Journal of Evolutionary Biology Research

Volume 7 Number 1, January 2015
ISSN 2141-6583

Academic Journals

ABOUT JEBR

The **Journal of Evolutionary Biology Research (JEBR)** (ISSN 2141-6583) is published Monthly (one volume per year) by Academic Journals.

Journal of Evolutionary Biology Research (JEBR) is a peer reviewed journal. The journal is published per article and covers all areas of of the subject such as: Mating Systems and Strategies, Trends in Ecology and Evolution, Genetical Evolution of Social Behaviour, Genetic drift and Biased mutation.

Submission of Manuscript

Please read the **Instructions for Authors** before submitting your manuscript. The manuscript files should be given the last name of the first author

Click here to Submit manuscripts online

If you have any difficulty using the online submission system, kindly submit via this email ajb@academicjournals.org.

With questions or concerns, please contact the Editorial Office at jebr@academicjournals.org.

Editors

Instructions for Author

Electronic submission of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

The **cover letter** should include the corresponding author's full address and telephone/fax numbers and should be in an e-mail message sent to the Editor, with the file, whose name should begin with the first author's surname, as an attachment.

Article Types
Three types of manuscripts may be submitted:

Regular articles: These should describe new and carefully confirmed findings, and experimental procedures should be given in sufficient detail for others to verify the work. The length of a full paper should be the minimum required to describe and interpret the work clearly.
Short Communications: A Short Communication is suitable for recording the results of complete small investigations or giving details of new models or hypotheses, innovative methods, techniques or apparatus. The style of main sections need not conform to that of full-length papers. Short communications are 2 to 4 printed pages (about 6 to 12 manuscript pages) in length.

Reviews: Submissions of reviews and perspectives covering topics of current interest are welcome and encouraged. Reviews should be concise and no longer than 4-6 printed pages (about 12 to 18 manuscript pages). Reviews are also peer-reviewed.

Review Process

All manuscripts are reviewed by an editor and members of the Editorial Board or qualified outside reviewers. Authors cannot nominate reviewers. Only reviewers randomly selected from our database with specialization in the subject area will be contacted to evaluate the manuscripts. The process will be blind review.
Decisions will be made as rapidly as possible, and the journal strives to return reviewers' comments to authors as fast as possible. The editorial board will re-review manuscripts that are accepted pending revision. It is the goal of the AJFS to publish manuscripts within weeks after submission.

Regular articles

All portions of the manuscript must be typed double-spaced and all pages numbered starting from the title page.

The Title should be a brief phrase describing the contents of the paper. The Title Page should include the authors' full names and affiliations, the name of the corresponding author along with phone, fax and E-mail information. Present addresses of authors should appear as a footnote.

The Abstract should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The Abstract should be 100 to 200 words in length.. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited.
Following the abstract, about 3 to 10 key words that will provide indexing references should be listed.

A list of non-standard **Abbreviations** should be added. In general, non-standard abbreviations should be used only when the full term is very long and used often. Each abbreviation should be spelled out and introduced in parentheses the first time it is used in the text. Only recommended SI units should be used. Authors should use the solidus presentation (mg/ml). Standard abbreviations (such as ATP and DNA) need not be defined.

The Introduction should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

Materials and methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer's name and address. Subheadings should be used. Methods in general use need not be described in detail.

Results should be presented with clarity and precision. The results should be written in the past tense when describing findings in the authors' experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the Results but should be put into the Discussion section.

The Discussion should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

The Acknowledgments of people, grants, funds, etc should be brief.

Tables should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph form or repeated in the text.

Figure legends should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or Powerpoint before pasting in the Microsoft Word manuscript file. Tables should be prepared in Microsoft Word. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

References: In the text, a reference identified by means of an author‘s name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author‘s name should be mentioned, followed by ’et al‘. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like ’a‘ and ’b‘ after the date to distinguish the works.

Examples:

Abayomi (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; 1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001) References should be listed at the end of the paper in alphabetical order. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text (e.g., A. Kingori, University of Nairobi, Kenya, personal communication). Journal names are abbreviated according to Chemical Abstracts. Authors are fully responsible for the accuracy of the references.

Examples:

Chikere CB, Omoni VT and Chikere BO (2008). Distribution of potential nosocomial pathogens in a hospital environment. Afr. J. Biotechnol. 7: 3535-3539.

Moran GJ, Amii RN, Abrahamian FM, Talan DA (2005). Methicillinresistant Staphylococcus aureus in community-acquired skin infections. Emerg. Infect. Dis. 11: 928-930.

Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

Short Communications

Short Communications are limited to a maximum of two figures and one table. They should present a complete study that is more limited in scope than is found in full-length papers. The items of manuscript preparation listed above apply to Short Communications with the following differences: (1) Abstracts are limited to 100 words; (2) instead of a separate Materials and Methods section, experimental procedures may be incorporated into Figure Legends and Table footnotes; (3) Results and Discussion should be combined into a single section.

Proofs and Reprints: Electronic proofs will be sent (e-mail attachment) to the corresponding author as a PDF file. Page proofs are considered to be the final version of the manuscript. With the exception of typographical or minor clerical errors, no changes will be made in the manuscript at the proof stage.

Fees and Charges: Authors are required to pay a $550 handling fee. Publication of an article in the Journal of Evolutionary Biology Research is not contingent upon the author's ability to pay the charges. Neither is acceptance to pay the handling fee a guarantee that the paper will be accepted for publication. Authors may still request (in advance) that the editorial office waive some of the handling fee under special circumstances

Submission of a manuscript implies: that the work described has not been published before (except in the form of an abstract or as part of a published lecture, or thesis) that it is not under consideration for publication elsewhere; that if and when the manuscript is accepted for publication, the authors agree to automatic transfer of the copyright to the publisher.

Journal of Evolutionary Biology Research

Table of Contents: Volume 7 Number 1, January 2015

ARTICLES

academicJournals

Vol. 7(1), pp. 1-6, January, 2015
DOI: 10.5897/JEBR2014.0059
Article Number: C33127849694
ISSN 2006 9871

http://www.academicjournals.org/JEBR

Journal of Evolutionary Biology Research

Full Length Research Paper

Improvement in cocoon yield induced by phytojuvenoid on the multivoltine mulberry silkworm (*Bombyx mori* Linn.)

Roli Srivastava* and V. B. Upadhyay

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

Received 9 February, 2014; Accepted 2 June, 2014

Impact of phytojuvenoid on commercial parameters in *Bombyx mori,* a monophagous insect, was studied. The variation in the phytojuvenoid concentration significantly influenced the length of cocoon and the number of larval treatment did not cause significant influence on the length of cocoon of *B. mori*. The length of cocoon increased from 2.88 cm (control) to the maximum level of 3.65 cm in 30% phytojuvenoid concentration - triple treated larvae and the volume of cocoon increased with increasing the number of larval treatment from single to triple in 10, 20 and in 30% phytojuvenoid concentration and the volume was highest (3.48 ml) in 30% phytojuvenoid concentration at triple treated larvae. The results show that topical application of bioactive phytojuvenoid improved the commercial parameters in *B. mori.*

Key words: Phytojuvenoid, silk producing potential, larvae, *Bombyx mori*, larval treatment.

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 effective, labour saving and eco-friendly. In order to increase, the production of silk, efforts have been made to study effect of ecological factor, photoperiod, artificial diet (Iwanvat and Ono, 1969), X-rays (Kanarev and Cham, 1985) etc. on the performance of silkworm. The Magnetization of eggs influences silk producing potential and incubation period of eggs (Upadhyay and Prasad, 2010b) and larval performance (Prasad and Upadhyay, 2011).

The phytoecdysteroid has been noticed to influence the

*Corresponding author. E-mail: dr.rolisri@gmail.com

development, growth, silk producing and reproductive potential of *B. mori* (Srivastava and Upadhyay, 2012a, b). The juvenile hormone (JH) analogue also has been noticed to influence the reproductive and commercial potential of *B. mori* (Srivastava and Upadhyay, 2013a, b, c; Nair et al., 2003). The JH analogues and mimics have been reported to have some hormonal influence on the growth of *B. mori* and cocoon production (Nair et al., 2006). 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 period, the more it gets converted and in 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; Trivedy et al., 1997). 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 shell weight (Garel, 1983). Some plants like *Pinus longifolia, Abies balsomea, Psorelea corylifolia* and *Azadiracta indica* act on *B. mori* larvae as bioactive juvenoid compounds (Nair et al., 1999). Keeping this in view, an attempt has been made to study the topical effect of bioactive phytojuvenoid on the improvement in the commercial parameters in this monphagous insect (*B. mori*), which is the aim of the present investigation.

MATERIALS AND METHODS

The seed cocoons (pupa enclosed in silken case) of multivoltine mulberry silkworm *(B. 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 5 cm) 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°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) 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°C and 80 ± 5% RH in 12 h / day dim light condition. After 4 h 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 for egg laying.

Oviposition

The gravid females laid eggs on the sheet of paper in the dark condition at 26 ± 1°C and 80 ± 5% RH. The egg laying moths were covered by open plastic cellules to prevent intermixing of egg masses deposited by different moths. After 24 h 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 magnifications for the detection of bacterial and protozoan pathogens.

Incubation of eggs and hatching

The disease free layings (D.F.L's), thus prepared, were treated with 2% formaline for 15 min 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.

Rearing of larvae

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°C and 80 ± 5% RH and 12 ± 1 h 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.

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, the needle of *Pinus longifolia* 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 40 h of extraction, a 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 thus obtained, was dissolved in distilled water as 5 g in 25 ml water and this solution was used for further experiment, as 100% concentration of phytojuvenoid. For further experiment, the suitable narrow ranges of *Pinus* phyto-juvenoid 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

Table 1a. Effect of phytojuvenoid treatment on the cocoon length (cm) in *Bombyx mori*.

Stage of treatment (Larval instar)	Phytojuvenoid concentration (%)					F_1-ratio; n_1 =4
	Control (X_1)	10 (X_2)	20 (X_3)	30 (X_4)	40 (X_5)	
Single(V)	2.88±0.02	3.05±0.01	3.28±0.04	3.41±0.01	3.06±0.03	19.05*
Double (IV-V)	2.88±0.02	3.15±0.06	3.38±0.03	3.53±0.04	2.85±0.02	
Triple(III-V)	2.88±0.02	3.29±0.05	3.49±0.03	3.65±0.02	2.78±0.01	

F2-ratio = 0.6546**; n2 = 2*P1< 0.01; ** Non significant. Each value represents mean+ S.E. of three replicates; X1, X2, X3, X4 and X5 are the mean values of the cocoon length (cm) in control, 10, 20, 30 and 40 % phytojuvenoid concentration respectively.

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 1 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, 100 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 100, 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 that is 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 that is 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. All the data obtained by the experiment were analyzed statistically by two-way ANOVA and Post- hoc test.

Length of cocoon

The cocoon obtained by the experiment was measured for length. The length of cocoon was taken by cutting the cocoon from the middle in length. The average length of cocoon (three batches of 10 cocoons in each batch) was recorded for each replicate. Three replicates of each experiment were made.

Volume of cocoon

To observe the volume of cocoon, healthy cocoons were tooken and cut slightly at the top end and the pupae was removed. The empty cocoon was filled with water with the help of pipette and the volume of required water was measured in milliliter. For the average volume of cocoon, 30 cocoons (three batches of 10 cocoons in each batch) were filled with water for each replicate. Three replicates of each experiment were made.

RESULTS

Length of cocoon

It is clear from the data given in the Table1a that the phytojuvenoid concentration and number of larval treatment caused notable influence on the length of cocoon. With the increasing number of larval treatment with 10, 20 and 30% phytojuvenoid concentration, the length of cocoon increased gradually and reached the maximum level of 3.65±0.02 cm in the case of triple treated larvae with 30% phytojuvenoid concentration. In the case of larval treatment with 40% phytojuvenoid concentration, the length of cocoon increased in single treated larvae but further increase in the number of larval treatment caused decline in the length of cocoon which reached to the minimum level of 2.78±0.01 cm in triple treated larvae. The trend of increase in the length of cocoon was almost of same fashion in 10, 20 and 30% phytojuvenoid concentration in relation to the number of larval treatment.

Two-way ANOVA indicates that variation in phytojuvenoid concentration significantly (P_1 < 0.01) influenced the length of cocoon. The Post-hoc test (Table1b) shows significant group difference in the length of cocoon in all group combinations in single treated larvae except in between control and 10%, 10 and 40% and 20 and 30% phytojuvenoid concentration. In the double and triple treated larvae, all the group combi-nations showed significant group difference in the length

Table 1b. Post - hoc test showing effect of phytojuvenoid treatment on the cocoon length (cm) in *Bombyx mori.*

Mean difference in between groups	Stage of treatment		
	Single	Double	Triple
X_1~X_2	0.17	*0.31	*0.41
X_1~X_3	*1.60	*0.50	*0.61
X_1~X_4	*0.53	*0.65	*0.77
X_1~X_5	*1.18	*0.97	*0.10
X_2~X_3	*0.23	*0.29	*0.20
X_2~X_4	*0.36	*0.34	*0.36
X_2~X_5	0.01	*0.34	*0.36
X_3~X_4	0.13	0.15	0.16
X_3~X_5	*0.22	*0.53	*0.71
X_4~X_5	*0.35	*0.68	*0.87

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

$$= \frac{5.05\sqrt{0.013}}{3}$$

$$= 0.19$$

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 cocoon volume (ml) in *Bombyx mori* in control, 10, 20, 30 and 40 per cent phytojuvnoid concentration respectively.

of cocoon except in between 20 and 30% phytojuvenoid concentration.

Volume of cocoon

The data presented in Table 2a clearly indicates that the phytojuvenoid concentration and the number of larval treatment of larvae caused notable influence on the volume of cocoon. With the increasing number of larval treatment with 10, 20 and 30% phytojuvenoid concentration, the volume of cocoon increased gradually and reached to the maximum level of 3.48±0.02 ml in the case of triple treated larvae with 30% phytojuvenoid concentration. In the case of larval treatment with 40% phytojuvenoid concentration, the volume of cocoon increased in single treated larvae but further increase in the number of larval treatment caused decline in the volume of cocoon which reached to the minimum level of 2.69±0.02 ml in triple treated larvae. The trend of increase in the volume of cocoon was almost of same pattern 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 volume of cocoon. The Post-hoc test (Table 2b) shows significant group difference in the volume of cocoon in between control and 20% control and 30%, 10 and 20%,10 and 30% and 20 and 40% at in case of single treated larvae. In the double and triple treated larvae, all the group combinations showed significant group difference in the volume of cocoon except in between control and 40% and 20 and 30% phytojuvenoid concentration.

DISCUSSION

Length of cocoon

The length of *B. mori* cocoon was influenced due to change in the phytojuvenoid concentration and the number of larval treatment. With the increasing number of larval treatment from single to triple, the length of cocoon increased in case of 10, 20 and 30% phytojuvenoid concentration while in 40% concentration, the length of cocoon increased in single treatment and further decreased with the increasing number of larval treatment. The reserpine of *Ranwolfia serpentina* plays a stimulative role in an increase in the length of cocoon (Sujatha and Rao, 2002b). The silkworm strains have been classified on the basis of cocoon length and other variables (Nakada, 1989, 1992a, 1992b). The cocoon length and width are important variables on account of the evolutionary aspects of the silkworm (Nakada, 1991, 1994). The variability existed in the polyvoltine germplasm stocks with regard to cocoon length (Rao and Nakada, 1998). The number of genes with regards to the expression of cocoon length has been identified and it was found that not many genes are involved in this (Gamo et al., 1985). In the present investigation, the length of cocoon is increased with increasing the application of phytojuvenoid concentrations up to 30% while the cocoon length decreased with 40% concentration of phytojuvenoid showing that the response is largely dose dependent. The higher phytojuvenoid concentration either resulted in the formation of vulnerable larvae or in pupal mortality. This seems to be due to the total disturbance in the endogenous hormone titre and concombinent disarrangements in the tissue metabolic activities. The lower phytojuvenoid concentrations may have influenced the metamorphic rhythm as well as economic traits as spinning process.

Volume of cocoon

The variation in the phytojuvenoid concentration and number of larval treatment influenced the volume of *B. mori* cocoon. The minimum volume of cocoon was

Table 2a. Effect of phytojuvenoid treatment on the cocoon volume (ml) in *Bombyx mori.*

Stage of treatment (Larval instar)	Phytojuvenoid concentration (%)					F_1-ratio; n_1 =4
	Control (X_1)	10 (X_2)	20 (X_3)	30 (X_4)	40 (X_5)	
Single(V)	2.83 ±0.03	2.92 ±0.03	3.15 ±0.05	3.28 ±0.04	2.93±0.01	17.5882
Double (IV-V)	2.83 ±0.03	3.04 ±0.01	3.27 ±0.03	3.37 ±0.04	2.81 ±0.02	
Triple(III-V)	3.83 ±0.03	3.17 ±0.03	3.39 ±0.04	3.48 ±0.02	2.69±0.02	

F2-ratio = 0.9150**; n2 = P1 > 0.01 ** Non significant. Each value represents mean + S.E. of three replicates. X1, X2, X3, X4 and X5 are the mean values of cocoon volume (ml) in control, 10, 20, 30 and 40 % phytojuvenoid concentration respectively.

Table 2b. Post - hoc test showing effect of phytojuevnoid treatment on the cocoon volume (ml) in *Bombyx mori.*

Mean difference in between groups	Stage of treatment		
	Single	Double	Triple
X_1~X_2	0.09	*0.21	*0.34
X_1~X_3	*0.32	*0.44	*0.56
X_1~X_4	*0.45	*0.54	*0.65
X_1~X_5	0.10	0.02	0.14
X_2~X_3	*0.23	*0.23	*0.22
X_2~X_4	*0.36	*0.33	*0.31
X_2~X_5	0.01	*0.23	*0.48
X_3~X_4	0.13	0.10	0.09
X_3~X_5	*0.22	*0.36	*0.70
X_4~X_5	0.45	*0.56	*0.77

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

$$= \frac{6.10\sqrt{0.011}}{3}$$

$$= 0.13$$

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 cocoon volume (ml) in *Bombyx mori* in control, 10, 20, 30 and 40 per cent phytojuvnoid concentration respectively.

noticed in case of the larvae treated with 40% phyto-juvenoid concentration - triple treated larvae, whereas the maximum volume of cocoon was recorded in the case of 30% phytojuvenoid concentration - triple treated larvae (Table 2a). The topical application of the juvenile hormone or of a structural analog is able to increase the silk production (Akai et al., 1971).

The reserpine of *Ranwolfia serpentine* plays a stimu-lative role in an increase in volume of cocoon (Sujatha and Rao, 2002a, b). Cocoon volume and width variables are important on account of the evolutionary aspects of the silkworm (Nakada, 1991, 1994) and the variability existed in the polyvoltine germplasm stocks with regard to cocoon volume (Rao and Nakada, 1998). The genes with regards to expression of cocoon volume have been identified (Gamo et al., 1985). Twenty-five eco-races of *Antheraea mylitta* alone reveal interesting variability in volume of cocoon (Rangaswami et al., 1987). The females spin larger cocoon than the males and thus the female cocoon volume is more than for male (Rangaswami et al., 1987). Thus, with the increasing phytojuvenoid concentration from 10 to 30%, the volume of cocoon increased. It seems that the increase in the volume of cocoon may be due to the conversion of additional leaf consumed during the extended period into the silk material and the direct stimulatory effect of phyojuvenoid compound on the protein synthesis in silk gland.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFFERENCES

Akai H, Kiguchi K, Mox K (1971). Increased accumulation of silk protein accompanies JH-induced prolongation of larval life in *Bombyx mori*. Appl. Entomol. Zool. 6:218-220.

Chowdhary SK, Raju PS, Ogra RK (1990). Effects of JH analogues on silkworm, *Bombyxmori* L. growth and development of silk gland. Sericolgia 30(2):155-165.

Gamo T, Satio S, Ohtsuka Y, Hirobe T, Tazima Y (1985). Estimation of combining ability and genetic analysis by dialed cross between regional races of silkworm (2) shape and size of cocoons. Tech. Bull. Seric. Exp. Stn. 126:121-135.

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

Iwanvat Y, Ono Y (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.

Jolly MS (1983). Organization of industrial bivoltinegrainage for tropics. Seric. Project No. 3. C.S.R. and T.I. Mysore, Govt. of India, 19-20.

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

Krishnaswamy S, Narasimhanna MM, Suryanaryan SK, Kumar RS

(1973) Sericulture Manual 2. Silkworm Rearing. F.A.O.Agric. Serves Bull. Rome. 15 (2):1-131.
Nair KS, Morison MN, Nirmal KSM, Sabita N (2006) Impact of hormonally induced larval maturation and uniform spinning on the cocoon and grainage traits of pure race of bivoltine silkworm *Bombyx mori* L. Indian J. Seric. 45(1):45-50.
Nair KS, Nair JS, Trivedy K, Vijayan VA (2003). Influence of bakuchiol, a JH analogue from bemchi (Psoraleacorylifolia) on silk production in silkworm, Bombyx mori L (Bombycidae:Lepidoptera). J. Appl. Sci. Environ. Manage., 7:31-38.
Nair KS, Nair JS, Vijayan VA, Trivedy K (1999). Juvenilomimic compounds for enhance productivity in silkworm, *Bombyx mori* - A screening. Indian J. Seric. 38(2):119-124.
Nakada T (1989). On the measurement of cocoon shape by use of image processing method, with an application of sex discrimination of silkworm,*Bombyx mori* L. Proc. Int. Congr. SABRAO, Japan, 957-960.
Nakada T (1992a). The principal component analysis regarding cocoon shape in the silkworm with special reference to the difference between female and male. Abstract, 62nd Congr. Jpn. Soc. Seric. Sci. 92.
Nakada T (1992b). Sequence of some cocoon traits in the progeny test after crossing between wild and domesticated silkworms. Wild Silkmoth 17:98-104.
Nakada T (1994). On the cocoon shape measurement and its statistical analysis in the silkworm, *Bombyx mori* L. Indian J. Seric. 33(1):100-102.
Nakada T, Maeda H, Murakami M (1991). Discriminant analysis related to sex difference of cocoon shape in the silkworm, *Bombyx mori* L. Proc. Inst. Statist. Math. 39(2):169-186.
Prasad S, Upadhyay VB (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.
Rangaswami G, Narasimhanna MN, Kashivisvanasthan K, Sastry CR (1987). Sericulture manual, 4:21.
Rao RMP, Nakada T (1998). Clustering of polyvoltine strains of the silkworm, *Bombyx mori* by image processing method: significance of cocoon size and weight variables. Indian J. Seric. 37(1):33-39.
Sakurai S, Okuda M, 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.
Srivastava R Kanchan, Upadhyay VB (2012a). Influence of Phytoecdysteroid on Weight of Ovary and Weight of Multivoltine Mulberry Silkworm (Bombyx mori Linn.). European J. Appl. Sci. 4(3):117-122.
Srivastava R Kanchan, Upadhyay VB (2012b). Effect of phytoecdysteroid on length of silk filament and non-breakable filament length of multivoltine mulberry silkworm *Bombyx mori* Linn. Acad. J. Entomol. 5(3):174-181.
Srivastava R, Upadhyay VB (2013a). Effect of bioactive phytojuvenoid on the reproductive potential of multivoltine mulberry silkworm *Bombyx mori* Linn. (Lepidoptera: Bombycidae). Acad. J. Entomol. 6(2):85-92.
Srivastava R, Upadhyay VB (2013b). Influence of Bioactive Phytojuvenoidon the Reproductive Potential of Multivoltine Mulberry Silkworm *Bombyx mori* Linn. (Lepidoptera: Bombycidae). Acad. J. Entomol. 6(3):139-145.
Srivastava R, Upadhyay VB (2013c). Influence of Bioactive Phytojuvenoid on the silk producing Potential of Multivoltine Mulberry Silkworm (*Bombyx mori* Linn.). IJFBS 1(2):26-31.
Sujatha K, Rao PA (2002a). Impact of certain phytochemical substance on the post cocoon characters of *Bombyx mori* L In: Abstract of National Seminar on mulberry Sericulture Research in India, KSSRDI, Thalaghattapura, Banglore, India, (135):26-28.
Sujatha K, Rao PA (2002b). Reserpine (A phytochemical) modulated enhancement of silk productivity in silkworm, *Bombyx mori* L J. Experimental Zool. India 5(1):57-60.
Trivedy K, Nair KS, Assan NM, data RK (1997). A Juvenile hormone mimic modulated enhancement of silk productivity in silkworm, *Bombyx mori* Ind. J. Serc. 360:35-38.
Upadhyay VB, 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.

Journal of Evolutionary Biology Research

Related Journals Published by Academic Journals

- *International Journal Of Genetics And Molecular Biology*
- *Journal Of Cell And Animal Biology*
- *Journal Of Developmental Biology And Tissue Engineering*
- *Journal Of Biophysics And Structural Biology*
- *International Journal Of Biodiversity And Conservation*
- *Journal Of Ecology And The Natural Environment*

academicJournals