EFFECT OF STARVATION ON CERTAIN BIOLOGICAL AND BIOCHEMICAL ASPECTS OF *SESAMIA CRETICA* LED. LARVAE THROUGH MASS REARING

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Abstract

Laboratory rearing of *Sesamai cretica* Led. larvae on an artificial diet is very important for pest control programs. However, in some conditions such as starvation might affect some biological aspects. So in this study, the authors found that starvation of larvae for 60 hours and 72 hours led to decrease larval duration to 26.7 and 28.6, days respectively in comparison to 30.03 days for control. While, adult longevity increased. Also, pupal malformation had been clearly increased when larvae were starved for 48 hours. Pupal duration was prolonged in case of 60 hours starvation (13.7 days) comparing with 10 days for 0 hours starvation. Pupal weight was decreased when larvae were starved for 60 hours (0.1411 and 0.1238 gm for male and female, respectively). Sex ratio was determined through out different starvation periods. On the other hand, starvation of *S. cretica* larvae caused significantly reduction in total soluble proteins, carbohydrates, lipids and carbohydrates hydrolyzing enzymes (invertase and amylase). Also, the results showed that invertase activity was higher than amylase activity in normal *S. cretica* larvae.

INTRODUCTION

Sesamia cretica Led. is a serious pest on sugar-cane and maize crops, causing great reduction in the final yield, in Egypt. So, there is a great need for further studies on this pest in order to use obtained result in integrated pest management program. Hence the laboratory rearing of *S. cretica* Led. on an artificial diet seems to be necessary, sometimes the culture may be exposed to some sudden unsuitable conditions such as starvation which might have unfavorable effect on some biological aspects and the contents of protein, glycogen, lipids and some carbohydrates hydrolyzing enzymes.

The present work is designed to clarify the impact of starvation conditions in different periods. In this respect, El-Sherif, 1994 and Khadr *et al.*, 1995 reported that larval and pupal duration of *Agrotis ipsilon* increased. While, pupal weight decreased with different starvation periods.

MATERIALS AND METHODS

Larvae of *S. cretica* were collected from the farm of Faculty of Agriculture, Benha University at Moshtohor and reared on diet prepared as El-Mitwally *et al.*, (1997), for some generations. The culture was maintained in Plant Protection Research Institute. Dokki. One hundred of 4th instar *S. cretica* larvae were divided into five groups, 20 larvae each, and were starved for 0, 24, 48, 60 & 72 hours. At the end of starvation for all the five groups, 5 larvae were taken from each treatment, kept at - 20°C , in order to determine the protein and lipids contents and some enzymes, the remaining of larvae were allowed to feed on the artificial diet until pupation. All techniques were carried out under laboratory conditions, 29 ± 2°C and 60 ± 5% R.H.

Preparation of samples for biochemical studies:

The starved larvae were homogenized in distilled water using a Teflon homogeizer-(MECHIANIKA PRECYZYJNA Warszawa type MPN-309-Poland)- surrounded with a jacket of ice for 3 minutes. Homogenates were cenrtrifuged at 6000 r.p.m for 10 minutes at 5°C –(by BECKMAN GS-6R Centrifuge)-, and the supernatants were directly used for determining the total soluble (proteins, carbohydrates and lipids) and enzymes assessment.

Enzymes assessments:

Carbohydrates hydrolyzing enzymes (invertase and amylase) were determined using the method of Ishaaya and Swiriski (1976) using sucrose and starch as substrates.

Determination of main metabolites:

Total soluble protein were determined according to the method described by Bradford 1976). Total soluble carbohydrates were determined according to Singh and Sinha (1977). While, total soluble lipids were determined according to Knight *et al.* (1972).

Statistical analysis:

The significance of the main effects was determined by analyzing of variance (ANOVA). The significance of various starvation periods was evaluated by Duncan's multiple range tests ($P< 0.05$). All analysis was preceded using a software package "Costat", a product of cohort software Ine. Berkley, California (Duncan, 1955).

Larval (calculated from the beginning of the 4th instar 11 days old), pupal adult duration and malformation besides, weight of pupae, sex ratio and longevity of adult were recorded. All statistically analysis using F test and L.S.D. values were done.

RESULTS AND DISCUSSION

1- Biological studies:

Data presented in Table (1) clearly showed that larval duration and adult longevity of *S. cretica* 4th instar larvae which were starved for 60 hours, had a significant difference than those of control which were not starved (0 hours). Larval duration was 26.7 days, adult longevity was 9.1 days for male and 12.4 days for female, while they were 30.03, 8.4 and 10.5 days respectively in control.

Pupal weight of male increased significantly (0.2013 gm) after starvation for 24 hours. Nerveless pupal weight of female had increased only after starvation for 48 hours (0.1762 gm.) as shown in table (1). However, pupal weight of female decreased after other starvation periods comparing with control.

Also, the results obtained in the same table clearly show that the starvation for 60 hours led to highest increase in the pupal duration being 13.7 days. At the same time pupal malformations were increased by 0.5, 2.5, 0.75 and 2%, after starvation for 24, 48, 60 and 72 hours, respectively.

The sex ratios for aduls resulting from larvae which were starved for 60, 48 and 72, days were 2 : 5, 3 : 6 and 3 : 5 for male : female, respectively, (Table 1). From these results, starvation of *S. cretica* larvae for 60 and 72 hours might be unfavorable for mass rearing.

These results are in agreement with those of Khadr *et al.* (1995), in which they found, that larval duration of *Agrotis ipsilon* was increased with starvation while mean weight of pupae had a remarkable decrease. Also, El-Mitwally *et al.* (1996) found similar results in which, they revealed that *S. littoralis* larvae which were starved for 3 or 4 days, had lower duration and weights in comparison with non-starved larvae.

2-Biochemical studies:

2-1- Carbohydrates hydrolyzing enzymes:

Table (2) and Fig. (1) show the changes in carbohydrates hydrolyzing enzymes (invertase and amylase), of *Sesamia cretica* larvae tested after different starvation periods. The data expressed as percentages of increase or decrease in the enzymes activity relative to control. The data obtained show that, generally the invertase activity in *Sesamia cretica* is higher than the amylase activity, control insects showed higher invertase and amylase activities than the starved insects all over the experiment periods, the invertase and amylase activities were gradually decreased at 24, 48 and 60 hrs starvation periods. After 60 hours starvation period, insects showed lowest invertase and amylase activities (58.24% and 46.55% relative to control)

respectively. After 72 hrs. starvation period, invertase and amylase activities seemed to be slightly recovered to 83.51% and 74.89%, respectively.

The previous data revealed that, carbohydrates hydrolyzing enzymes (invertase and amylase) are sensitive to the starvation processes, that they have received a great deal of attention in concern with digestion and utilization of carbohydrates in insects, Meisner *et al.*, (1978) stated that, they are two important digestive enzymes. Nakonieczny *et al.*, (2006) studied *Parnassius apollo* (Lepidoptera, Papilionidae) in numerous localities all over Europe and reported that α-Amylase plays the main role in utilization of carbohydrates, α-Amylolytic and other glycolytic activities indicate that larvae utilize starch and other carbohydrate compounds as energy sources. They also reported that, the digestive processes in the insect midgut should reflect adaptation to a specific food source, this is in agreement statement with Takeda *et al.*, (2006) who figured that, the midgut of the cockroach exhibits maximal a-amylase activity 3 hrs. after intake of starch, but not of non-nutrients.

2-2-The main metabolites:

Data in Table (3) and illustrated in Fig.(2) show the changes in total soluble (proteins carbohydrates and lipids) of *Sesamia cretica* adults after different starvation periods compared with control insects. The data expressed as percentages of increase or decrease in the contents relative to the control. The data obtained show that, in general, total soluble proteins content in larvae recorded the highest value of the soluble contents followed by the total soluble carbohydrates and then the total soluble lipids. The results obtained show gradual significant decrease in the total soluble proteins during the tested starvation periods started after 48- hrs. (the total soluble proteins was 77.61% relative to control). Continuous reduction was noticed, it reached the lowest level after 72- hrs. Regarding to the results of total soluble carbohydrates, the significant reduction in the content observed after 48- hrs. of starvation recorded 86.29% (relative to control) and continued significantly tell reached 49.27% (relative to control) after 72- hrs of starvation. The slight reduction in the total soluble lipids obtained significantly just after 60- and 72- hrs. of starvation they recorded (81.57% and 81.17%, respectively) relative to control.

The previous data revealed that, the content of soluble proteins had the highest pronounced reduction in content as a reflection of the starvation periods that insects of *Sesamia cretica* were suffered, it is obviously known that, proteins constitute the most complex compounds and at the same time the most characteristic properties of living matter. They are present in all viable cells and essential to the process of cell division. They act also as enzymes and hormones controlling many chemical reactions in the metabolism of cells. Agosin (1978) reported that, one of the characteristics of

animals is motility, which is associated with the presence of contractile proteins in insects, as well as other differentiated organisms. These contractile proteins are present in a single tissue muscle. Structural proteins and enzymes of the cuticle participate in the tanning process known as sclerotization.

The decrease in total soluble carbohydrate content of starved *Sesamia cretica* could be attributed to convert it into glucose for supporting all life processes, this is in agreement with Nakonieczny *et al.*, (2006), they reported that, the activity of carbohydrates hydrolyzing enzymes and other glycolytic activities indicate that larvae utilize starch and other carbohydrate compounds as energy sources. Carbohydrates are of vital importance since they can be utilized by the insect body for production of energy or conversion to lipids or proteins.

Table 1. Certain biological aspects of 4th *S. cretica* larvae after exposure to different starvation periods

	Mean of								
Starvation periods (hours)	Larval duration (days)	Pupal duration (days)	Pupal weight (gm)		pupal malformation %	Adult longevity (days)		Sex ratio	
			Male ♂	Female ♀		Male ♂	Female ♀	♂	♀
0	30.03	10	0.1608	0.035	0	8.4	10.5	3	: 4
24	30.8	9.8	0.2013	0.0342	0.5	9.9	10.3	3	: 4
48	29.7	6.8	1441	0.0445	2.5	10.1	9.5	3	: 6
60	26.7	13.7	0.1544	0.0317	0.75	9.1	12.4	2	: 5
72	28.6	7.7	0.1411	0.0293	2	11.3	11.8	3	: 5
F value =	5.66	4.5	10.48	7.14	14.35	12.68	5.19	-	-
L.S.D. =	3.68	5.34	0.031	0.031	1.19	1.3	1.06	-	-

Table 2. Changes of invertase and amylase activities in *Sesamia cretica* (Led.) larvae after different starvation periods.

	*Enzyme Activity (Mean ± SE)			
Starvation period/hr.	Invertase		Amylase	
	Activity	**%	Activity	**%
Control	440.54 ± 19.91 a	100.00	69.03 ± 2.47 a	100.00
24	378.55 ± 17.51 b	85.93	60.74 ± 1.84 b	87.99
48	317.06 ± 11.69 c	71.97	51.70 ± 1.81 c	74.90
60	256.55 ± 14.32 d	58.24	32.14 ± 1.84 d	46.55
72	367.90 ± 11.45 b	83.51	51.70 ± 1.70 c	74.89
Mean of activity	352.12		53.06	

* Enzyme Activity = µg glucose/min./g body weight

**%= percentage relative to control

-Values followed by the same letter are not significantly different (P<0.05; Duncan's multiple range test).

-L.S.D. of invertase activity = 46.16 and of Amylase activity = 6.15

Table 3. Changes of total soluble proteins, carbohydrates and lipids in *Sesamia cretica* (Led.) larvae after different starvation periods.

*Total soluble content (Mean ± SE)						
Starvation period/hr.	proteins		carbohydrates		lipids	
	Content	**%	content	**%	content	**%
Control	11.29 ± 0.17 a	100.00	4.61 ± 0.12 a	100.00	0.55 ± 0.02 b	100.00
24	10.56 ± 0.34 a	93.49	4.27 ± 0.15 ab	92.51	0.67 ± 0.02 a	121.60
48	8.76 ± 0.28 b	77.61	3.98 ± 0.12 b	86.29	0.49 ± 0.01 c	90.26
60	7.41 ± 0.27 c	65.66	2.87 ± 0.07 c	62.14	0.45 ± 0.01 d	81.57
72	4.59 ± 0.18 d	40.68	2.27 ± 0.09 d	49.27	0.44 ± 0.01 d	81.17
Mean of content	8.52		3.60		0.52	

* Total soluble content = mg content/g body weight
**%= percentage relative to control
-Values followed by the same letter are not significantly different (P<0.05; Duncan's multiple range test).
-L.S.D. of Total soluble protein content = 0.81
Total soluble carbohydrates content = 0.36
Total soluble lipids= 0.05

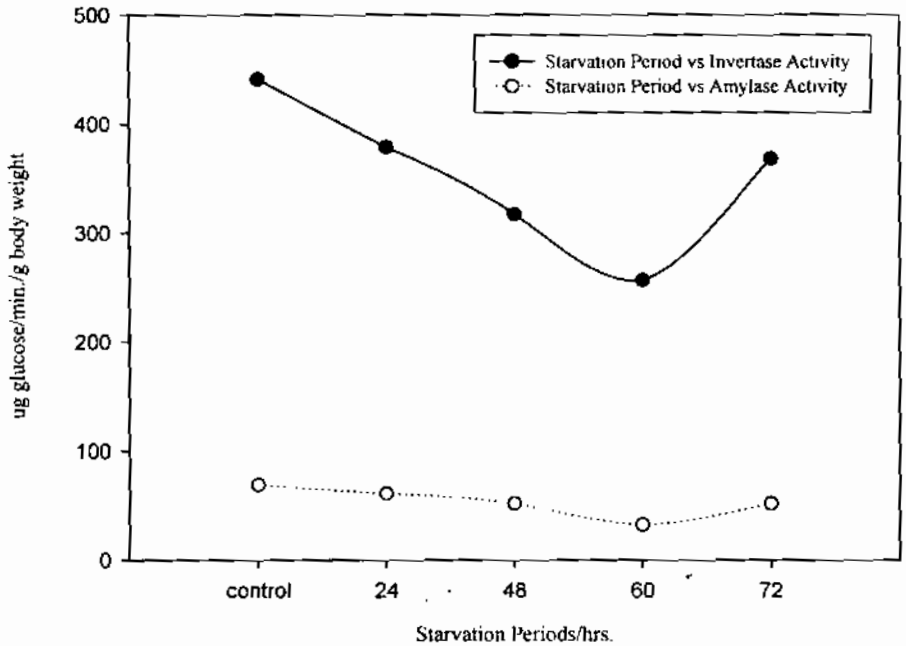

Fig. 1. Changes of invertase and amylase activities in *Sesamia cretica* (Led.) larvae after different starvation periods

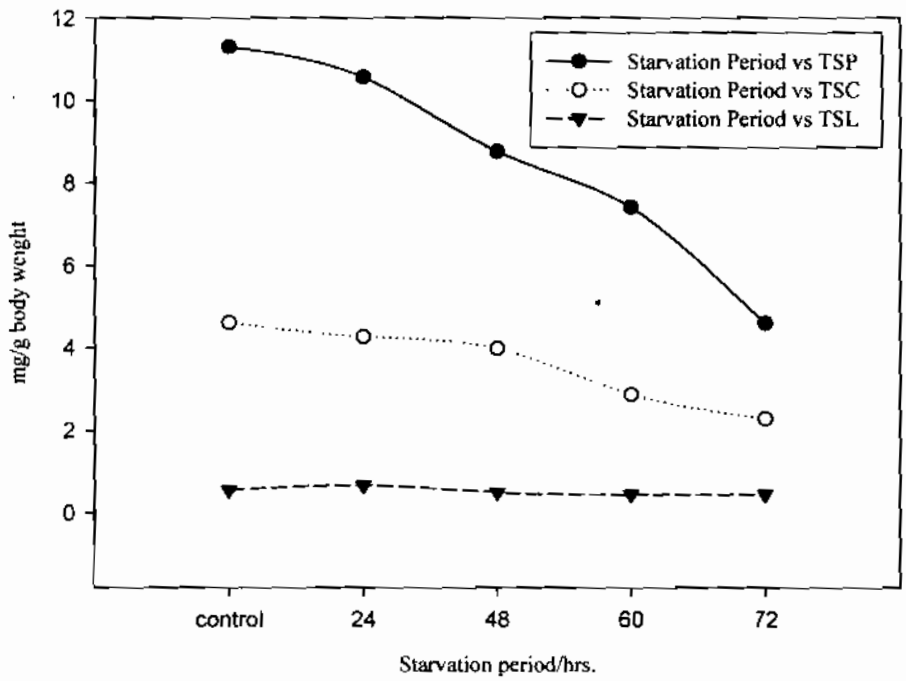

Fig. 2. Changes of total soluble proteins, carbohydrates and lipids in *Sesamia cretica* (Led.) larvae after different starvation periods.

TSP: Total Soluble Proteins, TSC: Total Soluble Carbohydrates and TSL: Total Soluble Lipids

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فعل التجويع على الخصائص البيولوجية والبيوكيميائية ليرقات دودة القصب الكبيرة خلال التربية الكمية

شنـــوده سيـــد يعقـوب ، أمـــاني ســـامي الحفنى ، احمد محمد احمد فرج

معهد بحوث وقـــاية النباتات – مركز البحـــوث الزراعيـــة – جيزة

تعد التربية المعملية لحشرة دودة القصب الكبيرة على البيئـــات الصـــناعية مـــن الامـــور الضرورية اللازمة لاجراء البحوث العملية فى مجال مكافحة الافات، ولكن هناك بعض الظروف التى قد تؤثر عليها ومنها التجويع الذى قد يؤثر على بعـــض الخصـــائص البيولوجيـــة والبيوكيميائيـــة للحشرة. وقد وجد الباحثون ان التجويع لفترة ٦٠ و ٧٢ ساعة يؤدى الى نقص فترة طـــول العمـــر اليرقى (٢٦,٧ و ٢٨,٦ يوم على الترتيب) مقابل ٣٠,٣ يوم لليرقات غير المجوعة. بينما زاد طـــول عمر الفراشات، وكان التشوه العذرى واضحا فى حالة التجويع لمدة ٤٨ ساعة. وقـــد طـــال العمـــر العذرى فى حالة ٦٠ ساعة (١٣,٧ يوم) مقابل ١٠ يوم فى حالة عدم التجويع. نقص وزن العـــذارى عند تجويع اليرقات لمدة ٦٠ ساعة (٠,١٢٣٨ جم) للاناث وفى حالـــة التجويـــع لمـــدة ٧٢ ســـاعة (٠,١٤١١ جم) للذكور، ودرست كذلك النسبة الجنسية فى فترات التجويع المختلفة. من ناحية اخرى أثبت الدراسات البيوكيميائية ان تجويع يرقات دودة القصب الكبيرة سبب انخفاضا معنويا فى المحتوى الكلى للبروتين والكربوهيدرات والدهون الذائبة وانزيمات تحليل الكربوهيدرات (انفرتيز واميليـــز). وأوضحت النتائج ان النشاط الانزيمى للانفرتيز كان اعلى عما هو فى الاميليز فى اليرقـــات غيـــر المجوعة.