

LED Lighting as a Modulator for *Bombyx mori* L. Egg Hatching and Biochemistry

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ABSTRACT

Bombyx mori L. rearers usually face the problem of non-synchronized egg hatching and subsequent low hatchability rate as they do not have equipment to control optimum temperature, humidity and light intensity. The experiment depends on focusing LED lighting on eggs with adjustment of temperature and humidity till hatching. The effect of continuous LED lighting on *B. mori*, on two hybrids (Egyptian and Bulgarian) embryos, for different periods (24, 72, 120, 168, 216 hrs.) was studied. The changes in total proteins, carbohydrates and lipids (mg/g egg weight) titers were estimated colorimetrically. The results showed that a gradual improvement in egg hatchability percentages with shortage in incubation period for treated eggs compared with control. The best results were achieved under the continuous LED lighting till hatching (216 hrs.). The embryos respond to LED exposure by modulating metabolism to adapt lighting stress for both the two hybrids. Also, response of embryos depends on the hybrid origin. It may be recommended to use LED light at the last stage of *B. mori*, embryogenesis as a non-emitting heat light source to enhance and unify hatchability and shorten incubation period.

Keywords: *Bombyx mori* L., eggs, LED (Light Emitting Diode), total protein, total carbohydrates, total lipids, metabolism.

INTRODUCTION

Intense efforts have been made on *B. mori* egg storage programs to find-out the appropriate techniques for achieving highest number of hatchable eggs. One of the used techniques is exposing eggs to different temperature and light regimes. Egg hatching is under endogenous control (Lazzari, 1991), whereas in others it is triggered by environmental factors (Saunders, 1982). The embryonic stage of an insect is a period of higher sensitivity to external stimuli (Tilton and Brower, 1983). LED (Light Emitting Diode) is a solid-state unit that converts electricity to light with minimal heat production therefore, it is a very efficient source of light. Light-emitting diode color can range from ultraviolet (350 nm) to infrared (700 nm) depending on the chemical composition of the LED (Humphreys, 2008). Damage effect of visible light with short wavelengths on insects had been reported. A gradual decrease in hatching percentages for Lepidoptera was reported by Faruki, *et al.* (2007) upon exposure to short wavelength UV-C (254nm) for different periods. Otherwise, positive effect of short wavelengths ranging from UV-A to green (500–560 nm) have been reported for spider mites. Whereas, irradiation with UV-A, blue, and green lights caused photo-reactivation of mites damaged by UV-B (Murata and Osakabe, 2014). Hori, *et al.* (2014) suggested that, the toxicity of visible light with short wavelengths is species-specific in insects, and that shorter wavelengths are not always be toxic. Diapause in *B. mori* eggs is determined by genetic characters and endocrinological mechanisms, mediated by environmental factors such as temperature and photoperiod (Singh and Saratchandra, 2002). Consequently, there were physiological, biochemical and metabolic changes associated with the initiation, maintenance and termination of diapause (Yaginuma *et al.*, 1990).

The present study aims to investigate the effect of using a LED lamp as a cheap and non-emitting heat source of light for improving *B. mori* egg hatchability.

MATERIALS AND METHODS

Bombyx mori eggs of two different hybrids (Egyptian and Bulgarian) were used in this experiment. Egyptian hybrid was obtained from the Sericulture Research Department (SRD) of Plant Protection Research Institute (PPRI). The Bulgarian hybrid was imported from Bulgaria. *B. mori* eggs incubation period was realized using the gradual raising of temperature as followed by the SRD routine work which is used especially for incubating eggs kept in the refrigerator and proceed for the second rearing season as follows; after removing the eggs from the

refrigerator 5 °C, they kept for three days at 15°C then balance between 27°C and 28°C till hatching.

Two wooden boxes with dimensions (100 X 100 X 100 cm) were used; one was provided with a commercial LED Lamp, Tirivina LED 15 W 6500 k 220 V 50/60 Hz as a non-emitting heat light source. The other one was kept in dark for control egg groups. The two boxes were provided with Hygro-thermometers for adjusting temperature (27–28 °C) and humidity (75–85 %) after removing from 15°C. Egg patches were exposed to LED lighting for different periods (24, 72, 120, 168, 192 and 216 hrs.) then transferred to dark box till hatching. Every 48 hrs. 80 eggs from both hybrids (4 replicates of 20 eggs) were transferred from the LED lighting box to the dark one. Another half gram of eggs was taken every 48 hrs. from each egg group for biochemical analysis. Daily follow-up and registration were done till complete egg hatching.

Biochemical Analysis:

Egg samples were homogenized in distilled water then centrifugated at 500 rpm. Homogenates were collected in cold tubes with crystals of phenylthiourea to prevent melanization, then centrifuged at 6000 rpm for 10 min. Supernatant fluid was divided into small aliquots and stored at –20 °C until analysis of main component (total proteins, total carbohydrates and total lipid).

1. Determination of total proteins content

Total proteins were determined by the method of Bradford (1976) using standard of Bovine serum albumin. The absorbance was measured spectro-photometrically at 595 nm. The optical densities is plotted against concentrations, thus a curve can be constructed.

2. Determination of total lipid content

Total lipid was estimated according to Knight, *et al.* (1972) spectro-photometrically at 620 nm using phosphovanillin reagent. The content of lipid was expressed as mg / gram body weight.

3. Determination of total carbohydrates content

Total carbohydrates were determined according to Singh and Sinha (1977) using Anthron reagent. The absorbance was recorded at 620 nm. The content of carbohydrates was expressed as mg/g body weight.

Statistical analysis

Collected data were recorded and analyzed using statistical analyzing system version 9.1 program proc. GLM (SAS Institute, 2003).

RESULTS AND DISCUSSION

The present study is introducing a simple technique by using wooden box containing a LED lamp and tested

exposure egg of two hybrids to different light periods (24, 72, 120, 168, 216 hrs.);

Egyptian hybrid:

As presented in Figure (1), Egyptian hybrid eggs control took about fifteen days till fully hatched. While, under the influence of LED lighting, a gradual improvement in hatchability percentages and reducing the incubation period to fourteen days. Hatching percentages at 216 hrs. LED lighting group were (44, 34, 4%) during (12, 13 and 14th day of incubation, respectively).

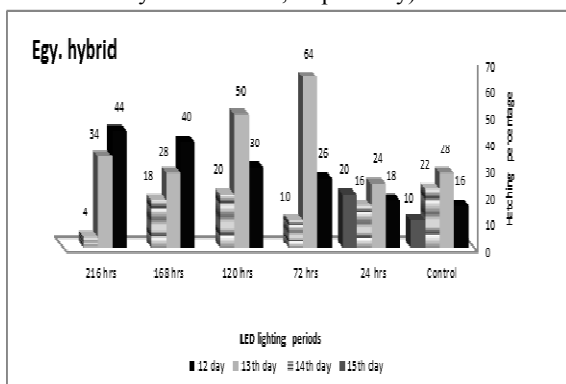


Figure 1. Hatching percentages in the Egyptian hybrid eggs under the effect of LED lighting for different periods

Bulgarian hybrid

Control group of Bulgarian eggs took about eleven days to complete hatching while kept at 27 °C. Under LED lighting for 24 hrs. there was a reduction in egg hatchability comparing with control (65 and 75.5 %, respectively). Then, a gradual improvement in hatchability with increasing LED exposure was detected. As well as, egg hatching started at 168hrs.

After nine days by 25 % then increased to 100 % at 216hrs. It could be concluded that, continuous exposure to the LED lighting increases the hatching rate with shortage of incubation period.

The hatchability improvement may be related to the effect of short wavelengths that penetrate egg shell to DNA of the embryo (Longcore *et al.*, 2015).

Embryonic diapause in *B. mori* is attributed to metabolic adjustment which serves to bring about a new physiological state (Singh and Saratchandra, 2002), which encourage the importance of studying the changes in metabolic parameters; proteins, carbohydrates and lipids under the effect of LED lighting. Insect eggs need to be provisioned with nutrients for successful embryonic development, lipids and proteins comprising the main

components as ascertained by Diss, *et al.* (1996). Short wavelengths indirectly affect lipids, proteins, and DNA by enhancing the production of reactive oxygen species (ROS) in microbial cells (Santos *et al.*, 2013). The present data clarified that Egyptian eggs are more sensitive and respond to LED lighting than Bulgarian ones. These findings suggest that light absorption by certain inner tissues of the embryo is wavelength-specific. The species-specific photo-sensitizers in insect tissues absorb specific wavelengths of light, thereby generating free radicals and subsequently die from tissue damage caused by free-radical formation as suggested by Hori, *et al.* (2014). Faruki, *et al.* (2007) found that young embryos of *Cadra cautella* (Lepidoptera) were more sensitive to UV rays than older ones. This is support our findings in the present study as the embryos at late stage did not negatively affected under the effect of LED waves.

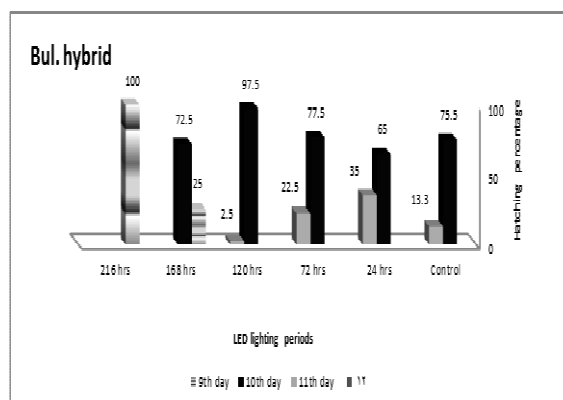


Figure 2. Hatching percentages of the Bulgarian hybrid eggs under the effect of LED lighting for different periods

Total protein:

Egg proteins consist mainly of vitellins, which are mostly transformed into structural elements during embryonic development, while rarely being used as energy sources (Gillot, 2005).

Table (1), represented the statistical analysis between the four tested groups after 24 hrs. following transferring from 15 °C to 27°C . Bulgarian LED lighting group significantly recorded the highest protein concentration (216.93±2.04 mg / g) comparing with the other tested groups. Observing the protein titer in all tested periods revealed that, the concentration increased gradually till 168hrs. then decreased significantly to (179.78±2.22 mg/g) at 216 hrs.

Table 1. Protein levels (mg/ g egg weight) in Egyptian and Bulgarian hybrid *B. mori* eggs under LED lighting for different periods (mean ± SE).

Lighting periods	Egyptian		Bulgarian		LSD
	LED	Control	LED	Control	
24 hrs	164.06 ± 1.78 b**	159.67 ± 1.62 b*	216.93 ± 2.04 a**	157.61 ± 1.95 b**	8.75
72 hrs	199.26 ± 2.48 b*	156.16 ± 2.69 d*	215.33 ± 3.34 a**	171.91 ± 3.1 c*	13.21
120 hrs	195 ± 3.09 b*	159.88 ± 2.94 c*	236.23 ± 3.92 a*	179.61 ± 2.45 c*	14.59
168 hrs	203.74 ± 3.16 b*	160.83 ± 3.41 c*	240.54 ± 4.26 a*	169.08 ± 3.84 c*	17.57
216 hrs	176.22 ± 3.45 a**	158.83 ± 1.87 c*	179.78 ± 2.22 a***	166.42 ± 2.58 b*	12.99
LSD	13.28	9.26	14.88	15.73	

Letters in same row represent the significance at P < 0.01 between the Egyptian and Bulgarian egg groups. Stars in the same column represent the significance at P < 0.01 of different periods in the same egg group.

After 24 hrs. of LED exposure, Egyptian LED lighting group showed significantly lower protein concentration (164.06±1.78mg/g) than Bulgarian LED group (216.93±2.04 mg/g). The protein titer increased gradually in

all tested periods then decreased significantly after 216hrs. of (176.22±3.45mg/g and 179.78 ± 2.22, respectively).

From the obtained data, it may be concluded that, LED lighting stimulate the production of proteins in embryos as a kind of modulation to the continuous LED

exposure till 168hrs. then the concentration remain stable without significant changes at 216hrs. While, in control groups protein titer mostly remain stable during all incubation period except Bulgarian control group showed slight increase.

The main egg protein of *B. mori* was defined by Irie and Yamashita (1983) is glycol-lipoprotein with a mol. wt of 125,000 Da. This molecule contained about 2 % carbohydrate and 4 % lipid, which may explain the high protein concentration comparing to carbohydrates and lipids in the present study. Zhu, *et al.* (1986) discovered that more than 95 % of the total protein consist of yolk proteins: vitellin (~40%), 30 KDa protein (~35%) and egg-specific protein (~20%). Egg-specific protein and 30K protein were gradually disappeared, which may explain the decrease in the protein concentration near egg hatching in the present study.

Total Carbohydrates

Changes in total carbohydrates levels (mg / g egg weight) in the tested egg groups are presented in Table (2). In all tested periods, total carbohydrate concentrations of both Egyptian and Bulgarian egg groups did not exceed 45 mg / g egg weight in LED exposure for 24 hrs. resulting in increment in carbohydrate concentrations of Egyptian and Bulgarian egg groups (37.31±0.44 and 39.16±0.52 mg/g) comparing with their controls (32.52±0.43 and 32.78±0.19 mg /g), respectively. The same trend was recorded under continuous exposure for 72hrs. (45.67±0.62 and 45.17±0.50) and their controls (41.87±0.72 and

41.38±0.50), respectively. After 168 hrs. carbohydrate concentration significantly decreased in LED lighting groups then increased after 216 hrs. of continuous exposure. Total carbohydrate and lipid concentrations increased at hatching time to enhance egg hatchability (Jaronik *et al.*, 2004). Miura and Shimizu (1987) suggested that, during embryogenesis the glycogen content gradually decreased for 5-6 days after oviposition, then there was a rapid decrease until the day of hatching.

Siraj, *et al.* (2007) suggested that as energy is a vital force in the biological system, breakdown of organic constituents mainly carbohydrates is required to meet the energy under the stress condition. It is generally accepted that carbohydrate catabolism predominates during early embryonic development of insects and that lipids are mainly utilized during the rest of the development (Agrell and Lundquist, 1973).

Total Lipids.

Total lipids (mg/g egg weight) concentrations among studied egg groups were showed in Table (3). Generally, continuous LED exposure stimulate the production of lipids in both Egyptian and Bulgarian egg groups and were significantly higher than their controls in all tested periods. A significant increase in lipid titers in control groups of both hybrids egg groups till 120 hrs. then non-significant decrease were recorded at hatching time (216 hrs.).

Table 2. Total carbohydrate levels (mg/ g egg weight) in Egyptian and Bulgarian hybrid *B. mori* eggs under LED lighting for different periods (mean ± SE).

Periods	Egyptian		Bulgarian		LSD
	LED	Control	LED	Control	
24 hrs	37.31±0.44 a***	32.52±0.43 b***	39.16±0.52 a**	32.78±0.19 b***	1.88
72 hrs	45.67±0.62 a*	41.87±0.72 b** and ***	45.17±0.50 a*	41.38±0.50 b**	2.68
120 hrs	46.95±0.81 a*	31.01±0.37 b***	45.73±0.78 a*	44.38±0.47 a*	2.88
168 hrs	42.04±0.47 ab**	43.99±0.63 a*	40.74±0.43 b**	44.41±0.64 a*	2.49
216 hrs	44.06±0.71 a**	42.55±0.47 a*	44.57±0.61 a*	43.73±0.64 a*	2.77
LSD	2.91	2.51	2.71	2.39	

Letters in same raw represent the significancy at P < 0.01 between the Egyptian and Bulgarian egg groups. Stars in the same column represent the significancy at P < 0.01 of different periods in the same egg group.

Table 3. Total lipids levels (mg/ g egg weight) in Egyptian and Bulgarian hybrid *B. mori* eggs under LED lighting for different periods (mean ± SE).

Light period	Egyptian		Bulgarian		LSD
	LED	Control	LED	Control	
24 hrs	43.71±0.49 a**	20.07±0.17 b***	43.32±0.54 a***	21.76±0.36 b***	4.59
72 hrs	44.30±0.66 a**	27.16±0.96 b**	47.86±2.65 a**	31.75±0.65 b**	4.53
120 hrs	45.10±0.8 a**	34.17±0.61 c*	46.47±1.42 a**	38.81±0.38 b*	4.48
168 hrs	43.75±0.89 ab**	34.92±0.64 bc*	45.33±1.36 a**	36.96±1.97 c*	7.64
216 hrs	48.49±0.37 a*	32.51±0.61 b*	50.12±1.32 a*	34.53±0.65 b*	2.82
LSD	3.49	5	4.78	6.02	

Letters in same raw represent the significancy at P < 0.01 between the Egyptian and Bulgarian egg groups. Stars in the same column represent the significancy at P < 0.01 of different periods in the same egg group.

Glycogen content decreased with the onset of diapause in *B. mori* eggs and reached a minimum level then increased again when the diapause was terminated (Miura and Shimizu, 1987). These observations showed that early utilization of carbohydrates and following concomitant consumption of triacylglycerol (TG) in late stage. The second major component of eggs is lipid, which is composed of triacylglycerol (~80%) and phospholipids (~20%). Most of the metabolic energy (approximately 70% of total energy) utilized during embryogenesis is derived from the oxidation of triacylglycerol (Yamashita and Yaginuma 1991).

Since ATP is the key energetic molecule in all cellular states, the effect of visible light with short wave lengths ranged from 655nm to 830 nm on ATP may be of biological significance when cells and tissues are irradiated (Amat *et al.*, 2005). Irradiation increases enzymatic and hexokinase reactions (phosphorylation of glucose), that initiates the metabolic pathway for glycolysis outside the mitochondria (Storey 1980). Glycolysis, where one

molecule of glucose is metabolized into two molecules of pyruvate and two molecules of ATP, begins with the hexokinase reaction (Pastorino and Hoek, 2003). Pyruvate can be further processed anaerobically to lactate by lactic acid fermentation (Robergs *et al.*, 2004). This reaction is a source of ATP when mitochondria are unable to synthesize ATP and when the cell has a low oxygen concentration (Westerblad and Allen 2003).

Recommendation

It may be recommended to use LED (Light Emitting Diode) lighting on *Bombyx mori* L. embryos at the last stage of diapause (after transferring egg boxes from 15 °C to 27 °C) to enhance hatchability and shorten incubation period.

REFERENCES

Agrell, I. P. S. and Lundquist, A. M. (1973). Physiological and biochemical changes during insect development. in "The Physiology of Insecta". Edited by Rockstein, M., Academic Press, New York, 1: 159-247.

- Amat, A., Rigau, J., Waynant, R. W., Ilev, I. K., Tomas, J., and Anders, J. J. (2005). Modification of the intrinsic fluorescence and the biochemical behavior of ATP after irradiation with visible and near-infrared laser light. *Journal of Photochemistry and Photobiology B: Biology*, 81(1), 26-32.
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72: 248- 254.
- Diss, A. L.; Kunkel, J. G.; Montgomery, M. E. and Leonard, D. E. (1996). Effects of maternal nutrition and egg provisioning on parameters of larval hatch, survival and dispersal in the gypsy moth, *Lymantria dispar* L. *Oecologia*, 106:470-477.
- Faruki, S. I.; Das, D. R.; Khan, A. R. and Khatun, M. (2007). Effects of ultraviolet (254nm) irradiation on egg hatching and adult emergence of the flour beetles, *Tribolium castaneum*, *T. confusum* and the almond moth, *Cadra cautella*. *J. Insect Science*, 7 (36):1- 6. Available online: insectscience.org/7.36.
- Gillott, C. (2005). *Entomology*. Springer Netherlands, PP-832, ISBN 978-1-4020-3183-0.
- Hori, M.; Shibuya, K.; Sato, M. and Saito, Y. (2014). Lethal effects of short-wavelength visible light on insects. *Scientific reports*, 4 : 7383. DOI: 10.1038/srep07383.
- Humphreys, C. J. (2008). Solid-state lighting. *MRS bulletin*, 33(4), 459-470.
- Irie, K. and Yamashita, O. (1983). Egg-specific protein in the silkworm, *Bombyx mori*: purification, properties, localization and titer changes during cogenesis and embryogenesis. *Insect Biochemistry*, 13(1): 71-80.
- Jaronik, V.; Kratochvil, L.; Honek, A. and Dixon, A. F. G. (2004). A general rule for the dependence of developmental rate on temperature in ectothermic animals. *Proc. R. Soc. Lond. B. Biol. Sci.*, 271: S219-S221.
- Knight, J. A.; Anderson, S. and Rawle, J. M. (1972). Chemical basis of the sulfo-phospho-vanillin reaction for estimating total serum lipids. *Clinical Chemistry*, 18(3): 199- 202.
- Lazzari, C. R. (1991). Circadian rhythm of egg hatching in *Triatoma infestans* (Hemiptera: Reduviidae). *J. Med. Entomol.*, 28(5): 740-741.
- Longcore, T.; Aldern, H. L.; Eggers, J. F.; Flores, S.; Franco, L.; Hirshfield-Yamanishi, E.; Petrinc, L. N.; Yan, W. A. and Barroso, A. M. (2015). Tuning the white light spectrum of light emitting diode lamps to reduce attraction of nocturnal arthropods. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, 370 (1667):2014-0125. <http://doi.org/10.1098/rstb.2014.0125>
- Miura, K. and Shimizu, I. (1987). Changes of triglyceride and glycogen content in the silkworm (*Bombyx mori*) eggs during diapause and embryogenesis. *Comp. Biochem. Physiol.*, 8611(4): 719-723.
- Murata, Y. and Osakabe, M. (2014). Factors affecting photo reactivation in UVB-irradiated herbivorous spider mite (*Tetranychus urticae*). *Exp. Appl. Acarol.*, 63: 253-265.
- Pastorino, J. G. and Hoek, J.B. (2003). Hexokinase II: the integration of energy metabolism and control of apoptosis. *Curr. Med. Chem. Aug.* 10 (16) : 1535-1551.
- Robergs, R. A., Ghiasvand, F. and Parker, D. (2004). Biochemistry of exercise induced metabolic acidosis. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 287 (3) : R502-R516.
- Santos, A. L.; Oliveira, V.; Baptista, I.; Henriques, I.; Gomes, N. C.; Almeida, A. and Cunha, A. (2013). Wavelength dependence of biological damage induced by UV radiation on bacteria. *Archives of microbiology*, 195(1): 63-74.
- SAS Institute 2003. SAS version 9.1. Cary, M. C.
- Saunders, D. S. (1982). *Insect clocks*. Pergamon Press, Oxford-Newyork, ISBN 0-08-02884-0, Second Edition. PP— 409.
- Singh, N. B. and Sinha, R. N. (1977). Carbohydrates, lipids and proteins in the developmental stages of *Sitophilus oryzae* and *S. granarius* (Coleoptera: Curculionidae). *Ann. Entomol. Soc. Amer.*, 70: 107-111.
- Singh, T. and Saratchandra, B. (2002). Biochemical changes during embryonic diapause in domestic silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae). *Int. J. Indust. Entomol.*, 5 (1):1-12.
- Siraj, M.; Balavenkatasubbaiah, M.; Chandrasekharan, K.; Nataraju, B.; Sharma, S. D.; Selvkumar, T. and Sudhakara Rao, P. I. (2007). Effect of BmDENV1 infection on the biochemical parameters in Densonucleosis type 1 resistant and susceptible breeds of silkworm, *Bombyx mori* L. *Acta Entomologica Sinica*, 50 (1): 74-78.
- Storey, K. B. (1980). Regulatory properties of hexokinase from flight muscle of *Schaistocerca americana gregaria*. Role of the enzyme in control of glycolysis during the rest-to-flight transition. *Insect. Biochem.* 10 (1980) 637-645.
- Tilton, E.W. and Brower, J. H. (1983). Radiation effects on arthropods. in: *Preservation of food by ionizing radiation*. Josephson, E.S. and Perterson, M. S. editors, CRC Press Inc., 2: 269-316.
- Westerblad, H. and Allen, D.G. (2003). Cellular mechanisms of skeletal muscle fatigue. *Adv. Exp. Med. Biol.* 538 (2003) 563-571.
- Yaginuma, T.; Kobayashi, M. and Yamashita, O. (1990). Distinct effects of different low temperatures on the induction of NAD-sorbitol dehydrogenase activity in diapause eggs of the silkworm, *Bombyx mori*. *J. comp. Physiol.*, (B) 160: 277-285.
- Yamashita, O. and Yaginuma, T. (1991). Silkworm eggs at low temperatures implications for sericulture. in: *Insects at low temperature*. Springer US, 424-445.
- Zhu, I.; Indrasith, L. S. and Yamashita, O. (1986). Characterization of vitellin, egg-specific protein and 30 kDa protein from *Bombyx* eggs and their fates during oogenesis and embryogenesis. *Biochim. Biophys. Acta*, 882:427-436.

إستخدام الإضاءة الليد كمنظم لعملية الفقس وتأثيره على الحالة البيوكيميائية لبيض فراشة الحرير التوتيه

رحاب حسني طه

معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الجيزة

يواجه مربين دودة القز مشكلة عدم تزامن فقس بيض فراشة الحرير التوتيه بالإضافة الي معدل الفقس المنخفض للبيض حيث أنهم لا يمتلكون معدات للتحكم في درجة الحرارة والرطوبة والإضاءة المثلى. تعتمد التجربة على تركيز الإضاءة الليد على بيض هجين مصري وأخر مستورد لفترات مختلفة (٢٤، ٧٢، ١٢٠، ١٦٨، ٢١٦ ساعة) مع ضبط درجة الحرارة والرطوبة. وكذلك تقدير التغيرات التي تطرأ على الأجنة في البروتينات والكربوهيدرات والدهون الكلية. وقد أظهرت النتائج تحسناً تدريجياً في نسب الفقس مع نقص في فترة التحضين للبيض المعامل مقارنة بالكنترول. وقد تحققت أفضل النتائج تحت الإضاءة الليد المتواصلة لمدة (٢١٦ ساعة). تستجيب أجنة كلا الهجينين إلى التعرض للإضاءة الليد عن طريق تنظيم عمليات الأيض للتكيف مع الإضاءة. أيضاً تعتمد استجابة الأجنة على أصل هجين. قد يكون من المستحسن إستخدام الإضاءة الليد في المرحلة الأخيرة من النمو الجنيني كمصدر ضوء لا ينبعث منه حرارة لتعزيز وتوحيد الفقس وتقصير فترة الحضنة.