



INTERNATIONAL
SERICULTURAL
COMMISSION

UNITED NATIONS REG. NO. 10418

ISSN 0250-3980

Volume 57 | Number 3 | 2017

SERICOLOGIA

Journal of Silkworms
A Quarterly Publication of
International Sericultural Commission





INTERNATIONAL
SERICULTURAL
COMMISSION

UNITED NATIONS REG. NO. 10410

SERICOLOGIA

Journal of Silkworms

MANAGING DIRECTOR

Mr. Rajit Ranjan Okhandiar

CHIEF EDITOR

Dr. Julia S. Nair

EDITOR - AT - LARGE

Dr. G. Chavancy

CHAIR, SCIENTIFIC COMMITTEE

Dr. K. Mandal

ADMINISTRATIVE COUNCIL

Mr. K. K. Shetty

Mr. Dileep Kumar R.

PUBLISHED BY

International Sericultural Commission (ISC)

Central Silk Board Complex, BTM Layout

Madiwala, Bengaluru - 560 068, INDIA

Tel: +91 80 26282191, 26680162, 26681663

Fax: +91 80 26681663

E-mail: sericologia@gmail.com

www.inserco.org

PRINTED AT

Ramya Reprographic Pvt. Ltd

No. 16/2B, Singasandra Village

Behind Dakshin Honda Show Room

Hosur Road, Bengaluru - 560 068

INDIA. Tel: +91 98440 36357

E-mail: ramyareprographic@gmail.com

EDITORIAL BOARD

Dr. Basavaraja H. K., India

Dr. Cappellozza S., Italy

Dr. Chen Y., China

Dr. Couble P., France

Dr. Dandin S. B., India

Dr. Daniel Dezmirean, Romania

Dr. Das B. C., India

Dr. Fonseca T. C., Brazil

Dr. Freddi G., Italy

Dr. Gapuz C. F., Philippines

Dr. Goldsmith M. R., USA

Dr. He K., China

Dr. Homidy H., Uzbekistan

Dr. Iatrou K., Greece

Dr. Jolly M. S., India

Dr. Kobayashi M., Japan

Dr. Kusakabe T., Japan

Dr. Lea H. Z., Korea

Dr. Machii H., Japan

Dr. Madyarov S., Uzbekistan

Dr. Mase K., Japan

Dr. Mauchamp B., France

Dr. Miranda J. E., Brazil

Dr. Rajkhowa R., Australia

Dr. Shimada T., Japan

Dr. Sivaprasad V., India

Dr. Somashekhar T. H., India

Dr. Sonwalkar T. N., India

Dr. Tenczek T., Germany

Dr. Tzenov P., Bulgaria

Dr. Xia Q., China

Dr. Yamashita O., Japan

Dr. Yungen M., China



Content

Volume 57 | Number 3 | 2017

Review Paper

Breeding in tasar silkworm, *Antheraea mylitta* Drury: A review

P. K. Kar, G. Lokesh, A. K. Sinha and Alok Sahay 122

Research Papers

Performance of F₁ population of tropical tasar silkworm, *Antheraea mylitta* D. (Lepidoptera: Saturniidae) on different primary food plants

G. Lokesh, A. K. Srivastava, P. K. Kar, P. P. Srivastava, A. K. Sinha and Alok Sahay 133

Allethrin and prallethrin based mosquito coil emission induces toxicity and alters the haemolymph proteins and cocoon traits of *Bombyx mori*

K. V. Rajini, M. Likhith Gowda, C. A. Sangma, K. A. Avinash, Punyavathi and
H. B. Manjunatha 143

Metabolic response of tasar silkworm, *Antheraea mylitta* Drury to increased tannin content

P. K. Kar, K. Jena, M. Bara and A. K. Sinha 150

Histopathological changes during the progressive infection of BmIFV in the susceptible / tolerant breeds of silkworm, *Bombyx mori* L.

M. Mamatha and M. Balavenkatasubbaiah

Brief Note

158

Treatment of waste water for residual dye removal in silk dyeing units

D. Sargunamani, K. Raghu, K. M. A. Khadar and Subhas V. Naik

Technical Report

164

Towards building a national sericulture (mulberry) innovation system in India - Possibilities and prospects

G. K. Rajesh and S. Ramaswamy

169





BREEDING IN TASAR SILKWORM, *ANTHRAEA MYLITTA* DRURY: A REVIEW

P. K. Kar^{1*}, G. Lokesh², A. K. Sinha² and Alok Sahay²

¹Regional Tasar Research Station, Central Silk Board, Prafulla Nagar, Baripada, Mayurbhanj, Odisha 757001, India.

²Silkworm Breeding and Genetics Section, Central Tasar Research and Training Institute,
Piska Nagri, Ranchi, Jharkhand 835303, India.

*E-mail: prasantakar@rediffmail.com

ABSTRACT

Tropical tasar silkworm, *Antheraea mylitta* Drury is commercially exploited in India for wild silk production that sustains the livelihood of thousands of poor rural families engaged in tasar culture. Many ecotypes of tasar silkworm are found to thrive in tropical forests where the populations perpetuate on their own, feeding on many types of food plants, thus they being outbred in nature. However, R & D efforts over the years have generated an extensive egg production system as well as package of rearing practices to be followed on systematically developed *Terminalia arjuna* and *T. tomentosa* plantations. Since 1960s, there have been attempts to develop breeds and lines of tasar silkworm and exploit the hybrid vigour. However, till date, large scale utilization of hybrid tasar silkworm remains a dream. This area of work awaits concerted and systematic efforts towards isolation and development of tasar silkworm breeds followed by exploitation of hybrid vigour for a tremendous transformation of the vanya silk production scenario of the country. This review being a comprehensive account of the silkworm breeding efforts undertaken at various pockets of tasar culture in India, may serve as potential background information towards any further serious attempt in this direction.

Key words: *Antheraea mylitta*, breeding, heterosis, hybridization, tasar silkworm.

INTRODUCTION

Tasar silkworm, *Antheraea mylitta* Drury is commercially exploited in India for vanya silk production. Tasar culture not only provides gainful employment to rural poor but also earns substantial foreign exchange for the country. Considering the potentiality and economic aspects of tropical tasar, there is enough scope of improvement. Nevertheless, there is limited information on the population structure and variability, genetic architecture, selection of right parents and genetic parameters. Although many studies have been undertaken in this species for genetic improvement, exploitation of hybrid vigour is not up to the expectations.

Genetic variability in tasar silkworm is highly essential to sustain the level of high productivity. Genetic uniformity with an insect is readily brought about by

using the same gene or gene complexes in breeding and by large scale extension of genetically related strains. When uniformity becomes the cause of genetic vulnerability, genetic diversity is the only insurance against it. The effectiveness of selection depends upon the range of genetic diversity in respect of economic characters, which are already present in the population (Wade, 1983). Almost all traits of interest in tropical tasar silkworm are highly influenced by environmental conditions. Breeding is a science concerned with predicting the consequences of forces such as selection, inbreeding, cross breeding *etc.* and devising optimum breeding policies for improvement in productivity in domestic animals or plants. The progress of breeding in such a heterogeneous population is primarily conditioned by the magnitude, nature, inter-relationship and genetic variation in the biological, commercial and technological characters (Srivastava *et al.*, 2000). Genetic parameters such as genetic advance

helps in partitioning the overall variability into its heritable and non-heritable components which is directly or indirectly associated with biological characters and yield, thus, assumes special importance with regard to breeding.

Diversity in *A. mylitta* is the result of adaptation of a species to a variable eco factor and interaction of genetic constitution. So far, 44 ecoraces of tasar silkmoth have been reported in India (Suryanarayana and Srivastava, 2005) (Figure 1, Table 1). Maximum number of ecoraces are available in Jharkhand followed by Chhattisgarh. Odisha is also bestowed with seven known ecoraces. Over a long period of time, several attempts were made towards partial domestication of wild tasar silkmoth besides their exploitation through different breeding plans. This review provides an exhaustive coverage on the existing tasar silkmoth ecoraces, studies conducted so far on improving them and the pros and cons of breeding and utilization of hybrid vigour in this sector.

Objectives of breeding

Improvement in such systems may be measured in



Figure 1: Map of India showing the distribution of ecoraces of tasar silkmoth, *A. mylitta*

Number within circle corresponds to the name of ecorace as serially listed in Table 1

Table 1: Distribution of tropical tasar silkmoth ecoraces in India

State/UT	Ecoraces	
Jammu & Kashmir	1. Sivalika	
Uttar Pradesh	2. Sultanpur	3. Monga
	4. Mirzapur	
Assam	5. Nowgong	6. NEI-95
Nagaland	7. NG - 94	
Meghalaya	8. NE2 - 95	
Manipur	9. Jiribam	
Jharkhand	10. Japla	11. Modia
	12. Kowa	13. Laria
	14. Barharwa	15. Munga
	16. Sarihan	17. Palma
	18. Daba	19. Lodhma
	20. Tira	21. Bankura
West Bengal		
Rajasthan	22. Tesera	
Odisha	23. Simlipal	24. Modal
	25. Nalia	26. Sukinda
	27. Boudh	28. Omarkote
	29. Sukly	30. Korbi
	31. Mandala	32. Kurudh
	33. Raily	34. Bhopalpatanam
Chhattisgarh	35. Jangbhir	
Madhya Pradesh	36. Jhabua	37. Piprai
	38. Seoni	39. Multai
Dadra & Nagar Haveli	40. Dadra & Nagar Haveli	
Maharashtra	41. Bhandara	
Andhra Pradesh (undivided)	42. Andhra	
Karnataka	43. Belgaum	
Kerala	44. KE - 02	

UT - Union territory

terms of increased quantity of production, superior quality of product and greater efficiency in relation to inputs. Realization of improvement depends upon the breeding value of parents, the extent of genetic variability, population size, mating design and selection efficiency.

For increased silk production, a holistic approach consisting of strategies viz., selective breeding to increase the inherent genetic potential of breeds, improvement in nutrition and management and effective disease control is required. In the first and foremost step, there is the need of collection of the best available tasar silkmoth strains. This may be followed by a heterosis breeding scheme to take

advantage immediately for improvement for which, either maximization of initial rates of improvement or maximization in the total improvement is required. The next step may be the adoption of selective breeding to maximize productivity where selection among populations and selection within each of several populations is must.

From breeders' viewpoint, the criteria for development of tasar silkworm breed and its further utilization are (a) selection of the appropriate breeds, (b) choice of the correct breeding plan and system, (c) estimation of selection parameters and economic value, (d) designing of mating system for selected individuals, (e) designing of evaluation system, (f) development of selection criteria and (g) designing a system for multiplication of evolved breed.

Progress of breeding in tropical tasar silkworm

With the establishment of Central Tasar Research and Training Institute (CTRTI) at Ranchi, Jharkhand, collection of genetic resources of tasar silkworm was initiated in the year 1965 and simultaneous efforts were made for maintenance, characterization and evaluation of genetic material.

The history of tasar silkworm breeding is relatively recent. Although tasar culture is old by centuries, it is still a virgin field that calls for breeding on systematic lines. The variability present in the species provides the understanding of the material and makes the breeding

methodology meaningful in achieving gains. Sen *et al.* (1976), Sengupta and Sengupta (1982), Sengupta *et al.* (1993), Sinha *et al.* (1993) and Singh and Srivastava (1997) have reported extremely high degree of genetic variability present in the ecoraces of *A. mylitta*. This is probably due to the wild nature of this insect. Various attempts were made to exploit the genetic architecture of *A. mylitta* (Jolly *et al.*, 1969, 1972; Bardaiyar *et al.*, 1976; Sengupta *et al.*, 1987; Siddiqui *et al.*, 1988; Sinha *et al.*, 1994 a; Sinha *et al.*, 1998). Reddy *et al.* (2010) have reviewed the application of parental selection for productivity improvement in tropical tasar silkworm. However, wild nature of insect and inadequate knowledge of genetics of tasar silkworm have puzzled the breeders with regard to the best type of breeding programme, which could be laid out to improve the existing material.

With a focus on the purity of material, a number of inbred lines were evolved at CTRTI, Ranchi. Some of these are: GB2, GB3, GB4, GB5, GB6, GB7, GB8, GB9, GE1, GE2, GF2, GF3, GF5, GB913, GB914, GB915, GB916, GB511, GE212, R57, L8, RS, S17, RF1, RF4, RF35, Nagril, Nagri2 and Nagri3. Diallel analysis with four mutant lines of Daba, based on larval body colour, were carried out by Jolly *et al.* (1969) and it was observed that all the hybrids were better in respect of hatching percentage than the respective better parents. Yellow and Almond hybrid combinations were found superior in shell weight. Hybridization studies were also made through single, double and three-way crosses involving four mutant lines and maximum heterosis was reported in double crosses in respect of fecundity, larval weight,

Table 2: Performance of hybrids during seed crop

Character	Sarihan x Laria		Modal x Sarihan		Modal x Daba		Daba (Check)
	Ranchi	Dumka	Ranchi	Dumka	Ranchi	Dumka	Ranchi
Fecundity	242	235	223	238	284	302	196
Hatching (%)	74.5	76.0	73.5	81.0	73.2	78.0	72.6
Cocoon yield/DFL	79	52	52	48	45	39	68
Cocoon weight (g)	11.26	11.70	10.83	12.00	10.53	9.44	10.18
Shell weight (g)	1.26	1.35	1.29	1.60	1.24	1.21	1.19
Shell %	10.82	11.54	11.91	13.33	11.77	12.82	11.71

cocoon weight, shell weight and silk percentage (Bardaiyar *et al.*, 1976).

Nine inbred lines – GB1, GB2, GB8, GB12, GB13, GE1, GE3, GE4 and GF4, were crossed with tester Daba (Jolly *et al.*, 1972) and the highest heterosis with regard to hatching percentage, cocoon weight and shell weight were recorded in cross combinations, GB12 x Daba, GE3 x Daba and GB13 x Daba, respectively.

Sengupta *et al.* (1987) reported the nature of heterosis from the performance of eight hybrids involving 8 selected lines *viz.*, Daba, Sukinda, Laria, Bmg, Ymg, GE1, R57 and L8. Heterosis over better parent for absolute silk yield ranged from 58.14 to 172.64 per cent. Overall study of the hybrid vigour revealed that Bmg x Ymg is the best cross followed by Laria x Sukinda for most of the desirable characters. During 1984, six lines *viz.*, Daba, RF1, RF4, RF35, RS and L8 were crossed in all possible combinations and RF1 was found an excellent general combiner for almost all the characters except fecundity and larval weight followed by RF4 for absolute silk yield, larval weight, shell weight and shell percentage. Daba was good combiner in respect of absolute silk yield, fecundity and ERR. In terms of specific combining ability, four combinations *viz.*, Daba x L8 (high x high), Daba x RF1 (high x high), RF1 x L8 (high x high) and RF4 x RF35 (high x low) were better (Siddiqui *et al.*, 1988).

Sinha *et al.* (1994 a, 1994 b, 1995) made inter-varietal, non-reciprocal crosses of eight inbred lines of *A. mylitta* *viz.*, R57, GE1, GE2, GE3, N1, N2, Ymg and L8 wherein N1 was rated the best combiner for larval weight, cocoon weight, shell weight and absolute silk yield; GE2 for fecundity and ERR, GE1 for hatching percentage and L8 for absolute silk yield. Maximum heterosis was observed for cross, GE3 x N1 in respect of absolute silk yield.

Inter-racial hybridization and its outcome

Freshly collected nine ecoraces of tasar silkworm, namely Daba, Ampatia, Barharwa, Bhandara, Modal, Nalia, Mugia, Laria and Raily were taken up for inter-racial hybridization during II crop of 1965. Among these, Ampatia x Barharwa and its reciprocal performed the best

and exhibited marked superiority over Daba (Anonymous, 1966). During 1966-67, seven races of previous year were again crossed with Daba in II crop. The results could not confirm findings of the previous year's and instead of Ampatia x Barharwa cross, Laria x Barharwa and Ampatia x Nalia emerged as better performers (Anonymous, 1967).

After a lapse of several years, the hybridization between ecoraces were again taken up in the year 1974-75 with Sarihan, Mugia, Patjharia, Raily and Modal. From these ecoraces, nine cross combinations were raised and Patjharia x Mugia, Sarihan x Raily and Sarihan x Patjharia showed marked gain over the two parents. However, none of the crosses were superior over commercially exploited variety, Daba (Anonymous, 1975). In the very next year, ecoraces, Raily, Barharwa, Laria and Modal were crossed with Daba where the cross combination Daba x Raily and its reciprocal expressed considerable gain in shell weight over check variety, Daba (Anonymous, 1976). The same crosses were repeated in the next year for both the crops. The results confirmed that Daba x Raily cross maintains the expression of heterosis (Anonymous, 1977). Subsequently, for the I crop of 1977-78, Laria, Barharwa, Raily and Daba were used for hybridization among themselves. But, none of the combinations could record a considerable gain (Anonymous, 1978).

It was only after a period of over a decade *i.e.*, in the year 1989-90, the hybridization studies picked up a fast momentum, involving ecoraces, Daba, Raily, Modal, Laria and Sukly and evolved lines- Ymg, R57, GE 1 and GF3. In Daba x Modal cross combination, improvement over superior parent was observed with regard to absolute silk yield (Anonymous, 1990).

Selection indices in tasar silkworm

Quantitative traits *viz.*, fecundity, hatching, larval duration, effective rate of rearing, yield per disease free laying (DFL), cocoon weight, shell weight and shell percentage of nine ecoraces of tasar silkworm maintained in Germplasm Bank were evaluated in the commercial crop. Observations recorded for these characters were statistically evaluated using multiple randomized complete block design to quantify the genetic component, namely genotypic and phenotypic variance, covariance,

heritability and correlation between characters. Based on the evaluation, it was recorded that the mean difference of each genotype for each character was highly significant ($p < 0.01$) except for larval duration. The phenotypic correlations between characters were positive and significant, which ranged from 0.632 to 0.809 except fecundity vs. cocoon yield/DFL. The highest index (17.65) was recorded for the ecorace Daba bivoltine followed by Daba trivoltine (17.07) and Sukinda (15.43). The lowest index (14.08) was recorded for Bhandara. The simultaneous selection analysis indicated that Daba is the best suited ecorace considering all the quantitative traits (Srivastava *et al.*, 2002).

Diallel cross analysis among ecoraces of tasar silkworm in Ranchi condition

Five ecoraces viz., Daba, Modal, Raily, Sarihan and Laria were crossed in diallel fashion. Twenty and 16 F_1 hybrids were generated during seed and commercial crop, respectively. Genetic components of parents and hybrids of seed crop were estimated by Griffing Method-II. Based on mean and general combining estimates, three parents viz., Raily, Modal and Daba were identified as better performers for almost all the traits. Performance of biological and commercial traits based on specific combining ability and heterosis over better parent indicated that the F_1 hybrids, Sarihan x Laria and Sarihan x Daba excelled for hatching, larval duration, female and male shell weight and male shell %.

Based upon specific combining ability estimates and heterosis, short listing of promising hybrids was done by combined trait index selection method. Subsequently, three promising hybrids, Sarihan x Laria (SL), Modal x Sarihan (MS) and Modal x Daba (MD) were identified. The hybrid, SL recorded maximum survival of 77.09 % (I crop) and 72.60 % (II crop). In respect of cocoon and shell weight, MS performed the best (12.08 g, 1.62 g) in seed crop whereas, during commercial crop, the highest cocoon weight (12.52 g) and shell weight (1.90 g) were recorded in the hybrid SL.

Data of seed and commercial crop, 2001 were analysed statistically by using RCBD technique. Important genetic components such as genotypic and

phenotypic variance, heritability and genetic advance were estimated. Also, simultaneous selection indices were applied by using total scores of eleven characters. Analysis of variance indicated that except hatching per cent and male larval weight in seed crop, all characters were significantly different ($p < 0.01$) from each other in both the crops (Anonymous, 2002).

The phenotypic and genotypic variance was comparatively higher in seed crop than commercial crop for most of the traits. The phenotypic coefficient of variation for different parameters was found to range from 0.531 to 28.885, and genotypic coefficient of variation from 0.51 to 20.251. The maximum heritability was recorded for fecundity (0.957, seed crop) and the lowest heritability (-0.009, seed crop) for hatching per cent. In seed crop, the lowest genetic advance observed was -0.080 for hatching per cent while the highest genetic advance estimated was 101.25 for fecundity. The simultaneous selection indices based on total scores for all the eleven characters during commercial crops showed maximum values for the hybrid, SL followed by MS and MD.

Rearing of three identified promising hybrids viz., Sarihan x Laria, Modal x Sarihan and Modal x Daba was conducted at Ranchi during seed and commercial crops. During seed crop, yield per DFL was 79, 52 and 45 respectively, whereas in commercial crop, it was 41, 38 and 33 cocoons, respectively for the hybrids. Trial rearing of the above hybrids was conducted at farmers' level at Dumka. Maximum fecundity (302) was reported for the hybrid MD whereas, the best hatching (81 %) was recorded in MS. The hybrid SL recorded the highest yield of 52 cocoons/DFL, followed by MS (48 cocoons/DFL) and MD (39 cocoons/DFL). Regarding cocoon weight, shell weight and shell %, the hybrids involving Sarihan as one of the parents performed better (Table 2). Hybrids of Sarihan x Laria and Modal x Sarihan were prepared at RTRS, Dumka and supplied to farmers for field trial. Farmers could harvest 45 cocoons/DFL for Sarihan x Laria while for Modal x Sarihan, it was 37 cocoons/DFL.

Hybridization studies involving the Raily ecorace

Raily ecorace of Bastar region of Chhattisgarh is characterized by high cocoon and shell weight along with

higher filament length. This race is not exploited for commercial production due to its racial/behavioural characters as it exhibit poor survivability and loose all the original characters under captivity. Hence, there was need for genetic improvement. Daba TV though is well established under semi-domestic conditions, the quality of cocoons is inferior in comparison to Raily. Hence, a breeding strategy was formulated to achieve a balance between viability and productivity traits involving Raily and Daba. Reciprocal backcross breeding method was used for introgression of domesticated nature of Daba into Raily ecorace. During I crop, superior heterosis over mid parent value (MP) was recorded in respect of mating and fecundity in BC1 and F1A combinations, however, it was negative in F1B combination. During II crop, BC2 and BC1 and F1A combinations exhibited superior heterosis over mid parent value in respect of mating whereas, it was negative in F1B (Daba x Raily) combination. Under popularization of Raily x Daba cross, five farmers were supplied with 50 DFLs each during I and II crop. An average yield of 62 and 36 cocoons/DFL were recorded during I and II crops, respectively (Anonymous, 2009).

Causes for non-exploitation of hybrid vigour

For exploitation of hybrid vigour, it is necessary to have luxuriant availability of pure stocks, a feature that is still lacking in *A. mylitta*. Many studies are yet to be done to understand wild nature of the insect and to help in commercial exploitation of tasar F₁ hybrids. It is seen that, not many ecoraces are easily amenable to human handling. Many of the ecoraces if reared *ex situ* cannot survive after a few generations. Based on the amenability, some important ecoraces are grouped in Table 3. Similarly, the merits and demerits of the wild ecoraces are enlisted in Table 4. In tasar silkworm, most of the traits are with high level of plasticity where, environmental interaction is more pronounced. There is remarkable deterioration in quantitative traits when the wild populations are reared *ex situ* (Tables 5 and 6), which is a concern inviting the intervention of appropriate breeding plan.

From the scanning of hybridization attempts undertaken in the course of all these years, it is obvious that a few promising hybrids were evolved but their

Table 3: Status of some of the races in relation to their adaptability

Ecorace	Status	Remarks
Daba (bivoltine), Daba (trivoltine) and Sukinda	Easily amenable	Wide range of habitat preference and adaptation
Sarihan, Bhandara and Andhra, Mandla	Moderately amenable but poor adaptability	Poor commercial characters but lower denier
Raily, Modal and Laria	The least amenable	Silk richness maximum <i>in situ</i> and quality deteriorates <i>ex situ</i>

Table 4: Merits and demerits of wild ecoraces

Merit	Demerit
Higher shell weight	The least amenable to handling
Higher cocoon weight	Erratic moth emergence
Higher shell percentage	Poor coupling under captivity
Higher filament length	Poor survivability
Higher fecundity	Inconsistent voltinism
Tolerance to biotic and abiotic stress	High plasticity in traits

exploitation could not be realized in the field. There must be some valid and strong reasons, which deterred the exploitation of F₁ hybrids in tasar silkworm up till now. Unless these reasons are clearly examined, it is difficult to work out a feasible plan for its further execution. Some of the probable hurdles in hybrid preparation and exploitation are grouped under the following two heads:

Plausible causes restricting the expression of hybrid vigour

- Inter-crossing of races in nature keep them at hybrid level, hence, further heterosis is not discernible.
- Inbreeding of ecoraces result in steep decline of commercial characters; when we utilize these for hybridization, we do get heterosis over the deteriorated parents, but on comparison with the natural population, such effect gets nullified particularly for cocoon weight and shell weight.

Table 5: Diversity in commercial characters of nature-grown cocoons

Race	Cocoon weight (g)		Shell weight (g)		Shell %	
	Male	Female	Male	Female	Male	Female
Daba	10.20±1.29 (5.69-12.94)	13.17±1.45 (9.86-16.39)	1.71±0.26 (1.15-2.47)	1.87±0.27 (1.25-2.65)	16.76	14.20
Sukinda	10.58±1.32 (9.08-12.38)	11.25±1.54 (9.38-12.36)	1.58±0.30 (0.97-2.21)	1.84±0.28 (1.05-2.48)	16.00	14.77
Raily	11.68±1.68 (7.00-12.89)	14.04±1.98 (11.16-18.58)	1.97±0.43 (1.18-2.96)	2.32±0.51 (1.44-3.42)	20.35	16.52
Modal	12.10±1.19 (9.60-16.54)	15.21±2.14 (11.59-20.1)	2.92±0.36 (2.25-3.92)	3.03±0.36 (2.25-3.92)	24.51	19.90
Laria	7.72±1.05 (5.56-10.18)	7.38±1.10 (6.06-10.09)	1.63±0.25 (1.03-2.45)	1.63±0.32 (0.91-2.26)	21.11	20.81
Sarihan	6.62±1.02 (5.20-8.34)	7.89±1.10 (6.64-9.98)	0.96±0.10 (0.70-1.25)	1.09±0.20 (0.77-1.46)	14.50	13.73
Bhandara	5.75±0.9 (3.99-6.7)	8.85±0.99 (7.23-9.98)	1.27±0.25 (0.75-1.61)	1.72±0.34 (1.18-2.36)	20.09	19.43
Andhra	6.78±1.54 (4.28-10.7)	9.94±1.91 (4.85-13.90)	1.21±0.36 (0.55-2.15)	1.52±0.43 (0.58-2.43)	16.02	17.85
Tira	5.20±0.9 (3.40-8.84)	6.44±1.09 (4.34-10.15)	0.85±0.17 (0.39-1.47)	0.99±0.85 (0.51-1.99)	16.35	15.35

Data are Mean±SD; Figures in parentheses indicate range.

Table 6: Diversity in commercial characters in *ex situ* (Ranchi) condition

Race	Cocoon weight (g)		Shell weight (g)		Shell %	
	Male	Female	Male	Female	Male	Female
Daba	8.92±1.05 (7.04-10.67)	11.26±2.07 (8.49-14.44)	1.15±0.25 (0.61-1.46)	1.28±0.28 (0.84-1.72)	12.89	11.37
Sukinda	7.92±1.10 (6.87-9.58)	10.62±1.17 (8.16-12.13)	1.00±0.20 (0.71-1.23)	1.12±0.158 (0.93-1.35)	12.63	10.55
Raily	7.80±1.33 (6.18-10.31)	11.01±1.70 (8.41-13.75)	0.89±0.25 (0.66-1.47)	1.12±0.34 (0.77-1.77)	11.41	10.17
Modal	7.76±1.26 (5.90-10.64)	10.79±0.83 (9.51-12.23)	0.91±0.22 (0.65-1.47)	1.15±0.21 (0.80-1.40)	11.73	10.66
Laria	5.80±0.66 (4.90-6.86)	8.11±1.52 (6.14-11.39)	0.70±0.17 (0.40-1.03)	0.79±0.19 (0.55-1.04)	12.07	9.74
Sarihan	5.77±0.57 (4.76-6.47)	8.48±0.71 (7.66-9.95)	0.68±0.07 (0.53-0.78)	0.85±0.15 (0.65-1.22)	11.79	10.02
Bhandara	5.79±0.61 (5.21-6.97)	9.12±1.62 (6.41-11.01)	0.64±0.08 (0.44-0.72)	0.97±0.21 (0.71-1.30)	11.05	10.64
Andhra	6.50±0.34 (4.90-8.09)	8.48±0.58 (7.74-9.44)	0.87±0.13 (0.65-1.05)	0.94±0.29 (0.72-1.52)	13.38	11.08
Tira	4.66±0.92 (1.96-6.09)	6.64±1.20 (3.15-8.75)	0.83±0.05 (0.71-0.98)	0.99±0.05 (0.90-1.20)	17.81	14.91

Data are Mean±SD; Figures in parentheses indicate range.

- iii) Available ecoraces are prone to intermixing due to migration and this disturbs the stability of ecoraces, which is the most required for hybridization programme.
- iv) Polyphagous nature of the tasar silkworm is very frequently changing the phenotypic expressions of commercial characters thus making the comparisons, loose. In other words, the phenotypic plasticity is high.
- v) Seasonal and environmental interaction has great influence on the expression of commercial characters.

Causes restricting the adoption of evolved hybrids at commercial level

- I) Studies made earlier remained sporadic and restricted to particular environment.
- ii) Lack of consistency in high yielding hybrid and parental stock.
- iii) Continuity of parents and large scale production of parents remained a problem due to following reasons:
 - a) Uncertainty of crop due to environmental rigors and attack of pests, parasites and predators as rearing of silkworm is conducted on trees in open habitat,
 - b) High incidence of mortality due to diseases in the introduced habitat,
 - c) Low multiplication rate of genetic material,
 - d) Non-synchronization of moth's emergence in different races make it difficult to obtain the F₁s,
 - e) Wild races are not amenable to handling and they lose their original characters consequent upon the change of habitat and
 - f) Lack of proper identification of genetic markers with phenotypic expression.

Genetic diversity analysis in tasar silkworm using molecular markers

Genetic variability in the living beings and their

response to various environmental components constitute the total variability and it may not be always possible to differentiate both genetic and environmental components (Ahlawat and Gupta, 2005). The genetic diversity in tasar silkworm was assessed by morphological, physio-genetic, behavioural and biochemical standards. The assessment through phenotypic representative in this insect is not a reliable measure to establish genetic variability due to the influence of environment on gene expression. The analysis of DNA allows direct assessment of genetic variability in genotypes since this is free from any environmental influence and independent of developmental stage of organism (Kar *et al.*, 2005).

Molecular tools can be useful in identifying the genetic make up, determining the population structure and genetic inter-relationship among different ecoraces of *A. mylitta* and some studies have been made in this regard also. Rao (2000) while dealing with molecular characterization of Daba and Andhra ecoraces, showed that there are certain bands which are common in both the ecoraces which is indicative of the fact that there is possible genetic proximity between two ecoraces. Chatterjee *et al.* (2004) by using 41 samples from five populations of Raily ecorace of *A. mylitta* along with six other ecoraces revealed considerable DNA polymorphism among these ecoraces based upon Inter-Simple Sequence Repeat (ISSR) markers. Gene flow and genetic diversity among the populations of Raily ecoraces depicted no sign of genetic drift among the fragmented populations, though sixty per cent of DNA polymorphism was detected among the populations.

Kar *et al.* (2005) studied the genetic diversity in the wild and semi-domesticated populations of Daba ecorace of *A. mylitta* in order to ascertain the distribution of variability within and among populations of semi-domesticated Daba-bivoltine, Daba-trivoltine and nature grown Daba wild populations, with ISSR markers. Considerable intra - and inter - population variability was found in all the three populations. Population structure analysis further suggests that the semi-domestic populations of Daba ecorace were at the threshold of differentiating themselves. The genetic variability present within wild Daba population is of much importance for

conservation and utilization in systematic breeding programme. Using 12 ISSR and 10 RAPD primers, Vijayan *et al.* (2005) studied genetic diversity within and between Raily, Daba and Modal ecoraces collected from Chhattisgarh, Jharkhand and Orissa states, respectively. It was observed by them that the genetic diversity among the ecoraces is not significant enough to make drastic genetic drift among these ecoraces in the near future. Saha and Kundu (2006) worked on molecular identification of ten ecoraces of silkworm, *A. mylitta* with RAPD and SCAR markers. It was observed that seven RAPD selected bands can identify eight ecoraces out of ten. These identified RAPD fragments were sequenced and primers were designed for SCAR markers. Out of seven sets of primers, a single primer pair produced polymorphic SCAR bands that diagnosed five of the ten ecoraces studied. Mahendran *et al.* (2006) studied genetic variation in nine ecoraces by using restriction fragment length polymorphism (RFLP) and reported that the phylogenetic relationship of different ecoraces obtained on the basis of RFLP pattern support the phenotypic and geographical isolations. In order to understand the genetic variability and phylogenetic relationship among the different ecoraces, Mahendran *et al.* (2005) characterized a repetitive DNA in tropical tasar silkworm, *A. mylitta*. Kar *et al.* (2013) have studied the genetic diversity among different populations of Raily ecorace of tasar silkworm. Very recently, Chakraborty *et al.* (2015) analyzed the population structure of eight different ecoraces using ten Amsat microsatellite loci. Hierarchical analysis of population structure using Analysis of Molecular Variance (AMOVA) revealed significant structuring ($F_{ST} = 0.154$) and considerable inbreeding ($F_{IS} = 0.505$). A significant isolation by distance was also observed. The Bayesian algorithm revealed two clusters whereas, self organization maps (SOM) showed the different ecoraces not to be clearly differentiated. They suggest that although there is a large degree of phenotypic variation among the different ecoraces of *A. mylitta*, genetically they are not very different, and the phenotypic differences may largely be a result of their respective ecology. However, large number of sample size must have been taken into account. These findings reveal that the phylogenetic analysis of the ecoraces is not congruent with the morphological

variations and their geographical distribution.

Conclusion

Much efforts should be devoted to breed better races as the silkworm breeding programme aim at evolving superior race for quantitative and qualitative characters. There are two distinct strategies in silkworm breeding viz., establishment of inbred lines by selection of quantitative and qualitative characters at successive generations and selection of suitable F_1 hybrids, which has excellent combination. No doubt, there is potential in tasar silkworm for improvement in quantitative traits, but the ways and means are to be worked out. It is seen that tasar silk moths outbreed in forest condition where males from distant populations fly over and mate with females of other population and this results in recombination and possibly responsible for better expression of quantitative traits in wild cocoons. From the perspective of its genetics, identifying the hybrid zones of different ecoraces and studying the population structure including those regions may give a finer understanding of their population genetics. Development of additional microsatellite or other molecular markers and employing a more extensive sampling regime including samples at a finer distance scale would improve the resolution of population genetic structure and, intra- and inter-race diversity of this species. Appropriate strategy must be made for systematic breeding programmes and for this, the population size, place and climatic conditions are of utmost importance.

REFERENCES

- Ahlawat S. P. S. and Gupta S. C. (2005) *Biotechnology for the assessment and conservation of animal biodiversity*. Training course on Molecular Techniques for Gene Characterization and Genome Analysis, NBAGR, Karnal, pp. 1-6.
- Anonymous (1966, 1967, 1975, 1976, 1977, 1978, 1990, 2002, 2009) *Annual Report*, Central Tasar Research and Training Institute, Ranchi, India.
- Bardaiyar V. N., Jolly M. S., Benchamin K. V. and Sinha B. R. R.

- P. (1976) Heterosis in relation to single, three-way and double crosses in *Antheraea mylitta* D. *Indian J. Seric.*, **15**: 15-20.
- Chakraborty S., Muthulakshmi M., Deena Vardhini P., Jayaprakash P., Nagaraju J. and Arunkumar K. P. (2015) Genetic analysis of Indian tasar silk moth (*Antheraea mylitta*) populations. *Sci. Rep.*, **5**, 15728; doi: 10.1038/srep15728.
- Chatterjee S. N., Vijayan K., Roy G. C. and Nair C. V. (2004) ISSR profiling of genetic variability in the ecotypes of *Antheraea mylitta* Drury, the tropical tasar silkworm. *Russian J. Genet.*, **40** (2): 152-159.
- Jolly M. S., Bardaiyar V. N., Narasimhanna M. N. and Razdan J. L. (1969) Diallel cross analysis of quantitative characters of four races of Indian Tasar silkworm *Antheraea mylitta* D. *Indian J. Seric.*, **8**: 25-33.
- Jolly M. S., Bardaiyar V. N., Sinha S. S. and Razdan J. L. (1972) Performances of four races of *Antheraea mylitta* D. in relation to triallel crossing system. *Indian J. Seric.*, **11**: 58-62.
- Kar P. K., Srivastava A. K., Lokesh G. and Sinha M. K. (2013) Population genetic analysis of Raily ecorace of *Antheraea mylitta* Drury using RAPD markers. *The Ecoscan*, Spl. Issue, Vol. **III**, pp. 107-110.
- Kar P. K., Vijayan K., Mohandas T. P., Nair C. V., Saratchandra B. and Thangavelu K. (2005) Genetic variability and genetic structure of wild and semi-domesticated populations of tasar silkworm (*Antheraea mylitta*) ecorace Daba as revealed through ISSR markers. *Genetica*, **125**: 173-183.
- Mahendran B., Padhi B. K., Ghosh A. K. and Kundu S. C. (2005) Genetic variation in ecoraces of tropical tasar silkworm, *Antheraea mylitta* D. using RFLP technique. *CurrSci.*, **90** (1): 100-102.
- Mahendran M., Acharya C., Dash R., Ghosh A. K. and Kundu S. C. (2006) Repetitive DNA in tropical tasar silkworm *Antheraea mylitta*. *Gene*, **370**: 51-57.
- Rao A. P. (2000) Some salient features of Andhra local ecorace of *Antheraea mylitta* Drury in relation to its conservation and multiplication. *Int. J. Wild Silkmoth & Silk*, **5**: 356-358.
- Reddy R. M., Sinha M. K. and Prasad B. C. (2010) Application of parental selection for productivity improvement in tropical tasar silkworm *Antheraea mylitta* Drury: A review. *J. Entomol.*, **7**: 129-140.
- Saha M. and Kundu S. C. (2006) Molecular identification of tasar silkworm (*Antheraea mylitta*) ecoraces with RAPD. *Biochem. Genet.*, **44**: 72-85.
- Sen S. K., Sengupta A. K., Das M. G. and Jolly M. S. (1976) Studies on genetic variability, correlations, path coefficient analysis and discriminant functions in *Antheraea mylitta* D. *Indian J. Seric.*, **15**: 9-14.
- Sengupta A. K. and Sengupta K. (1982) Ecoraces of tasar and their potentialities. Base paper 6, *Proceedings of Workshop on tasar culture and silk industry*, Central Tasar Research and Training Institute, Ranchi, India, pp. 1-14.
- Sengupta A. K., Siddiqui A. A., Das Mohapatra D. P., Kumar A. and Sengupta K. (1987) Studies on the potentials of heterosis in tropical tasar *Antheraea mylitta* D. *Sericologia*, **27**: 519-524.
- Sengupta A. K., Sinha A. K. and Sengupta K. (1993) Genetic reserves of *Antheraea mylitta* Drury. *Indian Silk*, **32**: 39-46.
- Siddiqui A. A., Sengupta A. K., Kumar A., Das Mohapatra D. P. and Sengupta K. (1988) Studies on the genetic architecture and gene action in diallel populations of tropical tasar silkworm *Antheraea mylitta* D. *Sericologia*, **28**: 107-113.
- Singh B. M. K. and Srivastava A. K. (1997) Ecoraces of *Antheraea mylitta* Drury and exploitation strategy through hybridization. Base paper 6, *Current Technology Seminar on Non-Mulberry Sericulture*, Central Tasar Research and

- Training Institute, Ranchi, India, January 8-9, 1997, pp. 1-39
- Sinha A. K., Sengupta A. K., Sharma K. K., Sinha B. R. R. P. and Sinha S. S. (1995) Yield components and their implication to better genotype of *Antheraea mylitta* Drury (Lepidoptera: Saturniidae). *Pers. Cytol. Genet.*, **8**: 617-626.
- Sinha A. K., Siddiqui A. A., Sengupta A. K., Sharma K. K. and Sinha B. R. R. P. (1993) Genetic variability in tasar silkworm *Antheraea mylitta*. *J. Cytol. Genet.*, **28**: 77-80.
- Sinha A. K., Siddiqui A. A., Sengupta A. K. and Sinha S. S. (1994 a) Genetics of certain silk yield traits in *Antheraea mylitta*—a diallel fashion. *J. Cytol. Genet.*, **29**: 29-33.
- Sinha A. K., Siddiqui A. A., Sengupta A. K. and Sinha S. S. (1994 b) Selection of best combiners through diallel cross of Indian tropical tasar silkworm *Antheraea mylitta* Drury (Lepidoptera: Saturniidae). *Ann. Entomol.*, **12**: 9-11.
- Sinha A. K., Srivastava A. K., Singh B. M. K., Kar P. K., Sinha B. R. R. P. and Sinha S. S. (1998) Hybrid vigour and hybrid breeding in tropical tasar silkworm *Antheraea mylitta* D. (Lepidoptera: Saturniidae). *Pers. Cytol. Genet.*, **9**: 625-629.
- Sinha B. R. R. P. and Srivastava A. K. (2002) Application of biotechnology to enhance quality tasar production. *Persp. Cytol. Genet.*, **11**: 39-50.
- Srivastava A. K., Naqvi A. H., Roy G. C. and Sinha B. R. R. P. (2000) Temporal variation in quantitative and qualitative character of *Antheraea mylitta* Drury. *Int. J. Wild Silkworm and Silk*, **5**: 54-56.
- Srivastava A. K., Sinha A. K. and Sinha B. R. R. P. (2002) Present status of tropical tasar silkworm germplasm management. *Proceedings of Workshop on Germplasm Management and Utilization*, Central Sericultural Germplasm Resources Centre, Hosur, pp. 116-122.
- Suryanarayana N. and Srivastava A. K. (2005) *Monograph on tropical tasar silkworm*. Central Tasar Research and Training Institute, Ranchi, India, p. 134.
- Vijayan K., Nair C. V., Kar P. K., Mohandas T. P., Saratchandra B. and Raje Urs S. (2005) Genetic variability within and among three ecotypes of the tasar silkworm *Antheraea mylitta* Drury as revealed by ISSR and RAPD markers. *Int. J. Indust. Entomol.*, **10** (1): 51-59.
- Wade M. J. (1983) The effects of genotypic interactions on evolution in structural populations. *Proc. Int. Congress of Genetic "Frontiers of Genetics" IV*, Oxford and IBH Publishing Co., New Delhi, India, pp. 283-290.



Research Paper

PERFORMANCE OF F₁ POPULATION OF TROPICAL TASAR SILKWORM, *ANTHRAEA MYLITTA* D. (LEPIDOPTERA: SATURNIIDAE) ON DIFFERENT PRIMARY FOOD PLANTS

G. Lokesh^{1*}, A. K. Srivastava², P. K. Kar², P. P. Srivastava², A. K. Sinha² and Alok Sahay²

¹Central Sericultural Germplasm Resources Centre, Central Silk Board, Hosur, 635109, India.

²Silkworm Breeding & Genetics, Central Tasar Research and Training Institute,
Central Silk Board, Ranchi, 835303, India.

*E-mail: lokesh10csb@gmail.com

ABSTRACT

The expression of heterosis in economic characters of semi-domesticated tasar silkworm is highly desirable from commercial point of view as well as in genetic improvement. Polyphagous tasar silkworm feeds on a wide range of food plants and expresses extensive phenotypic characters at different ecological niches. The F₁ populations of reciprocal crosses of two wild Sal based ecoraces viz., Raily and Laria, and semi-domesticated Daba crossed with Laria were produced. The performance of the F₁ hybrids on three primary food plants such as Arjun, Asan and Sal was studied and compared with their parent populations. Significant differences ($p < 0.001$) in the performance among the F₁ combinations and the parent populations were recorded with Laria x Daba cross displaying higher heterosis value for fecundity (+ 15.16 %) and higher hatching (83 ± 2.56 %). Significant variations were recorded in the cocoon yield and cocoon characters during first and second crop as well as among the different food plants involved. Pupal weight and fecundity were recorded higher in *Terminalia* fed tasar silkworms. Arjun and Asan plants have shown prominence in respect of cocoon yield over Sal. The F₁ populations fared better cocoon characters on Sal than other food plants. The results reveal immense potential for utilization of single hybrids (F₁) of tasar silkworm for boosting tasar cocoon productivity and simultaneous exploitation of the vast Sal flora.

Key words: *Antheraea mylitta*, Daba, heterosis, F₁ population, Laria, Raily, reciprocal cross.

INTRODUCTION

The tropical tasar silkworm rearing that contributes to vanya silk production provides a livelihood option for millions of tribes and aboriginals living in the forests of Central, Eastern and Northern India. *Antheraea mylitta* D., the tasar silkworm has rich genetic resources, with 44 ecoraces distributed in different ecological systems (Suryanarayana and Srivastava, 2005). It is polyphagous feeding mainly on three primary food plants and more than a dozen secondary food plants (Suryanarayana *et al.*, 2005). As quite well understood, the quality of leaf plays an important role in the nutrition of silkworm and in turn cocoons and silk production (Bongale and Chaluvachari, 1995). It is apparent that the expression of phenotypic

characters largely depends on interaction with host plant's biochemical constitution and the environment. The primary food plants such as *Terminalia arjuna* (Arjun) and *Terminalia tomentosa* (Asan) are widely utilized for the rearing and production of tasar cocoons. Many wild ecoraces forage on *Shorea robusta* (Sal) for its survivability and perpetuation (Reddy *et al.*, 2011). Many have reported that host plants influence the silkworm larval weight, survivability, growth and development of the larva, pupal weight, adult emergence, and fecundity (Pandey *et al.*, 1968; Singh and Byas, 1975; Dubey *et al.*, 1981). Sal flora provides the base for a large quantity of nature grown cocoons of some important ecoraces viz., Laria of Jharkhand, Modal of Orissa and Raily of

Chhattisgarh, wherein they are regularly collected from the thickly populated Sal forests (Srivastava *et al.*, 2003). The ecoraces breed better and maintain their physical and genetic characters in their natural habitat (forests) which hitherto deteriorate when reared *ex-situ* (Yadav *et al.*, 1992; Lokesh *et al.*, 2015). Lower survivability and inadequate cocoon yield on the Sal highly warrant interventions for the improvement of productivity, since, Sal covers 86.9 % of the total tasar flora in India as against 13.1 % of Arjun and Asan (Kar *et al.*, 2010). In this context, suitable approaches are needed to develop tasar silkworm breeds through genetic manipulation which can overcome the vagaries of Sal and deliver better output.

The breeding of silkworm since long has been aimed towards evolving of superior and hardy breeds either by means of selection alone or by combining outcrossing or backcrossing with selection in the subsequent generations in order to give rise to stabilized crops and to improve both quantity and quality of silk (Tazima, 1984). While evolving new breeds, the response of certain characters towards selection and its correlated changes with other economic traits are to be taken care (Gamo, 1976; Yokoyama, 1976, 1979). Similarly, general opinion is that improvement of silkworm races is possible by outbreeding with exotic races and improvement of cocoon quality, by repeated crossing (Tazima, 1964; Kovalov, 1970). Hybrid vigour is at its best in F_1 hybrid which decreases gradually in subsequent generations and is prone to nullify in about 14 generations in silkworm (Singh *et al.*, 2012). The selection of parents is always vital and hence, wild ecoraces which have proven vigour, sustainability under different conditions are choice of the breeders to enhance the adaptability, survivability and productivity of silkworm breeds. Crossing among

genetically divergent ecoraces for introgression of genes / traits of commercially viable characters *i.e.*, semi-domesticated with wild relative donor parent, establishes promising outcome (Moorthy *et al.*, 2007; Reddy *et al.*, 2009).

In view of this, in the present study, F_1 populations were prepared by crossing of wild Sal based ecoraces (Raily and Laria) and one of them with a semi-domesticated race (Daba) to envisage the exploitation of heterosis effect of the F_1 hybrids of tasar silkworm on different host plants. Emphasis was also put on the utilization of Sal flora through identification of better performing F_1 hybrids on Sal plants.

MATERIALS AND METHODS

Cocoons of semi-domesticated Daba were collected from Silkworm Breeding & Genetics section, Central Tasar Research and Training Institute (CTRTI), Ranchi. Nature grown cocoons of Laria were collected from Sal forest of Peterbar area of Jharkhand state and Raily cocoons were collected from its natural eco-niches *i.e.*, Sal forest of Bastar district of Chhattisgarh state. The cocoon characters *viz.*, cocoon weight, shell weight and silk percentage were recorded for the parental stock of all the three races (Table 1). The cocoons were preserved in separate out-door cages specified for the purpose at the Grainage area of CTRTI, Ranchi till the moth emergence.

Moths were collected and reciprocal as well as parental crosses *viz.*, Laria x Daba, Daba x Laria, Laria x Raily, Raily x Laria, Daba x Daba, Laria x Laria and Raily x Raily were prepared according to the plan. Except for semi-domesticated Daba x Daba, crossing process was

Table 1: General characteristic features of three ecoraces of *A. mylitta* selected for the preparation of reciprocal crosses

Ecorace	Native	Food plant	Fecundity	Cocoon traits	Voltinism
Daba (Semi-domestic)	West Singhbhum, Jharkhand Reared in 9 States in India	<i>Terminalia arjuna</i> (Arjun), <i>T. tomentosa</i> (Asan)	200 -220	Large sized shell with short peduncle, weighs 10-12 g, shell wt.1.5-2.0 g, medium denier (10) filament	Bivoltine & trivoltine
Laria (wild)	Peterbar forest area, Jharkhand	<i>Shorea robusta</i> (Sal)	160- 200	Medium sized shell, long peduncle and robust cocoons with low denier silk filament (7-8)	Uni, bi & trivoltine
Raily (wild)	Sal forest of Bastar Dist. Chhattisgarh	<i>Shorea robusta</i> (Sal)	250-350	Large dark grey coloured tough cocoon shell, weighs 11-12.5 g, shell wt.1.8-2.3 g, higher filament thickness (>11) with length >1200 m.	Uni, bi & trivoltine

induced through hand coupling. Coupling (mating) was maintained for 6 hours, after decoupling, mother moths were kept undisturbed for 72 hours in paper boxes for oviposition, under optimum conditions.

After 72 hours of oviposition, the fecundity (number of eggs laid by individual mother moth) was recorded (Sinha, 1998). Systematic mother moth examination was conducted to detect incidence of pebrine disease if any. The disease free layings (DFL) were selected and washed as per standard protocol. The DFLs were then subjected to optimum conditions of incubation.

Comparative hatching performance of different crosses was recorded. Three days' hatching performance was considered and presented in percentage (Sinha, 1998).

Silkworms of different F_1 crosses were brushed on the day of hatching separately on three food plants such as *T. arjuna* (Arjun), *T. tomentosa* (Asan) and *S. robusta* (Sal). Rearing was conducted and different batches were maintained on separate, labeled plants following standard tasar silkworm rearing practices. For the Sal rearing, initial brushing was performed on small Sal plants at CTRTI host plant farm and maintained up to the completion of 1st moult. Later, the whole batch of silkworm was shifted to coppice Sal plants at Palma forest area (Social forest) and continued rearing till cocoon spinning and harvesting. The mature larval weight was recorded during final stage of larva for different batches and also, the total cocoon yield for comparative study.

The quantitative characters of cocoons of different F_1 groups and parental lines such as cocoon weight, shell weight and silk % were recorded after harvesting and sorting of 100 good cocoons from each group.

Statistical analysis: One-way analysis of variance was used to test the significance of differences between the mean values of independent observations. Comparisons were performed with Duncan's Multiple Range Test (DMRT) to find out significance of differences among the batches. Differences were considered significant at $p < 0.05$ (Duncan, 1955).

RESULTS

Assessment of cocoon characters in the parent stock:

Cocoons of three different parental ecoraces of *A. mylitta* were initially assessed to acquire preliminary data of the stock. Raily cocoons exhibited superiority in cocoon characters (cocoon weight: 13.30 g, shell weight: 2.60 g, shell percentage: 19.54) compared to semi-domesticated Daba and wild Laria populations as presented in the Table 2.

Table 2: Cocoon characters of the parental populations of three selected ecoraces of *A. mylitta*

	Daba			Laria			Raily		
	Cocoon weight (g)	Shell weight (g)	Shell %	Cocoon weight (g)	Shell weight (g)	Shell %	Cocoon weight (g)	Shell weight (g)	Shell %
Mean	12.09	1.45	11.99	7.34	0.89	12.12	13.30	2.60	19.54
SD	2.32	0.26	1.25	1.51	0.28	0.65	2.99	0.44	1.38
Min	7.18	1.01	11.80	4.41	0.43	09.75	8.84	1.92	16.35
Max	16.15	2.03	12.57	12.34	1.61	13.05	19.89	3.65	22.6

Fecundity and hatching: The result revealed significant ($p < 0.001$) differences among the races and F_1 combinations studied in respect of quantitative parameters such as fecundity and larval hatching. Higher fecundity was obtained in the wild ecorace Raily (262 ± 9) over other races and F_1 populations. Among the reciprocal crosses, Laria x Daba recorded higher fecundity (243 ± 13) which was higher than that of Daba (control). Heterosis values were also estimated for fecundity, revealing positive increase of about 12 % over mid parent value and 2.9 % over better parent value. Similarly, other crosses such as Daba x Laria and Raily x Laria have also shown positive heterosis effect over the respective mid parent values (Table 3). Daba recorded higher hatching (88 ± 2 % and 87 ± 3 %, respectively for I and II crop) over other races as well as F_1 populations of different combinations. Laria x Raily showed higher positive heterosis value (+13.1%) in hatching among other cross combinations. Wild ecoraces, Laria and Raily parent lines showed poor hatchability compared to semi-domestic race and F_1 crosses (Table 3).

Table 3: Fecundity and larval hatching during first and second crop seasons in F₁ and parent population

Parent/ F ₁ cross	Fecundity (No.)		Hatching (%)	
	I crop	II crop	I crop	II crop
Daba	231 ± 5.4	230 ± 5.8	88 ± 2.03	87 ± 1.93
Laria	190.7 ± 7	205 ± 8.4	68.67 ± 2.39	66 ± 2.28
Raily	262 ± 4.8	278 ± 5.7	64.67 ± 2.63	68 ± 2.28
Laria x Daba	243 ± 13.2 (+15.16)	245 ± 6.7 (+12.0)	83 ± 2.56 (+5.73)	82 ± 1.59 (+4.45)
Daba x Laria	228 ± 7.2 (+8.06)	238 ± 6.7 (+6.0)	79 ± 2.39 (+0.63)	79 ± 2.90 (+0.06)
Raily x Laria	240 ± 8.7 (+5.96)	238 ± 5.4 (+0.80)	68 ± 3.16 (+1.5)	73 ± 2.90 (+5.79)
Laria x Raily	253 ± 9.8 (+11.7)	235 ± 2.5 (-1.20)	77 ± 4.83 (+14.9)	78 ± 5.55 (+13.1)
P Value	Races	2781.08***	399.56***	
	Seasons	244.65NS	11.00NS	

Data are Mean ± SD; Values in parentheses are heterosis percentage; * = p<0.05, ** = p<0.01, *** = p<0.001

Rearing performance: Variations were noted in the rearing behaviour and larval life span of different groups versus different food plants. The total larval span in the Sal rearing, recorded up to spinning stage was 33 -38 days & 42 - 50 days in first and second rearing season, respectively whereas, the total larval span on *T. tomentosa* / *arjuna* was 30-33 days in first crop and 38-45 days during second crop season for all the silkworms. No significant differences in the larval life span were recorded among different F₁ populations when reared on a particular food plant. Prevalence of pest population was noticed higher in both the *Terminalia* and Sal rearing sites. But, severe damage due to predators such as rats and lizards was maximum in the Palma coppice Sal plantation. The predator menace was observed throughout the rearing period in Sal. This factor has critically affected the yield of cocoons.

Quantitative traits

Larval weight: The variations in average larval weight recorded among different races and F₁ populations was significant @ p<0.05. Comparatively higher average larval weight was recorded during first crop season.

Table 4: Performance of F₁ and parent populations (Mean ± SD) during first and second crop seasons

Ecorace/ Combination	Food plant	Larval weight (g)		Cocoons/ DFL	
		I Crop	II Crop	I Crop	II Crop
Daba	Arjun	42.65±0.8	41.50±2.68	64±5	73 ± 5
	Asan	41.05±1.2	42.15±1.22	58±7	65 ± 8
Laria	Arjun	38.59±1.8	38.21±2.5	22±5	26 ± 5
	Asan	37.10±2.0	38.86±1.67	29±6	22 ± 4
Raily	Sal	33.34±2.4	33.90±2.20	9±8	17 ± 6
	Arjun	40.86±1.4	40.35±3.05	18±3	20 ± 8
Laria x Daba	Asan	41.10±1.5	38.80±3.50	21±5	21 ± 7
	Sal	39.68±3.0	35.90±3.50	14±6	15 ± 8
Daba x Laria	Arjun	42.39±1.1	41.72±1.50	39±6 (-9.3)	48±8(+09.10)*
	Asan	41.45±1.0	42.02±1.00	33±5 (-24.13)	42±5(+04.00)*
Raily x Laria	Sal	35.90±2.3	36.05±3.11	18±6 (+50.0)*	18±6(+ 05.90)*
	Arjun	42.59±1.3	39.50±2.05	41±5 (-4.65)	40±8(+3.63)*
Laria x Raily	Asan	41.62±1.5	39.90±2.45	38±5(-12.64)	40±8(+7.64)*
	Sal	36.30±1.9	36.85±3.20	12±6(+33.33)*	16 ± 7(-05.90)*
P Value	Arjun	42.25±2.0	42.02±3.30	28±3(+40.0)	30±8(+30.43)*
	Asan	40.80±3.5	42.30±3.50	25±4(+16.0)	33±7(+53.50)*
Races	Sal	36.45±2.8	37.20±3.35	16±5(+39.13)	15±5(-06.25)*
	Arjun	39.4±1.8	41.50±2.20	29±6(+25.0)	32±5(+48.83)*
Seasons	Asan	39.8±2.5	41.80±2.55	28±2(+12.0)	32±8(+49.00)*
	Sal	36.75±3.1	37.25±3.10	18± 3(+56.0)	19±6(+18.75)*
Food plants		0.0170*		0.000***	
		0.8964 NS		0.0261*	
P Value		0.5808NS		0.3483NS	

Values in parentheses are heterosis percentage; * = p<0.05; ** = p<0.01; *** = p<0.001

Terminalia particularly, Asan leaves contributed for heavier larvae. Among the different groups of silkworms, Daba recorded better performance in respect of larval growth (42.65±0.8 g). The performance of the larval weight with respect to the season /crop showed differences (Table 4) where the parent populations recorded higher weight during I crop season while the F₁ population recorded higher weight in the second crop season (43.30±3.5 g).

Cocoon yield: Seasonal variation in the cocoon yield was observed depicting significant ($p < 0.05$) influence of the season on the cocoon yield. The second crop season proved to be more favourable in respect of cocoon yield in all groups. The highest cocoon harvest was recorded with Daba (64 ± 5 & 73 ± 5 per DFL, for I & II crop, respectively) brushed on *Terminalia*. *T. arjuna* fared better for cocoon yield in the present study while Sal recorded comparatively lower yield. There were significant differences ($p < 0.001$) among the races / F_1 populations in cocoon yield and positive heterosis effect was evident in the present case where F_1 hybrids performed comparatively better than their parents particularly, on Sal plants. The combinations, Raily x Laria (+53.5 %) and Laria x Raily (+49 %) recorded considerably high heterosis.

Cocoon characters: In the present observation, no significant influence of season or race or food plant on the

average cocoon weight was found. However, positive heterosis values were recorded in the F_1 populations (Table 5). Maximum cocoon weight was recorded with the F_1 combination Laria x Daba (12.89 g) reared on *T. arjuna* compared with other groups and as well as food plants. F_1 populations stood out in terms of cocoon yield with better heterosis values as against parents. In general, it was Asan plants that supported well in achieving high cocoon weight. Significant variations ($p < 0.01$) in the shell weight among the silkworm races and F_1 populations were recorded. The influence of season was also found to be significant (Table 6). Higher shell weight (1.85 to 2.15 g) and correspondingly higher silk percentage (16 - 17) were recorded in respect of Sal. F_1 populations recorded higher shell weight and correspondingly higher shell percentage. Higher average cocoon weight was recorded during first crop season compared to second crop season. On the contrary, cocoons of second crop displayed more silk content.

Table 5: Performance of F_1 and parent populations (Mean $\pm$ SD) on quantitative cocoon characters during first and second crop seasons

Cocoon parameter	Crop	Daba			Laria			Raily			Laria x Daba			Daba x Laria			Raily x Laria			Laria x Raily		
		Arjun	Asan	Sal	Arjun	Asan	Sal	Arjun	Asan	Sal	Arjun	Asan	Sal	Arjun	Asan	Sal	Arjun	Asan	Sal	Arjun	Asan	Sal
Cocoon weight (g)	I	12.68 ± 2.56	12.36 ± 2.13	11.03 ± 2.65	10.98 ± 2.18	10.27 ± 2.75	12.35 ± 1.60	12.42 ± 1.50	12.38 ± 1.86		12.89 ± 1.83 (8.73)	12.40 ± 1.54 (6.25)	10.80 ± 2.01 (0.50)	12.53 ± 2.08 (5.69)	12.45 ± 2.10 (6.68)	10.76 ± 2.22 (4.77)	12.38 ± 2.05 (5.90)	12.53 ± 2.10 (7.09)	12.20 ± 1.65 (7.77)	12.30 ± 2.0 (5.21)	12.35 ± 1.85 (5.55)	12.40 ± 2.5 (9.54)
	II	11.65 ± 1.30	12.05 ± 1.55	10.19 ± 2.11	10.45 ± 2.08	10.28 ± 2.05	12.02 ± 2.90	12.55 ± 2.68	12.60 ± 3.33		12.35 ± 1.98 (13.09)	12.39 ± 2.15 (10.13)	11.01 ± 2.28 (7.10)	12.10 ± 1.25 (10.80)	12.25 ± 2.10 (8.89)	11.50 ± 2.25 (11.86)	11.90 ± 2.85 (7.20)	11.95 ± 2.98 (3.91)	12.20 ± 2.85 (6.64)	11.80 ± 2.50 (6.30)	11.85 ± 2.20 (3.04)	12.18 ± 3.08 (6.46)
P Value		Replicates: 0.000***, Food plants: 0.9016NS; Seasons: 0.2929NS; Races: 0.2918NS																				
Shell weight (g)	I	1.75 ± 0.31	1.68 ± 0.38	1.56 ± 0.22	1.54 ± 0.19	1.60 ± 0.28	1.75 ± 0.32	1.70 ± 0.25	2.01 ± 0.35		1.83 ± 0.14 (10.57)	1.69 ± 0.21 (4.96)	1.65 ± 0.25 (3.12)	1.76 ± 0.19 (6.34)	1.67 ± 0.21 (3.72)	1.58 ± 0.23 (-1.2)	1.70 ± 0.55 (2.71)	1.65 ± 0.50 (1.85)	1.90 ± 0.80 (5.55)	1.75 ± 0.45 (5.74)	1.70 ± 0.65 (4.90)	2.10 ± 0.50 (16.6)
	II	1.87 ± 0.32	1.81 ± 0.38	1.58 ± 0.42	1.51 ± 0.36	1.76 ± 0.48	2.01 ± 0.2	1.90 ± 0.25	2.15 ± 0.48		2.05 ± 0.34 (19.1)	1.95 ± 0.29 (17.46)	1.93 ± 0.33 (9.65)	1.85 ± 0.38 (7.58)	1.80 ± 0.32 (8.43)	1.90 ± 0.31 (7.95)	1.88 ± 0.40 (5.0)	1.72 ± 0.32 (11.76)	2.05 ± 0.38 (5.12)	1.90 ± 0.26 (6.14)	1.80 ± 0.29 (5.88)	2.15 ± 0.31 (10.25)
P Value		Replicates: 0.0672NS; Food plants: 0.0631NS; Seasons: 0.0016**; Races: 0.0039**																				
Shell %	I	13.80 ± 1.96	13.59 ± 1.72	14.13 ± 1.86	14.02 ± 1.62	15.27 ± 2.04	14.17 ± 1.8	13.68 ± 2.10	16.23 ± 2.25		14.19 ± 0.98 (1.64)	13.62 ± 1.59 (-1.4)	15.67 ± 1.87 (2.61)	14.04 ± 0.88 (0.57)	13.41 ± 1.21 (-2.8)	14.68 ± 1.93 (-3.8)	13.73 ± 3.50 (-2.9)	13.16 ± 2.75 (-4.9)	15.57 ± 3.05 (-1.14)	14.23 ± 3.1 (0.57)	13.76 ± 2.65 (-0.64)	16.93 ± 2.80 (7.50)
	II	16.05 ± 1.52	15.02 ± 1.58	15.50 ± 1.35	14.44 ± 1.41	17.12 ± 2.10	16.72 ± 2.20	15.13 ± 1.90	17.06 ± 2.50		16.60 ± 1.17 (5.26)	15.73 ± 1.55 (6.78)	17.53 ± 2.60 (2.39)	15.28 ± 1.30 (-3.1)	14.69 ± 1.35 (-0.2)	16.52 ± 2.50 (-3.5)	15.70 ± 2.30 (-2.5)	14.40 ± 2.20 (-2.5)	16.80 ± 2.50 (-1.7)	16.12 ± 2.25 (0.06)	15.19 ± 2.20 (2.7)	17.65 ± 2.11 (3.27)
P Value		Replicates: 0.0000***; Food plants: 0.00016***; Seasons: 0.0000***; Race: 0.16398NS																				

Values in parentheses represent heterosis over mid-parent value in percentage, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$; NS - Not significant.

Table 6: ANOVA for different parameters in F_1 and parent populations

	DF	MS values				
		Larval weight	Cocoon yield	Cocoon weight	Shell weight	Shell %
Replicates	2	2.547	288.3*	47.3***	0.102	9.777***
Food plants	1	2.280	56.679	0.057	0.130	11.50***
Seasons	1	0.127	332.012*	4.136	0.398**	57.06***
Food plant x Season	1	5.072	5.250	0.544	0.005	2.046
Race	6	21.171*	2755.58***	4.616	0.133**	1.044
Food plant x Race	6	0.338	43.929	0.065	0.002	0.093
Season x Race	6	6.453	58.595	0.142	0.020	0.627
Food Plant x Season x Race	6	1.541	17.833	0.068	0.003	0.120
General mean		40.805	35.702	12.045	1.762	14.647
C.V.		6.662	22.289	15.899	10.766	5.508

* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$

DISCUSSION

The success of cocoon crop in tasar industry is based on many factors, the important ones being (1) genetic background of the silkworm (2) quality of the host plant leaves and (3) environmental conditions particularly for the silkworm reared out-door. Silkworm population maintained under varying environment can be expected to have greater variance and higher fitness than population that has been existing in a constant environment. The suitability of a host plant to an insect depends upon the presence of balanced nutrition in the plants for proper growth and development of the insect (Beck, 1956). It was also reported that, the meta physiological activity in the life cycle of the tasar silkworm including reproduction are influenced to a great extent by the climate and the feeding quality of leaves (Rath *et al.*, 2006). Similarly, in the present case, different parameters studied in the silkworms of different groups revealed variations due to the influence of the biotic as well as abiotic factors. The fecundity and egg fertility are the most desired characters of silkworms from the sustenance point of view (Reddy *et al.*, 2010 a), healthy and robust silk moths are very important for higher fecundity. Imperative influence of genotype-environment interaction associated with host plant quality on the fecundity of the silkworms is reported (Venugopala Pillai *et al.*, 1987; Srivastava *et al.*, 2004). Also, the moths of different breeds (different genetic background) can influence the egg laying performance in

silkworms (Kumar and Paul, 1993; Krishnaprasad *et al.*, 2002). The fecundity of different reciprocal crosses recorded in the present study indicates the variability in the number of eggs laid. Higher fecundity (253 ± 8) was recorded with the combination of Laria x Raily compared to the Daba (231 ± 8). The effect of hybridization is very much apparent and is due to the inter-ecorace cross. The robustness and vigour of the wild relatives might have been critical in obtaining the higher fecundity and quality of the eggs in the progeny. In contrast, the hatching performance was low in the F_1 of different crosses compared to the Daba. This may be due to the compatibility barriers and fertility complications experienced when individuals of different ecoraces mate (Reddy *et al.*, 2009; Lokesh *et al.*, 2015).

The host plant is an important factor in regulating insect populations, food consumption, developmental time and survivability during larval period, this would greatly differ among different species of food plants (Schoonhoven *et al.*, 2005). The rearing performance of different groups of tasar silkworms reared on three primary food plants showed a variation in the parameters studied. However, it reiterates that, the performance of tasar silkworms is also influenced by the climate / season as evident from the present study. Generally, the larval life span of tasar silkworms appeared shorter during I crop season than the II (Deka and Kumari, 2013; Jena, 2016). Similar observations were made in the present study, the

larval life span being shorter on *Terminalia* food plants than *S. robusta*, irrespective of groups. The composition of the leaves of the host plant are very important for the consumption, digestibility and conversion efficiency of the insect which are imminent physiological activities that regulate the development and survivability (Shruti Rai *et al.*, 2006; Roy and Barik, 2013). No significant influence of the hybridization was visible in the larval life span. But, other quantitative characters such as larval weight, yield and cocoon traits were found varying in the different groups of tasar silkworms. Larval weight was recorded higher during I crop and Arjun plants found as the best for the rearing during I crop. On the other hand, Asan plants supported good larval weight during II crop season. The variations in the performance due to seasonal biochemical alterations in the host plant take place in response to the environment and similar reports are available (Saikia *et al.*, 2011; Jena, 2016). On the contrary, the F_1 populations recording higher larval weight during II crop season, could be due to the effects of gene introgression as well as higher conversion efficiency of food during second crop season. It was reported (Saikia *et al.*, 2011) that the wild silkworm *A. frithi*, at low altitude, exhibited more larval growth during winter and autumn seasons compared to other seasons. Dash *et al.* (1992) reported that first crop which generally falls during rainy season, encounters a fluctuating climate with high temperature, high humidity with higher precipitation in the tropical regions leading to lower performance and challenging survivability of the tasar silkworm. Higher larval survivability of wild tasar silkworm, Laria on Sal during autumn season is due to the congenial climate and availability of nutrition rich leaves with optimum moisture (Lokesh *et al.*, 2016). The present study is in concurrence with the earlier report (Srivastava *et al.*, 2003.) that Sal based silkworms showed better performance during second crop season.

The acquirement of the vigour in consumption of leaves and in turn, better efficiencies of conversion / digestibility by the cross breed silkworms could be the reason for better larval growth, survivability and cocoon yield. As observed in the present study, prevalence of pest and predator in the Sal natural plantation was high and this could be one of the crucial factors for low cocoon yield in the Sal rearing.

The variations observed in the cocoon weight, shell weight and respective shell percentage among F_1 s of different combinations feature the compatibility and genetic capability of these crosses in expression of higher cocoon traits compared to the Daba (control). It is also understood that crossing the commercially exploited domesticated silkworms with its wild counterparts would always yield better (Hansda *et al.*, 2008; Reddy *et al.*, 2010 b). At the same time, deterioration in some characters may also occur when distantly related silkworms are crossed due to incompatibility (Zhao *et al.*, 2007) as observed presently between Daba and Laria for commercial cocoon traits. Laria and Raily are wild ecoraces of tropical tasar silkworm and in nature, they got adapted on *S. robusta* as primary food plants, so this must be the reason behind their compatibility as a hybrid to deliver positive commercial cocoon traits.

Conclusion

F_1 crosses including reciprocals among 2 wild and 1 semi-domesticated ecoraces of tasar silkworm, *A. mylitta* D. were evaluated for their performance on different host plants during two crop seasons. F_1 crosses expressed their superiority in economic parameters over the parental races. *Terminalia* food plants offered better cocoon yield while, Sal grown cocoons excelled in other characteristics. This study gives an insight into the immense potential for utilization of the vast Sal flora through single hybrids (F_1) of tasar silkworms. Further efforts are needed to understand the pattern of heterosis expression to exploit the wild Sal flora to the maximum extent possible.

ACKNOWLEDGEMENT

We are thankful to Dr. A. S. Aruna, Scientist-C, Cold Storage Plant, Central Silk Board, Mysuru and Shri. S. Sekar, Assistant Director, Central Sericultural Germplasm Resources Centre, Hosur for their help in statistical analysis of the data.

REFERENCES

- Beck S. D. (1956) The European corn borer *Pyrausta nubilalis* (Hubh) and its principal host plant. II The influence of nutritional factors on larval establishment and

- development. *Ann. Entomol. Soc. Am.*, **49**: 582-588.
- Bongale U. D. and Chaluvachari (1995) Evaluation of eight mulberry germplasm varieties by leaf biochemical and bio-assay moulting studies. *Sericologia*, **35**: 83-94.
- Dash A. K., Nayak B. K. and Dash M. C. (1992) The effect of different food plants on cocoon crop performance in the Indian tasar silkworm *Antheraea mylitta* Drury (Lepidoptera: Saturniidae). *J. Res. Lepidoptera*, **31** (1-2): 127-131.
- Deka M. and Kumari M. (2013) Comparative study of the effect of different food plant species on cocoon crop performance of tropical tasar silkworm (*Antheraea mylitta* Drury). *Int. J. Res. Chem. Environ.*, **3** (1): 99-104.
- Dubey A. K., Mishra U. S. and Dixit S. A. (1981) Effect of host plant on the developmental stages of gram pod borer, *Heliothis armigera* (Hubner). *Indian J. Entomol.*, **43** (2): 178-182.
- Duncan D. B. (1955) Multiple range and multiple F- tests. *Biometrics*, **11**: 1-42.
- Gamo T. (1976) Recent concepts and trends in silkworm breeding. *Sericologia*, **24**: 394-415.
- Harsda G., Manohar Reddy R., Sinha M. K., Ojha N. G. and Vijayaprakash N. B. (2008) *Ex situ* stabilization and utility prospects of Jata ecorace of tropical tasar silkworm *Antheraea mylitta* Drury. *Int. J. Indus. Entomol.*, **17** (2): 169-172.
- Jena L. K. (2016) Host Plant specificity of Indian tasar silk insect *Antheraea mylitta* Drury (Saturniidae) during different seasons. *Imp. J. Interdis. Res.*, **2** (6): 1414-1420.
- Kar P. K., Srivastava A. K., Sinha R. B., Sinha M. K. and Vijayaprakash N. B. (2010) Sal based eco-races of tasar silkworm: Need for conservation. *Indian Silk*, **48** (12): 12-14.
- Kovalov P. A. (1970) *Silkworm breeding techniques*. Translated and published by Central Silk Board, Bombay, p. 233.
- Krishnaprasad N. K., Sannappa B. and Varalakshmi R. (2002) Effect of multiple mating on grainage performance of newly evolved bivoltine breeds of *Bombyx mori* L. *Bull. Indian Acad. Seric.*, **6** (2): 50-55.
- Kumar K. C. M. and Paul D. C. (1993) Effect of age of males on ejaculation and behaviour of spermatozoa in the internal reproductive organs of the female moth, *Bombyx mori* L. (Lepidoptera: Bombycidae). *Sericologia*, **33** (2): 219-222.
- Lokesh G., Srivastava A. K., Kar P. K. and Sinha M. K. (2015) Influence of cross breeding of wild and semi-domestic populations of tropical tasar silkworm *Antheraea mylitta* D. on grainage and silkworm rearing traits. *Int. J. Sci. Res. Pub.*, **5** (8): 1-7.
- Lokesh G., Srivastava P. P., Sinha A. K. and Alok Sahay (2016) Seasonal climatic influence on the leaf biochemicals of Sal (*Shorea robusta*) flora and *in situ* breeding behaviour of Laria ecorace of tropical tasar silkworm *Antheraea mylitta* Drury. *J. Entomol. Zool. Stud.*, **4** (6): 57-62.
- Moorthy, S. M., Das S. K., Kar N. B. and Urs S. R. (2007) Breeding of bivoltine breeds of *Bombyx mori* L. suitable for variable climatic conditions of tropics. *Int. J. Indus. Entomol.*, **14**: 99-105.
- Pandey N. D., Yadava D. R. and Teotia T. P. S. (1968) Effect of different food plants on larval and post larval development on *Diacrisia oblique* Walk. *Indian J. Entomol.*, **30** (3): 229-234.
- Rath S. S., Singh M. K. and Suryanarayana N. (2006) Change in rate of feeding and assimilation in *Antheraea mylitta* fed on two major food plants and its effect on silk production and reproduction. *Agric. J.*, **1** (1): 24-27.
- Reddy R. M., Rupesh Charan, Muniswamy Reddy P. M., Lokesh G., Reddy R. D., Reddy J. S., Reddy C. S., Angadi B. S. and

- Sivaprasad V. (2011) Pattern of silk yield and silk filament quality in ecoraces and F1 hybrids of Indian tropical tasar silkworm, *Antheraea mylitta* Drury (Lepidoptera: Saturniidae) in relation to crop seasons. *Academic Journal of Entomology*, **4** (3): 88-93.
- Reddy R. M., Sinha M. K. and Prasad B. C. (2010 a) Application of parental selection for productivity improvement in tropical tasar silkworm *Antheraea mylitta* Drury-A review. *J. Entomol.*, **7**: 129-140.
- Reddy R. M., Suresh Rai, Srivastava A. K., Kar P. K., Sinha M. K. and Prasad B. C. (2010 b) Heterosis pattern and commercial prospective of assorted F1 hybrids of Indian tropical tasar silkworm, *Antheraea mylitta* Drury. *J. Entomol.*, **7** (3): 160-167.
- Reddy R. M., Suryanarayana, Sinha M. K., Gahlot N. S., Hansda G., Ojha N. G. and Vijaya Prakash N. B. (2009) Silk filament progression with backcross breeding generations in tropical tasar silkworm, *Antheraea mylitta* D. *Int. J. Indus. Entomol.*, **19** (1): 187-192.
- Roy N. and Barik A. (2013) Influence of four host-plants on feeding, growth and reproduction of *Diacrisia casignetum* (Lepidoptera: Arctiidae). *Entomol. Sci.*, **16**: 112-118.
- Saikia P., Choudhury S., Brahma D., Sougrakpami N. and Dutta K. (2011) Rearing performance of a population of wild tasar silk moth, *Antheraea frithi* M. (Lepidoptera: Saturniidae) under in-situ outdoor and indoor captive conditions. *The Ecoscan*, Special issue, **1**: 275-279.
- Schoonhoven L. M., Van Loon J. J. A. and Dicke M. (2005) *Insect-Plant Biology*, Oxford University Press, Oxford.
- Shruti Rai, Aggarwal K. K. and Babu C. R. (2006) Influence of host plant (*Terminalia arjuna*) defences on the evolution of feeding behaviour in the tasar silkworm. *Curr. Sci.*, **91** (1): 68-71.
- Singh H. N. and Byas (1975) Larval development of *Prodenia litura* F in relation to certain cultivated plants. *Indian J. Entomol.*, **37** (1): 1-6.
- Singh Tribuwan, Singh P. K. and Sahaf K. A. (2012) The heterosis phenomenon in mulberry silkworm, *Bombyx mori* L. *Ann. Biol. Res.*, **3** (9): 4330-4336.
- Sinha B. R. R. P. (1998) Survey of genetic resources and study of the genetic divergence in *Antheraea mylitta* Drury. *Ph.D. Thesis*, Ranchi University, Ranchi, India.
- Srivastava A. K., Naqvi A. H., Sinha A. K., Vishwakarma S. R. and Roy G. C. (2004) Genotype and environment interaction in *Antheraea mylitta* Drury and its implications. *Perspectives in Cytology and Genetics*, **11**: 219-224.
- Srivastava A. K., Sinha A. K. and Sinha B. R. R. P. (2003) *Descriptor of tropical tasar silkworm, Antheraea mylitta Drury (Lepidoptera Saturniidae)*, CTRTI, Ranchi, pp. 1-14.
- Suryanarayana N., Kumar R. and Gargi (2005) *Monograph on Indian tropical tasar silkworm food plants (Terminalia Spp.)*. Central Tasar Research and Training Institute, Ranchi, Central Silk Board, India.
- Suryanarayana N. and Srivastava A. K. (2005) *Monograph on Tropical Tasar Silkworm*, Central Tasar Research and Training Institute, Ranchi, Central Silk Board, India.
- Tazima Y. (1964) *The genetics of the silkworm*, Logos Press London, p. 421.
- Tazima Y. (1984) *Silkworm moth, evolution of domesticated animals*, Longman, New York, pp. 416-424.
- Venugopala Pillai S., Krishnaswami S. and Kashivishwanathan K. (1987) Growth studies in silkworm, *Bombyx mori* L. under tropical conditions. II. Influence of agronomical methods of mulberry on growth, cocoon crop and fecundity of silkworm. *Indian J. Seric.*, **26** (1): 38-45.
- Yadav G. S., Siddiqui A. A., Borah A., Rajaram and Sengupta A. K. (1992) Studies on association of fecundity with cocoon weight, pupa weight and shell weight of muga silkworm

- Antheraea assama* Westwood during two commercial crop. *J. Assam Science Soc.*, **34 (2)**: 13-18.
- Yokoyama T. (1976) Breeding silkworm. *Sci. & Tech. Seric.*, **15 (4)**: 58-61.
- Yokoyama T. (1979) Silkworm selection and hybridization. In: *Genetics in relation to insect management. Working papers, The rockfeller Foundation Management*, pp. 71-83.
- Zhao Y., Chen K. and He S. (2007) Key principles for breeding spring and autumn silkworm varieties; from our experience of breeding 873 x 874. *J. Environ. Sci.*, **5**: 57-61.



ALLETHRIN AND PRALLETHRIN BASED MOSQUITO COIL EMISSION INDUCES TOXICITY AND ALTERS THE HAEMOLYMPH PROTEINS AND COCOON TRAITS OF *BOMBYX MORI*

K.V. Rajini, M. Likhith Gowda, C. A. Sangma, K. A. Avinash, Punyavathi and H. B. Manjunatha*

Proteomics and Genomics Laboratory, Department of Studies in Sericulture,

University of Mysore, Mysuru 570006, India.

*E-mail:manjunathahb@gmail.com

ABSTRACT

Burning of mosquito repellent coil is not uncommon in our daily life to safeguard from mosquito vector borne diseases. But, the effect of continuous, long term exposure to such insecticidal vaporizer environment, from the point of human health, is still on debate. Surprisingly, the personnel involved in silkworm (*Bombyx mori* L.) rearing for production of silk-cocoons is still uninformed on the safety of its use inside the rearing room. This study is first of its kind to examine the impact of mosquito coils of two popular commercial brands viz., Mortein nature guard and Allout, on silkworm growth, larval haemolymph proteins, silk-cocoon formation and economic traits by exposing larvae of CSR₂ and PM x CSR₂ breeds to mosquito coil smoke for 12 h daily, from day 1 of first instar until spinning. Consequently, there was a decline in the growth of larvae of treated lots of CSR₂ and PM x CSR₂ against their respective control batches. More importantly, a protein with molecular mass of 82 kDa, which is identified as key storage protein, was down regulated that resulted in development of deformed moths in CSR₂. In addition, the cocoon characteristics and yield were also more affected in the larvae of CSR₂ than PM x CSR₂ exposed to allethrin and prallethrin based mosquito coil emission. Thus, our investigation pertinently revealed that although both the type of mosquito coil emission was found toxic, allethrin based smoke induced more toxicity than prallethrin and between two silkworm breeds, CSR₂ exhibited higher sensitivity than PM x CSR₂. Accordingly, we suppose that farmers actively involved in silkworm rearing should be cautious while using mosquito coils in the rearing house to protect them from the mosquito bite causing infectious diseases, that might lead to cocoon crop loss.

Key words: Allethrin, *Bombyx mori*, cocoon, mosquito coil, prallethrin, protein.

INTRODUCTION

The common practice of protection from the mosquitoes that spread malaria disease and cause casualty of 500,000 people per year (Anonymous, 2017), is use of varied types of mosquito repellents. Among them, mosquito coil that is widely known as an efficient mosquito repellent is insecticide based. The most common active ingredients of the mosquito coils are pyrethrins and allethrin that account ~0.4 to 0.8 % of the coil mass whereas, the other ingredients are pyrethrum, esbiothrin, butylated hydroxyl toluene (BHT), piperonyl butoxide (PBO) and N-acetyl di-carboximide (Lukewa

and Chandiwana, 1998). Upon burning of mosquito coil without flame, the insecticides evaporate with the smoke that repel / kill the mosquitoes. Although, they have been recommended for use in outdoor or semi-enclosed patios and porches, coils are fairly used in sleeping quarters overnight. During this period, people tend to expose themselves to a chemically complex mosquito coil emission containing small particles (<1µm), metal fumes, and vapors that may reach the alveolar region of the lung. Furthermore, the sub micrometer particles can also reach the lower respiratory tract (Weili *et al.*, 2003) and the gas phase of mosquito coil smoke containing carbonyl compounds generate strong irritating effects on the upper

respiratory tracts when exposed to mosquito coils for a period of several months (Chang and Lin, 1998).

Toxicological reports have even evidently revealed the focal declination of the tracheal epithelium, metaplasia of epithelial cells, and morphologic alteration of the alveolar macrophages in rats due to the effects of mosquito coil (Liu and Wong, 1987; Liu and Sun, 1988). Additionally, studies conducted for a period of 30 days aptly demonstrated deposition of substantial amount of allethrin on ceiling and floor and predicted the probable exposure of humans to this through skin adsorption (Ramesh and Vijayalakshmi, 2001). The effect of exposure to mosquito coil involves morphological, cellular and hormonal changes that induce disturbance in oxidant and antioxidant functions, apoptosis, inflammation and manifestation of gene expression but, no information is available on the possible impact on proteins (Sheoran *et al.*, 1998). However, all these facts address the health-related issues in general but, its direct or residual impact in the personnel occupied in rearing of silkworm larvae is still unknown.

In spite of the fact that mosquito coil smoke has many potential adverse health effects, large populations in developing countries still use it consistently. In India, the sericulture farmers in particular, conduct silkworm rearing in their living houses because they can't afford to have separate rearing house, even if so, labourers tend to sleep in the young-age silkworm rearing centres or mass rearing house and use mosquito coils, being unaware of the deleterious effects of mosquito coil smoke on larval growth and cocoon productivity. Hence, the present investigation was undertaken with the key tasks, (a) to discover the toxicity effect of allethrin and prallethrin, (b) to evaluate the sensitivity / susceptibility of silkworm breeds to mosquito coil emission and (c) to assess the changes in the protein profile and commercial traits of the silkworm, *B. mori*.

MATERIALS AND METHODS

Experimental material

The silkworm breeds, PM x CSR₂ and CSR₂, possessing high and low innate tolerance, respectively

and commercially available mosquito coils - Mortein nature guard (0.05 % w/w prallethrin) and Allout (0.01 % w/w Allethrin) were used in the present investigation.

Maintenance of silkworm and experimental methods

Subsequent to hatching, healthy larvae crawled onto the chopped mulberry leaf bits, were divided into six different groups with three replications of 100 larvae each. Mosquito coil was burnt every day from 6.00 p.m. to 6.0 a.m. in the rearing room since day 1 of I instar until spinning of larvae, while control batches were maintained in separate rearing room. Other rearing conditions (Srinivasababu *et al.*, 2014) provided remained similar for both the treatment and control batches.

Analysis of biological and commercial traits

Larval mortality: Larval mortality was calculated based on the number of larvae mounted for spinning against number of larvae brushed and exposed subsequently to mosquito coil emission.

Larval weight: Six larvae were selected randomly from each replication on day 6 of fifth instar and their average weight was recorded to determine the impact of mosquito coil smoke on their growth and development.

Effective rate of rearing (ERR): The ERR was calculated based on the number of good cocoons spun by the number of larvae brushed to assess the extent of manifestation of physiological and biochemical processes in the larvae due to mosquito coil smoke.

Cocoon parameters: Six cocoons were selected randomly from each replication on day 6 after spinning to record average weight that denotes the biosynthetic capability of the larvae after exposure to mosquito coil smoke. The cocoon shell weight was recorded after removing the pupa from the cocoons that indicate the quantum of silk protein biosynthesised by the larvae after exposure to mosquito coil smoke. Cocoon shell percentage was calculated based on the shell weight of the respective cocoon weight.

Pupal weight was estimated from the values of cocoon

weight and shell weight. Any significant deviation in the average weight of the pupa refers to the manifestation of toxicity as influenced by mosquito coil smoke in the process of metamorphosis of larvae into pupae under the destined hormonal influence.

Data analysis: All the data derived from three replications were statistically analysed using one factor analysis program on the OPSTAT platform (<http://14.139.232.166/opstat/>, Sheoran *et al.*, 1998).

Protein analysis through electrophoresis (Sodium dodecyl sulphate - poly acrylamide gel electrophoresis, SDS-PAGE)

The caudal horn of the larva was incised to collect the haemolymph in the pre-cooled eppendorff tube containing a pinch of thiourea. The haemolymph protein sample was dissolved in extraction buffer (50 mM Tris buffer - pH 6.8, 1 mM PMSF, 100 mM DTT) at 4 °C. The homogenate was centrifuged at 4000 rpm for 20 min at 4 °C and the supernatant was used for SDS-PAGE (14 % gel) as described by Weber and Osborn (1969) with modifications (Vasudha *et al.*, 2006).

RESULTS

Larval mortality

Toxicity due to allethrin and prallethrin based mosquito coils emission was evident in terms of inefficiency of larvae to spin the cocoons. Interestingly, allethrin induced high toxicity with 58.66 and 10.00 % death of the larvae even at early and later instars of CSR₂ (Figure 1A) and PM×CSR₂, respectively. The corresponding figures in respect of prallethrin based mosquito coils were 16.33 and 1.67 %. This data clearly indicate the higher impact of allethrin than prallethrin and the higher susceptibility of CSR₂ compared to PM×CSR₂ (Figure 2 A) which is statistically significant at $p < 0.05$ (Table 1).

Larval growth

The larval weight recorded on day 6 of fifth instar from the allethrin exposed batch was 2.24 and 2.40 g as against 3.82 and 3.11 g in control groups of CSR₂ and PM×CSR₂,

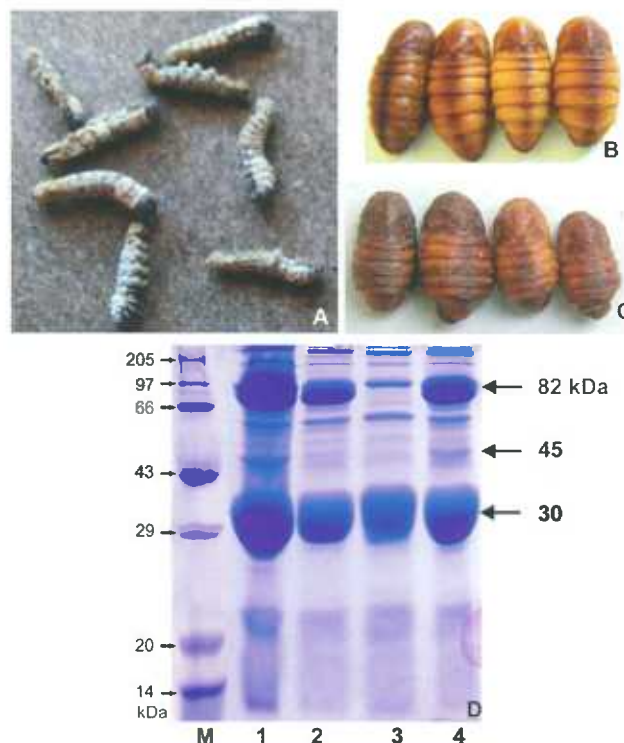


Figure 1: Allethrin and prallethrin based mosquito coil emission induced larval and pupal mortality and changes in the haemolymph protein of *Bombyx mori*.

A. Death of the II instar larvae (CSR₂) due to mosquito coil smoke; B. Normal pupae of CSR₂; C. Deformed pupae of CSR₂ due to mosquito coil smoke; D. Haemolymph protein profile (Lane 1- PM x CSR₂ Control; Lane 2 - PM x CSR₂ treated; Lane 3- CSR₂ treated; Lane 4 - CSR₂ Control; M-Marker).

respectively. In this regard, the prallethrin treated batch displayed comparatively higher values of 2.81 and 2.80 g, respectively (Figure 2 B).

Haemolymph protein profile

SDS-PAGE analysis of haemolymph revealed discrete changes in the protein pattern. Interestingly, intensity of a major protein band with molecular mass of 82 kDa was prominently low in CSR₂, while it was slightly less in PM×CSR₂ larvae exposed to allethrin based mosquito coil smoke compared to their respective control groups (Figure 1D). Concomitantly, a protein band with molecular weight of 45 kDa was also severely affected whilst the intensity of the band in control remained the same in both the silkworm breeds examined. Less impact of coil emission was evident on 30 kDa protein compared to control groups

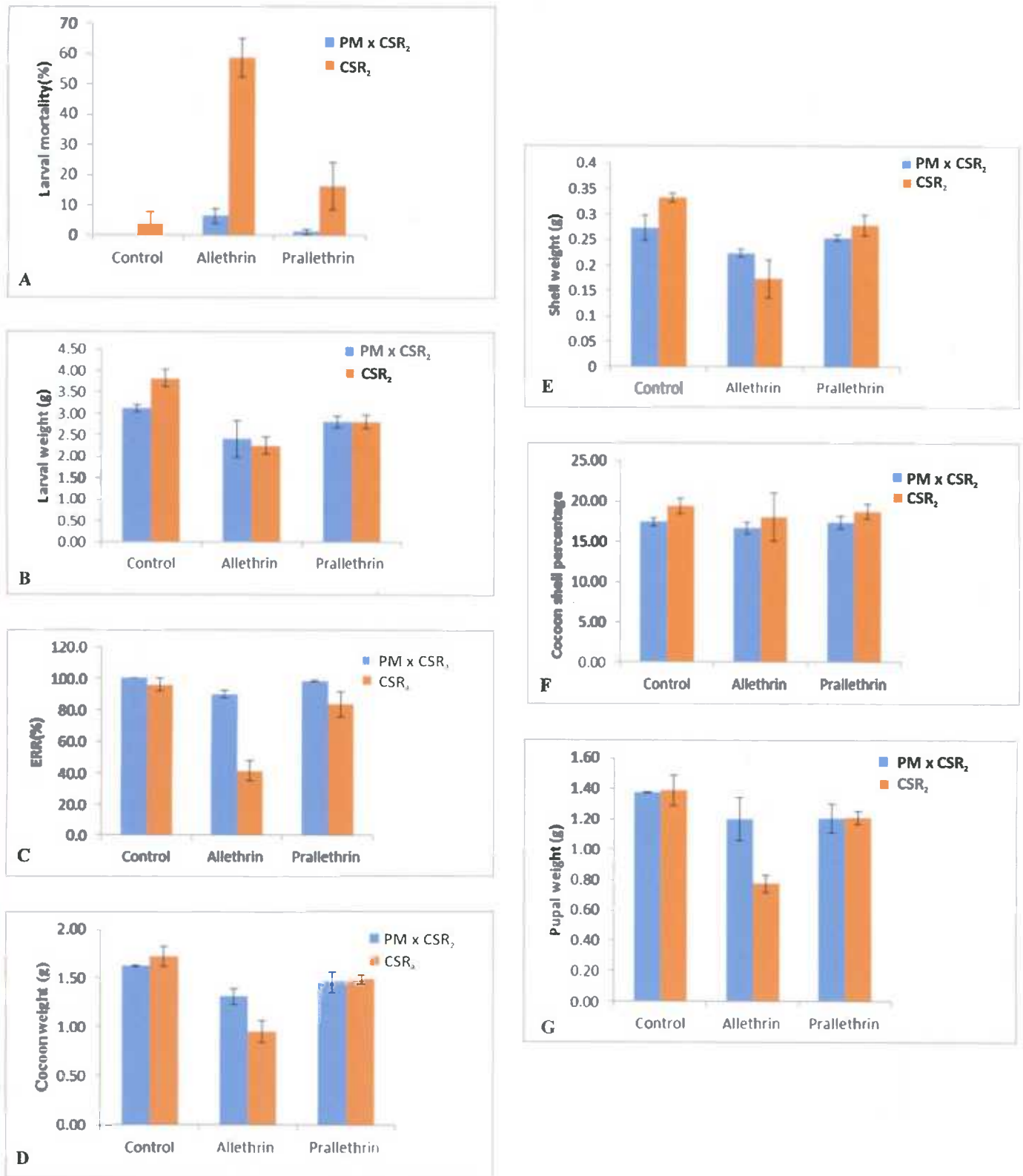


Figure 2: Effects of allethrin and prallethrin based mosquito coil smoke on biological and commercial parameters of PM x CSR₂ and CSR₂ breeds

Table 1: Statistical analysis of data on the impact of mosquito coil smoke on biological and commercial parameters of the silkworm, *Bombyx mori* breeds, PM x CSR₂ and CSR₂

	Larval weight (g)	Mortality (%)	ERR (%)	Cocoon weight (g)	Shell weight (g)	Pupal weight (g)	Shell (%)
PM x CSR₂							
F-test	*	*	*	*	*	N/A	N/A
±SE (m)	0.151	0.903	0.903	0.043	0.009	0.057	0.395
±SE (d)	0.213	1.277	1.277	0.061	0.012	0.08	0.559
C.D. at 5 %	0.531	3.184	3.184	0.153	0.03	N/A	N/A
C.V. (%)	9.396	40.20	1.627	5.153	5.98	7.818	3.992
CSR₂							
F-test	*	*	*	*	*	*	N/A
±SE (m)	0.107	3.616	3.616	0.052	0.017	0.042	1.096
±SE (d)	0.152	5.114	5.114	0.073	0.024	0.06	1.549
C.D. at 5 %	0.378	12.756	12.756	0.183	0.06	0.15	N/A
C.V. (%)	0.378	23.783	8.501	6.497	11.293	6.558	10.137

of CSR₂ and PM×CSR₂ while the intensity of other proteins remained unaltered (Figure 1D).

Effective rate of rearing

The per cent of good cocoons spun by the CSR₂ and PM×CSR₂ larvae reared under exposure to allethrin based mosquito coil smoke was 41.33 and 90, respectively. On the other hand, the larvae of CSR₂ and PM×CSR₂ reared under prallethrin based coil emission have spun 83.67 and 98.33 % good cocoons as against 96.00 and 100 % in control batches, respectively. Among all, allethrin induced damage was more significant in CSR₂ than PM×CSR₂ (Figure 2 C).

Cocoon parameters

The larvae exposed to allethrin and prallethrin based mosquito coil emission have spun cocoons with low weight compared to their respective control groups of CSR₂ and PM × CSR₂. However, average cocoon weight of the allethrin exposure batches of CSR₂ was 0.95 g while it was 1.49 g in prallethrin exposure batches as against 1.72 g in control. In the case of PM×CSR₂, weight of the cocoon was recorded as slightly affected by mosquito coils smoke *i.e.*, 1.31 and 1.46 g in allethrin and prallethrin treated batches, respectively as against 1.62 g

in control (Figure 2 D). Taken together, higher toxicity inflicted by allethrin smoke and the extreme sensitivity of CSR₂ to mosquito coil smoke irrespective of their active ingredients were obvious.

A significant decrease ($p < 0.5$, Table 1) in the weight of cocoon shell spun by CSR₂ larvae exposed to allethrin and prallethrin based mosquito coils smoke was recorded and it was 0.17 and 0.28 g respectively as against 0.33 g in control. Similar effect was also recorded in PM×CSR₂ with an account of 0.22 and 0.25 g for allethrin and prallethrin coil smoke, respectively as against 0.27 g in control groups (Figure 2 E). Correspondingly, the cocoon shell weight percentage was also affected, wherein it was 18.08 and 18.69 % in CSR₂ and 16.64 and 17.37 % in PM×CSR₂ larvae exposed to allethrin and prallethrin coils smoke in comparison with that of 19.39 and 17.4 % in control, respectively (Figure 2 F).

Pupal growth and development

Exposure of CSR₂ and PM × CSR₂ larvae to allethrin and prallethrin coils smoke eventually affected the growth and development that led to either deformed or dead pupae (Figures 1B, C). Even the good pupae of CSR₂ weighed less (0.77 and 1.20 g in allethrin and prallethrin treated batches, respectively compared to 1.38 g in control).

Comparatively, PM $\times$ CSR₂ showed slight tolerance to allethrin and prallethrin mosquito coils smoke in which the pupae weighed 1.19 and 1.20 g though it was less than their respective control groups (1.368 g, Figure 2 G).

DISCUSSION

During the present investigation, the larvae of CSR₂ and PM $\times$ CSR₂, the most exploited commercial silkworm breeds, were exposed to allethrin and prallethrin based mosquito coil emission for twelve hours daily from hatching until mounting, mimicking daily exposure of human to mosquito coil in normal setting. Although both the mosquito coil smoke induced mortality in both the silkworm breeds examined, allethrin is proved to be more toxic than prallethrin with the highest mortality accounting to 58.68 % in CSR₂ whereas, it was a meagre 10 % in PM $\times$ CSR₂, indicating differential effect that is associated with innate tolerance level of *B. mori* larvae. Eventually, continuous exposure of larvae to allethrin and prallethrin smoke exhibited loss of weight by 41.17 and 26.34 % in CSR₂ and 22.92 and 9.82 % in PM $\times$ CSR₂, which in turn had an influence on development of pupae whose weight was less by 44.20 and 13.04 % in CSR₂ and 12.79 and 12.28 % in PM $\times$ CSR₂, respectively. This manifestation could be the result of inhalation of allethrin released during burning of mosquito coils and consumption of allethrin residues deposited on mulberry leaf. It is reported that the sub micrometer particles of the mosquito coil smoke reach the lower respiratory tract (Weili *et al.*, 2003) and induce focal declination of the tracheal epithelium, metaplasia of epithelial cells, and morphologic alteration of the alveolar macrophages in rats (Liu and Wong, 1987; Liu and Sun, 1988).

More interestingly, it is evident for the first time from the present study that a key protein with molecular mass of 82 kDa known as storage protein that accumulate progressively as the larvae grow was recorded very low in CSR₂ and slightly less in PM $\times$ CSR₂, which is obvious with the intensity of the protein band in the SDS-PAGE gel. Since, intensity of the 82 kDa protein band, which is believed to support pupal growth, was less, it might have resulted in development of deformed moths in the case of exposure of larvae to allethrin. Besides, intensity of a protein band of 45 kDa was also found less in the

haemolymph of the larvae exposed to coil emission, but their biological significance remained obscure. These proteo-morphological changes observed might be due to increased free radical generation during prolonged exposure to mosquito coil smoke as has been reported in rat (Madhubabu and Yenugu, 2012). However, further investigation is warranted to substantiate it and would be a thrust area of research in development of vaccine for malaria disease (Anonymous, 2017).

Further, as it has been found that inhalation of allethrin-based mosquito coil smoke affect the cellular architecture of reproductive organs and sperm parameters in rat (Madhubabu and Yenugu, 2012), particulates inhaled by the *B. mori* larvae through spiracles or consumed along with mulberry leaves might also drastically disrupt the biosynthetic parameters as evidenced by the reduced output in the experimental breeds. Notably, about 56.94 % of total cocoon production was declined in CSR₂; however, the cocoons thus produced by the larvae exposed to allethrin based coil emission were of low quality with 44.76, 48.48, 44.20 and 6.756 % low in its cocoon, shell and pupal weight and shell percentage compared to the larvae reared under normal condition. Similar impact was also recorded from PM $\times$ CSR₂, with degree of effect comparatively being less than that of CSR₂, which indicate higher innate tolerance level to mosquito coil emission. With these interesting results, we therefore, suppose that silkworm larvae could be potent model to discover molecular mechanism underlying in differential response of larvae to different types of mosquito repellents in practice while their impact on human health is greatly neglected.

All these findings strongly support that allethrin based mosquito coil emission not only induce high toxicity but also affect the larval growth and development by altering the synthesis of a major storage protein, which is a major source of energy for cocoon spinning and growth and development of *B. mori* pupae. However, its implication on the health of the farmers who tend to expose to mosquito coil emission for longer period during rearing is enigmatic even though epidemiologic studies have proved that long-term exposure to mosquito coil smoke induce asthma and persistent wheeze in children (Azizi and Henry, 1991; Koo and Ho, 1994).

REFERENCES

- Anonymous (2017) Key molecule may bring malaria vaccine closer. *Deccan Herald-Cutting Edge*, March 20, p. 6.
- Azizi B. H. and Henry R. L. (1991) The effects of indoor environmental factors on respiratory illness in primary school children in Kuala Lumpur. *Int. J. Epidemiol.*, **120**: 144-150.
- Chang J. Y. and Lin J. M. (1998) Aliphatic aldehydes and allethrin in mosquito-coil smoke. *Chemosphere*, **36**: 617-624.
- Koo L. C. L. and Ho J. H. C. (1994) Mosquito Coil Smoke and Respiratory Health among Hong Kong Chinese: Results of Three Epidemiological Studies. *Indoor Environ.*, **3**: 304-310. <https://doi.org/10.1159/000463577>
- Liu W. K. and Sun S. E. (1988) Ultra structural changes of tracheal epithelium and alveolar macrophages of rats exposed to mosquito coil smoke. *Toxicol. Lett.*, **41**: 145-157.
- Liu W. K. and Wong M. H. (1987) Toxic effects of mosquito coil (mosquito repellent) smoke on rats. Biochemical and cellular changes in broncho alveolar lavaged samples from rats after inhalation of mosquito coil smoke. *Toxicol. Lett.*, **39**: 223-230.
- Lukewa N. and Chandiwana S. K. (1998) Efficacy of mosquito coils containing 0.3% and 0.4% pyrethrins against *An. gambiae sensu lato* mosquitoes. *Cent. Afr. J. Med.*, **44**: 104-107.
- Madhubabu G. and Yenugu S. (2012) Effect of continuous inhalation of allethrin-based mosquito coil smoke in the male reproductive tract of rats. *Inhal. Toxicol.*, **24**: 143-152.
- Ramesh A. and Vijayalakshmi A. (2001) Monitoring of allethrin, deltamethrin, esbiothrin, prallethrin and transfluthrin in air during the use of household mosquito repellents. *J. Environ. Monitor.*, **3**: 191-193.
- Sheoran O. P., Tonk D. S., Kaushik L. S., Hasija R. C. and Pannu R. S. (1998) Statistical Software Package for Agricultural Research Workers. In: *Recent Advances in information theory, Statistics & Computer Applications*, Hooda D. S. and Hasija R. C. (Eds.), Department of Mathematics Statistics, CCSHAU., pp. 139-143.
- Srinivasababu G. K., Singh G. B. and Chandrakanth K. S. (2014) Silkworm Rearing. In: *Hand book of Sericulture Technologies*, Dandin S. B. and Giridhar K. (Eds.), Central Silk Board, Bangalore, pp. 335-385.
- Vasudha B. C., Aparna H. S. and Manjunatha H. B. (2006) Impact of heat shock on heat shock proteins expression, biological and commercial traits of *Bombyx mori*. *Insect Sci.*, **9**: 93-101.
- Weber K. and Osborn M. (1969) The reliability of molecular weight determination by Dodecyl Sulphate Polyacrylamide Gel Electrophoresis. *J. Biochem.*, **244**: 4406-4412.
- Weili Liu, Zhang J., Hashim J. H., Jalaludin J., Hashim Z. and Goldstein B. D. (2003) Mosquito coil emissions and health implications. *Environ. Health Perspect.*, **111**: 1454-1460.



METABOLIC RESPONSE OF TASAR SILKWORM, *ANTHERAEA MYLITTA* DRURY TO INCREASED TANNIN CONTENT

P. K. Kar^{1*}, K. Jena, M. Bara and A. K. Sinha

Central Tasar Research and Training Institute, Nagri, Ranchi, Jharkhand 835303, India.

¹Regional Tasar Research Station, Prafulla Nagar, Baripada,

Mayurbhanj, Odisha 757001, India.

*E-mail: prasantakar@rediffmail.com

ABSTRACT

The present study aimed to investigate net photosynthesis, transpiration rate and stomatal conductance of *Terminalia tomentosa* leaves fortified with tannins besides oxidative stress, antioxidant defenses and commercial characters of the tasar silkworm fed on it. Supplementation of higher concentration of tannins negatively influenced the transpiration rate and relative humidity of host plant leaf while there was no effect on net carbon dioxide, stomatal conductance and photosynthesis. In the silkworm gut, α -amylase activity was found significantly reduced in tannin treated groups as compared to control. Further, modulation of oxidative stress (lipid peroxidation and total hydroperoxides) as well as presence of antioxidants (catalase, glutathione-s-transferase and ascorbic acid) were detected in tannin fed larvae. Interestingly, deterioration of commercial characters of male and female larvae was observed under tannin fed condition. Results suggest that tannins regulate the metabolism of tasar silkworm to a great extent, which may adversely affect the larval fitness and commercial characters of tasar silk. The findings could be helpful in framing strategies for effective utilization of widely available host plant *Shorea robusta* which is known to have more tannin content.

Key words: *Antheraea mylitta*, antioxidants, cocoon traits, phenols, photosynthesis, tannins.

INTRODUCTION

Tasar silkworm, *Antheraea mylitta* Drury is reared by farmers on *Terminalia arjuna* (Arjun) and *T. tomentosa* (Asan) for production of commercially important cocoons. Wild silkworm ecoraces viz., Modal, Raily, Laria etc. are also found in natural *Shorea robusta* (Sal) forests. However, rearing on Sal has not been successful and late stage mortality of silkworm is reported very often. On the other hand, vast stretches of Sal forests in tropical India could be utilized if the host plant and silkworm interaction is properly understood.

Plant - insect interaction is a dynamic system and an evolutionary process, which involves development of the defense mechanisms by the plants and counter

adaptations by the insects. Defense mechanisms involve either morphological barriers or elaborate array of phytochemicals which act as repellents, phagodeterrents and oviposition deterrents, thus exhibiting resistance. Polyphenols (tannins) and phenols are considered to play an important role in plant defense system. These secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of organisms. They often play an important role in plant defense against herbivores and other interspecies defenses.

Tannins are one of the most important classes of secondary plant compounds involved in the defense of deciduous plants against phytophagous insects. Exactly how tannins affect the herbivore is still open to debate, but

they are referred to as antidiigestive protein binding agents (Cardinal-Aucoin *et al.*, 2009). The adverse effects of tannins have been shown for different insect species, and during the course of evolution, insects have acquired various defense mechanisms against their harmful effects (Barbehenn and Constabel, 2011). The gypsy moth feeds on many tannin-containing tree leaves (Barbosa and Krischik, 1987), and studies have been devoted to the influence of tannins on its performance (Foss and Rieske, 2003; Barbehenn and Constabel, 2011). However, till date, the effect of tannin on the metabolism of tasar silkworm has not been studied, hence, the present study was planned.

In natural environment, herbivorous insect species are subjected to varieties of stress such as abiotic and biotic, fasting or nutritional deficiencies, xenobiotic compounds and toxic, plant secondary metabolites, which generates reactive oxygen species (ROS) (Schultz, 1989). ROS is toxic to living organisms, because it causes oxidative damage to proteins, nucleic acids and lipids. Insects are also equipped with both enzymatic and non-enzymatic antioxidant components (Jovanovic-Galovic *et al.*, 2004; Zhao and Shi, 2009). So, the oxidant and anti-oxidant status of the silkworm consequent upon tannin intake was also studied.

MATERIALS AND METHODS

Host plant, treatments and silkworm

Tasar silkworm host plant, *T. tomentosa* was selected for the study conducted during the third crop. Plants with identical growth under economic plantation were selected. Before the silkworm rearing, plants were sprayed with different concentrations of tannic acid. Treatment-1 (T-1) was sprayed with 0.1% of tannic acid whereas, Treatment-2 and 3 (T-2 & T-3) were sprayed with 0.5 and 1.0% of tannic acid, respectively. The control lot was sprayed with distilled water only. Each group consisted of three replications, each of 200 worms. The spray procedure continued for 5 days. Thereafter, tasar silkworm larvae (Daba race) just after the fourth moult, were reared outdoor. Quantitative traits of larvae and cocoons were recorded during V instar and seven days after formation of cocoons, respectively.

Medium (fourth leaf from the tip) leaves were used for studying photosynthesis, respiration and other related parameters. After taking the readings by automatic analyzer, the leaves were plucked and kept in -80°C deep freezer for further biochemical analysis.

Photosynthesis and related analysis

Photosynthesis rate, transpiration, stomatal conductance, leaf temperature and relative humidity were directly measured *in situ* using a handheld photosynthesis system (CI-340) from CID Bioscience (USA). Leaf moisture content was calculated from the fresh and dry weight after keeping it in an oven at 60°C till a constant weight was achieved.

Biochemical analysis

Leaf phenol was estimated by using Folin-Ciocalteu reagent (FCR) (Thimmaiah, 1999). Leaf tannin content was determined by using Folin-Dennis reagent (Thimmaiah, 1999). Gut α -Amylase activity was measured according to Bernfeld (1955). Lipid peroxidation (LPX) level was assayed by measurement of malondialdehyde (MDA), a decomposition product of polyunsaturated fatty acids. Hydro peroxides were determined by the TBA reaction as described by Heath and Packer (1968). Total hydroperoxides were determined spectrophotometrically according to the method of ferrous oxidation with xylenol orange (FOX1) (Wolff, 1994). Catalase activity was determined according to Aebi, (1974). Glutathione-S-transferase activity was measured according to Habig *et al.* (1974) using 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate. Ascorbic acid (ASA) content was determined according to the method of Mitusi and Ohata (1961). The protein content was estimated by the Bradford (1976) method using bovine serum albumin (BSA) as standard.

Economic parameters, such as the larval weight, silk gland weight, cocoon weight, shell weight, filament length and non-breakable filament length were recorded and analyzed as per standard protocols.

Statistical analysis

Significant variations in the parameters were investigated by using one-way ANOVA. Differences were considered statistically significant when $p < 0.05$. Tukey's post hoc test was done to establish the HSD or CD among the mean values. Regression and correlation analyses were also performed for different parameters in three tasar population samples.

RESULTS AND DISCUSSION

In general practice, tropical tasar silkworm is reared on Asan and Arjun plants for commercial cocoon production as well as for generating cocoons utilized for egg production and supply. On the other hand, there is report on cocoon collection from natural Sal forests. However, all the stretches of Sal forest are not habituated by tasar

silkworm. This indicates that there is selective response of tasar silkworm for specific ecopockets of Sal forests. Although wild tasar cocoons are found on Sal, rearing on Sal plants is difficult. There are cases where tasar silkworms in bulk die before cocoon formation. Sal leaves contain higher quantity of phenolics and tannins in comparison to Asan (unpublished data). It was reported earlier that phenols and tannins in higher concentration hamper the digestibility and assimilation rate of phytophagous insects (Barbehenn and Constabel, 2011). Dash *et al.* (1992) reported low ERR % in Sal fed larvae in comparison with that of Asan and Arjun plant in autumn and winter seasons. So, the present study was planned to observe the effect of tannin fortified on Asan, a normal food plant of tasar silkworm.

In the present study, the spray of tannic acid at 0.5 and 1.0 % on Asan leaves led to significant change in the

Table 1: Total phenol content in Asan leaves before and after spray with tannic acid

Status of Asan leaf		Phenol content (mg/g dry weight)			
		Mean	SEM	t value	P value
Control	Before	29.594	0.870	0.209	0.841
	After	29.805	0.505		
0.1 % Tannic acid	Before	28.280	1.553	0.880	0.413
	After	29.774	0.688		
0.5 % Tannic acid	Before	29.292	0.994	2.389*	0.048
	After	32.461	0.878		
1 % Tannic acid	Before	28.533	0.677	3.749**	0.009
	After	34.555	1.457		

Table 2: Total tannin content in Asan leaves before and after spray with tannic acid

Status of Asan leaf		Tannin content (mg/g dry weight)			
		Mean	SEM	t value	P value
Control	Before	28.883	0.405	0.339	0.746
	After	28.633	0.615		
0.1 % Tannic acid	Before	29.451	0.659	1.219	0.268
	After	30.394	0.404		
0.5 % Tannic acid	Before	29.986	0.214	4.659**	0.003
	After	32.380	0.467		
1 % Tannic acid	Before	29.661	0.139	8.042**	0.000
	After	34.027	0.525		

contents of total phenolics as well as tannins in the leaf (Tables 1, 2). The tannin content and total phenolics concentration in a plant is species specific and even varies with season and climatic conditions. It is reported that condensed tannins in mature leaves, are almost three times higher in the tropical forests (Turner, 1995). Since the tannic acid was applied on the leaves, it was thought essential to see the effect on the photosynthesis and other related metabolism in the leaves. There was no difference in the leaf temperature, net CO₂ content and photosynthetic rate (Table 3). However, there was significant reduction of transpiration rate of the 1% tannin treated leaves. Although there was decreased level of stomatal conductance in all the treated leaves, the difference was not significant. An important observation recorded was that the relative humidity (in and out) got affected by tannin treatment.

Retuerto *et al.* (2004) have stated that compensation responses to insects that do not destroy the photosynthetic machinery are more difficult to measure. Changes in photosynthetic performance may, however, offer a clue. Photosynthetic rate in holly trees (*Ilex aquifolium*) infested by scale insects (*Coccus* sp.), was measured

wherein the photosynthesis appeared to be increased compared with that in control plants, thereby compensating for losses of nutrients to herbivory. In the present case, tannin and phenolics do not seem to hamper the photosynthetic process. However, the difference in transpiration rate and humidity in tannin treated leaves as shown in this study needs further confirmation, because this is particularly important in the case of *S. robusta* which not only possesses less leaf water content but also release more water into the environment.

When cocoon quantitative traits in female and male tasar silkworm in relation to tannic acid treatment on Asan plant were concerned, there was gradual reduction in cocoon weight, shell weight and pupal weight, which was highly significant in Treatment-3 i.e., 1 % tannin spray (Tables 4, 5). Tannins are considered as anti-digestive protein-binding agents (Barbehenn and Constable, 2011). Higher tannins in leaves might have hindered the digestion process of silkworm. Due to less feeding response, metabolite uptake would have hampered which might have resulted in reduction of quantitative traits. Cork and Foley (1991) suggested that selection for different digestive strategies results primarily from nutritional

Table 3: Photosynthetic and related parameters in relation to tannic acid treatment on Asan plant (Mean $\pm$ SEM)

Parameter	Control	Tannic acid			F value	CD at 5 %
		0.1 %	0.5 %	1 %		
Leaf temperature (°C)	25.91 ^a $\pm$ 0.32	29.36 ^a $\pm$ 0.45	33.00 ^a $\pm$ 0.20	32.85 ^a $\pm$ 0.27	54.64	9.739
Carbon dioxide (CO ₂) net (ppm)	73.09 ^a $\pm$ 1.77	67.38 ^a $\pm$ 3.32	73.94 ^a $\pm$ 4.24	43.35 ^a $\pm$ 2.30	11.01	41.54
Net photosynthesis rate (mol/m ² /s)	20.87 ^a $\pm$ 0.51	19.54 ^a $\pm$ 0.96	21.39 ^a $\pm$ 1.29	12.71 ^a $\pm$ 0.67	9.811	11.652
Transpiration rate (millimol/m ² /sec)	4.86 ^a $\pm$ 0.10	3.59 ^a $\pm$ 0.12	4.74 ^b $\pm$ 0.19	2.79 ^b $\pm$ 0.10	27.687	1.866
Stomatal conductance (mmol/m ² /sec)	263.26 ^a $\pm$ 13.54	226.07 ^a $\pm$ 4.97	223.53 ^a $\pm$ 16.55	225.24 ^a $\pm$ 8.81	1.316	55.567
Relative humidity (in) (%)	51.35 ^a $\pm$ 0.55	43.14 ^a $\pm$ 1.55	32.90 ^b $\pm$ 0.36	31.20 ^b $\pm$ 0.34	60.49	17.273
Relative humidity (out) (%)	68.43 ^a $\pm$ 0.70	77.23 ^a $\pm$ 1.45	68.99 ^a $\pm$ 1.34	82.74 ^b $\pm$ 0.78	10.039	9.96

Table 4: Cocoon quantitative traits in female tasar silkworm in relation to tannic acid treatment on Asan plant (Mean $\pm$ SEM)

Trait	Control	Tannic acid			F value	CD at 5 %
		0.1 %	0.5 %	1 %		
Cocoon weight (g)	12.673 ^a $\pm$ 0.849	10.778 ^{ab} $\pm$ 0.962	10.138 ^{ab} $\pm$ 0.687	8.838 ^b $\pm$ 0.597	4.121**	2.312
Shell weight (g)	1.583 ^a $\pm$ 0.340	1.433 ^{ab} $\pm$ 0.173	1.225 ^{ab} $\pm$ 0.116	0.813 ^b $\pm$ 0.057	2.757*	0.484
Pupal weight (g)	11.090 ^a $\pm$ 0.564	9.345 ^{ab} $\pm$ 0.790	8.913 ^b $\pm$ 0.580	8.025 ^b $\pm$ 0.640	3.929*	1.865
Shell %	12.176 ^a $\pm$ 2.020	13.185 ^b $\pm$ 0.400	12.034 ^a $\pm$ 0.446	9.424 ^a $\pm$ 1.214	1.741	2.323

* P<0.05, ** P<0.01

Table 5: Cocoon quantitative traits in male tasar silkworm in relation to tannic acid treatment on Asan plant (Mean $\pm$ SEM)

Trait	Control	Tannic acid			F value	CD at 5 %
		0.1 %	0.5 %	1 %		
Cocoon weight (g)	11.763 ^a $\pm$ 0.390	9.550 ^b $\pm$ 0.334	8.948 ^b $\pm$ 0.158	8.870 ^b $\pm$ 0.108	24.376**	1.962
Shell weight (g)	1.868 ^a $\pm$ 0.044	1.363 ^b $\pm$ 0.103	1.198 ^b $\pm$ 0.052	0.995 ^b $\pm$ 0.050	31.451**	0.54
Pupal weight (g)	9.895 ^a $\pm$ 0.406	8.188 ^b $\pm$ 0.275	7.750 ^b $\pm$ 0.110	7.875 ^b $\pm$ 0.096	15.15**	1.442
Shell %	15.940 ^a $\pm$ 0.741	14.241 ^a $\pm$ 0.798	13.366 ^b $\pm$ 0.358	11.213 ^b $\pm$ 0.514	9.809**	2.849

* $P < 0.05$, ** $P < 0.01$ **Table 6: Status of oxidants, antioxidants and amylase activity in tasar silkworm gut when fed on control and treated Asan leaves (Mean $\pm$ SEM)**

Parameter	Control	Tannic acid			F value	CD at 5 %
		0.1 %	0.5 %	1 %		
Total hydroperoxide (nmol/mg protein)	0.448 ^a $\pm$ 0.040	0.690 ^b $\pm$ 0.040	0.943 ^{bc} $\pm$ 0.042	1.235 ^c $\pm$ 0.097	7.923	0.489
Lipid peroxidation (nmol TBARS/mg protein)	1.469 ^a $\pm$ 0.064	2.357 ^b $\pm$ 0.064	2.582 ^b $\pm$ 0.059	3.827 ^c $\pm$ 0.176	22.146	1.408
Catalase (CAT) (μ kat/mg protein)	1.005 ^a $\pm$ 0.036	0.984 ^a $\pm$ 0.038	1.306 ^{ab} $\pm$ 0.041	1.550 ^b $\pm$ 0.086	6.146	0.39
Glutathione-S- transferase (GST) (nmol CDNB formed/ min/mg protein)	203.12 ^a $\pm$ 5.51	387.98 ^b $\pm$ 17.48	404.96 ^b $\pm$ 15.94	159.35 ^a $\pm$ 13.85	20.224	182
Ascorbic acid (ASA) (μ g/mg protein)	0.648 ^a $\pm$ 0.019	0.886 ^b $\pm$ 0.027	0.956 ^b $\pm$ 0.014	0.655 ^a $\pm$ 0.038	9.078	0.229
Gut amylase (μ g maltose released/mg protein)	812 ^a $\pm$ 51	539 ^{ab} $\pm$ 31	457 ^b $\pm$ 20	346 ^c $\pm$ 43	6.742	304

factors including high fiber and phenolic contents. A wide range of effects of dietary phenolics on insects have been shown earlier which include acute toxicity (Lindroth and Peterson, 1988), feeding deterrence (Jones and Klocke, 1987), inhibition of digestion and growth (Isman and Duffey, 1982; Summers and Felton, 1994), and nutritive effects (Bernays *et al.*, 1983). Increased concentrations of condensed tannin in paper birch was correlated with decrease in tussock moth performance traits *viz.*, survival, pupal mass and development time (Agrell *et al.*, 2000). Shahida Perveen *et al.* (2001) reported that the bollworm diet incorporated with 0.1 % tannic acid and phenolic mixture showed phagodeterrency, weight retardation, mortality and less pupation rate. These results corroborate the findings of the present study.

Herbivorous insects are continuously challenged with exogenous ROS that are part of the plant defence against them (Orozo-Cardenas and Ryan, 1999). As a defense, plant produces phenolic compounds against herbivores, which lead to reduce insect's fitness due to ROS (such as semiquinone radical, peroxide and hydroxyl radical), thus generating cytotoxicity (Hagerman *et al.*, 2003; Barbehenn *et al.*, 2005). Total hydroperoxide and lipid peroxidation (LPX) level in silkworm fed with treated leaves increased significantly in a dose dependent manner (Table 6). Among the anti-oxidative enzymes, catalase (CAT) activity showed significant increase in T-2 and T-3 over control whereas, T-1 was at par with that of control. In the case of Glutathione-S-transferase (GST), the activity level increased significantly for T-1 and T-2 which came

down to control level in T-3 treatment. Ascorbic acid (ASA) content level also showed the similar trend as shown by GST. Specific gut amylase activity exhibited decreasing trend in a dose dependent manner.

An enhanced level of LPX and total hydroperoxide in the midgut of the larva suggests increased production of ROS, which might have also affected the quantitative traits of silkworm. The increased level of secondary metabolites (total phenolics and tannins) content in the corresponding plant could be the possible reason for the increase in the rate of ROS formation, resulting in oxidative stress in midgut tissues of *A. mylitta*. In the caterpillars of *Malacosoma disstria*, Barbehenn *et al.* (2008) found that the oxidative stress was significantly more when the larvae fed on tannin rich sugar maple leaves in comparison to that of red oak. Thus, there was a clear cut relationship between phenolic oxidation in the midgut contents of caterpillars and oxidative stress. Similarly, higher level of CAT activity was also detected in larvae (*Spodoptera littoralis*) fed with tannic acid (Krishnan and Sehna, 2006) corroborating present findings. GSTs are a group of detoxification enzymes, and increased GST activity in larvae at smaller doses followed by decline at heavy doses showed the utilization in detoxification. Earlier report suggested that ingestion of pro-oxidant rich food increases the GST activity (Peri-Mataruga *et al.*, 1997). Similar is the case of ASA.

Thus, from the above discussion, it is clear that tannin and the phenolics play an important role in regulating the metabolism of tasar silkworm to a great extent. In natural forests, the wild silkworm might have developed a strategy to counter the high tannin and phenolics of *S. robusta* either through timing of life cycle or physiological adjustment which needs a thorough study. Further, detailed investigations are required to know the intricacies of interaction of wild tasar silkworm and its host plant in order to utilize vast Sal flora.

REFERENCES

- Aebi H. (1974) Catalase. In: *Methods in Enzymatic Analysis*, Bergmayer H. U. (Ed.), Academic Press, New York, pp. 673-678.
- Agrell J., McDonald E. P. and Lindroth R. L. (2000) Effects of CO₂ and light on tree phytochemistry and insect performance. *Oikos*, **88**: 259-272.
- Barbehenn R. V., Cheek S., Gasperut A., Lister E. and Maben R. (2005) Phenolic compounds in red oak and sugar maple leaves have prooxidant activities in the midguts of *Malacosoma disstria* and *Orgyia leucostigma* caterpillars. *J. Chem. Ecol.*, **31**: 969-988.
- Barbehenn R. V. and Constabel C. P. (2011) Tannins in plant-herbivore interactions. *Phytochem.*, **72**: 1551-1565.
- Barbehenn R. V., Maben R. E. and Knoester J. J. (2008) Linking phenolic oxidation in the midgut lumen with oxidative stress in the midgut tissues of a tree-feeding caterpillar *Malacosoma disstria* (Lepidoptera: Lasiocampidae). *Environ. Entomol.*, **37**: 1113-1118.
- Barbosa P. and Krischik V. A. (1987) Influence of alkaloids on feeding preference of eastern deciduous forest trees by the gypsy moth *Lymantria dispar*. *Am. Nat.*, **130** (1): 53-69.
- Bernays E. A., Chamberlain N. J. and Woodhead S. (1983) Phenols as nutrients for a phytophagous insect *Anacridium melanorhodon*. *J. Insect Physiol.*, **29**: 535-539.
- Bernfeld P. (1955) Amylase, α and β . *Methods in Enzymol.*, **1**: 149-151.
- Bradford M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**: 248-254.
- Cardinal-Aucoin M., Baucé E. and Albert P. J. (2009) Preingestive detection of tannins by *Choristoneura fumiferana* (Lepidoptera: Tortricidae). *Ann. Entomol. Soc. Am.*, **102**: 717-726.
- Cork S. J. and Foley W. J. (1991) Digestive and metabolic strategies of arboreal mammalian folivores in relation to chemical defenses in temperate and tropical forests. In: *Plant*

- Defenses Against Mammalian Herbivory*, R. T. Palo and C. T. Robbins (Eds.), Boca Raton: CRC, pp. 133–166.
- Dash A. K., Nayak B. K. and Dash M. C. (1992) The effect of different food plants on cocoon crop performance in the Indian tasar silkworm *Antheraea mylitta* Drury (Lepidoptera: Saturniidae). *J. Res. Lepid.*, **31** (1-2): 127-131.
- Foss K. and Rieske L. K. (2003) Species-specific differences in oak foliage affect preference and performance of gypsy moth caterpillars. *Entomol. Exp. Appl.*, **108**: 87-93.
- Habig W. H., Pabst M. J. and Jakoby W. B. (1974) Glutathione-S-Transferases, the first enzymatic step in mercapturic acid formation. *J. Biol. Chem.*, **249**: 7130-7139.
- Hagerman A. E., Dean R. T. and Davies M. J. (2003) Radical chemistry of epigallocatechin gallate and its relevance to protein damage. *Arch. Biochem. Biophys.*, **414**: 115-120.
- Heath R. L. and Packer L. (1968) Photoperoxidation in isolated chloroplasts I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.*, **125**: 189-198.
- Isman M. and Duffey S. (1982) Toxicity of tomato phenolic compounds to the fruitworm, *Heliothis zea*. *Entomol. Exp. Appl.*, **31** (4): 370-376.
- Jones K. C. and Klocke J. A. (1987) Aphid feeding deterrence of ellagitannins, their phenolic hydrolysis products and related phenolic derivatives. *Entomol. Exp. Appl.*, **44**: 229-234.
- Jovanovic-Galovic A., Blagojevic D. P., Grubor-Lajsic G., Worland R. and Spasic M. B. (2004) Role of antioxidant defense during different stages of preadult life cycle in European corn borer (*Ostrinia nubilalis*, Hubn.): diapause and metamorphosis. *Arch. Insect Biochem. Physiol.*, **55**: 79-89.
- Krishnan N. and Sehna F. (2006) Compartmentalization of oxidative stress and antioxidant defense in the larval gut of *Spodoptera littoralis*. *Arch. Insect Biochem. Physiol.*, **63**: 1-10.
- Lindroth R. L. and Peterson S. S. (1988) Effects of plant phenols on performance of southern armyworm larvae. *Oecologia*, **75**: 185-189.
- Mitusi A. and Ohata T. (1961) Photooxidative consumption and photoreductive formation of ascorbic acid in green leaves. *Plant Cell Physiol.*, **2**: 31-44.
- Orozco-Cardenas M. and Ryan C. A. (1999) Hydrogen peroxide is generated systemically in plant leaves by wounding and system in via the octadecanoic pathway. *P. Nat. Acad. Sci. USA*, **96**: 6553-6557.
- Peri-Mataruga V., Blagojevi D., Spasi M. B., Ivanovi J. and Jankovi-Hladni M. (1997) Effect of the host plant on the antioxidative defence in the midgut of *Lymantria dispar* L. caterpillars of different population origins. *J. Insect Physiol.*, **43**: 101-106.
- Retuerto R., Fernandez-Lema B., Roiloa R. and Obeso J. R. (2004) Increased photosynthetic performance in holly trees infested by scale insects. *Funct. Ecol.*, **18**: 664-669.
- Schultz J. C. (1989) Tannin-insect interactions. In: *Chemistry and Significance of Condensed Tannins*, R.W. Hemingway and J. J. Karchesy (Eds.), Plenum Press, New York, pp. 417-433.
- Shahida Perveen S., Qaisrani T. M., Amin S., Perveen R. and Naqvi S. H. M. (2001) Biochemical basis of insect resistance in cotton. *Online J. Biol. Sc.*, **1** (6): 496-500.
- Summers C. B. and Felton G. W. (1994) Prooxidant effects of phenolic acids on the generalist herbivore *Helicoverpa zea* (Lepidoptera: Noctuidae): Potential mode of action for phenolic compounds in plant anti-herbivore chemistry. *Insect Biochem. Mol. Biol.*, **24**: 943-953.
- Thimmaiah S. R. (1999) *Standard Methods of Biochemical Analysis*, Kalyani Publishers, New Delhi.

- Turner I. M. (1995) Foliar defences and habitat adversity of three woody plant communities in Singapore. *Funct. Ecol.*, **9**: 279-284.
- Wolff S. P. (1994) Ferrous ion oxidation in presence of ferric ion indicator xylenol orange for measurement of hydroperoxides. *Method. Enzymol.*, **233**: 182-189.
- Zhao L. C. and Shi L. G. (2009) Metabolism of hydrogen peroxide between univoltine and polyvoltine strains (*Bombyx mori*). *Comp. Biochem. Physiol.*, **152**: 339-345.



Research Paper

HISTOPATHOLOGICAL CHANGES DURING THE PROGRESSIVE INFECTION OF BmIFV IN THE SUSCEPTIBLE / TOLERANT BREEDS OF SILKWORM, *BOMBYX MORI* L.

M. Mamatha and M. Balavenkatasubbaiah*

Central Sericultural Research and Training Institute, Mysuru 570008, Karnataka, India.

*E-mail: mbvsubbaiah@gmail.com

ABSTRACT

Infectious flacherie disease of silkworm is caused by the virus, BmIFV (*Bombyx mori* infectious flacherie virus). In India, this disease was first reported by Sato (1992) and later, many workers have taken up investigations on various aspects of disease in silkworm. There are a few reports available on the susceptibility status of different silkworm breeds / hybrids to BmIFV and so far, only a few tolerant and susceptible breeds have been identified against this pathogen. Our earlier studies indicated CSR2 as highly susceptible and 5N as the most tolerant breed of silkworm against BmIFV infection. This pathogen has been found to infect only the midgut tissue but not other tissues viz., haemolymph, fat body, silk gland, gonad and malpighian tubules in both the susceptible (CSR2) and tolerant (5N) breeds. In the present study, an attempt was made to understand the histological changes taking place in the midgut during the progressive infection (1-12 DPI) of BmIFV in both the susceptible and tolerant breeds. In CSR2, as the infection progressed, the number of goblet cells that got infected increased continuously and was discharged into the gut lumen and only a few regenerative *nidi* cells developed into new goblet and columnar cells which ultimately led to physiological damage as reflected by high larval mortality. But, in the tolerant breed, the infected goblet and cylindrical cells were less in number as the infection progressed. This may be because along with the process of discharging of infected cells into the gut lumen, there was regeneration of new goblet cells at a higher rate from the *nidi* cells, allowing physiological repair. The susceptibility / tolerance status of these breeds against BmIFV is discussed.

Key words: BmIFV, *Bombyx mori* L., histopathology, midgut, susceptible / tolerant breeds.

INTRODUCTION

Silkworm crop loss due to occurrence of diseases would be common if disease management practices are not followed meticulously during silkworm rearing. One of the major silkworm diseases viz., infectious flacherie is caused by a picornavirus, *Bombyx mori* infectious flacherie virus (BmIFV). This pathogen was first reported in Japan by Yamazaki *et al.* (1960) and in India, by Sato (1992) and Sivaprasad *et al.* (1993). Later, Nataraju and Datta (1999) developed a textile dye based-dipstick immunodiagnostic kit for the detection of infectious flacherie disease during silkworm rearing. Subsequently,

productive breeds of silkworm, susceptible and tolerant to BmIFV were identified and subjected to studies related to progressive morphological changes upon infected with BmIFV (Mamatha and Balavenkatasubbaiah, 2008 a, 2008 b). A few years later, we identified midgut as the target tissue of BmIFV infection in the silkworm (Mamatha and Balavenkatasubbaiah, 2017). However, the mechanism of susceptibility / tolerance along with histopathological, haematological and bio-chemical aspects of the susceptible / tolerant stocks are to be studied. Histology involves not only analysis of three dimensional structures but also the application of methods and techniques that help to understand structure, function and

cellular interrelationships in a more dynamic way (Vidyunmala, 2010). Since the diseases are primarily because of the abnormal activity in cells/tissues, the close relation between cytology one hand and pathology on the other should be exploited. The alimentary canal in silkworm is more or less a straight tube divided into three main parts, the foregut, midgut and hindgut. Main functions of the foregut are swallowing the diet, storing it for some time followed by its transportation into the midgut. Midgut is the largest part of the alimentary canal. It ranges between the second and the ninth body segment. The wall of the midgut is composed of a muscular layer, basal membrane, epithelium and peritrophic membrane. The epithelium is formed of cylindrical cells, goblet cells and regenerative *nidi* cells (which produce new cells). The cells of midgut are actively involved in enzyme production and secretion, as well as in absorption of nutrients (Vidyunmala, 2010). In the present study, an attempt was made to study the histopathological changes in the BmIFV infected tissue (midgut) of silkworm of CSR2 and 5N breeds and the results are presented and discussed.

MATERIALS AND METHODS

As per the earlier studies, CSR2 (susceptible) and 5N (tolerant) breeds were brushed *enmass* and reared following the standard method up to III instar. Immediately after III moult, one hundred newly ecdysed 4th instar larvae were per orally inoculated with an infective dose of BmIFV at $10^{-7.255}$ dilution of stock inoculums by smearing on mulberry leaf @ 1 ml/100 sq.cm leaf/100 larvae. The control batches were also maintained without any inoculation. The larvae were reared up to 12 days of post inoculation (DPI).

From both treated and control batches of CSR2 and 5N, larvae were dissected individually and midgut tissue was taken out carefully without inflicting damage, daily from 0 to 12 DPI and the histopathological work was carried out as per the standard methodology viz., fixation of tissues in Bouin's fluid, clearing, embedding, block making, section cutting (using rotator microtome) and mounting of tissue ribbons on glass slides as described by Goel (2006).

The sections of tissue were stained with haematoxylin

and counter-stained with eosin. The staining procedure was as follows.

- § Hydrated and de-paraffinized the slides by passing through a graded series of ethyl alcohol in descending order, *i.e.*, 100, 90, 80, 70, 50 and 30 % and water.
- § Stained the slides by keeping them in haematoxylin for about 2-5 min.
- § Washed the slides under running tap water for about 3-5 min.
- § Dehydrated the slides by passing through a graded series of alcohol in ascending order up to 70 %.
- § Counter stained in eosin by giving one or two quick dips in the stain solution and then washed them in fresh grade of 70 % alcohol.
- § Dehydrated further by passing through ascending grades of ethyl alcohol *i.e.*, 80 and 90 % rectified spirit and twice in absolute alcohol.
- § Transferred the slides to xylene for cleaning and mounted in DPX mountant.

These slides were observed carefully under microscope and recorded the changes in the epithelial cells of the midgut tissue from 0 to 12 DPI of both the breeds and compared with control batches by taking the micro photographs.

RESULTS

The histopathological changes in the midgut of susceptible breed (CSR2) as observed in the control batches, revealed no cellular changes from 0 to 12 days. The cross sections of midgut displayed healthy epithelium, columnar and goblet cells without any abnormal changes. In the case of BmIFV treated batches, from 0 to 3 DPI, there were no clear histopathological changes (Figure 1). On 4 DPI, histopathological changes were manifested and midgut goblet and columnar cells developed abnormality and appeared shrunken. These cells were strongly stained throughout the course of

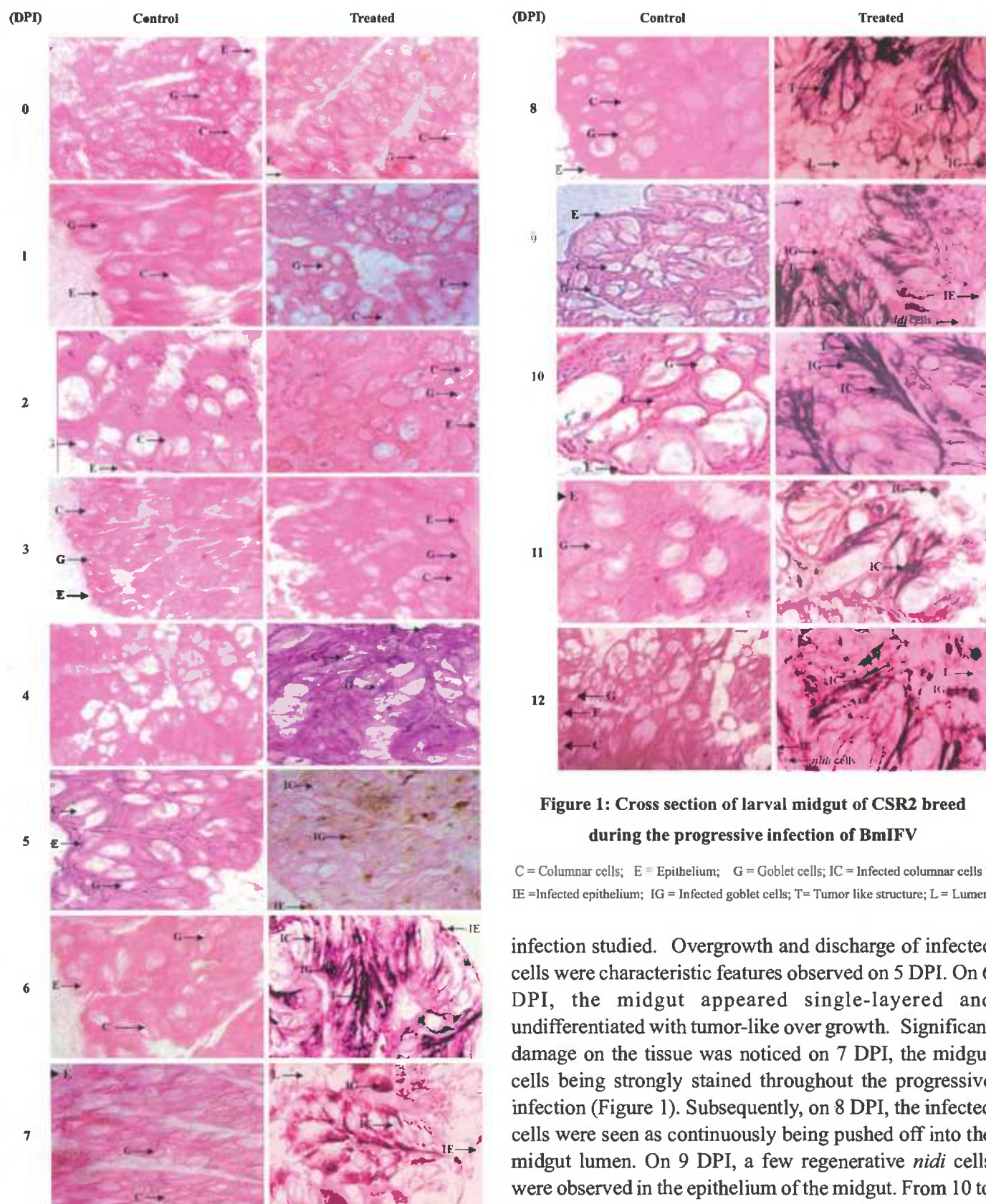


Figure 1: Cross section of larval midgut of CSR2 breed during the progressive infection of BmIFV

C = Columnar cells; E = Epithelium; G = Goblet cells; IC = Infected columnar cells; IE = Infected epithelium; IG = Infected goblet cells; T = Tumor like structure; L = Lumen.

infection studied. Overgrowth and discharge of infected cells were characteristic features observed on 5 DPI. On 6 DPI, the midgut appeared single-layered and undifferentiated with tumor-like over growth. Significant damage on the tissue was noticed on 7 DPI, the midgut cells being strongly stained throughout the progressive infection (Figure 1). Subsequently, on 8 DPI, the infected cells were seen as continuously being pushed off into the midgut lumen. On 9 DPI, a few regenerative *nidi* cells were observed in the epithelium of the midgut. From 10 to 12 DPI, which was an advanced stage of infection, more number of infected cells (goblet and columnar cells) were

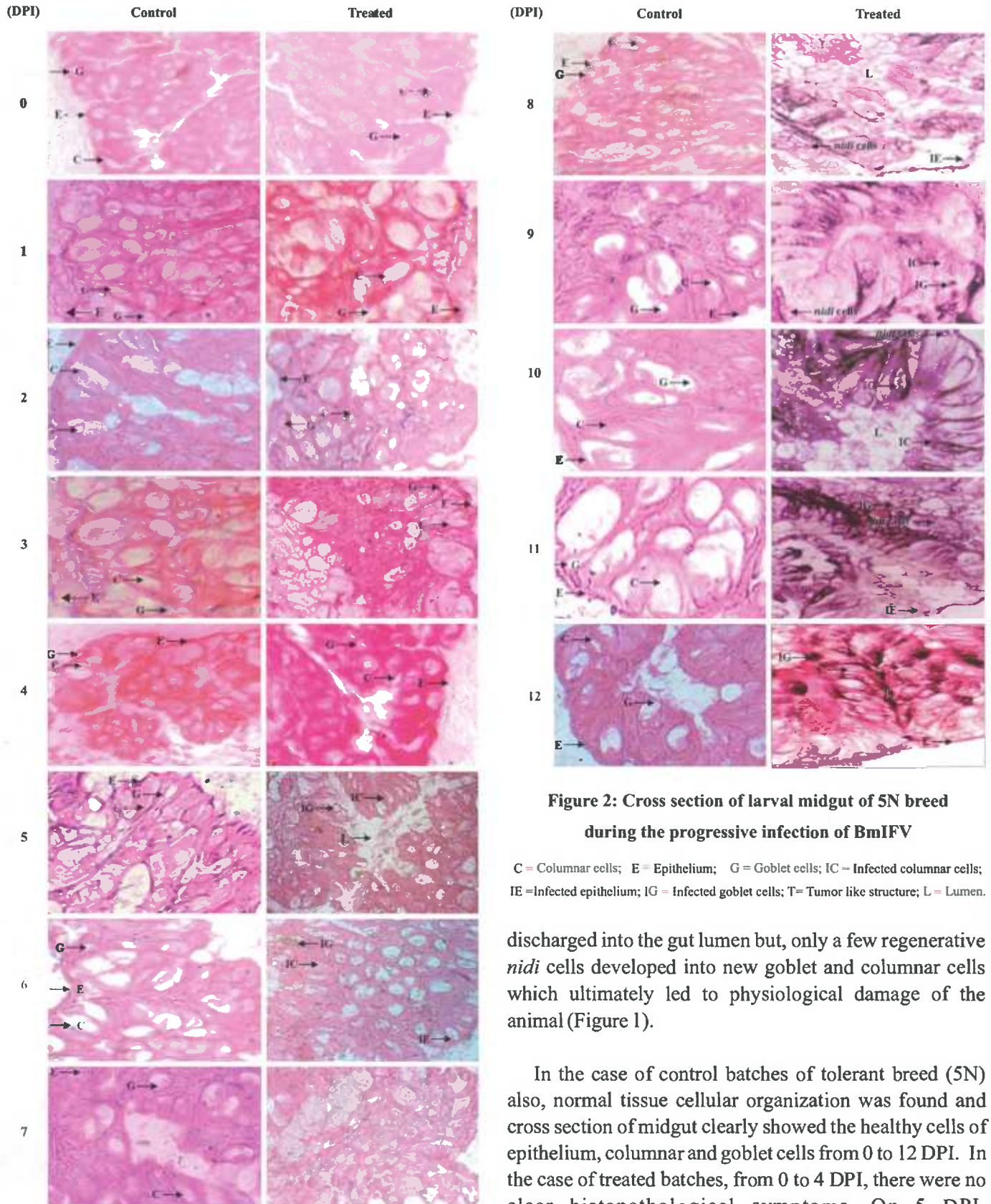


Figure 2: Cross section of larval midgut of 5N breed during the progressive infection of BmIFV

C = Columnar cells; E = Epithelium; G = Goblet cells; IC = Infected columnar cells; IE = Infected epithelium; IG = Infected goblet cells; T = Tumor like structure; L = Lumen.

discharged into the gut lumen but, only a few regenerative *nidi* cells developed into new goblet and columnar cells which ultimately led to physiological damage of the animal (Figure 1).

In the case of control batches of tolerant breed (5N) also, normal tissue cellular organization was found and cross section of midgut clearly showed the healthy cells of epithelium, columnar and goblet cells from 0 to 12 DPI. In the case of treated batches, from 0 to 4 DPI, there were no clear histopathological symptoms. On 5 DPI, histopathological symptoms were initiated and a few

midgut goblet and columnar cells developed abnormality and became shrunken. On 6 DPI, overgrowth and discharge of infected cells were observed. In contrast to the earlier case, these cells seemed less stained throughout the sequence of infection. On 7 DPI, multi-layered and undifferentiated regenerative *nidi* cells were found (Figure 2). On 8 DPI, the infected cells were found as continuously being pushed off into the midgut lumen due to newly developed immature cells of regenerative *nidi* cells. The number of regenerative *nidi* cells were noticed more in the tolerant breed (5N) compared to susceptible breed (CSR2). From 9 - 12 DPI, it could be seen that the damaged goblet cells were continuously replaced by the newly formed regenerative *nidi* cells followed by the replacement of cylindrical cells as a function of the physiological repair. Tumor like over growth of midgut epithelium was also observed in some places (Figure 2).

DISCUSSION

In the silkworm, epithelium of larval midgut is the sole target of cytoplasmic polyhedrosis virus, densonucleosis virus and infectious flacherie virus. After infection by these viruses, epithelial cells of the midgut show various cytopathic changes characteristic to respective viruses, and general outcome of the infected cells is either to be discharged into midgut lumen (Inoue, 1974) or to be sequestered into neighboring cells (Inoue and Ayuzawa, 1972; Matsui, 1973). These processes of infection and resulting cytopathic events are altered depending upon the different physiological status of the midgut epithelium (Choi *et al.*, 1989). In the present study, histopathological changes in the midgut of susceptible breed (CSR2) indicated that though there was infection in the midgut, there were no clear histopathological symptoms from 1 to 3 DPI. On 4 DPI onwards, histopathological symptoms were manifested and midgut goblet and columnar cells developed abnormality, became shrunken and got discharged into the lumen. From 10 to 12 DPI, *i.e.*, during the advanced stage of infection, more number of infected cells (goblet and columnar cells) were discharged into the gut lumen and only a few regenerative *nidi* cells developed into new goblet and columnar cells leading to physiological damage of the animal and high larval

mortality which was recorded in earlier studies.

The results also indicated that during the progressive infection of BmIFV, the virus infected and multiplied in both the susceptible (CSR2) and tolerant breeds. However, in CSR2, the number of infected goblet cells increased continuously while in the resistant breed (5N), the infected goblet cells were less in number as the infection progressed. This may be due to the discharging of infected cells into gut lumen and the regeneration of new goblet cells from the *nidi* cells allowing physiological repair. The mechanism of regeneration of midgut epithelial cells differed in different regions of the midgut. The regeneration of the goblet cells in place of infected cells helps in prolonging the life of infected larvae. The tolerance level depends on the regenerative capacity of midgut when the micro-organism invades the midgut, there is an increased mitotic activity in the regenerative *nidi* layer and the infected cells are normally extruded into the gut lumen. The midgut cells formed subsequently may be free from infection. The earlier studies of Samson (1987) indicated that lethal dose of *Bacillus thuringiensis* strains in silkworm caused rapid damages to epithelial cells of midgut. The regenerative capacity of epithelial cells of midgut differs in different silkworm strains and possibly is the basis for inter strain differences in their susceptibility to BmIFV. The rate of virus multiplication in the midgut is same in both susceptible and tolerant strains of silkworm. However, in the tolerant strain, the infected goblet cells are discharged into the lumen of the midgut at each moult and the regenerative cells rapidly develop into new goblet cells prolonging the larval life (Inou, 1974). Similar results were also reported by Mary flora *et al.* (2002) in their studies on the comparative susceptibility of different breeds of silkworm to infectious flacherie virus and histopathological changes due to infection. Nataraju *et al.* (2005) had also reported that the susceptibility status of silkworm to BmIFV is related to the ability of regeneration of midgut cells. Hence, differences in histopathological changes in the midgut are the basis for the differences observed in the larval mortality between susceptible and tolerant breeds of silkworm, due to BmIFV infection.

ACKNOWLEDGEMENT

First author wishes to express her thanks to the University Grants Commission, New Delhi for providing Rajiv Gandhi National Fellowship to carry out her research work. She is also thankful to the University of Mysore, Mysuru for permitting her to carry out this work and CSRTI, Mysuru for providing laboratory facilities and encouragement.

REFERENCES

- Choi H., Kobayashi M. and Kawase S. (1989) Changes in infectious flacherie virus-specific polypeptides and translatable mRNA in the midgut of the silkworm, *Bombyx mori* during larval molt. *J. Invertebr. Pathol.*, **53**: 128-131.
- Goel R. K. (2006) *Laboratory techniques in Sericulture*. A.P. H. Publishing Corporation, 5 Ansari Road, Darya Ganj, New Delhi, pp.161-173.
- Inoue H. (1974) Multiplication of an infectious flacherie virus in the resistant and susceptible strains of the silkworm, *Bombyx mori*. *J. Seric. Sci. Jpn.*, **43**: 318-324.
- Inoue H. and Ayuzawa C. (1972) Fluorescent antibody study of an infectious flacherie of the silkworm, *Bombyx mori*. *J. Invertebr. Pathol.*, **19**: 227-230.
- Mamatha M. and Balavenkatasubbaiah M. (2008 a) Identification of productive bivoltine silkworm breeds tolerant to BmIFV (*Bombyx mori* Infectious Flacherie Virus). *Uttar Pradesh J. Zool.*, **28** (3): 297-305.
- Mamatha M. and Balavenkatasubbaiah M. (2008 b) Comparative study on progressive morphological changes in the susceptible and tolerant breeds of silkworm infected with BmIFV. *Indian J. Seric.*, **47** (1): 64-69.
- Mamatha M. and Balavenkatasubbaiah M. (2017) Identification of target tissue(s) of BmIFV infection in the susceptible/tolerant breeds of silkworm, *Bombyx mori* L. through immunodiffusion test. *Sericologia*, **57** (1): 45-52.
- Mary Flora C. A., Sivaprasad V., Balavenkatasubbaiah M., Nataraju B., Thiagarajan V. and Datta R. K. (2002) Comparative susceptibility and histopathology of infectious flacherie virus in different breeds of silkworm, *Bombyx mori* L. In: *Advances in Indian Sericulture Research, Proceedings of National Conference on strategies for Sericulture Research and Development*, November 16-18, 2000, S. B. Dandin and V. P. Gupta (Eds.), CSRTI, Mysore, pp. 323-327.
- Matsui M. (1973) Electron microscope study on the flacherie virus infection in the goblet cell of midgut of the silkworm, *Bombyx mori* L. *J. Seric. Sci. Jpn.*, **42**: 11-16.
- Nataraju B. and Datta R. K. (1999) Application of textile dye based dipstick immune diagnostic kit for management of infectious flacherie in silkworm rearing. *The proceedings of XVII Congress of International Sericultural Commission*, Cairo, Egypt, pp. 283-288.
- Nataraju B., Sathyaprasad K., Majunath D. and Aswani Kumar C. (2005) *A text book on Silkworm Crop Protection*, Central Silk Board, Bangalore.
- Samson M. V. (1987) Bacterial diseases of silkworm and their control. *Ph. D. Thesis*, University of Mysore, Mysore.
- Sato T. (1992) *Report on the Silkworm Pathology*, Bivoltine Sericulture Technology Development Programme, Japan International Cooperative Agency (JICA), Central Sericultural Research and Training Institute, Mysore, India.
- Sivaprasad V., Nataraju B. and Balavenkatasubbaiah M. (1993) Study on the prevalence of non-occluded viral diseases of silkworm, *Bombyx mori* L. *Annual Report*, 1993, Central Sericultural Research and Training Institute, Mysore, India, p.76.
- Vidyunmala S. (2010) Studies on fluoride toxicity on some biochemical and histological aspects of silkworm, *Bombyx mori* L. Role on economic characters. *Ph.D. Thesis*, Sri Krishnadevaraya University, Anantapur, India.
- Yamazaki H., Sakai E., Shimodaira M. and Yamada T. (1960) Studies on the infectious flacherie (F-type) of silkworm, *Bombyx mori*. *Bull. Seric. Exp. Stn.*, **61**: 1-28.



TREATMENT OF WASTE WATER FOR RESIDUAL DYE REMOVAL IN SILK DYEING UNITS

D. Sargunamani^{1*}, K. Raghu¹, K. M. A. Khadar² and Subhas V. Naik²

Silk Conditioning and Testing House, Central Silk Technological Research Institute, Central Silk Board, Kancheepuram 631501, India.

²Central Silk Technological Research Institute, Central Silk Board, Bengaluru 560068, India.

*E-mail: sargunamani@gmail.com

ABSTRACT

The silk dyeing industry poses environmental hazards associated with residual dye discharged into the left over waste water. Hence, the effluent from the dyeing units deserves proper treatment before being discharged into the sewer to ensure biosafety of environment. The dyes used in the industry are usually dispersible, and the surplus or the residual dye is removed using various physiochemical, chemical, biological and electrochemical techniques. But, complete removal of the dye from the waste water still remains a difficult task. Many researchers have been working in the direction of more efficient and cost effective methods of dye removal. This study was undertaken with this backdrop wherein, an attempt was made adopting a new technology for dye removal from waste water discharged after dyeing of silk, using muffle furnace at high temperature of 800 °C with zero water discharge. The commercial dyes have revealed the presence of banned aryl amines when subjected to High Performance Liquid Chromatography (HPLC). Muffle furnace treatment of dye waste water at 800 °C for 3 minutes for branded dye (have known chemical groups/chemical abstract numbers and made by certified manufacturers) and at 800 °C for 9 minutes for commercial dye (dyes purchased from local market and difficult to identify composition of molecules) was found to be effective for colour disappearance and dye waste water evaporation. Thermogravimetry-differential scanning calorimetry (TG-DSC) and Gas chromatography-mass spectrometry (GC-MS) instrumental analyses were carried out to detect the components of the residue. Results showed that at 800 °C, it reduced to a higher extent with formation of many compounds evaporated in gaseous form such as 3-(2,2-dimethylpropylidene) bicyclo[3.3.1]nonane-2,4-dione, Butylated hydroxytoluene (BHT) *etc.*

Key words: Acid dye, dye waste water, GC-MS, silk, TG-DSC.

INTRODUCTION

The chemical wet processing operations of silk are degumming, bleaching, dyeing, printing and finishing. Dyeing is one such process that causes water pollution in which colour plays a major role. It is estimated that up to 50 % of dye that is not fixed in the silk yarn is released into water after dyeing and washing (<https://www.intechopen.com/books>). Some dyes are carcinogenic or give allergic effects and it is essential to follow the recommended guidelines while using them (Southern, 1995; Freeman *et al.*, 1996; Geisberger, 1997). Dyes of categories, such as acid, metal complex, basic,

reactive colours and natural dyes are used for dyeing of silk (Gulrajani, 1988; Deepti Bahl Gupta and Gulrajani, 1993; Teli, 1996; Sargunamani and Selvakumar, 2012; Sargunamani and Khadar, 2016) Some dyes contain banned aryl amines which are commercially analyzed using HPLC (Nadiger, 2001). After dyeing, waste water containing residual dye is discharged into the sewer and some of the units treat it for colour disappearance using conventional methods (bacteria / oxidising agents and so on). Therefore, a new method that leaves no waste water to be let out into sewer had been thought of. Hence, a pilot study on the concept of evaporation of dye waste water

using muffle furnace was carried out to find out optimum time and temperature of dye colour disappearance. Factorial experiments were conducted to understand the effects of each factor at different levels (temperature 600, 700 and 800 °C and time 3, 6 and 9 min) on the response variable (colour disappearance). TG-DSC and GC-MS instrumental analyses were carried out for dye mass reduction and to detect the components *viz.*, gases or any other chemical compounds after the treatment of dye waste water.

MATERIALS AND METHODS

Acid dye (Nitional yellow GL Spl) powder was bought from Vasanth Dyes and Chemicals Pvt. Ltd., Bengaluru and Red chilli, commercial dye (local dye), from local market of Kancheepuram. Acetic acid was procured from Jayam Scientific Suppliers, Chennai. The commercial method of silk yarn dyeing was followed (Sargunamani *et al.*, 2017) for branded and commercial dyes. After dyeing, the dye waste water was collected. A sample of (20 ml) the collected dye waste water was taken in a crucible and kept in muffle furnace (Figure 1) at high temperature ranging up to 800 °C for 3, 6 and 9 min. Factorial design method was used for carrying out experiments (<http://www.socialresearchmethods.net>) for optimizing conditions of colour disappearance. Detection of banned aryl amines if any in commercially used dye powders was done using High performance liquid chromatography (HPLC) at Central Silk Technological Research Institute



Figure 1: Muffle furnace with temperature option up to 1400 °C

(CSTRI), Bengaluru (Nadiger, 2001). The decomposed residue was analyzed using gas chromatography-mass spectrometry (GC-MS) at South Indian Textile Research Association, Coimbatore. TG-DSC (Thermogravimetry-differential scanning calorimetry) analysis was performed using TG-DSC instrument (NETZSCH STA 449 F3 Jupiter) at Indian Institute of Technology, Chennai.

RESULTS AND DISCUSSION

Table 1 shows the results of screening of popular commercial dyes for the presence of banned aryl amines that would degrade human health. It is seen that majority of dyes were free of banned aryl amines, whereas, three samples, namely Arakku, Black and Chrysophenine were detected with banned aryl amines of 45, 58 and 6 mg/kg, respectively, the eco-standard norms being 30 mg/kg. Since the banned aryl amines are carcinogens, use of such

Table 1: Detection of banned aryl amines in commercial dye samples of Kancheepuram

Acid dye	Result
ASD/COR	-
RED RR	-
ARAKKU	+ (45 mg/kg)
V.PAKKU	-
BLACK	+ (58 mg/kg)
RED CHILLI	-
BROWN PR	-
CHRY SOPHENINE	+ (6 mg/kg)
CH/Ext.cone	-
ORANGESE, RED G	-

+: Detected; -: Not detected

Table 2: Time taken for dye removal of acid dye waste water in muffle furnace

Nitional yellow GL Spl (Branded dye)				Red chilli (Commercial dye)			
Time (min)				Time (min)			
Temperature (°C)	3 min	6 min	9 min	3 min	6 min	9 min	
600	CNR	CNR	CNR	CNR	CNR	CNR	
700	CNR	CNR	CNR	CNR	CNR	CNR	
800	CR	CR	CR	CNR	CNR	CR	

CNR: Colour is not completely removed, CR: Colour is completely removed.

dyes may be avoided in the industry (www.greenrivertech.com).

Table 2 shows trials of treatment time versus temperature conducted in muffle furnace for effective dye removal. Various stages during the treatment of samples of dye waste water taken in the crucible and kept in muffle furnace and subjected to a temperature of 800 °C are shown in Figure 2 (a~d). Waste water containing residues of the dye, Nitronal yellow GL Spl (branded dye-BD) displayed faster colour disappearance and evaporation at 800 °C vs 3 min treatment compared to commercial dye which took 9 min at the same temperature when kept in muffle furnace, leaving a little ash which is easily removable.

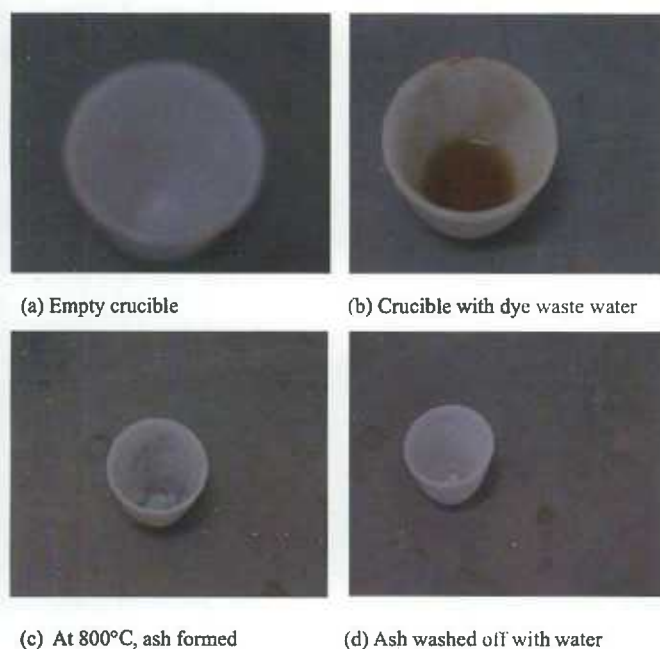


Figure 2: Various stages of dye waste water treatment in the crucible placed inside the muffle furnace

Since the major portion of dye waste effluent is water (>95 %) followed by dye and traces of acetic acid, the dye component is removed fast and evaporation happens in a shorter time. When dye waste water is subjected to 800 °C, which is quite above the boiling point [water: 100°C, acetic acid: 118.1 °C (<https://en.wikipedia.org/wiki/Boiling>) and acid dyes: 480°C (<https://en.wikipedia.org/wiki/Acidred88>), water evaporates and dye mass reduces leaving a little quantity

of ash which can be cleaned by a gentle wash.

The dye mass reduction has resulted in dye colour disappearance which has been confirmed with TG-DSC analysis (Figure 3). It shows that residual mass was 89.77 % at 490 °C and when increased to higher temperature, it turned into 27.19 %. It may be reasoned that dye molecules were fragmented to smaller size / disappeared on exposure to high temperature leading to dye mass reduction. The result is similar to that obtained by Kocaokutgen and Heren (1998) who reported that azo dyes exhibit thermal behavior of losing even 100 % mass. The amount of volatile pyrolysis product (%) at different temperatures from 50 to 790 °C showed 7.93 to 100 % loss (<http://journals.tubitak.gov.tr>). GC-MS analysis done for the chemical components showed the maximum peak retention time (time of injection to detection of a compound in GC-MS) as 13.79 min as shown in Figure 4, other retention time obtained are not considered in this case. Figure 5 shows the mass spectrum (relative abundance v/s m/z) pattern representing distribution of ions by mass for the maximum peak RT-13.79. Different compounds for maximum peak retention time 13.79 are obtained referring standard table of GC-MS. The possible dominant chemical compounds formed are 3-(2, 2-dimethylpropylidene) bicyclo[3.3.1]nonane-2,4-dione and Butylated hydroxytoluene (BHT). Among these, BHT is known for its antioxidant properties. Though, debate link it under cancer risk category, it is not yet confirmed and The National Cancer Institute determined in 1979, that it was non-carcinogenic in a mouse model (https://en.wikipedia.org/wiki/Butylated_hydroxytoluene).

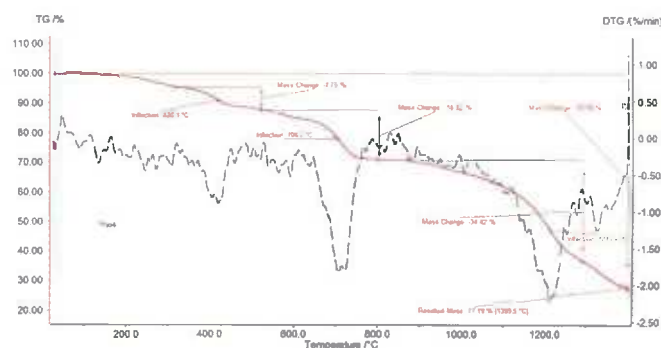


Figure 3: Combined plot of TG-DSC showing mass change at various temperature

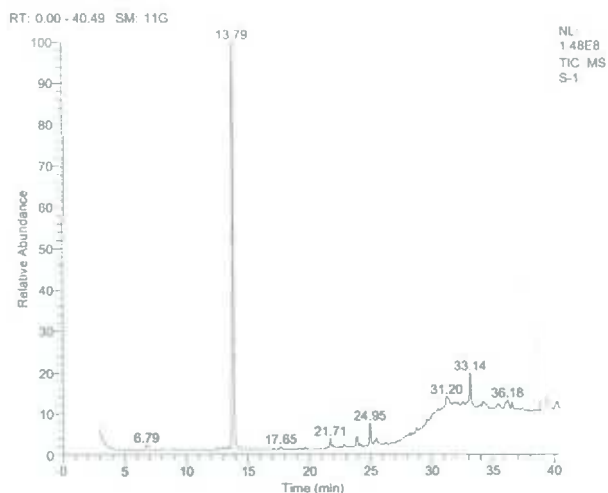


Figure 4: Relative abundance v/s time in GC-MS analysis of acid dye powder

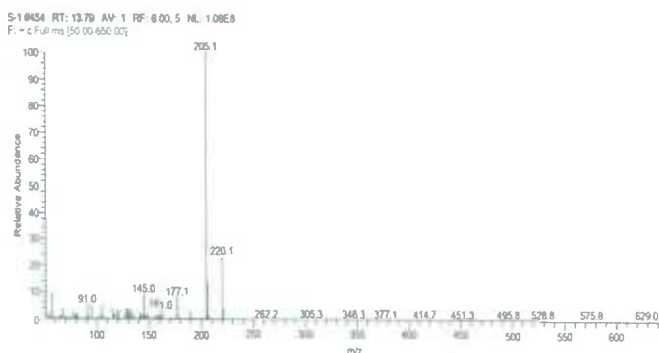


Figure 5: Relative abundance v/s m/z in GC-MS analysis of acid dye powder (RT: 13.79)

Conclusion

The present technique used for removal of dye from waste water work on the principles of adsorption, filtration, coagulation, precipitation, flocculation, chemical, photo and bio-degradation. This pilot study is an innovative method of dye removal and evaporation of dye waste water using muffle furnace at 800 °C for 3 min and 9 min for branded and commercial dye, respectively. TG-DSC results show major mass reduction at higher temperature. GC-MS revealed various chemical components, dominantly BHT, phenol products and other complex chemical compounds.

REFERENCES

- Deepti Bahl Gupta and Gulrajani M. L. (1993) Studies on dyeing with natural dye Juglone (5-hydroxy-1, 4- naphthoquinone). *Indian J. Fib. Tex. Res.*, **18** (4): 202-206.
- Freeman H. S., Hinks D. and Esancy J. (1996) *Physio-Chemical principles of Colour chemistry*, A. T. Peters and H. S. Freeman (Ed.), Chapman and Hall, U. K., p. 254.
- Geisberger A. (1997) Azo dyes and the law—an open debate. *J. Soc.Dyrs. Col.*, **113** (7–8): 197–200.
- Gulrajani M. L. (1988) *Silk dyeing, printing and finishing*, IIT, New Delhi, India.
- Kocaokutgen and Heren (1998) Thermal behaviour of some azo dyes containing sterically hindered and water soluble groups. *Turk. J. Chem.*, **22**: 403-408.
- Nadiger G. S. (2001) Azo ban, eco-norms and testing. *Indian J. Fib. Tex. Res.*, **26** (1): 55-60.
- Sargunamani D. and Khadar K. M. A. (2016) Overview of Kancheepuram silk saree, silk industry and role of SCTH, CSB for silk sector improvement – current scenario -2016. *29th National Convention of Textile Engineers, Contemporary approaches of make in India concepts in textiles and apparel industries, Make in India Conference, Bengaluru, India, 26-27 February, 2016*, pp. 8-9.
- Sargunamani D., Raghu K., Khadar K. M. A. and Subhas V. Naik (2017) Application of Total Quality Management (TQM) concepts for business improvement in a silk dyeing unit at Kancheepuram. *Seicologia*, **57** (1): 58-62.
- Sargunamani D. and Selvakumar N. (2012) Effect of ozone treatment on the dyeing properties of mulberry and tassar silk fabrics. *J. Eng. Fib. Fab.*, **7** (3): 21-27.
- Southern T. G. (1995) Technical solutions to the colour problem: a critical review. In: *Colour in Dye house effluent*, P. Cooper (Ed.), Society of Dyers and Colourists, U.K., p.73.

Teli M. D. (1996) New Developments in dyeing process control. *Indian J. Fib. Tex. Res.*, **21**: 41-49.

[https://www.intechopen.com/books/eco-friendly-textile-dyeing-and-finishing/textile dyes dyeing process and environmental impact](https://www.intechopen.com/books/eco-friendly-textile-dyeing-and-finishing/textile-dyes-dyeing-process-and-environmental-impact)

<http://www.socialresearchmethods.net>

www.greenrivertech.com.tw

<https://en.wikipedia.org/wiki/Boiling>

<https://en.wikipedia.org/wiki/Acidred88>

https://en.wikipedia.org/wiki/Butylated_hydroxytoluene



TOWARDS BUILDING A NATIONAL SERICULTURE (MULBERRY) INNOVATION SYSTEM IN INDIA - POSSIBILITIES AND PROSPECTS

G. K. Rajesh^{1*} and S. Ramaswamy²

Department of Economics, Gandigram Rural Institute - Deemed University, Madurai 624302, India.

*E-mail: gkrajeshrajesh@gmail.com

ABSTRACT

Sericulture is an important agro industrial in India with foremost socio-cultural implications. Silk is an important export commodity and the nation has bestowed matching importance to the sector from very early days. Over the years, the sector has undergone tremendous technological changes through innovations mainly focussed on improvement of the farm produce, namely cocoon. But, innovative efforts of this level were not received by the immediate processing sector, namely reeling industry, which has affected the quality of the raw silk fibre. This paper attempts to establish that Indian sericulture sector has all the necessary ingredients, features *etc.* and is characterised by complex networks and linkages, by way of economic, social, political, organizational and institutional factors influencing the development, diffusion and use of innovations; hence, qualify as a Sectoral Innovation System in its own right. However, its progress and development are limited by the inherent weaknesses of the system. This study identifies important weaknesses of the system and suggests ways for improvement. The two important aspects related to weakness of the system were detected as, poor participation of the private sector in R&D and over emphasis of the agricultural aspect of sericulture. Bulk of innovation efforts were focussed on farm sector without matching modernisation of the silk reeling sector. This has rendered the efforts on up-gradation of raw silk quality, ineffective.

Key words: Absorptive capacity, India, sectoral system of innovation, sericulture.

INTRODUCTION

The 'tendency to catch-up' as a fundamental human trait is exemplified by instances in the history of innovations. Developing countries often tend to import and directly apply policies and best practices for innovation from industrialised nations as a technological catch-up strategy. However, there are particularities in developing countries, which cannot be neglected when studying innovation dynamics in a systemic approach. At times, when this fact is neglected, the innovative attempt could lead to systems' disruption. This paper proposes to examine the technological evolution of Indian sericulture sector since the 18th century to current times, through the analytical framework of Sectoral System of Innovations. The objective is to analyse the complex technological evolution of the sector over three centuries, understand the innovation system that has come into being and to

suggest measures to strengthen it.

India is the second largest silk producer, the largest consumer and importer of silk and silk goods (UN COMTRADE data, 2007). Sericulture is important to Indian economy as a cottage industry employing six million people (Ministry of Textiles, Government of India, 2007). As a labour intensive activity practiced throughout the year, it is identified as a means for rural employment generation and as a remedy for seasonal unemployment (Jayaram *et al.*, 1998). Indian sericulture sector is constrained by low productivity, low quality of raw silk produced, price instability and import competition. With the demand pull (for high quality warp) created by sophisticated power looms and consequent to relaxation in exim policies, large quantities of high quality silk is being imported at prices lower than local silk. This has apparently lowered the demand for domestic silk, resulting

in downward spirals of cocoon and silk prices (Vasumathi, 2000). The reason for these constraints is the poor quantitative and qualitative performance of the prevalent, traditional, low yielding silkworm varieties. To ameliorate the local polyvoltine silkworms, a superior hybrid, namely 'Bivoltine Hybrid (BV)' and High Yielding Variety (HYV) technology, namely 'BV Hybrid Technology (BVHT)' were evolved as early as 1970s and systematic popularisation efforts began during 1990s. But, the hybrid technology did not diffuse well for nearly two decades. Recently, the innovation has started diffusing well creating favourable impact on the performance of the sector.

The bulk of innovative efforts undertaken in the Indian sericulture sector over the past three decades have been focussed on evolving productive silkworm hybrids capable of producing high quality silk. This paper attempts a comprehensive analysis of the scenario, through the analytical framework of Sectoral System of Innovations. The paper highlights the characteristics of the emerging Sectoral Innovation System and analyses the roles of key players and key enabling factors and drivers of competitiveness. In conclusion, some recommendations for building on this nascent Sectoral Innovation System for sericulture are suggested, with the aim of reducing the gap between knowledge generation and the absorptive capacity of the private sector. The scope of this paper is confined to the mulberry Sericulture Sector (soil to reeled silk) within India.

This article focuses only on bivoltine sericulture as a preliminary case study in applying the framework of Sectoral System of Innovations. A large number of innovations have been formed in mulberry sericulture sector. The paper doesn't address all these individual innovations but focus on the bivoltine hybrid technology. The bivoltine silk accounts for only about 20 % of the total mulberry silk production. This indicates that, the cross breed and multivoltine technology continue to dominate the sector and is very decisive. A large number of technologies have been developed for the cross breed sericulture and those innovations are very important for the sector which falls outside the scope of this paper. Bivoltine technology is the focal issue of the present study. For the discussion on the qualitative aspects of silk

as an industrial product in the local and export markets, this paper focuses only on raw silk. This is by considering the fact that raw silk is an internationally standardized commodity with comparable grades, and the quality of which is not extremely distorted by the complex value chain of subsequent processing industry that produces the basket of diversified products. Hence, the product category of made-ups and readymade garments (RMG) accounting for the greater part of India's silk exports is not considered here, which would require a more detailed and exclusive research. This paper has utilized the UN COMTRADE database, the largest depository of international trade data, considering its universal acceptance as a source of high quality data.

Sericulture and its strategic importance to India

Sericulture has important socio-cultural implications. Studies have established large scale employment generation potential and high income generation potential of sericulture (Hanumappa and Erappa, 1986). Jayaram *et al.* (1998) showed that every acre of sericulture practiced under irrigated conditions had a potential to employ 247 men and 193 women round the year (Jayaram *et al.*, 1998). They have also shown that the small scale mulberry farms provided ample scope for employment of owned family labour and suggested its potential to solve the problem of seasonal unemployment. Lakshmanan *et al.* (1999) found that female labour is quite dominant in all sericultural activities, to an extent of nearly 50 %. Saraswathi and Sumangala (2001) observed that in the indoor activity of silkworm rearing, women participation was as high as 94.67 % and that except for the peak period, the entire sericultural activity is conducted using family labour. Most of the activities in silk production are in the informal sector and menial in nature. Thus, about 90 % of the employment goes either to the landless or to the marginal farming families that hire out these labour, or to the sericulture families (Sinha, 1989). While considering the price spread in the whole industry, it can be seen that 48 % of it goes to farming sector (Mattigatti *et al.*, 2000).

Sericulture and silk production are labour-intensive at the village level, employing both men and women at all stages of production. In China, it occupies some 20 million farmers, as well as half a million in the silk processing

industry (ITC Silk Review, 2001). In India, sericulture is a cottage industry in 59,000 villages, providing full and part-time employment to about six million people from the farm sector, and silk processing industry (ITC Silk Review, 2001).

Silk as a textile fibre and its un-tapped potentials as a futuristic material

Silk is a protein secreted by the silkworm to spin a protective shell for its pupa. Silk has many unique properties which make it attractive as a textile fibre. Apart from its strength (greater than a steel wire of similar dimensions), it resists breakage, and can be stretched to more than 20 % of its original length. It is lower in density than cotton, wool and rayon, and is a highly moisture absorbent (Currie, 1997). After degumming, it has excellent dyeing properties and assumes a lustrous, semi-transparent sheen with a smooth surface that does not soil easily. It is more heat-resistant than wool. The fabric has excellent wrapping qualities and a good 'fall' which gives it an agreeable look on the wearer's body (ibid).

Silk constituted only 0.2 % of the total fibre and 0.5 % of the total natural fibres produced globally. While its

current production volumes are relatively small, the increased interest in tough composites, biodegradable composites, sustainable materials and bio mimetic high performance materials indicate that silk has the potential to play an important role in advanced bio-materials in future (Holland *et al.*, 2007). Silk fibre's mechanical properties and its behaviour as a textile are well known (Vollrath and Porter, 2009). Silk as a biodegradable material holds tremendous possibilities for India to reconsider its position from being an exclusive textile fibre to a material for the future. The increasing body of silk literature in the material sciences discipline point towards the necessity for a paradigm shift in the perspective of silk as an exclusive luxury textile fibre being used in India for more than 2500 years.

The fundamentals of silk farming and reeling have changed a little due to the industrial revolution (Ganga, 2003). Considering the significant rural development potential of silk (as detailed in the ensuing section), the central and state governments have been supporting the sector heavily. Silk farmers in India form an influential pressure group. On this account, the sector receives subsidies for primary inputs and other support. The silk reeling industry does not receive the same level of subsidy

Table 1: World silk production by important producers from 1938 to 2015 (select years; production in tonnes)

Country	1938	1970	1980	1985	1990	2010	2011	2012	2013	2014	2015
Bangladesh	-	-	-	-	20.00	40	38	43	43	44	44
Brazil	-	-	-	-	-	770	558	614	550	560	600
China (% aggregate)	4860.00	11120.00	37200.00	38960.00	50047.00	115000	104000	126000	130000	146000	170000
		(128.81)	(234.53)	(4.73)	(28.46)	(30.98)	(-9.57)	(21.15)	(3.17)	(12.31)	(16.44)
India (% aggregate)	1900.00	2260.00	2610.00	4900.00	10400.00	21005	23060	23679	26480	28708	28523
		(18.95)	(15.49)	(87.74)	(112.24)	(36.00)	(9.78)	(2.68)	(11.83)	(8.41)	(-0.64)
Indonesia	-	-	-	8.00	20.00	20	20	20	16	10	8
Iran	300.00	400.00	270.00	280.00	540.00	75	120	123	123	110	120
Japan	43150.00	20520.00	15640.00	9590.00	5700.00	54	42	30	30	30	30
Korea N.	1820.00	3020.00	3310.00	1440.00	1100.00	-	300	300	300	320	350
Korea S.	1820.00	-	2100.00	3500.00	4400.00	3	3	1.5	1.6	1.2	1
Thailand	1100.00	1700.00	890.00	1140.00	1350.00	655	655	655	680	692	698
Turkey	-	-	-	-	-	18	22	22	25	32	30
Uzbekistan	-	-	-	-	-	940	940	940	980	1100	1200
Vietnam	500.00	300.00	280.00	330.00	510.00	550	500	450	475	420	450
Others	2080.00	4860.00	5280.00	7490.00	9792.00	-	-	-	33.4	30.8	18
Total	57530.00	44180.00	67590.00	67650.00	83994.00	139100	129661	152846	159737	178058	202072

Source: Database of International Sericultural Commission

as farmers do, and the reelers operate on a far more independent and entrepreneurial basis, with low margins (Holland *et al.*, 2007).

Hence, from the discussion, the following concerns emerge as key issues, the system needs to address:

- Diffusion of the Bivoltine Hybrid Technology among farmers.
- Under exploited potentials of commercial utilisation of by-products.
- A need to shift attention towards equipping the system to produce high quality silk and silk products for use in material sciences.
- A need to focus on production of environmentally sustainable silk.

India in the global silk scenario

Table 1 summarises global silk production since 1938. Until late 1970s, Japan was the largest silk producer. During 1980s, China overtook Japan and has retained its position as the world's largest silk producer ever since. India came to the second position in 1987, crossing Japan and retained the position thereafter but, leaving a large production gap with that of China. India increased its output from 9720 tons in 1989 to 13200 tons by 1992, an increase of 3480 tons which represented a very high growth. However, since 1992, India's silk production exhibited a low growth rate. However, the total global silk production shows an increasing trend going by absolute values. So is the case with China and India especially since 2010, both the countries showed annual growth rate in two digits.

From 2002, China showed a very high growth rate of 37.9 % though it subsequently fell. Japan has been showing a negative trend in silk production throughout the period. According to Currie (1997), the Japanese silk production is still alive owing to the Japanese policy to import cocoon to keep the reeling plants in the country active. It can be seen that India's growth rate was comparatively better than that of China and world until 1999. After 2001, it fell far below, showing negative growth up to -6 % (2002) though started showing signs of recovery during 2004. Over all, it appears that the fall in Indian raw silk production has been utilized by China to

increase its supply. Thinking in the reverse order, probably, the fall in Chinese raw silk imports subsequent to hike in Chinese production during 2002-03 could have led to the tremendous decline in Indian silk production during that period.

Table 2 presents India's export, import and demand - supply gap of mulberry silk yarn (not thrown) during sixteen years from 2000. The annual production of mulberry raw silk in India was 20478 tons, in 2015, while India imported 3454 tons of raw silk and exported just 11 tonnes. Though the imports had steadily increased from

Table 2: India's raw silk production, export, import and demand - supply gap

Year	Silk production (tons)	Export (tons)	Import (tons)	Demand-supply gap (tons)	Export value (Million USD)	Import value (Million USD)
2000	15214	42	4975	4933	0.97	105.13
2001	15857	156	5490	5334	3.48	113.43
2002	17351	31	9449	9418	0.76	149.59
2003	16369	30	9464	9434	0.82	134.34
2004	14620	65	7476	7411	1.43	124.64
2005	15445	181	9167	8986	3.88	176.50
2006	16525	90	5986	5895	2.87	158.25
2007	16245	36	7639	7602	1.06	179.73
2008	15610	160	8107	7947	2.53	193.99
2009	16322	306	8004	7698	1.71	197.30
2010	16360	32	5915	5883	0.90	194.63
2011	18272	55	5597	5542	0.64	226.72
2012	18715	13	5235	5222	0.56	233.31
2013	19476	9	3609	3599	0.67	165.95
2014	21390	6	3403	3397	0.28	155.89
2015	20478	11	3454	3443	0.27	152.90
CAGR (%)	1.9	-8.7	-2.4	-2.4	-7.68	2.37

Source: UN COMTRADE, 2017

4975 (2000) to 9434 tons (2003), the trend declined further. Hence, the Compound Annual Growth Rate (CAGR) of raw silk imports remained (minus) 2.4 % over sixteen years. During this period, the raw silk exports have drastically fallen by a CAGR of (minus) 8.7 %. The domestic silk showed a CAGR of 1.9 %. While the decline in export quantity has produced a corresponding decline in export value (CAGR = -7.68 %), the decline in imports haven't brought down the import value, which went up by a CAGR of 2.37 %. This indicates that while the export price of Indian raw silk has been steady or declining, the

price of imported silk has been increasing disproportionately over the sixteen years. This analysis indicates that India has neither been able to meet the increasing demand for silk in the domestic market by increasing domestic silk production, nor to exploit the huge export potential; instead, resorted to raw silk imports to fill up the domestic demand - supply gap. This is a clear indication of the sub optimal quality of Indian raw silk that makes it less competitive in the global silk market.

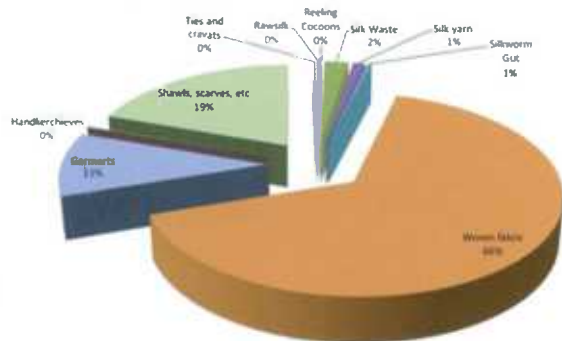


Figure 1: Profile of India's export of silk and silk products (1998 – 2016)

Source: UN COMTRADE data, 2017

Figure 1 illustrates the profile of India's export pattern of raw silk and all silk products. Major share (96 %) of India's export earnings is from three commodities viz., woven fabric (66 %), shawls, scarves etc. - not knit (19 %) and garments (Women & girl's blouses & shirts, of silk, not knit) - 11 %. The average annual growth rate of these export commodities during three decades from 1990, furnished in Table 3 shows a steady decline in growth of exports.

Table 3: Ten years' average of AGR (%) of export earnings from important segments of silk exports

Period	All silk products	Woven fabric	Garments	Shawls, scarves etc.
1990-99	7.3	7.2	19	17.4
2000-09	3.3	3.6	16.6	13.1
2010-16	-12.7	-14.9	-10.2	-2.7

Table 4 shows the average annual growth rate in Import and export values in US dollars for three decades from 1999 to 2016. Though both import and export have declined over the years, the export value has drastically and consistently fallen especially during the period from 2010 to 2016 (18 %).

Table 4: Average annual growth rate (%) in import and export

Period	Import	Export
1990-99	9.9	7.7
2000-09	14.9	3.7
2010-2016	-8	-18.8

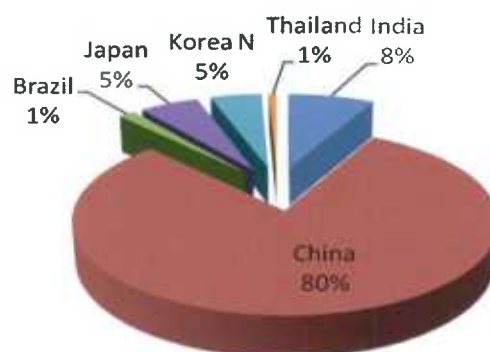


Figure 2: Share of total value in USD of global silk exports (2000 – 2015)

Source: UN COMTRADE data, 2017

Figure 2 presents the share of global silk exports (all silk goods) by six major silk producing countries that are key players in export. While China enjoys the biggest share of the pie, that is 80 % of the export trade by value, India has only 8 % of share. It will be interesting to analyze the impact of raw material imports on India's domestic sericulture sector and importance of technological innovations in improving its fate.

Impact of cheap imports on domestic industry

Apart from India's inability to exploit the export potentials and dependency on imported raw material, another grave issue is pertaining to cheap imports of raw materials ruining the domestic sericulture industry. It is reported that Chinese raw silk and silk fabrics are reportedly being imported into the country at very low prices (Tikku, 1999).

The growth rate in the value of imported silk (in Indian Rupee terms) has been lower than that of quantity of imports. The prices of domestic raw silk and cocoon are observed to be the worst affected during the years 2001-02 and 2002-03, the periods in which imports grew very high and prices of imported silk kept falling. Thomas *et al.* (2005 b) showed that the cocoon price gets influenced by

the yarn prices with a lag of six to ten days. During 2001-02 and 2002-03, when domestic raw silk prices fell by 4.9 and 24.1 %, respectively, the cocoon price also fell by 4.8 and 10.8 %. This has impacted on cocoon production, lowering it by 8.2 and 8.4 % during 2002-03 and 2003-04, respectively. The data show that the effect of imports influenced the mulberry plantation also. During 2002-03, in Karnataka alone, 23 % of the existing mulberry plantations were uprooted and in the subsequent year, another 10 % of uprooting occurred. 6780 charka reeling units were closed down over a span of six years whereas, the number of cottage basin reeling units have increased by 846 only. The number of multi end reeling units have also fallen by 59. This means a considerable labour displacement from the charka sector during the six years which is not likely due to upgradation of the charka units into cottage basins.

The fall in prices, quantity of cocoon production and mulberry area and labour displacement from the reeling sector cannot be completely attributed to the raw silk import. Many other socio-economic factors could be at play. However, it is seen that subsequent to a protectionist intervention of the government during 2003, the quantity of imports fell by 2.3 %, domestic raw silk prices increased from ₹805 to 984 per kg (22.2 % growth) and cocoon prices increased by 17.8 %. During the subsequent year, the quantity of cocoon production showed slight improvement (2.2 % growth) and the mulberry uprooting rate came down from 10.26 to 2.23 %. This indicates that the silk imports have had a deleterious effect on the domestic sericulture.

Table 5: Silk hand looms and power looms in India

Year	Raw silk import % growth rate		Hand looms (No.)	Power looms (No.)
	quantity	value		
1999-00	77.7	59.1	227701	29340
2000-01	-6.1	15.1	227701	29340
2001-02	44.5	31.5	258000	29340
2002-03	32.9	3.6	258000	29340
2003-04	2.3	-2.9	258000	29340
2004-05	-14.1	-3.4	258000	29340

Source: Central Silk Board data base

It is generally held that the imported raw silk is consumed by the power looms since power looms require qualitatively superior and strong yarn for the warp and the relatively poor quality local yarn is fit for only the weft (Thomas *et al.*, 2005 a; Vasumathi, 2000). Table 5

displays the growth rate of raw silk imports over 6 years from 1999-00 against number of hand looms and power looms in the country. It is seen that the number of power looms have stagnated at 29340 over the years and the number of hand looms have increased by 30299 during 2001-02. The raw silk imports have been on the increase. Thomas *et al.* (2005 a) found that only 50-53 % of the yarn requirement of the power loom sector is met from Chinese imports. Thus, it may be inferred that a considerable portion of the imported yarn is being absorbed by the hand loom sector also, which is traditionally known as the sole consumer of charka silk (Vasumathi, 2000).

From the above discussion, it is clear that Indian sericulture sector has been constrained by the poor quality of its silk which caused its unsatisfactory performance in the export market. The situation was that very high quality Chinese silk being imported at prices lower than domestic silk has almost disrupted the Indian domestic silk Industry until recently. During the last five years, there has been a decline in imports of raw silk indicative of decreasing dependency on Chinese raw silk. It is understood that this change has been brought about as a result of the improved diffusion of innovations, especially Bivoltine Hybrid Silkworms and Bivoltine Hybrid Technology among sericulture farmers. This is explained in the ensuing section of this paper.

While considering this fete as an achievement, the efforts spanning three decades in developing and popularising the innovation and the evolution of the Sericulture System of Innovations has to be appreciated. A proper understanding of the Indian Sericulture Innovation System and its historic evolution is necessary for appreciating the specific course, the sector has historically taken, to identify its shortcomings and in evolving corrective policy. The rest of the paper is devoted for viewing the sector through the Sectoral Innovation System perspective and to identify features that constrain the system and suggest corrections.

SECTORAL INNOVATION SYSTEMS

The concepts of Innovation Systems and National Systems of Innovation are understood as given by Edquist (2005) and Lundvall (1992), respectively and the Sectoral

System of Innovation by the exhaustive definition provided by Malerba (2002). While introducing the concept of Sectoral System of Innovation and Production, Malerba (2002) recognises two traditions of sectoral level studies, one dealing with the industrial economics literature and the other, dealing with sectoral case studies. Malerba identifies their limitations and substantiates the necessity for a unified analytical frame. Thus, the Sectoral System of Innovation combines tenets of evolutionary theory and systems approach and is capable of capturing the multidimensional, integrated and dynamic qualities of sectors. Innovation Systems approach highlights learning as a key determinant of innovation, places importance on inter-disciplinary approach and emphasizes the historical perspective (Edquist, 2005).

Over the course of the three centuries, the geographical boundary (ies) of India have transformed from princely states to colonial presidencies to constituent states of a Federal Republic Nation. Silk is an important natural textile fibre and India is its second largest producer, the largest consumer and importer. The Indian sericulture sector presents a complete picture of evolution of a primitive enterprise into an organized industry - characterised by complex networks and linkages, by way of economic, social, political, organizational and institutional factors influencing the development, diffusion and use of innovations.

The history of Indian sericulture evidences accumulation of knowledge and its application by heterogeneous agents through complex interactions that are conditioned by social and economic institutions.

INDIAN SERICULTURE INNOVATION SYSTEM (ISIS): Historical evolution and role

Table 6 presents a rough estimate of improvements, the Indian Sericulture Sector has witnessed over forty-five years. Since 1970, the per hectare productivity of raw silk increased by 400 per cent while the total raw silk output of the country by 900 per cent. This exemplifies the tremendous technological transformation, the Sericulture Sector of India has undergone over forty-five years. Hence, a historical analysis of the evolution of the system is necessitated in order to understand the complex

processes through which the sectoral innovation system has been shaped.

Table 6: Improvements in the Indian sericulture sector over 45 years since 1970

Parameter	1970	2015	% increase
Area under mulberry (hectare)	104885	208947	99
Total raw silk production (tonnes)	2046	20478	901
Productivity (kg silk per acre)	20	98	402

The mention of silk in ancient Indian scriptures indicate its production and use as early as 2000 BCE (Gopal, 1961). Recorded evidence of India's connection with the ancient Silk Route and her commercial silk transaction dates back to at least 200 BCE (UN, 1994). Innovative efforts in the Indian sericulture sector may have started at-least by the early 18th century. However, five phases in the evolution of Indian sericulture could be discerned from literature *viz.*, beginnings (1770-1840), age of unsuccessful experiments (1840-1910), transition phase (1910-1940), the consolidation phase (1940-1980) and the 'bivoltine hybrid' era (1980 onwards) (Lefroy and Ansorge, 1915; Charsley, 1982; CSB Annual Reports since 1980s).

The silk trade flourished in India during the medieval period. Under the Moughals, silks from Kashmir and Bengal were exported mainly by the Moors, who during the 14th and 15th centuries transmitted it to Europe (Nanavathy, 1990). The British had identified the qualitative shortcoming with Indian silk and tried to improve it by bringing experts to modernize the rearing and reeling techniques. In 1771, the 'China worm' was introduced with the idea of improving cocoon quality, which diffused well. Technology was substantially improved in conformity with the European know-how and practices so that British weavers accepted raw silk of Bengal; to which the overseas market responded favourably (Ray Indrajit, 2005). Another breakthrough was successful organization of sericulture as a cottage industry. The industry's technology and organization were thus thoroughly reformed by the close of the eighteenth century in tandem with the requirements of the European market. Consequent to the abolishment of British East India Company's monopoly on private trade, the company wound up its silk trade in 1833, leaving it to private

entrepreneurs. During the last quarter of 19th century, Bengal silk began to decline due to lack of proper organization, husbanding authority and the absence of technical know-how (Ray Indrajit, 2005).

Hanumappa and Erappa (1988) cites sericulture development in the princely state of Mysore as an example of the crucial role, the state can play in augmenting the sources of rural income. Sericulture flourished in Mysore during the 18th century under Tipu Sultan. The technology was transferred from Bengal. Japanese and Italian silkworm strains were imported and experts hired from these countries (Nanavaty, 1990). Spread of diseases during 1866 and the world depression in 1929 along with competition from imported silk and rayon led to downfall of Indian silk industry after World War II. A tariff protection commenced from 1934 to save the industry from imports of cheap silk (National Commission of Agriculture, 1976). During the World War II, the Indian silk industry surged again, mainly due to demand from the allies for silk for manufacture of parachutes.

The first authentic enquiry into the conditions of Indian silk industry was undertaken in 1914-15 by H. Maxwell Lefroy and E. C. Anson (Lefroy and Anson, 1915). In a report, they observed that the industry was scattered and unorganized producers were subjected to exploitation. They suggested formation of a central organization to address the needs of the industry (Lefroy and Anson, 1915). Subsequent recommendations by a Silk Panel in 1946 led to the formation of the commodity Board, namely Central Silk Board (CSB) in 1948.

Central Silk Board (CSB) is a statutory body, under the administrative control of the Ministry of Textiles, Government of India. One of the earliest commodity boards to be constituted by the Government of India, the Board coordinates the development of sericulture and advises the Government on policies governing export and import. It has the responsibility for pre-shipment inspection of silk goods exported from the country. The Board is also responsible for organizing sericultural research, training, basic seed (egg) production and collection of statistics pertaining to sericulture and silk industry (National Commission of Agriculture, 1976;

Gopalachar, 1978).

The Central Silk Board established a number of sericulture research institutions in 1960s. With systematic efforts, it became possible in 1970s, to develop a technology suitable for tropics. New mulberry varieties coupled with agronomical practices were made available to the farmers. Packages of practice were developed for silkworm rearing, besides realising new bivoltine races. Popularisation of the bivoltine hybrids was given priority. Since seed producers started using bivoltine as a male parent for the preparation of cross breeds, the traditional poor yield crosses could be replaced to the extent of 85 %. Consequent to this, mulberry sericulture was spread to non-traditional states viz., Kerala, Maharashtra, Rajasthan and Gujarat in the 1980s. While other crops (grains) perish due to very little precipitation, mulberry survives such acute situation where ground water is also not available for raising the crops, thus providing subsistence to a large number of farmers (National Commission of Agriculture, 1976).

Currently, in India, mulberry silk is chiefly produced in 5 states viz., Karnataka, Andhra Pradesh, Tamilnadu, West Bengal and Jammu & Kashmir, contributing to about 99 % of the total mulberry silk produced. Interestingly, the states of Andhra Pradesh and Tamilnadu with almost no silk production during 1960 (Vasumathi, 2000), currently occupy the second and fourth position, respectively. West Bengal at present, contributes about 11 % of the total cocoon / silk production, while Karnataka contributes the lion's share (48 %) with Andhra Pradesh and Tamilnadu contributing 25 and 9 %, respectively. Current production statistics is furnished in Table 7.

'*Tropical Sericultural Technology*' developed during 1970's helped India to increase her silk production from 969 tons (1950's) to 13970 metric tones in 2003-04 (Central Silk Board Database, 2007). The major thrusts of '*Tropical Sericultural Technology*' were introduction of bivoltine silkworm, breeding of new silkworm breeds especially suited to our tropical climate and development of new rearing technology. But only 40 % of the potential of bivoltine silkworms could be realized at the farmer's level. The yield gap analysis showed that in comparison to the multivoltines, the bivoltine silkworms are much less

Table 7: Raw silk production (tonnes) in major sericultural states in India

State	1985		2015		Change in % contribution
	Silk production (tons)	% contribution	Silk production (tons)	% contribution	
Andhra Pradesh	1028	14.6	5086	24.8	10.2
Karnataka	4300	61.2	9823	47.9	-13.3
Tamilnadu	833	11.8	1898	9.2	-2.6
West Bengal	750	10.7	2351	11.4	0.7
Others	118	1.7	1193	5.8	4.1
Total	7029		20478		

adapted to the tropical condition (Datta and Chatterjee, 1992). A breakthrough was achieved under the *Bivoltine Sericulture Technology Development Project* (1991-99), wherein, many productive and qualitatively superior bivoltine hybrids (BV hybrids) were developed at Central Sericultural Research and Training Institute, Mysuru by utilizing Japanese commercial hybrids as breeding resource material (Datta, 2003). The concept of cross breeds (CB) is much older, which came as a means to improve local multivoltine strains by crossing with bivoltines.

Table 8: Comparison of bivoltine and cross breed cocoon production

Item	BV Hybrid		Cross breed	
	₹ / acre	%	₹ / acre	%
Leaf cost	27864.49	32.13	18825.83	27.31
Silkworm seed	1782.44	2.06	3766.69	5.46
Disinfectants and materials	7301.97	8.42	3755.73	5.45
Labour	13566.9	15.64	11975.61	17.37
Depreciation on fixed capital	32972.45	38.02	25485.78	36.97
Other costs	3232.12	3.73	5122.92	7.43
Total cost	86720.37	100	68932.56	100
Revenue	123519.92		86175.33	
Net return	36799.55		17242.77	
B:C ratio	1.42 : 1		1.25 : 1	

Source: Kumaresan, 2002

Table 8 gives comparative farm level performance of Bivoltine hybrids and Cross Breeds. It shows that the bivoltine hybrid rearing though incur higher costs of production, yield far greater profits.

Efforts to popularize bivoltine hybrids

The Central Silk Board, with the support of governments of traditionally multivoltine growing states

of Karnataka, Andhra Pradesh, Tamilnadu and West Bengal, introduced bivoltine programme during the Fifth Five-Year Plan period. The bivoltine programme, however, deviated from its targeted objectives and bivoltine cocoons were used to produce improved crossbreed layings. However, the evolution of bivoltine breeds with a silk content of over 21-22 per cent led to a significant drop in the renditta of crossbreed cocoons from 13.2 in 1980-81 to 9.5 in 1986-87 (Mahadevappa, 1987). Intensive efforts were made to popularize bivoltine sericulture through massive sericulture development programmes, such as Intensive Sericulture Development Programme (ISDP), World Bank-assisted Karnataka Sericulture Project (KSP) and National Sericulture Project (NSP).

The Central Silk Board obtained technical cooperation from Japanese Experts through Japan International Cooperation Agency (JICA) to develop bivoltine sericulture technologies suitable for Indian conditions during 1989, as the Japanese have proven technologies for the production of quality silk. Bivoltine Sericulture Technology Development (BSTD) Project was formulated for five years from 1989, in which, new bivoltine silkworm hybrids and technology packages for rearing bivoltine hybrids were evolved. The technologies evolved under BSTD Project were demonstrated and test verified with the farmers at different locations during the second phase of the project, which is known as Promotion of Popularising the Practical Bivoltine Sericulture Technology (PPPBST) Project.

In spite of these efforts, the performance of bivoltine sericulture in India has been poor.

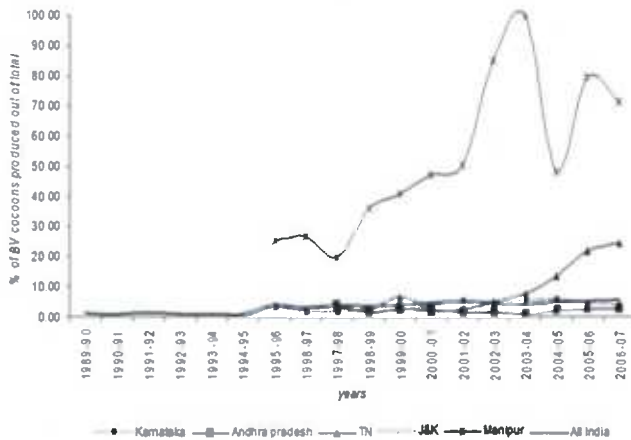


Figure 3: Percentage of bivoltine hybrid cocoons produced in 5 leading states over a period of 18 years

Figure 3 illustrates the diffusion of bivoltine hybrid technology in five states and in India, as a whole for 18 years from 1989 to 2007. It is seen that the national performance in diffusion of Bivoltine Hybrid which was below 1 % until 1995 has since risen but, during 2006-07, still remained as low as around 5 %.

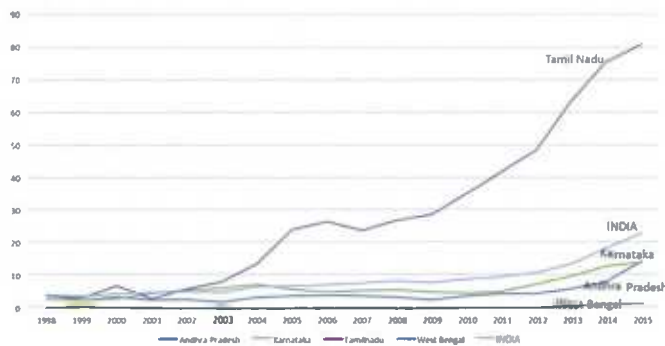


Figure 4: Diffusion of bivoltine hybrid technology in India by major silk producing states (1998 – 2015)

Note: Y axis presents percentage diffusion

Figure 4 illustrates the scenario until 2015. It can be seen that the Bivoltine Hybrid Technology has diffused further to above 20 % at national level and the state of Tamil Nadu has made tremendous progress by achieving 80 % diffusion. In spite of the long spell of dormancy of the innovation since 1990, the sector has started showing signs of receiving the beneficial impacts of the diffusion of this innovation.

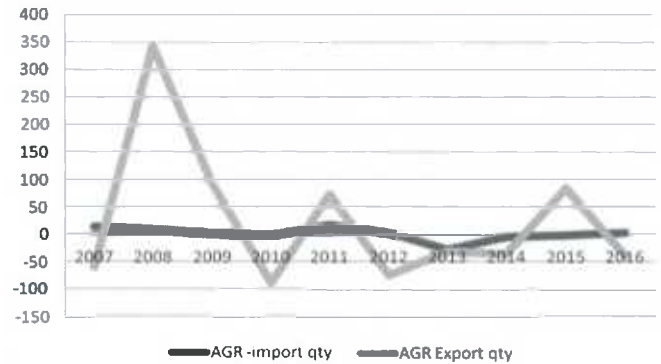


Figure 5: % AGR of import and export of raw silk (not thrown) by India (2007 – 2016)

Note: Y axis displays % AGR

Figure 5 depicts the annual growth rate of raw silk import and exports by India. The decreasing dependency of the domestic silk industry on imported silk is evident here, which could be attributed to the increased diffusion of Bivoltine Hybrid Technology recently.

Key components in the Indian Sericulture Innovation System

According to Malerba (2002), “. . . a Sectoral Innovation System includes a set of established and new products for specific uses and a set of agents supporting and carrying out technological, market, and non-market interactions for the creation, production, and sale of those products . . .”. Further, Schrepf *et al.* (2013) identifies the three important building blocks of Sectoral Innovation Systems viz., knowledge and technological domain, the actors and networks, and the institutions.

The Indian sericulture sector qualifies the criteria specified in this definition. It has a profound and well documented knowledge system with two thousand years of history. The sericulture knowledge base has been enriched periodically by new, time tested technologies. It has an array of technologies ranging from those related to land preparation, mulberry plantation & agronomy, silkworm raising, silkworm seed production, crop protection, reeling technology and yarn processing technology: with divisions and sub-divisions of activities. Sericulture sector has been highly technology intensive with a perpetual focus on product quality improvement through plant and breed improvement. The production

inputs of sericulture range from agricultural inputs for mulberry production and silkworm rearing, chemical inputs for crop protection of both the plant and animal, silkworm seed, numerous special equipment for silkworm rearing, silk reeling machinery *etc.* The demand for silk is well defined as, existing (and perpetually growing), emergent, and potential. The system comprises of both organizational and individual agents. There are firms (sericulture farmers, reelers, silkworm seed producers, chawki suppliers, inputs suppliers *etc.*) and non-firm organizations (Central and state government research stations, universities, banks and other financial agencies, central and state government departments and extension services, powerful farmer's and reeler's organizations and technical associations). There are institutions characteristic of the sericulture sector (specific laws and acts laid down for controlled extraction of resources, such as precious genetic material, regulated markets, social conventions governing extraction of natural resources, work division and crop rotation), contracts, traditions *etc.* Above all, the system is highly dynamic and sensitive to import and export of material from outside the system boundaries and finally characterised by an extremely complex network of players. All these match well with the definition provided by Malerba (2002). Hence, it may be argued that a well-defined Sericulture Innovation System is in place.

Key agents

The key agents of the Sericulture Innovation System are: Sericulture farmers (commercial sericulturists and seed cocoon producers), Input suppliers such as: Licenced Seed Producers (graineures), Chawki (young age worm) rearers, Fertilizers and chemicals suppliers, Suppliers of implements, silkworm rearing equipment *etc.*, State Government Departments and their sericulture extension system, Central Silk Board and its R&D and extension centres, State and Central Government owned Research and Training stations, Private manufacturers of inputs and their R&D wings, Universities offering sericulture courses and conducting sericulture research, Sericulture farmers' associations, Government silk markets, Silk reelers, manufacturers of reeling machinery, Suppliers of inputs to reeling industry, Government silk yarn markets, Silk testing and conditioning centres, Researchers,

Extension officers, Administrative set up and bureaucracy, Political leaders, agricultural labourers, the banking sector, private money lenders *etc.* An exhaustive listing of all the players and defining their roles is outside the scope of this paper. Therefore, a few important and the most visible ones are discussed briefly.

Commercial sericulturists: The commercial sericulture farmers are the backbone of the sector. The farmers, although belonging to various socio economic classes, together form a strong pressure group. The collective bargaining power of sericulture farmers has historically led to many policy initiatives protecting their interests. A recent example being Farmers' organizations campaign and several state-and national-level struggles seeking the restoration of import duty on silk at 30 per cent.

Seed cocoon producers: These are farmers who are selected and registered as rearers of parental races for the purpose of hybridisation. These are privileged farmers as they receive close technical guidance aimed at production of cocoons with specific quality standard and receive high prices for the seed cocoons they produce.

Registered seed producers: Private producers of commercial silkworm eggs need to obtain licenses, as a regulatory measure for the government to ensure quality and 'disease freeness' of silkworm eggs.

Chawki rearers: Chawki rearing is the massive and centralised rearing of young silkworms up to ten days, so that farmers could buy them instead of directly buying silkworm eggs. The technology was standardised as early as 1980s but, took a long time to diffuse.

Central Silk Board (CSB): Central Silk Board (CSB) is a statutory body established in 1948 by an Act of Parliament. It is working under the administrative control of Ministry of Textiles, Government of India. CSB possess an extensive research system (Figure 6) comprising of three Central Sericultural Research and Training Institutes (CSRTI), Central Tasar Research and Training Institute, Central Sericultural Germplasm Resources Centre, Seribiotech Research Laboratory, Central Muga Eri Research and Training Institute, 11 Regional Research Stations, 44 Research Extension Centres for Mulberry, Sub-RECs for

Mulberry and 1 Sub-REC for Muga, 1 Field Lab at Titabar, 3 P4 Stations at Kargikota, Chakradharpur & Saraiyahat for Tasar and 3 P4 Farms at Kalimpong, Hassan & Manasbal for Mulberry. The Central Silk Technological Research Institute (CSTRI), at Bengaluru provides research and extension support in the post-cocoon areas with the support of Zonal Offices, Regional Silk Technological Research Stations (RSTRS), Silk Conditioning and Testing Houses, Textile Testing Laboratories, Raw Silk Testing Centres and Demonstration cum Technical Service Centres (DCTSCs) and Cocoon Testing Centres. National Silkworm Seed Organization (NSSO) is the organization responsible for multiplication of basic seed evolved by the Research centres and Universities.

State Departments of sericulture: All the major silk producing states have their own sericulture departments looking after sericulture extension activities and R&D through State Government Research and Training Institutes.

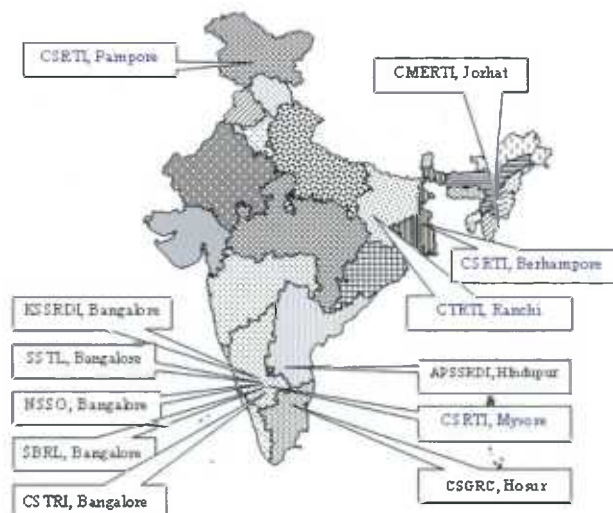


Figure 6: The network of central and state sericulture research institutes in India

Adopted from Muniraju (2008)

Government regulated cocoon markets: There is a well-established Cocoon Marketing System in India. By law, cocoon transaction is facilitated by Government owned markets where transaction is facilitated through auction. The Karnataka state has 34 commercial Cocoon Markets in different cocoon production areas, mostly located in the major reeling centers.

Government silk markets: To support raw silk marketing, silk testing centers are operated by the Central Silk Board. Recently, the state government has come up with a proposal to widen the silk testing centre network by establishing silk testing centers, in collaboration with the CSB, in all the major reeling clusters. The state has organized silk exchanges in major reeling centers to facilitate raw silk sales. It was mandatory for the reelers to transact the silk only in the silk exchanges under the provisions of the Karnataka Silkworm Seed, Cocoon and Silk Yarn (Regulation of production, Supply, Distribution and Sale) Act, 1959. This was backed by Silk enforcement.

Reeler cooperatives: Efforts to organize the reeler cooperatives to support reeling and address raw silk marketing issues have met with limited success. Almost 95 % of the reeler cooperative societies have become defunct, so also is the Karnataka Silk Marketing Federation.

Apart from these, many more players such as Manufacturers of reeling machinery, Suppliers of inputs to reeling industry, Government Silk yarn markets, Silk testing and conditioning centres, Researchers, Extension officers, Administrative set up and bureaucracy, Political leaders, Agricultural labourers, share croppers, The banking sector, private money lenders *etc.* play important roles in the sector.

Weakness of the Indian Sericulture Innovation System

The discussion hitherto were towards an argument in favour of the existence of a Sectoral Innovation System in Indian Sericulture. It appears that the system has all elements necessary for a functional and sustainable Sectoral Innovation System. However, the system is not without weaknesses. An important weakness of the sector is poor participation of the private sector in Research and Development activities. Figure 7 illustrates the per cent contribution of various research organizations in completed sericulture scientific projects for sixty two years for the period from 1945 to 2006. While the central government institutes dominate with nearly 75 % share of projects, the representation of private firms (categorised as other institutes) is just 0.1 %. Even the role of Universities (14 %) and State Institutes (7.8 %) are limited.

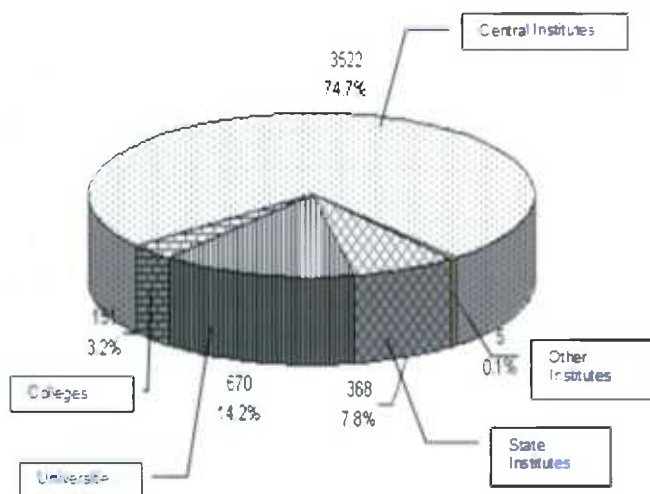


Figure 7: Organizational share of completed sericulture projects for 62 years (1945-2006)

Source: Muniraju (2008)

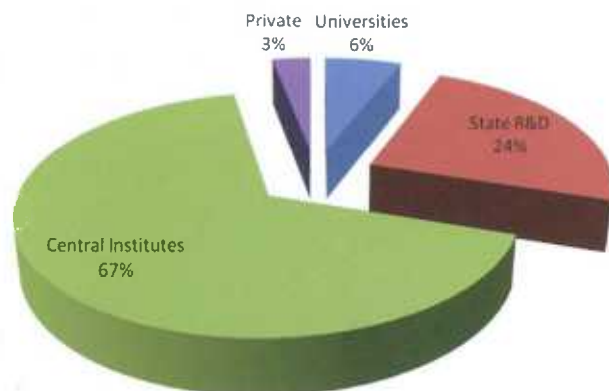


Figure 8: Sericultural technologies: share of various categories of institutes (1945 to 2006)

Source: Muniraju (2008)

Figure 8 illustrates share of release of various sericultural technologies by research institutes. While Central government owned institutes account for 67 %, private participation is only 3 %. State R&D institutes contribute to 24 % and Universities, 6 %. It is important that the Government device strategies to vitalise the private sector and encourage them to enter R&D.

Yet another weakness is with respect to the over emphasis placed upon the agricultural aspect and negligence of the industrial aspect of silk reeling and processing. In Indian sericulture, the farming community

forms a major pressure group capable of influencing policy decisions for advantage. While this is reasonable, the processing industry receives scanty attention with respect to creation of knowledge, technological innovations and diffusion. The Indian silk reeling sector is marked by low levels of diffusion of technologies which badly reflect upon the poor quality of silk being produced.

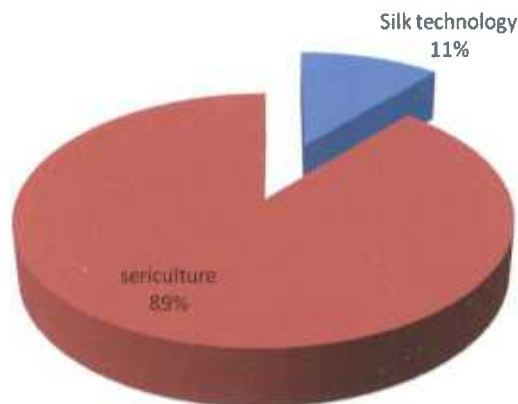


Figure 9: Number of experts in sericulture: discipline-wise (1945 to 2006)

Source: Muniraju (2008)

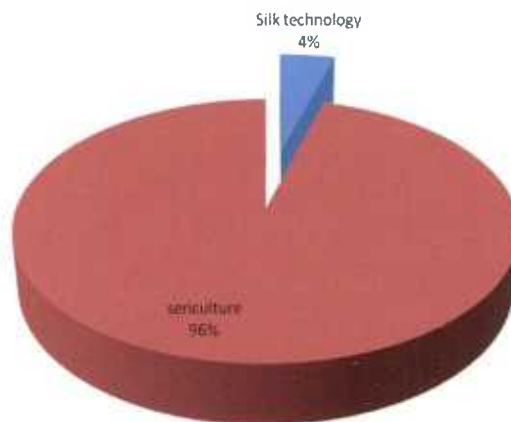


Figure 10: Publications in sericulture: discipline-wise (1945 to 2006)

Source: Muniraju (2008)

Figures 9 and 10 present the number of technical experts and number of publications, respectively in sericulture sector for 62 years from 1945 to 2006. While the lion's share of experts and publications were in the agricultural sector, the silk technological sector received scanty attention. The above discussion points to important

shortcomings of the Sectoral innovation System of sericulture in India. There is an urgent need to adopt strategies for rectifying these.

Summary and conclusion

This paper has attempted to establish through facts and figures that Indian sericulture sector has all the necessary ingredients as well as features and is characterised by complex networks and linkages, by way of economic, social, political, organizational and institutional factors influencing the development, diffusion and use of innovations; hence qualify as a Sectoral Innovation System in its own right. However, its progress and development are limited by its inherent weaknesses. This paper identifies two important weaknesses of the system 1. Poor participation of the private sector in R&D and 2. Over emphasis of the agricultural aspect of sericulture. The bulk of innovation efforts in the Indian sericulture sector over the past three decades have been focussed on evolving highly productive silkworm hybrids capable of producing high quality silk. In this process, traditional local varieties of the mulberry plant and silkworms were replaced by imported / evolved varieties. Matching modernisation efforts were not made in the silk reeling sector. This coupled with the sub-optimal technology absorptive capacity of the reeling sector rendered the attempts less effective. India imported and directly applied policies and best practices in sericulture from industrialised nations as a technological catch-up strategy. However, there are particularities in developing countries, which cannot be neglected when studying innovation dynamics in a systemic approach. At times, when this fact is neglected, the innovative attempt could lead to systems' disruption. Indian Sericulture Innovation System needs to replace the strategy of direct technology transfer from abroad and undertake an introspective innovation attempt. It is recommended that by bringing in more private participation, by increasing technology absorptive capacity of the silk reeling industry and by incentivising technology adoption in the silk reeling sector, the Indian Sericulture Innovation Sector can benefit.

REFERENCES

- Charsley S. R. (1982) *Culture and sericulture: Social anthropology and development in a South Indian livestock industry*, Academic Press, London.
- Central Silk Board (1980) Annual reports of central Silk Board. Central Silk Board URL : <http://www.csb.gov.in/publications/annual-report/>
- Central Silk Board database (2007) Sericulture data obtained by the author from Central Silk Board under Right to Information Act, India, 2005.
- Currie R. (1997) Global Silk Industry: Today and Tomorrow. *Indian Silk*, 35 (12): 5-7.
- Datta R. K. (2003) Silkworm Breeding in India and Its Future Needs. In: *Souvenir, National Conference on Tropical Sericulture for Global Competitiveness*, National Academy of Sericultural Sciences, India.
- Datta R. K. and Chatterjee S. N. (1992) Scope of biotechnology in improving productivity of Indian silk. *Perspectives in Cytology & Genetics*, 7: 241-256.
- Edquist C. (2005) *Systems of Innovation Technologies, Institutions and Organizations, Science, Technology and the International Political Economy*, Reprinted in 2005 by Routledge, First published in 1997 by Pinter A Cassell imprint, University of Ottawa.
- Ganga G. (2003) *Comprehensive Sericulture*. Oxford and IBH Publishers, New Delhi, Vol. 2.
- Gopal L. (1961) Textiles in Ancient India. *Journal of the Economic and Social History of the Orient*, 4 (1): 53-69.
- Gopalachar A. R. S. (1978) *3 Decades of Sericultural Progress (Sericulture Industry in India Potential and Prospects)*, Central silk Board, India.
- Hanumappa H. G. and Erappa S. (1986) Economic issues in

- sericulture: Study of Karnataka. *Economic and Political Weekly*, **20** (31): 1322-1324.
- Hanumappa H. G. and Erappa S. (1988) Sericulture in Princely Mysore (1914-1945) a Survey. *Seminar on South Indian Economy*, C. 1914 - C. 1945. CDS, Trivandrum, April 25-28, 1988.
- Holland C., Terry A., Porter D. and Vollrath F. (2007) Comparing the rheology of native spider and silkworm dope. *Nature Materials*, **5** (11): 870-874.
- ITC Silk Review (2001) *A survey of international trends in production and trade*, 6th Ed., Geneva (Switzerland) ITC.
- Jayaram H., Mallikarjuna V., Lakshmanan S., Ganapathi Rao R. and Geetha Devi R. G. (1998) Labour employment under different mulberry farm holdings - A comparative study. *Indian J. Seric.*, **37** (1): 52-56.
- Kumaresan P. (2002) Quality Silk Production: Some Economic Issues. *Economic and Political weekly*, September 28, pp. 4019-4022.
- Lakshmanan S., Mallikarjuna B., Ganapathi Rao R., Jayaram H. and Geetha Devi R. G. (1999) An empirical investigation on labour productivity in mulberry sericulture. *Indian J. Seric.*, **38** (1): 48-52.
- Lefroy M. H. and Ansorge E. C. (1915) *Report on an Inquiry into the Silk Industry in India*, Vol. I, Calcutta, India.
- Lundvall B. Å. (1992) *National Innovation Systems: Towards a Theory of Innovation and Interactive Learning*, Pinter, London.
- Mahadevappa (1987) Development of Silkworm Races - Problems and Prospects. *Proceedings of the Seminar on Sericulture*, Administrative Training Institute, Mysore, September 28, 1987.
- Malerba F. (2002) Sectoral systems of innovation and production. *Research Policy*, **31**: 247-264.
- Mattigatti R., Srinivasa G., Iyengar M. N. S., Datta R. K. and Geetha Devi R. G. (2000) Price spread in silk industry- an economic analysis. *Indian J. Seric.*, **39** (2): 163-164.
- Ministry of Textiles, GOI (2007) *Annual Report of Ministry of Textiles*, Government of India.
- Muniraju E. (2008) *Directory of Completed Sericulture Projects and Sericulture Experts of India*, Karnataka State Sericulture Research and Development Institute, Thalaghattapura, Bangalore, India.
- Nanavathy M. (1990) *Silk, Production, Processing and Marketing*, Wiley Eastern Ltd., New Delhi, 1990, ix, p. 176.
- National Commission of Agriculture (1976) *Report of the National Commission on Agriculture, sericulture and apiculture*, Government of India, Ministry of Agriculture and Irrigation, New Delhi, pp. 447-480.
- Ray Indrajit (2005) The Silk Industry In Bengal During Colonial Rule: The De-Industrialisation. Thesis Revisited. *Indian Economic Social History Review*, **42**: 339.
- Saraswathi J. M. and Sumangala P. R. (2001) Participation of farm women in sericulture enterprise. *Indian J. Seric.*, **40** (1): 86-91.
- Schrepf B., Kaplan D. and Schroeder D. (2013) National, Regional, and Sectoral Systems of Innovation - An overview, *Report for FP7 Project "Progress"*, progressproject.eu
- Sinha S. (1989) Development Impact of Silk Production, A Wealth of Opportunities. *Economic and Political Weekly*, January 21, pp. 157-163.
- Thomas J., Arun Kumar K. S., Lalith A. and Reddy S. (2005 a) Requirement of Quality Silk Yarn of the Power Loom Sector-A Quantitative Analysis. In: *Reading in Sericulture Economics Marketing and Management*, K. S. Arun Kumar, R. K. Datta and G. Srinivasa (Eds.), Santhosh Creations, Bangalore.

- Thomas J., Arun Kumar, K. S., Reddy and Lalith A. (2005 b) Quantification of Relationship Between Silk Cocoon and Silk Yarn Prices- an Application of ARIMA Model. In: *Reading in Sericulture Economics Marketing and Management*, K. S. Arun Kumar, R. K. Datta and G. Srinivasa (Eds.), Santhosh Creations, Bangalore.
- Tikku M. K. (1999) Tangled Threads: Silk Growers and Imports. *Economic and Political Weekly*, March 6-13, p. 578.
- UN COMTRADE (2007) United Nations International Trade statistics. URL: <https://comtrade.un.org/>
- United Nations (1994) "*Silks In Asia*," Economic and Social Commission for Asia and the Pacific (ESCAP), Bangkok, Thailand.
- Vasumathi (2000) An Analytical Study of the Silk Reeling Operations in Karnataka. *Ph. D. Thesis*, Department of Management Studies, Indian Institute of Science, Bangalore.
- Vollrath F. and Porter D. (2009) Silks as ancient models for modern polymers. *Polymer no.*, **50 (24)**: 5623-5632. doi: 10.1016/j.polymer.2009.09.068 Vollrath *et al.*

Republic of Uzbekistan enrolled as the 20th Member Country of ISC

The Republic of Uzbekistan has enrolled as the 20th member Country of International Sericultural Commission with effect from 22nd September, 2017. Uzbekistan is the third largest silk producer of the world. The ISC HQs has initiated necessary action for notifying the Membership among its Member Countries.



Schedule and host city finalized for ISC Congress-2019

The Japanese Government has decided to hold the 25th ISC Congress at Tsukuba, which is about 70 km (one hour drive) from Tokyo city centre. Tsukuba is the major centre of sericulture activities in Japan. The schedule of the Congress finalized in consultation with all Member Countries is 18th to 22nd November, 2019.

SAARC Agriculture Centre (SAC) delegation visits ISC

A two member delegation from SAARC Agriculture Centre (SAC) Dhaka, Bangladesh comprising of Dr. S.M. Bokthiar, Director and Dr. Tayan Gurung, Senior Programme Specialist visited the office of the International Sericultural Commission, Bangalore during 7th to 8th August, 2017. During the visit, detailed



deliberations were undertaken with the officials and consultants of ISC for the preparation of a Multilateral Sericulture Developmental Project involving Bangladesh, Bhutan and Nepal. The delegation visited the Basic Seed Farm, Hasan on 8th August 2017 to study the silkworm seed production network developed in India.

Visit of Uzbekistan Delegation to ISC

A 5 member Uzbek delegation led by Mr. Sh. Achilov, Deputy Chairman, Uzbekipaksanoat (an association of Uzbek silk industry stakeholders), Uzbekistan visited the sericulture areas of Karnataka India during 24th & 25th July, 2017 to study the successful model of commercial sericulture practice developed in India. The delegation has also visited the office of the International Sericultural Commission, Bengaluru and discussed the various procedures involved in the enrollment of Uzbekistan as a Member Country of ISC.



8th International Conference on Wild Silkmoths, 22nd to 24th January, 2018

International Society for Wild Silkmoths and Central Silk Board, Ministry of Textiles have decided to organize the 8th International Conference on Wild Silkmoths at Guwahati, India during 22nd to 24th January, 2018. The following are the scientific sections of the Conference:

Section	Theme
Tasar silkworm	All aspects and areas concerning Tasar, including food plants, by- product and other utilities of tasar silkworm and silk.
Eri and Muga silkworm	All aspects and areas concerning Eri and Muga, including food plants, by-product and other utilities of Eri and Muga silkworm and silk.
Other wild silkworms & domesticated silkworms	All aspects and areas concerning other wild silkworms and silk, its use and application in various areas and all domesticated silkworms and its related activities.
Post-cocoon Technology	Reeling, cocoon preservation, silk quality, gradation, weaving, product diversification, marketing, export, etc.
Gene Technology	Silkworm as a biological model, genetics, physiology, biochemistry, genetic-engineering, molecular biology, developmental biology and genomics.

Interested persons may send their research paper abstracts to [email: wildsilkmothconference2018@gmail.com](mailto:wildsilkmothconference2018@gmail.com)

The following are the important dates:

- | | | | |
|----|--|---|--------------------------------|
| 1) | Contributions (submission of Abstract Research Papers) | - | 15 th November 2017 |
| 2) | Completed Registration Forms with Registration Fee | - | 15 th November 2017 |
| 3) | Late Registration with late registration fee | - | 15 th December 2017 |

For detailed information on the Conference, please contact:

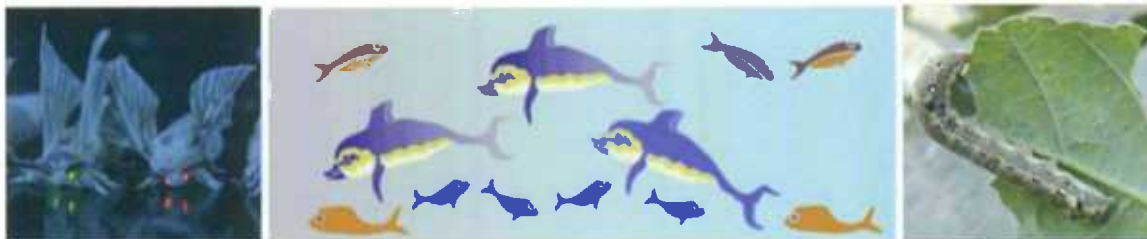
Coordinator- ICWS- 2018,

Central Silk Board- Ministry of Textiles, Govt. of India,

Silk Board Complex, BTM Layout, Madiwala, Bengaluru-560 068, INDIA

e-mail : wildsilkmothconference2018@gmail.com

Tel: 00 91 80 26680162; Fax: 00 91 80 26681663. Mobile: 00 91 9036536134



THE 10th INTERNATIONAL WORKSHOP ON MOLECULAR BIOLOGY AND GENETICS OF THE LEPIDOPTERA

Dear Colleagues,

With this circular we wish to inform you that the web site for the 10th International Workshop on the "Molecular Biology and Genetics of the Lepidoptera" on August 19-25, 2018, is now up and running at <https://web.uri.edu/lepidoptera>. In it, you will find all the information you need to have about your travel to Greece and the venue at the Orthodox Academy of Crete (OAC), the required registration and abstract submission forms and links to other useful information.

We plan to organize scientific sessions under the following broad areas:

- Genome Sequencing and Mapping
- Comparative Genomics, Evolution and Phylogeny
- Post-genomic and Functional Genomic Tools and Applications
- Development and Differentiation
- Endocrinology, Physiology and Biochemistry
- Receptors and Ligands
- Immunity
- Neurobiology
- Transgenesis and Paratransgenesis
- Viruses, Other Pathogens and Pest Control
- Chemical Ecology

Although by necessity we are keeping the titles of the sessions short, we are inviting your abstracts for presentations for a range of topics to be grouped under these session headings. Examples of such topics include the following:

- Genomics and Genetics
 - Genome and chromosome structure and evolution
 - Population genetics and systematics
 - Genomics-based tools for trait identification and population diagnostics
 - Positional cloning
 - Molecular mimicry and adaptation
 - Mono-, oligo- and polyphagy of herbivores
 - Insecticides, resistance and detoxification
 - Species adaptation, invasiveness and control

- Development and Physiology
 - Cell differentiation
 - Endocrinology and hormonal control of development and metamorphosis
 - Wing patterning
 - Sex determination
 - Reproduction
 - Immunity
 - Neurobiology and behaviour
- Functional genomics and post-genomic tools and applications
 - Tissue and cell-specific transcriptomics and proteomics
 - Basic regulatory mechanisms
 - Epigenetic control of gene expression
 - RNA-guided genome editing
 - RNA-mediated control of gene expression
 - RNA delivery tools (nanoparticles, RNPs, aerosols)
 - Recombinant protein expression and post-translational processing systems and applications
- New molecular tools (optogenetics, high throughput (nanopore) sequencing and DNA methylation, fluorescence tags for imaging and macromolecular interactions, genetic and optogenetic cell ablation)
- parasite and microbiotic control of insect physiology (Wolbachia, Polydnaviruses, gut flora)
- Pathology and pest control (pathogens, parasites and viruses)
- Genetic manipulation for plant eaters - understanding fundamental mechanisms
- Chemical ecology (olfaction, gustation, host plant recognition, other forms of chemical communication, interference with chemosensation)

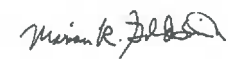
Finally, we also wish to organize a number of special afternoon sessions (to last from 1-2 hours each) with dedicated abstracts on the following topics:

- Progress on RNA-mediated control of gene expression (coordinator TBA)
- Genetic manipulation tools: progress, applications, problems and prospects (TALEN, CRISPR, off-target effects, improvements, somatic mutagenesis, etc.) (coordinator TBA)
- Environmental perspectives for genome editing and gene drive for pest control (coordinator TBA)
- Basic and applied research on genetically modified silkworms (*Bombyx mori* and other species) (coordinated by Toru Shimada)
- Networking and funding initiatives for emerging agricultural and forest pests and systems for pest control (coordinated by Emmanuelle d'Alençon, Astrid Groot, Michel Cousson and Paul Shirk)

Please note that in accord with the informal and collaborative nature of the Workshop we welcome additional timely suggestions for the program!

The detailed Workshop Program will become available after the Registration period.

Looking forward to a successful meeting in 2018,

 — 

Kostas Iatrou and Marian Goldsmith

INTERNATIONAL SERICULTURAL COMMISSION

An intergovernmental organization instituted in 1960 and registered with United Nations

APPLICATION FOR ASSOCIATE MEMBERSHIP

Name (Personal Associate Membership for Individuals)	
Person In-Charge (Collective Associate Membership for Institutions/Libraries etc)	
Address	
Country	
Tel	Fax
E-mail	
Privileges Personal Associate Member : Receives Sericologia Collective Associate Member : Receives Sericologia Receives Scientific and Technical information as and when sought Subsidized Registration fee for participation in ISC Congress	

I wish to become a Personal Associate/Collective Associate Member of ISC

New Application ☐

Renewal ☐

Rates : Personal Associate Members : US\$ 175 per year
 Collective Associate Members : US\$ 470 per year

Applicant's signature : _____

Date : _____

Membership fees can be remitted either by cheque or by Bank Transfer

Beneficiary : International Sericultural Commission

Account No. **3188283389**

Bank & Branch: Central Bank of India, CSB Branch, Bangalore, India

Bank Code: IFSC - CBIN0283975, SWIFT - CBININ8BMRB

For overseas transfer

(THROUGH SCBL US 33, STANDARD CHARTERED BANK, NEW YORK, USA TO A/C NO.3582-050570-001
 OF CENTRAL BANK OF INDIA, OVERSEAS BRANCH, MUMBAI, SWIFT : CBININBBOSB)

This form along with your cheque or the references of your Bank Transfer must be mailed to the office of the International Sericultural Commission whose address is given below.

Ground Floor, Central Silk Board Complex, B.T.M. Layout, Madiwala, Bengaluru – 560 068. INDIA Tel: +91 80 26680162,
 26282186 | Fax: +91 80 26681663 Email: iscbangalore@inserco.in

www.inserco.org

INFORMATION TO CONTRIBUTORS

SERICOLOGIA is a peer reviewed quarterly scientific journal dedicated to the science of sericulture, published by the International Sericultural Commission. Papers submitted to SERICOLOGIA should carry original contributions of scientific research (basic or applied) or reviews on any aspect of sericulture.

Submission of a paper to SERICOLOGIA implies that it has not previously been published, that it is not under consideration for publication elsewhere and that, if accepted in SERICOLOGIA, it will not be published elsewhere in the same form without the written consent of the Chief Editor.

Manuscripts and illustrations must be prepared in British English on a standard A4 size paper setting. It should be typed in 12 point Times New Roman or Arial font in double spacing and single column with well set margins on top, bottom, left and right. Page numbers should be given at the bottom centre of every page.

The typescript should contain the following features:

- **Title** of the paper should be in a 14 point font. It should be bold typed, centered and fully capitalized.
- **Authors'** Full name, address, mobile/fixed line numbers, and email/alternate email address should be in 10 point font and centered underneath the title. The first address should be of the centre at which the study was conducted. In the case of multiple ownership, the authors may indicate the correspondence address.
- **Abstract** must be the gist of the paper which should explain the background, aims, methods, results & conclusion in a single paragraph not exceeding 200 words.
- **Key words** should follow the abstract, subject to a maximum of five, arranged in alphabetic order.
- **Main text** should have serially arranged sections such as Introduction, Materials and Methods, Results and Discussion for an effective and systematic presentation of the contents. Acknowledgements may be included if relevant. Only standard abbreviations should be used. Whenever specialized abbreviations are used, it should firstly be given in full with the abbreviation indicated in parenthesis. Scientific names should be given for all species used in the investigation.
- **Tables** should be simple, centered, separately numbered & self explained, and titles must be above the tables. Sources of data if any, should be mentioned below the table. All tables and figures should be referred to in the text.
- **Illustrations:** All necessary illustrations should accompany the manuscript but should not be inserted in the text. Photographs, graphs and diagrams should be numbered consecutively in Arabic numerals in the order in which they are referred to in the

text. Digital/electronic version of the graphs and photographs should be submitted as separate files. Graphs should be in excel format. Photographs should be of high resolution in jpeg format. Legends to figures should be typed on a separate sheet and not at the back of the original.

- **References:** In the text, the referencing pattern should be as Dupont (1964)/ (Dupont, 1964)/ Dupont and Durand (1964) or (Dupont and Durand, 1964). When a citation includes more than two authors, e.g., Dupont, Durand and Martin, the paper should be cited in the text as Dupont *et al.* (1964) or (Dupont *et al.*, 1964). If papers by the same author(s) in the same year are cited they should be distinguished by the letters a, b, etc. following year of publication.
- At the end of the text, the list of references prepared following Harvard Style of Referencing should be alphabetically arranged. References not cited in the text should not be included. References should be cited in the following pattern:

Dollo M., Samal P. K., Sundriyal R. C. and Kumar K. (2009) Environmentally sustainable traditional natural resource management and conservation in Ziro Valley, Arunachal Himalaya, India. *J. Am. Sci.*, 5: 41–52.

Ceci S. J., Williams W. M. and Barnett S. M. (2009) Women's underrepresentation in science: Sociocultural and biological considerations *Psychol. Bull.*, 135: 218–261.

Russell E. W. (1961) Soil conditions and plant growth. (9th edition), John Wiley and Sons, New York.

Power J. F. (1990) Fertility management and nutrient cycling. In: *Dry land agriculture. strategies for sustainability. Advances in soil science*, Singh R. P., Parr J. F. and Stewart B. A. (Eds.), Springer Verlag, New York.

Fagoonjee I., Schmuterer R. and Ascher K. R. S. (1987) Use of neem in vegetable crop protection in Mauritius. *Proceedings of the 3rd International Neem Conference*, Nairobi, Kenya, 10-15 July, 1986, pp. 419-429.

Online Resources: Always indicate the date that the source was accessed, as online resources are frequently updated or removed.

Printing

Print-ready proof is sent electronically. Corrections to proof should be restricted to printer's errors only. The author should take a printout of the proof, mark the corrections legibly in red ink and send by speed post within 3 days of receipt. Alternatively, scanned images of the corrected proof can also be sent via email. If corrections are minimum, it may be indicated page-wise, column-wise and line-wise and intimated to the Chief Editor via email. If there are no corrections, the same may also be intimated to the Chief Editor.

Reprints

Sericologia sends the reprints electronically in the form of PDF to the principal or corresponding author.

The articles should be submitted in duplicate along with the soft copy to the Chief Editor, Sericologia, International Sericultural Commission, I Floor, Central Silk Board Complex, BTM Layout, Madiwala, Bengaluru - 560068, INDIA. Tel: +91 80 26232191, Fax: +91 80 26681663, E-mail: sericologia@gmail.com, www.inserco.org



Sericologia

International Sericultural Commission

CSB Complex

**BTM Layout, Madiwala, Bengaluru - 560068
Karnataka, India**

Phone: +91-80-26282191

+91-80-26680152

+91-80-26681653

Fax: +91-80-26681663

Email: sericologia@gmail.com

Website: www.inserco.org