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Relationship between temperature and some biological aspects and biochemical of *Earias insulana* (Boisd.) (Lepidoptera: noctuidae)

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ABSTRACT

The experiments were carried out under controlled conditions of temperatures (16, 21, 26, 32 and 37 ± 1ºC) and relative humidity 65-70 % R.H. to study the temperature effect on some biological aspects and biochemical of *Earias insulana* field strain when reared on okra pods. The incubation period of *E. insulana* eggs was 12.45 days at 16ºC. But on 37 ºC it shortened to 1.10 days. Larval duration decreased as temperature increased when reared on okra pods. Similar trend was recorded for pupal duration. Generation period lasted 38.27 days on 21 ºC but it shortened to 17.4 days on 32 ºC. The thresholds of development (T_o) were 15.95, 14.41, 13.32, 17.63 and 12.85 ºC for eggs, larvae, pupae, pre- oviposition and generation period of *E. insulana*, respectively. While, the average of thermal units required to complete the developmental stages were 23.15, 131.33, 90.79, 20.83 and 346.19 DD̊s, respectively, when reared on okra pods. The total eggs laid by female high significantly affected by all temperatures. The highest number of eggs laid at 26 followed by 21ºC binge 273.66 and 208.00 eggs/female. The low and high temperatures caused the highest reduction in total lipid and protein. The reduction in total lipid and protein caused reduction in the fecundity and fertility of *E. insulana* adult female.

Key words: *Earias insulana* (Boisd.), temperature, development and Biochemical

INTRODUCTION

The spiny bollworm (SBW), *Earias insulana* (Lepidoptera: Noctuidae) is one of the most dangerous pests attacking many plants of Malvaceae family, especially okra *Abelmos esculentaus.* It's one of the most important vegetable crops growing during the summer and kharif seasons. However under field conditions, constant temperatures are not maintained for along period and fluctuating temperatures can cause difference in development and survival (Nowierski *et al*., 1983; Liu and Meng, 1999 and Davis *et al*., 2006). Temperature is critical a biotic important factor affecting the seasonal fluctuation and population dynamics of this pest on different host plant in the field (Hoffmann *et al.,* 2003 and Tran *et al*., 2007). Temperature determines the likelihood of mortality and hence, population declines, especially at extremes (Chown and Terblanche, 2007).

Many authors recorded that relationship between the temperatures and many aspects of biological processes (Al-Mohammady, 2000 and Dhillon and Sharma, 2004). Ismail *et al.,* (2005) and Shah *et al.,* (2012) studied the effect of temperature on developmental times of the life history stage of the *E. insulana* and/ or *Earias vittella.* They recorded the low temperatures from 15 to 20 ºC caused prolonged larvae and pupal stages, increased the mortality, While the high temperature from 31 to 35 ºC the development of larvae was faster. The high rate of pupation failure suffered by fast-growing insects at 34 ºC (Shah *et al*., 2012). On the other hand, considering the optimum temperature ranged from 25 to 28 ºC, because the all stages can be

completed the development and eggs produced, reproductive was much higher at 27 ºC compared to 20 ºC (Czesak and Fox, 2003 and Fayez, 2011).

Also, effect of temperature on biochemistry, physiology, behavior and life-history traits studied by (Kingsolver, *et al.,* 2004). Developmental processes are controlled by temperature dependent on biochemical reactions, which are restricted by lower and upper thresholds (Sharpe and Demichele, 1977; Hochachka and Somero, 2002 and Domos and Soultani, 2008).

The objectives of the current study were to investigate the effect of constant temperatures and fed on okra pods on some biological aspects and biochemical of *Earias insulana* in order to determine the optimum temperature.

MATERIALS AND METHODS

The experiments were carried out to study the effect of different constant temperatures (16, 21, 26, 32 and 37 ± 1°C) and 65-70 % R.H. on some biological aspects and biochemical of *E. insulana* when fed on okra pods.

1- Insect used:

The spiny bollworm, *E. insulana:*

Full grown larvae of *E. insulana* used in the currant study was collected from okra field at Sharkia Governorate during September to November of 2011 season and transferred to Bollworm Research Department, Plant Protection Research Institute, Agricultural Research Centre (ARC).

Insect rearing

The SBW larvae collected from okra fields were fed separately on okra pods in glass tubes (3 × 10 cm) covered with absorbent cotton wool. The food was daily renewed until pupation. Pupae were transferred to clean glass tubes and incubated until moth emergence. The emerged moths were sexed and caged to eggs lay. Moths were provided with 10 % honey solution. The eggs were separated daily and placed in glass jars. Obtained eggs were used in experiments.

Development of different stages:

Eggs laid on the same day (< 24 old) were placed in glass jar and incubated under five constant temperatures (16, 21, 26, 32 and 37± 1°C) and 65-70% R.H. Three replicates of 100 eggs/each were used for each tested temperature degree. The number of eggs hatched was recorded and eggs incubation period was calculated. The hatched larvae in each temperature were used for studied the developmental at the respective temperature.

Newly hatched larvae were transferred individually to glass tubes (3 × 10 cm) containing cutting okra pod (about ½ okra pod), and changed with fresh anther every one day. Each tube was plugged tightly with absorbent cotton and placed in an incubator at the previous conditions. Four replicates of 40 larvae/ each were used for each tested temperature. Larvae were examined daily until pupation to record larval duration. Pupae were transferred to clean glass tubes and examined daily until moth emergence to record pupal duration. The weight of full grown larvae and pupal were recorded. When adults emerged, moths were sexed and caged to eggs laying. Moths were provided with 10 % honey solution. The cages were inspected daily until moth death. The pre-ovipostion, oviposition and post-ovipostion periods and longevity of adult females and males and the numbers of eggs and hatchability percentages were calculated.

Statistical analysis

One way analysis of variance (ANOVA) and Duncan's multiple range test of means were used (Duncan's, (1957).

Effect of different constant temperatures on different stages of *E. insulana.*

Data recorded in the present work were analyzed by simple regression analysis. The effects of the above mentioned conditions were tested on eggs, larvae, pupae and pre- oviposition period of *E. insulana* after rearing on okra. Except rat of SBW´s development at 16 and 37°C not included in our analysis because the adults emergence were very few number. The theoretical development thresholds and the thermal units required were determined according to Blunk (1923) as following:

$(y) = a + bx$

Y = rate of development = 1/ duration, a= constant temperature. and b = simple regression coefficient

T_0= - a/b and k = 1/b

T_0 estimated thermal units required to complete development of each stage, equation of thermal summation was used according to Blunk (1923).

$K = y (T – T_0)$.

K = Thermal units (degree days = DDs); y = duration (days); T_0 = threshold of development; T = temperature in degree centigrade used.

Biochemical analysis:

To study the effect of temperature on some biochemical parameters, 100 newly hatched larvae of SBW transferred individually to glass tubes (3X 10 cm) containing okra pods and kept under the previous controlled conditions. The samples of SBW larvae were collected after 20 days at 16 ºC, ten days at 21 and 26 ºC and 4 days at 32 and 37 ºC and kept in clean tubes in a refrigerator at 4 ºC for chemical analysis.

Determination of Biochemical parameters:

Total protein, total lipids, glucose and amylase, glutamic oxaloacetic transaminase (GOT = ALT) and glutamic pyruvic transaminase (GPT = AST) enzyme activities were determined colorimetrically according to Koller (1984), Drevon and Schmitt (1964), Henry and Chiamori (1960); Trinder (1969) and Murray (1984).

RESULTS AND DISCUSSIONS

Effect of temperature on different stages of *E. insulana*:

Egg hatchability:

Data presented in Table (1) and Fig (1) Showed that the *E. insulana* eggs was able to hatch at range of temperatures from 16 - 37ºC, but the highest hatchability was recorded at 26 flowed by 21 ºC. The reduction of hatchability at high temperature may be due to an adverse affect of high temperature on embryonic development.

Percentage of larval and pupal mortality:

Data presented in Table (1) and Fig (1) reveled that the high percentage of mortality recorded at 16, 32 and 37 ºC. The respective, mortalities were 37, 46 and 69 %. On the other hand, the pupal mortality was 82.9 % at 16 ºC and 93 % at 37 ºC. These resulted agree with Dooremalen, *et al.* (2011) recorded that the high temperature cause 50 and 90% mortality in both sexed adults.

Larval and pupal weight:

The average larval and pupal weights of SBW were high decreased to 0.004 and 0.003 g/ larva and 0.003 and 0.001 g/pupae at 16 and 37 ºC, respectively, while it was increased to 0.066 g/ larva and 0.043g/ pupa at 26 ºC. From these data can be concluded that the low and high temperature caused high reduction in weight larvae

and pupae from 21 to 22 time which lead to small in body size and caused failure in adult's emergence (Table, 1).

Lee and Roh (2010) observed that the main cause of mortality for larvae of *Spodoptera exigua* at 34 ºC was developmental failure during the process of the pupal metamorphosis. Nijhout (1994) Recorded that high rate of pupation failure suffered by fast-growing insects at 34 ºC could be due to greater of energy limitation during this critical, energy-demanding stage.

Percentage of adult emergence:

Data presented in Table (1) & illustrated in Fig. (1) clearly the high reduction in adult emergence to 17 and 7 % at 16 and 37 ºC, respectively, while, the average percent of adult emergence increased to 91% at 26ºC .

Table 1: Effect of temperature on different stages of *E. insulana*

Temp. ºC	Eggs		No. of larvae used	Larval stage		Pupal stage			% adult emergence
	No. used	Hatchability%		Mortality %	Weight (g)	Pupation %	Weight (g)	Mortality %	
16	300	54.00	160	37.00	0.004	63.00	0.003	82.9	17.10
21	300	89.90	160	21.00	0.048	79.00	0.029	37	63.00
26	300	93.00	160	8.00	0.066	88.00	0.043	8.9	91.10
32	300	83.10	160	46.00	0.006	73.00	0.002	63	37.00
37	300	53.60	140	69.00	0.003	17.00	0.001	93	7.00

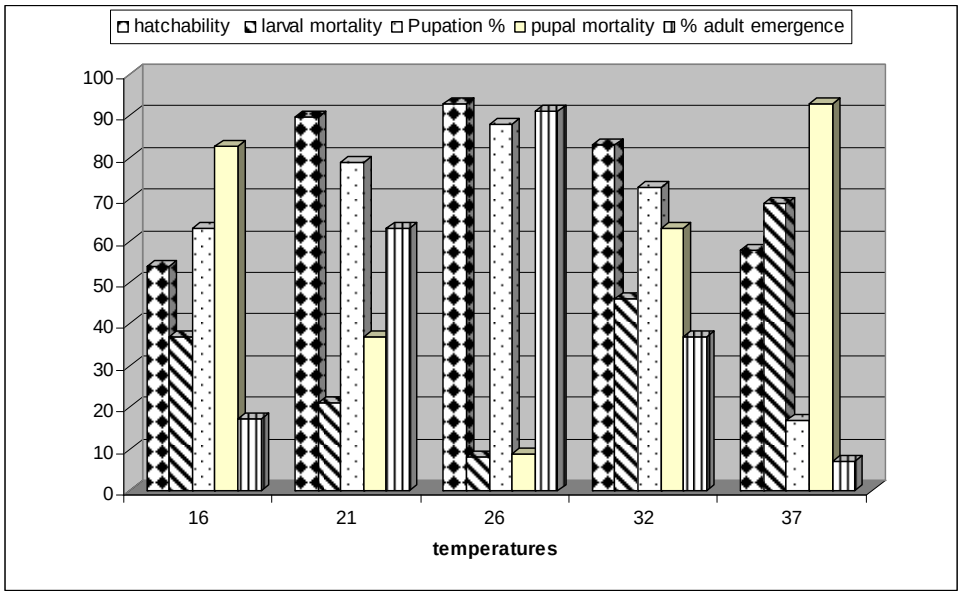

Fig. 1: Effect of temperature on different stages of *E. insulana*

Effect of temperature on developmental periods of *E. insulana*:

Incubation eggs:

Data in Table (2) recorded that the incubation period of *E. insulana* eggs was 12.45 days when reared on okra at 16ºC, while on 37 ºC they shortened to 1.10 days. Significant differences were found between the incubation periods of eggs as related to temperature, it indicated that embryonic development of the *E. insulana* was affected with tested temperatures. The threshold development (T_0) of eggs was 15.95 ºC while the average degree days (DD̊s) required for complete development was 23.15 DD̊s on okra pods. This resulted agreement with Al- Mohammady (2000) reported the lowest incubation period of *Earias vettella* ranged from 2 -1.15 days on 32.6 ºC and highest incubation period 3- 5 days on 23.2 ºC. (Shah *et al.* (2011) recorded that the lowest incubation period for *E. vittella* was 2 days on 35 ºC and the highest 4 days on 27 ºC.

Table 2: Duration, rat of developmental (RD), developmental threshold (T_0) and degree day ($DD^{\circ}s$) for *E. insulan*.

Tem.	Eggs				Larvae				Pupal				Pre-ovi.				Generation			
	Duration	RD	T_0	$DD^{\circ}s$	Duration	RD	T_0	$DD^{\circ}s$	Duration	RD	T_0	$DD^{\circ}s$	Duration	RD	T_0	$DD^{\circ}s$	Duration	RD	T_0	$DD^{\circ}s$
16	12.45± 0.37	0.080	15.95	23.15	45.56± 0.58	0.021	14.41	131.33	20.00± 1.76	0.05	13.32	90.79	-	-	17.63	20.83	-	-	12.85	346.19
21	5.70± 0.2	0.175			19.30± 0.4	0.052			10.13± 0.18	0.098			4.76± 0.8	0.21			38.27± 1.75	0.026		
26	3.30± 0.32	0.303			13.70± 0.1	0.073			9.86± 0.22	0.10			3.2.00± 0.12	0.313			30.11± 1.2	0.033		
32	1.27± 0.1	0.787			8.26± 0.29	0.121			5.90± 0.29	0.169			1.37± 0.3	0.72			17.40± 0.86	0.057		
37	1.10± 0.12	0.909			5.30± 0.06	0.189			3.27± 0.28	0.30			-	-			-	-		
LSD	0.73				0.75				5.53				1.12				1.84			
P	0.00**				0.000***				0.001**				0.001**				0.0001			

Duration and rat of developmental period of *E. insulana:*

Larval stage:

At 16, 21, 26, 32 and 37 ºC, the average durations were 45.56, 19.3, 13.7, 8.26 and 5.3 days, respectively, for *E. insulana* larvae reared on okra pods. Statistical analysis showed significant negative deference between the mean of larval durations and all tested temperatures. The correlation coefficient values (r = -0.89, R^2 = 0.042, $P< 0.05$), Table (2). The lower threshold of development (T_0) for the larval stage was 14.41 ºC. The average thermal unite required for larval development till pupation was 131.33 $DD^{\circ}s$. Can be concluded that at 16˚C development was much slower than other temperatures it tacked 8.58 time than the larvae reared at 37 ºC. The developmental period of SBW larvae was decreased as temperature increased. This resulted agree with Al- Mohammady (2000) reported that the larval durations were 5 and 12 days of *E. vitttella* reared at 31 and 27 ºC, respectively. Dhillon and Sharma (2004) observed that the *E. vittella* larval period was ranged between 9.2 – 12.2 days at 28 ºC, Fayez (2011) reported that the mean larval duration was 3.54 days at 33 ºC and 14.7 days at 26 ºC for *E. insulana*.

Pupal duration:

As shown in Table (2) the pupal duration of *E. insulana* decreased as temperature increased from 16 to 37 ºC. The average durations were 20.00 days on 16°C and 3.27 days at 37˚C, respectively. Can be concluded that high temperature reduced duration to 6.10 time at 37 ºC compared to 16 ˚C. The developmental threshold (T_0) for the pupal stage was 13.32 ºC and the average of thermal heat units required was 90.79 $DD^{\circ}s$ for *E. insulana* pupae completed developmental period.

Pre- oviposition period:

Data presented in Table (2) recorded that the mean of pre- oviposition period was 1.37 days at 32 ºC and 4.76 days at 21ºC when reared the SBW on okra pods. Statistical analysis showed significant negative relationships between the mean of pre–ovipositio period at the tested temperatures, the correlation coefficient values (r = -0.99; R^2 = 0.0.998, $P< 0.05$). The threshold development (T_0) of pre- oviposition preiod was 17.63 ºC, while, the average degree days ($DD^{\circ}s$) required for complete development to lay the first egg was 20.83 $DD^{\circ}s$ on okra pods Table (2). Can be concluded that the SBW taken 4 time period to lay the first egg when reared at low temperature 21 ºC compared with at high temperature 32 ºC**.**

The generation period:

The mean generation time at three constant temperatures (21, 26 and 32 ºC) could be estimated by summation the mean duration of different developmental stages (from eggs to pre-ovipostion) for *E. insulana* were 38.27, 30.10 and 17.40 days, respectively Table (2). Statistical analysis indicated that there were significant

negative relationships between the mean duration of generation and the tested temperatures (r = -99; R^2= 98; *P*< 0.05). According to the regression line equation the developmental threshold (T_0) for generation time of *E. insulana* estimated by 12.85˚C, while, the average degree days (DD̊s) required for complete generation was 346.19 DD̊s when fed on okra pods.

Adult stages

Oviposition period:

The oviposition periods for SBW female lasted 18.16, 13.30 and 5.66 days when reared at 21, 26 and 32 ˚C, respectively, (Table, 3). Can be concluded that the increase in temperature to 32 ºC, caused decreased in oviposition period to 3.2 time compared with 21 ºC.

Table 3: Effect of different constant temperatures on longevity, **fecundity** and fertility of ***E. insulana***.

Temp. ºC	Ovipositional period days ± S.E			Longevity days ± S.E		Total eggs/ female	% Hatchability
	Pre-ovi.	Ovi.	Post-ovi	♀♀	♂♂		
21	4.60±0.5	18.16±0.5	3.44±0.41	26.10±2.01	17.13±0.88	208.00±10.69	66.00
26	3.20±0.26	13.30±0.11	2.86±0.6	19.76±0.7	14.60±0.4	273.66±6.37	92.30
32	1.10±0.3	5.66±0.47	2.10±0.5	9.30±0.36	5.80±0.6	104.00±18.33	47.30
LSD	1.12	1.61	-	2.56	1.66	39.83	10.55
P	0.000	0.000	NS	0.000	0.000	0.0015	0.000

Post-oviposition period:

As shown in Table (3), the post oviposition periods of SBW were 3.44, 2.86 and 2.10 days /female at, 21, 26 and 32ºC, respectively.

Fecundity:

The mean number of eggs deposited by female of SBW was significantly affected at 26ºC

Compared to, 21, 32 ºC. The average numbers of eggs increased to 208.00 and 273.66 eggs/female when reared at 21 and 26ºC, respectively. The mean number of deposited eggs by one female was decreased to104.00 eggs/female by increasing the temperature to 32 ºC.

In addition, high temperature of 32 ºC caused redaction in female fecundity by 50 to 61.9 % compared with 21 and 26 ºC, respectively, Table (3). There was a significant negative relationship between temperature and mean number eggs / female (r = - 95; R^2 = - 65; *P*< 0.05). Fayez (2011) stated that the fecundity and reproduction potential of *E. insulana* female was higher at 18 than 26 ºC when larvae fed on okra pods. Mironidis and Soultani (2010) found that no eggs were laied by adults of *H. armigera* exposed to 40, 42.5 and 45 ºC,

Hatchability percentage:

The hatchability percentages of SBW eggs were 66.00 and 47.30% occurred at 21 and 32 ºC, respectively. While, the hatchability percentage highly increased to 92.30 % at 26 ºC (Table, 3). The low and high temperatures caused highly reduction in eggs hatched compared with 26 ºC.

Adult longevity:

Data in Table (3) showed that the shortest periods of longevity were 9.30 days/ female and 5.80 days/ male at 32ºC, but it prolonged to 26.10 days/ female and 17.13 days/ male at 21ºC. In addition, there was a significant negative relationship between

temperature and the longevity of female and male. The correlation coefficient values ($r = 0.92$; $R^2 = -0.99$; $P < 0.05$).

From the above mentioned data, it can be concluded that, the temperature play an important role for development of the different stages of SBW. It is better to rear the SBW at 26 ºC followed by 21ºC, where the fecundity was high. These resulted agree with Mironidis and Soultani (2010) recorded that mean adult longevity declined significantly with the increase in the temperature tested. The values of fecundity were few of the adults exposure to high temperatures.

Biochemical analysis:

Samples of larvae were collected from each tested temperature was chemically analyzed and results were as follows:

Total soluble protein:

Data presented in Table (4) & illustrated in Figure (2) reveal that the two tested temperatures 16 and 37 ºC caused high reduction in soluble protein in larvae compared to the larvae reared at 26 ºC. The totals soluble protein were 3.65 and 1.25 mg/ml at 16 and 37 ºC, respectively, compared with 7.37 mg/ml at 26 ºC**.**

Table 4: Effect of temperatures on some biochemical analyzed of ***E. insulana*.**

Temp.	Protein mg/ml	Lipid mg/ml	Glucose mg/ml	Enzyme activity		
				Amylase mg/ml	(ALT) mg/ml	(AST) mg/ml
16	3.65	2.15	11.08	68.97	21.00	31.00
21	5.52	4.89	44.50	18.19	16.00	10.00
26	7.37	8.07	48.11	27.80	27.3	42.90
32	5.10	2.88	7.36	8.73	13.00	10.00
37	1.25	1.69	2.15	8.13	11.00	10.00

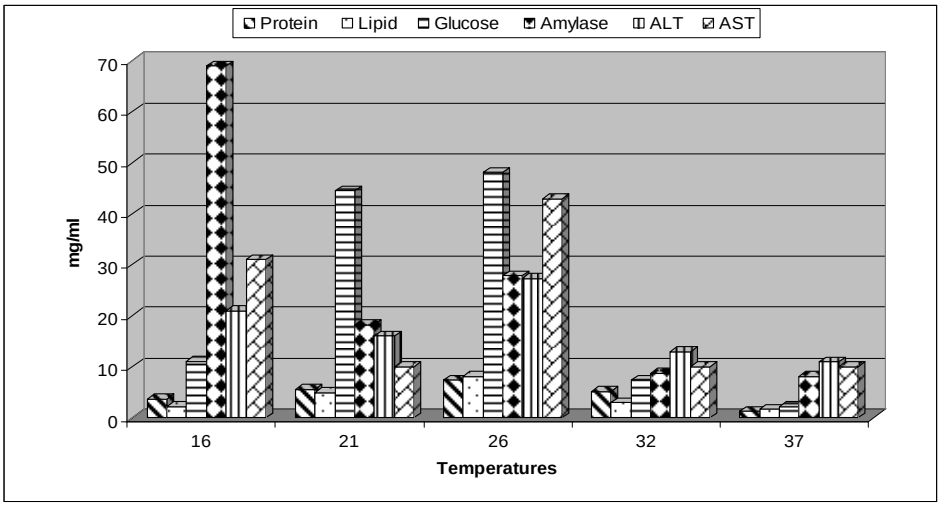

Fig. 2: Effect of temperatures on some biochemical analyzed of ***E. insulana***.

Total lipid:

Data in Table (4) indicated that the high reduction in total lipid contents were 2.15, 4.89, 2.88 and 1.69 mg/ml, when larvae reared at 16, 21, 32 and 37 °C, respectively, compared with 26 ºC, which recorded the high level of total lipid (8.07 mg/ml). From these data can be concluded that the high redaction in total lipid results from increase in total energetic expense with prolonged larval duration at (16 ºC), while, at 32 and 37 ºC, Rapidly growing larval are expected to invest 64.3 to 79.1 % from lipid to more energy intake to growth. These resulted agreement with Raikhel and Dhadialla (1992), Miller *et al.* (2005) and Lee and Roh (2010) showed that lipid

storage efficiency was lower in larvae of *Spodoptera* exigua at 18 than at 26 ºC, and was similar to those at 34 ºC. Dooremalen (2011), Lipid composition may be an important trait under lying fitness response to temperature, because it affects membrane fluidity as well as a viability of stored energy reserves. Keeley (1985), Kunkle and Nordin (1985), recorded that in insect adult females, the major function of the fat body is the synthesis and release of vitellogenic proteins and lipids for yolk formation during oocyte maturation. Also, decreased the total lipid caused decreased on the mean number of eggs and hatchability percentage for adult.

Glucose:

Data in Table (4) show that glucose activity greatly reduced in *E. insulana* larvae reared on okra at 16, 32 and 37 ºC. The levels of activity were 11.08, 7.36 and 2.15 mg/ml. comparing to 44.5 and 48.11 mg/ml at 21 and 26 ºC.

Transaminase enzymes (GOT and GPT or ALT and AST):

Data in Table (4) showed that the transaminase enzymes activity on larvae of *E. insulana* reared at 16, 21, 26, 32 and 37 ºC. The levels of GOT and GPT were highly increased to 27.30 and 42.90 mg/ml at 26 ºC, respectively. While, the highest reduction in GOT and GPT activity on *E. insulana* larvae fed on okra pods at 37ºC (11.00 and 10.00 mg/ml) followed by (13.00 and 10.00 mg/ml) at 32 ºC.

In conclusion, the chemical changes explain the relationship between the high and low temperature and reduction in all larval protein, lipid, glucose enzymes and prolonged and / or faster duration with less weight, small size and reduction or failure in some adults emergence. The reduction in protein, lipid, especially glucose, amylase, GOT and GPT caused inhibition in metabolism process in larvae as well as the reduction in reproductive potentiality of SBW adults resulted. High temperatures tend to kill insect's cells by denaturing proteins, altering membrane and enzyme structures and properties, and by loss of water (dehydration) and they offer a rich potential for pest management strategies (Chapman, 1998; Gullan and Cranston, 2005).

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ARABIC SUMMARY

العلاقة بين درجات الحرارة وبعض المظاهر البيولوجية والتحليل البيوكيميائى للسلالة الحقلية لدودة اللوز الشوكية

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أجريت دراسات معملية بمعهد بحوث وقاية النباتات- قسم بحوث ديدان اللوز لدراسة تأثير درجات الحرارة (16، 21، 26، 32، 37ºم) على تطور أطوار السلالة الحقلية لدودة اللوز الشوكية المرباه على عائل طبيعى (الباميا) أوضحت الدراسة أن فترات هذه الأطوار قد قلت بزيادة درجة الحرارة من 16 إلى 37 ºم كما أوضحت النتائج أن صفر النمو كان 15.95، 14.41، 13.32، 17.63، 12.85ºم درجة مئوية لطور البيضة، اليرقة، العذراء وما قبل البيض و مدة الجيل على التوالى وكانت الوحدات الحرارة اللازمة لإكتمال نمو الأطوار على التوالى كالآتى 23.18، 131.33، 90.79، 346.193 عندما ربيت الحشرة على الباميا كما أوضحت النتائج إنخفاض عدد البيض الموضوع على درجات الحرارة العالية مقارنة ب26ºم . ومن التحليل البيوكيميائى أوضح تأثير درجات الحرارة المنخفضة والمرتفعة على خفض كمية الدهون تليها البروتينات فى اليرقات وهذا مما أدى الى خفض فى الكفاءة التناسلية لأناث دودة اللوز الشوكية.