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GROWTH AND DEVELOPMENT OF THE SILKWORM (*BOMBYX MORI* L.) C301 WITH HEAT SHOCK TREATMENTS

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Abstract

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Growth and development of the silkworm larvae are strongly influenced by environmental changes such as temperature. The role of temperature on the growth and development of larvae is included as new studies on heat shock proteins. Studies on the growth and development of silkworm larvae by heat shock with different temperatures have been conducted in physiology animal *Laboratory Department of Biology* University of Sumatera Utara. The Research design used was a complete randomized design (CRD) with four treatments and five replications each consisting of 20 larvae. The treatment consists of administering heat shock at temperature of 34, 38 and 42°C, and without heat shock (ambient temperature). Heat shock was conducted in early 4th instar for 3 hours. The research results showed that of the heat shock can increase the percentage of mortality, accelerate the larval stage, reducing larvae weight by lowering the growth; consumption and digestibility. It also decrease productivity and lead to failure of the formation of cocoon, pupae and imago.

Key words: Bombyx mori; development; heat shock; growth

Introduction

The main factor that affects the physiology of insects is the temperature and humidity (Couret et al., 2014). Most insects can adapt to daily environmental temperature fluctuations (Damos and Savopoulou-Soultani, 2011; Chen et al., 2015). Fluctuation in environmental conditions is maintained by maintaining the internal temperature and water content, but it has a limit of tolerance (Singh et al., 2009). *Bombyx mori* is very sensitive to fluctuations in the environment, and cannot survive to the extreme temperature fluctuations. The ability to adapt in environmental conditions affects the physiology of larvae. Furthermore, it depends on a combination of environmental factors and developmental stage. Adaptations made by the larval are to extend the stage of metamorphosis, survival and multiplication rate. They also modify melanization processes, as well as increasing the levels of the lysozyme and phenoloxidase enzymes (PO) (Adamo and Lovett, 2011; Khaliq et al., 2014).

Temperature has a direct correlation with the growth of silkworm. The wide temperature fluctuations are harmful to the development of the larvae. The rise in temperature will affect the physiological development and decrease in temperature will cause a decrease in physiological (Rahmathulla et al., 2004). Increasing temperature during maintenance, accelerate larvae growth and shorten the period of larvae especially in late instar period. On the other hand, at low temperatures, the growth and larvae period is become slower. More than a third of herbivorous insects show a decrease in the level of consumption in high temperatures (Lemoine et

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al., 2014). Larvae reared to produce silk in a cocoon shape by eating mulberry leaves during the feeding period. Larvae are breaded to produce silk in a cocoon shape by eating mulberry leaves during the feeding period. Biological characteristics of insects in the chrysalis phase are strongly influenced by environmental temperature. The ability of individuals to survive in varied environments will provide a strong capital base for the organism to adapt in an unstable environment or even extreme. However, this situation will make an organism which unable to cope with stress, will damage or even cause death at the individual level and extinction at the level of population (Badyaev, 2005). Organisms face environmental stress with avoidance coping or remain in that environment with all its consequences. Some organisms are capable of coping with stress namely by changing physiology, morphology and behavior strategy (Adamo and Lovett, 2011; Lemoine et al., 2014). If the environmental stress is caused by biotic factors, then some changes, especially behavioral changes will be made in accordance with the interactions to the biotic factors, some are mutual, parasites, or even predators and prey (Badyaev, 2005). On the contrary, if the environment stress comes from abiotic factors, generally the response of organism will be physiological changes manifested by changes in phenotype, for example, shortening or lengthening the instar stage period in the life cycle as a coping strategy and continue the next life stage (Whitman and Agrawal, 2009).

Materials and Methods

The silkworm eggs used in this research are poly hybridization, derived from Candiroto silkworm breeding center, Temanggung, central java, Indonesian. The research was conducted using completely randomized design (CRD), which consists of four treatments with five replicates and each replicate consisted of 20 larvae. The treatments are heat shock, given to early instar 4th stage for 3 hours at a temperature of 34°, 38°, 42°C and without heat shock (ambient temperature). Mulberry leave which used as feed is *Morus sp*. Heat shock treatment is done by placing the larvae into the oven for 3 hours. After 3 hours the larvae are removed from the oven and then put back in rearing room. Variables measured include larval stage; late 4th and 5th instar the weight gain and feed efficiency (Scriber and Slansky, 1981). Pupa stage includes the weight of pupae, cocoon weight and cocoon shell weight and imago. Quantitative data (dependent variable) obtained, is tested the significance against the effects of the treatment group (independent variables) with the aid of computer statistics program *SPSS release 20*.

Results

Larval Stage

The percentage of mortality, length of the larval stage and larval weight gain by heat shock at a different temperature can be seen in Figure 1. The percentage of mortality in the treatment varies, the higher the heat shock temperature given, the mortality percentage rate is increased, which means a lot of dead larvae were found during rearing to be cocoon. Mortality percentage without heat shock treatment (ambient temperature range 28-31°C) is 0%, at the end of instar 4th and 5th stages, or no larvae were found dead. The percentage of mortality in late instar 4th and 5th with heat shock treatment temperature 34°C is 20%, from 20 larvae, there were four dead larvae.

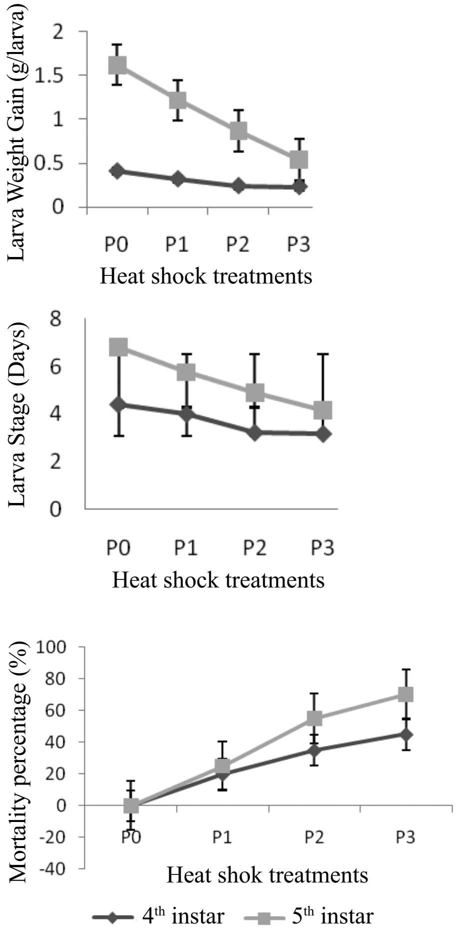

Fig. 1. Silkworm of *Bombyx mori* L. (C301) larval growth reared under various heat shock conditions. P0 = without heat shock (ambient temperature); P1 = heat shock temperature of 34°C; P2 = heat shock temperature of 38°C; P3 = heat shock temperature of 42°C

Overview of larvae feed efficiency by heat shock treatment with different temperatures can be seen in Table 1. The dry food material consumption in instar 4th at P0 (without heat shock) and P1 (heat shock temperature of 34°C) was not significantly different (P > 0.05). However, it different significantly from P2 (heat shock temperature of 38°C) and P3 (heat shock 42°C). The same effect was found in the efficiency conversion of feed digestibility (ECD) and feed conversion. ECD and the feed conversion without heat shock treatment (P0) and heat shock temperature of 34°C (P1) did not differ significantly, but significantly different with heat shock treatment temperature 38°C and 42°C.

The efficiency rate of food consumption in fifth instar stage can be seen in Table 2. The dry food material consumption in fifth instar, decreased in accordance with the increasing of temperature of the heat shock treatment. In the treatment without heat shock (P0) and heat shock temperature of 34°C (P1) is 2.19 and 1,94 g/larvae (P > 0.05) and significantly different from the heat shock 38 and 42°C temperature is equal to 1.47 and 1.28 g/larvae. Larvae digestibility value also decreased, the higher the temperature given, digestibility will decreases. Digestibility value significantly different among treatment without heat shock, with a shock heat treatment temperature of 34°C and with a heat shock temperature of 38 and 42°C. As well as for ECI, the higher the temperature, efficiency of conversion of feed ingested value will decreases. Larvae efficiency of conversion of ingested (ECI) value significantly different between treatment without heat shock, heat shock treatment temperature of 34°C, with a heat shock temperature of 38 and 42°C.

Efficiency conversion of feed digestibility (ECD) in fifth instar, decrease in accordance with increasing of the heat shock temperature needed. In the treatment without heat shock (P0) significantly different heat shock temperature of 34, 38 and 42°C. Similarly for the value of feed conversion is decreased, the higher the temperature, feed conversion will be decreases. Larvae feed conversion values significantly different between treatment; without heat shock, with a shock heat treatment temperature of 34°C, a heat shock temperature of 38 and 42°C.

Pupae Stage

Observations on the pupae stage (cocoon shell weight, cocoon larvae and pupae) of *Bombyx mori* (C301) by heat shock with different temperatures are presented in Table 3.

Bombyx mori larvae by heat shock with different temperatures have different effects on the picture cocoon and pupa. Weight of cocoon decrease in without heat shock treatment and showed a significantly different with heat shock treatment (P < 0.05). In without heat shock treatment, cocoon weight is 1.26 g. While on treatment by heat shock at a temperature of 34, 38 and 42°C are 1.10 g; 0.90 g and 0.61 g. In this case it can be said that the treatment of heat shock reducing the weight of cocoons.

Table 1

The nutritional indices parameters of fourth instar larvae silkworm of *Bombyx mori* L. (C301) reared under various heat shock conditions

Treatments	Dry Feed Material for Consumption (g/larvae)	Approximate Digestibility (%)	ECI (%)	ECD (%)	Feed Conversion
P0	0.55a	58.75a	14.38a	24.80a	1.31a
P1	0.43a	47.62b	12.12b	23.56a	1.17a
P2	0.40b	42.07b	11.38b	20.61b	0.99b
P3	0.32b	40.45b	10.63b	20.09b	0.91b

P0 = without heat shock (ambient temperature); P1 = heat shock temperature of 34°C; P2 = heat shock temperature of 38°C; P3 = heat shock temperature of 42°C; ECI = efficiencyof conversion of feed ingested; ECD = conversion efficiency of feed digestibility

Table 2

The nutritional indices parameters of fifth instar larvae silkworm of *Bombyx mori* L. (C301) reared under various heat shock conditions

Treatment	Dry Feed Material for Consumption (g/larvae)	Approximate Digestibility (%)	ECI (%)	ECD (%)	Feed Conversion
P0	2.19a	38.80a	14.35a	35.05a	1.49a
P1	1.94a	35.25a	12.33a	26.45b	1.10b
P2	1.47b	33.55b	11.80b	23.55c	1.03c
P3	1.28b	27.83bc	11.16b	20.83c	0.91c

P0 = without heat shock (ambient temperature); P1 = heat shock temperature of 34°C; P2 = heat shock temperature of 38°C; P3 = heat shock temperature of 42°C; ECI = efficiencyof conversion of feed ingested; ECD = conversion efficiency of feed digestibility

Table 3
The average of single cocoon, single shell and pupa weight larvae *Bombyx mori* (C301) by heat shock with different temperatures

Treatment	Single cocoon weight (g)	Single shell weight (g)	Pupa weight (g)
P0	1.26a	0.28a	0.99a
P1	1.10b	0.19a	0.89a
P2	0.90bc	0.09b	0.78b
P3	0.61c	0.08b	0.49c

In Table 3 also shows that cocoon shell weight and pupa weight was decreased too. The higher the temperature of the heat shock treatment, the cocoon and pupa shell weight is getting down. Cocoon shell weight reduction between without heat shock treatment and heat shock treatment temperature of 34°C is not different significantly; 0.28 and 0.19 g, yet it significantly different from heat shock treatment of 38 and 42°C degree; 0.09 and 0.08 g. Observation of the pupa weight between no heat shock and 34°C heat shock treatment was not significantly different but it significantly different when applied in heat shock treatment temperature of 38 and 42°C, the weight data in sequence are 0.99; 0.89; 0.78 and 0.49 g. From the above observations can be expressed that heat shock treatment affects quality of cocoons which is lowering the quality of cocoon and pupae.

Discussion

The changes in temperature rearing cause dead for larval. It also occurs in Sinha and Sanyal research (2013) exposure to heat at a temperature of 17°C and 33°C to silkworm is still able to be tolerated while the temperature 43°C cause deadly effect. This is happened due to the inability of larvae to adapt themselves against the heat shock. Deaths caused by the heat shock will give stress, inability to cope with stress can be fatal and cause death at the individual level and extinction at the level of population (Badyaev, 2005). High temperatures affect almost all biological processes, including biochemical and physiological reactions (Willmer et al., 2004; Regniere et al., 2012). Larval rearing at high temperatures can cause death (Khaliq et al., 2014). The optimum temperature for the normal growth of silkworm larvae is between 20 and 28°C, the desired temperature range for maximum productivity is from 23 to 28°C. Temperatures above 30°C directly affect the health of larvae. Effect of temperature and humidity on the growth and development of larvae including new studies on heat shock proteins (Neven, 2000; Rahmathulla, 2012; Sinha and Sanyal, 2013). Dead silkworm larvae characterized by declining growth and issued a yellow liquid. The dead larvae always issue a yellow liquid.The release of these fluids due to the inability of larvae in adapting to extreme environmental changes, causing inability in maintaining body fluid homeostasis.

The Figure 1 it also seen that the higher the temperature of the heat shock treatment, the faster the larval stage will be. In 4th instar, without long heat shock, larval stage period in an average of 4.40 days and with heat shock treatment of 34°C was 4 days ($P < 0.05$). Meanwhile in heat shock treatment of 38 and 42°C, instar stage period is 3.23 and 3.18 days. This was significantly different with no heat shock and temperature of 34°C. Without heat shock and temperature of 34°C does not give different effects, this is due to the larvae still able to tolerate heat shock of 34°C degree, compared to 38 and 42°C degree. According to Benchamin (1983), the larval stage can be done faster if the optimun temperature is still in a tolerable limit. The same effect was found in the fifth instar stage, without heat shock treatment and with heat shock treatment of 34°C did not differ significantly, but significantly different from larvae with heat shock treatment of 38 and 42°C. This shows that the treatment of heat shock accelerate the larval stage, which makes a lot of larvae died or can not meet the needs of the body because of the swift physiological processes and finally cannot survive. Shortage of instar stage cause larvae cannot meet their needs in preparation for entering the cocoon stage. According to Scriber and Slansky (1981), temperature can affect physiological processes of larvae, varied in temperature above the optimum temperature will cause the failure of physiological processes and cause growth disorders. Increasing temperature in silkworm rearing can reduce rearing time. Rearing time can be reduced for 10 days (Benchamin, 1983). According to Reddy et al. (2002), temperature and humidity provide synergistic effects on larval period. Changes in temperature and humidity have an effect on the molting period (Mishra and Upadhyay, 2002). A decrease in temperature increases the duration of larval moulting (Morohoshi, 1969; Kamili and Masoodi, 2004). Several studies have shown that silkworm larvae are more sensitive to high temperatures during instar 4th and 5th stages (Ueda and Lizuka, 1962; Tazima and Ohuma, 1995). The average weight gain in fourth instar larvae is diminishing at P0 (without heat shock) of 0,41 g/larvae and P1 (heat shock 34°C) of 0,32 g/larvae. This is significantly different from the treatment of heat shock 38 and 42°C. This phenomenon is also found in 5th instar. Increasing temperature of the heat shock treatment cause in a decrease of larval weight gain. In the treatment without heat shock and heat shock treatment at 34°C significantly different from the treatment of heat shock 38 and 42°C ($P < 0.05$). Without heat shock treatment at a temperature of 34°C were not significantly different, it is correspond with Scharf et al. (2015), temperature of 34°C had a positive influence on growth, imago and fertilization phase. In *Gryllus texensis*-cases, temperature of 33°C increase the number of eggs and weight (Adamo and Lovett, 2011).

The variation in temperature fluctuations cause the insects become unable to achieve optimal physiological performance and will be achieved it when placed in ideal and favorable condition. Natural selection due to less ideal environmental conditions, insects have a certain ability to evaluate the environment and make decisions in response involvesm in physiological, behavioral, and genetic aspects. This response often involves changes in consumption and food utilization, consumption levels and mealtimes, deviant behavior, metabolism, synthesis of enzymes, nutritional, physiological processes and other behaviors. Temperature treatment can affect physiological performance which different than a constant temperature. Fluctuating temperatures will stimulate growth performance (Scriber and Slansky, 1981; Jaworski and Hilszczanski, 2013).

The development of adaptation of various enzymatic and metabolic processes that allow the larvae to survive and thrive in a wide range of temperatures. Temperature acclimation, physiological and behavioral thermoregulation allows insects to compensate at various levels of environmental temperature changes (Heinrich, 1981). The 5th instar stage with stress temperature of 35±1°C increases catalase enzyme activity, especially in fat tissue but at a temperature of 40 ± 1°C decrease the activity (Nabizadeh and Kumar, 2010).

Larval growth embodied by the accumulation of organic materials which resulting from the balance between anabolic and catabolic reactions with nutrients absorbed after digestion of food. When the temperature exceeds 30°C, metabolic functions become erratic and causing health problem. At the same time when the temperature falls below 20°C, metabolic function is inactive leads to irregular growth and worse in health (Tanaka et al., 1971). Food consumption level and nutritional qualities affect larval growth, weight and survival (Murugan and George, 1992). Overall food consumption increases between 20 and 30°C temperature and when the temperature is increased feed consumption will decline (Lemoine et al., 2014).

Insects obtain energy and nutrients through eating to support growth and development. Pathway involved in the regulation of eating is the insulin signaling. Insulin signaling regulate food intake through crosstalk with sulfa neuropeptide quinine in *Tribolium castaneum*. Insulin-like Receptor (INR) decrease food intake in instar stage 4th and 5th which will lead to weight loss and death when the larvae metamorphosed into a pupa (Lin et al., 2016). According to Miranda et al. (2002) juvenile hormone can sustain the growth of larvae. Methoprene consumption (juvenile hormone analog) 48 hours after ecdysis of fourth instar stage in the 2nd integument dorsal thoracic segment increased instar 5th duration, the weight of the larvae, silk gland, cocoon skin and pupal weight. Moreover, it can control the increase in the production of silk about 24% and can reduce negative effects on cocoon around 12%.

Increasing temperatures on silkworm larvae rearing lowering some index such as the absorption of nutrients, digestibility and digestibility percentage (Rahmathulla et al., 2004). Index of nutrients in some larvae still can be maintained, this is possible because of α-amylase enzyme activity that still can help the body's metabolic processes. According to Kaur et al. (2014) under physiological conditions, α-amylase improve digestive performance of the insects that life under different conditions and improve the biological fitness. Secretion of α- amylase is depent on calcium and chloride ions for integrity and structural activity.

Several experiments were performed to determine the importance of α-amylase in the digestive process of the fourth instar stage of *P. Brassicae*. Among other things are suitability of α-amylase activity in different preparations on the mesenteron larvae stage, purification of the enzyme by three-step purification, characterization of α-amylase purification such as pH and optimum temperature as well as response to specific inhibitors that performed in vitro. The effect of acarbose on nutritional index and in vivo amylolytic activity and determining the responsible genes for the secretion of α-amylase. The last is response of larvae acarbose metabolic expression (Kaur et al., 2014). Effect of heat can cause damage to the nervous system and endocrine (Neven, 2000).

Table 1 can also be seen for the value of 4th instar silkworm digestibility between treatments without heat shock was significantly different from that given heat shock at a temperature of 34, 38, 42°C are 58.75%; 47.62%; 42.07% and 40.45%. It is also equal to the efficiency of conversion of feed ingested (ECI) value, without heat shock treatment was significantly different from that given heat shock. Nutritional analysis indexes such as the level of consumption, digestion, assimilation and conversion on larval development will be useful in understanding racial differences in the digestive system and the assimilation ability of silkworm larvae.

High temperatures affect almost all biological processes including the level of biochemical and physiological reactions (Hazel, 1995; Neven, 2000; Kumar, 2010) finally affect the quality and quantity of cocoon development. Physiological activity, food consumption, and economic parameters are influenced by silkworms body temperature. Increased consumption of mulberry leaves during late instar varies inversely with the decreasing of rearing temperature (Sigematsu and Takeshita, 1967; Kumar, 2010). Various environmental factors influence the nutrition and water needs of insects (Rahmathulla and Devi, 2001). Silkworms feed conversion are at maximum in low temperature (26°C) throughout the period of the larvae, it will protect viability better on caterpillar bivoltine (Muniraju, 1999).The conversion efficiency will be at high stats when the larvae are reared at a low temperature (Shen, 1986). Index parameters such as absorbed feed nutrients, ingested feed, digested forecasts, and the ratio of digestion will be goodunder optimum temperature (23-25°C) and humidity (65-70%). According to Rahmatullah (2003) humidity and nutritional indexs affect the development of the silk gland.

Temperature is an important factor in the enzymatic activity. Elevating temperature of one media from the optimal values increase the rate of enzyme catalyzed reactions becuse kinetic energy and the molecule becomes higher in frequency (Delkash-Roudsari et al., 2014). In the case of temperature, it was reported that the optimal value for α-amylase activity in studied insects are at 30-60°C (Zibaee, 2012).

If the temperature rises exceed 22-25°C during the formation of the cocoon, cocoon shell becomes very loose and not strong. It also changes the nature of sericin, induce filament silk and causes difficulty in forming a cocoon. Low temperature is slowing the secretion of silk yarn and resulting in large-sized cocoon. Furthermore, it takes a very long time to form a cocoon spun, relative humidity (60-70%) induce health and good quality cocoons. If the temperature is increased beyond the optimal temperature, larvae and pupae will be stressed and may be susceptible to interference in pupae and cocoon formatting that eventually lead to death at the pupae stage. Low humidity causes a double layered cocoon and cocoon loose. Excessive humidity will cause the release of gas from the feces and urine of silkworm during spinning. Temperature and humidity affect the larvae during the formation of the cocoon (Ramachandra et al., 2001; Manisankar et al., 2008). Different temperatures are very influential during the establishment of cocoon stage (Gowda and Reddy, 2007).

Imago Stage

Silkworm's fecundity with heat shock treatment differs when exposed to different temperatures. From the observation showed that heat shock as previously discussed may affect mortality, instar stage, growth and nutritional indices as well as the development of pupa stage which finally also affect the development of the imago. Fertilization and eggs producing is occurred in no heat shock treatment. Meanwhile, heat shock treatment given to five pairs of the inbred resulting in failure of fertilization. With the result of fewer eggs produced or even does not produce eggs at all. Especially at a temperature of 42°C heat shock. The level of egg production is varied due to temperature changes. Appropriate changes of temperature will be able to stimulate the production of eggs. But in otherwise, if the changes are not optimal, egg cannot be produced (Mathur and Lal, 1994). The minimum and maximum temperature range is closely connected with the development of the individual (Dixon et al., 2009). Limit temperature range for reproduction is much narrower than the temperature range for growth. Females (*migratory locust*) failed to form mature eggs when the temperature changes of day and night between 30°C and 20°C. Drosophila at a temperature of 32°C causes 50% of sterile female and 96% sterile male. According to Mathur et al. (1988) ovulation and optimum fertility of *Bombyx mori* is at 25.36±0.17°C temperatures and fluctuations of the optimal level will decrease ovulation, ovipositor, fecundity and increased retention of eggs. According to Mahmoud (2013) fecundity and fertility is strongly influenced by temperature, especially in male moths. Imago of *B. mori* would die when placed in a temperature of 40+5°C but could still lay eggs when placed in a temperature of 30+1°C. When they beds larvae spawn by 2494 number of eggs and die within 72 hours. At a temperature of 10+1°C larvae undergo diapause and delay in laying eggs but the number of eggs is relatively much more in 2619 grain (Wanule and Balkhande, 2013). *Gryllus texensis* which breed for 6 days at a temperature of 5-7°C above optimal temperature affect it reproduction rate (Adamo and Lovett, 2011). Temperature change also affects the color and the number of eggs as well as lead to vulnerability in the embryonic period (Kogure, 1933). The poikiloterm, insect physiology and metabolism is under the control of ambient temperature (Chu et al., 2016).

To get the size of the filament, cocoon even size and good quality, required heat-resistant larvae rear. Basic genetic and genetic variability in expression of quantitative and qualitative nature when exposed to high temperatures is an important step for the selection of the oldest thermotolerance resource potential for the breeding program. To achieve greater success, it's necessary to understand the molecular mechanisms of larval temperature tolerance, identification of various heat shock protein (Hsp) groups (Neven, 2000). It is necessary to find genes that are responsible for the induction of heat with the steps on finding their genomes in silkworm.

Conclusion

The development and growth of the silkworm larvae is greatly influenced by it temperature of rearing. The heat shock treatment given in early larval instar stage 4th led to a faster period of larval stage, reducing the weight of larvae and the index value of nutrition and productivity of cocoon and cause failure of development of the imago.

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