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Impact of thermal stress on the haemolymphal proteins, biological and economical characters of the silkworm, *bombyx mori* L.

Rehab Hosny Taha
Sericulture Research Department (SRD), Plant Protection Research Institute (PPRI), Agricultural Research Center (ARC), Egypt.
Rehab_hosny1@hotmail.com

ABSTRACT

The purpose of this study is to determine the impact of different thermal stresses on univoltine *Bombyx mori* embryos and grown larva in the terms of larval haemolymph proteins , biological and economical characters. The experiment was designed to expose egg groups to different temperatures as follows; a group exposed to 0°C for one hour, a group exposed to 0°C for two hours, a group exposed to 40°C for one hour and a group exposed to 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 larval groups. The larval haemolymph of tested groups were subjected to protein analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Data revealed that considerable alteration in temperature hamper the protein activities which affects the embryonic development and hatching of eggs. 40ºC for two hours was lethal dose for embryo. The highest measured parameters were recorded for eggs exposed to 40ºC for one hour. While for grown larvae, the highest results were recorded for larvae exposed to 0ºC for 1 hour. Thermal stresses induced protein changes with the appearance of new bands. The epigenetic degree of similarity recorded 50% between the control egg group and the egg groups exposed to 0ºC for 2 hrs and 40ºC for 1 hrs. as well as, for grown larva, between control larval group and larval group exposed to 40ºC for 2 hrs.

Keywords: *Bombyx mori*, egg, larva, thermal stress, haemolymphal proteins, biological, economical characters .

INTRODUCTION

The mulberry silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae) is a very heat sensitive organism. The differences in the environmental components considerably affect the genotypic expression in the form of phenotypic output of silkworm crop such as cocoon weight, shell weight and cocoon shell ratio (Rahmathulla, 2012). Therefore, intensive and careful domestication over centuries has apparently deprived this commercial insect of the opportunity to acquire thermo-tolerance.

Lack of thermo-tolerance may be attributed as one of the many factors responsible for poor performance of *B mori* strains at times as many quantitative characters decline sharply at higher/lower temperatures. Therefore, one of the key considerations in developing hybrids could be the need for thermo-tolerant strains (Kumari *et al*., 2011). The development and economic production of sericulture greatly depends on the metabolic modulations and physiological adaptability of silkworm, besides its genetic constitutions (Sinha and Sanyal 2013). The recent advances in silkworm breeding and those in stress-induced protein synthesis have opened up new avenues to evolve robust productive silkworm hybrids (Moghaddam *et al*., 2008). Such information my provide useful clues for the establishment of methods for prevention of viral diseases

in the silkworm (Kobayashi and Chaeychomsri, 1993).

Insects have evolved different biological and physiological strategies to overcome drastic and adverse changes in their surrounding environment (Singh *et al*., 2010). It depends on the molecular mechanism of the cell involving the rapid synthesis of special proteins, the heat shock proteins (Park *et al*., 2008). The term "heat shock protein" was introduced by Tissieres *et al*. (1974) as these proteins increased in synthesis due to sudden increases/decreases in temperature. They act as 'molecular chaperones' to ensure better survival under stressful conditions, including thermo-stress (Mosser and Morimoto, 2004) and have been implicated in immunogenicity to cancers/infectious diseases (Srivastava, 2002).

Generally, the heat shock response depends on the magnitude of temperature and duration of exposure, and is relative to the environmental temperature at which the organism normally survives as suggested by Lagerspetz (2006).

The determination of the electrophoretic patterns of the protein bands mobility and number (qualitative changes) and the intensity of some bands (quantitative changes) probably, reflect some of physiological processes (Hussein *et al*.,1993). Quantitative protein profiling is an essential part of proteomics and requires new technologies that accurately, reproducibly, and comprehensively identify and quantify the proteins contained in biological samples (Smolka *et al*., 2002). Screening for thermo-tolerance in the silkworm, is an essential prerequisite for the development of thermo-tolerant strains. The current study, aims to study the effect of temperature stress at the egg and fourth larval stages of *B. mori* in relation to its biochemical alternations and their subsequent impact on commercial traits.

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, following the standard methodology of rearing in SRD according to Krishnaswamy (1978). Larvae were fed on leaves of Kokuzo-27 mulberry variety.

A- Temperature stresses

Egg stage

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

Control- treatment 1 (T1) - 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.

All the treatment batches (T2~T5) after being exposed to thermal stress were kept at 15°C for two hours for recovery and finally placed at 27 °C till hatching.

Fourth larval stage

At the beginning of the fourth larval stage (grown larval stage), larvae were divided into 5 groups, with100 larvae/ replicate and the following treatments 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

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

Insect performance in all treatments was assessed based on pre- cocooning parameters such as hatching percentage, larval duration, and larval weight. Commercial characteristics such as cocoon weight, shell weight and shell percentage for female and male were also recorded and subjected to analysis of variance according to Snedecor and Cochran (1981).

At the sixth day of the fifth larval instar, haemolymph samples were collected from each treatment.

B- Sodium Dodecyl Sulphate (SDS)-polyacrylamide gel electrophoresis

SDS-polyacrylamide gel electrophoresis was performed in 12% acrylamide slab gels following the system of Laemmli, (1970). 10 microlitres of haemolymph sample was taken in an eppendrof tube and centrifuged at 5000 rpm for 10 min. Then a volume of 20 µl protein extract was added to 10 µl of treatment buffer (1M Tris-HCl pH6.8+ 10% (w/v) SDS + glycerol + 0.5M EDTA + Bromophenol blue + b-mercaptoethanol). Then 20 µl of each sample was loaded in the gel. After the electrophoresis run was completed, gels of SDS-protein was stained in Coomassie Brilliant Blue solution for 12-18 h, then rinsed in destaining solution until the dark background became colourless except the blue protein bands and photographed. The gels were scanned to calculate the relative mobility and concentration of identified bands using Gel-Pro Analyzer software (V.3).

C- Similarity Index:

The similarity index between all tested treatments were calculated according to Nei & Li (1979) using the formula

$$S.I = \frac{2N}{Na + Nb}$$

S.I: Similarity Index
N: the number of common bands
Na and Nb: the number of bands in individuals (a) and (b).

RESULTS

A- Effect of thermal stresses on *Bombyx mori* L. eggs

Egg groups exposed to 0 ºC for one and two hours and 40 ºC for one hr hatched normally, while exposure to 40º C for two hours resulted in death of embryos. The depressed eggs with fully formed dead larvae inside the eggs were found after high temperature stress as shown in Figure (1).

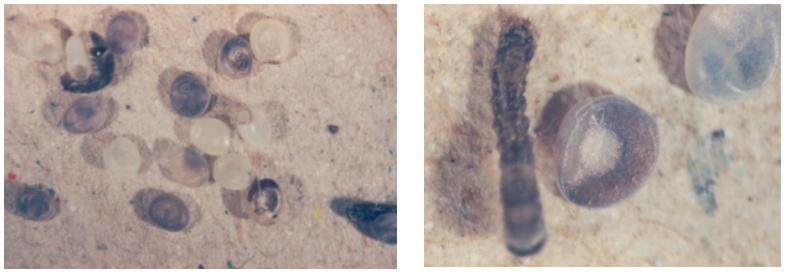

Fig. 1: egg group exposed to 40 C for two hours

Fourth larval groups which exposed to 0ºC for one and two hours were seen in a stillness case for about 15 minutes and then returned to normal condition. Nothing happened in larvae exposed to 40 ºC for one and two hours.

Data represented in Table (1) showed that, egg group exposed to 0 ºC for two hours decreased incubation period

significantly compared to the other groups. While, exposed to 40 ºC for one hour increased hatching percentage, single larval and cocoon weights and single silk ratio for both male and female.

Fourth larval stage exposed to 0ºC for one hour showed increment in single larval weight, single female and male cocoon weights and silk ratios . All larvae exposed to 0ºC and 40ºC for one and two hours resumed feeding and spun normal cocoons (Table 2).

Table 1: *Bombyx mori* L. pre and post-cocooning parameters of eggs exposed to different temperatures for different times

Parameters	Incubation period (hrs)	Hatching %	Larval weight (gm)	♀ cocoon weight (gm)	♀ silk ratio	♂cocoon weight (gm)	♂ silk ratio
Control	258 a	63.667 a	1.698 a	1.237 a	17.973 a	1.026 ab	20.440 b
0°C for 1 hr	249 ab	71 a	2.044 a	1.260 a	16.886 a	1.065 ab	20.761 ab
0°C for 2 hrs	**240 b**	75 a	2.125 a	1.315 a	18.525 a	1.093 a	22.236 ab
40°C for 1 hr	258 a	**78.333 a**	**2.191 a**	**1.351 a**	**18.820 a**	**1.120 a**	**22.555 a**
40°C for 2 hr	264 a	22.667 b	1.955 a	1.175 a	17.913 a	0.939 b	20.773 ab
LSD	16.929	22.854	0.638	0.229	2.055	0.143	2.006

The same small liters in a column means there is no significance between means.

Table 2: Pre and post-cocooning parameters of *Bombyx mori* L. fourth larval stage exposed to different temperatures for different times

parameters	Larval weight	♀ cocoon weight	♀ silk ratio	♂ cocoon weight	♂ silk ratio
Control	2.117 a	1.261 a	17.829 ab	1 a	21.798 ab
0°C for 1 hr	**2.220 a**	**1.282 a**	**18.912 a**	**1.016 a**	**23.440 a**
0°C for 2 hrs	1.870 a	1.150 a	17.246 a	0.964 a	20.762 b
40°C for 1 hr	2.180 a	1.213 a	17.556 b	0.965 a	21.120 b
40°C for 2 hrs	2.052 a	1.251 a	17.221 b	0.996 a	20.855 b
LSD	0.369	0.214	1.239	0.147	1.709

The same small liters in a column means there is no significance between means.

B-Electrophoretic studies

Fractionated protein

Data presented in Tables (3) and demonstrated in Figure (2) showed egg stage protein pattern of *Bombyx mori*, in all treatments the bands molecular weight ranged from 24.853 to 124.354 KDa. Six common bands no. 1, 8, 10, 17, 23 and 24 were detected in the control (T 1) and the different treatments. Percentages of protein bands amount were compared quantitatively among different treatments to clarify the quantitative changes.

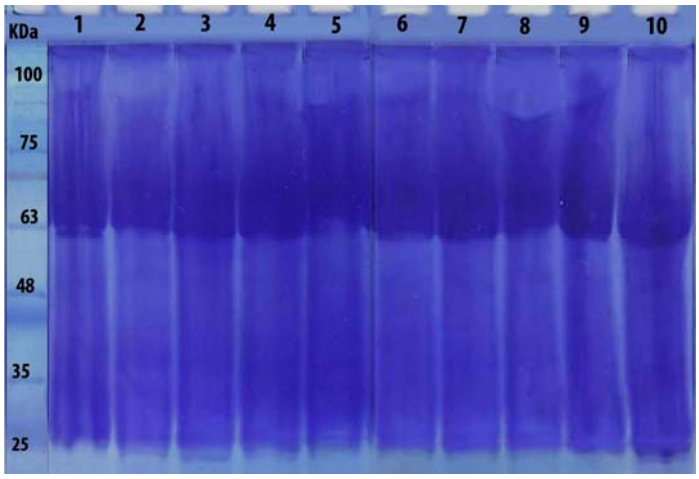

Fig 2: SDS- Polyacrylamide gel pattern of *Bombyx mori* L. larval haemolymph exposed to different temperatures for different times

*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: larval haemolymph protein pattern of *Bombyx mori* exposed to different temperatures for different times during egg stage.

	Lane 1			Lane 2			Lane 3			Lane 4			Lane 5		
row	Rf	Mw	Am%	Rf	Mw	Am%	Rf	Mw	Am%	Rf	Mw	Am%	Rf	Mw	Am%
R1	0.003	124.354	6.948	0.003	124.354	5.451	0.003	124.354	5.129	0.003	124.354	6.516	0.003	124.354	6.696
R2										0.009	109.69	3.252			
R3													0.02	106.8	3.001
R4	0.062	96.171	10.965												
R5				0.069	94.794	6.217									
R6				0.085	90.790	6.982									
R7							0.177	73.529	8.052						
R8	0.238	68.933	18.027	0.238	68.933	17.963	0.238	68.933	16.376	0.238	68.933	18.139	0.238	68.933	16.388
R9										0.249	67.792	16.392	0.249	67.792	17.837
R10	0.311	63.062	8.428	0.311	63.062	11.932	0.311	63.062	13.792	0.311	63.062	11.366	0.311	63.062	5.2
R11							0.317	62.149	12.402						
R12	0.321	61.102	8.414												
R13										0.371	55.744	3.999			
R14				0.387	53.663	5.451	0.387	53.663	5.101	0.387	53.663	5.220	0.387	53.663	11.890
R15										0.417	50.683	6.441			
R16							0.48	44.998	3.374						
R17	0.549	39.620	11.059	0.549	39.620	9.604	0.549	39.620	9.229	0.549	39.620	4.814	0.549	39.620	13.777
R18				0.563	38.620	13.566	0.563	38.620	6.631	0.563	38.620	15.752	0.563	38.620	10.329
R19							0.594	36.325	10.665						
R20	0.629	34.002	19.230	0.629	34.002	10.554									
R21				0.734	33.695	3.828									
R22	0.667	32	7.266												
R23	0.737	28.815	5.310	0.737	28.815	3.018	0.737	28.815	2.290	0.737	28.815	1.678	0.737	28.815	5.534
R24	0.807	25.549	4.353	0.807	25.549	2.901	0.807	25.549	4.923	0.807	25.549	3.367	0.807	25.549	5.606
R25				0.821	24.853	2.533	0.821	24.853	2.036	0.821	24.853	3.064	0.821	24.853	3.646

Relative fragmentation (RF), molecular weight(Mw) and amount percent (Am %)

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

Common band at R 10 appeared in (T 2, T 3 and T 4) were two times in amount percent comparing to control (T 1), the protein amount of the common band at R 17 in (T 4) declined ~ three times less than control (T 1). Also, the common band R 23, protein amount percent of control (T 1) and (T 5) were ~ two times than (T 2 and T 3) and four times than (T 4). The protein amount percent of the common band at R 24, of (T 2) showed the lowest density among the other samples.

The degree of similarity among the tested treatments was calculated and tabulated in Table (4). The Similarity was not less than 0.5, the highest similarity 0.8 was recorded between control (T 1) and (T 5).

Table 4: Epigenetic degree of similarity between the tested treatments of *Bombyx mori* exposed to different temperatures for different times during egg stage.

S.I					
Samples	**1**	**2**	**3**	**4**	**5**
1	----	0.6	0.5	0.5	0.6
2		---	0.7	0.7	0.7
3			---	0.7	0.7
4				---	**0.8**
5					---

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

Table 5: Larval haemolymph protein pattern of *Bombyx mori* grown larvae exposed to different temperatures for different times

	Lane 6			Lane 7			Lane 8			Lane 9			Lane 10		
	Rf	Mw	Am %	Rf	Mw	Am %	Rf	Mw	Am %	Rf	Mw	Am %	Rf	Mw	Am %
R1	0.003	124.354	6.567	0.003	124.354	5.756	0.003	124.354	6.367	0.003	124.354	9.077	0.003	124.354	6.211
R2	0.062	96.171	7.171												
R3				0.063	95.974	7.705									
R4										0.083	91.355	11.523			
R5							0.113	85.016	8.744						
R6													0.138	80.097	7.954
R7	0.238	68.933	17.435	0.238	68.933	14.644	0.238	68.933	24.474	0.238	68.933	16.649	0.238	68.933	8.211
R8										0.266	66.33	7.466	0.266	66.33	8.970
R9	0.311	63.062	8.319	0.311	63.062	12.617	0.311	63.062	7.320	0.311	63.062	14.386	0.311	63.062	7.453
R10	0.322	61.102	7.686	0.322	61.102	10.590									
R11													0.334	60.072	14.458
R12	0.34	59.261	5.689												
R13							0.35	58.065	6.482						
R14				0.367	56.124	6.701									
R15							0.38	54.946	9.598						
R16													0.387	53.881	5.959
R17				0.403	52.885	7.321									
R18										0.408	51.377	11.342			
R19	0.417	50.029	8.015												
R20													0.433	48.989	8.622
R12													0.493	43	5.830
R22	0.563	38.620	11.186	0.563	38.620	5.159	0.563	38.620	13.313						
R23	0.578	37.484	11.470	0.578	37.484	5.826	0.578	37.484	6.021						
R24													0.594	36.006	10.259
R25	0.737	28.815	6.779	0.737	28.815	9.661	0.737	28.815	9.703	0.737	28.815	12.764	0.737	28.815	11.253
R26	0.807	25.815	4.818	0.807	25.815	9.509	0.807	25.815	2.842	0.807	25.815	9.809	0.807	25.815	2.213
R27	0.821	24.853	4.865	0.821	24.853	4.511	0.821	24.853	5.136	0.821	24.853	6.984	0.821	24.853	2.586

Relative fragmentation (RF), molecular weight (Mw) and amount percent (Am %) of protein bands

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

Data presented in Table (5) and demonstrated in Figure (2) showed the protein pattern at fourth larval stage, six common bands no. 1, 7, 9, 25, 26 and 27were detected between the control (T 6) and the other treatments. The common band at R 7 of (T 8) showed the highest protein density among the other treatments. The common band at R 9, sample (T 9) showed the highest density ~ 2 times than (T 8) and (T 10). The protein density of band at R 25of (T 9) and (T 10) were 2 times than the protein of control (T 6). The protein densities of common band at R 26, T 7 and T 9 were ~ 2 times of control (T 6) and ~ 4 times of (T 8) and (T 10). The common protein band density at R 27 of (T 10) recorded the lowest density among the other tested samples.

Degree of similarity among the tested treatments are presented in Table (6). The similarity was not lowered than 0.5, the highest similarity was 0.7 and recorded between control (T 6) and treatments (T 7) and (T 8), as well as between (T 7) and (T 8).

Table 6: Epigenetic degree of similarity between the tested treatments of *Bombyx mori* grown larvae exposed to different temperatures for different times

S.I					
Samples	**6**	**7**	**8**	**9**	**10**
6	---	**0.7**	0.7	0.6	0.5
7		---	0.7	0.6	0.5
8			---	0.6	0.5
9				---	0.6
10					---

Sample 6 : T6 control
Sample 7 : T7 - 0°C for 1 hr Sample 8 : T8 - 0°C for 2 hrs
Sample 9 : T9 - 40°C for 1 hr Sample 10 : T10 - 40°C for 2 hrs

DISCUSSION

Temperature plays an important role and decides the fate of mulberry silkworm, *Bombyx mori* L. during embryonic and postembryonic development (Kumar *et al*., 2012). Nowadays, the spread and success of silkworm rearing was mainly due to the introduction of F1 hybrids of native multivoltine as female parent (for resistance) and bivoltine as male parent (for high quality silk). Even now it is a challenging task to develop not only stress and disease-resistant strains, but also to provide high yielding silkworm strains with improved stress tolerance (Manjunatha *et al*., 2010).

The heat stress treatments could be employed to determine the level of thermo-tolerance based on mortality (Loeschcke and Sorensen, 2005). Notably, thermotolerance increased as larval development proceeded, sequentially in the order of first instar > second instar > third instar > fourth instar > fifth instar (Vasudha *et al*., 2006). The highest mortality (21%) was observed in the first instar and 100% survival in the fifth instar larvae. Thermo-tolerance varies in different races of silkworms and other insects (Lohmann and Riddiford, 1992).

In the present study, thermo-tolerance varied from egg stage to larval stages as the embryos died at exposure to 40ºC for 2 hrs. and the hatching percentages were affected among the tested groups. Both thermal stresses at 0ºC for 2 hrs. and 40ºC for 1 hr at egg stage almost have the same effect on the pre-cocooning and post cocooning parameters. while in the grown larvae,

the highest values were recorded for the group exposed to 0ºC for 1 hr. The increased cocoon weights and cocoon shell weights over control, reflects the positive correlation between heat stresses response and silk protein content in the cocoon.

It is well known that both prokaryotic and eukaryotic cells respond to unfavorable environmental conditions by increased/decreased synthesis of stress proteins. It is a universal phenomenon in bacteria to human, but certain features of the response do vary from organism to organism (Craig, 1985).

Studying protein profile of an insect is of considerable importance for understanding different physiological processes. The separation and characterization of the individual proteins facilitates the study of the chemical nature and physiological function of each protein (Mohamed, 1990).

Data in the present study showed the disappearance of some protein bands or appearance of new ones with different exposure time. The disappearance in certain protein bands of tested treatments may be attributed the effect of thermal doses which inhibits the synthesis and expression process of these deleted proteins (qualitative effect). In addition, even the remained band after treatment usually differs in the amount of protein, this may be explained that, thermal stresses could not inhibit the synthesis of this protein type, but affect its quantitative amount.

Considerable changes in protein fractions were demonstrated electrophoretically. 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 processes (Hussein et al.,1993). In the present study the quantitative increase or decrease in common protein bands among the tested treatments 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.

The inhibition of some protein bands can explain some other experimental results such as reduction in weight, slow development, tissue degeneration and preventing adult emergence (Schloter, 1985).

The protein pattern differences may act as a tool to identify (estimate) the similarity and genetic distance between the control and treated samples as concluded by Rao *et al*. (1999) and Ayeed *et al*. (2001).

The present results revealed that, protein pattern monitors number of bands as a difference between the control and other treatments. This difference is translated as similarity degree , which recorded a low value 50% between the control egg group and the egg groups exposed to 0ºC for 2 hrs. and 40ºC for 1 hrs (which recorded the best economical characters). Also, for grown larval treatments, the similarity degree was 50% between control larval group and larval group exposed to 40ºC for 2 hrs. The data from the present study enhance knowledge for the development of thermo tolerant silkworm breeds/ hybrids and their effective commercial utilization in the sericulture industry.

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ARABIC SUMMARY

تأثير الضغط الحراري على بروتينات الدم و الخصائص البيولوجية و الإقتصادية لحشرة دودة القز ، بومبكس موراي إلـ .

رحـاب حـسنـي طــه

قسم بحوث الحرير- معهد بحوث وقاية النباتات – مركز البحوث الزراعيه – مصر .

الغرض من هذه الدراسه هو تحديد أثر الضغوط الحرارية المختلفه على أجنة و يرقات سلاله أحادية الجيل من حشرة البومبكس موراي من خلال دراسة النمط البروتيني للسائل الدموي و كذلك الخصائص البيولوجية و الإقتصادية للحشره.

وصممت التجربة على أن تعرض مجموعات البيض إلى درجات حراره كالتالي : مجموعة تعرضت لصفر درجه مئويه لمدة ساعه ، مجموعه تعرضت لصفر درجه مئويه لمدة ساعتين ، مجموعه تعرضت 40 درجه مئويه لمدة ساعه ، مجموعه تعرضت 40 درجه مئويه لمدة ساعتين ، بالإضافة إلى المجموعة الكنترول و تم تطبيق هذا أيضاً على مجوعات اليرقات الناضجه . وتم دراسة التباين في النمط البروتيني لعينات السائل الدموي المسحوبة من يرقات المجموعات تحت الدراسة بإستخدام التفريد الكهربي SDS-PAGE .

أسفرت النتائج عن أن التغير في درجات الحراره للبيض يؤثر على البروتينات التي بدورها تؤثر على معدل الفقس للأجنة و كانت 40 درجة مئوية جرعة مميته للجنين ، في حين أحسن نتائج بيولوجيه و إقتصاديه فكانت لمجموعة البيض التي تعرضت لجرعة 40 درجه مئوية لمدة ساعة واحده و مجموعة اليرقات التي تعرضت لجرعة صفر درجة مئوية لمدة ساعة واحدة سجلت أحسن النتائج ايضاً . هذه الضغوط الحرارية أدت إلى ظهور حزم بروتينيه جديده و التي بدورها أثرت على درجة التقارب بين المجموعات تحت الدراسه و المجموعة الكنترول على المستوى تحت الجيني . فكانت درجة التقارب 50 % بين مجموعة الكنترول و مجموعات البيض التي تعرضت إلى صفر درجه مئوية لمدة ساعتين و كذلك 40 درجه مئوية لمدة ساعة واحده . أما بالنسبه لليرقات ، فكانت درجة التقارب 50 % بين الكنترول و مجموعة اليرقات التي تعرضت لجرعة 40 درجه مئويه لمدة ساعتين .