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Research Paper

IMPACT OF HYDROGEL WITH DRIP IRRIGATION ON MULBERRY PRODUCTION IN ODISHA

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ABSTRACT

Mulberry in Odisha is generally a rainfed crop with close spacing. Water scarcity is considered as one of the major constraints for optimum mulberry productivity in the state. Present study was aimed to evaluate the effectiveness of hydrogel under different levels of drip irrigation in mulberry cultivation at Regional Sericultural Research Station of Central Silk Board, Koraput for two years (2017-2019) with four different crop seasons. Among all the treatments, alternate day irrigation with hydrogel (AIH) proved to be economical and produced effective leaf yield (4.78 t/ha/crop or 23.92 t/ha/yr) with BC ratio of 1.37, which is statistically at par with that of daily irrigation without (5.13 t/ha/crop) and with hydrogel (4.88 t/ha/crop). Further, AIH has recorded maximum water use efficiency (25 kg/ha/mm) as compared to rest of the treatments.

Key words: Drum kit drip, growth and yield, hydrogel, mulberry, water use efficiency.

INTRODUCTION

Mulberry (*Morus alba* L.) is a perennial, deep rooted, fast growing and high biomass producing plant used for silkworm (*Bombyx mori* L.) rearing and production of silk. The plant is known to respond extremely well to various inputs, particularly to the availability of optimum soil moisture and nutrients. Cultivation of mulberry for cocoon production in Odisha is practiced mostly under limited agronomic inputs. Insufficient water resources, erratic monsoon and long dry spell are some of the major limiting factors for the progress of mulberry sericulture in the state. Further, mulberry is being raised by the resource starved farmers with marginal lands having shallow depth, low water holding capacity and organic matter with poor native fertility. Even though, the state is blessed with sufficient cultivable land, sericulture farmers are facing tremendous challenges for expansion and maintenance of the existing mulberry area and its productivity. Water scarcity and its implications in agriculture as a whole and sericulture in particular has become very prominent under the era of climate change as maximum amount of water is used by agriculture sector (85 %) as compared to industry (10 %) and domestic sector (5 %). Under such situation, use of hydrogel to enhance water use efficiency for better productivity of mulberry can prove a suitable option for the sericulture farmers of the region.

Hydrophilic gels popularly called as hydrogels or super absorbent polymers are categorized into different groups, such as naturally occurring, semi synthetic or synthetic. Most of these polymers on an average can retain 332-465 times water to its weight and release it slowly during drought under light soil (Dehkordi, 2016). Due to its hydrophilic nature, hydrogels are subjected to swelling on coming in contact with water and release almost 95 per cent of stored water available for crop absorption (Johnson and Veltkamp, 1985). The process of retaining water and releasing the same by super absorbent gels may last for 2-5 years depending on the soil environment and cultivation

process but, ultimately in due course of time, it breaks down into CO_2, water, ammonia and potassium ions without any residue, thus, making it environment friendly (Trenkel, 1997). Hydrogels also act as soil ameliorant or conditioner by improving porosity, bulk density, soil permeability, compaction, infiltration rate *etc*. Even though the average annual rainfall of the region is around 1500 mm, steep topography, light soil texture, heavy runoff and high infiltration rate reduce the soil moisture content. Therefore, water becomes a major limiting factor for mulberry production in this region. Hence, in the current study, we tried to evaluate the impact of hydrogel under drum kit drip irrigation system on growth and yield of mulberry along with its water use efficiency.

MATERIALS AND METHODS

Climatology and soils

A field experiment was conducted for a span of two years (2017-2019) with four crop seasons at the Regional Sericultural Research Station (RSRS), Landiguda, Koraput, Odisha. The farm is situated at an altitude of 870 m above mean sea level, between 18.77º north and 82.74º east longitudes that comes under agro climatic zones of Eastern Ghat Highland in Odisha. The experimental site receives an annual average rainfall of 1500 mm with the long rains falling from July to August. Summer season begins in March and continues up to the second fortnight of June. Mean maximum and minimum temperature were recorded 34.1 and 9.5 ºC, respectively. The soils of the experimental site are red, low in available nitrogen (187 kg/ha), phosphorus (3.77 kg/ha) and high in available potash (63.45 kg/ha) with 0.31 % organic carbon content and moderately acidic pH (5.01) in reaction.

Treatment and experimental layout

The experimental plot was divided under facility of drum kit drip irrigation and laid out under randomized complete block design (RCBD) with four replications and six treatments *viz.,* Daily irrigation with hydrogel (DIH), Daily irrigation without hydrogel (DI), Alternate day irrigation with hydrogel (AIH), Alternate day irrigation without hydrogel (AI), No irrigation with hydrogel (NIH) and No irrigation without hydrogel (NI) as control. The quantum of water and hydrogel applied per plant was two litres and 3g respectively. Total number of plots was 24 having size of 4.5 x 4.5 sq. m with 90 x 90 cm spacing of S1635 mulberry variety established.

Maintenance of plantation

Saplings of S1635 were transplanted during August-September 2017 as per research design and left for establishment. During June 2018, trials were initiated and hydrogel treatments were imposed @ 3 g/plant along with Drum Kit Drip irrigation system. The mulberry plantation with a gross plot area of 527.31 sq. m (L x B of 27.9 m x 18.9 m) was used for the experimental purpose. The crop was maintained as per standard mulberry cultivation package of practices. Nitrogen, Phosphorous and Potassium were applied @150:50:50 kg/ha/yr. Plant protection measures particularly for powdery mildew disease were adopted by spraying 0.1 % of Carbendazim.

Bioassay

Bioassay with silkworm hybrid, Bcon1 × Bcon4 was conducted to test the nutritional efficacy of leaves from RCBD experiment as reflected on rearing performance of silkworm. During October-November 2019, rearing of silkworm was conducted with 3 replications each consisting of 300 larvae for each treatment by following the standard rearing practices (Krishnaswami, 1978).

Data recording

Collection of data was made by selecting five plants randomly from the centre of each plot, 60-65 days after pruning. Different yield and yield attributing characters of mulberry plant, namely number of primary branches, total shoot length, plant height, leaf yield and moisture content of leaves were recorded. Water use efficiency was computed from ratio of leaf yield (kg/ha) and total water used (mm) by the crop. Further, total water was calculated by adding amount of water applied and effective rainfall. Economics of different treatments of experiment was also worked out using partial budgeting technique (Manjunatha *et al*., 2018) to estimate cost effectiveness

and field utility of the outcome of the study.

Data analysis

Data recorded on yield and quality parameters (mulberry and silkworm) were subjected to analysis of variance (ANOVA) and comparisons were made among means of the treatments using least significant difference (lsd) values at $P \leq 0.05$.

RESULTS

Two years' pooled data pertaining to yield and plant growth attributes of mulberry under drip irrigation of hydrogel treatments (with or without) are presented in Table 1. Among the treatments, Daily irrigation [DI] recorded maximum leaf yield (5.13 t/ha/crop) followed by Daily Irrigation + Hydrogel [DIH] (4.88 t/ha/crop), Alternate day irrigation + hydrogel [AIH] (4.78 t/ha/crop), Alternate day irrigation [AI] (4.25 t/ha/crop), No irrigation + hydrogel [NIH] (3.44 t/ha/crop) and No irrigation [NI] (3.20 t/ha/crop). The mean plant height (172 cm) was significantly higher in AIH treatment compared to all other treatments except DIH (169.29 cm). Similarly, in the case of average number of primary branches, maximum was for AIH (7.29) followed by DI (6.79), AI (6.77), DIH (6.72), NI (6.39) and NIH (6.22). The average total shoot length was also the highest in AIH treatment (1056.99 cm) whereas, the average moisture content was maximum in DI treatment (81.04 %) followed by DIH (80.69 %), AIH (80.19 %), AI (80.18 %), NI (78.86 %) and NIH (78.73 %). Quantitatively, all the treatments across various parameters were found significantly different from each other statistically. It reveals AIH treatment an appropriate one across various growth and yield parameters of mulberry.

Water use efficiency (WUE) across various treatments on mulberry is presented in Table 2. The maximum water use efficiency was found in AIH (127.27 %) followed by DI, AI, DIH and NIH. AIH increases leaf yield of 12.46 % as compared to AI and further it consumed ~15 % less water when compared to daily irrigation (DI).

Table 1: Different irrigation and hydrogel treatments versus mulberry growth and yield

Treatment	Leaf yield t/ha/crop	No.of primary branches	Plant height (cm)	Total shoot length (cm)	Moisture (%)
AI	4.25	6.77	157.02	875.61	80.18
AIH	4.78	7.29	172.00	1056.99	80.19
DI	5.13	6.79	160.43	913.61	81.04
DIH	4.88	6.72	169.29	949.93	80.69
NI	3.20	6.39	141.93	711.71	78.86
NIH	3.44	6.22	157.61	777.49	78.73
SEm ±	0.13	0.22	3.20	32.41	0.31
lsd (P<0.05)	0.38	0.64	9.53	96.32	0.94
CV %	6.05	6.48	4.01	7.36	0.79

AI: Alternate day irrigation; DI: Daily irrigation; NI: No irrigation; H: Hydrogel.

Table 2: Water use efficiency of mulberry under different hydrogel treatments

Treatment	Total water applied (mm)	Leaf yield t/ha/yr	WUE kg/ha/mm	% improvement over control
AI	936	21.27	22	100.00
AIH	936	23.92	25	127.27
DI	1109	25.65	23	109.09
DIH	1109	24.41	22	100.00
NI	840	9.60	11	-
NIH	840	10.32	12	9.09

Bioassay with silkworm hybrid, BCon1 × BCon4 was conducted and the results are presented in Table 3. It revealed that no significant difference was observed across the treatments of the study and silkworm economic parameters. However, effective rate of rearing (ERR) by number (8667) and shell % (17.6) was maximum with the leaves fed from the treatment AIH.

Table 3: Bioassay results with silkworm hybrid, BCon1 x BCon4

Treatment	Weight of 10 larvae (g)	ERR		Cocoon weight (g)	Shell weight (g)	Shell %
		Number	Weight (kg)			
AI	38.33	8053	15.84	1.854	0.310	16.81
AIH	38.55	8667	16.08	1.827	0.321	17.68
DI	39.85	8213	16.32	2.030	0.330	16.24
DIH	38.94	7893	15.60	1.880	0.314	16.72
NI	37.44	8507	16.80	1.765	0.297	16.98
NIH	37.44	8267	16.08	1.883	0.325	17.27
SEm ±	1.53	259	0.62	0.078	0.011	0.85
lsd (P<0.05)	Non-significant					

Economics of mulberry production under different periods of drip irrigation with / without hydrogel was computed using budgeting technique (Table 4). The total cost involved was maximum in DIH (95591/-) followed by DI, AIH, AI and minimum in NI (43572/-). However, leaf yield from DIH (24.41 t/ha/yr) was at par with that of treatment AIH (23.92 t/ha/yr). Whereas, the return per rupee of investment of individual treatment was found high in DI (1.43:1) followed by AIH (1.37:1), AI (1.35:1) and the least in NIH (1.02:1). Moreover, return in AIH is just 4 % less of DI due to additional cost of hydrogel. Hence, the alternate day irrigation clearly proves an economical and effective way of leaf production system in the study, because of cost involved per unit area production from AI is less with more effective return as compared to other treatments.

Table 4: Economics of mulberry cultivation under different hydrogel treatments

Treatment	Cost of cultivation (/ha)	Leaf yield t/ha/yr	Gross return (/ha)	BC ratio
AI	78566.5	21.27	106250	1.35:1
AIH	86966.0	23.92	119500	1.37:1
DI	89791.5	25.65	128250	1.43:1
DIH	95591.5	24.41	122000	1.28:1
NI	43572.5	9.60	48000	1.10:1
NIH	50572.5	10.32	51600	1.02:1

Market rate of hydrogel @ 700/kg and mulberry leaf @ 5/kg

DISCUSSION

It is evident from the present study that daily irrigation and daily irrigation with hydrogel has recorded numerically more leaf yield as compared to the alternate day irrigation with hydrogel but, at the same time, it has consumed 15 % more water than alternate day irrigation treatment. On the other hand, particularly with respect to plant growth and associated attributes, AIH has shown superiority over all the others. Similar results were also reported by Waly *et al*. (2013) where treatment of 0.2 % hydrogel had shown superiority in all growth attributes than other treatments. With respect to plant height, AIH was found to be the best which may be attributed to water availability and indirect supply of nutrients provided by superabsorbent polymer, that might have increased the activity of cell division, cell expansion and cell elongation, ultimately leading to an increased plant height. Similar results have been reported by Al-Harbi *et al*. (1999) in cucumber, Sendur *et al*. (2001) in tomato, Sivalapan (2006) and Amiri *et al*. (2013) in soybean. Positive impact of hydrogel was also observed in the case of total shoot length which was the highest in AIH. The effect of hydrogels on stem elongation is reported by many authors; Brar *et al*. (2001) in maize and Anupama *et al*. (2007) in *Chrysanthemum* seedlings. Therefore, overall improvement in growth of mulberry with the application of polymer could be ascribed to its pivotal role in maintaining soil moisture during dry spell which is important for growth and development of plant. Further, water use efficiency was found to be maximum in the case of AIH compared to others which may be explained as the formation of water in the root zone, but not the amount of applied water. This result is in agreement with that of the experiment conducted at Hayatnagar (Hyderabad), where application of polymer at the rate of 25 kg/ ha with alternate week irrigation in tomato recorded the highest water productivity of 290 kg/ha/mm as compared to control (CRIDA, 2013). The result also agrees with those reported on banana (Ibrahim, 1993; Goenaga *et al*., 1995; Eckstein *et al*., 1998; More *et al*., 1999; Salvin *et al*., 2000; Ibrahim *et al*., 2012). However, mulberry grown without hydrogel but, with daily irrigation accrued the highest B:C ratio (1.43) that may be because of non-addition cost of hydrogel. In an experiment, Islam *et al*. (2011) indicated that dose of hydrogel in maize, higher or lower than optimum was neither be sufficient nor economical. Therefore, the present study indicates that hydrogel does have positive and significant effect on the growth and development of mulberry but, in view of higher cost, its' usage can be promoted only by considering an optimum dose and the number of plants / ha. To substantiate with, bio-assay with silkworm has also revealed no significant positive or negative effect on economic parameters of silkworm.

Conclusion

Topographical heterogeneity, light soil texture, excessive run off coupled with heavy infiltration are some of the

major hindrances for productivity of rainfed mulberry. Under such circumstances, the present study showed promising results in mulberry through application of superabsorbent polymer and this proved as a suitable option for the mulberry sericulture farmers of this region. Hydrogel application with reduced / alternative frequency of drip irrigation under wider spacing of mulberry would be an appropriate choice for economic productivity as well as sericulture sustainability in the state.

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Research Paper

IDENTIFICATION AND CHARACTERIZATION OF SERICIN ISO-FORMS EXTRACTED FROM COCOONS OF SILKWORM, *BOMBYX MORI*

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ABSTRACT

This study provides information on identification of different sericin isoforms from silkworm, *Bombyx mori*. The sericin fraction extracted from the silk cocoon shell was characterized based on identification of individual polypeptide fragments and their functions. Sericin fraction was extracted from four different breeds of mulberry silkworm cocoons *viz*., bivoltine (CSR2), multivoltine (PM), hybrid (PM x CSR2) and sericin rich hybrid. Initially, proteins were extracted from silk cocoons using distilled water, without addition of any chemical reagent or application of heat energy, to obtain them in the native form and separated through 8 % SDS-PAGE. The results indicated that sericin proteins extracted from all kinds of cocoons with distilled water, contained different iso-forms with molecular weight in the measure of ~ 200, 120 and ~50 kDa except for sericin rich cocoon. Interestingly, protein obtained from sericin rich cocoon showed single band with higher molecular weight (~350 kDa) whereas, proteins extracted from cocoons of bivoltine, multivoltine and hybrid silkworm with 6 M urea showed more types of protein bands (~ 280, ~200, ~180, ~120 and ~50 kDa). Some of the extracted sericin isoforms were analysed for antibacterial activity and the ~ 50 kDa protein band was found to be highly water soluble and with the highest antibacterial activity against *Staphylococcus* spp. Further, the selected ~50 kDa fragment (iso-form) of sericin extracted with water was confirmed by MALDI-TOF/MS analysis.

Key words: Antibacterial activity, *Bombyx mori*, cocoon, sericin.

INTRODUCTION

Silkworm, *Bombyx mori* cocoons are made-up of mainly two forms of proteins, such as fibroin (70 to 75 %) and sericin (20 to 25 %). During the degumming process of silk reeling, sericin fraction is removed from cocoons and discarded into the open field considering it as an inexpensive material or by-product. The contamination of surrounding water bodies or open field by this effluent generates a biological oxygen demand and causes environmental pollution due to the growth of bacteria and fungi. It was estimated that presently, over 50,000 tonns of sericin is being generated worldwide, annually. This has prompted many a study to explore ways of its utilization. The exploitation of sericin for broader range of biological application requires detailed understanding of its biochemical and physical characteristics.

Sericin fraction obtained from the cocoons of *B. mori* is combination of both water soluble and partially soluble globular glycoproteins having random coil and *β*-sheets (Liliam *et al.,* 2017). Even though sericin is a water soluble protein, it forms a jelly like substance in water, due to the presence of higher proportion of serine residues in its polypeptide and covalently linked oligosaccharide molecules on its surface causes an extensive intermolecular complex with adjacent sericin and water molecules (Shaw and Smith 1951; Cho *et al.,* 2003). Solubility of sericin also depends on pH and ionic strength of the solution as well as isoelectic pH of individual sericin fragments (Takasu *et al.,* 2010). According to solubility in water, sericin was classified into three main fractions (A, B and C), sericin A fraction is more soluble in water and is present in the outer layer of the cocoon thread, whereas water soluble B fraction occupies the middle layer and

water insoluble C fraction is seen adjacent to the fibroin filament (Kunz *et al.,* 2016). Sericin A fraction is known to be synthesized from Ser 3 gene and sericin B and C fractions are coded by Ser 1 gene (Garel *et al.,* 1997). The molecular weight distribution of sericin ranges from 10 to 400 kDa (Tao *et al.,* 2005). The molecular heterogeneity of sericin is generated by the alternative splicing of primary transcripts of Ser 1, Ser 2 and Ser 3 gene products in the posterior and middle areas of middle silk gland (Akai, 1983; Michaille *et al.,* 1990; Takasu *et al.,* 2002). However, some of the amino acid sequences are known to be repeated sequences in sericin synthesized from Ser 3 gene, hence, different molecular weight sericin fragments could have similar amino acid sequences (Takasu *et al.,* 2007).

Several studies have demonstrated that sericin has antioxidant, anti-inflammatory, anti-cancer, anti-ageing and antimicrobial activity (Aramwit *et al.,* 2012). Moreover, sericin nanoparticles have been applied as controlled drug-releasing biomaterials and sericin has also proved to be less immunogenic (Zhang, 2002). Sericin has great potential to be used in food, cosmetic and biopharmaceutical products (Aramwit and Sangcakul, 2007).

However, most of the biological characteristics of sericin was explained with mixed forms of sericin fractions / fragments / iso-forms, and not as an individual sericin polypeptide. However, specific physical and biochemical function associated with individual sericin molecule / polypeptide was not yet clearly understood. The present study was therefore, focused on the biological and molecular characteristics of individual sericin polypeptide extracted from *B. mori* cocoons.

MATERIALS AND METHODS

All chemicals and reagents used in the study were of analytical grade and were used without further purification. Sericin used in the study was extracted by different extraction methods from four different types of mulberry silkworm (*B. mori)* cocoons *viz*., bivoltine (CSR2), multivoltine (PM), multi and bivoltine hybrid (PM x CSR2) and sericin rich hybrid (sericin content is more than 80 %).

Sericin extraction by 6 M urea solution and distilled water

Two grams of chopped *B. mori* cocoon shells of bivoltine (CSR2), multivoltine (PM), hybrid (PM x CSR2) and sericin rich hybrid were separately weighed and transferred to respective beakers containing 20 ml of 6 M urea solution, another experimental set-up with the same procedure was followed using sterilized distilled water without any chemical agents. After two hours of soaking at room temperature, the cocoon shell samples were crushed in a clean mortar with pestle. The crushed samples were then squeezed by using fresh muslin cloth. The homogenate was collected and subjected for centrifugation at 8000 rpm for 15 min to settle down the debris and supernatant was collected. The proteins present in the supernatant were precipitated with ice cold acetone (5ml of supernatant was added to 45 ml of ice-cold acetone in a 100 ml beaker). The ice-cold acetone mixture was incubated for 24 hours in the refrigerator. Then the precipitated samples were centrifuged at 10,000 rpm for 10 min and supernatant was removed, the remaining acetone from the dislodge protein pellet was allowed to evaporate from the uncapped tube at room temperature. The pellet was mixed with 1 ml of freshly prepared tris HCl buffer (pH 7.4) and then collected in 2 ml eppendorf tube and preserved in refrigerator for further studies.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS PAGE) analysis of sericin

Molecular weight distribution of proteins extracted with sterile distilled water and 6M urea solution were studied by 8 % SDS-PAGE analysis using a vertical gel electrophoresis (Laemmli, 1970) coupled with a Power Pac (Tarsons, India).

Preparation of samples

To determine the molecular weight distribution and protein profile of extracted sericin proteins, 50 µl of crude sericin extract solution was added to 50 µl of Laemmli sample buffer [containing Tris-Base, 0.5 % (v/v) β-

mercaptoethanol, 4 % (m/v) sodium dodecyl sulfate, 10 % (v/v) glycerol, and 0.025 % (m/v) bromophenol blue] in a 2 ml Eppendorf tube (with its lid punctured by a needle) and kept in boiling water bath for 10 min.

Preparation of loading and separating gels

Two categories of gels were used *viz*., separating gel (8 %) and loading gel (5 %). The separating gel was added first to the casting tray and it consists of 8 % acrylamide / bisacrylamide (solidifying agent), 1.5 M Tris HCl buffer (pH – 8.8), 10 % SDS, 10 % APS (polymerizing agent), TEMED (catalyst) and distilled water (Table 1). The gel was allowed to stand for 30 minutes for polymerization to occur. Following this, loading gel was added, which consists of 8 % acrylamide / bisacrylamide (solidifying agent), 0.5 M Tris HCl buffer (pH 6.8), 10 % SDS, 10 % APS , TEMED and distilled water (Table 1). As soon as loading gel was added, combs were inserted to the gel for the formation of wells.

Table 1: Composition of SDS-PAGE gels

Chemical composition	Separating gel (8 %)	Loading gel (5 %)
Acrylamide / bisacrylamide (stock) (ml)	3.4	0.67
Distilled water (ml)	3.8	2.95
1.5 M Tris HCl (pH 8.8) (ml)	2.6	-
0.5 M Tris HCl (pH 6.8) (ml)	-	1.25
10 % SDS (ml)	0.1	0.05
10 % APS (ml)	0.1	0.05
TEMED (ml)	0.01	0.005

Running of SDS-PAGE gels

Then, for SDS-PAGE analysis, 20 µl of each sample and 20 µl of molecular weight markers (of pre-stained protein marker, with protein standards ranging from 20 to 250 kDa) were loaded into respective wells of loading gels and run at a constant voltage of 120 V, 80 mA per gel and 20 W, for 2 hours, after which the gels were stained with staining solution (Coomassie brilliant blue R -250, water: methanol: glacial acetic acid) followed by de-staining (water: methanol: glacial acetic acid in the ratio of 5:4:1). After destaining, molecular weights of unknowns were analysed using molecular weight markers of known kDa and the gels were then photographed for analysis.

MALDI-TOF/MS analysis of SDS PAGE separated proteins

Analysis of individual protein band for generation of peptide masses was carried out by MALDI-TOF/MS analysis at Indian Institute of Science (IISC), Bengaluru India. Peptide mass finger prints of each of the sample were analyzed by Mascot software server available at http://www.matrixscience.com, and protein blast was performed with public data base available with National Center for Biotechnology Information (NCBI) website.

Antibacterial assay of sericin

Antibacterial activity of bivoltine sericin extracted by the above mentioned methods was studied with *Staphylococcus* spp. and evaluated by agar well diffusion method. Lag phase bacterial cultures of *Staphylococcus* spp. grown in nutrient broth was spread on nutrient agar plates and 50 µl (100 µg/ml) of sericin was added into the respective labeled wells. Distilled water was used as negative control. The inoculated nutrient agar plates were incubated at 37 °C for 24 hours and zone of inhibition was measured qualitatively.

RESULTS

The protein profile of SDS-PAGE analysis indicated that crude sericin extracts from silkworm, *B. mori* cocoons, obtained by two different extraction procedures had different molecular weight ranges. The proteins extracted through distilled water displayed predominantly two to three bands (~ 200, ~120 and ~50 kDa) in all kinds of silkworm cocoons except those of sericin rich hybrid. Interestingly, protein obtained from sericin rich cocoon had single band with higher molecular weight (~350 kDa). Whereas, proteins extracted from bivoltine, multivoltine and multivoltine x bivoltine hybrid with 6 M urea have shown five to six different molecular weight bands in SDS-PAGE (~ 280, ~200, ~180, ~120, ~50, ~30 kDa *etc.*) (Figure 1).

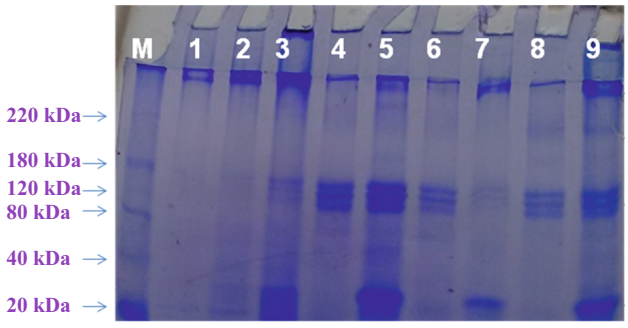

Figure 1: Analysis of sericin proteins on SDS- PAGE. M lane - Marker; Lane 1 - Sericin extracted from sericin rich hybrid cocoon with distilled water (W); Lanes 2 & 3 - Sericin extracted from sericin rich hybrid cocoon with 6 molar urea (U); Lane 4 - Sericin extracted from bivoltine cocoons with W; Lane 5 - Sericin extracted from bivoltine cocoons with U; Lane 6 - Sericin extracted from multivoltine cocoons with W; Lane 7 - Sericin extracted from multivoltine cocoons with U; Lane 8 - Sericin extracted from hybrid cocoons with W; Lane 9 - Sericin extracted from hybrid cocoons with U.

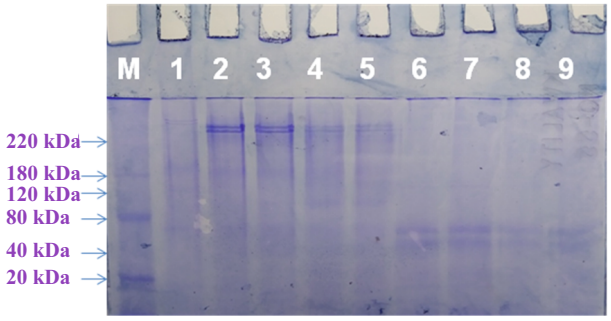

Figure 2: Analysis of sericin peptides after purification. M lane - Marker; Lanes 1, 2, 3, 4 & 5 - Crude fraction; Lanes 6, 7, 8 & 9 - Purified ~50 kDa water soluble fraction; each band was further analyzed by MALDI-TOF/MS.

The different molecular weight sericin fractions were separated (Figure 2) and tested for their antibacterial activity and results indicated that 50 kDa protein band was specifically associated with anti-bacterial activity against tested bacterial culture (Figure 3). This was assumed to be water soluble and showed antibacterial activity against *S. aureus* by forming bacterial aggregation. MALDI-TOF/MS analysis of the 50 kDa iso-form of sericin obtained from water extract (Figure 4) had similarity with reference sequence NCBI: XP: 021205348.1 sericin 1-like isoform X7 [*Bombyx mori*].

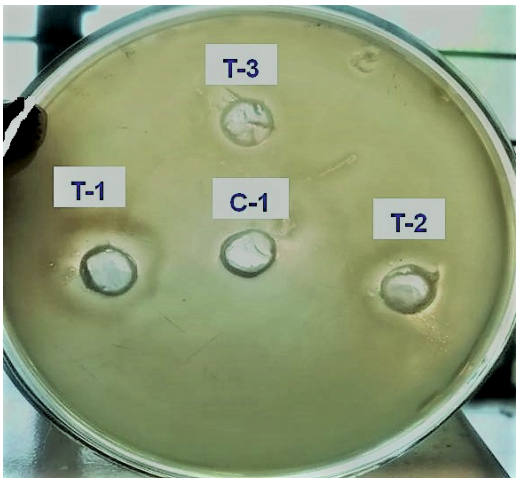

Figure 3: Antibacterial activity of ~50 kDa sericin against *Staphylococcus.* T1 - ~50 kDa water soluble sericin fraction; T2 - whole water extract; T3 - whole urea extract; C-1 –control, distilled water.

DISCUSSION

The iso-forms of sericin and molecular weight distribution of sericin molecules on SDS-PAGE are still being controversial. In the present investigation, five different forms of sericin peptides were detected when extracted with urea solution alone, whereas water treatment alone had predominantly two types of bands. It was assumed that, chemical treatment might have attacked the peptide bonds of the primary structure of the protein, and therefore, showed broader bands pattern due to the mixture of different molecular weight peptides when compared with distilled water treatment. Moreover, Takusa *et al*. (2002) concluded that sericin extracted from *B. mori* cocoons was found to mainly consist of three polypeptides with molecular masses of 400, 250, and 150 kDa. In the present investigation, it was observed that molecular weight distribution of sericin depends on the extraction method. It was presumed that, due to the difficulty in extraction of sericin iso-forms in native form from the rest of the sericin peptides, each study reported different kinds of molecular weight sericin (Kunz *et al.,* 2016).

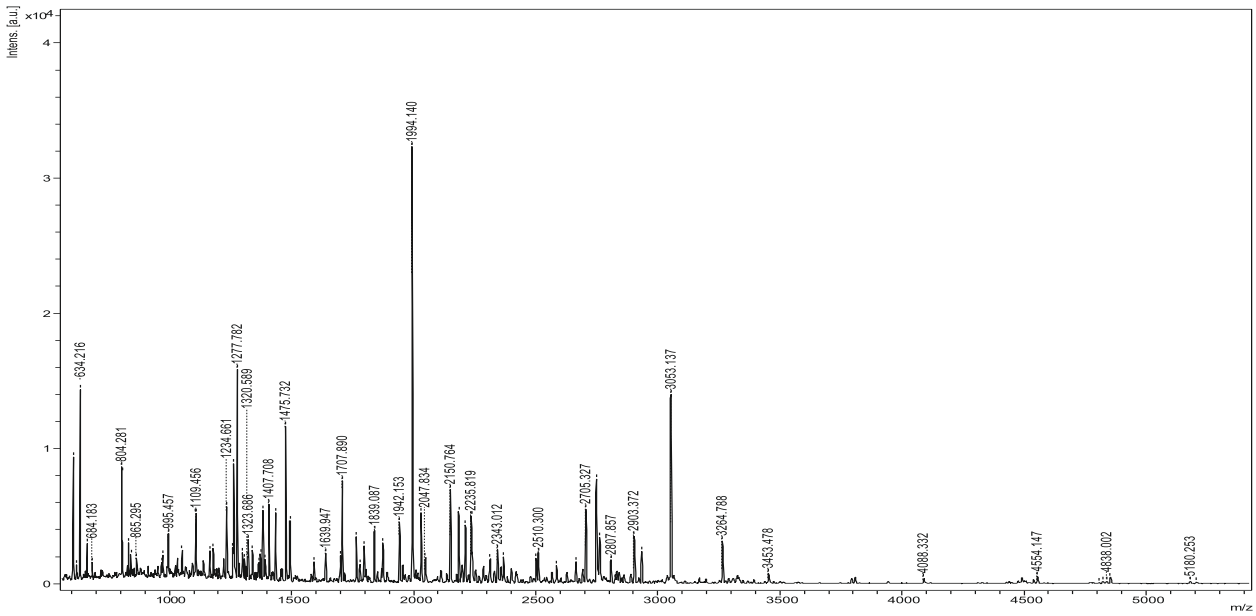

Figure 4: MALDI-TOF/MS analysis of ~50 KDa sericin isoform. Molecular masses matched partially to reference sequence NCBI: XP: 021205348.1 sericin 1-like isoform X7 (*Bombyx mori*).

-50 kDa sericin protein sample: >XP_021205348.1 sericin 1-like isoform X7 [*Bombyx mori*]: Sequence similarities are marked in yellow color.

MRFVLCCTLIALAALSVKAFGHHPGNRDTVEVKNRKYNAASSESSYLNKDNDSISAGAHR
AKSVEQSQDKSKYTSGPEGVSYSGRSQNYKDSKQAYADYHSDPNGGSASAGQSRDSSLRE
RNVHYVSDGEAVAASSDAR DENRSAQQNAQANWNADGSYGVSADRSGSASSRRRQANY
YSDKDITAASKDDSR ADSSRRSNAYYNRDSDGSESAGLSDRRASSSKNDNVFVYRTKDSIG
GQAKSSRSSHSQESDAYYNSSPDGSYNAGTRDSSISNKKKASSTIYADKDQIRAANDRYSS
KQLKQSSAQISSGPEGTSVSSKDRQYSNDKRSKSDAYVGRDGTVAYSNKDSEKTSR QSNT
NYADQNSVRSDSAASDQTSNSYDKGYSDKNTVAHSSGSR GSQNQKSSSYRADKDGFSSST
NTEKSRFSSSNSVVETSDGTSASRESSAEDTKSSNSNVQSDALSEDSSEVDIDLGNLGWWW
NSDNKAQRAAGGATKSEASSSTQATTVSGADDSADSYTWWWNPRRSSSSSSSASSSSSGS
NVGGSSQSSGSSTSGSNARGHLGTVSSTGSTSNTDSSSKSAGSRTSGGSSTYGYSSSHRGGS
VSSTGSSSNTDSSTKNAGSSTSGGSSTYGYSSSHRGGSVSSTGSSSNTDSSTKNAGSSTSGGS
STYGYSSSHRGGSVSSTGSSSNTDSSTKSAGSSTSGGSSTYGYSSRHRGGRVSSTGSSSTTD
ASSNSVGSSTSGGSSTYGYSSNSRDGSVSSTGSSSNTDSNSNSAGSSTSGGSSTYGYSSNSR
DGSVSSTGSSSNTDSNSNSAGSSTSGGSSTYGYSSNSRDGSVSSTGSSSNTDASTDLTGSSTS
GGSSTYGYSSDSRDGSVSSTGSSSNTDASTDLAGSSTSGGSSTYGYSSDCGDGSVSSTGSSS
NTDASTDLAGSSTSGGSSTYGYSSNSRDGSVSSTGSSSNTDASTDLTGSSTSGGSSTYGYSS
SNR
DGSVLATGSSSNTDASTTEESTTSAGSSTEGYSSSSHDGSVTSTDGSSTSGGASSSSASTAKS
DAASSEDGFWWWNRRKSGSGHKSATVQSSTTDKTSTDSASSTDSTSSTSGASTTTSGSSST
SGGSSTSDASSTSSSVSRSHRSGVNRLLHKPGQGKICLCFENIFDIPYHLRKNIGV

The previous studies demonstrated the antibacterial characteristics of sericin extracted from cocoons of *B. mori*. But, none of them could provide a clue on the specific sericin iso-form from the mixture of sericin peptides, that is responsible for antibacterial property (Aramwit *et al*., 2012). Moreover, the antibacterial properties of sericin samples are quite different from each other depending on concentration of protein, and type of chemical agents or heat applied for extraction procedure and the bacteria species tested, as apparent from the

varying growth inhibition halo diameters produced. Interestingly, in the present investigation, it was observed that, 50 kDa of sericin iso-form is mainly associated with antibacterial activity, due to bacterial aggregation property rather than lysis of bacterial cultures (Figure 3). Further, MALDI-TOF/MS analysis confirmed that the 50 kDa of sericin peptide was more similar to sericin 1 iso-form.

The present study could identify five different molecular weight sericin peptides from *B. mori* cocoons when treated with 6M urea, whereas with distilled water, only two or three predominant molecular weight sericin iso-forms were identified. Among those, the 50 kDa water soluble sericin isoform was ascertained to possess antibacterial activity.

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Research Paper

DEVELOPMENT OF VOID SPUN SILK FABRICS UTILIZING INNOVATIVE VOID SPUN SILK YARNS

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ABSTRACT

Spun silk is basically produced from silk waste in spun silk spinning units all over the world. The utilization of spun silk was limited to specific end uses. In order to render better surface appearance and superior fabric characteristics to the spun silk, a technique was designed to impart bulkiness in the yarns by spinning them along with Poly Vinyl Alcohol (PVA) yarns during the process of spinning and after weaving, the PVA component was dissolved so as to create void in the spun silk and hence, improve the fabric performance. Two varieties of PVA yarns *viz*., Japanese and Indian were used in the study. The PVA yarns were plied as 2, 4 and 6 ply and inserted in the spun yarn during the spinning process. These spun silk yarns were used as weft in the fabric manufacturing along with organzine bivoltine raw silk as warp. The fabrics thus produced were processed for removal of PVA yarn, so that void is developed in the fabrics with a unique surface appearance apart from improvement in functional, aesthetic and comfort properties compared with spun silk fabrics constructed without void. Based on the analysis, it was found that the fabric parameters of void spun silk fabrics were superior in terms of crimp, crease recovery, elongation, air permeability and drape characteristics. The ANOVA results indicate that all the characteristics of spun silk and void spun silk were significantly different at 1% level. Using the Z-score analysis, it could be found that void raw silk fabrics produced with 2 ply Indian PVA yarns and 4 ply Japanese PVA yarns have scored more thus indicating their superiority followed by void spun silk fabrics produced with 4 ply Indian PVA yarns and 2 ply Japanese PVA yarns in spinning process. The thermal comfort properties of these fabrics *viz.*, Q max values of spun and void spun silk fabrics did not show significant difference, whereas the void spun silk fabrics produced with 4 ply Japanese PVA yarns and 2 ply Indian PVA yarns in spinning process have shown maximum clothing insulation values (CLO) of 0.53 and 0.56, respectively. Thermal insulation value (TIV) of void spun silk are slightly higher than that of spun silk fabrics, with fabrics produced using 4 ply Japanese PVA yarns and 2 ply Indian PVA yarns in spinning process exhibiting maximum TIV values of 33.08 and 37.22, respectively. Thus it could be concluded that void spun silk fabrics produced in spinning process could be an effective alternative for new applications in the silk industry.

Key words: Functional, aesthetic and thermal comfort properties, PVA yarn, spun silk, void spun silk fabrics.

INTRODUCTION

The silk waste generated during the process of silk reeling is used for the production of spun silk yarn, which in turn is used in production of carpets, dress materials, furnishings *etc*. (Sonwalkar, 1993). The spun silk yarn is similar in appearance to that of cotton but possesses different characteristics *viz*., softness, lustre, durability, elegance *etc*. (Eric Oxtoby, 1987). Presently, use of spun silk is very limited in view of cost factor and its less utility. The production of hollow yarns in cotton and polyester has improved the characteristics and utility of the yarns to a great extent (Jerzy Andrysiak *et al*., 2014) and hence, it was thought that a similar effort in the case of spun silk would result in improving the performance of the spun silk yarns for varied applications. For this purpose, an

experiment was designed wherein the PVA yarn is inserted as core in the process of spun yarn spinning and the yarn is used in the production of fabrics as weft. These fabrics when processed (dyed), the PVA component is dissolved and hollowness / void is created inside the yarn / fabrics, which result in improving the functional, aesthetic and thermal comfort properties of void spun silk fabrics.

MATERIALS AND METHODS

Production of spun silk yarn

Raw material

Silk waste of good quality generated during the process of reeling was accumulated from the silk reeling units to be used as raw material for the spun silk yarn manufacturing in the spun silk spinning unit.

Procedure

The silk wastes were degummed using soda solution in the degumming bath in order to remove the sericin component present in it. The degummed silk waste was dried and stored.

The degummed silk waste was processed in the opening machine to open the silk fibres and form them into laps on the opening roller. These fibres were cut and removed as a lap for further processing. During the opening process, the impurities present in the silk waste were removed.

The laps were cut into required staple length and cut laps were once again processed in the opening machine to form into laps.

The laps were processed in the breaker carding machine initially, to convert the lap into sliver form. These slivers were combined in the lap former to form a small lap. Three such laps were once again fed into the finisher carding machine to form into sliver.

The slivers were processed in the roving frame to convert the sliver into roving bobbins. The rovings in the bobbins were drafted in the ring frame to convert them into spun silk yarn of desired count.

The spun silk yarn thus produced was converted into cones for further doubling of them so that spun silk yarns of the desired 2/40s count was produced.

Design of experiment: The PVA yarns of 2 varieties were sourced, one from M/s Savlon, Japan and another, from India, for the experiment. The details of quality of PVA yarns used for the study are presented in Table 1. These PVA yarns were prepared as 2, 4 and 6 plied yarns and introduced into the spun silk yarn manufacturing machine *i.e.,* in the front roller of the spinning frame so that spun silk along with PVA yarn would be produced as shown in Figure 1. The characteristics of spun silk and void spun silk yarns produced in ring spinning process are provided in Table 2.

Production of void spun silk fabrics

The spun silk and void spun silk yarns produced with 2, 4

Table 1: Quality of PVA yarns used for spinning void spun silk

PVA yarn	Content	Denier	Tenacity (g/d)	Elongation (%)
Japanese PVA yarn	Single	30.31	2.8	15.5
	2 ply	59.56	3.0	13.9
	4 ply	89.29	3.1	12.8
	6 ply	178.68	2.2	17.2
Indian PVA yarn	Single	91.0	1.7	12.4
	2 ply	189.0	2.4	19.2
	4 ply	367.0	2.3	20.0
	6 ply	576.0	2.1	20.4

Figure 1: Production of void spun silk in ring spinning frame

Table 2: Characteristics of spun silk and void spun silk yarns produced in ring spinning process

Characteristic	Spun silk (40s count)	Void spun silk					
		2 ply Japanese PVA	4 ply Japanese PVA	6 ply Japanese PVA	2 ply Indian PVA	4 ply Indian PVA	6 ply Indian PVA
Denier	261.0	313.4	383.8	480.8	479.7	723.1	944.6
Tenacity (g/d)	13.2	17.0	15.4	15.9	13.0	8.9	13.0
Elongation (%)	6.07	8.73	8.87	9.93	9.34	17.87	14.3
Microscopic cross section (microns)	231.6	340.3	229.22	276.47	284.4	353.7	389.8
Microscopic longitudinal width (microns)	304.6	273.4	276.6	373.8	500.5	399.6	421.0
Wicking (cm)	0.0	2.2	2.7	2.7	3.1	4.1	3.5

and 6 ply Japanese and Indian PVA yarns were doubled and twisted with required TPI and used as weft. The bivoltine raw silk was single twisted, doubled and double twisted and used as warp. Seven different varieties of fabrics were developed for characterization. They were then degummed and dyed in HTHP dyeing machine in order to remove the PVA component from the fabrics. The dyed fabrics were studied for functional, aesthetic and comfort properties. The fabrics were also assessed for thermal comfort properties using Kawabata Evaluation System (KES).

Data and analysis: The seven varieties of spun silk and void spun silk fabrics were studied for various fabric characteristics *viz*., fabric mass, fabric thickness, EPI, PPI, warp and weft count, cover factor, crimp warp / weft, twist warp / weft, tensile strength *viz*., breaking load and elongation warp / weft, crease recovery, flexural rigidity, abrasion resistance, air permeability, drape coefficient and bursting strength. The fabrics were also subjected for assessment of thermal comfort properties *viz*., Q max values, clothing insulation values (CLO) and thermal insulation value (TIV). The data were analyzed statistically.

The spun silk and void spun silk fabrics of different combinations were tested for various functional, aesthetic and comfort properties following the standard testing procedures *viz*., threads in cloths, IS 1963:1981; count of warp / weft, IS 3442:1980; crimp percentage of warp / weft, IS 1963:1981; tensile strength, IS 1969:1985; crease recovery, IS 4681:1981; flexural rigidity, ASTM D 1388-96 option A; drape coefficient percentage, IS 8357:1977; air permeability, IS 11056:1984 and fabric thickness, IS 7702:1975.

The data thus obtained were analyzed statistically using SPSS package.

RESULTS AND DISCUSSION

The fabric characteristics of the spun silk and void spun silk samples are furnished in Table 3 and Figure 2. The ANOVA results of all the characteristics of spun silk and void spun silk fabric samples are given in Table 4. The characteristics of spun silk and void spun silk fabric produced in ring spinning process were evaluated for Z score and put for subjective analyses and the results are provided in Tables 5 and 6. Table 7 indicates the thermal comfort characteristics of spun silk and void spun silk fabrics produced in ring spinning process.

Functional, aesthetic and comfort properties of spun silk / void spun silk fabric

Fabric mass and thickness: From Table 3 and Figure 2 a & b, it could be observed that fabric mass and thickness of spun silk fabric was 112.5 g/m^2 and 0.300 mm, respectively whereas, for 4 ply Japanese PVA void spun silk fabric, it was 96.8 g/m^2 and 0.351 mm and for 2 ply

Table 3: Characteristics of spun silk fabric produced in ring spinning process using Japanese and Indian PVA yarns

Characteristic	Control 40s spun silk	Void spun silk					
		2 ply Japanese PVA	4 ply Japanese PVA	6 ply Japanese PVA	2 ply Indian PVA	4 ply Indian PVA	6 ply Indian PVA
Fabric mass (g/m^2)	112.5	95.5	96.8	82.7	101.6	89.6	92.7
Fabric thickness (mm)	0.300	0.339	0.3505	0.3345	0.355	0.390	0.389
Threads in cloth							
Ends / inch	124	132	132	126	130	128	126
Picks / inch	68	56	59	48	56	46	46
Yarn count (denier)							
Warp	38.7	37.1	37.0	36.8	37.0	37.1	38.8
Weft	289	274	269	264	299	317	340
Crimp (%)							
Warp	16.2	19.4	19.7	17.0	20.9	18.2	17.6
Weft	2.2	5.6	9.4	8.2	6.3	8.9	8.7
Twist (TPM)							
Warp	792.0	770.8	774.4	774.0	751.6	764.4	760.0
Weft	615.6	635.6	573.4	588.4	639.0	684.2	619.2
Cover factor	21.1	22.4	22.5	21.6	22.1	21.7	20.7
Tensile strength							
Breaking load (kg)							
Warp	29.71	31.40	33.17	35.28	32.14	34.93	35.10
Weft	69.87	52.73	51.17	36.19	51.05	24.40	24.27
Elongation (%)							
Warp	27.20	23.07	23.85	23.39	25.05	24.87	32.10
Weft	10.19	13.21	16.27	12.50	15.41	14.43	13.95
Crease recovery angle (º)							
Warp	103.9	116.7	113.3	105.5	111.5	120.2	107.8
Weft	109.3	117.8	118.3	109.0	106.4	121.1	120.3
Total	213.2	234.5	231.6	214.5	217.9	241.3	228.1
Flexural rigidity (mg - cm)							
Warp	0.013	0.017	0.013	0.013	0.011	0.013	0.012
Weft	0.091	0.025	0.023	0.016	0.021	0.020	0.045
Overall	0.035	0.021	0.017	0.014	0.015	0.016	0.023
Abrasion resistance (Cycles)	3003	3167	2900	3200	3833	2800	1833
Air permeability (m^3/m^2/m)	22.84	54.21	57.68	82.12	68.60	120.88	126.87
Drape coefficient	23.86	23.82	21.31	21.66	22.45	22.65	21.91
Bursting strength (kg/cm^2)	13.90	12.80	14.70	12.00	14.85	13.05	15.75

Indian PVA void spun silk fabric, it was 101.6 g/m^2 and 0.355 mm, respectively. The fabric mass of void spun silk was less compared to spun silk fabrics due to removal of PVA yarn from fabrics.

Weft crimp and twist in weft: The weft crimp and twist in weft were 2.2 % in spun silk fabrics and 9.4 % in void spun silk fabrics on using 4 ply Japanese PVA yarn, whereas 6.3 %, in void spun silk fabrics when 2 ply Indian PVA yarn was used due to removal of PVA yarn during processing (Table 3). This may be attributed to the shrinkage of fabrics due to removal of PVA yarns in the weft.

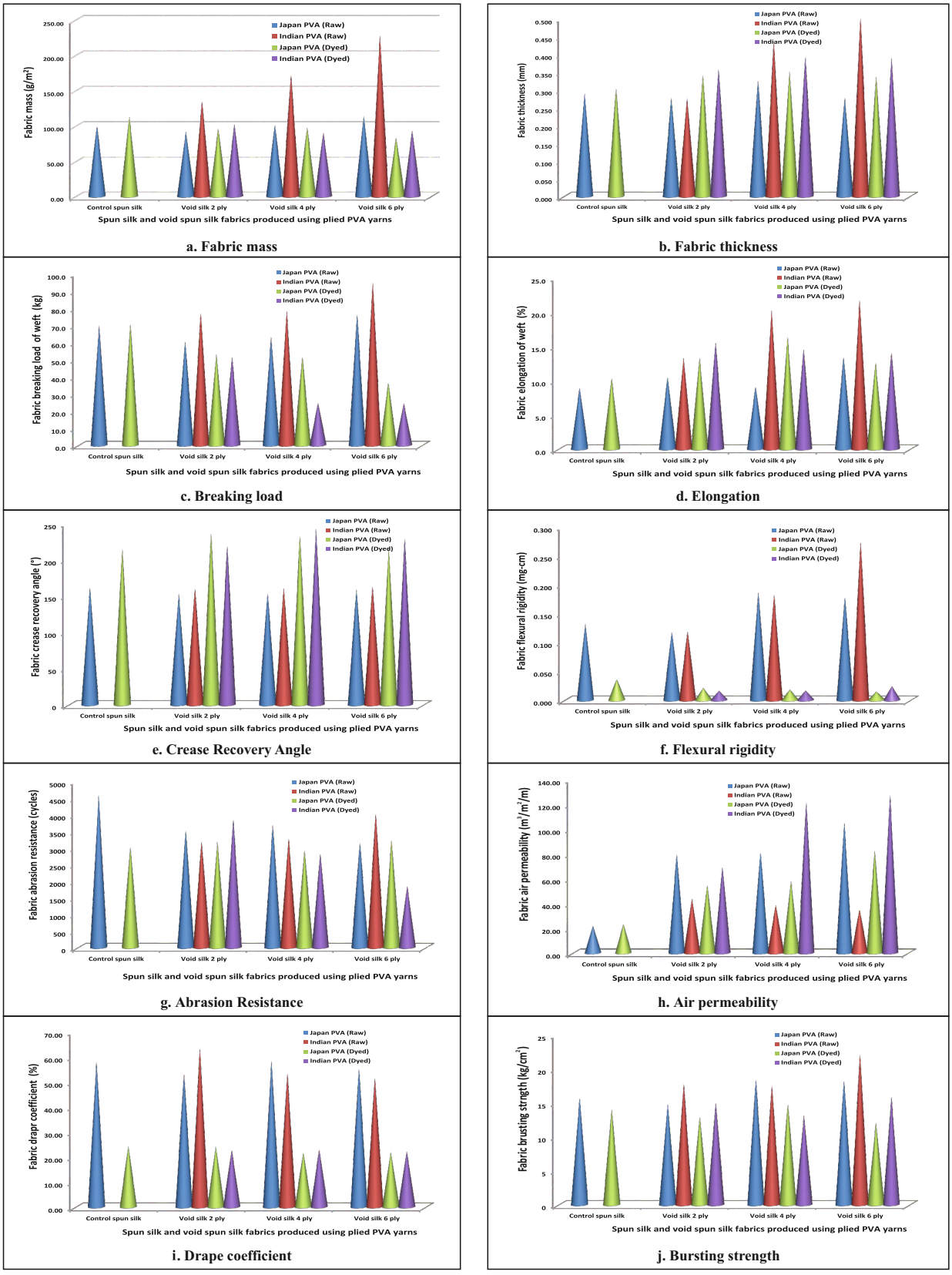

Figure 2: Characteristics of spun silk and void spun silk fabric produced in ring spinning process

Tensile properties: The breaking load and elongation of the void spun silk produced with both Japanese and Indian PVA yarns were significantly more compared to spun silk fabrics (Table 3 and Figure 2 c & d), thus revealing the much stronger and more stretchable nature of void silk fabrics which make them suitable for variety of applications.

Crease recovery: Crease recovery angle of the spun silk fabric was 213, which was significantly increased to the extent of 6.4 and 7.4 % in the case of void spun silk fabrics produced with both Japanese and Indian PVA yarns, respectively (Table 3 and Figure 2 e).

Flexural rigidity: The flexural rigidity of void spun silk fabrics produced with both Japanese and Indian PVA yarns were lower than that of spun silk fabrics to the extent of 50 %, indicating the more flexible and less stiff nature of void spun silk fabrics (Table 3 and Figure 2 f).

Abrasion resistance: The abrasion resistance of both spun silk and void spun silk fabrics produced in ring spinning process did not show significant difference (Table 3 and Figure 2 g).

Air permeability: The air permeability of the void spun silk produced with both Japanese and Indian PVA yarns were significantly more compared to spun silk fabrics (Table 3 and Figure 2 h). The air permeability of void spun silk fabrics produced using Japanese PVA yarn has shown about 180 % improvement over spun silk fabrics, whereas in the case of Indian PVA yarn, it was 350 % more which may be attributed to the more count of Indian PVA yarn which created more void in the fabrics.

Drape coefficient: Drape coefficient of the spun silk fabric was 23.26, which was significantly decreased by the extent of 6.35 and 6.69 % in the case of void spun silk fabrics produced with both Japanese and Indian PVA yarns, respectively (Table 3 and Figure 2 i).

Bursting strength: It could be observed that bursting strength was 13.9 kg/cm^2 in spun silk fabrics and 14.7 kg/cm^2 in void spun silk fabrics produced using 4 ply Japanese PVA yarn, whereas 14.85 kg/cm^2 in void spun silk fabrics on using 2 ply Indian PVA yarn (Table 3 and Figure 2 j).

Table 4: ANOVA results of characteristics of dyed spun silk and void spun silk fabrics produced in ring spinning process

Fabric characteristic	Degrees of freedom	Mean sum of Square	F - Value	Significance
Fabric thickness (mm)	7	0.004	59.19	0.000**
Cover factor	7	1.26	20.31	0.000**
Crimp - Weft (%)	7	25.71	156.60	0.000**
Twist - Weft (TPM)	7	3169.33	12.17	0.000**
Breaking load – Warp (kg)	7	16.07	7.43	0.000**
Breaking load – Weft (Kg)	7	966.45	246.55	0.000**
Elongation – Warp (%)	7	26.62	17.66	0.000**
Elongation – Weft (%)	7	14.96	23.36	0.000**
Crease recovery angle total (º)	7	5296.08	138.17	0.000**
Flexural rigidity (mg-cm)	7	0.00	185.13	0.000**
Abrasion resistance (cycles)	7	927776	3.55	0.017^{NS}
Air permeability ($m^3/m^2/m$)	7	1982.04	1340.43	0.000**
Drape coefficient (%)	7	3.27	4.43	0.000**
Bursting strength (kg/cm^2)	7	56.58	92.66	0.000**

**- Significant at 1% level, NS - Not significant.

Based on the analysis, it was found that the fabric characteristics of void spun silk fabrics were superior in terms of crimp, crease recovery, tensile properties, air permeability, bursting strength and drape characteristics.

Analysis of spun silk and void spun silk fabrics

The data (Table 4) revealed that the abrasion resistance of void spun silk fabrics produced in ring spinning process did not have significant difference, whereas all the other characteristics were found to be significantly different at 1 % level.

Arriving at the best trait fabric

In order to arrive at the best trait, an attempt was made to find out Z-scores for each character using the following formula (Muthulakshmi *et al*., 2016),

$$Z = (X - \mu) / \sigma$$

Table 5: Z score analysis of characteristics of dyed spun silk and void spun silk fabrics produced in ring spinning process

Particulars	Fabric thickness	Cover factor	Crimp Warp	Crimp Weft	Twist Warp	Twist Weft	Crease recovery	Flexural rigidity	Abrasion resistance	Air permeability	Drape coefficient	Bursting strength	Breaking load Warp	Breaking load Weft	Elongation Warp	Elongation weft	Z Score	Rank
Control spun silk	-1.67	-0.97	-1.41	-1.97	1.15	-0.18	-0.62	2.16	0.06	-0.56	1.16	0.02	-1.44	1.61	0.49	-1.73	-3.93	
Void spun silk 2 ply Japanese	-0.40	0.98	0.62	-0.58	-0.05	0.38	-0.36	0.05	0.28	0.27	1.13	-0.72	-0.73	0.53	-0.82	-0.25	0.34	2
Void spun silk 4 ply Japanese	0.00	1.16	0.80	0.97	0.37	-1.28	-0.19	-0.40	-0.90	0.79	-1.05	0.56	0.03	0.43	-0.57	1.26	2.80	1
Void spun silk 6 ply Japanese	-0.54	-0.28	-0.91	0.48	0.27	-0.92	-0.59	-0.87	0.33	1.73	-0.75	-1.26	0.93	-0.50	-0.71	-0.60	-4.21	
Void spun silk 2 ply Indian	0.78	0.64	1.56	0.08	-0.89	0.38	-0.51	-0.68	1.20	-0.79	-0.06	0.66	-0.41	0.43	-0.19	0.84	3.05	1
Void spun silk 4 ply Indian	1.26	-0.04	0.49	0.76	-0.62	0.03	-0.29	-0.63	-0.22	-0.85	0.11	0.80	0.78	-1.24	1.02	0.36	1.70	2
Void spun silk 6 ply Indian	1.22	-1.49	-0.51	0.66	-0.50	-0.08	-0.03	0.37	-1.56	-0.90	-0.53	1.30	0.85	-1.25	2.04	0.12	-0.29	

Where, X is the mean of each trait under each character and

μ is the population mean of the trait and

σ is the population Standard deviation.

These Z-Scores were calculated for each of the fabric characteristic to arrive at the best. From the results given in Table 5, it could be observed that void spun silk fabrics produced with 4 ply Japanese PVA yarn and 2 ply Indian PVA yarn in spinning process have scored the highest indicating that both are superior having better fabric characteristics followed by void spun silk fabrics produced with 2 ply Japanese PVA and 4 ply Indian PVA yarns. The void spun silk fabrics produced with 6 ply Japanese as well as Indian PVA yarns did not have better fabric characteristics and were difficult to spin when compared with other yarns used in the study, due to its thickness.

Subjective analysis of the void silk fabrics

In order to assess consumer preference of the void silk fabrics, about 2 meters of each variety of fabric were presented to different people for subjective assessment. The fabrics were assessed for appearance, lustre, surface finish, drape and bulkiness with scale of 1 to 5. In appearance, 1 means plain without crepe effect and 5 means prominent crepe effect, in lustre, 1 means dull, 5 means shiny, in surface finish, 1 means poor and 5 means very good, in drape, 1 means stiff, poor drape, 5 means good drape, in compression, 1 means thin and 5 means bulky. The data on the subjective assessment of the fabrics by various people like business men, consumers, researchers, traders, *etc*., were analysed based on weighted average method. The subjective assessment data are presented in Table 6 and it was found that the void spun silk fabrics produced with 4 ply Japanese PVA yarn and 2 ply Indian PVA yarn in spinning process has attracted many consumers.

Analysis of thermal comfort characteristics of spun silk and void spun silk fabrics

Q-max value: The Q-max value largely depends on the

Table 6: Subjective analysis of characteristics of dyed spun silk and void spun silk fabrics produced in ring spinning process

Particulars	Appearance	Lustre	Surface feel	Drape	Compression	Total	Rank
Control 40s spun silk	5.2	7.6	7.6	7.4	10.2	38.0	
Void spun silk 2 ply Japanese	9.2	9.8	10.7	12.4	10.1	52.2	
Void spun silk 4 ply Japanese	12.8	10.4	12.8	14.2	10.8	61.0	1
Void spun silk 6 ply Japanese	9.6	9.6	11.6	13.8	12.4	57.0	2
Void spun silk 2 ply Indian	13.6	11.6	14.0	13.7	12.5	65.4	1
Void spun silk 4 ply Indian	11.0	10.0	11.4	13.9	10.4	56.7	2
Void spun silk 6 ply Indian	5.8	7.6	9.4	9.4	10.6	42.8	

contact area between skin and fabric surface. Thus, the surface characteristic of a material is important to sensations on how warm or cool a fabric feels to an individual. The Q-max values of spun silk and void spun silk produced with 2, 4 and 6 ply Japanese and Indian PVA yarns in spinning process are provided in Table 7 and, it could be seen that, there is no significant difference between the Q-max values of spun silk and void spun silk fabrics produced in ring spinning process.

Clothing insulation value (CLO): The Clothing insulation values of spun silk and void spun silk produced with 2, 4 and 6 ply Japanese and Indian PVA yarns in spinning process are displayed in Table 7 wherein it is evident that, the CLO values of void spun silk fabrics are slightly higher than that of spun silk fabrics. The void spun silk fabrics produced with 4 ply Japanese PVA yarns and 2 ply Indian PVA yarns in spinning process have shown maximum CLO values of 0.53 and 0.56, respectively.

Thermal insulation value (TIV): From the results given in Table 7, it could be seen that, the TIV values of void spun silk are slightly higher than that of spun silk fabrics. The void spun silk fabrics produced with 4 ply Japanese PVA yarns and 2 ply Indian PVA yarns in spinning process have shown maximum TIV values of 33.08 and 37.22, respectively.

Factor analysis of the void spun silk fabric characteristics: In order to confirm the above results, the data were subjected to Factor analysis (principle component) and confirmatory factor analysis in order to reduce and group identical factors together, which in turn explains observed variations in the larger number of variables. Further, Kaiser Meyer Olkin (KMO) and Bartlett's tests (measures the strength of relationship among the variables) were attempted, which showed a value more than 0.5 (significant at 5 % level), indicating the acceptance of Barley.

Table 7: Thermal comfort characteristics of spun silk and void spun silk fabrics produced in ring spinning process

Fabric sample	Q – max (W / cm^2)	Clothing insulation value (CLO)	Thermal insulation value (TIV)
Spun silk	0.11 (0.002)	0.49 (0.001)	28.50 (0.46)
Void spun silk 2 ply Japanese	0.11 (0.002)	0.47 (0.001)	25.67 (0.62)
Void spun silk 4 ply Japanese	0.09 (0.001)	0.53 (0.001)	33.08 (0.46)
Void spun silk 6 ply Japanese	0.10 (0.002)	0.51 (0.001)	31.44 (0.46)
Void spun silk 2 ply Indian	0.09 (0.001)	0.56 (0.001)	37.22 (0.46)
Void spun silk 4 ply Indian	0.09 (0.002)	0.55 (0.001)	35.59 (0.46)
Void spun silk 6 ply Indian	0.10 (0.001)	0.42 (0.001)	15.32 (0.62)

Values in parentheses are standard deviation of mean values.

Eigen value reflects the number of extracted factors whose sum should be equal to number of items which are subjected to factor analysis. Eigen value of more than 1 has reduced the level of number of factors to 2 in the present study. Further, the rotation component matrix of void raw silk fabrics are given in Table 8.

From Table 8, it could be observed that the void spun silk produced with 4 ply Japan PVA yarns in spinning process has shown the highest positive loading on cover factor, crease recovery, abrasion resistance, air permeability, drape coefficient and bursting strength and negative

Table 8: Rotation component matrix of void spun silk fabrics produced in ring spinning process

Characteristic	Void spun silk produced with 4 ply Japan PVA yarn		Void spun silk produced with 2 ply Indian PVA yarn	
	Component 1	Component 2	Component 1	Component 2
Fabric thickness	-0.997			-0.996
Cover factor	0.998			0.827
Crimp warp		0.901	-0.639	0.769
Crimp weft		-0.918	0.998	
Twist warp		0.942	-0.919	
Twist weft		0.967	0.600	-0.800
Breaking load – warp		0.995		0.987
Breaking load – weft	-0.999			0.898
Elongation – warp		0.985		0.918
Elongation – weft	-0.997			0.984
Crease recovery	0.918		0.747	0.665
Flexural rigidity		0.997	0.992	
Abrasion resistance	0.998		0.986	
Air permeability	0.709	0.706	0.973	
Drape coefficient	0.827		-0.938	
Bursting strength	0.827		-0.938	

Extraction method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.

loading on fabric thickness, breaking load weft and elongation weft under component 1, and the highest positive loading on crimp warp, twist warp & weft, flexural rigidity, breaking load warp and elongation warp and negative loading on crimp weft under component 2. The void spun silk with 2 ply Indian PVA yarns has shown highest positive load on crimp weft, crease recovery, flexural rigidity, abrasion resistance, air permeability, crimp weft, twist warp, drape coefficient and bursting strength under component 1, and also has shown the highest load on cover factor, crimp warp, breaking load warp & weft, elongation warp & weft, fabric thickness and twist weft under component 2.

Conclusion

The present study has undoubtedly brought out the superiority of void spun silk fabrics in terms of crimp, crease recovery, tensile properties, air permeability, bursting strength and drape characteristics. The ANOVA results indicate that all characteristics except abrasion resistance were found to be significantly different at 1 % level among the 7 samples taken for study. The higher Z-score and subjective analyses' results revealed that void spun silk fabrics produced with 2 ply Indian PVA yarns and 4 ply Japanese PVA yarns are the best followed by void spun silk fabrics produced with 4 ply Indian PVA yarn and 2 ply Japanese PVA yarns in spinning process.

The unique appearance developed on the surface of the fabrics due to removal of PVA yarn, could definitely create an appeal among the consumers and exporters. Thus it could be inferred that void spun silk fabrics produced in spinning process could be an effective alternative for new silk applications in the silk industry.

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Research Paper

IN SILICO STUDY OF QUORUM SENSING INHIBITION OF TWO BACTERIA ASSOCIATED WITH *BOMBYX MORI*

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ABSTRACT

Flacherie is a complicated and disastrous disease affecting mulberry silkworm, *Bombyx mori* due to synergistic bacterial and viral pathogenesis. The present study comprised of an evaluation of endophytic fungus metabolite, farnesol in inhibition of quorum sensing transcription factors of bacterial species affecting *B. mori*. Molecular interaction studies showed that the farnesol bound with LuxR from *S. aureus* and SpnIR from *S. marcescens* have significantly high free energies (ΔG) of -4.99 and -7.02 kcal/mol, respectively. The binding energies were comparable with that of commercial QStatin molecule. Farnesol imposed high inhibition to LuxR and SpnIR with Ki 160 and 6.25 µM. At the binding site, amino acid residue, Asn98 was actively involved in hydrogen bonding with LuxR and weak interactions, such as vdW with Leu76 and Asp73, alkyl and pi-alkyl with Ile71, Arg72 and His75 were involved. Farnesol formed polar hydrogen bond with Trp54 in SpnIR before attaining a stable conformation. QPLD and MMGBSA analyses showed much higher binding energies of farnesol at the binding sites -5.01 and -13.4, and -7.90 and -19.21, respectively, with LuxR and SpnIR. MD simulation studies exhibited the stable conformation at farnesol bound state of LuxR than unbound state displaying the complex stability at dynamic environment. Therefore, it can be suggested that farnesol might pave a paradigm shift in controlling bacterial flacherie by anti-quorum sensing activity.

Key words: *Bombyx mori*, flacherie, inhibitor, MD simulation, MMGBSA, quorum sensing.

INTRODUCTION

Flacherie is a synergistic pathogenesis of virus and bacteria and hence, the most complicated and lethal to silkworm. There are many bacterial species usually associated with flacherie in *Bombyx mori e.g., Staphylococcus aureus*, *Streptococcus fecalis*, *Bacillus subtilis* and *Serratia marcescens* (Rosalind and Zengzhi, 2012; Vootla *et al.*, 2013). Viral species *viz.,* flacherie virus (BmIFV) and densovirus (BmDNV) are frequently indulging in viral flacherie in *B. mori*, alone or in association with bacterial species (Rosalind and Zengzhi, 2012). Bacterial colonization, biofilm formations and pathogenesis are regulated by concerted mechanism and physiological process known as quorum sensing mediated via small diffusible molecules (Li and Tian, 2012). The regulatory proteins, such as transcription factors, LuxR and VqmA in *Vibrio cholerie*, TraR of *Agrobacterium tumefaciens*, LuxS in *B. subtilis*, LuxR in *S. aureus,* SpnIR in *S. marcescens* and LasR in *Pseudomonas aeruginosa* play vital role in quorum sensing activation and processing (Wei *et al.,* 2006; White and Winans, 2007). Therefore, quorum sensing is a major concern in bacterial flacherie in *B. mori*. Several reports of anti-quorum sensing molecules have shown the inhibitory effects on the regulatory transcription factors that play a major role in activation of quorum sensing in pathogenic bacterial species. One such potent molecule is commercially available as QStatin [1-(5-bromothiophene-2-sulfonyl)-1H-pyrazole], which has evidenced high quorum sensing (QS) inhibition in *Vibrio* sp. by selectively inhibiting LuxR transcripton regulator (Asfour, 2018; Kim *et al*., 2018). There are many natural compounds of plant origin having anti-QS activities reported elsewhere

(Teplitski *et al*., 2000; Choo *et al*., 2006; Isaac-Abraham *et al*., 2011; Hayek *et al*., 2013). Fungi are potential reservoirs for bacterial-QS inhibitor molecules. *Phomopsis tersa*, an endophytic fungi was reported to synthesize 3-Isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione complex, identified in the crude extract which exhibited anti-QS and inhibition of biofilm formation in *P. aeruginosa* (Meena *et al*., 2020). Aquatic endophytic fungi*, Sarocladium* sp., *Fusarium* sp., *Epicoccum* sp. and *Khuskia* sp. isolated from marine coral organism were reported to produce anti-QS molecules displaying inhibiton against *Chromobacterium violaceum* CVO26 (Martín-Rodríguez *et al.,* 2014). Therefore, evidences suggested that fungal endophytes might serve as potent inhibitor molecules against bacterial quorum sensing involved in flacherie in *B. mori*. In the present study, an *in silico* approach was established to study the secondary metabolite, farnesol that involves in the squalene synthesis pathway of various endophytic fungi as potent anti-QS molecule. Molecular interaction studies were performed against quorum sensing protein regulators (transcription factors) to establish the effectiveness of farnesol in comparison with commercial anti-QS molecule.

MATERIALS AND METHODS

System information

For the experimental analysis, the computational approach was carried out in HP Workstation core i7, 3.9 GHZ processor, 4 GB NVIDIA Geforce graphics, 8 MB RAM, 1TB HDD.

Protein structure modeling and refinement

Bacterial flacherie in *B. mori* is potentially manifested by *S. aureus* and *S. marcescens* as evidenced from earlier reports (Wei *et al.,* 2006; White and Winans, 2007). As the X-ray crystal structure of quorum sensing protein (SpnIR) from *S. marcescens* is lacking in the protein data bank, the structure was homology modeled using the protein sequence (Uniprot ID: Q8GQM9) using Modeller vs 9.24 (Webb and Sali, 2016). Afterwards, the quality of the modeled structure was analyzed in PROCHECK web server (https://servicesn.mbi.ucla.edu/PROCHECK/) for verifying 3D, Errat plot and Ramachandran plot. The modeled SpnIR was further validated for any missing side chain residues in What If Interface (https://swift.cmbi.umcn.nl/) to reduce any ambiguity before it was considered for molecular interaction studies.

Molecular docking and MM/GBSA analysis

In order to analyze the inhibitory effect of farnesol, quorum sensing regulatory transcription factors were examined in this study. The three dimensional coordinates of LuxR (PDB ID: 3B2N) of *S. aureus* at X-ray 1.8-3 Å resolution was retrieved from RCSB Protein Data Bank. In addition, modeled structure of SpnIR from *S. marcescens* was also taken as target for the docking study. 3D structures of farnesol and QStatin were fetched from pubchem database (https://pubchem.ncbi.nlm.nih.gov/) in SDF format and subsequently converted into pdb format using OpenBabel 2.2.3 (O'Boyle *et al*., 2011). Molecular interaction studies were carried out using the help of Autodock version 4.2.1.

In order to obtain accurate binding affinities for quercetin, QM-Polarized Ligand Docking (QPLD) was performed using Schrodinger 2018-4 package (https://www.schrodinger.com/qm-polarized-ligand-docking). Quantum mechanics ligand docking gave accurate treatment of electrostatic charges to farnesol to avoid charge polarization induced by the LuxR and SpnIR environment. QPLD combines the docking power of Glide with the accuracy of QSite, Schrödinger's respected QM/MM software. In order to perform QM docking, glide dockings were performed within a particular grid size followed by addition of QM charges using Jaguar tool embedded in the Schrodinger 2018-4 package. Then redocking was performed in high precision with an approximate ligand van der Waals spacing of 0.8 Å and maximum atomic displacement, 1.3 Å. A with maximum of 10 ligand-docking poses generated with RMS deviation 0.5 Å.

The relative binding affinities of protein ligand complex was estimated employing Molecular mechanics with generalized born surface area (MM/GBSA). The relative binding free energy, ΔG_{bind} were estimated according to the following equation:

$$\Delta G_{bind} = E_{complex}(minimized) - [E_{ligand}(unbound, minimized) + E_{receptor}(unbound, minimized)]$$

G_{bind} is the calculated relative free energy that includes both ligand and receptor strain energy. $E_{complex}$ (minimized) is the MM/GBSA energy of the minimized complex, and E_{ligand} (unbound, minimized) is the MM/GBSA energy of the ligand after removal from the complex and allowed to relax. $E_{receptor}$ (unbound, minimized) is the MM/GBSA energy of protein after separating it from the ligand (Bathini *et al*., 2016). Prime MM/GMSA module was used for calculating the relative binding free energy for each molecule. The protein ligand complexes obtained from QPLD docking were subjected to MM/GBSA calculations. MM/GBSA calculations were performed to estimate relative binding affinity of ligands to the receptor. MM/GBSA calculations were performed for the best ranking molecules in XP mode. MM/GBSA was used for free energy based ranking of ligands belonging to a congeneric series. MM/GBSA and docking protocols employed in the present work for assessing ligand affinities to quercetin allow protein flexibility and therefore, give reliable result (Bathini *et al*., 2016).

Molecular dynamics and simulation (MD) and quantum mechanics studies

Molecular dynamics studies of LuxR docked with farnesol molecule were carried out using GROMACSv19.1 in a Linux based operating system (Ubuntu vs16.1), NVIDIA Geforece graphics, intel core i7 processor with 8 GB RAM. Before the simulation processes, the protein and ligand molecules were separated using VMD vs 1.9.3 (https://www.ks.uiuc.edu/Research/vmd/current/) using a Tcl console. Protein topology and force field parameters were set according to native AMBER99SB-ILDN force field integrated in GROMACS vs 5.1. Energy minimization was carried out applying both conjugate gradient and steepest descent algorithms for 100 ps. NPT and NVT ensembles were carried out at 310 K with a standard atmospheric pressure of 1 pa. The final molecular dynamics run was carried out for 10 ns time scale.

RESULTS

Homology modeled protein structure of SpnIR after structure quality analysis displayed a good architecture (Figure 1a). The outcomes from PROCHECK in the form of Errat plot analysis suggested >90 % amino acid residues qualified least errors at $p<0.05$ level of significance (Figure 1). Ramachandran plot of protein secondary structure analysis exhibited 92 % residues at most favored regions of Psi and Phi angles (Figure 1b). Moreover, 7 % residues were in additionally allowed region and 1 % in generously allowed regions (Figure 1b). Therefore, the overall predicted structure of SpnIR was of good quality with dope score, -0.2.

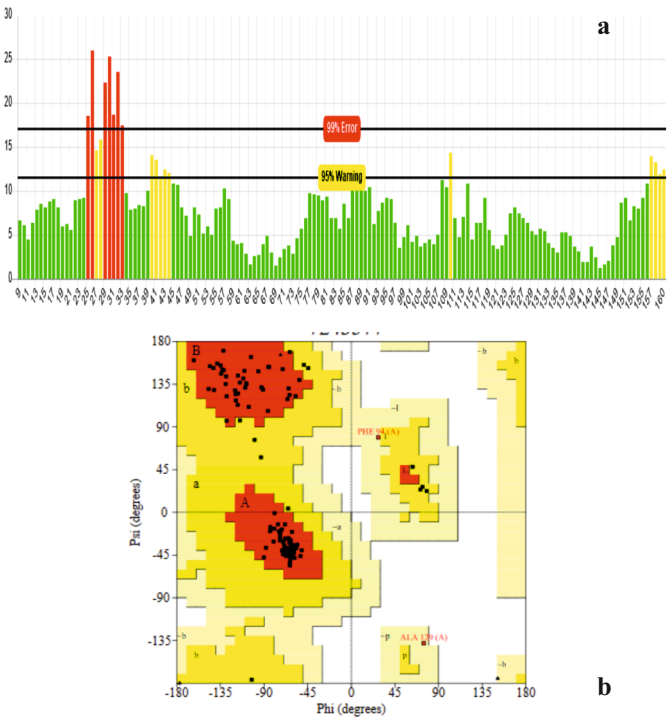

Figure 1: (a) Errat plot and (b) Ramachandran plot of homology modeled structure of SpnIR of *S. marscescens*

The molecular surface of LuxR from *S. aureus* at 1.8-3 Å atomic resolution and the homology modeled structure of SpnIR from *S. marcescens* are displayed in Figure 2. The ligand binding site of the transcription factor, LuxR displayed 227.01 $Å^3$ volume of binding cavity, 632.2 $Å^2$ of surface area and 0.73, druggability score. Similarly, SpnIR displayed 564.1 $Å^3$ of volume, 779.77 $Å^2$ of surface area and 0.72, druggability score. Ligand binding sites were predicted and displayed in Figure 3.

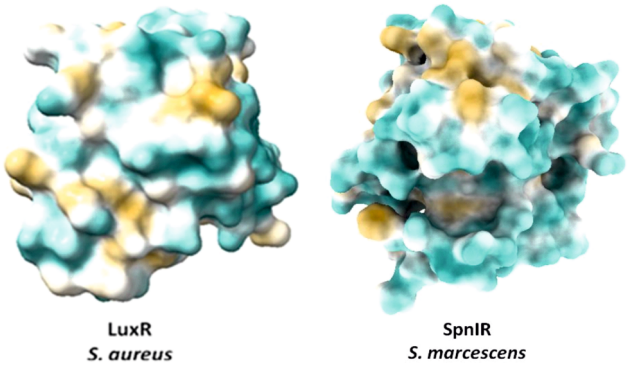

Figure 2: Surface view of LuxR from *S. aureus* at 1.8-3 Å atomic resolution and homology modeled SpnIR of *S. marcescens*

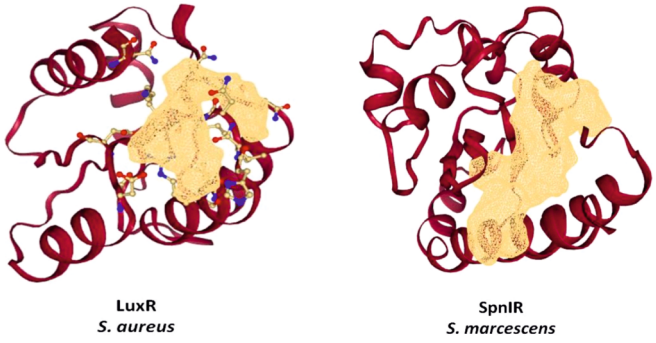

Figure 3: Predicted ligand binding sites for farnesol and QStatin in LuxR from *S. aureus* and SpnIR of *S. marcescens*

Molecular docking analysis of the transcription factors, LuxR and SpnIR with farnesol and commercial QStatin in autodock output confirmed the specific binding (Table 1). The best binding conformations of LuxR and SpnIR with farnesol are displayed in dock complex (Figure 4).

Binding of LuxR with farnesol displayed moderate binding energy (ΔG) of -4.99 kcal/mol, with inhibitory concentration (Ki), 160 µM. The total free energy of binding was associated with the contribution of other kinds of energy, such as Vander Waals (vdW) dissociation energy (ΔE) - 6.47 kcal / mol, electrostatic energy (eV), -0.17 and torsional energy, 2.39 (Table 1). Docked pose displayed the cavity of farnesol binding formed at the surface with saddle shaped structure (Figure 4 a). Close view of binding site of farnesol at LuxR showed only a single polar hydrogen bond formed with amino acid, Asn98 (Figures 4 b and 4 c). In addition to hydrogen bonding, some weak interactions occur, such as vdW with Leu76 and Asp73, alkyl and pi-alkyl with Ile71, Arg72 and His75 (Table 2). Whereas, SpnIR from *S. marcescens* displayed much higher binding with farnesol [(ΔG) -7.02 kcal/mol; Ki 6.25 µM] (Table 1, Figure 4 d). Binding site cavity displayed the conventional polar hydrogen bond formed between farnesol, and Trp54 of SpnIR (Table 2, Figures 4 e and 4 f).

On the other hand, QStatin displayed similar but little higher binding energies of (ΔG) -5.16 and -7.35 kcal/mol with LuxR and SpnIR, respectively than farnesol (Table 1, Figure 5).

The inhibitory concentrations (Ki) of QStatin with LuxR and SpnIR were 140 and 4.12 µM, respectively (Table 1).

The docking energies of farnesol-LuxR and SpnIR complex calculated in glide displayed the highest ligand binding affinity with -5.01 and -7.90 kcal/mol, respectively, whereas, QPLD with QM and MM optimized

Table 1: Molecular interaction energies of quorum sensing proteins with natural Farnesol and commercial QStatin compounds

Complex	Binding energy (ΔG) (kcal/mol)	Ligand efficiency	Inhibitory concentration Ki (µM)	vdW Dissociation energy (ΔE) kcal/mol	Electrostatic energy (eV)	Torsion energy
LuxR - Farnesol	-4.99	-0.26	160	-6.47	-0.17	2.39
LuxR - QStatin	-5.16	-0.38	140	-5.82	-0.04	0.60
SpnIR-Farnesol	-7.02	-0.40	6.25	-8.00	-0.08	0.77
SpnIR-QStatin	-7.35	-0.53	4.12	-7.84	-0.05	0.60

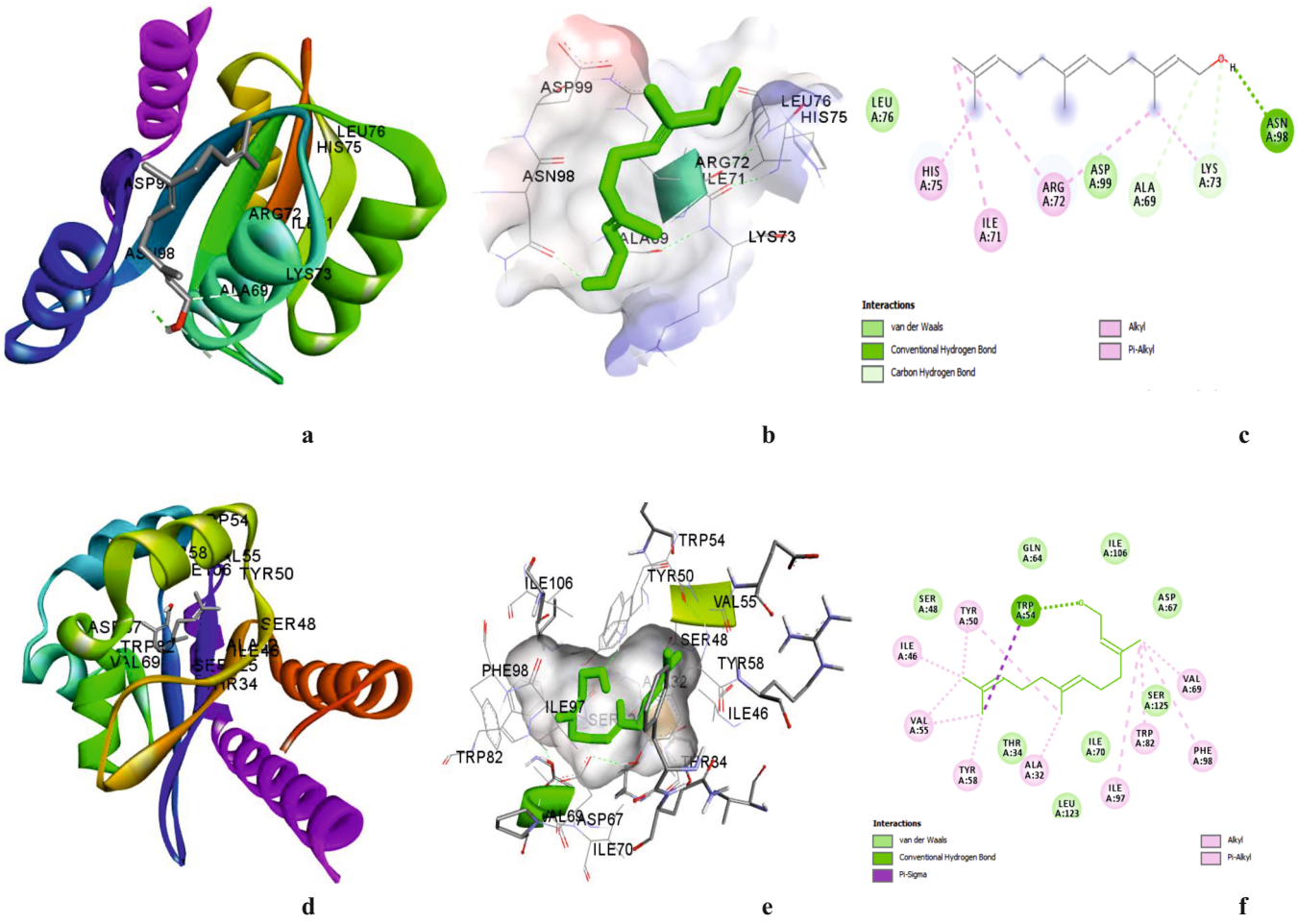

Figure 4: Molecular docking analysis exhibited docked pose (a, d), molecular surface of propensity hydrogen boding site (b, e) and wireframe view of molecular interactions of farnesol with the binding site residues (c, f) of LuxR and SpnIR, respectively.

redocking following extra precision protocol (XP) showed -5.77 and -9.1 kcal/mol, respectively, binding free energies at the same binding site used in autodock tool. The correlation between experimental free energy binding (ΔG_{bind}) from the best dock results as found in QPLD and with the predicted GBSA showed good correlation coefficient with R^2=0.82. From the correlation analysis, it could be deciphered that the docked farnesol made significant binding with LuxR and SpnIR with solvent accessible binding energy of -13.4 and -19.21 kcal/mol, repectively. In addition, $\Delta G_{H\text{-}bond}$ and $\Delta G_{bind\text{-}vdW}$ contributed the most significant energies during the complex formation between farnesol and LuxR and SpnIR, respectively (Table 2).

Molecular dynamics and simulation (MD) and quantum mechanics studies

Molecular dynamics and simulation study of the LuxR and LuxR-farnesol complex is displayed in Figure 6. The dynamics study showed slight distorted C-α backbone conformation of LuxR upon farnesol binding as compared to the energy minimized apo-protein up to 5 ns of simulation. However, final stability of LuxR achieved with farnesol bound state is at around 10 ns of simulation. After 10 ns, the total RMSD shift in quercetin bound complex was observed to be 0.1 which is much lesser as compared to the protein backbone without ligand at 310 K temperature (Figure 6 a). Root mean square fluctuations

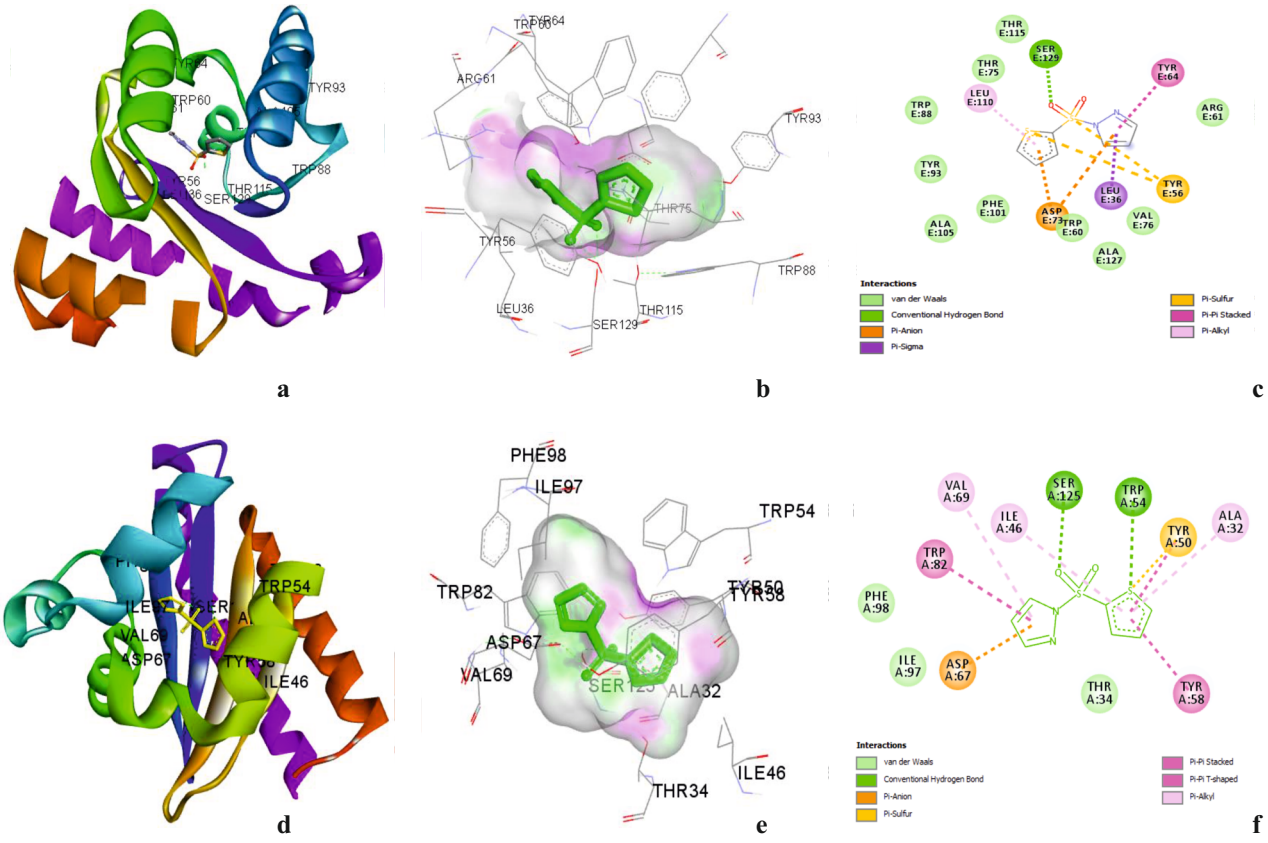

Figure 5: Molecular docking analysis exhibited docked pose (a, d), molecular surface of propensity hydrogen boding site (b, e) and wireframe view of molecular interactions of QStatin with the binding site residues (c, f) of LuxR and SpnIR, respectively.

Table 2: Comparative analyses of farnesol as inhibitor against various quorum sensing regulatory proteins (transcription factors) in bacteria

Transcription factor	PDB ID	Organism	Glide (ΔG) (kcal/mol)	MM/GBSA (ΔG) (kcal/mol)	Polar H-bonding
LuxR	3B2N	*Staphylococcus aureus*	-5.01	-13.40	Asn98
SpnIR	–	*Serratia marcescens*	-7.90	-19.21	Trp54

– = Modeled SpnIR protein

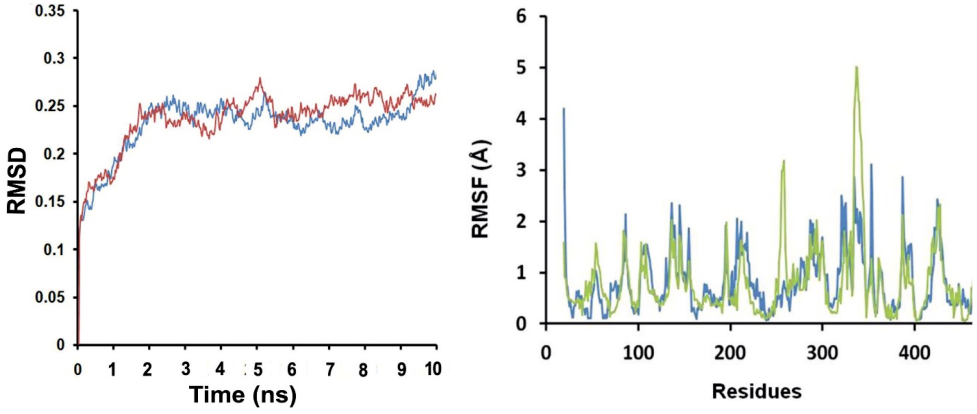

Figure 6: (a) RMSD plot of LuxR backbone at 310 K with respect to 10 ns time scale in molecular dynamics and simulations study; red line representing farnesol bound complex and blue line without farnesol, (b) RMSF plot of LuxR displaying the Cα-backbone fluctuations with farnesol bound (blue) and unbound (green) state.

(RMSF) of the amino acid residues forming the C-α backbone of protein chain displayed the stable conformation of quercetin farnesol bound LuxR as compared to unbound state (Figure 6 b). Therefore, on binding of farnesol with LuxR, forms a stable conformation, which is required for better inhibition activity.

DISCUSSION

Bacterial flacherie is of great concern to mulberry sericulture practicing farmers. The bacterial colonization within silkworm body facilitates the invasion by viral species too. *In silico* approach gives high throughput analysis of targeting the bacterial species against their quorum sensing behavior before colonization and pathogenesis. Farnesol is an intermediate metabolite as well as byproduct of squalene biosynthesis in fungi (Jacob *et al*., 2003). Molecular interaction studies in docking showed significant inhibition of quorum sensing transcription factors that may inhibit the bacterial colonization as reported earlier by Unnanuntana *et al*. (2009). Inhibitory concentrations of farnesol against quorum sensing regulatory transcription factors LuxR from *S. aureus* and SpnIR from *S. marcescens* exhibited significant inhibition when compared with commercial QStatin. Due to surface binding at the transcription factors, farnesol displayed lower ligand efficiency but, high inhibition. Higher inhibition of QStatin was due to small molecular architecture than farnesol. Interestingly, it was observed that although farnesol has a much bigger size, it displayed higher binding corroborating with commercial QStatin. MM/GBSA studies after quantum-polarized ligand docking showed much higher binding energies at the atomic level. This is the first ever report of farnesol from endophytic fungi as wide spectrum of anti-QS agent. Farnesol, in this study, exhibited much higher binding energies with the transcription factors as well as better inhibition as compared to the earlier evidence of FDA approved drugs (Aliyua *et al*., 2016; Mellini *et al*., 2019). Molecular dynamics and simulation study discern the role of farnesol at highest molecular vibrations and interactions conforming a stable architecture. RMSD and RMSF exhibited that farnesol at dynamic environment in water stabilizes the C-α backbone, which is an essential requirement to active inhibition of the transcription factors that stimulate quorum sensing. Therefore, *in silico* study of farnesol active against quorum sensing proteins may pave the future use for protecting *B. mori* from bacterial flacherie after formulation and experimental application in the field.

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Research Paper

SILK UNION (BACKED) FABRICS – A PROMISING PRODUCT FOR THE SILK APPAREL SECTOR

PART I: FABRIC HAND OF SILK UNION (BACKED) FABRICS

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ABSTRACT

Different varieties of Silk union (backed) fabrics with varied fabric construction parameters and different material for back were developed, evaluated, characterised and compared with respect to fabric Hand. Following the guidelines provided by Kawabata, based on the Total hand value (THV), their suitability for ladies wear for different seasons was evaluated and were graded accordingly. The results draw a conclusion that fabrics with sateen weave stand out as the best, followed by twill and the plain weave. Fabrics with both cotton and modal used as the backing perform equally well. From the grading, it is evident that all the varieties of fabrics developed have good suitability to ladies wear for winter than for summer season. Therefore, they can be a promising product for ladies wear and the silk apparel sector as a whole in the near future.

Key words: Backed fabrics, Dupion silk, fabric hand, modal, Soft silk, Total Hand Value (THV).

INTRODUCTION

The basic requirement of a clothing material is to provide a good fit to the human body and be adaptable to its movements. The primary function is to protect the human body from different environments and from injury to the skin caused by mechanical contact with external materials. Textile fabrics produced from natural fibres have been used by mankind since ages as the most suitable materials for clothing and at a later stage, man-made fibres have been added to this category (Kawabata and Masako Niwa, 1989).

The measurement of low stress mechanical properties using the Kawabata Fabric Evaluation system or the (KES-F) system and evaluation of fabric hand value and then assessing the suitability of fabrics in garment manufacture has become popular among textile researchers. This system is also expected to aid the development of newer products in the future. Total hand value (THV) represents an assessment of the overall quality of the fabric and is a measure of its value in the market or in other words, its selling appeal to the consumer (Behra and Shakyawar, 2000).

Silk fabrics are used in the preparation of ladies blouses by a large segment of Indian consumers of silk sarees and also the silk apparel sector. While converting some silk fabrics into ladies blouses and garments, a lining material is attached at the back of the fabric in order to provide the required body to the fabric while stitching and a proper garment fit to the consumer. Many a time, a cheaper cotton fabric is used as lining and though the silk face fabric has a colour with good fastness properties, due to the use of cheaper cotton lining material with not very good fastness properties, the lining colour bleeds on to the silk face fabric and in the process, damages the blouse or the whole garment itself. In addition to this, the lining attachment makes the blouse/garment heavier and hence uncomfortable to wear during summer. Further, the cost of

conversion into a garment with lining is higher than that of the cost of conversion of a single silk fabric. In order to address this problem, it was envisaged to develop a union (backed) single silk fabric which can eliminate the use of a separate lining material on one hand and at the same time, provide a material with better fastness and comfort properties and also save the cost of conversion into a blouse or a garment. Therefore, this study was aimed at developing and characterising silk union (backed) fabrics with varied fabric construction parameters and different material for back to cater to the production of ladies blouses and garments and also develop a database on structural, mechanical, low stress mechanical, garment related and comfort properties of silk union (backed) fabrics for the use of manufacturers, converters and consumers of silk.

The review of literature revealed that some research has been carried out with respect to evaluation and characterisation of pure silk fabrics, wool, cotton, linen and silk union fabrics (Ron Postle and Gu Ping, 1994; Sharma *et al*., 2000; Behra, 2007; Behra and Mishra, 2007) but, there is little information available on the development and characterisation of silk union (backed) fabrics.

For the present study, fabric samples consisting twelve Soft silk and twelve Dupion silk varieties with varied fabric construction parameters and different material for back to cater to the production of ladies blouses and garments were developed. These fabrics were subjected to testing of structural, functional, aesthetic, low stress mechanical and comfort properties objectively. From the data generated, the fabrics were characterised and compared. In this paper, the findings with respect to fabric hand are reported. The Total Hand value (THV) was calculated from the KES data and this was used to evaluate their suitability for ladies wear for various seasons with reference to the guidelines provided by Kawabata and were graded accordingly (Kawabata and Masako Niwa, 1982; INOUE Takako and NIWA Masako, 2003).

MATERIALS AND METHODS

Varieties of Soft silk and Dupion silk (backed) fabric samples were developed using good quality mulberry raw silk and Dupion silk yarn reeled on Automatic reeling machine (ARM) and good quality Cotton (combed and mercerized) and micro Modal yarn. The yarns were prepared in different plies, twist levels and shades.

The details of the structural parameters of Soft silk and Dupion silk (backed) fabrics are given in Table 1.

Table 1: Structural parameters of Soft silk (backed) & Dupion silk (backed) fabrics

Fabric Particulars / Weave	Plain	Twill	Sateen
Ends per inch	200	200	200
Picks per inch	80	100	100

Fabric construction parameters such as fabric sett, cover factor and weave, considerably change the performance of the fabric particularly in respect of low stress mechanical properties (Kawabata and Masako Niwa, 1989). With this backdrop, two sets of fabrics were developed. One set was of a single fabric and the other as two separate layers (face & back) keeping all the yarn and fabric construction parameters constant to facilitate the comparison of the samples. The idea of developing fabrics in two layers was to compare them with respect to the hand properties of the fabrics with a separate lining material. The samples developed consisted of twelve Soft silk and twelve Dupion silk varieties. The codes for Soft silk and Dupion silk fabrics woven with Cotton Back and Modal Back are provided in Table 2 and 2A, respectively.

These fabric samples were subjected to testing of structural, functional, aesthetic, low stress mechanical and comfort properties objectively, adopting standard methods for testing under controlled environmental conditions. From the data generated, the fabrics were characterised. The THV was calculated from the KES data and this was used to evaluate their suitability for ladies wear for both summer and winter seasons with reference to the guidelines provided by Kawabata and were graded accordingly. From these results, the conclusions were drawn.

Table 2: Fabric codes of Soft silk & Dupion silk (backed) fabrics woven with cotton backing

Fabric type	Single layer			Double layer		
	Plain	Twill	Sateen	Plain	Twill	Sateen
Soft silk	SS-PB-C(F)	SS-TB-C(F)	SS-SB-C(F)	SS-P-C(B)	SS-T-C(B)	SS-S-C(B)
	SS-PB-C(B)	SS-TB-C(B)	SS-SB-C(B)	SS-P-C(L)	SS-T-C(L)	SS-S-C(L)
Dupion silk	DUP-PB-C(F)	DUP-TB-C(F)	DUP-SB-C(F)	DUP-P-C(B)	DUP-T-C(B)	DUP-S-C(B)
	DUP-PB-C(B)	DUP-TB-C(B)	DUP-SB-C(B)	DUP-P-C(L)	DUP-T-C(L)	DUP-S-C(L)

Table 2A: Fabric codes of Soft silk & Dupion silk (backed) fabrics woven with modal backing

Fabric type	Single layer			Double layer		
	Plain	Twill	Sateen	Plain	Twill	Sateen
Soft silk	SS-PB-M(F)	SS-TB-M(F)	SS-SB-M(F)	SS-P-M(B)	SS-T-M(B)	SS-S-M(B)
	SS-PB-M(B)	SS-TB-M(B)	SS-SB-M(B)	SS-P-M(L)	SS-T-M(L)	SS-S-M(L)
Dupion silk	DUP-PB-M(F)	DUP-TB-M(F)	DUP-SB-M(F)	DUP-P-M(B)	DUP-T-M(B)	DUP-S-M(B)
	DUP-PB-M(B)	DUP-TB-M(B)	DUP-SB-M(B)	DUP-P-M(L)	DUP-T-M(L)	DUP-S-M(L)

(F) – Face, (B) – Back, of single layer fabric; (B) – Body layer, (L) - Lining layer, of double layer fabric

RESULTS AND DISCUSSION

1. Fabric weight, thickness & cover factor of Soft silk (backed) fabrics

1A. The details of the fabric weight and thickness of Soft silk (backed) fabrics woven as single layer are given in Figures1 and 2, respectively and fabric cover factor, in Figure 3.

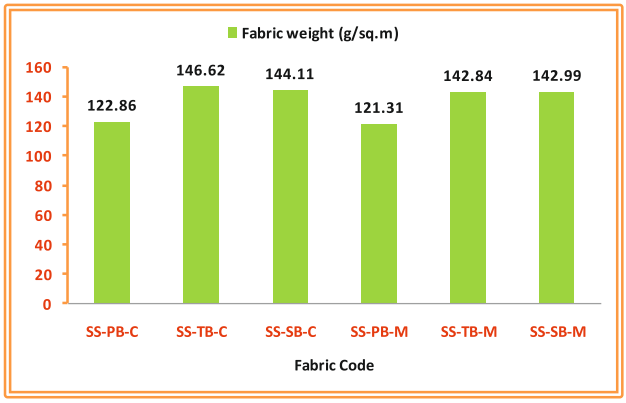

Figure 1: Weight of Soft silk (backed) fabrics (single layer)

It can be observed from Figure 1 that the weight of the fabric samples ranged between 121 and 147 grams. Soft silk fabrics woven with plain weave are lighter than the fabrics woven with twill and sateen weave and the difference is statistically significant at 95 % level of confidence [One way Anova - F (2, 27) = 977.906, p = 0.000]. Whereas, there is no significant difference (p = 0.051) between the weight of the fabrics woven with twill and sateen weave. Further, there is no significant difference in weight between the Soft silk fabrics of similar weave woven with cotton and modal for the backing [One way Anova - F (1, 28) = 0.298, p = 0.590].

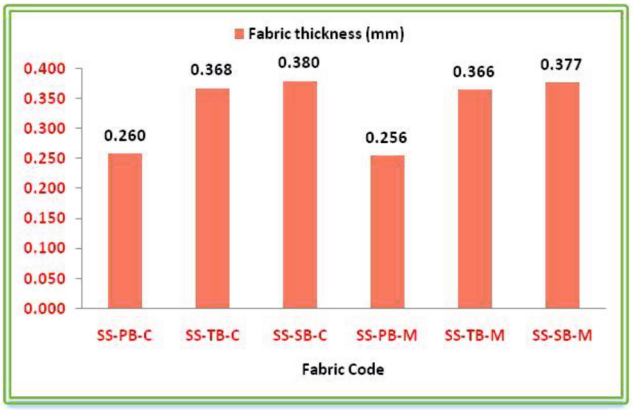

Figure 2: Thickness of Soft silk (backed) fabrics (single layer)

As seen in Figure 2, fabric thickness ranged between 0.260 and 0.380 mm and the difference in thickness is statistically significant at 95 % level of confidence among the fabric samples with different weaves [One way Anova - F (2, 57) = 3285.069, p = 0.000]. Whereas, there is no significant difference in thickness between the Soft silk fabrics of similar weave woven with cotton and modal for the backing [One way Anova - F (1, 58) = 0.057, p = 0.813] as all the yarn and fabric structural parameters were kept constant to facilitate the comparison between different

varieties of fabrics.

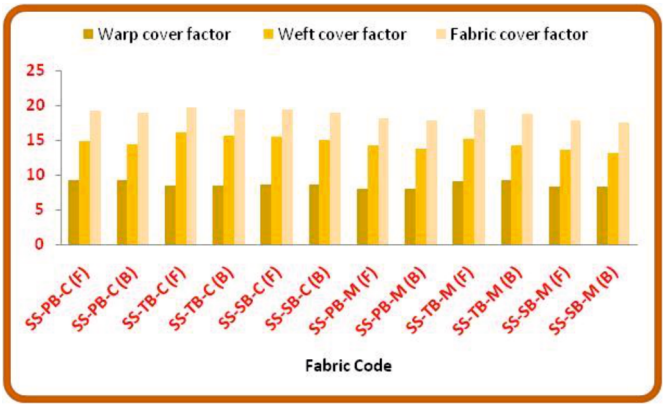

Figure 3: Cover factor of Soft silk (backed) fabrics (single layer)
(F) – Face, (B) – Back, of single layer fabric

Figure 3 reveals that there is no significant difference in the cover factor among the Soft silk fabrics woven with different weaves [Anova - F (2, 9) = 2.06, p = 0.183]. In the case of Soft silk fabrics woven with different material for the backing, the difference is statistically significant at 95 % level of confidence [Anova - F (1, 10) = 11.144, p = 0.008].

1B. The details of the weight and thickness of Soft silk (backed) fabrics woven as two layers are given in Figures 4 and 5, respectively and fabric cover factor, in Figure 6.

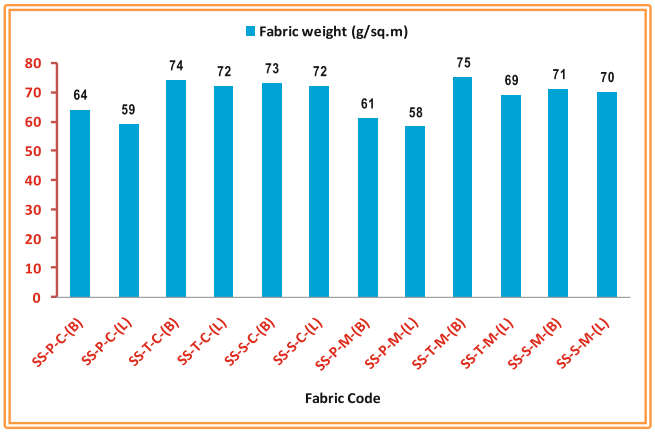

Figure 4: Weight of Soft silk (backed) fabrics (two layers)

The weight of the fabric samples ranged between 119 and 146 grams (Figure 4). The weight in this case is the sum of body layer (B) and lining layer (L). Even in this case, soft silk fabrics woven with plain weave are lighter than the fabrics woven with twill and sateen weave and the difference is statistically significant at 95 % level of confidence [One way Anova - F (2, 57) = 215.064, p = 0.000]. Whereas, there is no significant difference between the weight of the fabrics woven with twill and sateen weave (p=0.125). There is no significant difference in weight between the Soft silk fabrics of similar weave woven with cotton and modal for the backing [One way Anova – F (1, 58) = 1.229, p = 0.272].

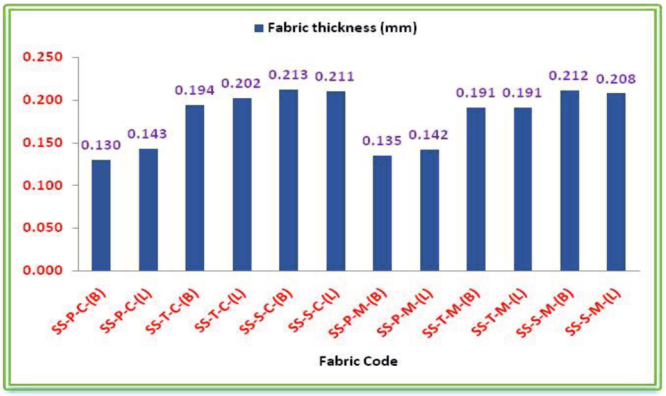

Figure 5: Thickness of Soft silk (backed) fabrics (two layers)

As depicted in Figure 5, fabric thickness (sum of body layer and lining layer) ranged between 0.273 and 0.424 mm. The difference in thickness is statistically significant at 95 % level of confidence [One way Anova - F (2,117) = 2427.110, p = 0.000] among all the fabric samples woven with different weaves. There is no significant difference between the Soft silk fabrics of similar weave woven with cotton and modal for the backing [One way Anova - F (1,118) = 0.159, p = 0.691], as all the yarn and fabric structural parameters were kept constant to facilitate the comparison between different varieties of fabrics.

Figure 6 reveals that there is no significant difference between the cover factor of the Soft silk fabrics woven with different weaves and different material for the backing and as separate layers at 95 % level of confidence [Anova - F (2, 9) = 3.940, p = 0.059].

2. Fabric weight, thickness and cover factor of Dupion silk (backed) fabrics

2A. The details of the fabric weight and thickness of

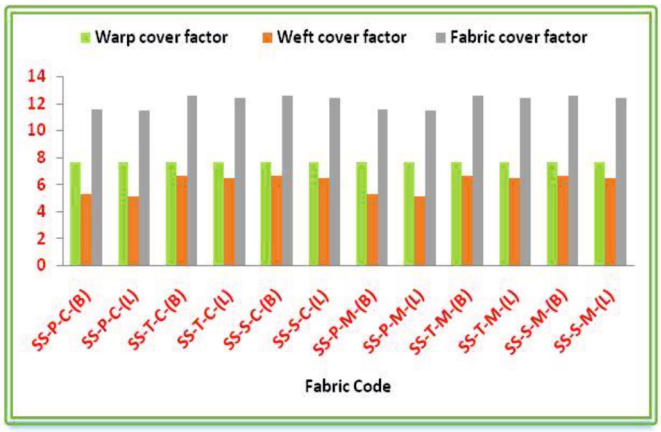

Figure 6: Cover factor of Soft silk (backed) fabrics (two layers)

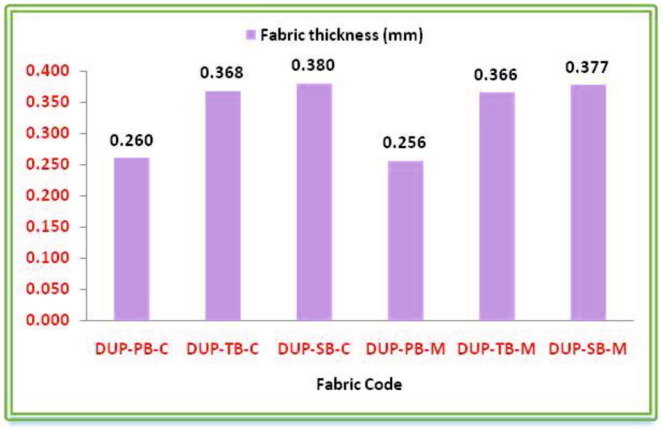

Figure 8: Thickness of Dupion silk (backed) fabrics (single layer)

Dupion silk (backed) fabrics woven as single layer are given in Figures 7 and 8, respectively and fabric cover factor, in Figure 9.

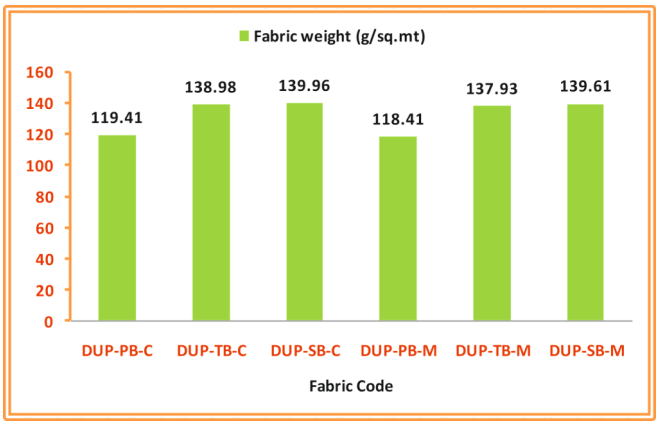

Figure 7: Weight of Dupion silk (backed) fabrics (single layer)

It can be observed from Figure 7 that the weight of the fabric samples ranged between 118 and 140 grams. Dupion silk fabrics woven with plain weave are lighter than the fabrics woven with twill and sateen weave and the difference is statistically significant at 95 % level of confidence [One way Anova - F (2, 27) = 7780.500, p = 0.000]. Whereas, there is no significant difference between the weight of the fabrics woven with twill and sateen weave.

As seen in Figure 8, fabric thickness ranged between 0.260 and 0.380 mm and the difference in thickness is statistically significant among all the fabric samples woven with different weaves [One way Anova - F (2, 57) = 2703.318, p = 0.000]. There is no significant difference between the Soft silk fabrics of similar weave woven with cotton and modal for the backing [One way Anova - F (1, 58) = 0.000, p = 1.000] as all the yarn and fabric structural parameters were kept constant to facilitate the comparison between different varieties of fabrics.

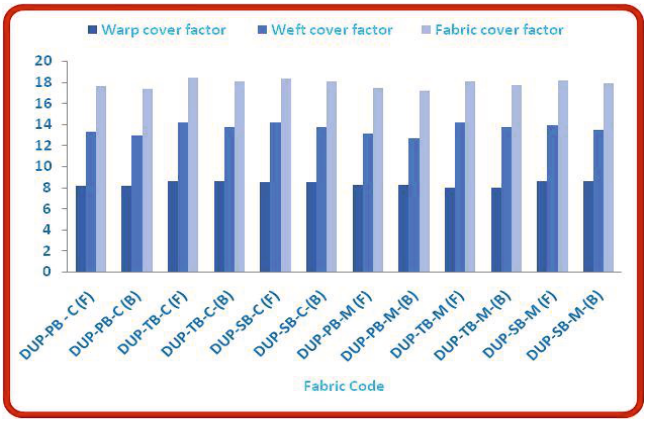

Figure 9: Cover factor of Dupion silk (backed) fabrics (single layer)

Figure 9 indicates warp, weft and fabric cover factor and the difference in the cover factor among the Dupion silk fabrics woven with different weaves is statistically significant at 95 % level of confidence [Anova - F (2, 9) = 13.374, p = 0.002]. Whereas, the difference between the fabrics woven with different material for backing is statistically not significant at 95 % level of confidence [Anova – F (1,10) = 0.762, p = 0.403].

2B. The data pertaining to fabric weight and thickness of Dupion silk (backed) fabrics woven as two layers are presented in Figures 10 and 11, respectively and that on fabric cover factor, in Figure 12.

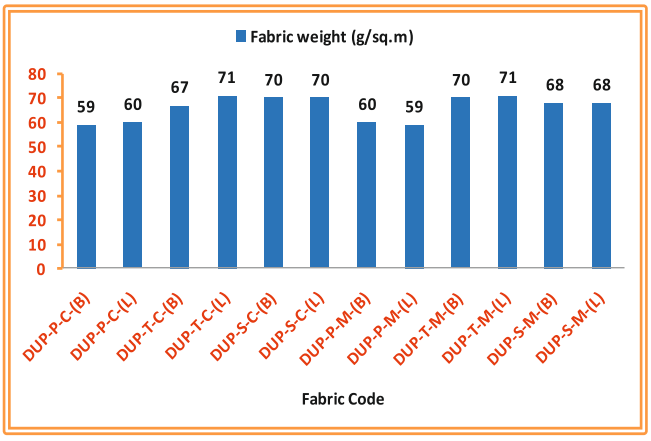

Figure 10: Weight of Dupion silk (backed) fabrics (two layers)

The weight of the fabric samples ranged between 119 and 141 grams (Figure 10). The weight in this case is the sum of body layer and lining layer. Even in this case, Dupion silk fabrics woven with plain weave are lighter than the fabrics woven with twill and sateen weave and the difference is statistically significant at 95 % level of confidence [One way Anova - F (2, 58) = 531.185, p = 0.000]. There was no significant difference in weight between the Dupion silk fabrics of similar weave woven with cotton and modal for the backing [One way Anova - F (1,59) = 0.031, p = 0.862].

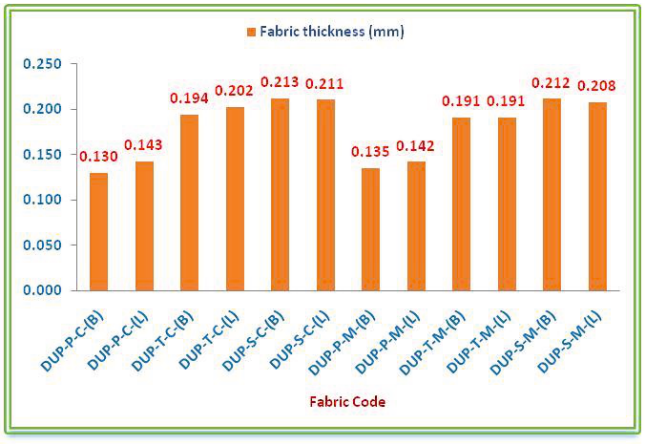

Figure 11: Thickness of Dupion silk (backed) fabrics (two layers)

As seen in Figure 11, fabric thickness (sum of body layer and lining layer) ranged between 0.273 and 0.424 mm. The difference in thickness is statistically significant at 95% level of confidence among all the fabric samples woven with different weaves [One way Anova - F (2,117) = 2383.081, p = 0.000].

Whereas, there is no significant difference between the Dupion silk fabrics of similar weave woven with cotton and modal as the backing [One way Anova - F (1,118) = 0.051, p = 0.822] as all the yarn and fabric structural parameters were kept constant to facilitate the comparison between different varieties of fabrics.

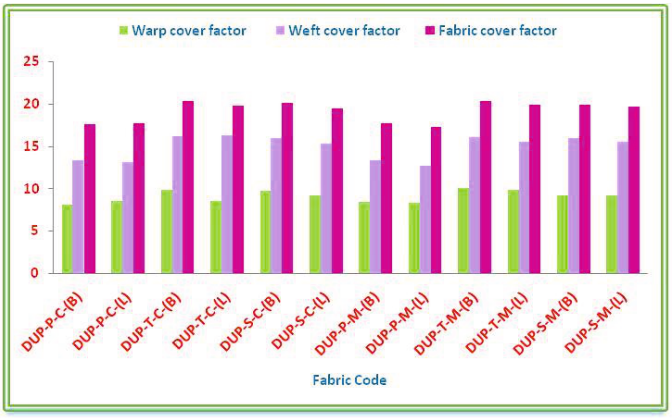

Figure 12: Cover factor of Dupion silk (backed) fabrics (two layers)

Figure 12 indicates warp, weft and fabric cover factor and the difference in the cover factor among the Dupion silk fabrics woven with different weaves is statistically significant at 95 % level of confidence [Anova - F (2, 9) = 108.878, p = 0.002]. Whereas, the difference between the fabrics woven with twill and sateen weave is not significant (p = 0.135). The difference between the fabrics woven with different material for backing is statistically not significant at 95 % level of confidence [Anova - F (1,10) = 0.002, p = 0.963].

3. Fabric Hand

The results of fabric Hand of Soft silk (backed) fabrics are displayed in Figures 13 to 16.

Figures 13 and 14 reveal that the THV of soft silk (backed) fabrics woven as a single layer ranged between 2.16 and 4.62 for winter and from 0.46 to 1.37 for summer.

THV in the case of Soft silk (backed) fabrics woven as two layers ranged from 2.00 to 4.29 for winter and between 0.32 and 1.29 for summer as indicated in Figures 15 and 16.

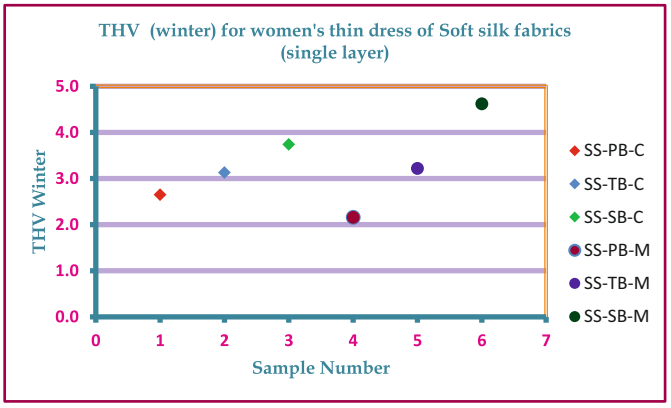

Figure 13: THV (winter) for women's thin dress of Soft silk (backed) single layer fabrics

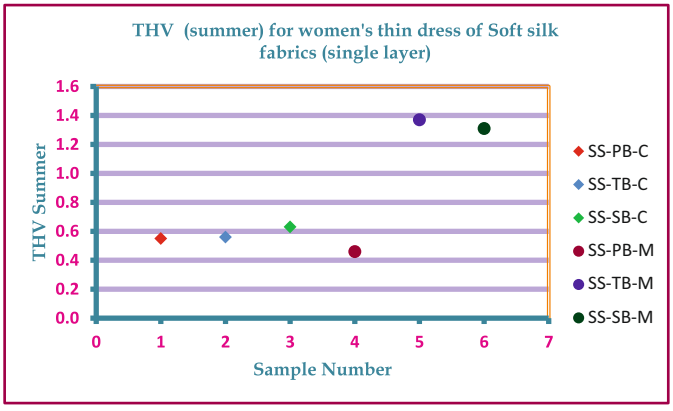

Figure 14: THV (summer) for women's thin dress of Soft silk (backed) single layer fabrics

The results of fabric Hand of Dupion silk (backed) fabrics are presented in Figures17 to 20.

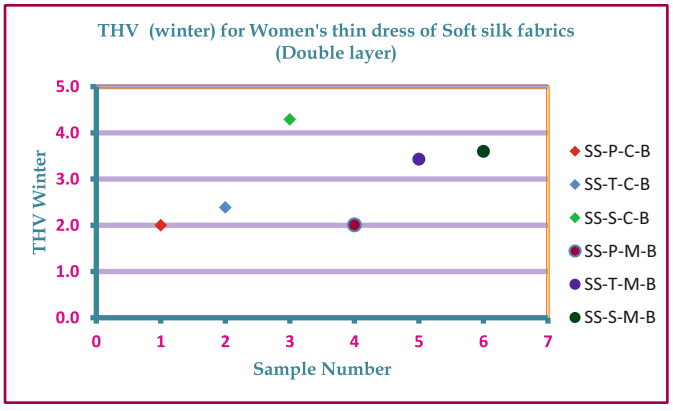

Figure 15: THV (winter) for women's thin dress of Soft silk (backed) double layer fabrics

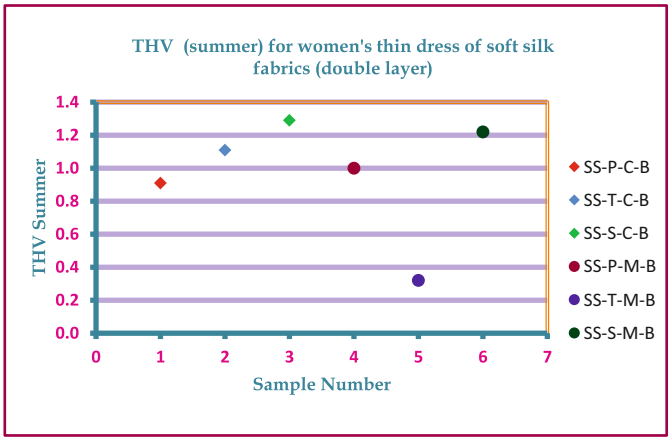

Figure 16: THV (summer) for women's thin dress of Soft silk (backed) double layer fabrics

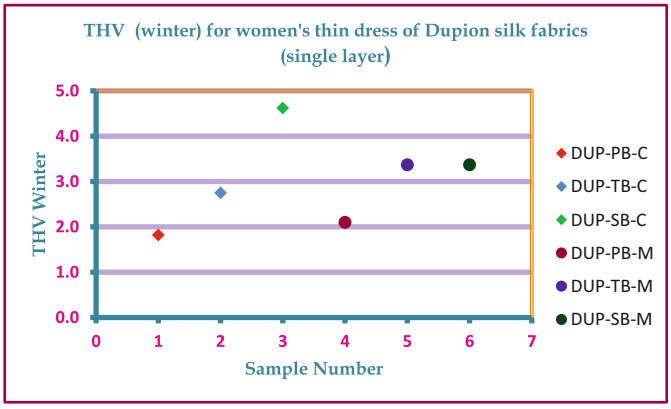

Figure 17: THV (winter) for women's thin dress of Dupion silk (backed) single layer fabrics

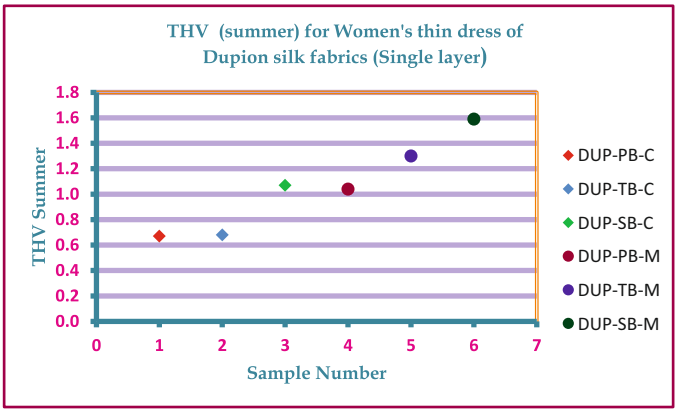

Figure 18: THV (summer) for women's thin dress of Dupion silk (backed) single layer fabrics

The THV of Dupion silk (backed) fabrics woven as a single layer ranged between 1.82 and 4.62 for winter and from 0.67 to 1.59 for summer (Figures 17 and 18).

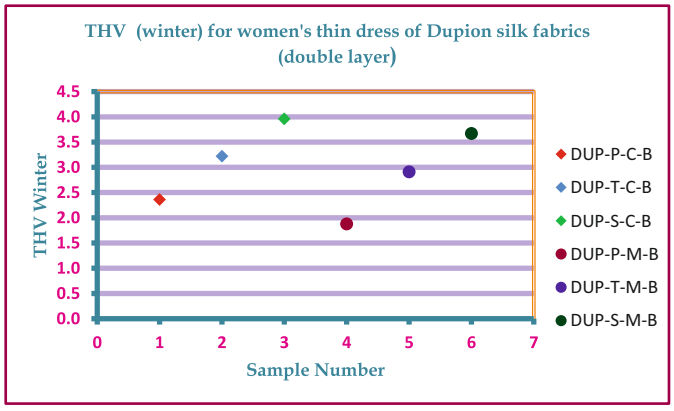

Figure 19: THV (winter) for women's thin dress of Dupion silk (backed) double layer fabrics

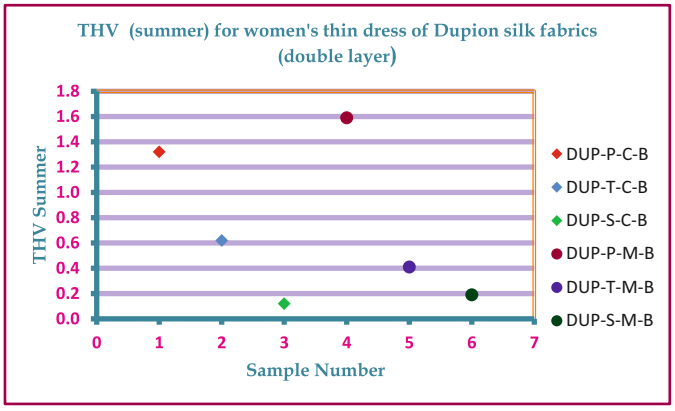

Figure 20: THV (summer) for women's thin dress of Dupion silk (backed) double layer fabrics

THV in the case of Dupion silk (backed) fabrics woven as two layers ranged between 1.88 and 3.96 for winter and from 0.12 to 1.59 for summer as indicated in Figures 19 and 20.

4. Evaluation of fabrics for suitability to summer & winter for ladies blouse, using the KES data

In order to evaluate the suitability of these fabrics for ladies thin dress for summer and winter season depending on the Total Hand Value (THV), the grading system developed by Kawabata was used. This grading system has a scale of zero to five (0-5). A value of '0' indicates 'No use' which means not at all suitable and THV grades of 1, 2, 3, 4 and 5 indicates 'Poor', 'Fair', 'Average', 'Good' and 'Excellent' suitability of the fabrics, respectively. The grades for Soft silk and Dupion silk backed fabrics are presented in Tables 3-4 and 5-6, respectively.

Table 3: Grades for Soft silk with cotton backing

Sample	Grade (Winter)	Grade (Summer)
SS-PB-C	Average	Poor
SS-TB-C	Good	Poor
SS-SB-C	Good	Poor
SS-P-C-B	Fair	Poor
SS-T-C-B	Average	Fair
SS-S-C-B	Excellent	Fair

The grades presented in Table 3 indicate that Soft silk fabric (cotton backed) with Sateen weave in both single layer and double layer has the best grade of 'Good' and 'Excellent' amongst the fabrics evaluated followed by soft silk fabric (cotton backed) with twill weave and soft silk fabric (cotton backed) with plain weave with respect to their suitability for winter season whereas, the grades of all the fabrics are below average with respect to their suitability for summer season.

Table 4: Grades for soft silk with modal backing

Sample	Grade (Winter)	Grade (Summer)
SS-PB-M	Average	Poor
SS-TB-M	Good	Fair
SS-SB-M	Excellent	Fair
SS-P-M-B	Average	Poor
SS-T-M-B	Good	Poor
SS-S-M-B	Good	Fair

The grades listed in Table 4, indicate that Soft silk fabric (modal backed) with sateen weave in both single layer and double layer has the best grade of 'Excellent' and 'Good' amongst the fabrics evaluated and listed above followed by soft silk fabric (modal backed) with twill weave and soft silk fabric (modal backed) with plain weave with respect to their suitability for winter season whereas, the grades of all the fabrics are below average with respect to their suitability for summer season.

Table 5: Grades for Dupion silk with cotton backing

Sample	Grade (Winter)	Grade (Summer)
DUP-PB-C	Fair	Poor
DUP-TB-C	Average	Poor
DUP-SB-C	Excellent	Fair
DUP-P-C-B	Average	Fair
DUP-T-C-B	Good	Poor
DUP-S-C-B	Good	Poor

The grades shown in Table 5 point out that Dupion silk fabric (cotton backed) with sateen weave in both single layer and double layer has the best grade of 'Excellent' and 'Good' amongst the fabrics tested followed by Dupion silk fabric (cotton backed) with twill weave and Dupion silk fabric (cotton backed) with plain weave with respect to their suitability for winter season whereas, the grades of all the fabrics are below average with respect to their suitability for summer season.

Table 6: Grades for Dupion silk with modal backing

Sample	Grade (Winter)	Grade (Summer)
DUP-PB-M	Average	Fair
DUP-TB-M	Good	Fair
DUP-SB-M	Good	Fair
DUP-P-M-B	Fair	Fair
DUP-T-M-B	Average	Poor
DUP-S-M-B	Good	Poor

The grades recorded in Table 6 indicate that Dupion silk fabric (modal backed) with sateen weave in both single layer and double layer and Dupion silk fabric (modal backed) with twill weave in single layer has the best grade of 'Good' amongst the fabrics tested followed by Dupion silk fabric (modal backed) with twill weave in double layer and Dupion silk fabric (modal backed) with plain weave with respect to their suitability for winter season whereas, the grades of all the fabrics are below average with respect to their suitability for summer season.

Conclusion

Amongst the varieties of Soft silk and Dupion silk (Backed) fabrics developed with different weaves, fabrics with Sateen weave stand out as the best with respect to fabric hand and their suitability to ladies wear, followed by twill and the plain weave. This implies that the change in fabric weave keeping all other structural parameters constant has significant influence on the fabric low stress mechanical properties and in turn, fabric hand. The reason is that the twill and the sateen weave fabrics are more flexible, smooth and soft compared to the plain weave fabrics which enhances the THV of these fabrics. Fabrics with both cotton and modal used as the backing, perform equally well in various aspects. All the varieties of fabrics developed have good suitability to ladies wear for winter season than for summer. Therefore, they can be a promising product for ladies wear and the silk apparel sector as a whole in the near future.

ACKNOWLEDGEMENT

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Research Paper

PHYSICAL, FUNCTIONAL AND AESTHETIC PROPERTIES OF MULBERRY AND MUGA SPUN SILK YARN WOVEN FABRICS

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ABSTRACT

The present study forms a part of attempts towards exploring means of utilization of pierced and cut silk cocoons as well as silk reeling waste of mulberry and muga varieties. These were successfully spun on worsted system of spinning and produced 2/60, 2/80 and 2/120 $^{s}N_{m}$ spun silk yarns, and plain fabrics were woven out of these on power loom using 2/60 $^{s}N_{m}$ yarn in warp and 2/60, 2/80 and 2/120 $^{s}N_{m}$ yarn in the weft in each variety of silk. These yarns and fabrics were subjected to international standard testing methods for assessment of their mechanical properties. The results were compared and analyzed in line with the requirement of the industry. Muga spun silk yarn displayed better weaving performance similar to mulberry spun silk yarn and fabrics produced out of it presented improved mechanical properties *viz*., bursting strength, drape coefficient, flexural rigidity, elongation *etc*. The fabric was on par with that of mulberry in respect of properties, such as water permeability, pilling resistance, abrasion resistance, thermal and tensile parameters. Hence, it is established that both varieties of spun silk fabrics are highly suitable for women and men dress materials for all seasons.

Key words: Abrasion resistance, air permeability, breaking strength, bursting strength, drape coefficient, muga spun silk yarn and fabric, stiffness, thermal insulation value.

INTRODUCTION

Muga, the golden yellow colour silk is prerogative of India and the pride of Assam state. Presently, about 18000 ha of muga plantation are available and about 50,000 families are engaged with the industry. Annual production of muga raw silk is 220 MT which is used to produce high value products *viz*., sarees, mekhalas, cheddars, dress materials *etc*.

After reeling out the silk from the cocoons, the un-windable portion of the entangled mass or shorter filaments which account for about 60 % is termed as silk reeling waste constituting about 370 MT per annum. Large quantity of pierced and cut cocoons of muga and mulberry and reeling waste were degummed and subjected to mill spinning in worsted system to produce superior quality spun silk yarn (Kariyappa *et al*., 2006, 2011). Using 2/60 $^{s}N_{m}$ yarn in warp and 2/60 , 2/80 and 2/120 $^{s}N_{m}$ spun yarn in the weft, plain mulberry and muga fabrics were produced on power loom.

With the new trends of fashion directed towards quality, muga spun silk fabric owing to its natural golden color, elegance and comfort properties, has of late, gained great prestige and reputation. It is therefore, necessary to raise the awareness among the users, of the unmatched qualities of muga spun silk fabrics, in order to promote usage of muga waste for production of international quality spun silk yarn.

Thus, the present study was carried out to ascertain the physical, functional and aesthetic properties of muga spun silk fabrics and compare it with that of mulberry. The specifications of fibres (Kariyappa *et al.,* 2008) used for the study are mentioned in Table 1.

Table 1: Mulberry and muga fiber parameters

Parameter	Mulberry	Muga
Fiber diameter (μ)	14.33	19.85
CV %	27.56	34.75
Denier	1.901	3.650
Specific weight	1.3067	1.3074
Moisture regain (dry) %	10.694	10.485
Moisture content (wet) (%)	9.661	9.490
Elongation %	18.10	41.90
CV %	20.92	30.90
Tenacity (gpd)	3.91	2.79

MATERIALS AND METHODS

Muga silk waste procured from Assam and mulberry cut cocoons taken from CSTRI degummed lot were utilized to produce 2/60 , 2/80 and 2/120 $^{s}N_{m}$ counts of spun silk yarns at M/s Kareem silks, on worsted system of spinning (Kariyappa *et al.,* 2011).

Utilizing these yarns, plain fabrics were woven on two by one under pick power loom at CSTRI with particulars of, Reed 60, PPI 42 and width 54 inch as given below.

Muga spun silk fabrics

- 2/60 $^{s}N_{m}$ x 2/60 $^{s}N_{m}$, 4.55 meters
- 2/60 $^{s}N_{m}$ x 2/80 $^{s}N_{m}$, 4.52 meters
- 2/60 $^{s}N_{m}$ x 2/120 $^{s}N_{m}$, 4.21 meters

Mulberry spun silk fabrics

- 2/60 $^{s}N_{m}$ x 2/60 $^{s}N_{m}$, 4.35 meters
- 2/60 $^{s}N_{m}$ x 2/80 $^{s}N_{m}$, 4.29 meters
- 2/60 $^{s}N_{m}$ x 2/120 $^{s}N_{m}$, 4.37 meters
- 2/60 $^{s}N_{m}$ x 2/140 $^{s}N_{m}$, 4.02 meters

Tests

The following properties were tested according to the standard procedures as indicated against each of them.

- **Yarn**

Unevenness and imperfection - Uster 3, 5 replications per sample; yarn speed, 400 meter per min; time 1 min; tension 37.5 %.

- Tensile strength and elongation % - Instron tensile tester model no. 5000R using IS 1670-1991 testing method - 500 mm GL 20 s (20 samples of yarn each).

Fabric

Test	Standard method
Weight per square meter (4 specimens)	IS 1964-2001
Thickness (4 specimens)	IS 7702-1975
Air permeability - PROLIFIC air permeability tester (10 specimens)	IS 11056-1984
Bursting strength (4 specimens)	IS 1966-1975
Tearing strength (5 specimens)	IS 6489-1993
Tensile strength and elongation - Instron 50 x 200 mm strip 300 m/min (5 specimens)	IS 1969-1985
Drape (4 specimens)	IS 8357-1977
Abrasion resistance-Martindale abrasion tester (4 specimens)	IS 12673-1989
Crease recovery	IS 4681-1981
Bending length and flexural rigidity (15 specimens)	IS 6490-1971
Water repellency (4 specimens)	IS 390-1975
Pilling resistance (4 specimens)	IS 1097-1964
Thermal insulation value - Thermolabo-II Wind speed -1m/sec (3 samples per specimen)	Guarded hot plate method

Statistical analysis

Results obtained on yarn and fabric parameters were analyzed through two-way analysis of variance (ANOVA) *i.e.*, general linear model, multivariate post hoc, least significant difference and descriptive statistics, using SPSS 11.5 and for critical difference, windostat package.

RESULTS AND DISCUSSION

Yarn properties

Evenness, imperfection and hairiness properties

The values in respect of yarn evenness, imperfection,

hairiness and index of irregularities are presented in Table 2. The absolute evenness of mulberry spun silk yarn stands significantly higher than that of muga, there are no significant differences between the two types of spun silk yarns in the case of thin places, thick places at (+ 100 %) and neps (+ 280, + 400). The mulberry spun silk yarn has significantly less thick place at (+ 50 %) and neps (+ 140 and + 200). The mulberry spun silk yarn has significantly higher index of irregularity and hairiness. In both the spun silk yarns, yarn evenness, yarn imperfection, hairiness and index of irregularity increased with increase of yarn count. The yarn quality is directly influenced by the fibre quality. The mulberry spun silk yarn has higher evenness and less imperfection than muga spun silk yarn because mulberry fibre is finer and more uniform than muga fibre. Finer fibre of mulberry accommodates more number of fibres in yarn as seen in cross section thereby enhancing evenness of mulberry spun silk yarn. The hairiness of spun silk yarn is significantly influenced by the fibre length and uniform length distribution. As the muga fibres possess higher effective mean length and more uniform length distribution, muga yarns present significantly less hairiness (Kariyappa *et al.,* 2011).

Tensile properties

The results show that mulberry spun silk yarn has significantly higher breaking force, tenacity and Young's modulus than muga spun silk yarn (Table 2). The muga spun silk yarn has significantly higher elongation

Table 2: Muga and mulberry spun silk yarn properties

	Count 2/60 $^{s}N_{m}$		Count 2/80 $^{s}N_{m}$		Count 2/120 $^{s}N_{m}$		Count 2/140 $^{s}N_{m}$	CD at 5 %
Evenness								
Per 100 km	Mulberry	Muga	Mulberry	Muga	Mulberry	Muga	Mulberry	
Um %	7.40	9.55	8.62	9.80	9.65	12.21	10.71	0.4681
Cvm %	9.57	12.14	11.05	12.31	12.32	15.43	13.83	0.7444
Cv (1 m) %	5.74	6.00	6.67	6.22	7.45	6.62	7.50	1.3550^{NS}
Imperfection								
Thin places (- 50 %)	0	0	0	2	0	2	2	1.2250^{NS}
Thick places (+ 50 %)	11	13	20	18	30	22	32	5.6885
Thick places (+100 %)	1	2	2	2	3	3	4	1.3998^{NS}
Neps (+ 140 %)	35	58	25	46	84	95	190	18.5043
Neps (+ 200 %)	18	32	12	18	36	36	55	9.6186
Neps (+ 280 %)	12	15	9	10	22	16	26	5.8832^{NS}
Neps (+ 400 %)	7	6	6	6	4	8	10	2.7195^{NS}
Index (-)	1.27	1.33	1.32	1.56	1.40	1.78	1.40	0.1304
Hairiness (-)	2.62	1.95	2.88	2.25	3.27	2.34	3.32	0.2335
Sh (-)	0.77	0.58	0.83	0.74	0.83	0.83	0.92	0.0789
Mechanical								
Breaking force (gf)	1026.70	856.20	666.20	655.30	447.10	352.90	370.30	35.6200
Elongation (%)	8.39	16.41	7.73	16.00	6.92	14.48	6.76	0.7291
Tenacity (gf / den)	3.42	2.85	2.96	2.91	2.98	2.35	2.88	0.1708
B-Work (gf.cm)	2697.20	4436.80	1625.80	3341.60	990.20	1710.40	793.80	2.1565
Young's modulus	58.00	39.70	71.50	45.70	80.80	48.00	69.90	2.6279
Yarn shrinkage (%)	2.85	12.32	2.77	10.15	2.43	8.75	1.75	0.4305

Table 3: Physical and comfort properties of mulberry and muga spun silk fabrics

Fabric property		Mulberry				Muga			CD at 5 %
		2/60 x 2/60 ^{s}N$_m$	2/60 x 2/80 ^{s}N$_m$	2/60 x 2/120 ^{s}N$_m$	2/60 x 2/140 ^{s}N$_m$	2/60 x 2/60 ^{s}N$_m$	2/60 x 2/80 ^{s}N$_m$	2/60 x 2/120 ^{s}N$_m$	
Linear Density (N$_m$)	warp	2/60^s	2/60^s	2/60^s	2/60^s	2/60^s	2/60^s	2/60^s	1.00
	weft	2/60^s	2/80^s	2/120^s	2/140^s	2/60^s	2/80^s	2/120^s	1.00
Threads	Ends/in	62	62	62	62	62	62	62	1.00
	Picks/in	44	44	44	44	44	44	44	1.00
Breaking strength (kgf)	Warp	1014.70	1045.66	1085.45	1089.17	779.49	962.48	1004.00	46.87
	Weft	819.24	525.82	197.37	264.98	770.67	463.26	324.02	41.235
Elongation (%)	Warp	25.4	20.20	16.00	15.80	50.40	48.20	33.80	1.475
	Weft	18.30	15.00	9.50	7.30	26.92	26.86	22.90	1.3952
Tearing strength (g)	Warp	5500	5400	5300	5250	5500	5400	5300	
	Weft	4314	4250	2560	2509	4672	3750	2701	168.02
Crease recovery angle (degrees)		182.8	183.2	191.2	192.0	161.20	182.40	182.9	5.279
Fabric mass (g/m^2)		163.5	144.88	125.40	121.20	163.6	147.32	126.36	0.4969
Air Permeability (m^3/m^2/min)		20.31	22.98	28.64	29.64	25.31	29.97	35.96	2.2864
Water permeability rating		0	0	0	0	0	0	0	1.00
Pilling resistance (rating)		5	5	5	5	5	5	5	1.000
Abrasion resistance (no. of rubs)		750	685	622	525	690	540	475	114.88
Drape coefficient (%)		41.26	32.31	28.15	27.37	45.74	39.09	38.00	4.2879
Bursting strength (kgf/cm^2)		18.3	14.3	12.00	11.80	18.92	13.40	12.92	0.4309
Thickness (mm)		0.39	0.38	0.32	0.32	0.43	0.41	0.37	0.0084
Flexural rigidity- Stiffness (mg-cm)	Warp	147.37	158.51	186.26	180.15	325.08	161.43	194.83	49.78
	Weft	219.69	162.99	61.24	63.07	501.68	105.14	48.67	24.498
	Overall	178.48	160..67	106.04	105.93	403.23	129.69	97.07	31.28
Thermal insulation value (Tog)		0.8576	0.8568	0.8521	0.8519	0.8438	0.8418	0.8100	0.0014
Warp way shrinkage		5.16	3.75	3.50	3.25	9.50	8.83	8.05	0.4633
Weft way shrinkage		1.25	1.25	1.30	1.00	7.10	5.80	5.70	0.3916

percentage than that of mulberry. These properties are in fact influenced by the fibre properties. The higher strength of mulberry silk fibre is because they are composed of very linearly folded and very crystalline polymers of beta configuration having more hydrogen bond formed in a regular manner than muga fibre. The higher elongation percentage of muga fibre is attributed to its polymer structure, higher visco-elasticity and more bulky side groups as compared to mulberry fibre.

Yarn shrinkage properties

The muga spun silk yarn has significantly higher shrinkage percentage than mulberry spun silk yarn. The yarn shrinkage percentage decreased with increase of yarn count in both varieties of spun silk yarn. Yarn shrinkage may be due to interlocking of fibers during hot water treatment, which leads to reduction in yarn length. Muga silk has substantial amount of amino acid with bulky side groups, higher hydrophilic to hydrophobic amino acid ratio (9.06 to 9.85) compared to mulberry variety (5.29 to 6.22) and maximum voids present in muga silk is responsible for higher moisture absorption (Kushal Sen and Murugesh Babu, 2004). Fibers swell as they absorb moisture leading to an increase in the diameter of the fibre that makes up the yarn.

Fabric properties

Breaking strength

Breaking strength (kg) is the maximum amount of tensile stress that it can be subjected to, before failure. The definition of failure can vary according to type of material. Table 3 displays breaking strength of mulberry fabric in warp way and weft way as marginally higher than that of muga spun silk fabric irrespective of weft yarn count, but not significant. The breaking strength of fabric in warp way increased with increase of weft yarn count in the fabric, this is due to the fact that as weft count increases, the weft assistance in the fabric reduces resulting in higher warp breaking strength (Rengaswamy *et al*., 2008). The breaking strength of fabric, weft way, reduced with increase of weft count in both varieties of spun silk fabric because the number of fibers gets reduced as count increases. This is because the tensile strength of fibre and yarn has influenced the fabric strength in both varieties of fabrics.

Elongation %

Elongation percentage is the extensibility of fabric at breaking point, the definition being variable depending on the type of material. The results (Table 3) show that the muga spun silk fabric possessed significantly higher elongation percentage than that of mulberry fabric in both warp and weft way which might be attributed to higher fibre elongation percentage of muga than mulberry fibre. The elongation percentage of spun silk fabric in warp way is significantly more than that of weft way and also it decreased with increase of weft count in both the varieties of fabrics. The elongation in warp way was observed more than that of weft way because, during the weaving process, warp yarn gets more strained than the weft yarn.

Tearing strength

Tearing strength is the measure of the resistance of either the warp or weft series of yarns in the fabric. The fabric that tears easily is regarded as an inferior product. The data on tearing strength of both the experimental spun silk fabrics are presented in Table 3. Both the varieties of spun silk fabrics presented jaw slippage in the warp way. This might be due to use of 2/60 ^{s} spun silk yarn in the warp as a result of which the tearing strength of the fabric exceeded the machine capacity.

Tearing strength of muga spun silk fabric in weft way is significantly higher than that of mulberry spun silk fabric irrespective of weft yarn count, also, the tearing strength decreased with increase of weft count (*i.e.*, 2/60 $^{s}N_{m}$ to 2/120 $^{s}N_{m}$) in the fabric, as it becomes thinner. The work of rupture and elongation of muga fibre and yarn are significantly higher than that of mulberry and, as these properties directly influence fabric tearing strength, muga spun silk fabrics displayed higher tearing strength.

Fabric mass

This is the measure of weight of fabric in grams per square meter. Muga fabrics displayed significantly higher weight than mulberry spun silk fabrics (Table 3). The fabric weight decreased with increase of weft yarn count in both the varieties of spun silk fabrics. This is because, it is established that density and fineness of the fibre play a very important role in deciding the fabric weight.

Air permeability

Air permeability is reciprocal 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 is high (Behera *et al*., 1997). The amount of air that passes through a unit fabric area per unit time is mentioned in Table 3. The results show that muga spun silk fabrics permit significantly higher air permeability than mulberry fabrics. The air permeability increased with increase of weft yarn count in both the varieties of fabrics. The weft yarn count also significantly influenced air permeability of fabrics. Since the fineness of fibre play a very important role in air permeability, finer fibre of mulberry silk provided higher packing density in the yarn than coarser fibre of muga fabrics, and as a result, reduced the porosity in the yarn and fabric. Hence, coarse fibre of muga offered higher air permeability than finer fibre of mulberry spun silk fabrics (Booth, 1996).

Water repellency

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. The values of water repellency are provided in Table 3. These values remained the same for both the varieties of fabrics as both types of fabrics are hydrophilic in nature. The polymers of both fibers have hydroxyl in peptide groups; with polarity in nature which attract water molecules making fabrics more comfortable to wear.

Pilling resistance

Pilling is tufts of entanglement of fibers that look like small pills attached to the surface of the cloth, which appear after some usage of fabrics. The results (Table 3) revealed moderate pilling owing to yarn hairiness, yarn content and fibre type. The values of pilling resistance were observed same for all experimental fabrics.

Abrasion resistance

Abrasion is just one aspect of wear and is the rubbing away of the component fibers and the yarn of the fabric during usage. Table 3 reveals the results obtained in respect of abrasion resistance. Mulberry spun silk fabric is observed to possess marginally higher abrasion resistance than muga spun silk fabrics, the difference being insignificant. The Abrasion resistance decreased with increase of weft yarn count in both the experimental fabrics. The fibre cross section shape, strength and yarn fineness are determining factors of abrasion resistance. The shape of muga fibre is more prone to abrading and this substantiates the present finding of less abrasion resistance of muga fabric than mulberry fabrics. Both the fabrics were found to be durable.

Drape coefficient

Aesthetic appeal of apparel has always been important for human being. Drape, one of the most important properties of fabric, has played significant role in providing graceful aesthetic effects to garment. 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 folds or pleats as results of force of gravity. The data on drape coefficient of experimental spun silk fabrics are given in Table 3. In terms of drape coefficient, muga spun silk fabrics were superior to mulberry spun silk fabrics irrespective of yarn counts. The triangular cross sectional shape and coarseness of muga fibre contributes to make the fabric more stiff.

The drape coefficient decreased with increase of weft yarn count in both varieties of spun silk fabrics. The fibre cross section, thickness and weight of the fabric were found to affect the drape properties of spun silk fabrics. The drape coefficient has been developed to describe the degree of drape and drape shape (configuration, modality). A lower drape coefficient value means the fabric is softer, and its drapability is better. In other words, the higher the drape coefficient**,** the stiffer the fabric with less drapability.

Bursting strength

Bursting strength is the measure of fabric resistance to multidirectional force. Table 3 reveals the results obtained in respect of bursting strength of both spun silk fabrics as more or less same. The bursting strength of fabric reduced with increased yarn count in both the varieties of spun silk fabrics. It is further observed from the data that weft yarn count in both cases has got a significant influence on bursting strength of fabrics. This is due to the fact that the silk fibre and yarn strength and elongation influence the fabric bursting strength.

Fabric thickness

Thickness is an important property of the fabric. The thickness has a great impact on the drape and application of the fabric. Table 3 reveals that the thickness of muga spun silk fabric is significantly higher than that of mulberry. The fabric thickness reduced with increased yarn count in both the varieties of spun silk fabrics and also, the weft yarn count in both the fabrics is found to significantly influence fabric thickness. This is attributed to the influence of fibre fineness and density. The muga fibre being coarser than that of mulberry, naturally results

in thicker fabric.

Flexural rigidity (Stiffness)

Fabric stiffness indicates the resistance of the fabric to bending. Stiffness is a key factor in the study of hand and drape. The results (Table 3) revealed muga spun silk fabric as having significantly higher fabric stiffness than mulberry spun silk fabrics. The flexural rigidity of fabric is found to reduce with increase of weft yarn count in both the varieties. The lesser thickness of mulberry fabric accounts for the lesser flexural rigidity of it than muga spun silk fabrics or in other words, muga spun silk fabrics are stiffer than mulberry spun silk fabrics. The fabrics having smaller values of flexural rigidity also hang down freely and cling to a curved surface easily. Fabric thickness, weight and fibre fineness have got a combined effect on the flexural rigidity of spun silk fabrics.

Crease recovery angle

Crease resistance is the property of fabric which causes it to recover from folding deformation that normally occur during its use. Higher the crease recover angle, the more resistant the fabric to crease and *vice versa*. The values of crease recovery angle of fabric are shown in Table 3. Mulberry spun silk fabric presented significantly higher crease recovery angle than muga. The crease recovery angle increased with increase of weft yarn count in both kinds spun silk fabrics. Here also, the weft yarn count is observed to significantly influence the crease recovery angle in both the varieties of fabrics. This parameter is highly influenced by the fineness of fibre, fibre and yarn elongation percentage and fabric thickness. Higher values of these provide higher fabric crease recovery angle.

Fabric shrinkage

Fabric shrinkage is a parameter of testing textile fabrics to measure changes in length and width after washing. Shrinkage failing materials are dimensionally unstable and they can cause deforming of the garments or products made out of those materials. The results of the present study (Table 3) indicated the superiority of mulberry spun silk fabrics in both warp and weft way. Fabric shrinkage decreased with increase of weft yarn count in both the varieties of spun silk fabrics. It was also observed that warp way shrinkage is significantly higher than weft way shrinkage. This is because warp yarn gets more strain than weft yarn during weaving and hence, results in more shrinkage.

Muga silk is characterized with higher moisture absorption capacity (Kushal Sen and Murugesh Babu, 2004). Silk fibers swell as they absorb moisture leading to an increase in the diameter of the yarns that make up the fabric. But surprisingly, as the yarns expand at high moisture levels, the fabric itself shrinks. Higher swelling leads to higher yarn and fabric shrinkage. Due to swelling, the dimensions of fabric enhances because of moisture absorption. But when moisture is evaporated, the extended dimension is not returned back in original form but to a less extent as compared to initial dimension, which causes shrinkage (Pocobene and Hodkinson, 1992). Besides, in spinning operation, fibers get strained the same way during weaving operation, the warp and weft yarns get strained. During washing, strain in the fiber gets relaxed and, as a result, yarn and fabric shrink (Ernest, 1953).

Thermal insulation value

Thermal insulation value provided by fabrics depends on fibre type, fabric thickness, air permeability of fabric, bulk density of fibre, 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 property of fabric (Behera, 2007). Thermal insulation properties of various fabric samples measured on thermo lobo (KE-FB5) are presented in Table 3.

The results show that thermal property of mulberry spun silk fabrics is significantly higher than muga spun silk fabrics. The thermal insulation value decreased with increase of weft yarn count in both spun silk fabrics. Mulberry fibers being finer and more or less circular in cross section, gives good packing density in yarn. It may be concluded that both fabrics can provide the desired protection to human body against climate fluctuations.

Conclusion

The present results draw a conclusion that, from muga silk

waste, spun silk yarn of international quality similar to that of mulberry spun silk, can be produced, and silk fabric can be woven on high speed power loom without any hindrance. Muga yarn displayed better weaving performance and fabrics produced from muga yarn presented improved mechanical properties *viz*., bursting strength, drape coefficient, flexural rigidity, elongation *etc*. The fabric was on par with that of mulberry in respect of properties, such as water permeability, pilling resistance, abrasion resistance, thermal and tensile parameters. The muga spun silk fabrics were found stiffer compared to the softer mulberry fabrics. Both sorts of fabrics were proven durable, with reasonably good aesthetic, tactile and thermal comfort properties. In view of this, both kinds of spun silk fabrics are recommended as the most suitable for women and men dress material for all seasons.

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Research Paper

IMPLEMENTATION OF SIX SIGMA METHODOLOGIES IN MULTIEND AND DOMESTIC BASIN SILK REELING UNITS FOR THE IMPROVEMENT IN QUALITY OF RAW SILK

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ABSTRACT

The domestic basin and multiend silk reeling units operating in Siddlaghatta silk reeling clusters formed the centers for this study. The process parameters adopted by the units for the production of raw silk were noted and data collected on quality of raw silk produced by the units were analyzed adopting the six sigma methodologies *viz*., root cause analysis and failure mode and effect analysis. Based on the findings, technical guidance on process parameters were provided to the reelers of the units on a regular basis. Significant improvement in raw silk quality in terms of winding breaks, size deviation and maximum size deviation characteristics of raw silk was found on adoption of six sigma methodologies in both domestic basin and multiend reeling units. The high quality raw silk eventually fetched better price enhancing their profitability significantly. Adoption of six sigma methodologies would help the silk reeling industry to further prosper well amidst the prevailing fluctuations in the market.

Key words: Domestic basin, Multiend reeling, six sigma, size deviation.

INTRODUCTION

Modern quality improvement tools have the capacity to overcome many stumbling blocks in the silk industry. Processes, particularly those involving human handling are more susceptible to errors, manifested in the end products (Sathyaraju and Sowdamini, 2015). Unlike other manufacturing industries, silk industry is yet to adopt various quality tools. Statistical quality control, statistical process control and total quality management are some of the tools, which other industries have utilized and poised to use much better innovative tools. One such innovative tool is six sigma. It's a unique one which was adopted initially by the manufacturing industries like Motorola and General Electric and reaped many benefits with remarkable impact on quality, cost reduction and cycle time reduction. In the textile sector, from spinning yarn to weaving and garment manufacturing, the six sigma tools could be used. The textile exporters and the textile research bodies have remarkably promoted this tool into textile industries and the benefits are considered significant. Chattopadhyay *et al*. (2008) have studied the textile spinning mill and identified the different processes which contribute significantly on the quality improvement using six sigma principles. In silk sector, David Raja *et al*. (2012) studied the influence of silk cocoon quality using total quality management techniques and established that farmers are to be educated on the production of quality cocoons. However, presently, the importance of quality of cocoons and its relation with price fetched, has been established and overall improvement in cocoon quality has been achieved. Nivedita *et al*. (2013) stated that consumers should know that they play an important role in maintaining healthy trade practices as they constitute the last link in the supply chain. Their knowledge of quality silk, varieties, blends *etc.*, will deter the unscrupulous trade practices and they get genuine silk products for the price paid. A little effort on their part can ensure them

quality merchandise and also help safeguard the interest of the silk industry at large.

In this context, it was thought that silk reeling industry is the major juncture for conversion of cocoons into quality silk and any improvement achieved in the production of quality raw silk in reeling sector would benefit the remaining chain of the industry. Hence, it was decided to select one reeling cluster and also some of the silk reeling units *viz*., domestic / cottage basin and multiend reeling units for implementing the six sigma tools *viz*., root cause analysis and failure mode and effect analysis to assess the manifestations thereon.

MATERIALS AND METHODS

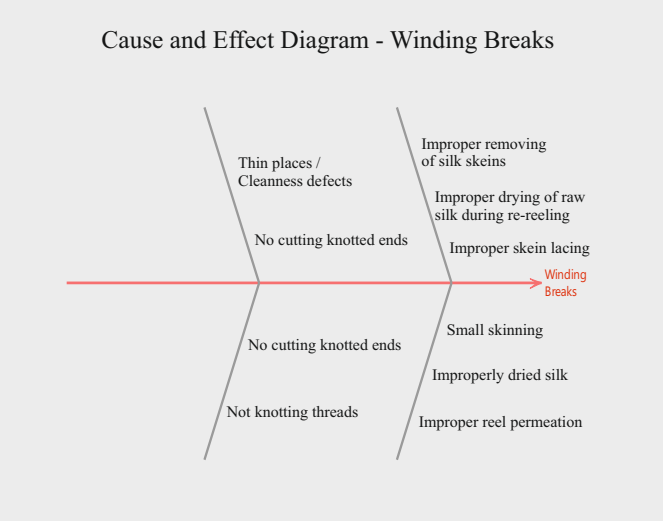
Domestic basin silk reeling unit: The Italian version of silk reeling machine is called as Domestic basin silk reeling machine in India. In these units, stifling of cocoons are carried out using steam and the cooking and reeling are carried out separately (Sonwalkar, 1993). The cooked cocoons are converted into raw silk on to the standard size skeins of 1.5 meter circumference in the reeling machine itself. The domestic basin silk reeling machine has 5 ends per basin and 6 ~ 12 basins per unit. Each basin is run by one reeler and for two basins, one cooker supplies cocoons. Together, around 1 kg of raw silk is produced per basin per shift of 8 hours. The domestic basin silk reeling units were selected for the study based on the experience and continuous evaluation of silk quality. The process parameters being adopted by the domestic basin silk reeling units were evaluated regularly before adopting the six sigma principles.

Multiend basin silk reeling unit: Central Silk Technological Research Institute (CSTRI), Bengaluru has developed multiend silk reeling technology package for the production of international quality raw silk from the Indian cocoons (Somashekar and Kawakami, 2003). The green cocoons procured from the markets were dried using hot air drier and cooked effectively using two pan system of cooking and reeled on multiend reeling machine on to small reels. The multiend silk reeling machine has 10 ends per basin and 10 basins per unit. Each basin is run by one reeler and for two basins, one cooker supplies cocoons. The raw silk reeled on small reels are converted into standard size skeins on re-reeling machine. About 1 ~ 1.2 kg of raw silk is produced per basin per shift of 8 hours in Multiend silk reeling unit. A few such reeling units were selected for the study based on the unit capability to produce superior quality raw silk continuously as well as regular assessment of silk quality.

Root cause analysis: Cause and effect diagram helps the user to understand and list out all possible causes and effect or desired effect. Prof. Ishikawa of University of Tokyo developed this tool in the year 1943. Since the diagram is drawn in the shape of skeleton of a fish, it is also called fish bone diagram. The tip of the fish depicts the effect to be achieved, like improving reelers' satisfaction or problem to be resolved, bringing down the cost of production or improving the quality of raw silk and so on. The effect can be achieved through a number of causes. The causes are written in the bone portion of the diagram. Based on the data collected on the quality of raw silk of the selected silk reeling units, a cause and effect diagram was drawn and presented (Figure 1).

Failure mode and effect analysis: Failure mode and effect analysis (FMEA) is a systematic group of activities intended to, recognize and evaluate the potential failure of a product / process and the effects of that failure and identify actions that could eliminate or reduce the chance of occurrence of potential failure. FMEA is proactive tool, technique and quality method that enables the identification and prevention of process or product errors before they occur. The FEMA analysis of the occurrence of size deviation and winding breaks of raw silk in selected silk reeling units is presented in Table 1 (Hariraj *et al.,* 2013).

Assessment of silk quality: Quality of raw silk is influenced by five major factors *viz*., quality of cocoons, reeling contrivances, process parameters, workers' skill and quality of water (Subhas Naik *et al*., 2012). Raw silk testing is an essential tool to assess the quality parameters,



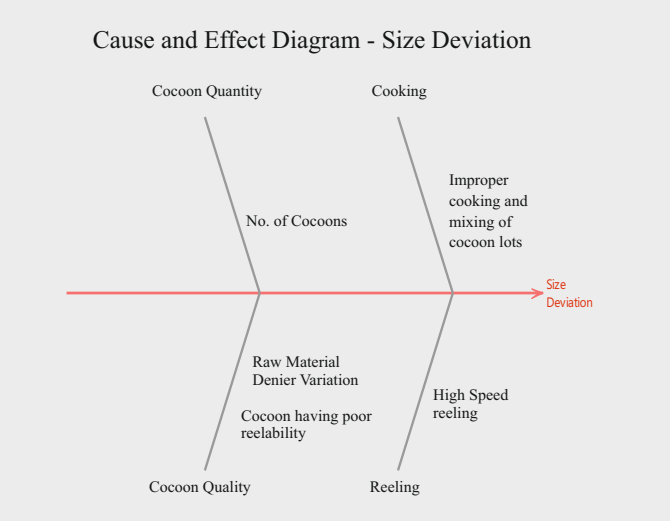


Figure 1: Root cause analysis for the occurrence of size deviation and winding breaks of raw silk in silk reeling processes

Table 1: Failure mode and effect analysis for the occurrence of size deviation and winding breaks of raw silk in silk reeling process

Process	Potential failure mode	Potential failure effect	Severity	Potential causes	Occurrence	Current process control	Deductibility	Risk priority number
Silk reeling	Winding breaks	Improper maintenance of cocoons in silk reeling process resulting in lower or higher denier of silk than the targeted one	8	1. Threads are knotted 2. Improper drying resulting in gum spots. 3. The knotted ends are not cut and long ends are left out as they are. 4. Improper reel permeation resulting in reels dried during re-reeling process.	7	1. Use of denier indicating device 2. Maintain temperature at 35-40 °C and humidityof 40-45 % in re-reeling machine. 3. Pre-soaking and adequate reel permeation 4. Proper traverse is the most important factor in reducing the number of winding breaks.	10	560
	Average size and size deviation	Results in weft bars and warp stripes. Maximum deviation causes a lot of breaks during the winding process in twisting factory.	8	1. Improper mixing of different cocoon lots. 2.Cocoons having poor reelability. 3.Maintaining high speed during reeling. 4.Improper cooking resulting in breakages.	4	1. Cocoon should have more silk content, less cocoon filament denier variation from outer to inner layers. 2. Skill of the reeler in maintenance of cocoons per end in all the ends.	10	320

based on which the end-user requirements can be decided. Apart from the visual and tactual examination of raw silk for determining its nature, uniformity, colour and general finish, the raw silk produced in the selected domestic basin and multiend silk reeling units were assessed were assessed by conducting the winding test and denier or size test, to know the winding breaks per skein per hour, average size of raw silk, size deviation and maximum size deviation of raw silk (Book of standards 1958-62). Based on the values obtained in the tests, the raw silks were graded as per the Bureau of Indian Standards norms by the CSTRI sub unit in Siddlaghatta, before and after providing technical inputs to the reeling units.

Data collected: The process parameters *viz*., cooking basin and reeling basin water temperature, water pH, total dissolved solids *etc*., and raw silk quality parameters *viz*., winding breaks, size deviation and maximum size deviation were analyzed before and after six sigma applications. The data thus obtained were analyzed statistically.

RESULTS AND DISCUSSION

Analysis of raw silk quality reeled at silk reeling units

The raw silk produced in domestic basin and multiend reeling units were subjected to testing at CSTRI sub unit, Siddlaghatta on a regular basis both before and after providing technical guidance to the reelers. The data thus obtained were analyzed statistically using T- test and the results pertaining to each reeler are depicted in Figures 2 and 3 and in Tables 2 & 3. As seen, there is a significant improvement in the quality of raw silk on adopting technical inputs generated using six sigma principles in both domestic basin and multiend reeling units. It could be observed (Table 2) that winding breaks has shown significant reduction of 50 ~ 60 % and size deviation and maximum size deviation has shown 30 % improvement in the case of multiend silk reeling units, which may be attributed to proper maintenance of cocoons during reeling, knotting the broken ends, reduction in gum spots due to proper processing *etc*. In the case of domestic basin silk reeling units, as seen from Table 3, similar trend is observed in the quality characteristics studied. Further, from the Figures 2 & 3, it could be seen that before implementation of process improvements, number of winding breaks were between C & E Grade and after implementation, overall mean was shifted towards A & 2A, which is a significant improvement. Thus, the study reveals that continuous monitoring of the silk reeling units and constant interaction with the reelers on the silk quality requirements; enhance the quality of raw silk produced in the domestic basin and multiend silk reeling units.

Table 2: Quality parameters of silk reeled on Multiend reeling units (Shri. Dada Peer - case 1 & Smt. Muniyamma - case 2) before and after six sigma implementation

Particulars	Winding breaks		Size deviation		Maximum size deviation	
	Before	After	Before	After	Before	After
Case 1						
Mean	12.4	4.20	2.57	1.76	3.85	2.59
SD	3.07	0.88	0.44	0.22	0.85	0.39
t-value	12.7		8.7		7.3	
p-value	0.02**		0.00**		0.00**	
** Significant at 5 %						
Case 2						
Mean	7.27	3.43	3.46	2.30	4.69	3.55
SD	1.78	1.17	1.48	0.37	1.42	0.69
t-value	8.8		4.0		3.6	
p-value	0.02**		0.00**		0.00**	
** Significant at 5 %						

Table 3: Quality parameters of silk reeled on Domestic basin silk reeling units (Shri. Asadulla Sheriff - case 3 & Shri. Amjad Pasha - case 4) before and after six sigma implementation

Particulars	Winding breaks		Size deviation		Maximum size deviation	
	Before	After	Before	After	Before	After
Case 3						
Mean	11.6	3.95	2.58	1.78	3.86	2.59
SD	2.49	1.22	0.58	0.09	0.99	0.23
t-value	16.1		10.2		9.0	
p-value	0.02**		0.00**		0.00**	
** Significant at 5 %						
Case 4						
Mean	11.8	4.08	2.46	1.62	3.57	2.36
SD	1.77	1.05	0.33	0.22	0.55	0.34
t-value	28.9		14.2		12.5	
p-value	0.00**		0.00**		0.00**	
** Significant at 5 %						

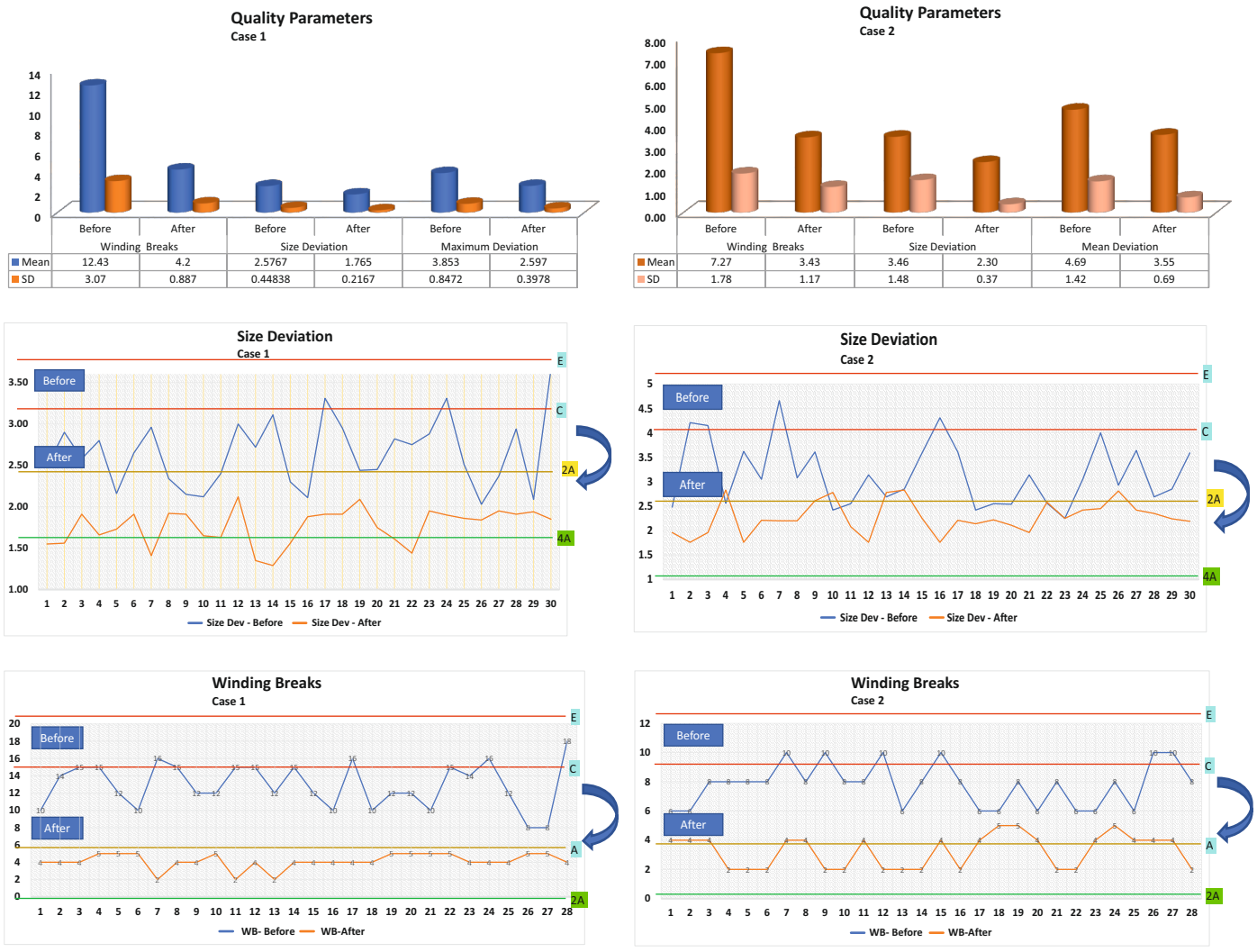


Figure 2: Improvement in quality parameters of raw silk reeled in Multiend silk reeling machine before and after six sigma implementation

Paired t-tests were attempted to know the variations in winding breaks, size deviation and maximum deviation before and after application of six sigma methodologies. The variations being placed at 5 % significance level, interprets that major quality parameters of silk yarn have improved drastically after the application of six sigma methodologies which is evident from mean values and further, SD values in each case has reduced significantly, which is an indication of the stability of the silk yarn properties due to application of six sigma methodologies.

Failure Mode and Effect analysis (FMEA) The domestic basin and multiend silk reeling units were assessed for reasons of more winding breaks and size deviation and the remedial action to be taken were provided to the reelers of the units. The quality

Table 4: Calculation of Risk Propriety Numbers (RPN) of the quality characteristics of raw silk reeled on Domestic basin / Multiend silk reeling unit before and after six sigma implementation

Quality characteristic	Particulars	Severity	Occurrence	Deductibility	Risk Priority Number
Winding breaks	Before	8	6	8	384
	After	8	4	6	192
Size deviation	Before	8	7	10	560
	After	8	5	8	320

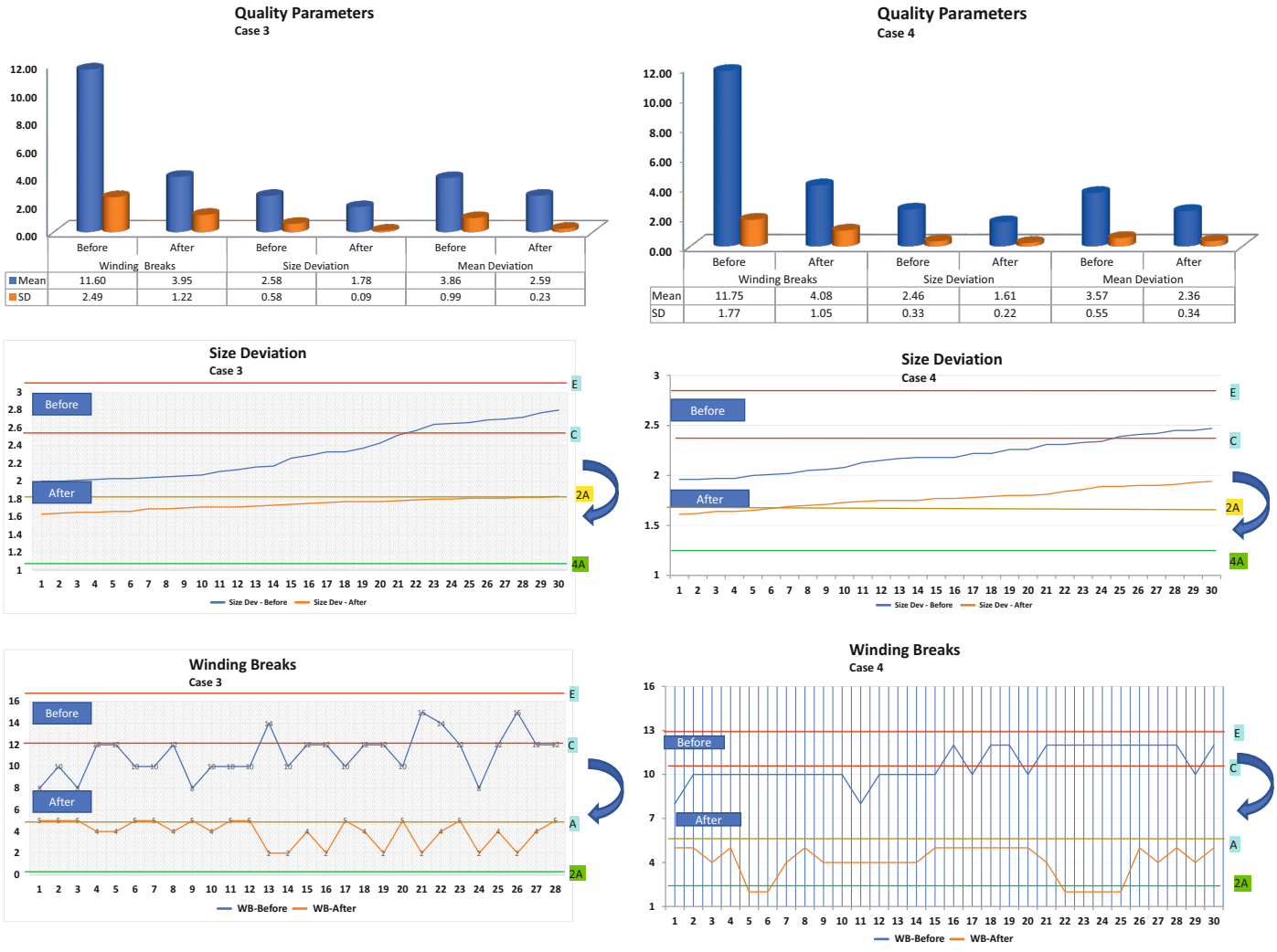


Figure 3: Improvement in quality parameters of raw silk reeled in Domestic basin silk reeling machine before and after six sigma implementation

performance results were collected and based on the severity, occurrence of the defects and deductibility, the risk priority number was calculated before and after six sigma implementation (Table 5).

Table 5: Paired t-test for winding breaks before and after six sigma implementation in Domestic basin / Multiend silk reeling unit

Quality characteristic	Particulars	N	Mean	Standard deviation	Standard error mean
Winding breaks	Before	40	11.6	2.489	0.394
	After	40	3.950	1.218	0.193
	Difference	40	7.65	3.009	0.473

95 % CI for mean difference is (6.688, 8.612)
T- test of mean difference = 0 (vs =/= 0)
T - Value is 16.08 and p value is 0.000.

From the results (Table 6), it could be observed that risk priority number in respect of winding breaks of multiend reeling units were 384, which could be considerably reduced using six sigma principles to 192 by identifying the causes and rectifying the same. Similarly, the risk priority number detected for size deviation characteristics of raw silk reeled on multiend reeling units were 560, which could be reduced to 320 using six sigma principles. The results were subjected to paired t-test (Table 6) and found that there is significant improvement in quality characteristics of raw silk produced on both domestic basin and multiend reeling units since p value is less than 0.05.

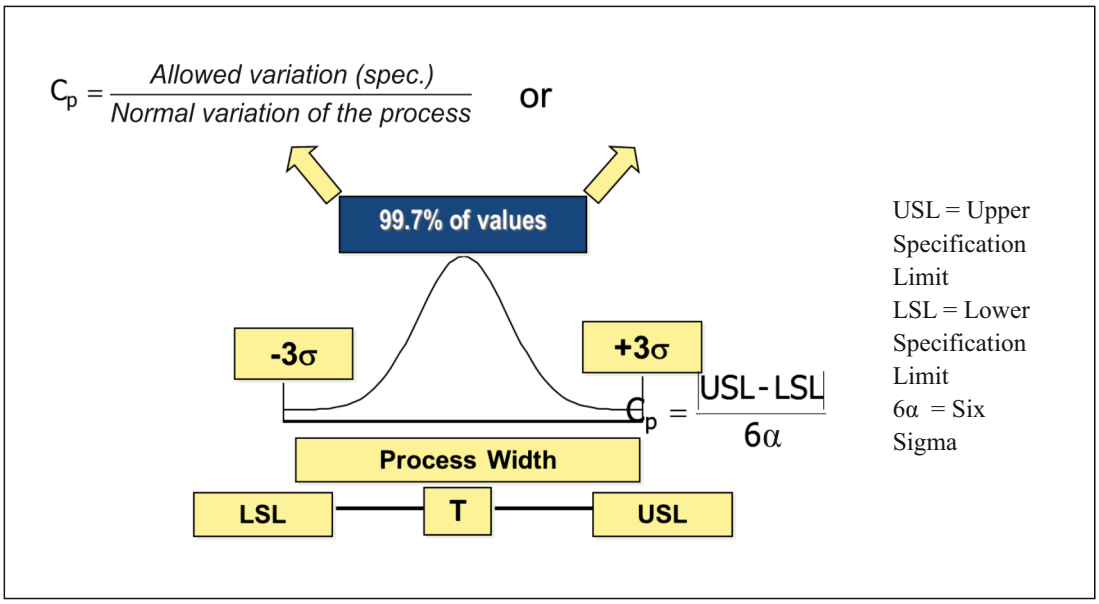


Figure 4: Process Capability Index

The fundamental requirement of any process is that it should be stable. That is, performance of the process should be within upper and lower specification limits. If the performance is out of these limits, it is out of control and there will be process variation. Then, the process should be stopped to find out the cause, and eliminate it. The key process parameters, such as pH of reeling water, cooking temperature, basin temperature and hardness of water (total dissolved solids) were tested with the help of pH meter, Digital Thermometer and TDS meter. Both Multiend and domestic basin silk reeling units were studied for various process parameters. It was found that most of the values measured are within the norms *i.e.*, pH of water was 7.5 as against norms of 7 ~ 7.5, cooking temperature was 95 °C (Norms 90 ~ 95 °C), basin water temperature was 40 °C (Norms 40 ~ 45 °C) and total dissolved solids was 490 ppm (Norms 500 ppm). Based on the assessment, the temperature of basin water of a few reeling units are depicted in Table 8 and Figure 6.

Table 6: Analysis of basin water temperature for process capability index

Process parameter	Case - 1	Case - 2	Case - 3	Case - 4
Basin water temperature (°C)	36.13	39.87	36.22	36.45
Standard deviation	0.68	0.65	0.69	0.68
Upper limit	45.00	45.00	45.00	45.00
Lower limit	40.00	40.00	40.00	40.00
Six sigma	4.11	3.90	4.15	4.06
Cp	1.22	1.28	1.21	1.23
Interpretation	Adequate	Adequate	Adequate	Adequate
CPk (USL)	2.025	1.111	2.022	1.929
CPk (LSL)	-1.884	-0.067	-1.824	-1.748

Note: If Cp<1.0, process variation is higher than specification and has more defects;
If Cp lies between 1.0 to 1.33, the process is acceptable and meet the specification with a few defects;
If Cp >1.33, process variation is less than specification and assumed to be ideal state.

Tangible benefits

On implementation of six sigma principles and interaction with the reelers, a significant improvement could be brought in on raw silk quality. Based on the details provided by the reelers, it was observed that the multiend reelers were benefitted to the extent of 150 ~ 200 / - per kg of raw silk due to improvement in raw silk quality, whereas the domestic basin reelers are benefited to the extent of 100 / - per kg of raw silk as could be seen in Table 9. On continuous implementation of six sigma principles in the reeling units, the reelers would be benefited to the tune of 30000/- per annum in multiend reeling units, whereas in domestic basin reeling units, to the tune of 9000/- per annum.

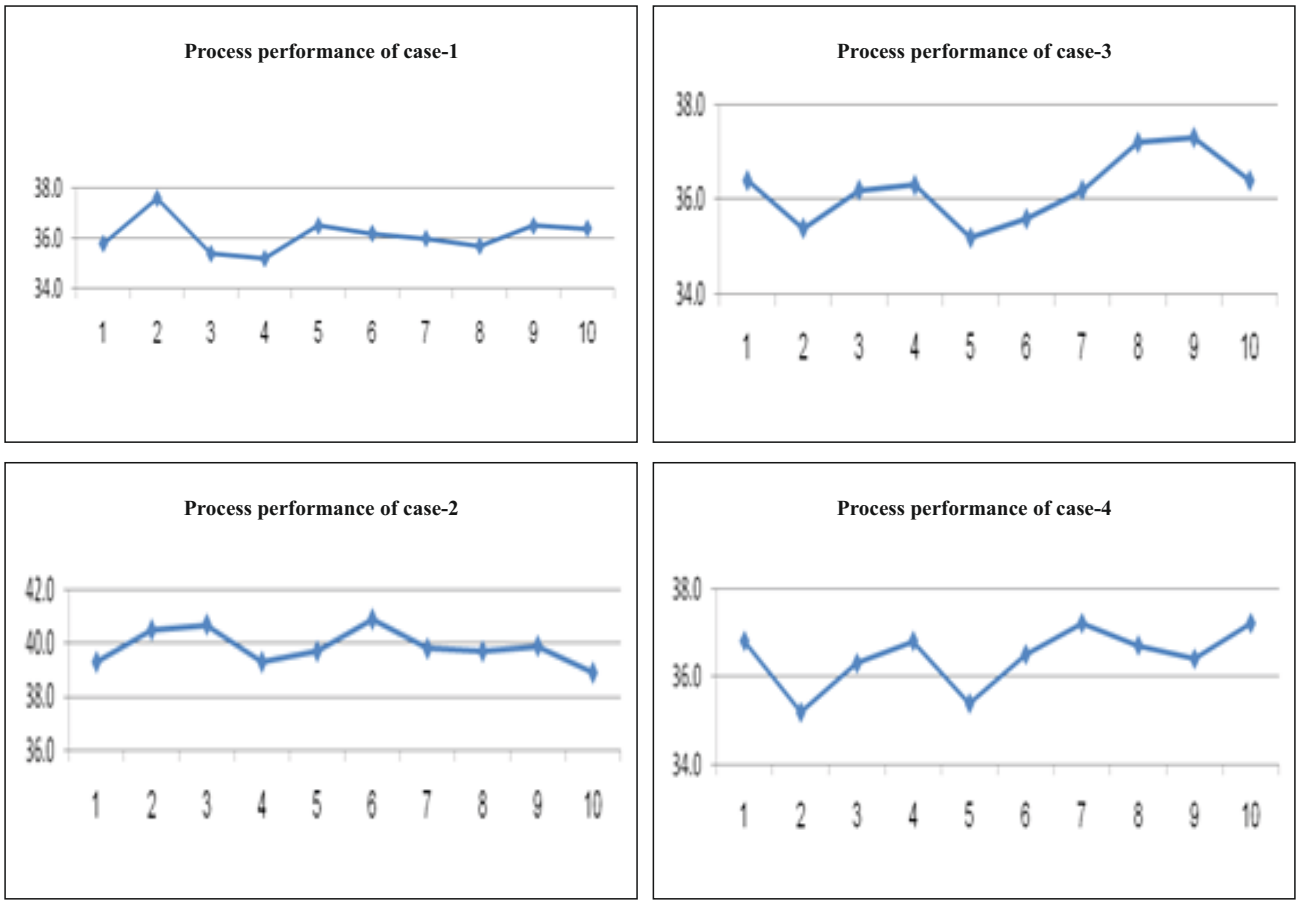


Figure 5: The basin water temperature of the reeling units assessed on individual basins

Table 7: Overall improvement in income level of the reelers as a result of improvement in silk quality

Reeler	Unit	Selling price of raw silk ( )		Production / day (kg)	Total sales / day ( )		Profit @ 5 % ( )		Incremental annual benefit (INR)
		Before	After		Before	After	Before	After	
Muniyamma	Multiend	3350	3500	12	40200	42000	2010	2100	27000
Dada Peer	Multiend	3300	3500	12	39600	42000	1980	2100	36000
Asadulla	Domestic	3100	3200	6	18600	19200	930	960	9000
Amjad Pasha	Domestic	3100	3200	6	18600	19200	930	960	9000

Conclusion

Thus, the study reveals that the multiend and domestic basin silk reeling units can be effectively advised to adopt improved processing techniques continuously for the production of superior quality raw silk. Propagation of raw silk testing continuously in the CSTRI sub centers would benefit the reelers in the long run. The adoption of six sigma principles in the silk reeling units would enable the reelers to achieve better production, high quality of raw silk as well as more income. The reelers should be educated to adopt the root cause analysis, failure mode and effect analysis to achieve tangible benefits in the silk production activity, so that the entire chain of sericulture and silk production activities would be benefitted.

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Research Paper

PHOTOPERIODIC EFFECT ON DIAPAUSING PUPAE OF *ANTHERAEA PROYLEI* DURING SEED COCOON PRESERVATION PERIOD

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ABSTRACT

Antheraea proylei exhibits facultative diapause controlled by photoperiod. To ascertain the exact requirement of photoperiod to maintain the pupal diapause, different photoperiodic treatments *viz*., 8, 12 and 24 h photophase and 24 h scotophase were tested by preserving the pupae from June to January (255 ± 9.99 days) under normal room conditions. The meteorological data pertaining to temperature and humidity along with the parameters indicative of the pupal developmental stage, such as pupal body weight loss, moth emergence and other grainage attributes were recorded. The total weight loss was observed more in male (10.81 - 14.72 %) than female (8.55 - 10.49 %) pupae, showing a significant difference among the male ($P<0.05$) and female ($P<0.01$) pupae preserved at 8, 12 & 24 h photophase and 24 h scotophase. However, the percentage of weight loss was maximum in both male (12.71 - 14.72 %) and female pupae (10.22 - 10.49 %) preserved at 24 h photophase and scotophase, resulting in increased erratic moth emergence to the tune of 53.03 – 71.20 % compared to 10.89 – 11.48 % at 8 and 12 h photophase, showing a significant difference among the male ($P<0.01$) and female ($P<0.01$) in all the four treatments. Variations in abiotic factors have shown a considerable impact on treatments of seed cocoon preservation at 24 h photophase and 24 h scotophase and no influence on 8 and 12 h photophase preservation. Grainage performance revealed no significant differences in coupling percentage and fecundity, but the cocoon-DFL ratio has shown significant differences among the treatments. Hence, based on the present results, it is proposed that oak tasar seed cocoons should be preserved at 8 to 12 h photophase for maintaining the pupal diapause whereas, preservation at 24 h photophase and scotophase terminates the pupal diapause under normal room conditions.

Key words: *Antheraea proylei,* emergence, grainage, photophase, preservation, scotophase.

INTRODUCTION

Insects hibernate during specific developmental stages with suspended animation called diapause (Yamashita and Hasegawa, 1985). Diapause is not merely a pause in development or a passive response to undesirable environmental conditions but rather an active adaption, the onset of which precedes adverse conditions. Thus diapause is such a sophisticated biological phenomenon that there is great diversity in the expression of the diapause syndrome among insect species (Denlinger, 1985). The expression of diapause is triggered by a set of environmental signals, such as photoperiod, temperature and humidity (Beck, 1968; Saunders, 1981; Saunders, 1982) allowing the insect to anticipate approaching seasonal changes (Tauber and Tauber, 1973; Saunders 1976, 1981; Masaki 1980; Hodek and Hodkova, 1988). Photoperiod is considered to be the primary ecological factor responsible for the induction and termination of diapause in most insects (Danilevskii, 1965; Beck, 1968). The photoperiodic time-measurement system of insects perceives seasonal changes in photoperiod and transduces them through the changes in hormonal milieu (Denlinger, 1985), into the expression of ecologically relevant developmental modes, such as continuous development or diapause.

In some Saturniids, such as *Hyalophora cecropia* and *A.*

polyphemus, however, diapause can be terminated by chilling or long-day photoperiod (Williams and Adkisson, 1964; Mansingh and Smallman, 1967). *A. proylei* exhibits facultative diapause and remains under diapause in pupal stage for a period of 8 to 9 months and adult emergence occurs after 255 ± 9.99 days of diapause (Reeta *et al*., 2003). Light treatment was generally used for raising additional crops of *A. proylei* during summer and autumn seasons (Kumar, 1981; Reeta *et al*., 2005, 2010). Much work has been done in various Lepidopteran pupae, such as estimation of the pupal weight loss in *Diacrisia obliqua* Wlk. by Goel and Kumar (1983) and in *Bombyx mori* Linn. by Kumar *et al.* (2000). *A. proylei* pupa during its developmental arrest (diapause) faces abiotic / physical factors *viz*., temperature, humidity, photoperiod, *etc* which widely fluctuate in different seasons during preservation period. Moreover, several metabolic activities and biochemical parameters undergo changes during preservation period (diapause), with implications in the course of development, metamorphosis and reproduction (Tripathi *et al*., 1990). However, relevant scientific information on oak tasar silkworm, *A. proylei* being meagre, it was felt necessary to study the photoperiodic effect during seed cocoon preservation and its impact on erratic moth emergence under normal room condition.

MATERIALS AND METHODS

Rearing of oak tasar silkworm, *A. proylei* was initiated during March and cocoons were harvested during April-May. The selected seed cocoons were garlanded and hung inside the wired mesh cage for preservation. They were preserved under four different treatments *viz.,* 8, 12 and 24 h photophase and 24 h scotophase w.e.f. 1st June to January under normal room condition. One hundred number each of male and female pupae in triplicate were numbered properly and weighed individually in an electronic balance (Model MX-7301A), every first week of the month till emergence of moth in all the four treatments. The parameters recorded during the period of preservation were meteorological data, pupal weight and erratic emergence of moths. The remaining seed cocoons of all the treatments after preservation were subjected to grainage operations separately during the month of February. The grainage performance was recorded in terms of male and female moth emergence, coupling per cent, fecundity, cocoon-DFL ratio and grainage span. The experiment was repeated for three consecutive years. Pooled data of three years were analysed by following standard statistical methods separately for male and female in each treatment and for all the treatments for a conclusive interpretation.

RESULTS AND DISCUSSION

The oak tasar seed cocoon preservation period lasted from June to January *i.e*., for about 255±9.99 days for 8, 12 and 24 h photophase and 24 h scotophase treatments under normal room condition. The female pupa was observed heavier with an average initial weight of 6.61±0.12, 6.61±0.30, 6.58±0.14 and 6.60±0.05 g, than male with corresponding weight of 4.86±0.16, 4.97±0.10, 4.80 ±0.07 and 4.82±0.12 g and subsequent final pupal weight of 6.00±0.15, 6.05±0.3, 5.89±0.26 and 5.93±0.14 g in female and 4.29±0.14, 4.43±0.56, 4.19±0.22 and 4.11±0.09 g in male preserved at 8, 12 and 24 h photophase and 24 h scotophase, respectively (Table 1) under normal room condition (Table 2). Similar trend was also observed in the pupae of *D. obliqua* (Goel and Kumar, 1983); *Lymantria marginata* Wlk. (Singh and Goel, 1985); *Phesia orichalcea* Fabr. (Kumar and Goel, 1985) and *B. mori* (Kumar *et al.*, 2000).

The progressive weight loss percentage over the months from June to January was recorded more in male than female with significant variation between them irrespective of treatments. The gradual increase noticed in the pattern of pupal weight loss during the pupal diapause probably corroborates with the phenomena of histolysis and histogenesis and also changes in the total amount of protein (Chen, 1966; Agrell and Landquist, 1973). The present finding is in conformity with those of Tripathi *et al*. (1990) in *A. mylitta* and Goel and Kumar, 1983, in *D. obliqua*. The total weight loss percentage in male pupa was significantly more (11.73, 10.86, 12.71 and 14.73 %) than female pupa (9.22, 8.47, 10.49 and 10.15 %) in all the four respective treatments (Table 3). This probably signifies the accumulation of energy by female for ovipositional

Table 1: Total weight loss percentage during seed cocoon preservation in *A. proylei*

Parameter	Total weight loss percentage (Mean ± S.D.)							
	8 h photophase		12 h photophase		24 h photophase		24 h scotophase	
	M	F	M	F	M	F	M	F
Initial pupal weight (g)	4.86±0.16	6.61±0.12	4.97±0.10	6.61±0.30	4.80±0.07	6.58±0.14	4.82±0.12	6.60±0.05
Final pupal weight (g)	4.29±0.14	6.00±0.15	4.43±0.56	6.05±0.31	4.19±0.22	5.89±0.26	4.11±0.09	5.93±0.14
Total weight loss (%)	11.73	9.22	10.86	8.47	12.71	10.49	14.73	10.15
Calculated 't' value	3.93		5.14		4.98		9.02	

Significant at 5 % level (Student's't' test).

activities. However, the weight loss percentage in both male and female were comparatively higher in the seed cocoons preserved at 24 h photophase and 24 h scotophase than those preserved at 8 and 12 h photophase under normal room condition. Successive increase in the weight loss percentage points towards the culmination of the pupal stage as high rate of metabolic activities takes place. The higher weight loss percentage in both male and female preserved at 24 h photophase and 24 h scotophase corresponded with more erratic moth emergence to the tune of 53.03 – 71.21 % than in seed cocoons preserved at 8 and 12 h photophase which recorded very low erratic emergence, to the extent of only 10.89 – 11.48 %.

The erratic moth emergence percentage during preservation was noticed progressive over the period from June to January and also more in male moths than in female for all the four treatments (Table 4). Short photoperiod or permanent darkness often causes uninterrupted growth (Lees, 1956). Tanaka (1950, 1951) has almost conclusively asserted that among external causes, day length exerts a decisive effect on the diapause of *A. pernyi.* According to him, all *A. pernyi* pupae can be induced to hibernate or emerge by simply controlling the day length in the larval stage irrespective of breed, season, culture or other external factors. *A. proylei* pupal diapause is also regulated by the environmental factors, such as photoperiod and temperature, as the pupal brain contains a photoperiodic receptor, which is linked to the neuro-secretory cells of the brain that produces prothoracicotropic hormone (PTTH). The seed cocoon preserved at 24 h photophase and 24 h scotophase experienced long day and initiated growth and development slowly, due to the production of PTTH-ecdysone. Whereas, since the seed cocoon preserved at 8 and 12 h photophase experienced short day, the PTTH secretion from the brain was shut off. In the absence of PTTH, the prothoracic glands stop producing the growth

Table 2: Meteorological data recorded during seed cocoon preservation under normal room condition

Month	Meteorological data (Mean ± S.D.)											
	8 h photophase			12 h photophase			24 h photophase			24 h scotophase		
	Temperature (°C)		RH (%)	Temperature (°C)		RH (%)	Temperature (°C)		RH (%)	Temperature (°C)		RH (%)
	Max.	Min.		Max.	Min.		Max.	Min.		Max.	Min.	
June	26.75±0.47	24.29±0.39	83.69±0.15	26.77±0.51	24.36±0.64	83.89±0.53	26.97±0.49	24.86±0.57	84.36±0.62	27.13±0.49	24.77±0.54	84.87±0.46
July	27.46±0.32	25.00±0.26	87.31±0.69	27.23±0.46	24.97±0.49	88.27±0.42	27.63±0.52	25.44±0.61	88.57±0.57	27.73±0.58	25.57±0.46	88.79±0.37
August	27.27±0.41	24.39±0.61	88.31±0.46	27.21±0.38	24.60±0.58	88.57±0.29	27.58±0.40	24.90±0.37	89.12±0.84	28.09±0.27	25.48±0.33	89.38±0.42
September	26.28±0.27	23.83±0.17	85.13±0.61	26.77±0.54	23.78±0.62	84.85±0.25	26.89±0.61	24.78±0.46	85.45±0.58	27.34±0.66	24.94±0.58	85.62±0.19
October	23.98±0.36	22.61±0.53	84.27±0.29	24.04±0.57	22.32±0.57	84.73±0.48	24.93±0.54	22.82±0.49	85.23±0.46	25.18±0.75	23.14±0.62	85.45±0.57
November	20.32±0.81	18.26±0.56	83.84±0.33	19.97±0.42	17.80±0.89	84.22±0.69	21.17±0.38	18.70±0.78	84.82±0.54	21.62±0.49	18.68±0.77	84.97± 0.52
December	16.52±0.29	14.36±0.47	83.56±0.41	16.39±0.71	14.96±0.66	83.86±0.37	16.86±0.43	15.53±0.72	84.36±0.64	17.27±0.58	15.81±0.25	85.29±0.62
January	15.90±0.64	13.66±0.43	83.02±0.50	15.80±0.78	13.73±0.47	83.09±0.52	16.10±0.62	14.37±0.29	83.86±0.35	16.70±0.49	14.26±0.17	84.35±0.68
Mean	23.06±0.40	20.80±0.43	84.89±0.43	23.02±0.54	20.82±0.62	85.19±0.45	23.52±0.50	21. 42±0.53	85.72±0.58	23.88±0.53	21.58±0.54	86.09 ±0.48

Table 3: Pupal weight loss percentage during seed cocoon preservation in *A. proylei*

Month	Progressive pupal weight loss (%) ± S.D.							
	8 h photophase		12 h photophase		24 h photophase		24 h scotophase	
	M	F	M	F	M	F	M	F
June	1.85 ±0.22	1.48±0.05	1.60±0.07	1.33±0.20	1.71±0.33	1.44±0.09	1.87±0.22	1.37±0.20
July	1.50±0.08	1.07±0.17	1.51±0.47	1.02±0.12	1.63±0.09	1.23±0.05	1.69±0.25	1.27±0.11
August	1.44±0.06	1.11±0.17	1.24±0.17	1.06±0.03	1.75±0.29	1.67±0.10	1.67±0.22	0.969±0.04
September	1.32±0.33	0.89±0.03	1.10±0.12	0.97±0.06	1.34±0.11	1.31±0.18	1.96±0.27	1.49±0.23
October	1.11±0.06	0.98±0.08	0.96±0.08	0.68±0.16	1.27±0.13	0.98±0.25	1.54±0.15	1.18±0.23
November	1.04±0.16	0.88±0.07	1.03±0.16	0.87±0.07	1.51±0.21	1.07±0.13	1.59±0.23	1.11±0.09
December	1.18±0.04	1.18±0.36	1.19±0.02	0.99±0.11	1.39±0.07	1.14±0.08	2.13±0.24	1.45±0.27
January	2.34±0.44	1.57±0.22	2.18±0.06	1.63±0.05	2.11±0.11	1.65±0.12	2.27±0.16	1.39±0.06

C. D. at P < 0.05 - Male = 0.43; C. D. at P < 0.01 - Female = 0.24

Table 4: Emergence pattern during seed cocoon preservation in *A. proylei*

Month	Progressive emergence pattern (%) ± S.D.							
	8 h photophase		12 h photophase		24 h photophase		24 h scotophase	
	M	F	M	F	M	F	M	F
June	0.05±0.08	0.19±0.33	0.10±0.17	0.24±0.30	0.00±0.00	0.00±0.00	2.80±0.93	2.21±0.38
July	0.05±0.08	0.14±0.25	0.10±0.17	0.46±0.20	6.00±0.54	4.43±0.35	8.86±0.29	6.29±0.38
August	0.00±0.00	0.05±0.08	0.00±0.00	0.05±0.08	6.57±0.44	5.57±0.20	7.65±0.91	6.48±0.67
September	0.56±0.96	0.55±0.88	0.06±0.10	0.00±0.00	4.94±0.89	3.86±0.91	5.10±0.86	6.57±0.87
October	0.09±0.08	0.14±0.25	0.84±0.25	0.10±0.17	3.29±0.42	3.14±0.32	4.44±0.27	4.49±0.43
November	0.80±0.75	0.22±0.22	0.60±0.93	0.29±0.49	3.29±0.51	4.00±0.65	3.07±0.54	2.91±0.30
December	1.75±0.56	0.52±0.46	1.20±0.81	1.25±0.72	2.94±0.76	1.43±0.81	2.17±0.45	2.22±0.60
January	3.58±0.78	2.79±0.98	3.04±0.55	2.56±0.94	2.43±0.80	1.14±0.25	4.25±0.58	1.69±0.90

C. D. at P < 0.01 - Male = 4.14; C. D. at P < 0.01 - Female = 3.75

promoting hormone, ecdysone. The lack of ecdysone has been shown to be responsible for induction and maintenance of diapause (Chippendale, 1983). Pupal diapause in Lepidoptera is caused by a lack of ecdysone and is terminated either by supplying ecdysone or creating conditions under which the prothoracic glands secrete ecdysone (Williams, 1952). When the oak tasar pupae under preservation experience low temperature during winter, the prothoracic glands are activated to produce ecdysone, as a result, normal emergence of moths occur after winter without any photoperiodic treatment (Pandey *et al*., 1992). The above described physiological manifestations indicate that oak tasar seed cocoon can be preserved at 8 and 12 h photophase which maintains the pupal diapause, whereas, the seed cocoon preserved at 24 h photophase and 24 h scotophase terminates the pupal diapause under normal room condition. Similarly, in *A. pernyi,* diapause is completely prevented in total darkness (Tanaka, 1950). Lees (1956) refers that both light and dark phases of the cycle of illumination are involved in the control of diapause. In *Antheraea* and majority of insect species, long photoperiod or permanent illumination exercises its effect by preventing diapause, while 8 or 12 hours of it induces diapause (Chowdhury, 1962). In silkworm, *B. mori*, light effect is just reverse unlike Saturniids, a long day of more than 16 hours generates egg diapause and shorter day of 12 hours and below gives non-diapause eggs.

The grainage performance of the treated preserved seed cocoons revealed no significant differences in coupling percentage and fecundity among the treatments (Table 5). Similar findings were reported by Benchamin *et al*., 1990, in *B. mori* that different durations of photoperiodic

Table 5: Grainage performance of preserved seed cocoons

Treatment	Emergence (%)			Coupling (%)	Fecundity (no.)	Cocoon-DFL ratio	Grainage span (days)
	Male	Female	Total				
8 h photophase	45.45	40.72	86.17	83.89	115	3.34	17
12 h photophase	43.15	41.00	84.15	84.17	114	3.87	16
24 h photophase	20.25	23.61	43.86	81.22	113	5.43	16
24 h scotophase	11.53	13.83	25.36	80.38	112	6.60	15
CD at 5 %	12.64	7.84	16.98	NS	NS	2.77	NS

treatment failed to affect fecundity and fertility as most of the oocyte maturation and spermatogenesis are completed before the treatment, which starts on the 6th day of spinning. However, significant difference was observed in cocoon - DFL ratio among the treatments.

Further, the abiotic factors *viz*., maximum & minimum temperature and relative humidity were found not to have much impact on the loss of pupal weight in *A. proylei,* irrespective of treatments as also reported in *B. mori* (Kumar *et al*., 2000).

While the fluctuations in abiotic factors exerted an influence on the erratic moth emergence percentage in seed cocoons preserved at 24 h photophase and 24 h scotophase, it didn't have a bearing on treatments of 8 h and 12 h photophase (Table 7). These results provide a convincing indication that *A. proylei* seed cocoons should be preserved at 8 to 12 h photophase to maintain the diapause and at 24 h photophase and 24 h scotophase, diapause is terminated at a slow rate under normal room condition. The Chinese oak silkworm, *A. pernyi* is considered exceptional (Lees, 1968), because the photoperiodic induction of diapause is reported to be virtually independent of temperature (Tanaka, 1944). Indeed, the termination of diapause in this Saturniid is unique among known instances, regardless of whether the pupae are previously exposed to low temperature treatment or not, adult development is initiated only under long day photoperiod (Williams and Adkisson, 1964). Hodek and Cerkasov (1960) have shown experimentally that multivoltine *Coccinellidae* show a correlation between the photoperiod and temperature in the induction of imaginal diapause, the photoperiod being more significant, long photoperiod appears to prevent diapause and a short one induces diapause. Mansingh and Smallman (1971) reported that although photoperiod can exert a dominant role in the induction and termination of diapause, particularly with *A. pernyi*, nevertheless, slight rise in the temperature during critical developmental stage can modify or nullify the photoperiodic effects.

Hence, oak tasar seed cocoons should be preserved at 8 to 12 h photophase for maintaining the pupal diapause, whereas, preservation at 24 h photophase and 24 h scotophase terminate the pupal diapause at a slow rate under normal room condition.

Table 6: Correlation between abiotic factors and pupal weight loss in *A. proylei*

Parameter	Correlation co-efficient (r)							
	8 h photophase		12 h photophase		24 h photophase		24 h scotophase	
	M	F	M	F	M	F	M	F
Max. temp. (ºC) Vs pupal weight loss (%)	-0.20	-0.33	-0.30	-0.29	-0.22	-0.07	-0.61	-0.30
Min. temp. (ºC) Vs pupal weight loss (%)	-0.24	-0.36	-0.35	-0.36	-0.30	-0.02	-0.62	-0.29
R. H. (%) Vs pupal weight loss (%)	-0.30	-0.42	-0.20	-0.32	-0.02	-0.15	-0.35	-0.55

Table 7: Correlation between abiotic factors and moth emergence in *A. proylei*

Parameter	Correlation co-efficient (r)							
	8 h photophase		12 h photophase		24 h photophase		24 h scotophase	
	M	F	M	F	M	F	M	F
Max. temp. (ºC) Vs emergence (%)	-0.95	-0.91	-0.84	-0.81	0.37	0.45	0.59	0.77
Min. temp. (ºC) Vs emergence (%)	-0.96	-0.91	-0.86	-0.84	0.36	0.45	0.58	0.77
R. H. (%) Vs emergence (%)	-0.63	-0.60	-0.57	-0.49	0.85	0.80	0.88	0.75

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Research Paper

DEVELOPMENT OF NEW BIVOLTINE DOUBLE HYBRIDS OF SILKWORM, *BOMBYX MORI* (L.) UNDER SEMI TEMPERATE CONDITIONS OF NILGIRIS

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ABSTRACT

The present study embodies a breeding programme designed for evaluation and evolution of productive bivoltine silkworm double hybrids using the newly identified fourteen potential Foundation Crosses (FCs), including eight oval cocoon FCs, *viz*., CSR 2 x D 1, CSR 2 x D 7, CSR 2 x Gen 3, CSR 2 x CSR 50, SLD 1 x SLD 2, D 1 x D 7, D2 x D 7 and CSR 50 x CSR 27 and six dumbbell cocoon FCs, *viz*., CSR 51 x CSR 6, CSR 51 x CSR 26, D 11 x CNR 3, D 11 x SLD 8, D 13 x CNR 3 and D 13 x SLD 8, as resource material. The study was conducted at Satellite Silkworm Breeding Station, Central Silk Board, Coonoor. The 14 Foundation Crosses were reared following the prescribed standard bivoltine rearing technologies, selected cocoons and prepared layings of forty eight new combinations of double hybrids in dumbbell FC (D x D) x oval FC (O x O) fashion among them in all possible combinations of direct crosses. Evaluated their performance in two levels, *viz*., level I and II, in three seasons each under controlled laboratory conditions. The popular double hybrid in the field (CSR 6 x 26) x (CSR 2 x 27) was reared along with them as control. Studied their efficiency on rearing and reeling performance based on the economically important fourteen cocoon and silk yield parameters; seven each of pre-cocoon, *viz*., fecundity, larval duration, effective rate of rearing (ERR) by no. (based on pupation rate) and ERR by weight, cocoon weight, shell weight and shell percentage and post cocoon traits, *viz*., average filament length (AvFL), non breakable filament length (NBFL), raw silk (RS) %, raw silk recovery (RSR) %, reelability, neatness and renditta. Analyzed the data using the Multiple Trait Evaluation Index method and short listed the hybrids from the initial 48 to 14 under Level – I. Further evaluated the 14 double hybrids in three seasons (Level – II) and identified two productive and potential double hybrids, namely (CSR 51 x 26) x (CSR 2 x D1) and (CSR 51 x 26) x (CSR 2 x Gen 3) as better than the control hybrid. The potential of these hybrids could be exploited in the field in large scale for the benefit of sericulture industry.

Key words: Bivoltine silkworm, double hybrids, evaluation.

INTRODUCTION

The sericulture industry in India mainly focused on the exploitation of hybrids of multivoltines and cross breeds (multi x bi) in the field for many decades. The possibility and potentiality of bivoltine sericulture were realized in the country in the early nineties and since then, the efforts were being made for promotion of bivoltine sericulture in India. During the last three decades, increase in silk production benefited, to a great extent, from the application of genetic principles in the silkworm breeding programmes and development of technologies over a period of time. Bivoltine Sericulture has an added advantage over multivoltines and cross breed in terms of quantitative and qualitative improvement in cocoon productivity and raw silk. The recent evolution and popularization of CSR double hybrids and their foundation crosses have made a tremendous improvement in survival and cocoon yield, sustenance of sericulture, stability in cocoon crops and raw silk quality.

Consequently, over the years, the concept of single hybrids paved the way for the development of Foundation Crosses and identification of double hybrids in the field. Many bivoltine double hybrids were evolved and authorized in India which has revolutionized the Indian silk industry and the economic status of the farmers. The bivoltine double hybrids coupled with the proven sericulture technologies in the form of package of practices for mulberry cultivation and silkworm rearing have made bivoltine sericulture, a luring occupation with high returns among the farmers (Basavaraja, 1996; Nirmal Kumar *et al*., 1998; Datta *et al.,* 2000; Yamaguchi, 2000; Mal Reddy *et al.,* 2005; Pallavi and Basavaraja, 2007; Rajalakshmi and Sakthivel, 2019).

Keeping this in view, a study was taken up to evolve new potential and productive bivoltine double hybrids utilizing the newly evolved, fourteen promising Foundation Crosses at Satellite Silkworm Breeding Station, Coonoor (Rajalakshmi and Sakthivel, 2019). Coonoor, being a semi temperate congenial zone in Nilgiris, has the record of evaluation and evolution of many bivoltine breeds and hybrids which were later authorized (Rajalakshmi *et al.,* 1997, 1998; Rajalakshmi, 2014; Chauhan *et al.,* 2000).

MATERIALS AND METHODS

Newly identified, fourteen Foundation Crosses (FCs) comprising of six dumbbell FCs, namely CSR 51 x CSR 6, CSR 51 x CSR 26, D 11 x CNR 3, D 11 x SLD 8, D 13 x CNR 3 and D 13 x SLD 8 and eight oval FCs, *viz*., CSR 2 x D 1, CSR 2 x D 7, CSR 2 x Gen 3, CSR 2 x 50, SLD 1 x SLD 2, D 1 x D 7, D 2 x D 7 and CSR 50 x CSR 27 were used as resource material for the study. These FCs were reared following standard bivoltine rearing technologies, selected cocoons and prepared layings of forty eight new combinations of double hybrids in dumbbell x oval fashion among them in all possible combinations of direct crosses (Table 1) and assigned the respective codes (DH 1 ~ DH 48) for ease of further description in this article.

The silkworm rearing trials were conducted following the standard package of practices of bivoltine rearing. The

Table 1: Direct crosses of new double hybrid combinations

Crosses: Dumbbell x Oval ⟹ Oval FCs - 8 (Males)

⇩ Dumbbell FCs - 6 (Females)	(CSR 2 x D 1)	(CSR 2 x D 7)	(CSR 2 x Gen 3	(SLD 1 x 2)	(D 1 x D 7)	(D 2 x D 7)	(CSR 2 x 50)	(CSR 50 x 27)
(CSR 51 x CSR 6)	DH 1	DH 2	DH 3	DH 4	DH 5	DH 6	DH 7	DH 8
(CSR 51 x CSR 26	DH 9	DH 10	DH 11	DH 12	DH 13	DH 14	DH 15	DH 16
(D 11 x CNR 3)	DH 17	DH 18	DH 19	DH 20	DH 21	DH 22	DH 23	DH 24
(D 11 x SLD 8)	DH 25	DH 26	DH 27	DH 28	DH 29	DH 30	DH 31	DH 32
(D 13 x CNR 3)	DH 33	DH 34	DH 35	DH 36	DH 37	DH 38	DH 39	DH 40
(D 13 x SLD 8)	DH 41	DH 42	DH 43	DH 44	DH 45	DH 46	DH 47	DH 48

study was conducted in two levels – Level – I, screening and shortlisting from a large no. of crosses and Level – II, testing the short listed hybrids from Level – 1 along with the control hybrid in the field (CSR 6 x CSR 26) x (CSR 2 x CSR 27), in three seasons each. Mass rearing of five disease free layings (DFLs) of each cross was carried out and after 3rd moult, the larvae were replicated into three batches of 250 larvae each. Studied their efficiency on rearing and reeling performance based on fourteen (Level -1) economically important cocoon and silk yield parameters; seven each of i) rearing and cocoon traits, *viz*., fecundity (egg no.), larval duration (fifth age and total) for Level – II, ERR by No. (based on the pupation rate by counting the cocoons with live pupae inside) and ERR by weight for cocoon yield, cocoon weight, shell weight and shell percentage and ii) post cocoon traits, *viz*., average filament length (AvFL), non breakable filament length (NBFL), renditta, reelability, raw silk %, raw silk recovery % and denier and neatness for level (I).

The data on the performance of forty eight double hybrids (DHs) at Level - I and Level – II, were analyzed using the widely accepted "Multiple Trait Evaluation Index" (Mano *et al*., 1993) method followed in sericulture to short list and identify the best combinations from a large number of crosses based on a number of characters of economic importance, as per the formula given below.

Evaluation Index (E.I.) = ((A–B) / C) x 10) + 50

A - Individual value of a hybrid for a particular trait (e.i.),

B - The Mean value of all hybrids for a particular trait,
C - Standard Deviation for a trait,
10 = Standard unit and 50 = Fixed value.

The data on rearing parameters of short listed hybrids were subjected to ANOVA and estimation of coefficient of variation over seasons. The data of the foundation crosses of the two identified double hybrids and their parents along with that of the control hybrid is also presented.

RESULTS AND DISCUSSION

A) Trial Level 1: Testing of 48 hybrids and short listing

The average data of three trials on the rearing, cocoon and reeling performance of forty eight Double Hybrids along with the control are presented in Table 2. The Multiple Trait Evaluation Index (E.I.), individual (e.i.) and overall average E.I. values of the hybrids are presented in Table 3.

i) Fecundity

Significant variation ($P < 0.05$) in fecundity (*i.e.*, number of eggs / laying) was observed among double hybrids. The data showed that the fecundity ranged from 521 in DH 30 to 690 in DH 33 with an average of 626. Thirteen hybrids recorded a fecundity of above average value (Table 2).

The highest individual e.i. value for fecundity was 69.85 recorded by DH 33, followed by 67.06 (DH 39) and 61.48 (DH 4, DH 10, DH 20) (Table 3). Twenty five hybrids recorded e.i. > 50 for fecundity, the most important trait in view of cocoon yield.

ii) Effective rate of rearing (ERR) by No. (Pupation rate)

The rate of pupation *i.e.*, the number of cocoons with live pupa inside, or otherwise called survival, is considered one of the important traits in cocoon crops, as far as farmers are concerned because it fetches them more money. Significant variation was registered in the survival ($P < 0.05$) of the hybrids. It registered the highest of 97.79 % (DH 16) followed by 97.71 (DH 5) to the lowest value of 92.0 % (DH 36) *i.e.*, all hybrids recorded > 90 %, with an average of 96.63 % of which 29 hybrids recorded more than average (Table 2). There is a significant improvement in the rate of pupation in double hybrids, a highly desirable trait for the farmers.

The individual e.i. value of > 50 was recorded by 29 hybrids with the highest value of 58. 80 (DH 16) followed by 58.20 (DH 5), 57.29 (DH 9) and 57.06 (DH 25).

iii) Effective rate of rearing (ERR) by weight (Cocoon yield / 10000 larvae)

Cocoon yield in terms of weight recorded significant difference ($P <0.05$), and is considered the most important trait, from farmers' angle. It ranged from the lowest of 19.673 (DH 36) to the highest of 23.96 kg (DH 9). Three hybrids recorded cocoon yield below 20 kg and twenty two hybrids, more than the average of 21.55 kg of which three hybrids (DH 3, DH 7 and DH 9) recorded more than 23 kg (Table 2). Overall, a significant improvement in the cocoon yield is visible in double hybrids, a highly desirable trait from farmers' point of view.

Twenty two hybrids registered the individual e.i. value of > 50, the highest being 83. 03 (DH 9) followed by 71.40 (DH 7), 70.58 (DH 3), 65.55 (DH 8) and 65.42 (DH 13).

iv) Cocoon weight

The hybrids did not register any significant difference ($P <0.05$) for cocoon weight and all forty eight hybrids registered > 2 g. It ranged from 2.028 (DH 34) to 2.272 g (DH 3), with an average of 2.162 g. Sixteen hybrids recorded > 2.2 g and 24 hybrids, > mean value.

The individual e.i. value of > 50 was recorded by twenty two hybrids with the highest value of 68.33 (DH 3),

Table 2: Performance of new double hybrids (Level – 1)

Double Hybrid	Fecundity (No.)	ERR by		Cocoon weight (g)	Shell weight (g)	Shell %	AvFL (m)	NBFL (m)	Denier	Renditta	Raw silk %	R.S.R. %	Reelability %	Neatness (p)
		No.	Weight (kg)											
DH 1	578	9504	22.543	2.241	0.487	21.71	1148	1025	3.16	5.44	18.42	84.28	83.12	94
DH 2	635	9720	22.191	2.213	0.486	22.08	1161	1050	3.20	5.57	18.45	80.94	83.84	93
DH 3	656	9720	23.047	2.272	0.518	22.80	1152	1052	3.19	5.45	18.57	80.18	87.57	93
DH 4	663	9691	21.536	2.204	0.490	22.22	1195	1019	3.21	5.23	19.15	86.27	85.24	93
DH 5	637	9771	22.311	2.199	0.465	22.33	1137	962	3.09	5.75	17.50	85.25	84.54	93
DH 6	646	9673	21.590	2.151	0.48	22.31	1168	997	3.20	5.18	19.32	86.34	85.43	93
DH 7	631	9623	23.107	2.219	0.496	22.34	1201	1007	3.08	5.58	17.96	79.10	83.81	94
DH 8	653	9752	22.680	2.151	0.496	22.39	1190	1033	3.22	5.27	19.00	83.59	86.81	94
DH 9	642	9759	23.956	2.259	0.505	22.35	1197	1048	3.09	5.71	17.62	77.96	87.70	93
DH 10	663	9717	22.087	2.134	0.481	22.51	1217	1052	3.19	4.98	20.09	86.10	86.74	93
DH 11	636	9744	22.063	2.143	0.472	22.00	1223	954	3.12	5.08	18.41	84.27	83.79	94
DH 12	601	9711	21.964	2.141	0.472	22.06	1151	982	3.09	5.51	18.20	81.90	85.26	94
DH 13	583	9718	22.671	2.248	0.499	22.39	1225	1037	3.17	5.20	19.26	85.05	84.64	94
DH 14	600	9666	22.621	2.202	0.499	22.61	1208	1023	3.16	5.18	19.32	84.83	84.65	93
DH 15	603	9751	22.568	2.203	0.494	22.44	1139	964	3.18	5.49	18.24	81.12	84.63	94
DH 16	609	9779	22.418	2.208	0.496	22.46	1213	1028	3.21	5.14	19.46	86.37	84.67	93
DH 17	652	9635	21.469	2.224	0.489	21.97	1164	967	3.21	5.38	18.59	83.58	83.12	94
DH 18	629	9640	21.024	2.118	0.459	21.66	1130	953	3.11	5.57	18.06	82.90	84.63	94
DH 19	620	9686	21.333	2.134	0.472	22.11	1111	948	3.16	5.66	17.76	80.79	85.37	94
DH 20	663	9479	19.951	2.162	0.468	21.63	1103	949	3.12	5.67	17.65	81.17	86.08	93
DH 21	641	9653	21.700	2.128	0.466	21.89	1141	926	3.14	5.39	18.56	84.87	81.18	94
DH 22	625	9673	20.527	2.092	0.460	21.98	1280	1073	3.14	5.45	19.72	88.45	83.74	93
DH 23	628	9701	20.824	2.141	0.463	21.63	1160	979	3.02	5.56	18.04	82.82	84.59	94
DH 24	608	9699	21.609	2.161	0.480	22.19	1146	960	3.18	5.30	18.90	84.37	83.86	93
DH 25	580	9756	22.453	2.228	0.497	22.30	1195	1010	3.22	5.35	18.72	83.31	84.59	93
DH 26	609	9632	20.896	2.141	0.477	22.28	1216	1020	3.19	5.01	19.95	87.80	83.84	93
DH 27	582	9712	21.809	2.163	0.482	22.26	1167	1030	3.14	5.38	18.63	82.74	88.40	93
DH 28	621	9589	20.716	2.100	0.465	22.01	1162	969	3.19	5.26	19.00	85.20	83.34	94
DH 29	615	9714	22.311	2.245	0.502	22.34	1221	1049	3.13	5.43	18.42	81.54	85.92	94
DH 30	521	9697	21.204	2.108	0.474	22.47	1141	927	3.20	5.26	19.08	83.77	81.73	93
DH 31	645	9590	21.293	2.224	0.491	22.07	1195	1019	3.23	5.24	19.11	86.73	85.29	94
DH 32	644	9688	22.113	2.240	0.507	22.61	1204	1027	3.17	5.18	19.33	84.70	85.35	94
DH 33	690	9654	21.561	2.210	0.490	22.19	1223	1049	3.16	5.25	19.13	86.32	86.06	94
DH 34	637	9597	20.262	2.028	0.457	22.5	1184	996	3.12	5.04	19.88	87.50	83.89	94
DH 35	646	9471	20.467	2.095	0.460	21.96	1161	1036	3.10	5.37	18.63	84.88	89.17	93
DH 36	638	9210	19.673	2.031	0.415	20.41	1080	957	3.16	5.41	18.55	90.87	88.44	94
DH 37	650	9576	19.847	2.075	0.468	22.56	1187	1030	3.07	5.16	19.41	84.67	86.84	94
DH 38	657	9688	20.000	2.064	0.456	22.12	1137	970	3.02	5.41	18.53	83.73	85.28	93
DH 39	681	9700	21.100	2.13	0.476	22.33	1192	1034	2.98	5.45	18.40	82.60	86.95	94
DH 40	642	9710	21.313	2.127	0.473	22.22	1197	1006	3.08	5.36	19.72	84.73	84.60	93
DH 41	593	9735	21.895	2.204	0.494	22.40	1184	1022	3.05	5.55	18.05	79.92	86.07	94
DH 42	611	9664	20.593	2.086	0.471	22.59	1176	1021	3.09	5.23	19.15	84.80	86.80	93
DH 43	656	9631	20.874	2.180	0.486	22.29	1143	983	3.17	5.48	18.28	81.64	86.04	94
DH 44	561	9683	20.644	2.133	0.462	21.65	1191	1007	3.17	5.22	19.18	87.27	84.56	93
DH 45	655	9691	21.337	2.166	0.485	22.31	1194	1046	2.99	5.63	17.76	79.10	87.57	94
DH 46	587	9692	20.509	2.080	0.468	22.48	1179	987	3.36	5.51	18.19	80.64	83.86	94
DH 47	640	9660	22.178	2.194	0.486	22.14	1190	1025	3.15	5.30	18.89	84.47	86.15	93
DH 48	606	9632	21.338	2.171	0.488	22.47	1181	1008	3.06	5.44	18.39	81.06	85.46	94
Average	626	9663	21.545	2.162	0.480	22.18	1176	1002	3.14	5.37	18.71	83.80	85.19	94
CD at 5 %	9.97	29.49	0.29	0.02	0.01	0.12	11.26	11.47	0.02	0.06	0.20	0.83	0.53	0.15

Data are average of three trials; ERR – Effective rate of rearing; AvFL – Average filament length; NBFL – Non – breakable filament length; R.S.R. % - Raw silk recovery %.

Table 3: Evaluation Index (E.I.) values of new double hybrids (Level – 1)

Double Hybrid	Fecundity (No.)	ERR by		Cocoon weight (g)	Shell weight (g)	Shell %	AvFL (m)	NBFL (m)	Denier	R.S. %	R.S.R. %	Reelability %	Neatness (p)	Avg. E.I. values	Renditta
		No.	Weight (kg)												
DH 1	53.10	37.93	63.67	63.17	53.50	41.30	40.96	58.74	51.67	45.32	51.50	36.82	60.00	49.21	54.06
DH 2	52.79	54.33	58.85	58.50	53.00	48.15	45.00	68.64	56.11	48.33	40.98	43.65	50.00	51.98	42.19
DH 3	59.31	54.33	70.58	68.33	69.00	61.48	42.26	69.42	55.56	47.66	38.58	65.16	50.00	57.82	54.69
DH 4	61.48	52.13	49.88	57.00	55.00	50.74	55.97	56.41	57.78	57.02	57.79	50.32	50.00	54.73	40.94
DH 5	53.41	58.20	60.49	56.17	42.50	52.78	37.42	34.27	44.44	30.48	54.56	45.86	50.00	47.74	73.44
DH 6	56.20	50.76	50.62	48.17	50.00	52.41	47.42	47.86	56.11	59.76	58.01	51.53	50.00	52.22	38.13
DH 7	51.55	46.96	71.40	59.50	58.00	52.96	57.91	51.75	42.78	37.82	35.17	41.21	70.00	52.08	62.81
DH 8	58.37	56.76	65.55	48.17	58.00	53.89	54.52	62.04	58.33	54.60	49.32	60.29	60.00	56.91	43.75
DH 9	54.96	57.29	83.03	66.17	62.50	53.15	56.62	67.67	43.89	32.42	31.58	65.99	50.00	55.79	71.25
DH 10	61.48	54.10	57.42	45.33	50.50	56.11	63.07	69.42	55.00	72.26	57.26	59.87	50.00	57.83	25.63
DH 11	35.11	56.15	57.10	46.83	46.00	46.67	65.17	31.36	47.78	45.16	51.48	41.08	70.00	51.76	31.88
DH 12	42.25	53.64	55.74	46.50	46.00	47.78	41.93	42.23	43.89	41.77	44.01	50.45	70.00	48.17	58.75
DH 13	36.66	54.17	65.42	64.33	59.50	53.89	65.81	63.59	53.33	58.79	53.94	46.50	60.00	56.61	39.06
DH 14	41.94	50.23	64.74	56.67	59.50	57.96	60.16	57.96	52.22	59.76	53.25	46.53	50.00	54.69	38.13
DH 15	42.87	56.68	64.01	56.83	57.00	54.81	37.90	35.05	53.89	42.34	41.53	46.43	60.00	49.95	57.19
DH 16	44.73	58.80	61.96	57.67	58.00	55.19	61.78	60.10	57.22	62.10	58.09	46.66	50.00	56.33	35.63
DH 17	58.06	47.87	48.96	60.33	54.50	46.11	45.97	36.41	57.78	47.98	49.31	36.82	60.00	50.01	50.63
DH 18	50.93	48.25	42.86	42.67	39.50	40.37	35.00	30.78	46.11	39.44	47.15	46.43	60.00	43.81	62.50
DH 19	48.14	51.75	47.10	45.33	46.00	48.70	29.03	28.83	51.67	34.68	40.49	51.11	60.00	44.83	67.81
DH 20	61.48	36.03	28.16	50.00	44.00	39.81	26.28	29.42	47.78	32.82	41.70	55.64	50.00	41.78	68.75
DH 21	54.65	49.24	52.12	44.33	43.00	44.63	38.71	20.29	50.00	47.58	53.36	24.43	60.00	44.80	50.94
DH 22	49.69	50.76	36.05	38.33	40.00	46.30	83.40	77.38	49.44	66.21	64.67	40.76	50.00	53.31	51.56
DH 23	50.62	52.88	40.12	46.50	41.50	39.81	44.68	40.87	36.11	39.19	46.91	46.18	60.00	45.03	61.56
DH 24	44.42	52.73	50.88	49.83	50.00	50.19	40.16	33.69	54.44	53.06	51.78	41.53	50.00	47.90	45.63
DH 25	35.73	57.06	62.44	61.00	58.50	52.22	55.97	52.91	58.33	50.08	48.44	46.15	50.00	52.99	48.44
DH 26	44.73	47.65	41.11	46.50	48.50	51.85	62.91	56.80	55.56	70.00	62.62	41.40	50.00	52.28	27.50
DH 27	36.35	53.72	53.62	50.17	51.00	51.48	46.93	60.68	50.00	48.63	46.66	70.45	50.00	51.51	50.31
DH 28	48.45	44.38	38.64	39.67	42.50	46.85	45.32	37.18	55.00	54.60	54.40	38.18	60.00	46.55	43.13
DH 29	46.59	53.87	60.49	63.83	61.00	52.96	64.52	68.25	48.89	45.24	42.85	54.62	70.00	55.24	53.75
DH 30	17.43	52.58	45.33	41.00	47.00	55.37	38.71	20.68	56.11	55.97	49.89	27.96	50.00	42.93	42.81
DH 31	55.89	44.46	46.55	60.33	55.50	47.96	56.13	56.41	60.00	56.45	59.23	50.64	60.00	54.58	41.88
DH 32	55.58	51.90	57.78	63.00	63.50	57.96	59.04	59.71	52.78	60.00	52.84	50.99	60.00	57.31	38.13
DH 33	69.85	49.32	50.22	58.00	55.00	50.19	65.17	68.25	52.22	56.69	57.93	55.54	60.00	57.57	42.19
DH 34	53.41	44.99	32.42	27.67	38.50	55.93	52.58	47.48	47.22	68.79	61.67	41.72	60.00	48.64	29.38
DH 35	56.20	35.43	35.23	38.83	40.00	45.93	45.16	63.01	45.00	48.63	53.41	75.35	50.00	48.63	50.00
DH 36	53.72	15.62	24.36	28.17	17.50	17.22	19.02	32.33	51.67	47.34	72.29	70.67	70.00	39.99	52.50
DH 37	57.44	43.40	26.74	35.50	44.00	57.04	53.55	60.68	41.67	61.29	52.73	60.48	70.00	51.12	36.56
DH 38	59.62	51.90	28.84	33.67	38.00	48.89	37.42	37.38	36.11	47.02	49.76	50.54	50.00	43.78	52.19
DH 39	67.06	52.81	43.90	44.67	48.00	52.78	55.00	62.23	32.22	44.92	46.20	61.18	70.00	52.38	54.69
DH 40	54.96	53.57	46.82	44.17	46.50	50.74	56.78	51.36	42.78	66.21	52.92	46.21	50.00	51.00	49.38
DH 41	39.76	55.46	54.79	57.00	57.00	54.07	52.58	57.77	39.44	39.27	37.74	55.61	60.00	50.81	61.25
DH 42	45.35	50.08	36.96	37.33	45.50	57.59	49.84	57.18	43.89	57.02	53.15	60.22	50.00	49.55	40.94
DH 43	59.31	47.57	40.81	53.00	53.00	52.04	39.35	42.62	52.78	42.98	43.19	55.41	60.00	49.39	56.88
DH 44	29.84	51.52	37.66	45.17	41.00	40.19	54.68	51.94	53.33	57.50	60.95	45.99	50.00	47.67	40.31
DH 45	59.00	52.13	47.15	50.67	52.50	52.41	55.81	66.89	33.33	34.68	35.16	65.16	60.00	51.14	66.25
DH 46	37.90	52.20	35.81	36.33	44.00	55.56	50.81	44.17	73.89	41.61	40.02	41.53	70.00	47.99	58.75
DH 47	54.34	49.77	58.67	55.33	53.00	49.26	54.52	58.93	51.11	52.90	52.10	56.08	50.00	53.54	45.31
DH 48	43.80	47.65	47.16	51.50	54.00	55.37	51.45	52.33	40.56	44.76	41.36	51.69	70.00	50.12	54.38
Average	50	50	50.01	49.98	49.82	50.09	50	50	49.94	49.98	50.00	50.00	56		49.73
SD	9.95	10.00	9.96	9.55	9.68	10.09	10.00	10.00	10.32	9.99	10.00	9.99	9.90		9.90

R.S. % - Raw silk %

followed by 66.17 (DH 9), 63.83 (DH 29) and 63 (DH 32).

v) Shell weight

Significant difference (P <0.05) was not registered for cocoon shell weight too, the values ranged from the minimum of 0.415 (DH 36) to the maximum of 0.518 g (DH 3), with an average value of 0.480 g. Twenty six hybrids registered values > average and four hybrids (DH 3, DH 7, DH 29 and DH 32) recorded more than 0.50g.

Twenty three hybrids registered individual e.i. value of >50 with the highest value of 69 (DH 3) followed by 63.5 (DH 32), 62.5 (DH 9) and 61 (DH 29).

vi) Shell percentage

No significant difference was registered among hybrids for cocoon shell percentage. It registered the highest of 22.80 % (DH 3) to the lowest, 21.63 % (DH 20, DH 23). Thirty one hybrids recorded above the average of 22.18% and 9, below 22 %.

The individual e.i. value of > 50 was recorded by 26 hybrids with the highest value of 61.48 (DH 3) followed by 57.96 (DH 14, DH 32), 57.59 (DH 42) and 57.04 (DH 37).

vii) Average filament length (AvFL)

The length of cocoon filament showed significant difference (P< 0.05) among the hybrids and it ranged from 1080 (DH 36) to 1280 m (DH 22). Twenty eight hybrids recorded more than the average value of 1176 m, of which eleven hybrids exceeded 1200 m.

Twenty four hybrids recorded the individual e.i. value of > 50 with the highest value of 83.40 (DH 22) followed by 65.81 (DH 13), 65.17 (DH 11, DH 33), 64.52 (DH 29) and 63.07 (DH 10) .

viii) Non breakable filament length (NBFL)

The non-breakable filament length showed significant variation within the range of 926 (DH 21) to 1073 m (DH 22). Twenty nine hybrids registered values above the average of 1002 m but, 19 of them recorded below 1000 m.

The individual e.i. value of > 50 was recorded by twenty eight hybrids with the highest value of 77.38 (DH 22) followed by 69.42 (DH 3, DH 10), 68.25 (DH 29, DH 33), 67.67 (DH 9) and 66.89 (DH 45).

ix) Denier (Filament size)

Filament size did not show significant variation among the hybrids and the values were closer to the average value of 3.14 d with the range of 2.98 (DH 39) to 3.23 d (DH 31). The lesser the denier, more beneficial the silk and hence, selection is based on the lowest values.

The individual e.i. value of < 50 was recorded by 19 hybrids with the lowest value of 32.22 (DH 39) followed by 33.33 (DH 45), 36.11 (DH 23, DH 38), 40.56 (DH 48) and 42.71 (DH 7).

x) Renditta

Significant difference (P < 0.05) was observed for renditta among the hybrids. It ranged from the highest value of 4.98 (DH 10) followed by 5.01 (DH 26), 5.04 (DH 34), 5.08 (DH 11), 5.14 (DH 16) and 5.18 (DH 6), to the lowest of 5.71 kg (DH 9). Twenty one hybrids recorded better (lesser) than the average value of 5.37 kg. Lesser the value of renditta, it's more beneficial for the reeler.

The individual e.i. value of < 50 in the order of merit was recorded as 25.63 (DH 10), 27.50 (DH 26), 29.38 (DH 34), 31.56 (DH 11), 35.63 (DH 16) and 36.56 (DH 37). Twenty one hybrids registered the values < 50, qualifying them for selection.

xi) Raw silk

Significant difference (P< 0.05) was registered for raw silk % and the data varied from 17.62 (DH 9) to the highest of 20.09 % (DH 10) with a mean value of 18.71 %. Twenty two hybrids recorded above the average.

The individual e.i. value of > 50 recorded by twenty four hybrids in the order of merit are 72.26 (DH 10), 70.0 (DH 26) , 68.0 (DH 34), 66.21 (DH 22, DH 40) and so on.

xii) Raw silk recovery (RSR)

The hybrids registered a significant difference (P< 0.05) for raw silk recovery % and the data varied from the lowest of 77.96 (DH 9) to the highest of 90.87 % (DH 36) with an average of 83.80 %. Twenty five hybrids recorded values above the average.

The individual e.i. value of > 50 recorded by 25 hybrids in the order of merit are 72.29 (DH 36), 64.67 (DH 22), 62.62 (DH 26), 61.67 (DH 34) and so on.

xiii) Reelability

A significant difference (P<0.05) was registered for reelability % with the highest of 89.17 % (DH 35) and the least of 81.18 % (DH 21). Twenty six hybrids registered more than the mean value of 85.19 %.

The individual e.i. value of > 50 was recorded by twenty one hybrids with the highest value of 75.35 (DH 35), followed by 70.67 (DH 36), 70.45 (DH 27), 65.99 (DH 9) and 65.16 (DH 3).

xiv) Neatness

There was no significant variation (P<0.05) for neatness points. It ranged from 93 to 94 and twenty six hybrids recorded 94 points. All the double hybrids evolved under the salubrious climatic conditions of Nilgiris showed good neatness.

The individual e.i. value of > 50 was recorded by twenty six hybrids with the highest value of 70 with DH 7, DH 11, DH 12, DH 29, DH 36, DH 37, DH 39, DH 46 and DH 48. Seventeen hybrids registered > 60.

The non-significant, negatively correlated and less economically important characters such as denier and neatness were not included for the Level – II test trial to have more reliable selection based on important traits. Instead, the data of the larval duration (fifth age duration and total duration) were considered while screening and identifying the hybrids. Also, the shortlisted hybrids were tested thrice along with the popular control hybrid (CSR 6 x 26) x (CSR 2 x 27) to have a stable concurrent result. Phenotypic observation of cocoon uniformity, a very important character while selecting and short listing was also studied separately.

B. Trial - Level II: Testing and evaluating the hybrids shortlisted from Level - I

The short listed fourteen double hybrids were further tested under Trial level – II for three seasons along with the control and the average data (Table 4), individual evaluation index (e.i.) and average Evaluation Index (E.I.) values (Table 5) and the ANOVA (Table 6) are presented.

1. Pre cocoon traits

Six hybrids recorded a **fecundity** of above the average value of 629 (Table 4). The highest value of 655 with an e.i. value of 66.35 (DH 11) was followed by 649 and 62.58 (DH 10), 647 and 61.19 (DH 32), 646 and 60.38 (DH 7) and 634 with 53.14 (DH 9, DH 13) (Table 5). Enhanced fecundity of more than 600 / laying, is one of the main advantages of double hybrids which ensure more cocoon yield upon good survival.

Larval duration, especially of fifth age is important as far as silk productivity per day and selection of suitable hybrids under semi temperate climatic conditions prevailing in the Nilgiris are concerned. Six hybrids (DH

Table 4: Performance of short listed double hybrids (Level – II)

Double Hybrid	Fecundity (No.)	Larval duration (Days: Hours)		ERR by No.	ERR by weight (kg)	Cocoon weight (g)	Shell weight (g)	Shell %	AvFL (m)	NBFL (m)	Renditta	R. S. %	R. S. R. %	Reelability %
		V age	Total											
DH 2	612	7:22	24:16	9694	21.297	2.143	0.469	22.04	1116	930	5.28	18.27	84.02	85.41
DH 3	620	7:20	24:14	9708	22.320	2.220	0.485	21.88	1110	931	5.19	19.88	88.96	83.92
DH 4	613	7:13	24:16	9667	21.956	2.228	0.479	21.53	1064	881	5.80	19.92	83.01	82.86
DH 7	646	7:20	24:14	9692	21.866	2.192	0.466	21.69	1100	977	5.33	18.77	86.39	86.15
DH 8	629	7:20	24:14	9707	21.422	2.196	0.478	21.77	1172	923	5.38	18.60	85.03	83.93
DH 9	634	7:20	24:14	9697	22.497	2.274	0.517	22.74	1165	1012	5.13	19.46	85.77	87.23
DH 10	649	7:16	24:12	9695	21.111	2.151	0.468	21.73	1109	960	5.46	18.67	84.13	85.23
DH 11	655	7:20	24:14	9711	22.118	2.213	0.497	22.46	1155	956	5.11	19.44	84.01	85.32
DH 13	634	7:22	24:20	9705	21.953	2.188	0.476	21.74	1086	958	5.48	18.34	82.73	86.25
DH 14	610	7:22	24:20	9718	21.667	2.194	0.473	21.57	1089	903	5.77	17.38	79.71	82.95
DH 29	629	7:18	24:20	9699	21.129	2.163	0.467	21.59	1203	1033	5.42	18.45	81.91	85.78
DH 31	629	7:16	24:20	9713	21.616	2.186	0.478	21.87	1121	964	5.43	18.43	83.50	85.99
DH 32	647	7:16	24:20	9697	20.745	2.129	0.471	21.90	1126	997	5.51	18.19	77.12	88.40
DH 42	601	7:16	24:20	9737	21.686	2.123	0.466	21.96	1169	1023	5.71	17.52	76.85	87.56
Control	624	7:20	24:14	9723	22.080	2.201	0.485	22.03	1116	969	5.48	19.29	82.87	86.21
Average	629	7:19	24:17	9704	21.698	2.186	0.478	21.90	1127	961	5.43	18.71	83.07	85.55
CD at 5 %	8.76	0.02	0.02	8.85	0.27	NS	NS	0.18	21.10	23.87	0.12	0.42	1.78	0.88
CV	0.03	0.00	0.00	0.00	0.02	0.03	0.02	0.02	0.03	0.05	0.04	0.04	0.04	0.02

The data are average of three trials

3, DH 7, DH 8, DH 9, DH 11 and control) registered the fifth age larval duration slightly higher than the average value of 7 days and 19 h with the total duration of 24 days and 14 hours.

As far as **survival rate** is concerned, eight hybrids registered higher than the mean value of 9704 and individual e.i. value > 50. The highest value of 9737 with an e.i. value of 70.52 was registered by DH 42, followed by 9723 with e.i., 61.82 (Control), 9718; 58.71 (DH 14), 9713; 55.60 (DH 31), 9711; 54.35 (DH 11), 9708; 52.49 (DH 3), 9707; 51.87 (DH 9) and 9705; 50.62 (DH 13). Pupation rate or survival percentage is directly co-related with the cocoon yield and hence, more importance has to be given for survival while evaluating the hybrids and identifying them for field exploitation (Pallavi and Basavaraja, 2007; Bharat Kumar *et al*., 2019).

The **effective rate of rearing (ERR) by weight,** the direct parameter which determines the cocoon yield and returns to farmers is very important while selecting the hybrids for the benefit and sustenance of the sericulture industry. Though **s**even hybrids registered more than the average value of 21.698 kg, only three hybrids (DH 9, DH 3 and DH 11) recorded higher values than the control. DH 9 recorded the highest of 22.497 kg with e.i. value 66.31 (DH 9), followed by DH 3 (22.320; 62.69) and DH 11 (22.118; 58.57), these being higher than the control (22.080; 57.80). Values above the mean were recorded by DH 4 (21.956; 55.27), DH 13 (21.953; 55.20) and DH 7 (21.866; 53.43).

11. Cocoon traits

For c**ocoon weight,** four hybrids registered higher values than the control hybrid. The highest value of 2.274 g recorded with e.i. value of 72 (DH 9) was followed by 2.228 g and e.i. value, 60.38 (DH 4), 2.215 g with e.i., 57.31 (DH 3), 2.213 g with e.i., 56.75 (DH 11) and control hybrid with 2.201 g with e.i. value, 53.75. Cocoon weight is yet another character having direct co-relation with the cocoon yield and thereby, with the financial return for the farmers. This improvement in cocoon weight is directly related to the cocoon yield in double hybrids, a

Table 5: Evaluation Index (E.I.) values of shortlisted double hybrids (Level – II)

Hybrid	Fecundity	Larval duration		ERR by No.	ERR by weight	Cocoon weight (g)	Shell weight (g)	Shell %	AvFL (m)	NBFL (m)	R. S. %	R. S. R. %	Reelability %	Avg. E.I.	e.i. Renditta
		V age	Total												
DH 2	39.31	60.00	46.67	43.78	41.82	39.29	42.33	54.09	47.04	42.72	44.29	52.93	49.13	46.4	42.86
DH 3	44.50	53.33	40.00	52.49	62.69	57.31	56.63	49.43	45.46	43.02	65.19	68.18	39.83	52.3	38.57
DH 4	39.94	30.00	45.00	26.99	55.27	60.38	52.25	37.78	33.56	31.55	65.71	49.81	33.19	43.2	67.62
DH 7	60.38	53.33	40.00	42.54	53.43	51.50	39.33	43.69	42.96	53.69	50.78	60.25	53.75	49.6	45.24
DH 8	50.00	53.33	40.00	51.87	44.37	52.37	51.00	46.04	61.74	41.23	48.57	56.03	39.89	48.9	47.66
DH 9	53.25	53.33	40.00	45.65	66.31	72.00	89.00	75.49	59.91	61.76	59.74	58.32	60.50	61.2	35.71
DH 10	62.79	40.00	33.33	44.40	38.02	41.25	40.50	44.85	45.17	47.72	49.45	53.29	48.00	45.4	51.43
DH 11	66.35	53.33	40.00	54.35	58.57	56.75	69.00	66.97	57.17	48.90	59.48	52.89	48.56	56.3	34.76
DH 13	53.14	60.00	60.00	50.62	55.20	50.59	49.00	45.19	39.17	49.37	45.13	48.96	54.39	50.8	52.43
DH 14	37.74	60.00	60.00	58.71	49.37	52.00	46.38	39.35	40.15	36.68	32.69	39.61	33.72	45.1	66.09
DH 29	50.00	46.67	60.00	46.89	38.39	44.13	40.00	40.00	69.83	66.65	46.62	46.42	51.44	49.5	49.52
DH 31	50.00	40.00	60.00	55.60	48.33	50.06	50.00	49.09	48.43	50.69	46.36	51.33	52.75	50.2	50.00
DH 32	61.19	40.00	60.00	45.65	30.55	35.75	44.40	49.88	49.61	58.19	43.18	31.64	67.78	47.4	53.57
DH 42	32.39	40.00	60.00	70.52	49.74	34.25	38.67	51.92	60.96	64.30	34.55	30.80	62.56	48.4	63.33
Control	46.86	53.33	40.00	61.82	57.80	53.75	57.00	53.94	47.00	51.90	57.53	49.37	54.09	52.6	52.31

basic achievement in the concept of development of double hybrids.

With regard to **shell weight**, only two hybrids; DH 9 (0.517 g, e.i. value, 89.00), the highest and DH 11 (0.497 g; 69.0), recorded higher values than control (0.485 g; 58.0). DH 3 (0.485 g; 56.63) was at par with the control. Cocoon prize is being fixed based on the value of shell percentage. More the shell weight, higher the shell percentage and the price of cocoons.

For **shell percentage,** only two hybrids registered higher values than control. The highest value of 22.74 % with an e.i. value, 75.45 (DH 9) was followed by 22.46 with 66.97 (DH 11),

This is in conformity with the earlier findings that though there is significant improvement in the cocoon weight and corresponding shell weight in the double hybrids, the ratio of shell weight to cocoon weight *i.e*., shell percentage, remain less compared to that of the parents and single hybrids because of more cocoon weight in double hybrids. Hence, cocoon yield will have good improvement (Pallavi and Basavaraja, 2007; Moorthy *et al.,* 2011; Rajalakshmi and Sakthivel, 2019).

111. Post cocoon traits

The traits concerned with the reelers are also to be given utmost importance while selecting and identifying the hybrids. The **Average filament length (AvFL)** registerd for five hybrids *viz*., DH 29 (1203 m; 69.83), DH 8 (1172 m; 61.74), DH 42 (1169 m; 60.96), DH 9 (1165 m; 59.91) and DH 11 (1155 m; 57.17) and **Non breakable filament length (NBFL)** recorded in DH 29 (1033 m, e.i. 66.61), DH 42 (1023 m, e.i. 64.30) and DH 9 (1012 m, e.i. 61.76), showed that the hybrids performed better for rearing and cocoon traits have a different order of merit as far as reeling parameters are concerned.

Renditta, the number of kg of cocoons required to be reeled for one kg of raw silk, decides the turn over of the reeler. Seven hybrids registered higher values *i.e*., less than the mean value of 5.43 as following: 5.11, e.i., 34.76 (DH 11), 5.13, e.i., 35.71 (DH 9), 5.19, e.i., 38.57 (DH 3), 5.28, e.i., 42.86 (DH 2), 5.33, e.i., 45.24 (DH 7), 5.38, e.i., 47.66 (DH 8) and 5.42, e.i., 49.52 (DH 29). The hybrids having higher value of rearing and cocoon traits lead in the order of merits for renditta. The lesser value of renditta is one of the beneficial traits for the reeler.

Table 6: ANOVA for the characteritics of short-listed double hybrids

Hybrid	Fecundity (No.)	ERR by Number	ERR by weight (kg)	Cocoon weight (g)	Shell weight (g)	Shell %	AvFL (m)	NBFL (m)	Renditta	Raw silk %	R.S.R. %	Reelability %
DH 2	612.3±14	9694±96.9	21.3±2.5	2.1±0.1	0.47±0.03	22±0.5	1116.1±73.4	930.3±51.5	5.3±0.2	18.3±0.9	84±4.1	85.4±2.9
DH 3	619.5±49	9708.2±105.7	22.3±1.5	2.2±0	0.48±0.01	21.9±0.1	1110.3±12.1	931.3±32.9	5.2±0.1	19.9±0.3	89±0.7	83.9±1.7
DH 4	613±17.1	9666.7±49.3	22±0.7	2.2±0	0.48±0.01	21.5±0.4	1064.3±30.7	880.7±35.2	5.8±0.2	19.9±0.3	83±1.0	82.9±0.6
DH 7	646±23.5	9692±83.0	21.9±1.7	2.2±0.2	0.47±0.03	21.7±0.4	1100±49.7	977±33.9	5.3±0.3	18.8±0.7	86.4±3.1	86.1±3.0
DH 8	655±57.7	9707.1±108.4	21.4±1.1	2.2±0	0.48±0.02	21.8±0.9	1172.3±40.3	922.7±34.6	5.4±0.1	18.6±0.5	85±3.6	83.9±1.2
DH 9	633.7±40.7	9696.7±65.1	22.5±0.5	2.3±0.1	0.52±0.04	22.7±0.9	1164.7±88.7	1012.3±66.5	5.1±0.1	19.5±0.7	85.8±4.2	87.2±1.8
DH 10	649.1±39.5	9695.3±49.4	21.1±0.6	2.2±0.1	0.47±0.01	21.8±0.5	1108.7±84.9	960.3±34.2	5.5±0.2	18.7±0.6	84.1±3.7	85.2±3.7
DH 11	628.7±28.4	9711.3±19	22.1±1.4	2.2±0.1	0.5±0.02	22.5±0.6	1154.7±28.4	996±16	5.1±0.1	19.4±0.5	84±1.3	85.3±2.1
DH 13	633.7±18.7	9705.3±27.5	22±0.3	2.2±0.1	0.48±0.02	21.7±0.1	1085.7±53.2	957.7±59.8	5.5±0.2	18.3±0.8	82.7±2.6	86.3±2.3
DH 14	609.7±17.6	9718.3±45.4	21.7±1	2.2±0.1	0.47±0.02	21.6±0.4	1089.3±82	903±88.8	5.8±0.2	17.4±0.4	79.7±2.2	83±1.2
DH 29	628.7±28	9698.6±45.1	21.1±0.8	2.2±0	0.47±0.01	21.6±0.2	1202.7±86.1	1032.7±76.5	5.4±0.2	18.5±0.5	81.9±3.2	85.8±1.1
DH 31	628.8±33.4	9713.2±8.6	21.6±0.4	2.2±0	0.48±0.01	21.9±0.6	1120.7±29.5	964±47.2	5.4±0.2	18.4±0.3	83.5±1.7	86±2.7
DH 32	647±60.5	9697.4±51.1	20.7±1.8	2.1±0.1	0.47±0.03	21.9±0.2	1125.7±59.5	996.7±5.1	5.5±0.1	18.2±0.6	77.1±1.7	88.4±0.9
DH 42	601.3±13.5	9737.4±55	21.7±0.5	2.1±0.1	0.47±0.02	22±0.5	1168.7±43.2	1022.7±31.4	5.7±0.2	17.5±0.5	76.9±3	87.6±2.1
Control	624±34.2	9723.1±42.9	22.1±0.1	2.2±0.1	0.48±0.03	22±0.8	1116±21.7	968.7±32.1	5.5±0.1	19.3±0.3	82.9±1.6	86.2±1.5
F-value	0.616^{ns}	0.195^{ns}	0.517^{ns}	0.548^{ns}	1.115^{ns}	1.135^{ns}	1.306^{ns}	2.534^{*}	4.799^{**}	5.826^{**}	4.174^{**}	1.753^{ns}

ns non-significant; * significant at 0.05 level; ** significant at 0.01 level; Values following ± are SD.

With regard to **Raw silk percentage,** four hybrids recorded values higher than the control *i.e*., 19.92 with e.i. of 65.71 (DH 4), 19.88, e.i., 65.19 (DH 3), 19.46, e.i., 59.74 (DH 9), 19.44, e.i., 59.48 (DH 11) and 19.29, e.i., 57.53 (control). For **Raw silk recovery** percentage, eight hybrids were found to be better than control, and the values ranged from the highest of 88.96 % and e.i., 68.18 (DH 3), followed by 86.39, e.i., 60.25 (DH 7), 85.77, e.i., 58.32 (DH 9), 85.03, e.i., 56.03 (DH 8), 84.13, e.i., 53.29 (DH 10), 84.02, e.i., 52.93 (DH 2), 84.01, e.i., 52.89 (DH 11) and 83.50, e.i., 51.33 (DH 31).

The **Reelability** percentage also registered a different order of merit. Four hybrids recorded higher than the control. The highest of 88.40 % with e.i. value, 67.78 (DH 32) was followed by 87.56 %; 62.56 (DH 42), 87.23 %; 60.50 (DH 9), 86.25 %; 54.39 (DH 13) and 86.21 %; 54.09 (control).

One way Analysis of variance was done for comparing the performance of hybrids. The rearing and cocoon traits did not show any significant variation among hybrids or it could be interpreted that the short listed hybrids are on par with the existing hybrid in the field. But, significant variation among hybrids was noted in the reeling parameters, *viz*., NBFL (at 0.05 level), renditta, raw silk % and R.S.R. % (at 0.01 level) which showed that the identified hybrids could boost the end product, *i.e*., raw silk to a considerable extent. In the case of variable in which f-value in the ANOVA was found to be significant, pair wise comparison was done by using Duncan Multiple Range Test (DMRT). With regard to NBFL, DH29 displayed a significantly higher value compared to DH2, DH3, DH4, DH8 and DH 14. In the case of renditta, the most desired trait for raw silk for the benefit of the industry, hybrids with significantly higher values (0.01 level) in the order of merit are DH 11 the best, followed by DH 9 and DH 3 and all these three were found to be superior to control. Variations in raw silk percentage is significant at 0.01 % level and recorded the highest in DH4 followed by DH3, DH9 and DH11, all of these performing over control.

Table : 7 Data on rearing performance of parents and foundation crosses

Parental breed	Fecun-dity (No.)	V age larval duration (Days : hours)	Total larval duration (Days : hours)	ERR %	Cocoon weight (g)	Shell weight (g)	Shell %	Cocoons / Litre (No.)
D 1	532	7.09	23.09	92.53	1.844	0.402	21.78	80
GEN 3	556	7.09	24.10	95.5	1.676	0.396	23.62	88
CSR 2	520	7.00	23.12	90.7	1.7	0.388	22.82	82
CSR 27	534	7.06	23.18	96.8	1.817	0.42	23.12	78
CSR 6	538	7.00	24.00	94.6	1.783	0.372	20.84	110
CSR 26	563	6.22	24.04	93.6	1.725	0.363	21.02	108
CSR 51	513	7.10	24.00	88.8	1.756	0.373	21.59	113
Foundation cross								
CSR 51 x 26	577	7.04	24.04	96.40*	1.802*	0.393*	21.78	96
CSR 2 x D1	549	7.02	24.04	93.80	1.89	0.437	21.95	64
CSR 2 x Gen 3	533	7.02	23.06	97.20*	1.821	0.407	22.35	63
CSR 2 x CSR 27	556	7.02	24.04	95.04	1.985	0.449	22.64	65
CSR 6 x CSR 26	543	7.04	24.04	93.52	1.76	0.373	21.19	103

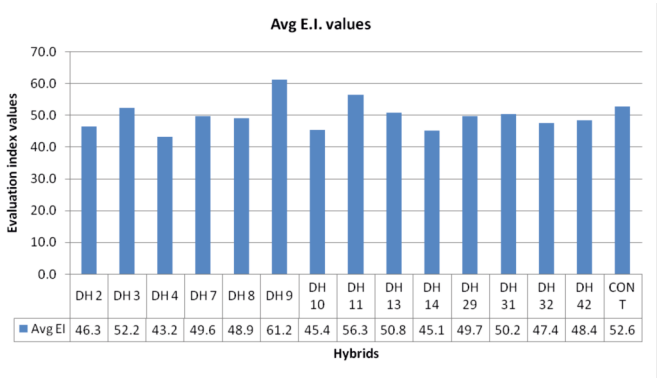

Figure 1: Evaluation Index values (Level – II trial)

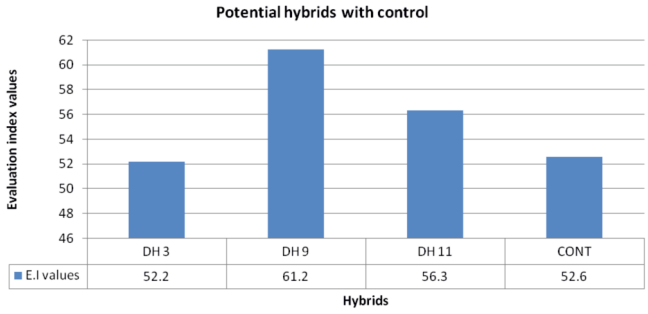

Figure 2: Potential double hybrids identified against control

The two levels of testing (Level I and II) has clearly brought out the elaborative details of the new double hybrid crosses. In silkworm, the success of the cocoon crop is dependent on many characters, of which the fecundity, larval duration (fifth age and total), survival percentage (pupation), cocoon yield (weight) and shell percentage stand for the benefit of the farmers and post cocoon parameters, for the benefit of the reelers. Hence, a thorough study in different seasons and analyzing the data by giving importance to all characters are essential. The hybrids which are very good in rearing aspects, mainly fecundity and survival may not present a similar picture for cocoon and reeling traits. References are already available that foundation crosses are superior to parental breeds in terms of fecundity, pupation rate and stability (Table 7). It is obvious that though the parental breeds are superior, if the same is not reflected in the foundation crosses or hybrids, they are of no much use. Therefore, in silkworms, a large number of hybrids are evaluated and the promising ones are selected based on the improvement in the economic characters for exploitation in the field for the benefit of the silk industry. The main advantages of double hybrids are enhanced fecundity, ease of rearing, stability in cocoon yield and adaptability to the fluctuating climatic conditions prevailing in the field, which could be obtained through good and stable Foundation Crosses and their heterosis. (Pallavi and Basavaraja, 2007; Nair Suresh Kumar and Harjeet Singh, 2011, Rajalakshmi and Saktivel, 2019, Sivaprasad, 2019). The well known fact of comparatively better performance of double hybrids than single hybrids was once again confirmed by the study which is in conformity with the earlier ones (Nirmal Kumar *et al*., 1998; Mal Reddy, 2005; Pallavai and Basavaraja, 2007; Rajalakshmi, 2014; Bharath Kumar *et al*., 2019).

The concept of developing suitable double hybrids by utilizing the genetically and phenotypically similar breeds (for foundation crosses) as four parents, make the foundation strong and stable so that the double hybrids prepared out of these would survive and adapt well to the fluctuating climatic conditions prevailing in the field than

Figure 3: Cocoons of DH 9 (CSR 51 x 26) x (CSR 2 x D1)

Figure 4: Cocoons of DH 11 (CSR 51 x 26) x (CSR 2 x Gen 3)

single hybrids because of its wide stable genetic base of four parents. Superior quality silk of > 3 A grade is produced from double hybrids in countries, such as India, China , Bulgaria *etc*. (Sivaprasad, 2019).

Average E.I. values registered by the hybrids showed improvement in their overall performance (Figure 1). Three hybrids, namely, DH 9 ((CSR 51 x 26) x (CSR 2 x D 1)) with the highest E.I. value (61.2) and individual e.i. value of renditta (35.71) , DH 11 (CSR 51 x 26) x (CSR 2 x Gen 3) (56.3 and 34.76) and DH 3 (CSR 51 x 6) x (CSR 2 x Gen 3) (52.2 and 38.57) performed better than the control (52.6 and 52.31) in the order of merit (Figure 2). Thus, the equally rated best two double hybrids, DH 9 (CSR 51 x 26) x (CSR 2 x D 1) (Figure 3) and DH 11 (CSR 51 x 26) x (CSR 2 x Gen 3) (Figure 4) were selected as promising, newly evolved double hybrids.

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