Comparative study to determine food consumption of cotton leafworm, *Spodoptera littoralis*, on some cotton genotypes

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Abstract: A study was done on the feeding behaviour, development indices, and biochemical response of 4th instar larvae of *Spodoptera littoralis* in which seven cotton genotypes were used: Giza86, Giza88, Giza92, 10229 × Giza 86, H10, Suvin, and Karshenseki. Castor bean leaves were used as the control. All the tested cotton genotypes decreased the feeding behavior of *S. littoralis* larvae in terms of consumption percentages, consumption rate (CR), growth rates (GR), efficiency of conversion of ingested and digested food (ECI and ECD), approximate digestibility (AD), and feeding deterrence (FDI) compared to the control. Additionally, all the tested cotton varieties prolonged the time taken for larval and pupal duration and reduced both the pupation percentages and the weight of the resulting pupae, as compared to the control. Giza86 recorded the lowest values of both larval growth index and fitness (7.31 and 1.05, respectively) while the genotype Suvin recorded the lowest standardised growth index (0.020) more than other genotypes and the control that gave 10.16, 1.53, and 0.032, respectively. There was a significant inhibition in the level of both total soluble protein and total lipids, and activities of amylase and the trehalase enzymes in all the tested varieties, compared to that found in the control.

Key words: biochemical, consumption, cotton genotypes, *Spodoptera littoralis*

Introduction

Cotton (*Gossypium barbadense* L.) occupies an important position among crops in Egypt. Cotton plants are infested by several pests, and the cotton leafworm, *Spodoptera littoralis* (Boisduval), is one of the most destructive agricultural lepidopterous pests. The cotton leafworm causes a variety of damage as a leaf feeder, sometimes as a cutworm on seedlings, and occasionally destructing the bolls (Darvishzadeh 2014). Many populations of *S. littoralis* are extremely resistant to pesticides. If a populations becomes well-established, they can be exceptionally difficult to control (USDA 1982).

The infestation caused by *S. littoralis* differs depending on the cotton variety (Kamel 1965). This difference may be attributed to plant leaf morphology or to the chemical contents of the leaves. Quantitative analysis of the consumption of host plants by *S. littoralis* is an important factor used in studying and verifying larval preferences of plant varieties (Scriber and Slansky 1981).

The quality and quantity of food consumed by the pest can affect its entire biology, rate of growth, development, reproduction, and history (Bavaresco *et al.* 2004). The aims of the present study focus on the *S. littoralis* larvae's quantified consumption rate of some cotton cultivars grown in Egypt and the effect of these cultivars on the pest's entire biology. Additionally, the relationship between different cotton cultivars and some biochemical change-attributes were studied.

Materials and Methods

Tested cotton genotypes

The seven tested cotton varieties (*G. barbadense*) used in this study were grown on the experimental farm of Sakha Station, Kafr El-Sheikh Governorate, Egypt. The cotton varieties where provided by the Cotton Research Institute, Agricultural Research Center, Egypt (Table 1). Leaf samples of different cotton varieties were picked, sealed in paper bags, and transferred to the laboratory where they were offered to 4th instar larvae (L4) of cotton leafworm.

Culture of the cotton leafworm, *Spodoptera littoralis*, rearing technique

A laboratory (susceptible) strain of *S. littoralis* was reared away from any insecticidal contamination, at the division of cotton leafworm, Branch of Plant Protection Research Institute at Zagazig, Sharkia Governorate, Egypt. The provided insects were used in the present investigation.

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Table 1. List of the seven cotton genotypes and related information

No.	Genotype	Abbreviation	Pedigree	Category	Current position	Region
1	Giza86	G86	Giza77 x Giza81	long stable	commercial variety	Egypt
2	Giza88	G88	Giza77 x Giza45	extra long stable	commercial variety	Egypt
3	Giza92	G92	Giza84x (Giza74 x Giza68)	extra long stable	commercial variety	Egypt
4	10229xGiza86	10xG86	Australian 10229 x Giza86	long stable	new promising hybrid	Australia – Egypt
5	[G84 x (G70 x G51b)] S62	H10	[G84x (G70 x G51b)] S62	extra long stable	new promising hybrid	Egypt
6	Suvin	Suvin	Sujata x Vincent	long stable	commercial variety	India
7	Karshenseki	Karsh.	Karshenseki	long stable	commercial variety	Russia

Egg-masses were reared on leaves of castor bean, *Ricinus communis*, according to El-Defrawi *et al.* (1964) under constant conditions: 26±1°C and 70±5% relative humidity (RH) *R. communis* is a widely distributed wild plant in Egypt. *Ricinus communis* is cheap, and considered the easiest source to feed cotton leafworm. *Ricinus communis* is one of the most preferred hosts to this insect under laboratory conditions. Thus, we used *R. communis* as a control or as a reference in comparison with the cotton genotypes used in our experiment.

Food consumption and antifeeding behaviour

The experiment was conducted to determine the effect of seven tested cotton genotypes as well as castor leaves, *R. communis* (as a reference), on the nutritional indices of the newly molted *S. littoralis* L4 larvae. The larvae were previously starved for 3–4 h before treatment to ensure an empty intestine. A total of 50 larvae were evaluated on each cotton genotype (10 larvae per replicate per cotton variety).

As a contrast, fresh leaves of each treatment were kept in clean jars without larvae, under the same conditions, to determine the natural loss of moisture, which was used for calculating the corrected weight of the consumed leaves. After 24 h, feces were weighed and removed from the leaves and the remaining leaves weighed again. The jars were cleaned and the newly weighed leaves were given to the larvae.

The procedure was carried out daily for seven days (which was the time period for the end of the larval stage and the beginning of the prepupae formation). The laboratory conditions were 26±1°C and 70±5% RH. The following parameters were calculated according to Waldbauer (1968) (Table 2).

Additionally, consumption percentages were calculated using the formula of Saleh *et al.* (1986) as follow:

$$\frac{\text{Mean weight of eating leaves (cotton varieties)}}{\text{Mean weight of } \textit{R. communis} \text{ leaves}} \times 100.$$

The feeding deterrence index was calculated according to Isman *et al.* (1990):

$$FDI = (C - T)/(C + T) \times 100,$$

where: C – area of castor leaves fed to the larvae, T – area of cotton varieties fed to the larvae.

Development indices

Development indices of *S. littoralis* L4 larvae were studied by feeding the larvae seven different genotypes of cotton varieties, as well as castor leaves (as a reference). Larval and pupal periods, percent of pupation, and pupal weight were determined. Additionally, larval growth index, standardised growth index, and fitness index were

Table 2. The formulae of the nutritional indices calculated for *Spodoptera littoralis* (Waldbauer 1968)

Abbreviation	Nutritional index	Formula
RCR	relative consumption rate	$E/(A \times T)$
RGR	relative growth rate	$P/(A \times T)$
AD	approximate digestibility	$[(E - F)/E] \times 100$
ECI	efficiency of conversion of ingested food	$(P/E) \times 100$
ECD	efficiency of conversion of digested food	$[P/(E - F)] \times 100$

P – dry weight gain (mg), A – initial and final mean dry weights of the larvae during feeding period (mg), E – dry weight of food ingested (mg), T – duration of feeding period (days), F – the dry weight of feces produced (mg)

calculated using the following equations (Pretorius 1976; Itoyama *et al*. 1999):

$$\text{Larval growth index} = \frac{\text{Pupation}}{\text{Larval period (days)}}$$

$$\text{Standardized growth index} = \frac{\text{Pupal weight}}{\text{Larval period (days)}}$$

$$\text{Fitness index} = \frac{\text{Pupation [\%]} \times \text{pupal weight}}{\text{Larval period} + \text{pupal period}}$$

Biochemical studies

Preparation of samples

The preparation of samples involved the use of healthy *S. littoralis* larvae after seven days of feeding on all the tested cotton varieties as well as castor leaves. These larvae were placed in clean jars, starved for 4 h and then homogenised in distilled water using a Teflon homogeniser surrounded with a jacket of crushed ice, for 3 min. Homogenates were centrifuged at 3,500 r.p.m. for 10 min at 5°C to remove haemocytes that were assayed to determine the total lipids. The supernatants were used directly for the other biochemical analysis. Three replicates were used for each assay.

Total soluble protein

Colorimetric determination of total soluble protein in the total homogenate of larvae was carried out as described by Gornall *et al*. (1949).

Total lipids

The total lipids were estimated according to the method of Schmit (1964).

Carbohydrate hydrolysing enzymes

Carbohydrate hydrolysing enzymes (amylase and trehalase) were determined using the method of Ishaaya and Swiriski (1976) using starch and trehalase as the substrates.

Statistical analysis

The significance of the main effects was determined by analysis of variance (ANOVA). The significance of various treatments was evaluated by Duncan's multiple range test ($p < 0.05$) (Snedecor and Cochran 1980). Data were subjected to statistical analyses using the software package Costat® Statistical Software (2005) a product of Cohort Software, Monterey, California, USA.

Results

Feeding performance

Larval consumption percentages

The data in table 3 showed that the daily food consumption percentages of *S. littoralis* larvae which fed on seven cotton varieties were reduced when compared to the control larvae (feeding on castor leaves). Maximum peaks in consumption occurred on the third day in all the different varieties with the exception of G86 that gave a maximum value (88.92%) at day one post feeding. The highest peaks were recorded (90.85, 94.94, 94.78, 89.39, 99.12, and 91.68%) for varieties 10xGiza86, H10, G88, G92, Suvin, and Karsh., respectively.

Consumption rate (CR)

All tested cotton varieties reduced the mean CR after feeding *S. littoralis* L4 larvae for seven days on different varieties other than castor leaves (Fig. 1A). Data showed that G88 was the most effective in reducing the mean CR of L4 larvae (2.243 g · larva⁻¹) followed by H10 (2.34 g · · larva⁻¹), Suvin (2.42), G86 (2.42), Karsh. (2.43), G92 (2.44), and 10xGiza86 (2.49), in comparison to the control (2.61 g · larva⁻¹).

Growth rates (GR)

Data representing the mean GR showed a decline for all the cotton varieties during the seven days of feeding which was below that seen in the control (0.69 g) (Fig. 1B). Feed-

Table 3. Daily consumption percentages of *Spodoptera littoralis* larvae through seven days of feeding on different cotton genotypes

Cotton genotypes	Consumption at days [%]						
	1	2	3	4	5	6	7
G86	88.92	71.40	88.74	55.32	67.44	79.29	66.37
G88	79.32	74.40	94.78	62.00	79.27	69.47	80.77
G92	76.66	78.53	89.39	79.98	93.63	83.68	75.52
H10	81.33	86.39	94.94	72.41	94.22	90.01	61.21
10xG86	85.07	71.58	90.85	59.26	80.81	90.36	66.34
Suvin	67.30	78.67	99.12	52.75	80.59	82.43	89.28
Karsh.	64.67	76.03	91.68	68.05	95.84	84.82	85.38

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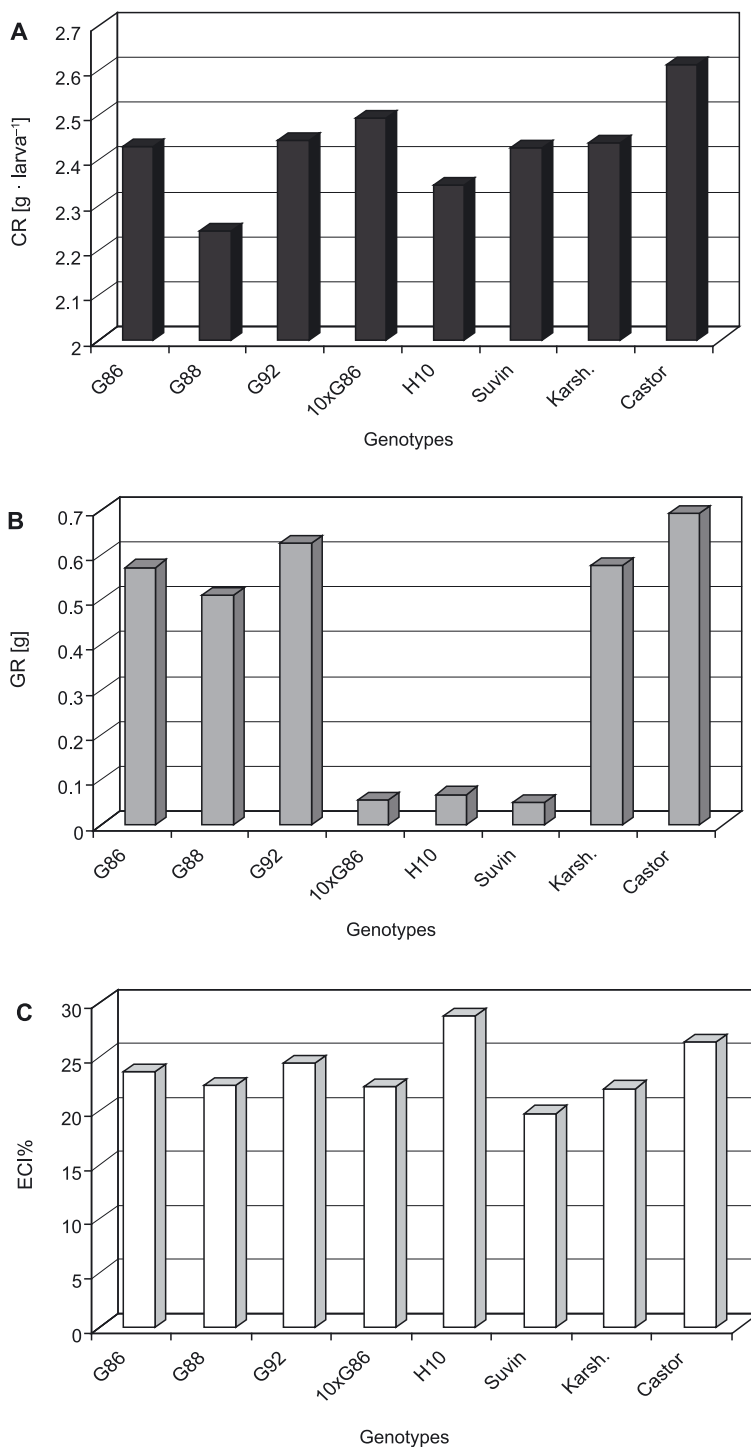
Fig. 1. Different feeding performance parameters: CR – consumption rate, GR – growth rate and ECI – efficiency of conversion of ingested (A–C), of *Spodoptera littoralis* larvae through seven days of feeding on different cotton genotypes

ing on Suvin, G88, 10xGiza 86, G86, Karsh., G92, and H10 was recorded as 0.50, 0.51, 0.55, 0.57, 0.57, 0.62, and 0.67 g, respectively.

Efficiency of conversion of ingested food (ECI%)

Data in figure 1C indicated that there was variation between the effect of the cotton varieties on the conversion of ingested food to one unit of body substance, and larvae feeding on castor leaves. The averaged values of ECI%

were recorded as 19.75, 22.07, 22.21, 22.38, 23.59, 24.39, and 28.75% for larvae fed on Suvin, Karsh., 10xGiza 86, G88, G86, G92, and H10, respectively, compared to the control larvae (26.39%) after seven days of feeding.

Efficiency of conversion of digested food (ECD%)

Suvin again showed the highest potentiality in reducing the mean conversion of digested food to one unit of body substance (22.36%) throughout seven days of feeding.

Followed by Karsh. (25.34%), 10xGiza86 (25.42%), G86 (28.29%), G88 (26.89%), G92 (29.49%), and H10 (34.40%). While castor leaves gave (31.518%) (Fig. 2A).

Approximate digestibility (AD%)

The tested cotton varieties had a slight effect on the approximate digestibility compared to the control (86.12%) as shown in figure 2B. The approximate digestibility per cent values ranged between a minimum value of 82.65% for G.86 to a maximum value of 85.93% for Suvin.

Feeding deterrence index (FDI)

The mean feeding deterrence index of *S. littoralis* larvae were calculated throughout seven days of feeding with the tested seven cotton genotypes. Both G86 and Suvin caused the highest percent of deterrence compared to the
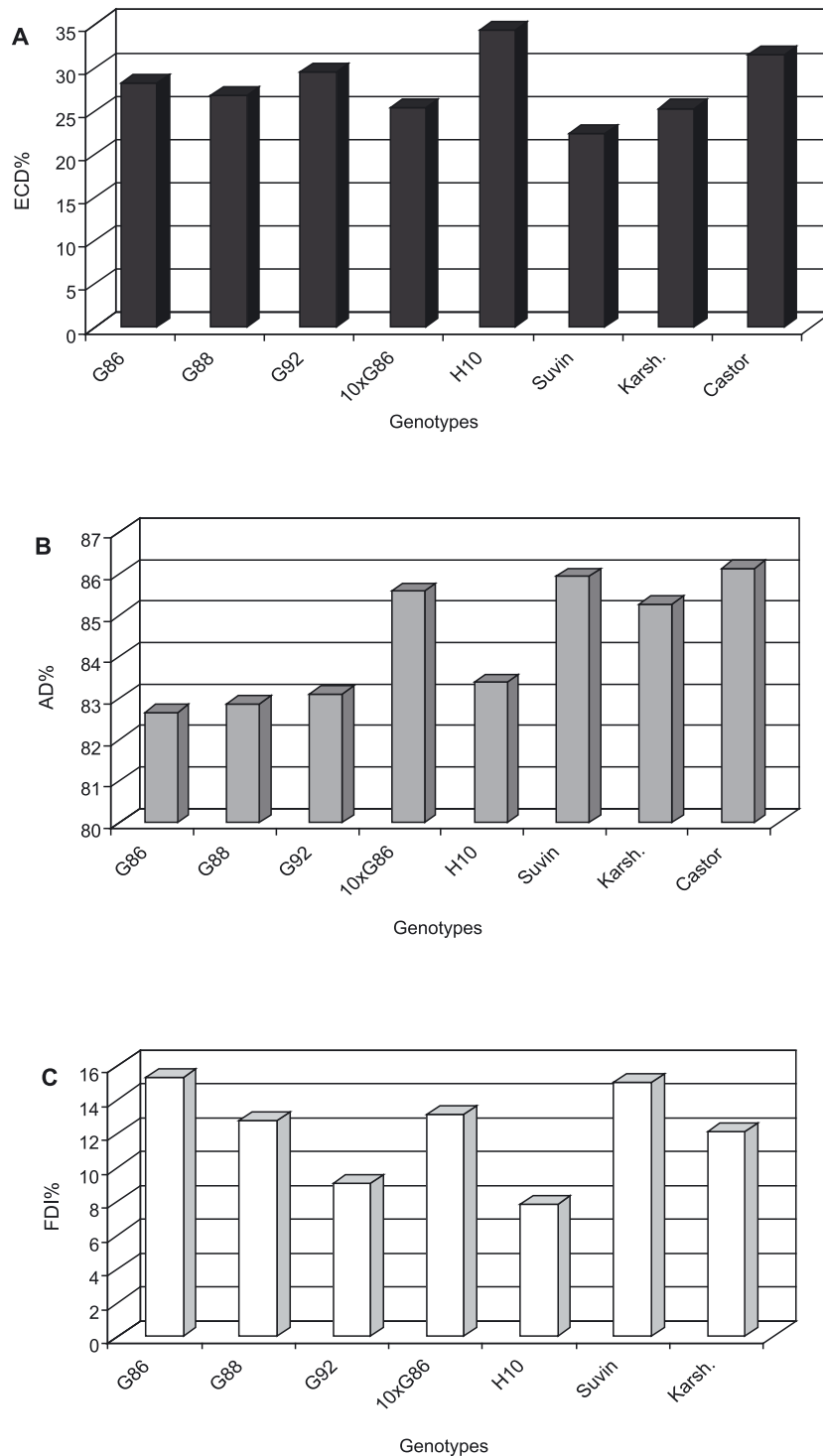

Fig. 2. Different feeding performance parameters: ECD – efficency of conversion of digested food, AD – approximate digestibility and FDI – feeding deterrence index of *Spodoptera littoralis* larvae through seven days of feeding on different cotton genotypes

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Table 4. Development indices of *Spodoptera littoralis* larvae fed on tested cotton genotypes

Genotypes	Average larval duration [days]	Pupal formation [%]	Weight of pupae [mg]	Average of pupal duration [days]
G86	11.76±0.28 b	86.00±1.41 b	0.2557±0.02 a	9.10±0.18 cd
10xG86	11.53±0.14 b	90.00±2.10 ab	0.2720±0.01 a	10.00±0.16 ab
H10	10.70±0.18 c	80.00±1.41 c	0.2810±0.02 a	10.20±0.11 a
G88	11.35±0.16 bc	90.00±2.19 ab	0.2464±0.01 a	9.14±0.19 cd
G92	11.04±0.14 bc	94.00±1.42 a	0.2799±0.02 a	9.80±0.20 abc
Suvin	12.56±0.17 a	94.00±1.90 a	0.2577±0.24 a	9.27±0.23 cd
Karsh.	11.30±0.20 bc	86.00±1.43 b	0.2695±0.02 a	9.47±0.19 bcd
Castor bean leaves	9.44±0.15 d	96.00±0.89 a	0.3027±0.01 a	9.00±0.14 d
LSD$_{0.05}$	0.521	4.733	0.0466	0.511
p	0.0000	0.0000	0.3299 ns	0.0000

Data expressed as mean ±SE, mean under each variety having different letters in the same column denote a significant different (p ≤ 0.05), ns – not significant

other genotypes (15.30 and 15.03%, respectively). Whereas, G92 and H10 recorded the lowest percentages (9.04 and 7.83%, respectively) (Fig. 2C).

Development indices

Spodoptera littoralis L4 larvae were fed on Suvin cotton cultivars, as well as castor leaves, to record the time taken for larval and pupal duration (days), pupation percentages, and weight of pupae (g).

Among the tested cotton genotypes, the maximum significant time taken for the larval period was recorded in Suvin (12.56±0.17 days) and the minimum significant value of 10.70±0.18 days in H10. The maximum significant time taken for pupal duration was registered for H10 (10.20±0.11 days) and the minimum for G86 (9.10±0.18 days), p < 0.0001. However, in all the tested cotton genotypes, the larvae showed more significant time for both the larval and pupal duration, when compared to control (9.44±0.15 and 9.00±0.14 days, respectively) (Table 4).

The percent of pupation was significantly reduced in larvae which fed on all the tested cultivars when compared to larvae which fed on castor leaves (96.00±0.89%). The maximum pupal formation was recorded as 94.00±1.42 and 94.00±1.90% for both G92 and Suvin, respectively. While the least pupal formation was 80.00±1.41% for H10 (Table 4). The average weight of the resulting pupae (gm) from different cotton varieties were lighter than that ob-

tained from castor leaves (0.3027±0.01 g) without any significant difference, p > 0.05 (Table 4). The average weight ranged between 0.2464±0.01 g for G88 to 0.2810±0.02 g for H10.

The growth indices of *S. littoralis* larvae on different cotton cultivars are shown in table 5. The results showed that the highest and lowest values of the larval growth index were on G92 (8.514) and G86 (7.312), respectively. Castor leaves gave 10.169. The standardised growth index of *S. littoralis* showed differences among the tested cotton varieties, being the highest on H10 (0.026) and the lowest on Suvin (0.020). The control recorded 0.032 (Table 5). In addition, the results indicated that the tested cotton varieties used as larval food had a remarkable effect on the fitness index of *S. littoralis*, which was highest for G92 (1.26) and lowest for G86 (1.05), compared to castor bean which gave 1.53 (Table 5).

Biochemical responses

The results of total soluble protein, total lipids, and both amylase and trehalase enzymes in L4 larvae after seven days of feeding on seven cotton varieties, and the control's seven days of feeding on castor bean leaves are depicted in figure 3.

The mean total soluble protein in all the tested varieties showed a significant decrease. The control recorded 1342.09±25.14 mg · ml⁻¹. The highest and lowest decreases

Table 5. Growth index, standardized growth index and fitness index of *Spodoptera littoralis* on tested cotton genotypes

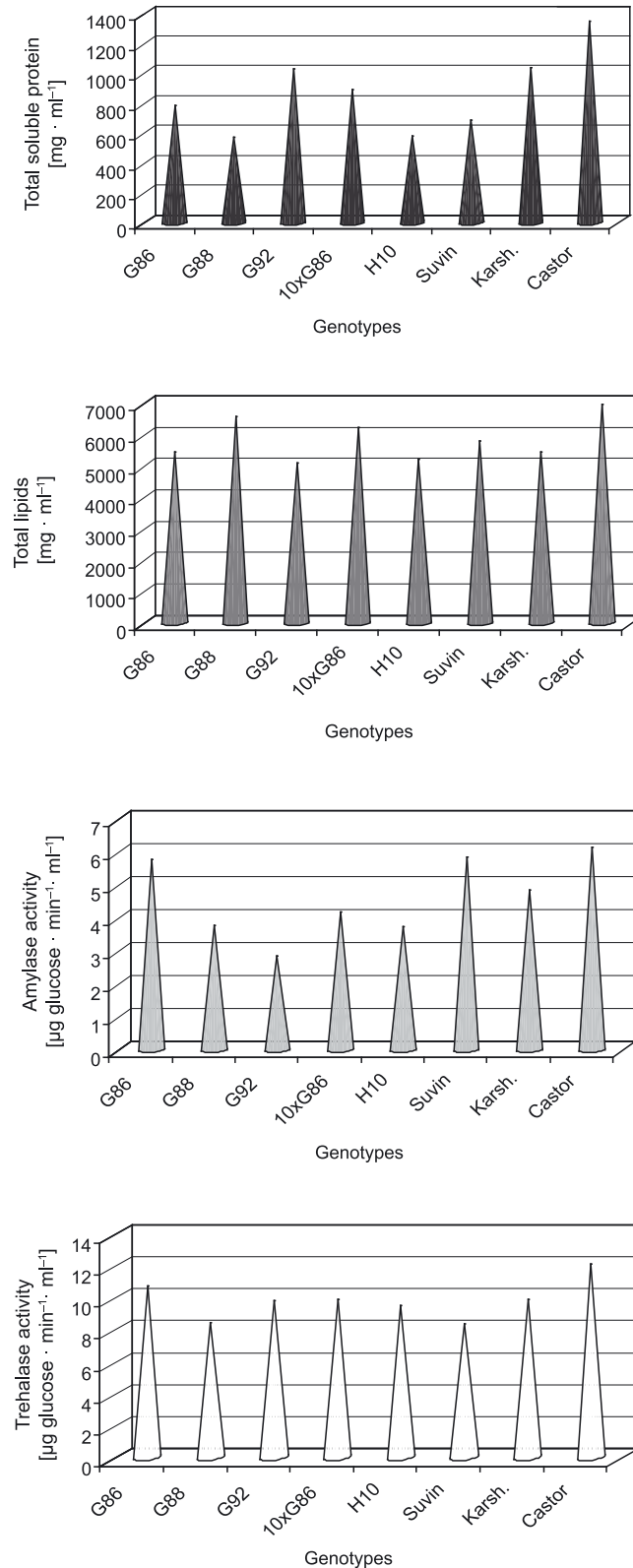| Genotypes	Larval growth index	Standardized growth index	Fitness index
G86	7.31	0.021	1.05
10xG86	7.80	0.023	1.13
H10	7.47	0.026	1.07
G88	7.92	0.021	1.08
G92	8.51	0.025	1.26
Suvin	7.48	0.020	1.10
Karsh.	7.61	0.023	1.14
Castor bean leaves	10.16	0.032	1.53

Fig. 3. Changes in the levels of measured biochemical parameters in the homogenated 4th larval instar of *Spodoptera littoralis* after seven days of feeding on different cotton genotypes

were recorded as 567.669±20.42 and 1033.937±46.80 mg · ml⁻¹ for G88 and Karsh., respectively (Fig. 3A).

Similarly, the mean total lipids were significantly reduced as affected by all the tested varieties compared to the control. The control showed 6978.877±243.00 mg · ml⁻¹.

The mean total lipids decreased between a minimum value of 6555.556±262.37 mg · ml⁻¹ for G88 to a maximum value of 5111.11±236.98 mg · ml⁻¹ for G92, p < 0.001 (Fig. 3B).

The same reduction trend was obtained in both amylase and trehalase enzymes, where amylase activity of

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S. littoralis larvae recorded the highest significant reduction in larvae fed on G92 (2.833±0.19 µg glucose · min^{-1} · · ml^{-1}) and the lowest reduction in larvae fed on Suvin (5.811±0.18 µg glucose glucose · min^{-1} · ml^{-1}), compared to the control: 6.114±0.27, p < 0.001 (Fig. 3C).

As for trehalase activity, the highest significant reduction caused 8.374±0.45 µg glucose · min^{-1} · ml^{-1} ml for Suvin and the lowest significant reduction of 10.737±0.95 µg glucose · min^{-1} · ml^{-1} ml for G86, compared to control: 12.138±0.84, p < 0.001 (Fig 3D).

Discussion

It is known that there are a great number of food sources which *S. littoralis* larvae can choose from to aid their development. The development of the *S. littoralis* larvae is largely influenced by the quality of the food. In the current study, we showed that the various cotton varieties had a remarkable effect not only on the feeding performance but also on some biological and biochemical aspects of *S. littoralis.*

Generally, the consumption percentages of *S. littoralis* larvae varied among different cotton genotypes. This percentage variation may be due to the quality and quantity of food. Any substance which reduced food consumption by the larvae can be considered as antifeedants or feeding deterrent (Isman 2002). Such antifeedants offer the first line of crop protection against chewing insects. Both ECI and ECD are usually used as indicators for food quality. The index ECI, is a measure of an insect's ability to incorporate the ingested food into biomass (Nathan *et al.* 2005). Suvin exhibited the lowest ECI and ECD values of *S. littoralis* larvae compared to other varieties and to the control; resulting from the low efficiency in converting ingested food into growth. The decrease in ECI is associated with energy-consuming physiological activities, the molting process, and the approach of development (Carne 1966). Also, Luthy and Wolfersbrenger (2000) reported that the decrease in ECD (24.9 and 15.9%) on two different varieties of cotton by *S. frugiperda* (J.E. Smith) could be due to allocation of energy for regeneration of the damaged midgut epithelium.

A lower GR was obtained from *S. littoralis* larvae which fed on all the tested cotton varieties, compared to the control, with special reference to Suvin and G86 varieties that showed a low quality of food – probably acting as an inhibitor. Dahlman (1977) suggested that reducing the conversion efficiency of ingested and assimilated food might give a growth rate depression.

The lower GR could be due to irreparable damage to the midgut lumen cellular surfaces (Jansen and Groot 2004). Lower GR, ECI, and ECD probably lead to a delay in larval development, and percent of pupation, and probably lead to the formation of smaller pupae. Abdel-Rahman and Al-Mozini (2007) found severely reduce GR, CR, and ECI in *S. littoralis* larvae treated with three plant extracts. These authors reported that reduction of digestion resulted from covalent bands with food proteins or digestive enzymes. In general, all the tested cotton genotypes caused a slight decrease in AD, especially G86 and G88. These low AD values in the larvae indicated that

food was not being retained for long in the larva intestine and that could be the cause of the disruption in the metabolism rate.

The feeding deterrence index in G86 and Suvin reached 15.305 and 15.032%, respectively, and gave the lowest value 7.832% in H10. The higher FDI in *S. littoralis* larvae was perhaps due to a rapid deterrence evoked by chemical sensila on the mouth parts of larvae or retracted pulses coming from the stomodael nervous system after ingestion (Sadek 2003), moreover, because of the presence of a number of chemical compounds like flavonoids, terpenes, tannins, and sterols (Salama and Sharaby 1988) or toxic effects after ingestion. These antifeedant varieties can be described as allomone substances which inhibit feeding and do not kill the larvae directly, but rather limit its developmental and growth parameters. Therefore, the reason for the prolongation of the larval and pupal duration as well as the reduction in both pupal formation and weight in all seven cotton genotypes (except the control) may be the result of the reduction in ECI, ECD, AD, GR, and FDI obtained in this study. Santos and Boiça (2001) reported that cotton genotypes affect *Alabama argillacea* (Hübner) pupal weight and development. In field conditions, deterrence retards the growth stage of the insect, and prolonging the insect's search for food increases the probability of insect mortality (Khosravi *et al.* 2010). The same author added that lower ECD, ECI, GR probably lead to a delay in larval growth, and lead to the formation of smaller pupa which have a direct relation to fecundity and longevity of adults which results in susceptibility to diseases and natural enemies.

Growth and fitness of herbivorous insects are directly related to food quality, quantity and secondary metabolites. In many defoliator larvae, measurements of pupal weight and adult size are strongly correlated with potential fitness. Higher concentrations of toxic secondary metabolites and lower food quality will decrease growth and fitness of lepidoptera insects (Aryal *et al.* 2007).

In our study, G86 recorded the lowest values of larval growth index and fitness while Suvin recorded a lower standardised growth index than other cotton varieties and the control. Nasirian *et al.* (2014) reported that the highest larval growth index observed in *Plodia interpunctella* (Hübner) (Lepidoptera) correlated with a higher larval survival rate and shorter larval period when feeding on different wheat cultivars. While the highest fitness index in these larvae can probably be attributed with a higher percentages of pupation or higher pupal weight. Therefore, we suggest that the two cotton varieties G86 and Suvin are unsuitable as diets for the feeding and development of *S. littoralis* larvae.

To gain more practical information in this study, the activity of amylase and trehalase enzymes, total soluble protein, and total lipids in *S. littoralis* larvae that feed on different cotton genotypes were also evaluated. In the present investigation, both total soluble protein and total lipids were decreased in larvae which fed on different varieties than what the control fed on. It is logical, that the larvae degrades proteins to resultant amino acids to let them into the TCA cycle as a keto acid to compensate for the lower energy caused by stress (Nath *et al.* 1997). As for

lipids, these are an important source of energy reserved in fat bodies. The lipid amounts can vary depending on the growth stage and feeding conditions (Chapman 1998). The lipid reserves during the feeding period increase but are reduced in the non-feeding stage. In this investigation lipid reduction is variety-dependent.

The lower amylase activity (digestive enzyme) of *S. littoralis* larvae on different cotton varieties may be attributed to the lower starch contents of cotton, when compared with the control. Some possible reasons for such variation may be because of the physiological differences of cotton varieties on *S. littoralis* larvae. Trehalose might be an interesting target in the development of new techniques controlling insects (Silva *et al*. 2004). In many organisms, changes in trehalase activity are closely linked to an alteration in the physiological conditions or development, indicating that certain enzymes play an important role in such biological functions as homeostasis and developmental events (Temesvari and Cotter 1997).

Reduced activities of amylase and trehalase enzymes, and the level of both total soluble protein and total lipids could be related to a strong deterrence effect in *S. littoralis* fed on different cotton varieties. The same trend was obtained by Etebari and Matindoost (2004) who showed that starvation might reduce biochemical components in haemolymph.

In conclusion, the two cotton varieties; Giza86 and Suvin, showed a pronounced effect on *S. littoralis* larvae and caused a reduction in the growth rate, a lower food efficiency, and reduced key metabolic components. Such findings may help in the control strategies of *S. littoralis*.

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