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IMPROVING THE COHESION CHARACTERISTICS OF MULTIBIVOLTINE RAW SILK USING BIVOLTINE SERICIN COATING IN REELING PROCESS

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ABSTRACT

Cohesion characteristics of multibivoltine raw silk are significantly inferior to that of bivoltine which may be attributed to the racial character. In an attempt to improve the cohesion characteristics of multibivoltine raw silk, sericin extracted from bivoltine silk was coated over multibivoltine raw silk during the reeling process. Pure bivoltine sericin powder for this purpose was extracted from the cocoon shells by using the pressurized degumming machine developed by Central Silk Technological Research Institute (CSTRI), Bengaluru, India without using any chemical. Two methodologies of impregnations of sericin were tried. In the first one, the bivoltine sericin powder was mixed in the reeling basin water during the process of reeling and the second method was to reel multibivoltine raw silk in reeling machine and during the process of small reel permeation before re-reeling process, sericin powder was impregnated in the raw silk using vacuum pressure. Further, two pan cooking method used in both the methods has shown significant improvement (at 1 and 5 % levels) in terms of less waste generation and better size characteristics. Both the processes of sericin coating have resulted in significant improvement in cohesion of raw silk. However, sericin coating (3 gpl) during small reel permeation is recommended in view of better adoptability.

Key words: Cohesion, mutliend reeling, sericin, two pan cooking, vacuum permeation.

INTRODUCTION

Cohesion is one of the important characteristics of raw silk which has significant influence on performance of raw silk during preparatory and weaving processes and on the quality of fabric. Research conducted at Central Silk Technological Research Institute (CSTRI), Bengaluru, India reveals that in order to achieve required efficiency in weaving, particularly when the silk is used in the warp without twist (for the production of particular fabrics like crepes), raw silk with good cohesion is very essential (Subhas *et al.*, 1995).

From the studies conducted and the results of raw silk quality testing conducted at CSTRI and its sub units, it is observed that cohesion of multibivoltine raw silk is significantly less (20 ~ 60 strokes) as compared to that of bivoltine raw silk (60 ~ 150 strokes).

Exhaustive studies conducted in this line reveal that cohesion of raw silk is significantly influenced by the adhesive property of sericin which in turn is influenced by race / breed, atmospheric humidity conditions during cocoon spinning and cocoon cooking conditions and croissure length and reeling basin water temperature maintained during reeling to swell and soften the sericin (Liang Jan Zhu *et al.*, 1995; Subhas *et al.*, 1995; Subhas and Somashekar, 2003 a, 2003 b, 2004 a, 2004 b, 2005, 2006, 2007, 2008).

Significantly higher cohesion expressed in the case of bivoltine raw silk is attributed to the more sticky nature of bivoltine sericin along with other factors, such as better drying and proper cooking of cocoons and reeling with more croissure length.

CSTRI has developed a pressurized degumming machine

to degum the silk without chemicals. The sericin thus extracted from this machine was utilized for the study (Joseph *et al.*, 2014).

In this context, it was felt necessary to study the improvement of cohesion characteristics in multibivoltine raw silk after coating it with sericin during reeling process in reeling water as well as during reel permeation process after production of raw silk and before re-reeling process.

MATERIALS AND METHODS

The multibivoltine cocoons procured from Government cocoon market, Kolar, Karnataka were used for the study. The quality characteristics of experimental cocoons are listed in Table 1. The cocoon samples were divided into batches and subjected to drying, cooking and reeling.

Table 1: Characteristics of multibivoltine cocoons

Particulars	PM x CSR2 race cocoons
Cocoon weight (g)	1.925
Shell weight (g)	0.400
Shell %	20.7
Defective cocoons (%)	1.8

Cocoon drying with hot air drier: Cocoons were hot air dried to the optimum level in Batch type hot air drier. Each batch of cocoons was dried for 5 hours as per the following temperature profile.

First hour	- 100 °C
Second hour	- 90 °C
Third hour	- 85 °C
Fourth hour	- 70 °C
Fifth hour	- 55 °C

Cocoon sorting: Defective cocoons were sorted out from reeling cocoons using cocoon sorting machine.

Cocoon cooking: The multibivoltine cocoons were cooked using two methods viz., open pan cooking and two pan cooking.

Open pan cooking: The cocoons were immersed in hot water at 90 °C and cooked for about 4 minutes using the ladle. Cold water was then sprinkled on to the cocoons to reduce the temperature of water to 80 °C and were brushed in the same pan at 80 °C. The cooked cocoons were transferred to the multiend reeling machine for reeling.

Two pan cooking: The dry cocoons (200 nos.) were filled in the cocoon cooking cage. Using the two pan cooking equipment, the cocoon cage was immersed in the first pan at 60 °C for about 1 minute and then transferred on to the second pan and treated at 90 °C for about 1 minute. Later, the cocoons were treated at 60 °C for about 1 minute in the first pan followed by treatment at 95 °C for about 2 minutes and then, cold water was sprinkled on to the cocoons to reduce the temperature of water to 80 °C and subsequently, the cocoons were brushed in the same pan at 80 °C. The cooked cocoons were transferred on to the multiend reeling machine for reeling.

Multiend silk reeling machine: The brushed cocoons were picked to get the true ends and reeled on multiend reeling machine. Eight cocoons were maintained per end in the reeling machine. The following process parameters were maintained during the process of reeling.

Reeling speed:	120 m/min
Croissure length:	8 cm
Basin water temperature:	40 °C

Small reel permeation: The silk reeled on multiend reeling machine, on small reels were treated in reel permeation chamber for better water absorption by silk before re-reeling. Small reels were subjected to vacuum permeation pressure of 300 mm Hg and this process was repeated for 3 cycles.

Re-reeling: Re-reeling of raw silk skeins were carried out on closed type of re-reeling machine. Re-reeling process was carried out by adopting 120 rpm and by maintaining 35 ~ 40 °C temperature and 45 % RH inside the re-reeling machine.

Production of bivoltine sericin powder: The bivoltine cut cocoon shells were degummed at pressurized degumming machine developed by CSTRI at 1:10 material liquor ratio (Joseph *et al.*, 2014). The degummed liquor was collected and water particles were evaporated using spray drying technique. The resultant material was dried in hot air drier at 100 °C and the dried sericin was ground to produce bivoltine sericin powder. For coating the sericin powder on to silk, it was dissolved in boiling water and as per requirement, 1, 2 and 3 gpl solutions were prepared and used in the reeling process.

Sericin impregnation: The experiment was designed as to coat the multibivoltine raw silk with bivoltine sericin during the reeling process and during the reel permeation process. The process variables comprised of cooking methods *viz.*, open pan cooking and two pan cooking and varying concentrations of sericin (1, 2, 3 gpl) in reeling basin / reel permeation chamber.

Coating bivoltine sericin on multibivoltine raw silk in reeling basin: According to the quantity of water used in reeling basin, the required quantity of sericin solution (1, 2 and 3 gpl) was mixed and reeling was carried out with CSTRI standard parameters (Lakshmipathaiah *et al.*, 2000). In each case, 4 replications were conducted. After reeling the raw silk, the reeling parameters were collected. The raw silk was subjected to quality tests and quality characteristics were assessed.

Coating bivoltine sericin on multibivoltine raw silk in small reel permeation chamber: The multibivoltine raw silk was reeled on multiend reeling machine using two methods of cooking *viz.*, open pan and two pan cooking. After the reeling process, the reeling parameters were collected. The raw silk reeled was converted into standard skeins in re-reeling machine. During the process, the small reels with raw silk were subjected to small reel permeation process before re-reeling to wet the silk in order to unwind the same effectively. Based on the quantity of water used in reel permeation chamber, the required quantity of sericin solution (1, 2 and 3 gpl) was mixed and permeation was carried out with CSTRI

standard parameters (Lakshmipathaiah *et al.*, 2000). During the small reel permeation process, bivoltine sericin powder was impregnated in the raw silk using vacuum pressure and then the small reels were re-reeled and silk was assessed for quality parameters. In each case, 4 replications were conducted. The raw silk thus produced was subjected to quality tests and quality characteristics were assessed.

Data collected: The reeling parameters *viz.*, renditta, raw silk recovery percentage and waste percentage on silk weight were collected while reeling the cocoons. The multibivoltine raw silk coated with sericin was subjected to size test, serigraph test and cohesion test following standard methods. The testing parameters *viz.*, average size, size deviation, maximum size deviation, tenacity, elongation and cohesion characteristics were studied. The data were analyzed statistically, using SPSS package.

RESULTS AND DISCUSSION

Coating bivoltine sericin on multibivoltine raw silk in reeling basin

The two pan cooked cocoons have shown improvement in reeling performance compared to open pan cooked and reeled multibivoltine cocoons (Table 2). Further, the sericin coated multibivoltine raw silk has shown significant improvement in cohesion characteristics as could be observed from ANOVA (Tables 2 and 3). Compared to lower sericin powder concentrations, 3 gpl sericin coating in reeling basin has proved to offer significant improvement towards cohesion characteristics.

Coating bivoltine sericin on multibivoltine raw silk in small reel permeation chamber

The results presented in Table 4 substantiates the superior reeling performance of two pan cooked cocoons over open pan cooked and reeled multibivoltine cocoons. Significant reduction in waste generation during two pan cooking is an added advantage. Moreover, the sericin powder (3 gpl) coating on silk has enhanced the cohesion characteristics

Table 2: Reeling performance and quality characteristics of multibivoltine raw silk reeled with different concentrations of sericin in reeling basin

Particulars	Control		1 gpl		2 gpl		3 gpl	
	Open pan	Two pan	Open pan	Two pan	Open pan	Two pan	Open pan	Two pan
Renditta	8.1	7.8	8.5	8.0	8.2	7.8	8.2	7.8
Raw silk recovery (%)	23.6	24.6	22.6	23.9	23.3	24.5	23.4	24.6
Waste (%) on silk weight	20.2	17.6	19.6	17.0	17.9	16.1	15.9	14.2
Average size	24.1	23.8	23.0	24.2	23.2	24.2	23.0	23.7
Size deviation	1.44	1.62	1.2	1.04	0.96	0.92	1.36	1.06
Maximum size deviation	2.4	2.8	1.7	1.4	1.4	1.4	1.4	0.95
Tenacity (g/d)	3.5	3.6	3.5	3.4	3.5	3.3	3.5	3.5
Elongation (%)	22	22	23	23	21	23	22	21
Cohesion (strokes)	38	37	41	48	49	58	45	63

Table 3: Analysis of variance (ANOVA) results of characteristics of multibivoltine raw silk coated with sericin in reeling basin

Particulars	Comparison	Sum of square	Degree of freedom	Mean square	F- Value	Significance
Renditta	Between group	0.366	3	0.122	1.855	0.160 ^{NS}
	Within group	1.842	28	0.066		
	Total	2.209	31			
Raw silk recovery (%)	Between group	2.957	3	0.986	1.759	0.178 ^{NS}
	Within group	15.689	28	0.56		
	Total	18.646	31			
Waste (%) on silk weight	Between group	71.111	3	23.704	7.921	0.001 ^{**}
	Within group	83.787	28	2.992		
	Total	154.899	31			
Average size	Between group	1.165	3	0.388	0.567	0.642 ^{NS}
	Within group	19.19	28	0.685		
	Total	20.355	31			
Size deviation	Between group	1.442	3	0.481	19.972	0.000 ^{**}
	Within group	0.674	28	0.024		
	Total	2.116	31			
Maximum size deviation	Between group	6.898	3	2.299	15.861	0.000 ^{**}
	Within group	4.059	28	0.145		
	Total	10.957	31			
Tenacity (g/d)	Between group	0.096	3	0.032	2.058	0.129 ^{NS}
	Within group	0.435	28	0.016		
	Total	0.531	31			
Elongation (%)	Between group	7.094	3	2.365	2.110	0.121 ^{NS}
	Within group	31.375	28	1.121		
	Total	38.469	31			
Cohesion (strokes)	Between group	631.125	3	210.38	3.332	0.034 [*]
	Within group	1767.75	28	63.134		
	Total	2398.88	31			

* The mean difference is significant at the 0.05 level.

** The mean difference is significant at the 0.01 level.

NS - Not significant at 5 % and 1 % level

Table 4: Quality characteristics of multibivoltine silk coated with bivoltine sericin during small reel vacuum permeation process

Particulars	Control		1 gpl		2 gpl		3 gpl	
	Open pan	Two pan	Open pan	Two pan	Open pan	Two pan	Open pan	Two pan
Renditta	8.1	7.8	8.1	7.8	8.2	7.8	8.2	7.8
Raw silk recovery (%)	23.6	24.6	23.7	24.6	23.3	24.5	23.4	24.6
Waste (%) on silk weight	19.2	17.9	18.8	16.8	17.9	16.1	16.4	14.7
Average size	24.1	23.5	22.8	22.5	23.5	23.2	22.9	22.5
Size deviation	1.44	1.52	1.67	1.55	1.45	1.54	1.60	1.45
Maximum size deviation	2.4	1.8	2.6	2.2	1.8	1.4	2.3	1.4
Tenacity (g/d)	3.5	3.68	3.4	3.73	3.4	3.75	3.5	3.75
Elongation (%)	22	20	21	21	23	21	22	21
Cohesion (strokes)	38	45	52	65	61	65	67	76

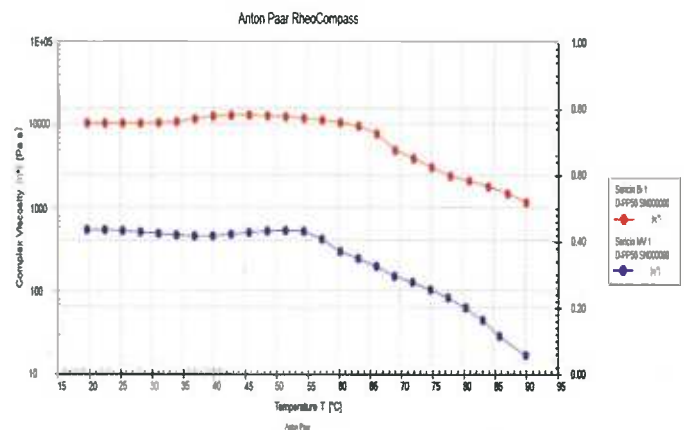
significantly (Table 4) to enable it to be used as warp.

Further from the ANOVA (Table 5), it could be observed that, waste percentage on silk weight, average size, maximum size deviation and cohesion characteristics have significant differences at 1 % level, whereas, the other characteristics did not show significant differences among the variables selected for the study.

Though both the methodologies of impregnations *i.e.*, by mixing the bivoltine sericin powder solution in the reeling basin water during the process of reeling and by sericin coating in small reel permeation with 3gpl, have given good results, it is recommended to use small reel permeation methodology in view of easy adoption and effective coating. The multibivoltine raw silk coated with bivoltine sericin could be used as warp on power looms due to its improved cohesion characteristics.

It is to be noted that better cohesion in the case of multibivoltine raw silk coated with bivoltine sericin is due to better adhesive property of bivoltine sericin. In order to study the adhesive properties of sericin, the sericin samples from multibivoltine and bivoltine cocoons were extracted. The shells of the cocoons were boiled in water for about 5 minutes and the resultant liquor was used for the study. The liquor samples were tested in Rheometer. It was observed that the bivoltine sericin has shown more viscosity than multibivoltine sericin when heated up to 90 °C and cooled up to 20 °C. Further, the coagulation point

of multibivoltine sericin was noticed lower than that of bivoltine sericin (Figure 1).

**Figure 1: Viscosity of multibivoltine and bivoltine sericin**

Conclusion

The study has brought out clearly that cohesion characteristics of multibivoltine raw silk can be significantly enhanced by coating it with bivoltine sericin either in reeling basin or in small reel permeation chamber. CSTRI intends to popularize the small reel permeation technique of sericin coating in view of easy adoption, effective coating and better economics as the same liquor can be reused. The institute has also planned for the production and continuous supply of bivoltine sericin powder for its use in silk industry as well as for other applications.

Table 5: Analysis of variance (ANOVA) results of characteristics of multibivoltine raw silk coated with sericin in small reel permeation chamber

Particulars	Comparison	Sum of square	Degree of freedom	Mean square	F- Value	Significance
Renditta	Between group	0.046	3	0.015	0.337	0.799 ^{NS}
	Within group	1.274	28	0.045		
	Total	1.32	31			
Raw silk recovery (%)	Between group	0.403	3	0.134	0.325	0.807 ^{NS}
	Within group	11.553	28	0.413		
	Total	11.955	31			
Waste (%) on silk weight	Between group	40.457	3	13.486	7.278	0.001 ^{**}
	Within group	51.882	28	1.853		
	Total	92.34	31			
Average size	Between group	7.396	3	2.465	4.705	0.009 ^{**}
	Within group	14.671	28	0.524		
	Total	22.067	31			
Size deviation	Between group	0.079	3	0.026	1.75	0.180 ^{NS}
	Within group	0.42	28	0.015		
	Total	0.498	31			
Maximum size deviation	Between group	2.981	3	0.994	4.854	0.008 ^{**}
	Within group	5.731	28	0.205		
	Total	8.712	31			
Tenacity (g/d)	Between group	0.018	3	0.006	0.215	0.885 ^{NS}
	Within group	0.785	28	0.028		
	Total	0.803	31			
Elongation (%)	Between group	1.094	3	0.365	0.188	0.904 ^{NS}
	Within group	54.375	28	1.942		
	Total	55.469	31			
Cohesion (strokes)	Between group	3825.5	3	1275.2	27.297	0.000 ^{**}
	Within group	1308	28	46.714		
	Total	5133.5	31			

** The mean difference is significant at the 0.01 level.

NS - Not significant at 5 % and 1 % level

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Article scientifique

AMELIORATION DES CARACTERISTIQUES DE COHESION DE LA SOIE GREGE MULTI-BIVOLTINE PAR ENROBAGE DE SERICINE BIVOLTINE PENDANT LA FILATURE

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RESUME

Les caractéristiques de cohésion de la soie grège multibivoltine sont très inférieures à celles de la soie bivoltine ce qui est attribué aux caractères de la race. Afin d'améliorer ces caractéristiques, de la sérécine extraite de soie bivoltine a été déposée sur la soie multibivoltine au cours de la filature. A cet effet, de la poudre de sérécine bivoltine a été extraite de coques de cocons en utilisant une machine à décreuser sous pression développée par le Central Silk Technological Research Institute (CSTRI) à Bangalore, sans ajout de produits chimiques. Deux méthodologies d'imprégnation de sérécine ont été testées. Dans la première, la poudre de sérécine bivoltine est mélangée dans l'eau de la bassine de filature et dans la seconde, la poudre de sérécine est imprégnée pendant la perméation avant le reflottage, ceci sous vide. De plus, deux méthodes de cuite donnent une amélioration significative (à 1% et 5%) en termes de génération de déchets et de titre. Les deux méthodes d'enrobage de sérécine améliorent significativement la cohésion de la soie grège. Nous recommandons l'enrobage par la sérécine pendant la perméation avec 3 gpl.

Mots clés: Cohésion, filature multibouts, sérécine, cuisson à deux bacs, perméation sous vide.



PROSPECTIVE HYBRIDS OF BULGARIAN UNI-BIVOLTINE SILKWORM BREEDS AND INDIAN POLYVOLTINE BREED, C. NICHII

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ABSTRACT

This study was carried out during the period, 2017 – 2018 at the Scientific Center on Sericulture, Vratsa, Bulgaria, as a part of an Indo-Bulgarian research project, involving utilization of the Indian polyvoltine silkworm breed C. Nichi. The project primarily aimed at instilling robustness into the breeding lines of Bulgaria. In the spring silkworm rearing season of 2017 (May/June), crosses between the Indian polyvoltine breed and Bulgarian uni-bivoltine breeds, Vratsa 35 and B1 of Japanese type and Merefa 2 and Svila 2 of Chinese type were made. During the autumn season of the same year (September/October), the pure Bulgarian breeds were reared along with the newly generated F₁ crosses. The autumn rearing season in Bulgaria is less favorable due to the worse mulberry leaf quality. The F₁ crosses between the Bulgarian uni-bivoltine breeds and C. Nichi manifested much higher pupation rate but lower fresh cocoon and silk shell weight and shell percentage compared with the pure uni-bivoltine breeds. The selected individuals from the F₁ crosses were back-crossed with highly productive Bulgarian pure uni-bivoltine breeds and further tested in the spring season of 2018. The results revealed that the backcrosses with uni-bivoltine breeds of the Japanese type *i.e.*, ♀ (B1 x C. Nichi) x ♂ Vratsa 35 and its reciprocal, had cocoon weight values close to, and silk shell weight and shell percentage lower than those of parental uni-bivoltine breeds, while the backcrosses with the Chinese type uni-bivoltine breeds [♀ (Svila 2 x C. Nichi) x ♂ Merefa 2 and its reciprocal] manifested higher cocoon weight (2344 mg and 2346 mg), silk shell weight (452 mg and 451 mg) values at par and lower shell percentage (19.28 and 19.22), compared with that of the pure uni-bivoltine breeds. It may be concluded that the hybrid population between Bulgarian uni-bivoltine pure breeds and Indian polyvoltine breed C. Nichi are promising for further breeding programme.

Key words: *Bombyx mori* L., breeds, crosses, polyvoltine, silkworm, uni-bivoltine.

INTRODUCTION

The silkworm, *Bombyx mori* L. populations of uni and bivoltine origin have comparatively higher productive potential, but at the same time, possess lower tolerance to adverse rearing conditions (Murakami and Ohtsuki, 1989). Unlike them, the polyvoltine, tropical silkworm populations are characterized by higher sturdiness, which makes them suitable as donors of genes for higher survivability (Kantaratnakul *et al.*, 1987; Kim Le Thi, 1987; Murakami, 1989). Moorthy *et al.* (2007) have developed several bivoltine silkworm lines, having higher tolerance to high temperature conditions by series of

back-crosses with polyvoltine strain. A widely used method adopted for imparting tolerance to adverse rearing conditions in India and Thailand is by making F₁ crosses between hardy polyvoltine and highly productive bivoltine breeds (Kantaratnakul *et al.*, 1987; Rao *et al.*, 2001; Sudhakara Rao *et al.*, 2001; Rao and Umadevi, 2012; Mukhopadhyay *et al.*, 2013).

In Bulgaria, the breeding work for creation of uni-bivoltine silkworm breeds with higher tolerance to adverse rearing conditions was initiated in 1998 by crossing highly productive Bulgarian silkworm breeds with polyvoltine breeds Bonde 517 and Cambodge (Tzenov *et al.*, 1998,

1999, 2000; Nacheva *et al.*, 2004). Using this method, two pure breeds VB1 and HB2, having higher tolerance to adverse rearing conditions and form parents of the commercial four-way Bulgarian F₁ hybrid, VB1xVr.35 x HB2xMerefa2, recognized by the Government in 2012, were created. As the tropical polyvoltine breeds, used in this breeding work had yellow-greenish cocoon color and flossy silk shell, it took a long course of time to develop breeds with pure white color and normal nature of grains of the silk shell.

The present study aimed to investigate the possibilities of using the Indian polyvoltine breed C. Nichi for imparting higher sturdiness into the breeding lines in Bulgaria.

MATERIALS AND METHODS

The breeding programme was worked out during the period, 2017 – 2018 at the Scientific Center on Sericulture, Vratsa, Bulgaria, as a part of an Indo-Bulgarian research project, allowing obtaining the Indian polyvoltine silkworm breed C. Nichi, having white cocoon color. In the spring rearing season of 2017 (May/June), the Indian polyvoltine breed was reared in parallel with the Bulgarian uni-bivoltine breeds, Vratsa 35 and B1 of Japanese type and Merefa 2 and Svila 2 of Chinese type; later, the F₁ crosses, Svila 2 x C. Nichi and B1 x C. Nichi were made.

During the autumn season of the same year (September/October), the highly productive pure Bulgarian breeds Vratsa 35 and Merefa 2 were reared together with the newly developed F₁ crosses. The autumn rearing season in Bulgaria is less favorable due to the worse mulberry leaf quality.

After the autumn rearing, the following back-crosses with the highly productive Bulgarian breeds were prepared:

♀ (B1 x C. Nichi) x ♂ Vratsa 35
 ♀ Vratsa 35 x ♂ (B1 x C. Nichi)
 ♀ (Svila 2 x C. Nichi) x ♂ Merefa 2
 ♀ Merefa 2 x ♂ (Svila 2 x C. Nichi)

The above crosses were tested in the spring silkworm rearing season of 2018. Fifteen disease free layings of each silkworm hybrid were incubated and the hatched larvae reared together until the end of 2nd instar. Each silkworm hybrid was reared in 4 replicates, containing 200 larvae, counted after the 2nd moult and further reared until the cocoon spinning.

The silkworm larvae were reared following the standard method, accepted in Bulgaria (Panayotov and Ovesenska, 2002), and fed “ad libitum” with the leaves of rain-fed Bulgarian mulberry variety No.106. The data obtained were processed statistically (Lidanski, 1988) and compared with that of the Bulgarian pure breed, Vratsa 35.

RESULTS AND DISCUSSION

The data on the performance of pure breeds as well as hybrids are presented in Tables 1, 2 and 3. The Bulgarian uni-bivoltine silkworm breeds performed much higher in respect of fresh cocoon and silk shell weight and shell percentage than the Indian polyvoltine breed C. Nichi (Table 1).

Table 1: Performance of pure uni-bivoltine Bulgarian silkworm breeds and the Indian polyvoltine breed C. Nichi during the spring rearing season of 2017

Breed	Pupation rate (%)	Fresh cocoon weight (mg)	Silk shell weight (mg)	Silk shell %
Vratsa 35	82.00	2152	468	21.75
B 1	89.88**	1890**	425	22.49
Merefa 2	83.75	2132	462	21.67
Svila 2	91.38***	2022	427	21.12
C. Nichi	91.33***	1163***	156***	13.41***

Table 2: Performance of pure uni-bivoltine Bulgarian silkworm breeds versus F1 crosses during the autumn rearing season of 2017

Breed / hybrid	Pupation rate (%)	Fresh cocoon weight (mg)	Silk shell weight (mg)	Silk shell %
Vratsa 35	44.00	1517	324	21.36
Merefa 2	41.83	1617*	314	19.42**
Svila 2	82.50***	1407**	226***	16.06***
Svila 2 x C. Nichi	82.50***	1407**	226***	16.06***
B 1 x C. Nichi	94.00***	1268***	220***	17.35***

*P < 5 %; **P < 1 %; ***P < 0.1 %

The F₁ crosses between the Bulgarian uni-bivoltine breeds and C. Nichi (Table 2), reared during the unfavorable autumn rearing season in Bulgaria manifested a much higher pupation rate, but lower fresh cocoon and silk shell

Table 3: Performance of pure uni-bivoltine Bulgarian silkworm breeds and F1 back-crosses during the spring rearing season of 2018.

Breed / hybrid	Pupation rate (%)	Fresh cocoon weight (mg)	Silk shell weight (mg)	Silk shell %
Vratsa 35	76.00	2077	460	22.15
B1	90.38***	1915	451	23.55**
Merefa 2	79.75	2262**	479	21.18*
Svila 2	89.25***	2020	454	22.48
♀ (B1 x C. Nichi) x ♂ Vratsa 35	90.50***	2066	396**	19.17***
♀ Vratsa 35 x ♂ (B1 x C. Nichi)	82.50*	2037	388**	19.05***
♀ (Svila 2 x C. Nichi) x ♂ Merefa 2	86.00**	2344***	452	19.28***
♀ Merefa 2 x ♂ (Svila 2 x C. Nichi)	80.75	2346***	451	19.22***

*P < 5 %; **P < 1 %; ***P < 0.1 %

weight and shell percentage values, compared with the pure uni-bivoltine breeds, Vratsa 35 and Merefa 2. The selected individuals from the F₁ crosses were back-crossed with the Bulgarian pure uni-bivoltine breeds Vratsa 35 and Merefa 2 and further tested in the spring season of 2018. The results (Table 3) revealed that the backcrosses with uni-bivoltine breeds of the Japanese type *i.e.*, ♀ (B1 x C. Nichi) x ♂ Vratsa 35 and its reciprocal had cocoon weight values close to, and silk shell weight and shell percentage significantly lower than those of parental uni-bivoltine breeds, while the backcrosses with the Chinese type uni-bivoltine breeds [♀ (Svila 2 x C. Nichi) x ♂ Merefa 2 and its reciprocal] manifested higher cocoon weight (2344 mg and 2346 mg), silk shell weight (452 mg and 451 mg) values at par and lower shell percentage (19.28 and 19.22), compared with the pure uni-bivoltine breeds.

Conclusion

It may be concluded that the hybrid populations between highly productive Bulgarian uni-bivoltine pure breeds and Indian polyvoltine breed C. Nichi are promising for further breeding work.

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Article scientifique

HYBRIDES PROMETTEURS ENTRE DES LIGNEES DE VERS A SOIE BULGARES MONO-BIVOLTINS ET DES LIGNEES INDIENNES POLYVOLTINES C. NICHII

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RESUME

Cette étude a été réalisée pendant la période 2017-2018 au Centre scientifique sur la sériciculture de Vratsa en Bulgarie dans le cadre du projet de recherche Indo-Bulgare comprenant l'utilisation de lasouche de vers à soie polyvoltins C. Nichi. A l'origine, le projet avait pour but d'instiller de la robustesse dans les lignées bulgares monovoltines. Pendant la période d'élevage de printemps de 2017 (Mai/Juin) des croisements entre la lignée polyvoltine indienne et les lignées bulgares monovoltines Vratsa 15 et B1 de type japonais et Merefa 2 et Svila 2 de type chinois ont été effectués. A l'automne de la même année les lignées pures bulgares ont été élevées avec les F1 nouvellement obtenues. Les croisements F1 manifestent un taux de pupaison plus élevé mais un poids de cocons frais et de coque soyeuse ainsi qu'une richesse soyeuse plus faibles comparés aux lignées bivoltines. Les individus sélectionnés parmi des hybrides F1 ont été croisés en retour avec des lignées pures bulgares mono-bivoltines et testés pendant le printemps 2018. Les croisements de retour effectués avec des lignées de type japonais comme femelle (B1xC.Nichi) x mâle Vratsa 35 et son réciproque donnent des valeurs de poids de cocon et des coques soyeuses proches, plus faibles que les parents mono-bivoltins, alors que le croisement de retour avec des types chinois manifestent un poids de cocon supérieur (2344 mg et 2346 mg) un poids de coque voisin (452 mg et 451 mg) mais une richesse soyeuse plus faible (19,28 et 19,22) comparés aux lignées pures monovoltines. On peut conclure que les croisements entre lignées pures monovoltines bulgares et C. Nichi sont prometteurs pour les programmes de lignage futurs.

Mots clés: *Bombyx mori* L., lignées, croisements, polyvoltin, ver à soie, mono-bivoltin.



FUNCTIONAL, AESTHETIC AND COMFORT PROPERTIES AND HAND BEHAVIOUR OF RED ERI SILK-WOOL BLENDED SPUN YARN WOVEN FABRICS

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ABSTRACT

Long staple fibres of red eri silk were blended with wool fibre in different proportions for producing ring spun yarn on worsted system of spinning and the yarns produced were converted into plain woven fabrics on power loom. Various fabric properties viz., functional, aesthetic and thermal including low-stress mechanical properties and hand values were determined on Kawabata fabric evaluation system. Blending of eri with wool improved the fabric durability, aesthetics and wear comfort performance. Fabrics produced from eri-wool blends at ratios of 70:30 and 60:40 are suitable for summer and winter dress material, respectively.

Key words: Comfort, crispiness, firmness, fullness, Hauteur, red eri silk, stiffness, total hand value.

INTRODUCTION

Eri silk is one of the four varieties of silk produced in India with two types under it i.e., red and white, possessing distinct fibre characteristics. The eri silk is extruded from silkworm which feeds on castor / tapioca plants and wool is an animal fiber which is sheered from the sheep, both of these being protein fibres (Kariyappa *et al.*, 2006 a).

Finer wool fibre (14- 16 microns) is presently not available in India to produce fine and soft quality of woollen fabrics. Indian woollen industry imports the more expensive 14-16 microns wool from foreign countries for this purpose (Kariyappa *et al.*, 2006 b). Cost of 16 microns wool is about ₹1000-1200 per kg compared to ₹700-750 per kg of eri cocoons. In order to reduce the cost, eri silk fibre (14-16 microns) may be used as an alternative in place of finer micron wool and blended with the domestic wool. By doing so, the fabric properties such as smoothness, luster, durability, dimensional stability, pilling properties and crease resistance can be improved as eri silk stands better in terms of these properties than wool. In addition, the thermal properties of eri silk are also aligned to that of wool fabric (Kariyappa *et al.*, 2001, 2009).

Both types of eri silk fibres are finer and fabrics made from these fibres are highly comfortable for wearing (Kariyappa *et al.*, 2014). The clothing comfort has three aspects, such as physiological, tactile and thermal comfort. Physiological comfort is mainly related to the latest fashion trend that is acceptable in the society. The tactile comfort has relationship with fabric surface and mechanical properties. The thermal comfort is related to the ability of fabric to maintain temperature of skin through transfer of heat and perspiration generated within the body (Matsudaira and Kawabata, 1988).

In the present work, red eri silk – wool blended yarns were produced following different blend ratios. The blends were deliberately selected as to be eri silk rich to develop fabrics with better hand value. The properties of eri silk - wool blended yarn were tested and yarns were converted into plain woven fabrics on power loom. These fabrics were tested for various conventional fabric properties including thermal as well as hand value on Kawabata fabric evaluation system to examine the changes in hand value due to blending of silk and wool.

MATERIALS AND METHODS

Eri silk and wool fibre having the following specifications were used for the study.

Fibre property	Eri	Wool
Fibre dia. (microns)	16	25
Breaking tenacity (g/tex)	29.4	12.5
Elongation %	11	10
Effective mean length (H_{mm})	54.5	45.6

Methods

Yarn preparation

Four types (70:30, 60:40, 50:50, 30:70) of eri silk -wool blended yarns (2/68[°]), 100 % eri silk yarn and 100 % wool yarn were prepared on worsted system of spinning.

Yarn quality evaluation

1. Unevenness and imperfections

The measurements of yarn unevenness and imperfection were done on Uster tester -4. The yarn irregularity in terms of U_m % and the imperfections in terms of thick places, thin places and neps were evolved.

2. Yarn count

The yarn count was measured with the help of wrap reel and electronic balance.

3. Breaking strength, elongation %, Young's modulus, tenacity and work of rupture

The breaking strength, elongation %, Young's modulus, tenacity and work of rupture were measured on Instron-5500R tester using the standard procedure in accordance with ASTM D 2256-2015.

Fabric preparation

The plain woven fabrics were made on power loom using same yarn in warp and weft with parameters of ends per inch, 60; picks per inch, PI42 and width of fabric, 54

inches.

Fabric properties

1. Functional properties

The functional properties of the fabric were measured through standard methods as following.

Test	Standard method
Weight per square meter	IS 1964-2001
Thickness of fabric	IS 7702-1975
Air permeability (PROLIFIC air permeability tester - 10 specimens)	IS 11506-1984
Bursting strength	IS 1966-1975
Tearing strength	IS 6489-1993
Tensile strength and elongation (Instron 50 x 200 mm strip 300 m/min)	IS 1969-1985
Drape coefficient (4 specimens for each sample)	IS 8357-1977
Abrasion resistance (Martindale abrasion tester)	IS 12673-1989
Crease recovery	IS 4681-1981
Bending length and flexural rigidity	IS-6490-1971
Water repellency (4 specimens for each sample)	IS-390-1975
Pilling resistance	IS-1097-1964
Thermal insulation value (Thermolabo-II Wind speed -1m/sec - 3 samples per specimen]	Guarded hot plate method

2. Low stress mechanical properties

The fabrics were tested for low-stress tensile, bending, shear, surface and compressional properties on Kawabata fabric evaluation system. The primary hand value and total hand value (THV) were estimated from these results using Kawabata system of equations. For the evaluation of hand values, 16 low stress mechanical fabric attributes were determined (Table 1).

RESULTS AND DISCUSSION

Yarn properties

1. Unevenness, imperfection and hairiness properties

The values of unevenness, imperfections and hairiness and index of irregularity are presented in Table 2. The absolute unevenness, imperfections, hairiness and index of irregularity appears to be at higher side in 100 % wool spun

Table 1: Fabric mechanical attributes

Parameter / Instrument	Symbol	Characteristic value	Unit	Test method
KES –FB1	LT	Linearity load –Extension curve	None	1. Sense: 5 x 5
Tensile properties	EMT	Extensibility	%	2. Velocity: 0.2 mm/sec
(Shear and tensile tester)	WT	Tensile energy	gf.cm/cm ²	3. Elongation: 25 mm/10V
	RT	Tensile resilience	%	4. Tensile force: 500 gf/cm
				5. Sample size: 5 cm x 20 cm
KES –FB1	G	Shearing stiffness	gf.cm/deg	1. Sense: 2 x 5
Shear properties	2HG	Hysteresis of shear force at 0.50 of shear angle	gf/cm	2. Rate of shearing: 0.417 mm/sec
(Shear and tensile tester)	2HG5	Hysteresis of shear force at 50 of shear angle	gf/cm	3. Angle: 8 deg
				4. Weight: 200 g
				5. Size: 5 cm x 20 cm
KES –FB2	B	Bending rigidity	gf.cm ² /cm	1. Sense: 2 x 1
Bending properties				2. Rate of bending: 0.5 cm ⁻¹ /s
(Pure bending tester)	2HB	Hysteresis of bending moment	gf.cm/cm	3. B: K=0.5. 1.5 cm ⁻¹
				4. 2HB: K=1.0 cm ⁻¹
				5. Size: 20 cm x 1 cm
KES –FB3	LC	Linearity of compression –thickness curve	None	1. Sense: 2 x 5
Compression properties	WC	Compression energy	gf.cm/cm ²	2. Velocity: 50 sec/mm
(Compression tester)	RC	Compression resilience	%	3. Stroke: 5 mm/10V
	T	Thickness	mm	4. Area: 2 cm ²
	Wt	Fabric weight	mg/ cm ²	5. Maximum compression load: 50 gf/cm ²
KES –FB4	MIU	Coefficient of friction	None	1. Sense: 2 x 5 for both MIU & SMD
Surface properties	MMD	Mean deviation of MIU	None	2. Fabric tension: 20 g
(Surface tester)	SMD	Geometrical roughness	Micron (µm)	3. Load on roughness detector: 10 g
				4. Load on surface friction detector: 10 g
				5. Fabric speed: 1 mm/second
				Maximum sweep: 3 cm

Primary hand expressions and their meaning are as follows:

Koshi: Stiffness/firmness; Shari: Crispiness; Hari: Anti drape stiffness/hardness;

Fukurami: Fullness/softness; Numeri: Smoothness.

yarn. The addition of eri silk fibre in spun silk yarn could bring in significant reductions in unevenness, imperfections, hairiness and index of irregularity. The improvement in quality of blended spun silk yarn is due to characteristics of eri silk, such as more evenness and longer fibres (Kothari *et al.*, 2002). Since the wool fibres are shorter than those of eri, they are prone to exhibit more of hairiness on the spun yarns.

2. Tensile properties

The results show that values of breaking force, tenacity, elongation percentage, Young's modulus and B-work significantly improved along with an increase of the amount of eri silk component in the blended yarn. The tensile properties of blended yarn were higher than that of wool fibre due to higher tenacity and elongation percentage of eri silk fibre which influenced the yarn properties. The higher strength of eri silk fibre is because

they are composed of linearly folded and very crystalline polymers of beta configuration having more hydrogen bonds formed in regular manner. The low tensile strength of wool is due to relatively few and shorter hydrogen bonds. The higher elongation percentage of eri silk fibre is due to polymer structure of eri silk fibre which has higher visco-elasticity and more bulky side group compared to wool polymer. Eri silk fibres as well as spun silk yarn has higher tenacity and elongation than wool. This is due to the more easy flow of polymer segment in eri silk fibre than that of wool fibre.

3. Optical properties

The values of optical properties are provided in Table 2. The optical properties *viz.*, whiteness index, yellowness index and brightness index improved with increase of eri silk component in blended yarn. This is due to the fact that light falling on the silk fibre is uniformly reflected from

triangular configuration of silk fibre, whereas in wool, the circular surface of fibre scatter the light rays and hence, results in reduced optical properties.

Fabric properties

1. Breaking strength, tearing strength and elongation percentage

Table 3 displays the data on breaking strength, tearing strength and elongation percentage. These values are higher in warp way fabric than weft way in all varieties of fabrics. This is because of the higher warp density of fabric. These values indicated a significant increase with increase of eri silk component in blended yarn with the maximum in 70:30 eri-wool blend fabrics and the least in 100 % wool fabric. Eri silk fibre has higher strength and elongation percentage than wool which naturally reflects in the fabrics.

2. Air permeability ($\text{m}^3/\text{m}^2/\text{min}$)

Air permeability is the reciprocal effect of the air resistance. The resistance of the fabric to the flow of air is a measure of the initial warmth / cool feeling when the garment is worn. The higher the air flow value, the greater the intensity of warm / cool feeling will be. The effect of air permeability on comfort properties is much greater when the speed of air flow is high. The amount of air that passed through unit fabric area per unit time is presented in Table 3. The results show that blending of eri silk and wool fibre has significantly influenced the characteristic of air permeability.

Higher air permeability noticed in 70:30 eri-wool blended fabric as compared to other blend ratios is because of lower cover factor and lower hairiness in the yarn due to longer eri fibres used. Lower cover factor of fabric and low hairiness in the yarn will surely allow more air to pass through the fabrics. Hence, the fabrics made from 70:30 eri-wool blended yarn are considered the most suitable for summer wear.

3. Water repellency and pilling resistance (rating)

High and uniform absorbency of water is a desirable quality of the fabric. Absorbency of fabrics is influenced by their wicking ability. The absorbency is also very important in view of quick evaporation of perspiration in under garment, absorption of water by towel, cleaning cloth, diapers, gauge and bandages. Pilling is tufts of entanglement of fibers attached to the surface of the cloth after some usage of fabrics and they look like small pills. The values of water repellency and pilling resistance rating are given in Table 3. These values remain same for all blended and pure fabrics due to the fact that all varieties of fabrics are hydrophilic in nature. The polymers of both the fibres have hydroxyl in peptide group, with polarity which attract water molecules making the fabrics more comfortable to wear. The results revealed moderate to severe pilling owing to yarn hairiness, yarn content and fibre type.

4. Abrasion (no. of rubs) resistance and bursting strength (kgf/cm^2)

Abrasion is just one aspect of wear and is the rubbing away of the component fibers and the yarn of the fabric during usage. Bursting strength is a measure of fabric resistance to multidirectional force. Table 3 reveals the results obtained in respect of abrasion resistance and bursting strength. It is observed that abrasion resistance and bursting strength increased significantly with increase in eri silk fibre component in the blended yarn. Abrasion resistance and bursting strength registered the highest values in respect of 70:30 eri silk:wool fabric on account of maximum share of eri silk fibre and higher thickness. The pure wool fabric exhibited low values due to lower strength and elongation percentage of wool fibre than eri silk fibre.

5. Drape coefficients (%)

Drape is the ability of the fabric to assume a graceful appearance while in use. It is an important factor of textile materials, which allows fabric to orient itself into graceful

Table 2: Yarn unevenness, imperfections, hairiness and tensile properties

Per 40C km	100 % E (67.6)	30 % W:70 % E (74)	40 % W:60 % E (65.8)	50 % W:50 % E (78.4)	70 % W:30 % E (76)	100 % W (61.2)	C. D. at 5 %
Um %	10.16	13.30	14.21	15.35	15.64	16.59	1.206
Cv m %	12.99	18.06	19.07	19.52	19.89	21	1.509
Cvm (1m) %	4.76	5.78	6.28	6.84	8.25	8.96	1.728
Thin Places (-50 %)/km	2	118	131	225	347	372	60.42
Thick places (+50 %)/km	46	161	180	248	252	292	42.418
Thick places (+100 %)/km	1	5	8	12	12	14	4.036
Neps (+ 140 %)/km	150	240	395	408	524	613	61.591
Neps (+ 200 %)/km	42	59	78	128	131	163	25.59
Neps (+ 280 %)/km	14	24	28	53	72	98	14.027
Neps (+ 400 %)/km	7	8.00	11	23	25	61	8.243
Hairiness (-)	2.19	2.65	3.05	3.09	3.11	5.55	0.183472
Index of irregularity	1.09	2.49	2.52	2.52	2.59	3.04	0.2007
Mechanical properties							
Breaking force (gf)	726.66	424.40	411.60	319.00	260.10	191.10	18.78
Elongation (%)	16.530	16.400	15.330	14.650	13.650	12.17	1.91
Tenacity (gf / den)	2.24	1.57	1.39	1.38	0.96	0.57	0.0780
Young's modulus	382.283	287.432	282.87	245.488	245.488	231.02	20.7
B-Work(kgf.cm)	35.444	20.329	19.864	15.611	10.359	9.488	2.449
Yellowness	21.993	21.6	21.263	21.112	21.11	19.423	
Weightness Index	46.285	44.663	43.713	43.489	43.299	42.688	
Brightness index	49.935	46.451	44.834	44.039	43.735	43.084	

E – Eri silk fibre; W – Wool fibre; Values in brackets denote liner density in metric count (Nm).

folds or pleats as a result of force of gravity. The data on drape coefficient of experimental blended fabrics are given in Table 3. The results indicate that the drape coefficient of blended fabric significantly decreased with increase of eri silk component in the blended yarn. Among the blended fabrics, 70:30 eri silk-wool blended fabric showed lower value of drape coefficient and 100 % pure wool fabric has shown higher value. Fabrics with lower values of drape coefficient are considered to hang down well and cling to a curved surface easily. This is because the combined effect of fabric thickness, weight and fibre fineness affects the drape properties of blended fabrics.

6. Flexural rigidity [Stiffness (mg-cm)]

Fabric stiffness indicates the resistance of the fabric to bending. Stiffness of the fabrics is a key factor in the study of hand and drape. The results revealed higher flexural rigidity of weft than warp in all six varieties of fabrics

(Table 3). This is because the warp density in the fabric is higher than weft density. Among the blended fabrics, 70:30 eri-wool blended fabric has shown lower value of flexural rigidity and 100 % pure wool fabric displayed higher value. Similar to the case of drape coefficient, fabrics characterized with smaller values of flexural rigidity also hang down freely and cling to a curved surface easily. This is due to the combined effect of fabric thickness, weight and fibre fineness on the flexural rigidity of blended fabrics.

7. Thermal insulation value (TIV)

Thermal insulation value provided by fabrics depends on fibre type, fabric thickness, bulk density of fibre arrangement and the compressibility of fabric structure. The mechanism of heat transfer involves dry heat transmission through conduction, convection and radiation. Latent heat transfer due to water vapor transport and liquid water transport also determines the thermal

Table 3: Mechanical properties of eri - wool blended fabrics

Character		E 100 %	W/E (30:70)	W/E (40:60)	W/E (50:50)	W/E (70:30)	W 100 %
Linear density (Nme)	Warp	67.6	74	65.8	78.4	7.6	61.2
	Weft	67.6	74	65.8	78.4	75.6	61.2
Threads	Ends/inch	62.9	61.9	62.5	63.21	62.7	63.9
	Picks/inch	51.3	44.7	53.0	51.02	45.2	44.7
Fabric mass (g/m ²)		131.2	122	139.3	125.1	124	136.4
Thickness (mm)		0.357	0.407	0.359	0.332	0.392	0.377
Cover factor		19	18.4	19.5	18.9	18.5	19.6
Tensile strength							
Breaking strength (kg)	Warp	39.1	53.6	42.4	33.7	44.1	29.1
	Weft	31.2	48.5	36.5	27.3	31.3	23.9
Elongation %	Warp	29.2	30.9	32.1	29.2	28.5	23.8
	Weft	22.2	21.9	22.4	18.2	20.2	21.5
Tearing strength (g)	Warp	5453	5568	5018	4813	3686	3763
	Weft	4646	5325	4070	4582	2675	2765
Water repellency (rating)		0	0	0	0	0	0
Air Permeability (m ³ /m ² /min)		71.4	94.6	38.3	62.6	85.9	52.6
Pilling resistance (rating)		4	4	4	4	4	4
Abrasion resistance (no. of rubs)		355	472	344	270	263	275
Drape coefficient (%)		43.2	42.5	48.3	48.5	45.9	51.8
Bursting strength (kgf/cm ²)		8.0	10.8	9.0	8.5	8.4	7.5
Stiffness (mg-cm)	Warp	141.6	122.6	207.4	129.9	150.9	188.6
	Weft	157.6	150.2	305.8	168.7	193.8	213.1
	Overall	149.4	135.7	251.8	135.7	171	200.5
Thermal insulation value (TIV)		23.60	23.90	28.00	27.402	27.30	26.90

E – Eri silk fibre; W – Wool fibre.

property of fabric (Behara, 2007). The thermal insulation properties of various fabrics measured on the rmo lobo (KE-FB5) are shown in the Table 3. Thermal insulation values were observed as significantly influenced by blending of eri silk with wool. Thermal insulation value depends upon type of fibre air permeability and thickness of fabric. Among the blended fabrics, 60:40 eri-wool blend fabric possessed higher thermal insulation value due to higher thickness and higher fabric cover factor. It may be concluded that eri-wool blended fabric can provide the desired protection to the human body against climatic fluctuations. Besides this, the flat stature of eri fibre will help to trap air between the fibres resulting in varying TIV in blended fabric depending upon the blend.

Low stress mechanical properties

1. Tensile properties

Tables 4-7 are depicted with the results of low stress mechanical properties of the fabrics. Table 4 shows various tensile properties attributed to blended fabrics. EMT is tensile strain biaxial extension. The larger the

EMT value, the greater the wear comfort (Sharma *et al.*, 2000). EMT of fabric increased along with the proportion of eri silk component in blended yarn. Among the blended fabrics, 60:40 eri - wool combination has shown higher EMT value than other fabrics, which is attributed to higher areal density, fabric thickness value and higher eri silk component. This implies that the heavy fabrics might be of better hand value due to their extensibility at low stress deformations. This may be attributed to the flexibility of the fabrics due to eri silk which has got an elongation percentage higher than that of wool.

LT represents linearity of stress-strain curve. It has some correlation with hand value of the fabric. A lower r value of LT, is supposed to be better from comfort point of view. Among the blends, 70:30 eri:wool blended fabric has shown the lowest value and hence, is more comfortable to wear. LT value depends on the elongation percentage of spun silk yarn. 100 % eri spun silk yarn has high elongation percentage than wool spun yarn.

WT represents tensile energy per unit area (*i.e.*, area under

Table 4: Low stress tensile and shear properties

Particulars		Tensile property				Shear property		
		LT	WT	RT	EMT	G	2HG	2HG5
W 100 %	Warp	0.78	7.35	62.22	3.76	1.37	4.55	5.31
	Weft	0.71	8.92	51.54	5.06	1.29	4.09	5.00
	Avg	0.74	8.14	56.88	4.41	1.33	4.32	5.16
WE (70:30)	Warp	0.34	10.65	50.84	5.81	0.96	6.19	6.54
	Weft	0.68	5.15	62.68	3.05	0.98	5.53	6.22
	Avg	0.71	7.90	56.76	4.43	0.97	5.86	6.38
WE (50:50)	Warp	0.76	10.2	58.82	5.36	1.30	5.16	5.94
	Weft	0.68	5.00	65.01	2.95	1.26	4.29	5.19
	Avg	0.72	7.60	61.92	4.16	1.28	4.72	5.56
WE (40:60)	Warp	0.78	11.20	50.78	5.72	1.68	8.31	9.01
	Weft	0.70	4.82	61.14	2.76	1.52	7.69	8.65
	Avg	0.74	8.01	55.96	4.24	1.60	8.00	8.83
WE (30:70)	Warp	0.70	9.55	48.60	5.45	0.91	5.69	6.88
	Weft	0.70	4.50	63.91	2.59	0.80	4.26	5.63
	Avg	0.70	7.03	56.26	4.02	0.85	4.98	6.25
E 100 %	Warp	0.76	10.65	53.44	5.61	1.35	6.54	7.35
	Weft	0.75	5.28	61.14	2.82	1.41	6.15	7.19
	Avg	0.75	7.96	57.29	4.22	1.38	6.34	7.27

Table 5: Bending and surface properties

Particulars		Bending property		Surface property		
		B	2HB	MIU	MMD	SMD
W 100 %	Warp	0.1509	0.1256	0.148	0.013	11.23
	Weft	0.1236	0.2744	0.193	0.030	8.49
	Avg	0.1373	0.2000	0.171	0.026	9.86
WE (70:30)	Warp	0.0930	0.1723	0.146	0.025	13.22
	Weft	0.1180	0.2080	0.186	0.034	10.06
	Avg	0.1055	0.1901	0.166	0.030	11.64
WE (50:50)	Warp	0.1104	0.1326	0.170	0.097	9.84
	Weft	0.1088	0.1426	0.184	0.034	7.33
	Avg	0.1096	0.1411	0.177	0.065	8.58
WE (40:60)	Warp	0.1132	0.1706	0.158	0.071	8.861
	Weft	0.1643	0.2596	0.181	0.033	8.728
	Avg	0.1388	0.2151	0.170	0.050	8.794
WE (30:70)	Warp	0.0730	0.1217	0.148	0.020	13.26
	Weft	0.0816	0.1360	0.157	0.033	15.11
	Avg	0.0773	0.1288	0.152	0.026	14.19
E 100 %	Warp	0.1104	0.1737	0.161	0.105	11.25
	Weft	0.1394	0.2116	0.178	0.030	9.93
	Avg	0.1249	0.1927	0.170	0.068	10.59

the load elongation curve). It is strongly related with the movement of the body parts in particular garment and

fabrics. Lower value of WT is better in this respect. It is observed from the Table that in this regard, 70:30 eri-wool fabric is the best among the blended fabrics. The value is seen decreasing with increase of eri silk component in blended yarn due to varying load bearing capacity of fibres in the yarn.

RT represents the recovery from tensile deformation. The higher value of RT makes the fabric more elastic. Among the blended fabrics, 50: 50 eri-wool blend showed the highest value of RT which revealed an increasing trend with increase of eri silk component in blended yarn. This is because the fabric has higher mass.

Table 6: Compress properties

Particulars	Compression property				
	LC	WC	RC	Thickness (T)	Fabric (weight)
W (0:100)	0.311	0.248	59.28	0.721	14.77
E:W (30:70)	0.345	0.255	44.70	0.726	13.38
E:W (60:40)	0.267	0.273	48.38	0.824	13.73
E:W (50:50)	0.340	0.271	50.13	0.725	14.82
E:W (70:30)	0.327	0.231	43.27	0.694	11.62
E (100:0)	0.276	0.255	49.82	0.767	13.82

Table 7: Hand and total hand value of fabrics (summer and winter)

Variety	Hand value				THV (summer)	Hand value			THV (winter)
	Koshi	Shari	Fukurami	Hari		Koshi	Numari	Fukurami	
Wool	5.70	5.16	3.83	7.19	3.01	4.80	0.44	3.42	2.11
W/E70:30	4.75	5.52	3.85	6.44	3.31	4.41	2.13	5.02	2.18
W/E50:50	5.02	4.83	3.86	6.60	3.02	4.51	3.13	4.96	2.22
W/E40:60	6.02	5.32	3.72	7.74	2.90	4.72	2.12	3.87	2.55
W/E30:70	3.81	5.83	3.73	5.56	3.53	4.28	3.48	4.39	2.16
Eri	5.69	5.82	3.42	7.53	3.05	4.80	0.44	3.42	2.11

2. Shear properties

Shear rigidity (G) of blended fabric was the lowest for 70:30 eri - wool blended fabric (Table 4). The shear rigidity of fabric depends mainly on the mobility of warp / weft threads in the fabrics, while the mobility depends upon the frictional property of the constituent fibre, yarn diameter and surface properties of yarn. The lower value of G is preferred for better hand behaviour of the fabric. Therefore, 70:30 eri - wool blended fabric is the best in terms of comfort to the wearer.

2HG and 2HG5 are the hysteresis of shear force at 0.5° and 5°, respectively. The hysteresis of blended fabrics significantly reduced along with more fraction of eri silk in the blend yarn. Among the blended fabrics, the hysteresis was reported the lowest (recovery is better from shear deformation) for 70:30 eri-wool blended fabric. The high value of hysteresis gives trouble in tailoring and also shows wrinkling during wear.

3. Bending properties of the blended fabric

Bending is a measure of indication on how easily fabric can bend, bending rigidity (B) of fabric depends upon the bending rigidity of fibre, yarn and the mobility of warp and weft threads within the fabric. Table 5 shows that bending rigidity is the lowest for 70:30 eri - wool blended fabric, and incidentally, superior Koshi value (this indicates stiffness of the fabric *i.e.*, primary hand value) which is noticed the highest for 100 % pure wool fabric. The eri fibres being more fine and flexible by nature, contributes to lighter and smoother fabric.

The hysteresis of bending moment 2HB has also shown the same trend as bending rigidity and the explanation in that regard holds relevant here also.

4. Surface properties

Table 5 shows that the coefficient of friction (MIU) is the lowest for 70:30 eri-wool blended fabric and is higher in 50:50 eri-wool blended fabric. This may be attributed to the fineness of eri silk fibre and evenness of blended yarn that influences the MIU. The coefficient of friction depends upon contact area of the fabric with that of the body. MMD is a measure of the variation of MIU.

5. Compressional properties

There is no clear trend noticed in compressional properties (Table 6). However, linearity of compression (LC) is slightly higher for the fabrics prepared from 70:30 eri-wool blended yarn and the lowest for 50:50 eri-wool blended fabric. The compressional energy (WC) is noticed slightly lower for 70:30 eri - wool blended fabric and slightly higher for 50:50 eri - wool blended fabric. The value of compressional resilience (RC) is observed slightly higher for 100 % wool fabric. The compressional property of fabrics mainly depends on compressional behaviour of fibre, yarn and fabric thickness.

Fabric hand value

From the above low stress mechanical properties, the primary hand value and total hand value (THV) were estimated using Kawabata system equation considering the fabric for summer wear. THV was estimated using

primary hand value such as KOSHI (Fabric hardness), SHARI (fabric crispness), HARI (fabric stiffness) and FUKURAMI (fabric fullness) (Senthil Kumar and Jambagi, 2008). For the THV of winter wear, three primary hand values, KOSHI, NUMERI (fabric surface smoothness) and FUKURAMI were used. The calculated primary and total hand values for winter and summer are given in Table 7. Total hand value is the measure of tactile comfort provided by the clothing. THV is found to be higher for 70:30 eri - wool blended fabric in summer wear and 60:40 eri - wool blended fabric in winter wear.

Conclusion

From the study, it is revealed that blending of eri silk with wool fibre in spinning helps in improving evenness, tensile strength, Young's modulus, elongation, work of rupture and reducing the imperfections and hairiness. The improvement in yarn properties is clearly reflected in fabric properties, such as increase in fabric tensile, extensibility, tearing and bursting strength, air permeability, thermal properties, reduction in drape coefficient and flexural rigidity. Blended fabrics display good moisture absorbent and pilling resistant characteristics. From this, it is concluded that fabrics made from eri-wool blended yarn are durable and soft with good aesthetic, tactile and thermal comfort properties. From the Kawabata analysis, it is noticed that fabric made from 70:30 eri-wool blend ranks higher in these properties, is more comfortable and is the most suitable for summer garments and fabrics made from 60:40 eri - wool blend is more comfortable for winter wear as this fabric has higher hand value in winter, higher thermal and low air permeability values.

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Article scientifique

COMPORTEMENT FONCTIONNEL, DE CONFORT ET MAIN DES TISSUS MELANGES DE LAINE ET DE SOIE FILEE ERI

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RESUME

Des fibres à base de soie rouge eri mélangées à des fibres de laine en différentes proportions pour produire des filés sur un système de filage et les fils produits sont convertis en tissus de soie sur un métier à tisser manuel. Des propriétés diverses comme esthétique, thermiques, mécaniques et la main ont été déterminées sur un système d'évaluation Kawabata. Le mélange laine et soie eri améliore la durabilité, l'esthétique et le confort. Les tissus produits à partir de eri/laine 70/30 et 60/40 conviennent aux vêtements d'été et d'hiver respectivement.

Mots clés: Confort, craquant, fermeté, ampleur, Hauteur, soie rouge eri, tenue, valeur totale de la main.



NITROGEN FIXING EFFICIENCY OF *AZOTOBACTER* STRAINS ISOLATED FROM RHIZOSPHERE OF ARJUN AND ASAN PLANTS OF WEST SINGHBHUM, JHARKHAND, INDIA

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ABSTRACT

Application of bacterial biofertilizers for nutrient management and growth in tasar food plants is an eco-friendly, economic and sustainable approach. Hence, the present study was aimed at isolating efficient nitrogen fixing *Azotobacter* strains from rhizosphere of tasar host plants and explore their use as biofertilizers. Ten rhizosphere soil samples were collected from Arjun (*Terminalia arjuna*) and Asan (*Terminalia tomentosa*) plants of forest and block plantations in tasar silkworm rearing areas of West Singhbhum, Jharkhand. About 28 *Azotobacter* strains were isolated and screened for their nitrogen fixing ability in three day old pure strain liquid cultures using micro-Kjeldahl method. Wide range of nitrogen fixation was observed from 2.3 to 23.0 $\mu\text{g N ml}^{-1}$ with an average of 12.3 $\mu\text{g N ml}^{-1}$. Some *Azotobacter* isolates viz., A11 (23 $\mu\text{g N ml}^{-1}$), A26 (19.5 $\mu\text{g N ml}^{-1}$), A24 (18.5 $\mu\text{g N ml}^{-1}$), A12 (17.4 $\mu\text{g N ml}^{-1}$), A13 (16.8 $\mu\text{g N ml}^{-1}$), A4 (16.3 $\mu\text{g N ml}^{-1}$) and A2 (16.3 $\mu\text{g N ml}^{-1}$) have shown high nitrogen fixing ability. It was noticed that strains with high nitrogen fixing ability were derived irrespective of species of host plant, type of plantation and geographical regions. These potential native *Azotobacter* strains could be explored as biofertilizers to improve tasar host plant nutrition.

Key words: *Azotobacter*, nitrogen fixing ability and biofertilizer, rhizosphere, tasar culture.

INTRODUCTION

Tasar culture is a forest based agro-industrial avocation and livelihood for 1.25 lakhs tribal families residing by the edge of forest by Northern and North-East India (Reddy *et al.*, 2015). India is the largest producer of tropical tasar silk with the production of 3268 MT (CSB, 2016-17). In recent decades, tasar culture has taken a new dimension of rearing under systematic commercial plantation unlike previous forest based rearing and thus changing subsistence tasar culture to commercial rearing. The area under block plantation has been increasing over the years by establishment of plantation in forest and government waste land through various government schemes to facilitate tasar farmers (Gargi *et al.*, 2015). But, of late, the tasar cocoon quality has been deteriorating in block plantations. Decrease in leaf

nutrient quality is one of the major factors, which in turn is due to decrease in soil nutrient status (Shantakar Giri *et al.*, 2016) owing to continuous silkworm rearing without / rare application of manures and fertilizers as tasar farmers are economically poor (Singhvi, 2014). Moreover, as tasar culture is mainly practiced in forest area, application of chemical fertilizer is not advisable.

It has been established that, beneficial microorganisms known as plant growth promoting rhizobacteria (PGPR) have pivotal role in nutrition, phyto stimulation and biological control in mulberry (Fan *et al.*, 2010; Nithya *et al.*, 2011; Baqual and Das, 2012; Rajaram *et al.*, 2013; Chowdary *et al.*, 2014) and other agricultural crops. In tasar host plants also, PGPR could have an important role in nutrition and other beneficial effects on plant growth and development. Therefore, use of PGPR as biofertilizer

offers great potential.

Among PGPR, *Azotobacter* spp. are predominantly found in soil. They are free-living aerobic bacteria, which fix atmospheric nitrogen non-symbiotically. Furthermore, they produce growth promoting substances, such as gibberellins, auxins and cytokinins (Aquilantia *et al.*, 2004) and are found to be antagonistic to pathogens. They are heterotrophic bacteria capable of fixing an average of 20 kg N/ha/year and the most efficient strain could fix about 30 kg N/ha/year (Chennapa *et al.*, 2016). Different strains of *Azotobacter* species show varying capacity of nitrogen fixation (Ridvan Kizilkaya, 2009; Diego *et al.*, 2011; Bashir *et al.*, 2013). Under *in vitro* conditions, the species of *Azotobacter* are known to fix 10 mg of N/g of carbohydrate on an average.

In the selection of bacteria (as fertilizer) for inoculation of crops, indigenous isolates might be preferred (Bhattarai and Hess, 1993) as they adapt to local ecological conditions easily and can be more competitive than the non-indigenous strains. Therefore, the present study was aimed to isolate *Azotobacter* strains from rhizosphere soil of primary tasar host plants (*Terminalia arjuna* and *T. tomentosa*) from tasar rearing areas of West Singhbhum (Jharkhand) and to test them for their nitrogen fixing ability under *in vitro* condition.

EXPERIMENTAL MATERIALS

Rhizosphere soil sample collection

A total of 28 rhizosphere soil samples of tasar host plants (Arjun, Asan and Sal) were collected from different tasar rearing areas (Table 1) of West Singhbhum, Jharkhand, India. Soil samples were derived from both forest and block (commercial) plantations including silkworm reared and non-reared plants.

Method of soil sample collection

Rhizosphere soil adhering to roots along with roots was collected at one foot depth, one meter apart from the tree

Table 1: Physico-chemical parameters of soil samples collected from tasar rearing areas of West Singhbhum

Place	Host plant	Block/Forest plantation	pH	E.C. (dSm ⁻¹)
Bandagao forest	Sal	Forest	5.3	0.46
Champua forest	Sal	Forest	6.4	0.72
CKP Bansai	Asan	Forest	6.5	0.35
CKP Indirwa (1)	Asan	Forest	5.6	0.62
CKP Indirwa (2)	Asan	Forest	6.5	0.63
CKP Indirwa (3)	Arjun	River bank	6.3	0.47
CKP PPC, Chelabeda (1)	Arjun	Block	4.8	0.58
CKP PPC, Chelabeda (2)	Arjun	Block	6.5	0.64
PPC Kharsawan (1)	Arjun	Block	7.2	0.99
PPC Kharsawan (2)	Arjun	Block	7.0	0.88
PPC Kharsawan (3)	Arjun	Block	5.4	0.46
Kharsawan (Farmer field) (1)	Asan	Block	5.5	0.41
Kharsawan (Farmer field) (1)	Arjun	Block	5.4	0.44
Kharsawan (Farmer field) (2)	Asan	Block	5.9	0.36
BSMTC Kharsawan (1)	Arjun	Block	5.8	0.58
BSMTC Kharsawan (2)	Asan	Block	5.9	0.61
PPC Hatghamaria (1)	Arjun	Block	5.7	0.45
PPC Hatghamaria (2)	Arjun	Block	5.7	0.75
REC Hatghamaria (3)	Sal	Dense forest	6.0	0.59
REC Hatghamaria (4)	Arjun	Block	5.2	0.43
REC Hatghamaria (5)	Arjun	Block	5.5	0.31
REC Hatghamaria (6)	Asan	Natural forest	5.0	0.25
REC Hatghamaria (7)	Asan	Dense forest	6.4	0.34
PPC Debrasai (1)	Arjun	Forest	5.0	0.18
PPC Debrasai (2)	Arjun	Forest	5.2	0.15
PPC Debrasai (3)	Asan	Forest	5.8	0.34
Thalaburu	Asan	Mixed	6.5	0.41
Thalaburu	Arjun	Mixed	6.7	0.44

Numbers in parentheses indicate soil samples drawn at different spots of same place; CKP - Chakradharpur; PPC - Pilot Project Centre; BSMTC - Basic Seed Multiplication & Training Centre; REC - Research and Extension Center.

trunk. In each sampling location, three rhizosphere soil samples were collected by following three-point sampling method (Malik *et al.*, 1982), which were then mixed to form representative sample of respective location. Soil samples were collected in polythene covers, brought to laboratory in cooling box and stored in refrigerator till further use. Apart from this, general soil samples were drawn from each location by following V shaped pit method (Mohan and Sangeetha, 2015) and soil pH and electrical conductivity were recorded.

Isolation and purification of *Azotobacter* strains

Azotobacter strains were isolated from rhizosphere soil samples by serial dilution technique, where 100 µl of diluted soil inoculum (10^{-4} and 10^{-5}) was inoculated on Jensen agar (Jensen, 1951) (sucrose 20.0g, K_2HPO_4 1.0 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, NaCl 0.5 g, $FeSO_4$ 0.1 g, Na_2MoO_4 0.005 g, $CaCO_3$ 2.0 g, Agar 15.0 g, distilled water 1 l, pH 7.2) by pour plate method. Petri dishes were then incubated at 30 °C for 48 hours. Based on colony morphology, different isolates were purified by streaking and single colonies were stored in slants for further use.

Estimation of atmospheric Nitrogen fixing ability among *Azotobacter* isolates in liquid culture

The pure *Azotobacter* isolates were cultured in 1 ml nutrient broth for overnight at 30 °C at 140 rpm, and 100 µl of which was inoculated in 30 ml Jensen's broth and kept under shaking incubator at 37 °C and 140 rpm for 3 days. Total Nitrogen fixed by *Azotobacter* strain was determined by using micro-Kjeldahl analysis. Three day old liquid culture was digested in micro Kjeldahl flask containing 10 ml of conc. H_2SO_4 and 1g of catalytic mixture (5:1 K_2SO_4 & $CuSO_4$), initially at 100 °C for one hour to avoid frothing followed by 360 °C for 1 and a half hours or till the solution appeared clear. After digestion, 100 ml of distilled water was added for cooling and 10 ml of 40 % NaOH was added. In an Erlen-Mayer flask, 10 ml of 4 % boric acid and 5 drops each of methyl red (pH: 4.3-6.3) and bromocresol green (pH: 3.6-5.2) indicators were added and distillation was done as per the set protocol in Kelplus system. The solution, boric acid and mixture of indicators containing the distilled off Ammonia (NH_3) was titrated against 1/30 N H_2SO_4 till color changed from blue to pink. The percentage of N_2 in the sample was calculated by the given formula.

$$\text{Nitrogen fixation (\%)} = \frac{(\text{titer value} - \text{blank value}) \times N \text{ of } H_2SO_4 \times 0.04}{\text{Volume of the sample (ml)}} \times 100$$

RESULTS AND DISCUSSION

Estimation of soil pH revealed that, majority of test regions were having acidic pH (~5.89) except PPC, Kharsawan (neutral pH). The pH range was from 4.8 (PPC, Chelabeda) to 7.2 (PPC, Kharsawan). The electrical conductivity of soil samples ranged from 0.15 to 0.99 dSm^{-1} . It was noticed that EC value of forest soil was lower as compared to block plantation (Figure 1), indicating less salt content in forest soil.

Morphological characters of *Azotobacter* isolates

Using Jensen media, 57 *Azotobacter* strains were isolated from rhizosphere soil samples of West Singhbhum region. The isolates have shown wide range of colony morphological characteristics as shown in Table 2 & Figure 2. Most of the isolates presented whitish (cream color) to light brown colony color and displayed various texture (flat/raised slimy, smooth), shape (round, spherical, irregular, shining) and size (3-8 mm diameter colonies). Out of 57, one representative *Azotobacter* isolate from each sample spot was used to test their Nitrogen fixing ability. Gram staining revealed that, all 28 isolates were gram-negative.

Nitrogen fixing ability of *Azotobacter* isolates

Nitrogen fixing ability of 28 *Azotobacter* strains tested

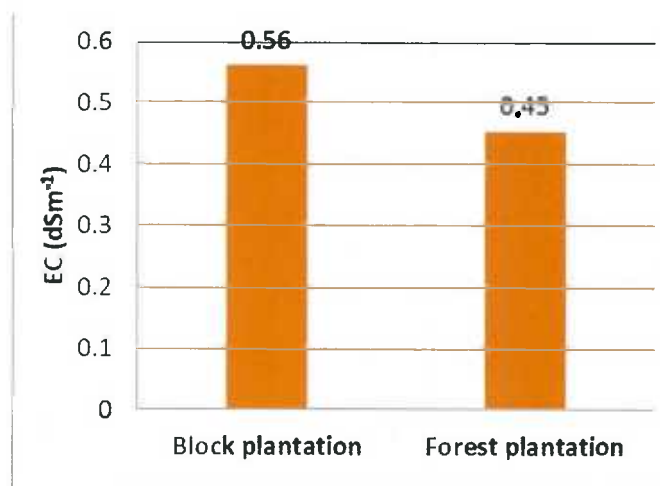


Figure 1: Electrical conductivity of soil of block plantation and forest plantation

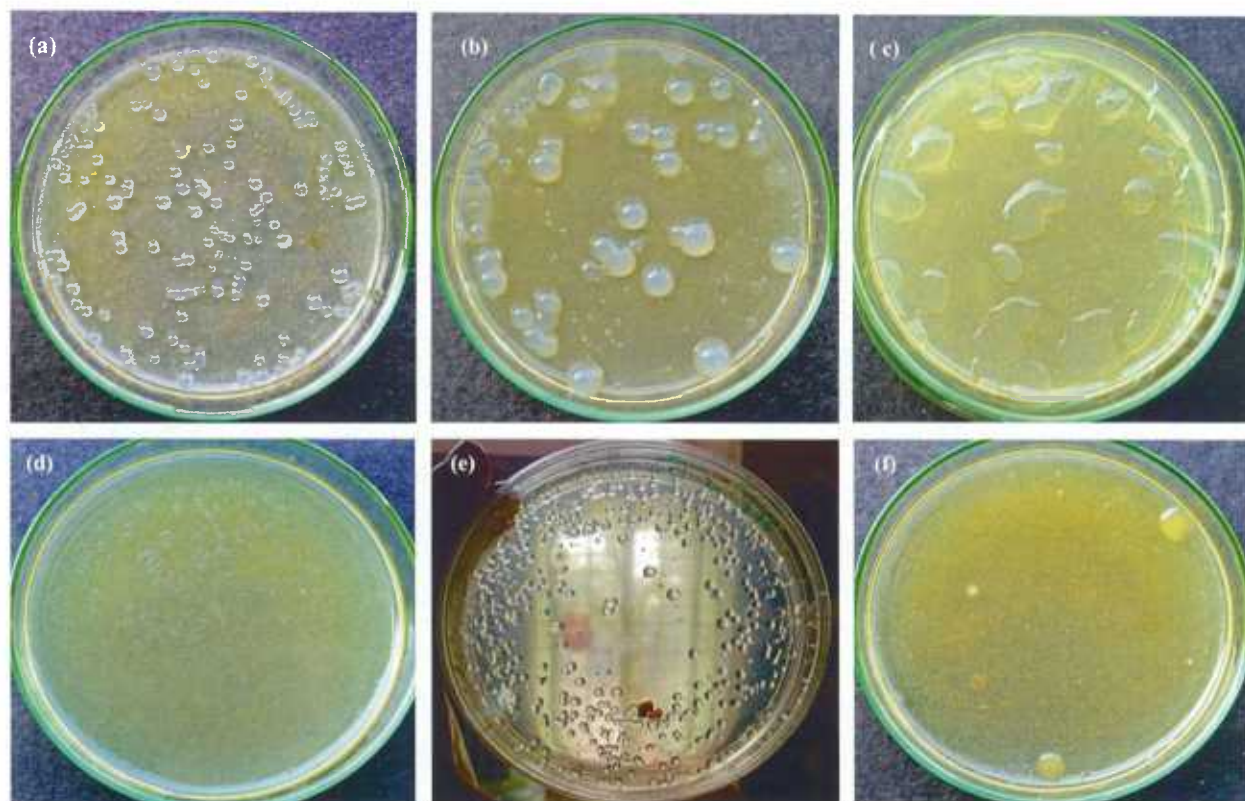


Figure 2: Variation in colony morphology of *Azotobacter* isolates derived from different tasar rearing regions. Isolates with varied colony morphology derived from Champwa Sal forest (a), Banasai Asan forest (b), Bandagao Sal forest (c), Indirwa Arjun (d), REC, Hatghamaria Arjun plantation (e) and Banasai Asan forest (f).

in liquid pure culture using micro-Kjeldahl method revealed wide variation for their nitrogen fixation (Table 3). The range for nitrogen fixation spread from 2.3 to 23.0 $\mu\text{g N ml}^{-1}$ with an average of 12.3 $\mu\text{g N ml}^{-1}$. The species of *Azotobacter* are known to fix on an average, 10 mg of N/g of carbohydrate under *in vitro* conditions (Chennappa *et al.*, 2016). In the present study, nine *Azotobacter* isolates have shown nitrogen fixation ability of below 10 $\mu\text{g N ml}^{-1}$, eighteen strains belonged to a range between 10 and 20 $\mu\text{g N ml}^{-1}$ and one strain exhibited above 20 $\mu\text{g N ml}^{-1}$. This clearly indicates that, majority of *Azotobacter* strains isolated have good nitrogen fixation ability. It was noticed that high nitrogen fixing strains were derived irrespective of host plant species, plantation type and geographical regions. *Azotobacter* isolates identified with high nitrogen fixing ability are A11 (23 $\mu\text{g N ml}^{-1}$, PPC, Kharsawan, Arjun block plantation), A26

and A24 (19.5 & 18.5 $\mu\text{g N ml}^{-1}$, respectively, PPC, Debrasai, Arjun forest plantation), A12 & A13 (17.4 & 16.8 $\mu\text{g N ml}^{-1}$, respectively, Kharsawan farmer's block plantation), A4 (16.3 $\mu\text{g N ml}^{-1}$, Indirwa Asan forest) and A2 (16.3 $\mu\text{g N ml}^{-1}$, Champwa sal forest). Thus these isolates need to be explored further for crop improvement as biofertilizers.

In an earlier attempt, Ridvan Kizilkaya (2009) had isolated 55 *Azotobacter* isolates to determine the nitrogen (N) fixing capacity in pure culture. The N fixation capacities of native 3 day old *Azotobacter chroococcum* strains added to Ashby Media varied from 3.50 to 29.35 $\mu\text{g N ml}^{-1}$ with an average of 10.24 $\mu\text{g N ml}^{-1}$. Majority of isolates have shown nitrogen fixation in the range of 3.5-20 $\mu\text{g N ml}^{-1}$. The range of nitrogen fixation ability is comparable with the results of present study.

Table 2: Morphological characteristics of *Azotobacter* isolates

Sample code	Colony morphology	Colour	Shape	Gram's test
A1	Flat slimy, transparent	Light brown	Round	Negative
A2	Raised, slimy, transparent	Transparent	Irregular	Negative
A3	Raised, spherical, slimy/water drops	White/cream	Spherical	Negative
A4	Raised, slimy	Brown	Spherical	Negative
A5	Raised, slimy	Cream white	Round	Negative
A6	Raised, semi-curve, watery	Cream white	Round	Negative
A7	Flat, rough surface	Light brown	Irregular	Negative
A8	Flat, rough surface, dry	Light brown	Irregular	Negative
A9	Flat, rough surface, dry	Light brown	Irregular	Negative
A10	Flat, rough surface	Light brown	Irregular	Negative
A11	Raised, slimy	Brown	Spherical	Negative
A12	Raised, spherical, slimy/water drops	White/cream	Spherical	Negative
A13	Flat, slimy, transparent	Light brown	Round	Negative
A14	Flat, rough surface	Light brown	Irregular	Negative
A15	Flat, rough surface, dry	Light brown	Irregular	Negative
A16	Flat, rough surface, dry	Light brown	Irregular	Negative
A17	Raised, slimy	Brown	Spherical	Negative
A18	Raised, slimy	Cream white	Round	Negative
A19	Raised, semi curve, watery	Cream white	Round	Negative
A20	Raised, spherical, slimy/water drops	White/cream	Spherical	Negative
A21	Raised, slimy	Cream white	Round	Negative
A22	Flat, rough surface, dry	Light brown	Irregular	Negative
A23	Raised, slimy	Cream white	Round	Negative
A24	Raised, semi curve, watery	Cream white	Round	Negative
A25	Raised, spherical, slimy/water drops	White/cream	Spherical	Negative
A26	Flat, rough surface, dry	Light brown	Irregular	Negative
A27	Raised, slimy	Cream white	Round	Negative
A28	Raised, spherical, slimy/water drops	White/cream	Spherical	Negative

Conclusion

Under the present study, seven *Azotobacter* isolates were identified with good nitrogen fixing ability under *in vitro* condition. These could be evaluated further in the field condition (*in vivo*) for their colonization and nitrogen fixation. Potential isolate could be utilized in the form of biofertilizer to improve the tasar plant nutrition and thereby to enhance tasar silk cocoon quality.

ACKNOWLEDGEMENT

The authors are thankful to Central Silk Board for funding

Table 3: Nitrogen fixing capacity of *Azotobacter* spp. strains in Jensen liquid culture

Isolate	Place	Jenson media, $\mu\text{g Nml}^{-1}$
A1	Bandagao forest	11.7
A2	Champua forest	16.3
A3	CKP, Bansai	11.7
A4	CKP, Indirwa (1)	16.3
A5	CKP, Indirwa (2)	2.8
A6	CKP, Indirwa (3)	6.9
A7	CKP, PPC, Chelabeda (1)	2.3
A8	CKP, PPC, Chelabeda (2)	6.9
A9	PPC, Kharsawan (1)	11.7
A10	PPC, Kharsawan (2)	15.7
A11	PPC, Kharsawan (3)	23.0
A12	Kharsawan (Mani Oraon)	17.4
A13	Kharsawan (Mahesh Oraon) (1)	16.8
A14	Kharsawan (Mahesh Oraon) (2)	11.8
A15	BSMTC, Kharsawan	9.2
A16	BSMTC, Kharsawan	12.2
A17	PPC, Hattghamaria	13.5
A18	PPC, Hattghamaria	14.8
A19	REC, Hattghamaria	5.9
A20	REC, Hattghamaria	6.4
A21	REC, Hattghamaria	13.9
A22	REC, Hattghamaria	8.6
A23	REC, Hattghamaria	9.4
A24	PPC, Debrasai	18.5
A25	PPC, Debrasai	16.2
A26	PPC, Debrasai	19.5
A27	Thalaburu (Mangal Singh)	12.8
A28	Thalaburu (Mangal Singh)	13.7

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Article scientifique

EFFICACITE DE FIXATION D'AZOTE PAR DES SOUCHES D'*AZOTOBACTER* ISOLEES DE LA RHIZOSPHERE D'ARJUN ET D'ASAN DU SINGHBHOM DE L'OUEST, JHARKHAND, INDE

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RESUME

L'application d'engrais biologiques pour la nutrition et la croissance des plants nourriciers du tasar is écologique, économique et durable. Cette étude a été entreprise pour isoler des souches d'*Azotobacter* fixatrices d'azote à partir de la rhizosphère des plantes nourricières du tasar. Dix échantillons de rhizosphère ont été collectés sur Arjun (*Terminalia arjuna*) Asan (*Terminalia tomentosa*) dans la forêt et les plantations des zones d'élevage du tasar dans le Singhbhum de l'ouest au Jharkhand. Environ 28 souches d'*Azotobacter* ont été isolées et triées pour leur capacité à fixer l'azote dans des cultures liquides de souches pures de trois jours en utilisant la méthode de micro Kjeldahl. Une large fourchette de fixation a été constatée de 2,3 à 23,0 $\mu\text{g N ml}^{-1}$ avec une moyenne de 12,3 $\mu\text{g N ml}^{-1}$. Quelques isolats d'*Azotobacter* tels que, A11 (23 $\mu\text{g N ml}^{-1}$), A26 (19,5 $\mu\text{g N ml}^{-1}$), A24 (18,5 $\mu\text{g N ml}^{-1}$), A12 (17,4 $\mu\text{g N ml}^{-1}$), A13 (16,8 $\mu\text{g N ml}^{-1}$), A4 (16,3 $\mu\text{g N ml}^{-1}$) and A2 (16,3 $\mu\text{g N ml}^{-1}$) montrent une forte capacité à fixer l'azote. Ceci indépendamment de la plante d'origine, du type de plantations et de la zone géographique. Ces potentiels des souches natives d'*Azotobacter* peuvent être explorés comme engrais biologique pour améliorer la nutrition des plantes nourricières tasar.

Mots clés: *Azotobacter*, capacité à fixer l'azote et engrais biologique, rhizosphère, culture du tasar.



IMPACT OF THE SILKWORM EGG PRODUCTION SEASON IN BULGARIA ON THE MAIN QUANTITATIVE CHARACTER VALUES IN THE NEXT GENERATION

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ABSTRACT

This study comprised of the Bulgarian commercial hybrids viz., SN1xI1 x M2xN2 and Super 1 x Hesa 2 and their reciprocal crosses authorized by the Government. The silkworm eggs were produced in two different seasons, namely spring, extending from early May to the end of June and autumn, spread from early September to the end of October. A fraction of the silkworm eggs, produced from the spring season and meant for rearing in the succeeding autumn season of the same year were HCl treated and the rest were allowed to undergo hibernation, for rearing during the spring of the next year. The silkworm eggs, produced during the autumn season were hibernated and designated for rearing during the next year's spring and summer-autumn seasons. The silkworm egg testing manifested that in the spring silkworm rearing, the main quantitative character values, such as pupation rate, fresh cocoon weight, silk shell weight and shell percentage were not influenced significantly by the egg production season. On the other hand, during the autumn rearing season, the fresh cocoon weight in the hybrid SN1xI1 x M2xN2 and the reciprocal tended to be lower for the hibernated eggs than in the HCl treated ones, while in the hybrid Super 1 x Hesa 2 and its reciprocal, the differences were negligible. The results obtained bring out that the silkworm eggs, produced during the less favorable autumn season in Bulgaria manifest lower cocoon weight in some hybrids, if reared during the autumn season of the next year.

Key words: *Bombyx mori* L., eggs, F₁ hybrids, rearing seasons, silkworm.

INTRODUCTION

In Bulgaria, generally, there are two silkworm rearing seasons, namely spring, which starts by the end of April / first half of May and summer - autumn season, starting during the second half of August or early September. The summer-autumn silkworm rearing in Bulgaria is advised to commence not later than the mid of September (Tzenov, 2015).

In fact, in Bulgaria, if we go for only one crop from mulberry, it is possible to have the summer rearing within a stipulated period, starting from the beginning of July. However, in practice, two crops from mulberry are usually taken and hence, the decision on summer-autumn

rearing necessitates ensuring enough growth and sufficiency of mulberry foliage after the spring bottom pruning. As the spring bottom mulberry pruning in Bulgaria is usually performed from the beginning to the mid of June, and considering that mulberry needs about 3 months to sprout and form new shoots, suitable for top pruning, it may be ascertained that the beginning of the summer autumn silkworm rearing should not be earlier than the mid of August so that the top pruning may be performed not earlier than early September. In India and Thailand, one of the best cocoon crops are obtained during the so called "winter period" i.e., from December to February (Boonchoo, 2006; Nagaraja, 2006). In any case, the mulberry leaf quality has a very big influence not only on the main productive character values, such as fresh

cocoon and silk shell weight, but also on the pupal weight, the number of eggs laid by the moth and even on the single egg weight in the batch (Tzenov *et al.*, 1995). According to some authors, the silkworm egg cold storage is possible for a period from 75 (in order to break the diapause) to 600 days (maximum term to still have viable eggs), depending on the embryo development stage and temperature (Chaturvedi and Upadhyay, 1990; Chuprayoon *et al.*, 2002, Anonymous, 2003; Shiota, 2004; Luo *et al.*, 2006; Cappellozza *et al.*, 2007). In Bulgaria, in the traditional egg cold storage method, the silkworm eggs, laid at the end of June / beginning of July are stored at 2 °C from the beginning of December to the end of April / beginning of May, by when it is the start of incubation. Before this period, the eggs are preserved at a schedule of gradually decreasing temperature from 25 to 8 °C from July to the end of November. The eggs produced in the autumn season and directed for spring rearing however, are stored at 25 °C for two weeks after the oviposition, then at 10 °C for one week and after that, at the same temperature schedule as for the eggs laid in June / July. Some of the eggs, produced in October are preserved at a different regime and suitable for rearing during the period, July – October of the next year.

The present study was undertaken in order to compare the quality of the silkworm eggs, produced in June / July and those, produced in October, one part of them directed for rearing during the next year's spring season and the other part directed for rearing in the summer - autumn season of the next year.

MATERIALS AND METHODS

The breeding programme was conducted during the period of 2014 – 2017 at the Agricultural University, Plovdiv and Scientific Center on Sericulture, Vratsa, Bulgaria. The Bulgarian commercial hybrids, SN1xI1 x M2xN2, Super 1 x Hesa 2 and their reciprocal crosses authorized by the Government were selected for the study. The hybrid, SN1xI1 x M2xN2 is sex-limited for larval markings. The silkworm eggs were produced during two different seasons, namely spring, wherein the silkworm rearing starts in early May and finishes the papionage by

the end of June / beginning of July and autumn season, extending from early September to October. The silkworm eggs, produced from the spring season were both acid treated and hibernated for rearing during the autumn season of the same year and spring of the next year, respectively. The silkworm eggs, produced from the autumn season were hibernated for rearing during the next year's spring and summer-autumn seasons.

Two grams of eggs of each silkworm hybrid were incubated and larvae reared together until the end of 2nd instar. At the beginning of 3rd instar, 4 replicates of 200 larvae each were counted from each hybrid and reared further until the cocoon spinning.

The silkworm larvae were reared following the standard method for spring rearing in Bulgaria (Panayotov and Ovesenska, 2002), and fed "ad libitum" with mulberry leaves of No. 106 Bulgarian variety. The mulberry plants were maintained as rain fed plantation. The data obtained were processed statistically (Lidanski, 1988).

RESULTS AND DISCUSSION

The major findings of the study are presented in Tables 1 and 2. The silkworm egg testing manifested that in the spring silkworm rearing, the main quantitative character values, such as pupation rate, fresh cocoon weight, silk shell weight and shell percentage were not influenced significantly by the egg production season (Table 1). The excellent quality of mulberry leaf during the spring season in Bulgaria might have been a contributory factor for this, resulting in comparatively high fresh cocoon and silk shell weight values (2103 ~ 2350 mg and 419 ~ 474 mg, respectively). Another factor that has been instrumental might be the shorter duration of egg storage of the hibernated silkworm eggs, produced in the autumn of the previous year and designated for rearing in the spring of the next year. In fact, these eggs are younger, meanwhile, the eggs laid at the end of June are preserved for 10 months up to the beginning of incubation, while those laid at the end of October are preserved only for 6 months. However, owing to the lower cocoon and pupal weight, the autumn parental moths lay not only less number of eggs, but also of less weight. We suppose that this lower single egg weight

is partly compensated during the spring rearing season by the superior quality of mulberry leaf and shorter period of egg storage from the point of oviposition to the beginning of incubation.

Table 1: Main quantitative character values in spring silkworm rearing of silkworm eggs, produced during the spring and autumn seasons of the previous year

Hybrid / egg production season	Pupation rate (%)	Fresh cocoon weight (mg)	Silk shell weight (mg)	Silk shell %
SN1xI1 x M2xN2 spring	94.55	2224	437	19.65
SN1xI1 x M2xN2 autumn	93.98	2103	419	19.92
td	0.88	4.13**	1.34	0.87
Hesa 2 x Super 1 spring	93.77	2267	460	20.29
Hesa 2 x Super 1 autumn	94.22	2246	442	19.68
td	0.91	1.11	1.23	1.03
Super 1 x Hesa 2 spring	96.13	2115	435	20.57
Super 1 x Hesa 2 autumn	95.18	2350	474	20.17
td	0.89	4.98**	0.84	0.75

During the autumn rearing season, the fresh cocoon weight in the hybrid SN1xI1 x M2xN2 and the reciprocal which is sex-limited, tended to be lower for the hibernated eggs than for the HCl treated ones. In this hybrid & its reciprocal, the fresh cocoon weight for HCl treated eggs was 1580 and 1588 mg, respectively, while for the hibernating eggs produced in the autumn of the previous year, the cocoon weight was 1494 and 1524 mg, respectively. On the contrary, in the hybrid, Super1 x Hesa 2 and its reciprocal, the differences between two types of eggs were negligible.

In fact, there is difference in the hibernation duration of silkworm eggs, used in the spring and autumn rearing. Those used in the spring rearing is stored for 6 months from the time of oviposition to the initiation of incubation, while the source of eggs for the autumn rearing is stored for 10 months. Whereas, the HCl treated eggs, used in this experiment however, were stored for only 2 months. Our results manifest that in some silkworm strains, the autumn egg production season and the longer term of egg storage from the oviposition to the beginning of incubation can result in decrease of cocoon weight.

Table 2: Main quantitative character values in autumn silkworm rearing of silkworm eggs, produced in the spring season of the current year and HCl treated and hibernated eggs, produced in the autumn of the previous year

Hybrid / egg production season	Pupation rate (%)	Fresh cocoon weight (mg)	Silk shell weight (mg)	Silk shell %
M2xN2 x SN1xI1 spring of the current year, HCl treated	96.75	1580	330	20.89
M2xN2 x SN1xI1 autumn of the previous year, hibernated	90.17	1494	318	21.29
td	3.22 ***	4.14**	2.12	0.68
SN1xI1 x M2xN2 spring of the current year, HCl treated	86.50	1588	312	20.47
SN1xI1 x M2xN2 autumn of the previous year, hibernated	87.17	1524	316	20.73
td	0.86	3.12*	1.22	0.75
Hesa 2 x Super 1 spring of the current year, HCl treated	84.00	1688	330	19.55
Hesa 2 x Super 1 autumn of the previous year, hibernated	95.75	1704	344	20.19
td	5.17***	1.25	0.92	0.93
Super 1 x Hesa 2 spring of the current year, HCl treated	82.83	1724	344	19.95
Super 1 x Hesa 2 autumn of the previous year, hibernated	89.67	1721	348	20.22
td	4.23**	1.13	1.27	0.88

Conclusion

The results make it apparent that the silkworm eggs, produced during the less favorable autumn season in Bulgaria manifest lower cocoon weight in some hybrids, if reared during the autumn season of the next year.

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Article scientifique

IMPACT DE LA SAISON DE PRODUCTION DES OEUFs DE VER A SOIE SUR LES CARACTERES QUANTITATIFS DE LA GENERATION SUIVANTE EN BULGARIE

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RESUME

Cette étude concerne les hybrides bulgares commerciaux SN1xI1 et Super1xHesa2 ainsi que leurs croisements réciproques autorisés par le gouvernement. Les œufs de ver à soie sont produits au cours de deux saisons différentes, au printemps du début mai à la fin juin et l'automne de début septembre à fin octobre. Une partie des œufs produits au printemps et destinés à l'élevage d'automne de la même année sont traités à l'HCl, le reste étant soumis à hibernation pour l'élevage du printemps suivant. Les œufs produits en automne sont hibernés et utilisés pour les élevages du printemps et de l'automne suivants. Pendant l'élevage de printemps, les caractères quantitatifs principaux tels que le taux de pupaison, le poids de cocons frais, le poids de la coque soyeuse et la richesse soyeuse ne sont pas

significativement influencés par la saison de production des œufs. Pendant l'élevage d'automne, le poids de cocons frais chez l'hybride SN1xI1 x M2xN2 et son réciproque tend à être plus faible avec les œufs hibernés qu'avec les œufs traités à l'HCl alors que chez Super1xHesa2 et son réciproque, les différences sont négligeables. Les résultats montrent que les œufs de ver à soie produits pendant la saison défavorable d'automne en Bulgarie donnent un poids de cocons plus faible chez certains hybrides s'ils sont élevés à l'automne de l'année suivante.

Mots clés: *Bombyx mori* L., oeufs, hybrides F1, saisons d'élevage, ver à soie.



TARGETING IMMEDIATE EARLY GENE (IE-1) FOR INDUCING VIRUS RESISTANCE AGAINST GRASSERIE DISEASE CAUSED BY BMNPV BY RNA INTERFERENCE TECHNOLOGY

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ABSTRACT

Bombyx mori L. (Lepidoptera: Bombycidae) nucleopolyhedrosis virus (BmNPV) is a highly pathogenic virus that causes grasserie infection in silkworms and effective management of the virus has been a challenge because of its sturdy nature and the lack of control strategies. RNA mediated silencing technology (RNAi) has become the tool of choice for induction of virus resistance in many organisms. A significant feature of this technology is the presence of double stranded RNA (dsRNA). In this study, *Escherichia coli* were engineered to produce dsRNA against ie-1 (immediate early gene of BmNPV) from a plasmid (L4440) containing gene of interest under the control of double T7 promoter which efficiently produces dsRNA when it is transformed into *E. coli* HT115 host strain and upon induction with IPTG. Our study targeted the BmNPV ie-1 gene involved in viral multiplication. The viral copy number analysis revealed that larva fed with 50 µg of *E. coli* expressing ie-1 dsRNA showed lower copy number (100 copies) of the Gp41 gene against 1x10⁵ copies of Gp41 in the NPV infected silkworms. The qPCR analysis of viral genes showed 5-6-fold decrease in the viral gene expression in midguts of dsRNA fed silkworms compared to that of NPV infected larvae and showed increased survivability of > 50 % compared to infected silkworms. These results demonstrate the successful use of *E. coli* expressing dsRNA as an efficient and alternative tool for insect pest management.

Key words: Bioassay, BmNPV, double stranded RNA, oral RNAi, RNA interference, viral copy number.

INTRODUCTION

Silk known as “Queen of Textiles”, is historically, one of India's most important industrial output characterised with unique properties of unparalleled grandeur, natural sheen and inherent affinity for dyes, high absorbance, light weight, soft touch and high durability. India has a large domestic market for silk goods and about 85 % of silk goods produced are sold in the domestic market. Due to the large domestic consumption, the need for large scale production of silk is the biggest challenge for silk farmers because there is a major gap in the actual yield potential of each crop with respect to its harvested yield, the gap in production and batch to batch variation is

primarily due to disease outbreak, environmental hazards and lack of quality seed.

Silkworms are prone to different kinds of diseases very often as their immune systems are incapable of resisting infections. The most common pathogens infecting the silkworm, *Bombyx mori* are microsporidians, nucleopolyhedrosis virus (NPV), densovirus, infectious flacherie virus (IVF), cytoplasmic polyhedrosis virus (CPV) and various bacteria. Among the pathogens, virus causes serious deleterious effects due to their systemic infections. Out of these nuclear polyhedrosis caused by *B. mori* nuclear polyhedrosis virus (BmNPV) can cause considerable crop loss up to 33-55.3 % (Nataraju *et al.*,

1998) of the total loss due to different silkworm diseases, the occurrence of it being the most common in summer (Christi and Sohaf, 1990).

RNA interference

RNA mediated silencing technology has emerged as an important tool for the study of gene functions through silencing specific genes by degrading mRNA before it is translated. RNA interference (RNAi) refers to double-stranded RNA mediated gene silencing, to prevent the expression of specific genes without affecting the expression of other genes. In 1998, Fire and his coworkers announced the discovery of RNA interference in *Caenorhabditis elegans*. Since then, it has developed as a powerful tool for functional genomics and widely used in insect genetic research. RNA mediated silencing technology has now become the tool of choice for induction of virus resistance in animals, plants and microorganisms.

Bacterial vectors for RNAi delivery

Although RNAi technology holds great promise for the advancement of new gene directed therapies, the difficulty of delivering siRNA to target cells has impeded the rapid expansion of RNAi-based therapies. Therefore, most of the current studies aim at improving existing methodologies and adopting innovative technologies to increase the efficiency of RNAi. Studies conducted by Timmon and Fire, 1998 and Timmon *et al.*, 2001 have shown that systemic gene silencing could be attained in the nematode *C. elegans* when it ingested *E.coli* engineered to produce interfering RNAs. This discovery created an extra method to introduce dsRNA into organisms for triggering RNAi. The use of viral vectors, nano particles, liposomes and chemical modifications to siRNA have been exploited to deliver RNAi and delivery of therapeutic RNA interference has become a field of its own right in the biotechnology industry.

Bacteria are increasingly recognized for vast potential as therapeutics and efforts are being made in various areas of

therapeutic drug development based on live-attenuated or otherwise modified bacteria. Bacteria have also been found to be useful for the delivery of short interfering RNA. Bacteria are versatile gene vectors and have been shown to be an effective, safe and inexpensive measure for delivering RNAi to target cells with high specificity and high selectivity

This technology involves cloning of the selected BmNPV genes into *E. coli* vector that can express dsRNA against selected BmNPV genes. Once the clones are developed, they are transformed, to non-pathogenic *E. coli* host system for producing large quantities of dsRNA against NPV genes. Development of efficient dsRNA construct that is able to combat the viral infection will help in reducing the disease incidence to a major extent. By this stable and cost effective technique, RNAi can be activated *in-vitro* and *in-vivo* by non-pathogenic bacteria engineered for synthesis and delivery of silencing shRNA to the target cells. The technology developed is non-conjugative, non-pathogenic and environment friendly.

MATERIALS AND METHODS

Collection of infected larvae and purification of BmNPV polyhedra

The BmNPV infected 5th instar silkworm larvae were collected from the rearing house. The infected haemolymph (a white milky fluid) was collected in sterile tubes containing a few specks of phenylthiourea by puncturing the prolegs of the infected larvae and filtered through two layers of cheese cloth / non-absorbent cotton pad. The polyhedra were pelleted by centrifugation at 5000 rpm for 10 min at 4 °C. The polyhedral pellet was suspended in distilled water and the pellet was washed repeatedly thrice by centrifugation at 5000 rpm for 10 min at 4 °C followed by pellet wash using 1 M NaCl thrice, a white pellet adhering on to walls of the tubes indicates purified polyhedra. The purified polyhedra were suspended in distilled water and stored at 4 °C and diluted as per requirement.

Viral DNA extraction, PCR amplification, and sequencing

The viral DNA was isolated from the purified occlusion bodies using 2PK buffer containing 200 mM Tris, 25 mM EDTA, and 300 mM NaCl and 2 % SDS followed by proteinase K for 30 min and DNA was extracted with phenol:chloroform:isoamyl alcohol (25:24:1), precipitated in 2.5 volumes of ethanol, washed with 70 % ethanol, and suspended in 10 mM Tris - 1 mM EDTA. Thirty-five nanograms of viral DNA was used to amplify the viral genes (Table 1). The PCR amplification was performed using the following conditions: initial denaturation of 5 min at 94 °C; 30 cycles of 30 s at 94 °C; 30 s at 52 °C; 1 min at 72 °C; and a final extension of 10 min at 72 °C. The PCR products were separated in 1.2 % agarose gel along with 100 bp DNA ladder. For DNA sequencing, 50 ng of PCR product was used in a sequencing reaction. The sequences obtained were verified in the National Centre for Biological Information (NCBI) database using BLASTN search algorithm.

Vector construction and dsRNA expression

To construct a plasmid that expresses dsRNA corresponding to Immediate early factor-1 (ie-1), the gene sequences of ie-1 was obtained from NCBI database (Accession Number L33180) and checked the entire length of sequence for siRNA sequences using Whitehead siRNA analysis software (Yuan *et al.*, 2004). After analysis, ~310 bp fragments were PCR amplified using BmNPV viral DNA as a template. Amplification reactions comprised 30 cycles at 94 °C for 60 s, 58 °C for 30 s, and 72 °C for 60 s, with a final extension step of 72 °C for 8 min. PCR products were confirmed by separation on 1 % agarose gels and visualized by ethidium bromide staining. The PCR product was then cloned into the plasmid L4440 at XhoI and XbaI sites. The L4440 plasmid has two T7 promoters in inverted orientation flanking the multiple cloning sites. The resulting recombinant clone L4440-ie1 and L4440 (without the insert) were introduced into competent HT115 (DE3) cells lacking RNase III. To induce the production of dsRNA, single colonies of Ht115

Table 1: List of primers selected for the analysis of viral gene expression

Primer	Primer sequence '5-3'
ie-1	FP-CGCTCCGGAGCCGACACGGTAGTATC
	RP-ACGCGTCGACGGCCACCACACTTTGTGAAT
lef-1	FP-CGCGGATCCGGTACAAGGCCTTCGAAA
	RP-CCGTCCGGACCGGACCACATCCACCAATTCCAT
Gp41	FP-CGTAGTAGTAGTAATCGCCG
	RP-AGTCGAGTCGCGTCGCTTT
Gp64	FP-CCTTCAGCCATGGAAGTG
	RP-GACGACCTCGAATTTTGG
lef-3	FP-ACGCGTCGACTTGTTTTGAAGTCGCG
	RP-TGCTCTAGACTTGCGTTTGTGCAATTTTGCA
GAPDH	FP-GCTGCCTCCTTGACCTTTTGC
	RP-CAATCCGCGTCCCTGTTGCTAAT
ie-2	FP-ATGAGTCGCCAAATCAA
	RP-TTAAGGTTTAGACATCTCA
p35	FP-TGTGTAATTTTCCGGTAGAAATCGAC
	RP-TTATTAATCATGTCTAATATTACATTTTGG

(DE3) bacteria containing L4440-ie-1 and L4440 without the insert were grown with shaking in LB with 100 µg/ml ampicillin plus 12.5 µg/ml tetracycline at 37 °C till the cells attained OD 595=0.4. The T7 polymerase was induced by addition of IPTG (Isopropyl thio galactosidase) to 0.25 mM and the bacteria were incubated with shaking for an additional 4 h at 37 °C. After 4 h of growth, the cells were harvested by centrifuging at 8000rpm for 10 min. To confirm the expressed dsRNA, cDNA was synthesized from induced bacterial cells of dsRNA ie-1 along with L4440 vector cultures. The cDNA was amplified and analyzed using gene-specific primers to confirm the presence of the gene and also induction.

Bioassay to validate the effect of DsRNA in BmNPV infected silkworm

For conducting bioassays, 1 % of the overnight grown *E. coli* culture was transferred to 500 ml LB, and cultured as described above. The cultures were then grown at same conditions until they attained an OD 595 of 0.4-0.6. Thereafter, cultures were induced with 0.25 mM IPTG and grown for 4 h. The cultures were then harvested and re-suspended in 1 ml DNase- RNase-free water (Invitrogen,

USA). NPV polyhedral and 50 µl of bacterial culture was spread uniformly on the freshly collected mulberry leaf and allowed to dry completely. The 5th instar *B. mori* larvae were then released over the mulberry leaves. The experimental set up consisting of larvae fed on fresh mulberry leaves (control), larvae infected with NPV polyhedra spread on mulberry leaves (NPV infected), NPV infected larvae fed with bacteria containing only L4440 vector (L4440+NPV) and NPV infected larvae fed with bacteria containing dsRNA ie-1 were monitored for the effects on insect growth and development. For each set, 50 larvae were used and the whole experiment was done in triplicate. After having consumed the feed fully, fresh leaves were offered to larvae twice, daily. Larvae were collected 24 h after feeding. Mid gut samples were dissected out from 10 larvae for semi quantitative analysis, and the remaining larvae were reared till the cocoon formation to test the survivability of dsRNA fed infected silkworms. Genomic DNA was isolated using standard protocol and the target gene was then amplified by PCR analysis using gene specific primers and Gp41 viral multiplication primer.

Analysis of viral copy number using Gp41 gene specific primer

Total DNA was extracted from the midguts of the BmNPV-infected *B. mori* as well as dsRNA treated BmNPV-infected *B. mori* at 24 pi by using standard genomic DNA isolation protocol. DNA samples that were obtained from ten larvae per treatment group were used as the template for PCR amplification. One pair of Gp41 primers (forward primer: 5'CGTAGTAGTAGTAATCGCCGC3' and reverse primer: 5'AGTCGAGTCGCGTCGCTTT3') were designed based on the BmNPV Gp41 gene sequence (GenBank accession no. BAA03365) (Yan-Yuan Bao *et al.*, 2009). As an internal control, the *B. mori* GAPDH gene (GenBank accession no. ABA43638) was also analyzed using the following primers: 5'GCTGCCTCCTTGACCTTTTGC3'(forward primer) and 5'CATTCCGCGTCCCTGTTGCTAAT3' (reverse primer). The specificity of the primers was confirmed by

using NCBI BLAST algorithms (<http://www.ncbi.nlm.nih.gov/>). The reactions were conducted on an Agilent Technologies Stratagene MX3005P Real-Time PCR system using the SYBR Premix Ex Taq Kit (TaKaRa), according to the manufacturer's protocol. Total DNA and specific BmNPV primers were used to amplify the BmNPV Gp41 gene sequence. Each amplification reaction was performed using a 20 µl reaction mixture, under the following conditions: denaturation at 95 °C for 30 s, followed by 40 cycles at 95 °C for 5 s and at 60 °C for 34 s. The fluorescent signals yielded by the PCR products were detected by subjecting the products to a heat-dissociation protocol (temperature range, 60-95 °C) during the last step of each cycle. Following amplification, melting curves were constructed, and data analysis was performed by using the ABI 7500 system SDS software. Two microliters of genomic DNA from BmNPV-infected samples and no-template control (NTC) were used as templates. The reaction was carried out in triplicate, and the average threshold cycle (Ct) value was used to quantify the relative BmNPV copy number. The results were standardized using the expression level of the *B. mori* glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene.

Confirmation of differentially expressed genes by performing real-time qPCR

Trizol reagent (TaKaRa) was used to extract total RNA from the midguts of the BmNPV-infected *B. mori* as well as dsRNA treated BmNPV-infected *B. mori* at 24 pi. RNA was treated with 10 U of DNase I (TaKaRa), following the manufacturer's instructions. The concentration of DNase-treated RNA was adjusted with DEPC H₂O to 1 µg/µl, and 1 µg of DNase I-treated RNA was reverse transcribed in a 10 µl reaction system using the First strand cDNA synthesis kit (TaKaRa). Real-time qPCR was performed using 2 µl of diluted first-strand cDNA (1/10) in each 20 µl reaction mixture. Specific primer sets were designed for genes that encoded BmNPV (ie-1, ie-2, lef-1, lef-3, p35 and Gp64) (Table 1). The conditions used for the real-time qPCR were as described under investigation of BmNPV proliferation by performing real-time quantitative PCR.

The results were standardized to the expression level of the constitutive *Bombyx* GAPDH gene. An NTC sample was run to detect contamination and to determine the degree of dimer formation. A relative quantitative method ($\Delta\Delta Ct$) was used to evaluate quantitative variation. Statistical significance of differences of values were determined with student's t test.

RESULTS

BmNPV infection and isolation of essential viral gene(s) involved in BmNPV multiplication

The grasserie diseased larvae became obvious after infection with BmNPV in the rearing trays and on mountages with shiny white translucent skin, swollen inter-segmental membrane and swelled body segments. At an advanced stage of infection, the body wall ruptured and white turbid hemolymph containing a large number of OBs oozed out. The cadavers look white at the time of death and became dark within 3–5 h as they putrefied (Figure 1A). PCR amplification using *ie-1* primers with genomic DNA isolated from the polyhedra yielded 310-bp as expected, (Figure 1B). Further, the sequencing of the *ie-1* PCR product confirmed that the amplified product was of BmNPV origin. No differences were noticed at the nucleotide sequence level in the isolated genes of *ie-1* against the NCBI accession number L33180.1.

Validation of expression analysis of dsRNA *ie-1* produced inside the bacteria

The confirmed clones were transformed into *E. coli* HT115 bacterial host strain for inducing the dsRNA *ie-1* production. The induction of dsRNA was confirmed by isolating total RNA from induced L4440 vector alone and L4440 vector containing *ie-1* gene of interest. The cDNA was synthesised and the cDNA was analysed using the *ie-1* gene specific primers. The induced L4440 vector containing *ie-1* gene of interest samples showed amplification of *ie-1* gene at 310 bp while there was no such amplification observed in the induced L4440 vector

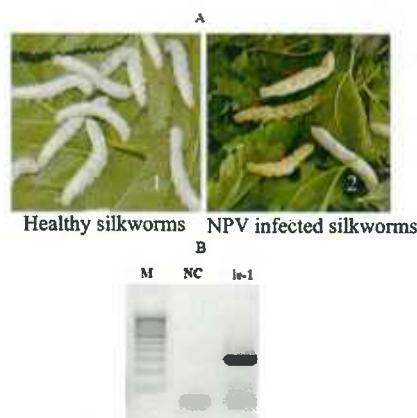


Figure 1: Analysis of viral genes in silkworms infected with *Bombyx mori* Nucleopolyhedrosis virus (BmNPV). A: 1–5th instar healthy silkworms; 2-BmNPV infected silkworms with swollen integuments and putrefied body; B: Viral gene *ie-1* amplified from the genomic DNA isolated from the infected silkworm for cloning into dsRNA producing L4440 vector; Lane M: DNA Ladder, NC: Negative control and Lane *ie-1*: viral gene *ie-1* amplified using gene specific primer.

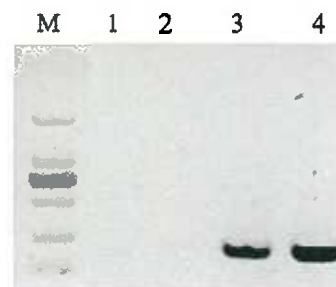


Figure 2: Semi quantitative analysis of dsRNA *ie-1* expression in bacteria. Lane M: ladder; Lane 1: negative control; Lane 2: induced L4440 vector; Lane 3: induced vector with *ie-1* gene; Lane 4: positive control.

cDNA samples. This clearly indicated expression of dsRNA *ie-1* in bacteria (Figure 2). The dsRNA *ie-1* clones were cultured in 500 ml conical flasks and induced with IPTG as mentioned above and cells were harvested after induction for feeding the silkworms.

Copy number analysis of Gp41 gene in dsRNA *ie-1* fed silkworms

The genomic DNA was isolated from the mid-gut tissue of

the NPV Infected, Vector (L4440+ NPV) fed and dsRNA fed infected silkworms to analyze virus multiplication using Gp41 primers at 24 hpi. The analysis of genomic DNA with Gp41 primers showed virus multiplication in the mid gut of all the samples. The Real time PCR was carried out to analyze the copy number of Gp41 in the above mentioned samples. The relative copy numbers that corresponded to the BmNPV GP41 gene was approximately 1×10^5 copies at 24 hpi in the NPV infected as well as L4440+NPV fed larvae (Figure 3). In contrast, the viral proliferation rate was extremely low in the larval mid gut of the dsRNA ie-1 fed, infected larval mid gut, with the relative copy numbers remaining below 100 copies until 24 hpi (Figure 3). These results revealed that BmNPV invaded the mid gut tissue of both NPV infected as well as dsRNA ie-1 fed silkworms, but the viral proliferation in the mid gut of the dsRNA ie-1 fed silkworms was significantly inhibited by RNAi mechanisms. Therefore, the results clearly indicated a significant decrease in the relative copy number of Gp41 gene in the dsRNA ie-1 fed silkworms compared to NPV

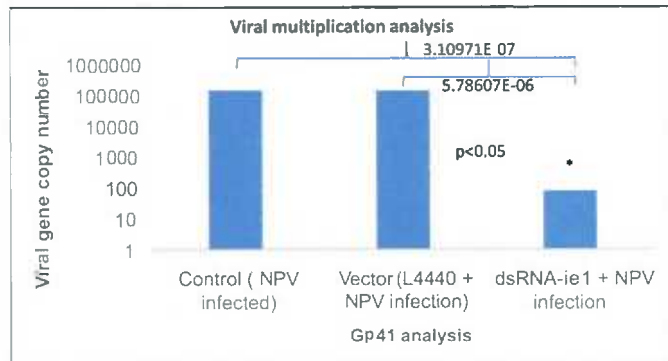


Figure 3: Genomic DNA analysis of the bioassay samples to validate the viral copy number using Gp41 primer

infected silkworms.

qPCR analysis of the viral genes associated with BmNPV infection in dsRNA fed silkworms

In order to determine the up-regulated expression of those genes that are activated by BmNPV infection and to understand the gene expression variations that are coupled with viral proliferation, we performed time

course analysis in the larval mid gut by real-time qPCR (Figure 4). We focused on the genes that were most likely expressed during viral multiplication in the NPV infected and dsRNA ie-1 treated, infected silkworms. Based on this consideration, we selected primers, ie-1, ie-2, lef-1, lef-3, p35 and gp64 to determine their transcript levels following dsRNA treatment in the infected *B. mori* strains. Total RNA was isolated at 24 hpi from the mid gut samples of the NPV infected and NPV+L4440 vector alone treated silkworm and NPV infected silkworms fed with ie-1(dsRNA ie-1) bacterial cells. Our Real time quantitative PCR (qPCR) analysis of mid gut tissues revealed differential expression of viral genes. The transcript levels of viral genes *i.e.*, ie-1, ie-2, lef-1, lef-3, p35 and Gp64 were significantly higher in the NPV infected and NPV+L4440 fed silkworms at 24 hpi. In the dsRNA ie-1 fed silkworms, the transcript levels of ie-1, ie-2, lef-1, lef-3, p35 and Gp64 significantly decreased at 24 hpi. Among the early viral genes, the expression levels of gene ie-1, attained maximum of 6.5 fold and ie-2, 7.5 fold and the late genes, lef-1, lef-3 p35 and Gp64 attained a maximum of 6-6.5 fold gene expression in the NPV infected and L4440+ NPV infected larval mid guts. In contrast, the dsRNA ie-1 fed, infected silkworms showed approximately 5-6 fold decrease in the gene expression, being a significant decrease in the transcript in dsRNA fed silkworms. We chose 24 hpi time point because according to studies conducted regarding BmNPV proliferation, the early phase genes, ie-1, and ie-2 could be detected as early as 2 h post infection whereas, the transcript levels of several genes associated with viral DNA replication and the lefs as well as structural genes *viz.*, Gp64 were detected by 6 hpi (Asha *et al.*, 2002), taking all the above factors into consideration, 24 hpi was taken as the time point to study the expression levels of viral genes.

Analysis of survival percentage of dsRNA fed NPV infected silkworms

To check the survival percentage of dsRNA fed BmNPV infected larvae, the number of dead larvae against the survived larvae was calculated for all the trays till all the

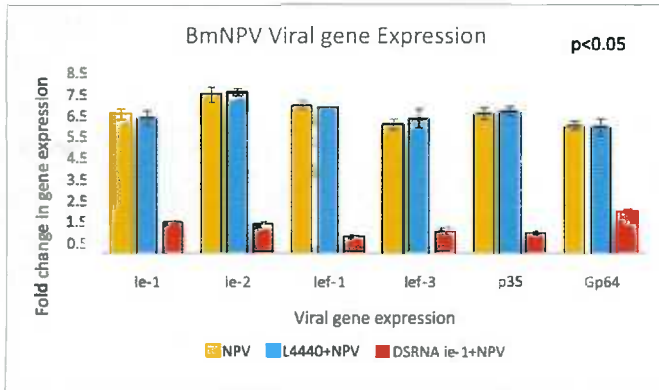


Figure 4: Fold change in gene expression of viral genes involved in NPV multiplication

larvae started spinning cocoons. The control larvae did not display symptoms of any infection and appeared healthy with 94.3 % survival. In larvae fed with BmNPV, 12.3 % and L4440 + NPV, only 25 % of the larvae looked healthy and all the other larvae showed the disease symptoms. The ie-1dsRNA fed BmNPV infected larvae showed 70 % survivability and the results obtained were significant ($p < 0.05$) (Figures 5, 6). The survivability may be attributed to the RNAi mechanism triggered by the siRNA in the mid gut tissue upon virus infection. The bioassay results indicated that the dsRNA fed to silkworms was effective against the virus and infected silkworms were able to resist and survive the infection.

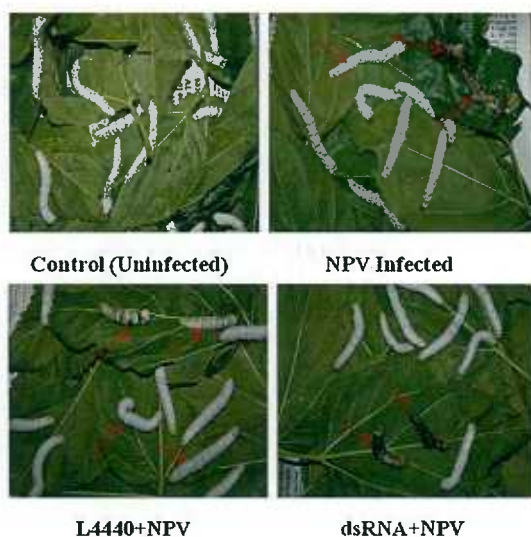


Figure 5: Bioassay to validate the effect of dsRNA lef-1 and dsRNA ie-1 in BmNPV infected silkworms

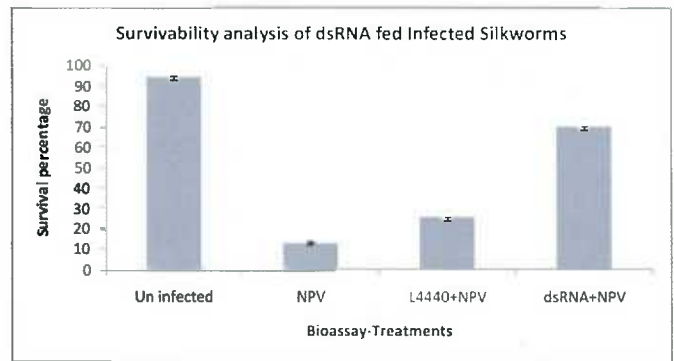


Figure 6: Graphical representation of bioassay results showing survivability of dsRNA lef-1 fed NPV infected silkworms in comparison with NPV infected silkworms

DISCUSSION

The BmNPV is a highly pathogenic virus, often causing severe loss to silk cocoon production of tropical countries. Its sturdy nature and the lack of effective control strategies have been persisting challenges in the field. RNAi is a recently developed technology used to inhibit BmNPV multiplication either by targeting the essential genes of BmNPV or by introducing an endogenous or exogenous antiviral gene into the silkworm genome to suppress virus multiplication. In the present study, we have demonstrated that dsRNA expressed in bacteria can be used effectively for viral gene silencing in *B. mori*. The study was aimed at a stable and efficient method to produce dsRNA to elicit RNA interference against BmNPV genes involved in viral infection in silkworm *B. mori*. The targeted gene was cloned in an *E. coli* vector L4440 having double T7 promoter flanking the gene of interest. The confirmed clones were transformed into *E. coli* host lacking RNase III activity and induced with IPTG for production of dsRNA. The production of dsRNA was confirmed by semi-quantitative PCR before feeding the bacterial cells to silkworms. The confirmed results indicated the efficient production of dsRNA against NPV ie-1 gene in bacteria. Our qPCR results revealed decrease in the relative copy number of Gp41 gene in the dsRNA fed silkworms, but the copy number of Gp41 gene was 6.6-7.0 fold in NPV infected silkworms, this shows that BmNPV invaded the mid gut tissues of treatment groups, but its

proliferation was greatly slowed down in the dsRNA ie-1 fed silkworm mid gut (Figure 1). This indicated that the antiviral mechanism that occurred in the *B. mori* was not due to resistance against BmNPV entry but rather to the inhibition of BmNPV proliferation by RNA interference mechanisms. Similar studies conducted in honey bees against antiviral defence mechanism showed that bees fed with Israeli acute paralysis virus (IAPV) and IAPV-specific dsRNA had reduced IAPV level as compared to bees fed with only virus (Laura and Michelle, 2015). RNAi is a phenomenon of selective mRNA destruction leading to gene knockdown (Fire *et al.*, 1998). RNAi has been demonstrated in many insect species including the silkworm, *B. mori* (Riu *et al.*, 2004).

The QPCR analysis of the viral genes showed decreased transcripts of immediate early genes ie-1 and ie-2 in the dsRNA fed silkworms. The highest gene expression levels of ie-1 and ie-2 genes in the NPV infected was 6.5 fold and 7.5 fold, respectively and in the dsRNA fed silkworms, the fold level decreased to 1.5 and 1.0 indicating 4 fold decrease in the gene expression of ie-1 and 6.0 fold decrease in ie-2 expression in dsRNA fed silkworms. Our study targeted the important gene involved in viral DNA replication *i.e.*, ie-1 (Immediate-early factor). ie-1 oligomerizes within the cytosol (Olson *et al.*, 2002) and gets involved with the viral DNA replication machinery, thus, ie-1 is an appropriate target for RNA interference (RNAi)-mediated gene silencing (Kanginakudru *et al.*, 2007; Subbaiah *et al.*, 2012). Therefore, targeting immediate early genes *viz.*, ie-1 by producing dsRNA against it is advantageous due to the fact that the early genes are transcribed prior to viral DNA replication and mostly, the immediate early (ie) genes are involved in regulation of viral replication. ie-1 has been shown to activate promoters of early genes including ie-2, 39K and p35 and is directly or indirectly involved in the expression of late genes (Guarino and Summers, 1986; Nissen and Friesen, 1989; Passarelli and Miller, 1993). Therefore, the results are clearly evident that the relative decrease in the expression of the viral genes especially lef-1, lef-3 and Gp64 mostly belong to late genes. Hence, the decrease in the expression levels may be attributed to

the time of expression of the early genes. Therefore, targeting the early genes is the key for the success of RNAi effect in the host silkworm, *B. mori*. This indicates that the construct developed for dsRNA against ie-1 is more effective in eliciting RNAi against virus. The dsRNA produced from L4440 vector alone without any cloned genes was used as control in all the experiments conducted to show that the vector alone did not have any contribution towards the RNA interference effect against the viral genes.

This study has shown that feeding bacterially expressed dsRNA led to a significant reduction in the transcript levels of viral genes especially early genes and restricted the viral multiplication in the host as compared to control. The study has shown that feeding 50 µl of ie-1 dsRNA (~50 µg dsRNA) at 5th instar to silkworms was effective in silencing the early as well as late gene expression during BmNPV infection in silkworm. Similarly, larvae fed with *E. coli* expressing ie-1 dsRNA showed a substantial reduction in the expression of targeted viral genes involved in the BmNPV multiplication and showed significant increase in the survivability of infected silkworms. The survived silkworms also spun cocoons indicating that the dsRNA ie-1 was able to control the virus multiplication if the silkworms are fed with the dsRNA at the early stage.

In summary, the antiviral mechanism that occurs in the dsRNA fed silkworms is not due to resistance against the BmNPV invasion but rather to the RNA interference mechanism triggered in the larval mid gut after feeding dsRNA. The defense processes against BmNPV infection that occur in the larvae might be regulated via interactions involving multiple genes. Other similar studies in honey bee have shown that in addition to inducing RNAi, dsRNA is also engaged in a previously uncharacterized non-sequence-specific immune pathway *i.e.*, the involvement of innate immune pathways (*i.e.*, Jak-Stat, Toll, and Imd) and non-sequence-specific dsRNA-mediated immune responses. Since microorganisms infecting honey bees are common to *B. mori* also, the involvement of innate immune system may also be activated by dsRNA in

response to viral infection (Laura and Michelle, 2015). The pathways involved in these interactions are currently unclear. Therefore, the study showed that dsRNA fed silkworms increased the tolerance level to the virus infection by producing siRNA specific to viral mRNA and initiate gene silencing which is a characteristic feature of RNA interference. Therefore, feeding dsRNA at a regular interval of 24 hours enhances the efficacy of RNAi in the mid gut of *B. mori*. Further, it was observed that feeding dsRNA is better in terms of specificity and reduced chances of secondary siRNA production in the target organism, which may lead to off-target effects. Oral delivery of dsRNA has several advantages over other methods. It is less labour intensive, cost effective and can easily be performed. Oral delivery of dsRNA is a high-throughput method for RNAi delivery in insects intolerant to injection and for large scale field applications for RNAi-mediated pest control. Hence, bacteria expressing the desired dsRNA(s) against viral gene(s) in insects may be effectively used for combating viral infections in silkworm, *B. mori*. Our data provide a global view of effect of feeding bacteria produced dsRNA against viral genes in *B. mori* and its effect on viral multiplication and insights for further investigations on the complex interactions involved in the RNA interference against viral genes in silkworm, *B. mori*.

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Article scientifique

CIBLAGE PAR ARN INTERFERENCE DU GENE IMMEDIATE EARLY (IE-1) POUR INDUIRE LA RESISTANCE VIRALE CONTRE LA GRASSERIE CAUSEE PAR BMNPV

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RESUME

Le virus de la polyèdrose nucléaire (BmNPV) de *Bombyx mori* L. (Lepidoptera: Bombycidae) est très pathogène et cause l'infection de la grasserie chez le ver à soie, et son contrôle a été un défi en raison de sa nature et de l'absence de stratégie. La technologie d'inhibition par ARN (RNAi) est un outil de choix pour induire la résistance à un virus chez de nombreux organismes. Un fait significatif de cette technologie est la présence d'ARN double brin (dsARN). Dans cette étude, *E. coli* a été modifiée pour produire du dsARN contre ie-1 (gene immediate early de BmNPV) à partir d'un plasmide (L4440) contenant le gène d'intérêt sous le contrôle du promoteur T7 qui produit du dsARN lorsque il est intégré chez la souche HT115 de *E. coli* sous induction avec IPTG. Notre étude cible ie-1 impliqué dans la multiplication virale. L'analyse montre qu'une larve nourrie avec 50µg de dsARN ie-1 ne contient qu'un petit nombre de copies de Gp41 (100 copies) alors qu'il y a 1x10⁵ copies de Gp41 chez les vers à soie infectés. L'analyse par qPCR des gènes viraux montre une décroissance de 5-6 fois de l'expression des gènes viraux dans l'intestin moyen des vers à soie nourris avec le dsARN comparée à celle des larves infectées par BmNPV et une survie supérieure de 50%. Ceci montre que l'expression de dsARN chez *E. coli* est un outil efficace pour le contrôle des maladies chez les insectes.

Mots clés: Essai biologique, BmNPV, ARN double brin, ARNi oral, interférence par ARN, nombre de copies virales.



IMPACT OF PRE-STEAMING TECHNIQUE ON IMPROVING THE QUALITY OF BIVOLTINE RAW SILK

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ABSTRACT

Silk twisting activity is carried out either separately or as multiend reeling cum twisting enterprise. The winding performance of Indian raw silk is often affected by the prevalent conditions in the twisting factory. Twisters always find it difficult to adopt further suitable methodology to improve the winding performance, owing to the additional cost. With this backdrop, Central Silk Technological Research Institute, Bengaluru, India has developed a suitable cost effective technology, which can be adopted utilizing the existing facilities available with twisting units. The field trials conducted in silk twisting factory revealed significant improvement in the winding performance and elongation of bivoltine raw silk subjected to saturated steam inside the steaming cabinet for 15 minutes duration in the case of skeins weighing below 100 g, and 20 minutes duration for skeins weighing more than 100 g. SEM studies indicated that pre-steaming process of bivoltine raw silk has smoothened the silk filaments and caused the swelling of surface adhering sericin which in turn reduced gum spots. Hence, the pre-steamed, well hydrated bivoltine raw silk with reduced gum spots has improved winding performance with fewer breaks. The cost benefit analysis of pre-steaming technique has established that pre-steaming significantly improved the potential revenue of the twisting unit.

Key words: Bivoltine raw silk, pre-steaming conditions, SEM studies, steaming cabinet, winding performance.

INTRODUCTION

The silk reeling and twisting sectors in India are basically tiny and small scale industries that require lot of assistance for development. The silk reeling and silk twisting units sometimes work in tandem but mostly have been working separately, so far. Central Silk Technological Research Institute (CSTRI), Bengaluru, India, after thorough studies and analysis, formulated modules to be incorporated in twisting units along with multiend silk reeling units in order to improve the profitability of the sector (Nagaraj *et al.*, 2013). While this has widely been accepted by the silk reeling units, improving the performance of the twisting units has also become the prime criterion. The studies conducted by CSTRI reveal that quality of raw silk produced by multiend reeling units, by and large are of superior grade

and much better than that produced in cottage basin silk reeling units (Hariraj *et al.*, 1992 b; Subhas Naik *et al.*, 2002).

Silk being a hygroscopic material, is affected by the temperature and humidity maintained in the twisting unit. Since the twisting units are of small-scale sector, it is difficult to maintain the temperature and humidity in the twisting shed. This creates major problem while winding the raw silk skeins and results in more number of winding breaks. The soaking process, which can be used, is costly and not readily acceptable by the small-scale twisters. In view of this, it was thought that the pre-steaming process can be used as an alternative method to impart bulkiness in raw silk, which in turn assists in improving the winding performance of raw silk as pre-steaming was tried in the case of cocoons (Hariraj *et al.*, 1992 a).

CSTRI has standardized the pre-steaming techniques for bivoltine raw silk in terms of temperature to be used, duration of steaming *etc.*, based on the feedback on winding performance of raw silk. The present study comprised of field trials conducted at twisting unit, Ramanagaram following the standardized conditions, for assessing the quality of pre-steamed bivoltine raw silk vis-à-vis raw silk without pre-steaming. For the purpose, a steaming cabinet was fabricated which can handle 10 kg of raw silk per batch for pre-steaming. Also, the surface modification of the pre-steamed bivoltine raw silk was characterized using scanning electron microscope. The economics related to pre-steaming processes was also worked out for popularizing the process among the twistors (Hariraj *et al.*, 2017).

MATERIALS AND METHODS

Raw material: Bivoltine raw silk of 20~22 denier, single ply produced in the multiend reeling unit with standard process parameters was used for the study.

Pre-steaming procedure: The raw silk samples were assembled on parallelly set rods and about 10 kg of raw silk could be accommodated on 10 ~ 12 rods. The rods holding silk were kept within the steaming cabinet, covered with a gunny cloth and closed with a lid. Steaming operation was done by heating the water inside the cabinet. The raw silk was steamed for about 15 minutes and later removed from the cabinet and stored for converting skeins on to bobbins in winding machine. The silk skeins were immediately wound on winding machine. The procedural steps are illustrated in Figures 1, 2, 3 & 4.

Field trials were conducted in reeling cum twisting unit, of one of the entrepreneurs in Chennanahalli, Ramanagaram. The raw silk produced in the silk filature was subjected to pre-steaming followed by an immediate winding process in the twisting unit of the entrepreneur. Simultaneously, the raw silk skeins reeled in the filature were also wound on the winding machine without pre-steaming and the winding breaks occurred during the process was noted.

Testing of raw silk: The tensile characteristics of raw silk skeins wound on to the bobbins with and without pre-steaming were studied. The pre-steamed and untreated raw silk skeins were tested for tenacity, elongation and cohesion characteristics following the standard testing procedures of IS 15090 Part1:2002 (Book of standards, 1968). The data thus obtained were analyzed statistically using SPSS package.



Figure 1: Skeins are arranged for pre-steaming



Figure 2: Skeins are placed in the pre-steaming cabinet



Figure 3: Pre-steaming of raw silk under progress

The results of mass scale pre-steaming of bivoltine raw silk are provided in Table 1 and data of statistical analysis are presented in Table 2.

SEM studies: The bivoltine raw silk samples (with and without pre-steaming) were tested in JOEL 100 X II ASID 4D S scanning electron microscope under 800 magnification. The sample for SEM were fixed for 2 hours in glutaraldehyde in 0.2 M cacodylate buffer (pH 7.2), dehydrated in graded ethanol-acetone series and dried in critical point using CO₂ as transition fluid. After drying, the samples were mounted on to copper stubs, coated with gold and examined using scanning electron microscope at 20 kv. The results are depicted in Figure 5.



Figure 4: Pre-steamed bivoltine raw silk ready for winding

Table 1: Field data on quality parameters of bivoltine raw silk, with and without pre-steaming

Particulars	Group	No. of samples	Mean	Standard deviation	Standard error mean
Winding breaks / 10 skeins / hour	Not pre-steamed	5	9.20	1.095	0.490
	Pre-steamed	5	2.40	1.673	0.748
Tenacity (g/d)	Not pre-steamed	5	3.64	0.063	0.028
	Pre-steamed	5	3.69	0.076	0.034
Elongation (%)	Not pre-steamed	5	19.80	1.643	0.735
	Pre-steamed	5	22.80	1.304	0.583

Cost benefit analysis: Studies were conducted in the twisting unit continuously for assessing the cost benefit analysis. Considering the various costs involved in the pre-steaming activities and the benefits obtained by pre-steaming the cocoons, the cost benefit ratio was worked out. Based on the studies, the potential revenue obtained

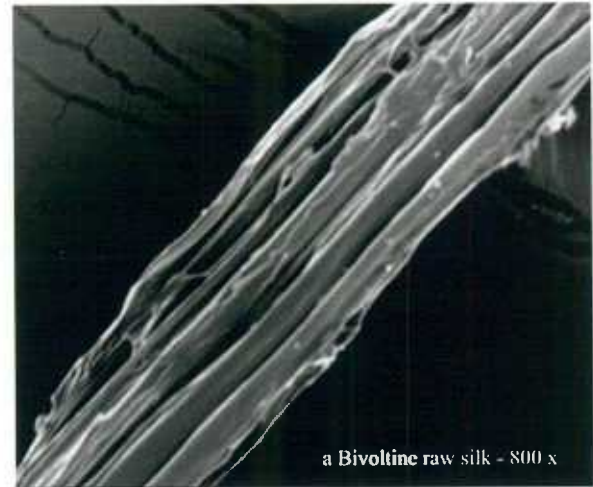


Figure 5: SEM images of bivoltine raw silk, without steaming (a) and after pre-steaming (b)

for a small scale twisting unit which can twist raw silk reeled from 10 basin multiend reeling unit was also worked out and presented in Table 4.

RESULTS AND DISCUSSION

Pre-steaming of bivoltine raw silk

The field trial performance of pre-steamed and subsequently wound bivoltine raw silk indicated significant improvement in winding performance compared to silk wound without pre-steaming. It could also be observed that elongation characteristics of pre-steamed bivoltine silk were significantly superior to that of silk that has not undergone pre-steaming (Table 2).

From Table 3, it could be observed that both winding performance and elongation of raw silk were significantly improved by pre-steaming technique; at 1 % level in the case of winding breaks and at 5 % level, in the case of elongation percentage. The results indicate that the quality of bivoltine raw silk was significantly improved apart from increasing labour productivity and the silk also fetched better price.

Scanning electron microscopic studies of pre-steamed bivoltine raw silk

The SEM images were taken at 800 magnifications for both pre-steamed and non-steamed bivoltine and multibivoltine raw silk. From the SEM images shown in Figure 5, it was found that pre-steaming process of raw silk has smoothened the silk filaments, surface adhering sericin was swollen due to steaming and might have reduced gum spots. Hence, the pre-steamed, well hydrated silk with reduced gum spots has improved winding performance with fewer breaks.

Cost benefit analysis of pre-steamed bivoltine raw silk

The cost benefit analysis of pre-steaming technique has

Table 2: Statistical analysis of field data on quality parameters of bivoltine raw silk, with and without pre-steaming

Particulars	t-value	Degree of freedom	Significance (2-tailed)	Mean Difference	Std. Error Difference
Winding breaks / 10 skeins / hour	7.603	8	0.000**	6.800	0.894
Tenacity (g/d)	-1.089	8	0.308 ^{NS}	-0.048	0.044
Elongation (%)	-3.198	8	0.013*	-3.000	0.938

** - Significant at 1 % level; * - Significant at 5 % level; NS - Not significant at 1 % and 5 % levels.

Table 3: Cost benefit analysis of pre-steaming technique

Particulars	Amount in ₹.
COST	
Cost of pre steaming per kg (Labour + Electricity)	30.00
BENEFIT	
Increase in labour productivity (~3 %)	12.00
Increase in raw silk weight (~1 %)	35.00
TOTAL	47.00
Benefit cost ratio (BCR)	1.57
Potential incremental revenue per day	238.00

established that pre-steaming does not require any additional equipment if the multiend reeling works with twisting unit as steaming chamber is provided along with twisting machinery package and improved the potential revenue of the multiend reeling unit. However, in the case of pre-steaming of large quantities of silk skeins at a time, a cabinet can be used to handle about 10 kg of raw silk at a time.

The economics worked out for the technique as presented in Table 4, reveals that the benefit of pre-steaming technique is significantly more than cost involved in the operation. The potential revenue obtained for a small scale twisting unit which can twist raw silk reeled from 10 basin multiend reeling unit is ₹238/ day, which would significantly benefit the twister. Further, the twister is benefited with improved quality of twisted raw silk without any chemical used in the process and better marketability of the product.

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Article scientifique

IMPACT DE LA PREVAPORISATION SUR L'AMELIORATION DE LA QUALITE DE LA SOIE GREGE BIVOLTINE

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RESUME

Le moulinage est effectué soit séparément soit dans une filature multibouts équipée de moulinage. La performance de la soie grège indienne bivoltine est souvent affectée par les conditions existantes dans les moulinages. Les mouliniers trouvent souvent difficile d'adopter des méthodologies appropriées pour améliorer le flottage en raison du coût additionnel. Le Central Silk Technology Research Institute à Bengaluru en Inde a développé une technologie appropriée à coût raisonnable qui peut être adoptée avec les possibilités existantes dans les moulinages. Les essais conduits dans les moulinages révèlent qu'une amélioration significative est obtenue dans les performances de flottage et d'élongation de la soie grège bivoltine saturée en vapeur pendant 15 minutes avec des flottes pesant moins de 100 g et pendant 20 minutes pour les flottes pesant plus de 100 g. Des études de microscopie électronique à balayage indiquent que la prévaporisation assouplit les filaments de soie, la sérécine en surface étant gonflée ce qui réduit les bourrons de grès et hydrate la soie grège bivoltine ce qui améliore la performance de flottage avec moins de ruptures. L'analyse du coût établit que la vaporisation améliore significativement le revenu potentiel du moulinage.

Mots clés: Soie grège bivoltine, conditions de pré-vaporisation, microscopie électronique à balayage, étuve, flottage.

ISC hosted the APSERI-2019 at Mysore, India



ISC hosted the prestigious 6th Asia Pacific Congress on Sericulture and Insect Biotechnology (APSERI-2019) at Mysore, India during 2nd to 4th March 2019. The programme was organized with the support of Central Silk Board, Ministry of Textiles, Govt. of India. The Congress has been conducted as one of the most spectacular events organized by ISC so far. There were all-round appreciation to ISC for organizing the Congress in a splendid manner. The following are some of the highlights and takeaways of the Congress:

#	Particulars	Number
1	Total delegates participated	246
2	Countries represented	19
3	Research papers presented	166
	Oral	72
	Poster	94
4	Collaborative initiatives	5
5	Countries shown interest to join ISC (Azerbaijan, Uganda, Paraguay)	3

As a side-line event of the Congress, an international exhibition on silk products was held from 2nd to 6th March 2019 in collaboration with Silk Mark Organization of India, Central Silk Board, Bangalore. On 4th March 2019, the Congress delegates were taken to farmers' fields for experiencing the successful model of Indian sericulture practice. They also visited the premier R&D Institute on sericulture (CSRTI) at Mysore, and the historic places in and around Mysore.





Executive Committee of ISC held at Bukhara, Uzbekistan

The Executive Committee of the ISC was held at Bukhara, Uzbekistan during 5th and 6th April 2019. The Meeting was chaired by the Hon'ble Minister of Silk Industry of Uzbekistan, Mr. Sharipov Bakhrom Kurbanovich. Fifteen delegates from 7 countries attended the Meeting. The Executive Committee discussed and finalized the activities for the year 2019, approved the budget, reviewed the arrangements for ISC Congress at Japan during November 2019 and approved the notification of Louis Pasteur Award and Excellence in Sericulture Science (ESS) Award among the Member Countries and Associate Members. The delegates were taken for field visit in Bukhara and Samarkand as part of the EC Meeting.





ISC delegation attended 9th BACSA Conference

Based on the invitation of Black Caspian Sea and Central Asia Sericulture Association (BACSA), an ISC delegation comprising Mr. R. R. Okhandiar, Secretary General and Mr. Dileep Kumar R., Programme Coordinator attended the 9th International Congress of Black Caspian Sea and Central Asia Sericulture Association (BACSA) held at Batumi, Georgia during 8th and 9th April 2019. The Secretary General presented a report on the success story of Indian Silk Industry that can be replicated in BACSA member countries. The Programme Coordinator presented a paper on the "Role of ISC for the development of sericulture and silk industry across the globe" with the aim of enrolling BACSA member countries as member countries of ISC. ISC has already received positive responses from Azerbaijan, Tajikistan and Georgia to enrol as Member Countries of ISC.



Global Silk Production (Metric Tonnes)

#	Country	2014	2015	2016	2017	2018
1	Bangladesh	44.5	44	44	41	41
2	Brazil	560	600	650	600	650
3	Bulgaria	8	8	9	10	10
4	China	1,46,000	1,70,000	1,58,400	1,42,000	1,20,000
5	Colombia	0.5	0.5	-	-	-
6	Egypt	0.8	0.8	1.2	1.1	1.25
7	India	28,708	28,523	30,348	31,906	35,261
8	Indonesia	10	8	4	2.5	2.5
9	Iran	110	120	125	120	110
10	Japan	30	30	32	20	20
11	North Korea	320	350	365	365	350
12	South Korea	1.2	1	1	1	1
13	Philippines	1.1	1.2	1.82	1.5	2
14	Syria	0.5	0.3	0.25	0.25	0.25
15	Thailand	692	698	712	680	680
16	Tunisia	4	3	2	2	2
17	Turkey	32	30	32	30	30
18	Uzbekistan	1,100	1,200	1,256	1,200	1,800
19	Vietnam	420	450	523	520	680
20	Madagascar	15	5	6	7	7
	Total	178057.60	202072.80	192512.27	177507.35	159648.00

COMMISSION SERICOLE INTERNATIONALE

An inter-governmental organization instituted in 1960

No. ISC/2/10/2016

25.04.2019

ANNOUNCEMENT

The International Sericultural Commission (ISC), Bangalore, India, an inter-governmental organization engaged in the development of sericulture and silk industry, has been conferring the prestigious Louis Pasteur Award on persons who have made outstanding contributions to the development of silk industry in their country or across the world. The award is given away once in three years for three persons selected from across the globe. The Louis Pasteur Award consists of a Citation and a Medal. The Award for 2019 shall be presented during the 25th ISC Congress on Sericulture and Silk Industry scheduled to be held at Tsukuba, Japan during 19-22 November 2019.

International Sericultural Commission hereby invite nominations for the Louis Pasteur Award-2019 from the ISC Member Countries (Afghanistan, Bangladesh, Brazil, DPR Korea, Egypt, France, Ghana, Greece, India, Iran, Indonesia, Japan, Kenya, Madagascar, Nepal, Romania, Syria, Thailand, Tunisia, Uzbekistan). The nominated candidates should have involved with the development of sericulture industry in their country or across the world. Each Member Country can nominate up-to two candidates for the Award. The candidates may be identified by an Expert Committee among the applications received from interested candidates through open advertisement. The nominations of each candidate should be submitted along with the following documents:-

- (a) Duly filled-in "Application Form for Louis Pasteur Award". The Application Form is attached.
- (b) Brief self-appraisal report, not exceeding five pages, chronologically listing the applicant's contributions to the development of sericulture industry.
- (c) A one page write-up indicating why they consider themselves fit for consideration to the Award.

The nominations along with all relevant details from the Member Countries should be sent to the Secretary General, International Sericultural Commission, Bangalore, India, by 10th August 2019, through E-mail: iscbangalore@inserco.in.

The nominations received after the due date shall **NOT** be considered.



25.4.19
R.R.OKHANDIAR
SECRETARY GENERAL



COMMISSION SERICOLE INTERNATIONALE

An inter-governmental organization instituted in 1960

No. ISC/1/30/ESS/2019

01.05.2019

ANNOUNCEMENT

The International Sericultural Commission (ISC), Bangalore, India, an inter-governmental organization engaged in the global development of sericulture and silk industry, has decided to introduce a new International Award namely "Excellence in Sericulture Science(ESS)" in the following 4 categories of sericulture science:

#	Category	Areas covered
1	Silkworm Host Plant Development	Host plant development for mulberry, tasar, eri and muga sericulture – agronomy, breeding, pest and disease management, molecular biology, cultivation, tissue culture, machinery development, etc.
2	Silkworm Development	Silkworm breeding, silkworm protection, physiology, silkworm rearing practices, seed production, extension, machinery development, etc.
3	Post cocoon and Post yarn Technology	All activities in post cocoon and post yarn development, machinery development, extension, fashion industry, marketing, promotion, etc.
4	Silkworm and Silk in Non-Textile Industry	Use of silkworm and silk in frontier areas of research including biotechnology, biomaterial, pharmaceutical, by-product utilisation, etc.

The award shall be given to scientists, professionals and other stakeholders associated with the activities related to silkworm host plant and all silkworms and all silks including mulberry, non-mulberry, spiders, bees, hornets, and the other silk insects. Any specific invention, research, action or any other efforts of the persons/group resulted/which can result significant impact to the specified areas of silk and related industries shall be considered for the award. The achievement must have field applicability and contribute to the development of silk industry in any manner. Louis Pasteur Awardees are excluded from applying for this award. A person/group can apply for any one categories of the award.

The Award shall be presented during the 25th ISC Congress scheduled at Tsukuba, Japan during 19th to 22nd November 2019. The ESS Award consists of a Citation and a Medal. Candidates from 20 Member Countries and Associate Members are eligible to apply for the award. The applications shall be evaluated by the International Award Committee of ISC comprising eminent scientists drawn from 5 countries. One of the Award Committee Member shall be a Louis Pasteur Awardee and function as the Chairperson of the Committee. The Committee shall evaluate the contributions of the

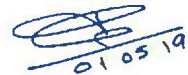


applicant purely based on the industry impact. One Awardee from each of the above four categories would be selected for the year 2019 among the nominations received from ISC Member Countries and Associate Members.

Accordingly, International Sericultural Commission hereby invite nominations for the **ESS Award-2019** from the ISC Member Countries and Associate Members. Each Member Country can nominate up-to two candidates each for the above 4 categories of the Award (total 8 nominations) and Associate Members can nominate one candidate each for the 4 categories of the Award (total 4 nominations). The candidates may be identified by an Expert Committee among the applications received from interested candidates through open advertisement. The nominations of each candidate should be submitted along with the duly filled-in Application Form for "**Excellence in Sericulture Science (ESS)**" Award 2019. The Application Form is attached.

The nominations along with all relevant details from the Member Countries and Associate Members should be submitted to the Secretary General, International Sericultural Commission, Bangalore, India, by **10th August 2019**, through E-mail: iscbangalore@inserco.in.

The nominations received after the due date shall **NOT** be considered.



R.R.OKHANDIAR
SECRETARY GENERAL

- 1) National Delegates of 20 Member Countries
- 2) Associate Members



INTERNATIONAL

SERICULTURAL

COMMISSION

UNITED NATIONS REG. NO. 10418

INTERNATIONAL SERICULTURAL COMMISSION

An intergovernmental organization instituted in 1960 and registered with United Nations

APPLICATION FOR ASSOCIATE MEMBERSHIP

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

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

New Application

☐

Renewal

☐

Rates : Personal Associate Members
Collective Associate Members

: US\$ 175 per year
: US\$ 470 per year

Applicant's signature :

Date :

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

Beneficiary : International Sericultural Commission

Account No. **3188283389**

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

Bank Code IFSC – CBIN0283975, SWIFT – CBININBBMRB

For overseas transfer

(THROUGH SCBL US 33, STANDARD CHARTERED BANK, NEW YORK, USA TO A/C NO.3582-050570-001

OF CENTRAL BANK OF INDIA, OVERSEAS BRANCH, MUMBAI, SWIFT : CBININBBOSB)

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

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INFORMATION TO CONTRIBUTORS

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

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

The typescript should contain the following features:

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

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

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

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

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

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