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Effects of Temperature OnThe Development, Food Consumption and Utilization Parameters of the Last Two Larval Instars of *Spodoptera littoralis* (Boisd.)

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ABTRACT

***Spodoptera littoralis* (Boisd.) is one of the most destructive agricultural lepidopteran pests in Egypt. About 90 to 95% of total defoliation occurs during the last two larval instars. Sets of caterpillars were raised on artificial diet from hatching at one of four constant temperatures 15, 20, 25, or 30°C. The effect of temperature on the development and food utilization during the last two larval stages were examined. The duration of development of both penultimate and ultimate instars significantly decreased as the temperature increased from 15 to 30ºC. At each temperature, the duration of penultimate instar was significantly shorter than the ultimate ones. The development of both penultimate and ultimate instars of *S. littoralis* larvae was significantly fastest at 30ºC and slowest at 15ºC. The insect had an extra moult at 20 (23.7%) and 25ºC (8.5%) before pupation. The various rearing conditions affected the different nutritional values. The data of nutritional indices for the fifth and sixth instars of *S.littoralis* are not generally consistent with each other. Approximate digestibility (AD) values of the last two instars were highest at 25ºC and lowest at 30ºC.So, *S.littoralis* is better developed at 25°C, than any of the other rearing temperature.**

Key words: *Spodoptera littoralis*, Constant temperature, larval critical weight, Instar number, growth and Nutritional parameters.

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INTRODUCTION

During the last decade Egypt is facing a fast-growing population requiring significant improvement of agriculture and food production. One of the major obstacles in sustaining and rising agriculture production is the damage caused by pests, especially the cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera, Noctuidae).The cotton leaf worm, is a very serious pest due to the damages it causes, and control difficulties (Azab et al., 2001). It occurs throughout most of the Mediterranean region, large parts of Africa and Madagascar. It is a polyphagous insect, in Egypt, 73 host plants were recorded for *S. littoralis* (Bishara, 1934; Willcocks and Bahgat, 1937; Moussa et al., 1960). It is one of the most important cotton pests in Egypt. Due to its wide host range, it attacks several important crops all year round. It has shown to lower cotton yields by as much as 75% (Hosny and Isshak, 1967). The pest exists in the soil during cold spells without undergoing diapause (Sidibe and Lauge, 1977). On several crops, damage arises from extensive feeding by larvae, leading to complete stripping of the plants. On cotton, the larvae feed on the leaves creating holes of irregular shape and usually all that remains are the bigger veins.
The larvae may also bore into the bud or young boll and feed on the whole contents, causing them to be shed or dry up (Bishara, 1934). About 90% of total defoliation

occurs during the last two larval instars. It is active all the year round and attacks cotton, maize, clover, and several vegetable crops. Depending on the climate of the region, *S. littoralis* can have from two to seven generations per year. As the insect develops, it completes six instars (Salem and Salama, 1985). In Egypt, Hafez (1972) reported that, it has seven generations per year; 4 generations on clover, 2 to 3 generations on cotton, and possibly one generation on maize. So, the insect develops under different climatic conditions, for example, changes of temperature. Since temperature affects all life processes of *S.littoralis* it also affects the physiology of food consumption and food utilization. The present study was conducted to elucidate the effect of 4 constant temperatures on development and nutritional parameters of the last two instars of *S. littoralis* larvae.

MATERIALS AND METHODS

In 2014/2015, a series of experiments were conducted in the IPM laboratory at the Department of Economic Entomology, Faculty of Agriculture, Alexandria University, Egypt using the cotton leaf worm *S. littoralis* which was mass reared on semi-artificial diet according to (Hegazi et al., 1977). The diet consisted of: kidney beans 160 grams, Medical dried yeast 35 grams, Methyl-p-hydroxybenzoate 3.5 grams, ascorbic acid 3.5 grams, Agar 13 grams, Formaldehyde 2.5 ml, Water (total) 700 ml. Four cultures were established at 4 constant temperatures of 15, 20, 25 and 30 ±1ºC at 60 ± 5% RH and a photoperiod of 12L: 12D. The larvae were reared individually in petri dishes (3.5 cm in diameter) (n=25-30 larvae x 4 trials/temp). Larval instars were determined by checking the shed head capsules (Mironidis and Savopoulou-Soultani, 2008). 5th -instar larvae in the premolt stage from the above cultures were held overnight without food, and those which molted within 12 h were used in the experiments (4 x 21 larvae/temp), which were continued until the prepupal stage was reached. Thus, all larvae were similar in physiological conditions, size and age.

The guts of the newly molted larvae were almost free of residual fecal material. The advantages of using the starved newly-molted insects in growth and feeding studies were first pointed out by Waldbauer (1962, 1964). The maximum weight of a larva during the last larval instar was taken as its final weight. Measurements of the present study were made on dry weight basis. Larvae were tested individually, each being held in a 3.5 cm in diameter petri dish. The dry weight of the introduced food was indirectly determined. The food was divided into portions each ca 1.2 g. Two adjacent portions were weighed and used at 24 h intervals for each larva. One was given to a larva and the other was used to determine the dry weight of the former. Faeces were also collected at 24 h intervals. The left-over and uneaten portions as well as the faeces of larva were dried separately in a hot air oven (60ºC) and weighed and in turn the dry weight of food consumed by a larva could easily be estimated. Since a direct measurement of the dry weight of the tested larvae at the beginning of the experiments was not possible, the mean dry weight was estimated from samples of 10 newly molted, starved 5th and/or 6th instar larvae.

The difference between the latter and the dry weight of a newly formed next stage of the tested larvae represented the dry weight gained by a larva during the experimental period. Nutritional indices proposed by Waldbauer (1964, 1968) were used .These indices were calculated as follows: Consumption index (CI) =wt. food ingested/duration of feeding period (days) x mean wt. of larva during feeding period. Approximate digestibility (AD) =wt. food ingested - wt. of faeces excreted/. wt. food ingested x 100. Growth rate (GR) =wt. gain of larva during feeding period /duration of feeding period (days) x mean wt. of larva during feeding period. Efficiency of conversion of ingested food (ECI) to biomass =wt. gain of larva during feeding period/wt. of food ingested during feeding period x100. Efficiency of conversion of digested food (ECD) to biomass=wt. gain of larva during feeding period/wt. of food ingested - wt. of faeces excreted x 100.

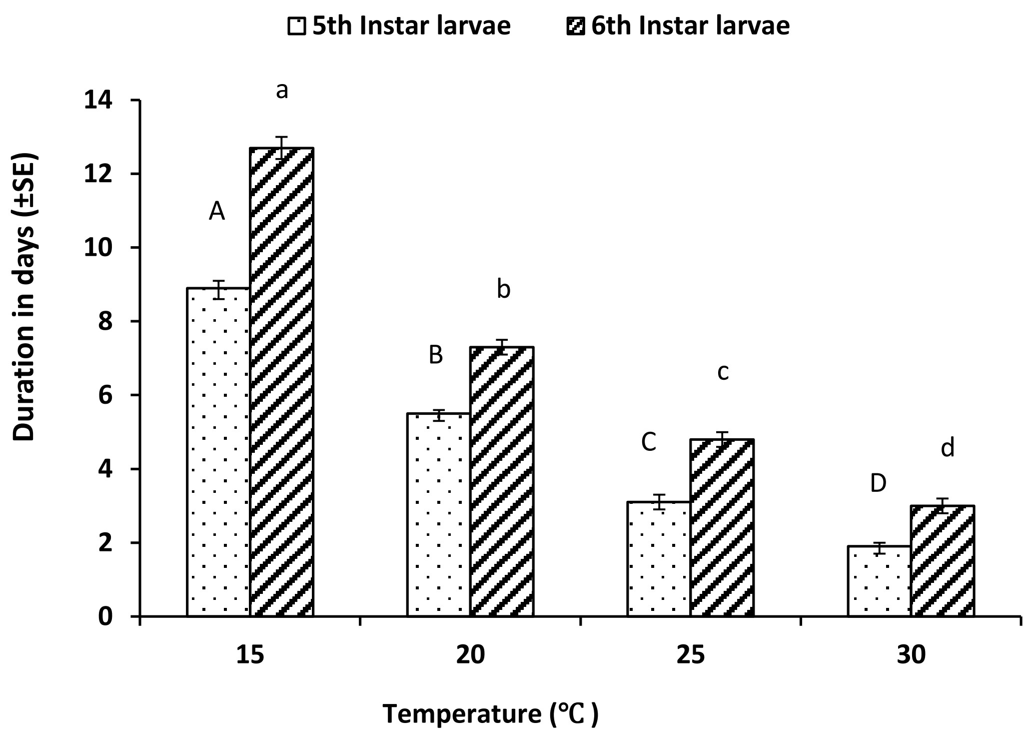
Statistical Analysis

Percentage values were subjected to arcsine square root transformation to increase the homogeneity of variance and normality. Data were analyzed by one-way analysis of variance (ANOVA) and means were compared by least significant difference (LSD) or Student's t-test. The SPSS 8.0 software (SPSS Inc., Chicago, IL) was used for statistical analyses.

RESULTS

Development Time and Growth

The results of this study showed that temperature had significant effects on the developmental durations of the last two instars of *S. littoralis* larvae (Figure 1). For the same developmental stage, the development duration significantly decreased as the temperature was increased from 15 to 30ºC (5^{th} instar: F = 581.9, df = 3, 80, $P < 0.05$, 6^{th} instar F = 402.9, df = 3, 80, $P < 0.05$). At each temperature, the duration of 5^{th} instar larvae was significantly shorter than the 6^{th} instar (t=10.38 at 15ºC, t = 9.5 at 20ºC, t=8.5 at 25ºC, t=6.25 a 30ºC, $P < 0.05$). Typically, the duration of 5^{th} and 6^{th} instar larvae was 8.9±0.2 vs. 12.7±0.3 days at 15ºC, 5.5±0.1 vs.7.3±0.2 days at 20ºC, 3.1±0.2 vs. 4.8±0.2days at 25ºC and 1.9 ±0.1vs. 2.9 ±0.1days at 30ºC, respectively. The occurrence of extra moult differed significantly between rearing temperatures. At 20 and 25ºC, 23.7 and 8.5% of



Fig**ure 1.** Duration in days (±SE) of the last two instars of *S.lilttoralis* larvae at different constant rearing conditions. Bars with the same uppercase or lowercase letter are not significantly different (P<0.05).

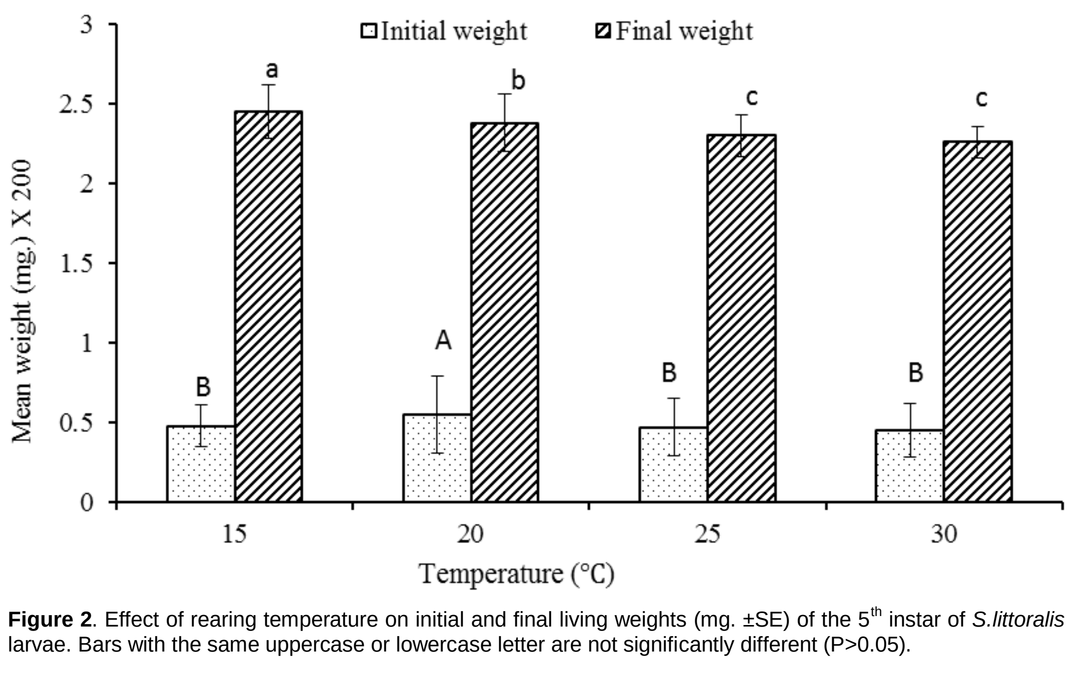


Figure 2. Effect of rearing temperature on initial and final living weights (mg. ±SE) of the 5th instar of *S.littoralis* larvae. Bars with the same uppercase or lowercase letter are not significantly different (P>0.05).

S. littoralis larvae developed through an extra instar (normally six) before pupation, in respect, vs. 0% at 15 or 30ºC.

The initial living weight of 5th (F = 5.45, df = 3,36, *P* < 0. 05) or 6th (F = 2.9, df = 3,36, *P* < 0. 05) instar larvae was heavier at 20ºC (Figures 2 and 3). Typically, a 5th instar larva weighed an average of 55.3 ±2.4 mg (n = 10 x 3 reps.), while the 6th instar larva averaged 271.4±7.2 mg

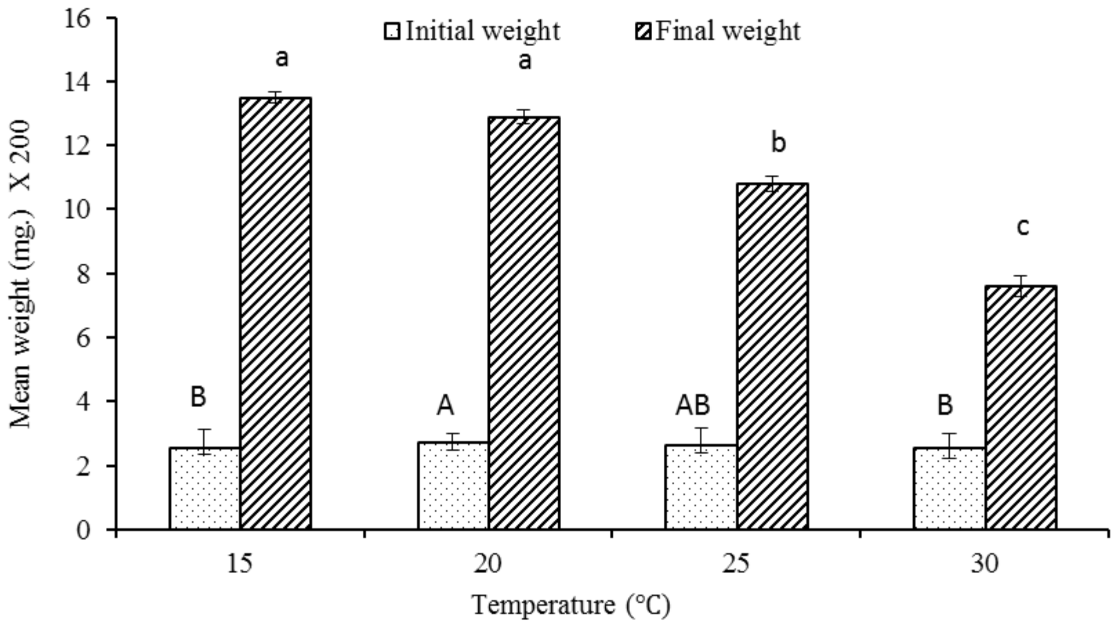


Figure 3. Effect of rearing temperature on initial and final living weights (mg. ±SE) of the 5th instar of *S.littoralis* larvae. Bars with the same uppercase or lowercase letter are not significantly different (P>0.05).

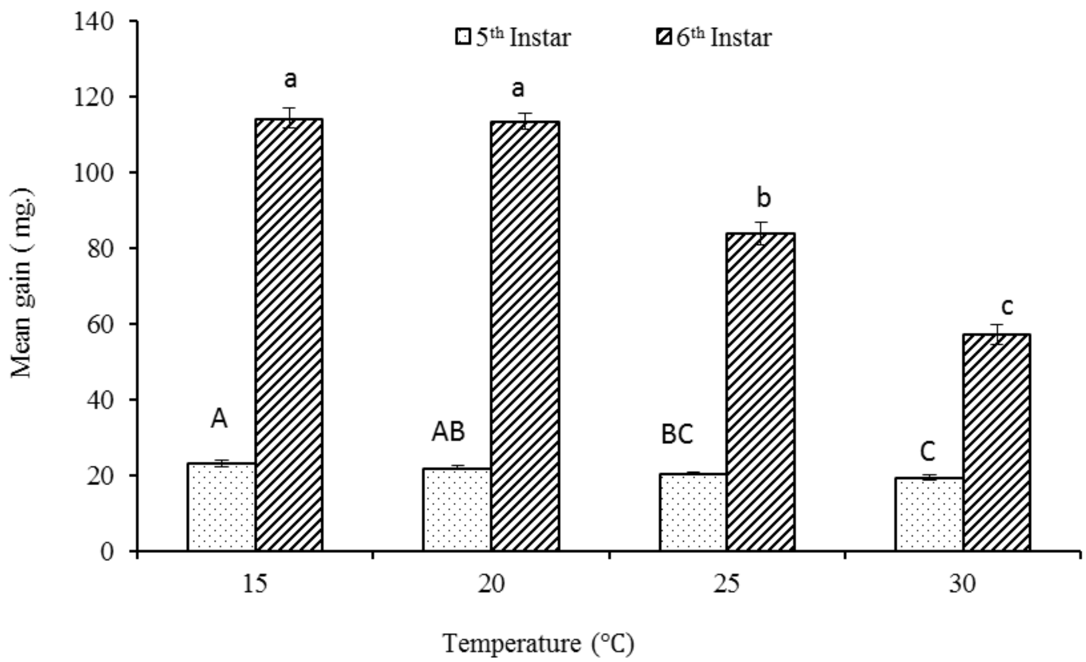


Figure 4. Effect of rearing temperature on mean dry mass gain (mg. ±SE) during the 5th and 6th instar of *S.littoralis* larvae. Bars with the same uppercase or lowercase letter are not significantly different (P>0.05).

(n = 10 x 3 reps.). However, the maximum living larval weights was heavier at 15ºC for both 5th (F = 33.3, df = 3,36, P < 0. 05) or 6th (F = 113.9, df = 3,36, P < 0. 05) instar larvae. They weighted averages of 245.58±1.7 and 1349.5±18.4 mg, respectively. Dry weight gain was found to be significantly less in higher temperature in the case of 5th instar (F = 5.9, df = 3, 36, P = 0. 002) or 6th (F=109.5, df = 3, 36, P < 0. 05) instar larvae compared to lower temperature (Figure 4).The mean gains in dry weight of the 5th instar were 23.2±0.93, 21.9±0.55, 20.4±0.55 and 19.4±0.6 mg at 15, 20, 25 and 30ºC, respectively. For the 6th instar larvae the mean gains in dry weight at 15ºC (114.2±2.7 mg) and at 20ºC 113.3±2.1mg) significantly decreased to 83.7±2.9 and

Table 1. Consumption and food utilization parameters of the last two instar of *S. littoralis* larvae at different temperatures.

Trail	Instar	Temperature ºC			
		15	20	25	30
C.I	5th	1.31 ± 0.02 C	1.70 ± 0.10 C	2.30 ± 0.20 B	3.1 ± 0.20 A
	6th	0.75 ± 0.02 D	1.40 ± 0.02 C	2.30 ± 0.10 B	2.5 ± 0.04 A
A.D	5th	31.30 ± 0.60 C	34.2 ± 0.70 B	36.6 ± 0.60 A	28.6 ± 0.70 D
	6th	34.60 ± 0.70 B	38.7 ± 0.60 AB	40.4 ± 1.00 A	30.2 ± 0.70 C
G.R	5th	0.12 ± 0.01 D	0.36 ± 0.01 C	0.53 ± 0.04 B	0.71 ± 0.10 A
	6th	0.08 ± 0.02 D	0.21 ± 0.02 C	0.35 ± 0.01 B	0.44 ± 0.02 A
E.C.I	5th	6.10 ± 0.11 C	20.34 ± 0.60 B	22.99 ± 0.69 A	21.03 ± 0.84 B
	6th	11.88 ± 0.22 C	15.87 ± 0.52 B	12.87 ± 0.55 C	17.96 ± 0.74 A
E.C.D	5th	20.60 ± 1.14 D	52.65 ± 1.60 B	63.35 ± 1.90 A	42.52 ± 1.50 C
	6th	39.75 ± 0.66 B	40.39 ± 1.05 B	32.97 ± 0.87 C	60.67 ± 2.51 A

Means within a row followed by the same letter are not significantly different, ($P < 0.01$).

(57.2± 2.6 mg at 25 and 30ºC, in respect.

Food Consumption and Utilization

The results of the nutritional indices of 5th and 6th larval instars of *S. littoralis* are shown in Table 1. Nutritional indices of the 5th instar larvae of *S .littoralis* were significantly different at various rearing range of tested temperatures (p <0.05). The larvae reared at 30ºC showed the highest values of CI (F = 43.71, df = 3, 36, p <0.05) (3.1 ± 0.18) and GR (F = 27.65, df = 3. 36, p <0.05) (0.71 ± 0.1). While the lowest values of CI (1.31± 0.02) and GR (0.12 ± 0.01) was at 15ºC. However, the lowest value of AD was for larvae reared at 30ºC (28.6 ± 0.7). While, the highest value of AD (F = 26.8, df = 3, 36, p <0.05) (36.6± 0.6) was for larvae reared at 25ºC compared with the other larvae reared at the tested range of temperatures. The highest (22.99 ± 0.69) and lowest (6.1 ± 0.11) ECI values (F = 157.5, df = 3, 36, p <0.05) of the 5th instar larvae of *S. littoralis* were for those larvae reared at 25 and 15ºC, respectively.

The highest value of ECD (63.35 ± 1.9) and lowest one (20.6± 1.14) (F = 136. 17, df = 3. 36, p <0.05) were observed for larvae reared at 25 and 15ºC, respectively, (Table 1). Also, the nutritional indices of the 6th instar larvae of *S. littoralis* were significantly different at rearing range of tested temperatures (p <0.05). As in the case of 5th instar (Table 1), the larvae reared at 30 and 15ºC had the highest (2.5 ± 0.04) and lowest (0.75 ± 0.02) values of CI, respectively (F = 599.7, df = 3.36, p <0.05). Our results indicated also that, the highest (0.44 ± 0.02) and lowest (0.08 ± 0.02) values of GR (F = 234. 16, df = 3. 36, p <0.05) were for larvae reared at the same rearing temperatures (30 and 15ºC), respectively. However, the last instar *S. littoralis* larvae showed the highest (40.4 ±1.1) and lowest (30.2 ± 0.75) values of AD (F = 12, df = 3. 36, p <0.05) at 25 and 30ºC, respectively. Values of ECI, and ECD among 6th, instars are compared in Table 1. The highest values of ECI (17.9 ± 0.7) (F = 25.7, df = 3.36, p <0.05) and ECD (60.7 ± 2.5) (F = 66.6, df = 3. 36, p <0.05) were for larvae reared at 30ºC, respectively. Generally, at the tested temperatures of 15, 20 and 25ºC, the AD values of the 5th instar of *S. littoralis* had lower values compared with those of the 6th instars (t=3.4 at 15ºC, t=4.8 at 20ºC and t=3.3 at 25ºC, p <0.05), but no significant AD values were found among the 5th and 6th instar larvae reared at 30ºC.

DISCUSSION

Detailed knowledge of the insect life cycle and how it responds to ecological factors, is the necessary raw material for forecasting, and for developing successful control programs (Nylin, 2001). Several studies have examined the developmental responses of insects to environmental conditions, especially photoperiod and temperature (Hegazi and Schopf, 1984; Liu et al., 2008; Li-Tao et al., 2013). About 90 to 95% of total defoliation occurs during the last two larval instars. The development of both penultimate and ultimate instars of *S. littoralis* larvae was significantly fastest at 30ºC and slowest at 15ºC. But, the larvae consumed less food at 30ºC (E.M.Hegazi, personal communication). At 15ºC the larval 5th and 6thinsars are prolonged that is, the growth rate of the insect is reduced. This affected the dry weight gain of the insect larvae. Dry weight gain of the last two instars of *S. littoralis* larvae was found to be significantly less at 30ºC, compared with those larvae reared at 15 or 20ºC.

It was reported that *S. littoralis* larvae had 6 instars (Salama and Shoukry, 1972). However, we have shown here that the number of instars of *S. littoralis* was variable and 7 instars could be observed at rearing temperature of 20 and 25ºC where 23.7 and 8.5% of *S. littoralis* larvae developed through an extra instar before pupation, in respect. Baker and Miller (1974) reported similar observation on the effect of low temperatures in addition to the larval food “chrysanthemum” of *S. littoralis* larvae on producing extra instars. Kingsolver (2007) found that the frequency of individuals of *Manduca sexta* with six instars decreased with increasing rearing temperature. Duodu and Biney (1981) mentioned that *S. littoralis* larvae had an extra molt on two (cotton and *Urena*) of four tested food plants. Esperk et al. (2007), reported that the most common factors influencing insect instar number are temperature, photoperiod, food quantity and quality, humidity, injuries, inheritance and sex. Low temperature combined with short photoperiod induces slow development together with additional instars in several species (Ballmer and Pratt, 1989; Shintani and Ishikawa, 1997). However, Naser et al. (1974) found that high temperatures and high humidity’s tended to shorten the egg and larval stages of *S.littoralis.*

The nutritional indices, of *S. littoralis* reared at different temperatures were significantly different, suggesting that the various rearing conditions affected the different nutritional values and initial and final weight of insect larvae. The data of nutritional indices for the 5th and 6th instars of *S. littoralis* are not generally consistent with each other. This is because the nutritional requirements of the insect change through insect’s life and such differences typically result in changes in food consumption and feeding behavior (Browne, 1995). The dry weight-fresh weight consumption index (CI) calculated from dry weight of food eaten and fresh weight of animal is an indicator of relative intake of nutrients. Both of CI and GR were found to be significantly less at 15ºC compared with those reared at 30ºC. ECI is a general index of an insect’s ability to use the food consumed for growth and development, and ECD is an index of the efficiency of conversion of digested food into growth (Nathan et al., 2005). The low AD sat 30ºC were accompanied with higher ECIs and ECDs. The much higher weight increase achieved by the 5th or 6th larval instars of *S. littoralis* at 15 or 20ºC than at the other conditions is apparently due to the prolongation of the larval duration followed by the highest amount of diet eaten (E. M. Hegazi, personal communication) and a relatively high efficiency with which this diet was digested. At 25ºC, the AD, ECI and ECD are almost similar to those reported for the same pest on cotton leaves at temperatures fluctuating between 27 and 33ºC (Duodu and Biney, 1981).

On the other hand when larvae of *S. eridania* (Scriber, 1982), *S. litura* (Babu et al., 1979) or *S. littoralis* (Duodu and Biney,1981) were bred on natural food other than cotton leaves, higher values of ADs were obtained than those observed in the present study. The ADs were significantly highest at 25ºC and least at 30ºC. The significant decline in AD at 30ºC was perhaps due to the combination of a highest CI and a quick food passage through the insect gut. It seems that *S. littoralis* reformed this condition by highest efficiency in converting digested food into body matter; its low dry weight gain (19.4 and 57.2 mg, for the 5th and 6th instars, respectively) was due to the limited period during which the larva has to complete its development (1.9 and 3.0 days, for the 5th and 6th instars, respectively). So, it seems that *S. littoralis* is better developed at 25ºC, than any of the other rearing temperatures. The results suggest that there is a great variation in the nutritional indices among the 5th and 6th instars of *S. littoralis* larvae under different rearing conditions.

CONCLUSION

The study showed how *S. littoralis* responds to constant temperature. The larval development was significantly faster at high temperatures and nutritional indices gave contradicting results. The insect is better adapted at 25ºC than any of the other three temperatures. So, the study may represent necessary raw material for forecasting, and for developing successful control programs.

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