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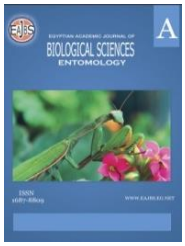

Influence of Temperature on the Development, Growth Rate, and Life Table Parameters of *Earias insulana* Boisd. (Lepidoptera: Noctuidae) under Laboratory Conditions

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ABSTRACT

Experiments were performed at five constant temperatures of (12, 18, 23, 27, and 33 ± 2ºC) and relative humidity 65±5 % R.H. to estimate the threshold of development and thermal units' requirements for laboratory strain of *Earias insulana* Boisd. (Lepidoptera: Noctuidae) reared on an artificial diet without agar. Results of the total developmental period, percentages of adults' emergence, and longevity of females are significantly affected when reared at different temperatures. The total developmental period of the immature stages was shorter at high temperatures than that reared at low temperatures. The incubation period of egg significantly affected by different temperatures which was 9.9 days at 12 °C but it was 2.4 days when eggs incubated at 33ºC, respectively. The lower threshold of embryonic development found to be 12.092°C. The average thermal units expressed as degree-days required for completing the development of the incubation period for eggs was 49.31 DD's. Also, tested temperatures significantly affected the larval duration and weight, the mean larval duration was 50.6 days at 12 °C but it was 10 days at 33 ºC. Also, mean larval weight affected with tested temperatures which were 0.00063 g at 12 °C, but it was 0.0017 g at 32 ºC. The lower threshold of development (T_0) for the larval stage was 10.97 ºC, The average thermal unite required for larval development till pupation was 215.1 DDs. Tested temperatures significantly affect on the pupal duration, the mean pupal duration was 26.3 days at 12 °C, but it was 5.5 days at 33 ºC. Also, mean pupal weight affected by tested temperatures, which were 0.0005 and 0.0014 g at 12 °C, and 33 ºC, respectively. The developmental threshold (T_0) for the pupal stage was 12.7044 ºC and the average of thermal heat units for pupal stage was 110.063. The highest adult emergence percentage was occurred at 27°C, which being 92%; also pre oviposition period, significantly affected by temperature, which were 4.9 and 24, 27 days at 18 °C and 33 ºC, respectively. The threshold development (T0) of pre-oviposition preiod was 14.13 ºC, while, the average degree days (DDs) required for complete development to lay the first egg was 33.94 DDs. Generation time also, significantly affected by tested temperatures, which were 57.1 and 19.3 days at 18 °C and 33 ºC , respectively , the developmental threshold (T0) for generation of *E. insulana* was 11.15°C, while, the average degree days (DDs) required for complete generation was 413.4 DDs when fed on previous artificial diet. Also ovi-position, post ovipositoin and longevity of adult were significantly affected. Results of the life tables for *E.insulana* showed shortage in generation periods for that reared at high temperatures and vice versa occurred at low temperatures. The present study was conducted to explore the effect of different temperature on some biological parameters of *E.insulana.*

INTRODUCTION

The spiny bollworm, *Earias insulana* (Lepidoptera: Noctuidae) is considered the most dangerous pests in the fields of cotton, okra, and many other plant families**.** The spiny bollworm attacking many plants of Malvaceae family, especially cotton, okra . Larvae are considerable the major stage attack the cotton or okra fields. It causes serious damage in cotton bolls and great loss as in both the quality and quantity of cotton yield. Temperature is one of the abiotic factors that affect the growth and development of insects, including pink boll worm, where the temperature in the fields vary from time to time and may lead to significant changes in the stages of growth and development of the insect and also in the process of predicting the presence of this insect in the fields. (Moustafa *et al.,* 2015) (Adly , *et al.,* 2016) and high affected in Development, survivorship, and reproduction of insects (El-Sayed , 2014).

The duration of the life cycle of *Earias insulana* depends on the temperature degrees and thus varies with the season. There is no diapause, though development is retarded in cold weather. *E. insulana* tolerates a range of temperatures, but it does not adapt well. Many researchers studied the effect of constant temperature and its effects on many insects such as spiny bollworm. Generally, they found that low temperatures prolonged the life cycle of immature stages and increased mortality percentages, conversely, the temperature increased. The optimum temperature was ranged from 25 to 28, where the best growth and development of all stages occurred, (Kandil, 2013 and El-Sayed, 2014).

The current study aims to investigate thermal degree days of *Earias insulana*, and calculate zero development, also investigate the effect of constant temperatures on some biological and life table parameters.

MATERIALS AND METHODS

The experiments were carried out at the Faculty of Agriculture, Menoufia University during June 2019 to study the effect of different constant temperatures (12, 18, 23, 27 & 33 ± 2°C) and 65±5 % R.H. on the duration of different stages. Calculate zero development and thermal degree day's units of *E. insulana*.

Rearing Insect:

The culture of *E. insulana*, larvae used in this experiment was obtained from laboratory rearing of the Bollworms Research Department, Plant Protection Research Institute, reared for several generations at 26 ± 2 °C and 65±5 % R.H. on artificial diet (Tables1&2) that described by (Amer, 2015). This artificial diet doesn't contain agar and less expensive**.**

Effect of Temperature on The Development of Different Stages:

Eggs laid on the same day (< 24 old) were placed in a glass jar and incubated under five constant temperatures (12, 18, 23, 27 & 33± 2°C) and 65±5% R.H. Three replicates of 100 eggs/each were used for each tested temperature degree. The number of eggs hatched was recorded and the incubation period was calculated.

Immature Stages:

First instars' larvae resulted from previously treated eggs were transferred individually to glass tubes (3 × 10 cm) containing 3gm of artificial diet (tables 1&2) described by (Amer, 2015). Each tube was plugged tightly with absorbent cotton and placed in an incubator at the previous conditions. Three replicates of 30 larvae/ each were used for each tested temperature except for the temperature of 12 °C in which the number of larvae was insufficient. 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. The newly

emerged females were observed and sexd then caged to laying eggs. Moths were supplied with 10 % honey solution. The cages were observed daily until the death of moths. The pre-oviposition periods were calculated until laying the first egg for each female. The mean generation times were determined as the total of mean durations of different developmental stages, i. e. incubation period, larval, pupal, and pre-oviposition (Adly *et al*., 2016). Also, adult longevities and number of eggs were calculated. A temperature of 12°C was excluded due to incomplete adult stage.

Table 1: The contains of the artificial diet which described by (Amer, 2015)

Ingredients	Quantity
Kidney beans (g)	250.00
Wheat grated (g)	125.00
Dry active yeast (g)	49.00
Ascorbic acid (g)	3.00
Sorbic acid (g)	1.75
Methyl parahydroxy benzoate (g)	1.75
Liquid milk (ml)	100.00
A mixture of vitamins(ml)	8.00
Formaldehyde 34-38%(ml)	2.50

Kidney beans, *Phaseolus vulgaris*. Wheat grated, *Triticum aestivum* L.

Table 2: Ingredients of vitamins mixture (Grand Vit with Iron Syrup), Produced by Sigma Pharmaceutical industries for Sina Pharm.

Each 5ml contains:

Ingredients	Quantity
Vitamin A	1200 I.U
Vitamin D3	100 I.U
Vitamin B1	1 mg
Vitamin B2	1 mg
Vitamin B6	0.5 mg
Vitamin C	50 mg
Vitamin E	1 mg
Nicotinamide	5 mg
Panthenol	2 mg
Calcium gluconate	25 mg
Calcium phospholactate	25 mg
Ferrous gluconate	43.2 mg

Statistical Analysis:

All experiments contained three replicates. The results were analyzed by one – way analysis of variance (ANOVA) using COSTAT statistical software (Cohort Software, Berkeley). When the ANOVA statistics were significant ($P < 0.01$), the means were compared by Duncan's multiple range test (1957).

The theoretical development threshold (t_0), rate of development and the accumulated thermal units (K) were determined according to Blunk (1923), also the regression line equation using Microsoft office excel worksheet (version7) :

$Y = a + bx$ $T_0 = - a/b$ $k = 1/b$

Where Y = rate of development = 1/ duration in days

a = constant temperature b = simple regression coefficient

Life table parameters were calculated according to Birch (1948) using Life 48 basic computer program (Abou-Setta *et al*., 1986) and Euler-Lotka equation: $\infty\Sigma 0$ e-rm Lx mx dx = 1, Where: (X) Age in days, (Lx) Age specific survival rates, (Mx) Female fecundity, ($R_\circ$) Net reproductive rate($R_\circ = \Sigma$ lx*mx), (T)Generation time (T = (Σ lx* mx*x) / $R_\circ$), (rm) Intrinsic

rate of increase (rm= log $R_\circ$ / T), (λ)Finite rates of increase (λ = exp. rm) and (Dt)Doubling time(Dt = log (2) /rm)).

RESULTS AND DISCUSSION

Data presented in Tables (3 & 4) shows those Periods of different stages of *E. insulana* were affected by different constant temperatures, where egg incubation periods were 9.9, 7.9, 4.5, 3.6, and 2.4 days when eggs incubated at 12, 18, 23, 27, and 33ºC, respectively. Larval mean durations were 50.6, 29, 18.6, 14.1and 10 days at 12, 18, 23, 27, and 33 ºC, respectively. As in egg incubation periods and the larval durations, also in the pupal duration, pre- oviposition and generation time, short periods with high temperatures, where the mean pupal durations were 26.3, 15.3, 9.3, 7.5 and 5.5 days at 12, 18, 23, 27 and 33 ºC, respectively. Also, the means of pre-oviposition period were 4.9, 3.3, 2.6, and 1.6 days at 18, 23, 27, and 33 ºC, respectively. The mean generation times for *E. insulana* were 57.1, 35.4, 27.8, and 19.3 days at 18, 23, 27, and 33 ºC, respectively. Finally, oviposition, post-oviposition, and adult longevity affected by tested temperatures, which were 20.21 .17.6, 12.6, and 6.11 days for oviposition periods, 5.11, 3.11. 2.55 and 2.31 days for post-oviposition periods, 30.2, 23.97,18.05, and 10.02 days for adult longevities at 18, 23, 27, and 33, respectively. The previous parameters increased by increasing the temperature and reached the maximum rate at 27 ^{o}C, but the opposite occurred at 33 ^{o}C.

Generally, given data indicated that an increase occurred in different periods of *E. insulana* at low temperatures and the opposite occurred at high temperatures. It was observed that there was an inverse relationship between temperatures and different periods of *E. insulana* **(**Guo, *et al.,* 2013; Li, *et al.,* 2013; Kandil, 2013**;** El-Sayed, 2014; Adly, *et al.*, 2016 and Khafagi, *et.al.,* 2016). It is clearly showed that the constant temperature in the range of 23 to 27^{o}C is the favorable zone for *E. isulana* immature and adult stages (Kandil, 2013 and El-Sayed, 2014).

Table 3: Effect of different constant temperatures on immature stages of *E. insulana.*

Factor	Temp.	Egg stage			Larval stage			Pupal stage		
		Egg hatch %	n.	Egg incubation period (days)	n.	Larval period (days)	Larval weight (g)	n.	Pupal period (days)	Pupal weight (g)
Actual	12	10	30	9.9±1.04 a	10	50.6±3.2a	0.0013	5	26.3±3.8a	0.0005
	18	63	189	7.9±0.8 b	60	29±1.66 b	0.0071	20	15.3±1.2b	0.0023
	23	72	216	4.5±0.6 c	80	18.3±0.33c	0.0214	65	9.3±0.7 c	0.0214
	27	96	288	3.6±0.57 c	85	14.1±0.6 d	0.0556	80	7.5±0.28d	0.0546
	33	40	120	2.4±0.57 d	70	0.0017 e	0.0017	40	5.3±0.8 e	0.0017
LSD		-	-	1.101	-	3.41		-	1.33	
Rate	12	0.101			0.020			0.038		
	18	0.127			0.034			0.065		
	23	0.222			0.055			0.108		
	27	0.278			0.071			0.133		
	33	0.417			0.100			0.189		
Regression Values	a	-0.124			-0.033			-0.061		
	b	0.016			0.004			0.007		
	to	7.888			8.365			8.166		
	K	63.413			250.788			133.544		
	R2	0.9299			0.9733			0.9752		
	P value	0.008			0.0019			0.0017		

Values with different letters are significantly different at P< 0,05 (Duncan test).

Results are presented as mean ±SE

Table 4: Effect of different constant temperatures on the adult stage of *E. insulana*

Factor	Temp.	Adult stages						
		n.	Pre oviposition period (days)	n.	Generation time (days)	Oyi-position	Post - oviposition	Adult longevity (♀)
Actual	12	-	-	-	-	-	-	-
	18	10	4.9±0.5 a	10	57.1±3.1 a	20.21±0.62a	5.11±0.3a	30.2±2.3a
	23	40	3.3±0.41 b	40	35.5±2.51b	17.56±0.31b	3.11±0.41b	23.97±0.91b
	27	80	2.6±0.53 c	80	27.8±1.41c	12.60±0.33c	2.55±0.32c	18.05±0.62c
	33	20	1.6±0.32 d	20	19.3±0.52d	6.11±0.36d	2.31±0.41c	10.02±0.31d
LSD	-	-	**0.431**	-	**7.51**	**1.32**	**0.31**	**4.32**
Rate	12	-		-		-	-	-
	18	0.204		0.018				
	23	0.303		0.028				
	27	0.385		0.036				
	33	0.625		0.052				
Regression Values	a	-0.354		-0.027				
	b	0.029		0.002				
	to	12.074		11.151				
	K	34.088		414.795				
	R2	0.9417		0.9833				
	P value	0.0296		0.0084				

Values with different letters are significantly different at P< 0,05 (Duncan test).

Results are presented as mean ±SE

The relationship between temperature and developmental rats of different stages of *E. inulana* becomes clear in Figure (1). In general, the growth rate has increased with higher temperatures and therefore there is a positive correlation between temperature and growth rate, this positive correlation became clear at different stages.

The threshold of embryonic development (T_0) was determined and it was found to be 7.888^{o}C as indicated in Figure (1), the average thermal units (K) required for completing the embryonic development in the incubation period was 63.413 units. The rate of egg development at tested temperature degrees, gave a good idea to calculate the regression line equation Y=0.016 X -0.124.

For the larval stage, the lower threshold of development (T_0) for the larval stage was 8.365 ºC. The average thermal units (K) required for larval development until pupation was 250.788. Previous results showed that the developmental rate of the larval stage at 12°C was much slower than larvae reared at 33 ºC. The developmental rate of larvae was used to calculate the regression line equation Y=0.004 X -0.033.

For the pupal stage, the developmental threshold (T_0) was 12.7044 ºC and the average of thermal heat units for pupal completed development was 110.063 units. The equation of the regression line was Y=0.008 X -0. Previous results agree with many previous studies which showed that incubation periods, larval and pupal duration of the spiny bollworm decreased as temperatures increased, also increasing of developmental rates were obtained at high temperatures and the most favorable temperatures for development of the immature stages of *E. insulana*, reared on an artificial diet in the laboratory were 27 to 33^{o}C, generally, durations of developmental stages were shorter than at lower temperatures (Spana *et al.*, 2017; Kandil ,2013 and El-Sayed , 2014).

The threshold development (T_0) of the pre-oviposition period was 14.13 ºC, while, the mean thermal units (K) required for complete development to lay the first egg was 33.94. The

equation for the regression line between the developmental rate in pre-oviposition and temperature was Y=0.029 X -0.354. It could be concluded that the optimum zone of temperature for Adult development and maturity of ovaries to lay eggs was between 23 and 27^{o}C for *E.insulana*, where *raising* of temperature accelerated the rate of development of the female ovary and reached faster to maturation (Abdel-Salam,2013; Kandil,2013; El-Sayed, 2014 and Adly, *et al.*, 2016).

According to the regression line equation, the developmental threshold (T_0) for the generation of *E. insulana* estimated by 11.15°C, while, the mean thermal units (K) required for the complete generation were 413.4 units. The equation for the regression line between the developmental rate for generation and temperature was Y=0.002 X -0.0.26 (Fig.1). The previous results refer to the acceleration of developmental rates for the generation with the increase of temperatures, where it reached the maximum at 33C^{0} (Adly *et al.*, 2016 and Kandil, 2013).

Generally, Insect pests affected by abiotic factors in nature especially by temperature, and their biology, behavior, and fitness are greatly affected (Jaleel, *et al.*, 2019). There are various studies showing the effect of temperature on the development of different insects such as (Garrad, *et al.*, 2016 and Jaleel, *et al.*, 2018)

Based on the current study, it is concluded that constant temperatures affect the different stages of *E. insulana* reared on an artificial diet at the laboratory conditions. The development rate increased with the increase in temperature, leading to the shortening of the different periods and the opposite occurred when the temperature decreased. Also, it could be concluding that the optimal zone of temperature for the growth and development of spiny bollworm 23 to 27 degrees in the laboratory conditions.

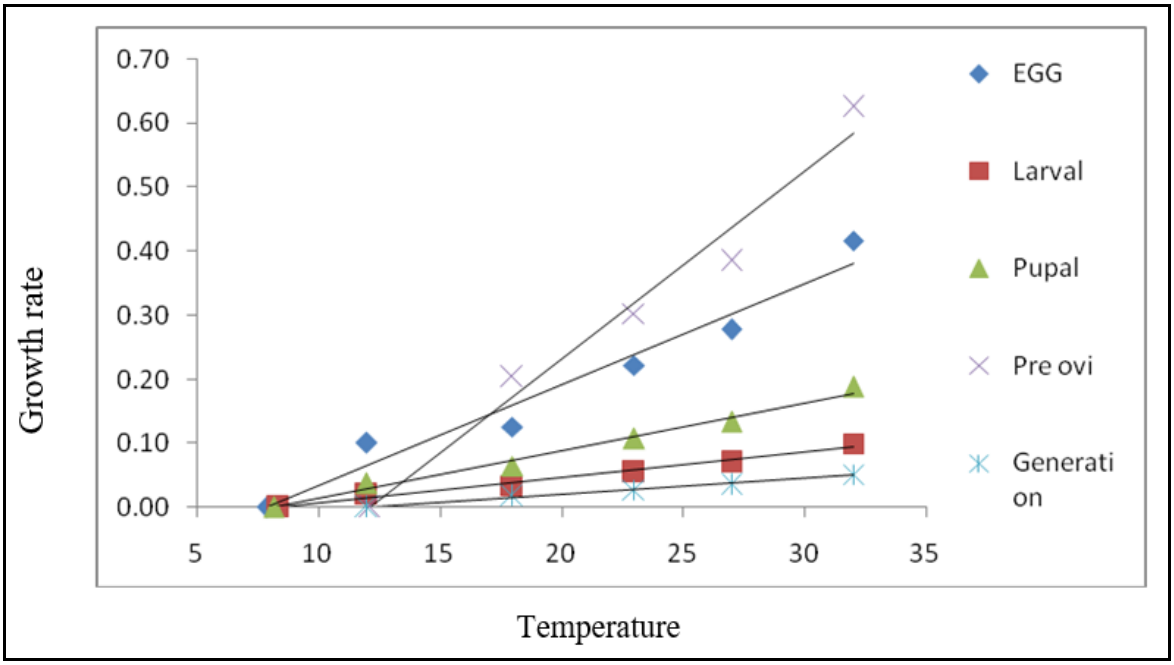

Fig. 1: Regression line of relationship between development rates of *E. inulana* different stages at different constant temperatures.

Effect of Temperature on Life Table Parameters:

Results obtained from life table parameters are presented in Table 5 and Figure 2. Tested temperature affected survival rates of immature stages to the adult stage.

Survival rates were 0.51, 0.82, 0.85, and 0.70 when reared at 18, 23, 27 and 33 **ºC**, respectively. These results showed that a low survival rate occurred at 18 ºC but the higher

rate occurred at 27 ºC. Obtained results showed an increase in survival rates with the increase of temperature but the excessive increase in temperatures led to a decrease in survival rates again. The temperature also affected the sex ratio (female/total, but the effect is weak, the obtained sex ratios were 0.5, 0.53, 0.57, and 0.51when the insect reared at 18, 23, 27, and 33 **ºC**, respectively.

The net reproductive rate (Ro) varied according to the temperature used. (Ro) were 2.77, 7.09, 8.056 and 2.33 females/female at 18, 23, 27and 33 **ºC**, respectively. The results showed that a temperature of 27 **ºC** is the most favorable or the optimum temperature for female's reproduction; it has the highest value of the net reproductive rate.

Evident differences were observed in the mean generation time (T) between tested temperatures. The mean values were 64.85, 43.53, 33.70, and 21.78 at 18, 23, 27, and 33 ºC, respectively**.** The results indicated that the generation time decreased with the increase of temperatures, but this decrease in the generation period is accompanied by a decrease in the oviposition period and consequently a decrease in the number of eggs/female. The results showed that a temperature of 27 **ºC** was a preferred degree for the female, despite the relatively long generation time, as the oviposition period was also relatively long, and consequently, thus increasing the number of eggs for each female.

The intrinsic rates of increase (rm) were 0.015, 0.019, 0.031 and 0.039 at 18, 23, 27 and 33 ºC, respectively. When these were converted into finite rates of increase (λ), the population capacity of *E. insulana* increased. The values were 1.02, 1.05, 1.09, and 1.03 times/female/day when *E. isulana* reared at 18, 23, 27, and 33 ºC, respectively; showing the highest capacity to increase at 27 ºC. Population doubling times (DT) was 20.06, 15.84, 9.71 and 7.72 days for 18, 23, 27 and 33 ºC, respectively.

Table 5: Effect of different constant temperatures on life table parameters of *E.insulana*.

Parameter	Temperatures			
	18 ºC	**23 ºC**	**27 ºC**	**33 ºC**
Survival to maturity	0.51	0.82	0.85	0.70
Sex ratio (females/total)	0.5	0.53	0.57	0.51
The net reproductive rate (R_o)	2.77	7.09	8.056	2.33
Mean generation time (T)	64.85	43.53	33.70	21.78
The intrinsic rate of increase (r_m)	0.015	0.019	0.031	0.039
The finite rate of increase (exp. r_m)	1.02	1.05	1.06	1.07
Time of population doubling ((In2 / r_m))	20.06	15.84	9.71	7.72

Previous results indicated that temperature affected the biology of tested insects. Exposure to low and high temperatures led to changes in biological indicators, as low temperatures increased biological aspects, conversely, the high temperature led to a decrease in these indicators (Cui *et al.,* 2018).

Insects are heterothermic organism which is very sensitive to temperature changes during different developmental periods and may affect insect growth, development, and reproduction, etc. (Messenger 1959, Li *et al.,* 2017).

Our results agreed with (Howe, 1967 and Lu *et al.,* 2009), they indicated that growth and development of insects tend to accelerate with a temperature increase in the favorable temperature range but are adversely affected when the temperature range becomes unfavorable and that both sharp low and high temperature can have adverse effects on survival of different stages and reproduction (Qin *et al*., 2017), this may be due to the effect of temperatures on the consumption and digestion of food, where lower temperatures lead to lower rates of nutrition and vital metabolism, and vice versa it occurs when temperatures rise, (Wang *et al.,* 2016).changes in temperature reflected on the sexual ratio, The net reproductive

rate (Ro), mean generation time and time of population doubling (DT), but at certain limits, while sharp decrease or increase leads to the death of the insect(Kuang et al. 2010), Xiang *et al.*, 2015), (Qin *et al.*, 2017).

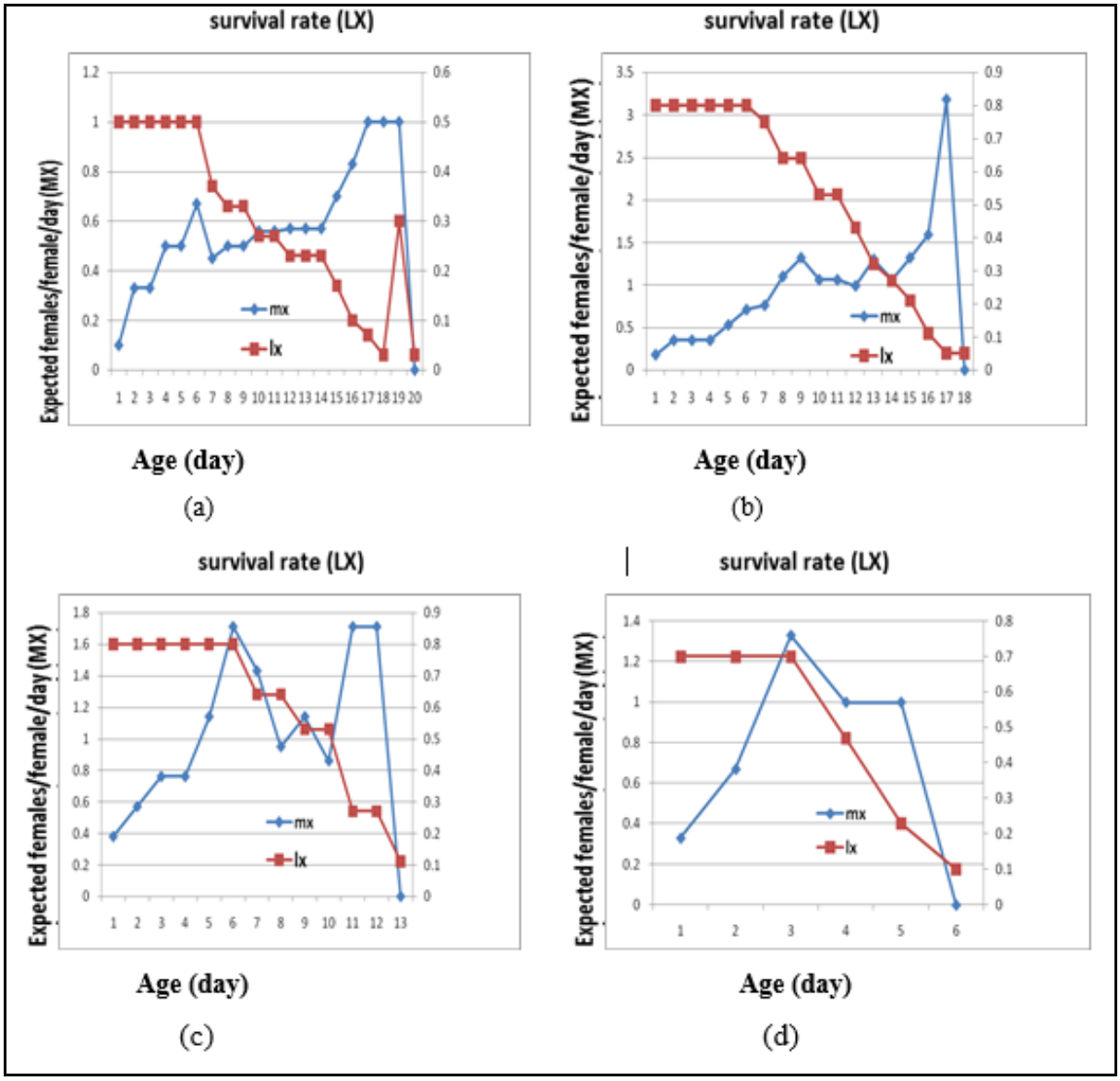

Fig. 2: Age- specific fecundity and survival rate of *E. insulana*. at : (a) 18 ºC , (b) 23 ºC , (c) 27 ºC and (d) 33 ºC . Where lx: Survival rate and mx: Female fecundity.

Conclusion

It could be concluded from the results that constant temperatures affect the different stages of *E. insulana* reared on the previous artificial diet described by (Amer 2015). The optimal zone of temperature for the growth and development of spiny bollworm was 23 to 27 degrees in the laboratory conditions. Also, the effect of different temperatures on life table parameters was studied; the results indicated that the preferred temperature for growth and development in the laboratory was 27. Also, the temperature should be taken into account to more clearly compare more biological and physiological effects on insects**.**

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