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Influences of Sudden Thermal Stress on Certain Physiological Aspects and Protein Profiles of Silkworm, *Bombyx mori* (Lepidoptera: Bombycidae)

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ABSTRACT

The temperature influences silk productivity. Recently, the sudden increase in temperature that reached between 40-45°C for a whole day affected the breeding of the silkworm. The current study tested the effects of sudden thermal stresses on physiological and protein profiles of a silkworm, *Bombyx mori*. The fourth and fifth silkworm larval instars were exposed to a high temperature (40°C) for five periods (3, 5, 7, 9 and 12 h) in an incubator. The obtained results indicated that the responses of exposed larvae were different according to the exposure periods and larval instar, where the relatively high level of total protein was recorded in the fourth instar larvae that were exposed to the period of 5 h followed by that exposed for 7 h. In addition, the catalase enzyme was found to have decreased in exposed larvae in comparison with that of control. As a result of electrophoresis, the protein bands varied in numbers and molecular weight with the exposure period and larval instar. The findings suggested that exposure to high temperatures for specific periods may benefit the physiological properties and generation of the protein component of silk produced by silkworms.

Key words: High-temperature, physiological properties, enzyme level, protein electrophoresis

INTRODUCTION

Temperature is one of the most important environmental factors affecting the activity and distribution of living organisms, including insects (Sun *et al.,* 2022).The constant rise in temperature, as well as the period of exposure to hot temperature exhibits profound effects on genotypic articular as phenotypic yield of silkworm product (Parrey, 2018). In Egypt, the climate changes are one of the most difficult challenges of agricultural production including silkworm breeding for silk production, where the daily temperatures suddenly reach 45°C especially throughout the period of silkworm breeding for silk production.

Natural silk is produced by feeding silkworm larvae on mulberry leaves. The climatic factors, especially elevated temperature decreased the viability of worms, increased the percentage of deaf and black cocoons, which in turn affects productivity indicators such as percentage, dry weight of cocoons, percentage of defective cocoons (Umarov *et al.,* 2021; Batirova and Umarov, 2023). Also, external environmental factors affected silkworm larvae during feeding periods, and this led to diversity in the response of larvae to these conditions which finally influenced the productivity and quality of silk (Jumagulov *et al.*, 2021).

Low thermo-tolerance affects many quantitative characteristics of silkworms at higher/lower temperatures. The biological characteristics of the silkworm cocoons are directly affected by the changes in temperature of rearing places. Therefore, functioning of the same hybrids, the estimation index and secondary function values differed according to the environmental factors and location of the rearing governorate (Fouad and Gad, 2023). The environmentally induced quality parameters must not be ignored when analyzing and deploying silk cocoons, silk filaments or silk-derived biopolymers. Specifically, temperature affects cocoon morphology, its stiffness and strength (Offord *et al.*, 2016). Therefore, the importance of studying the effect of microhabitat temperature in silkworm rearing rooms is attributed to reaching heat-resistant strains,

which are good in terms of environmental adaptation, which benefits the silk production from the silkworm (Parrey, 2018). The importance of the current study comes because of the climate changes with the continuous temperature rising in Egypt. Where, the effect of certain exposure periods to high temperature on cocoon characteristics such as, cocoon weight, shell weight, cocoon shell ratio, the silk filament characterizes, physiological characteristics and protein profiles of mulberry silkworms are impacted by exposure periods to high temperatures. Further, silkworm breeders in Egypt do not have temperature or humidity regulating devices in breeding rooms, so the study is of importance in terms of temperature changes.

MATERIALS AND METHODS

The eggs of silkworm hybrid (H1 × KK × G2 × V2) reared in the spring season obtained from the Sericulture Research Department of Plant Protection Research Institute, Agriculture Research Center (ARC), Egypt and incubated till hatching. The larvae were reared until 4th instar under laboratory conditions (28±2°C and 70±5% relative humidity) following the standard methodology of rearing, according to Elyamani *et al.* (2020). The larvae fed on fresh mulberry leaves three times/day in a plastic tray (30 larvae/tray). Upon reaching the fourth and fifth larval instar, six larvae groups each of 30 larvae for each instar were sorted, split to separate trays and replicated four times after 24 h of start the feeding. Five groups of each instar were incubated at 40 °C for periods of 3, 5, 7, 9 and 12 h and the last group was left under room condition as control. After the heat treatment, the tested larvae were transferred to ordinary rearing conditions at 25-28 °C and fed until mounting.

Different biological characteristics were measured such as cocoon weight, cocoon shell weight, and cocoon silk ratio. For technological measurements, five cocoons of each treatment were dried in an oven at 60°C for 8 h to be reeled individually. The length, weight and size of reeled silk filament were measured for each cocoon.

Samples of insect's hemolymph were obtained by removing a thoracic leg for tested larvae and collected in an Eppendorf tube 1.5 ml containing few of phenyl-thiourea (PTU) crystals to prevent the pigmentation of samples and the tubes were kept at 20 °C. The blood samples were centrifuged at 10000 rpm for 10 min at 5°C. The supernatant was immediately assayed to determine *aspartate* transaminase *(AST)*, alanine aminotransferase (ALT) activities, total soluble protein (TSP) and catalase as an antioxidant enzyme.

Phosphate buffer stock solution (0.2M, pH 7.1), 8.8 g NaH_2PO_4-$2H_2O$ and 51.6 g Na_2 HPO4-$12H_2O$ were dissolved in a suitable amount of distilled water. Two g of SDS were added and the solution was completed to 1 l with distilled water. This solution was suitable to be used up to one week, and it was kept in a brown bottle at 4°C. Electrode buffer solution, one volume of phosphate buffer stock solution (a) was diluted with one volume of distilled water. Phosphate sample buffer 0.01M, pH 7.1: 5 ml phosphate buffer stock solution (a), 1 ml 2-mercaptoethanol, 1 g SDS and the solution was completed to 100 ml with distilled water. Acrylamide solution 22.2 g acrylamide and 0.6 g Bisaerylamide were dissolved in 100 ml distilled water and filtered through Whatman No. 1 filter paper. This solution was suitable to be used within one month and kept in a brown bottle at 4°C. Ammonium persulphate solution; 150 mg ammonium persulphate was dissolved in 10 ml distilled water. This solution was suitable for use for one week in a brown bottle at 4°C. Bromophenol blue 0.25%: 25 mg Bromophenol blue were dissolved in 10 ml phosphate sample buffer (C).

The gel solution (10%) was made by mixing 33 ml phosphate buffer stock solution with 29.7 ml acrylamide solution in a vacuum flask. The solution was desecrated under vacuum for a few minutes, then 3.2 ml ammonium persulphate solution and 0.1 ml N, N, N', N'-tetramethylenediamine (TEMED) were added and mixed avoiding introducing much air. The glass electrophoresis tubes were filled with gel solution. The polymerization reaction was completed within 40 min. When polymerization occurred rapidly, the amounts of TEMED and/or persulphate were decreased.

The basic principle of protein electrophoresis is the movement of the charged protein molecules through a supporting medium towards an electrode with the opposite charge. Upon electrophoresis, silk gland proteins normally separate into five fractions: Albumin, $\alpha 1$, α, β and γ globulins. Albumins are closest

to anode and γ globulins to the cathode. The other three fractions lie between the two extremes in the above-mentioned order. After electrophoresis, the protein fractions were visualized by staining with Coomassie Brilliant Blue (COBB) stain which was specific for proteins. The stained fractions were then interpreted by densitometry using a DCD-16 Gelman densitometer. Refraction of protein bands by SDS electrophoresis in a solution of Sodium Dodecyl Sulphate (SDS) and 2-mercaptoethanol, proteins dissociated into subunits with rod-like shape, in which the diameter of the rods was thought to be constant, while the long axis varied in proportion with the molecular weight. The latter value was determined by comparing the electrophoretic of unknown proteins with the mobility of known protein markers.

Statistical analysis was performed to measure variance (ANOVA) and the means were compared using Duncan's test (≤ 0.05) according to Kumar and Shanker (2017) using Costat program.

RESULTS AND DISCUSSION

The highest weight of fresh cocoons was 1.58 g for cocoons from larvae which were exposed to high temperatures for a period of 5 h when treated larvae at the fourth instar (Table 1). While the weight of fresh cocoons resulted from larvae that were exposed to high temperature for 7 h recorded 1.40 g. On the other hand, the larvae treated with the fifth larval instar gave the highest weight of the fresh cocoons 1.57 g for the larvae that were exposed for a period of 5 h, followed by those exposed to high temperature for 7 h, recorded 1.45 g, compared to 1.18 and 1.24 g which recorded for control during the larval treatment at the fourth and fifth instar, respectively. Statistical analysis showed that there were high significant differences between the averages of larval treated with the fourth and fifth instar. On the other hand, the tabulated data showed that the highest weight of the cocoon shell resulted from the larvae were exposed to high temperature for 5 h at the fourth larval instar was 0.306 g, followed by 0.293 g for those larvae exposed to high temperature for a period of 7 h compared to the control that recorded 0.260 g. At the fifth larval instar, the highest weight of the cocoon shell, which resulted from the larvae that were exposed to high temperature for 5 and 7 h recorded 0.283 and 0.240 g, respectively, compared to the control that recorded 0.260 g (Table 1). Statistical analysis showed that there were no significant differences between the averages of shell cocoon weight for larvae exposed to high temperatures in the larval fourth instar, whereas the very highly significant differences of the larvae exposed to high temperatures in the fifth larval instar. The silk ratio in the cocoon shell was affected by heat treatment, where the highest silk ratio was recorded 22.36% for cocoons produced from the larvae treated at the fourth instar larvae for a period of 5 h, followed by those cocoons that resulted from the larvae that were exposed for a period of 7 h (20.39%). While the larvae treated at

Table 1. Cocoon characteristic of silkworm, *Bombyx mori* on fourth and fifth larval instar exposed to high temperature (40°C) at different periods

Exposure time (h)	Cocoon index					
	Fresh cocoon weight (g)		Shell cocoon weight (g)		Silk ratio %	
	4th	5th	4th	5th	4th	5th
3	1.31^{bc}	1.17^{e}	0.250^{bc}	0.220^{cd}	17.91^{b}	18.75^{a}
5	1.58^{a}	1.57^{a}	0.306^{a}	0.283^{a}	22.36^{a}	19.36^{a}
7	1.40^{b}	1.45^{b}	0.293^{a}	0.240^{bc}	20.39^{ab}	18.03^{a}
9	1.29^{bc}	1.36^{c}	0.263^{abc}	0.233^{c}	19.31^{ab}	16.41^{b}
12	1.23^{c}	1.28^{d}	0.226^{c}	0.210^{d}	18.42^{b}	16.09^{b}
Control	1.18^{c}	1.24d^{e}	0.260^{abc}	0.260^{b}	21.90^{a}	17.13^{a}
F test	10.962	43.441	2.919	15.015	2.702	8.964
P	0.0004***	0.0001***	0.0596^{NS}	0.0001***	0.0735^{NS}	0.0010***
LSD	0.132	0.068	NS	0.021	NS	1.444

***Highly significant at P=0.001. NS–Non-significant at P=0.05.

fifth instar and exposed to high temperature for 5 h gave the highest percentage of silk recorded 19.36%, followed by those that exposed to a temperature for 3 h recorded 18.75% compared to the control that recorded 21.90 and 17.13% for fourth and fifth instar, respectively.

The silkworm was influenced by ambient temperatures and seasonal changes, and that's back to the large changes in the climate, temperatures and humidity in the world. In Egypt, where the current study was conducted, the temperatures changed throughout in the last few years as the daily temperature reached 40°C. Differences in environmental conditions from day to day and from season to others highlighted the need to manage temperature and relative humidity to produce a good cocoon. The results of cocoon characteristics were affected by the exposure periods to high temperatures and larval instar (Umarov *et al.,* 2021).

Also, cocoon and shell weight significantly increased to 17.52 and 19.44% over control, respectively as also reported by Batirova and Umarov (2023). Indicators in different rearing regions were reflected in the correlation with the natural climatic conditions of each region during the worm feeding season. Also, they found that the hot climate had a positive effect on the biological and cocoon productivity indicators of the hybrids. Whereas Mir and Qamar (2018) and Chakraborty and Dastidar (2022) found that the survival rates were inversely proportional to the high temperatures 42-48°C, as the larval weight, pupal weight, egg-laying, hatching percentage, and cocoons characteristics were affected by decreasing the temperature when they studied the effects of high temperature on growth, yield and production of the Eri silkworm (*Samia ricini* D.). In contrast, Tanjung and Lenny (2019) explained that the application of thermal shock using the temperature of 42° for larvae of the fifth instar led to the failure of the cocoon formation and the irregularity of the shape of the cocoon.

The longest silk filament was recorded for the control 1143.60 m, followed by the larvae that were exposed to high temperature for a period of 5 hr, recording 1141.85 m (Table 2). All the exposure times gave filament silk that was less than control. Moreover, the longest filament silk was recorded for the larvae exposed to a temperature at the fifth larval instar was 1172.65 m for larvae that were exposed for 5 h, followed by those larvae that were exposed to high temperature for a period of 7 h, and recording silk filament length 1111.65 m, compared to the control that recorded 1022.80 m. Statistical analysis showed that there were highly significant differences between the averages of filament length. The weight of filament silk was affected by the time of exposure to high temperatures and the larval instar. Moreover, the control recorded 0.237 g weight of silk filament equal to the silk filament produced from the larvae that were exposed to high temperature for a period of 5 h at fourth larval instar, while all treatments recorded a lower silk filament weight of the control. In contrast, the treatment of the fifth larval instar,

Table 2. Silk filament characteristic of silkworm, *Bombyx mori* on fourth and fifth larval instar exposed to high temperature (40°C) for different periods

Exposure time (h)	Filament characters					
	Filament length (m)		Filament weight (g)		Filament size (dn)	
	4th	5th	4th	5th	4th	5th
3	989.10^{bc}	899.80^{d}	0.227^{ab}	0.212^{ab}	2.16^{a}	1.97^{ab}
5	1141.85^{a}	1172.65^{a}	0.237^{a}	0.352^{a}	2.19^{a}	3.48^{a}
7	1068.35^{a}	1111.65^{ab}	0.225^{ab}	0.252^{ab}	1.78^{b}	2.12^{ab}
9	937.20^{c}	966.20^{cd}	0.195^{bc}	0.212^{ab}	1.69^{b}	1.59^{b}
12	877.8^{c}	934.90^{cd}	0.165^{c}	0.197^{b}	1.65^{c}	1.94^{ab}
Control	1143.6^{a}	1022.80^{bc}	0.237^{a}	0.220^{ab}	1.88^{b}	1.93^{ab}
F test	8.42	11.623	6.878	1.311	7.227	1.548
P	0.0003***	0.0001***	0.0009***	0.3031^{NS}	0.0007***	0.2249^{NS}
LSD	112.401	92.414	0.032	NS	0.256	NS

***Significantly different at P=0.001. NS–Non-significant at P=0.05.

the highest weight of the silk filament was 0.335 g for larvae that exposed to high temperature for a period of 5 h, followed by larvae that were exposed to high temperature for a period of 7 h recorded 0.252 g, compared to the control 0.220 g. There were no significant differences among the average weight of filament in case of treatment at the fifth instar, whereas the averages recorded high significant differences in the fourth larval instar (Table 2).

The highest silk filament size was recorded as 2.19 dn for larvae that were exposed to high temperature for a period of 5 h, followed by 2.16 dn recorded for larvae exposed to high temperature for 3 h at treatment in the fourth instar. In the fourth larval instar, the highest silk filament was recorded 3.48 dn for larvae exposed to high temperature for a period of 5 h followed by 2.12 dn for larvae exposed to high temperature for a period of 7 h, compared to control that recorded 1.93 dn (Table 2). Statistical analysis showed that there were highly significant differences between the averages of silk filament size of the larvae exposed at the fourth larval stage, while the significant differences were not significant for the larvae exposed at the fifth larval instar.

The total protein in tested larvae hemolymph was affected by a certain exposure period under the tested temperature degree (40°C) and larval instar (Fig. 1). The relatively high total soluble protein content 2.42 g/dl was recorded for the fourth instar larvae exposed for 5 h, followed by 2.30 g/dl for that exposed to 7 h. In the case of the fifth instar, the relatively highest total soluble protein content of 2.28 g/dl was recorded for larval exposed for 7 h and followed by 2.12 g/dl recorded for that exposed to 5 h. In the case of catalase enzyme, the larvae of control treatment manifested the highest enzyme content of 282.0, followed by 211.0 µ/ml recorded for fifth instar larva exposed for 3 h at the tested temperature. The last treatments gave intermediate levels of catalase enzyme and ranged 93-211.0 µ/ml. Regarding the alanine transaminase (ALT) enzyme, the highest activity level of ALT of 3.01 µ/ml was recorded for control larvae, while 2.59 and 3.84 µ/ml were recorded for the larvae of fourth and fifth instars exposed for 9 h under 40°C, respectively. On the other hand, the lowest activity level of 1.78 mg/ml was recorded for the fourth instar larvae exposed for 3 h. In the same trend, the aspartate transaminase

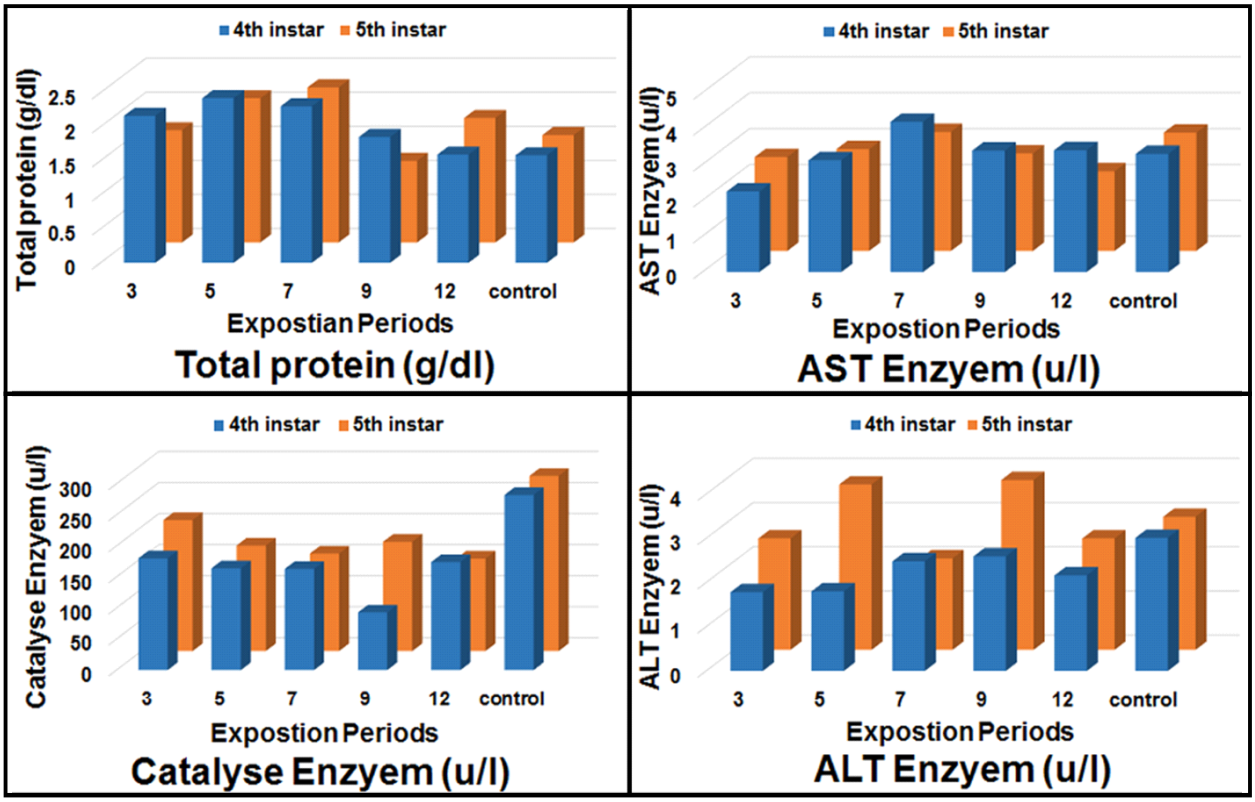

Fig. 1. Physiological parameters of the silkworm, *Bombyx mori* fourth and fifth instar larvae exposed to constant temperature (40°C) at certain exposure periods.

(AST) enzyme activity was influenced by each exposure period and larvae instar where the relatively high activity levels of 4.19 and 3.32 µ/ml were recorded for the fourth and fifth instars exposed for 7 h under-tested temperature, respectively, than compared with control 3.01 µ/ml.

The high temperature caused significant changes in the hemolymph total protein levels of the treated larvae. Also, the periods of exposure under-tested high temperature affected the total protein level. In contrast, the catalase enzyme level decreased in treated larvae than that in untreated, where the control larvae recorded the highest level of the enzyme, which enhanced the ability of the larva to resist diseases and remain strong until the formation of the cocoon. Concerning the aspartate transaminase (AST) enzyme activity, it was affected by exposure periods under high temperature. In the case of the alanine transaminase (ALT) enzyme, the activity level decreased in the fourth instar treated larvae compared to the control, whereas the highest level was recorded in the treated fifth instar larvae compared to the control too. So, the high temperature affected the physiology of silkworms, which was reflected in the productive and biological characteristics of the silkworm.

For the generation of molecular markers based on protein polymorphisms, the most common technique is the electrophoretic separation of proteins, then specific staining of a unique protein subclass. The clear difference in protein band formation due to exposure to high temperatures illustrated the susceptibility of silkworms to high temperatures. In addition, the different times of exposure to high temperature led to a variation in the appearance of protein bands, which were not like protein bands in the control.

The electrophoretic patterns of protein in the hemolymph of silkworm at the end of the fourth and fifth larval instar exposed to 40°C temperatures throughout periods of 3, 5, 7, 9 and 12 h were observed in Fig. 2 with distinct variation in the movement of some protein bands of silkworms' hemolymph. The protein patterns on SDS-PAGE cleared that most of the treatments gave four protein bands except the fourth instar larvae exposed to the tested temperature for 5 h and recorded five protein bands. While some treatments recorded three

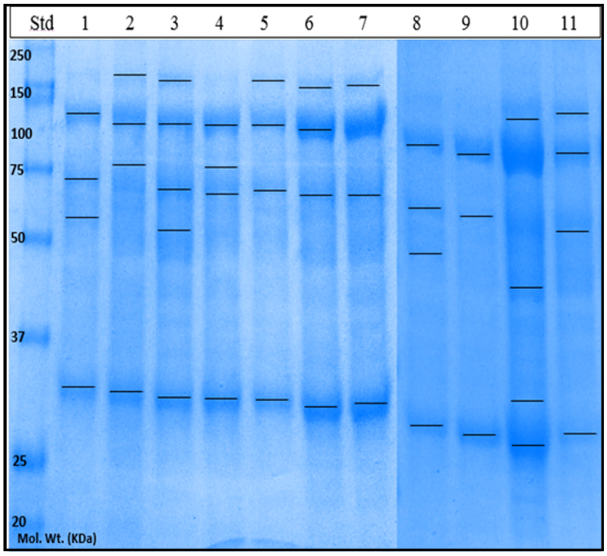

Fig. 2. SDS-Page pattern of hemolymph protein of silkworm, *Bombyx mori*, 4th and 5th larvae instars exposed to high temperature for periods 3, 5, 7, 9 and 12 h. Std–Standard marker; Lane 1: Control, Lanes: 2, 3, 4, 5 and 6 in 4th instar larvae exposed for 3, 5, 7, 9 and 12 h under 40°C, respectively. Lanes: 7, 8, 9, 10 and 11 at 5th instar larvae exposed for 3, 5, 7, 9 and 12 h under-tested temperature, respectively.

protein bands for silkworm larvae (fourth instar exposed for 9 h, fifth instar exposed for 3 and 7 h under-tested temperature degree). The hemolymph protein profile of control was seen in four bands with molecular weights of 125, 72, 57 and 31 KDa.

As shown in Table 3, the fourth instar larvae exposed to a tested temperature for the period of 3 h was characterized with protein bands having molecular weights of 195, 117 and 82 KDa, while that exposed for 5 h, was distinguished by bands with molecular weight of 52 KDa. Also, shared the protein band had molecular weight 116 KDa recorded for that exposed for 7 h and the larvae of the same instar exposed for periods of 5 and 12 h shared the protein band with a molecular weight of 175 KDa, and those exposed for periods of 5, 7 and 9 h shared the protein band with molecular weight 30 KDa. Regarding fifth instar larvae exposed for certain tested periods the molecular weights of protein bands were recorded as follows; those that exposed for a 3 and 5 h demonstrated some characteristic bands with molecular weights of 174 and 65 KDa than 95, 60 and 48 KDa, respectively; while shared the protein band had molecular

Table 3. Molecular weights of hemolymph protein of silkworm, *Bombyx mori*, 4th and 5th instars larvae exposed for certain tested periods under 40°C temperature

Mol. wt. (KDa)	Control	4th instar (h)					5th instar (h)				
		3	5	7	9	12	3	5	7	9	12
250	-	-	-	-	-	-	-	-	-	-	-
195	-	+	-	-	-	-	-	-	-	-	-
190	-	-	-	-	+	-	-	-	-	-	-
175	-	-	+	-	-	+	-	-	-	-	-
174	-	-	-	-	-	-	+	-	-	-	-
160	-	-	-	-	-	-	-	-	-	-	-
150	-	-	-	-	-	-	-	-	-	-	-
127	-	-	-	-	-	-	-	-	-	-	+
125	+	-	-	-	-	-	-	-	-	-	-
123	-	-	-	-	-	-	-	-	-	+	-
117	-	+	-	-	-	-	-	-	-	-	-
116	-	-	+	+	-	-	-	-	-	-	-
114	-	-	-	-	+	-	-	-	-	-	-
105	-	-	-	-	-	+	-	-	-	-	-
100	-	-	-	-	-	-	-	-	-	-	-
95	-	-	-	-	-	-	-	+	-	-	-
89	-	-	-	-	-	-	-	-	+	-	-
84	-	-	-	-	-	-	-	-	-	-	+
82	-	+	-	-	-	-	-	-	-	-	-
78	-	-	-	+	-	-	-	-	-	-	-
72	+	-	-	-	-	-	-	-	-	-	-
68	-	-	+	-	+	-	-	-	-	-	-
66	-	-	-	+	-	+	-	-	-	-	-
65	-	-	-	-	-	-	+	-	-	-	-
60	-	-	-	-	-	-	-	+	-	-	-
59	-	-	-	-	-	-	-	-	+	-	-
57	+	-	-	-	-	-	-	-	-	-	-
55	-	-	-	-	-	-	-	-	-	-	+
52	-	-	+	-	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-	-	-	-	-
48	-	-	-	-	-	-	-	+	-	-	-
43	-	-	-	-	-	-	-	-	-	+	-
37	-	-	-	-	-	-	-	-	-	-	-
31	+	+	-	-	-	-	-	-	-	+	-
30	-	-	+	+	+	-	-	-	-	-	-
29	-	-	-	-	-	+	+	+	-	-	-
27	-	-	-	-	-	-	-	-	+	-	+
26	-	-	-	-	-	-	-	-	-	+	-
25	-	-	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-	-	-

weight 29 KDa for that exposed for 12 h. As well as, exposed for 7 and 12 h shared the protein band with molecular weight 27 KDa (Table 3).

This appearance of these specific new bands which were characteristic for each treatment were attributed to changing into one or more of the other protein components of the larvae hemolymph, or to the increase of the rate of digestive (amylase) and oxidizing (succinate dehydrogenase) enzymes which helped in the utilization of exogenous food materials, leading finally to more production.

The study also showed that the exposure of the 4th instar larvae to high temperatures for certain periods affected the release of protein bands, especially when exposed for 5 h than those of the 5th instar. The study agreed with that of Chakraborty and Dastidar (2022) who found that heat stress resulted in additional protein types in the larvae hemolymph. The kinetics of 72 KDa was completely different within and between the fourth and fifth instars larvae hemolymph after exposure to three temperatures ranging from 18 to 44°C for three consecutive days for one hour. The expression of thermal shock proteins varied in different cases of larval instar and the protein increased as a result of thermal shock in the fifth instar hemolymph. Heat shock proteins were discussed as molecular markers for the evaluation and development of heat-tolerant

silkworm strains in tropical regions. Moreover, Matsuoka and Sakamoto (2018) found that exposure to 40.0°C for 4 h, substantially elevated tolerance to the threshold heat shock and simultaneously increased levels of 70 and 27 kDa proteins, whereas eexposure to 10.0°C for 4 h lowered heat tolerance and did not alter the expression of 70 and 27 kDa proteins. These putative heat-shock proteins of 70 and 27 kDa might be involved in the effect of mild temperature hardening on heat tolerance. Farahani *et al.* (2020) stated that the activity of two antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT), increased with heat stress, suggesting that these enzymes served a protective role for the insects. These results showed the differential induction of the two HSPs' transcripts with heat stresses. These results suggest application of sudden heat thermal in breeding strategy for inducing robustness in productive breeds, and this result agreed with that of Fang *et al.* (2021) who found that increasing of resistance to heat shock was noticed as larval development proceeds and increased thermotolerance was achieved with the induction of heat shock protein 72 h in the 5th instar larval hemolymph and it was significantly improved over control when the larvae were exposed to heat shock temperatures of 34, 38 and 42°C for 3 h. In a similar study by Tanjung and Lenny (2019) exposed the fifth instar larvae to thermal shock for 3 hr at 42°C until the cocoon was formed. The resulting pupae were analyzed in terms of total protein, total fat and carbohydrates. The results of protein 80.4%, total fat 2.13% and 6.58% carbohydrates for exposed larvae, while the protein in cocoons was not affected.

CONCLUSION

The present study demonstrated the importance of exposure of silkworms to high temperatures for certain periods and its effect on biological, productive, physiological characteristics and protein profile of the silkworm, *Bombyx mori*. The results varied according to the exposure periods to the high temperature as well as the larval age, which the silk ratio in the cocoon shell was affected by high temperature, where the highest silk ratio was produced from cocoons that resulted from fourth instar larvae which treated by high temperature for 5 h, followed by those cocoons that resulted from the larvae that exposed to high temperature for 7 h. The results indicated that exposure to high temperatures for specific periods may benefit the biological, technological characteristics, and generation of the protein component of silk, till the exposure period does not exceed more than 9 continuous hours.

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