in August and July 2016 at an average temperature of 30.4 and 30.1°C, respectively. Then it increased to 37.76 days during September 2016 with decrease of the temperature to 28.5°C. The longest duration was recorded in winter months (January, February, March and December 2016) where it reached to 126.91, 117.29, 80.64 and 86.01 days when the monthly temperature was 14.2, 14.6, 17.7 and 15.1°C, respectively. The average duration of life cycle from January to December 2016 was 61.78 days when the mean annual temperature was 23.29°C and the number of annual generations was 7.143 generations with an average of 0.595 generation per month.

The rate of generation development was calculated as follows:

Rate of generation development (G)=No. of days per month (n)  $\times$  [temperature (t) – developmental zero (x)] / thermal constant (y).

For instance, in January, heat units required for insect development (from egg to egg) G=n (t-x)/y i.e.,  $31 \times 6 / 774.1 = 0.24$ .

So, to determine the expected rate of annual generation development of the insect in the field, it is assumed that the duration of each cycle throughout the year is correlated with the thermal constant (y) of the adult female which amounts to 774.1 DDs. So, calculations take into consideration that the accumulation of heat units started on the  $1^{st}$  of January 2016. From the data given in Table 4 it appears that the cycles for the insect occur throughout the year and that determination of one generation development at any time represents the expected time for the insect emergence.

Analysis of the data clearly indicate that the expected time of the first generation was 6.546 in April 2016. This cycle passes through the remaining 23.546 days of April where the rate of generation development was 0.5; this cycle extends through the first 21.87 days in the following month (May) to attain 0.5 of the generation to complete the  $2^{nd}$  generation cycle in 21.87, May 2016. Again the  $3^{rd}$  cycle extends through remaining 9.13 days within the same month where the rate of the  $3^{rd}$  generation development was about 0.21 and then extends until 29.733 days of June where the rate was 0.79 to complete the  $3^{rd}$  generation in the day 29.733 of June, 2016.

This phenomenon was continued until the end of December 2016, when the 7<sup>th</sup> generation cycle was detected in the mid of December 2016.

## DISCUSSION

The data obtained during these investigations are important to know more about the biology of cotton mealybug *P. solenopsis* and to estimate the thermal constant and developmental zero aiming to calculate the expected frequencies of annual generations for proper insect management. The sex of this species is separate; it moults three times to give females and four times to give males. The incubation period of eggs showed to very short and the crawlers were observed on the same day of deposition giving the impression of viviparous reproduction. It was  $7.7 \pm 1.22$  h at 20°C and then it decreased significantly to  $4.31 \pm 0.41$  h at 30°C. This coincides with previous authors; Nikam et al., [27] observed that the incubation period ranged between 35 to 60 min with an average of  $39.00 \pm 7.57$  min at a range of temperature 25-30°C. Vennila et al., [28] reported that the maximum incubation period of eggs was 120 min with a mean of  $68.5 \pm 33.0$  min under laboratory conditions. Arif et al., [32] reported that the incubation period of eggs lasted 4-6 h in the laboratory. While, Hameed et al., [33] showed reduction in egg hatching period from  $32 \pm 0.22$  to  $0.57 \pm 0.15$  h at 20 to  $40^{\circ}$ C. Also, Singh and Kumar [34] recorded few minutes ( $3.634 \pm 0.7$ ) for hatching the deposited eggs of *P. solenopsis* under laboratory conditions. In contrast, high effects of temperature on egg incubation period (more than 3 days) after oviposition have been observed in other mealybug species as *Paracoccus marginatus* [22] and *Phenacoccus Madeirensis* [35].

Our laboratory observations indicated that, the neonate crawlers (1<sup>st</sup> nymphal instar) emerged from ovisacs. This instar is very active but became less active after crawlers settled on its feeding site. The successful moulting was confirmed by the presence of exuivum on the leaf or on the posterior end of molted nymphs with changes in colour, shape and size of each nymphal instar as reported by Nikam et al. [27] and Hameed et al. [33]. Females pass through three immature nymphal instars only prior to reaching the adulthood.

Females are wingless, flat and oval, body covered with white waxy layer. There are two parallel rows of dark spots on the dorsal side (thorax and abdomen) and numerous short white waxy filaments on the outer margin of the body. Anal filaments are a bit longer. While, adult males pass through three immature nymphal instars and pre-male stage (pupa). Winged adult males emerged from the puparia. Males appeared to be thin and elongate in shape, grey in color and characterized by the presence of a single pair of fore transparent wings. Two long waxy filaments were present at the abdominal end of body and resembled cotton whitefly adults in shape and size with rudimentary mouth parts.

	Days	Heat	t units	Rate of generation development		Cycles No.
Months ( n	(n)	(t-x)	n ( t-x )		Expected date of annual generations	
January	31	6	186	0.24		
February	29	6.4	185.6	0.24		
March	31	9.5	294.5	0.38		1
April	6.546	16.5	108.009	0.14		
Total	97.546		774.1	1	6.546, April, 2016	
April	23.454	16.5	387	0.5		
May	21.87	17.7	387.1	0.5		2
Total	45.324		774.1	1	21.87, May, 2016	
May	9.13	17.7	161.6	0.21		
June	29.733	20.6	612.5	0.79		3
Total	38.863		774.1	1	29.733, June, 2016	
June	0.267	20.6	5.5	0.007		
July	31	21.9	678.9	0.88		
August	4.04	22.2	89.7	0.116		4
Total	35.307		774.1	1	4.04, Aug., 2016	
August	26.96	22.2	598.5	0.77		
September	8.65	20.3	175.6	0.23		5
Total	35.61		774.1	1	8.65, Sep., 2016	
September	21.35	20.3	433.41	0.56		
October	18.516	18.4	340.69	0.44		6
Total	39.866		774.1	1	18.516, Oct, 2016	
October	12.484	18.4	229.7	0.297		
November	30	14.7	441	0.57		
December	14.99	6.9	103.4	0.134		7
Total	57.474		774.1	1	14.99, Dec., 2016	]
December	16.01	6.9	110.47	0.143		

Table 4: The expected frequencies and heat units required for cycles of annual generations of <i>Phenacoccus solenopsis</i> during 2016 in Sharkia
governorate

Temperature is a major environmental factor that affects development, survival and abundance of mealybugs [22,36-38]. When the temperature is low, development occurs at a much slower rate [39] and, as a result, time spent in each developmental stage increases.

In the same direction, the present investigation indicated that temperature significantly influenced the development of all nymphal instars and total nymphal development. The developmental periods of  $1^{st}$ ,  $2^{nd}$  and  $3^{rd}$  in stars were  $10.2 \pm 0.31$ ,  $10.24 \pm 1.69$  and  $12.32 \pm 1.2$  days, respectively at 20°C, increase of temperature to 30°C shortened significantly the nymphal durations being  $4.4 \pm 0.22$ ,  $5.5 \pm 0.43$  and  $4.99 \pm 0.28$  days, respectively. The total developmental duration for nymphal stage was  $32.76 \pm 2.29$  days at 20°C and then significantly decreased to  $14.89 \pm 0.5$  days with increase of temperature to 30°C. The developmental periods became shorter for every instar with increase in temperature. This may be due to increase in metabolism which resulted in increase of the growth rate and development time of *P. solenopsis* [33].

It appears that the rate of both pupation (pre-male stage) and male emergency was lower than that of females under the tested temperatures. Our results similar with results obtained by Hameed et al. and Kumar et al. [33,34]. The pre-oviposition periods lasted 12.48  $\pm$  0.69 and 4.85  $\pm$  0.78 days. The longevity of females was 32.84  $\pm$  2.07 and 20.62  $\pm$  1.11 days, while the post-oviposition period lasted 5.07  $\pm$  2.86 and 2.46  $\pm$  0.93 days at 20 and 30°C, respectively. The ovisacs produced by a single female ranged from 2-5 with an average of 3.92  $\pm$  0.95 ovisacs at 20°C.

While at 30°C, the ovisacs produced by a single female ranged from 2-4 with an average of  $3.58 \pm 0.64$  ovisacs. The highest egg production was observed at 30°C (239.92 ± 32.65 eggs/female) and 95.31% hatchability compared with 191.08 ± 31.34 eggs/female and 65.32% hatchability at 20°C.

Similarly, Venneila et al. [28] reported 344 eggs/female. Nikam et al. [27] found that longevity of female of the same species was  $33.67 \pm 1.19$  days compared with  $8.70 \pm 0.79$  days for the male at temperature and relative humidity range from 25 to 30 °C and 75 to 80%, respectively. Also, Yong et al. [40] reported 458 eggs per female. While, the fecundity rate of the female ranged from 300 to 750 eggs and the longevity of female was higher (24.44  $\pm$  2.33 days) than the male (1.96  $\pm$  0.84 days) at room temperature (24.3  $\pm$  31.2°C) [41]. Whereas, Hameed et al. [33] recorded 232 eggs/female at 20°C, then reduced to 136 eggs at 40°C. This variation may be due to difference in the food source.

The importance of temperature for the development of various insects has been reported by many workers e.g. Bodenheimer; Hamdy; Salama et al.; Kim et al. [30,37,42]. According to Zalom and Wilson [42] the rate of insect development is based on the accumulation of heat measured in physiological rather than chronological time. Zalom et al. [24] reported that the thermal units provide a valuable tool for insect pest control, in forecasting infestations monitoring and timing of insecticide applications. Accumulated thermal units have been used to predict the seasonal development and emergence of various insects [25-26,43]. Data obtained in the present investigations can help in predicting *P. solenopsis* annual generations and expected times of frequency of annual generations under current and expected future climate changes depending on the accumulated thermal requirements. The thermal requirements represent the limiting and required conditions for development of each stage. In order to determine the thermal threshold (below which development was inactivated) and thermal constant for each of the insect developmental stages.

Our results showed that the thermal constant and developmental zero of *P. solenopsis* were calculated to be 7.29°C and 79.9 DDs for the eggs, 11.67°C and 272.9 DDs for the nymphal stage, 11.06°C and 46.4 DDs for the males and 3.31°C and 554.1 DDs for the females, respectively. The duration of life cycle was  $65.6 \pm 10.36$  days at 20°C then decreased to  $35.51 \pm 1.12$  days at 30°C and the thermal requirements to complete the insect development for one generation were 8.2°C for the developmental zero and 774.1 DDs for the thermal constant. Based on the thermal requirements values, the average life cycle duration during one year from January to December 2016 was 61.78 days and the number of annual generations was 7.143 with an average of 0.595 generation per month when the average annual temperature was 23.29°C and the maximum rate of generation development was 8.77 and 8.9 in July and August 2016 when the average temperature was 30.1 and 30.4°C, respectively.

Taking in consideration that the accumulation of heat units started on 1<sup>st</sup> January 2016. So, the expected time of the first generation was 6.546 in April 2016. This cycle passes through the remaining 23.546 days of April where the rate of generation development was 0.5; this cycle extends through the first 21.87 days in the following month (May) to attain 0.5 of the generation to complete the 2<sup>nd</sup> generation cycle. Again the 3<sup>rd</sup> cycle extends through the remaining 9.13 days within the same month where the rate of the 3<sup>rd</sup> generation development was about 0.21 and then extends until 29.733 days of June where the rate was 0.79 to complete the 3<sup>rd</sup> generation cycle was detected in the mid of December 2016. In this concern Sreedevi et al. [29] stated that the thermal constants required for completion of cumulative development of female and male nymphs and the whole generation of *P. solenopsis* on hibiscus were 222.2, 237.0, 308.6 DDs. While, Prasad et al. [44] reported that males required 363.6 DDs. for their cumulative development compared to females (317.5 DDs). The estimated lower threshold for crawlers to adult development in the females of *P. solenopsis* was 11.7°C, while it was 7.4°C for *P. madeirensis* [22]. Our calculations in this study indicated that the maximum rate of generation development was 0.877 and 0.89 in July and August 2016 when the average temperature was 30.1 and 30.4°C, respectively. This coincides with Dhawan et al. [45], who stated that the peak field infestation of *P. solenopsis* was observed during 23<sup>rd</sup> July to 26<sup>th</sup> August with the mean temperature range of 28.2-32.3°C.

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