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Original Article

Biological and Biochemical Impacts of Temperature on *Spodoptera littoralis* (Boisduval)

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ABSTRACT

Background: The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), is one of the most destructive agricultural lepidopteran pests in Egypt, attacking several important crops all year round. *S. littoralis* can have two to seven generations per year, depending on the climate of the region; for example, changes in temperature affect all life processes of *S. littoralis*.
Methods: The development, survival, and fecundity of *S. littoralis* at one of five constant temperatures as well as their effects on the biochemical impacts were investigated.
Results: The results showed that the duration of developmental stage (eggs, instars, pupae), longevity, and fecundity significantly decreased with increasing temperature from 15 to 35 ºC. Larvae emerged fastest from the eggs at 25 °C. The percentage of oviposited females was 46, 58, 74.5, 66.5, and 50% at 15, 20, 25, 30, and 35 °C, respectively. The results also showed that the fourth larval instars of *S. littoralis* at 25 °C had a higher level of proteins, lipids, carbohydrates, and free amino acids in comparison with those larvae at 35 °C. Therefore, temperature rise leads to increasing metabolic rate, and decreasing the development period.
Conclusion: Thus, 25 °C was the optimum temperature for development, fecundity, rates of biochemical and physiological reactions of *S. littoralis*.

Key words: Biochemical impacts, Constant temperature, Fecundity, *Spodoptera littoralis*

1. Introduction

Several environmental variables contribute to the growth rate. Temperature is one of the most prevailing environmental effects on growth and nearly all other biological processes in organisms. The developmental rate in insects is highly contingent on external temperature conditions. Hence, the temperature is generally considered as the single most significant environmental factor is influencing behavior, distribution, development, survival, and reproduction in insects [1].

Knowledge of environmental factors that can affect the increase in insect populations is crucial for predicting potential changes in population dynamics and developing sustainable and environment-friendly pest control strategies [2]. The cotton leafworm, *Spodoptera littoralis* (Boisduval) (*Lepidoptera: Noctuidae*) is

economically an important pest of major crops across the world. It is a serious pest for cultivated crops primarily in tropical and subtropical regions in Africa, [3]. In Egypt, *S. littoralis* larvae are polyphagous, feeding on about 73 vegetables and numerous field crops species belonging to 44 botanical families and are mainly harmful to cotton; severe infestations commonly reduce yields [4].

Therefore, the main objective of the present study is to explore the effect of five constant temperatures on development, survival, and fecundity in *S. littoralis*, as well as examine their effects on the biochemical parameters such as total proteins, lipids, carbohydrates, and free amino acids of fourth larval instars of *S. littoralis*.

2. Experimental

2.1. Experimental insects

The stock culture of the Egyptian cotton leaf worm, *Spodoptera littoralis,* was maintained for several years without exposure to any pesticides in the laboratory. They were kept on at room temperature (25 ± 1 °C), ≈70% humidity, with a 16: 8 light: dark photoperiod. The larvae fed on castor oil leaves (*Ricinus communis* L.), which were fresh and clean leaves and were supplied daily. Newly emerged adults were transferred into plastic jars for mating and egg-laying. Adults fed on a 10% sucrose solution impregnated onto cotton wool. Egg masses deposited by females of the stock culture were collected daily, and the hatched larvae were placed individually into Petri dishes.

2.2. Biological studies

The newly hatched larvae were reared individually in Petri dishes (3.5 cm in diameter) (n=30 larvae x 4 trials/temperature). Survival pupae were counted, sexed, and weighed at 24 hr-old, and kept until adult emerged. Freshly emerged moths were paired; each pair (male and one female) was placed in a glass jar and fed on 10% fresh sucrose solution. A folded sheet paper was placed in the jar to provide suitable sites for oviposition. The effect of five various temperatures on development, survival, fecundity (total number of eggs/female), fertility (hatchability percentages of eggs), and the longevity of adults of both sexes were determined. The mating cups were checked daily, and egg masses were removed until the female died. The total number of eggs/female for each mating and hatched eggs percentages were evaluated.

2.3. Biochemical studies

2.3.1. Sample preparation

Survived newly hatched 4th larval instars of *S. littoralis* from each rearing temperature degrees were collected in a chilled glass. These larvae were then homogenized in phosphate buffer (PH 7) using a Teflon tissue homogenizer surrounded by a jacket of crushed ice. After homogenation, supernatants were kept in a freezer at -20 ^{o}C to use for biochemical assays. The insects were homogenized in distilled water (50 mg /1 ml). The homogenates were centrifuged at 8000 rpm for 15 min at 5 ^{o}C. The supernatant was kept in a deep freezer till used for the determination of the total soluble protein [5], total carbohydrates [6], total lipids [7] and free amino acid [8] as described in previous research.

2.4. Data analysis

Data from all experiments were subjected to analysis using the SPSS 20.0 Software Package (SPSS Inc., Chicago, IL, USA). One-way ANOVA followed by the Tukey's HSD test in JMP 11.1.1. (SAS Institute Inc. 2013, Cary, NC, USA).

3. Results and discussion

3.1. Effect of temperature on development developmental duration, longevity, and fecundity

Effect of five constant temperature on the duration of each developmental stage (eggs, instars, pupae), longevity, and fecundity are shown in Table 1. At 15 to 35 °C, the duration of the egg stage, instars, and pupae was significantly affected by temperature: increasing the temperature decreased the duration. The duration of the egg stage was the shortest at 25 °C. At a temperature of 25 °C or higher, the larvae emerged from the eggs in less than 15 and 20 °C. At temperatures lower than 25 °C, larval development took longer. At 25, and 30 °C, pupal developmental duration was 12.31, and 12.74 d, respectively, but the differences were not significant. At 15 and 35 °C, pupal developmental duration was 31.10, and 22.53 d, respectively, which was longer compared to all other temperatures.

Females lived longer than males, and female longevity was the longest at 15 and 20 °C. Male longevity was not influenced by temperature. The temperature influenced the reproductive traits significantly in *S. littoralis*. Female fecundity was the lowest at 15 °C (133.2) and peak egg-laying at 25 °C (596.5). Concerning the oviposition, the ratio of *S. littoralis* also varied greatly at tested temperatures. As shown in Table 2, temperature also had a significant effect on the percentage of larvae that formed pupae at the sixth instar, where larvae had six instar before pupation for example 9.77–12.95% of the sixth instar at 15–20 °C.

The highest percentage of larvae that formed pupae was 95.7% and the lowest percentage was 0.67%, both observed at 30 °C. The percentage of the larvae that reached the sixth instar was higher at 30 °C than at 25 °C. Temperature also had a significant effect on pupal weight. Pupal weight was significantly lowest at 35 °C. The emergence rate of the adults from the pupae was positively correlated with the 20–35 °C temperature range. In contrast, 15 °C had a significant negative effect on the emergence rate of adults. Results showed that the under suitable temperature conditions, *S. littoralis* larvae grew well to pupae stage.

The adults are often more fertile, leading to rapid reproduction within a short period, where the developmental duration of each instar decreased as the temperature increased. Both high and low temperatures (15 °C and 35 °C) had adverse effects on the survival of *S. littoralis* larvae and the fertility of adults. The results showed that temperature had a significant effect on the growth and reproduction of *S. littoralis*, reporting a longer developmental duration at low temperatures and a shorter developmental duration at high temperatures. Insects are heterothermic poikilotherms, which are very sensitive to temperature changes. These changes may affect insect growth, development, reproduction, distribution, population dynamics, *etc*. [9], which is consistent with the many studies on the effect of temperature on insect growth and development reporting a longer developmental duration at low temperatures and a shorter developmental duration at high temperatures [10, 11].

The oviposition ratio of *Spodoptera litura* also varied greatly at different temperatures. The highest percentage of females which did not lay eggs was 41.34% at 15 °C, followed by 15.39% at 34 °C, indicating that both low and high temperature could have adverse effects on adult reproduction [12]. Temperature changes can also have profound effects on many aspects of the

biology of insects, such as body weight and mating [13].

Table 1. Effect of temperature on the duration of the different developmental stages of *S. littoralis*

Developmental stage	Developmental duration (days)				
	15 ºC	20 ºC	25 ºC	30 ºC	35 ºC
Egg	4.78^{e}±1.95	3.74^{d}±1.01	1.87^{a}±1.23	2.11^{b}±1.07	2.24^{c}±1.15
First instar	4.47^{d}±1.47	3.64^{c}±1.17	2.35^{a}±1.45	2.41^{a}±1.11	2.61^{b}±1.17
Second instar	3.97^{d}±1.10	3.13^{c}±1.84	1.16^{a}±1.34	1.23^{b}±1.17	2.27^{b}±1.20
Third instar	4.03^{e}±1.17	2.27^{c}±1.15	1.22^{a}±1.07	1.95^{b}±1.26	3.08^{d}±1.12
Forth instar	4.21^{d}±1.06	3.14^{c}±1.13	1.79^{a}±1.20	1.89^{a}±1.15	2.98^{b}±1.14
Fifth instar	3.87^{e}±1.11	3.01^{c}±1.02	2.02^{a}±1.60	2.19^{b}±1.45	3.50^{d}±1.07
Sixth instar	5.15^{d}±1.17	4.09^{c}±1.25	2.78^{a}±1.17	2.98^{b}±1.05	4.00^{c}±1.15
Larva	25.7^{c}±1.65	19.28^{b}±1.67	11.32^{a}±1.12	12.69^{a}±1.77	18.44^{b}±1.11
Pupa	31.10^{c}±1.25	14.20^{a}±1.26	12.31^{a}±1.11	12.74^{a}±1.51	22.53^{b}±1.18
Female adult (♀)	22.75^{c}±1.26	19.01^{c}±1.14	7.86^{a}±1.16	8.76^{ab}±1.21	9.39^{b}±1.24
Male adult (♂)	11.34^{b}±1.83	9.24^{a}±1.60	6.47^{a}±1.23	6.95^{a}±1.84	8.20^{a}±21.10
Fecundity (eggs/ female)	133.2^{a}±1.83	321.85^{c}±1.60	596.5^{e}±1.23	478.8^{d}±1.84	193.1^{b}±21.10
Ratios of oviposited females (%)	46.0	58.0	74.5	66.5	50.0

The data in the table are the means ± SE. Different letters in the same row indicate significant differences at $P < 0.05$

Table 2. Effect of temperature on pupation of *S. littoralis*

Temperatures (ºC)	Normal pupae (%)	Deformed pupae (%)	Pupal mean weight (mg/pupa)	Pupation (%)
15	35.0^{a}±1.15	9.77^{bc}±1.95	126.4^{a}±1.95	41.5
20	78.4^{b}±1.66	12.95^{c}±1.73	240.1^{c}±4.33	69.3
25	90.5^{d}±1.20	1.36^{b}±0.66	293.6^{e}±3.82	98.1
30	95.7^{d}±1.83	0.67^{a}±0.15	275.0^{d}±2.91	82.0
35	86.9^{c}±1.31	3.20^{b}±1.16	182.5^{b}±3.22	77.6

The data in the table are the means ± SE. Different letters in the same column indicate significant differences at $P < 0.05$

3.2. Effect of temperature on survival

Effect of temperature on egg hatching and survival rate of the larval instar stages of *S. littoralis* is presented in (Table 3). No significant effect of temperature on egg hatching was observed. At a temperature of 25 or 30 °C, the highest percentage of larvae emerged from eggs. Larvae at the first and sixth instar stages were more affected by temperature. At 15 & 35 °C, the survival rate of the first instars was 72.10 & 81.25%, respectively, which is significantly lower compared with 25 °C (82.59%). At 25 & 30 °C, the survival rate of the sixth instars was higher compared with the other temperatures. The highest rates of larval survival were observed at 25 °C. Larvae at the 1st and last instar stages were more susceptible to the influence of temperature. Within the temperature range of 15–35 °C, the relationship between the survival rate and temperature positive or negative. 25 °C was the optimum temperature. These results show that the frequency of insect molting and temperature are closely related [14]. Qin *et al*. [12] suggested that the increase in the number of larvae that molt at low or high

temperatures might be a physiological response to adverse environmental conditions.

Table 3. Effect of temperature on egg hatching and survival rate of the larval instar stages of *S. littoralis*

Temperatures (ºC)	Egg hatchability (%)	Survival rate (%)					
		First instar	Second instar	Third instar	Fourth instar	Fifth instar	Sixth instar
15 ºC	79.22	72.10	90.44	90.53	91.76	93.08	77.00
20 ºC	82.54	80.04	96.12	96.40	94.31	94.35	83.18
25 ºC	84.90	82.59	98.25	97.75	97.96	95.31	86.92
30 ºC	83.61	81.62	97.00	96.64	95.72	94.67	85.80
35 ºC	80.25	74.37	93.81	91.00	93.77	94.81	81.12

3.3. Biochemical impacts of temperature on *S. littoralis* larvae

Biochemical impacts were performed to determine the effects of constant tested temperatures on the amount of total proteins, lipids, carbohydrates, and free amino acids in the haemolymph of the 4th instar larvae of *S. littoralis*.

The data (Table 4 and Figure 1) showed that the total amount of proteins, lipids, carbohydrates and free amino acids increased significantly by raising temperatures to reach their maximum activities at 25 ºC, were 59.62, 18.65, & 28.32 mg/g.b.wt, respectively, as for free amino acids recording 787.5 (µg alanine/ml).

There was a severe reduction in total lipids and carbohydrates at 35 ºC, 7.51 & 6.86 mg/g.b.wt, respectively. On the other hand, the levels of total lipids and carbohydrates decreased significantly at temperatures lower than 15 ºC, was 5.14 & 8.20 mg/g.b.wt respectively, and no significant changes were detected between other temperatures. Our study showed temperature effect on total proteins, lipids, carbohydrates, and free amino acids, led to variations in growth rates, as insect use lipids and stored carbohydrates as the main energy and development source. Further, our study showed the fourth larval instars of *S. littoralis* at 25 ºC had a higher level of proteins, lipids, carbohydrates and free amino acids in comparison with those larvae at 35 ºC; therefore, temperature rise led to increasing metabolic rate, and decreasing the development period. Our results agree with [15] findings which reported that high temperature affected all biological process, including the structure of proteins and biological members and rates of biochemical and physiological reactions.

Moreover, high temperature tended to kill insect's cells by denaturing proteins, altering membrane, enzyme structures and properties, and by the loss of water (dehydration) and they offer a rich potential for pest management strategies. Sonmez and Gulel [16] concluded that a low temperature decreases the total carbohydrates and protein amounts of the pest *Acanthoscelides obtectus*. They recommended that storage should be kept at 10-15 ºC so that, *A. obtectus* and other possible pests give minimal damage crops.

Lee and Roh [17] concluded that lipids storage efficiency was lower in larvae of *Spodoptera exigua* at 18 ºC than at 26 ºC and was similar to those at 34 ºC. Lemoine and Shantz [18] revealed that the decline of protein of *Spodoptera exigua* at 30oC due to nitrogen digestibility was almost 25% at 30 ºC compared with 25 ºC. Reduced nitrogen digestibility, coupled with increased cellular division and somatic growth rates, may be responsible for the observed strengthening of protein limitation at high temperatures. Lepidopteran larvae may be particularly

vulnerable to environmental warming because their rapid growth rates demand high protein foods.

Table 4. Effect of different constant temperatures on biochemical of 4th *S. littoralis* larval instar

Temperatures (°C)	Total proteins (mg/g.b.wt)	Total lipids (mg/g.b.wt)	Total carbohydrates (mg/g.b.wt)	Free amino acids (µg alanine/ml)
15	39.55^{b}±1.47	5.14^{a}±1.17	8.20^{a}±1.37	355.5^{b}±8.08
20	48.79^{b}±1.02	10.40^{b}±1.73	16.65^{b}±1.15	601.4^{c}±11.6
25	59.62^{c}±1.76	18.65^{b}±1.21	28.32^{c}±1.73	787.5^{e}±14.7
30	54.38^{c}±1.52	14.67^{b}±1.36	20.07^{c}±0.57	743.1^{d}±9.82
35	20.88^{a}±1.24	7.51^{a}±0.86	6.86^{a}±0.21	62.0^{a}±7.62

The data in the table are the means ± SE. Different letters in the same column indicate significant differences at $P < 0.05$

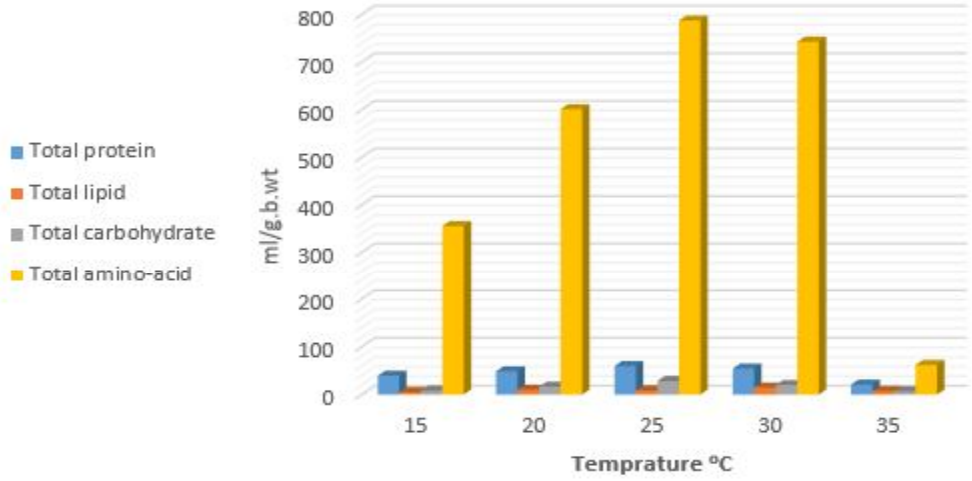

Figure 1. Effect of different constant temperatures on biochemical of 4th *S. littoralis* larval instar

4. Conclusions

In conclusion, the temperature is generally considered the single most significant environmental factor influencing pest population suppression through an effect on behavior, distribution, development, survival, and reproduction in insects. This study showed that temperature had a significant effect on the developmental durations of *S. littoralis* stages. The data obtained in this study could contribute to the development of pest forecasting programs and the improvement of current management strategies for this important insect pest.

Abbreviation

Not applicable

Conflict of interest

Not applicable

Consent for publications

The author(s) read and approved the final manuscript for publication.

Availability of data and material

All data generated during this study are included in this published article.

Authors' contributions

The author contributed to the production and writing of the manuscript.

The author(s) read and approved the final manuscript.

Ethics approval and consent to participate

The manuscript does not contain any studies involving human participate, Human data or human tissue.

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