Biolife 2(2):502-510
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
ISSN (online): 2320-4257
www.biolifejournals.com
BIOLIFE RESEARCH ARTICLE

APPLICATION OF PHYTOJUVENOID ENHANCES THE AMINO ACIDS IN THE MULTIVOLTINE MULBERRY SILKWORM (*BOMBYX MORI* LINN.)

Roli Srivastava[1*] and V.B. Upadhyay[2]

[1-2] Silkworm laboratory, Department of Zoology, D.D.U. Gorakhpur University Gorakhpur-273009, India

E-mail: dr.rolisri@gmail.com

ABSTRACT

Impact of phytojuvenoid on total free amino acids content in the haemolymph in *Bombyx mori*, a monophagous insect, was studied. Total free amino acids content in the haemolymph of *Bombyx mori* larvae at the initial and final stage of spinning increased with the increasing number of larval treatment in 10, 20 and 30% phytojuvenoid concentration which reached to the maximum level of 34.64 ± 0.05 µg/mg in the initial and 11.70 µg/mg in the final stage of spinning while in 40% phytojuvenoid concentration the total free amino acids content increased in single treatment of larvae but further increase in the number of larval treatment caused considerable decline. The results show that topical application of bioactive phytojuvenoid improved the commercial parameters in *Bombyx mori* L.

Key words : Phytojuvenoid, amino acid content, haemolymph, Larvae, *Bombyx mori.*

.

INTRODUCTION

Silk, the natural fiber that spells splendor lusture 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 economic enterprise positively favoring the rural poor in the unorganized sector. Nistari is a resistant variety of multivoltine mulberry silkworm (*Bombyx mori*) which contributes up to a great extent in the commercial production of cocoon. The efforts are being made to evolve new technologies that are cost effective, labour saving and eco-friendly. In order to increase, the production of silk, efforts have been made to study effect of ecological factor (Upadhyay et al., 2004.), temperature (Upadhyay and Mishra 1991) etc on the performance of silkworm. The Magnetization of eggs influences silk producing potential (Upadhyay and Prasad 2010a,), incubation period of eggs (Upadhyay and Prasad 2010b) and larval performance (Prasad and Upadhyay, 2011). The phytoecdysteroid has been noticed to influence the development, growth, silk producing and reproductive potential of *B. mori* (Upadhyay and Pandey 2012, Pandey and Upadhyay 2012, 2013, Srivastava and Upadhyay 2012a, b, 2013). Nutrition plays an important role in improving the growth and development of the silkworm, *Bombyx mori* L. like other organism. It is well known that silk production is dependent on the larval nutrition for production of good quality of cocoons. Nutrient supplementation is one of the strategies by which cocoon and silk productivity can be increased and the quality can be enhanced and maintained. Nutritional supplements can include minerals, vitamins, proteins, amino acids and sugars (Goudar and kaliwal 1999, Sengupta et. al. 1972). The juvenile hormone analogue

also has been noticed to influence the commercial potential of *Bombyx mori* (Nair et al., 2003, Trivedy et al 1997). Some plants like *Pinus longifolia, Abies bolsomea, Psorelea corylifolia* and *Azadirachta indica* act on *Bombyx mori* larvae as bioactive juvenoid compounds (Nair et al., 1999). The response of silkworm to very small quantities of phytojuvenoids or its analogues may extend the larval maturation events and influence the spinning process. The synchronized maturation of larvae and simultaneous spinning of cocoon is very important in the sericulture industry.

However, the response to such treatment varies depending on the dosage of compounds showing duration and number of applications (Chowdhary et al., 1990). The more food ingested during this prolonged period gets converted and it turn contributes to silk protein. Delay in moulting is probably due to the inhibitory action of JH on ecdysone synthesis in *B. mori* (Sakurai et al., 1986). JH is claimed to inhibit protein synthesis in early treated larvae with later on region protein synthesis resulting in bigger silk gland and the result is improvement of cocoon (Garel 1983). The phytojuvenoid caused beneficial effect on the reproductive and commercial traits and the life pattern of silkworm (Srivastava and Upadhyay 2013a, b, c). Keeping this in view, an attempt has been made to study the topical effect of bioactive phytojuvenoid on the improvement in the amino acids in the monphagous insect (*Bombyx mori*), which is the aim of the present investigation.

MATERIALS AND METHODS

The seed cocoons (pupa enclosed in silken case) of multivoltine mulberry silkworm *(Bombyx mori* nistari), a native of West Bengal in India, were obtained from the silkworm grainage, Directorate of sericulture, Behraich Uttar Pradesh and were maintained in the plywood trays (23 x 20 x 5cm) under the ideal rearing conditions (Krishnaswamy *et al*. 1973) in the silkworm laboratory, Department of Zoology D.D.U. Gorakhpur University, Gorakhpur. The temperature and relative humidity were maintained at 26±1^{0}C and 80±5% RH respectively till the emergence of moths from the seed cocoons. The moths emerged generally in the morning at around 4 A.M. The tray, in which seed cocoons were kept, was suddenly illuminated by light in the morning at 4 O'clock on 9th and 10th day of spinning.

The newly emerged moths 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 by Krishnaswamy *et al*. (1973); N. Achaiah (2013) and Jolly (1983).

Copulation:

Moths have a tendency to pair immediately after emergence, therefore, the female moths required to copulate with the male moths, were allowed their mates for copulation. Sufficient pairs, each containing one male and one female from newly emerged moths were allowed to mate at 26±1^{0}C and 80±5% RH in 12 hour / day dim light condition. After four hours of mating, the paired moths were detached manually by holding the female moths 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 egg laying. The disease free layings (D.F.L's), thus prepared, were treated with 2% formaline for 15 minute to increase the adhesiveness of eggs on the paper sheet and surface disinfection. Thereafter, the egg sheets with eggs laid on were thoroughly washed with running water to remove formaline and the eggs were dried in shade. The dried eggs were transferred to the incubator for hatching. After two consecutive days of hatching, the silkworm larvae were collected with the help of feather of birds and reared to maintain a stock culture in the silkworm laboratory at 26±1^{0}C and 80±5% RH and 12±1 hours light a day. Four feedings of the small pieces of fresh and clean leaves of *Morus alba* were given to the larvae and care was taken that food always remained in excess in the rearing trays. These larvae were taken for the purpose of experiments.

Experimental Design:
For extraction of phytojuvenoid the needle of *Pinus* were collected, washed thoroughly with distilled water and dried in incubator at 37^0C. The dried materials were powdered separately with the help of mechanical device. Further, 50 gm powder was subjected to extraction separately through soxlet apparatus with 250 ml distilled water for 40 hours. After 40 hours of extraction a little amount of concentrated solution of plant extract was obtained. The concentrated solution was dried and 6.45 gm material was obtained in powdered form. The dried powder thus obtained, was dissolved in distilled water as 5 gm in 25 ml water and used this solution for further experiment, as 100% concentration of phytojuvenoid. For further experiment the suitable narrow ranges of *Pinus* phytojuvenoid concentrations viz. 10, 20, 30 and 40% were taken. Thus, four phytojuvenoid concentrations were applied topically by spraying as 1 ml on to 100 larvae separately. Three sets of experiments were designed viz., single, double and triple treatment of larvae.
Single treatment of larvae: Single treatment of larvae was performed at the initial stage of fifth instar larvae just after fourth moulting. One hundred larvae of fifth instar at the initial stage were taken out from the BOD incubator and treated with one ml of 10% concentrated solution of *Pinus* needle extract by sprayer.

Double treatment of larvae:
Double treatment of larvae was started from the initial stage of fourth instar larvae. In the first treatment, one hundred larvae of fourth instar were treated by 1 ml of 10% concentrated solution of *Pinus* needle extract by spraying. The treated larvae were then transferred in BOD incubator for rearing and development. Further, similar second treatment for the same larvae was given at the initial stage of fifth instar larvae. Thus, in double treatment, fourth and fifth instar larvae were treated.

Triple treatment larvae:
For triple treatment, the third instar larvae in the initial stage were separated from BOD incubator. In the first treatment one hundred, third instar larvae, were treated by 1 ml of 10% concentrated solution of *Pinus* needle extract by sprayer and kept in BOD for rearing. The second treatment of same larvae was done just after third moulting i. e. at the initial stage of fourth instar larvae and transferred in BOD incubator for rearing. Third treatment was given at the initial stage of fifth instar i.e. just after fourth moulting of the same treated larvae as earlier. Thus, in the triple treatment third, fourth and fifth instar larvae were treated. Similar experiments were performed by 20, 30 and 40% concentrations of phytojuvenoid obtained from *Pinus* needle extract. A control set was always maintained with each set of experiment.

Estimation of total free amino acids content:
Estimation of total free amino acids content in tissues were made according to Spies et al., (1957) as modified by Singh and Agarwal (1989). For the estimation of total free amino acids content level in the haemolymph of larvae at the initial and final stage of spinning the larvae were dissected in distilled water and haemolymph was taken out separately. Took 0.5 gm (0.62 mg) haemolymph and homogenized in 2ml of 96% ethyl alcohol. The homogenate were centrifuged at 20,000 rpm and saved the supernatant. Further, took 0.1ml of supernatant in which 0.1ml distilled water and 2.0ml ninhydrin reagents were added. The mixture was mixed thoroughly. Ninhydrin reagent was prepared by mixing 1.0 gm ninhydrin in 25ml of absolute ethanol and 0.04 gm stannous chloride in 25ml citrate buffer (pH 5.0)

The reaction mixture was kept on boiling water bath for exactly 15 minutes. Further, 2 ml of 5% alcohol was added to the above boiled mixture after cooling. A violet colour was developed which was measured at 575 nm. Standard curve using the same procedure was drawn with known amount of glycine. The value of free amino acid has been expressed as µg/mg of respective tissues. Six replicates of each experiment were made.

Statistical analysis:
All the data obtained by the experiment were analyzed statistically by two-way ANOVA and Post- hoc test.

RESULTS

Total free amino acids content in the haemolymph of larvae at the initial stage of spinning:

The data given in table-1a clearly indicates that the phytojuvenoid concentration and number of larval treatment influenced the total free amino acids content in the haemolymph of the larvae at the initial stage of spinning. With the increasing number of larval treatment with 10, 20 and 30% phytojuvenoid concentration, the total free amino acids content in the haemolymph of the larvae at the initial stage of spinning increased gradually and reached to the maximum level of 34.64±0.05 µg/mg in case of triple treated larvae with 30% phytojuvenoid concentration.

In case of the larval treatment with 40% phytojuvenoid concentration, the total free amino acids content in the haemolymph of larvae at the initial stage of spinning increased in single treated larvae but further increase in the number of larval treatment caused decline in the total free amino acids content in the haemolymph of the larvae at initial stage of spinning which reached to the minimum level of 28.42±1.01 µg/mg in triple treated larvae.

The trend of increase in the total free amino acids content in the haemolymph of the larvae at initial stage of spinning was almost same in 10, 20 and 30% phytojuvenoid concentration in relation to the number of larval treatment.

Two-way ANOVA indicates that variation in the phytojuvenoid concentration significantly ($P_1 < 0.01$) influenced the total free amino acids content but the number of larval treatment did not cause significant influence on the total free amino acids content in the haemolymph of the larvae at initial stage of spinning. The Post–hoc test (table-1b) shows significant group difference in the total free amino acids content in between all group combinations except in control and 10%, control and 40% and 10 and 40% in single treated larvae. In the double treated larvae significant group difference was noticed in between all group combinations except in control and 40% and in triple treated larvae significant group difference in the total protein content was recorded in between all the group combinations.

Total free amino acid content in the haemolymph at the final stage of spinning:

It is clear from the data given in the table-2a that

Table-1a: Effect of phytojuvenoid treatment on the total free amino acids content (µg/mg) in the haemolymph of *Bombyx mori* larvae at the initial stage of spinning

	Phytojuvenoid concentration (%)					
Stage of treatment (Larval instar)	**Control X_1**	**10 X_2**	**20 X_3**	**30 X_4**	**40 X_5**	**F_1-ratio n_1 =4**
Single	29.60	29.92	31.88	33.18	30.12	
(V)	±1.07	±0.95	±2.03	±0.02	±1.04	
Double	29.60	30.85	32.56	33.97	29.45	**22.02***
(IV-V)	±1.07	±1.03	±1.06	±0.07	±1.02	
Triple	29.60	31.76	33.64	34.64	28.42	
(III-V)	±1.07	±0.94	±0.93	±0.05	±1.01	

F_2-ratio = 0.7917 ** ; **n_2**=2

P_1**< 0.01; * Non significant**

Each value represents mean ± S.E. of six replicates

X_1, X_2, X_3, X_4 and X_5 are the mean values of the total free amino acids content (µg/mg) in the haemolymph in control, 10, 20, 30 and 40 % phytojuvenoid concentration respectively.

the phytojuvenoid concentration and number of larval treatment influenced the total free amino acids content in the haemolymph of the larvae at the final stage of spinning. With the increasing number of larval treatment with 10, 20 and 30% phytojuvenoid concentration, the total free amino acids content in the haemolymph of larvae at the final stage of spinning increased gradually and reached to the maximum level of 11.70± 0.46 µg/mg in case of triple treated larvae with 30% phytojuvenoid concentration.

Table-1b: Post-hoc test showing effect of phytojuvenoid treatment on the total free amino acids content in the haemolymph of *Bombyx mori* larvae at the initial stage of spinning.

Mean difference in between groups	stage of treatment		
	Single	**Double**	**Triple**
X_1~X_2	0.32	*1.25	*2.16
X_1~X_3	*2.28	*1.96	*4.04
X_1~X_4	*3.58	*4.37	*4.37
X_1~X_5	0.52	0.15	*1.18
X_2~X_3	*2.96	*1.71	*1.88
X_2~X_4	*3.26	*3.12	*2.88
X_2~X_5	0.20	*1.40	*3.34
X_3~X_4	*2.30	*1.41	*1.00
X_3~X_5	*1.76	*3.11	*5.22
X_4~X_5	*3.06	*4.52	*6.22

Honesty Significant difference (HSD) =

$$= q\sqrt{\frac{MS\ within}{n}}$$

$$= 6.10\sqrt{\frac{0.524}{6}}$$

$$= 0.74$$

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 mean values of free amino acids content in the haemolymph of *Bombyx mori* larvae in control,10, 20, 30 and 40 per cent phytojuvenoid concentration respectively.

In case of larval treatment with 40% phytojuvenoid concentration, the total free amino acids content in the haemolymph of the larvae at final stage of spinning increased in single treated larvae but further increase in the number of larval treatment caused decline in the total free amino acids content in the haemolymph of the larvae at the final stage of spinning which reached to the minimum level of 9.85±0.34 µg/mg in triple treated larvae. The trend of increase in the total free amino acids content in the haemolymph of the larvae at the final stage of spinning was almost same in 10, 20 and 30% phytojuvenoid concentration in relation to the number of larval treatment.

Two-way ANOVA indicates that variation in the phytojuvenoid concentration significantly ($P_1 < 0.05$) influenced the total free amino acids content and the number of larval treatment did not cause significant influence on the total free amino acids content in the haemolymph of larvae at the final stage of spinning. The Post–hoc test (table-2b) shows significant group difference in the total free amino acids content in the haemolymph of larvae at the final stage of spinning in all the group combinations except in between control and 10%, control and 40% and 10 and 40% in single treated larvae. In double treated larvae significant group difference in the total free amino acids content was noticed in between all the group combinations except in between control and 40%. In triple treated larvae significant group difference in the total free amino acids content was recorded in between all the group combinations except in between 20 and 30% phytojuvenoid concentration.

The total free amino acids content in the haemolymph of larvae at the initial and final stage of spinning was considerably influenced by the variation in the phytojuvenoid concentration and the number of larval treatment. Total free amino acids content in the haemolymph increased with the increase in the phytojuvenoid concentration from 10 to 30% concentration while in 40% concentration it was declined for the same number of larval treatment. The increase in the free amino acids level of the body fluid was noticed in 5^{th} instar larvae in

Table-2a: Effect of phytojuvenoid treatment on the total free amino acids content (µg/mg) in the haemolymph of *Bombyx mori* larvae at the final stage of spinning

	Phytojuvenoid concentration (%)					
Stage of treatment (Larval instar)	**Control X_1**	**10 X_2**	**20 X_3**	**30 X_4**	**40 X_5**	**F_1-ratio n_1 =4**
Single (V)	10.55 ±0.29	10.88 ±0.69	10.96 ±0.24	11.01 ±0.68	10.64 ±0.37	
Double (IV-V)	10.55 ±0.29	11.23 ±0.42	11.31 ±0.34	11.35 ±0.57	10.28 ±0.46	**6.96***
Triple (III-V)	10.55 ±0.29	11.58 ±0.33	11.66 ±0.45	11.70 ±0.46	9.85 ±0.34	

F_2-ratio = 0.7759 ** ; **n_2**=2
P_1**< 0.01; * Non significant**
Each value represents mean ± S.E. of six replicates
X_1, X_2, X_3, X_4 and X_5 are the mean values of the total free amino acids content (µg/mg) in the haemolymph in control, 10, 20, 30 and 40 % phytojuvenoid concentration respectively.

Philosamia cynthia ricini (Kerakai and Buck, 1964; Chai, 1964) while the total free amino acids level of the body fluid was enhanced during the late 5th instar due to thermal acclimatization (Doria, 1968). In silkworms silk fibroin is derived from four amino acids i.e. alanine, serine, glycine and tyrosine (kirimura, 1962) which come from their dietary source of protein and amino acids (Ito, 1983). Silkworms obtained 72-86% of their amino acids from mulberry leaves and more than 60% of the absorbed amino acids are used for silk production (Lu and Jiang, 1988). The sharp increase in the total free amino acids content in the haemolymph was observed in the early days of the spinning due to the hydrolysis of integument and gut protein (Terra *et al.*, 1975) while decrease in the total free amino acids in the haemolymph of *Bombyx mori* was noticed toward the end of spinning (Inokuchi, 1972). The change in the free amino acids level was observed in the haemolymph of *Antheraea pernyi* during termination of diapause (Mansingh, 1967). JHA isolated from Bemchi (*Psoralea corylifolia*) significantly increased the total free amino acids content in the haemolymph of *Bombyx mori* (Nair et al., 2009).

Table-2b: Post-hoc test showing effect of phytojuvenoid treatment on the total free amino acids in the haemolymph of *Bombyx mori* larvae at final stage of spinning.

Mean difference in between groups	**stage of treatment**		
	Single	**Double**	**Triple**
X_1~X_2	0.18	*0.48	*0.84
X_1~X_3	*1.23	*0.56	*1.98
X_1~X_4	*1.58	*1.07	*2.27
X1~X_5	0.17	0.18	*0.68
X_2~X_3	*1.05	*1.08	*1.14
X_2~X_4	*1.40	*1.45	*1.43
X_2~X_5	0.01	*0.66	*1.52
X_3~X_4	*0.35	*0.37	0.29
X_3~X_5	*1.06	*1.74	*1.66
X_4~X_5	*1.41	*2.11	*1.95

Honesty Significant difference (HSD) =

$$= q\sqrt{\frac{MS\ within}{n}}$$

$$= 6.10\sqrt{\frac{0.109}{6}}$$

$$= 0.34$$

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 mean values of free amino acids content in the haemolymph of *Bombyx mori* larvae in control,10, 20, 30 and 40 per cent phytojuvenoid concentration respectively.

Thus, it may be concluded that the treatment of *Bombyx mori* larvae with phytojuvenoid concentration prolongs the larval period in the life cycle and also increases the food consumption by the larvae. This may be due to increase in the metabolic rate of larvae under the influence of the phytojuvenoid. It is observed that the food consumption by the larvae increases when the treatment is with low concentrations. The increased food consumption may increases the free amino acids in the haemolymph tissues of larvae; finally resulting in the increase in free amino acids content in tissues. However, the treatment of larvae with higher phytojuvenoid concentration may cause adverse effect on the consumption of food by the larvae, resulting in the declined of total free amino acids content in the tissues.

REFFERENCES

1. Chai, Chi – Hui., (1964) Studies on the protein metabolism in the haemolymph of *Philosamia cynthia ricini*. Acta Entomol. Sinica, 13 (4): 482 – 492.
2. Chowdhary, S.K., Raju, P.S. and Ogra, R.K., (1990). Effects of JH analogues on silkworm, *Bombyx mori* L. growth and development of silk gland. Sericolgia, 30(2): 155-165.
3. Doira, B., (1968) Developmental and sexual differences of blood proteins in the silkworm, *Bombyx mori*. Sci. Boll. Fac. Agr. Kyushu Univ., 23 (4) : 205 – 214.
4. Garel, J. P., (1983). The physiology and biology of spinning in *Bombyx mori* V Endocrinologcal aspects of silk production. Experientia, 39(5):461-466.
5. Goudar, K.S. and Kaliwal, B.B., 1999. Effect of cortisone on the economic parameters of the silkworm, *Bombyx mori* L. sericologia, 39:555-561.
6. Inokuchi, T., (1972) Qualitative changes and sexual differences of free amino acids in haemolymph of the Vth instar larvae in the silkworm, *Bombyx mori.* J. Seric. Sci. Japan, 41 (1): 44-50.
7. Ito, T., (1983). Nutrition of the silkworm and the artificial diet (In Japanese) Japan seric. J. press, Tokyo, JP. 292.
8. Jolly, M.S., (1983). Organization of industrial bivoltine grainage for tropics. Seric. Project No. 3. C.S.R. and T.I. Mysore, Govt. of India, 19-20.
9. Karakai, M. and Buck, J., (1964). Electrophoritic studies on blood proteins of *Samia cynthia ricini* during development. Biol. J. Nora Women Univ., 18: 32 – 34.
10. Kirimura J., (1962). Studies on the amino acids composition and chemical structure like protein by microbiological determination (In Japanese with English summary) bulletin of sericultural experiment station. 17:447-522.
11. Krishnaswamy, S., Narasimhanna, M. M., Suryanaryan, S.K. and Kumar Raja, S., (1973). Sericulture Manual 2. Silkworm Rearing. F.A.O.Agric. Serves Bull. Rome, 15 (2): 1 –131.
12. Lu, S.L. and. Jiang, Z.D., (1988). Absorption and utilization of amino acids in mulberry leaves by *Bombyx mori* L. acta sericologia sancta, 14:198-204.
13. Mansingh, A. (1967) Change in the free amino acids of the haemolymph of *Antherarea pernyi* during induction and termination of diapause J. Insect Physiol., 13 (11): 1645 – 1655.
14. N. Achaiah (2013). Study On Free Amino Acid Levels In *Raillietina Tetragona* (Molin, 1858). Biolife. 1(3); 96-98.
15. Nair, K.S., Nair, J. S., Kamble, C.K., Vijayan, V.A., (2009). Protein metabolism in the fifth instar larvae of *Bombyx mori* L. mediated by a juvenile hormone analogue isolated from bemchi (*Psoralea corylifolia*). Allopathy Journal, 23(2): 63-71.
16. Nair, K.S., Nair, J. S., Vijayan, V.A. and Trivedy, K., (1999). Juvenilomimic compounds for enhance productivity in silkworm, *Bombyx mori* – A screening. Indian J. Seric. 38(2): 119-124.

17. Nair, K.S., Nair, J.S., Trivedy, K. and Vijayan, V.A., (2003). Influence of bakuchiol, a JH analogue from bemchi (Psoraleacorylifolia) on silk production in silkworm, *Bombyx mori* L (Bombycidae:Lepidoptera). J. Applied Sci. Environl. Manage., 7: 31-38. 33.
18. Pandey, P. and Upadhyay, V.B., (2012). Influence of phytoecdysteroid on silk producing potential of Multivoltine mulberry silkworm (*Bombyx mori* Linn.). J.Appl. Biosci., 38(2): 187-191.
19. Pandey, P. and Upadhyay, V.B., (2013). phytoecdysteroid treatment on the Larval performance of multivoltine mulberry Silkworm *Bombyx mori* Linn. Malays. Appl. Biol., 42(1): 51-60.
20. Prasad, S. and Upadhyay, V.B., (2011). Biotechnological importance of cocoon magnetization with particular reference to the larval performance of multivoltine mulberry silkworm (*Bombyx mori* Linn.). Middle-East J. Sci. Res., 10(5): 565-572.
21. Sakurai, S., Okuda, M. and Ohtaki, T., (1986). Juvenile hormone inhibits ecdysone secretion and responsiveness to prothracicotropic hormone in prothrocic gland of *Bombyx mori.* Gen. Comp. Endocrinol., 75:220-230.
22. Sengupta, K., Singh, B.D. and mustafi, J.C., (1972). Nutrition of silkworm *Bombyx mori* L. I. Studies on the enrichment of mulberry leaves with various sugars, protein, amino acids and vitamins for vigorous growth of the worm and increased cocoon crop protection. Indian j. Sericulture 11(1): 11-27.
23. Singh, D.K. and Agarwal, R.A., (1989). Toxicity of piperoryl butoxide carbaryl synergism on the snails *Lymmaea* (Radix) acumineta. Int. Rev. Gest. Hydrobiol., 74: 689-699.
24. Spice, J.R., (1957). Colorimetric procedure for amino acid. In Method in Enzymology (Eds: Calomick, S.P. and Kalplam, N.O) Academic Press. 12: 711-718.
25. Srivastava R. and Upadhyay, V.B., (2013a). Effect of Bioactive Phytojuvenoid on the Reproductive Potential of Multivoltine Mulberry Silkworm *Bombyx mori* Linn. (Lepidoptera: Bombycidae). Academic Journal of Entomology 6 (2): 85-92, 2013.
26. Srivastava R. and Upadhyay, V.B., (2013b). Influence of Bioactive Phytojuvenoidon the Reproductive Potential of Multivoltine Mulberry Silkworm *Bombyx mori* Linn. (Lepidoptera: Bombycidae). Academic Journal of Entomology 6 (3): 139-145, 2013.
27. Srivastava R. and Upadhyay, V.B., (2013c). Influence of Bioactive Phytojuvenoid on the silk producing Potential of Multivoltine Mulberry Silkworm (*Bombyx mori* Linn.). IJFBS 2013; 1 (2): 26-31.
28. Srivastava, K. and Upadhyay, V.B., (2012a). Influence of Phytoecdysteroid on Weight of Ovary and Weight of Multivoltine Mulberry Silkworm (*Bombyx mori* Linn.). European Journal of Applied Sciences, 4(3): 117-122.
29. Srivastava, K. and Upadhyay, V.B., (2012b). Effect of Phytoecdysteroid on Length of Silk Filament and Non-Breakable Filament Length of Multivoltine Mulberry Silkworm *Bombyx mori* Linn. Academic Journal of Entomology, 5(3): 174-181.
30. Srivastava, K. and Upadhyay, V.B., (2013). Phytoecdysteroid changes protein content in the larvae of *Bombyx Mori.* J. Appl. Biosci., 39(1): 49-52, June, 2013.
31. Terra, W.R., Ferreora, C. and De Bianchi, A.G., (1975). Distribution of nutrient reserves during spinning in tissue of the larvae of fly *Rhynchosciara americana*. J. Insect Physiol., 21(8): 1501-1510.
32. Trivedy, K., Nair, K.S., Assan, N. M. and Datta, R.K., (1997). A Juvenile hormone mimic modulated enhancement of silk productivity in silkworm, *Bombyx mori* Indian J. Serc., 360: 35-38.
33. Upadhyay, V.B. and Mishra, A.B., (1991). Nutritional ability of bivoltine silkworm *Bombyx mori* Linn. larvae at higher temperature regimes. J. Adv. Zool., 12(1): 56-59.
34. Upadhyay, V.B. and Prasad, S., (2010a). Magnetization for the improvement of silk producing potential in multivoltine mulberry silkworm (*Bombyx mori L*inn). The Bioscan, 5(2): 285-289
35. Upadhyay, V.B. and Prasad, S., (2010b). Biotechnological importance of

cocoon magnetization with particular reference to the reproductive potential of multivoltine mulberry silkworm (*Bombyx mori* Linn). Sericologia, 50(4): 461-472.
36. Upadhyay, V.B. and Pandey, P., (2012). Influence of the phytoecdysteroid on pupal performance of multivoltine mulberry silkworm (*Bombyx mori* linn). The Bioscan., 7(3): 401-407.
37. Upadhyay, V.B., Gaur K.P. and Gupta, S.K., (2004). Effect of ecological factors on the silk producing potential of the mulberry silkworm (*Bombyx mori* Linn.) Malays. Appl. Biol. 33(1):13-18.

DOI:
https://dx.doi.org/10.5281/zenodo.7205044
Received: 3 April 2014;
Accepted; 20 May 2014;
Available online : 10 June 2014