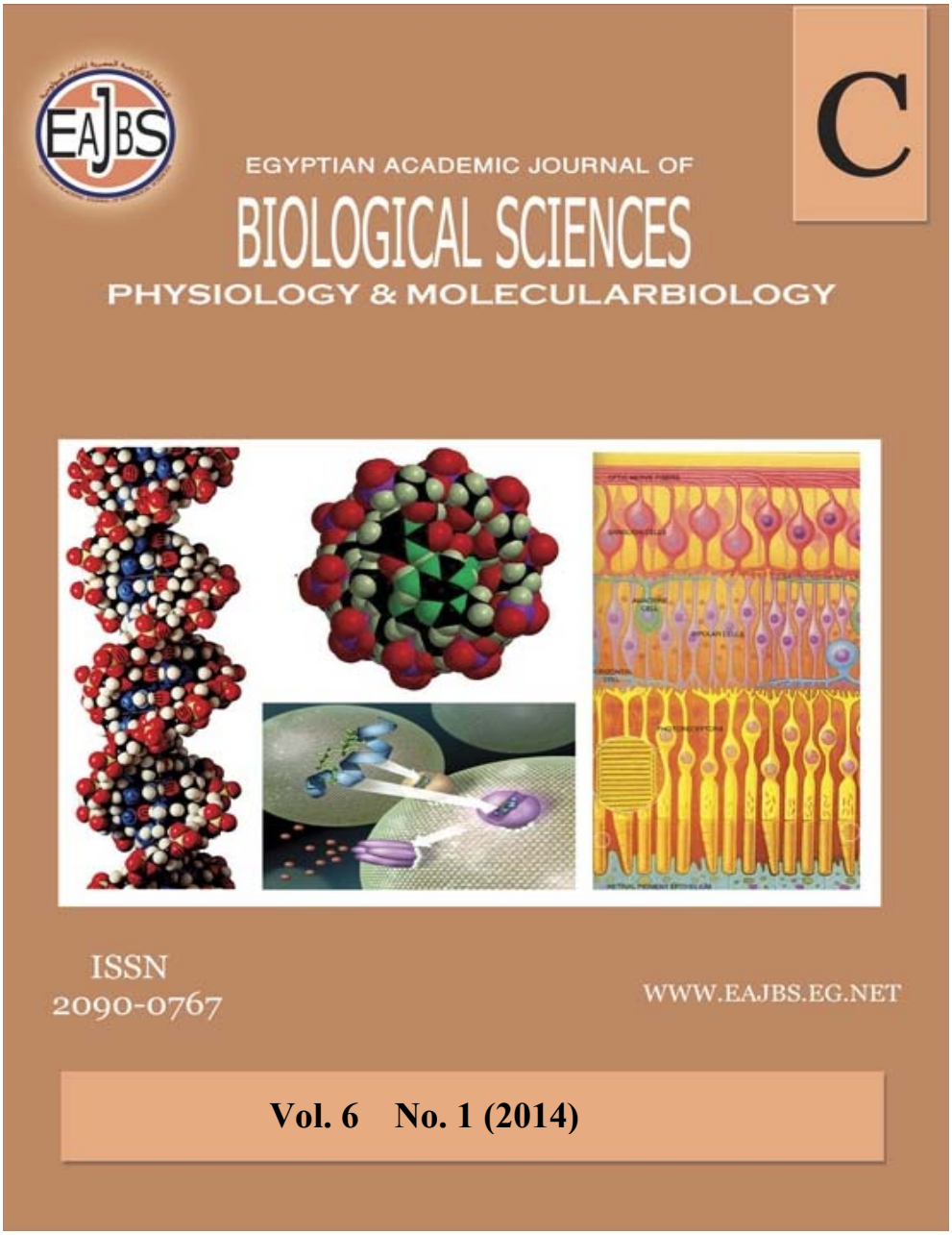

Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences ,Department of Entomology ,Faculty of Sciences Ain Shams University .

Physiology & molecular biology journal is one of the series issued twice by the Egyptian Academic Journal of Biological Sciences, and is devoted to publication of original papers that elucidate important biological, chemical, or physical mechanisms of broad physiological significance.

www.eajbs.eg.net

Egypt. Acad. J. Biolog. Sci., 6(1): 71 – 81 (2014)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology

ISSN 2090-0767
www.eajbs.eg.net

Influence of Sudden Thermal Stresses on Growth Rate and Enzymatic Identification of *Bombyx mori* L. Larvae

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ARTICLE INFO

Article History
Received: 15/2/2014
Accepted: 1/4/2014

Keywords:
Silkworm
Thermal stress
Egg
Larvae
Growth rate
Effective rate of rearing
Enzymes

ABSTRACT

The purpose of this study is to determine the impact of different thermal stresses for different periods on univoltine *Bombyx mori* embryos and grown larvae in terms of larval growth rates, effective rate of rearing and the enzymatic proliferation of larval haemolymph. Egg groups was exposed to different temperatures; 0°C for one hour, 0°C for two hours, 40°C for one hour and 40°C for two hours, followed by 2 hours recovery at room temperature in addition to a control group, the same was applied to the grown larvae. Larval haemolymph of tested groups were subjected to enzyme electrophoretic analysis by native polyacrylamide gel electrophoresis (PAGE) for phosphatases and esterases identification. Exposure to sudden thermal stress of 0°C for one hour at larval stage recorded the highest growth rate. The sudden thermal stresses did not significantly affect the effective rate of rearing. However, thermal stresses increased ACPase and ALKPase activities and decreased α and β esterases activities comparing to control. These results suggest applying sudden heat thermal proteins in breeding strategy for inducing robustness in productive breeds.

INTRODUCTION

Silkworm is one of the most important animals which produce silk thread in the form of cocoon by consuming mulberry leaves during larval period. Growth and development of silkworm is greatly influenced by environmental factors such as temperature and relative humidity (Hussain *et al*., 2011). The development of silkworms also depends on its metabolic modulations and physiological adaptability, besides its genetic constitution (Chatterjee *et al*.,1993) and (Thiagarajan *et al*., 1993). The seasonal differences in the environmental components considerably affect the genotypic expression in the form of phenotypic output such as cocoon weight, shell weight and cocoon shell ratio (Nacheva and Junka, 1989). High temperature affects nearly all biological processes including the rates of biochemical and physiological reactions and ultimately affecting the quality and quantity of cocoon crops (Hazel,1995 and Willmer and Johnston, 2004).

The silkworms likely adapt to low temperature by different mechanisms involving synthesis of low molecular weight proteins, heat shock proteins and regulation of activities of certain key enzymes which plays crucial roles in insect metabolism (Singh *et al*., 2013).

The wide occurrence of phosphatases in insect tissues is thought to be associated with the (a) transport of metabolites, (b) metabolism of phospholipids, phosphoproteins, nucleotides and carbohydrates and (c) synthesis of proteins (Ray *et al.*, 1984 and Chaubey *et al*., 2010).

Among the various isozymes, esterases have been studied extensively since they are the group of enzymes involved in metabolic and defense functions and are found in both soluble and membrane bound forms. Their involvement in resistance/stress to various kinds of insecticides and thermal stresses (Eguchi and Yoshitake,1967) and also studied for their function in the digestion, nutrition and detoxification in insects (Kasim and Ahmed,1980), reproduction (Richmond *et al*., 1980) and as a tool for genetic relationships analysis among *B. mori* races and hybrids (Mahmoud *et al*., 2011).

Although temperature and humidity have been known to play important role in silk production, no much information are available on the effect of temperature stresses on silkworm physiology and commercial traits. The present study was undertaken to study the effect of different thermal stresses for different periods at the egg stage (late embryo stage or blue stage) and at the beginning of fourth larval stage (grown larval stage) of *B. mori* in relation to its enzymatic activity and their subsequent impact on larval growth and effective rate of rearing.

MATERIAL AND METHODS

Silkworm eggs were obtained from the Sericulture Research Department (SRD) of Plant Protection Research Institute (PPRI), Agricultural Research Center (ARC), Giza, Egypt. Rearing technique was done in the lab. under the hygro-thermic conditions 28 ± 1 °C and 75 ± 5% RH, according to Krishnaswamy (1978). Larvae were fed on leaves of Kokuzo-27 mulberry variety.

Temperature stresses

Egg stage:

At the first day of raising temperature from cold storage at 5°C to 27°C, 100 eggs at late embryo stage (blue stage) within three replications were kept in egg hatching boxes under four temperature stress conditions (T2 to T5) along with control (T1).

Control- treatment 1 (T1) – maintain at 27°C till hatching.

Treatment -2 (T2) - 0 degree Celsius for one hour in an incubator.

Treatment -3 (T3) - 0 degree Celsius for two hours in an incubator.

Treatment -4 (T4) - 40 degree Celsius for one hour in an incubator.

Treatment -5 (T5) - 40 degree Celsius for two hours in an incubator.

After being exposed to thermal stress all treatments (T2~T5) were kept at 15 °C for two hours for recovery then placed at 27 °C till hatching.

Fourth larval stage (grown larval stage):

At the beginning of the fourth larval stage (grown larval stage), larvae were divided into 5 groups in three replicates, with100 larvae/ replicate and the same above temperature were imposed:

Control -Treatment -6 (T6) - room temperature.

Treatment -7 (T7) - 0 degree Celsius for one hour in an incubator.

Treatment -8 (T8) - 0 degree Celsius for two hours in an incubator.

Treatment -9 (T9) - 40 degree Celsius for one hour in an incubator.

Treatment -10 (T10) - 40 degree Celsius for two hours in an incubator.

(T7 ~T10) were maintained at 15 °C for two hours for recovery, then the rearing technique was continued normally at room temperature.

Insect performance was assessed based on larval growth (GR) rate and effective rate of rearing (ERR).

Growth was calculated was calculated according to (Thyagaraja *et al*., 1991):
GR = (body weight of freshly ecdysed larvae/ body weight of newly hatched larvae) / larval duration by days.

The effective rate of rearing (ERR %) was recorded according to Chanu and Ibotombi (2011).
ERR (%) = (no. of cocoon harvested / total no. of larvae reared) X 100

The data were recorded and subjected to analysis of variance according to Snedecor and Cochran (1981).

Enzymatic determination:

At the sixth day of the fifth larval instar, haemolymph samples were collected from each treatment and deepfreeze in eppendrof tubes until use for electrophoretic studies.

Phosphatases and Esterases electrophoresis:

Isozymes were separated in 10 % Native-polyacrylamide gel electrophoresis as described by Stegemann *et al*. (1985). Wendel and Weeden (1989) technique was used for phosphatases. Gel staining protocols of Scandalios (1964) were used for esterases.

RESULTS

Growth rate and effective rate of rearing:

Among the egg groups exposed to sudden thermal stresses, the highest performance was recorded for control and egg group exposed to 40°C for one hour as presented in Table (1).

Table 1: Larval growth rate (GR) & effective rate of rearing (ERR) for *B. mori* eggs exposed to sudden thermal stresses for different periods

Parameters	Control group	0 ºC for one hr.	0 ºC for two hrs.	40 ºC for one hr	40 ºC for two hrs	LSD
GR 1st instar	0.297 ab	0.267 b	0.327 ab	**0.386** **a**	0.267 b	0.103
GR2nd instar	**1.155** **a**	1.079 ab	1.060 ab	0.927 bc	0.832 c	0.211
GR 3rd instar	**5.124** **a**	4.597 ab	4.736 ab	4.485 bc	3.944 c	0.583
GR 4th instar	**16.114** **a**	14.155 ab	13.780 ab	14.687 a	11.562 b	3.021
GR 5th instar	44.760 a	45.555 a	49.351 a	**51.165** **a**	45.316 a	7.268
ERR %	80.318 a	82.494 a	88.173 a	**90.890** **a**	77.495 a	19.808

In a row, means followed by same letter (s) are not significantly different at 0.01 by LSD

For grown larvae, the highest growth rate was observed for larvae exposed to 0 ºC for one hr. No significant difference was observed among all treatments, (Table 2). Thermal stresses did not significantly affect survival or effective rate of rearing.

Table 2: Larval growth rate (GR) & effective rate of rearing (ERR) of *B. mori* fourth larval groups exposed to sudden thermal stresses for different periods

parameters	Control group	0 ºC for one hr.	0 ºC for two hrs.	40 ºC for one hr	40 ºC for two hrs	LSD
GR 4th instar	14.803 bc	**16.921** **a**	15.636 ab	13.714 c	14.154 Bc	1.698
GR 5th instar	54.633 a	**55.242** **a**	43.992 b	55.058 a	54.133 A	9.530
ERR %	88.889 a	**98.444** **a**	87.778 a	99 a	95 A	19.808

In a row, means followed by same letter (s) are not significantly different at 0.01 by LSD

B- Electrophoretic analysis of Phosphatases and Esterases:

B- 1 Acid phosphatase (ACPase):

Egg stage

A common band exist at Rf (0.065) in all treatments except control (Fig.1). The quantitative changes was calculated to be (1.684) in acid phosphatase pattern under exposure to 0 ºC for one and two hours, (Table 3). The enzyme activity increased and showed 1, 2, 2 and 3 new bands in treatments 2,3,4 and 5, respectively comparing to control.

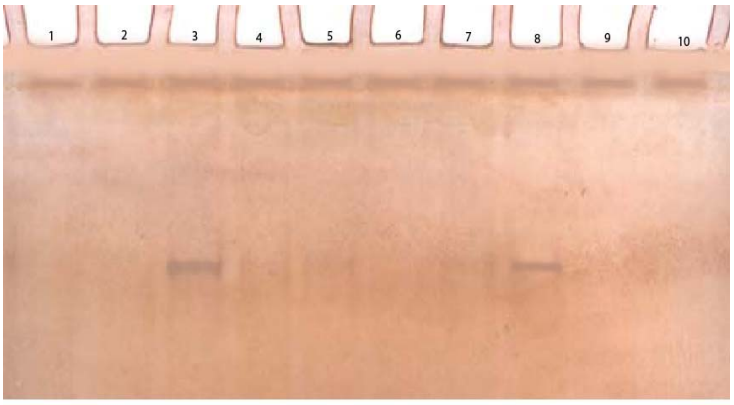

Fig 1: Haemolymphal ACPase activity for all tested treatments of *B. mori* exposed to sudden thermal stresses for different periods at egg and fourth larval stages

*Egg stage
Lane 1: T1 control
Lane 2: T2 - 0°C for 1 hr Lane 3: T3 - 0°C for 2 hrs
Lane 4: T4 - 40°C for 1 hr Lane 5: T5 - 40°C for 2 hrs

*Fourth larval stage
Lane 6: T6 control
Lane 7: T7 - 0°C for 1 hr Lane 8: T8 - 0°C for 2 hrs
Lane 9: T9 - 40°C for 1 hr Lane 10: T10 - 40°C for 2 hrs

Table 3: Haemolymphal ACPase activity with bands relative fragmentation (Rf) and amount percentage (Am%) at egg stage exposed to sudden thermal stresses for different periods.

	Lane 1		**Lane 2**		**Lane 3**		**Lane 4**		**Lane 5**	
Rows	**Rf**	**Am%**	**Rf**	**Am%**	**Rf**	**Am%**	**Rf**	**Am%**	**Rf**	**Am%**
R1			0.065	56.329*	0.065	33.441	0.065	26.951	0.065	21.197
R2	0.078	100								
R3									0.147	24.155
R4							0.15	23.955		
R5					0.166	23.011				
R6			0.172	43.671						
R7									0.670	27.287
R8					0.675	43.548				
R9							0.7	49.094		
R10									0.782	27.361

*Egg stage
Lane 1: T1 control
Lane 2: T2 - 0°C for 1 hr Lane 3: T3 - 0°C for 2 hrs
Lane 4: T4 - 40°C for 1 hr Lane 5: T5 - 40°C for 2 hrs

*Fourth larval stage
Lane 6: T6 control
Lane 7: T7 - 0°C for 1 hr Lane 8: T8 - 0°C for 2 hrs
Lane 9: T9 - 40°C for 1 hr Lane 10: T10 - 40°C for 2 hrs

Larval stage:

As represented in Table (4), No common bands were found between the tested samples. Sudden thermal stresses at the beginning of fourth larval stage increased the enzyme activity which resulted in the appearance of 2, 3, 1 and 1 new bands in treatments 7, 8, 9 and 10, respectively comparing to control.

Table 4: Haemolymphal ACPase activities with bands relative fragmentation (Rf) and amount percentage (Am%) at fourth larval stage exposed to sudden thermal stresses for different periods.

	Lane 6		**Lane 7**		**Lane 8**		**Lane 9**		**Lane 10**	
Rows	**Rf**	**Am%**	**Rf**	**Am%**	**Rf**	**Am%**	**Rf**	**Am%**	**Rf**	**Am%**
R1									0.05	40.201
R2							0.065	44.173		
R3			0.068	41.714						
R4					0.071	26.068				
R5	0.078	100								
R6							0.160	55.827	0.160	59.799
R7					0.163	21.184				
R8			0.669	58.286						
R9					0.681	52.748				

*Fourth larval stage
Lane 6: T6 control
Lane 7: T7 - 0°C for 1 hr Lane 8: T8 - 0°C for 2 hrs
Lane 9: T9 - 40°C for 1 hr Lane 10: T10 - 40°C for 2 hrs

B-2 Alkaline Phosphatase (ALKPase):

Egg stage:

A common band exist in all treatments and control at Rf (0.062), which increased quantitatively in control and treatment 2 (egg group exposed to 0ºC for one hr) to 3.6 ~ 4.6 over the other treatments (Table 5 and Fig.2). Thermal stress at 0 ºC for two hrs and at 40 ºC for one hr induced the production of two new characteristic bands. While thermal stress at 40 ºC for 2 hrs reduced enzyme activity which resulted in the appearance of one characteristic band only.

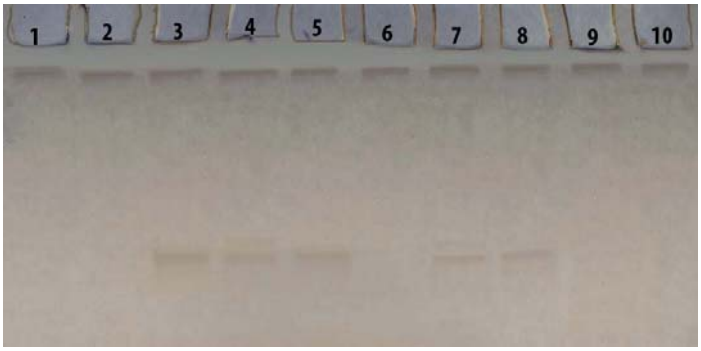

Fig. 2: Haemolymphal ALKPase activity for all tested treatments of *B. mori* exposed to sudden thermal stresses for different periods at egg and fourth larval stages

*Egg stage
Lane 1: T1 control
Lane 2: T2 - 0°C for 1 hr Lane 3: T3 - 0°C for 2 hrs
Lane 4: T4 - 40°C for 1 hr Lane 5: T5 - 40°C for 2 hrs

*Fourth larval stage
Lane 6: T6 control
Lane 7: T7 - 0°C for 1 hr Lane 8: T8 - 0°C for 2 hrs
Lane 9: T9 - 40°C for 1 hr Lane 10: T10 - 40°C for 2 hrs

Table 5: Haemolymphal ALKPase activity with bands relative fragmentation (Rf) and amount percentage (Am%) at egg stage exposed to sudden thermal stresses for different periods.

	Lane 1		Lane 2		Lane 3		Lane 4		Lane 5	
Row	Rf	Am%	Rf	Am%	Rf	Am%	Rf	Am%	Rf	Am%
R1	0.062	100*	0.062	100*	0.062	21.478	0.062	27.030	0.062	25.851
R2							0.525	47.972		
R3					0.601	57.809				
R4					0.66	20.713				
R5							0.669	24.998		
R6									0.737	74.149

*Egg stage
Lane 1: T1 control
Lane 2: T2 - 0°C for 1 hr Lane 3: T3 - 0°C for 2 hrs
Lane 4: T4 - 40°C for 1 hr Lane 5: T5 - 40°C for 2 hrs

Larval stage

As shown in Table (6), a common band exist in all treatments and control at Rf (0.062), which increased quantitatively in control and treatments 40 ºC for one and two hours groups to (~ 3.6) over the other treatments. Thermal stresses for one and two hrs at 0 ºC induced the production of 2 new characteristic bands.

Table 6: Haemolymphal ALKPase activity with bands relative fragmentation (Rf) and amount percentage (Am%) at fourth larval stage exposed to sudden thermal stresses for different periods.

Row	Lane 6		Lane 7		Lane 8		Lane 9		Lane 10	
	Rf	Am%	Rf	Am%	Rf	Am%	Rf	Am%	Rf	Am%
R1	0.062	100*	0.062	27.101	0.062	28.498	0.062	100*	0.062	100*
R2			0.59	25.001						
R3					0.654	51.698				
R4			0.657	47.898						
R5					0.821	19.804				

*Fourth larval stage
Lane 6: T6 control
Lane 7: T7 - 0°C for 1 hr Lane 8: T8 - 0°C for 2 hrs
Lane 9: T9 - 40°C for 1 hr Lane 10: T10 - 40°C for 2 hrs

B-3 alpha (α) esterase:

Egg Stage

No common bands were observed between the tested treatments as shown in Table (7) and Fig. (3). Sudden thermal stresses at egg stage induced the production of only one band in all treatments while the control sample showed three characteristic bands.

Fig. 3: Haemolymphal α esterase activity for all tested treatments of *B. mori* exposed to sudden thermal stresses for different periods at egg and fourth larval stages

*Egg stage
Lane 1: T1 control
Lane 2: T2 - 0°C for 1 hr Lane 3: T3 - 0°C for 2 hrs
Lane 4: T4 - 40°C for 1 hr Lane 5: T5 - 40°C for 2 hrs

*Fourth larval stage
Lane 6: T6 control
Lane 7: T7 - 0°C for 1 hr Lane 8: T8 - 0°C for 2 hrs
Lane 9: T9 - 40°C for 1 hr Lane 10: T10 - 40°C for 2 hrs

Table 7: Haemolymphal α esterase activity with bands relative fragmentation (Rf) and amount percentage (Am%) at egg stage exposed to sudden thermal stresses for different periods.

Rows	Lane 1		Lane 2		Lane 3		Lane 4		Lane 5	
	Rf	Am%	Rf	Am%	Rf	Am%	Rf	Am%	Rf	Am%
R1									0.047	8.457
R2							0.05	6.711		
R3			0.055	6.153	0.055	8.396				
R4	0.058	7.266								
R5	0.239	17.949								
R6	0.435	16.038			0.435	15.714	0.435	13.447	0.435	11.675
R7			0.441	17.634						
R8	0.679	58.747								
R9					0.697	75.890				
R10			0.706	76.213			0.706	79.842	0.706	79.868

*Egg stage
Lane 1: T1 control
Lane 2: T2 - 0°C for 1 hr Lane 3: T3 - 0°C for 2 hrs
Lane 4: T4 - 40°C for 1 hr Lane 5: T5 - 40°C for 2 hrs

Larval stage

Electrophoretic analysis for the haemolymphal samples of different treatments revealed that, new characteristic bands were 2, 1, 3, 2, and 4 in treatments 1, 2, 3, 4 and 5 respectively as shown in Table (8).

Table 8: Haemolymphal alpha esterase activity with bands relative fragmentation (Rf) and amount percentage (Am%) at fourth larval stage exposed to sudden thermal stresses for different periods.

	Lane 6		Lane 7		Lane 8		Lane 9		Lane 10	
Rows	**Rf**	**Am%**	**Rf**	**Am%**	**Rf**	**Am%**	**Rf**	**Am%**	**Rf**	**Am%**
R1	0.052	6.741	0.052	11.485						
R2							0.064	16.887		
R3					0.067	7.661				
R4									0.073	9.039
R5					0.126	8.133				
R6									0.156	9.652
R7									0.23	13.892
R8							0.239	17.978		
R9	0.311	14.037								
R10	0.435	10.654					0.435	13.138		
R11					0.456	12.912				
R12									0.459	10.618
R13			0.494	10.814						
R14							0.676	51.997	0.676	56.798
R15			0.685	77.701	0.685	71.294				
R16	0.691	68.568								

*Fourth larval stage
Lane 6: T6 control
Lane 7: T7 - 0°C for 1 hr Lane 8: T8 - 0°C for 2 hrs
Lane 9: T9 - 40°C for 1 hr Lane 10: T10 - 40°C for 2 hrs

3-b Beta (β) esterases

Egg stage

Egg groups exposed to 0°C for one and two hours showed three and four characteristic bands, respectively. Egg groups exposed to 40°C showed four characteristic new bands (Table 9 and Fig. 4).

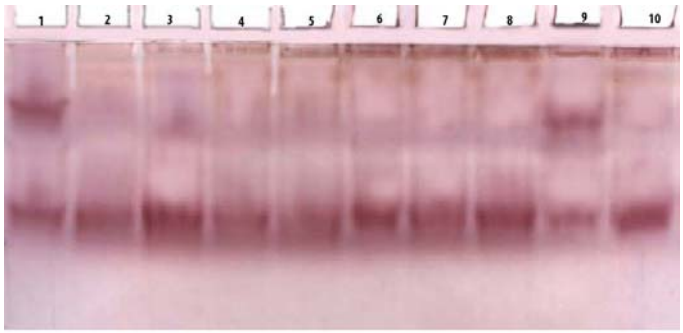

Fig. 4: Haemolymphal β esterase activity for all tested treatments of *B. mori* exposed to sudden thermal stresses for different periods at egg and fourth larval stages.

*Egg stage
Lane 1: T1 control
Lane 2: T2 - 0°C for 1 hr Lane 3: T3 - 0°C for 2 hrs
Lane 4: T4 - 40°C for 1 hr Lane 5: T5 - 40°C for 2 hrs

*Fourth larval stage
Lane 6: T6 control
Lane 7: T7 - 0°C for 1 hr Lane 8: T8 - 0°C for 2 hrs
Lane 9: T9 - 40°C for 1 hr Lane 10: T10 - 40°C for 2 hrs

Table 9: Haemolymphal β esterase activity with bands relative fragmentation (Rf) and amount percentage (Am%) at egg stage exposed to sudden thermal stresses for different periods.

	Lane 1		**Lane 2**		**Lane 3**		**Lane 4**		**Lane 5**	
rows	Rf	Am%	Rf	Am%	Rf	Am%	Rf	Am%	Rf	Am%
R1							0.0412	7.298		
R2					0.044	6.522				
R3									0.05	11.741
R4	0.061	6.545								
R5			0.079	7.118						
R6							0.091	6.385		
R7									0.121	7.591
R8			0.135	11.052	0.135	8.727				
R9					0.238	9.141				
R10	0.241	19.843								
R11			0.317	18.291						
R12	0.32	17.262								
R13									0.326	24.495
R14					0.349	12.668				
R15							0.354	17.619		
R16	0.663	56.350								
R17			0.674	63.539						
R18					0.677	62.942				
R19							0.686	68.698		
R20									0.7	56.173

*Egg stage
Lane 1: T1 control
Lane 2: T2 - 0°C for 1 hr Lane 3: T3 - 0°C for 2 hrs
Lane 4: T4 - 40°C for 1 hr Lane 5: T5 - 40°C for 2 hrs

Larval stage

As shown in Table (10). One, two, four and five new characteristic bands were observed in treatments 7, 8, 9 and 10, respectively.

Table 10: Haemolymphal β esterase activity with bands relative fragmentation (Rf) and amount percentage (Am%) at fourth larval stage exposed to sudden thermal stresses for different periods.

	Lane 6		**Lane 7**		**Lane 8**		**Lane 9**		**Lane 10**	
rows	Rf	Am%	Rf	Am%	Rf	Am%	Rf	Am%	Rf	Am%
R1					0.029	19.696				
R2							0.047	15.878		
R3	0.05	11.132	0.05	16.922						
R4									0.052	11.168
R5									0.132	13.451
R6									0.244	8.537
R7							0.259	24.574		
R8									0.331	8.071
R9	0.337	9.152								
R10	0.371	14.552	0.371	17.728	0.371	17.571				
R11							0.418	15.903		
R12									0.474	10.525
R13	0.512	12.693								
R14							0.677	43.645		
R15					0.68	62.733				
R16			0.686	65.350						
R17										
R18	0.669	52.471							0.669	48.248

*Fourth larval stage
Lane 6: T6 control
Lane 7: T7 - 0°C for 1 hr Lane 8: T8 - 0°C for 2 hrs
Lane 9: T9 - 40°C for 1 hr Lane 10: T10 - 40°C for 2

DISCUSSION

All groups showed increased growth from the first day of exposure till the end of larval stage. The results revealed that, the highest performance was recorded for 40ºC for one hr and 0 ºC for one hr for egg and grown larvae, respectively. Effective rearing rate (ERR) indicates the survivability of silkworm during the rearing period showed no significant differences between all treatments. The survivability of silkworm larvae at heat stresses extremes related to the

production of proteins serving as molecular chaperons assisting in refolding of denaturated proteins as suggested by Samad *et al*.(2005).

Exposure grown larvae to sudden thermal stresses for different periods achieve growth rate better than eggs treatments. Differential levels of tolerance to thermal exposure (other than their normal growth temperature) were reported to be dependent on the life stages of the silkworm (Joy and Gopinathan, 1995). Manjunatha, *et al*. (2013) displayed that, the mild heat stress between 35 and 40 °C for two hours facilitate the silkworm larvae to overcome and withstand the fluctuating natural environmental conditions and perform better. Obviously, this physical state is supported by expression of one or many proteins, heat shock proteins (HSPs) in a given generation with the activation of some genes located in their chromosomes (Hong *et al*., 2010 and Sosalegowda *et al*., 2010).

Phosphatases exhibited a positive relationship to the cocoon quality of the silkworm. Therefore, it suggests that ALKPase may be used as a biochemical index to evaluate the health and economic characteristics of the silkworm, *Bombyx mori* L. as well as different stress and disease result in considerable decrease in the ALKPase activity (Miao, 2002). These findings disagree with the present results as the thermal stresses in egg and larval stages induced the production of new enzyme bands.

Esterases are very large class of enzymes, all of which can break an ester bond with the help of a water molecule. Most enzymes of this class hydrolyze endogenous substances and are important in intermediary metabolism (Sivakumram and Maya, 1991). Esterases activity can be regarded as an indicator for thermotolerance in the silkworm generations (Wu and Hou, 1993).

In the present study thermal stresses affect the pattern of the studied isozymes (phosphatases and esterases) in both egg and larval stages. The disappearance of some bands in the tested treatments may be attributed the effect of thermal doses which inhibits the gene expression and synthesis of these deleted proteins (qualitative effect). The difference in bands densities (quantitative effect) may be due to the presence of different numbers of iso-genes responsible for the production of this protein type or due to the prolongation of the genes related to this protein in their action compared to the other treatments (Taha, 2013). The changes in the protein mobility and number of bands (qualitative determination) and the intensity of some bands (quantitative determination) probably, reflect some of physiological and biological processes (Hussein *et al*.,1993 and El-Banna, 2009). These differences were due to better adaptation of one group over another (Mahadav *et al*., 2009).

Finally, our conclusions agree with Sinha and Sanyal (2013) which recommended the practical application of sudden temperature stresses systematically in sericulture to achieve better growth, performance and cocoon production.

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ARABIC SUMMARY

تأثير الضغوط الحرارية المفاجئه على معدل النمو و تحديد النشاط الإنزيمي في دودة الحرير التوتيه *Bombyx mori* L.

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الغرض من هذه الدراسه هو تحديد أثر الضغوط الحرارية المفاجئة لفترات مختلفه على أجنة و يرقات سلاله أحادية الجيل من دودة الحرير التوتيه من خلال دراسة معدل نمو اليرقات و معدل كفاءة التربية و تحديد النشاط الإنزيمي في السائل الدموي لليرقات .

تعرضت مجموعات البيض لدرجات حرارة مختلفه ؛ صفر درجه مئويه لمدة ساعة ، صفر درجه مئوية لمدة ساعتين ، 40 درجة مئويه لمدة ساعة و 40 درجة مئويه لمدة ساعتين تليها ساعتين للإنتعاش في درجة حرارة الغرفه ، هذا بالإضافة إلى مجموعة الكنترول . و قد تم تطبيق نفس الشيء على اليرقات الناضجه (بداية عمر رابع) . و تعرض السائل الدموي ليرقات العمر الخامس لكل المجموعات تحت الإختبار إلى التفريد الكهربي native polyacrylamide gel electrophoresis (PAGE) لتحديد نشاط إنزيمات الفوسفاتيز و الإستيراز .

سجل التعرض الحراري المفاجئ لدرجة صفر درجه مئويه لمدة ساعة واحده أعلى معدلات نمو . الضغوط الحرارية المفاجئه لم تؤثر معنوياً على معدل كفاءة التربيه . و مع هذا ، أدت الضغوط الحرارية المفاجئة إلى زيادة نشاط إنزيمات الفوسفاتيزز (الحامضي و القلوي) و خفض إنزيمات الإستيرز (α و β) مقارنة بالكنترول . من هذه النتائج يقترح أن تطبق إستراتيجية الإعتماد على بروتينات الناتجه من الضغوط الحرارية المفاجئه في برامج التربيه لزيادة قوة السلالات المستخدمه .