Effect of Bioactive Phytojuvenoidon the Reproductive Potential of Multivoltine Mulberry Silkworm (*Bombyx mori* Linn.) (Lepidoptera: Bombycidae)

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Abstract: The application of phytojuvenoid on *Bombyx mori* larvae has been proved to be significance in the sericulture industry. Variation in the phytojuvenoid concentration significantly ($P_1 < 0.01$) influenced the reproductive potential of *B. mori* in terms of fecundity and hatchability of eggs. The fecundity and hatchability increased with the increasing number of larval treatment of 10, 20 and 30% phytojuvenoid concentration. The maximum level of fecundity (365±5.60 eggs) and hatchability (97.22±0.98 %) was noticed in case of triple treatment by 30% phytojuvenoidconcentration. The minimum level of fecundity and hatchability was noticed in case of triple treatment by 40% phytojuvenoid concentration. The larvae were treated with the phytojuvenoidconcentration (obtained from *Pinus* needle extract) of 10, 20, 30 and 40% just aftermoulting, four phytojuvenoid concentrations were applied topically by spraying on larvae separately. Three sets of experiments were designed. A control set was always maintained with each set of experiment.Phytojuvenoid hormone interactions when applied tactfully may be useful for boosting up the sericulture industry as well as the economy of silkworm rearing.

Key words: Phytojuvenoid · Reproductive Potential · Larvae · *Bombyx mori* · Larval treatement

INTRODUCTION

The Indian silk industry is an integral part of Indian textile industry and is among the oldest industries in India. The silk industry in India engages around 60 lack workers and it involves small and marginal farmers in unorganized sector. It is well known for its low investment and quick and high return which make it an ideal industry fitting well into the socio-economic frame of India. 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 effective, labour saving and eco-friendly. In order to increase, the production of silk, efforts have been made to study effect of temperature [1], relative humidity [2], photoperiod [3], artificial diet [4], X-rays [5] etc on the performance of silkworm. The magnetization of silkworm larvae also influences the performance of silkworm [6]. The Magnetization of eggs influences silk producing potential [7, 8] and incubation period of eggs [9 and 10] and larval performance [11]. In insects, the process of

growth and development is regulated by circulating hormones viz., prothoracicotropic hormone (PTTH), juvenile hormone (JH) and ecdysone, which directly and indirectly manifest the phenomenon of moulting and metamorphosis. The pattern of insect development can be regulated artificially by the mimics or analogues of these circulating hormones. The JH analogues and mimics have been reported to have some hormonal influence on the growth of *B. mori* and cocoon production. The exogenous application of JH delays larval maturation and increases the silk yield. However, the response to such treatment varies depending on the dosage of compounds showing duration and number of applications [12]. The more food ingested during this 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* [13, 14]. JH is claimed to inhibit protein synthesis in early treated larvae with later on region protein synthesis resulting in bigger silkgland and the result is improvement of cocoon shell weight [15]. The plants like *Pinuslongifolia, Abiesbalsomea, Psoraleacorylifolia* and *Azadiractaindica* have been

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identified to have juvenile hormone and *Pinus* (Pineacea) contains terpertine oil. Thus, it is hypothesized that the application of phytojuvenoid on *Bmori* larvae may enhance the duration of larval instar resulting in the utilization of more mulberry leaves which may improve the quality as well as quantity of silk on the commercial scale.

MATERIALS AND METHODS

The seed cocoons of multivoltine mulberry silkworm (*B. mori*Nistari) were obtained from the silkworm grainageBeharich, Directorate of sericulture Uttar Pradesh and were maintained in the plywood trays (23x20x5 cm) under the ideal rearing conditions [16] in the silkworm laboratory. The temperature and relative humidity were maintained in the BOD (Biological Oxygen Demand) incubator at $26 \pm 1°C$ and $80 \pm 5\%$ RH, respectively until the emergence of moths from the seed cocoons. The newly emerged moths were quickly picked up and kept sex-wise in separate trays to avoid copulation. The whole grainage operation was performed as per description given by Krishnaswamy *et al.* [16] andJolly [17] and eggs were obtained.

After completion of fifth instar, the ripe worms ceased feeding and ready for spinning. Now small mountages were provided to the ripe worms. The ripe worms soon begin the mounting which was completed within three days. Thus, sufficient number of cocoons was obtained from the silkworm larvae reared in our laboratory.

Design of Experiment for extraction of phytojuvenoid compound the needle of *Pinus* were collected, washed thoroughly with distilled water and dried in incubator at 37°C. The dried materials were powdered separately with the help of mechanical device. Further, 50 g powder was subjected to extraction separately through soxlet apparatus with 250 ml distilled water for 40 h. After thata little amount of concentrated solution of plant extract was obtained. The concentrated solution was dried and 6.45 g material was obtained in powdered form. The dried powder was dissolved in distilled water as 5 g 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 onto 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 moultingi. 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.

Fecundity: For determining the fecundity, 15 layings (three batches of 5 layings in each batch) were taken for each replicate. Thus, average of five layings was taken as representative number of eggs laid by a female moth in case of each set of experiment. Three replicates of each experiment were made.

Hatchability: After complete hatching (third day from the beginning of larval hatching) the disease free layings were counted to collect the data in respect to the total number of eggs laid per female moth, number of unfertilized eggs and number of hatched eggs per laying. The average hatching of 10 layings were taken as representative hatchability percentage per laying in case

of each batch of the study. Thirty layings (three batches of the 10 layings in each batch) were counted for each replicate. Three replicates of each experiment were made.

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

RESULTS

Fecundity: The data presented in table-1a clearly indicates that the phytojuvenoid concentration and number of larval treatment influenced the fecundity. With the increasing number of larval treatment with 10, 20 and 30% phytojuvenoid concentration, the fecundity increased gradually and reached to the highest level of 365.40±5.60 in case of triple treated larvae with 30% phytojuvenoid concentration. In case of the treatment with 40% phytojuvenoid concentration, the fecundity increased in single treated larvae but further increase in the number of larval treatment caused decline in the fecundity which reached to the lowest level of 312.42±3.50 in triple treated larvae. The trend of increase in the fecundity was almost of same pattern in 10, 20 and 30% phytojuvenoid concentration in relation to the increasing number of larval treatment.

Two-way ANOVA indicates that variation in the phytojuvenoid concentration significantly ($P_1 < 0.01$) influenced the fecundity while number of larval treatment has no significant influence. The Post–hoc test (table-1b) indicates significant group difference in the fecundity in between control and 20%, control and 30%, 10 and 20%, 10 and 30% and 30 and 40% in single treated larvae. In the double and triple treated larvae the significant group difference in the fecundity were recorded in

between all the group combinations except in between control and 40% and 20 and 30% phytojuvenoid concentration.

Hatchability: The data presented in the table-2a indicates that the phytojuvenoid concentration and number of larval treatment influenced the hatchability. With the increasing number of larval treatment with 10, 20 and 30% phytojuvenoid concentration, the hatchability increased gradually and reached to the maximum level of 95.50±0.98% in case of triple treated larvae with 30% phytojuvenoid concentration. In case of the treatment with 40% phytojuvenoid concentration, the hatchability increased in single treated larvae but further increase in the number of larval treatment caused decline in the hatchability which reached to the minimum level of 86.88±1.10% in triple treated larvae. The trend of increase in the hatchability was almost same in 10, 20 and 30% phytojuvenoid concentration in relation to the number of larval treatment. Two-way ANOVA indicates that the variation in the phytojuvenoid concentration significantly ($P_1 < 0.01$) influenced the hatchability while number of larval treatment has no significant influence. The Post–hoc test (table-2b) indicates significant group difference in the hatchability in between control and 30% and 10 and 30% in single treated larvae. In the double treated larvae significant group difference in the hatchability was noticed in between control and 30%, 10 and 30%, 10 and 40%, 20 and 40% and 30 and 40% phytojuvenoid concentration. In the triple treated larvae significant group difference in the hatchability were recorded between all the group combinations except in between control and 10%, 10 and 20%, 10 and 30% and 20 and 30% phytojuvenoid concentration.

Table 1a: Effect of phytojuvenoid treatment on the fecundity in of *Bombyx mori* moth.

| Stage of treatment (Larval instar) | Control X_1 | Phytojuvenoid concentration (%) | | | | |
		10 X_2	20 X_3	30 X_4	40 X_5	F_1-ratio $n_1 = 4$
Single (V)		322.4 ±6.5	329.30 ±5.80	343.60 ±4.03	352.96 ±5.43	335.55 ±4.35
Double (IV-V)	322.4 ±6.5	336.10 ±6.83	349.80 ±3.75	359.63 ±6.30	320.68 ±3.43	12.24*
Triple (III-V)		322.4 ±6.5	344.80 ±5.25	356.93 ±4.53	365.40 ±5.60	312.42 ±3.50

F_2-ratio = 0.2684** $n_2 = 2$

*$P_1 < 0.01$ ** Non significant

Each value represents mean $\pm$ S.E. of three replicates

X_1, X_2, X_3, X_4 and X_5 are the mean values offecundity in control, 10, 20, 30 and 40 % phytojuvenoidconcentrationrespectively.

Table 1b: Post - hoc test showing effect of phytojuvenoidtreatmenton the fecundity in *Bombyx mori*moth.

Mean difference in between groups	stage of treatment		
	Single	Double	Triple
$X_1 \sim X_2$	6.90	*13.70	*22.40
$X_1 \sim X_3$	*21.20	*27.40	*34.53
$X_1 \sim X_4$	*30.56	*37.23	*43.00
$X_1 \sim X_5$	13.15	1.72	9.98
$X_2 \sim X_3$	*14.30	*13.70	12.13
$X_2 \sim X_4$	*23.66	*23.53	*20.60
$X_2 \sim X_5$	6.25	*15.42	*32.38
$X_3 \sim X_4$	9.36	9.83	8.47
$X_3 \sim X_5$	8.15	*29.12	*44.51
$X_4 \sim X_5$	*17.41	*38.95	*52.98

Honesty Significant difference (HSD) = $q\sqrt{MS\ within}$

$n = 5.05\sqrt{65.834}$

3 = 3.66

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 fecundity in *Bombyx mori*incontrol, 10, 20, 30 and 40 per cent phytojuvenoidconcent respectively.

Table 2a:Effect of phytojuvenoid treatment on the hatchability (%) of eggs in*Bombyx mori.*

Stage of treatment (Larval instar)	Control X_1	Phytojuvenoid concentration (%)				
		10 X_2	20 X_3	30 X_4	40 X_5	F_1-ratio n_1 =4
Single (V)		91.60 ±1.03	92.30 ±1.02	93.14 ±0.93	94.47 ±0.91	93.20 ±0.93
Double (IV-V)	91.60 ±1.03	92.80 ±1.01	93.86 ±1.24	94.93 ±0.94	89.90 ±1.12	19.39*
Triple (III-V)		91.60 ±1.03	93.19 ±1.04	94.30 ±1.12	95.50 ±0.98	86.88 ±1.10

F_2-ratio = 1.3262** n_2 = 2

*P_1<0.01 **   Non significant

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

X_1, X_2, X_3, X_4 and X_5 are the mean values of hatchability (%) in control, 10, 20, 30 and 40 % phytojuvenoid concentration respectively.

Table 2b:Post - hoc test showing effect of phytojuvenoidtreatmenton the hatchability in *Bombyx mori*moth.

Mean difference in between groups	stage of treatment		
	Single	Double	Triple
$X_1 \sim X_2$	0.70	1.20	1.59
$X_1 \sim X_3$	1.20	1.54	*2.26
$X_1 \sim X_4$	*2.87	*3.33	*3.90
$X_1 \sim X_5$	1.60	1.67	*4.72
$X_2 \sim X_3$	1.84	1.06	1.11
$X_2 \sim X_4$	*2.17	*2.13	1.31
$X_2 \sim X_5$	1.10	*2.87	*6.31
$X_3 \sim X_4$	1.33	1.07	1.20
$X_3 \sim X_5$	0.06	*3.93	*7.42
$X_4 \sim X_5$	1.27	*3.00	*6.62

Honesty Significant difference (HSD) = $q\sqrt{MS\ within}$

$n = 5.05\sqrt{1.540}$

3 = 2.09

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 hatchability of eggs in *Bombyx mori*incontrol, 10, 20, 30 and 40 per cent phytojuvenoid concentration respectively.

DISCUSSION

Fecundity is a hereditary character [18]. Fecundity of moths emerged from the pupae of refrigerated eggs [19] and refrigerated pupae [20, 21] has been noticed to be negatively influenced showing the sharp decline in the eggs laying potential of silkworm. The relationship between the fecundity and adult density of *Philosamiaricini* was inversely proportional to the number of moth pairs in the container [22]. The fecundity of *Bombyx mori* varies basically due to variation in the race of silkworm [23, 24]. Highly significant positive correlation of pupal weight with the fecundity has been noticed in some other sericogenous moth viz., *Antheraeamylita* [25], *Philosamiaricini* [26] and *Samiacynthiaricini* [27]. The heavy fecundity was noticed in the moths, obtained from *B. mori* larvae feeding on ascorbic acid treated mulberry leaves [28]. The quality of mulberry leaf was recognized to be the main factor for good fecundity in *B. mori*[29]. At higher range of relative humidity, the fecundity of *B. mori*declined with the storage duration of male and female moth [30]. The production of eggs has been noticed to be influenced by the mating duration in *B. mori* [31]. The heat treatment of *B. mori*caused an increase in the fecundity of silkworm [32]. The exposure of gamma radiation of *B. mori* eggs caused an increase in the fecundity [33]. The treatment of seed with magnetic energy enhanced the crop yield [34]. The application of magnetic field in the biological system caused enhancement of metabolic activities [35] and also accelerated the 'electron transport rate constant' in the cytochrome oxidase reaction in a biological system [36]. The low magnetic field caused stimulatory effect, whereas, higher magnetic field caused inhibitory effect on fecundity [37]. The effect of induced precocious metamorphosis by way of AHJ treatment on the reproductive capacity of silkworm, *B. mori*has not been elucidated so far except on egg size and fecundity [38]. The reproductive capacity of the silkworm was curtailed to a great extent by the JHA, apart from the significant difference in the economic traits [39]. The insect reproductive activity is controlled by juvenile hormone [40] and ecdysone [41]. JHA significantly decreased the fecundity in the treated silkworm as compared to the control [39]. Fortification of mulberry leaves with IAA has stimulatory effect on the fecundity [42]. JH plays a key role in the ovariol development, oocyte maturation ect., with an equally important role by ecdysone

released from the prothoracic gland (PG) in silkworm, *B. mori* [43]. The vitellogenic female protein necessary for the growth of oocytes is already abundant in the haemolymph of *B. mori*pupae before the maximum secretion of ecdysone before moultingfrom the prothoracic glands [44]. 20-hydroxyecdysone hormone may cause certain beneficial effects on the life pattern and the fecundity and increased production of good cocoon [45].

The phytojuvenoid treatment caused prolongation of the larval period and in this period, the larvae consume more leaf which may be principal cause of higher fecundity level in the moth, obtained from the treated larvae. Due to the additional consumption of leaf, the protein level becomes high which accelerates the oogenesis when *B. mori*is treated with the low concentration of phytojuvenoid. To the contrary, treatment with high concentration of the same phytojuvenoid seems to generate stress response, causing decline in the fecundity.

The survival and development of insects are at the mercy of nature and developmental activities are restricted in accordance with the prevailing ecological conditions and to a certain extent to their genetic built up [16, 18, 46]. The multiple and single mating made no significant difference in the per cent hatching of *B. mori*eggs [47], whereas, the treatment of *B. mori*eggs with HCl (6N) caused higher hatchability of eggs [48, 49]. The increasing duration of refrigeration of *B. mori*eggs caused notable decline in the hatching per cent [50, 19], whereas, the refrigeration of *B. mori*eggs at blue stage resulted in the reduction of hatching per cent [51]. At higher duration (50 and 70 days) of the refrigeration of *B. mori*eggs, hatchability declined sharply [19]. The ecological factors also affected the hatching per cent of *B. mori* eggs [52]. The refrigeration of eggs of early stage has adverse effect on the hatchability of insect eggs [53, 54]. The low magnetic field caused the activation of enzyme activity due to conformational change in the enzyme molecule, whereas, high level of magnetic field caused inhibitory changes in the enzyme molecule and thus, the enzyme activity inhibited [55]. 20-hydroxyecdysone may cause certain beneficial effects on the life pattern and the hatchability and increased production of good cocoon [45].

Thus, it may be concluded that with the increasing phytojuvenoid concentration up to 30%, the hatchability per cent of eggs increases due to activation of enzyme activities in embryonic stage. This seems to be due to

increase in the metabolic rate of embryonic cells leading to supply of more protein. The higher phytojuvenoid concentration more than 30% may cause stress response causing decrease in the enzyme activities as a result hatchability level decreased.

REFERENCES

1. Upadhyay, V.B. and A.B. Mishra, 1991. Nutritional ability of bivoltine silkworm Bombyx moriLinn.larvae at higher temperature regimes. J. Adv. Zool., 12(1): 56-59.

2. Mishra, A.B. and V.B. Upadhyay, 1992. Nutritional efficiency of bivoltine, Bombyx mori Linn. larvae at higher regimes of relative humidity. J. Adv. Zool., 13(1 and 2): 16-18.

3. Mishra, A.B. and V.B. Upadhyay, 1993. Nutritional efficiency of bivoltine Bombyx mori Linn.larvae at different photoperiod. Proc. 80 session Indian Science Congress Assoc. Goa., pp: 54-55.

4. Iwanvat, Y. and Y. Ono, 1969. Rearing experiments on artificial diet composed mainly on the mulberry beat powder harvested in the late autumn and after. J. Seric. Sci., 38: 307-315.

5. Kanarev, G. and G.T. Cham, 1985. Effect of laser irradiation of silkworm eggs on silkworm *Bombyx mori* development and productivity. Zhivotnov Nauki, 22: 47-53.

6. Chaugale, A.K. and N.K. More, 1992. Effect of magnetization on the developmental period and cocoon characters of the Bombyx mori linn. Indian J.Seric., 31(2): 115-122.

7. Upadhyay, V.B. and S.K. Tripathi, 2006. Effect of the magnetization of eggs on the silk producing potential of multivoltine mulberry silkworm (Bombyx moriLinn.) Sericologia, 46(3): 269-278.

8. Upadhyay, V.B. and S. Prasad, 2010a. Magnetization for the improvement of silk producing potential in multivoltine mulberry silkworm (Bombyx mori Linn). The Bioscan, 5(2): 285-289.

9. Tripathi, S.K. and V.B. Upadhyay, 2005. Magnetization of eggs influences the incubation period of multivoltine mulberry silkworm Bombyx mori Linn Eggs. J. Adv. Zool., 26(1): 24-28.

10. Upadhyay, V.B. and S. Prasad, 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.

11. Prasad, S. and V.B. Upadhyay, 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.

12. Chowdhary, S.K., P.S. Rajuand R.K. ogra, 1990. Effects of JH analogues on silkworm, Bombyx mori L. growth and development of silk gland. Sericolgia, 30(2): 155-165.

13. Sakurai, S., M. Okuda and T. Ohtaki, 1986. Juvenile hormone inhibits ecdysone secretion and responsiveness to prothracicotropic hormone in prothrocic gland of Bombyx mori. Gen. Comp. Endocrinol., 75: 220-230.

14. Trivedy, K., K.S. Nair, N.M. Assan and R.K. data, 1997. A Juvenile hormone mimic modulated enhancement of silk productivity in silkworm, Bombyx mori Indian J. Serc., 360: 35-38.

15. Garel, J.P., 1983. The physiology and biology of spinning in Bombyx mori V Endocrinologcal aspects of silk production. Experientia, 39(5): 461-466.

16. Krishnaswamy, S., M. M. Narasimhanna, S.K. Suryanaryan and S. Kumar Raja, 1973. Sericulture Manual 2.Silkworm Rearing. F.A.O. Agric. Serves Bull. Rome, 15(2): 1-131.

17. Jolly, M.S., 1983. Organization of industrial bivoltinegrainage for tropics. Seric. Project No. 3. C.S.R. and T.I. Mysore, Govt. of India, pp: 19-20.

18. Robertson, F.W., 1957. Studies on qualitative inheritance genetics and environmental correlation between body size and egg production in Drosophila melanogaster (Dip). J. Genet. 55: 428-443.

19. Pandey, A.K. and V.B. Upadhyay, 2001. Effect of refrigeration of eggs and prerefrigerationperiods on the fecundity of Bombyx moriL. Lepidoptera. J. Exp. Zool. India, 4(2): 249-254.

20. Singh, K., 1984. Studies on the techniques of silkworm (Bombyx mori) seed production suitable for Tarai area of Uttar Pradesh. Ph. D. thesis submitted in the Deptt. of Zoology, Gorakhpur University, Gorakhpur.

21. Goel, R.K., A.K. Goel and T.K. thangavelu, 1993. Effect of preservation period of seed and age of males on oviposition and fertilization in Oak tassar. J. Seric., 1(2): 39-44.

22. Srivastava, A.D. and S.D. Mishra, 1985. Effect of crowding on fecundity of eri silkworm, Philosamiaricini Hutt., Indian J. Seric., 24(1): 41-43.

23. Mathur, S.K. and G. Subba Rao, 198795. The saga of Murishidabad Silk industry. Indian Silk, 26(5): 16-17.

24. Thomas, G. and D. Dale, 1997. Fecundity of mulberry silkworm at various climates. Insect Environment, 3(3): 62-63.

25. 2Siddique, A.A., A.K. Debnath and K. Sengupta, 1985. Variability and correlation studies of shell weight and their contribution traits in Anthrerea D. Sericologia, 25(1): 45-50.

26. Singh, Y.R. and B. Prasad, 1987. Correlation among body weight and fecundity in eri silkworm, Philosamiaricini Hutt. (Lep. :Saturnidae). Sericologia, 27(1): 49-60.

27. Nagalakashmamma, K., B.L.V. Gowda and M.C. Deviah, 1988. Relationship of pupal weight on fecundity and hatching in eri moth, Samiacynthiaricini Boisduval (Abst.). International Congress on tropical sericulture practiced, Bangalore, 17(23): 26.

28. Rahman, S.M., L.A.M. Khanmand J. Ferdous, 1990. Effect of various concentrations of ascarbic acid on the oviposition and hatchability of silkworm, Bombyx mori L. Bulletin of Sericultural Research, Bangladesh, 1: 47-50.

29. Kumar, C.S., B.M. Sekharappa and S.K. Sarangi, 1997. Influence of temperature and leaf quality on rearing performance of silkworm, Bombyx mori L. Indian J. Seric., 36(2): 116-120.

30. Kamble, C.K., 1997. Fertility in Bombyx mori L. Indian Textile Journal, 108(2): 56-59.

31. Tripathi, P.N., T.T. Singh and T. Singh, 1995. Studies on mating duration and its effect on fecundity and fertility in mulberry silkworm moth Bombyx mori L. (Lepidoptera: Bombycidae). Bioved., 6(1): 15-18.

32. Singh, B.D. and K. Sengupta, 1980. Temperature during pupation influences oviposition, fecundity and hatchability of silkworm, Bombyx mori L. Science and Culture, 46(5): 192-193.

33. Adbel Salam, K.A. and S.M. Mahmoud, 1995. Stimulating effects of low levels of 60 Co gammarayson the silkworm, Bombyx mori (L). Anzeigerfuer Schaedling skunde pflanzens chutzumwelts chutx., 68(7): 147-150.

34. Todoron, S.I., A.I. Goron and I.M. Gemischer, 19667. Stimulative biomagnetic effect under action of a magnetic field and magnetized water upon the seeds of Cucurbita maxima Duch God. Sofiiskiya. Unic. Biol. Fank. Kn. 2 Bot. Mirobial. Fizol. Biokhim. Rast., 61: 239-250.

35. Alexander, M.P. and S. Ganeshan, 1990. Electromagnetic fieldinduce in-vitro pollen germination and tube growth. Curr. Sci., 59: 276- 277.

36. Blank, M., 1998. Cytochrom oxidase in electric and magnetic field. Columbia University, New York, Cellular Biophysics, 3: 1-4.

37. Mullay and Mulay, 1964. Effect on Drosophila melanogaster and S-37 tamour cells. Postulates for magnetic field interpreation.In biological effect of magnetic fields.M.F.Barnothy (Ed.) Plenum Press, New York.

38. Kimura, K., M. Kiuchi and H. Akai, 1986. Effect of JH analogue and anti juveniletraetemnts on the number and size of silkworm eggs. J. Seric Sci. Jpn., 55: 335-337.

39. Nair, K.S., K. Trivedy, J.S. Nair and R.K. Datta, 1998. Manifestations related to the developmental determination and reproductive capacity of silkworm induced by an anti-juvenile hormone agent, KK-42. Indian J. Seric., 37(2): 127-132.

40. Doane, W.W., 1973. Role of hormones of insect developmental system. insects, S.J. counce and C.H. waddinton (eds.), Academic press, new york, pp: 291-497.

41. Parlak, O., S. Sakurai, M. Kaya and T. Ohotaki, 1992. Content and possible role of ecdysteroids in the larval ovary of the silkworm *Bombyx mori*. Invertebr. Repord. Dev., 21: 1-6.

42. Bharthi, D. and Y. Miao Yungen, 2003. Administration of Plant growth hormone, Indole-3-acetic on growth and economic charaters of silkworm. (*Bombyx mori*L). J. Adv. Zool., 24(1&2): 1-740.

43. Hagdorn, H.H. and J.G. Kunkel, 1979. Vitellogeninvite in insects. annu. Rev.Entomol., 24: 475-505.

44. Kawaguchi, Y. and H. Doira, 1973. Genetic analysis of the soft and elongated trunk maturation in *Bombyx mori*. J. Seric. Sci. Jpn., 64: 41-34.

45. Prasad, S. and V.B. Upadhyay, 2012. Effect of 20-hydroxyecdysone on the reproductive potential of multivoltine mulberry silkworm (*Bombyx mori* Linn.). Academic Journal of Entomology, 5(2): 86-93.

46. Andrewartha, H.G. and L.C. Birch, 1954. The distribution and abundance of animals. UnivIst. Ed. Chicago Press, U.S.A., 4(2): 1123-1132.

47. Singh, R., H.K. Chaturvedi and R.K. Datta, 1994. Fecundity of mulberry silkworm, *Bombyx mori* L. in relation to female cocoon weight and repeated matings. Indian J. Seric., 33(1): 70-71.

48. Tiburcio, J.B., 1985. Effect of different acid on the hatchability of egg and development of silkworm (Philippines), CLSU (Central Luzon State University). Scientific Journal (Philippines), 5(2): 16-36.

49. Hurkadli, H.K., 1998. Hatching of silkworm eggs. Indian Textile Journal, 108(9): 72-74.

50. Venkataramn, C.V. and C.J. Prabhakar, 1980. Refrigeration studies. Annual Report. C.S.R. and T.I. Mysore, India, pp: 125-127.

51. Govindan, R. and T.R. Narayanswamy, 1986. Influence of refrigeration of eggs ofmultivoltine silkworm, *Bombyx mori* L. at eye spot stage on rearing performance. Sericologia, 26(2): 151-155.

52. Gaur, K.P. and V.B. Upadhyay, 2002. Influence of ecological factors on thepupalduration and pupal weight of *Bombyx mori* L. J. Exp. Zool. India, 5(1): 113-118.

53. Govindan, R., M.C. Deviah, H.R. Rangaswamy and C. Thipupeswamy, 1980. Effect of refrigeration of eggs of eri silkworm. *Samiacynthiaricini* (Boisd) on hatching, Indian J. Seric., 19: 13-15.

54. Krishaba, A.N. and T.J. Henneberry, 1966. Effect of cold storage on egg viability ofcabbagelooper and some aspects of the biology of progeny surviorous. J. Econ. Ent., 59(5): 1169.

55. Young, W., 1969. Magnetic field and *in-situ* acetylcholine-esterase in the vagal heart system. in. Biological effect of magnetic field (Ed. Barnothyet) & Plenum Press, pp: 79-102.