Academic Journal of Entomology 6 (2): 66-73, 2013
ISSN 1995-8994
© IDOSI Publications, 2013
DOI: 10.5829/idosi.aje.2013.6.2.74115

Effect of Phytoecdysteriod Treatment on Glucose Content in the Silk Gland, Fat Body and Haemolymph of Multivoltine Mulberry Silkworm *Bombyx mori* Linn (Lepidoptera: Bombycidae) at Initial Stage of Spinning

Purnima Pandey and V.B. Upadhyay

Department of Zoology, Silkworm laboratory,
D.D.U. Gorakhpur University,
Gorakhpur- 273009 India

Abstract: Phytoecdysteroid are being used commercially to shorten the larval duration for synchronize spinning of cocoons and labour saving. In this experiment *Achyranthes aspera* plant extract containing phytoecdysteroid activity was taken. Experiments were performed by 40, 50, 60 and 70% concentration of phytoecdysteroid obtained from Achyranthes leaf extract. A control set was always maintained with each set of experiment.Variation in the number of treatment (P_1>0.05) of *Bombyx-mori* larvae significantly influenced theglucose content in the silk gland, fat body and haemolymph of multivoltine mulberry silkworm *B. mori* Linn at initial stage of spinning. The maximum glucose content in the haemolymph of larvae at the initial stage of spinning was noticed to be 7.540±0.396 µg/mg in case of double treatment of larvae by 60% phytoecdysteroid concentration.With the increasing number of larval treatment from one to two times, the glucose content in the fat body and silk gland of larvae at the initial stage of spinning increased in case of 40, 50 and 60% phytoecdysteroid treatment but the triple treatment of larvae caused notable decline in the glucose content in the fat body and silk gland of larvae at initial stage of spinning in all the above concentrations. The maximum glucose content in the fat body (14.952±0.030 µg/mg) and in silk gland (15.133±0.100 µg/mg) of larvae at the initial stage of spinning was noticed in case of double treatment of larvae by 60% phytoecdysteroid.

Key words: Bioactive · Hormone · Larvae · Moulting · Silk Fibre

INTRODUCTION

Silk, the natural fiber that spells splendourlusture and elegance, has been an inseparable part of Indian culture and tradition, over thousands of years. Mulberry sericulture in India is a commercially attractive and sustainable farm based economicenterprise positively favoringthe rural poor in the unorganized sector.

Bombyxmori nistari is a resistant variety of multivoltine mulberry silkworm contributing to a great extent in the commercial production of cocoon in India. The silk gland, haemolymph and fat body of silk worm is the most important factor which influence the production of silk on commercial scale. Attempts have been made to study the effects of ecological factors [1], relative humidity [2], refrigeration of eggs [3], pupae [4] and cocoons [5] and magnetization of eggs [6-8] and cocoons [9, 10] and temperature [11, 12] on the performance of silkworms. The phytoecdysteroidhas been noticed to influence the development, growth, silk producing andreproductive potential of *B. mori* [13-17].

The outcome of this investigative study is expected to have tremendous applied significance and the knowledge derived from the study will be helpful in the rearing of silkworm at farmer's level. The study is important from academic as well as economic point ofview.Apart from adding to our knowledge of insect development and growth,it may also be helpful to device suitable rearing program of multivoltine mulberry silkworm to increase the production of silk and generate more employment opportunities.

Corresponding Author: PurnimaPandey, Silkworm laboratory, Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur- 273009 India.

MATERIALS AND METHODS

Seed Cocoon: The seed cocoons (pupa enclosed in silken case) of multivoltine mulberry silkworm (*Bombyxmori* nistari), a native of West Bengal in India, were obtained from the silkworm grainageBehraich, Directorate of Sericulture Uttar Pradesh and, were maintained in the plywood trays (23 X 20 X 5 cm) under the ideal rearing conditions [18] in the silkworm laboratory, Department of Zoology, DeenDayalUpadhyay Gorakhpur University, Gorakhpur. The temperature, relative humidity and photoperiod were maintained at 26±1°C, 80±5% RH and 12±1 hours light a day respectively till the emergence of moths from the seed cocoons. The moths emerged generally in the morning at around 4 am. The tray in which seed cocoons were kept, were suddenly illuminated by light in the morning at 4 O'clock on 9th and 10th day of spinning.

The newly emerged moths, from seed cocoons, were quickly picked up and kept sex–wise in separate trays to avoid copulation. The male moths were smaller in size but more active than the female moths which were comparatively larger and less active. The whole grainage operation was performed as per description given as [18].

Copulation: Moths have tendency to copulate immediately after emergence, therefore, the female moths, required to copulate with the male moths, were allowed their mates for copulation. First of all, 130 pairs, each containing one male and one female from newly emerged moths, were allowed to mate at 26±1°C, 80±5% RH and 12±1 hour/day dim light condition. After four hours of mating, the paired moths were decoupled manually by holding the female moth between the thumb and middle finger gently and pushing the male away by the fore finger. The male moths were discarded while the female moths were allowed to lay eggs.

Oviposition: Just after separation, the gravid female laid eggs on the sheet of paper in the dark condition at 26±1°C and 75±5% RH. The egg laying moths were covered by open plastic cellules to prevent intermixing of eggs masses deposited by different moths. After 24 hours of egg laying, the female moths were individually examined for their disease freeness. The females were crushed individually in mortar with pestles and blood smears were examined by microscope under 15 X 45 magnification for the detection of bacterial and protozoan pathogens.

The disease free laying (DLF's), thus prepared, were treated with 2% formaline for 15 minutes to increase the adhesiveness of eggs on the paper sheet, with the eggs laid on, were thoroughly washed with running water to remove formaline and the eggs were dried in shade. The dried eggs, thus obtained, were taken for various experimental conditions.

Design of Experiment: Some plants like *Achyranthesaspera* act as bioactive phytoecdysteroid compound on *B.mori* larvae [19]. In the present study *A.aspera* was taken for experiment due to their good availability. To observe the influence of bioactive phytoecdysteroid hormone on the performance of *B.mori,* the experiments were performed with different concentrations of phytoecdysteroid hormone with respect to the treatment of IIIrd, IVth andVth instar larvae.

A. aspera were collected, washed thoroughly with distilled water and dried in incubator at 37°C. The dried leaves were powdered separately with the help of mechanical device. Further, 50g powder, thus obtained was subjected to extraction separately through soxlet apparatus with 250 ml distilled water for 40 h. After that, a little amount of concentrated solution was obtained which was dried and 6.75 g powdered material was obtained. The dried powder was dissolved in distilled water as 5g in 25 ml water and used this solution for further experiment as 100% concentration of phytoecdysteroid.

The survival of larvae and their growth performance was satisfactory at 50% concentration of *Achyranthes* extract. Therefore, for further experiment the suitable narrow range of *Achyranthes*phytoecdysteroid concentration viz; 40, 50, 60 and 70% were taken.

Thus, four phytoecdysteroid concentrations were applied topically by spraying as 10 ml on 100g mulberry leaves and the larvae were fed on the treated leaves. Three sets of experiments were designed viz., single, double and triple treatment of larvae.

Single Treatment: Single treatment of larvae was performed with the Vthinstar larvae just before two days of the beginning of larval spinning. 100 larvae were taken out from the BOD incubator and the mulberry leaf treated with 40% concentration of *Achyranthes*leaf extract, was given as food. Further, the treated larvae were given normal mulberry leaf for food.

Double Treatment: Double treatment of larvae was started from the final stage of IVth instar larvae. In the first treatment, 100 larvae of IVth instar were treated just before two days of IVthmoulting, by providing treated mulberry leaves as food with 40% concentration of *Achyranthes* leaf extract. The treated larvae then transferred in BOD

incubator for further rearing and development. Further, second treatment for the same larvae was given at the final stage of Vthinstar larvae i.e. just before two days of spinning.

Triple Treatment: -For triple treatment, the third instar larvae just before IIIrdmoulting, were separated from BOD incubator. In the first treatment, 100 larvae of IIIrd instar were treated by providing 40% extract treated mulberry leaf and kept in BOD incubator for rearing. The second treatment of same larvae was done just before two days of IVthmoultingie. at the final stage of IVthinstar larvae and transferred in BOD incubator for further rearing. The third treatment was given to Vth instar larvae, two days before the start of spinning. Thus, in the triple treatment IIIrd, IVthand Vth instar larvae were treated.

Similar experiments were performed by 50, 60 and 70% concentration of phytoecdysteroid obtained from *Achyranthes* leaf extract. A control set was always maintained with each set of experiment.

Estimation of Glucose: The glucose content in different tissues at different developmental stages, obtained in continuation of the earlier experiment was estimated.

The glucose level was estimated according to Mendel *et al*. [20] method. For determining the glucose content, the tissues viz. 31 mg of haemolymph, 25 mg of fat body and 30 mg of silk gland were deproteinized separately with 5% TCA, containing 0.1% silver sulfate. The content thus obtains were centrifuged at 10,000 rpm for 10 minutes. Further, in 0.5 ml of deproteinized supernatant, 4.5 ml. of H_2SO_4 was added which was mixed thoroughly by shaking. Now, content was boiled in water bath for 6 minutes and the mixture was cooled at room temperature. The pink colour obtained, was read against at 520 nm in the spectrophotometer. The blank consisted of 0.5 ml of 5% TCA containing 0.1% silver sulfate and 4.5 ml of molar H_2SO_4 which was given the same treatment as that of the experimental samples. Standard curve was prepared with different concentrations of pure glucose solution. The glucose content in different tissues were expressed as µg/mg of tissue.

The experiment was conducted for different stages of the development. Six replicates of each experiment were made.

The O.D. were measured at 420 nm. Blank enzymes were inactivated by the addition of NaOH before mixing with the incubation mixture. O.D. was compared with the standard. Standard were prepared by different concentrations of p-nitrophenol solution. Six replicates of each experiment were made.

Similarly other series of experiment were performed with the larvae with the phytoecdysteroid at different larval stages and with different concentrations separately to observe various parameters of the glucose constituents in different tissues at different developmental stages. All the data obtained were analyzed statistically by two way ANOVA and Post-hoc test.

RESULTS

Glucose Content (Mg/mg) in the Haemolymph of *Bombyx mori* Larvae at the Initial Stage of Spinning: The data presented in Table 1a and Fig 1 shows that change in the phytoecdysteroid concentration and the number of larval treatment influenced the glucose content in the haemolymph of larvae at the initial stage of spinning. With the increasing number of larval treatment from one to two times, the glucose content in the haemolymph of larvae at the initial stage of spinning increased in case of 40, 50 and 60% concentration of phytoecdysteroid treatment but the triple treatment of larvae caused notable decline in the glucose content in the haemolymph of larvae at the initial stage of spinning in all the above concentrations. The 70% phytoecdysteroid treatment caused notable decline in the glucose content in the haemolymph of larvae at the initial stage of spinning with increase in the number of larval treatment from single to triple. The trend of increase in the glucose content in the haemolymph of larvae at the initial stage of spinning with the increasing number of larval treatment has been recorded to be almost similar in case of 40, 50 and 60% phytoecdysteroid treatment. The maximum glucose content in the haemolymph of larvae at the initial stage of spinning was noticed to be 7.540±0.396 µg/mg in case of double treatment of larvae by 60% phytoecdysteroid concentration and the minimum glucose of 4.56±0.088 µg/mg was recorded in case of triple treatment of larvae by 70% phytoecdysteroid concentration.

Two way ANOVA indicates that the number of larval treatment significantly (P_2<0.05) influenced the glucose content in the haemolymph of larvae at the initial stage of spinning. The post–hoc test (Table-1b) indicates significant group difference in the glucose content in the haemolymph of larvae at the initial stage of spinning, in the single treatment, in between control and 60% and 60 and 70%. In the double treatment of larvae significant group difference was noticed in between control and 60%, 40 and 60% and 60 and 70% concentration of phytoecdysteroid treatment. In the triple treatment of larvae no significant group difference was noticed in any of the group combinations.

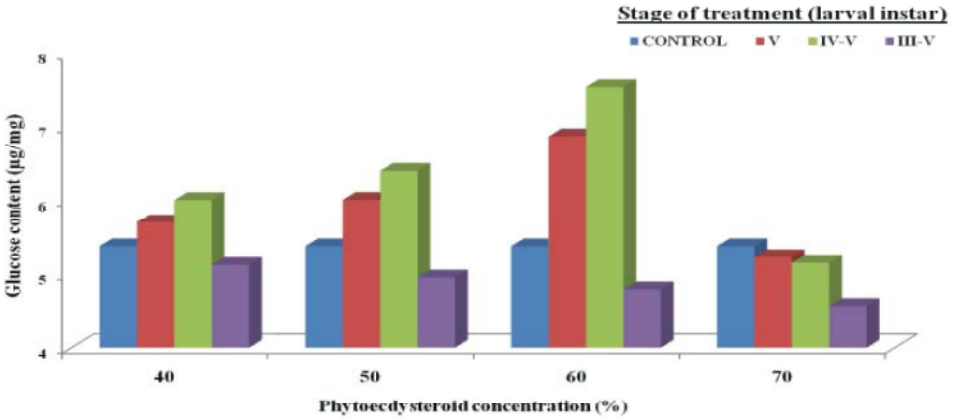

Fig. 1: Effect of phytoecdysteroid treatment on the glucose content (µg/mg) in the haemolymph of Bombyx mori larvae at the initial stage of spinning.

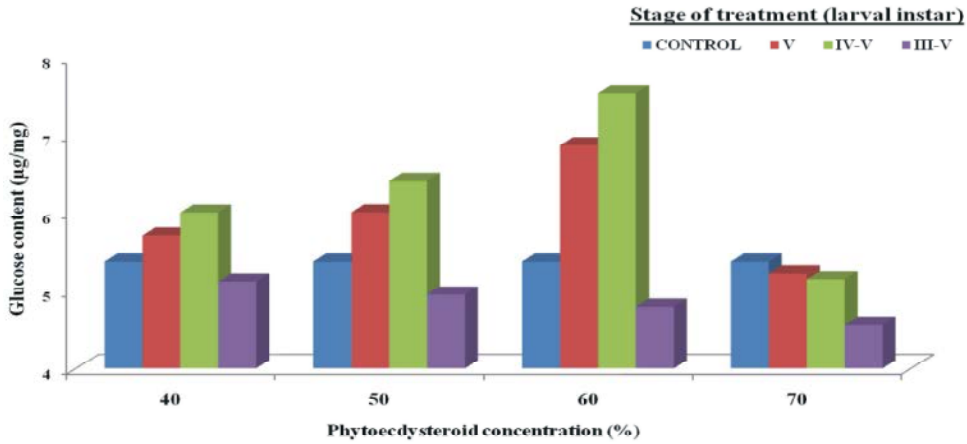

Fig. 2: Effect of phytoecdysteroid treatment on the glucose content (µg/mg) in the fat body of Bombyx mori larvae at the initial stage of spinning.

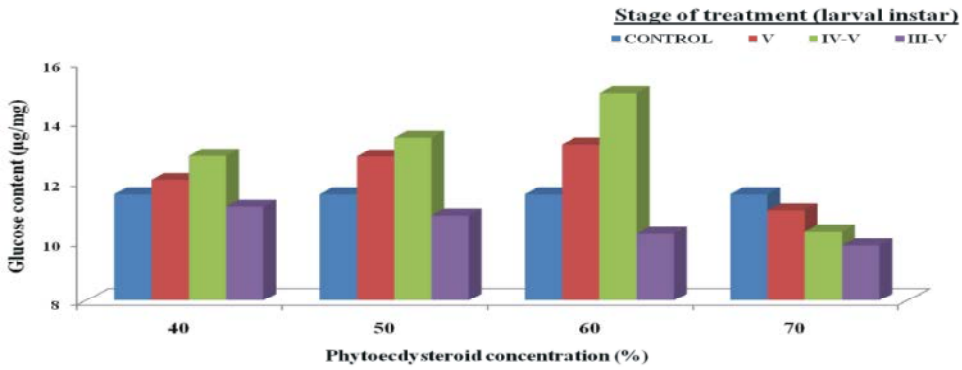

Fig. 3: Effect of phytoecdysteroid treatment on the glucose content (µg/mg) in the silk gland of Bombyx mori larvae at the initial stage of spinning.

Glucose Content (Mg/mg) in the Fat Body of *Bombyx mori* Larvae at the Initial Stage of Spinning: The data presented in Table-2a and Fig 2 shows that change in the phytoecdysteroid concentration and the number of larval treatment influenced the glucose content in the fat body of larvae at the initial stage of spinning. With the increasing number of larval treatment from one to two times, the glucose content in the fat body of larvae at the

Table 1b: Post-hoc test showing effect phytoecdysteroid treatment on the glucose content(µg/mg) in the haemolymph of Bombyx mori larvae at the initial stage of spinning.

Mean difference in between groups	Phytoecdysteroid concentration (%)		
	Single	Double	Triple
$X_1 \sim X_2$	0.33	0.63	0.26
$X_1 \sim X_3$	0.64	1.03	0.43
$X_1 \sim X_4$	*1.50	*2.17	0.59
$X_1 \sim X_5$	0.15	0.23	0.82
$X_2 \sim X_3$	0.31	0.40	0.17
$X_2 \sim X_4$	1.17	*1.53	0.33
$X_2 \sim X_5$	0.48	0.86	0.56
$X_3 \sim X_4$	0.86	1.13	0.16
$X_3 \sim X_5$	0.79	1.26	0.39
$X_4 \sim X_5$	*1.65	*2.39	0.23

Honestly significant difference (HSD) $q=\frac{\sqrt{\text{MS within}}}{n}=6.10\frac{\sqrt{0.296}}{6}=1.36$

MS = Mean square value of ANOVA table

q= Studentized range static

n = No. of replicates

*= Shows significant group difference

X_1, X_2, X_3, X_4 and X_5 are the mean values of the glucose content(µg/mg) in the haemolymph of Bombyx mori larvae at the initial stage of spinning in control, 40%, 50%, 60% and 70% phytoecdysteroid concentration respectively.

Table 2b: Post-hoc test showing effect of phytoecdysteroid treatment on the glucose content (µg/mg) in the fat body of Bombyx mori larvae at the initial stage of spinning.

Mean difference in between groups	Phytoecdysteroid concentration (%)		
	Single	Double	Triple
$X_1 \sim X_2$	0.49	1.29	0.42
$X_1 \sim X_3$	1.26	1.89	0.71
$X_1 \sim X_4$	1.67	*3.40	1.32
$X_1 \sim X_5$	0.54	1.25	1.70
$X_2 \sim X_3$	0.78	0.61	0.29
$X_2 \sim X_4$	1.18	2.12	0.90
$X_2 \sim X_5$	1.02	*2.53	1.19
$X_3 \sim X_4$	0.41	1.52	0.61
$X_3 \sim X_5$	1.79	*3.14	1.00
$X_4 \sim X_5$	2.20	*4.65	0.39

Honestly significant difference (HSD) $q=\frac{\sqrt{\text{MS within}}}{n}=6.10\frac{\sqrt{0.940}}{6}=2.414$

MS = Mean square value of ANOVA table

q= Studentized range static

n = No. of replicates

*= Shows significant group difference

X_1, X_2, X_3, X_4 and X_5 are the mean values of the glucose content (µg/mg) in the fat body of Bombyx mori larvae at the initial stage of spinning in control, 40%, 50%, 60% and 70% phytoecdysteroid concentration respectively.

Table 3b: Post-hoc test showing effect of phytoecdysteroid treatment on the glucose content (µg/mg) in the silk gland of Bombyx mori larvae at the initial stage of spinning .

Mean difference in between groups	Phytoecdysteroid concentration (%)		
	Single	Double	Triple
$X_1 \sim X_2$	0.56	1.06	0.33
$X_1 \sim X_3$	1.32	1.60	0.73
$X_1 \sim X_4$	*2.45	*3.36	1.32
$X_1 \sim X_5$	0.63	0.92	*2.43
$X_2 \sim X_3$	0.76	0.55	0.40
$X_2 \sim X_4$	1.89	2.31	0.99
$X_2 \sim X_5$	1.19	1.98	2.10
$X_3 \sim X_4$	1.13	1.76	0.59
$X_3 \sim X_5$	1.95	*2.52	1.70
$X_4 \sim X_5$	*3.08	*4.28	1.11

Honestly significant difference (HSD) $q=\frac{\sqrt{\text{MS within}}}{n}=6.10\frac{\sqrt{0.861}}{-6}=2.311$

MS = Mean square value of ANOVA table

q= Studentized range static

n = No. of replicates

*= Shows significant group difference

X_1, X_2, X_3, X_4 and X_5 are the mean values of the glucose content (µg/mg) in the silk gland of Bombyx mori larvae at the initial stage of spinning in control, 40%, 50%, 60% and 70% phytoecdysteroid concentration respectively.

initial stage of spinning increased in case of 40, 50 and 60% concentration of phytoecdysteroid treatment but the triple treatment of larvae caused notable decline in the glucose content in the fat body of larvae at initial stage of spinning in all the above concentrations. The 70% phytoecdysteroid treatment caused notable decline in the glucose content in the fat body of larvae at initial stage of spinning with increase in the number of larval treatment from single to triple. The trend of increase in the glucose content in the fat body of larvae at initial stage of spinning with the increasing number of larval treatment has been recorded to be almost similar in case of 40, 50 and 60% phytoecdysteroid treatment. The maximum glucose content in the fat body of larvae at the initial stage of spinning was noticed to be 14.952±0.030 µg/mg in case of double treatment of larvae by 60% phytoecdysteroid concentration and the minimum glucose content of 9.855±0.017 µg/mg was recorded in case of triple treatment of larvae by 70% phytoecdysteroid concentration.

Two way ANOVA indicates that the number of larval treatment significantly ($P_2<0.05$) influenced the glucose content in the fat body of larvae at initial stage of spinning. The post–hoc test (Table-2b) indicates

ignificant group difference in the glucose content in the fat body of larvae at initial stage of spinning, in the double treatment of larvae in between control and 60%, 40 and 70% and 50 and 70% and 60 and 70% concentration of phytoecdysteroid treatment. In single and triple treatment of larvae no significant group difference was noticed in any of the group combinations.

Glucose Content (µg/mg) in the Silk Gland of *Bombyx mori* Larvae at the Initial Stage of Spinning: The data presented in Table 3a and Fig - 3 shows that change in the phytoecdysteroid concentration and the number of larval treatment influenced the glucose content in the silk gland of larvae at the initial stage of spinning. With the increasing number of larval treatment from one to two times, the glucose content in the silk gland of larvae at the initial stage of spinning increased in case of 40, 50 and 60% concentration of phytoecdysteroid treatment but the triple treatment of larvae caused notable decline in the glucose content in the silk gland of larvae at the initial stage of spinning in all the above concentrations. The 70% phytoecdysteroid treatment caused notable decline in the glucose content in the silk gland of larvae at the initial stage of spinning with increase in the number of larval treatment from single to triple. The trend of increase in the glucose content in the silk gland of larvae at the initial stage of spinning with the increasing number of larval treatment has been recorded to be almost similar in case of 40, 50 and 60% phytoecdysteroid treatment. The maximum glucose content in the silk gland of larvae at the initial stage of spinning was noticed to be 15.133±0.100 µg/mg in case of double treatment of larvae by 60% phytoecdysteroid concentration and the minimum glucose content of 9.35±0.076 µg/mg was recorded in case of triple treatment of larvae by 70% phytoecdysteroid concentration.

Two way ANOVA indicates that the number of larval treatment significantly (P_2<0.05) influenced the glucose content in the silk gland of larvae at the initial stage of spinning. The post–hoc test (Table-18b) indicates significant group difference in the glucose content in the silk gland of larvae at the initial stage of spinning, in the single treatment of larvae in between control and 60% and 60 and 70% phytoecdysteroid concentration. In the double treatment of larvae significant group difference in the glucose content was noticed in between control and 60%, 50 and 70% and 60 and 70% and in the triple treatment of larvae significant group difference in glucose content was noticed in between control and 70% concentration of phytoecdysteroid treatment.

DISCUSSION

The glucose content in the haemolymph of *Bombyxmori* at the initial stage of spinning is influenced due to variation in the phytoecdysteroid concentration and number of larval treatment. The glucose content in the haemolymph at the initial stage of spinning increased with the increasing number of larval treatment from single to double in case of 40, 50 and 60% phytoecdysteroid concentration but triple treatment of larvae caused notable decline in all the above concentrations. An increase in the trehalose content of the haemolymph, during the progressive larval development, was noticed in *Antheraeamylitta* [21]. During the period of spinning of the cocoon and pupation in wax moth, the disappearance of trehalose in the haemolymph was observed [22]. A constant lower level of glucose content in the haemolymph has been noticed in *B.mori* [23, 24] and *Achetadomestica* [25]. The plant extract elevate the growth of the silkworm and thus increases the synthesis of biochemical constituents (glucose, glycogen, trahalose) and hereby enhances the silk production indices [26].

The change in the phytoecdysteroid concentration and the number of larval treatment of*B.mori* influenced the glucose content in the fat body of*B.mori* at the initial stage of spinning. The highest level of glucose content in the fat body was noticed to be 14.952 ± 0.030 µg/mg at the initial stage of spinning and in case of double treated larvae by 60% phytoecdysteroidconcentration. A number of reactions of intermediary metabolism related to carbohydrate, protein, fat and nucleic acid have been demonstrated to occur in insect fat body [27, 28]. Changes in the level of glucose content in the fat body of *B.mori* would be of physiological significance in order to understand the problem of energy supply associated with accumulation and conversion of nutrient reserves during the development and metamorphosis. The insect fat body has been recognized as the main site of trehalose biosynthesis from glucose which may be readily supplied through breakdown of the glycogen reserves maintained in the tissues [29, 30]. The synthesis and catabolism of trehalose is the process of some importance in the economy of insects and that this process is intimately

linked to the metabolism of glucose and glycogen [31]. An inverse relationship between the glycogen and trehalose in the fat body of silkworm has been noticed [32].

The glucose content in the silk gland has been noticed to be of increasing trend with the increasing phytoecdysteroid concentration up to 60% while with 70%, the glucose content decreased from single to triple treatment of larvae. The maximum level (15.133±0.100 µg/mg) of the glucose content was noticed in case of 60% concentration - double treatment of larvae at the initial stage of spinning. Sufficient amount of glucose in 5th instar larvae may be made available through glycogenolysis and also through trehalose hydrolysis during the 4 thecdysis in *Philosamiaricini* [33]. The major proportion of the glucose resulting from trehalose hydrolysis and glycogen breakdown is probably utilized for the metabolic processes associated with the preparation for spinning in *Philosamiaricini* [33]. The significant variation in the level of glucose and trehalose in different tissues like silk gland, fat body and haemolymph characterized the thermal adaptation of eri silkworm [34].

As discussed, a number of factors influenced the utilization and synthesis of glucose content in different tissues of silkworm at varying stages of the development. In last phase of 5th instar when silk gland is ready for synthesizing the silk fibers, the posterior silk gland cells synthesized large amount of fibroin. Such high glucose synthetic activity may imply coordinated functioning of all the elements of the cells machinery devoted to fibroin assembling and maturation. The variation in the number of larval treatment and phytoecdysteroid concentration caused such physiological and biochemical changes which may have influenced the glucose level in the fat body and haemolymph at the initial stage of spinning and the silk gland of *B.mori* larvae, whereas, high concentration of phytoecdysteroid, triple treatment of larvae may cause stress causing decline in the glucose content in *B.mori.*

Thus, it is concluded that double treatment *B.mori* larvae with 60% phytoecdysteroid is most suitable for the commercial rearing of multivoltine mulberry silkworm..

REFERENCES

1. Upadhyay, V.B., K.P. Gaur and S.K. Gupta, 2004. Effect of ecological factors on the silk producing potential of the mulberry silkworm (*Bombyxmori*Linn.) Malays. Appl. Biol., 33(1): 13-18.
2. Upadhyay, V.B. and A.B. Mishra, 2002. Influence ofrelative humidity on the nutritive potential of mulberry silkworm Bombyxmori. Linn. larvae J. Adv.Zool., 23(1): 54-58.
3. Pandey, A.K. and V.B. Upadhyay, 2000. Impact of refrigeration of eggs and pre-refrigeration period on the weight of shell of *Bombyxmori*Linn. J. Adv.Zool., 21(2): 98-101.
4. Upadhyay, V.B., R. Singh and S. Prasad 2006. Refrigeration of pupae influenced the hatchability and fecundity of *Bombyxmori* Linn. Malaysian Jour. Appl. Biol., 35(2): 13-19.
5. Upadhyay, V.B., R. Singh and S. Prasad, 2009. Refrigeration of cocoons influences the pupalcharacteristics of multivoltine mulberry silkworm (*Bombyxmori* Linn.) J. Appl. Biosci., 35(2): 166-168.
6. Upadhyay, V.B. and S.K. Tripathi, 2006. Effect of magnetization of eggs on the silk producing potential of multivoltine mulberry silkworm (*Bomby xmori* Linn.). Sericologia, 46(3): 269-278.
7. Tripathi, S.K. and V.B. Upadhyay, 2005. Magnetization of eggs influences the incubation period of multivoltine mulberry silkworm (*Bombyxmori* Linn.) eggs. J. Adv. Zool., 26(1): 24-28.
8. Upadhyay, V.B. and S. Prasad, 2010a. Magnetization for the improvement of silk producing potential in multivoltine mulberry silkworm (*Bombyxmori* Linn).The Bioscan., 5(2): 285-289.
9. Upadhyay, V.B. and S. Prasad, 2010b. Biotechnological importance of cocoonmagnetization with particular reference to the reproductive potential of multivoltine mulberry silkworm (*Bombyxmori* Linn). Sericologia., 50(4): 461-472.
10. Upadhyay, V. B. and S.K. Tripathi, 2005. Magnetic field influences protein content in the larvae and pupae of *Bombyxmori* Linn. J. Appl. Biosci., 31(2): 160-165.
11. Gupta, S.K., K.P. Gaur and V.B. Upadhyay, 2005. Influence of temperature on the total amino acids content in the silk gland of multivoltine mulberry silkworm (*Bombyxmori*Linn.) J. Ecophysiol. Occup. Hlth., 31(2): 160-165.
12. Upadhyay, V.B., S.K. Gupta, K.P. Gaur, C. Srivastavaand and S. Prasad, 2007. Influence of temperature on nucleic acid content in the silk gland of multivoltinemulberry silkworm (*Bombyxmori* Linn.) J. Appl. Biosci., 33(1): 63-65.

13. Upadhyay, V.B. and P. Pandey, 2012. Influence of phytoecdysteroid on pupal performance of multivoltine mulberry silkworm (*Bombyxmori* linn). The Bioscan., 7(3): 401-407.
14. Pandey, P. and V.B. Upadhyay, 2012. Influence of phytoecdysteroid on silk producing potential ofMultivoltine mulberry silkworm (*Bombyx mori*L.). J. Appl. Biosci., 38(2): 187-191.
15. Pandey, P. and V.B. Upadhyay, 2013. Impact of phytoecdysteroid treatment on the Larval performance of multivoltine mulberry Silkworm *Bombyx mori*Linn.Malays. Appl. Biol., 42(1): 51-60.
16. Srivastava, K. and V.B. Upadhyay, 2012a. Influence of Phytoecdysteroid on Weight of Ovary and Weight of Egg of Multivoltine Mulberry Silkworm *Bombyxmori* Linn. European Journal of Applied Sciences, 4(3): 117-122.
17. Srivastava, K. and V.B. Upadhyay, 2012b. Effect of Phytoecdysteroid on Length of Silk Filament and Non-Breakable Filament Length of Multivoltine Mulberry Silkworm *Bombyxmori* Linn. Academic Journal of Entomology, 5(3): 174-181.
18. Krishnaswamy, S., M.M. Narasimhanan and S.K. Suryanarayana, 1973. Sericulture Manual 2 silkworm rearing F.A.O. Agric. Services Bull., 15(2): 1-131.
19. Lafont, R., P. Beydon, C. Blais, M. Garcia, F. Lachaise, F. Riera, G. Somme and J.P. Girault, 2004. Ecdysteroid metabolism: a comparative study. Insect Biochemistry, 16(1): 11-16 (1986).
20. Mendel, B., A. Kemp and D.K. Mayers, 1954A. colorimetric micro method for the determination of glucose. Biochem, J., 56: 639-645.
21. Poonia, F.S. and S.D. Mishra, 1975. Quantitative changes in the level of carbohydrates in the food plant, haemolymph and excreta in the tasar silkworm, *Antheraeamylitta* D. (Lepidoptera) during the post embryonic stage. Indian J. Seric., 15(1): 31-34.
22. Lenartowicz, E. and S. Niemierko, 1968. The effects of low temperature and starvation on carbohydrate metabolism in larvae of *Galleria mellonella* L., J. Insect physiol., 14(4): 451-462.
23. Yanagava, H., 1973. Effect of dietary levels of glucose on the amount of trehalose, glycogen, lipids and free amino acids in the silkworm, (*Bombyxmori* L.). Bull. Seric. Exp. Stn., (Tokyo), 25(5): 267-285.
24. Kimura, S., 1974. On the metabolic fate of the carbohydrates during the larval-larval transformation in the silkworm, (*Bombyxmori* L.) with special reference to the lyctic and synthetic system of polysaccharides. Japan J. Appl. Ent. Zool., 18(4): 183-188.
25. Wang, C.M. and R.L. Patton, 1969. Carbohydrates in the haemolymph of cricket. *Achetadomesticus*. J. Insect physiol., 15: 861-866.
26. Sumathi, S. and G. Singaravelu, 2009. Supplementation of plant extracts on physiological studies in silk gland of silkworm *Bombyxmori*. J. Adv. Zool, 30(2): 88-92.
27. Kilby, B.A., 1963. The biochemistry of insect fat body. "Advances in insect physiology". (J.W.L. Beatment *et al*., eds,). Acad. Press London and New York, 1: 111-174.
28. Wyatt, G.R., 1974. Regulation of protein and carbohydrate metabolism of insect fat body. Verh. Dtsch. Zool. Ges., 67: 209-226.
29. Candy, D.J. and B.A. Kilby, 1959. Site and mode of trehalose biosynthesis in the locust. Nature London, 183: 1594-1575.
30. Candy, D.J. and B.A. Kilby, 1961. The biosynthesis of trehalose in the locust fat body. Biochem. J., 78: 531-536.
31. Prosser, C.L., 1973. Tempeature. In Prosser, C.L. Ed. Comparative Animal Physiology, 3rd ed. Saunders Co. Phil., 1: 362-420.
32. Saito, S. ;1963. Trehalose in the body fluid of the silkworm, *Bombyx mori* L. J Insect Physiol., 9: 509-519.
33. Singh, S.P. and J. Singh, 1978. Tissue specific glucose-trehalose variations during spinning in the eri-silkworm, *Philosamia ricini*. Arch. Int. Physiol. Biochem., 86: 557-561.
34. Singh, O.P., 1980. Certain changes in protein metabolism in eri silkworm, *Philosamia ricini* (Ward.) during development and thermal acclimation in relation to spinning process. Thesis submitted for Ph. D. Degree of Gorakhpur University, India.