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STRUCTURAL CHARACTERISTICS OF ERI SILK COCOONS AND FIBRES

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ABSTRACT

Eri is one of the wild varieties of silk produced by the silkworm, *Philosamia ricini* (Lepidoptera: Saturniidae). The eri cocoons are soft and fibrous with white and brick red shades. Brick red eri cocoons are produced in Kokrajhar district of Assam state whereas, white cocoons are produced in other states of north-eastern region as well as some other places in India. No significant differences in cocoon quality parameters such as average cocoon weight and shell weight were observed between these two varieties. Degumming loss was reported 10 % for white variety and 12 % for brick red variety. Both the cocoon varieties consist of five distinct layers viz., floss, upper, middle, lower and pelade. Major quantum of i.e., about 90 % of silk fibres are present in the floss and upper layers, 5 % in the middle layer and the remaining distributed in the lower and pelade layers. Significant differences existed for silk fibre weight, single fibre denier and single fibre tenacity in different layers of eri silk cocoon. But no difference was noticed for single fibre breaking elongation in different layers. Regression equations between each of the parameters (average values) such as weight of degummed cocoon silk fibre, single fibre denier, single fibre tenacity, and single fibre breaking elongation vs the particular trait (average values) in different layers were established. Estimated R² and 't' values confirmed the significance of these regression equations.

Key words: Bave, cocoon wax, degumming, denier, floss, gland, pelade, shell, spinneret.

INTRODUCTION

The silkworm, *Philosamia ricini* (Lepidoptera: Saturniidae) produces the wild eri silk. It feeds mainly on leaves of castor (*Ricinus communis*) and kesseru (*Heteropanax fragrans*). In India, eri silk cocoons are produced in north-eastern states i.e., Assam, Meghalaya, Nagaland etc. The eri cocoon shells are soft and fibrous due to the presence of floss layer similar to that of mulberry. Eri cocoons cannot be reeled to produce yarn like in mulberry, tasar and muga due to the discontinuous filament as these are open at one end (Jolly *et al.*, 1979).

Studies have revealed that seven ecoraces of eri

silkworm, *Philosamia ricini* are available in north-eastern states of India along with six pure line strains. Borduar eco-race has higher cocoon and shell weight followed by Kokrajhar variety (Singh *et al.*, 2003). Rearing performance of eri silkworm on castor and kesseru in different seasons revealed that mature larval weight, cocoon weight as well as shell weight were maximum when fed on castor as compared to kesseru during autumn, winter, spring and summer seasons. Shell weight was recorded the highest during spring followed by autumn, summer and winter seasons (Singh *et al.*, 2011). Reddy and Yang (2010) studied the structure and properties of cocoons and silk fibres produced by *Hyalophora cecropia*, the wild Saturniid silk moth and reported that the cocoons

are made up of three distinct layers and alkaline degumming is necessary to extract fibres from the layers which can be used for spinning only. The outer and inner layers of *cecropia* cocoons have similar tensile properties whereas the intermediate layer has higher strength and modulus. Mechanical properties and structure of domesticated *Bombyx mori* (mulberry) as well as wild counterparts, *Antheraea assamensis* (muga), *Antheraea pernyi* (temperate tasar) and *Antheraea mylitta* (tropical tasar) silkworm cocoons showed that, wild silkworm cocoons have superior inter laminar peel resistance, out of plane compressive modulus and hardness than the domestic type. Calcium oxalate crystals are deposited on the outer layers of wild cocoons which do not contribute for improvement of inter laminar fracture toughness but provides superior hardness, forming the first defensive layer (Zhang *et al.*, 2013).

The assessment of mechanical properties of mulberry silkworm cocoon revealed that the elastic modulus, strength and thermo-mechanical parameters vary according to different layers and overall, both the static and dynamic properties of cocoons are superior (Zhao *et al.*, 2005). Eri (*P. ricini*) cocoons are soft and fibrous and the cocoon shells consist of multiple, thin and paper like layers (Komatsu, 1980). Analysis of cross section images of eri silk fibres and its secondary structures after treatment with different organic solvents reported that, eri silk fibres consist of porous as well as non-porous structure which may be due to the alanine residue composition as well as impact of temperature of the cocoon rearing places (Prasong *et al.*, 2011). Evaluation of physical characteristics and structure of Indian silk fibres revealed that from the outer layer to the inner layer of the cocoon shell, the fibres show a slight initial increase in linear density and then exhibits steady decrease for mulberry, temperate tasar, eri and muga varieties. Mulberry is the finest in linear density followed by eri, temperate tasar and muga. Cross section of mulberry silk fibre is triangular as against the rectangular structure of non- mulberry silk fibres. Mulberry silk has the highest density and more compact structure as compared to non-mulberry varieties. Birefringence data revealed a more of

oriented molecular arrangement in mulberry silk than the non- mulberry silks (Gupta *et al.*, 2000).

It was confirmed from the earlier studies that no experimental work was carried out so far regarding distribution of different layers in eri silk cocoon shell as well as quality characteristics of silk fibres in different layers of eri silk cocoons. Hence, the present study was taken up to ascertain these aspects.

MATERIALS AND METHODS

White eri silk cocoons of Kamrup district (sample S1) and brick red ones of Kokrajhar district (sample S2) of Assam State, India were used for this study. Sodium carbonate and neutral soap Extran MA02 (pH 6 to 8) of laboratory reagent grade from Merck, Germany were utilized for degumming of cocoons.

Measurement of cocoon and shell weight

The cocoon and shell weight of both white and brick red varieties were measured using Sartorius high precision electronic balance with capacity of minimum of 1 mg and maximum of 10 g. The shell percentage was estimated from the above values.

Fifty observations were made in total for each of the sample and the average, standard deviation as well as coefficient of variation were estimated.

Degumming of cocoons

For both white and brick red cocoons, degumming was carried out using 10 % sodium carbonate and 10 % neutral soap solution based on cocoon weight keeping material to liquor ratio as 1 : 20 (Chattopadhyay *et al.*, 2016). The duration of boiling was kept at 1 hour and 200 g cocoon sample was boiled for each variety. After degumming, the cocoon samples were washed thoroughly using hot and cold water, twice each. Then the samples were hot air dried and conditioned. Degumming loss (%) was estimated as following:

$$\text{Degumming loss (\%)} = \frac{(W_1 - W_2)}{W_1} \times 100$$

Where W_1 is the initial weight of conditioned eri cocoon samples and W_2 is the weight of samples after degumming and conditioning.

Conditioning of fibre samples

The fibre samples were kept at standard atmospheric conditions of 65 ± 2 % relative humidity and 20 ± 2 °C temperature for 24 hours before testing.

Cocoon layers

Due to the presence of sericin and cocoon wax (Komatsu, 1980), it is difficult to separate different layers of eri silk cocoon. During withdrawal, two or more layers slip out at once. Therefore, degummed eri silk cocoons which did not exhibit this behavior were used for this assessment. At first, fibres from the floss layers were withdrawn from single degummed cocoon. Weight of floss layer fibres was noted by using precision electronic balance. Then the fibres from subsequent layers of degummed eri cocoons were separated by withdrawing slowly with extreme care so that fibres from remaining layers did not slip out at once. Another four distinct layers of fibres were detected excluding the floss layer; the different layers being designated as floss (L1), upper (L2), middle (L3), lower (L4) and pelade (L5) layers. Weight of fibres of the remaining four layers of eri silk separated from degummed cocoon was measured with proper care.

Single fibre denier

The denier of single fibre from different layers of eri silk cocoon was measured by using Lenzing Vibroscope instrument (ASTM D 1577- 07, 2012). Single fibre of 20 mm length was placed in the instrument under the tension weight of 100 mg using tweezers with proper care so that no damage or stretching occurs. The denier of single fibre was estimated by the following expression (Booth, 1996).

$$M = \frac{Wg}{\lambda^2 f^2} \times 9 \times 10^5$$

Where, W is the pretension weight, g is the gravitational force, λ is the wavelength for oscillation and f is the natural fundamental frequency. In total, 50 observations were recorded for each fibre sample and the statistical parameters *i.e.*, average, standard deviation, minimum value, maximum value and co efficient of variation (%) were estimated by using software interface of the instrument.

Single fibre tenacity and breaking elongation

Single fibre tenacity and breaking elongation for different layers of eri silk cocoon were measured by using Lenzing Vibrodyn Instrument (ASTM D 3822- 14, 2014). The principle of the testing instrument is constant rate of extension (CRE). The gauge length was kept at 20 mm with pretension weight of 100 mg. The testing speed was maintained at 12 mm/min for all samples. Single fibre was mounted in between the jaws of the instrument by tightening the clamps after removing slack, without stretching the specimen. Extreme care was taken to ensure that the fibre specimen lies on the line of action between the force measuring device and the point where the fibre leaves the moving jaw face. A total of 50 readings were noted for each sample and the statistical parameters were estimated by using software interface of the instrument.

Analysis of data

The experimental data were analyzed using MINITAB17 statistical software for different quality parameters.

RESULTS AND DISCUSSION

Cocoon and shell weight

The cocoon weight, shell weight and shell percentage of both white and brick red eri silk are presented in Table 1. No significant differences existed for cocoon quality

Table 1: Cocoon parameters of eri silk

Parameter	White eri	Brick red eri
Cocoon weight (g)	3.10	3.20
Shell weight (g)	0.40	0.45
Shell percentage	12.90	14.10

Table 2: Fibre weight in different cocoon layers

Particulars	White eri	Brick red eri
Mean sum of squares	0.628	0.611
F- Value	1092.78**	522.13**
F- Value (table)		
5 % confidence level	2.37	2.37
1 % confidence level	3.32	3.32
R ² value	0.979	0.991
't' value	47.432**	73.611**

** Significant both at 5 and 1 % level of confidence.

parameters, between white and brick red eri silk varieties.

Degumming loss

The degumming loss was observed as 10 % for white and 12 % for brick red eri cocoons. Although this was not up to the level of a significant difference between the two varieties, the higher loss for brick red eri may be presumably due to removal of natural coloring substance from cocoon surface in addition to extraction of sericin, wax and total ash content (Komatsu, 1980).

Fibre weight

The fibres from five different layers extracted from degummed eri silk cocoons *i.e.*, floss (L1), upper (L2), middle (L3), lower (L4) and pelade (L5) layers are depicted in Figures 1 and 2 for white and brick red varieties, respectively.

The major quantum of silk fibres were present in floss and upper layers whereas, the lower and pelade layers possessed very little amount of silk fibres. Measurement of weight indicated that about 90 % of silk fibres were present in the floss and upper layers, 5 % in the middle

layer and the remaining in the lower and pelade layers for both varieties. Table 2 presents the results for weight of fibres in five different layers for both white and brick red varieties of eri cocoons. The interval plots for weight of fibres in different layers of white and brick red eri cocoons are shown in Figure 3.

Significant differences were observed (both at 5 and 1 % level of confidence) among fibre weight in different cocoon shell layers for both white and brick red eri silk cocoons. Interval plots (Figure 3) depicted that significant differences existed among floss, upper, middle, lower and pelade layers for fibre weight except between that of lower and pelade layers. Estimation for differences of mean values by Fisher LSD method confirmed the significance both at 5 and 1 % of statistical confidence level. Since the volume of liquid silk available in silk gland is of higher quantity, the silkworm throws out large amount of silk during the initial stage of spinning. But as the spinning

Table 3: Single fibre denier in different cocoon layers

Particulars	White eri	Brick red eri
Mean sum of squares	0.610	0.909
F- Value	43.99**	30.06**
F- Value (table)		
5 % confidence level	2.69	2.69
1 % confidence level	4.02	4.02
R ² value	0.996	0.986
't' value	36.222**	18.664**

** Significant both at 5 and 1 % level of confidence.

Table 4: Single fibre tenacity in different cocoon layers

Particulars	White eri	Brick red eri
Mean sum of squares	0.359	0.627
F- Value	2.43N	6.32**
F- Value (table)		
5 % confidence level	2.69	2.69
1 % confidence level	4.02	4.02
R ² value	0.913	0.939
't' value	7.225**	8.785**

N; Not significant; ** Significant both at 5 and 1 % level of confidence

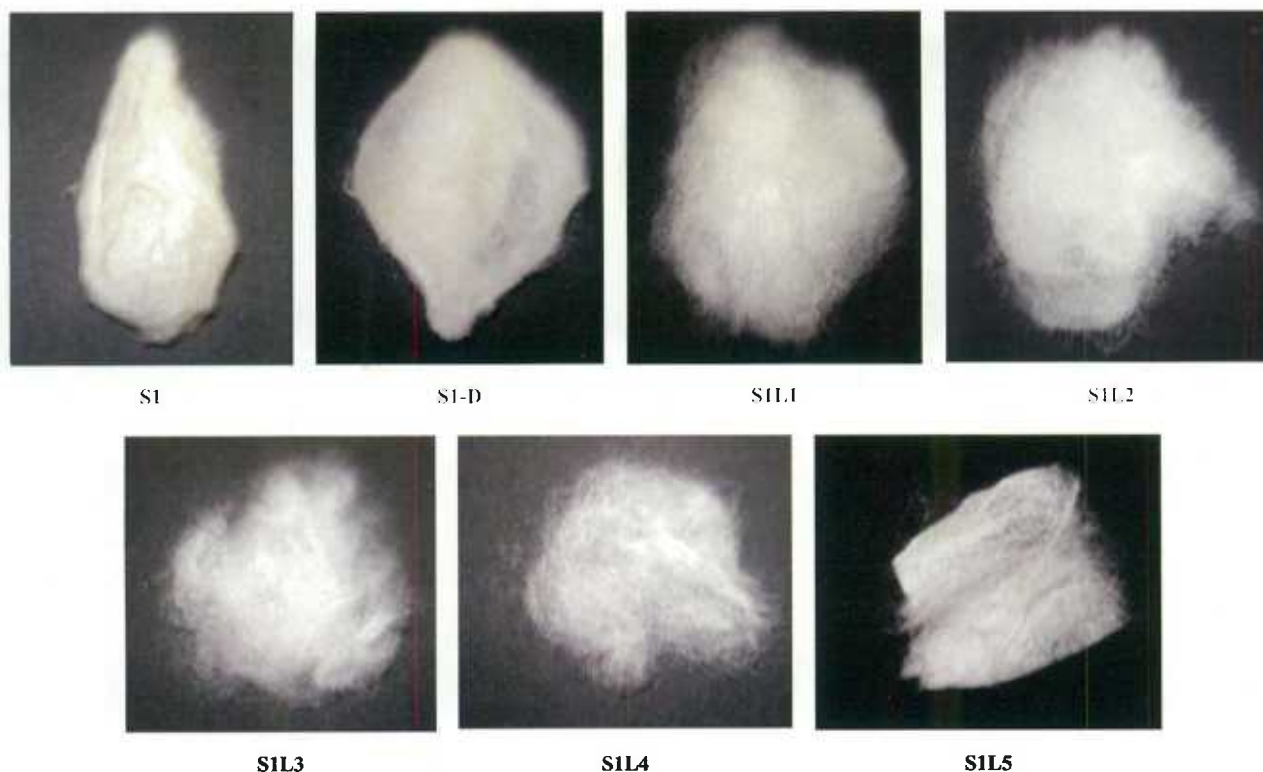


Figure 1: Fibres extracted from different layers of white eri silk cocoon

S1: White eri cocoon; S1- D: Degummed white eri cocoon; S1L1: Floss layer S1L2: Upper layer; S1L3: Middle layer; S1L4: Lower layer; S1L5: Pelade layer

process progresses, the volume of silk in the gland reduces thereby depositing less quantum of fibre in the cocoon shell. Minimum volume of liquid silk remains in the gland during the last phase of spinning and hence, the lower and pelade layers display less amount of silk fibres as compared to other layers. Studies revealed that the size (denier) of fibre gets finer from outer to inner layer of mulberry cocoons (Iizuka 1980; Gupta *et al.*, 2000). Hence, there is a decreasing trend being observed for silk fibre weight from floss to pelade layer.

The regression equation obtained between shell weight of degummed cocoon and weight of fibres in different layers for white variety is as follows.

$$Y_1 = 1.016W_1 + 1.009W_2 + 1.037W_3 + 0.270W_4 + 1.300W_5 + 0.001$$

Where, Y_1 is the average fibre weight of degummed

cocoon whereas W_1, W_2, W_3, W_4 and W_5 are the weight of fibre for floss, upper, middle, lower and pelade layers, respectively.

Similarly, in the case of brick red eri silk cocoons, the regression equation between shell weight of degummed cocoon and weight of fibre in different layers is as mentioned below.

$$Y_2 = 1.025W_6 + 0.997W_7 + 1.199W_8 + 0.596W_9 + 1.034W_{10} - 0.001$$

Where, Y_2 is the average fibre weight of degummed cocoon whereas, W_6, W_7, W_8, W_9 and W_{10} are the weight of fibre for floss, upper, middle, lower and pelade layers, respectively.

The R^2 value for white eri silk cocoon is 0.979 and for brick red variety is 0.991 (Table 2). The 't' values were

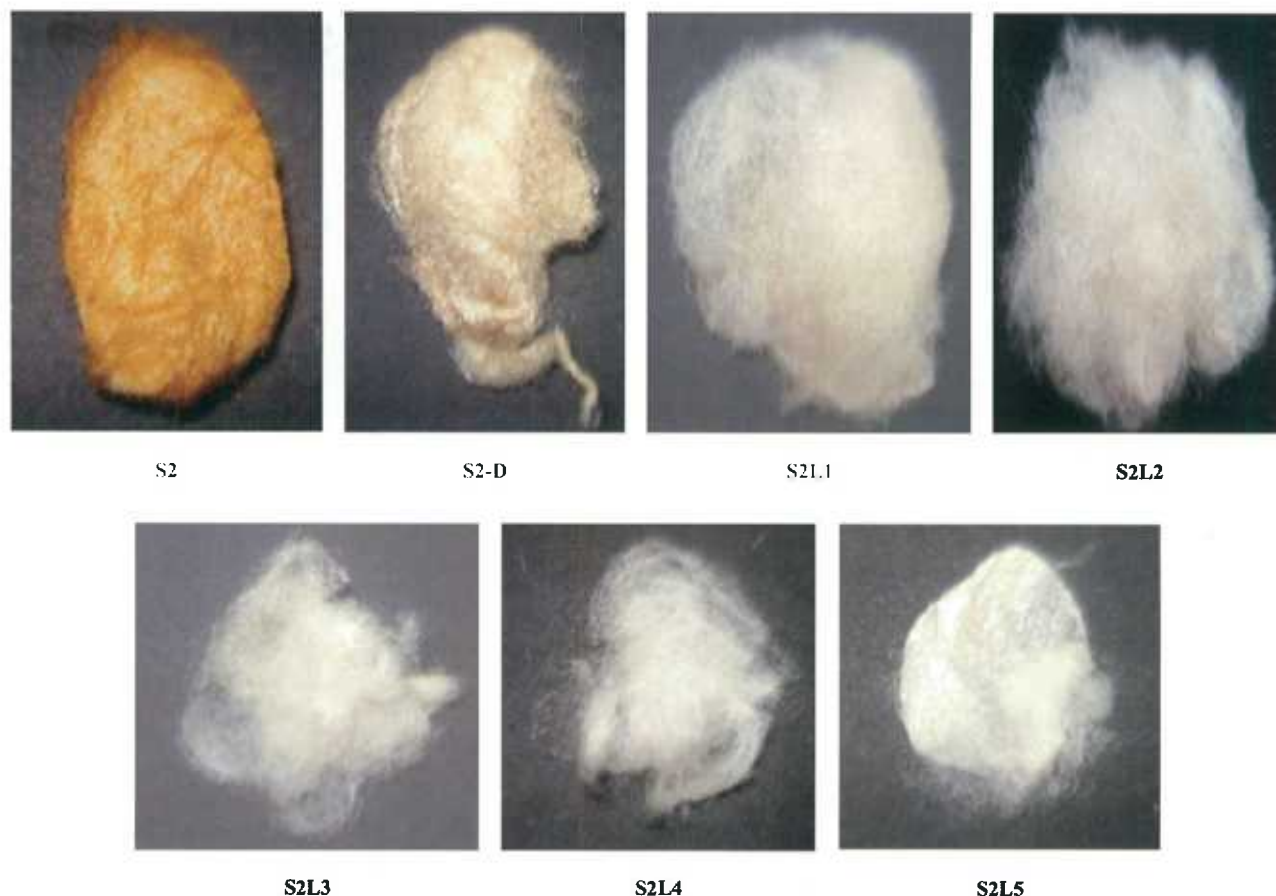


Figure 2: Fibres extracted from different layers of brick red eri silk cocoon

S2: Brick red eri cocoon; S2- D: Degummed brick red eri cocoon; S2L1: Floss layer S2L2: Upper layer; S2L3: Middle layer; S2L4: Lower layer; S2L5: Pelade layer

estimated at 47.432 and 73.611 for white and brick red cocoons, respectively (Panneerselvam, 2010). Both 't' values were significant at 5 and 1 % statistical confidence level at d.f. of 48. This is indicative of a strong correlation existing between the shell weight of degummed cocoon and fibre weight in different layers for both varieties of cocoon.

Single fibre denier

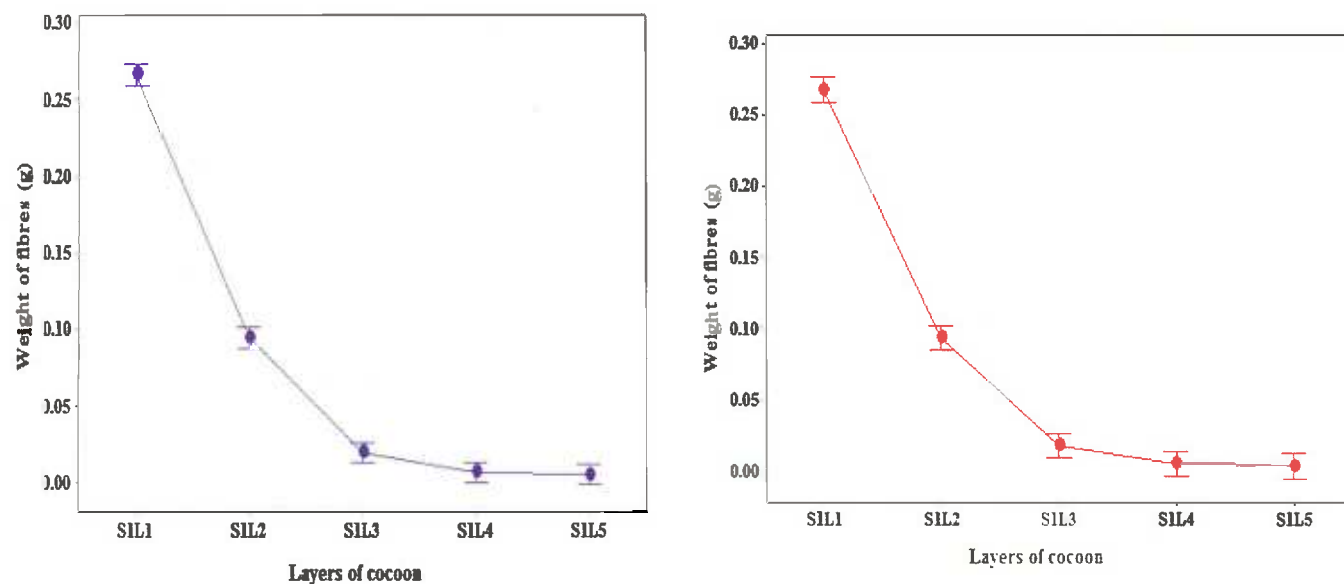
The average values for single fibre denier of different layers of degummed white and brick red eri silk cocoons at 95 and 99 % confidence level are shown in Figure 4.

It was observed that the denier of single fibre reduces

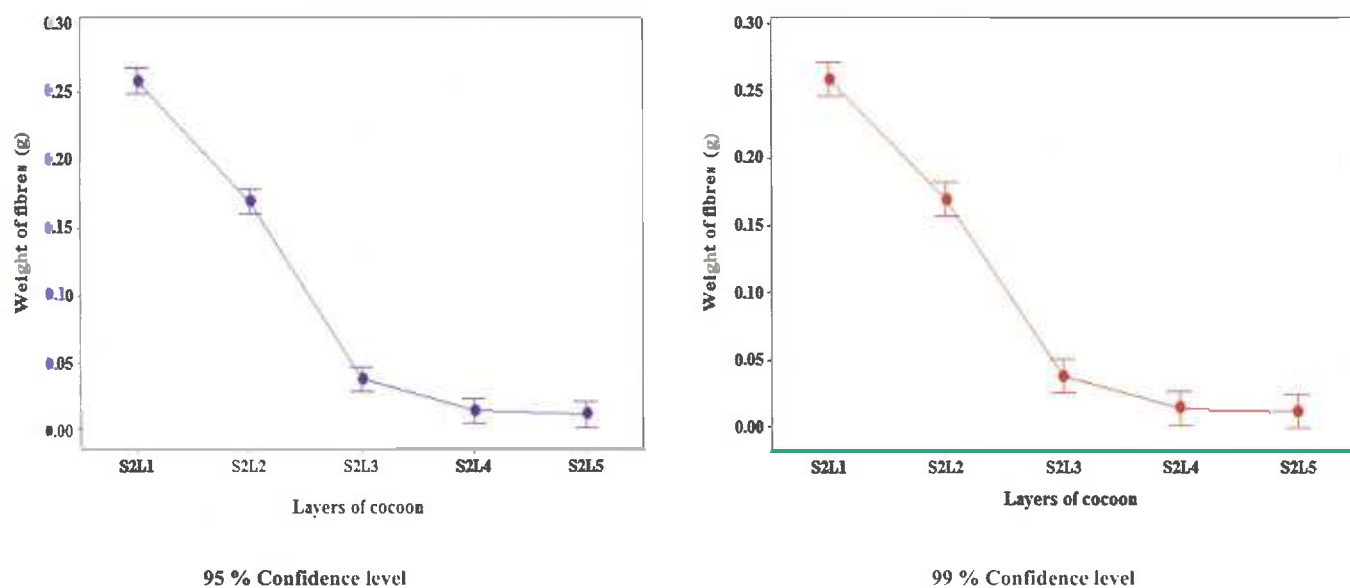
from floss to pelade layer in both the cases. The single fibre denier in different layers of white and brick red eri cocoons is presented in Table 3.

Significant differences (both at 5 and 1 % level of confidence) existed for single fibre denier among the five different layers of both white and brick red eri silk cocoons. Also, Fisher LSD method confirmed the significant difference for denier of single fibre in different layers both at 5 and 1 % level of confidence. For mulberry silkworm cocoons, the relationship between spinning speed and the thickness of cocoon fibre (Iizuka, 1980) is as following.

$$V = V_0 x e^{kd}$$



3 a: White eri cocoons



3 b: Brick red eri cocoons

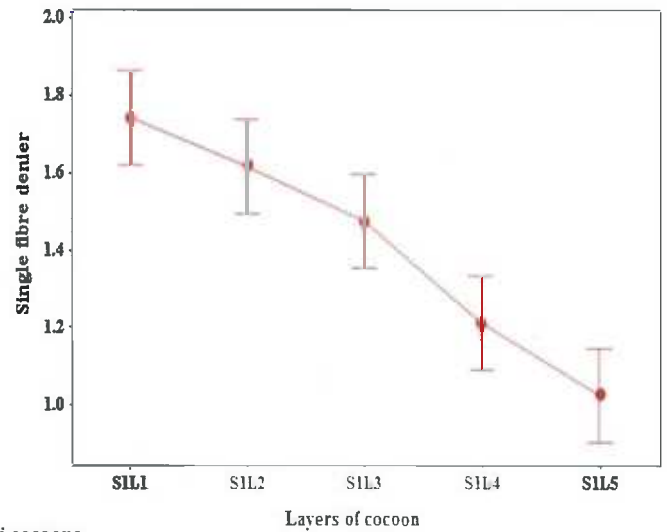
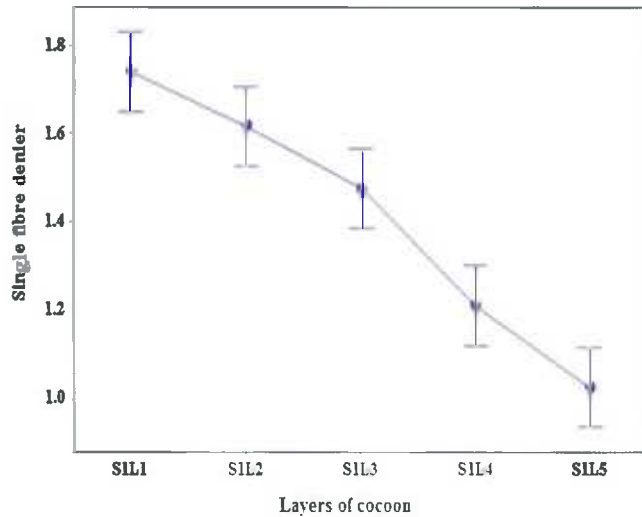
Figure 3: Interval plots for weight of fibres in different layers of eri cocoons

Where, V is the spinning speed, d is the thickness of cocoon fibre and V_0 and k are the constants. As the spinning rate decreases, the size of the bave (denier) gets finer. The spinning rate is higher at the initial stage and diminishes as the cocoon formation continues. Also, the size (denier) of bave gets finer from outer to inner layer of mulberry cocoons (Gupta *et al.*, 2000).

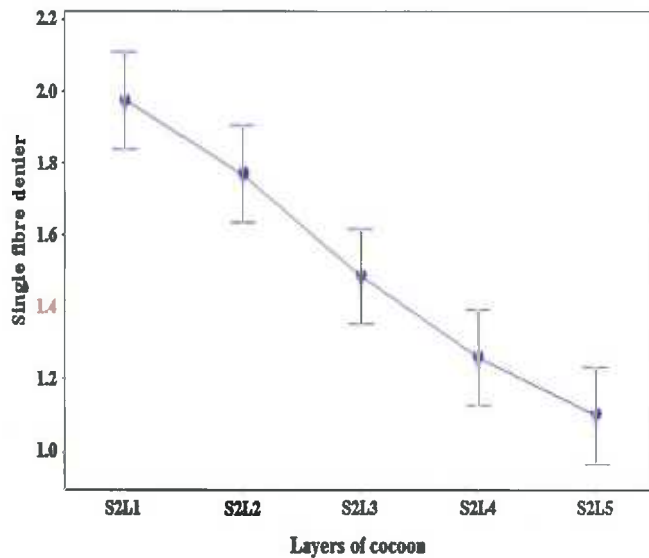
The regression equation obtained between average fibre denier and fibre denier in different layers of white eri cocoon is as follows:

$$Y_3 = -1.445D_1 + 3.586D_2 - 0.183D_3 - 2.989D_4 + 1.279D_5 + 0.919$$

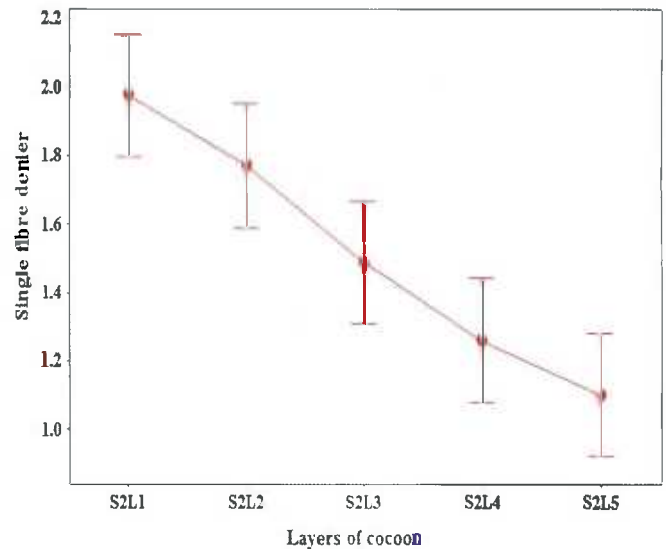
Where, Y_3 is the average fibre denier and D_1, D_2, D_3, D_4



4 a: White eri cocoons



95 % Confidence level



99 % Confidence level

4 b: Brick red eri cocoons

Figure 4: Interval plots for single fibre denier in different layers of eri cocoons

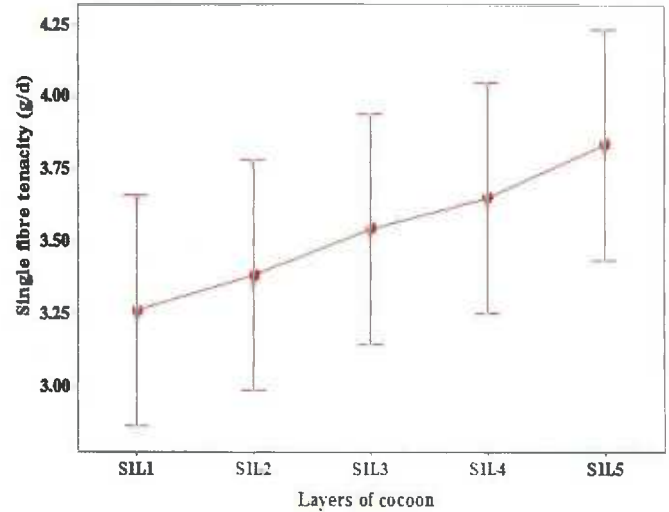
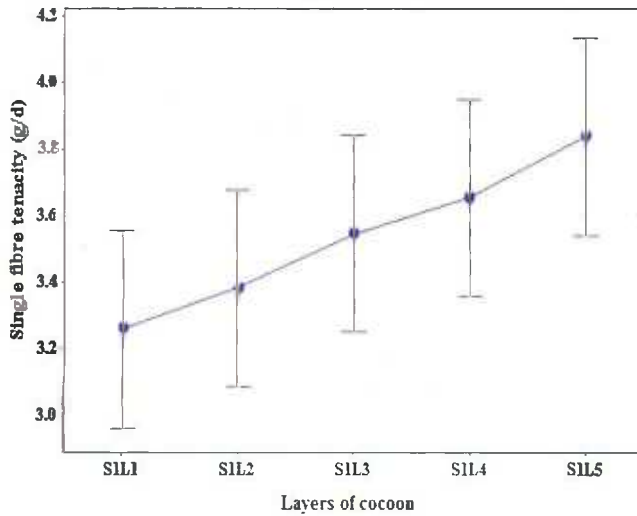
and D_5 are the denier of floss, upper, middle, lower and pelade layers, respectively.

Similarly, the regression equation worked out for brick red eri silk cocoons, between average fibre denier and denier in different layers is as follows:

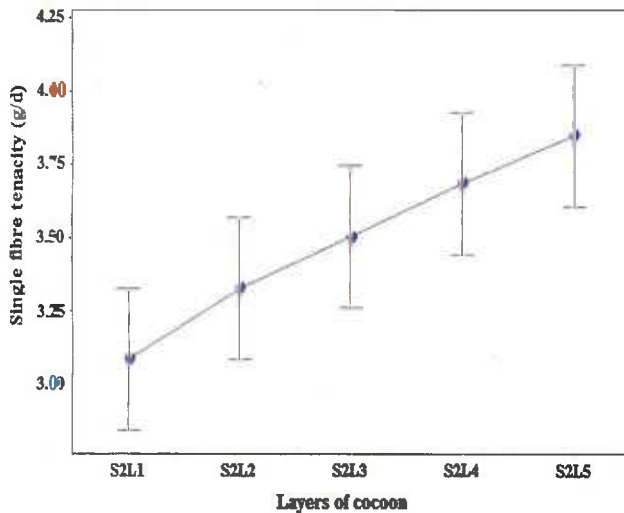
$$Y_4 = 0.809D_6 + 0.606D_7 - 0.866D_8 + 1.208D_9 + 0.988D_{10} - 2.210$$

Where, Y_4 is the average fibre denier and D_6 , D_7 , D_8 , D_9 and D_{10} are the denier of floss, upper, middle, lower and pelade layers, respectively.

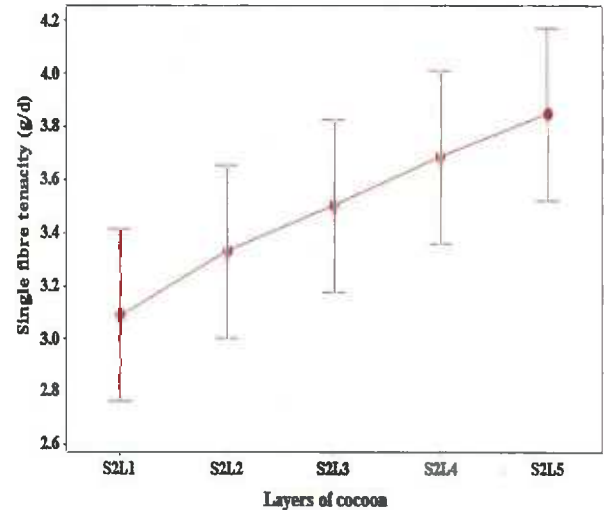
The R^2 values for white and brick red eri cocoons are 0.996 and 0.986, respectively (Table 3). The respective 't' values are 36.222 and 18.664. Both 't' values were



5 a: White eri cocoons



95 % Confidence level



99 % Confidence level

5 b: Brick red eri cocoons

Figure 5: Interval plots for single fibre tenacity in different layers of eri cocoons

significant at 5 and 1 % statistical confidence level at degrees of freedom of 5 which points towards a strong correlation between average single fibre denier and single fibre tenacity in different layers of both white and brick red eri silk cocoons.

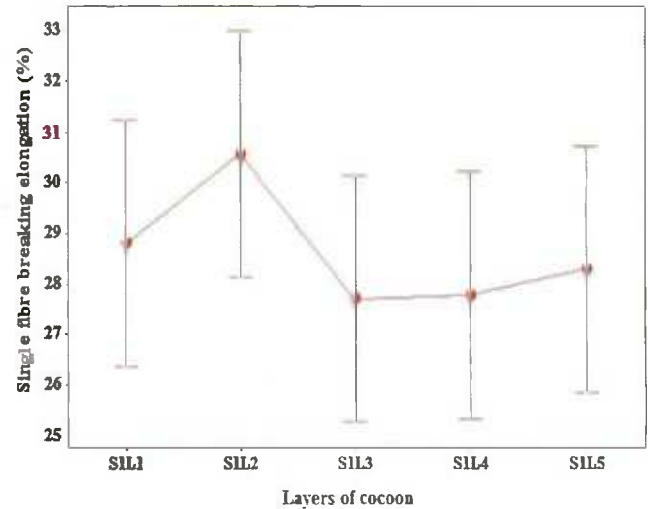
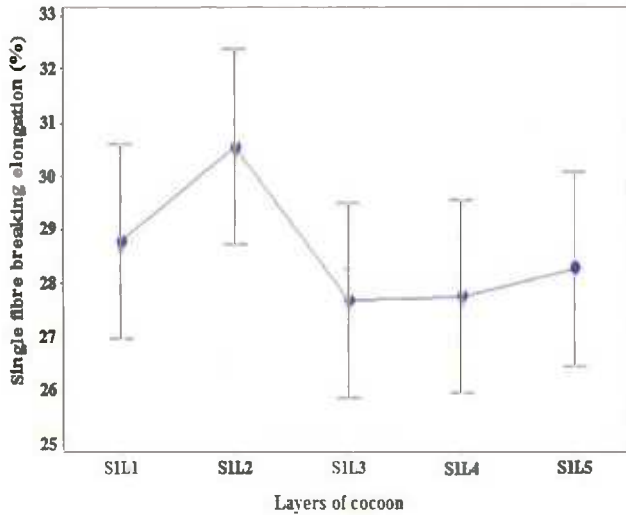
Single fibre tenacity

The mean values for single fibre tenacity of different layers of white and brick red eri silk cocoons at 95 and

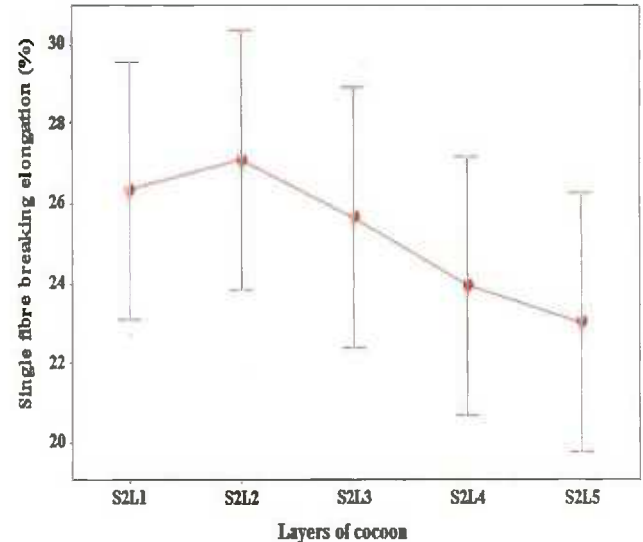
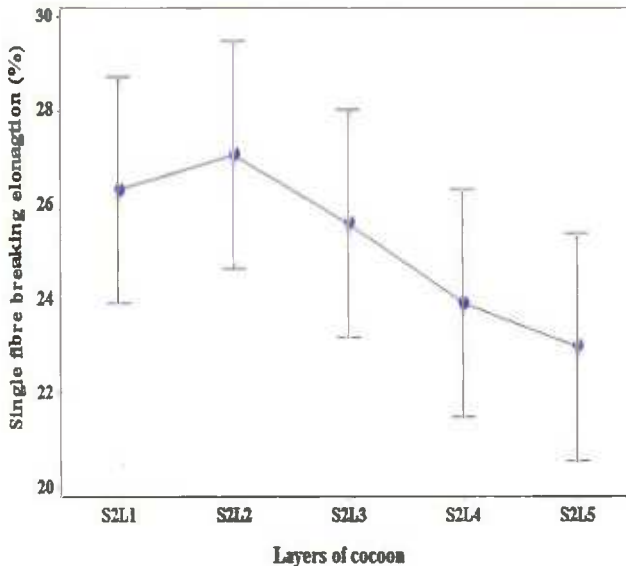
99 % confidence level are shown in Figure 5.

It is evident that the single fibre tenacity increases from floss to pelade layer for both the white and brick red eri silk cocoons. The ANOVA for single fibre strength in different layers of white and brick red eri cocoons is presented in Table 4.

No significant differences were noticed for single fibre tenacity among five different layers for white eri



6 a: White eri cocoons



6 b: Brick red eri cocoons

Figure 6: Interval plots for single fibre breaking elongation in different layers of eri cocoons

cocoon. But for brick red variety, significant differences were observed among the layers both at 5 and 1% level of confidence which was also confirmed by estimation of differences of mean values by Fisher LSD method.

In the case of white eri cocoon, significant difference was observed only between floss and pelade layer as well

as upper and pelade layer whereas, in the case of brick red eri cocoon, significant differences existed between floss and middle, lower and pelade layers; upper and lower and pelade layers and middle and pelade layers. So the 'F' value was not significant for white eri cocoon but for brick red variety, it was significant both at 5 and 1 % level of confidence. During initiation of cocoon formation, higher

volume of liquid silk is extruded from spinneret of silkworm. Due to higher volume, the extrusion pressure remains less. So, the orientation of molecular chain in silk filament is less which yields comparatively lower tensile strength. But as the spinning process continues, the volume of liquid silk gets reduced and extrusion pressure keeps increasing which results in a more oriented structure in silk filament. Hence, there is an ascending trend of silk fibre tenacity as observed from floss to inner layer. Studies reported that the increasing trend of tenacity for bivoltine mulberry cocoons suggests a probable improvement in the arrangement of molecular chains in the filament from outer to inner layer (Chattopadhyay *et al.*, 1997). A thinner thread is more crystalline and/or more ordered since the thinner thread has been produced through narrower anterior divisions of silk gland and thus under high stress (Iizuka *et al.*, 1994). These studies support the findings regarding increasing trend for single fibre tenacity of both white and brick red eri cocoons in different layers.

The regression equation obtained between average single fibre tenacity and single fibre tenacity in different layers for white eri silk cocoon is as follows:

$$Y_5 = -0.61T_1 + 0.68T_2 + 0.61T_3 + 1.04T_4 - 0.81T_5 + 0.23$$

Where, Y_5 is the average single fibre tenacity of white eri cocoons and T_1 , T_2 , T_3 , T_4 and T_5 are the single fibre tenacity for floss, upper, middle, lower and pelade layers, respectively.

Similarly, in the case of brick red eri cocoons, the regression equation is:

$$Y_6 = 1.52T_6 + 2.74T_7 - 3.04T_8 - 2.15T_9 + 0.47T_{10} + 6.17$$

Where, Y_6 is the average single fibre tenacity of brick red eri cocoons and T_6 , T_7 , T_8 , T_9 and T_{10} are the single fibre tenacity in floss, upper, middle, lower and pelade layers, respectively.

High R^2 values and significant 't' values (Table 4) establish the significant correlation between the average

single fibre tenacity and single fibre tenacity in different layers.

Single fibre breaking elongation

No significant difference was noticed for single fibre breaking elongation in the case of both white and brick red eri cocoons in different layers (Figure 6). This may be due to the slippage of chain molecules during extension of fibre. Studies reported that the extensibility is influenced by the changing rate in the fluid region exceeding the yielding point (Hirabayashi, 1980). The regression equation established between overall single fibre breaking elongation and single fibre breaking elongation in different layers is as mentioned below:

$$Y_7 = -0.757E_1 + 1.541E_2 - 1.512E_3 + 2.707E_4 + 1.510E_5 - 68.90$$

Where, Y_7 is the overall single fibre breaking elongation whereas, E_1 , E_2 , E_3 , E_4 and E_5 are that for floss, upper, middle, lower and pelade layers, respectively.

Similarly, for brick red eri cocoons, the regression equation is:

$$Y_8 = -0.782E_6 + 0.034E_7 + 0.652E_8 + 4.83E_9 - 1.878E_{10} - 41.89$$

Where, Y_8 is the overall single fibre breaking elongation and E_6 , E_7 , E_8 , E_9 and E_{10} are that for floss to pelade layers, respectively.

Very strong correlation was established for these equations due to higher R^2 and 't' values (0.948 and 9.575 for white eri and 0.992 and 25.244 for brick red eri, respectively) which were significant both at 5 and 1 % level of confidence.

Conclusion

Spun silk mills as well as decentralized cottage spinning units can use both white and brick red eri silk cocoons because there are no significant differences existing for cocoon quality parameters as well as silk fibres

between them. The comparatively higher degumming loss for brick red variety is due to the presence of natural colouring matter. Hydrogen peroxide bleaching may be required in the case of brick red eri cocoons to make the fibres pure white because after degumming, the colour of fibres remains as pale cream. Significant differences for weight of fibres existed among five different cocoon layers for both the varieties. Decreasing trend for single fibre denier from floss to pelade layers with significant differences among five different layers was observed for both white and brick red eri cocoons. An ascending trend for single fibre tenacity was revealed for the two cocoon varieties. No difference was seen in the case of single fibre breaking elongation for both varieties. So, it may be opined that the quality characteristics of eri silk spun yarn can be attributed based on properties of floss and upper layer fibres of cocoons.

Regression equations between degummed cocoons silk fibres' weight and weight of silk fibres in different layers; single fibre denier and denier of single fibre in different layers; single fibre tenacity and tenacity of single fibre in different layers as well as single fibre breaking elongation and breaking elongation of single fibre in different layers were established. Estimated R^2 and 't' values confirmed the significance of these regression equations.

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Article scientifique

CARACTERISTIQUES STRUCTURELLES DES FIBRES DE SOIE ET DES COCONS ERI

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R É S U M É

Eri est une variété sauvage de soie produite par le ver à soie *Philosamia ricini* un Lépidoptère de la famille des Saturnidés. Les cocons eri sont doux et fibreux avec des ombres blanches ou rouge brique. Les cocons rouge brique sont produits dans le district de Kokrajhar en Assam alors que les cocons blancs sont produits dans d'autres états du nord-est. Aucune différence significative n'est observée dans les paramètres de qualité comme le poids moyen du cocon et le poids de la coque soyeuse entre les deux variétés. La perte au décreusage est d'environ 10 % pour la variété blanche et de 12 % pour la variété rouge brique. Les deux variétés de cocons présentent cinq vestes, la bourre, la supérieure, la médiane, l'inférieure et la pélette. Environ 90% des fibres de soie sont présents dans la bourre et les vestes supérieures, 5 % dans la veste médiane et le reste dans la veste inférieure et la pélette. Des différences significatives existent pour le poids d'une fibre, le titre d'une fibre et sa ténacité dans les différentes couches du cocon. Mais aucune différence n'apparaît pour l'élongation à la rupture d'une fibre dans les différentes couches. Les équations de régression entre chacun des paramètres comme le poids de fibres de soie du cocon décreusé, le titre d'une fibre, la ténacité d'une fibre et l'élongation à la rupture en fonction du caractère particulier dans les différentes couches ont été établies. Les R^2 et 't' estimés confirment la signification de ces équations de régression.

Mots-clés: Bave, cire de cocoon, décreusage, titre, bourre, glande, pélette, coque soyeuse, filière.



Research Paper

EVALUATION OF CASTOR (*RICINUS COMMUNIS* L.) ACCESSIONS AS FEED FOR ERI SILKWORM (*SAMIA CYNTHIA RICINI* BOISDUVAL) AT JIMMA, SOUTH WEST ETHIOPIA

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ABSTRACT

The quality of feed has been proven to play a remarkable role in the growth and development of silkworms and ultimately on the economic traits of cocoons. The present study was undertaken to evaluate thirty two castor accessions for their leaf yielding performance and suitability as feed for eri silkworm rearing. The field experiment was laid out using RCBD design and the laboratory experiment was arranged following CRD design. Plain white eri silkworm breed was used for the rearing experiment in the laboratory. Among castor accessions tested, Acc 219668 registered 13890.9 kg/ha of fresh leaf yield followed by Acc 200361 with 13472.2 kg/ha. During growing season, brown leaf spot disease was encountered and higher disease severity (60.36 %) was recorded on Acc 219662/1. When the host plant superiority was assessed in terms of silkworm rearing performance, shorter larval duration (17.5 days), higher larval weight (7.6 g) and higher fecundity (351.45) were noticed for Acc 200361 while higher hatchability (96.75 %) was recorded for Acc 201067. Acc 200361 was also superior in terms of cocoon weight (3.55 g), shell weight (0.509 g), shell percentage (14.33) and ERR (98.6 %).

Key words: Castor accessions, eri silkworms, rearing performance.

INTRODUCTION

In Ethiopia, agricultural production is of a subsistence nature. Poverty and unemployment are the main challenges to the population. Poverty alleviation and employment generation, therefore, requires additional on - farm and off - farm income generation technologies like raising of silkworms (sericulture) (Metaferia *et al.*, 2007). About 80 % of Ethiopian population resides in rural areas. Agricultural exports are the country's source of foreign exchange. Hence, the Ethiopian government's development policy emphasizes agricultural sector development led industrialization (Berhanu, 2004). In

order to meet this policy, sericulture being an important agro based industry, is given a thrust as it provides employment at various levels *i.e.*, host plant cultivation, silkworm rearing, reeling, spinning and weaving thereby instilling much impact on the improvement of rural economy (Kavane, 2014).

Being polyphagous, eri silkworm (*Samia cynthia ricini* Boisduval) feeds on leaves of many food plants including castor (*Ricinus communis*), dokima/bedessa (*Heteropanax fragrans*), korch (*Evodia flaxinifolia*), cassava (*Manihot utilissima*), jatropa (*Jatropha curcas*), papaya (*Carica papaya*) *etc.* All the food plants are not

equally good for eri silkworm rearing. Their responses as well as yield differ when raised on different food plants. The primary host plant for eri silkworm is castor (*R. communis*) (Naika *et al.*, 2003; Kumar and Gangwar, 2010) and jatropa and cassava are secondary host plants (Kumar and Gangwar, 2010). Eri silkworms reared on castor leaves yield large cocoons rich in silk (Gezahegn *et al.*, 2005; Mitalee, 2012). However, castor is primarily important for production of industrial oil (Sarmah *et al.*, 2011). Castor grows widely and abundantly in many parts of Ethiopia. In addition to cultivated plants, it is also found in wild and waste lands, fallow fields along road side and irrigation canals and other deserted areas (Metaferia *et al.*, 2007).

The quality of feed is an essential component determining the growth and development of the silkworm and ultimate yield of cocoons (Ravikumar, 1988; Bongale *et al.*, 1997; Solanki and Joshi, 2001; Hazarika *et al.*, 2005). It has been further observed that growth, development and cocoon yield are influenced by the castor accession and quality of leaves fed to the worms (Solanki and Joshi, 2001; Chandrashekhar and Govindan, 2010). Therefore, the present study was undertaken to evaluate the available castor accessions and ascertain their foliage quality in terms of agronomical and leaf yield attributing traits and rearing performance of eri silkworms.

MATERIALS AND METHODS

Study area

The research work was undertaken during the rainy seasons of 2015/2016 at Eladale Research Station of Jimma University College of Agriculture and Veterinary Medicine, southwest Ethiopia (N 7°40' E 36°50', 1753 m AMSL), with long-term average annual rainfall of 1500mm, average monthly relative humidity of 91 % and average daily maximum and minimum temperature of 26.8 and 11.8 °C (Abera *et al.*, 2011). The dominant soils of the area are Nitisol and Cambisol which are well recognized as the most productive soils in Ethiopia (Mesfin, 1998) possessing drainage and other favorable

physical properties for agricultural practices.

Experimental materials

The experimental accessions were selected out of hundred castor accessions collected from various parts of the country based on their yield performance and are presently being maintained at Melkasa Agricultural Research Center (MARC).

Table 1: List of castor accessions used as experimental treatments

Sl. No.	Notation	Accession	Sl. No.	Notation	Accession
1	T ₁	Acc 200361	17	T ₁₇	Acc 212534
2	T ₂	Acc 200377	18	T ₁₈	Acc 212631
3	T ₃	Acc 200390	19	T ₁₉	Acc 219627
4	T ₄	White castor	20	T ₂₀	Acc 21963
5	T ₅	Acc 200355	21	T ₂₁	Acc 219645
6	T ₆	Acc 203644	22	T ₂₂	Acc 219647
7	T ₇	Local (Jimma)	23	T ₂₃	Acc 219648
8	T ₈	Acc M-16	24	T ₂₄	Acc 219650
9	T ₉	Acc 219662/1	25	T ₂₅	Acc 219653
10	T ₁₀	Acc Hiruy(Gk-sel-1)	26	T ₂₆	Acc 219654
11	T ₁₁	Acc GE-sel-1/63-271	27	T ₂₇	Acc 219662
12	T ₁₂	Acc 208950/2	28	T ₂₈	Acc 219665
13	T ₁₃	Acc 106501	29	T ₂₉	Acc 219668
14	T ₁₄	Acc 106564	30	T ₃₀	Acc 219671
15	T ₁₅	Acc 201067	31	T ₃₁	Acc 219673
16	T ₁₆	Acc 203241	32	T ₃₂	Acc 219682

Experimental procedure

The field experiment was arranged using RCBD, replicated twice. Castor accessions were planted and managed similarly in each and every plot except for the differences in their genetic make-up. The silkworm rearing room and equipment were cleaned, washed and disinfected with 2 % formalin solution at the rate of 800ml/10 m² before the commencement of the experiment (Dayashankar, 1982). Eri silkworm (*S. c. ricini*) was reared in the laboratory following completely randomized

design (CRD) with two replications for each treatment. In each tray of 9.2 sq. ft, young age (chawki) silkworms were reared uniformly. Tender and semi tender leaves were offered three times a day and the bed cleaning was done once in the morning. Twenty selected late age worms in each tray were allowed to complete their larval period on the selected castor accession. The same aged castor leaf of equal weight was offered to the worms as per the recommendation for tropical countries (Dandin *et al.*, 2003). Mature leaves were provided three to four times per day to the late age worms and the rearing bed was cleaned twice a day. Cocoon harvesting was carried out on the fifth or sixth day after spinning.

Data

Agronomic variables such as plant height (m), inter-node length (cm), leaf area (m²), fresh leaf yield (kg/ha) and disease incidence (%) and severity (%) and also silkworm rearing variables *viz.*, larval duration (days), mature larval weight (g), fecundity (No.), hatchability (%), cocoon weight (g), shell weight (g), shell percentage and ERR were recorded. The collected data were subjected to analysis of variance using SAS statistical software package 9.2 (SAS, 2008). The differences between mean data of treatments were compared using least significance difference (LSD) test at 5 % level of significance.

RESULTS AND DISCUSSION

Plant height and inter-node length

Significant variations were observed in plant height and inter-node length among accessions. Plant height of the test accessions ranged from 1.38 (Acc 106564) to 2.90 m (Acc 219627). Inter-node length varied from 15.375 cm for Acc 219653 to 28.875 cm for Acc 219662. On the other hand, medium plant height and inter-node length (198.13 cm and 16.625 cm, respectively) were recorded in local castor accession (Table 2) as observed earlier by Kedir, 2011 and Sarmah *et al.*, 2013. Further, varied plant height in different accessions in the present study is in conformity with the findings of Govindan *et al.*

(2003). The variations in plant height and inter-node length point towards the existence of heterogeneity among castor accessions for agronomic characteristics. Plant height and inter-node length are controlled by inherent genetic constitution of plant (Abdellah, 1991).

Leaf area

Analysis of variance revealed that leaf area was significantly affected by varietal differences as it ranged from 3547.5 cm² (local check) to 12000 cm² (Acc 200361) (Table 2). The results are indicative of possible inherent variability that exists within the accessions. The present findings are in agreement with that of Kedir (2011) and Sarmah *et al.* (2011) who observed differences in leaf area among different castor genotypes.

Fresh leaf yield

Significantly higher fresh leaf yield (13922.2 kg/ha) was obtained from Acc 200361 whereas, minimum fresh leaf yield (6115.8 kg/ha) was recorded for local check. Similar study was conducted by Kedir (2011) in Ethiopia, Melkassa and found differences in fresh leaf yield among eight different castor genotypes. Besides, Sannappa *et al.* (2016) also observed significant variations in fresh leaf yield among five castor hybrid varieties in India. In addition, Kalantri *et al.* (2007) reported average castor fresh leaf yield of 14,400 kg/ha.

Disease incidence and severity

During the course of the study, brown leaf spot (*Cercospora ricinella*) was a serious problem. The highest disease incidence was recorded on white castor (48.205%) and the least incidence on Acc 200355 (12.955%). However, the maximum disease severity was recorded in Acc 219662/1 (60.36 %) and the minimum in Acc 208950/2 (9.81 %) followed by local check (14.03 %). The differences in disease response may be due to the genetic variation in disease resistance of those accessions. Similarly, Sarmah *et al.* (2011) observed higher disease severity of *Alternaria* leaf spot (25 %) in Ac 30 and

Table 2: Agronomic performance and leaf spot disease reaction of different castor accessions

Castor accession	PH (cm)	INL (cm)	LA (cm ²)	FLY (kg/ha)	DI (%)	DS (%)
Acc 200361	205.75 ^{cdefghu}	27.625 ^{ab}	12000 ^a	13472.2 ^b	14.650 ^{mno}	13.03 ^{kl}
Acc 200377	162.75 ^{ghu}	19 ^{cde}	5011.4 ^{no}	10928 ^{hi}	25.56 ^{ghijkl}	22.38 ^{hijkl}
Acc 200390	195 ^{cdefghu}	17.625 ^{de}	5490.8 ^m	10370.7 ^{jk}	33.18 ^{efg}	30.35 ^{cdefg}
White castor	208 ^{cdefghi}	21.875 ^{abcd}	6310.8 ^k	8132.3 ^{no}	48.805 ^{bc}	47.205 ^{ab}
Acc 200355	190.25 ^{cdefghu}	21.25 ^{bode}	9724.2 ^{ef}	8194.3 ^{lm}	15.64 ^e	12.955 ^{kl}
Acc 203644	216.88 ^{bodefgh}	15.375 ^d	6922.7 ^{ij}	12334.9 ^{ef}	39.62 ^{cdk}	36.225 ^{cd}
Local	198.13 ^{defghu}	16.625 ^{de}	3547.5 ^q	6115.8 ^r	20.03 ^{lmn}	14.96 ^{kl}
Acc M-16	188.88 ^{cdefghi}	15.875 ^e	5484.5 ^{mn}	8766.1 ^{lm}	50.11 ^{ab}	47.83 ^{ab}
Acc 219662/1	287.5 ^{ab}	28.875 ^a	11008.6 ^b	1255.7 ^{de}	60.36 ^a	48.82 ^a
Acc Hiruy (Gk-sel-1)	172.88 ^{cdefghu}	19.375 ^{cde}	4592.4 ^{op}	10485.3 ^{jk}	32.18 ^{cdefghij}	25.48 ^{cdefghij}
Acc GE-sel-1/63-271	150.25 ^{hi}	21.125 ^{bode}	11435 ^b	13628.6 ^{ab}	17.995 ^{mno}	14.475 ^{kl}
Acc 208950/2	167.63 ^{ghu}	15.25 ^{de}	4369.5 ^p	90391.1 ⁱ	16.18 ^{mno}	9.5 ^l
Acc 106501	195.25 ^{cdefghij}	21.25 ^{bode}	5057.1 ^{no}	10293.9 ^k	31.43 ^{cdefghij}	30.35 ^{cdefgh}
Acc 106564	138.13 ⁱ	19.875 ^{cde}	7706.2 ^b	11342.1 ^g	23.175 ^{hijklmn}	22.48 ^{ghijk}
Acc 201067	212.38 ^{cdefgh}	18.75 ^{cde}	7425.9 ^{hi}	8691.9 ^m	23.23 ^{ijklm}	22.38 ^{cdefghij}
Acc 203241	216.38 ^{bodefgh}	21 ^{bcde}	8499.1 ^g	9064.2 ^l	16.16 ^{mno}	14.775 ^{kl}
Acc 212534	243.5 ^{abode}	19.375 ^{cde}	10290.4 ^{cd}	13126 ^c	33.98 ^{cdefghu}	30.35 ^{bodefgh}
Acc 212631	223.75 ^{abodefgh}	25.875 ^{abc}	8761.9 ^g	13485.4 ^b	50.45 ^{ab}	37.76 ^c
Acc 219627	290 ^a	23.500 ^{abcd}	5725.9 ⁱ	13541.9 ^b	21.2 ^{klmno}	16.38 ^{ijkl}
Acc 21963	202.38 ^{cdefghu}	28.250 ^{ab}	6073.7 ^{kl}	7415.5 ^q	36.5 ^{def}	25.52 ^{cdefghij}
Acc 219645	226.88 ^{abodefgh}	18.75 ^{cde}	6528 ^{jk}	12310.9 ^{ef}	25.01 ^{ghijkl}	23.155 ^{cdefghijk}
Acc 219647	162.25 ^{ghi}	17.25 ^{de}	5392.2 ^a	10698.6 ^j	35.63 ^{defg}	32.035 ^{cde}
Acc 219648	148 ^{hi}	16.5 ^{de}	7521.8 ^b	1643.8 ^{po}	31.618 ^{cdefghij}	28.57 ^{cdefg}
Acc 219650	189.5 ^{cdefghu}	19.5 ^{cde}	11169.9 ^b	10485.2 ^k	45.55 ^{bcd}	33.25 ^{cde}
Acc 219653	198.75 ^{defghu}	15.375 ^e	9323.5 ^f	12858.4 ^{cd}	30.865 ^{cdefghu}	22.43 ^{ghijk}
Acc 219654	241.75 ^{abode}	19 ^{cde}	10475.8 ^c	101986.6 ^l	25.56 ^{ghijklm}	25.03 ^{cdefghu}
Acc 219662	191.25 ^{cdefghu}	15.75 ^c	9840.4 ^{def}	12815.8 ^{cd}	20.455 ^{klmno}	18.925 ^{ijkl}
Acc 219665	225.75 ^{abodefgh}	25.375 ^{abc}	6217.7 ^{kl}	11264.3 ^{gh}	28.85 ^{cdefghijk}	20.27 ^{cdefg}
Acc 219668	271.75 ^{abc}	21.875 ^{abcd}	10250.4 ^{cde}	13890.9 ^a	23.670 ^{ghkmn}	18.99 ^{ijkl}
Acc 219671	270.13 ^{abcd}	21.125 ^{bode}	7397.7 ^{hi}	7850.3 ^m	39.6 ^{cde}	25.4 ^{cdefghij}
Acc 219673	237.88 ^{abodefgh}	19.25 ^{cde}	6071.1 ^{kl}	111928 ^{hi}	17.49 ^{klmno}	16.56 ^{ijkl}
Acc 219682	194.25 ^{cdefghu}	22.125 ^{abcd}	8482.9 ^g	12097.4 ^f	26.56	23.0 ^{cdefghu}
LSD (5 %)	72.792	7.5808	527.15	342.54	10.759	10.357
CV (%)	17.24272	18.28127	3.388224	1.561020	18.60608	18.90926

PH=plant height, INL=inter-node length, LA=leaf area, FLY=fresh leaf yield, DS=disease severity, DI=disease incidence LSD: least significant difference; CV: coefficient of variation. Means sharing the same letter (s) in each column do not differ significantly at 5 % significant level according to the LSD test.

Cercospora leaf blight (73 %) in Ac 20 and lower severity in Ac 04 (11.5 %, and 35.5 %, respectively). On the other hand, Lopes *et al.* (2014) in their study to evaluate castor bean genotypes sown in winter and summer at low altitude

in Brazil, reported that among ten genotypes of castor, G1 stood out for the low incidence of gray mold disease which inflicts an adverse effect on the yield of castor plants in many regions of Brazil.

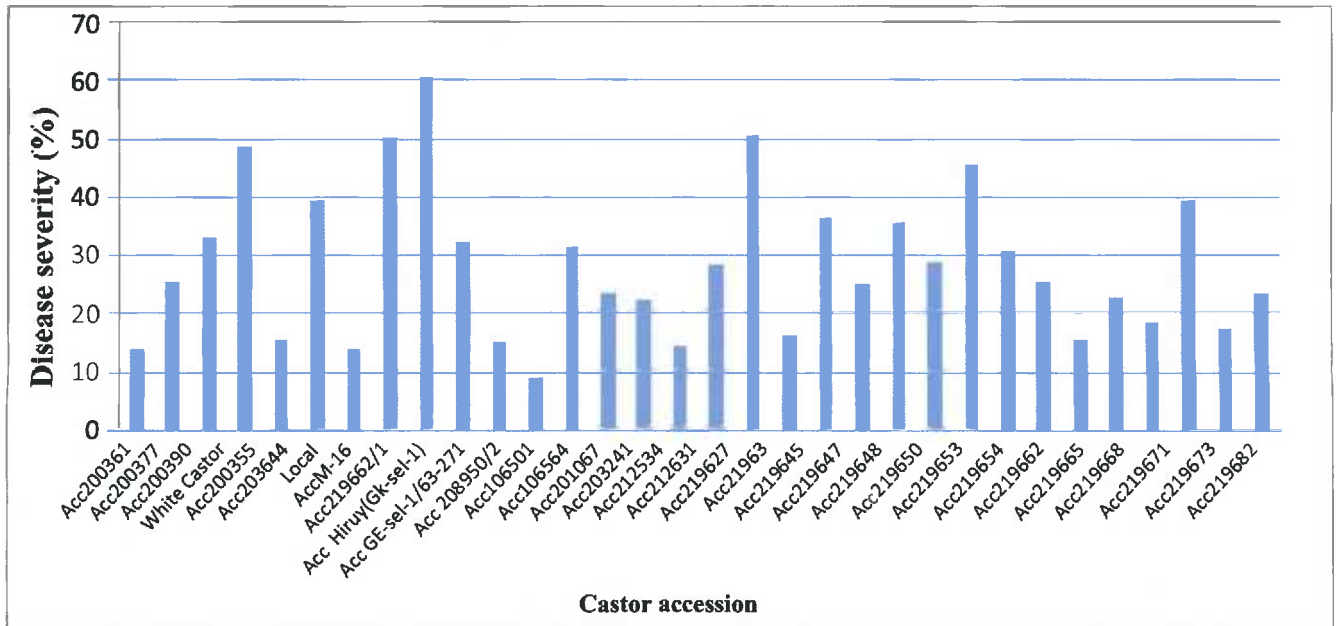


Figure 1: Severity of brown leaf spot disease in castor accessions

Larval parameters

Significant variations were evident in respect of larval duration and mature larval weight among treatments. Shorter larval duration (17.5 days) was noticed in the silkworms fed with Acc 200361 and Acc 212534 and longer larval duration (21.0 days) against Acc 200355, Acc 208950/2, Acc 201067, Acc 219645 and Acc 219673. On the other hand, larvae attained 7.6 and 7.4 g of mature weight when fed with Acc 200361 and Acc 219654, respectively whereas, lower weight of 5.6 g was observed with Acc 219665 and Acc 200390. Similar observations were reported by Jayaramaiah and Sannappa (2000 a); Govindan *et al.* (2002); Hazarika *et al.* (2003); Ramakrishna *et al.* (2003); Sannappa *et al.* (2007); Dasari *et al.* (2013) and Rajasri and Lakshmi (2015) who observed differences in larval parameters among silkworms because of differences in castor genotypes. The above variations observed might be due to the differential compositions of foliar constituents.

Grainage parameters

Silkworms fed on Acc 200361 showed significantly higher fecundity (351.45) followed by Acc 212534 (345).

The lowest fecundity (113) was recorded against Acc106501 followed by Acc208950/2 (116). The differences in feed quality were well reflected in the hatching percentage, showing significant variations among castor accessions studied. Acc 201067 was noticed to result significantly higher hatching percentage (96.75 %) followed by Acc Hiruy (Gk-sel-1) and Acc 200361 (96.5 %). The local check castor accession which was medium performer as compared to the other accessions was the lowest in terms of hatching percentage (88.25 %). These observations are in line with those of Sannappa *et al.* (2007) and Kedir *et al.* (2014) who reported variations in fecundity and hatchability of eri silkworm due to differences in castor genotypes.

Cocoon parameters

Percentage ERR showed significant variation when eri-silkworms were fed on different castor accessions. ERR varied from 82.5 to 98.6 %. Larva fed on Acc 200361 showed maximum ERR (98.6 %) followed by Acc 212534 (98.5 %). The least ERR was recorded from larvae fed on Acc 200377, Acc 219662 and Acc 219665 (82.5 %). The variation in ERR of silkworm fed with different castor accessions may be due to the differences in foliar

Table 3: Performance of eri-silkworm fed on different castor accessions

Castor accession	Larval duration (days)	Mature larval weight (g)	Survival rate (%)	Fecundity (No.)	Hatchability (%)	ERR (%)	Cocoon weight (g)	Shell weight (g)	Shell %
Acc 200361	17.5 ^a	7.6 ^a	98 ^{abc}	351.45 ^a	96.5 ^a	98.6 ^a	3.55 ^a	0.509 ^a	14.33 ^a
Acc 200377	19 ^{de}	6.1 ^{kl}	98 ^{abc}	217.55 ⁱ	96.5 ^a	82.5 ^d	3.2 ^{bcd}	0.4275 ^{efghi}	13.32 ^{def}
Acc 200390	19 ^{de}	5.6 ^m	98 ^{abc}	133.75 ^a	96.0 ^{abc}	98 ^a	3.4 ^{ab}	0.4625 ^{bcd}	13.57 ^{cde}
White castor	20 ^{bc}	7.0 ^{cdef}	97.5 ^{bcd}	269.4 ^a	95.25 ^{abcde}	95 ^{abc}	3.3 ^{abc}	0.453 ^{bcd}	13.75 ^{bcd}
Acc 200355	21 ^a	7.0 ^{cdef}	98 ^{abc}	262.05 ^e	96.15 ^{ab}	95.5 ^{ab}	3.15 ^{bcd}	0.3915 ⁱ	12.425 ^h
Acc 203644	19 ^{de}	6.4 ^{jk}	97.5 ^{bcd}	234.8 ^e	95.15 ^{abcde}	97 ^a	3.5 ^a	0.4635 ^{bcd}	13.235 ^{efg}
Local	20 ^{bc}	6.9 ^{defg}	96.5 ^{def}	191.5 ^m	88.25 ^k	92.5 ^{abc}	3.2 ^{bcd}	0.4015 ^{hi}	12.54 ^h
Acc M-16	18 ^{fg}	6.7 ^{fghi}	97 ^{cde}	211.5 ^j	94.9 ^{abcde}	97.5 ^a	3.5 ^a	0.4475 ^{bcd}	12.78 ^{ab}
Acc 219662/1	19 ^{de}	6.5 ^{hij}	95.5 ^f	195.15 ^m	96.3 ^{ab}	96.5 ^a	3.5 ^a	0.4765 ^{ab}	13.615 ^{cde}
Acc Hiruy(Gk-sel-1)	20 ^{bc}	6.7 ^{fghi}	99 ^a	208.75 ^j	96.5 ^a	97 ^a	3.1 ^{cde}	0.4525 ^{bcd}	14.585 ^a
Acc GE-sel-1/63-271	20 ^{bc}	6.55 ^{hij}	97 ^{cde}	150.3 ^q	95.55 ^{abcd}	92.5 ^{abc}	3.3 ^{abc}	0.4545 ^{bcd}	13.76 ^{bcd}
Acc 208950/2	21 ^a	6.2 ^{jk}	98 ^{abc}	116 ⁱ	93.5 ^{defgh}	97 ^a	3.1 ^{cde}	0.4235 ^{efghi}	13.33 ^{def}
Acc 106501	19 ^{de}	7.2 ^{bcd}	97.5 ^{bcd}	113.0 ⁱ	93.1 ^{efghi}	92.5 ^{abc}	2.8 ^f	0.349 ^j	12.46 ^h
Acc 106564	20.5 ^{ab}	7.3 ^{abc}	98 ^{abc}	193.5 ^m	95.5 ^{abcd}	95 ^{abc}	3.1 ^{cde}	0.4285 ^{defghi}	13.775 ^{bcd}
Acc 201067	21 ^a	7.0 ^{cdef}	96 ^{def}	343.75 ^b	96.75 ^a	96 ^{ab}	3.3 ^{abc}	0.4745 ^{abc}	14.375 ^a
Acc 203241	19 ^{de}	7.2 ^{bcd}	97.5 ^{bcd}	249.65 ^f	92.9 ^{fghi}	92.5 ^{abc}	3.5 ^a	0.476 ^{ab}	13.39 ^{de}
Acc 212534	17.5 ^a	6.9 ^{defg}	97.5 ^{bcd}	345.95 ^b	94.5 ^{abcde}	98.5 ^a	3.1 ^{cde}	0.413 ^{ghi}	13.325 ^{def}
Acc 212631	19 ^{de}	7.0 ^{cdef}	97 ^{cde}	180.7 ^a	93.8 ^{cdefgh}	92.5 ^{abc}	3.1 ^{cde}	0.437 ^{bcd}	14.09 ^{abc}
Acc 219627	18.5 ^{ef}	6.8 ^{efgh}	97 ^{cde}	246.8 ^f	96.5 ^a	97 ^a	3.3 ^{abc}	0.441 ^{bcd}	13.365 ^{def}
Acc 21963	20 ^{bc}	6.5 ^{hij}	97.5 ^{bcd}	263.95 ^e	95.1 ^{abcde}	95 ^{abc}	3.2 ^{bcd}	0.425 ^{efghi}	13.275 ^{defg}
Acc 219645	21 ^a	6.9 ^{defg}	98.5 ^{ab}	235.425 ^g	96.25 ^{ab}	97.5 ^a	3.3 ^{abc}	0.477 ^{ab}	14.455 ^a
Acc 219647	18 ^{fg}	7.4 ^{ab}	96.5 ^{def}	203.5 ^k	90.1 ^{jk}	88 ^{cd}	3.1 ^{cde}	0.4455 ^{bcd}	14.37 ^a
Acc 219648	19 ^{de}	6.8 ^{efgh}	98 ^{abc}	177.5 ⁿ	93.2 ^{efghi}	96 ^{ab}	3.3 ^{abc}	0.45 ^{bcd}	13.665 ^{cde}
Acc 219650	20.5 ^{ab}	5.8 ^{lm}	97 ^{cde}	302.9 ^c	95 ^{abcde}	89 ^{bcd}	3.5 ^a	0.4685 ^{bcd}	13.385 ^{de}
Acc 219653	19 ^{de}	6.1 ^{kl}	98 ^{abc}	220.3 ^{hi}	92.25 ^{ghij}	92.5 ^{abc}	3.1 ^{cde}	0.4265 ^{efghi}	13.75 ^{bcd}
Acc 219654	19 ^{de}	7.4 ^{ab}	97.5 ^{bcd}	223.325 ^b	95.5 ^{abcd}	92.5 ^{abc}	3.2 ^{bcd}	0.4345 ^{cdefgh}	13.57 ^{cde}
Acc 219662	19.5 ^{cd}	6.7 ^{fghi}	98 ^{abc}	199.25 ^l	95.65 ^{abcd}	82.5 ^d	2.5 ^g	0.307 ^k	12.315 ^b
Acc 219665	19 ^{de}	5.6 ^m	98.5 ^{ab}	172.05 ^o	95.9 ^{abc}	82.5 ^d	3 ^{def}	0.4275 ^{efghi}	14.25 ^{ab}
Acc 219668	18.5 ^{ef}	6.5 ^{hij}	97 ^{cde}	149.125 ^q	94.15 ^{bcd}	96 ^{ab}	3.2 ^{bcd}	0.416 ^{fghi}	12.84 ^{gh}
Acc 219671	20 ^{bc}	7.1 ^{bcd}	97 ^{cde}	152.125 ^q	91.25 ^{ij}	92.5 ^{abc}	3 ^{def}	0.405 ^{hi}	13.48 ^{de}
Acc 219673	21 ^a	6.9 ^{defg}	97.5 ^{bcd}	158.65 ^p	92 ^{bij}	96 ^{ab}	3.3 ^{abc}	0.451 ^{bcd}	13.67 ^{cde}
Acc 219682	18 ^{fg}	6.4 ^{jk}	98 ^{abc}	141.75 ^r	93.5 ^{cdefghi}	97.5 ^a	2.9 ^{def}	0.348 ^j	12.425 ^b
LSD (5%)	0.6714	0.3813	1.2548	3.7677	2.2627	7.4496	0.2798	0.06	1.4603
CV (%)	1.72115	2.785924	0.631123	0.867365	1.174217	3.894718	4.015186	5.801148	4.813193

LSD = least significant difference; CV = coefficient of variation. Mean values sharing the same letter (s) in each column do not differ significantly at 5 % P level according to the LSD test.

composition and nutrients availability in different accession which contribute to the growth and development of silkworms. Similar findings were reported by Chandrashekhara and Govindan (2010); Dasari *et al.* (2013) and Kedir *et al.* (2014); in all cases,

they observed variations in ERR because of variations in castor genotypes.

Cocoons formed by the worms fed on leaves of selected castor accessions exhibited significant variations in

cocoon traits in the first rearing. Significantly higher cocoon weight (3.55 g) and shell weight (0.509 g) were recorded from larvae fed on Acc 200361. However, the higher shell percentage (14.585) was recorded from Acc Hiruyi Gk-sel-1) closely followed by Acc 219645 (14.455 %), Acc 201067 (14.375 %), Acc 219647 (14.37 %) and Acc 200361 (14.33 %). On the contrary, lower cocoon weight (2.500 g), shell weight (0.307 g) and shell percentage (12.315) were recorded from larvae fed on leaves of Acc 219662. The worms fed on local check scored medium values for cocoon weight (3.2 g) and shell weight (0.4015 g) and lower shell percentage (12.54 %) as compared to other accessions. These results are in agreement with the findings of Patil *et al.* (2000), Pandey (2003), Ahmed (2015) and Sarkar *et al.* (2015) who recorded variations in cocoon traits when different castor genotypes were offered as food.

The variations noticed in cocoon characters may be a reflection of the nutritional status of the castor accessions as evidenced by the positive and significant correlation coefficients worked out between the foliar constituents and eri silkworm cocoon traits. Many previous workers have reported such significant correlation between castor leaf nutritional status and eri silkworm cocoon traits (Jayaramaiah and Sannappa, 2000 b; Chandrappa *et al.*, 2005; Sannappa *et al.*, 2007; Sarmah *et al.*, 2011).

The present results emphasize the impact of host plant on eri silkworm rearing performance and underscore the need of identifying an ideal castor accession to exploit maximum possible silk production. In terms of agronomic performance as well as rearing performance of eri silkworm, Acc 200361 was adjudicated as the best and hence, recommended for eri silkworm rearing and future sericulture development activities in Ethiopia.

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Article scientifique

EVALUATION DE VARIETES DE RICIN (*RICINUS COMMUNIS* L.) COMME ALIMENT DU VER A SOIE ERI (*SAMIA CYNTHIA RICINI* BOISDUVAL) A JIMMA AU SUD OUEST DE L'ETHIOPIE

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R É S U M É

Il est prouvé que la qualité de l'alimentation joue un rôle remarquable dans la croissance et le développement des vers à soie et finalement sur les caractères économiques du cocon. Notre étude a été entreprise pour évaluer trente deux souches de ricin quant à leur rendement en feuilles et leur aptitude à servir de nourriture pour l'élevage du ver à soie eri. L'expérimentation sur le terrain s'est faite en utilisant le schéma RCBD et l'expérimentation au laboratoire avec le schéma CRD. Une lignée de ver à soie eri entièrement blanche a été utilisée pour l'élevage en laboratoire. Parmi les ricins testés, le 219668 a fourni 13890,9 kg/ha de feuilles fraîches devant le 200361 avec 13472,2 kg/ha. Pendant la période de croissance végétative la maladie de la tache brune a été observée, le plus sévèrement touché à (60.36 %) étant le 219662/1. En terme de performance d'élevage de ver à soie, on note la plus faible durée larvaire (17,5 jours), le poids larvaire le plus élevé (7,6 g) et la fécondité la plus élevée (351,45) avec 200361 alors que l'éclosion la plus élevée (96,75 %) est obtenue avec 201067. De plus, les vers à soie eri nourris avec avec 200361 donnent le poids de cocon (3,55 g), le poids de la coque soyeuse (0,509 g), la richesse soyeuse (14,33) et l'ERR (98,6 %) les plus élevés..

Mots-clés: lignées de ricin, vers à soie eri, performance d'élevage.



SEASONAL INCIDENCE AND INTENSITY OF PARASITIZATION OF UZI FLY ON *ANTHRAEA PROYLEI* UNDER DIFFERENT CLIMATIC ZONES OF MANIPUR

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ABSTRACT

Two species of uzi flies viz., *Blepharipa sugen* Wied and *Exorista sorbillans* Wied (Diptera: Tachinidae) were found infesting *Antheraea proylei* (Lepidoptera: Saturniidae), the oak tasar silkworm, throughout the year in Manipur. Maximum population of both the species was observed during the month of June with infestation percentage of 67.85 and 63.11, respectively when the maximum temperature was 30°C and relative humidity was 77.6%. Both maximum and minimum temperature as well as relative humidity showed significant positive correlation with the population build-up of the uzi flies. The infestation of *E. sorbillans* was higher (55.9 to 63.4%) than that of *B. sugen* (38.6 to 44.1%) in other locations.

Key words: *Antheraea proylei*, correlation, population dynamics, uzi fly, weather.

INTRODUCTION

Uzi flies are important endo-larval parasitoids (Diptera: Tachinidae) which parasitize a number of insect hosts, including the economically important sericigenous insects. Important uzi fly species that infest and breed on silkworms include the Japanese uzi fly (*Crossocosmia sericariae* Rondani), the hime uzi fly (*Ctenophorocera pavidus* (Meigen)), the tasar uzi fly, *Blepharipa zebina* Walker (Tanaka, 1964; Jolly, 1967; Devaiah, 1993) and Indian uzi fly, *Exorista bombycis* Louis (Narayanaswamy and Devaiah, 1998). Uzi flies are distributed over a wide range of geographical areas across the world (Vander, 1896; Ghosh, 1933; Ayuthaya, 1972). Though the uzi fly species *E. sorbillans* (= *E. bombycis*) has been recorded in India since the last century, it was recognized as a serious endo-parasite of silkworm in Karnataka only during 1990's (Jamil and Murthy, 1993). They pose a threat to sericulture industry causing 40 to 80 % cocoon crop loss in different parts of the traditional sericultural states in India (Krishnaswami *et al.*, 1964). Ample amount of information is available on uzi fly infestation on different

species of silkworms viz., *Bombyx mori* L. (mulberry silkworm), *Antheraea assama* Westwood (muga silkworm), *Philosamia ricini* Hutt (Eri silkworm) and *Antheraea mylitta* Drury (tasar silkworm) (Thangavelu and Singh, 1993). Preliminary investigations on uzi fly infestation with regard to the oak tasar silkworm (*Antheraea proylei* Jolly) were carried out by Bidyapati *et al.* (1994). The present work was initiated with an intention to record the seasonal incidence as well as intensity of parasitization of the uzi fly on *A. proylei* under different climatic zones of Manipur.

MATERIALS AND METHODS

The parasitic behavior of uzi fly was studied by rearing *Antheraea proylei* throughout the year during 2013-2015 at the Regional Tasar Research Station (RTRS), Imphal, Manipur, India. The per cent infestation of uzi fly occurring naturally, was recorded by counting the number of infested silkworms, having either uzi eggs or even scars. For this, a total of one hundred worms irrespective of

instars were randomly selected *i.e.*, 25 worms each from four different locations of east, west, north and south of the rearing field. The per cent incidence of the pests were categorically graded in a scale as 'nil' (0), 'trace' (< 1 %), 'low' (1-5 %), 'moderate' (6-20 %), 'high' (21-60 %), 'severe' (61-80 %) and 'extremely severe' (>80 %).

The distribution of uzi parasite was studied by randomly collecting one thousand uzi infested cocoons, each from different locations *viz.*, Tumuyum Khullen (Field Laboratory), Yaikhongpao (Research Extension Center) and the Regional Tasar Research Station, Imphal during the month of April-May (spring crop). The infested cocoons were cut open and the number of uzi maggots was recorded. The data were statistically analyzed to find out the correlation between the uzi fly population and the weather parameters.

RESULTS AND DISCUSSION

The population dynamics during the current study showed that adult uzi fly species, *viz.*, *B. sugen* and *E. sorbillans* were present throughout the year, parasitizing larvae of the sericiginous host, *A. proylei*. The incidence of both species of the uzi flies was severe *viz.*, 63.11 and 67.85 % during the wet and warmer months of May to June, when the atmospheric temperature was in the range of 28.9-30.6 °C (max) and 19.9-22.3 °C (min) with RH of 68.9-76.3 % (Table 1). Later, the populations abruptly declined to moderate levels at 14.8 % (*B. sugen*) and 25.70 % (*E. sorbillans*) in July. The incidence of *B. sugen* was the lowest during October (4.3 %) and was stabilized at moderate intensity (10.4-8.41 %) during November-December. Similar rise and fall in the incidence of *E. sorbillans* was also seen during the corresponding months, but at a slightly higher rate than that of *B. sugen* under natural outdoor rearing conditions. Chakraborty *et al.* (1996) also reported similar pattern of population dynamics with respect to *E. bombycis* on the mulberry silkworm, *Bombyx mori* in West Bengal. Rao *et al.* (1993) also reported that there was 60 % infestation of *E. sorbillans* on *Bombyx mori* in Karnataka during the monsoon season. Bhattacharya *et al.* (1993) recorded that

Table 1: Seasonal incidence of uzi flies on *A. proylei* silkworm and weather parameters during 2013-15

Month	% Incidence of uzi fly sp. (Mean $\pm$ SD)		Weather parameters		
	<i>Blepharipa sugen</i>	<i>Exorista sorbillans</i>	Max°C	Min°C	RH %
January	-	-	22.2	5.5	67.3
February	-	-	24.0	9.2	64.5
March	6.63 $\pm$ 2.0	9.36 $\pm$ 2.8	26.0	11.7	64.8
April	7.45 $\pm$ 1.7	5.27 $\pm$ 2.1	27.6	16.3	66.6
May	25.50 $\pm$ 10.2	32.64 $\pm$ 8.3	28.9	19.9	68.9
June	63.11 $\pm$ 8.5	67.85 $\pm$ 7.9	30.4	21.6	77.6
July	14.82 $\pm$ 2.6	25.70 $\pm$ 5.3	24.5	21.6	77.3
August	12.51 $\pm$ 1.5	18.47 $\pm$ 2.5	30.6	22.3	76.8
September	7.05 $\pm$ 1.6	10.96 $\pm$ 5.2	29.6	21.2	78.4
October	4.34 $\pm$ 1.9	8.53 $\pm$ 1.6	29.3	17.7	75.3
November	10.42 $\pm$ 0.6	14.26 $\pm$ 1.1	25.7	12.1	71.7
December	8.41 $\pm$ 1.7	12.64 $\pm$ 1.9	22.7	6.8	70.3

Data are mean of three years (2013-2015); - Silkworm not reared

Table 2: Correlation (r) values between seasonal incidence of uzi flies on *A. proylei* silkworm and weather parameters

Species	Maximum temperature	Minimum temperature	Relative humidity
<i>Blepharipa sugen</i>	0.534*	0.605*	0.548*
<i>Exorista sorbillans</i>	0.497*	0.579*	0.517*

*significant at 5 % level

temperature within the range of 27.2 to 32.2 °C and 80.3 to 88.7 % of RH are congenial for generation survivability of the uzi fly. Many researchers observed that uzi fly preferably lays eggs on the 4th and 5th instar silkworms (Patil and Savanurmah, 1990; Chakraborty *et al.*, 1996; Rath, 2005). However, Jamil and Murthy (1993) found that uzi fly can infest any stage of the silkworm, when there is no availability of suitable host. The present findings also suggest that 3rd to 5th instar silkworms were infested by the uzi flies, with preference to the 5th instar.

Correlation studies showed that weather parameters *viz.*, maximum, minimum temperature and relative humidity had a significant (at 5 %) influence on the population densities of both the species of uzi fly (Table 2, Figure 1). Our findings on the impact of abiotic factors on the population of uzi fly also conform to the observations made by other researchers. The host worm (*A. proylei*) was strictly reared outdoor on oak plant leaves. Since it is a

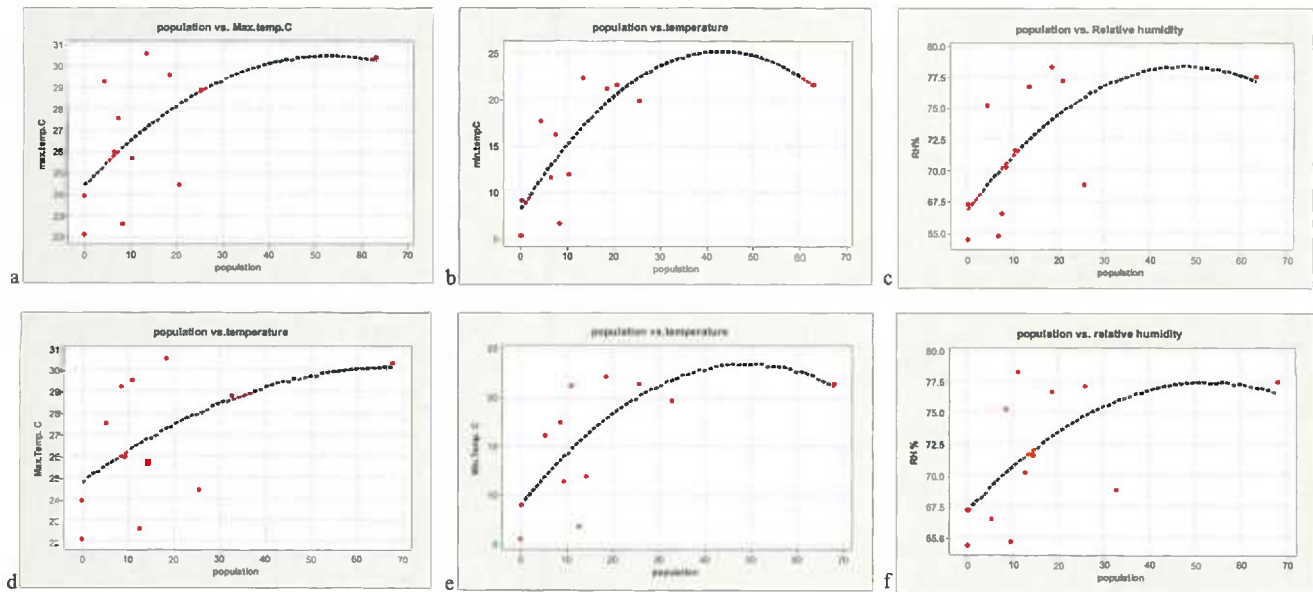


Figure 1(a-f): Graphical representation of correlation values (r) between seasonal incidence of uzi flies on *A. proylei* silkworm and weather parameters: (a-c) *Blepharipa sugen*; (d-f) *Exorista sorbillans*.

deciduous tree, it sheds its leaves during winter and hence obviously due to non-availability of food, there were no worms upon which the uzi flies could parasitize during the months of January to February. Besides different species of silkworms, alternatively, uzi flies have a wide host range to carry out their parasitic activities to complete their life cycle throughout the year. They have a multiple insect host range from different families of Lepidoptera and Coleoptera. Rajagopal *et al.* (1993) observed that besides *Bombyx mori*, the uzi fly *E. sorbillans* exhibited various degrees of parasitism, infesting more than 40 different lepidopterans belonging to 36 genera. Persoons (1989) observed that the uzi fly, *E. sorbillans* can complete its life cycle on a number of alternate hosts under laboratory conditions. However, no uzi fly infested larva among agricultural pests has been found in nature. On the contrary, Narayanaswami *et al.* (1993) reported a few insect pests of agricultural crops such as *Spodoptera litura* (tobacco caterpillar), *Heliothis armigera* (pod borer) and *Achoea janata* (castor semi-loopers) as the alternate hosts of the uzi fly, *E. bombycis*. The recovery of the uzi population on the host silkworms after a complete relapse of two months (January – February) suggests that other means of survival or preferred host for parasitization are very much available to the uzi flies. Siddappaji (1985)

observed that uzi fly assumes the role of parasitoids on larva of the silkworm host *Antheraea paphia* L. and later parasitize the pupae of the host.

The population density and dynamics of uzi flies in general depends largely on the reproductive strategy adopted by them. Most of them show either a high degree of multiplication or a high standard of survival ability to maintain their population in equilibrium status in nature (Savanurmath and Patil, 1993). Data presented in Table 3 clearly define the parasitism strategy adopted by them for this purpose. The uzi fly species parasitized the silkworm host cocoons, irrespective of the geographical and climatic variation where the worms were found. The per cent cocoon infestation of *E. sorbillans* was severe in the range of 61.6 – 63.4 % and exceeded that of *B. sugen* which recorded only a high level at 38.4 – 44.1 %, in all the three different locations of T. Khullen (Research Field Laboratory), Yaikhongpao (Research Extension Centre) and the Regional Tasar Research Station, Imphal (Research Field). Similarly, the average number of maggots in each pupa of the infested cocoons of *E. sorbillans* (7.60 max) exceeded that of *B. sugen* (1.97 max.). It is further observed (Table 4) that maximum number of cocoons infested by *B. sugen* (86) and *E.*

Table 3: Parasitic distribution pattern at different locations

Location	Per cent uzi infested cocoons		Average no. of uzi maggot per infested cocoon (Mean $\pm$ SD)	
	<i>Blepharipa sugen</i>	<i>Exorista sorbillans</i>	<i>Blepharipa sugen</i>	<i>Exorista sorbillans</i>
T. Khullen (Field Laboratory)	38.6	63.4	1.97 $\pm$ 1.18	6.63 $\pm$ 5.72
Yaikhongpao (REC)	44.1	55.9	1.38 $\pm$ 0.74	4.04 $\pm$ 3.93
RTRS, Imphal (Research Field)	38.4	61.6	1.84 $\pm$ 1.13	7.60 $\pm$ 7.50

Data are mean of three years (2013- 2015)

Table 4: Egg laying behavior of uzi fly in uzi-infested cocoons of *A. proylei*

Uzi fly species	Number of infested cocoons and maggots present									
	(1-3)	(4-6)	(7-9)	(10-12)	(13-15)	(16-18)	(19-21)	(22-24)	(25-27)	(> 27)
<i>B. sugen</i>	86	4	1	—	—	—	—	—	—	—
<i>E. sorbillans</i>	55	33	20	9	12	4	4	2	2	5

Data are mean of 3 years (2013-2015); Figures in parentheses are range of no. of maggots present in infested cocoons

sorbillans (55) had uzi maggots in the range of 1-3. In a few cases, the number of maggots was reported as 1-7 in *B. sugen* infested cocoons. However, with respect to *E. sorbillans*, up to 28 maggots were found inside individual cocoons. Similar variation in the number of maggots per infested cocoon was also observed by Patil and Savanurmah (1990). They opined that the differential oviposition by the gravid female uzi fly was dependent on the availability of suitable host and host's health as well as vigor. They also reported that the tasar uzi fly, *B. zebina* did not cross-infest mulberry silkworm in the presence of tasar silkworm, *A. paphia* (= *A. mylitta* Drury) larvae. Further, Patil and Govindan (1986) maintained that the time of oviposition of *E. sorbillans* differed from 2-6 days and has attributed the variation due to climatic conditions of the location. These findings strongly suggested the difference in the egg laying behavior of the two different uzi fly species on infesting the silkworms. Further, the silkworm uzi fly, *E. bombycis* completes its life cycle within 32 days during June–July and takes 58 days during winter (November–December) in the north-eastern regions of India (Thangavelu and Sahu, 1986).

The population dynamics of uzi flies is not only exclusive to their genotypic and phenotypic characteristics but, the influence of both the abiotic and biotic factors is also equally important. They are also

subjected to hyper-parasitization by a number of natural enemies which take their toll on the host during anytime of the span of their life cycle (Kumzuk, 2010). These biological agents are the most potential weapon for insect population control in nature.

It is therefore concluded that the distribution and population of uzi flies are not confined to any geographical or climatic regions. It is found and expected to be constantly associated with the economically important sericiginous insect species and therefore pose an inevitable threat to the sericulture industry. The relevance of this threat is more significant in Manipur, where the prospect of the commercialized hybrid oak tasar silkworm *A. proylei* [cross between Indian species *A. roylei* Moore and Chinese species *A. pernyi* Guerin (Jolly et al., 1969)] can prematurely run at stake. Information drawn from the current studies would therefore, support the exploration of new vistas of grainage and rearing schedules to avoid peak uzi infestation periods and to formulate control options against them, to suit multivoltine prospects of the specific oak tasar silkworm *A. proylei*.

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Article scientifique

INTENSITE ET INCIDENCE SAISONNIERES DU PARASITISME DE LA MOUCHE UZY SUR *ANTHRAEA PROYLEI* DANS DIFFERENTES ZONES CLIMATIQUES DE MANIPOUR

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RÉSUMÉ

Deux espèces de mouche uzy, *Blepharipa sugen* Wied et *Exorista sorbillans* Wied (Diptera: Tachinidae) infestent *Antheraea proylei* (Lepidoptera: Saturniidae), le ver à soie tasar du chêne, tout au long de l'année à Manipour. La population maximale des deux espèces est observée pendant le mois de juin avec un pourcentage d'infestation de 67.85 et 63.11 % respectivement alors que la température maximale est de 30 °C et l'humidité relative de 77.6 %. Aussi bien la température maximale et la minimale que l'humidité relative donnent une corrélation positive significative avec la population de mouches uzy. L'infestation par *E. sorbillans* est plus élevée (55.9 à 63.4 %) que celle de *B. sugen* (38.6 to 44.1 %) dans d'autres localités.

Mots-clés: *Antheraea proylei*, corrélation, dynamique des populations, mouche uzy, météorologie.



PHYSICAL PROPERTIES OF MICROWAVE CURED POLYCARBOXYLIC ACID CROSS-LINKED SILK CATALYZED WITH NANO TiO₂ PHOTO CATALYST AND POTASSIUM SODIUM TARTRATE

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ABSTRACT

The present research attempted to accomplish a cross-linking finish on the bleached mulberry silk fabric by using esterification catalyst and nano titanium dioxide photo-catalyst which can effectively operate under microwave curing. In this study, the bleached silk fabrics were treated with cross-linking agent acrylic acid (AA) and with nano TiO₂ photo-catalyst in presence of different esterification catalysts viz., potassium sodium tartrate, tri sodium citrate, sodium formate, sodium di-hydrogen phosphate and sodium succinate separately and then cured at two different conditions: microwave and high temperature (thermal). Combination of acrylic acid (AA) and potassium sodium tartrate (esterification catalyst) and nano TiO₂ photo-catalyst proved to be the best finish and, hence, it was studied under microwave and thermal curing condition. The spectra of Fourier transform infrared (FTIR) spectrophotometry revealed the crosslinking reaction between the carboxylic acid group of acrylic acid and the hydroxyl group of silk polymer; this reaction could form ester and ether groups in the presence of esterification catalyst and nano TiO₂ photo-catalyst under microwave and thermal curing treatment. The micrographs of scanning electron microscopy (SEM) indicated the surface deposition of acid cross-linking agents on the finished fibers separately. With conventional curing system, the microwave curing system was found advantageous in production of silk fabrics with easy care properties without high losses in strength properties.

Key words: Acrylic acid (AA), cross-linking, esterification, micro wave curing, photo catalyst.

INTRODUCTION

Silk, commonly suffer from poor dimensional stability and wrinkle/crease resistance which make them unsuitable and inconvenient for use in many textile applications and also ready susceptibility to attack by strong acids and alkalis. Wet wrinkle recovery property of silk is considered to be a property of high importance particularly in view of manufacturing high end fashion garment. Silk in wet state becomes highly susceptible to creasing/wrinkling due to consequent breakage of salt linkages of polymer. Such creasing tendency of silk in wet state cause great inconvenience in maintaining essential features of silk high end garment (Sen, 1993; Tuskada *et al.*, 2001; Leksophee *et al.*, 2004).

Wrinkle/crease resistance finishing of silk textile using resins from amine formaldehyde condensate results in some odd disadvantages in respect of relatively poor tensile strength retention despite significant improvement in wrinkle recovery. Such amine formaldehyde condensate resin finishes are also associated with the disadvantages of formaldehyde splitting during processing and use, endangering the health of processors and users. Finishing of silk with polycarboxylic acid as formaldehyde free finishing agent appears to be much more prospective in this respect (Munshi, 2014). Such compounds have evoked immense interest in the recent past in view of their environment friendly and non-toxic characters. However, sodium salts of phosphorous

containing mineral acid used as esterification catalyst with such polycarboxylic acid are reported to be not environment-friendly. Such catalysts containing sulphur influence reproduction of fish and favours growth of a kind of seaweed which consumes large amount of oxygen from water (Choi *et al.*, 1994). Also, high curing temperature of about 170 °C employed for finishing of silk with such compound adversely affects physico-chemical properties, whiteness index and flexibility of silk.

Durable press finishing involves the use of chemical cross-linking agents that can covalently ester link fibroin polymer chains within the silk fibers. Polycarboxylic acid cross-linking agents that have been used for durable press finishing viz., 1,2,3,4 butane tetra carboxylic acid (BTCA), maleic anhydride (MA), citric acid (CA) and succinic acid (SUA) were investigated actively during recent years. The cross-links not only results in the fabrics wrinkle resistance but also in discolouration and impairment of fabric strength and other mechanical properties (Kang *et al.*, 1998; Vukusic *et al.*, 2003). Cross-linking between phenolic –OH and primary hydroxyl groups, respectively of tyrosine and serine amino acids of silk macromolecular network causes fiber embrittlement that reduces the treated fabrics mechanical strength (Munshi, 2014).

At the same time, the use of nano TiO₂ photo-catalyst was investigated of late, to enhance the catalytic effect on the improvement of some physical properties (Xu *et al.*, 2000; Wang *et al.*, 2005). It is well known that the electron of the nano TiO₂ particle in the valence band will be excited to the conduction band under the radiation of UV light whose wavelength is lower than 400 nm for a few microseconds (μs) to raise the electron to the surface of the nanometer particle (Linsebigler *et al.*, 1995; Wang *et al.*, 2002). This phenomenon is called photo-reduction. This photo-reduction phenomenon may also be expected in microwave (wave length: 1mm – 1m) for a few seconds. It can reduce the water molecule and hydroxyl ion that are absorbed on the surface of other nanometer material in the reaction system to create a series reaction

by generation of free-radicals shown in Figure 1's Eq. 2(a) (i) – (ii). The carboxylic acid group and the vinyl double bond of the acid cross-linking agents could be activated by those radicals (Linsebigler *et al.*, 1995; Li 1997) and therefore, could react with hydroxyl and phenolic –OH groups, respectively of serine and tyrosine amino acids of silk (ester cross-link) and react with each other (condensation).

A conventional curing system may also contribute to the decrease in cross-linking mechanical properties. This conventional curing system involves heat transfer to the fabric by convection, conduction and radiation. The surface of the silk fabric becomes hotter than the interior leading to baking and non-uniform crosslink distribution throughout the fabric material (Kang *et al.*, 1998).

Microwave curing is a possible alternative to conventional curing for improving the mechanical properties of cross-linked textile materials. This method can generate heat uniformly throughout the textile substrate. Microwave radiation is absorbed by molecules having resonant frequencies in the microwave spectral region. When an electric field is applied at microwave frequencies, polar molecules rotate to align their dipole moment with the charging electrical field. Energy is absorbed and heat is generated by the internal friction between the rotating molecules (Zhang *et al.*, 2003).

One of the most outstanding advantages of microwave technology is volumetric heating which minimizes the damage from over drying. Since heat energy is transferred through the material electromagnetically, and not as a heat flux, it is not limited by the volume of the material (Vukusic *et al.*, 2004). Microwave heating of the textile fabric is almost instantaneous throughout the material, so that the fabric's environment insulates it against heat loss from the surface thereby allowing curing.

In this study, efforts were made to use microwave technology for curing silk fabrics treated with non-formaldehyde finishing agent, acrylic acid in the presence of sodium or potassium salts of acrylic acid as

esterification catalyst along with nano TiO₂ photo catalyst and to evaluate attainable changes or improvements in the fabric nature and properties

EXPERIMENTAL MATERIALS

Raw mulberry silk fabric with 358 end dm⁻¹ and 314 picks dm⁻¹, 54 denier warp and 54 denier weft having average area density 46 g m⁻² were used for the present study. Commercial grade acrylic acid (AA) obtained from Macromols Polymer India Pvt. Ltd. was used without any treatment or purification. Textile grade Nano TiO₂ obtained from Ahura Chemicals Ltd., India, was used. All other chemicals (E. Merck, India) used were of laboratory reagent grade.

METHODS

Degumming of silk

To remove silk gum from the raw silk fabric, the latter was degummed at 90 °C for 1.5 h in an aqueous solution containing 6 g l⁻¹ olive oil soap and 2 g l⁻¹ sodium carborate at fabric to liquor ratio of 1: 20. Degummed fabric was washed using water at 70 °C for 10 min, cold washed and finally dried in air.

Application of acrylic acid (AA) on silk

Application of acrylic acid (AA) on silk fabric was done by padding technique in a laboratory two-bowl padding mangle. After two successive dipping in the aqueous AA formulation, the fabric was passed through a pair of squeezing rollers. The rollers were adjusted to enable an overall pick-up of 100 %. The aqueous solution of AA contained different dose levels of AA as specified and also contained specific doses of esterification catalyst viz., potassium sodium tartrate, tri sodium citrate, sodium formate, sodium di-hydrogen phosphate and sodium succinate separately and nano titanium dioxide. Immediately after padding, half of the samples were simultaneously dried and cured in a microwave oven at 160W/2 min. The other half of fabric samples were dried

in an oven at 90 °C for 5 min and then cured at 140 °C for 5 min. All the samples were soap washed following ISO-I wash method, washed further with water and dried in air. Aqueous formulations of AA as described above were prepared using CO₂ – free distilled water.

Testing and analysis

The weight gain percentage was calculated on the basis of initial dry weight of the degummed silk (W₂) using the following relationship: Weight gain (%) = (W₁ – W₂)/W₂ × 100 where W₁ is the specific finishing chemical treated sample (Das *et al.*, 2008). The wrinkle recovery angle (WRA) of a selected fabric sample was measured using a SASMIRA Crease Recovery Tester in accordance with ASTM-D-1295-67 (1972). Breaking load and elongation at-break of some selected fabric samples were measured in Instron (KAO-TIEH) Universal Tensile Testing Machine, according to a method prescribed by ASTM-D-1682-64. Fabric stiffness, as expressed by bending length, was measured as per IS: 6490-1971 in a SASMIRA Cantilever Stiffness Tester. Tear strength measurement of unmodified/modified silk samples were done according to a method prescribed by IS: 6489-1971 in an Elmendorf Tearing Tester.

IR spectra of unmodified and selectively modified silk samples were obtained following KBr pellet technique as detailed elsewhere (Munshi *et al.*, 2014) using Perkin-Elmer Fourier Transform IR spectrophotometer. The dried fibre samples were crushed to a size finer than 20 mesh before pelletizing with KBr (each 0.3 g). Each of such test KBr pellets contained 1 % powdered fibres.

Surface morphology of selected unmodified/modified silk fibre was examined using FEL Quanta 200 (Netherland) Scanning Electron Microscope. Selected fibre samples taken out from the relevant fabrics were subject to coating with palladium with coating thickness of 7 nm using a vacuum coater (FEL-200 Autofine Coater). The observations were made at an operating voltage of 20kV using different specified magnifications.

RESULTS AND DISCUSSION

Influence of nano TiO₂ photo-catalyst and esterification catalyst potassium sodium tartrate on acrylic acid (AA) finished silk under microwave/thermal curing

It would be helpful, if we consider, at the outset, the mechanism of modification of mulberry silk (Figure 1) with AA under the influence of (i) potassium sodium tartrate (KNaC₄H₄O₆, 4H₂O) used as the esterification catalyst and (ii) Nano TiO₂ used as the photo catalyst to induce free-radical polymerization and graft copolymerization of AA on silk. The sequence of reactions shown in Figure 1 would be expected to take place, ultimately leading to notable weight gain and changes in the chemical nature of silk during the overall process. Potassium sodium tartrate is known to be an efficient esterification catalyst and reaction 1(a) producing AA ester of silk would be the direct consequence of its catalytic action. However, the AA ester of silk may then react with the monomeric acid as revealed by Fig. 1's Eq. 1 (b) (i) and to subsequent crosslinking of silk as shown by Eq. 1(b) (ii) during the microwave/thermal drying and curing step. Direct consequence of esterification under the influence of KNaC₄H₄O₆, 4H₂O is, therefore, unlikely to enhance –COOH group content of silk even though it would lead to enhancement in the ester group of the product. However, unanalyzed thermal polymerization of AA ultimately leading to grafting of poly(acrylic acid) chains on the chain molecules of silk and ultimate cross-linking (Eq. 1(b)) potentially occurs in addition to induced free-radical polymerization of AA (Eq. 2(a)–2(c)) when nano TiO₂ is also included in the catalyst system.

The microwave/thermal graft copolymerization and cross-linking as shown by Fig. 1's Eq. 1(b) (i) and (ii) would cause measurable enhancements in the –COOH content of the product from which removal of the unbound (not chemically bound to silk) monomeric and polymeric AA were ensured by solvent (water) extractions.

On the other hand, direct influence of the photo catalyst nano TiO₂ at microwave/thermal drying and curing would cause substantial enhancement in the

–COOH content of the modified silk, as a consequence of graft copolymerization without enhancement in the ester group. However, some purely thermal self-catalyzed esterification involving –OH groups in the chain molecules of the silk and finishing agents, AA during microwave/thermal drying and curing (95–1400C), even in the total absence of the esterification catalyst potassium sodium tartrate cannot be altogether precluded. Any enhancement in the ester group of the AA modified silk in absence of the esterification catalyst should thus be taken as a consequence of this self (AA) catalyzed microwave/thermal esterification.

Under the influence of the two catalysts taken together (KNaC₄H₄O₆, 4H₂O and Nano TiO₂) for the curing of the silk, all the reactions shown and discussed above are likely to take place simultaneously or successively leading to a weight gain for the fibre–fabric system. Moreover, additional reactions leading to further esterification and complex network formation (due to limited establishment of ester linkages between the carboxylic (–COOH) groups of a polycarboxylic chain bound to a particular silk molecule and alcoholic (–OH) groups of the same or other neighbouring silk molecules) may also take place.

At any event, it is very likely that microwave energy heats the fabric uniformly from inside out. As a consequence a uniform distribution of cross-links and polymerization occurs throughout the microstructure (Vukusic *et al.*, 2004). In addition, such heating uniformity prevents serious losses in strength without impairing the desired crease resistant durable press properties. This, indeed, affords considerable advantages compared to conventional surface heating, which is a non-uniform outside-in heating and might cause the surface, edges and corners to be much hotter than the inside of the fabric. Consequently, the quality of conventionally heated materials varies and frequently does not reach the desired level.

Effect of different esterification catalysts on silk

Among the different types of catalysts used in this study, potassium sodium tartrate performed better in terms of attainable weight gain, wrinkle recovery, retention of tearing strength, breaking load, elongation at-break and

Table 1: Effect of different curing catalysts on AA finished silk fabrics under microwave/thermal curing

Degummed Silk finished	Catalyst used*	Heating /curing condition	Weight gain (%)	Wrinkle recovery angle (W+F) ^o		Breaking load (N/cm)	Elongation at-break (%)	Tear strength retention (%)	Bending length (cm)
				Dry	Wet				
with	Nil	Thermal	-	178	190	48	17	100	1.4
		Microwave	-	178	190	48	17	100	1.4
None	Nil	Thermal	1.8	184	196	44	16.2	92	1.4
		Microwave	2.4	198	210	45	16.8	94	1.4
6 % AA	Potassium sodium tartrate	Thermal	7.02	248	258	34	13.0	78	1.4
		Microwave	7.57	260	272	36	13.2	82	1.4
-do-	Tri-sodium citrate	Thermal	6.98	236	260	34	13.8	72	1.4
		Microwave	7.39	254	270	36	14.5	84	1.4
-do-	Sodium formate	Thermal	6.55	214	226	29	10.23	47	1.4
		Microwave	7.26	222	234	23	11.4	49	1.4
-do-	Sodium di-hydrogen phosphate	Thermal	6.21	224	248	34	10.5	72	1.4
		Microwave	7.18	238	262	36	15	81	1.4
-do-	Sodium succinate	Thermal	5.23	206	214	36	14	80	1.4
		Microwave	7.03	212	222	37	14	85	1.4

*Curing catalyst: 4.8 % (on weight of fabric); Nano TiO₂: 2.4 % (on weight of bath), Microwave heating: 160W/2min. and/or Thermal heating: 90 – 140°C

Table 2: Comparison of properties of silk fabrics finished with AA under microwave and thermal curing

Degummed Silk finished with	Wrinkle recovery angle (W+F) ^o		Breaking load retention (%)	Elongation at-break (%)	Tear strength retention (%)	Bending length (cm)
	Dry	Wet				
None	178	190	100	17	100	1.4
6 % AA under thermal curing	248	258	70	13	78	1.4
6 % AA under microwave curing	260	272	75	13.2	82	1.4

*Curing catalyst: 4.8 % (on weight of fabric); Nano TiO₂: 2.4 % (on weight of bath), Microwave heating: 160W/2min. and/or Thermal heating: 90 – 140°C

bending length of the silk fabric. Improvement in wrinkle recovery of silk fabric via AA treatment is found to be the least under catalytic influence of sodium succinate though weight gain data remains at high level. Sodium formate appears to be unsuitable in view of its hydrolytic effect that caused cleavage of poly-peptide chains of silk as indicated by common fall of tearing strength and breaking load of the same below 55 % of the original value (Table 1). Elongation at-break for use of sodium formate as catalyst for esterification also suffered the most among the catalysts considered in this study. The potentials of tri-sodium citrate and sodium di-hydrogen phosphate when

used as catalysts for AA treatment of silk in improving wrinkle recovery and retention of mechanical properties are comparable though the effect is found to be marginally better for tri-sodium citrate in respect of improvement of wrinkle recovery property of silk fabric. The potential of sodium succinate in improving wrinkle recovery of silk is presented in Table 1.

Esterification of silk using AA in the absence of catalyst (control experiment) caused minimal improvement in wrinkle recovery property and change in tear strength, breaking load and elongation at-break of silk

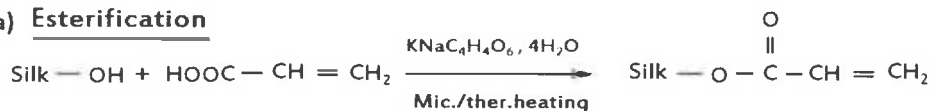
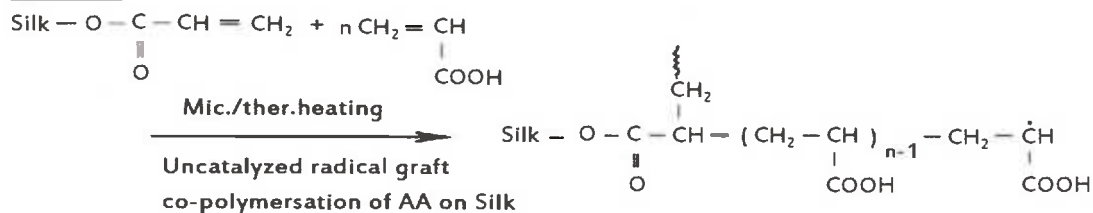
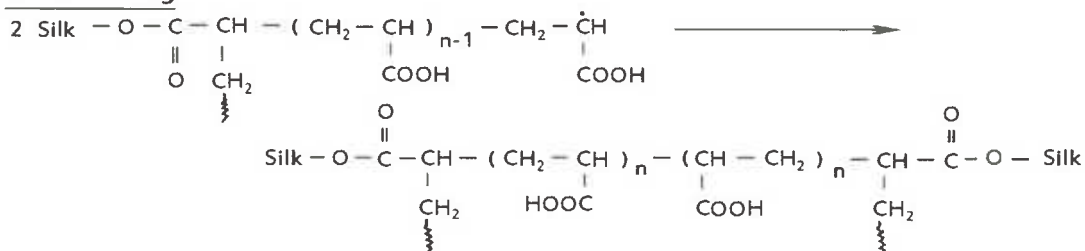
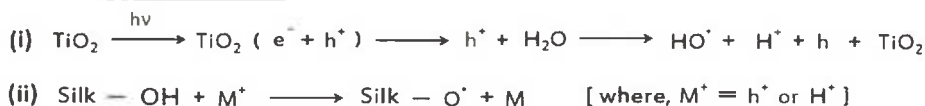
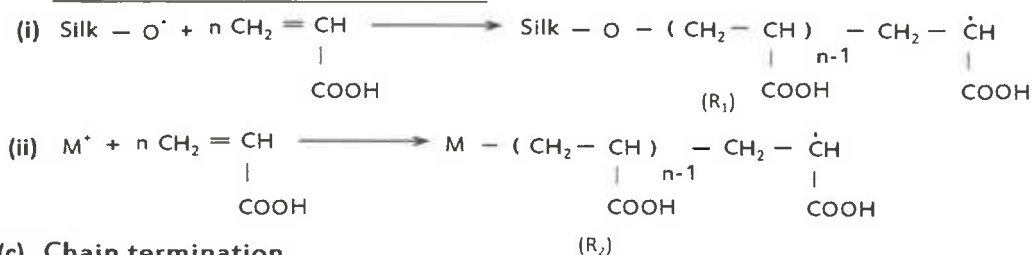
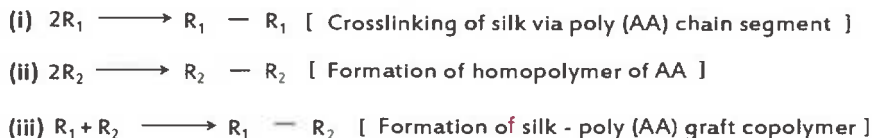
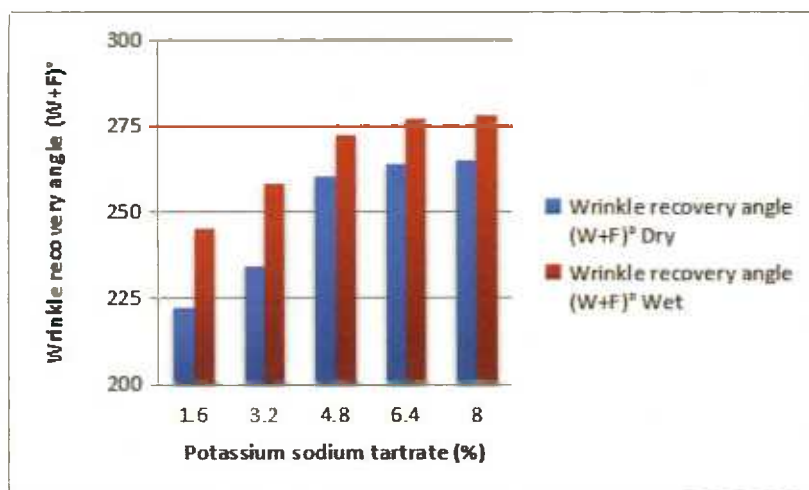
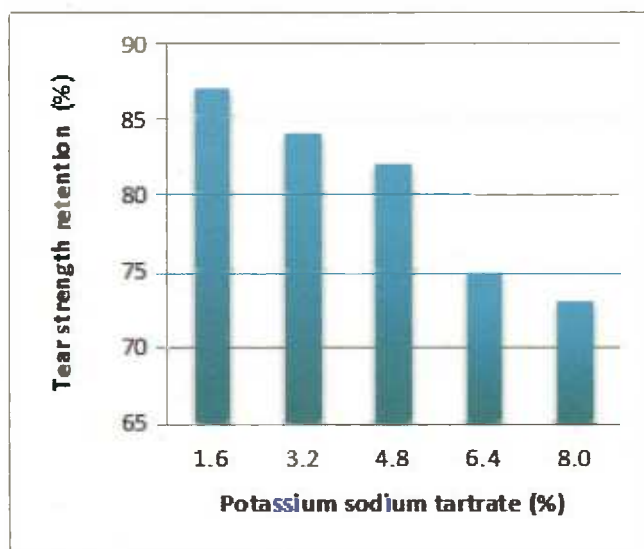
1 (a) Esterification(b) Microwave / Thermal free-radical polymerization of AA leading to :(i) Grafting(ii) Cross-linking2 Nano TiO₂ induced free-radical graft copolymerization (Mic./ther. heating):(a) Radical generation(b) Chain initiation and propogation(c) Chain termination

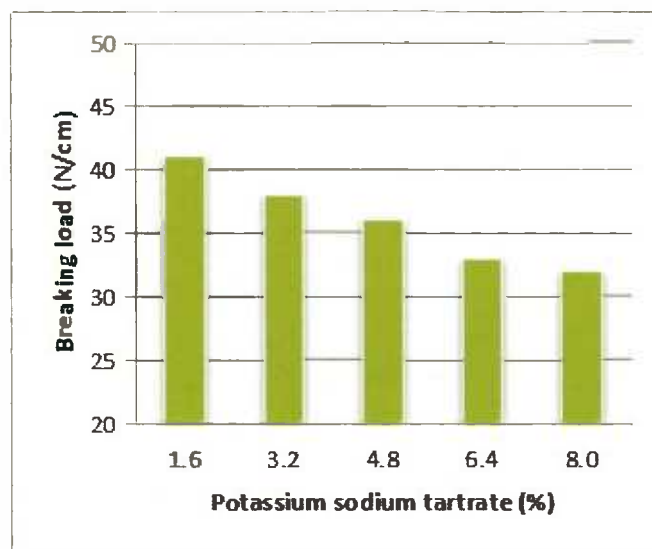
Figure 1: Reaction scheme showing modification of silk with acrylic acid (AA) in presence of potassium sodium tartrate as esterification catalyst and nano TiO₂ as photo catalyst under microwave/thermal curing



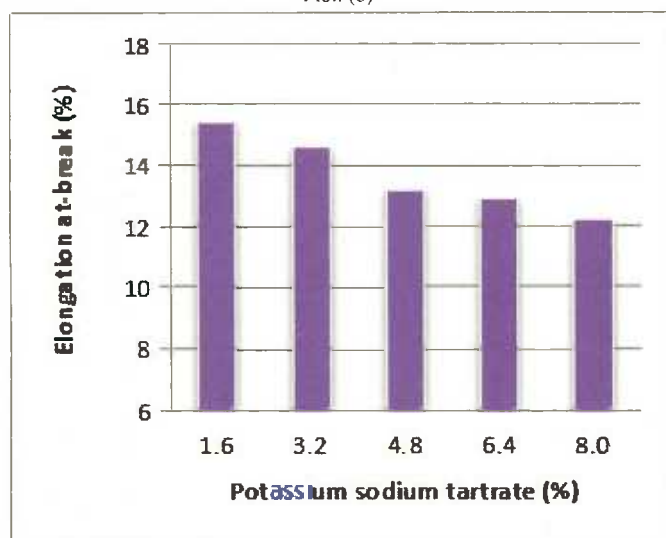
Plot: (a)



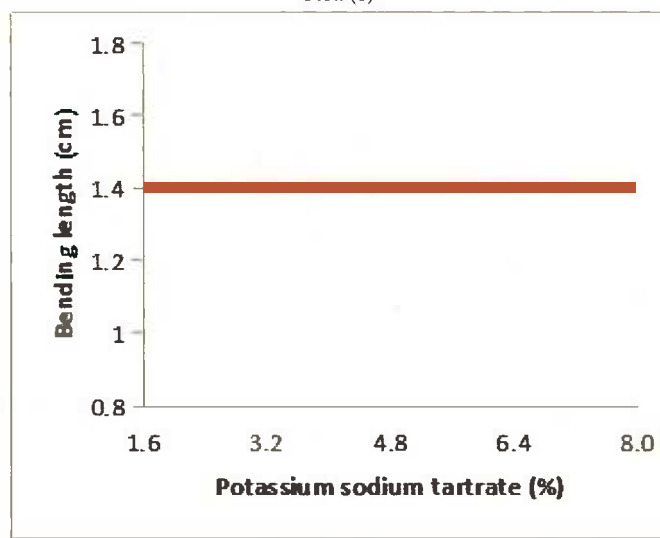
Plot: (b)



Plot: (c)



Plot: (d)

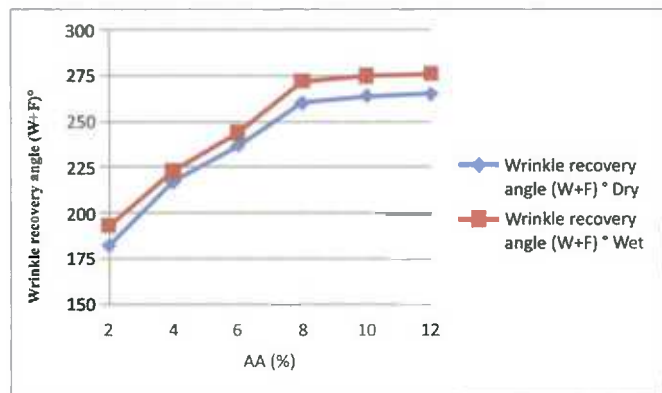


Plot: (e)

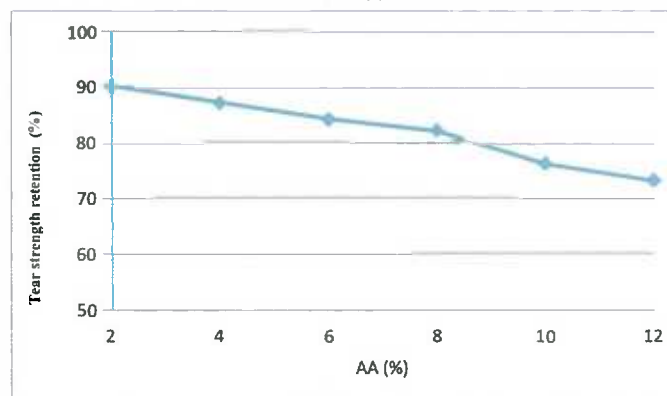
Figure 2: Effect of variation of dose of esterification catalyst on silk fabric under microwave curing

fabric indicating that potential of only AA to un-catalysed esterification process are low or simply marginal. Data (Table 1) indicate that improvement in wrinkle recovery property of the silk fabric on AA treatment for different catalysts used in this study was in the order – potassium sodium tartrate> tri-sodium citrate> sodium di-hydrogen phosphate> sodium formate> sodium succinate. It appears that attainment of higher wrinkle recovery of silk on treatment with AA is associated with higher loss of

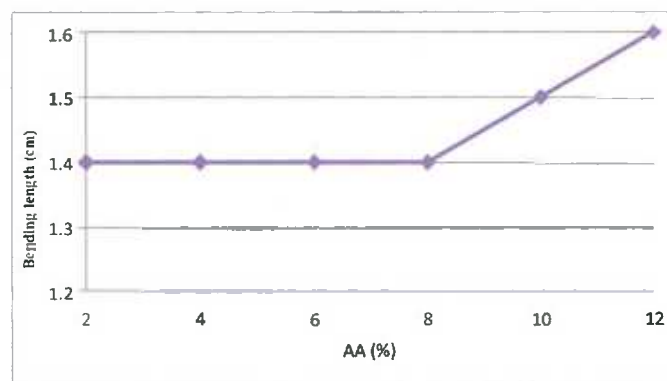
strength and elongation at-break although flexibility as given in terms of bending length remains unaltered for treatment of silk with AA in the presence of all the catalysts considered in this study. AA finishing of silk via microwave curing is the best affected when dual catalysts of potassium sodium tartrate and nano TiO₂ were used than that of thermal curing (pad-dry-cure technique) (Table 1).



Plot: (a)



Plot: (b)



Plot: (c)

Figure 3: Effect of variation of AA dose level on silk fabric under microwave curing

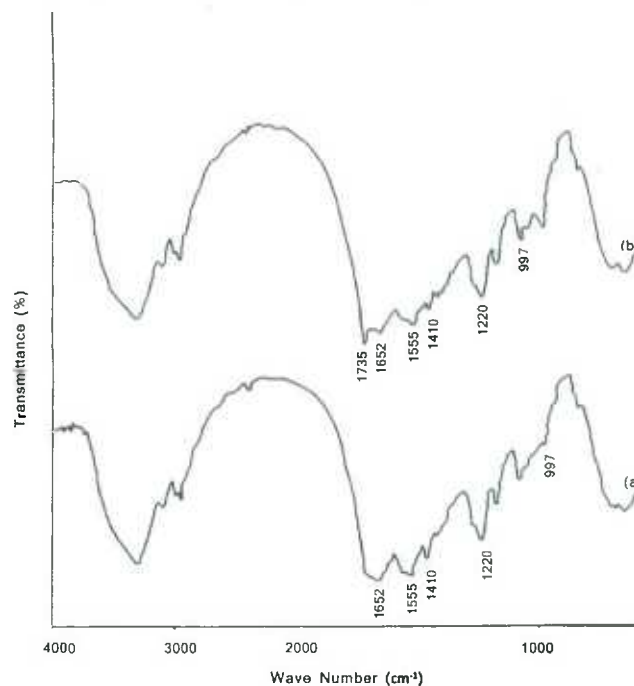


Figure 4: IR spectra of (a) Unmodified silk fibre and (b) Silk fibre modified with AA using potassium sodium tartrate and nano TiO₂ under microwave curing

Effect of variation of dose of esterification catalyst on silk

Data on 6 % AA application with the use of different doses of potassium sodium tartrate as esterification catalyst, each on the basis of weight of the fabric are depicted in Figure 2 with a common application of 2.4 % nano TiO₂. Plots of wrinkle recovery on different doses of catalyst show that higher doses of catalyst commonly produce higher improvement in wrinkle recovery property in view of consequent higher degree of esterification. Higher doses of application of catalyst caused higher degree of cross-linking in microwave curing which ultimately showed increasing trends in wrinkle recovery

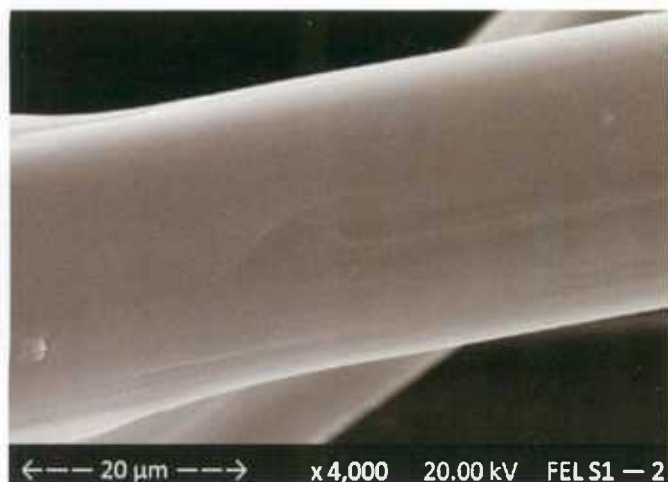


Figure 5 (a): Scanning electron micrograph of degummed silk

property and a falling trend in tenacity (breaking load), elongation at-break and tearing strength for the fabric up to a common application level of 4.8 % (Figure 2). Improvement in wrinkle recovery property beyond a common catalyst dose level of 4.8 % as shown in Figure 2 a is however observed to be low or marginal. Considering all data for the treated fabrics in Figure 2 including stiffness character [(bending length plot (e))], wrinkle recovery angle and tensile properties, application of 6 % AA with 4.8 % esterification catalyst (each on the basis of fabric weight) in the presence of 2.4 % nano TiO_2 (on the basis of bath weight) produced optimum or the most balanced improvements in the properties of silk fabrics.

Effect of variation of AA dose level on silk

Relevant data are shown in Figure 3. For this study, the ratio of dose of application of AA and potassium sodium tartrate catalyst was commonly maintained at 1: 0.8 (w/w), in the presence of 2.4 % nano TiO_2 (on the basis of bath weight). With increasing dose of AA as well as that of esterification catalyst (Figure 3) by maintaining their dose ratio fixed, wrinkle recovery property [plot: (a)] follows a common increasing trend with almost levelling of effect for 8 % application of AA based on weight of fabric. Tearing strength [plot: (b)], however, suffers a morotonic fall with increasing dose of AA as well as that of catalyst. Stiffness values (bending length [plot: (c)]) suffer a sudden change for AA application just beyond 8 % of weight of fabric. On overall assessment, 6 % AA application produced the most balanced effects in

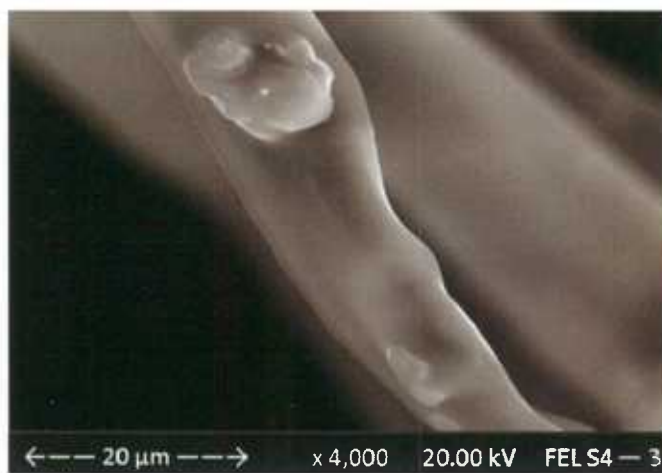


Figure 5 (b): Scanning electron micrograph of degummed silk modified with AA under microwave curing

improving properties of silk fabric in the presence of potassium sodium tartrate esterification catalyst and nano TiO_2 photo catalyst under microwave curing.

Comparison of properties of silk fabrics finished with AA under microwave and thermal curing

Textile related properties for i) unfinished, ii) AA finished silk under thermal curing and iii) AA finished silk under microwave curing are furnished in Table 2. It clearly indicates that AA finished silk fabric under microwave treatment displayed overall improvements in the fabric quality; AA specifically and commonly imparts higher retention of tear strength, breaking load and elongation at-break. Much higher retention of tear strength and breaking load makes the silk fabric much more durable and in this context, the AA finished silk under thermal curing is inferior in comparison, as related results in Table 2 reveal. A good cohesive and somewhat uniform hydrophilic film forming capacity of AA under microwave curing, its polar nature and ductility of its polymer film make all the differences in the properties of the correspondingly treated and finished fabrics covering mechanical strength, elongation, wrinkle recovery and bending length.

IR analysis

Infrared spectra of unmodified silk fiber [Spectrum (a)] and of the silk fiber modified with 6 % AA using both nanomer TiO_2 photo catalyst and potassium sodium tartrate as the esterification catalyst [Spectrum (b)] under

microwave treatment are shown in Figure 4. A broad absorption band over $3400\text{--}3000\text{ cm}^{-1}$ characteristic of hydrogen bonded N–H stretching vibration and an absorption band at 1652 cm^{-1} characteristic of amide stretching vibration are common to both the spectra. Two notable absorption bands at 1555 cm^{-1} and at 1410 cm^{-1} appearing in different intensities in the spectrum of unmodified silk [spectrum (a)] are characteristic of carboxylate anion stretching and phenolic –OH bending, respectively. Carboxylate anion stretching accounts for presence of free carboxylic acid group at the end of polypeptide chains and phenolic –OH accounts for presence of residues of tyrosine fraction of amino acids, respectively in the unmodified silk. A strong absorption band at 1220 cm^{-1} also appears in the spectrum of unmodified silk and is attributed to (C–N) vibration of amine groups present at the end of polypeptide chains of silk. In the spectrum of unmodified silk, absorption bands at 1735 cm^{-1} (ester stretching) and 997 cm^{-1} (vinyl unsaturation) are practically not existent.

AA finish on silk under the influence of potassium sodium tartrate and nanomer TiO_2 [spectrum (b)] results in the appearance of a strong absorption band at 1735 cm^{-1} characteristic of ester stretching and substantial weakening/disappearance of band 1410 cm^{-1} corresponding to phenolic–OH bending due to significant disappearance of phenolic–OH groups. Thus it can possibly be assumed that an interaction has taken place between the carboxylate anion of AA and phenolic –OH of tyrosine of silk fibre. Intensification of absorption bands at 1555 cm^{-1} and 1220 cm^{-1} in the AA modified silk as expected, are also due to the additional presence of unreacted carboxylate (anion) groups of AA moieties incorporated in the silk, induced by microwave curing and catalytic action of potassium sodium tartrate and nanomer TiO_2 . This trend of change in IR analysis functional group pattern is in accordance with the set of reactions given in mechanism proposed [Figure 1, Eq. 1(a) and 2(b) (i)–(ii)].

Examination of surface morphology of untreated and AA treated silk fabric by scanning electron microscopy

Scanning electron micrographs of degummed silk

with/without modification by AA finish are shown in Figure 5 (a) and Figure 5 (b), respectively. The micrograph (a) for simple degummed silk clearly shows smooth rod like structure with no apparent defect on its surface. AA finish of silk however, causes deposition of cross-linked poly (carboxylic acid) film on its surface. Such deposition is however found to be intense and gritty due to large scale polymerization and grafting of AA via esterification on the surface of the silk fibre. Such deposition in some places is so intense that the white coverage of silk surface by smooth film of AA is interfered by gritty deposition and an accumulation of AA on the surface as seen in the micrograph (b).

Conclusion

Establishment of ester linkages and relevant cross-linkages in the silk fabric under microwave/thermal curing technique was studied using AA in presence of potassium sodium tartrate, tri-sodium citrate, sodium formate, sodium di-hydrogen phosphate and sodium succinate as esterification catalyst and application of nano TiO_2 photo catalyst as a common factor in all cases. Combination of Potassium sodium tartrate and nano TiO_2 photo catalyst produced the most balanced effects. Treatment with 6 % AA and 4.8 % potassium sodium tartrate (on weight of fabric) along with 2.4 % nano TiO_2 (on weight of bath) photo catalyst at microwave curing of 160 W/2min. produced optimum improvements in the properties such as wrinkle recovery and weight gain with retention of 75 % of original strength. Micro wave curing of AA finished silk was found to be superior to conventional thermal curing (pad–dry–cure technique) at 140°C for 5 min. considering improvements in resistance of breaking strength, extensibility, tear strength and flexibility for a comparable improvement in wrinkle recovery property. IR analysis indicated that potassium sodium tartrate allowed esterification of AA with tyrosine and serine amino acids of silk in presence of nano TiO_2 photo catalyst; the said process ultimately led to some degree of cross-linking of chain polymers of silk under microwave treatment than that of thermal curing.

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Article scientifique

PROPRIETES PHYSIQUES DE SOIE LIEE A L'ACIDE CARBOXYLIQUE PAR MICROONDES ET CATALYSE PAR PHOTO CATALYSE AU TiO₂ ET AU TARTRATE DE POTASSIUM ET DE SODIUM

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RÉSUMÉ

La présente étude a pour objectif d'accomplir une finition par liaison sur de la soie mûrier blanchie en utilisant une catalyse d'estérification et une photo-catalyse au dioxyde de nano titane, qui pourrait être effectuée sous micro-ondes. Les tissus de soie blanchis ont été traités avec l'acide acrylique (AA) et avec le photo-catalyseur nano en présence de différents catalyseurs d'estérification comme le tartrate de potassium et de sodium, le citrate trisodique, le formiate de sodium, le phosphate de sodium di-hydrogène et le succinate de sodium séparément et séchés ensuite dans deux conditions : microondes et haute température. La combinaison d'acide acrylique (AA) et du tartrate de potassium et sodium (catalyse d'estérification) et du catalyseur nano TiO₂ montrent qu'ils sont les meilleurs finisseurs et donc sont étudiés sous microondes et séchage thermique. La spectrophotométrie de la transformée infrarouge de Fourier (FTIR) révèle la liaison entre le groupe carboxylique de l'acide acrylique et le groupe hydroxyle de la soie, cette réaction pouvant former des groupes ester et éther en présence de catalyseur d'estérification et de photocatalyseur TiO₂ sous microondes et traitement thermique. Les photographies de microscopie électronique à balayage (SEM) indiquent un dépôt des agents de liaison sur les fibres après finition séparément. Avec les systèmes conventionnels de chauffage, le système de microondes s'avère avantageux pour produire des tissus de soie avec sécurité sans perte de résistance.

Mots-clés: Acide acrylique (AA), liaison croisée, estérification, chauffage microondes, photo catalyseur.



DEVELOPMENT OF PRESERVATION TECHNIQUES FOR ERI SILKWORM EGGS (*SAMIA RICINI* DONOVAN: LEPIDOPTERA – SATURNIIDAE)

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ABSTRACT

Of late, eri sericulture has been steadily picking up in India. For the sustenance and full exploitation of the industry under Indian conditions, appropriate egg handling techniques need to be formed. This ensures improvement in the quality of both cocoons and egg production as well. All these years, the eri scenario lacked a suitable egg preservation technology for its adoption in the field. Under this backdrop, series of studies have been conducted at Silkworm Seed Technology Laboratory (SSTL), Bengaluru on the embryonic development, identification of suitable embryonic stages for eri egg preservation and exploitation of different levels of temperature and their combinations with single step and double step refrigeration techniques to develop ideal preservation schedules. Three embryonic stages of 48, 55 and 60 h were identified, that can withstand short-term cold storage. The eggs of these 3 stages were preserved under single step at 5, 10, 15 and 20 °C for different durations of 5, 10, 15 and 20 days. Eggs which were not subjected to preservation served as control. Egg preservation at 5 and 10 °C for 10 to 15 days had detrimental effect on hatching in all the 3 age groups, while at 20 °C, hatching was better (>60 %). The poor hatching of eggs under single step prompted to employ double step preservation technique. Preservation of eggs under double step for 11 or 12 days with the different temperature combinations resulted in significant increase in hatching with temperature combinations of “15+20+2.5 °C” and “15+20+10 °C for 5+1 or 2+5 days. To verify the validity of hatching, bioassay was conducted for the promising treatments. The rearing performance did not show significant difference in any of the rearing parameters between that of control and experimental batches indicating that eri eggs can safely be preserved for 11 to 12 days under double step refrigeration technique.

Key words: Double step preservation, embryonic stage, eri egg preservation, hatching.

INTRODUCTION

Now-a-days, ericulture has drawn attention of the non-traditional states viz., Andhra Pradesh, Gujarat, Uttar Pradesh and Jharkhand. The traditional area of eri is the North-eastern belt of India. It contributes for additional income to the farmers. Eri silk production registered a positive change during the last seven years, from 2038 MT in 2008-09 to 5060 MT in 2015-16. Though there was an increase of 52 % in eri silk production, substantial level of progress is yet to be achieved. Either partial adoption of known techniques or inappropriate seed

technologies are identified as main reasons for low silk production. As the rearers themselves produce the required quantity of seed, the quality may suffer. There are differences in the findings of those who attempted working in this field earlier. For example, Areekul *et al.* (1982) reported that the egg preservation at 10-15 °C for 4 weeks results in reduction in hatching from 87.4 to 59 %. Govindan *et al.* (1980) reported that the refrigeration of eggs of *Samia cynthia ricini* at 3 ± 1 °C beyond 5 days has adverse effects on hatching. Vishwakarma (1982-83) indicated that the hatching of 1-3 days old eggs in summer and 1-5 days old eggs in winter can be delayed for 4-5 days

by refrigerating at $7 \pm 2^\circ\text{C}$ without any detrimental effect. Eggs of 3 and 5 days old were found the most suitable for refrigeration depending upon the season by Nangia and Nageshchandra (1988). Sarkar *et al.* (2012) witnessed hatching of eri eggs (72 %) after 25 days of oviposition *i.e.*, with 17 days of preservation at 5°C and 8 days of incubation. The hatching is postponed naturally up to 20-21 days in winter due to low temperature of $6-7^\circ\text{C}$ prevailed in the northeast areas during December-January. As such, the demand for quality seed in the field has not been met with by the Central Silk Board / state Department of Sericulture units. Hence, with an aim to fill the gap between production and demand for the seed, the present experiment was taken up to develop suitable preservation schedules for the eri seed in order to streamline the egg production and distribution channel.

MATERIALS AND METHODS

The eggs obtained from Eri Egg Production Centre, Hosur were used for the study. To begin with, the experiment was focused on embryonic development during incubation of eggs at 25°C and 75-80 % RH. Both KOH (potassium hydroxide) and hot water methods were adopted for isolation of embryos. In early developmental stages (36-120 h), eggs were fixed in hot water (75°C) for 4 minutes and later fixed on the egg sheet with fevicol gum. After about 30 minutes when the the gum dried up, the embryos were isolated by cutting the egg shell open with a sharp surgical blade followed by squirting of the dissected eggs with hot water (60°C) by using pasture pipette. The embryos were collected and observed under microscope. After 120 h of age, only KOH method was employed for isolation of embryos as this method is easy in later developmental stages of eggs (*i.e.*, after blastokinesis stage). The embryonic stages from 36 h of age to blue egg stage were identified and embryonic chart was prepared (Rajanna *et al.*, 2011- 12).

The eggs of different stages were preserved at 10, 15 and 20°C for 5, 10, 15 and 20 days for screening under single step to identify the cold hardy stages based on hatchability. Eggs subjected to incubation at 25°C temperature and 75-80 % RH with proper aeration served as control. Embryonic stages of 48 (early appendages

formation stage) 55 (late appendages formation stage) and 60 h (shortest embryo stage) were identified for cold storage of eggs and further studies.

To improve hatchability, the eggs were preserved under double step technique, which is normally employed in the preservation of bivoltine *Bombyx mori* eggs. The double step treatments followed are, 1. $5+15+2.5^\circ\text{C}$ for 5+1+5 days; 2. $5+15+2.5^\circ\text{C}$ for 5+2+5 days; 3. $5+15+10^\circ\text{C}$ for 5+1+5 days; 4. $5+15+10^\circ\text{C}$ for 5+2+5 days; 5. $10+15+2.5^\circ\text{C}$ for 5+1+5 days; 6. $10+15+2.5^\circ\text{C}$ for 5+2+5 days; 7. $10+15+10^\circ\text{C}$ for 5+1+5 days; 8. $10+15+10^\circ\text{C}$ for 5+2+5 days; 9. $15+20+2.5^\circ\text{C}$ for 5+1+5 days; 10. $15+20+2.5^\circ\text{C}$ for 5+2+5 days; 11. $15+20+10^\circ\text{C}$ for 5+1+5 days; 12. $15+20+10^\circ\text{C}$ for 5+2+5 days; 13. 25°C (Control). The eggs incubated at 25°C temperature and 75-85 % humidity served as control. The hatching and bioassay data for promising treatment was analyzed statistically.

RESULTS AND DISCUSSION

The data on hatching pertaining to single step egg preservation technique are depicted in Figures 1-5 and that of double step preservation technique in Table 1. The data revealed marked depletion in hatching of eggs as the duration of preservation increased beyond 5 days at 5, 10 and 15°C compared to 20°C and control batch (25°C) indicating cold susceptibility of eggs to low temperature. The hatchability was affected markedly in 48, 55 and 60 h eggs preserved at low temperature of 5°C compared to all other levels of temperature ($5^\circ\text{C} < 10^\circ\text{C} < 15^\circ\text{C} < 20^\circ\text{C}$), indicating that the multivoltine eri egg preservation is favorable at moderately high temperature for obtaining better hatching.

In 48 h old eggs, hatching ranged from 48.10-17.00 %; 51.49 - 8.70 %; 74.01-11.72 % and 71.81 - 58.25 % when preserved at 5, 10, 15 and 20°C , respectively. In the control batch, the hatching recorded was 89.65 % (Figure 1). In 55 hour old eggs, hatching ranged from 62.50 - 17.21 %, 76.68 - 11.36 %, 75.02 - 15.59 % and 76.72 - 61.90 % when preserved at 5, 10, 15 and 20°C , respectively (Figure 2). In the case of 60 h eggs, the hatching ranged from 60.70 - 17.49 %, 76.26-16.19 %; 68.48-10.82 % and 84.05-61.39 % for the corresponding temperature and duration (Figure 3).

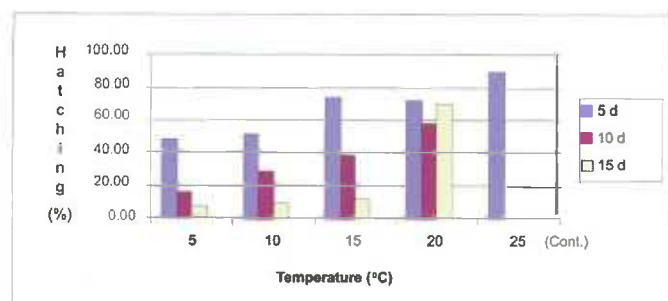


Figure 1: Effect of temperature and preservation duration on hatching in 48 h eggs – single step

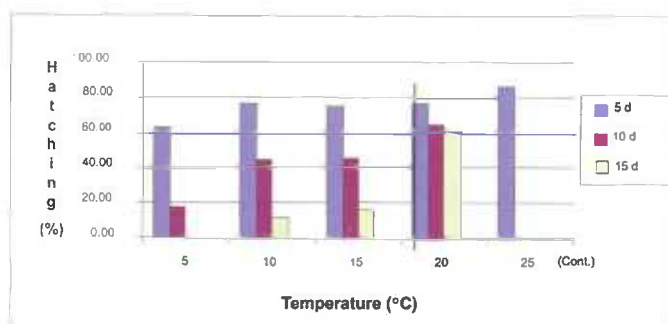


Figure 2: Effect of temperature and preservation duration on hatching in 55 h eggs – single step

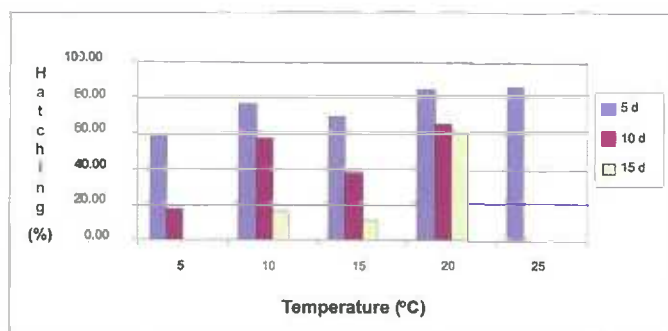


Figure 3: Effect of temperature and preservation duration on hatching in 60 h eggs – single step

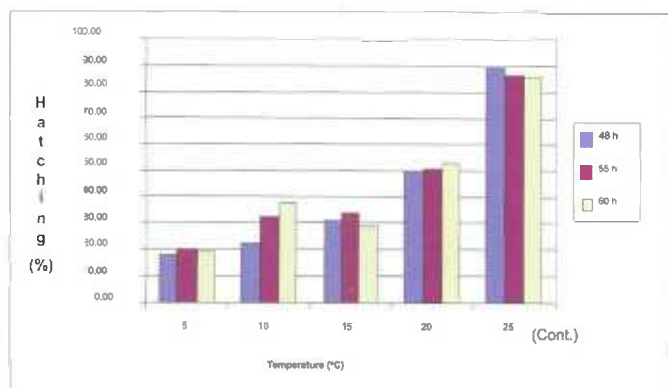


Figure 4: Effect of preservation temperature on hatching – single step

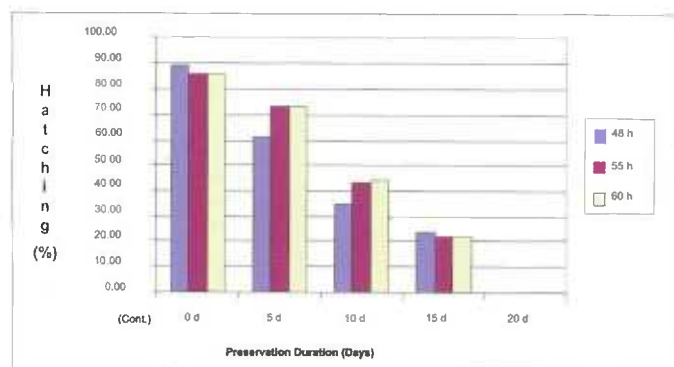


Figure 5: Effect of preservation duration on hatching – single step

Effect of preservation temperature on hatching: Under the single step, the average hatching percentage recorded in 48 h eggs was 17.77, 22.24, 31.16, 49.86 % and in 55 h eggs, it was 19.93, 33.08, 33.93 and 50.87 % at 5, 10, 15 and 20 °C, respectively. In the case of 60 h eggs, the hatching recorded was 19.55, 37.20, 29.11 and 52.71 %, respectively for the above stated temperature regime (Figure 4). The average hatching reflects the limited cold tolerance level at low temperature preservation than at moderately high temperature of 15 and 20 °C.

Effect of preservation duration on hatching: Hatchability was observed depleting as the duration of preservation increased from 5 to 20 days. Hatching in 5 days preserved eggs was 61.35, 72.73 and 72.37 % in 48, 55, and 60 h eggs, respectively. As the duration increased, the hatching rate declined to 35.44, 42.87 and 44.10 % for 10 days and to 24.24, 22.21 and 22.10 % for 15 days. However, in the case of 20 days preserved eggs, hatching was not observed as the eggs either hatched by 15 days itself or did not hatch at all (Figure 5).

Hatchability in double step preservation: Data showed significant differences in hatching among the treatments and control batches except in treatments 9 and 10 where there was no significant difference in hatching compared to control. The hatching recorded in these two treatments was 83.92 and 82.71 % in 48 h eggs, 85.18 and 78.45 % in 55 h and 81.69 and 80.53 % in 60 h eggs and 89.6, 85.93 and 85.04 % in control batches of 48, 55 and 60 h eggs. Hatchability results indicated that the double step preservation has an advantage over single step as the hatching is on par with control in these two treatments (Table 1).

Table 1: Hatchability (%) under double step preservation schedule in eri eggs

Treatment		Age of eggs		
Preservation temperature (°C)	Preservation period (days)	48 h	55 h	60 h
5+15+2.5	5+1+5	24.64 ± 3.20	27.88 ± 2.21	29.74 ± 5.14
5+15+2.5	5+2+5	10.62 ± 3.18	10.27 ± 1.00	11.18 ± 2.41
5+15+10	5+1+5	27.24 ± 8.10	30.70 ± 2.47	29.60 ± 0.75
5+15+10	5+2+5	11.16 ± 4.09	12.57 ± 3.89	12.81 ± 2.26
10+15+2.5	5+1+5	45.30 ± 8.89	55.26 ± 6.77	51.39 ± 10.99
10+15+2.5	5+2+5	37.87 ± 6.99	49.54 ± 4.62	50.72 ± 1.96
10+15+10	5+1+5	25.12 ± 8.89	23.29 ± 6.11	22.67 ± 5.45
10+15+10	5+2+5	19.71 ± 8.73	20.74 ± 3.13	22.06 ± 1.31
15+20+2.5	5+1+5	83.92 ± 7.62	85.18 ± 9.83	81.69 ± 11.88
15+20+2.5	5+2+5	82.17 ± 13.99	78.45 ± 16.06	80.53 ± 7.83
15+20+10	5+1+5	80.91 ± 12.12	74.94 ± 12.58	76.55 ± 5.11
15+20+10	5+2+5	73.25 ± 10.93	73.84 ± 12.75	67.60 ± 7.95
25 (Control)	0	89.66 ± 5.43	85.93 ± 6.88	85.04 ± 7.79

Sample: 5 dfls per treatment; No. of trials: Three; CD @ 5 % - 6.773.

Table 2: Rearing performance of preserved eri eggs

Treatment	Hatching (%)	Larval weight (g)	ERR (%)	ERR by weight (kg)	Cocoon weight (g)	Shell weight (g)	Shell %	Pupation (%)
1	82.92	67.08	93.23	24.33	2.47	0.37	14.84	90.39
	±2.49	±2.31	±2.19	±0.19	±0.19	±0.04	±0.50	±0.82
2	82.36	67.14	92.96	22.96	2.33	0.33	14.01	88.16
	±2.49	±1.14	±1.77	±1.77	±0.17	±0.03	±0.54	±3.32
3	82.48	70.47	93.26	24.01	2.46	0.35	14.31	89.44
	±2.42	±2.80	±1.72	±1.72	±0.16	±0.03	±0.32	2.23
4	78.06	69.60	92.26	24.32	2.50	0.34	13.76	90.54
	±2.36	±2.95	±2.48	±2.48	±0.18	±0.02	±0.16	±1.53
5	80.39	70.56	92.46	24.25	2.49	0.36	14.25	91.25
	±1.37	±3.06	±2.68	±2.68	±0.23	±0.04	±0.73	±0.92
6	77.56	65.29	90.46	22.20	2.29	0.31	13.67	87.00
	±1.35	±2.54	±1.91	±1.91	±0.16	±0.02	±0.96	±1.507
7 (Control)	84.22	71.59	94.91	24.97	2.52	0.37	14.77	91.87
	±2.18	±1.99	±0.60	±0.60	±0.04	±0.02	±0.65	±1.37
CD @ 5 %	3.520	NS	NS	NS	NS	NS	NS	NS

Sample: 5 dfls per treatment; No. of trials: Three; Treatments: Temperature; Duration: 1. 48 h: 15+20+2.5 °C; 5+1+5 days; 2. 48 h: 15+20+10 °C; 5+1+5 days; 3. 55 h: 15+20+2.5 °C; 5+1+5 days; 4. 55 h: 15+20+10 °C; 5+1+5 days; 5. 60 h: 15+20+2.5 °C; 5+1+5 days; 6. 60 h: 15+20+10 °C; 5+1+5 days; 7. Control: 25 °C.

Bio-assay: Silkworm rearing was conducted for 6 treatment batches along with control batch to validate the hatching and rearing performance compared to control (Table 2). The rearing performance in respect of larval weight, ERR, cocoon characters and pupation showed non-significant differences between that of control and

treatments. Overall, the hatching and bioassay results indicated that the eri eggs can be preserved safely for 11-12 days.

The most promising eri egg preservation schedule was evolved with the support of embryonic studies, by

exploiting different temperature combinations, trying out different preservation durations and verifying hatchability and other life parameters through bioassay to confirm the validity for its reliability.

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Article scientifique

DEVELOPPEMENT DE TECHNIQUES DE CONSERVATION DES ŒUFS DU VER A SOIE ERI (*SAMIA RICINI* DONOVAN: LEPIDOPTERA – SATURNIIDAE)

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RÉSUMÉ

La sériciculture eri en Inde a été améliorée dernièrement. Pour la durabilité et l'exploitation complète de l'industrie dans les conditions indiennes, des techniques de manipulation appropriées des œufs sont nécessaires. Ceci permet une amélioration de la qualité de production à la fois des cocons et des œufs. Toutes ces années, le scénario eri manquait d'une technique appropriée de conservation des œufs pour son adoption sur le terrain. Une série d'études a été conduite au Silkworm Seed Technology Laboratory (SSTL) à Bangalore sur le développement embryonnaire, l'identification de stades embryonnaires appropriés pour la conservation des œufs eri et l'exploitation de différents niveaux de température ainsi que leurs combinaisons avec des techniques de réfrigération à une ou deux étapes afin de développer un

processus de conservation idéal. Basé sur l'éclosion, trois stades ont été identifiés, 48, 55 et 60 h qui peuvent résister à une conservation au froid de courte durée. Les œufs de ces 3 stades ont été conservés en une seule étape à 5, 10, 15 et 20 °C pour des durées variables allant de 5, 10, 15 à 20 jours. Des œufs non soumis à ces conditions servent de témoins. La conservation à 5 et 10 °C pendant 10 et 15 jours a un effet néfaste dans les 3 groupes, alors qu'à 20°C l'éclosion est meilleure (>60%). La faible éclosion obtenue avec une seule étape a conduit à utiliser la technique de conservation en deux étapes. La conservation pour 11 ou 12 jours avec différentes combinaisons de température donne un accroissement significatif de l'éclosion avec des combinaisons de température de "15+20+2.5°C" et "15+20+10°C pour 5+1 & 2+5 jours. Des essais ont été conduits pour valider les éclosions avec les traitements prometteurs. Il n'y a pas de différences au niveau des performances d'élevage entre les œufs traités et les témoins ce qui signifie que les œufs eri peuvent être sainement conservés pendant 11 à 12 jours avec une technique de double réfrigération.

Mots-clés: Conservation en deux étapes,, stade embryonnaire, conservation des oeufs eri, éclosion.



SERIFIT- A DISINFECTANT FOR EFFECTIVE SANITATION IN INDIAN SERICULTURE

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ABSTRACT

The Silkworm diseases are one of the constraints in silk production in Indian sericulture. The diseases are best prevented through effective disinfection of rearing house, its surroundings and rearing appliances as well as maintenance of hygiene. A suitable disinfectant is the primary need to eliminate the persistent pathogen load from the rearing environment. Formalin, Bleaching powder, Chlorine dioxide (Sanitech/Serichlor and Sanitech Super) and Asthra have been used as disinfectants in Indian sericulture for disinfection and to maintain hygiene. In this direction, Serifit, a new disinfectant (a chlorine based inorganic compound) manufactured by M/s Sree Rayalaseema Hi-Strength Hypo Limited, Kurnool, Andhra Pradesh, India was evaluated for its germicidal effect against the pathogens of silkworm. *In vitro* studies on the efficacy of different concentrations of Serifit against *Bacillus thuringiensis* and *Beauveria bassiana* proved the effectiveness of Serifit as a germicide at 0.15 to 2 %. *In vivo* studies of different concentrations of Serifit against all common pathogens of silkworm, viz., polyhedra of BmNPV, spores of *Nosema bombycis* and *B. thuringiensis* and conidia of *B. bassiana* also confirmed its efficacy. Based on effective concentration, 0.20 % of Serifit was selected and this concentration was found effective for disinfection of contaminated rearing house, rearing appliances and silkworm egg surface. A technology on "Disinfection and Hygiene using Serifit" was tested both at laboratory and field conditions. In laboratory study, Serifit (0.20 %) was found effective for disinfection of rearing house and also for hygiene maintenance. Field trials of technology conducted at farmers' level in different sericultural areas of Karnataka, Tamil Nadu and Andhra Pradesh also indicated its effectiveness. The disinfectant is stable, non-hazardous, the least corrosive and suitable for effective sanitation.

Key words: *Bombyx mori* L., disinfectant, pathogens, sericulture, serifit, silkworm.

INTRODUCTION

The mulberry silkworm, *Bombyx mori* L., an economically important insect, has been domesticated for several centuries and the domestication presumably has weakened and made the insect highly susceptible to pathogenic infections that lead to diseases. All the major pathogenic microbes (viruses, bacteria, fungi and microsporidia) cause diseases in silkworm. The diseases are one of the major constraints in cocoon production. There are several reports on the crop loss due to diseases in silkworm. A total loss of 10 % was reported in Japan during the sixties (Yokoyama, 1963; Nanavaty, 1965). In

India, Vaidya (1960) reported 30-40 % crop loss per year due to silkworm diseases but after the advent of improved rearing technology and disease control technology, the loss due to diseases was significantly reduced to 15 -20 % during 1980s (Baig and Kumar, 1987). Selvakumar *et al.* (2002) reported an estimated loss of 12-15 kg/100 DFLs (disease free layings) of rearing due to diseases. Silkworm diseases are better prevented than cured. Disinfection of rearing house and appliances using effective disinfectant and maintenance of hygiene during rearing are considered to be the most suitable methods. Different disinfectants viz., formalin (Kagawa, 1980), bleaching powder (Kobayashi *et al.*, 1968), iodine compounds (Kawakami,

1970) and calcium hydroxide (Iwashita and Zhou, 1988) have been recommended for disinfection in other countries. In India, formalin was used as a disinfectant in sericulture for the past several decades. Owing to its demerits, bleaching powder solution was tested later as disinfectant (Balavenkatasubbaiah *et al.*, 1994). Subsequently, Chlorine dioxide (commercially available as Sanitech, Serichlor and Sanitech Super), a disinfectant used in poultry was introduced into sericulture (Balavenkatasubbaiah *et al.*, 1996; Balavenkatasubbaiah *et al.*, 2006). Recently, Asthra (Chlorine based disinfectant) was tested and recommended to the field to use as a disinfectant for disinfection of rearing house, appliances and maintenance of hygiene (Balavenkatasubbaiah *et al.*, 2014). However, the recommended disinfectants have some constraints being either hazardous, unstable or costly. With this backdrop, in the present study, Serifit - a Chlorine based inorganic compound manufactured by M/s Sree Rayalaseema Hi-Strength Hypo Ltd., Kurnool, Andhra Pradesh, India was evaluated for its suitability and adoptability in Indian sericulture.

MATERIALS AND METHODS

Serifit, a Chlorine based inorganic compound manufactured by M/s Sree Rayalaseema Hi-Strength Hypo Ltd., Kurnool, Andhra Pradesh, India was evaluated as disinfectant in the present study. The germicidal activity of the disinfectant was tested against silkworm pathogens and the pathogen concentrations were prepared by the following methods:

Nuclear polyhedrosis virus

The haemolymph of the grasserie [a viral disease caused by Nuclear Polyhedrosis Virus (BmNPV) infection] affected silkworm larvae was collected in sterilized distilled water, incubated overnight at room temperature and centrifuged at 5,000 rpm for 10 min. The pellet was washed 4-5 times with distilled water followed by centrifugation using percoll. After centrifugation with Percoll, the pellet obtained was again washed 4-5 times with distilled water by centrifugation and final suspension containing 1×10^6 polyhedra/ml was

prepared in sterile distilled water.

Nosema bombycis

The spores of *Nosema bombycis* were collected from pebrine infected silkworms and were purified by centrifugation using percoll (Sato and Watanabe, 1980). The spore suspension containing 1×10^6 spores/ml was prepared in sterile distilled water.

Bacillus thuringiensis

Bacillus thuringiensis subsp. *sotto* spores with parasporal inclusion (crystal toxin) complex were harvested from 60 h old culture and a suspension containing 1×10^8 spores/ml was prepared in sterile distilled water.

Beauveria bassiana

Beauveria bassiana conidia were harvested from 96 h old culture and a suspension containing 1×10^7 conidia/ml was prepared in sterile distilled water.

Efficacy of Serifit against silkworm pathogens

Different concentrations of Serifit (2.0 to 0.05 %) solution were prepared by dissolving the required quantity of Serifit granules in water and germicidal activity was tested *in vitro* against the bacterial and fungal pathogens of silkworm. Overnight incubated inoculum of *B. thuringiensis* (1×10^8 spores/ml) in nutrient broth was used for the plating on nutrient agar plates. *Beauveria bassiana* conidia (1×10^7 conidia/ml) in sterile distilled water were used for plating on potato dextrose agar plates. The test organisms were spread on the plates using a sterile L spreader. On the lawn prepared, three paper discs of 5 mm diameter cut from Whatman No.1 filter paper dipped in the Serifit solution of known concentration were kept in a triangular shape. *B. thuringiensis* inoculated plates were incubated at 30°C for 24 hours and those of *B. bassiana* were incubated at $25 \pm 1^\circ\text{C}$ for 2 days. The development of zone around the disc was taken as the anti-bacterial/anti-

fungal property of the disinfectant. The radius of the zones was measured. For both the pathogens, discs dipped in sterile distilled water were kept as control.

The germicidal effect of the different concentrations of Serifit was tested *in vivo* against silkworm pathogens viz., BmNPV polyhedra (1×10^6 polyhedra/ml), spores of *N. bombycis* (1×10^6 spores/ml) and *B. thuringiensis* (1×10^8 spores/ml) and conidia of *Beauveria bassiana* (1×10^7 conidia/ml). As mentioned above, these pathogens were suspended separately in different concentrations of Serifit (2.0 to 0.05%) for different durations (10, 20 and 30 min) at $25 \pm 1^\circ\text{C}$. After the treatment duration, the pathogens were centrifuged, washed and used for bioassay to determine the infectivity of the pathogens. 1.0 ml of these pathogens was per orally inoculated to 100 third instar larvae (immediately out of II moult). Three replications were maintained for each treatment and reared for a period of 10 days. In the case of *B. bassiana*, the treated conidia were inoculated per cutaneously. Observations were recorded for disease development and mortality. Inoculated control (inoculated with viable silkworm pathogens) was maintained for the comparative study.

Efficacy of Serifit as rearing house, appliances and egg surface disinfectant

Based on the results of *in vitro* and *in vivo* studies, 0.20 % of Serifit solution was test verified for its effectiveness in disinfection of silkworm rearing house, appliances and egg surface. The silkworm pathogens were exposed separately, in petriplates (containing 3 ml of air dried pathogens of same concentrations as used in the other experiments) to the disinfectant @ 1.5 liter/sq. m floor area. The pathogens were collected 30 min and 24 hours after disinfection, suspended in sterile distilled water, sedimented and washed by repeated centrifugation. The pathogens were inoculated as described earlier. A control, inoculated with pathogen from the prepared solution but not exposed to disinfectant was maintained for comparison.

In the case of tray disinfection, 5 ml each of pathogen solution of the above concentration were sprayed individually on separate rearing trays and air-dried. These contaminated trays were disinfected with 0.20 % Serifit disinfectant. To test the efficacy of the disinfectant, second instar silkworms (3 replications of 100 larvae each) were reared in these trays up to 10 days and observations were made on disease development and mortality during the rearing. For comparison of results, larvae reared in trays contaminated by smearing with pathogen solutions were maintained.

To test the efficacy of the disinfectant in silkworm egg surface sterilization, silkworm eggs were surface contaminated with silkworm pathogens and air dried before head pigmentation stage (0.5 ml pathogen/DFL). These contaminated layings were dipped in the disinfectant (0.20 % Serifit solution) for 10 min, air-dried and incubated at 25°C and 80 % RH till hatching. The hatched larvae were brushed and reared up to 10 days. The disease development and mortality during rearing was recorded.

Development of a technology on 'Disinfection and Hygiene using Serifit' or 'Serifit- A disinfectant for effective Sanitation in Sericulture'

A technology 'Disinfection and Hygiene using Serifit' or 'Serifit - a disinfectant for effective Sanitation in Sericulture' was developed and validated both at laboratory and field level. The detail of the technology is as follows:

Preparation of disinfectant solution

Prepared 0.20 % Serifit disinfectant solution by adding 200 g of Serifit granules in 100 liters of water (2.0 g / l of water). After adding Serifit, the solution was stirred thoroughly with a stick and kept for 30 min for dissolution of the disinfectant.

Disinfection

The Serifit solution was sprayed in a rearing house of

standard height (3 meters or 10 ft height) @ 1.5 l / sq. m floor area or 0.140 l / sq. ft floor area for effective disinfection.

If the height of the rearing house is more than that of the standard, additional disinfectant solution may be added @ 0.5 l/sq. m or 0.014 l/sq. ft to the total disinfectant solution for every additional meter or feet of height, respectively. Power sprayer was used to ensure uniform spray both inside and outside the rearing house. Disinfection was conducted twice *i.e.*, once after completion of rearing and another one before initiation of rearing.

Maintenance of hygiene

- Sanitized the hands and feet with 0.20 % Serifit solution before entering into the rearing house.
- The rearing house floor was wiped with 0.20 % Serifit solution after every instance of bed cleaning.
- The diseased/dead larvae if any were collected daily in a basin containing 0.20 % Serifit solution and destroyed by burning.

Validation of the technology at laboratory level

Validation of the technology was conducted at laboratory level. 0.20 % Serifit solution was prepared and conducted disinfection twice (as mentioned above) by spraying the solution in a rearing house of standard height (3 meters or 10 ft height) @ 1.5 l / sq. m floor area or 0.140 l/sq ft floor area and on rearing equipment.

Validation of the technology at the field level

Validation of the technology was conducted at farmers' level during the period October, 2015 to November, 2015 in sericultural areas of Karnataka (Ithandanahalli cluster, Research Extension Centre, Madivala), Tamil Nadu (Pollachi cluster, Research Extension Centre, Udumalpet) and Andhra Pradesh (V. Kota cluster, Research Extension Centre, V. Kota) against the ruling spray disinfectant as control. A total of 108 crops were tested with Serifit

disinfectant and crops of 108 farmers who were using the popular disinfectants were kept as control.

RESULTS

In vitro studies

In vitro studies on the efficacy of different concentrations of Serifit against *B. thuringiensis* and *B. bassiana* indicated the effectiveness of Serifit as a germicidal formulation at lower concentration of 0.15 % (Table 1). The data on the efficacy is presented in Table 1. In the case of *B. thuringiensis*, inhibition zones were observed for 2.0 to 0.15 % concentration of Serifit indicating the effectiveness of Serifit against the pathogen. However, in 0.1 and 0.05 % concentrations, no inhibition zones were observed. Similar results were also observed in the case of *B. bassiana*. Inhibition zones were observed from 2.0 to 0.15 % concentrations of Serifit and no inhibition zones for treatments of 0.1 and 0.05 %. In both the cases, no inhibition zones were observed for control.

In vivo studies

In vivo studies on the efficacy of different concentrations of Serifit against silkworm pathogens revealed the effectiveness of Serifit against BmNPV polyhedra, spores of *N. bombycis* and *B. thuringiensis* and conidia of *B. bassiana* at concentrations of 0.20 % and above (Table 2). The Polyhedra of BmNPV and spores of *N. bombycis* (1×10^6 spores/ml) and *B. thuringiensis* (1×10^8 spores/ml) and conidia of *B. bassiana* (1×10^7 conidia/ml) when exposed to different concentrations of Serifit solution *viz.*, 2.0, 1.0, 0.5, 0.4, 0.3, 0.2, 0.15, 0.10 and 0.05 % in three different exposure periods *viz.*, 10, 20 and 30 min were having the same effect. The solution was effective against all the pathogens up to 0.15 % irrespective of the exposure time. No mortality due to infection was observed up to 0.15 % in all the three exposures.

Use of Serifit as surface disinfectant

As surface disinfectant, 0.2 % Serifit solution was effective against surface contaminated layings with the concentrations of pathogens as used in *in vivo* studies,

Table 1: *In vitro* testing on the germicidal activity of Serfit

Pathogen	Concentration (%) of Serfit								
	2.00	1.00	0.50	0.40	0.30	0.20	0.15	0.10	0.05
<i>B. thuringiensis</i>	-	-	-	-	-	-	-	+	+
Control	+	+	+	+	+	+	+	+	+
<i>B. bassiana</i>	-	-	-	-	-	-	-	+	+
Control	+	+	+	+	+	+	+	+	+

(-) Effective (inhibition zone observed); (+) Not effective (No inhibition zone)

Table 2: Results of the *in vivo* testing on the efficacy of Serfit against silkworm pathogens

Concentration of Serfit (%)	10 min exposure time				20 min exposure time				30 min exposure time			
	% mortality/infection due to				% mortality/infection due to				% mortality/infection due to			
	Bm NPV	<i>N.b.</i>	<i>B. t.</i>	<i>B. b.</i>	Bm NPV	<i>N.b.</i>	<i>B. t.</i>	<i>B. b.</i>	Bm NPV	<i>N.b.</i>	<i>B. t.</i>	<i>B. b.</i>
2.00	-	-	-	-	-	-	-	-	-	-	-	-
1.00	-	-	-	-	-	-	-	-	-	-	-	-
0.50	-	-	-	-	-	-	-	-	-	-	-	-
0.40	-	-	-	-	-	-	-	-	-	-	-	-
0.30	-	-	-	-	-	-	-	-	-	-	-	-
0.20	-	-	-	-	-	-	-	-	-	-	-	-
0.15	-	-	-	-	-	-	-	-	-	-	-	-
0.10	+	+	+	+	+	+	+	+	+	+	+	+
0.05	+	+	+	+	+	+	+	+	+	+	+	+
Inoculated control	+	+	+	+	+	+	+	+	+	+	+	+
Normal control	-	-	-	-	-	-	-	-	-	-	-	-

(-) Effective (No mortality/infection); (+) Not effective (mortality/infection)

when exposed for 10 min. 0.2 % Serfit solution was effective as a surface disinfectant against all the tested pathogens.

Efficacy of Serfit solution for disinfection of rearing house and appliances

0.20 % concentration of Serfit solution was selected for further studies and tested for disinfection of rearing trays and rearing house, contaminated with pathogens. When the silkworm rearing was conducted in the contaminated trays disinfected using 0.2 % Serfit solution, no disease incidence was observed till second

moult. The worms inoculated with the pathogens exposed at the time of rearing room disinfection also did not develop any disease till second moult. The disinfectant solution was found to be effective for disinfection of contaminated rearing trays and rearing house (Tables 3, 4 and 5).

Laboratory testing of the technology

Laboratory testing was conducted on the technology "Disinfection and hygiene using Serfit" and the results indicated (Table 6) that Serfit (0.20 %) was an effective agent for disinfection and maintenance of hygiene in

Table 3: Efficacy of Serifit as surface disinfectant against the surface contaminated layings

Treatment	% mortality in surface contaminated layings			
	BmNPV	<i>N. b.</i>	<i>B. t.</i>	<i>B. b.</i>
0.20 % Serifit	-	-	-	-
Inoculated control	+	+	+	+

(-) No mortality/infection; (+) Mortality/infection

Table 4: Efficacy of Serifit in disinfecting the contaminated rearing trays

Treatment	% mortality/infection due to			
	BmNPV	<i>N. b.</i>	<i>B. t.</i>	<i>B. b.</i>
0.20 % Serifit	-	-	-	-
Inoculated control	+	+	+	+

(-) No mortality/infection; (+) Mortality/infection

Table 5: Efficacy of Serifit in disinfection of contaminated rearing house

Exposure time	% mortality/infection due to			
	BmNPV	<i>N. b.</i>	<i>B. t.</i>	<i>B. b.</i>
30 min	-	-	-	-
24 h	-	-	-	-
Inoculated control	+	+	+	+

(-) No mortality/infection; (+) Mortality/infection

sericulture.

Field trials

The results indicated an improvement of 6.73 kg /100 DFLs in cocoon yield by the use of Serifit over the existing ruling disinfectant used in sericulture (Table 7), with superior cocoon characters.

DISCUSSION

There are many disinfectants reported to be effective against silkworm pathogens. However, only a few of them are in practical use with several constraints associated with their use. In India, formalin is effective in a separate and closed rearing house under high humid

condition. It is a carcinogenic having pungent odour. Though Bleaching powder is suitable to all types of rearing houses, it is unstable and highly corrosive. Later, Chlorine dioxide (Sanitech/Serichlor), was introduced in sericulture (Balavenkatasubbaiah *et al.*, 1996; Balavenkatasubbaiah *et al.*, 2006) which was effective and could overcome the constraints associated with formalin and bleaching powder but its higher cost is a constraint. Recently, another Chlorine based disinfectant, Asthra was tested and recommended to the field to use as a disinfectant for disinfection of rearing house, appliances and maintenance of hygiene (Balavenkatasubbaiah *et al.*, 2014). In the present study, Serifit- a Chlorine (60-65 %) based inorganic compound, manufactured by M/s Sree Rayalaseema Hi-Strength Hypo Ltd., Kurnool, Andhra Pradesh was evaluated for its suitability and adoptability in Indian Sericulture.

The studies on the efficacy of different concentrations of Serifit against the silkworm pathogens indicated the effectiveness of Serifit as a germicide at 0.15 to 2.00 %. Based on effective concentration, 0.20 % of Serifit was selected, tested and found effective as a disinfectant for surface disinfection of silkworm eggs, rearing trays and rearing house. This concentration (0.20 %) was found less corrosive too. A technology on "Disinfection and hygiene using Serifit" was developed and evaluated both at laboratory and field level. It is very effective as a disinfectant for disinfection of rearing house and hygiene maintenance and the disinfection has no hazardous effect on larval health and cocoon characters. Field evaluation of the technology also indicated that Serifit (0.20 %) is an effective disinfectant which has been accepted by farmers.

The salient features of Serifit

- Serifit has germicidal activity against all silkworm pathogens
- It is a strong oxidizing agent
- Preparation of the disinfectant solution is easy
- It is easy to handle and transport
- It is highly soluble, does not form slurry and do not block sprayer nozzle
- It suits to all types of rearing houses under all environmental conditions and is less corrosive at recommended concentration
- It is stable with a shelf life of one year

Table 6: Silkworm rearing performance in the laboratory

Treatment	No. of larvae kept after III moult	% Pupation	Cocoon weight (g)	Shell weight (g)	Shell %	% of diseases			
						Grasserie	Flacherie	Muscardine	Total
0.20 % Serifit	200	95.00	1.820	0.400	21.97	1.50	3.50	0.00	5.00
Control (Other disinfectant)	200	94.00	1.800	0.390	21.67	3.00	3.00	0.00	6.00

Table 7: Performance of Serifit as disinfectant at different sericultural areas

Area	No. of farmers	No. of DFLs reared	Yield/ 100 DFLs (kg)	Cocoon weight (g)	Shell weight (g)	Shell %	% of diseases				Cocoons Rate/kg (₹)
							Grasserie	Flacherie	Muscardine	Pebrine	
Serifit											
Karnataka	35	8,300	86.13±6.48	1.87±0.09	0.38±0.03	20.48±0.69	0.03	0.02	0.03	0.00	251.40±10.90
Tamil Nadu	35	5,675	78.27±1.06	1.90±0.04	0.40±0.02	20.98±0.55	0.02	0.18	0.00	0.00	234.54±20.85
Andhra Pradesh	38	11,175	73.41±7.40	2.02±0.13	0.42±0.03	20.96±1.46	0.00	1.52	0.41	0.00	258.11±17.64
Total/ Average	108	25,150	78.71±7.93	1.94±0.12	0.40±0.03	20.80±1.05	0.02	0.60	0.15	0.00	248.21±19.45
Control (Other disinfectant)											
Karnataka	35	6,590	69.43±10.62	1.74±0.05	0.32±0.06	18.27±3.26	0.12	0.10	0.14	0.00	232.94±18.75
Tamil Nadu	35	5,975	78.11±1.31	1.89±0.04	0.39±0.01	20.65±0.41	0.06	0.22	0.00	0.00	227.09±17.40
Andhra Pradesh	35	10,075	70.02±4.29	2.02±0.08	0.41±0.02	20.24±0.77	0.03	1.81	0.83	0.00	256.84±30.86
Total/ Average	108	22,640	71.98±7.75	1.89±0.13	0.37±0.05	19.78±2.17	0.07	0.74	0.34	0.00	239.45±26.30

Based on the laboratory and field evaluation results, Serifit has been licensed and recommended to use as a disinfectant for effective sanitation in Sericulture.

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Article scientifique

SERIFIT- UN DESINFECTANT POUR UN ASSAINISSEMENT EFFICACE EN SERICICULTURE INDIENNE

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RÉSUMÉ

Les maladies du ver à soie sont une des contraintes de la production séricicole indienne. La prévention des maladies est obtenue par une désinfection efficace des pièces d'élevage, de leur environnement et du matériel ainsi que par le maintien de l'hygiène. Un désinfectant approprié est le besoin essentiel pour éliminer les pathogènes persistants de la magnanerie. Le formol, la chaux, le dioxyde de chlore (Sanitech/Serichlor et Sanitech Super), et Asthra ont été utilisés comme désinfectants en sériciculture indienne. De ce point de vue, Serifit, un désinfectant nouveau (composé minéral à base de chlore) produit par M/s Sree Rayalaseema HI-Strength Hypo Limited, Kurnool, Andhra Pradesh, a été évalué pour ses effets germicides contre les pathogènes du ver à soie. Des études *in vitro* sur l'efficacité de différentes concentrations de Serifit contre *Bacillus thuringiensis* et *Beauveria bassiana* prouvent l'efficacité de Serifit comme germicide de 0.15 à 2 %. Des études *in vitro* de différentes concentrations de Serifit contre tous les pathogènes communs du ver à soie, c'est-à-dire les polyèdres de BmNPV, les spores de *Nosema bombycis* et de *B. thuringiensis* et les conidies de *B. bassiana* confirment son efficacité. Basé sur une

concentration efficace, 0.20 % de Serifit a été sélectionnée et cette concentration est efficace pour la désinfection des locaux contaminés, les matériels et la surface des œufs. Une technologie de « désinfection et d'hygiène utilisant Serifit » a été testée au laboratoire et sur le terrain. Au laboratoire, Serifit (0.20 %) se trouve efficace pour la désinfection de la magnanerie et pour le maintien de l'hygiène. Les essais de terrain chez les fermiers de différentes zones séricicoles du Karnataka, du Tamil Nadu et d'Andhra Pradesh montrent la même efficacité. Le désinfectant est stable, non dangereux, le moins corrosif et approprié pour un assainissement efficace.

Mots-clés: *Bombyx mori* L., désinfectant, pathogènes, sériciculture, serifit, ver à soie



DETERMINANTS OF BIVOLTINE SERICULTURE TECHNOLOGY ADOPTION IN SERICULTURAL CLUSTERS OF SOUTHERN INDIA- AN ANALYSIS

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ABSTRACT

Though India is endowed with the production of all the four types of commercial silks, mulberry silk is considered to be qualitatively superior and many efforts have been made to popularize bivoltine sericulture in a larger scale. Central Silk Board in association with the Departments of Sericulture in various states has introduced the concept of Cluster Promotion Programme with an ambitious plan of producing 5000 MT of bivoltine silk annually by the end of XII five year plan of Government of India. Many studies conducted in this direction have indicated that the socio-economic conditions of sericulturists play an important role in adoption of bivoltine sericulture technology. The present study has made such an attempt with an objective of understanding the factors influencing adoption of bivoltine cocoon production by the farmers. For the purpose, a sample of 30 farmers each from the cluster areas of Kolar and Chikballapur district were interviewed personally using a structured schedule at random. The data were analysed using "Logit Model". The results indicated that the factors viz., contacts with extension personnel, participation in extension programmes, cash incentives and irrigation facility have influenced the adoption of bivoltine cocoon production in the study area apart from income obtained from sericulture. The study concluded on a note that, for enhancing the bivoltine silkworm rearing, extension efforts have to be strengthened in terms of technical support. Further, the new entrants into the field of bivoltine sericulture may be trained at farmers' level through farm schools and on the spot training by extension staff for efficient adoption and sustenance of bivoltine sericulture technology.

Key words: Adoption, bivoltine sericulture, Logit model, random sampling.

INTRODUCTION

India with its varied climatic conditions is fortunate with the production of all the four types of commercially known silks, namely mulberry, tasar, eri and muga. Over the years, the sericulture industry has made a clear impact on the farmers of southern states through its low gestation period. The much talked about qualitatively superior bivoltine silk production is yet to make a dent inspite of many efforts made during the world bank assisted National Sericulture Project (NSP) during 1986-97 and the JICA project (1998-2012).

In spite of efforts made by Central Silk Board and the state departments of sericulture, the practice of bivoltine

sericulture could not pick up to the expected level in India in general and Karnataka in particular. Hence, a new attempt towards development of bivoltine sericulture through cluster approach was planned and implemented. The present study was taken up with an objective of understanding the factors influencing bivoltine technology adoption by the farmers in Karnataka and working out of the probability of adoption of bivoltine silkworm rearing in sericulture dominant areas of Karnataka state.

METHODOLOGY

The present investigation was carried out in Kolar and Chikballapur districts of Karnataka state as these two

areas represent the unique methods of practicing sericulture in the state. Further, Tekal and Yeldur clusters out of the experimental districts were selected based on the concentration of mulberry area and the progressive results. From each of the selected clusters, three villages and 10 farmers from each village were selected at random. Thus a total of 60 farmers constituted the sample size of the study.

The primary data to meet the objectives of the study were collected from the selected farmers by using personal interview method with the help of a structured schedule prepared for the purpose. The data collected include, information on friends and social contacts, contacts made with the extension workers, cash incentives, irrigation facility and participation in extension programmes.

Statistical tools

The data thus generated from the sericulturists were tabulated and analysed using the tabular analysis in terms of numbers and frequencies. Scores were assigned to quantify the qualitative data and were subjected to Logit analysis to work out the probabilities of adoption of bivoltine sericulture. The use of logit model, which give maximum likelihood estimators, can overcome most of the problems associated with Linear Probability model and provide parameter estimators which are efficient. The model was estimated using maximum likelihood procedure.

The functional form of the logit model is specified as :

$$\text{Log} \frac{P_i}{1-P_i} = Z_i = a + b_i X_i + u_i$$

Where,

a = Intercept

P_i = The probability that a farmer will adopt

b_i = Logit coefficient

X_i = Independent variables

u_i = The error term

P_i

———— = The odd's ratio in favour of adoption

$1-P_i$

The variables included in the model were:

1. Friends and social contact (index),
2. Irrigation facility (index),
3. Voluntary contacts with extension workers (index),
4. Participation in extension programmes (index) and
5. Cash incentives (index).

RESULTS AND DISCUSSION

The variables such as irrigation facility, income from sericulture, participation in extension programmes and economic motivation in terms of cash incentive which influenced the farmers to adopt bivoltine sericulture among small farmers had a 0.78 probability of adoption of bivoltine silkworm rearing. The factors such as area under mulberry, income from sericulture, participation in extension programmes, contact with extension workers and economic motivation influenced adoption of bivoltine rearing by medium size farmers. On an average, there was a 0.63 probability that medium farmers would adopt bivoltine technology under the existing situation. Among large farmers, income from sericulture, contacts with CDFs (Cluster development facilitators) and economic motivation favoured the adoption of bivoltine silkworm rearing with a probability of 0.70.

From the foregoing analysis, it is amply clear that the farmers have an inherent preference for bivoltine race and the probability of adoption of bivoltine race was rather low among medium farmers (Feder and O'mara, 1982). However, among large farmers, bivoltine was preferred (with 0.70 probability) mainly due to higher levels of profit. It is also disconcerting to note that participation in

Table 1: Factors influencing adoption of bivoltine silkworm rearing technology by farmers in the area

Factor	Small farmers (n = 24)			Medium farmers (n = 20)			Large farmers (n=16)		
	Coefficient	Mean	t-value	Coefficient	Mean	t-value	Coefficient	Mean	t-value
Irrigated land (Acre)	1.33	1.03	2.02*	1.53	0.75	1.08	0.05	2.82	1.65
Area under mulberry (Standard acre)	0.32	0.84	1.70	0.24	0.55	1.02*	0.11	2.31	1.97
Friends & social contact (Index)	0.01	0.35	0.95	0.14	1.15	1.26	0.04	0.96	0.36
Income from sericulture (₹)	2.33		2.35**	3.15		2.28**	3.42		2.12**
Participation in extension programmes (Index)	0.64	4.70	2.35**	0.75	3.60	2.05*	0.40	2.06	1.98
Contact with CDFs (Index)	0.14	2.20	1.46	0.34	3.27	2.48**	0.93	4.05	3.45**
Cash incentives (Score)	0.41	0.34	2.33**	0.09	0.40	2.00*	0.31	0.20	2.10**
Probability of adoption		0.78			0.63			0.70	

* - Significant at 10 per cent level; ** - Significant at 5 per cent level; CDF - Cluster development facilitator.

extension programmes has not contributed much to the adoption of bivoltine. They preferred and depended more on individual and personal contacts for adoption of technology. Therefore, it is necessary to use different extension methods for different category of farmers to give a boost to the adoption of bivoltine technology. It is needless to mention that the infrastructure required to support larger production of bivoltine cocoons needs to be created in the absence of which the entire effort could be a failure. In other words, strengthening the backward and forward linkages for bivoltine rearing is necessary, which will also strengthen the hands of extension machinery for effective implementation of the bivoltine cocoon production programme. The adoption of bivoltine race is associated with the availability of the family labour on the farm. This is borne out of the fact that sericulture enterprise is labour intensive. (Sinha, 1990).

The non-adoption of bivoltine hybrid rearing is mainly attributed to its failure levels due to lack of adoption of proper disinfection methods and improved rearing practices, attitude of farmers and fluctuation in the market prices. This calls for adoption of package of practices of rearing bivoltine hybrids, which can provide a stable yield and income to the farmers. These findings are in line with the results recorded by Blankenburg (1984); Just and Zilberman (1983) and Hiebert (1974). As the bivoltine hybrid is not suitable for all conditions and for all categories of farmers, steps may be thought of to

popularize the same among the identified category of farmers, who have facilities such as separate rearing house, irrigation facility, improved mulberry variety and the mindset to adopt bivoltine hybrid rearing package. Also, the availability of cocoons in the market throughout the year may be ensured to avoid fluctuations in cocoon price.

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Rapport technique

DETERMINANTS DE L'ADOPTION DES TECHNIQUES DE SERICICULTURE BIVOLTINE DANS DES GROUPES DE L'INDE DU SUD – UNE ANALYSE

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RÉSUMÉ

Bien que l'Inde soit engagée dans la production des quatre types de soie commerciaux, la soie mûrier est considérée comme qualitativement supérieure et beaucoup d'efforts ont été réalisés pour vulgariser la sériciculture bivoltine à une plus large échelle. Le Central Silk Board associé aux Départements vde Sériciculture de plusieurs états a introduit le concept de Programme de Promotion de Groupements avec l'ambition de produire 5000t de soie bivoltine par an à la fin du XIIème plan quinquennal du Gouvernement indien. Beaucoup d'études menées dans cette direction ont montré que les conditions socio-économiques des sériciculteurs jouent un rôle important qui influence l'adoption de la production de cocons bivoltins par les fermiers. Notre étude est une telle tentative avec l'objectif de comprendre quels sont les facteurs qui influencent l'adoption de la production de cocons bivoltins par les fermiers. A cette fin un échantillon de 30 fermiers issus des zones du groupement du district de Kolar et Chikkaballapur ont été interrogés individuellement à l'aide d'un modèle structuré au hasard. Les données ont été analysées en utilisant le « Modèle Logit ». Les résultats indiquent que les facteurs tels que les contacts avec le personnel de formation, la participation aux programmes de formation, les motivations et les possibilités d'irrigation influencent fortement l'adoption de la production de cocons bivoltins sans tenir compte des revenus engendrés par la sériciculture. L'étude conclut que pour stimuler l'élevage de vers à soie bivoltins, des efforts de formation doivent être faits en terme de support technique. De plus, les nouveaux entrants dans la sériciculture bivoltine doivent être formés au niveau de la ferme à travers des écoles fermières et une formation sur le tas par un personnel spécifique pour une adoption efficace et durable de la technologie de sériciculture bivoltine.

Mots-clés: Adoption, sériciculture bivoltine, modèle Logit, échantillonnage au hasard.



MYCELIUM PRODUCTION OF *CORDYCEPS MILITARIS* THAI STRAINS ON THAI RICE VARIETIES MIXED WITH YOLK AND PUPAL POWDER OF THAI SILKWORM, *BOMBYX MORI*

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ABSTRACT

Four new isolates of *Cordyceps militaris* (BCC18247, BCC17991, BCC17944 and BCC27815) and *Paecilomyces tenuipes* were investigated for mycelium production on various culture media. We used fungal pathogen of *Bombyx mori*, *Beauveria bassiana* as positive control. *C. militaris* strains and *P. tenuipes* were grown on the PDA for 5 days and transferred to 5 recipes of culture media of different varieties of Thai rice viz., plain rice, black rice, brown rice, glutinous rice and rice bran, supplemented with pupal powder of Thai silkworm, Nang Lai and yolk of chicken (*Gallus gallus domesticus*). The dead pupae were used as insect host of fungi. Mycelial growth of *Cordyceps* strains was determined by fresh weight of mycelia. The mycelial growth of *C. militaris* strains was detected on dead pupae of silkworm and on the medium comprising of pupal powder, yolk and glutinous rice on the 14th and 4th day, respectively, after fungal inoculation. Only the fruiting body of *P. tenuipes* was found growing up on pupal cadavers but not of *C. militaris* strains. The most effective recipe for mycelial growth was observed as glutinous rice + pupal powder + yolk for all *C. militaris* strains with the maximum growth reported for BCC17944.

Key words: *Bombyx mori*, *Cordyceps militaris*, mycelium, Thai rice.

INTRODUCTION

Of late, sericulture doesn't find its use restricted only to the field of textiles, but also in other areas viz., production of cosmetics, supplementary food etc. and for pharmaceuticals. The polyvoltine races of *Bombyx mori* exist in tropical countries such as Thailand and India. But, the bivoltine races are temperate in origin being found in Japan, China etc. Cocoons of Thai silk moth are yellow and small but rich in sericin (Hiroshi, 1996). In Thailand, after silk reeling, the left over pupae are used for fish meal and poultry feed (Jintasataporn, 2012). Likewise, Thailand has been well known for Thai rice (*Oryza sativa*) production. There are many Thai rice varieties such as plain rice, black rice, brown rice and glutinous rice. Elemental composition was reported as comprising of three minor, such as Ca, K and Mg and

eight trace elements such as Al, As, Br, Cl, Cu, Fe, Mn and Zn (Busamongkol *et al.*, 2009).

Cordyceps fungus, an entomopathogen, has many insect hosts belonging to orders, Hymenoptera and Lepidoptera (Hywel-Jones and Evans, 1996; Sung and Spatafora, 2004). In Thailand, by nature, *Cordyceps* spp. are found in low temperature areas and usually on the mountainous terrains (Hywel-Jones and Sivichai, 1995). They grow at a slow pace and are found only in high altitude areas. Among *Cordyceps*, *Cordyceps militaris* has been used as food and in herbal medicine. The *C. militaris* contains cordycepin (Radwan and Wilson, 1980; Huang *et al.*, 2009) and polysaccharide component (Yan *et al.*, 2008), which are believed to be beneficial for treating cancer (Jin *et al.*, 2008; Jeong *et al.*, 2013) and possessing anti-aging effects (Yue *et al.*, 2013).

Cordycepin can stimulate the immune system and has effects on both innate immunity and adaptive immunity and also on gut immune system (Zhou *et al.*, 2009). It is produced both in fruiting body and in the mycelium of *Cordyceps* (Xie *et al.*, 2009). As Thailand has been known for its silk production and rice varieties and export through the world, the objective of this study was to find the suitability of the pupal powder of *B. mori* as an ingredient of culture media consisting of yolk and either one of 5 varieties of Thai rice viz., plain rice, black rice, brown rice and glutinous rice and rice bran for inducing the mycelial growth of *C. militaris* and its anamorph, *P. tenuipes*.

MATERIALS AND METHODS

Preparation of silkworm (*B. mori*) pupal powder

We used Thai silkworm, Nang Lai larvae for this experiment. They were reared by feeding on mulberry leaves, under the standard rearing conditions, at a temperature of 25-28°C with 75 ± 5 % humidity. Young silkworm larvae were fed on natural food, mulberry leaves until they moulted, and transformed into the pupal stage. Ten days old silkworm pupae were used for fungal inoculation. On the sixth day of development, the pupae were collected by cutting open the cocoons. The pupae were used in 2 groups; the first group was dried at 70 °C for 12 hours in an oven, and then ground with a pestle and mortar to prepare pupal powder. The second group was used for comparing the growth of *C. militaris* on live and killed silkworm pupae. The pupae were killed by heating in an oven at 70 °C for 6 hours.

C. militaris strains

Four *C. militaris* strains (BCC18427, BCC17991, BCC 17944 and BCC27815) were collected from Thailand and *P. tenuipes* was provided by BIOTEC, National Science and Technology Development Agency, Thailand. These *C. militaris* strains as mentioned above were investigated in various sources for mycelium and fruiting body (stroma) propagation. The fungal pathogen of silkworm, *Beauveria bassiana* was used as a positive

control. *C. militaris* strains and their anamorph, *P. tenuipes* were grown on the potato dextrose agar (PDA) for 5 days in 250 ml flasks. After 4 days, they were inoculated on culture media.

Preparation of fungal media

The fungal culture media comprised of 5 recipes of Thai rice varieties mixed with pupae powder and yolk viz., 1) plain rice+pupal powder+yolk, 2) black rice+pupal powder+yolk, 3) brown rice+pupal powder+yolk, 4) glutinous rice+pupal powder+yolk and 5) rice bran +pupal powder+yolk. Five grams each of plain rice, black rice, brown rice, glutinous rice, and rice bran were soaked in distilled water overnight, then ground and mixed with 5 g of silkworm pupal powder of Nang Lai strain, 15 ml of water and 10 ml of yolk. The mixtures were homogenized by using a blender. Each mixture was placed in a wide mouthed jar with lid, of 8 ounce in size, 6 cm in diameter, 11 cm in depth, and 210 ml in volume (Figure 1A). Then they were autoclaved at 121 °C for 20 minutes under 15 pounds-force per square inch (psi). Four days old inoculum was transferred on to these media and incubated at 24-25°C under 80-90 % humidity for the fungal growth. The mycelium growth of *C. militaris* strains and *P. tenuipes* were determined.

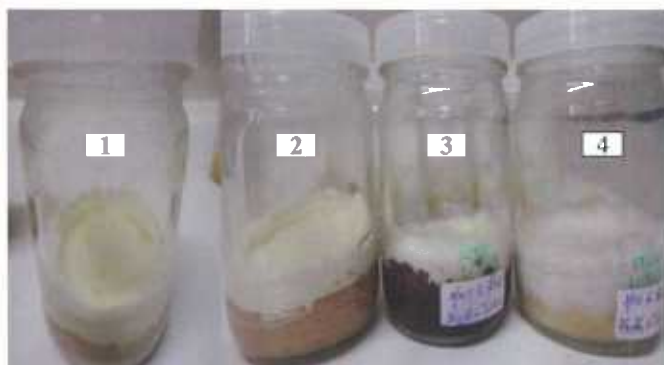
Statistical analysis

The present experiment followed the complete randomized design pattern with three replications in each treatment combination. The sampling units were considered homogenous among the treatments of rice types in the culture media. The mean data on mycelial weight were tested using the Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Tests (DMRTs) if the ANOVA indicated differences in at least one pair of mean data ($p < 0.05$). Shapiro-Wilk and Levene's F tests (data not included) were carried out. The distribution of mycelial weight in all treatment groups did not deviate from normal distribution (except for *B. bassiana*, where $p < 0.047$ was considered as acceptable for being normally distributed), and the variances among

all treatment groups were homogenous (except for BCC 17944 and BCC 27815 on rice bran where, p-values of Levene's F were < 0.05 , but considered homogenous because the distribution in normal Q-Q plots indicated small p-values due to similar mycelial weight among the replications). All statistical analyses were performed in IBM SPSS Statistics 22®.

RESULTS AND DISCUSSION

The mycelial growth could be seen within 4 days after fungal inoculation. The mycelial growth of *C. militaris* strains were determined by measurement of the mycelial weight (mg). The results of mycelial growth are shown in Figure 2. Glutinous rice + pupal powder + yolk was found the most suitable medium for mycelial growth followed by black rice + pupal powder + yolk and brown rice +



A



B

C

Figure 1: A-The mycelial growth of *Cordyceps militaris* strain, BCC 17944 on culture media comprising of pupal powder of Thai silkworm, Nang Lai and yolk with different types of Thai rice: 1. glutinous rice, 2. brown rice, 3. black rice and 4. plain rice; Fruiting body of *Paecilomyces tenuipes* on glutinous rice+pupal powder+yolk (B) and on dead silkworm pupae, Nang Lai (C).

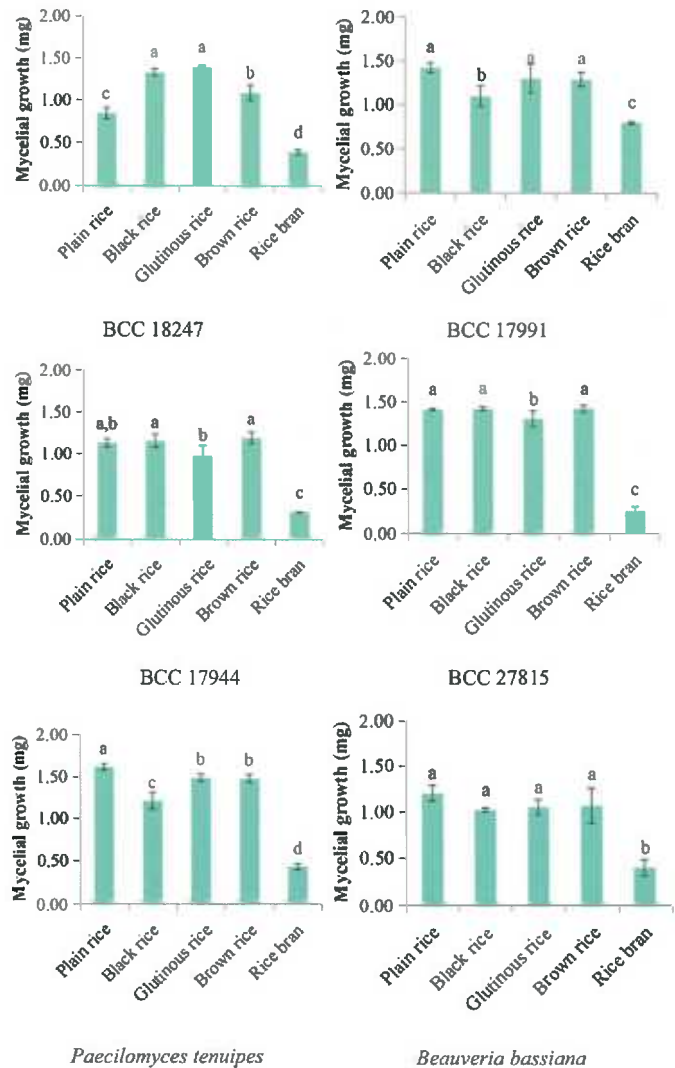


Figure 2: Mycelial weight of different *C. militaris* strains, *P. tenuipes*, and *B. bassiana* cultured in media enhanced with pupal powder, yolk, and different rice types

Error bars represent 1 unit of standard deviation. Mean values with the same letter are not statistically different ($p < 0.05$) according to DMRTs.

pupal powder + yolk. The highest mycelial weight was recorded against *C. militaris* BCC17944.

The killed pupae were more prone to penetration by *Cordyceps* spp. than live pupae. These findings suggest that plain rice, glutinous rice, brown rice and black rice mixed with pupal powder can be used as recipes for mycelial growth of *Cordyceps* spp. The anamorph of *Cordyceps*; *P. tenuipes* produced stroma on glutinous rice

mixed with pupal powder and yolk and the killed Nang Lai pupae (Figure 1B and 1C).

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Brève Communication

PRODUCTION DE MYCELIUM DES SOUCHES THAI DE *CORDYCEPS MILITARIS* SUR DES VARIETES DE RIZ THAI MELANGEES AVEC DU JAUNE D'ŒUF ET DE LA POUDRE DE PUPES DE VER A SOIE THAI, *BOMBYX MORI*

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R É S U M É

Quatre nouveaux isolats de *Cordyceps militaris* (BCC18247, BCC17991, BCC17944 et BCC27815) et de *Paecilomyces tenuipes* ont été étudiés pour la production de mycélium sur différents milieux de culture. Nous avons utilisés les pathogènes fongiques de *Bombyx mori*, avec *Beauveria bassiana* comme témoin positif. Les souches de *C. militaris* et *P. tenuipes* ont été cultivés sur PDA pendant 5 jours et transférés vers des milieux de culture de 5 recettes contenant différentes variétés de riz Thaï comme le riz simple, le riz noir, le riz brun, le riz gluant et du son additionnées de poudre de pupes de vers à soie Thaï, de Nang Lai et de jaune d'œuf. Les pupes mortes ont été utilisées pour héberger des champignons et des insectes. La croissance du mycélium des souches de *Cordyceps* a été évaluée par le poids de mycélium frais. La croissance du mycélium de *C. militaris* a été détectée sur des pupes de ver à soie mortes et sur des milieux comprenant de la poudre de pupes, du jaune d'œuf et du riz gluant après 14 et 15 jours d'inoculation respectivement. Seules les fructifications de *P. tenuipes* poussent sur les cadavres de pupes mais pas ceux des souches de *C. militaris*. La recette la plus efficace pour la croissance du mycélium s'avère être riz gluant + poudre de pupes + jaune d'œuf pour toutes les souches de *C. militaris* avec un maximum obtenu avec BCC17944.

Mots-clés: *Bombyx mori*, *Cordyceps militaris*, mycélium, riz thaï.

MoU between International Sericultural Commission and SAARC Agriculture Centre signed



A Memorandum of Understanding for collaboration between International Sericultural Commission, Bengaluru, India and SAARC Agriculture Centre, Dhaka, Bangladesh was signed on 25th August 2016 at India International Centre, New Delhi. The MoU aims at promoting the development of regional cooperation in the fields of research and training related to the improvement of production techniques and extension of sericulture and silk industry in all the 8 SAARC member countries. The expected output of the collaboration are:

- a) network for sericulture research and development in SAARC region;
- b) accelerated diffusion of sericultural technologies;
- c) maximized targeting of farmers in the shortest possible time;

- d) enhanced silk productivity and production in the region;
- e) enhance the livelihood of rural poor through development and dissemination of sericulture technologies.

As a follow-up of this, a three member expert team identified by ISC has been deputed to the sericultural areas and facilities of Bangladesh to undertake a feasibility study during 14th to 24th January 2017. Similar evaluation studies would be undertaken in other SAARC countries with the main objective of developing a sericulture development project for the SAARC region.

Venue of the 25th ISC Congress on Sericulture and Silk Industry finalized

The Govt. of Japan has accepted the request of International Sericultural Commission to organize the 25th ISC Congress on Sericulture and Silk Industry during 2019 in Japan. The Ministry of Agriculture, Forestry and Fisheries, Japan would be the nodal Ministry for organizing this prestigious event. The first Announcement of the Congress is expected to be released by June 2017. This is the first time that an ISC Congress would be organized in Japan. The theme of the Congress - 2019 would be on the frontier areas of research using silkworm and silk.





Programme Coordinator, ISC participated in the 9th China International Silk Conference

The 9th China International Silk Conference was held at Liuzhou, PR China during 8th to 10th August 2016. Mr. Dileep Kumar, R., Programme Coordinator was invited to attend the Conference as a Guest Speaker on Indian Silk Industry. The Conference was jointly organized by Soochow University, Soozhou and Guangxi University of Science and Technology, Liuzhou. The Conference witnessed the participation of 275 delegates from 17 countries. 115 research papers were presented on the following areas:

- (1) Sericulture bioengineering
- (2) Fiber materials & Textile products
- (3) Process technologies & System engineering of textiles and clothing
- (4) Ecotextile & Dyeing and finishing technologies
- (5) Finery science and technologies & Fashion clothing
- (6) Trade & Culture of textiles and clothing
- (7) History & Resource protection of mulberry silk
- (8) Other relevant researches in textiles and silk science.

The Programme Coordinator, ISC held initial discussions with the Chinese authorities on the enrollment of PR China as a Member Country of ISC.

ISC Volunteer Experts Visit Bangladesh

Responding to the request made by the Bangladesh Sericulture Development Board (BSDB), Government of the People's Republic of Bangladesh, ISC deputed 3 Volunteer Experts (Dr. B. S. Angadi, Dr. S. Nirmalkumar and Dr. M. Siva Kumar), to take up 'Reconnaissance Study on Sericulture Research and Development in Bangladesh' for a period of 9 days from 15th to 23rd January, 2017. During their visit, the experts visited different field units of Rajshahi, Chapai Nawabganj, Rangpur and Dhaka divisions. Experts visited the Bangladesh Sericulture Research and Training Institute (BSR & TI), Rajshahi, observed the progress made in the development of silkworm breeds/hybrids, mulberry varieties and offered suggestions for narrowing the gap between the laboratory results and the field performance. They had extensive interactions with the departmental technical staff, reelers, weavers, fabric manufacturers and exporters. Wrap up meeting was held with Mr. Shubhashish Bose, Secretary in Charge, Ministry of Textiles and Jute, Government of the People's Republic of Bangladesh, Dr. Mohammad Jalal Uddin, Executive Chairman, BARC, Dr. S.M. Bokhtiar, Director, SAARC Agriculture Centre, Dr. Tayan Raj Gurung, Senior Programme Specialist, SAARC Agriculture Centre, Mr. Anis-Ul-Haque Bhuiya, Director General, BSDB, Mr. Nazibul Islam, Member, BSDB and the other scientists of SAC, BARC, BRAC and BSDB. Experts gave suggestions for adoption of advanced technologies in the field of silkworm seed production, mulberry cultivation, silkworm breeding, silkworm rearing, post cocoon areas and identified potential clusters for developing as 'Soil to Silk Clusters'.

NEWS CORNER



Seen with Mr. Asaduzzaman Noor (6th from left) Bangladesh Cultural Minister, Mr. Dewan Kamal Ahmed Mayor of Nilphamari (Extreme left) and Mr. Md. Zakir Hossain, Deputy Commissioner of Nilphamari (second from left)



At the venue of wrap meeting team with Dr. Tayan Gurung



At one of the Bangladesh nurseries



Team with women sericulturists of Taraganj village



With women CRC entrepreneurs of Shailabari village (Near Sirajganj)



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