BIOCHEMICAL EFFECTS OF CAMPHOR HONEY ON 4TH AND 5TH INSTAR OF SILKWORM B. MORI

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Abstract: The present study is aimed to assess various biochemical changes, [glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), total soluble protein (TSP), and lipid peroxidation (MDA) in haemolymph of silkworm *Bombyx mori* larvae cultivated on mulberry leaves treated with honey as dietary additives in the fourth and fifth instars with five different concentrations. Results displayed that at 4% and 5% concentrations of honey treatment is vital in the enhancement of immunity and survival compared with control. The larvae raised on leaves supplemented with these food concentrations showed a significant rise in GPT, GOT and TSP and a substantial decrease in lipid peroxidation (MDA) level. It can be concluded that, mulberry leaves supplemented with honey at concentrations of 4th & 5th were confirmed to be more proficient in rearing silkworm as it improved protein enzymes activities (GPT & GOT) and total soluble protein of the larvae.

Keywords: Biochemical Changes, Food Supplements, Bombyx Mori.

Introduction: Silkworm noshes merely on mulberry leaves. An increase in protein contentin mulberry leaves might lead to enhancements in silk quality productivity. The making of high quality and quantity of natural silk depends primarilyon suitable environmental conditions, larval feeding, and protection from diseases. [1] [2] [3] [4] The dietary value of the mulberry leaves can be enhanced by stirring them with extra nutrients to increase the larval growth and to improve cocoon characteristics. [5]

The use of honey as beneficial substance has been re-experienced by the medical profession. In recently also gained the acceptance as an antibacterial substance for treatment of some ailments. [6] [7] Hence, the present effort is focused on assessment of the effects of camphor honey as nutritional additives on various biochemical aspects like Glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT) enzyme activities and Total soluble protein (TSP) during the 4th and 5th larval instars. Studies are also performed on the oxidative stress or biological damage (MDA level) occurred in lipids and proteins of larval haemolymph because of free radical production during the 4 th and 5th larval instars.

Materials and Methods: The present study was carried out during the spring autumn of 2014 in the laboratory of Sericulture Research Department, Magadh University, Gaya, Bihar (India). I- ***Materials:*** Eggs of mulberry silkworm, (Egyptian hybrid Giza). The honey concentrations (5%, 4% and 1%) were prepared by dissolving honey in appropriate amount of distilled water.

Silkworm Rearing Technique: Rearing of silkworm was carried out under laboratory conditions (28 ± 2°c and 70 ± 5% R.H.) according to the technique of Krishna swami (1978). Chicken egg cartons plates were used as montages for cocoon spinning. [8] Experimental larvae were divided two main groups. Each group again divided into five groups to feed on mulberry leaves supplemented with various concentrations of camphor honey. One group of the two main groups was fed on the first day of the 4th instar after molting and the other group on the first day of the 5th instar, using 3 replicates (100 larvae) for each concentration. Mulberry leaves were dipped in the five concentrations of treated honey for 5 minutes and left to dry then offered to larvae. The control group of leaves was treated only with distilled water.

Biochemical Measurements: Samples were made by removing one of the thoracic legs of the 4th and 5th instar larvae and bending the body to expose the sternum at the position of the removed leg. This ensured proper flow of the haemolymph and prevents the internal tissue damage. The haemolymph of each treatment was collected in Eppendorf tubes 1.5 ml with small crystal of phenyl thiourea (PTU) to prevent pigmentation of sample. [9] (Mahmoud, 1988). The tubes were kept at -20°C. The blood samples were centrifuged at 10000 r.p.m for 10 minutes at 5°c. The supernatant was immediately assayed to determine glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) activities according to the method of Reitman and Frankel. [10] Total soluble protein (TSP) as described by Gornall et al. [11] , Lipid peroxidation (MDA) according to the method of Sharma and Wadhwa .[12] and protein carbonyl content (PCC) as described by Levine et al.[13]

Statistical Analysis: Numerical computations like arithmetic mean, standard deviation and standard error, correlation and regression analysis were calculated by using XLSTAT 2010 Excel add-in Window software. [14] The obtained results were subjected to statistical analysis of variance and the data were presented as means.

Results and Discussion: Results of this work revealed a significant increase in physiological and biochemical aspects in both 4th and 5th instars of silkworm than control, detailed results were as follows.

Glutamic Pyruvic Transaminase (GPT): Results clearly revealed that larvae fed mulberry leaves supplemented with camphor honey and oil with different concentrations in the 4th instar showed a significant increase in enzyme GPT in all concentrations of honey and oil than control especially 4% and 5% concentrations (2.86 and 3.36 mg/L) than in control (0.89mg/L). This trend is continued in the 5th instar also in which 4% and 5% of honey supplement increased the enzyme activity significantly (P> 0.01) (4.74 & 4.89 mg Pyruvate/ml) than control 0.61 mg Pyruvate/ml. The correlation regression analysis shows a significant positive relation with concentration of food supplements. The value of R^2 for GPT and other enzymes is R^2 = 0.987 in 4^{th} instar larvae while it is R^2 = 0.898 in 5^{th} instar larvae.

Glutamic Oxaloacetic Transaminase (GOT): Like GPT, the values of GOT increased in the larvae of experimental groups fed with honey and oil In the 4th instar stage (P> 0.01, Table, 1). The increase was much higher in experimental groups of D and E (7.18 mg/L and 8.74mg/L) (4% and 5%) than the control (3.58mg/L). The trend of increase was gradual along with concentration of food supplement.

Total Soluble Proteins (TSP): Results (Table 1&2) clearly shown that larvae of the 4th larval instar fed on mulberry leaves treated with the 4% and 5% concentrations of camphor honey recorded the highest TSP values (17.12 & 21.77 mg/ml of serum) compared with control (11.49 mg/ml of serum). The 5th larval instar fed on camphor honey shown signs of significant increase in TSP values than control (15.10 mg/ml of serum) especially the concentrations of honey 4% and 5% (31.42 & 38.43 mg/ml of serum) respectively.

The highest level of soluble proteins after fourth molt and immediate gradual increase in the free amino acids in the silk gland up to the end of larval stage reflected the possibility of active function of the protein synthesis mechanism in the silk gland .[15] Besides, the dietary supplements in the diet influenced the accumulation of stored proteins in the haemolymph of larvae that implies the amount of reserved proteins in the larvae fed on low protein were less than the standard diet ones, but the larvae fed on optimal level of protein showed a higher levels of storage protein. [16]

Lipid Peroxidation (MDA): The levels of lipid peroxidation of larval haemolymph in the 4th and 5th larval instars of larvae reared on enriched mulberry leaves with camphor honey with different concentrations compared with control were shown in Tables (1, 2). In the 4th instar, the MDA content in larvae reared on mulberry leaves supplemented with different concentrations of camphor honey were significantly decreased as shown in Table (1) especially the 4% and 5% concentrations (11.48& 7.79 nmol./ ml of serum) respectively compared with control (30.00 nmol./ ml of serum). However, the 5th instar larvae reared on mulberry leaves supplemented with camphor honey revealed a significant decrease in means of MDA content in all concentrations compared with control (30 nmol./ ml of serum), especially the 4% and 5% concentrations of honey (15.73 & 14.88 nmol./ ml of serum) respectively. The correlation regression of lipid peroxidation is significantly negatively correlated with concentration of food supplements. It is R^2 = 0.992 in 4^{th} instar and R^2 = 0.939 5^{th} instar larva.

Bee-honey may be a strong factor for antioxidant activity and free radical depression. [6] These biochemical effects of bee-honey feeding might be owing to the presence of various components which represent antioxidant and antibacterial agents. [17] (Wang *et al*. 2002). The dietary supplementation of antioxidant components might enhance the larvae immunity to oxidative stress and enhanced larval vivacity. [18] (Gheldof *et al.* 2002).

Conclusions: From the above results, it can be concluded that, mulberry leaves supplemented with honey at concentrations of 4th & 5th were confirmed to be more proficient in rearing silkworm as it improved protein enzymes activities (GPT & GOT) and total soluble protein of the larvae. This enhancement was completely associated with decreased free radicals (MDA) and increased antioxidant defense in the larval haemolymph, hence, the viability of larvae increased.

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Table (1): Effect of Camphor Honey on Biochemical Aspects of the 4th Instar of Silkworm B. Mori

Group	Honey Conc.	GPT	GOT	TSP	MDA
Control	--	**0.89±0.02**	**3.68± 0.16**	**8.43± 0.29**	**34.13± 1.13**
A	1%	1.49±0.09	3.66± 0.12	12.17± 0.09	27.79± 0.96
B	2%	1.77±0.07	3.87± 0.27	11.77± 0.91	24.48± 0.76
C	3%	2.18±0.18	8.44± 0.63	14.40± 0.47	17.42± 0.79
D	4%	2.86±0.87	8.19± 0.06	17.12± 0.97	11.48± 0.47
E	5%	3.36±0.77	8.95± 0.16	21.77± 0.78	7.79± 0.94
Mean		8.972	6.622	15.446	17.792

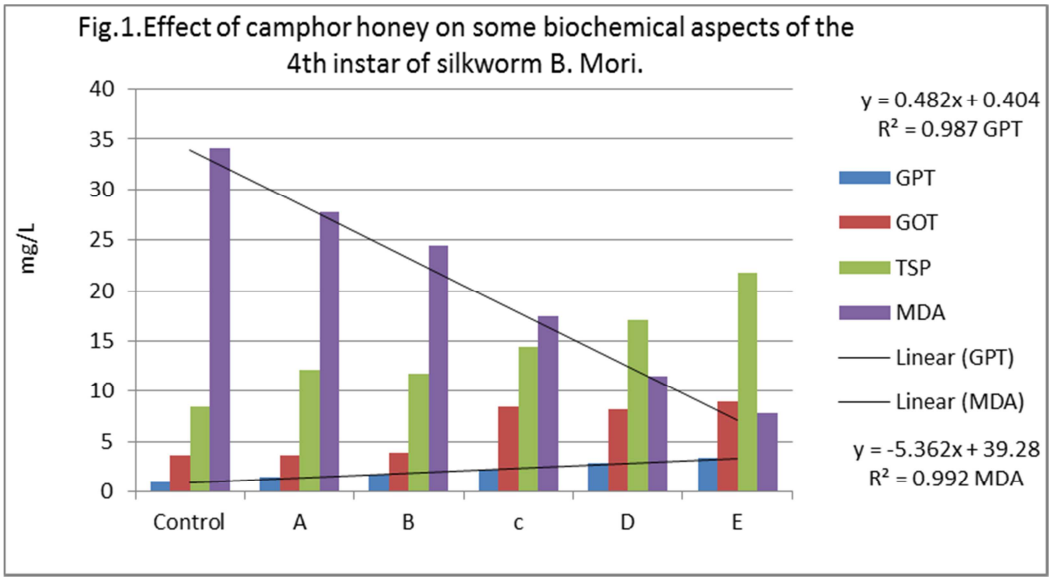
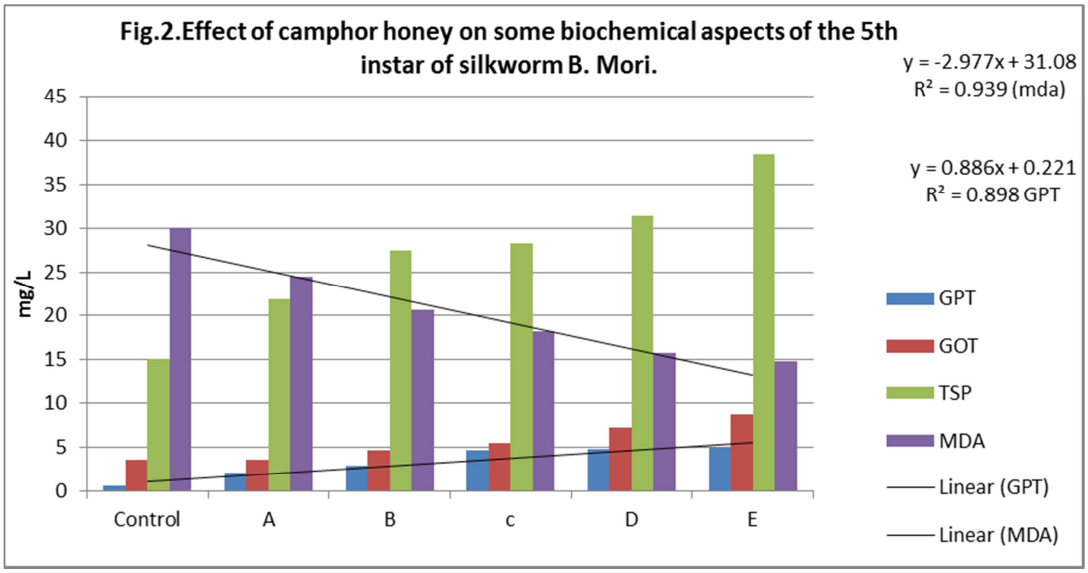


Table (2): Effect of Camphor Honey on Some Biochemical Aspects of the 5th Instar of Silkworm B. Mori

Group	Honey Conc.	GPT	GOT	TSP	MDA
Control	--	**0.61± 0.12**	**3.58± 0.13**	**15.10± 0.76**	**30.00± 0.90**
A	1%	2.14± 0.09	3.64± 0.09	21.84± 0.44	24.44± 0.29
B	2%	2.87± 0.13	4.61± 0.04	27.48± 0.73	20.72± 0.31
C	3%	4.69± 0.27	5.48± 0.19	28.27± 0.79	18.24± 0.46
D	4%	4.74± 0.15	7.18± 0.29	31.42± 1.04	15.73± 0.34
E	5%	4.89± 0.41	8.74± 0.48	38.43± 1.73	14.88± 0.76
Mean		3.866	6.33	29.488	18.802



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