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PREPARATION AND CHARACTERIZATION OF METHYLATED ANTHOCYANINS FROM MULBERRY FRUIT WITH IODOMETHANE AS A DONOR

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ABSTRACT

Anthocyanins are common active substances in plants that are often used as colorants and nutraceuticals in functional foods and beverages. This paper describes the process of methylation of anthocyanins from mulberry fruit with iodomethane. The highest conversion rate (84.6 %) was obtained by using iodomethane as the methyl donor. A Box–Behnken design of response surface methodology was conducted to analyze the response of the anthocyanins to donor ratio, methylation time, and methylation temperature, under pH 1.0, on the anthocyanins extracted from the mulberry fruit. We found that, using iodomethane, the optimum methylation conditions were obtained at an anthocyanin to iodomethane mass ratio of 1:1.95, methylation time of 2.92 h, and methylation temperature of 38.69°C. Structural analysis of methylated anthocyanins revealed that iodomethane introduces a C-H bond and methyl group. The thermostability and light resistivity of mulberry anthocyanins was significantly enhanced after methylation, and the antioxidant activity of the methylated anthocyanins was improved. This study provides a novel method for stabilizing anthocyanins in mulberry fruit, thereby enabling their use as a commercial food ingredient.

Key words: Anthocyanins, iodomethane, methylation, mulberry fruit, stability.

INTRODUCTION

Anthocyanins are a chemically diverse class of secondary metabolites that provide the color in most flowers and fruits. They are essential antioxidants, and therefore, commonly find a place in human foods. They are thought to offer a wide variety of health benefits, such as providing antioxidant (Ghiselli *et al.*, 1998) and antitumor properties (Kamei *et al.*, 1998), protecting against liver damage (Yoshimoto *et al.*, 1999), preventing oxygen free radical generation, reducing lipid peroxidation (Jankowski *et al.*, 2000), inhibiting NO production (Wang and Mazza, 2002), and stabilizing DNA triple-helical complexes (Mas *et al.*, 2000).

However, anthocyanins are extremely unstable and degrade during processing (Liu *et al.*, 2018). They have very high thermal sensitivity. They become highly unstable and lose their color when the temperature reaches 60 °C (Fracassetti *et al.*, 2013). Their color is relatively unaffected by changes in pH (Awika, 2008). Abdel-Aal and Hucl (2003) reported that anthocyanins in blue wheat were thermally most stable at pH 1.0. Their rate of degradation was slightly lower at pH 3.0 than at pH 5. Besides temperature and pH, their stability is affected by light (Kearsley and Rodriguez, 2007) and oxygen levels (Avizcuri *et al.*, 2016), and the content of sugar (Queiroz *et al.*, 2009), hydrogen peroxide (Özkan *et al.*, 2002), and metallic ions (Ratanapoompinyo *et al.*, 2017).

Anthocyanins consist of three aromatic rings that can be substituted with hydroxyl, sugar, acyl, and methyl groups in a variety of patterns, depending on the plant species. To improve anthocyanin stability, several modification methods have been studied, including glycosyl acylation (Zhao *et al.*, 2017), glycosylation (Yawadio and Morita, 2007), microencapsulation (Khazaei *et al.*, 2014), metal chelation (Buchweitz *et al.*, 2013), and the use of liposomes (Feng *et al.*, 2015). However, to date, no information is available in the literature on the methylation of anthocyanins from mulberry fruit.

Anthocyanin methylation could be an option to prevent the adverse quality changes if any, occurring during storage and processing. The purpose of this study was to select a methyl donor, and to optimize the anthocyanin methylation conditions. The thermostability and light resistivity of methylated anthocyanins were investigated, and the antioxidant effects of methylated anthocyanins were evaluated.

MATERIALS AND METHODS

Materials

Mulberry fruits were harvested from the plantation of the National Mulberry Orchard (Zhenjiang, China), freeze-dried (EYELAFDU-2100, Japan) and ground to powder.

The cyanidin 3-O-glucoside standard was purchased from Putian Genesis Biotechnology Co., Ltd (Beijing, China). Iodomethane, dimethyl carbonate, dimethyl phosphate, and acetonitrile were purchased from Sangon Biotech Co., Ltd (Shanghai, China). All other reagents used were of analytical grade.

Anthocyanin extraction and analysis

The extraction and determination of anthocyanins from mulberry fruit were performed using our previously described method (Ajaykrishna *et al.*, 2018). Anthocyanins were extracted using acidified methanol (methanol and 1.0 N HCl, 85:15, v/v), assisted by ultrasound. The partially purified extracts were

evaporated to dryness at 50 °C, using a rotating evaporator, and were re-dissolved in acidified methanol. Individual anthocyanins were separated and quantified using high-performance liquid chromatography (HPLC), equipped with a Waters 600 controller, dual-wavelength UV-visible (UV/vis) detector, and a data acquisition system (Millennium Chromatography Manager version 2.15.01). A 25 cm × 4.6 mm i.d. reversed-phase Supelcosil LC-18-DB column (Waters) was used, and the device was operated at room temperature.

Preparation for anthocyanin methylation

Methyl donors, including dimethyl carbonate, dimethyl sulfate, and iodomethane, were selected for anthocyanin methylation. First, the anthocyanin extracts were dissolved in 50 % ethanol. The reaction system was then mixed with 2 ml pyridine and anthocyanins, and with a methyl donor, under designed conditions (Table 1). Methylated anthocyanins were then evaporated at 40 °C, using a rotating evaporator to remove the pyridine and ethanol, and dried using a vacuum freeze dryer.

Table 1: Box-Behnken design levels

Factor	−1	0	1
Anthocyanin to donor ratio (mg:ml)	1:1	1:2	1:3
Time (h)	2.5	3	3.5
Temperature (°C)	30	40	50

Optimization of anthocyanin methylation conditions

The following three-step procedure was used to optimize the anthocyanin methylation indices: 1) Plackett-Burman (PB) design, to screen the 3 most influential factors that are known to affect anthocyanin methylation; 2) Single-factor assays, to determine the level of each factor that significantly improves methylated anthocyanins production; and 3) Box-Behnken design to optimize the conditions for anthocyanin methylation using response surface methodology. These virtual variables were used to estimate standard error. Based on the results obtained using the PB design, the 3 most influential factors that cover a wide range were selected to estimate variation in anthocyanin methylation (Ajaykrishna *et al.*, 2018).

Spectrophotometric measurements

The absorption spectra were recorded using a UV-visible spectrophotometer (UV-2450, SHIMADZU, Japan), scanning the visible range from 200 to 450 nm. The methylated anthocyanins were separated by their absorption peaks.

Infrared spectrometer measurements

Characterization of the structures of the methylated anthocyanins was achieved using a Fourier Transform Infrared Spectrometer, scanning the infrared absorption spectrum range from 4000 to 400 cm^{-1} .

Calculation of conversion yield

The methylated anthocyanin conversion yield was measured using HPLC, as described above. The methylated anthocyanin conversion yield was calculated as previously described (Yan *et al.*, 2016).

$$C = \frac{A_1}{A_1 + A_0} \times 100$$

Where C is the methylated anthocyanin conversion yield (%); A_0 is the nonmethylated anthocyanin peak area; and A_1 is the methylated anthocyanin peak area.

Stability tests

Thermostability test

The stability of methylated anthocyanins was evaluated using 0.8 mg/ml of purified anthocyanin dissolved in 0.01% aqueous HCl solution (pH 1.0). The effect of temperature on anthocyanin content was investigated in a water bath at 70, 80, and 90 °C for 10 h under dark condition. Anthocyanin content was determined using a UV-2450 UV/vis spectrophotometer, by measuring absorbance at 520 nm. Anthocyanin retention rate (R , %) was calculated as follows (Liu *et al.*, 2018):

$$R = (A_t/A_0) \times 100$$

Where A_t represents absorbance at the start of heating ($t=0$) and A_0 represents absorbance at time t .

Light resistivity test

To evaluate the light stability of the methylated anthocyanins, the anthocyanin solution (0.6 ml) was placed in open glass cuvettes under 450 W light at 20 °C. After exposure to the light for 2, 4, 6, 8, 10, and 12 d, the anthocyanin retention rate was determined as described above.

Antioxidant activities

Antioxidant activity was represented by the DPPH·-free radical scavenging capacity, total reducing power, and ferrous ion (Fe^{2+}) chelating capacity, assayed according to Wu *et al.* (2012).

Statistical analysis

The same experiment was repeated three times, and the results are expressed as mean values. Design-Expert v. 8.6 and GraphPad Prism v. 7 were used for experimental design and data analysis.

RESULTS

Structural characterization of anthocyanin methylation

The identification of methylated anthocyanins was carried out by HPLC. The methylated and nonmethylated anthocyanins were detected at 2.698 min and 11.115 min, respectively (Figure 1). The conversion yield of methylated anthocyanin was calculated according to the peak area.

To examine changes in the molecular structure, UV-visible spectrophotometry was used to scan the visible range from 200 to 450 nm. Figure 2A shows that the absorption peak position of methylated anthocyanins differs greatly from

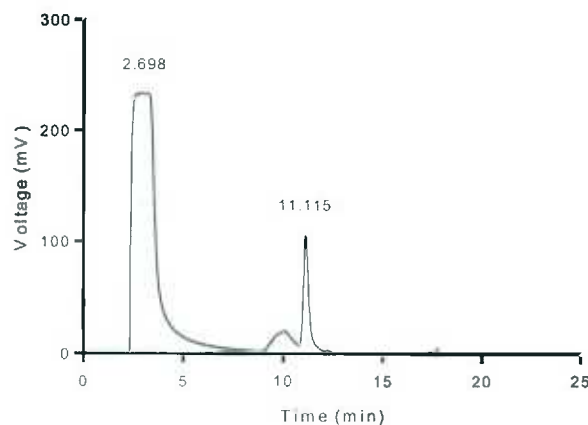


Figure 1: HPLC analysis of anthocyanins methylation. The value 2.698 indicates the time at which methylated anthocyanins were detected, and 11.115 the time at which nonmethylated anthocyanins were detected.

that of nonmethylated anthocyanins, indicating that the structure of methylated anthocyanins is significantly different. The characteristic absorption peak of methylated anthocyanins at 261 nm indicates the presence of a methyl group. This proves that the methylation of anthocyanins was realized by introducing the methyl group.

Infrared spectrometry was performed by scanning in absorption spectrum range from 4000 to 400 cm^{-1} (Figure 2B). The absorption peaks of nonmethylated and methylated anthocyanins differed significantly in location and fluctuation in three regions. The first region is from 3200 to 3400 cm^{-1} . The hydroxyl fluctuation peaks of nonmethylated and methylated anthocyanins are located at 3280 and 3366 cm^{-1} , respectively. The second region is close to 1639 cm^{-1} , the vibration absorption peak of the carbonyl group. The stretching vibration of methylated anthocyanins is enhanced relative to that of nonmethylated anthocyanins. The third region is located from 1043 to 1639 cm^{-1} , which is the characteristic absorption peak region in methylated anthocyanins. The stretching vibration region of the C-H bond is located at 1395 cm^{-1} , proving that methylation with iodomethane introduced the C-H bond and methyl group.

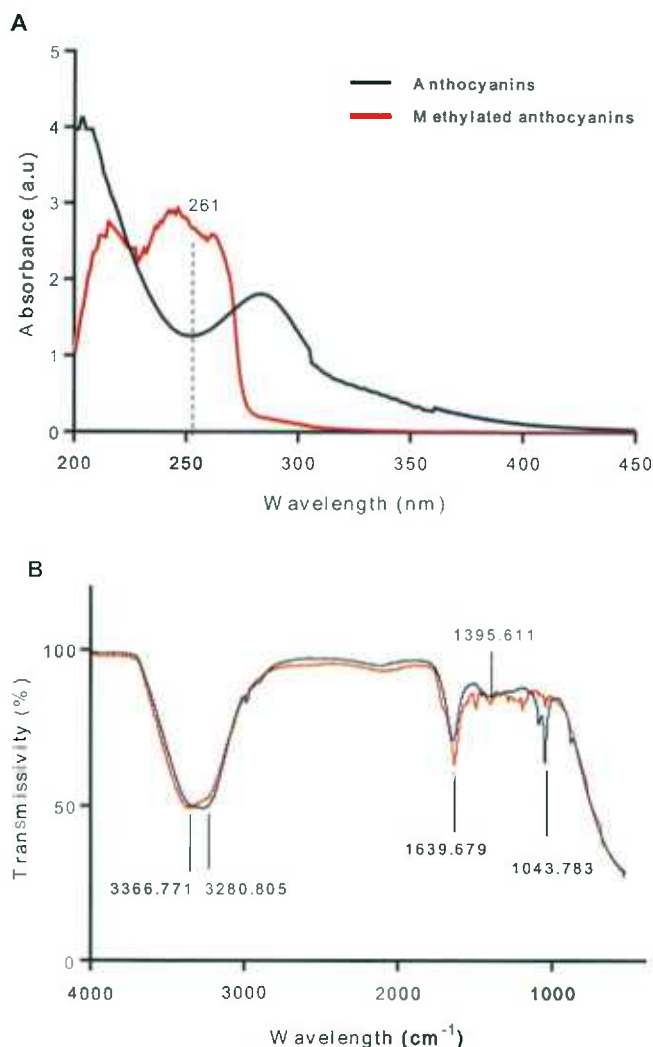


Figure 2: Structural characterization of anthocyanin methylation. (A) UV-visible spectrophotometry scan of the visible range from 200 to 450 nm; (B) Infrared spectrometry absorption spectrum range (4000 to 400 cm^{-1}).

Anthocyanin methylation process optimization

Based on a previous study (Wang *et al.*, 2013), certain extents of potential factors were performed to confirm the influencing internal tendency. We conducted single-factor tests of the methyl donors (dimethyl carbonate, dimethyl sulfate and iodomethane), anthocyanin to donor mass ratios (1:1, 1:2, 1:3, 1:4 and 1:5), methylation time (2.5, 3.0, 3.5, 4.0 and 4.5 h), and methylation temperatures (30, 40, 50, 60, and 70 $^{\circ}\text{C}$), to assess anthocyanin content.

Response selection is a critical stage in the optimization

Table 2: ANOVA results for the regression response surface model

Source	Sum of Squares	df	Mean Square	F Value	P value	Prob> F
Model	0.094	9	0.010	15.29	0.0008	**
x_1	3.001×10^{-4}	1	3.001×10^{-4}	0.44	0.5282	
x_2	1.596×10^{-3}	1	1.596×10^{-3}	2.34	0.1698	
x_3	1.625×10^{-3}	1	1.625×10^{-3}	2.38	0.1666	
x_1x_2	7.29×10^{-4}	1	7.290×10^{-4}	1.07	0.3355	
x_1x_3	7.832×10^{-3}	1	7.832×10^{-3}	11.49	0.0116	*
x_2x_3	9.025×10^{-5}	1	9.025×10^{-5}	0.13	0.7267	
x_1^2	0.049	1	0.049	72.17	0.0001	**
x_2^2	7.822×10^{-3}	1	7.822×10^{-3}	11.47	0.0116	*
x_3^2	0.017	1	0.017	25.58	0.0015	**
Residual	4.722×10^{-3}	7	6.817×10^{-4}			
Lack of Fit	3.497×10^{-3}	3	1.166×10^{-3}	3.66	0.1212	
Pure Error	1.275×10^{-3}	4	3.187×10^{-4}			
Cor Total	0.099	16				
R^2	0.9845					
Adj- R^2	0.9686					

* $P < 0.05$, ** $P < 0.01$.

process. Based on the single-factor experiments and the principles of Plackett-Burman design, the mass ratio (x_1), methylation time (x_2), and temperature (x_3) were chosen as the independent variables, and anthocyanin concentration as the response variable. Response surface analysis was adopted to optimize the process conditions of anthocyanin methylation.

The results of the regression analysis are shown in Table 2. Using multiple regression analysis, the results were fitted to a second-order polynomial equation. The regression equation between Y and x_1 , x_2 , and x_3 is $Y = 0.41 - 6.2510^{-3}x_1 - 0.014x_2 - 0.014x_3 - 0.014x_1x_2 + 4.7510^{-3}x_2x_3 - 0.11x_1^2 - 0.043x_2^2 - 0.064x_3^2$.

Table 2 shows the significance of the linear relationship between the response and independent variables. The model accounted for 96.86 % of the variability in the response, indicating that it is a good fit. The non-significance of the lack-of-fit parameter shows that the quadratic regression equation is a good estimator of the response.

The “Prob> F” value was less than 0.001 (Table 2),

indicating that the model is significant. The model calibration coefficient value, $R = 0.9686$, indicates that the model explains 96.86 % of the variability in the response, and only about 3.14 % of the total variability is not explained by it. This suggests that the most critical independent factors were the mass ratio (x_1), methylation time (x_2), and methylation temperature (x_3), in that order (Figure 3).

To determine the optimal methylation conditions, plotted graphs were made comparing the influential parameters (Figure 4). The response surface graphs, each relating two out of the three factors, consist of ellipses with one center, regardless of their orientation; the edges of the surfaces formed vaults with peaks. The optimum methylation conditions using iodomethane were as follows: anthocyanins to iodomethane mass ratio 1:1.95, methylation time 2.92 h, and methylation temperature 38.69 °C. Under these conditions, the predicted conversion yield was 82.34 %, and the validation value, 82.7 %, showing high reliability.

Stability tests

To investigate the effect of methylation on the thermostability of anthocyanins using iodomethane as a donor, thermo-stability was measured at 70, 80, and 90 °C. Thermostability of methylated anthocyanins was evaluated following storage at these different temperatures for 10 h in darkness. The retention rates of methylated anthocyanins were 98.33 %, 91.56 %, and 63.48 %, respectively and those of nonmethylated anthocyanins were 87.49 %, 72.15 %, and 27.44 % (Figure 5A). This illustrates that the methylated anthocyanins were relatively more stable than nonmethylated anthocyanins at high temperatures, and that mulberry fruit anthocyanins can be preserved well at these temperatures.

Light significantly affects anthocyanin stability (Kearsley and Rodriguez, 2007). After exposure to 400 W light for 2, 4, 6, 8, 10, and 12 d at 20 °C, nonmethylated anthocyanin retention rate decreased steeply from 90.82 to 65.03 % over 10 d. However, the methylated anthocyanin retention

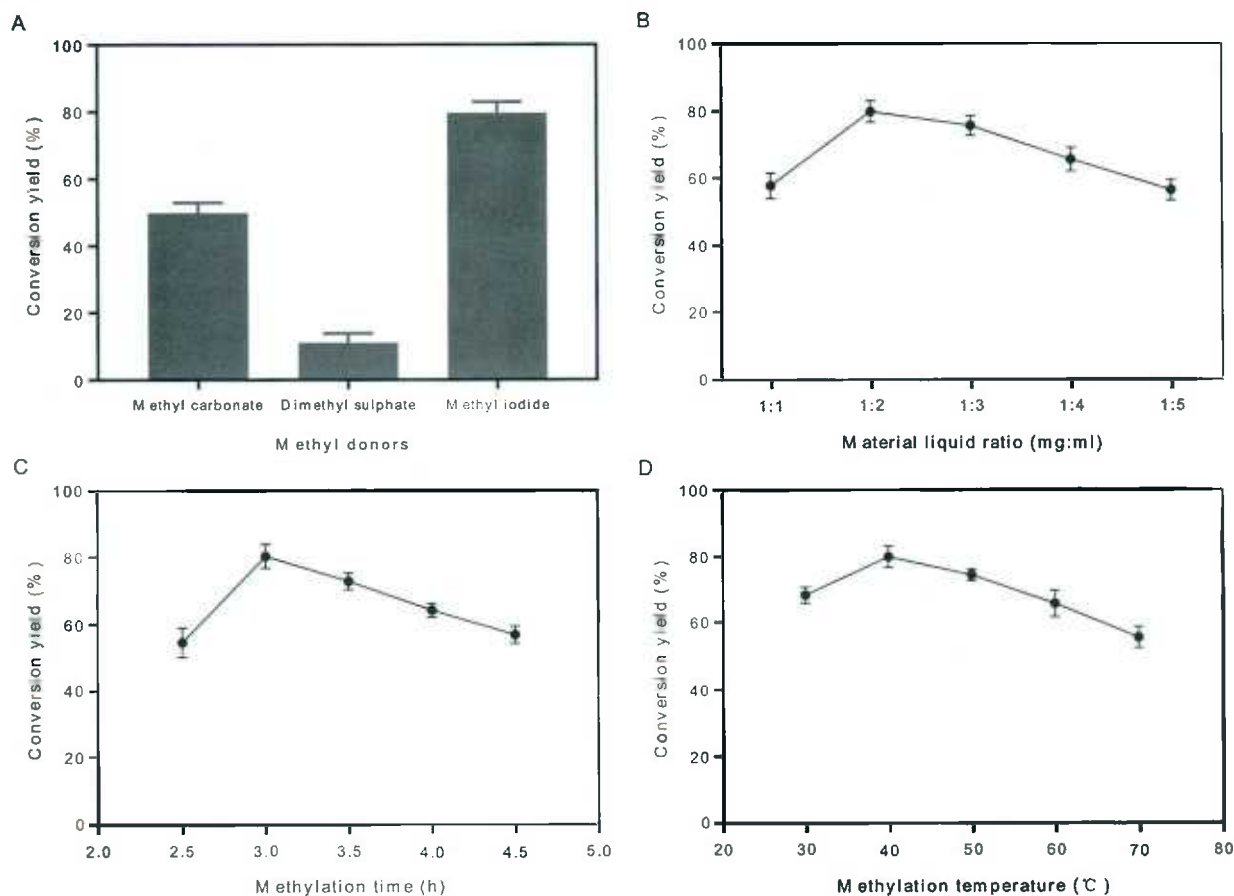


Figure 3: Selection of potential explanatory factors for anthocyanins methylation. (A) Methyl donors (methyl carbonate, dimethyl sulfate, and iodomethane); (B) Anthocyanin to methyl donor mass ratios (iodomethane as the donor, methylation time 3 h, and temperature 40 °C); (C) Methylation time (volume ratio 1:2, and temperature 40 °C); and (D) Methylation temperature (mass ratio 1:2, methylation time 3 h).

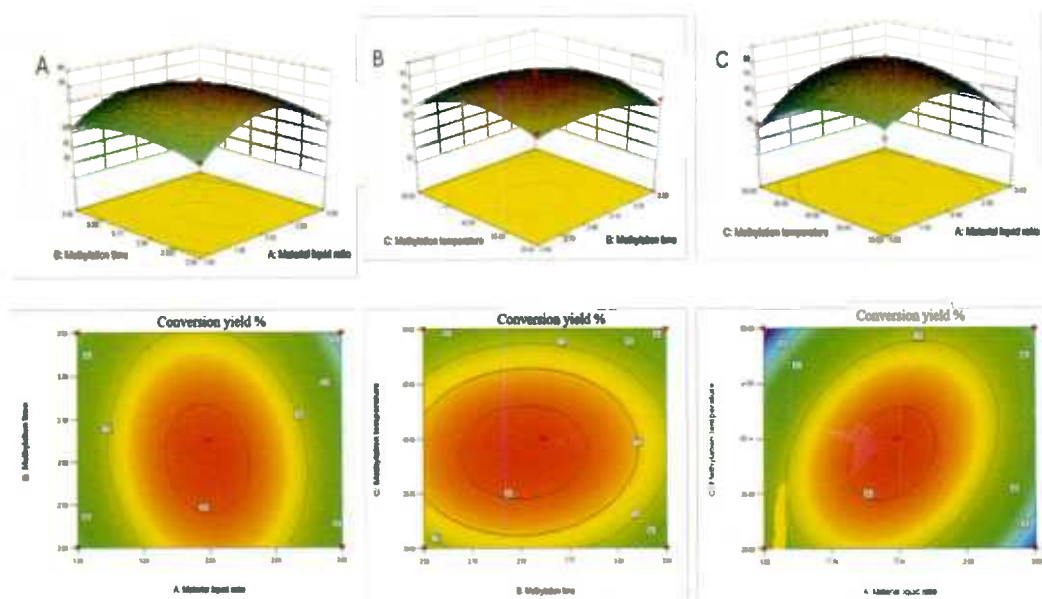


Figure 4: Response surface and contour plots for anthocyanin methylation. (A) Interaction of volume ratio and methylation time; (B) Interaction of methylation time and temperature; and (C) Interaction of mass ratio and methylation temperature.

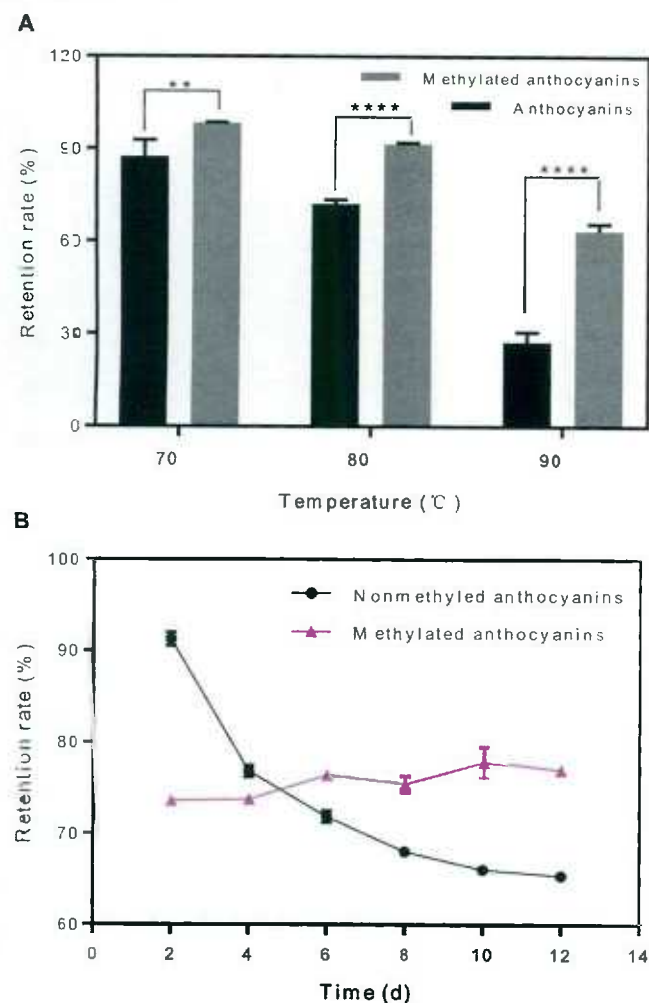


Figure 5: Stability test of anthocyanins. (A) Thermostability at 70, 80, and 90 °C, for 10 h in darkness; (B) Light resistivity under 400 W light.

rate was 73.7 % for the 2 d period and 77.1 % for 12 d period, without significant difference (Figure 5B).

Antioxidant activities

The antioxidant properties that were assessed *in vitro* were DPPH radical scavenging, Fe^{2+} -chelating capacity and reducing power (Figure 6). Nonmethylated and methylated anthocyanins exhibited these properties in a concentration dependent manner. DPPH radical scavenging activity and reducing power were at par in the two groups, without significant differences (Figure 6A, B). However, Fe^{2+} -chelating capacity was poor at low concentrations of Fe^{2+} , and increased rapidly with increasing concentration. At 0.8 mg/ml Fe^{2+} ,

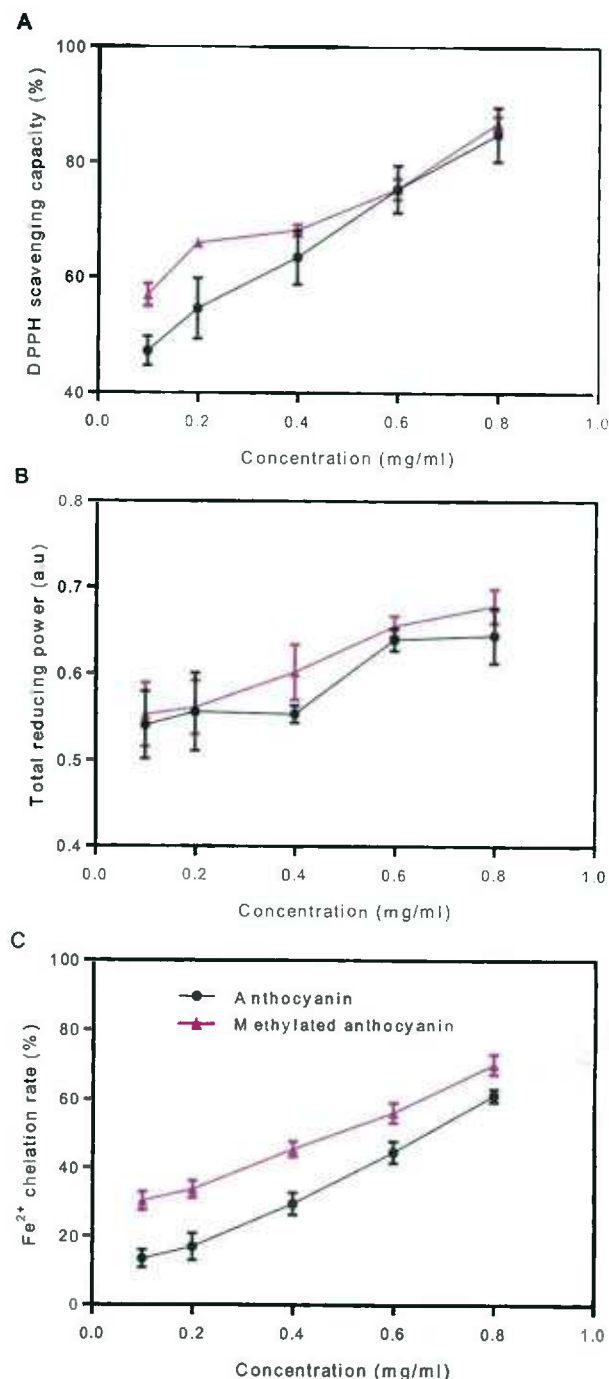


Figure 6: Antioxidant abilities of anthocyanins. (A) DPPH- free radical scavenging; (B) Total reducing power; and (C) Fe^{2+} -chelating capacity.

nonmethylated and methylated anthocyanins reached levels of 62.4 % and 73.3 %, respectively (Figure 6C). The Fe^{2+} chelating capacity of methylated anthocyanins was significantly higher than that of nonmethylated anthocyanins.

DISCUSSION

Anthocyanins have great potential for application in various food, pharmaceutical, and cosmetic industries, due to their attractive colors and broad-spectrum safety (Castañeda-Ovando *et al.*, 2009). However, the relatively poor chemical stability of anthocyanins *in vitro* and *in vivo* (in plant cells or in the digestive tracts of animals) has been a critical drawback and primary obstacle to commercial application (Zhao *et al.*, 2017). It is known that anthocyanins can be stabilized by co-pigmentation with flavonoids, organic acids, metal ions, or even other anthocyanins (Ratanapoompinyo *et al.*, 2017).

In this paper, we present the first study on the methylation of anthocyanins isolated from mulberry fruit, using iodomethane as a donor. Under optimal conditions, the conversion yield decreased from 84.64 % (using iodomethane), to 49.87 % (using dimethyl carbonate), and to 11.13 % (using dimethyl sulfate). Structural analysis of methylated anthocyanins revealed that iodomethane introduces a C-H bond and methyl group in the stretching vibration region of the C-H bond, located at 1395 cm^{-1} . In the case of acylation, anthocyanin stability was enhanced significantly through hydrophobic and “ π - π ” interactions (Giusti *et al.*, 1999).

Temperature is one of the main factors affecting anthocyanin stability. The timing and degree of anthocyanin decay were dependent on temperature. Fracassetti *et al.* (2013) observed that anthocyanin decay occurred slowly (up to 3 %) on day 14 at 25 °C and 42 °C, whereas it was faster on day 3 at 60 °C and 80 °C. Thermal treatment is believed to break down anthocyanins or their conjugated sugars into small molecules, such as aldehydes, benzoic acid derivatives or synonymous anthocyanidins (Sadilova *et al.*, 2006; Butelli *et al.*, 2012). In our study, the anthocyanin preservation rate decreased rapidly from 87.49 % at 70 °C to 27.44 % at 90 °C after 10 h. However, the preservation rate of methylated anthocyanins decreased from 98.33 % at 70 °C to 63.48 % at 90 °C. These results are consistent with those of previous studies (Xu *et al.*, 2015; Yan *et al.*, 2016).

Many studies have shown that acylation can improve anthocyanin stability. During processing and storage, acylated anthocyanins are more stable than their nonacylated analogs. In terms of light resistivity, acylated anthocyanins showed greater light resistivity than nonacylated ones in fruit juices (Inami *et al.*, 1996), and the acylated anthocyanins synthesized by lipase-catalyzed transesterification were more stable than their non-acylated glucosides under illumination with white fluorescent light (Nakajima *et al.*, 2003). Several studies have reported the potential ability of acyl groups to donate electrons to anthocyanins (Dangles *et al.*, 1993; Stintzing *et al.*, 2002), enhancing the stability of acylated anthocyanins under light irradiation. Light resistivity of acylated anthocyanins is dependent on the structure of the acyl moiety (Yan *et al.*, 2016). Here, we demonstrated that methylated anthocyanins possess significantly improved light stability.

Temperature and light degrade anthocyanin stability significantly and reduce antioxidant activity (Aramwit *et al.*, 2010). Methylated anthocyanins have better antioxidant capacity, in terms of Fe^{2+} -chelating capacity, than nonmethylated anthocyanins. However, the exact mechanisms whereby methylation enhances anthocyanin stability will be studied further.

In summary, iodomethane has been selected as a methyl donor for anthocyanin methylation, to improve anthocyanin stability. The optimized methylation conditions were as follows: anthocyanin to iodomethane mass ratio of 1:1.95, methylation time 2.92 h, and methylation temperature 38.69 °C. The methylation of anthocyanins by iodomethane may be attributed to the formation of a C-H bond and methyl group, through a mechanism that is not yet well understood. Our thermostability and light resistivity results show that methylation significantly enhances the preservation rate of anthocyanins, and improves their antioxidant activity. Thus, methylation of anthocyanins may be a novel method for stabilizing anthocyanins in mulberry fruit, and to enable their use as a commercial food ingredient.

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PREPARATION ET CARACTERISATION D'ANTHOCYANES METHYLEES A PARTIR DU FRUIT DU MURIER EN UTILISANT LE IODOMETHANE COMME DONNEUR

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RESUME

Les anthocyanes sont des substances actives communes chez des plantes souvent utilisées comme colorant et nutriments dans des aliments et des boissons. Cet article décrit le processus de méthylation des anthocyanes des fruits du mûrier avec du iodométhane. Le plus fort taux de conversion (84,6 %) est obtenu en utilisant le iodométhane comme donneur de méthyle. Un schéma Box-Behnken de la méthodologie de réponse a été conduit pour analyser la réponse des anthocyanines extraites du fruit du mûrier. Nous avons trouvé que les conditions optimales de méthylation sont obtenues lorsque le rapport de masses anthocyanines sur iodométhane est de 1 ; 1,95, le temps de méthylation de 2,92 h et la température de 38,69 °C. Les analyses structurales révèlent que le iodométhane introduit une liaison C-H et un groupement méthyle. La thermostabilité et la résistivité à la lumière des anthocyanines de mûrier sont significativement accrues après méthylation et l'activité antioxydante est améliorée. Cette étude fournit une nouvelle méthode de stabilisation des anthocyanines du fruit de mûrier ce qui les rend utilisables comme ingrédient d'aliment commercial.

Mots clés: Anthocyanines, iodométhane, méthylation, fruit du mûrier, stabilité.



UTILIZATION OF PATERNAL INHERITANCE OF DIAPAUSE INHIBITING SEX-LIMITED NON-DIAPAUSE BREED, HNID OF SILKWORM, *BOMBYX MORI*

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ABSTRACT

Diapause is generally established as a maternally inherited character and males do not have any role in determining the voltinism of the offspring. However, an exception to this rule was recorded where bivoltine females of silkworm, *Bombyx mori* L. lay non-hibernating eggs when they are mated with males of special race, KS-10. This kind of paternal influence has opened up many interpretations on the phenomenon of diapause in *B. mori* emphasizing on its expression under tropical conditions. In an effort to profitably exploit such paternal inheritance pattern, a breeding program was designed with the ultimate objective of obtaining naturally non-hibernating eggs from the bivoltine female. From the bivoltine sex-limited race HND, a new breed, HNID was developed at this institute. This is probably, a rare polyvoltine diapause inhibiting sex-limited race in the sericulture industry in India. Crosses with promising bivoltine races were conducted to study the pattern of voltinism and rearing performance of the resultant hybrids namely, BRO-2 x HNID, NK-2 x HNID and (BRO-2 x NK-2) x HNID. The superiority of these hybrids over the ruling hybrid in terms of parameters viz., fecundity, hatching percentage, larval duration, larval weight, yield/10,000 larvae by number and weight, cocoon weight, shell weight, cocoon shell percentage and pupation rate was ascertained along with other advantages.

Key words: Diapause inhibiting character, HND, HNID, polyvoltine, sex-limited breed.

INTRODUCTION

The Indian sericulture scenario, with the supremacy of its tropical climate, generally uses polyvoltine silkworm races as female parent and bivoltine as male, in the preparation of multi-bi hybrid seed for commercial use. About 90 % of the raw silk in India is produced from this kind of a combination. When it comes to the state of Karnataka, 80-90 % of the raw silk is produced from PM x CSR2 multi-bivoltine hybrid. The reciprocal cross of the same hybrid is not utilized because the seed undergoes hibernation as the mother inherits the diapause character. For the first time, in the sericulture industry, Rajendra Mundkur *et al.* (2004, 2010 a, b, c) could develop a new race KS-10 with unique characteristics. It behaves as

polyvoltine, but breaks diapauses when crossed with bivoltine female. Similar observations have been made with the sex-limited polyvoltine race HNID, which has been developed later using the race, HND. The diapause inhibiting behavior of the males of race HNID when crossed with females of different bivoltine races maintained at Karnataka State Sericulture Research and Development Institute (KSSRDI), was confirmed over a period (Mahadevappa *et al.*, 2018 a, b). This finding has a great scope in the present set-up of the industry. Presently, the CSR-2 bivoltine female cocoons, which are being rejected after sorting out the cocoons for sex separation, can be used after crossing it with male of HNID. In Karnataka, about 8.68 crores of cross breed layings of PM x CSR-2 are produced per year. This requires about 17.36

crores of CSR-2 bivoltine cocoons. Out of these cocoons, females amount to about 8.68 crores. These female cocoons can be effectively used in the hybrid preparation of CSR-2 X HNID for commercial exploitation. Assuming 70 % egg recovery rate, there is a scope of producing 6.1 crores of non-hibernating but bivoltine quality eggs. In order to study the feasibility of using these males of HNID, the present study was undertaken.

Further, Karnataka Government is popularizing the bivoltine hybrid combinations under various schemes. One of the bottlenecks in the implementation of bivoltine program is ensuring the proper hatching of bivoltine eggs. The widely used acid treatment, subjects the bivoltine eggs to stress and also may weaken the developing embryo. In the present attempt, we have probed the possibility of natural hatching of bivoltine silkworm eggs as in the case of multivoltines without any processing but, through the involvement of HNID race as the male component.

MATERIALS AND METHODS

Two bivoltine races spinning oval cocoons namely, BRO-2 and NK-2 and HNID, a sex-limited non-diapausing race spinning dumbbell cocoons were selected for the study. Using these races, the hybrid layings were prepared by crossing bivoltine female with HNID males. Three crosses namely, BRO-2 x HNID, NK-2 x HNID and (BRO-2 x NK-2) x HNID were prepared, brushed and reared in three replications each by adopting standard rearing method (Krishnaswamy, 1978) along with the ruling hybrid, PM x CSR2 as control. Silkworm larvae were reared by feeding V₁ variety of mulberry. Data were collected on the rearing parameters, such as fecundity, hatching percentage, larval duration, larval weight, yield/10,000 larvae by number and weight, cocoon weight, shell weight, cocoon shell percentage and pupation rate and statistically analyzed. The percentage of improvement with respect to the above parameters of these hybrids over the control cross breed, PM x CSR2 was determined.

RESULTS AND DISCUSSION

The rearing performance of hybrids showed highly significant differences among them for the traits, fecundity, yield/10,000 larvae by weight and cocoon weight and no significant differences were observed with regard to the remaining traits (Table 1). The three way cross, (BRO-2 x NK-2) x HNID had recorded higher values of fecundity (523), yield/10,000 larvae by weight (15.667 kg) and pupation rate (92.52 %) when compared to the other two single crosses.

All the three hybrids exhibited higher degrees of improvement over the control hybrid with regard to fecundity, yield/10,000 larvae by weight, shell weight, cocoon shell percentage and pupation rate as indicated in Table 2. The three way cross (BRO-2 x NK-2) x HNID exhibited the highest percentage of improvement of 23.5 with regard to fecundity and shell weight, of 12.17 %. The single hybrid, BRO-2 x HNID recorded the improvement of 16.70 % and 8.53 % for the traits, fecundity and shell percentage, respectively. Similar studies were also conducted with the hybrids prepared by using diapause inhibiting race KS-10, characterized by plain larvae and oval cocoons, as male parent and bivoltine female spinning oval cocoons by Rajendra Mundkur *et al.* (2016).

Further, the data were also collected for all the hybrids under study for the reeling parameters *viz.*, filament length, denier and reelability (Table 3). Compared to control, PM x CSR2, the hybrids, BRO-2 x HNID, NK-2 x HNID and (BRO-2 x NK-2) x HNID exhibited higher filament length and reelability. Among the hybrids, the three way cross presented the highest filament length (988m) and reelability (90 %).

Voltinism in silkworm is known to be genetically controlled (Murakami, 1990) and is described by Toyama (1906, 1913) as a maternally inherited biological event under the control of sex-limited genes. However, a type of voltinism that is not maternally determined was reported by Katsumata (1968). Late Professor Akio Murakami of National Institute of Genetics, Japan, in one of his

Table 1: Performance of the hybrids of bivoltine females with HNID males

Race	Fecundity	Hatching (%)	Larval duration (Days : Hours)	Larval weight (g)	Yield/10,000 larvae		Cocoon weight (g)	Shell weight (g)	Cocoon shell %	Pupation rate (%)
					No.	Weight (kg)				
BRO-2 x HNID	496	95.00	26:18	36.40	9521	15.365	1.665	0.338	20.35	91.48
NK-2 x HNID	491	96.22	26:20	35.73	9399	14.821	1.637	0.342	20.86	90.81
(BRO-2 x NK-2) x HNID	523	97.44	26:18	38.43	9477	15.677	1.743	0.350	20.32	92.50
Mean	503	96.22	26:18	36.66	9466	15.360	1.680	0.340	20.52	91.60
SD ±	15.30	1.28	7.63	1.38	83.30	0.47	0.05	0.01	0.29	0.98
F- value	83.03	6.43	0.06	10.81	2.10	1715.90	11.96	8.85	15.73	3.83
CD @ 0.005	8.00	2.02	21.17	1.80	178.80	0.20	0.25	0.03	1.21	1.82
CD @ 0.001	16.99	4.28	64.96	3.82	379.79	1.05	1.29	0.17	6.21	3.87
Inference	HS	NS	NS	NS	NS	HS	HS	NS	NS	NS

HS - Significant at 0.01 %; S - Significant at 0.05 %.

Table 2: Improvement of experimental hybrids over the ruling multi-bi hybrid, PM x CSR2

Race	Fecundity	Hatching (%)	Larval duration (Days : Hours)	Larval weight (g)	Yield/10,000 larvae		Cocoon weight (g)	Shell weight (g)	Cocoon shell %	Pupation rate (%)
					No.	Weight (kg)				
BRO-2 x HNID	496(16.70)	95.00(-2.16)	26:18(1.90)	36.40(2.53)	9521(0.231)	15.365(4.05)	1.665(0.301)	0.338(3.31)	20.35(8.54)	91.48(1.85)
NK-2 x HNID	491(15.5)	96.22(-0.90)	26:20(2.22)	35.73(3.60)	9399(1.063)	14.821(0.371)	1.637(-1.385)	0.342(9.61)	20.86(11.25)	90.81(0.564)
(BRO-2 x NK-2) x HNID	523(23.5)	97.44(0.35)	26:18(1.90)	38.43(8.25)	9477(-0.242)	15.677(6.169)	1.743(5.00)	0.350(12.17)	20.32(8.37)	92.50(2.43)
PM x CSR2 (Control)	425	97.10	26:06	35.50	9500	14.766	1.660	0.312	18.75	90.30

Values in parentheses indicate the percentage difference from the control.

investigations utilizing multivoltine Cambodge, in the year 1988, reported that, non-diapause traits, such as *pnd* and *npnd* depend on the male parent. He stated that genetic factor responsible for multivoltinism and non-pigmented eggs in multivoltine Cambodge race is located on sex chromosome (X Chromosomes). This is one of the important contributions one can apply to KS-10 race deciphering the dominance role of *Id* gene at molecular level, which is encoding diapause inhibiting factors aptly located on sex chromosome (Subramanya, 2016)

Yamamoto *et al.* (1978) described that in the pigmented and non-diapausing egg mutant (*pnd*), there is a single recessive mutation of the gene in the 11th chromosome as a

result of which the embryo never enters diapause. Katsumata (1968), Sonobe and Otake (1986) indicated that when such homozygous *pnd* type of female is crossed with normal diapausing type male, the eggs show normal diapause behavior. They proposed two theories related to the embryonic diapause in silkworm; (i) diapause is the phenomenon predetermined by the diapause factor during embryogenesis and (ii) diapause is the process determined by genetic factor during embryogenesis. They opined that there is a possibility that, in the eggs which have been exposed to diapause factor during ontogenesis, the specific protein synthesized by *pnd*⁺ gene (about 22 kda) may stimulate the synthesis of diapause associated proteins.

Table 3: Reeling parameters

Hybrid	Filament length (m)	Denier	Reelability (%)
BRO-2 x HNID	935	2.3	87
NK-2 x HNID	924	2.3	89
(BRO-2 x NK-2) x HNID	988	2.4	90
PM x CSR2 (Control)	785	2.6	82

Rajendra Mundkur *et al.* (2004) described that, when normal diapausing type of female is crossed with males of silkworm race, KS-10, diapause was prevented in the eggs. The authors suggested that silkworm race, KS-10 has dominant inhibitor of diapause gene, *Id* which supersedes the process predetermined by the diapause factor during embryogenesis. Andrewartha (1952) opined that, diapause is a convergent trait that has evolved independently several times in the course of evolution. Rajendra Mundkur *et al.* (2004) proposed a new theory related to diapause, specially taking paternal influence into account, "Diapause is the phenomenon determined by environment, maternal genes and paternal genes synergistically or exclusively". When any normally diapausing type of female is mated with KS-10 males, it lays non-diapausing eggs irrespective of the environmental cues (Rajendra Mundkur *et al.*, 2004). The results of crossing experiment conducted in this work using sex-limited HNID race, spinning white, dumbbell cocoons, with female bivoltine race are in conformity with the findings of the above author.

The non-diapause eggs laid by bivoltine females after crossing with males of HNID were subsequently brushed on hatching and reared for identification of suitable combinations for commercial exploitation. Similar studies were conducted on single hybrids by Rajendra Mundkur *et al.*, 2016, using diapause inhibiting race KS-10 as male component and bivoltine as female component. This race helps graineur in sex separation since it can be done in the larval stage itself based on the markings. Additionally, it also maximizes the egg production because of effective usage of female bivoltine cocoons which are otherwise not being used at present.

Based on the overall rearing performance and percentage of improvement over the control, the three way cross (BRO-2 x NK-2) x HNID and single cross BRO-2 x HNID, were identified for commercial exploitation.

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Article scientifique

UTILISATION DE L'HEREDITE PATERNELLE D'UNE SOUCHE NON DIAPAUSANTE LIEE AU SEXE, HNID CHEZ LE VER A SOIE *BOMBYX MORI*

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RESUME

La diapause est généralement un caractère hérité maternel, les mâles n'ayant aucun rôle dans le déterminisme du voltinisme de la descendance. Cependant une exception a été enregistrée lorsque des femelles bivoltines du ver à soie, *Bombyx mori* L. pondent des œufs non-hibernant lorsqu'elles sont accouplées à des mâles d'une race particulière, KS-10. Ce type d'influence paternelle a ouvert beaucoup d'interprétations du phénomène de la diapause chez *B. mori* mettant l'accent sur l'expression dans des conditions tropicales. Afin d'exploiter cette hérédité paternelle, un programme de croisements a été initié avec comme objectif d'obtenir des œufs naturellement non-hibernant à partir de femelles bivoltines. A partir d'une race bivoltine limitée au sexe HND, une nouvelle lignée, HNID, a été développée dans notre institut. C'est une race polyvoltine à inhibition de diapause limitée au sexe rare en Inde. Des croisements avec des races bivoltines prometteuses ont été effectués afin d'étudier le voltinisme et les performances d'élevage des hybrides BRO-2xHNID, NK-2xHNID et (BRO-2xNK-2)xHNID. La supériorité de ces hybrides en terme de fécondité, pourcentage d'éclosion, durée larvaire, poids des larves, rendement /10000 en nombre et en poids, poids du cocon, poids de la coque, richesse soyeuse et taux de pupaison sont certains ainsi que d'autres avantages.

Mots clés: Caractère d'inhibition de diapause, HND, HNID, polyvoltine, souche limitée au sexe.



Research Paper

IMPACT OF DURATIONS OF MATING AND MALE STAYING WITH FEMALE ON THE REPRODUCTIVE EFFICIENCY OF *TRICHOMALOPSIS UZIAE* SURESHAN & NARENDRA KUMAR (HYMENOPTERA: PTEROMALIDAE), A PUPAL PARASITOID OF THE SILKWORM UZI FLY, *EXORISTA BOMBYCIS* (LOUIS) (DIPTERA: TACHINIDAE)

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ABSTRACT

Trichomalopsis uziae Sureshan & Narendra Kumar (Hymenoptera: Pteromalidae) is a newly reported pupal parasitoid of the silkworm uzi fly, *Exorista bombycis* (Louis) (Diptera: Tachinidae). As it is a new report, no information is available on any of its biological characteristics. Hence in the present study, an attempt was made to know the impact of both mating duration as well as male in copula with female on the reproductive efficiency of the parasitoid. The results revealed that mating duration (<30 sec, 30-60 sec, and >60 sec) doesn't have a noticeable impact on reproductive performance, but, male in copula with female after termination of mating had a significant negative impact on progeny production and progeny sex ratio. In other words, the values for these parameters were higher when female stayed alone after completion of mating.

Key words: *Exorista bombycis*, progeny production, reproductive performance, sex ratio, *Trichomalopsis uziae*

INTRODUCTION

Sex allocation is an important reproductive decision for parents. However, it is often assumed that females have substantial control over sex allocation decisions and in many cases, especially in haplo-diploids, insect females apparently determine sex by deciding whether to fertilize an egg (to produce diploid daughter) or not (to produce a haploid son) (Shuker *et al.*, 2006). However, whether duration of mating will have any impact on reproductive efficiency is the subject that is not studied much in parasitoids.

But presence or absence of a male and its staying duration with female is realized to have a great say in the reproductive efficiency as well as sex allocation of a parasitoid. In an experiment by Singh (1998) with *Trichomalopsis apantelectena* Crawford, it was found that when a male and a female were confined together,

there was a reduction in the egg laying by the female, which has been attributed to hindrance.

With dearth of the above information in the newly reported pupal parasitoid, *Trichomalopsis uziae* (Hymenoptera: Pteromalidae) (Sureshan *et al.*, 2014; Narendra Kumar *et al.*, 2015), the present study was taken up to study the impact of mating duration as well as male staying duration with female on a few reproductive parameters, such as parasitism, progeny production, brood allocation, and sex ratio.

MATERIALS AND METHODS

Impact of mating duration on the reproductive efficiency of *T. uziae*

Virgin females and unmated males were collected by making minute exit holes with fine needle on the pupal

case, about 15-20 h prior to anticipated day of emergence. The males and females thus emerging through the exit holes were confined to separate test tubes with honey solution as feed. Later, they were paired in separate test tubes @ one pair per test tube and allowed to mate. Based on the duration of copulation, they were placed in 3 groups, namely <30, 30-60 and >60 seconds mated ones and males were removed instantly after natural decoupling by them, with 10 replications in each group. All the mated females were provided with five uzi pupae (3 days old) and exposed for 3 days so as to oviposit. Later, uzi pupae parasitized by each female of all the groups were maintained separately in test tubes waiting for the emergence of parasitoids, after which data on reproductive parameters, such as per cent parasitism, brood allocation (numbers), progeny production (numbers), and sex ratio (females/male) were recorded.

Impact of staying duration of male with the female on the reproductive efficiency of *T. uziae*

In order to know the effect of the parasitoid male staying duration with the female on its reproductive efficiency, males were allowed to stay with the freshly mated females in the following manner: a) male staying with female for 1 day, b) male staying with female for 2 days, c) male staying with female for 3 days, and d) male staying with female for 4 days. Against each of the above treatments, a control comprising the mated female alone was also maintained. The experiment was conducted with ten replications and five uzi pupae (3 days old) were provided to each female for oviposition by exposing for 1, 2, 3 & 4 days depending on the treatments.

The host pupae were removed and maintained in test tubes. They were observed for recording per cent parasitism, developmental duration (days), brood allocation (numbers), progeny production (numbers), and sex ratio (females/male).

Above investigations were carried out in the laboratory at 23-28 °C and 70-80 % RH. The accrued data were analyzed by one-way ANOVA (version 21) followed by

DMRT for expressing whether or not the results were significantly different from each other at 1 or 5 % among the treatments as per the methods outlined by Snedecor and Cochran (1979).

RESULTS AND DISCUSSION

Impact of mating duration on the reproductive efficiency

When the mating pairs of the parasitoid were allowed to mate for durations of <30 sec, 30-60 sec, and >60 sec, it was observed that the per cent parasitism of the host ranged from 88.00 ± 6.19 (<30 and 30-60 sec) to 92.00 ± 6.19 % (>60 sec). The parasitoid developmental duration varied between 12.20 ± 0.20 (30-60 sec) and 12.40 ± 0.24 days (<30 and >60 sec). With regard to total brood allocation, it fluctuated between 26.11 ± 0.17 (> 60 sec) and 27.65 ± 0.20 (30-60 sec). Insofar as the total progeny production goes, it ranged from 117.60 ± 0.84 (<30 sec) to 123.40 ± 0.70 (30-60 sec). The sex ratio of the parasitoid progeny varied from 4.87 ± 0.26 (<30 sec) to 4.95 ± 0.52 (30-60 sec) females/male. Comparison of mean values for the above parameters revealed a non-significant variation, except for total progeny production ($P < 0.05$) (Table 1).

Based on impact of mating duration on various reproductive parameters, including total progeny production of *T. uziae*, what becomes discernible is that duration of mating (from <30 sec to >60 sec), that had been allowed, didn't have a perceptible impact. Though the total progeny production with the medium (123.40 ± 0.70 numbers with 30-60 sec) and the longest durations (120.20 ± 0.49 numbers with 30-60 sec) being on par, the values are significantly higher when compared with that of the shortest duration (117.60 ± 0.84 numbers with <30 sec). Still, it didn't make much of a difference between the shortest and the longest durations in terms of numbers, which is only a marginal one with a difference of mere 6 in number. Hence, such a difference could as well be treated as negligible. In view of this, it can be understood that mating duration doesn't have any perceptible impact on

Table 1: Influence of mating duration on the reproductive performance in *Trichomalopsis uziae* Sureshan & Narendra Kumar

Duration of mating (Sec.)	Per cent parasitism @	Developmental duration (days)	Total progeny production (No.)			Brood allocation (No.)			Sex ratio (♀/♂)
			Male	Female	Total	Male	Female	Total	
<30	88.00±6.19	12.40±0.24	20.40±0.39	97.20±0.75 ^b	117.60±0.84 ^b	4.52±0.12	21.62±0.22	26.14±0.25	4.87±0.26
30- 60	88.00±6.19	12.20±0.20	20.60±0.17	102.80±0.70 ^a	123.40±0.70 ^a	4.69±0.06	22.96±0.21	27.65±0.20	4.95±0.52
>60	92.00±6.19	12.40±0.24	21.60±0.39	98.60±0.36 ^b	120.20±0.49 ^a	4.66±0.06	21.45±0.12	26.11±0.17	4.92±0.53
F value	NS	NS	NS	4.055**	3.98*	NS	NS	NS	NS

^a Out of 5 uzi fly pupae provided for parasitism

Values given in the Table are the mean of 10 replications (Mean ± SE);

** P≤0.01; * P≤0.05; NS-Non-significant

Mean values followed by the same superscript in columns are statistically not significant

any of the reproductive parameters under consideration in the current investigation.

Even though fertilization obviously directly depends on the presence of sufficient, viable spermatozoa, the quantification of the sperm transfer process itself has not received the attention it deserves. Weggelaar *et al.* (2019) revealed that there are clear instances where the process of sperm transfer is not a linear process, *i.e.*, longer copulation duration does not necessarily imply more transfer of sperm. Amin (2009) also revealed that in the case of the bumble bee, *Bombus terrestris*, colony development was not affected by short copulation duration.

Impact of staying duration of male with the female on the reproductive efficiency of *T. uziae*

The mean results for rate of parasitism when males and females stayed together for 1, 2, 3, and 4 days were 48.00±4.90, 68.00±10.20, 84.00±7.48, and 88.00±4.90, respectively. The corresponding figures for the females when they stayed alone were 52.00±4.90, 68.00±8.00, 92.00±4.90, and 88.00±8.00. The results with respect to per cent parasitism in both the cases increased significantly with increase in duration of staying in treatment and duration of exposure of host pupa in the control batch. The parasitoid developmental periods for the durations (1, 2, 3 & 4 days) when males and females were together varied from 12.20±0.20 (2 days) to

12.60±0.24 days (3 days) as against 12.20±0.20 (2 & 4 days) and 12.60±0.24 days (1 day) when females stayed alone. Taking brood allocation into cognizance, the mean values among treatments with male and female together, stood at 6.46±0.38, 20.70±2.46, 29.65±2.51, and 30.53±0.56 for 1, 2, 3, and 4 day treatments, respectively. The corresponding figures in treatments with female alone were 9.23±0.45, 26.92±1.74, 31.95±0.69, and 38.90±2.94. The analysis of the values for these two sets of treatments revealed a highly significant variation. The mean data for progeny production for males and females staying together stood at 15.20±0.86, 69.20±8.84, 124.00±14.27, and 136.80±6.98 for 1, 2, 3, and 4 days, respectively, while those for females alone were 22.00±2.02, 89.40±5.75, 146.40±5.57, and 174.80±24.39, respectively. A highly significant variation was noticed when the mean values among the treatments were compared. The mean results for sex ratio among treatments with males and females together for 1, 2, 3, and 4 days were 5.80±0.82, 4.46±0.37, 3.57±0.23, and 3.48±0.27, respectively as against 7.05±0.43, 5.88±0.40, 5.36±0.36, and 4.96±0.61 with females alone, showing significant difference (Table 2).

In an attempt to know whether staying duration (from 1 to 4 h at a space of 1 h) of *T. uziae* male with its female counterpart following mating for 3 h on its reproductive efficiency, what we observed was that there was an appreciable increase in rate of parasitism, brood allocation, progeny production, and sex ratio for both male staying with female as well as female staying alone with

Table 2: Effect of staying duration of male with female on the reproductive effectiveness of *Trichomalopsis uziae* Sureshan & Narendra Kumar

Male staying duration with female (days)	Per cent parasitism @	Developmental duration (days)	Progeny production (No.)			Brood allocation (No.)			Sex ratio (♀/♂)
			Male	Female	Total	Male	Female	Total	
M + F (1 day)	48.00±4.90 ^c	12.40±0.24	2.40±0.40 ^d	12.80±0.86 ^c	15.20±0.86 ^d	1.00±0.11 ^c	5.47±0.42 ^d	6.46±0.38 ^d	5.80±0.82 ^{ab}
F (1 day)	52.00±4.90 ^c	12.60±0.24	2.80±0.37 ^d	19.20±1.69 ^c	22.00±2.02 ^d	1.17±0.11 ^c	8.06±0.37 ^d	9.23±0.45 ^d	7.05±0.43 ^a
M + F (2 days)	68.00±10.20 ^{bc}	12.20±0.20	12.20±0.80 ^c	55.00±7.27 ^d	69.20±8.84 ^c	3.90±0.59 ^b	16.81±1.92 ^c	20.70±2.46 ^c	4.46±0.37 ^{bcd}
F (2 days)	68.00±8.00 ^{bc}	12.20±0.20	13.20±1.20 ^c	76.20±4.97 ^{cd}	89.40±5.75 ^c	3.97±0.38 ^b	22.94±1.52 ^b	26.92±1.74 ^b	5.88±0.40 ^{ab}
M + F (3 days)	84.00±7.48 ^{ab}	12.60±0.24	27.20±3.17 ^{ab}	96.80±11.51 ^{bc}	124.00±14.27 ^b	6.51±0.50 ^a	23.14±2.15 ^b	29.65±2.51 ^b	3.57±0.23 ^{cd}
F (3 days)	92.00±4.90 ^a	12.40±0.24	23.20±0.97 ^b	123.20±5.81 ^{ab}	146.40±5.57 ^{ab}	5.11±0.39 ^b	26.84±0.54 ^b	31.95±0.69 ^b	5.36±0.36 ^b
M + F (4 days)	88.00±4.90 ^{ab}	12.40±0.24	31.00±2.49 ^a	105.80±5.46 ^b	136.80±6.98 ^b	7.04±0.39 ^a	23.49±0.72 ^b	30.53±0.56 ^b	3.48±0.27 ^d
F (4 days)	88.00±8.00 ^{ab}	12.20±0.20	29.20±4.03 ^{ab}	143.60±20.74 ^a	174.80±24.39 ^a	6.64±0.68 ^a	31.86±2.56 ^a	38.90±2.94 ^a	4.96±0.61 ^{bc}
F - value	6.089 ^{**}	NS	29.887 ^{**}	25.371 ^{**}	27.535 ^{**}	29.598 ^{**}	36.321 ^{**}	41.086 ^{**}	6.654 ^{**}

^a- Out of 5 pupae provided for parasitism; M + F – Male kept with female; F – Female alone;

Values given in the Table are the means of 10 replications (Mean ± SE);

Mean values followed by the same superscript in the columns are statistically not significant;

** P≤0.01; * P≤0.05; NS-Non-significant

increase in duration. When the treatments with male staying with female were compared with those having female alone, what could be understood was that the treatments with female alone were superior to those with male and female together for same duration. When the impact of male staying duration with female *vis-a-vis* female alone for reduction in the reproductive parameters was quantified, no perceptible increase or decrease was recorded for the respective durations for rate of parasitism. By contrast, brood allocation, progeny production, and sex ratio did decrease with duration of male staying with female as against female alone. The decrease was of the order of 1.43, 1.30, 1.08, and 1.30 times for 1, 2, 3, and 4 days treatments, respectively for brood allocation. The corresponding values for decline in progeny production were 1.45, 1.30, 1.18, and 1.28 times. With regard to progeny sex ratio, the reduction registered during 1-4 days male staying with female was of the order of 1.22, 1.32, 1.50, and 1.43 times. The contribution of female progenies towards decline in the above parameters was far more than that by males that were more or less identical in number for the corresponding treatments.

When a comparison of treatments with male and female staying together or female staying alone for varying durations for different parameters, including rate of parasitism, was brought out, there was a highly perceptible increase with every additional day of male staying with female or otherwise (female alone) when compared with the preceding duration, which was the highest with the longest duration (4 days) and the least with the shortest duration (1 day). Admittedly, the increase in rate of parasitism with the longest duration vs the shortest duration for treatment with male and female staying together was 1.83-folds, while it was 1.69-folds for female staying alone. Similarly, improvement at 4 days against 1 day for brood allocation and progeny production in treatments with male and female staying together as well as female alone was of the order of 4.73 & 4.21 and 9.00 & 7.95-folds, respectively. Interestingly, the progeny sex ratio registered a dip which was to the tune of 1.67 and 1.42 times when the results for the longest and the shortest durations involving both male and female staying together and female alone were compared.

Presence or absence of a male and its staying duration with female is realized to have a great say in the reproductive efficiency as well as sex allocation of a parasitoid. In a detailed experiment conducted by Singh (1998), in the case of *T. apanteloctena*, it was recorded that when a male and a female were confined together, there was a reduction in the egg laying by the female, which has been attributed to hindrance. The hindrance was caused by male insect for either chasing the female for mating or for antennal encounters during oviposition. Thus, the presence of male parasitoid reduced the total number of eggs laid and daily egg output of the female parasitoid. The mean fecundity of female in the presence of male was considerably less than that in its absence. In addition, the longevity (in days) of the female in presence of male was somewhat more than that in its absence, while it maintained significantly higher mean sex ratio when isolated than that when mixed with male. Similar results have also been reported in pteromalid parasitoids by Legner (1967) in *Spalangia endius*, *S. cameroni*, *Muscidifurax raptor* and *Nasonia vitripennis* and King and Richard (1969) in *N. vitripennis*. Nonetheless, the experiment conducted by Shuker *et al.* (2006) in *N. vitripennis*, suggested that males can influence female sex ratios and contribute to the variation around the sex ratios optimal for females. However, the influence is not large, thus once again suggesting that females have greater influence on sex allocation than do males.

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Article scientifique

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MOUCHE UZY DU VER A SOIE, *EXORISTA BOMBYCIS* (LOUIS)
(DIPTERE: TACHINIDE)**

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RESUME

Trichomalopsis uziae Sureshan & Narendra Kumar (Hymenoptère: Ptéromalidé) est un parasitoïde pupal nouvellement décrit de la mouche uzy du ver à soie, *Exorista bombycis* (Louis) (Diptère: Tachinidé). Comme nouvelle espèce aucune information n'est disponible sur ses différentes caractéristiques biologiques. Nous tentons ici de déterminer l'impact de la durée de l'accouplement et de la présence du mâle avec la femelle sur l'efficacité reproductive du parasitoïde. Les résultats montrent que la durée de l'accouplement (<30 sec, 30-60 sec et >60sec) n'a pas d'effet notable sur la reproduction mais la copulation après l'accouplement a un effet significativement négatif sur la production d'œufs et le sex-ratio. La valeur de ces paramètres est plus élevée lorsque les femelles restent seules après l'accouplement.

Mots clés: *Exorista bombycis*, production de progéniture, performance reproductive, sex ratio, *Trichomalopsis uziae*.



OPTIMIZATION OF COCOON SOFTENING PROCEDURE FOR TASAR ECO- RACES TO ACHIEVE HIGHER SILK RECOVERY, QUALITY AND RETENTION OF NATURAL COLOUR

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ABSTRACT

It's inevitable for silken cocoons to undergo a cooking / softening process in preparation of extraction of its filament yarn. Under the conventional process, higher concentration of sodium carbonate along with neutral soap is used for softening of tropical tasar cocoons by which yarn extraction becomes easy along with the retention of natural colour. But, the higher alkali concentrations, in addition to the act of removal of sericin, adversely affect the fibroin quality resulting in lower tensile characteristics of silk filament. Enzymatic degumming processes have been found to overcome this disadvantage, but, unfortunately have not become popular for tasar silk units due to the long duration of soaking as well as not so attractive techno economics. Hydrogen peroxide and soap boiling of tasar cocoons is another method in practice which enables adequate sericin elimination without affecting fibroin component but, due to oxidative bleaching, the natural colour is removed from yarn. In the present study, it was found that adequate cooking efficiency can be achieved with the combination of sodium carbonate and sodium bi-carbonate used during softening of tropical tasar cocoons (Daba BV, Modal and Raily) due to better elimination of sericin from cocoon shells and at the same time, this treatment takes care of retention of the natural brown colour of yarn. It was observed that, combination of 5 g/l each of sodium carbonate and sodium bi- carbonate facilitates better softening of Daba cocoons whereas, 8 g/l each for Modal and 10 g/l each for Raily variety. The required concentrations vary among three ecoraces due to variations in shell thickness as well as presence of minerals and natural colouring matter in addition to sericin.

Key words: Crossover, Epprouvette, fibroin, non-broken filament length, raw silk recovery, reelability, sericin.

INTRODUCTION

Tropical tasar cocoons are spun by *Antheraea mylitta* Drury species, placed under order Lepidoptera and family Saturniidae. Mulberry silk, known for its luster, beauty, elegance and sensuality is owned by *Bombyx mori*. But, with respect to the ease of reeling, tasar stands next only to mulberry. After harvesting, tasar cocoons are subjected to a process of softening / cooking to reel continuous filament yarns of desired denier. The traditional practice followed for tropical tasar cocoon softening is soda (sodium carbonate - Na_2CO_3) cooking process. In this process, tasar cocoons are boiled in alkaline solution for about 30 min using 10 to 15 g/l of Na_2CO_3 . Sometimes,

neutral soap (sunlight / lux) is also used for reduction of surface tension as well as cleaning of adhered materials from cocoon shells (Jolly *et al.*, 1979; Sonwalkar, 1993). But, this process because of its high pH level (above 11), affects the quality of silk fibroin resulting in lower yarn tenacity. But, it has got the advantage of retention of natural yarn colour. Another recommended procedure *i.e.*, enzymatic treatment at temperature between 30 and 40 °C enables cleavage of polypeptide bonds of sericin and thereby makes tasar cocoons soft and suitable for reeling / spinning process (Jolly *et al.*, 1979; Sonwalkar, 1993). Biopril 50 and Anilozyme P (proteolytic type) enzymes are used here for softening purpose. But, the process couldn't pick up well due to the long duration involved and the

deleterious effect noticed on fibroin in case, the schedule time exceeds. The lacuna of deterioration of silk fibroin can be overcome by using hydrogen peroxide and neutral soap combination for cooking process wherein the residual sericin can be retained in filament to offer better cohesion characteristics. Also, the yarn can be dyed without bleaching which totally eliminates the natural colour of tasar cocoons (Mitra *et al.*, 2013). Usage of pressure cooker for hydrogen peroxide and neutral soap cooking process facilitates saving of time and fuel significantly (Gahlot *et al.*, 2012). Boiling of tasar cocoons in plain water followed by soaking in hydrogen peroxide, sodium carbonate and sodium silicate solution at 55 °C facilitates better softening followed by wet reeling as in mulberry silk (Gahlot *et al.*, 2012).

Comparative cooking and reeling performance of tasar cocoons using sodium carbonate along with sunlight soap, sodium carbonate and Biopril – 50 proteolytic enzyme revealed that enzymatic softening process facilitates cooking efficiency, productivity / raw silk recovery and tenacity of silk filament (Das *et al.*, 1994). Use of 1.5 % of tamarind for cooking of tasar cocoons in solution helped in attaining maximum yield (Chandrashekar *et al.*, 1996). Improved wet reeling of tropical tasar Daba cocoons enables better tenacity, neatness, cleanness and cohesion characteristics (Das *et al.*, 1992). Sodium carbonate along with glycerin soap helps better yield as well as superior raw silk quality characteristics for temperate (oak tasar) variety (Manna and Tikoo, 1990). Devi *et al.* (2012) observed that enzymatic cooking enhances the reelability (%), raw silk recovery (%) and quality characteristics of silk yarn of temperate tasar (oak) cocoons. Sodium carbonate along with hydrogen peroxide cooking is highly suitable for both non-mulberry (tasar and eri) as well as mulberry fabrics with respect to better absorbency, dye uptake, whiteness *etc.* (Vyas and Shukla, 2016). Tasar plus which is a uniform mixture of sodium carbonate and sodium bi-carbonate in 6:5 proportion can be well utilized for softening of tasar cocoons (Ghosh, 2019). About 4 litres of water is required for 400 cocoons and 11 g tasar plus is added per litre of water with proper dissolution. The

cocoons are boiled for 30 min followed by steaming for another 30 min and subjected for reeling operations. Tasar plus can well be utilized for wet reeling where sodium silicate and hydrogen peroxide are used. However, the optimization technique has not been described for alkali concentration as well as boiling and steaming durations.

From the above findings, it is revealed that, natural colour is retained when sodium carbonate is used for the degumming process whereas, the same is eliminated while following hydrogen peroxide cooking process. Enzymatic degumming process has not become popular for tasar silk sector due to its long duration as well as non-feasibility from techno-economic point of view. Hence, in order to retain the natural colour of tasar silk yarn along with the tensile characteristics by maintaining pH of cooking solution, an attempt was made to use sodium carbonate along with sodium bi-carbonate for softening of tropical tasar cocoons.

MATERIALS AND METHODS

Materials

Cocoons of different ecoraces of tropical tasar silkworm, *A. mylitta* viz., Daba Bi-voltine (BV), Raily and Modal of 2nd crop were considered for this study. Daba Bi-voltine (BV) cocoons were procured from Raw Material Bank, Central Silk Board, Chaibasa, Raily from Regional Sericultural Research Station, Jagdalpur, Chhattisgarh and Modal from Regional Sericultural Research Station, Baripada, Odisha. Cocoon and shell weight for the above varieties of tropical tasar cocoons are presented in Table 1.

Table 1: Cocoon characteristics of tropical tasar cocoons

Parameter	Daba BV	Raily	Modal
Cocoon weight (g)	12.70	13.30	9.60
Shell weight (g)	1.70	2.60	2.80

Methods

Optimization of softening / cooking process parameters

The experimental trials were carried out following Central

Table 2: Design of experiment

Parameter / Material	Tasar eco-race		
	Daba BV	Modal	Raily
Optimization of alkali concentrations			
Sodium carbonate (g/l)	2, 5, 8	2, 5, 8	5, 10, 15
Sodium bi-carbonate (g/l)	2, 5, 8	2, 5, 8	5, 10, 15
Constants – Process duration	Boiling - 20 min	Boiling - 20 min	Boiling - 15 min
	Steaming - 30 min	Steaming - 30 min	Steaming - 45 min
Optimization of boiling and steaming durations			
Boiling duration (min)	10, 20 & 30	10, 20 & 30	10, 15 & 20
Steaming duration (min)	15, 30 & 45	15, 30 & 45	30, 45 & 60
Constants	Optimized concentrations of sodium carbonate and sodium bi-carbonate.		

Randomized Design (CRD) and the results were subjected to Analysis of Variance (ANOVA). At first, the alkali concentration was optimized followed by boiling and steaming durations (Table 2).

Single cocoon reeling

After softening of tasar cocoons of three different eco-races as per procedure mentioned in Table 2, filament was withdrawn from cocoons under semi moist conditions by using an Epprouvette and breaks were noted. The total length of yarn from single cocoon was estimated from the number of revolutions noted from counter meter of Epprouvette. In total, 30 observations were taken for each experimental trial and three replications were carried out. After drying with hot air dryer for 20 minutes at 105 ± 3 °C (BS 4784-1973, 1974), yarn weight was measured using precision electronic balance. Yarn denier and non-broken filament length (NBFL) were estimated by standard procedures (Sonwalker, 1993).

Reeling performance

The reeling for each experimental trial was conducted using motorised reeling cum twisting machine (MRTM). The yarn withdrawal was carried out following dry reeling technique from semi moist cocoons with the speed of about 30 m / min. Breakage during the reeling performance was noted and 50 cocoons were used for each experimental trial and three replications were

maintained. Yarns as well as different wastes generated during filament withdrawal like, floss, reeling and basin / pelade wastes were collected. Yarns as well as wastes generated during reeling were dried using hot air oven at 105 ± 5 °C for 20 min (BS 4784- 1973, 1974). The reelability (%) and raw silk recovery (%) were estimated by standard procedures (Sonwalker, 1993).

Yellowness index of yarn

Yellowness index of reeled yarn samples obtained from cocoons softened by sodium carbonate, hydrogen peroxide along with neutral soap as well as sodium carbonate and sodium bi-carbonate combinations were evaluated following standard procedure (AATCC TM 110- 2015) using Gretag Macbeth 7000A Spectrophotometer.

Single fibre tensile characteristics

Single fibre denier of tasar silk extracted through conventional cooking / softening processes using sodium carbonate, hydrogen peroxide along with neutral soap and the newly proposed sodium carbonate and sodium bi-carbonate combinations was assessed as per the standard procedure (ASTM D 1577- 07, 2012) using Lenzing Vibroscope Instrument. Similarly, for tensile characteristics, single fibre was measured following standard procedure (ASTM D 3822/3822 M- 14, 2014). The tensile characteristics were measured using Lenzing Vibrodyn Instrument. The gauge length was kept at 20 mm

with pretension weight, 350 mg and testing speed was maintained at 12 mm/min for all observations. In total, 50 readings were taken for both denier and tensile characteristics assessment.

Conditioning of yarn samples

The yarn samples were kept under standard atmospheric conditions *i.e.*, 65 ± 2 % relative humidity and 27 ± 2 °C temperature for 24 hours and (ASTM D 1766/1766 M, 2015) subjected for assessment of filament weight, tensile characteristics and yellowness index.

Analysis of experimental data

The experimental data were analysed for parameters *viz.*, filament length, filament denier, non-broken filament length (NBFL), reelability (%) and raw silk recovery (%) using MS Excel Software.

RESULTS AND DISCUSSION

Optimization of process parameters for softening / cooking of tropical tasar cocoons

The process parameters as discussed under for softening / cooking of tropical tasar cocoons were optimized based on single cocoon reeling and reeling performance assessment.

1. Optimization of alkali concentrations

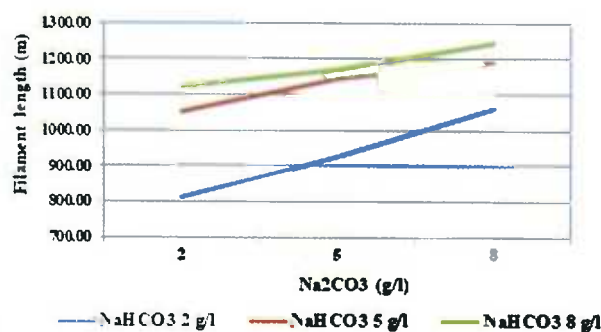
A. Daba BV

The filament length, non-broken filament length, reelability (%) and raw silk recovery (%) of Daba BV cocoons softened by different concentrations of sodium carbonate (Na_2CO_3) as well as sodium bi-carbonate (NaHCO_3) are shown in Figure 1, keeping boiling and steaming durations constant at 20 and 30 min, respectively.

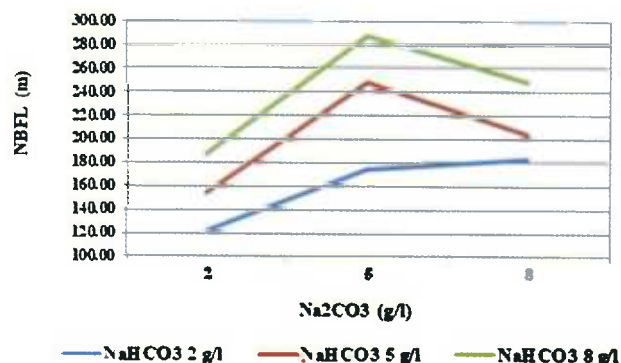
It is observed that with increase of alkali concentration, the filament length improves. From 2 to 5 g/l as well as 2

to 8 g/l of both Na_2CO_3 and NaHCO_3 concentrations, significant improvement of filament length was noticed. The sericin molecules present on the surface as well as at crossover points of cocoon shells get eliminated by action of alkali and this may be the reason for higher filament length during single filament withdrawal. The Analysis of Variance (ANOVA) along with critical difference estimation (Table 3) confirms the significant increase of filament length with increase of alkali concentrations. Ascending trend of non-broken filament length was noticed between 2 and 5 g/l of sodium carbonate concentration whereas, the same was found reducing with more alkali concentration (Figure 1). Sodium carbonate facilitates cleavage of peptide bonds of sericin and thereby eliminate it from cocoon shells. Higher alkali concentration helps rapid separation of sericin. Softening of silk cocoons was observed very effective between pH range of 9.5 and 11 whereas, above pH 11, fibroin gets affected. Below 9.5, the degumming happened to be very slow and absolutely no separation of sericin occurred below pH value 8.0 (Gulrajani, 1992). So, there can be chance of fibroin damage with higher alkali concentrations (8 g/l) as the pH value was estimated as 11.0. Under such situations, excessive breaks occurred during withdrawal of filament causing lower NBFL. Among the different quality characteristics of silk cocoons, NBFL is an important parameter that decides speed of reeling (Lee, 1999; Chattopadhyay *et al.*, 2018). The maximum NBFL (286.80 m) was obtained for the treatment of 5 g/l Na_2CO_3 and 8 g/l NaHCO_3 combination followed by 5 g/l each of Na_2CO_3 and NaHCO_3 (248.50 m). Since there exists no significant difference between these two values, combination of 5 g/l each of Na_2CO_3 and NaHCO_3 may be considered for Daba BV cocoons softening. The ANOVA for NBFL confirms the present findings (Table 3).

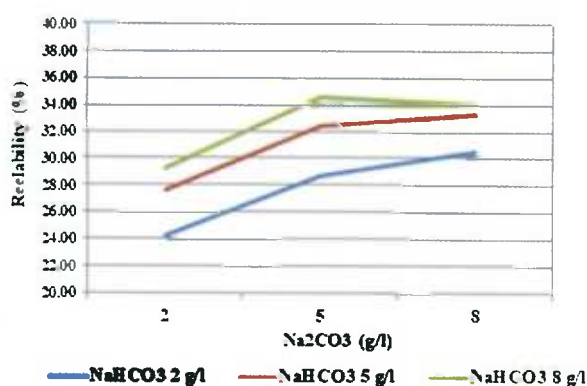
Ascending trend was observed for reelability of Daba BV cocoons from 2 to 5 g/l of alkali concentrations (Na_2CO_3 and NaHCO_3), but no considerable difference was noticed between 5 and 8 g/l. ANOVA also confirms these findings (Table 9). Reelability (%) is directly proportional to the NBFL of silk cocoons (Jolly *et al.*, 1979; Lee, 1999). So, 5



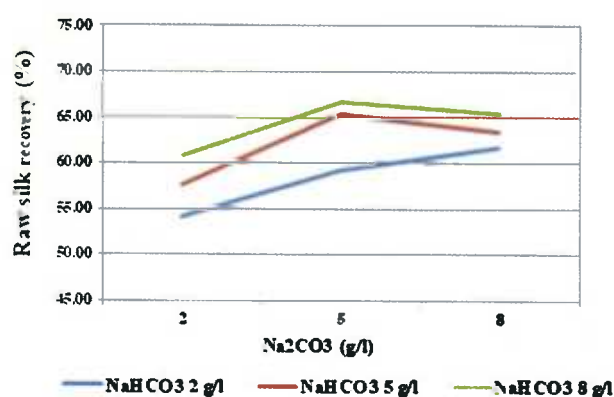
1 (a): Filament length



1 (b): Non- broken filament length (NBFL)



1 (c): Reelability



1 (d): Raw silk recovery

Figure 1: Quality characteristics and reeling performance of Daba BV cocoons subjected to various softening treatments

(Constant: Boiling and steaming durations as 20 and 30 min, respectively)

Table 3: Analysis of variance (ANOVA) for Daba BV cocoon quality characteristics and reeling performance

Source of variation	Cocoon parameters						Reeling performance					
	Filament length (m)			Non- broken filament length (NBFL) (m)			Reelability (%)			Raw silk recovery (%)		
	F value	Critical difference (CD)		F value	Critical difference (CD)		F value	Critical difference (CD)		F value	Critical difference (CD)	
		5 %	1 %		5 %	1 %		5 %	1 %		5 %	1 %
Between NaHCO ₃	39.32	244.11	403.93	18.55	111.07	183.80	44.64	4.30	7.11	17.60	8.71	14.41
Between Na ₂ CO ₃	16.73			20.36			68.56			22.90		

F (Tab) values are 6.94 (5 % confidence level) and 18.00 (1% confidence level); Constants: Boiling and steaming durations as 20 and 30 min, respectively.

g/l each of the combination of Na₂CO₃ and NaHCO₃ facilitates maximum reelability as well as non- broken filament length of Daba BV cocoons. It is also observed from Figure 1 that raw silk recovery improves along with an increase in alkali concentration from 2 to 5 g/l whereas,

no improvement was found between 5 and 8 g/l as confirmed by ANOVA (Table 3). Although higher concentration of alkali facilitates more softening of tropical tasar cocoons, there is an optimum level of sericin elimination. This may be the reason for no significant

Table 4: Denier of tasar silk filament versus different softening treatments

NaHCO ₃ (g/l)	Na ₂ CO ₃ (g/l)		
	2	5	8
2	10.84	9.47	11.52
5	10.93	10.53	10.60
8	11.54	11.86	11.24

improvement of silk recovery at a concentration of more than 5 g/l of alkali.

The denier of filament determined for different softening experiments is presented in Table 4. It is observed that no significant difference existed among different softening trials. With increase as well as decrease of filament length, weight of filament also changes proportionally. So, the filament denier remained at par for all the trials.

Hence, from the above studies, it can be opined that the combination of 5 g/l each of sodium carbonate and sodium bi-carbonate provides better softening of tropical tasar Daba BV cocoons as reflected in cocoon

characteristics as well as reeling performance.

B. Modal

Modal cocoons were softened using sodium carbonate and sodium bi- carbonate with concentration levels of 2, 5 and 8 g/l as per formulated design of experiment, keeping boiling duration of 20 min followed by that of steaming at 30 min as constant. Filament length, non- broken filament length, reelability (%) and raw silk recovery (%) resulted are illustrated in Table 5 along with Analysis of Variance (ANOVA). It is observed that cocoon quality characteristics (filament length and non- broken filament length) as well as reeling performance (reelability and raw silk recovery percentage) improved significantly with increase of alkali concentrations from 2 to 5 g/l and 5 to 8 g/l. The shell weight of Modal tropical tasar cocoons is higher as compared to Daba BV cocoons (Table 1) because it is reared on sal (*Shorea robusta*) vis-à-vis Daba BV silkworms which are reared on arjun (*Terminalia arjuna*) and asan (*Terminalia tomentosa*) plants. Although no significant difference was noticed in terms of crude protein

Table 5: Optimization of alkali concentrations during cooking / softening of Modal cocoons

Sodium carbonate (Na ₂ CO ₃) (g/l)	Sodium bi- carbonate (NaHCO ₃) (g/l)			F - values (estimated)		F - values (table)		Critical difference (CD)	
	2	5	8	Na ₂ CO ₃	NaHCO ₃	5 %	1 %	5 %	1 %
Filament length (m)									
2	665.50	697.14	1077.50	211.60	53.40	6.94	18.00	207.20	342.90
5	732.85	852.00	1245.00						
8	877.70	952.50	1380.00						
Non- broken filament length (m)									
2	126.87	131.74	164.68	8.00	13.90	6.94	18.00	71.50	118.30
5	143.26	177.48	190.96						
8	176.74	185.81	194.05						
Reelability (%)									
2	15.40	16.66	20.00	9.60	298.40	6.94	18.00	5.60	9.20
5	31.25	31.46	32.94						
8	30.00	31.11	32.30						
Raw silk recovery (%)									
2	38.92	49.40	54.24	11.20	15.60	6.94	18.00	21.40	35.40
5	49.24	59.67	64.57						
8	58.92	60.50	64.36						

Constant: 20 min boiling and 30 min steaming durations

content among these host plants, sal tree leaves possess comparatively higher quantity of crude fibre (Sinha and Jolly, 1971) resulting in maximum cocoon and shell weight.

At concentrations of more than 8 g/l of alkali, chances of bursting of cocoons are more as well as deterioration of silk fibroin resulting in inferior NBFL and reelability. Hence, for adequate and efficient softening of Modal tasar cocoons, 8 g/l each of sodium carbonate and sodium bi-carbonate may be maintained.

C. Raily

As per softening procedure discussed in the preceding sections, Raily cocoons were softened using sodium carbonate and sodium bi-carbonate at concentration levels of 5, 10 and 15 g/l each, keeping boiling duration at 15 min and steaming period at 45 min as constant. Higher concentrations of alkali were considered because preliminary trials revealed that cocoons are not softened properly (only about 70 % cooking efficiency) at less than 5 g/l alkali concentrations (Na_2CO_3 and NaHCO_3).

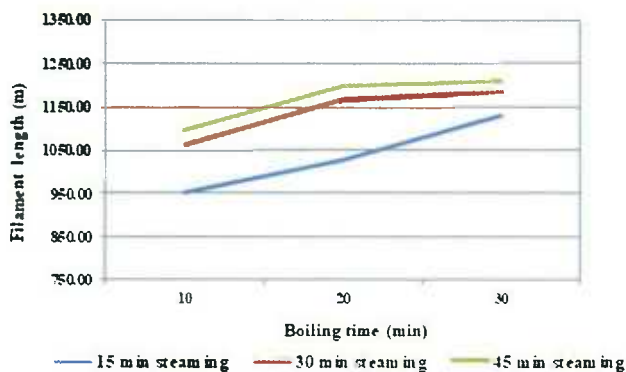
Similarly, boiling for more than 20 min may enhance chances of bursting of cocoons. Hence, different boiling durations were tried as 10, 15 and 20 min whereas, for better elimination of sericin from cocoon shells, steaming durations were tried with 30, 45 and 60 min. Results on important parameters *viz.*, filament length, non-broken filament length, reelability (%) and raw silk recovery (%) are mentioned in Table 6 along with Analysis of Variance (ANOVA).

It is revealed that cocoon quality characteristics (filament length and non- broken filament length) as well as reeling performance (reelability and raw silk recovery percentage) increase significantly with rising alkali concentrations from 5 to 10 g/l (Table 6). But, more than 10 g/l of alkali concentration (Na_2CO_3 and NaHCO_3) did not facilitate better test results. The shell weight of Raily tropical tasar cocoons is higher as compared to Daba BV cocoons (Table 1) with an obviously thicker shell. Raily ecorace is reared on sal (*Shorea robusta*) tree which possesses higher foliar crude fibre content (Sinha and Jolly, 1971) as compared to arjun and asan. With combination of crude fibre, sericin along with mineral

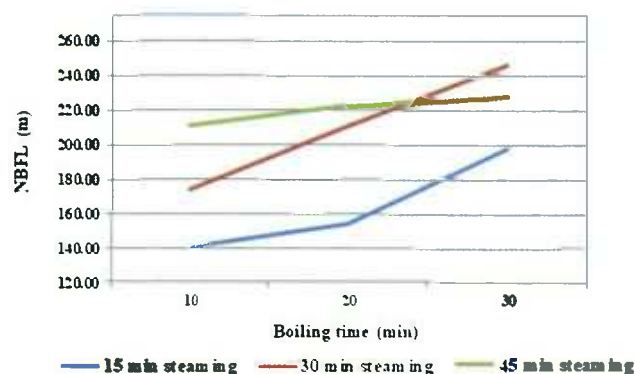
Table 6: Optimization of alkali concentrations during cooking/softening of Raily cocoons

Sodium carbonate (Na_2CO_3) (g/l)	Sodium bi- carbonate (NaHCO_3) (g/l)			F - values (estimated)		F - values (table)		Critical difference (CD)	
	5	10	15	Na_2CO_3	NaHCO_3	5 %	1 %	5 %	1 %
Filament length (m)									
5	954.00	1250.00	1360.00	30.50	11.90	6.94	18.00	346.70	573.70
10	1171.00	1450.00	1438.00						
15	1231.00	1430.00	1456.00						
Non- broken filament length (m)									
5	109.50	136.40	154.50	21.20	10.90	6.94	18.00	69.30	114.70
10	125.70	190.50	179.90						
15	138.60	185.60	181.60						
Reelability (%)									
5	19.50	22.00	22.20	19.10	18.40	6.94	18.00	4.40	7.20
10	21.50	25.60	24.90						
15	22.60	24.20	25.10						
Raw silk recovery (%)									
5	55.20	62.00	63.00	24.10	9.40	6.94	18.00	7.30	12.10
10	58.90	64.70	64.20						
15	61.20	65.10	65.00						

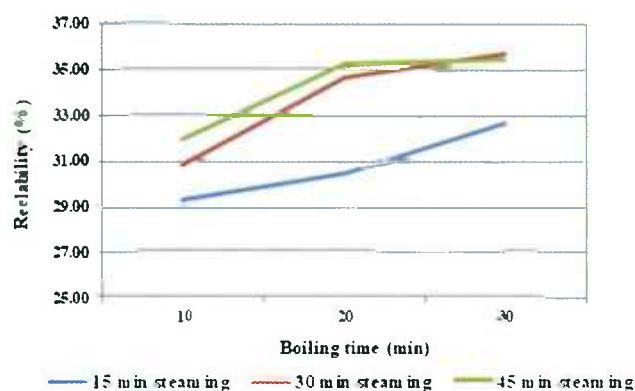
Constant: 15 min boiling and 45 min steaming durations



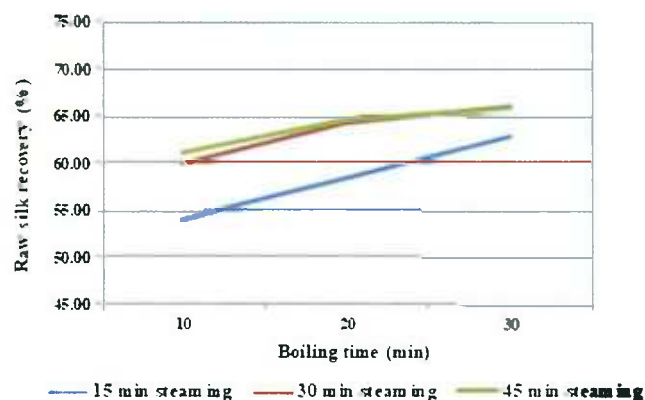
2 (a): Filament length



2 (b): Non- broken filament length (NBFL)



2 (c): Reelability



2 (d): Raw silk recovery

Figure 2: Quality characteristics and reeling performance of Daba BV cocoons subjected to various softening treatments
(Constant: 5 g/l each of Na_2CO_3 & NaHCO_3)

calcium oxalate and natural colouring matter tannin make the cocoon shells very tough and hence, higher concentrations of alkali are required during cooking. Analysis of variance (ANOVA) confirmed that significant differences in quality parameters exist between the results of treatments of 5 and 10 g/l (each Na_2CO_3 and NaHCO_3) but, above 10 g/l, no difference was found. So, for Raily cocoons, 10 g/l each of Na_2CO_3 and NaHCO_3 may be considered for softening.

2. Boiling and steaming durations

A. Daba BV

Keeping sodium carbonate and sodium bicarbonate

concentrations at 5 g/l each, trials were conducted with boiling duration of 10, 20 and 30 min followed by steaming for 15, 30 and 45 min as per formulated design of experiment. The results for filament length, non-broken filament length, reelability (%) and raw silk recovery (%) are illustrated in Figure 2 and result of Analysis of Variance (ANOVA) is provided in Table 7. An ascending trend is revealed for filament length, NBFL, reelability (%) and raw silk recovery (%) when duration of boiling increased from 10 to 20 min. Between 20 and 30 min boiling duration, no significant improvement was found. There is an optimum level of softening of tasar cocoons with a particular boiling duration which afterwards, remains at par due to total elimination of sericin from cocoon shells. Similarly, with an increase of steaming

Table 7: Analysis of variance (ANOVA) for Daba BV cocoon quality characteristics and reeling performance

Source of variation	Cocoon parameters						Reeling performance					
	Filament length (m)			Non- broken filament length (NBFL) (m)			Reelability (%)			Raw silk recovery (%)		
	F value	Critical difference (CD)		F value	Critical difference (CD)		F value	Critical difference (CD)		F value	Critical difference (CD)	
		5 %	1 %		5 %	1 %		5 %	1 %		5 %	1 %
Between boiling	20.90	180.00	297.90	11.80	103.10	170.60	17.60	5.20	8.60	22.20	7.60	12.50
Between steaming	20.90			7.70			20.50			26.00		

F (Tab) values are 6.94 (5 % confidence level) and 18.00 (1 % confidence level); Constant: 5 g/l each of Na_2CO_3 and NaHCO_3

duration from 15 to 30 min, significant improvement was noted which remained same after 30 min. Moreover, excess boiling may cause bursting of cocoon shells making them unreelable. So, it can be opined that, 20 min of boiling duration followed by 30 min of steaming will be ideal for Daba BV tropical tasar cocoons.

B. Modal

With sodium carbonate and sodium bicarbonate concentrations at 8 g/l each, trials were conducted with boiling duration of 10, 20 and 30 min followed by steaming for 15, 30 and 45 min as per formulated design of experiment. The results in respect of parameters viz., filament length, non- broken filament length, reelability (%) and raw silk recovery (%) are illustrated in Table 8 along with Analysis of Variance (ANOVA).

It is noted that, filament length, NBFL, reelability (%) and raw silk recovery (%) increased along with an extension in duration of boiling from 10 to 20 min. However, beyond this level, i.e., between 20 and 30 min boiling duration, no significant difference was found. During softening of tasar cocoons, sericin is eliminated by cleavage of peptide bonds at optimum pH level (between 9.5 to 11) with stipulated boiling duration (Gulrajani, 1992). So, by an increase in boiling duration beyond a particular level, one may not significantly enhance cocoon quality parameters as well as reeling performance. Similarly, increased steaming duration from 15 to 30 min, could offer significant improvement which remained at par after 30 min steaming duration. Moreover, as already mentioned, excess boiling causes bursting of cocoon shells and make them unreelable. So, it

can be presumed that, 20 min boiling followed by 30 min steaming will be an ideal package for Modal tropical tasar cocoons.

C. Raily

With sodium carbonate and sodium bicarbonate concentrations at 10 g/l each, trials were conducted with boiling durations of 10, 15 and 20 min followed by steaming periods of 30, 45 and 60 min as per design of experiment. The results for filament length, non- broken filament length, reelability (%) and raw silk recovery (%) are depicted in Table 9 along with Analysis of Variance (ANOVA).

The data reveal that, filament length, NBFL, reelability (%) and raw silk recovery (%) increased as duration of boiling increased from 10 to 15 min, beyond which, there were no significant differences. The optimum pH level for softening the cocoons was determined as between 9.5 and 11, with stipulated boiling duration (Gulrajani, 1992). So, a further increase in boiling duration did not enhance cocoon quality parameters as well as reeling performance significantly. Similarly, with increase of steaming duration from 30 to 45 min, significant improvement was noted which remained at par after 45 min. Moreover, excess boiling caused bursting due to increase in volume of air inside cocoon shells rendering them unfit for reeling. Hence, the steaming duration was maintained higher for adequate softening of Raily cocoons. So, it can be opined that, 15 min of boiling duration followed by 45 min of steaming would be an appropriate treatment package for Raily tropical tasar cocoons.

Table 8: Optimization of boiling and steaming durations for cooking / softening of Modal cocoons

Steaming time (min)	Boiling time (min)			F - values (estimated)		F - values (table)		Critical difference (CD)	
	10	20	30	Steaming time (min)	Boiling time (min)	5 %	1 %	5 %	1 %
Filament length (m)									
15	725.50	895.00	1077.50	15.70	46.60	6.94	18.00	331.80	549.10
30	802.50	1120.00	1164.00						
45	877.70	1210.00	1275.00						
Non- broken filament length (m)									
15	138.60	149.60	166.30	21.30	9.50	6.94	18.00	36.90	61.00
30	155.90	169.20	178.40						
45	176.74	179.80	184.30						
Reelability (%)									
15	25.34	29.60	32.90	15.40	18.90	6.94	18.00	7.50	12.40
30	30.50	34.50	34.80						
45	31.25	35.10	35.20						
Raw silk recovery (%)									
15	46.80	57.30	62.30	10.30	24.20	6.94	18.00	14.90	24.60
30	52.70	63.50	65.40						
45	58.92	65.40	66.10						

Constant: 8 g/l each of Na_2CO_3 and NaHCO_3 .

Table 9: Optimization of boiling and steaming durations for cooking / softening of Raily cocoons

Steaming time (min)	Boiling time (min)			F - values (estimated)		F - values (table)		Critical difference (CD)	
	10	15	20	Steaming time (min)	Boiling time (min)	5 %	1 %	5 %	1 %
Filament length (m)									
30	1054.00	1205.00	1306.00	21.30	30.40	6.94	18.00	326.90	540.90
45	1170.00	1501.00	1525.00						
60	1260.00	1490.00	1510.00						
Non- broken filament length (m)									
30	125.60	145.20	162.10	13.30	20.20	6.94	18.00	56.70	93.70
45	139.40	189.60	182.90						
60	152.80	191.20	188.60						
Reelability (%)									
30	19.80	22.30	23.60	31.30	44.60	6.94	18.00	3.50	5.70
45	21.60	25.70	25.60						
60	23.10	25.90	26.10						
Raw silk recovery (%)									
30	55.30	62.70	64.00	8.90	33.00	6.94	18.00	7.50	12.30
45	59.10	65.20	65.70						
60	61.40	65.50	66.00						

Constant: 10 g/l each of Na_2CO_3 and NaHCO_3 .

Yellowness index of tasar yarns produced under different cooking / softening processes

The yellowness index of tasar yarns produced following

conventional cooking / softening processes viz., sodium carbonate, hydrogen peroxide along with neutral soap and the newly proposed sodium carbonate and sodium bicarbonate combinations are given in Table 10.

Table 10: Yellowness index of tasar yarns

Cooking / softening process	Yellowness index		
	Daba BV	Modal	Raily
Sodium carbonate (conventional)	52.50	51.80	53.10
Hydrogen peroxide and neutral soap	47.60	48.50	49.40
Sodium carbonate and sodium bi-carbonate (developed)	51.20	52.40	54.00

It is observed that due to bleaching action of hydrogen peroxide during softening, the natural colour eliminates from yarn and hence, the yellowness index value is lower. But in the case of tasar yarns produced from conventional sodium carbonate as well as sodium carbonate and sodium bi-carbonate process, the natural brown colour is retained. Although no significant difference exists between the yellowness index values, the yarns display a creamy colour when treated with hydrogen peroxide and neutral soap. Hence, for dyeing of yarns or fabrics in light and medium shade, another bleaching is carried out for removal of the remaining colour. It can be opined that, the presently developed softening process can well be followed for softening of tropical tasar cocoons.

Denier and tensile characteristics of single tasar fibre

Denier and tensile characteristics of single tasar cocoon fibre extracted after different cooking / softening processes are depicted in Table 11.

It is revealed that, the tenacity is significantly lower for conventional sodium carbonate cooking / softening process. During boiling of tasar cocoons using sodium carbonate, the pH value of solution reaches 11 and above due to reduction of water along with higher amount of alkali usage (10 to 15 g/l). So, the polypeptide bond of silk fibroin deteriorates which affect the tenacity. No difference existed for fibre tenacity between treatments of hydrogen peroxide along with neutral soap and presently developed sodium carbonate and sodium bi-carbonate. Hydrogen peroxide is a bleaching agent with pH value of about 5.0 but at higher temperature, due to decomposition into per-hydroxyl and oxygen ion, the alkalinity rises to about 10.50 and hence, sericin dissolves and get

Table 11: Denier and tensile characteristics of single tasar fibre of Daba

Cooking / softening process	Single fibre quality characteristics		
	Denier	Tenacity (g/d)	Breaking elongation (%)
Daba BV			
Sodium carbonate (conventional)	9.50 (1.10)	1.25 (0.80)	25.60 (12.30)
Hydrogen peroxide and neutral soap	11.20 (0.90)	2.35 (0.75)	30.50 (9.50)
Sodium carbonate and sodium bi-carbonate (developed)	10.70 (0.60)	2.42 (0.70)	35.10 (11.40)
Modal			
Sodium carbonate (conventional)	11.50 (1.30)	1.45 (0.80)	28.60 (13.40)
Hydrogen peroxide and neutral soap	10.90 (1.00)	2.53 (0.60)	31.50 (11.50)
Sodium carbonate and sodium bi-carbonate (developed)	10.50 (0.90)	2.46 (0.50)	34.20 (12.40)
Raily			
Sodium carbonate (conventional)	11.80 (1.40)	1.37 (0.80)	29.40 (15.30)
Hydrogen peroxide and neutral soap	12.20 (1.10)	2.51 (0.75)	30.50 (12.50)
Sodium carbonate and sodium bi-carbonate (developed)	11.10 (1.20)	2.38 (0.90)	35.10 (13.50)

Values in parentheses indicate the standard deviation of the corresponding quality parameter.

eliminated from cocoon shells. Similarly, sodium bi-carbonate is an edible alkali with pH value less than 9.0 *vis-à-vis* about 11.00 for sodium carbonate. The pH remains below 11.00 when both are used in equal proportion. As per research work carried out, it was observed that, degumming is very effective at pH value above 9.50 but, in case, it exceeds beyond 11.00, silk fibroin is affected. Similarly, degumming is less effective below 9.50 and when it fell below 8.00, no separation of sericin occurred (Gulrajani, 1992). Hence, by using both sodium carbonate and sodium bi-carbonate, single fibre tenacity is not adversely affected because pH remains within the optimum range. So, the newly proposed softening process is highly appropriate for tasar reeling industry.

Cost of cooking / softening for developed procedures

The expenditure for softening of cocoons of three ecoraces (Daba BV, Modal and Raily) following the developed procedure (sodium carbonate and sodium bi-carbonate) as

Table 12: Cost of softening for tropical tasar cocoons (1000 no.)

Traditional sodium carbonate and soap (without pressure)	Hydrogen peroxide and soap (without pressure)	Sodium carbonate and sodium bi-carbonate (without pressure)
Daba BV		
Water: 40 litres Sodium carbonate: 10 g/l Soap: 10 g/l Boiling: 1 hour	Water: 30 litres Hydrogen peroxide: 10 ml/l Soap: 10 g/l Boiling: 20 min Steaming: 30 min	Water: 30 litres Sodium carbonate: 5 g/l Sodium bi- carbonate: 5 g/l Boiling: 20 min Steaming: 30 min
Cost of cooking Sodium carbonate: ₹36.00 Soap: ₹46.00 Fuel: ₹20.00	Hydrogen peroxide: ₹45.00 Soap: ₹34.00 Fuel: ₹20.00	Sodium carbonate: ₹14.00 Sodium bi-carbonate: ₹15.00 Fuel: ₹20.00
Total cost ₹102.00	₹99.00	₹49.00
Modal		
Water: 40 litres Sodium carbonate: 15 g/l Soap: 10 g/l Boiling: 1 hour	Water: 30 litres Hydrogen peroxide: 15 ml/l Soap: 10 g/l Boiling: 20 min Steaming: 30 min	Water: 30 litres Sodium carbonate: 8 g/l Sodium bi- carbonate: 8 g/l Boiling: 20 min Steaming: 30 min
Cost of cooking Sodium carbonate: ₹54.00 Soap: ₹46.00 Fuel: ₹20.00	Hydrogen peroxide: ₹68.00 Soap: ₹34.00 Fuel: ₹20.00	Sodium carbonate: ₹22.00 Sodium bi-carbonate: ₹24.00 Fuel: ₹20.00
Total cost ₹120.00	₹122.00	₹66.00
Raily		
Water: 40 litres Sodium carbonate: 15 g/l Soap: 10 g/l Boiling: 1 hour	Water: 30 litres Hydrogen peroxide: 15 ml/l Soap: 10 g/l Boiling: 20 min Steaming: 30 min	Water: 30 litres Sodium carbonate: 10 g/l Sodium bi- carbonate: 10 g/l Boiling: 15 min Steaming: 45 min
Cost of cooking Sodium carbonate: ₹54.00 Soap: ₹46.00 Fuel: ₹20.00	Hydrogen peroxide: ₹68.00 Soap: ₹34.00 Fuel: ₹20.00	Sodium carbonate: ₹27.00 Sodium bi-carbonate: ₹30.00 Fuel: ₹20.00
Total cost ₹120.00	₹122.00	₹77.00

compared to conventional processes are provided in Table 12.

It is evident that the cost involved in the present softening process for tropical tasar cocoons is substantially lower as compared to traditional sodium carbonate and soap as well as hydrogen peroxide and soap processes generally followed in tasar reeling sector. For the sodium carbonate and sodium bi- carbonate cooking process, about 50 % of the cost of cooking was found reduced for Daba BV

cocoons followed by 45 % for Modal ecorace and 35 % for Raily ecorace, thereby proving it economically viable. In tasar sector, reelers usually get ₹1000/- as conversion charge inclusive of chemicals and fuel cost for 1 kg of tasar yarn in which cocoons are provided by stake holders / traders. As the cost of softening is substantially lower, they can earn an extra profit over this for production of yarn. Moreover, the natural colour can be retained in yarn through sodium carbonate and sodium bi-carbonate cooking process.

Conclusion

Conventionally, sodium carbonate of high concentration between 10 and 15 g/l is used for cooking / softening of tropical tasar cocoons which result in inferior tensile characteristics of silk fibre. In order to overcome this disadvantage, enzymatic softening processes have been attempted but, the long duration was recognized as drawback along with the quality of fibroin getting affected if proper timing is not maintained. In yet another method using Hydrogen peroxide and soap, it does not affect the fibroin, but the natural brown colour of tasar silk yarn is eliminated. The present method has the advantage of easy availability of sodium carbonate (washing soda) and sodium bi-carbonate (edible soda) even in remote rural areas where the tasar silk yarn is usually produced by reelers. The conclusions that can be drawn are as following:

- 5 g/l of sodium carbonate and sodium bi-carbonate each, with 20 min boiling followed by 30 min steaming facilitates best single cocoon quality characteristics and reeling performance with cooking efficiency of about 96 %, reelability of 35 % and raw silk recovery of 65 % in the case of Daba cocoons.
- In the case of Modal cocoons, sodium carbonate and sodium bi-carbonate of 8 g/l each, with 20 min boiling followed by 30 min steaming facilitates adequate softening with cooking efficiency of about 92 %, reelability of about 26 % and raw silk recovery of about 62 %.
- Similarly, 10 g/l of sodium carbonate and sodium bi-carbonate each with 15 min boiling and 45 min steaming provides better single cocoon quality characteristics and reeling performance with cooking efficiency of about 90 %, reelability of 25 % and raw silk recovery of 60 % in the case of Raily cocoons.
- Higher value of yellowness index of tasar silk yarn produced using the presently developed cooking

process comprising of sodium carbonate and sodium bi-carbonate, indicates the retention of natural colour but, no difference was observed in comparison to hydrogen peroxide cooking process.

- Single fibre tenacity was significantly lower in the case of conventional sodium carbonate cooking / softening process as compared to hydrogen peroxide as well as sodium carbonate and sodium bi-carbonate procedure due to deterioration of silk fibroin at higher pH of degumming solution. But no difference was noticed between the other two softening techniques.
- About 50 , 45 and 35 % cost of cooking is reduced for Daba, Modal and Raily ecoraces, respectively while using sodium carbonate and sodium bi- carbonate softening process.

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OPTIMISATION DE LA PROCEDURE DE CUIITE DES COCONS TASAR POUR AUGMENTER LA RECUPERATION DE SOIE ET SA QUALITE AINSI QUE LA RETENTION DE LA COULEUR NATURELLE

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RESUME

Il est impératif de procéder à la cuite des cocons de soie afin de préparer l'extraction de son fil. Selon le processus conventionnel, une forte concentration de carbonate de sodium avec un savon neutre est utilisée pour ramollir les cocons tasar tropicaux et obtenir une extraction de fil facile et une rétention de la couleur naturelle. Cependant, les fortes concentrations alcalines non seulement élimine la sérécine mais altèrent la qualité de la fibroïne ce qui diminue les caractéristiques textiles du filament de soie. Le décreusage enzymatique permet de vaincre cet inconvénient mais n'est pas devenu populaire dans les unités de soie tasar en raison de la durée de trempage et des obstacles technico-économiques. Le peroxyde d'hydrogène et la cuisson au savon des cocons tasar est une autre méthode qui permet une élimination adéquate de la sérécine sans affecter la fibroïne mais ne conserve pas la couleur naturelle à cause du blanchiment oxydatif. Dans notre étude, nous montrons qu'une cuite efficace est obtenue en combinant le carbonate de sodium et le bicarbonate de sodium pendant le ramollissement des cocons tasar tropical (Daba BV, Modal et Raily) avec une meilleure élimination de la sérécine et la rétention simultanée de la couleur brune naturelle du fil. Une combinaison de 5 g/l des deux sels de sodium facilite une meilleure cuite des cocons Daba alors que 8 g/l et 10 g/l sont requis pour Modal et Raily respectivement. Ces concentrations varient en raison de l'épaisseur de la coque et de la présence de minéraux et aussi de la matière colorante naturelle associée à la sérécine.

Mots clés: Crossing over, Eprouvette, fibroïne, longueur du filament sans rupture, rendement en soie grège, dévidabilité, sérécine.



PRELIMINARY REPORT ON NUTRIENT COMPOSITION AND ANTIOXIDANT ACTIVITY IN THE PUPAE OF ERI SILKWORM, *SAMIA RICINI*

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ABSTRACT

Of late, eri culture has become popular in the northern part of West Bengal, particularly in the Cooch Behar district and its adjoining areas. The eri pupa is considered a delicacy and consumed mainly by the rural tribal communities as a protein supplement. Besides, serving as a source of food, eri culture offers an excellent livelihood option thus, contributing significantly to the sustainable environment and socio-economic development of the farmers. The present study analyzed the nutrient composition in the pupae of the eri silkworm, *Samia ricini* through proximate analysis. The result revealed presence of high amount of protein (62.85 ± 1.75 %), fat (22.13 ± 0.25 %) and carbohydrate (3.36 ± 0.002 %) in the pupae of eri silkworm. The antioxidant activity was determined by using standard antioxidant assays viz., DPPH, ABTS and superoxide radical. The methanolic pupae extract (MPE) of eri silkworm showed good extent of DPPH, ABTS and superoxide radical scavenging activity with IC_{50} values of 13.63 ± 0.81 , 65.68 ± 1.37 and 118.57 ± 0.94 $\mu\text{g/ml}$, respectively. The scope of relying on eri pupae as natural resource of antioxidant in the development of protein rich food products is discussed.

Key words: Antioxidant activity, eri pupae, food product, proximate analysis.

INTRODUCTION

North-eastern region of India including West Bengal is home to many species of edible insects including a diverse silkworm fauna (Sarmah, 2011). All the five types of commercially exploited silk varieties comprising of eri and muga are produced in this region. Eri culture has been in practice mainly in Assam and its neighbouring states i.e., Meghalaya, Nagaland, Manipur, Mizoram and Arunachal Pradesh since time immemorial. It has been a short period since eri culture has become popular in the northern part of West Bengal, particularly in Coochbehar and its adjoining areas. Non-mulberry sericulture has a close link with the socioeconomic life of the people. The eri (*Samia ricini*) pupa is considered a delicacy and consumed mainly by the rural tribal communities as a protein supplement. On the other hand, the muga (*Antheraea assamensis*) pupae are found to be suitable as high protein substitute for fish meal used for chick diet.

The possibility of feeding silkworms to provide edible animal protein with high quality during long-term spaceflights and lunar-based missions was investigated on several aspects (Yang *et al.*, 2009). Recently, Montana State University in the United States has been doing some pioneering work to promote insect as food and feed item (Van Huis *et al.*, 2013). Though the importance of silkworm as an alternative source of animal protein has been realized, perusal of literature revealed that little efforts have been made in India on the estimation of biochemical parameters in eri silkworm. Therefore, the present study was undertaken to evaluate the nutritional composition and antioxidant activities in the pupae of eri silkworm, *S. ricini* through biochemical analysis.

MATERIALS AND METHODS

The pupae of the eri silkworm, *S. ricini* collected from the farmers of Coochbehar district of West Bengal and

authenticated by a renowned sericulturist, were used for the present study. The following methodologies were adopted.

Preparation of sample

The collected eri silkworm pupae were first washed thoroughly to remove foreign particles and then dried in hot air oven at 60 °C overnight. The dried pupae were collected, ground into powder and subjected to proximate analysis. The dried pupae powder was mixed with distilled water and centrifuged at 3000 rpm for 20 min, spray-dried and ground into fine powdered material to prepare the pupae extract as per the method of Deori *et al.* (2014). Ten grams of the fine pupa powder was dissolved in 100 ml of methanol and filtered after 48 hours. The filtrate was concentrated under reduced pressure using a rotary evaporator at low temperature (<40 °C) and the methanolic pupae extract (MPE) thus prepared was kept at 4 °C for assessing its antioxidant properties through DPPH, ABTS and superoxide radical scavenging assays.

Evaluation of nutritional composition

Moisture, crude fibre and ash contents were assayed by different methods (Sadasivam and Manickam, 1996). The dried pupae were weighed and kept in a hot air oven at 60°C until a constant weight was obtained to calculate moisture content of the sample. The difference between initial and final weight was calculated and the moisture content is represented as percentage. The total crude fat content was determined following the soxhlet extraction method (ACAC, 1984). The total crude fat content was determined following the soxhlet extraction method (AOAC, 1984). The ash content was estimated in defatted pupae powder ignited in a muffle furnace at 600 °C until a constant weight was obtained and per cent value was calculated. The defatted pupae powder was used to estimate the total nitrogen content following the micro-Kjeldahl method (Kjeldahl, 1883; Goel, 2007) and then multiplied by the factor, 6.25 to determine the actual crude protein content. The carbohydrate content was obtained by subtracting the sum of moisture, protein, fat, ash and crude fibre from hundred.

Assessment of antioxidant properties

The antioxidant properties of the MPE was assessed through various methods *viz.*, DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) and superoxide radical scavenging assays as described below.

DPPH assay

The free radical scavenging activity of MPE was determined through DPPH assay following the method of Brand-Williams *et al.* (1995). The solution of DPPH in methanol (6×10^{-5} M) was prepared prior to spectroscopy. The sample of MPE was added to DPPH solution in 1:1 ratio followed by vortexing. The reaction was allowed to take place in dark condition at room temperature under nitrogen atmosphere. The absorbance at 517 nm was measured in UV-Vis Spectrophotometer at different time intervals keeping ascorbic acid as control. A decreasing intensity of the purple color was taken as increasing scavenging activity.

ABTS assay

The spectrophotometric analysis of ABTS⁺ radical cation(s) scavenging activity was carried out following the method of Re *et al.* (1999) with slight modification. The method is based on the ability of antioxidants to quench the ABTS⁺ radical cation, a blue/green chromophore with characteristic absorption at 734 nm. The ABTS⁺ was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulphate at a ratio of 1:1 (v/v). The mixture was allowed to react in dark condition for about 12 h at room temperature. The ABTS⁺ solution was diluted to get an absorbance of 0.750 ± 0.025 at 734 nm with sodium phosphate buffer (0.1 M, pH 7.4) just before usage. Approximately, 1 ml sample of MPE was added to 2 ml of ABTS⁺ solution and the absorbance value was measured at 734 nm comparing with trolox as standard.

Superoxide radical assay

The superoxide radical scavenging activity was measured following the method of Nishikimi *et al.* (1972) with slight modification. Superoxide radicals were generated in a PMS-NADH system by oxidation of NADH and assayed through reduction of NBT. The reaction mixture contained 1 ml of MPE, 1 ml of NBT solution and 1 ml of NADH solution. Approximately, 100 µl of PMS was added to the reaction mixture after 5 min of incubation. The reactant was incubated at 25 °C for 30 min and absorbance was measured at 560 nm against ascorbic acid as a control.

The inhibition percentage of radical scavenging activities of DPPH, ABTS and superoxide was calculated using the following formula.

$$\text{Inhibition (\%)} = \{(A_0 - A)/A_0\} \times 100$$

where,

A_0 is the absorbance of assay without sample and

A is the absorbance of assay with sample.

The result was expressed as the IC_{50} value. The IC_{50} value (concentration providing 50 % inhibition) was calculated from the plotted graph of scavenging activity against the concentrations of the sample.

RESULTS AND DISCUSSION

Nutritional composition

The nutrient composition as determined through proximate analysis on a dry weight basis in the pupae of eri silkworm, *S. ricini* is presented in Table 1. The crude protein content estimated was 62.85 % which is found even higher than the earlier reports (54 %) (Longvah *et al.*, 2011). A comparatively higher content of protein was also reported in the waste pupae of mulberry and tasar silkworm (Rao, 1994; Zhou and Han, 2006). Estimation of fat is one of the important steps towards nutritional evaluation of any sample (Ayaz *et al.*, 2006). The presence of an appreciable content of crude fat (22.13 %)

Table 1: Proximate analysis of pupae of eri silkworm (per 100 g)

Nutrient component	Mean ± SD
Moisture (%)	8.59 ± 1.31
Protein (%)	62.85 ± 1.75
Fat (%)	22.13 ± 0.25
Ash (%)	1.34 ± 0.13
Crude fibre (%)	1.73 ± 0.67
Carbohydrate (%)	3.36 ± 0.002

Note: Values are mean ± standard deviation of triplicate analyses

in the pupae of eri silkworm demonstrates its promising nutritional attributes for dietary purposes. The fat content at this high level also represents a good source of oil. An almost equal degree of fat content (20 %) was detected earlier in the pupae of the eri silkworm by Deori *et al.* (2014). The high content of protein and fat in the pupae of eri silkworm may offer immense potential for mitigating the calorie and protein deficiency encountered in the diet in a developing country, such as India.

Antioxidant properties

The antioxidant activity of methanolic extract of the eri pupa was assessed through various free radical scavenging assays *viz.*, DPPH, ABTS and superoxide with characteristic absorption at 517, 734 and 560 nm, respectively. Antioxidants prevent formation of free radicals via donating proton to these radicals, hence, the

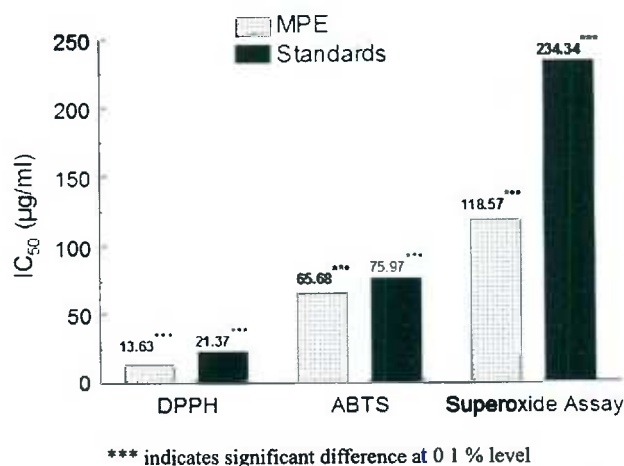


Figure 1: Free radical scavenging activity of MPE of eri silkworm pupa

absorption of scavenging activity tend to decrease. The decrease in the absorbance was taken as a measure to assess the radical scavenging potency of the MPE of eri silkworm. A lower IC_{50} value indicates a higher free radical scavenging activity. The results exhibited strong scavenging activity of MPE on DPPH, ABTS and superoxide radicals with IC_{50} values of 13.63, 65.68 and 118.57 $\mu\text{g/ml}$, respectively (Figure 1). In the case of DPPH and superoxide free radical scavenging assays, MPE was compared with the standard, ascorbic acid. The scavenging activity of MPE and ascorbic acid showed percentage inhibition of 59.32 and 68.92 %, respectively at a concentration of 32 $\mu\text{g/ml}$ in DPPH assay while, 22.69 and 17.25 %, respectively at a concentration of 40 $\mu\text{g/ml}$ in superoxide assay (Figures 2 and 3). The free radical scavenging activity of MPE was compared with trolox through ABTS radicals and maximum percentage inhibition of 65.36 (MPE) and 62.75 % (trolox) was

recorded at a concentration of 100 $\mu\text{g/ml}$ (Figure 4).

DPPH has been used extensively to determine the free radical scavenging activity of various samples due to its relative stability (Jha *et al.*, 2014). Deori *et al.* (2014) reported the IC_{50} value of methanolic pupae extract of muga silkworm to be of 25.83 $\mu\text{g/ml}$. High DPPH scavenging capacity at IC_{50} value of 60.58 $\mu\text{g/ml}$ was recorded in the silkworm pupae powder (Pachiappan *et al.*, 2016). Wu *et al.* (2011) observed a high DPPH scavenging activity of protein hydrolysates from larval instars of silkworm at IC_{50} value of 57.91 $\mu\text{g/ml}$. The antioxidant properties in the peptides derived from seedlings of wheat and mung bean have been studied and the small sized peptides reported to be more effective scavengers of free radical, such as ABTS, DPPH, nitric oxide and superoxide (Jha *et al.*, 2016).

The interest in antioxidants of natural origin as food and

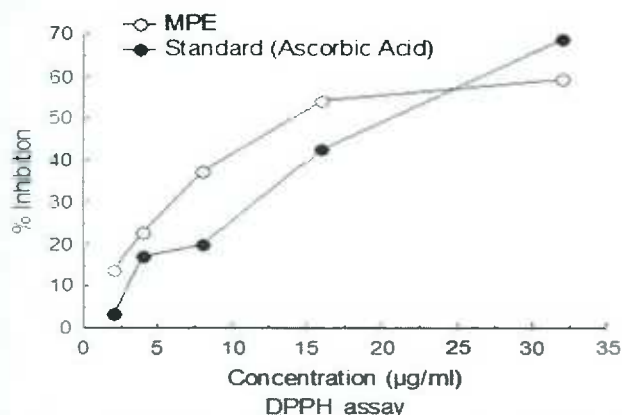


Figure 2: DPPH radical scavenging activity of MPE of eri silkworm pupa and ascorbic acid

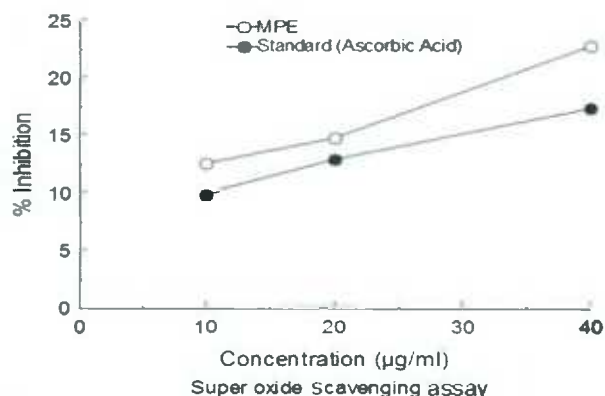


Figure 3: Superoxide radical scavenging activity of MPE of eri silkworm pupa and ascorbic acid

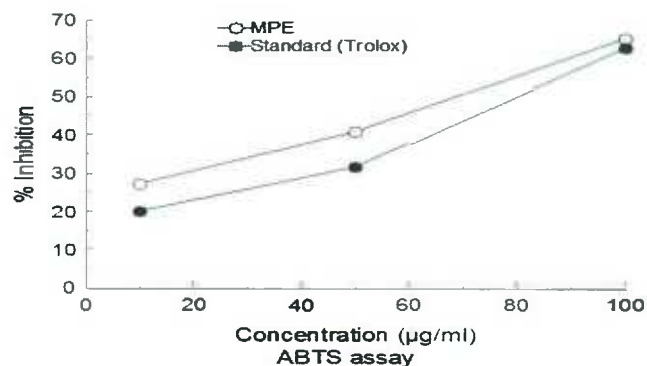


Figure 4: ABTS radical scavenging activity of MPE of eri silkworm pupa and trolox

health supplements has been rising greatly since the last few decades because of their potential to reduce the risk of several disease outbreaks in the human body without any toxic effect (Aruoma, 2003). The silkworm, in particular, has attracted the attention of scientists and academicians from across the globe mainly due to their important pharmacological properties in curing several ailments (Kwon *et al.*, 2012). The high protein content in the pupa of eri silkworm with strong antioxidant properties as revealed in the present study clearly indicates prospects of eri pupa as a valuable source of edible insect protein, rendering them profitable and applicable for food industry.

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RAPPORT PRELIMINAIRE SUR LA COMPOSITION NUTRITIONNELLE ET L'ACTIVITE ANTIOXYDANTE CHEZ LES PUPES DU VER A SOIE ERI, *SAMIA RICINI*

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RESUME

L'élevage eri est devenu populaire dans la partie nord du Bengale de l'Ouest, particulièrement dans le district Cooch Behar et les zones environnantes. La pupe de eri est considérée délicate et consommée dans les communautés tribales rurales comme complément protéique. Comme source alimentaire l'élevage de éri est une option excellente qui contribue à un environnement durable et un développement socio-économique des fermiers. Notre étude analyse la composition nutritionnelle de la pupe du ver à soie éri, *Samia ricini*. Les résultats montrent une forte quantité de protéines ($62,85 \pm 1,75\%$), de lipides ($22,13 \pm 0,25\%$) et d'hydrates de carbone ($3,36 \pm 0,002\%$) chez la pupe du ver à soie éri. L'activité antioxydante a été déterminée par l'analyse standard DPPH, ABTS et radical superoxyde. L'extrait méthanolique (MPE) montre un bon niveau de DPPH, ABTS et radical superoxyde avec des valeurs IC_{50} de 13,63, 65,68 et 118,57 $\mu\text{g/ml}$. Nous discutons l'utilisation de la pupe de éri comme ressource naturelle d'antioxydant dans un aliment riche en protéines.

Mots clés: Activité antioxydante, pupe éri, produit alimentaire, analyse.proxi.



TECHNOLOGICAL EFFICIENCY IN SERICULTURE AS AN IMPACT OF CLUSTER PROMOTION PROGRAMME IN BELLARY DISTRICT, KARNATAKA, INDIA

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ABSTRACT

The technological efficiency accomplished in sericulture as a function of implementation of CPP (Cluster Promotion Programme) was assessed by employing technological decomposition model following Bisalaiah (1972). The results elucidated remarkably high increase in the net profit to the tune of 87.39 % per acre per year. The overall impact of CPP in terms of technological efficiency in sericulture was to the degree of 64.43 %. The areas identified as deserving more attention were water harvesting and management and soil health improvement. Since labour use efficiency in sericulture was found higher, farmers need to be convinced of paying higher wages to attract labour and in order to exploit labour use efficiency in sericulture production.

Key words: Cluster promotion programme (CPP), technological efficiency.

INTRODUCTION

Sericulture is a labour intensive and agro-based activity with high employment potential as well as higher income turnover. That is how sericulture has flourished in India, the state of Karnataka being its leading producer. Traditionally, sericulture in Karnataka is mainly concentrated in the southern part. Of late, there has been a setback in sericulture with reduction in mulberry acreage in southern Karnataka due to urbanisation and labour shortage leaving lot of scope for development of sericulture in northern Karnataka. In Karnataka, the major production is multivoltine silk of inferior quality which does not match with the international grade. To meet the international grade of silk, promotion of bivoltine silk is must. In this regard, efforts have been made in the 11th five year plan to promote bivoltine sericulture programme through cluster approach by selecting 22 clusters in Karnataka. This cluster promotion programme (CPP) turned out to be successful and prompted bivoltine sericulture throughout sericulture states in India during

the 12th five year plan. Likewise, 46 clusters have been identified in Karnataka of which, 14 clusters are located in northern Karnataka.

The cluster promotion programme was planned and initiated during 2012-13. It's noteworthy that North Karnataka especially, Bellary district has responded very well to this programme with successful completion of 2 years of the 12th plan. On an average, Karnataka state produces 1235 MT of raw silk per year of which, Bellary district alone contributes 98.20 MT of raw silk (2016-17). Under this backdrop, the present study was taken up to assess the technological efficiency as an impact of CPP on sericulture development in north Karnataka clusters, by selecting Bellary district as a case.

METHODOLOGY

From Kudligi and Hagaribommanahally clusters of Bellary district, 60 farmers (30 farmers per cluster) were selected at random. Both primary as well as secondary data

regarding sericulture development in the study area were collected and subjected to critical analysis using statistical models. Averages, percentages and indices were used for analysing the data.

For technological efficiency, Cobb-Douglas production function analysis was employed followed by the technological decomposition model as described by Bisalaiah (1972). Production function analysis was employed as an analytical tool for assessing the relationship between the net profit per acre per year (Y) and seven independent variables. However, before identifying the independent variables for analysis, zero order correlation matrices were worked out and the correlation coefficients were examined for multicollinearity between independent variables. The variables identified for the functional analysis were as follows.

$$Y = f(X_1, X_2, X_3, X_4, X_5, X_6, X_7)$$

Where, Y = Gross profit from sericulture in rupees per acre per year

- X_1 = Cost of fertilizers per acre per year
- X_2 = Cost of FYM per acre per year
- X_3 = Cost of chemicals per acre per year (mulberry + silkworm)
- X_4 = Cost of labour per acre per year (mulberry + silkworm)
- X_5 = Number of silkworm crops
- X_6 = DFLs brushed per acre per year
- X_7 = Price of cocoon per kg

Taking into account the economic and statistical considerations, Cobb-Douglas production functions were fitted to establish the functional relationship between the dependent variable (Y) and the independent variables (X_i 's).

$$Y = \alpha X_1^{\beta_1} \cdot X_2^{\beta_2} \cdot X_3^{\beta_3} \cdot X_4^{\beta_4} \cdot X_5^{\beta_5} \cdot X_6^{\beta_6} \cdot X_7^{\beta_7} \cdot e^u$$

Where ' α ' is the constant, β_i 's are the regression

coefficients which also represent the production elasticity coefficients and 'u' is the random disturbance term independently distributed with zero mean and finite variance. The parameters of the function ' α ' and β_i 's were estimated using least square regression analysis by converting the functions into log linear forms.

$$\ln Y = \ln \alpha + \beta_1 \ln X_1 + \beta_2 \ln X_2 + \beta_3 \ln X_3 + \beta_4 \ln X_4 + \beta_5 \ln X_5 + \beta_6 \ln X_6 + \beta_7 \ln X_7 + U$$

.....(1)

Separate production functions were fitted for the data related to years 2013 (before CPP) and 2016 (after CPP). In order to study the technological difference between 2013 and 2016, the model following Bisalaiah (1972) was adopted to decompose the technological change into different components.

Chow's-F test was employed to test the difference between two production functions (2013 and 2016). The formula given by Chow (1960) is as below.

$$\text{Chow's } F = \frac{\sum u^2 - (\sum u_1^2 + \sum u_2^2)/k}{(\sum u_1^2 + \sum u_2^2)/(n_1 + n_2 - 2k)}$$

Where, $\sum u^2$ = Unexplained residual sum of squares of the pooled samples of both 2016 and 2013

$\sum u_1^2$ = Unexplained residual sum of squares of samples corresponding to 2016 yields

$\sum u_2^2$ = Unexplained residual sum of squares of samples corresponding to 2013 actual yields

k = The number of parameters included in the regression model.

n_1 = Sample size for 2013

n_2 = Sample size for 2016

($n_1 = n_2$ in the present analysis)

The F-test is carried out at k, ($n_1 + n_2 - 2k$) degrees of freedom

Difference between two production functions is as follows.

$$[\ln Y_m - \ln Y_n] = [\ln \alpha_m - \ln \alpha_n] + \sum [\beta_{mi} \ln X_{mi} - \beta_{ni} \ln X_{ni}] + [U_m - U_n] \dots\dots\dots(2)$$

Subscripts 'm' and 'n', indicate 2016 and 2013, respectively of CPP data. Subscript $i = 1 \dots 7$ (independent variables).

By simultaneously adding and subtracting $\sum \beta_{mi} \ln X_{mi}$ in (2), and rearranging the terms, we get the following equation (3).

$$[\ln Y_m - \ln Y_n] = [\ln \alpha_m - \ln \alpha_n] + [\sum (\beta_{mi} - \beta_{ni}) \ln X_{mi}] + \sum [\beta_{mi} (\ln X_{mi} - \ln X_{ni})] + [U_{mi} - U_{ni}] \dots \dots (3)$$

The resultant equation (3), helped in decomposing the total percentage change in the net profits (Y) into the components *viz.*, (i) neutral technological efficiency, (ii) non-neutral technological efficiency and (iii) efficiency / change due to input use as represented by the first three bracketed expressions on the right hand side of each equation. The last bracketed expression indicates the differences in the error term.

RESULTS

Input-output analysis in sericulture

Table 1: Input-output analysis

Particulars	2013	2016	% increase
Gross returns per acre per year (₹)	120755	198556*	64.43
Mulberry farm size (Acre)	1.53	1.59	3.92
X1 Cost of fertilizer (₹)	5162	4218*	-18.29
X2 Cost of FYM (₹)	24621	45863*	86.28
X3 Cost of chemicals (₹)	4123	5712*	38.54
X4 Cost of labour (₹)	12012	13167*	9.62
X5 No. of crops	4.31	4.39	1.86
X6 DFLs /ac/yr	640	756	18.13
X7 Price (₹)	342	356	4.09
Labour MD	52	57	9.62
Labour wage/ MD (₹)	153	231	50.98
Cocoon price (₹)	356	392	10.11
Cocoon yield/100 DFLs (kg)	53	67	26.42
Total cost /ac/yr (₹)	61278	87104	42.15
Net profit (₹)	59477	111452	87.39

*Prices discounted for 2016 wherever necessary

Table 1 presents input-output analysis in sericulture. The net profit as impact of CPP has risen from ₹59,477.00 (2013) to ₹1,11,452.00 (2016) registering 87.39 % increase per acre per year. This was mainly due to enhanced cocoon yield levels in response to intensive farming as indicated by increased cost of FYM (86.28 %) and decreased cost of fertilizers (18.29 %). Further, there was 18.13 % increase in the no. of DFLs taken up for rearing per acre per year. Because of increase in DFLs consumption, the labour requirement has gone up by 9.62 %.

Table 2: Cobb-Douglas production function coefficients

Particulars	2013	2016
Intercept	0.58	1.67
X1 Cost of fertilizers	0.18	0.16
X2 Cost of FYM	0.55	0.60
X3 Cost of chemicals	0.32	0.58
X4 Cost of labour	0.56	1.03
X5 No. of crops	0.83	0.63
X6 DFLs /acre/year	0.14	0.08
X7 Price	0.52	0.57
R ²	73 %	68 %
F - Test (5 %)	S	S

Table 2 Indicates coefficients of Cobb-Douglas production functions fitted to explain the relationship between inputs and output as explained in the methodology, for the data of 2013 and 2016. Both the production functions significantly explained the relationship to the extent of 73 % (2013) and 68 % (2016). Further, technological decomposition analysis was undertaken and presented in Table 3.

Neutral technological change which is due to change in the intercept, accounted for 8.25 % of the total change. The non-neutral technological change was 50.93 % which was mainly due to technological efficiency in the use of labour (34.46 %) followed by chemicals (16.89 %). Technological efficiency due to change in input use was to the extent of 5.25 % which was mainly due to increased use of FYM (2.91 %). Total technological efficiency in sericulture as an impact of CPP was estimated to be 64.43 %.

Table 3: Technological decomposition on the impact of CPP in sericulture, 2013 and 2016

Source of technology change		% Change (2013 & 2016)
1. Neutral technological change		8.25
2. Non - neutral technological change		
X1	Cost of fertilizers	-1.33
X2	Cost of FYM	3.95
X3	Cost of chemicals	16.89
X4	Cost of labour	34.46
X5	No. of crops	-2.28
X6	DFLs /acre/year	-3.03
X7	Cocoon price	2.28
Sub Total 2		50.93
3. Change due to input use		
X1	Cost of fertilizers	-0.25
X2	Cost of FYM	2.91
X3	Cost of chemicals	1.48
X4	Cost of labour	0.74
X5	No. of crops	0.09
X6	DFLs /acre/year	0.10
X7	Cocoon price	0.18
Sub Total 3		5.25
GRAND TOTAL		64.43

Conclusions and policy implications

The study reveals a significant impact of CPP which resulted in increased net profit to the extent of 87.39 % and the gross returns by 64.43 %. Neutral technological efficiency accounted for 8.25 % of the total change. The non-neutral technological change was 50.93 % which was mainly due to technological efficiency in the use of labour (34.46 %) followed by chemicals (16.89 %). Technological efficiency due to change in input use was to the extent of 5.25 % which was mainly due to extensive use of FYM.

Further, it's worth mentioning that the areas which need a thrust are water harvesting and management and soil health improvement. In the present days, labour problem is reported everywhere in agriculture in general and sericulture in particular. Since labour use efficiency in sericulture is higher, the farmers need to be convinced of paying higher wages (either in cash or in kind) in order to attract labour so that it can be exploited for sericulture production.

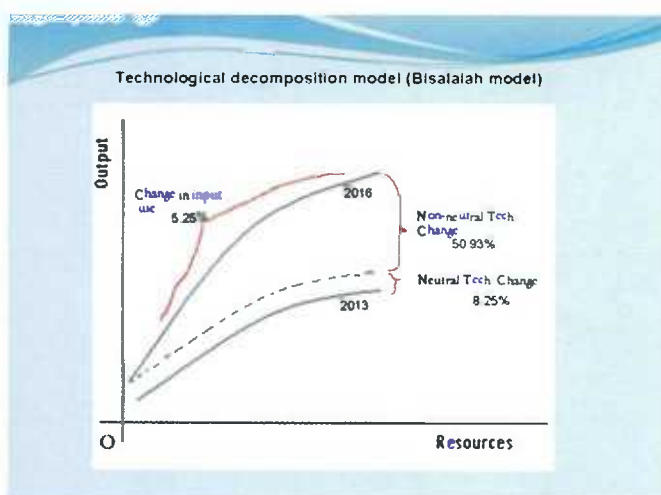


Figure 1: Technological decomposition analysis for sericulture as an impact of CPP

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EFFICACITE TECHNOLOGIQUE EN SERICICULTURE COMME IMPACT DE PROGRAMME DE PROMOTION DE GROUPE DANS LE DISTRICT DE BELLARY, KARNATAKA, INDE

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RESUME

L'efficacité technologique atteinte en sériciculture comme support du CPP (Cluster Promotion Program) a été obtenue en employant le modèle selon Bisalaiah (1972). Les résultats montrent clairement une forte croissance du revenu net de l'ordre de 87,39 % par acre et par an. L'impact global du CPP en termes d'efficacité technologique en sériciculture est de l'ordre de 64,43 %. Les domaines identifiés comme méritant plus d'attention sont la récolte et la gestion de l'eau et l'amélioration de la santé du sol. Alors que l'efficacité de l'utilisation du travail est très élevée les fermiers ont besoin d'être convaincus de payer de meilleurs salaires pour attirer le travail afin d'exploiter son efficacité en sériciculture.

Mots clés: Cluster promotion programme (CPP), efficacité technologique.



KARYOTYPE CHARACTERISTICS IN THREE SPECIES OF MORACEAE

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ABSTRACT

This study reveals the karyotype characteristics of three species of mulberry namely, *Morus cathayana*, *Morus nigra* and *Morus multicaulis*. Among these, the first two are diploids ($2n=28$) and the third one is uneuploid ($2n=30$). These species were found to possess three types of chromosomes with their size ranging between 0.98 and 2.90 μm . Both the diploid as well as the uneuploid species displayed two mega chromosomes. In all the species, invariably, the chromosomes at metaphase exhibited characteristic clumping and secondary association indicating the sticky nature of their terminal ends and also their hybrid nature.

Key words: Chromosome numbers, karyotype, mitosis, mulberry (*Morus* spp.).

INTRODUCTION

Many taxa of Moraceae are well known for their high economic value as they are resources that find use in sericulture, industries, medicine manufacture, aquaculture, agro-forestry, water-shed management and drought prone area developmental programmes (Bari, 1990; Kannan and Misri, 1990; Dandin *et al.*, 1992; Munirajappa *et al.*, 1995). Genetic improvement of mulberry is an important step to enhance its leaf quality and productivity. Since, the cytological information at the species level is much limited, generation of basic information in this line is highly essential. Triploids ($2n=42$) developed through natural or controlled hybridization between diploid and tetraploid parents are considered superior to diploids in leaf yield and nutritive quality (Venkatesh and Munirajappa, 2012). The information on polyploidy and breeding system of different mulberry ployploids are useful in evolving elite genotypes required for commercial cultivation. In the present study, chromosome numbers and karyotype of three species are discussed.

MATERIALS AND METHODS

For studying the somatic chromosome numbers and karyotype, young root tips were collected between 9.30 to 10.30 am and pretreated with 0.5 % colchicine for 3-4 hours at 10 °C. Then they were washed in water and fixed in 3:1 ethanol-acetic acid and the shoot tips were fixed in 6:3:1 Methanol:Chloroform:Propionic acid. Root tips were hydrolyzed in 1N HCl at 60 °C for 10 minutes and stained with Feulgen stain. Well stained root tips were squashed in 45 % acetic acid and covered with a cover slip. The temporary slides were observed and well spread metaphase plates were selected for karyotype analysis. The chromosome pairs were classified based on arm ratio according to nomenclature system for chromosome type, of Levan *et al.* (1964)

RESULTS AND DISCUSSION

Observations recorded in respect of somatic chromosome numbers and absolute chromatin length of haploid set of chromosomes as well as karyotype formulae of the three species are presented in Table 1.

Table 1: Karyotype characteristics of different *Morus* spp.

Species	Somatic chromosome number (2n)	Karyotype formulae	Absolute chromatin length of haploid set (µm)
<i>Morus nigra</i> var. <i>nigra</i>	2n=28	7M+5m+2sm	19.14
<i>M. cathayana</i> var. <i>cathayana</i>	2n=28	9M+3m+2sm	23.06
<i>M. multicaulis</i> var. <i>multicaulis</i>	2n=30	7M+4m+4sm	27.84

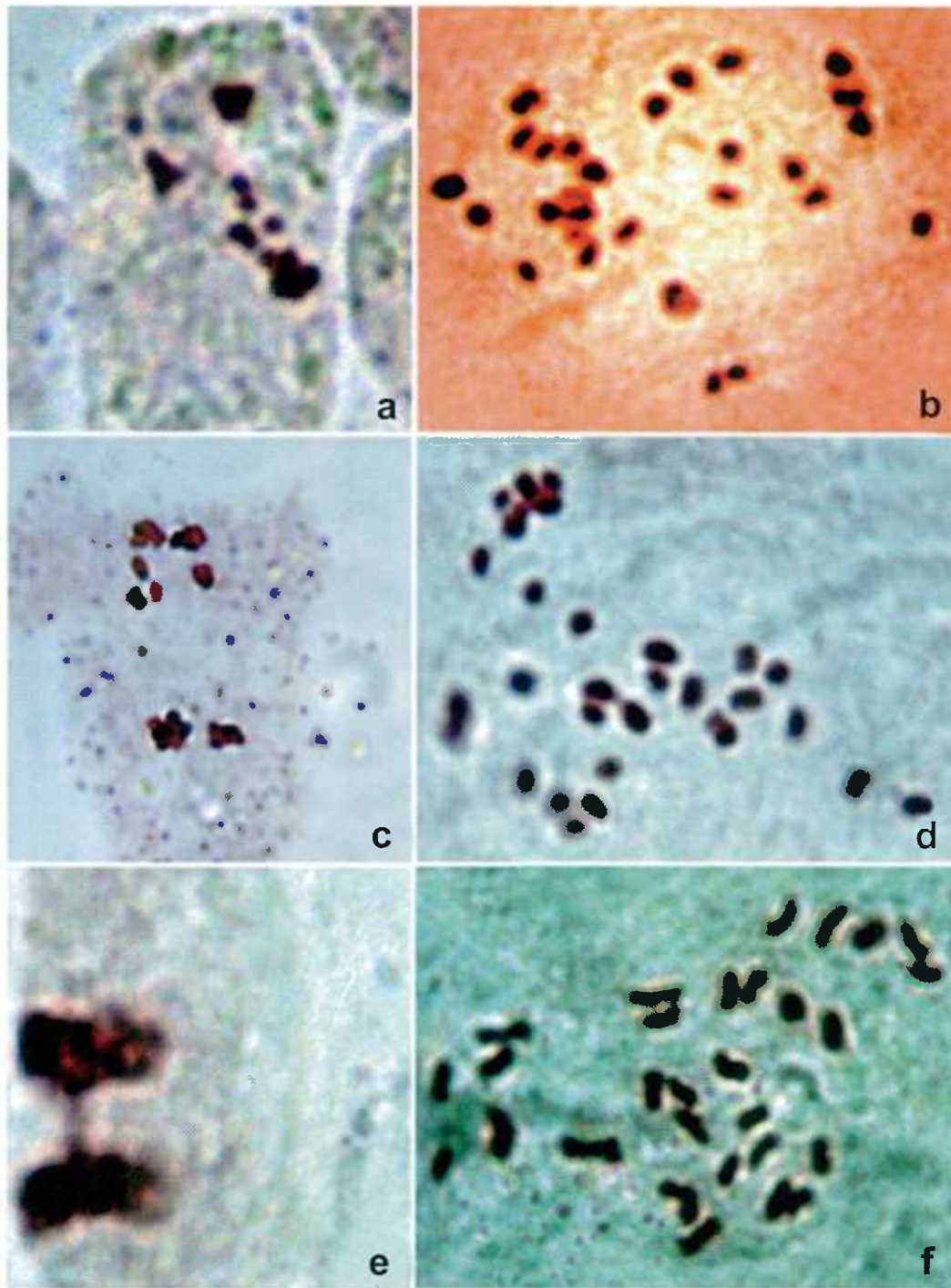
The concept of karyotype was developed by Levitsky (1931). The karyotype represents the amount of genetic and non-genetic chromatin and may characterize cluster of species within a genus, the entire genus or a group of genera (Jones, 1984). The genus *Morus* as a whole is characterized by small chromosomes. The present study also showed that the length of chromosomes vary between 0.98 and 2.90 µm. The longest chromosomes were recorded in *M. multicaulis* while the shortest ones in *M. nigra*. A wide gap in length existed between that of the shortest and the longest chromosomes, the range in variation being gradual among the group. The chromosome compliments in all the three species showed only three types *i.e.*, M, m and sm-type (Table 1) and in all these cases, they were having median centromere (M-type). This is in confirmation with previous reports. The number of SAT chromosome also varied between one and three pairs. Dandin *et al.* (1987), Susheelamma *et al.* (1990) and Venkatesh (2007) have reported bi-model type karyotype with only M-type and sm-type of chromosomes, which is contrary to the present investigation. Stabbins (1971) opined that bi-modal karyotype represent a specialized genomic architecture. Chakraborti *et al.* (1999) have also reported three or four types of chromosomes in a complement and established clearly the varietal differences in karyomorphology of some popular cultivars as reported in the present study. Levan *et al.* (1964) reported that the location of the centromere is a constant in each variety.

All the three species showed characteristic karyotypes. The chromosomes were comparatively longer in *M.*

multicaulis and the shortest in *M. nigra*. Naturally, the absolute chromatin length is reported high in uneuploid and low in the case of diploids. The uneuploid *M. multicaulis* showed slightly higher total chromatin than that of the diploids. Among the diploids, the total chromatin length was more in *M. cathayana* and less in *M. nigra*. At the species level, the karyomorphological features were distinct. *M. cathayana* showed significant differences from *M. nigra* by having more number of M-type chromosomes. Evolution of the taxa was attributed to structural changes in the karyotype and phylogenetic relationship among the varieties of each species based on their karyotypes.

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Figures 1: Display of chromosomes during mitotic metaphase: Association and clumping of chromosomes at mitotic metaphase of *Morus nigra* var. *nigra* (a), *Morus cathayana* var. *cathayana* (c) and *Morus multicaulis* var. *multicaulis* (e). Root tip cell showing 28 chromosomes ($2n=28$) each of *Morus nigra* var. *nigra* (b) and *Morus cathayana* var. *cathayana* (d) and 30 chromosomes ($2n=30$) of *Morus multicaulis* var. *multicaulis* (f) at mitotic metaphase.

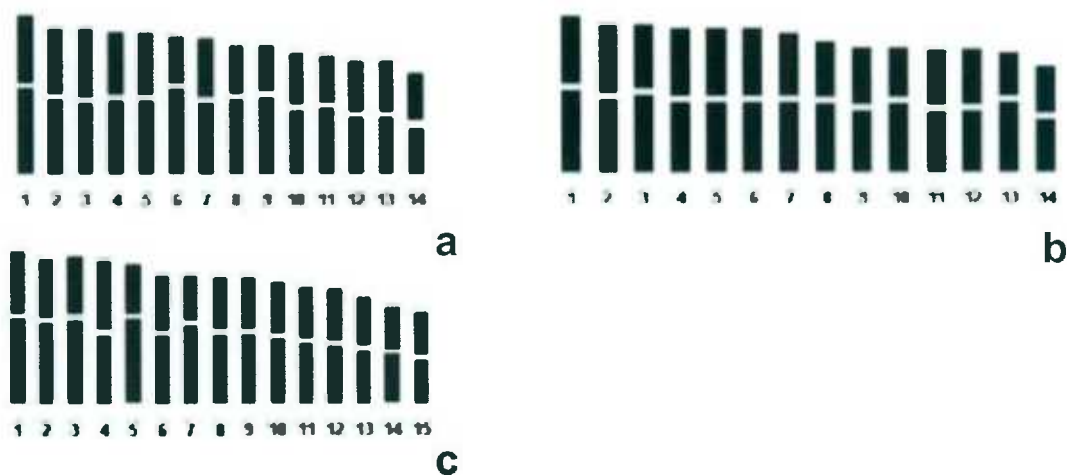


Figure 2: Ideograms of *Morus* spp.: (a) *Morus nigra* var. *nigra*, (b) *Morus cathayana* var. *cathayana* and (c) *Morus multicaulis* var. *multicaulis*.

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CARACTERISTIQUES DU CARYOTYPE CHEZ TROIS ESPECES DE MORACEES

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RESUME

Cette étude donne des informations sur le nombre de chromosomes, et les caractéristiques des caryotypes de trois espèces de mûrier, *Morus cathayana*, *Morus nigra* et *Morus multicaulis*. Les deux premiers sont diploïdes ($2n=28$) et le troisième aneuploïde ($2n=30$). Ces espèces possèdent trois types de chromosomes de tailles variant de 0,98 à 2,90 μm . Toutes les espèces possèdent deux mégachromosomes. A la métaphase les chromosomes montrent des touffes et des associations secondaires indiquant la nature adhésive de leurs extrémités et leur nature hybride.

Mots clés: Nombre de chromosomes, caryotype, mitose, mûrier (*Morus* spp.).



Report

INDIAN SILK INDUSTRY: EXPLORING NEW HORIZONS

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ABSTRACT

In the backdrop of many sericulture countries witnessing a decline in silk production, a plethora of opportunities open up before countries like India to excel in the world market but for the challenges, especially the quality. To cross this quality barrier, the country needs a shift from the present multivoltine to bivoltine silk production. Product design and diversification, reviving traditional products, adoption of weaving / printing techniques / skills, color contrasts, design vocabulary, use of natural dyes, development of new products, such as silk stretch fabric, light weight denims and silk knitted fabrics would serve as alternative strategies that can attract the global market. There is a vast scope for generating by-products of industrial importance and application in food, feed, defense, cosmetics, pharmaceuticals and nutraceuticals as well.

Key words: Bivoltine silk, design vocabulary, nutraceuticals, product diversification, silk stretch fabric.

INTRODUCTION

Silk is one of the oldest natural fibers known to the mankind and remains as the most loved one the world over. With its unparalleled grandeur, silk has reigned as the unconquered '*Queen of Textiles*' for centuries. Luxury, elegance, class, natural sheen, inherent affinity for dyes and rich colors, light weight, poor heat conduction, resilience and excellent drape are some of its irresistibly endearing qualities. Though silk is a high value textile and consumed more by the higher strata of the society, its production has been regarded as a vital tool for the economic development of a country because of highly employment oriented, low capital, labour intensive and remunerative nature of the enterprise. There are five major types of silk of commercial importance, obtained from different species of silkworms which in turn feed on a number of food plants. They are mulberry, tropical tasar, oak tasar, eri and muga.

The silk industry is unique with a base of agricultural output *i.e.*, cocoons which are variable in nature. It is a highly labour intensive, decentralized traditional industry. The production and processing approaches

applicable to other textile sectors do not go with the silk industry. The nature of the silk industry is also quite complex that encompasses reeling, twisting, weaving, knitting, wet processing comprising degumming, dyeing, printing and finishing besides ancillary support mechanism of market research, exports, garment and machinery manufacturing. There is also a vast scope for generating by-products of industrial importance and application.

Global Silk Industry

Silk is produced by more than 60 countries in the world, of which the Asian countries account for more than 90 % of mulberry silk production and almost 100 % of the non-mulberry silk production (Datta and Mahesh Nanavaty, 2005). China is the dominant player in world silk market with silk output of around 1,20,000 MT during 2018 (Table 1). It accounts for almost 75 % of the global silk production and enjoys a formidable position in the world silk market (Kosh, 2003). India is the second largest producer of mulberry silk with the share of about 22 % of the global raw silk production. China and India together accounted for about 98 % of the global raw silk production

of 1,59,855 MT during 2018. Uzbekistan, Thailand, Brazil, Vietnam, North Korea and Iran are the other major silk producing countries.

Table 1: Global raw silk production during 2011 to 2018

Country	Raw silk production (MT)							
	2011	2012	2013	2014	2015	2016	2017	2018
China	104000	126000	130000	146000	170000	158400	142000	120000
India	23060	23679	26480	28708	28523	30348	31906	35468
Uzbekistan	940	940	980	1100	1200	1256	1200	1800
Thailand	500	450	475	692	698	712	680	680
Vietnam	655	655	680	420	450	523	520	680
Brazil	558	614	550	560	600	650	600	650
North Korea	300	300	300	320	350	365	365	350
Iran	120	123	123	110	120	125	120	110
Others	153	149	149	148	132	133	116	117
Total	130286	152910	159737	178058	202073	192512	177507	159855

Source: International Sericultural Commission

The major consumers of the silk products are Western European countries and the United States of America (USA). These countries do not produce raw silk. However, the European countries *viz.*, Italy, France, Germany, the United Kingdom (UK) and Switzerland import raw silk and convert it into finished products for their domestic as well as export requirements.

Global silk industry is going through a transition. Barring a few other countries, such as India and Uzbekistan, silk production has been on a decline during recent years as sericulture is being replaced by other industries. Hence, the present situation offers an opportunity for India to excel in the world market. The global trade in silk goods accounts for less than 0.2 % of the world textiles fiber trade (International Trade Centre, 1999). China, with its huge silk production base is undoubtedly world leader in silk trade. It has almost 40 % of the share in the global trade in silk products followed by Italy, France and India (Kumaresan, 2016).

Silk production in India

Sericulture is an age-old practice and India has the unique distinction of producing all the five commercially known

varieties of silk. The annual production of raw silk in India was 35,468MT, of which mulberry raw silk alone aggregated to 25,344 MT in 2018-19 and the rest is *Vanya* silks, which include tasar, eri and muga. Bulk of the mulberry silk produced in India is of multivoltine, which is of low quality compared to that of bivoltine silk (Kumaresan, 2002; Rajesh, 2012). In addition to superiority in production of quality silk, the bivoltine silkworms yield significantly higher quantities of cocoons and thus supposed to be more remunerative to farmers. Therefore, India has been making consistent efforts to broaden the bivoltine base and has met with a significant success. The bivoltine silk production has been growing in the country at an incredible rate of 19.46 % per annum during 2011-12 to 2018-19 (Okhandiar and Kumaresan, 2019). The fast pace of growth in bivoltine silk production resulted in steep increase in the share of bivoltine silk production in total mulberry silk production in the country from 3.69 % in 1995-96 to 27.57 % in 2018-19. The *Vanya* silk production has also grown substantially in India from 4788 MT in 2011-12 to 10124 MT in 2018-19 at a compound growth rate of 9.81 % per annum.

Exploring new horizons

The authorities concerned are looking for other alternatives to make Indian silk more sellable at the global market. Product and design diversification, reviving traditional products, adoption of weaving / printing techniques / skills, development of new products and value addition to the by-products are the other ways to catch up the global market.

1. Unique products from low grade silk

In such vulnerable context, the term *market* demands further qualification. As we all know, the international market as it exists now is distinctly divided into two horizontal tiers based on the raw silk quality. The demand for industrially utilizable raw silk (*i.e.*, quality silk) grade is independent of demand for low grade silk *i.e.*, silk *sans* quality tag which is generally hand-woven. However, the lion share of such ungraded silk is domestically siphoned

off by our weaving clusters mainly for the production of *saris*. And interestingly, the defects *viz.*, slugs, striations *etc.*, are taken as special effects in the famous weaving traditions like *Kanjeevaram*! Secondly, coming back to the term market, available supply of silk cannot be shifted from one tier to another if they don't fulfill the technical requirements of that particular tier. As such, certain kind of upgradation is required may be through a conversion of this low-grade yarn to products with unique characteristics that attract a new and specialized market. The need of the hour is to consolidate the research output and convert the traditional art forms into contemporary exclusives suiting the world market. Further, there is a dire need to widen the export market base with new range of quality products beyond customers' expectations. In other words, evolving or inventing new products which are exempted from the minimum quality restrictions of the global market is the pressing priority besides popularizing the bivoltine silk. The harnessing of such *not much exploited* areas have already proved their worth elsewhere. Experience clearly hints that innovative producers who are able to identify and enlarge demand gaps have largely been overlooked by big producers! For example, Thailand could promote its low grade silk into highly appealing *Mutnee* silk and *tie-dye*. Likewise, the Kashmir carpet weaving out of silk waste is an excellent display of Indian expertise! Therefore, as has been already proved in the Thai and Kashmiri cases, the producers of excess quantities of low grade silk are able to produce unique products and carve for themselves a *niche* market which at the same time is a highly specialized one.

2. Exploiting traditional weaving skills

Basically, Indian silk industry has certain inherent strength. Thanks to the four varieties of silk and also its traditional weaving skills and expertise. India is known for its excellent traditional weaving, dyeing and printing skills. But so far, the trading of these products has been limited to the local markets. As such, offering a new look and market to these products could break the monotony. As far as printing is concerned, colour contrasts, design vocabulary and use of natural dyes have made our clusters

viz., *Nimach*, *Bagru*, *Bagh*, *Bhairogarh*, *Kalamkari*, *etc.*, unique. Using our much famed printing techniques, such as *Tie-dye*, *Patola*, *Bandhani* and *Batics* on different silk materials can go a long way in the Western market. Imitating the famous *Mekhala & Chaddar* design and diversification by infusing newer design sense into the traditional sari by replacing golden *Zari* with muga silk could enhance the market demand. Demand for diversified sari is obvious to exist.

The situation of hand loom industry is no different. Our traditional hand loom clusters need to be provided with innovative ideas in the areas, such as Tapestry weaving, *Jala* and multi shuttle techniques to churn out exclusive products for the ever demanding market. Besides, exploiting Indian hand arts *viz.*, *Kasuthi*, *Fulkari*, *Kuch*, *Zardosi*, *Chicken*, *Kanta* and *Cashmere* works add to the heritage silks. Today, the industry is being armed with special functional finishes *viz.*, oil and water repellence, aroma, anti microbial *etc.*, to catch the market. Keeping these aspects in view, a range of new products in terms of design, construction and end use have been developed and being developed by Central Silk Technological Research Institute (CSTRI), Bengaluru, India, as well.

3. Development of new products

Apart from reviving traditional products, the CSTRI and Product Development Cell of Central Silk Board (CSB) have developed a range of products with newer design concepts, construction and end use. To cite an example, the development of silk stretch fabric has widened the possibility of western fashion merchandising Indian silk blend dress material, stoles, upholstery & garments that are in demand. Attempts have also been made for production of light weight denims using mulberry and eri silk & its blends as warp and weft combinations to cater to the needs of ladies and kids wear. Silk denim garments are soft, light in weight and comfortable to wear in all seasons. The cell has explored the possibilities of developing silk knitted fabrics from Indian reeled silk as well.

Nowadays, though garment export is increasing on one

hand, import of high end garment domestically is also on the increase. However, to compete in the international market, we need to upgrade and stabilize the silk garment sector in terms of raw materials, stitch accessories, new designs equipped with high end finishing techniques. In other words, our export market base has to be widened with a range of quality products that reflect newness. Apart from showcasing the unique products it has developed, the CSB also helps the concerned to visualize / conceptualize and acquire the required technical know-how to develop such products. Their display includes exclusive silk apparels, home furnishings, life style products, knit wear, swatches, silk handicrafts *etc.* Further, ensuring purity of all the silk products through Silk Mark and popularizing them by participating in national and international exhibitions would only add new dimension to the promotion of Indian silk.

4. Value addition to the by-products

A considerable amount of waste is generated in sericulture industry. It is estimated that 16.49 million MT of by-products in terms of rearing waste is available from the present mulberry growing areas alone whereas pupa, which is a major by-product of reeling sector, is about 1,11,481 MT, available for proper value addition (Savithri *et al.*, 2016). While a part of the waste is converted into by-products, the majority of it remains underutilized or unutilized. Though the improved production / processing practices, skills and the state of the art machinery have helped reduce the production of waste to some extent, there is a dire need to intensify the efforts towards the better utilization of such waste. Efforts are being made by CSTRI to exploit the possibility of using silk / silk waste in the industries like cosmetics, pharmaceuticals, bio-medical, defense and nutraceutical apart from the routine textile uses. The rearing waste, such as pierced, cut, double, black, melted, flimsy, stained and *uzi* pierced cocoons and the wastes generated during cooking and reeling make major chunk of reeling waste. Apart from it, re-reeling waste and twisted silk wastes are also produced during winding, throwing and weaving. Silk being the low volume but high value item demands more attention

towards the best utilization and validation through by-product generation. The diversification of silk and silk by-products and silk waste utilization have a great potential to open up new vistas for the Indian silk industry.

Summary and conclusion

The steps like consolidating the research output and converting the art forms into contemporary exclusives, widening the export market base with new range of quality products, upgrading the low-grade yarn to products with unique characteristics that attracts a new and specialized market go a long way. In other words, evolving or inventing new products which are exempted from the minimum quality restrictions of the global market is the current need. Efforts are on to harness traditional skills coupled with improved technologies for different end uses which offer a wide range of opportunities for the entrepreneurs for design development, product diversification and generic promotion of Indian silk; promoting silk mark, collaborative R&D programs; *Vanya* silk market promotion through specific *Vanya* silk logo; brand promotion both nationally & internationally *etc.* However, with opportunities of growing demand for Indian silk products in the domestic as well as overseas markets; reduction in production of silk by traditional silk producing countries *viz.*, Japan, South Korea *etc.*; shifting of silk production activities like weaving, printing, garment manufacturing to Asian countries owing to high labour costs in the West, India could benefit enormously. CSTRI has already explored the possibilities of making specialized products, such as eri thermal wear, eco-products, bridal dresses, carpets, foot wear and umbrella, eri socks, made ups, home furnishings, life style products *etc.* The efforts of social enterprises like Bengaluru based *Kale -Nele* and *Vimor* of Bengaluru also deserve to be appreciated in conserving and redefining the traditional arts and crafts that encompass weaving as well.

By using social media also, one can effectively bring about a sea change by creating awareness about the innovative silk products, sharing the new concepts of design and development of newer products, and marketing them. And

for sure, Indian silk industry has got a bright future in the years to come.

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25th International Congress of ISC on Sericulture and Silk Industry

The '25th International Congress of ISC on Sericulture and Silk Industry' was held at Tsukuba, Japan during 19th to 22nd November 2019. The following are the takeaways of the Congress:

#	Particulars	Numbers
1	Total delegates	242
2	Countries represented	23
3	Research papers presented	150
3.1	Oral	105
3.2	Poster	45
4	International Awards presented	7
5	Enrolment of Countries in ISC - Uganda	1

The Congress was inaugurated by Mr. Rajit Ranjan Okhandiar, Secretary General, ISC. The inaugural ceremony was also addressed by Mr. Ko-ichi KADOWAKI, Vice-President of National Agriculture and Food Research Organisation, Japan and Ms. Yoko HASHIMOTO, Director for Regional Products & Industrial Crops, Agricultural Production Bureau, Ministry of Agriculture, Forestry and Fisheries, Japan. A video film on "Life of Silkworm" made by Dr. A. M. Babu, Scientist-D, (Retd.), Central Silk Board, India was released during the event.

Seven researchers from three countries were recognized by giving away the prestigious "Louis Pasteur Award" and the Award on "Excellence in Sericulture Science (ESS)". The profile of the awardees are given below:



A) Louis Pasteur Award - 2019



Dr. Keiko Kadono-Okuda
Japan

Major Contributions: An outstanding seri-biotechnologist with more than 30 years of experience in silkworm research. Her major contributions are,

- Findings that the imidazole compound (KK-42) synthesized as an anti-JH compound has inhibitory effect on ecdysone-synthesis
- Linkage analysis of EST cDNA clones covered all twenty-eight chromosomes in the silkworm, *Bombyx mori*
- Genome analysis of the silkworm, *Bombyx mori*, in the international consortium.
- Identification of DNV-2 (Bidensovirus)-resistance gene (nsd-2) and DNV-1 (Densovirus)-resistance gene (nsd-1) in the silkworm, *Bombyx mori*.
- Genome analysis of the common cutworm, *Spodoptera litura*, in the international consortium.



Dr. Thallapally Mogili
India

Major Contributions: An outstanding researcher and extension worker with more than 30 years of experience in sericulture sector. His major contributions are;

- Developed two high yielding mulberry varieties namely, G2 and G4.
- Developed and disseminated advanced package of practices for mulberry cultivation which substantially helped to enhance the bivoltine productivity in India.
- Developed a commercially viable model cluster for bivoltine, which has been recognised as the best bivoltine cluster in India. Consequently, the developmental model of this cluster is being replicated all over the country.
- Outstanding improvement at field level in all productivity parameters of commercial sericulture practice
- This initiation triggered in substantially improving the bivoltine silk production in India with annual growth rate of 12% for the last 7 years



Ms. Siriporn Boonchoo
Thailand

Major Contributions: Outstanding contribution for the development of Sericulture and silk industry in Thailand with more than 37 years of experience. Her major contributions are,

- Development of Silk Industry through enduring research and development, sericulture conservation and standard conformity assessment, post cocoon technology, sericulture economy and marketing, and Thai Silk Craft and Silk industry.
- Involved in establishment of germplasm bank.
- Introduced silk industry in 92 schools to create awareness
- Worked as a leader for ASEAN countries for sericulture development.
- Introduced smart agriculture to sericulture farmers thereby enhancing production, productivity and quality of silk.
- Strategic effort to stimulate demand of THAI silk in world market.
- Innovative silk marketing strategies like twinning products, cooperation between THAI silk and GUCCI, Italy, seri contract farming system, silk auction market, etc

B) Excellence in Sericulture Science (ESS) Award - 2019

Host Plant Development	 Mr. Prachachat Noppaseney Thailand	Achievement: Developed and released "Sakonnakhon" mulberry variety which is superior in leaf yield and widely accepted in the field.
Silkworm Development	 Dr. Tetsuya Iizuka Japan	Achievement: Development of silkworm races; "Hakugin" & "Hakurei" which produce extra fine silk, "Sericin Hope" that produces sericin, "Syurimaru" which produces large numbers of double cocoons and transgenic silkworm races which produces fluorescent silks.
Post Cocoon and Post Yarn Technology	 Mr. Thanupong Tanchailertthakul Thailand	Achievement: Developed various machineries required for silk under small and large scale utilization in Thailand, Laos, Cambodia and Philippines.
Silkworm and Silk in Non-textile Industry	 Dr. Hidetoshi Teramoto Japan	Achievement: Development of "Clickable Silk" for the first time in the world, which is expected to have a big impact on the industry. This study, the genetic code expansion of <i>B. mori</i> and the development of clickable silk, opened up a completely novel, versatile, and easy strategy to obtain functionalized silk materials, which would contribute to wider applications of <i>B. mori</i> silk in industrial and biomedical fields.

Executive Committee Meeting of ISC

The Executive Committee Meeting of ISC was held at "Conference Room No.-201" of International Congress Centre EPOCHAL, Tsukuba, Japan on 18th November, 2019. The meeting witnessed the participation of thirteen delegates from eight countries. The following are the major decisions taken in the EC meeting:

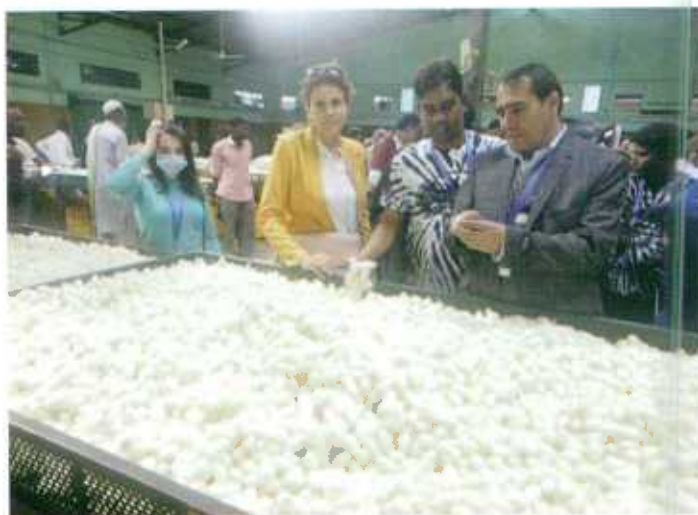
- 1) Formal enrolment of Uganda as the 21st Member Country of ISC.
- 2) Romania to organise the 26th ISC Congress at Cluj-Napoca during 17-22 May, 2022 under the theme; "Seritech- New Concepts in Sericulture".
- 3) The Govt. of Kenya to host the "International Workshop on "Development of Sericulture and Silk Industry in African Region" under the "Focus Africa Programme".



Visit of Programme Coordinator ISC to Philippines



As per the invitation of the Philippine Textile Research Institute (PTRI), Manila, Philippines, Mr. Dileep Kumar R., Programme Coordinator ISC attended the "Philippine Silk Summit" on 29-30 January, 2020. The Programme Coordinator presented a paper on the Role of ISC for the development of Sericulture and Silk Industry as a lead paper of the Summit. This occasion was also utilised to extensively campaign for the enrolment of Philippines as a Member Country of ISC. The visit was sponsored by the PTRI, Philippines.



Training on Post Cocoon Technology

The one month training on Post Cocoon Technology was held at Central Silk Technological Research Institute, Bangalore during 6th January to 2nd February, 2020. Twenty three trainees from thirteen countries; Afghanistan, Argentina, Bangladesh, Ethiopia, Ghana, Lithuania, Madagascar, Nigeria, Nepal, Syria, Tunisia, Uganda and Vietnam, attended the training. The training was fully sponsored under the Indian Technical and Economic Co-operation (ITEC) programme of Ministry of External Affairs, Govt. of India. Apart from the classroom and practical training classes, the trainees were also taken to the sericulture areas and facilities to understand the successful model of commercial sericulture practice developed in India for replication in their respective countries.





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