



INTERNATIONAL
SERICULTURAL
COMMISSION

UNITED NATIONS REG. NO. 10418

ISSN 0250-3980

Volume 54 | Number 4 | 2014

SERICOLOGIA

Journal of Silkworms
A Quarterly Publication of
International Sericultural Commission





INTERNATIONAL
SERICULTURAL
COMMISSION

UNITED NATIONS REG. NO. 10418

SERICOLOGIA

Journal of Silkworms

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Fax: +91 80 26681663, E-mail: sericologia@gmail.com, www.inserco.org

Printed at Ramya Reprographic Pvt. Ltd, No. 16/2B, Singasandra Village, Behind Dakshin Honda Show Room,
Hosur Road, Bengaluru - 560 068, INDIA. Tel: +91 98440 36357, E-mail: ramyareprographic@gmail.com

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DEVELOPMENT OF GENETIC LINKAGE MAP OF MULBERRY USING MOLECULAR MARKERS AND IDENTIFICATION OF QTLs LINKED TO YIELD AND YIELD CONTRIBUTING TRAITS

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ABSTRACT

A study was undertaken towards construction of genetic linkage map of mulberry by pseudo-test cross strategy with an objective of mapping yield and yield contributing traits. Two parent specific linkage maps were constructed by genotyping 150 F₁ segregating progeny from a cross between Mysore Local (low yielding, ♀) and V-1 (high yielding, ♂) varieties using 369 polymorphic random markers. On Mysore Local, a total of 73 markers were mapped on 21 linkage groups (LGs) covering 1414.8 cM distance. The V-1 map covered a distance of 1351.4 cM with 74 marker loci on 20 LGs. Composite Interval Mapping was performed with an empirical LOD threshold (≥ 2.5) utilizing data recorded in three years. QTLs for NBR and TSL were mapped on LG2 based on the data of October 2004. Besides, major QTLs (DLWL and FLWL) with high R^2 value (19 and 14 %, respectively) and LLS ($R^2 = 10$ %) were located on LG3 and LG13, respectively. QTLs for ALA and TSW on LG3 and QTL for MC on LG13 were identified based on the data of April 2005. Further, QTLs for ALA and TSL on LG3, MC on LG8 and LLS on LG17 were mapped based on the phenotyping in October 2006. The study is the first attempt to map QTLs in mulberry and one among the few in development of molecular linkage map of mulberry.

Key words: Linkage mapping, mulberry, QTLs, random markers, yield and yield contributing traits.

INTRODUCTION

Mulberry (*Morus* spp.) is a perennial, dioecious, outbreeding tree species, mainly grown for its foliage for feeding the domesticated silkworm, *Bombyx mori* L. Mulberry productivity is a predominant factor influencing the sericulture economics as the quantity and quality of mulberry has a direct bearing on the silkworm cocoon crop. It is estimated that 60 % of the cost of cocoon production goes into the cultivation of mulberry (Ullal and Narasimhanna, 1987). Hence, improvement in yield and quality of mulberry is one of the thrust areas in sustenance of silk industry. Sericulture is a viable employment generator, especially in the labour rich rural India, providing attractive financial returns and foreign exchange (CSB, 2013). Earlier, Indian mulberry breeding

programs have concentrated on improving the leaf yield. Perceptible success in higher leaf yield has been achieved by evolving varieties such as K-2, S-36, V-1, G-4 etc. (Sarkar, 2009). However, these gains are mainly due to random chance events and involved laborious breeding efforts. To be competitive, there is a need to concentrate on improving yield and quality of mulberry by developing varieties that are well adapted to local environmental conditions. This objective can be achieved with precision and speed by modern approaches of plant breeding like Marker Assisted Selection (MAS). This necessitates the molecular dissection of agronomically important traits and identification of markers linked to them.

Linkage maps are the primary requirement for localization of chromosomal regions controlling

polygenic traits (Collard *et al.*, 2005). Genetic linkage maps have been constructed for several plant species and have formed the basis for advanced genetic studies that provide a better understanding of the inheritance, identification and isolation of genes (Roose *et al.*, 2000). Venkateswaralu *et al.* (2006) constructed linkage maps of S-36 and V-1 using two way pseudo-test cross strategy by utilizing a small number (50 full-sibs) of progeny. However, the study was too preliminary in nature and without any attempt on mapping QTLs for traits of interest in mulberry. The present study was undertaken with objectives of constructing a framework genetic map of mulberry and mapping QTLs controlling yield and yield contributing traits.

MATERIALS AND METHODS

Development of mapping population

The mapping population was generated by crossing Mysore Local (♀), a land race and V-1 (♂), an elite mulberry variety which are contrasting for yield and several yield contributing traits. For a detailed characterization of the parental genotypes, refer Naik *et al.* (2002). The seeds of progeny were produced from a controlled cross and seedlings were raised in the glass house under controlled irrigation and nutrient supplementation (July 2001). Six months old, hardened seedlings were transplanted and 2666 F₁ progeny were established in the field (February 2002). A total of 150 randomly selected F₁ progeny were planted (six clonal replicates per progeny) along with the parents (as checks) in an Augmented Random Block Design (ARBD) for phenotypic and genotypic analysis.

Phenotypic characterization

The mapping population of 150 progeny and parents represented by three ramets were subjected to phenotyping in October 2004, April 2005 and October 2006 as per Thangavelu *et al.* (1997) after two years of establishment. The morphological parameters observed were leaf size, colour, texture, thickness, lobation, glossiness, stem posture, lateral branches and sex of the

plants. The quantitative characters recorded were average leaf area (ALA), fresh leaf weight of the longest shoot (FLWL), dry leaf weight of the longest shoot (DLWL), moisture content (MC), number of branches (NBR), length of the longest shoot (LLS), total shoot length (TSL), inter-nodal distance (IND), leaf yield per plant (LYP) and total shoot weight (TSW). Statistical analysis of the phenotypic data was carried out using SPSS statistical software. Analysis of variance (ANOVA) was performed using the general linear model (GLM) to test the significance of the genotype effect.

Genomic DNA isolation and quantification

High molecular weight genomic DNA was extracted from freshly collected young leaves using Nucleon Phytopure Kit (Amersham Biosciences, UK) as per the manufacturer's instruction. The quality and quantity of DNA was assessed on 0.8 % agarose gel stained with 0.5 µg/ml of ethidium bromide. The DNA stock solutions were diluted to a uniform concentration of 10 ng/µl.

PCR amplification

RAPD and ISSR-PCR reactions were performed as described by Naik and Dandin (2005). The PCR amplifications were carried out in 0.2 ml PCR tubes with 20 µl reaction volume on DNA Engine PTC-200 (MJ Research, USA). The RAPD Primers were obtained from Operon Technologies Inc., Alameda, USA. The ISSR primers were sourced from University of British Columbia, Vancouver, Canada. The amplified markers were resolved on 1.5 % (RAPD) and 2 % (ISSR) agarose gels in 1X TAE, stained with ethidium bromide (Sambrook and Russel, 2001) and gel images were recorded using Gene Genius gel documentation system (Syngene, UK).

Segregation analysis and linkage map construction

Sizes of the amplified DNA markers were estimated by comparing with λ DNA/*EcoRI*+*HindIII* ladder (Fermentas, USA) and markers were scored as '0' for absence and '1' for presence. All PCR reactions were performed twice and only reproducible bands in the range of 300 to 3500 bp were scored. All markers present in one parent and absent in the other were tested for goodness of

fit to the 1:1 segregation ratio in the progeny by a χ^2 test ($p < 0.05$). Only the markers without a distorted segregation ratio were assigned to linkage groups. Estimation of recombination fraction (r), linkage analysis and map construction were carried out in two steps. In the first step, the parent specific markers fitting to the test cross configuration were grouped. In the second step, two parent specific framework maps were constructed using the simplex markers. The framework markers were integrated into the map using MAPMAKER/EXP 3.0 (Lander *et al.*, 1987; Lincoln *et al.*, 1993). Linkages between markers were calculated using odds ratio expressed as Logarithm of Odds (LOD) (Risch, 1992). Distances in centimorgans (cM) were calculated using the mapping function described by Kosambi (1943), using a minimum LOD score of 3.0. Graphical representation of the map was generated using the statistical tool MapChart (Voorrips, 2002).

QTL mapping

QTL analysis was carried out by Composite Interval Mapping (CIM) using WinQTL Cartographer 2.5 (Wang *et al.*, 2007). A LOD score of 2.5 was used for suggesting the putative QTLs. The CIM analysis (Jiang and Zeng, 1995) was performed using backcross and forward regression with a window size of 5 cM Kosambi function and walking speed of 2 cM precision interval. To identify an accurate significance threshold for the trait, an empirical threshold was determined at 1000 permutations of the phenotypic data according to Churchill and Doerge (1994) and Doerge and Rebai (1996). A significance level of $p < 0.05$ was used to select associated markers to detect putative QTLs. Multiple regression analysis was used to determine the contribution of the QTLs detected. In addition, the additive effect and percentage of variation explained by an individual QTL were also estimated.

RESULTS AND DISCUSSION

Phenotypic evaluation

Morphological characters such as leaf size, shape, texture, phyllotaxy, stem posture and branching nature have a bearing on the yield and quality of leaf. Most of the

morphological characters exhibited normal frequency distribution among the segregating progeny (data not shown). Among the characters studied, leaf texture, lobation, stem posture, branches and sex expression segregated in 1:1 ratio. However, phyllotaxy (1/2, 2/5) segregated in 3:1 Mendelian ratio. The male parent V-1 is a high yielding variety and was manifested by significantly higher leaf area, weight, number of branches and shoot length. The leaf yield per plant was significantly higher in V-1 in all the three data recording seasons. Fresh and dry leaf weight showed significant variation in the year 2004. ALA was considerably higher in the second season compared to first and third and, TSW recorded maximum in the third year. The NBR and LYP showed gradual increase in the second and third year. Variation in MC was minimal in all the three years. Majority of the characters with positive skewness indicate the predominance of segregants towards the superior parent. The sharp kurtosis and positive skewed distribution of the TSL and DLWL traits indicate maximum number of progeny that have higher value than V-1.

Genotyping

Out of the 222 RAPD primers screened, a total of 148 primers (66.67%) were found to amplify 345 polymorphic markers among the parents, with an average of 2.33 markers/primer. A total of 10 ISSR primers (37.04%) out of the 27 primers screened amplified 24 polymorphic markers, with an average of 2.4 markers/primer. The primers amplifying polymorphic markers were utilized for genotyping and segregation analysis.

Construction of parent specific linkage maps

Map construction in allogamous plant species for which heterozygous individuals are available can make use of single dose polymorphic markers behaving as dominant markers in an F_1 segregating population (1:1) for the presence or absence of the fragment (Ritter *et al.*, 1990). The same mating configuration was tested and used while analyzing segregation of RAPD and ISSR markers in the F_1 population.

The linkage map of Mysore Local was developed using 170 (158 RAPD and 12 ISSR) female specific markers. χ^2

analysis for goodness-of-fit showed 102 markers (96 RAPD and 6 ISSR) followed a typical test cross segregation ratio (1:1) and 16 RAPD and 2 ISSR markers followed a monohybrid (3:1) ratio. The female linkage map covered 1414.8 cM with 68 RAPD and 5 ISSR markers. All the 73 markers were grouped into 21 linkage groups and the longest linkage group (LG3) had coverage of 180.8 cM with 9 markers and the shortest LG6 with 2 markers spanning a total distance of 12.1 cM. The average map density was 19.38 cM/marker and with an average distance of 67.36 cM per linkage group.

In the V-1 parent, 184 RAPD and 10 ISSR male specific markers were subjected to χ^2 analysis. Among them, 120 (113 RAPD and 7 ISSR) markers which followed the test cross ratio (1:1) were used for map construction. A total of 12 markers followed 3:1 ratio. Linkage map analysis resulted in a male map covering a total distance of 1351.4 cM with 71 RAPD and 3 ISSR markers. At LOD 3.0, all the 74 informative markers were grouped into 20 linkage groups with the longest LG11 spanning 189.8 cM with 7 markers and the shortest was LG16 with 2 markers mapped at a distance of 4.9 cM. The maximum number of 9 markers were aligned on LG2 with a length of 149.7 cM. The average distance between two markers on linkage groups was 18.26 cM, covering an average of 67.57 cM map distance per linkage group. Forty-six markers that originated from male parent remained unlinked at LOD 3.0.

An excess number of linkage groups in relation to the haploid chromosome number ($n = 14$) were observed in mulberry. This phenomenon has been reported in other species *viz.*, lettuce (Kesseli *et al.*, 1990) and bean (Nodari *et al.*, 1993). Ideally, the number of linkage groups must be equivalent to the haploid number. Factors such as non-random distribution of markers and variation of frequencies of recombination along the length of the chromosomes may have contributed to the fragmentation of genetic maps (Hartl and Jones, 2001).

QTL analysis

CIM was performed separately utilizing all the three data sets (of October 2004, April 2005 and October 2006)

Table 1: Statistical output of individual QTL effect and estimated location from the first data record (October 2004)

Trait	LG	Position	LOD*	Additive effect	R ² (%) [*]	Marker
FLWL	3	0.6621	4.4	-33.92	14	OPA07 ₁₇₀₀ OPL15 ₅₀₀ OPJ07 ₅₅₀
DLWL	3	0.6421	5.5	-11.90	19	OPA07 ₁₇₀₀ OPL15 ₅₀₀ OPJ07 ₅₅₀ OPL19 ₈₂₅
NBR	2	0.4891	2.9	-0.74	7	OPL19 ₈₅₀ OPD07 ₁₂₅₀
NBR	8	0.0601	2.7	-0.79	8	OPH20 ₁₀₀₀ OPQ09 ₁₇₅₀
LLS	3	0.0201	3.7	12.85	10	OPF04 ₁₁₀₀ OPO11 ₁₅₂₅
TSL	2	0.4891	3.7	-155.90	9	OPL19 ₈₅₀

Table 2: Statistical output of individual QTL effect and estimated location from the second data record (April 2005)

Trait	LG	Position	LOD*	Additive effect	R ² (%) [*]	Marker
ALA	3	0.6821	3.9	-36.28	10	OPA07 ₁₇₀₀
MC	13	0.0001	4.1	1.99	11	OPF04 ₁₁₀₀
TSW	3	0.0601	2.8	116.12	10	OPA07 ₁₇₀₀ OPF20 ₈₅₀

Table 3: Statistical output of individual QTL effect and estimated location from the third data record (October 2006)

Trait	LG	Position	LOD*	Additive effect	R ² (%) [*]	Marker
ALA	3	0.6221	2.85	-21.13	10	OPA07 ₁₇₀₀ OPL15 ₅₀₀
MC	8	0.5231	3.17	2.70	12	OPB01 ₂₀₀₀ OPQ09 ₂₀₀₀
LLS	17	0.3401	2.98	12.61	8	OPD16 ₇₅₀ OPD18 ₂₂₀₀
TSL	3	0.1801	3.43	296.78	13	OPF20 ₈₅₀ OPA07 ₁₇₀₀

* LOD score calculated by QTL Cartographer at $p < 0.05$ level probability.
^{*} Percentage of the phenotypic variance explained by the marker genotypes at the locus.

FLWL – Fresh leaf weight of the longest shoot; DLWL – Dry leaf weight of the longest shoot; NBR – Number of branches; LLS – Length of the longest shoot; TSL – Total shoot length.

ALA – Average leaf area; MC – Moisture content; TSW – Total shoot weight.

following empirical LOD threshold (≥ 2.5). The statistical analysis of individual QTL effect (year-wise) and their estimated location on linkage group along with LOD value, additive effect, coefficient of determination (R^2) and linked marker(s) are presented in the Tables 1, 2 and 3.

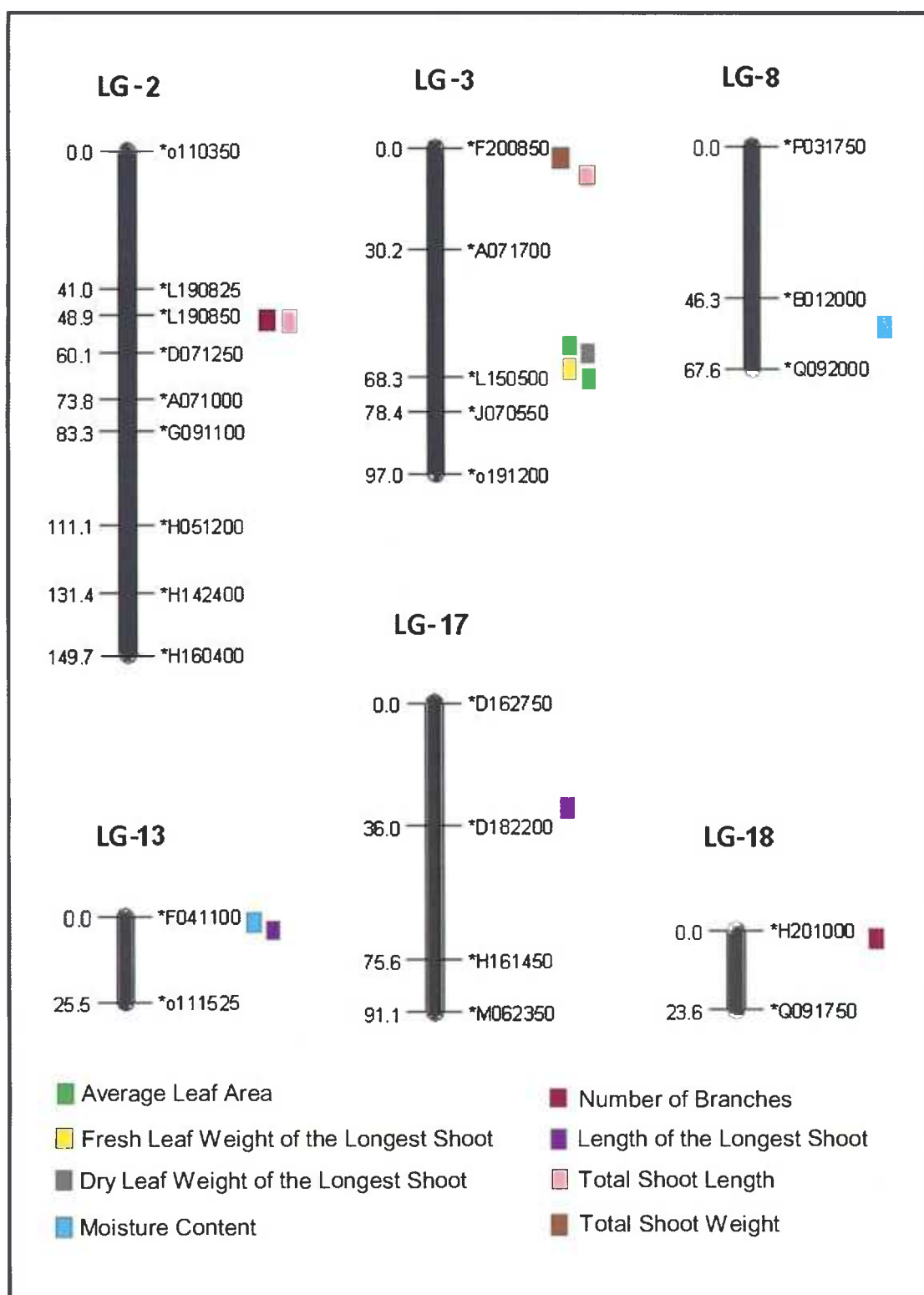


Figure 1: Hypothetical output showing QTL locations for yield traits on the male (V-1) linkage map based on Composite Interval Mapping. The maximum likelihood position of the QTL is indicated with a coloured box

Ten quantitative characters subjected to the QTL analysis identified 13 QTL loci controlling eight yield traits (ALA, FLWL, DLWL, MC, NBR, LLS, TSL and TSW) on six linkage groups at LOD values ranging from 2.5 to 5.5. Among them, QTL representing LLS, MC, TSL and TSW exhibited positive additive effects suggesting that the characters were inherited from the dominant parent, which can be used as a tag for positive expression of these characters in breeding programmes. In several instances, CIM analysis revealed significant marker trait association and detected QTLs above the LOD threshold of 2.5. Figure 1 represents the overall QTLs and their relative positions on the linkage map of V-1.

Segregation ratios for yield traits in the F_1 population that we studied suggest that the yield contributing parameters NBR and TSL (LOD = 2.9 and 3.7 respectively) tagged by the QTL locus (OPL19₈₅₀) located on LG2 in close proximity (48.91 cM) indicates that the genes controlling these traits are closely related. The QTLs with the largest additive effect (296.78) were at 18.01 cM explaining 13 % of the total phenotypic variation, considered to be the major QTL flanking the TSL followed by TSW with 10 % phenotypic variation. CIM analysis detected a total of 9 major QTLs (R^2 value ranging from 10-19) and 4 minor QTLs. QTLs with smaller additive effects, each explaining < 10 % of the total phenotypic variation were considered to regulate the traits moderately. The major QTLs (QTLs explaining >10% phenotypic variation) may refer to QTLs that are stable across environments, whereas minor QTLs may refer to QTLs that may be environmentally sensitive (Collard *et al.*, 2005). The QTL on LG3 was consistently identified from two sets of phenotypic data indicating the presence of dominant genes controlling the trait ALA. Many QTLs with high LOD values (>2.5) but with small and negative additive values were observed in the analysis. As some of the QTLs expressed in one season were not expressed in the other seasons (Tables 1, 2 and 3), the regulation of these characters/QTLs may be very complex. The effects of each QTL were moderate to significant (R^2 = 7 to 19). Two of the traits (IND and LYP) were not assigned to any chromosomal region in the

analysis, indicating that they are controlled by more complex genetic mechanism or may require a bigger population size with more linked markers for mapping.

It is possible that additional QTLs remain undetected since only a maximum of 19 % of phenotypic variation has been accounted. The size of the individual QTL effects is difficult to assess with this initial genome scan for several reasons. It is likely that some estimated QTL effects have been inflated since a common problem in QTL studies is that those loci where the effect by chance is over estimated are more likely to reach statistical significance (Göring *et al.*, 2001). It is also possible that individual QTL effects have been over estimated because they represent the effect of two or more linked QTLs, each with a smaller individual effect. Another possible bias in estimating the QTL effects may be caused by the fact that a distortion of the segregation lines due to sampling error and population size may also diminish the estimated effects (Yu *et al.*, 2007). The association of QTLs with corresponding traits identified by the marker system suggests the potential of RAPD and ISSR markers in mulberry breeding programmes. With the incorporation of more markers and a larger population size, additional QTLs may be identified (Hackett *et al.*, 1998). Vales *et al.* (2005) conducted a study on the effect of population size for detection of QTLs in barley and reported that the number of QTLs increased as the population size increased. They also found that QTLs with large effects can be detected with small populations, but it is necessary to increase the population size to be able to detect QTLs with small effects.

Plant yield traits often have QTLs mapped at similar genomic locations. Multiple traits can be correlated due to linkage or close association of the genes controlling the traits. Overlapping of multiple QTLs representing various yield traits (ALA, FLWL, DLWL, NBR, TSL and TSW) described in LG2 and LG3 indicates that these traits are closely linked and inherit together. Common marker (Tables 1, 2 and 3) representing different QTLs denote that the same gene is affecting different traits. Multiple QTLs representing a single character (NBR and MC)

confirm the association of more coding regions for the specific trait. Further studies are required to provide a better understanding of the genomic organization of QTLs associated with mulberry yield characters.

MAS can be used in mulberry for improvement of yield contributing traits by conversion of flanking RAPD markers to SCAR. The information thus generated may also be useful for genomic analysis of other species of *Morus* and related genera. The identified QTL locations can be fine mapped using co-dominant markers for the construction of consensus map and identification of tightly linked markers for application in Marker Assisted Breeding. Integration of genome maps in mulberry is another important task. Besides the construction of genetic maps, development of physical map is also important in mulberry genome research, which will help us in understanding the genomic architecture of mulberry and aid in positional cloning of genes of interest.

ACKNOWLEDGEMENT

Authors acknowledge the financial assistance received from Department of Biotechnology (Ministry of Science and Technology, Govt. of India), New Delhi (Grant No. BT/PR1171/PB/10/38/98, dated: 31/03/2000 and No. BT/PR3535/PBD/19/084/2002, dated: 23/12/2003). B. Mathithumilan was supported with a Senior Research Fellowship under the Grant. Marian Vincent Pinto acknowledges the financial assistance received in the form of Junior Research Fellowship from the Council of Scientific and Industrial Research, New Delhi.

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DEVELOPPEMENT D'UNE CARTE DE LIAISON GENETIQUE DU MURIER A L'AIDE DE MARQUEURS MOLECULAIRES ET IDENTIFICATION DES LOCI DES CARACTERES QUANTITATIFS LIES AU RENDEMENT ET DE CEUX CONTRIBUANT AU RENDEMENT

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R E S U M E

Une étude a été entreprise pour construire une carte de liaison génétique du mûrier par croisement avec l'objectif de cartographier les caractères du rendement et ceux contribuant au rendement. Deux cartes de liaison spécifiques de parents ont été construites par génotypage de 150 progénies ségrégantes F1 d'un croisement entre Mysore Local (faible rendement femelle) et V-1 (haut rendement mâle) en utilisant 369 marqueurs polymorphiques aléatoires. Chez Mysore Local, 73 marqueurs ont été cartographiés sur 21 groupes de liaison (LGs) couvrant une distance de 1414,8 cM. La carte V-1 couvre une distance de 1351,4 cM avec 74 loci marqueurs dans 20 LGs. La cartographie par Intervalle Composite a été réalisée avec un seuil LOD empirique ($2,5 < \text{en utilisant les données enregistrées en trois ans}$). Les Loci des Caractères Quantitatifs (QTLs) pour NBR et TSL ont été cartographiés sur LG2 à partir des données d'Octobre 2004. Les QTLs majeurs (DLWL et FLWL) avec une valeur R^2 élevée (19 et 14% respectivement) et LLS ($R^2 = 10\%$) ont été localisés respectivement sur LG3 et Lg13. Des QTLs pour ALA et TSW sur LG3 et un QTL pour MC% sur LG13 ont été identifiés à partir des données obtenues en Avril 2005. De plus, des QTLs pour ALA et TSL sur LG3, pour MC% sur LG8 et pour LLS sur GL17 ont été cartographiés à partir du phénotypage d'Octobre 2006. Cette étude est la première tentative pour cartographier des QTLs chez le mûrier et l'une parmi les rares à développer une carte de liaison moléculaire chez le mûrier.

Mots-clés: Carte de liaison, mûrier, loci des caractères quantitatifs (QTLs), marqueurs aléatoires, caractères du rendement et lies au rendement.



PRE-TREATED MULBERRY FRUIT AS A CEREBROPROTECTIVE IN ANIMAL MODEL OF ALZHEIMER'S DISEASE

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ABSTRACT

Alzheimer's disease (AD) is becoming quite prevalent in the modern society affecting considerable number of the elderly population globally. At this juncture, identification of a cerebroprotective agent against AD will be a remarkable breakthrough. Based on the beneficial effect of anthocyanins in this field, anthocyanins-rich substances have of late gained much attention. In this study, we aimed to determine the protective effect of mulberry fruit on memory impairment, neurodegeneration, cholinergic function and oxidative stress status in cognitive deficit rats, induced by AF64A. Adult male Wistar rats were given dried powder of mulberry fruits orally at doses of 2, 10 and 50 mg/kg BW for 7 days before and 7 days after the intracerebroventricular injection of AF64A bilaterally. Then, they were assessed for spatial memory, cholinergic neurons density and AChE activity together with oxidative stress markers including MDA level and the activities of SOD, CAT and GSH-Px in hippocampus. The results showed that mulberry fruits could recover memory deficit, improve oxidative stress and the cholinergic function in rats with memory deficit induced by AF64A. This indicates that mulberry fruit is a potential cerebroprotectant against Alzheimer's disease. Further research is necessary to elucidate the active ingredients.

Key words: Alzheimer's disease, cerebroprotective effect, mulberry fruit.

INTRODUCTION

Alzheimer's disease (AD), the most prevalent neurodegenerative disease in the aged population, is characterized by the impairment in memory and behavior (Johnson *et al.*, 2000). It has been reported to be a multi-etiological disease that involves the malfunctions of various biochemical pathways (Perry *et al.*, 2003; Citron, 2004). Accumulative lines of evidence have demonstrated that the underlying mechanisms are associated with oxidative stress and the deficits of neurotransmitters and receptors such as cholinergic

system (Guan, 2008). To date, the drugs approved for treating mild to moderate AD target at cholinergic deficit by trying to enhance the acetylcholine level in the brain via the suppression of acetylcholinesterase (AChE) (Heinrich and Teoh, 2004). However, the adverse effects such as hepatotoxicity, nausea, vomiting and diarrhea are presented (McGleenon *et al.*, 1999; Heinrich and Teoh, 2004;). Therefore, development of a novel therapeutic agent against AD is required. Based on the crucial role of oxidative stress on the pathophysiology of AD as mentioned earlier, the beneficial effects of substances

possessing antioxidant to protect against AD have gained attention. It has been demonstrated that alpha tocopherol consumption at dose of 2000 IU per day for 2 years can slow down the progress of AD (Sano *et al.*, 1997). However, the benefit of antioxidant treatment is still controversial and no current drugs can completely cure AD. Since AD is a multi-etiology disease, the multi-targets approach can increase the health benefit due to the synergistic effect. Hence, a hypothesis is put forth that a prophylaxis treatment with substance which acts at multi-target sites should protect against AD.

Mulberry (*Morus* spp.) belonging to family Moraceae, is an economic plant supporting sericulture in the North and Northeast of Thailand. Ripe fruits of mulberry, Chiang Mai variety, are rich in anthocyanins, a substance possessing many health benefits including the antioxidant and AChEI effects (Pervin *et al.*, 2014). Recently, mulberry fruit extract was noticed to enable recovery of memory impairment following cerebral ischemia. In addition, it also enhanced densities of neuron and cholinergic neuron but decreased oxidative stress (Braak and Braak, 1998). Therefore, we hypothesized that mulberry fruit could protect against Alzheimer's disease. Since supportive evidence are still very limited, we aimed to determine the effect of mulberry fruits on memory deficit and neurodegeneration in animal model of Alzheimer's disease. To explore the possible underlying mechanisms, the effects of mulberry fruits on AChE and oxidative stress markers in hippocampus were also investigated.

MATERIALS AND METHODS

Animals

Adult male Wistar rats (180-220 g, 8 weeks old) were obtained from National Laboratory Animal Center, Salaya, Nakhon Pathom and they were housed in groups of 5 per cage in standard metal cages at $22 \pm 2^\circ\text{C}$ and 12:12 h light-dark cycle. All animals were given access to food and water *ad libitum*. The experiments were performed after ensuring minimum animal suffering in accordance

with the internationally accepted principles for laboratory use and care of European Community (EEC directive of 1986; 86/609/EEC).

Preparation of dried powder of mulberry fruits

All ripe mulberry fruits used in this study were collected from Queen Sirikit Sericulture Center, UdonThani. All berries were picked at the commercially ripe stage and selected according to uniformity in color. The fruits were dried at 60°C for 48-72 h and ground to powder.

Study protocol

Rats were randomly divided into various groups described as following: 1) Vehicle+AF64A, rats in this group were orally given vehicle for 7 days before and 7 days after the intracerebroventricular administration of AF64A bilaterally 2) Donepezil +AF64A, rats were treated with donepezil, an acetylcholinesterase inhibitor, at a period of 7 days before and 7 days after the intracerebroventricular administration of AF64A bilaterally 3)-5) Mulberry fruit+AF64A, rats in these groups were orally given mulberry fruit powder at doses of 2,10 and 50 mg/kg BW, respectively. After the above treatments, rats were subjected to the procedure of determination of spatial memory. The hippocampus was isolated and used for the determination of cholinergic neurons. In addition, malondialdehyde (MDA) level and the activities of AChE, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in the area just mentioned were also determined.

Morphological analysis

Five coronal rat brain sections in each group were studied quantitatively. Neuronal counts in hippocampus were performed visually under 40x magnification with final field of $255 \mu\text{m}^2$ according to the following stereotaxic coordinates: AP, 4.8 mm; lateral, $\pm 2.4-6$ mm and depth, 3-8 mm. The observer was blind to the treatment at the time of analysis. Viable stained neurons were identified on the basis of a stained soma with at least

two visible processes. Counts were made in five adjacent fields and the mean number extrapolated to give total number of neurons per $255 \mu\text{m}^2$. All data are represented as number of neurons per $255 \mu\text{m}^2$.

Determination of oxidative stress markers and acetylcholinesterase (AChE) activity

Oxidative stress markers including MDA level and the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) and the activity of AChE in hippocampus were assessed *via* colorimetric method.

Statistical analysis

All data are presented as mean $\pm$ standard error of mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by LSD post hoc test. Probability levels less than 0.05 were regarded as significant.

RESULTS

The rats with memory deficit induced by AF64A and thereafter treated with either donepezil or mulberry fruits powder at doses of 2, 10 and 50 mg/kg BW exhibited significant decrease in escape latency (p -value < 0.001 all; compared to vehicle+AF64A) (Figure 1). The effect of mulberry fruits on retention time was also investigated and results are shown in Figure 2. It was found that rats with memory deficit induced by AF64A, followed by treatments of either donepezil or mulberry fruit powder at doses of 2 and 50 mg/kg BW revealed significant increase in retention time (p -value < 0.01 , 0.01 and 0.05 , respectively; compared to vehicle+AF64A).

Based on the crucial role of cholinergic system function on the pathophysiology of AD, the effects of mulberry fruit powder on density of cholinergic neuron and AChE activity in hippocampus were assessed and results are shown in Figures 3-4. Figure 3 indicates that memory deficit rats which received donepezil displayed the enhanced cholinergic neurons density in CA1, CA2

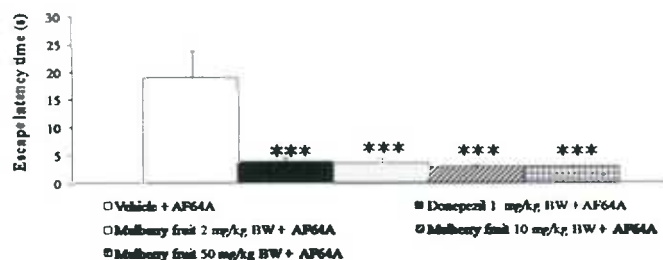


Figure 1: Effect of mulberry fruit on escape latency time in Morris water maze test

Values are expressed as mean $\pm$ SEM (n=8). Significance was determined using ANOVA followed by Tukey test. *** $p < 0.001$ compared to vehicle treated group.

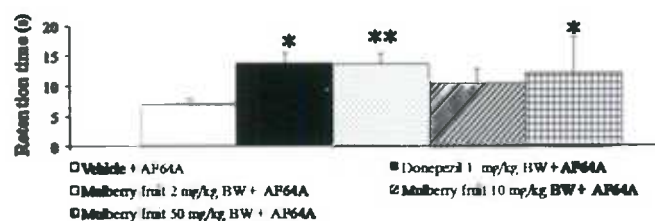


Figure 2: Effect of mulberry fruit on retention time in Morris water maze test

Values are expressed as mean $\pm$ SEM (n=8). Significance was determined using ANOVA followed by Tukey test. *, ** $p < 0.05$, 0.01 , respectively compared to vehicle treated group.

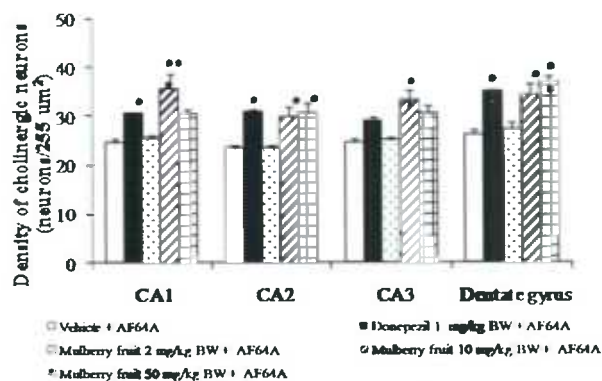


Figure 3: Effect of mulberry fruit on the density of survival neurons in various subregions of hippocampus

Values are expressed as mean $\pm$ SEM (n=8). Significance was determined using ANOVA followed by Tukey test. *** $p < 0.05$ and 0.01 , respectively compared to vehicle treated group.

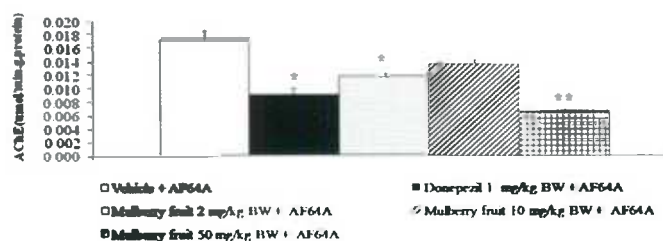


Figure 4: Effect of mulberry fruit on the activity of acetylcholinesterase enzyme in hippocampus

Values are expressed as mean $\pm$ SEM (n=8). Significance was determined using ANOVA followed by Tukey test. *** $p < 0.05$ and 0.01 , respectively compared to vehicle treated group.

Table 1: Effect of mulberry fruit on the level of malondialdehyde (MDA) and scavenging enzyme in hippocampus

Treatment	Malondialdehyde (nmol/mg protein)	Superoxide dismutase (U/mg protein)	Catalase (U/mg protein)	Glutathione peroxidase (U/mg protein)
Vehicle + AF64A	0.19±0.02	2.43±0.31	1.73±0.31	0.48±0.21
Donepezil 1 mg/kg BW + AF64A	0.11±0.03**	8.11±0.92*	13.60±0.96***	0.88±0.17***
Mulberry fruit 2mg/kg BW + AF64A	0.11±0.03**	6.43±0.24*	2.87±0.36*	1.91±0.30**
Mulberry fruit 10 mg/kg BW + AF64A	0.14±0.02	6.73±0.43*	5.89±1.12**	2.00±0.34**
Mulberry fruit 50 mg/kg BW + AF64A	0.08±0.01**	4.88±0.45	3.90±1.56	1.81±0.78**

Values are expressed as mean ± SEM (n=8). Significance was determined using ANOVA followed by Tukey test. ***, ** p < 0.05; 0.01 and 0.001, respectively compared to vehicle treated group.

and dentate gyrus of hippocampus (p-value <0.05, 0.01 and 0.01, respectively compared to vehicle+AF64A). Mulberry fruits at dose of 10 mg/kg BW significantly enhanced cholinergic neurons density in CA1, CA2, CA3 and dentate gyrus of memory deficit rats (p-value <0.001, 0.05, 0.01 and 0.05, respectively compared to vehicle+AF64A) whereas mulberry fruits at dose of 50 mg/kg significantly enhanced cholinergic neurons density only in CA2 and dentate gyrus (p-value <0.05 and 0.01, respectively compared to vehicle+AF64A). Figure 4 reveals the effect of mulberry fruit on AChE activity in hippocampus. Our results show that both donepezil and mulberry fruit at dose of 2 and 50mg/kg BW significantly suppress AChE activity in hippocampus (p-value <0.05; 0.05 and 0.01, respectively compared to vehicle+AF64A).

Results presented in Table 1 show the effect of mulberry fruit powder on oxidative stress markers including MDA level and the activities of SOD, CAT and GPx. Memory deficit rats subjected to donepezil treatment had decreased MDA level but enhanced levels of both SOD and CAT in hippocampus (p-value <0.01, 0.01 and 0.001, respectively compared to vehicle+AF64A). Low dose of mulberry fruits decreased MDA level but enhanced SOD, CAT and GPx activities in hippocampus (p-value <0.01, 0.05, 0.05 and 0.01, respectively compared to vehicle+AF64A). Medium dose of mulberry fruits also enhanced SOD, CAT and GPx

activities in hippocampus (p-value <0.05, 0.01 and 0.01, respectively compared to vehicle+AF64A) whereas high dose of mulberry fruits also decreased MDA level but enhanced only GPx activity in the mentioned area (p-value <0.01 all; compared to vehicle+AF64A).

DISCUSSION

The current study clearly demonstrated that mulberry fruit powder recovered memory deficit and enhanced cholinergic functions both via the enhanced cholinergic neurons and via the suppression effect on AChE. In addition, mulberry fruits also decreased oxidative stress in hippocampus.

It has been reported that hippocampus, a critical area for learning and memory, is vulnerable to damage at early stages of Alzheimers disease (AD) (Braak and Braak, 1998). In AD, the degeneration of neuronal cells in CA1, CA2, CA3 and DG were observed markedly (Fukutani *et al.*, 2000; Zarow *et al.*, 2005; Padurariu *et al.*, 2012). The decreased neuron density in the mentioned area in turn decreased learning and memory capacity in AD (Padurariu *et al.*, 2012). In addition, the decreased cholinergic status in hippocampus also produced spatial memory impairment (Craig *et al.*, 2008). Since our data have clearly revealed that medium dose of mulberry fruit powder increased cholinergic neuron density and enabled recovery of memory deficit without the significant changes of AChE and MDA, we suggest that the cognitive

enhancing effect of the medium dose of mulberry fruits might be due to the increased cholinergic status in hippocampus via the increased cholinergic neurons density which in turn increased spatial memory. The increased neuron density in CA1, CA2, CA3 and DG of hippocampus might not be related with the decreased oxidative stress. Other factors such as various types of growth factors, neurotransmitters and neuromodulators which play the roles on brain plasticity (Johansson, 2000) might have a function. The cognitive enhancing effect of high dose of mulberry fruits might also occur via the increased cholinergic status in hippocampus but the enhanced cholinergic status occur both via the suppression of AChE and the enhanced cholinergic neurons density. The increased GPx activity in hippocampus observed in memory deficit rats treated with the high dose of mulberry fruits induced the decreased MDA level in the mentioned area and might contribute a role on the enhanced cholinergic neuron density. However, the decreased oxidative stress might not be the principal factor which induced the enhanced cholinergic neuron density in hippocampus because the enhanced cholinergic neurons density in hippocampus was still observed even when no reduction of MDA level was observed. In addition, memory deficit rats treated with low dose of mulberry fruit showed the enhanced SOD, CAT and GPx together with the decreased MDA level but failed to show the enhanced cholinergic neurons density in hippocampus.

Brain synaptic density has been reported to play an important role in learning and memory (Geinisman, 2000). Synaptic brain density in hippocampus can be modified by oxidative stress (Ansari *et al.*, 2008). Therefore, the fact that the memory deficit rats which received the low dose of mulberry fruits showed the memory enhancing effect together with the decreased oxidative stress without the changes of cholinergic status might be associated with the decreased oxidative stress which in turn enhanced synaptic density in hippocampus. However, this requires further investigation.

CONCLUSION

Mulberry fruit is a potential functional food to protect against AD. The possible underlying mechanism depends

on dose of the mulberry fruits. Low dose might exert the effect via the decreased oxidative stress status whereas, medium dose might exert the effect via the increased cholinergic status in hippocampus and high dose might exert its effect via both mechanisms mentioned earlier. It is noteworthy that mulberry fruit produces the benefit at the same magnitude as a standard drug used nowadays, and at the same time is cheap, safe and easily available. However, further research is required to provide the understanding about the precise mechanism and the possible active ingredient.

ACKNOWLEDGEMENT

This study was supported by The Queen Sirikit Department of Sericulture, Ministry of Agriculture and Cooperatives and Integrative Complementary Alternative Medicine Research and Development Center, KhonKaen University, Thailand.

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LE FRUIT DE MURIER PRETRAITE COMME PROTECTEUR CEREBRAL CHEZ UN ANIMAL MODELE DE LA MALADIE D'ALZHEIMER

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RESUME

La maladie d'Alzheimer (AD) est devenue très fréquente dans les sociétés modernes affectant une proportion très importante de la population des personnes âgées. Dans ce contexte, l'identification d'un protecteur cérébral contre l'AD sera une percée remarquable. En raison de l'effet bénéfique des anthocyanines dans ce domaine, les substances riches en anthocyanines ont reçu plus d'attention. Dans notre étude, nous avons visé à déterminer l'effet protecteur du fruit du mûrier (la mûre) sur l'affaiblissement de la mémoire, la neuro-dégénérescence, la fonction cholinergique et le statut du stress oxydatif chez des rats déficients sur le plan cognitif induits par AF64A. Des rats mâles adultes Wistar ont reçu de la poudre sèche de mûres oralement à des doses de 2, 10 et 50 mg/Kg pendant 7 jours avant et 7 jours après l'injection cérébro-ventriculaire bilatérale de AF64A. Ils ont ensuite été testés pour la mémoire spatiale, la densité de neurones cholinergiques et l'activité AChE en même temps que les marqueurs du stress oxydatif incluant le niveau MDA et les activités SOD, CAT et GSH-Px dans l'hippocampe. Les résultats montrent que les mûres pourraient restaurer un déficit mémoriel, améliorer le stress oxydatif et la fonction cholinergique chez des rats ayant un déficit mémoriel induit par AF64A. Ceci indique que la mûre est un protecteur potentiel du cerveau contre la maladie d'Alzheimer. Des recherches supplémentaires sont nécessaires pour définir les éléments actifs.

Mots-clés: Maladie d'Alzheimer, effet cérébro-protecteur, fruit du mûrier.



DONORS & NGOS IN SERICULTURE: INSTITUTIONS, EMPOWERMENT & DEPENDENCY

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ABSTRACT

What should donors and non-governmental organizations (donors & NGOs) do in order to successfully promote inclusive development in sericulture? Although the answer to this question could have important implications for how development cooperation may contribute to poverty reduction in sericulture, the question has so far received very limited attention in the existing literature. Therefore, this study aims to answer this question by utilizing the concepts of institutions, empowerment and dependency for the purpose of analyzing information collected from a large number of publications, as well as from interviews with over thirty organizations involved in sericulture. In total, the study investigates twenty-nine interventions of donors & NGOs, taken place over the past twenty-five years, in the mulberry silk sectors of twenty countries. In the light of this analysis, the study concludes that development cooperation can promote inclusive development in sericulture if donors & NGOs: i) have an adequate focus on supply and demand factors affecting silk farmers' incomes; ii) cooperate with each other, and with stakeholders in recipient countries; iii) avoid to impose dependency; and iv) commit before exiting.

Key words: Dependency, donors & NGOs, empowerment, institutions, mulberry silk.

INTRODUCTION

Over the past few decades, a debate has emerged concerning the effectiveness of development cooperation. While some argue that development cooperation contributes to poverty reduction (Krishna *et al.*, 1997; Uphoff *et al.*, 1998; Sachs, 2005; Stokke, 2009), others are more critical (Moyo, 2009; Holmén, 2010). Viewing this debate in terms of sericulture, an activity known for its poverty reducing characteristics (Bhaskar *et al.*, 2008; Anitha, 2011; Cunvong, 2011), it is paramount to consider whether and how development cooperation may promote inclusive development in sericulture. Hence, this study analyzes interventions of donors & NGOs in the mulberry silk sector by addressing the

following research question: To what extent can donors & NGOs promote the empowerment of silk farmers? In order to answer this research question, the concept of empowerment must be discussed, which is done in the next section. Thereafter, the study conducts an empirical analysis. Following is a conclusion.

CONCEPTUAL FRAMEWORK

An essential concept that has permeated the debate on development cooperation is that of empowerment, a concept that can be argued to have two dimensions. The first dimension relates to economic empowerment and involves people's skills and capabilities, as well as their access to assets, resources and markets, *i.e.*, factors

Disclaimer: Any views or opinions expressed in this study are solely those of the author and do not necessarily represent those of the interviewed organizations, or the Department of Economics at Lund University School of Economics and Management.

affecting the opportunity of higher income (Rowlands, 1995; Luttrell *et al.*, 2009). The second dimension refers to social empowerment, comprising the power and control that people have over their lives, *i.e.*, factors affecting the opportunity to independently undertake long-term planning. Consequently, empowerment cannot be imposed from outside, but is a process that has to come from within (Kilby, 2006), whereby donors & NGOs may act as catalysts in the empowerment process by ensuring the participation of the poor and marginalized (Cornwall, 2000; Chambers, 2008).

Since empowerment is connected to opportunities, donors & NGOs should aim to foster the development of an institutional infrastructure that minimizes the risks and maximizes the gains associated with sericulture, thereby providing appropriate incentives for silk farmers. Therefore, donors & NGOs ought to bear a few factors in mind. Firstly, donors & NGOs should have an adequate focus on supply and demand factors affecting silk farmers' incomes; interventions should tackle context-based issues (Holmén, 2010), enabling silk farmers to increase the quality, quantity, and sales of their output. Secondly, donors & NGOs should cooperate with each other, and with stakeholders in recipient countries, *e.g.* the government, as that could make more resources available for investments in the supply-chain (Chandy and Kharas, 2011; Woods, 2011). Thirdly, donors & NGOs should aim not to substitute existing stakeholders in the supply-chains of recipient countries since donors & NGOs otherwise may impose dependency on the recipients, *i.e.*, donors & NGOs may become the only safety for the recipients (Bräutigam and Botchwey, 1999; de Wit and Berner, 2009; Holmén, 2010). For silk farmers, such a situation can become detrimental; the exit of donors & NGOs could jeopardize the sustainability of sericulture in the recipient country as the silk farmers involved are left without a safety net. However, there is also a fourth factor that is important, particularly in reference to the case of dependency, *viz.*, the commitment of donors & NGOs (Birdsall, 2004). Donors & NGOs should commit before exiting so that a favorable institutional infrastructure is allowed to develop; incentives for sericulture might

require some time to get a foothold as other activities may be associated with fewer risks (Boserup, 1965). In this regard, donors & NGOs may initially have to impose dependency on silk farmers due to a lack of supply-chain linkages in the recipient country, being sustainable if donors & NGOs display long-term commitment for the purpose of ensuring self-sufficiency through strengthening such linkages. Commitment can thus be viewed as crucial, especially when dependency cannot be avoided.

In brief, it could be argued that the empowerment of silk farmers can be promoted if donors & NGOs: i) have an adequate focus on supply and demand factors affecting silk farmers' incomes; ii) cooperate with each other, and with stakeholders in recipient countries; iii) avoid to impose dependency; and iv) commit before exiting. The last factor is particularly important when dependency cannot be avoided. By dint of these factors, the next section analyzes to what degree interventions of donors & NGOs in sericulture have been successful by using an evaluation criterion, namely by investigating to what extent the involved silk farmers' incomes have improved.

EMPIRICAL ANALYSIS

Using information collected from literature, and from interviews with various organizations, the study analyzed twenty-nine interventions, taken place over the past twenty-five years, in the mulberry silk sectors of twenty countries, all of which being presented in Table 1. The impact of the interventions has varied, ranging from only slight to exceptional improvements in silk farmers' incomes. Accordingly, Table 1 divides the interventions into two categories.

In the first category, the degree of success can be argued to be low since the rise in silk farmers' incomes has been constrained by the factors highlighted in the conceptual framework. Many of the interventions in this category did not succeed in addressing issues relating to supply and demand factors. In Bangladesh, Madagascar, Ghana, and

Donors & NGOs in sericulture

Table 1. Silk Sector Interventions by Donors & NGOs

Country	Donors & NGOs Involved in Implementing the Intervention	Intervention Types*	Degree of Success**
Afghanistan	United States Agency for International Development (USAID), Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ), Agricultural Cooperative Development International and Volunteers in Overseas Cooperative Assistance (ACDI/VOCA)	CBF, DFF, DPF, ISH, PIF, PCP, PMA	High
Bangladesh	World Bank, Bangladesh Silk Foundation (BSF), Local NGOs	AFO, CBF, CBT, DPF, PCP, PMA	Low
Cambodia	Agence Française de Développement (AFD)	CBF, DPF, IMV, ISH, PIF, PCP, PMA	Low
	Food and Agriculture Organization (FAO)	CBF, CBT, DFF, DPF, ISH, IMV, PIF	Low
	Economic and Social Relaunch of Northwest Provinces in Cambodia (ECOSORN), Khmer Silk Villages (KSV)	CBF, PCP, PMA	Low
Colombia	Instituto Italo-Latinoamericano (IILA), Red Latinoamericana de la Seda (RELA SEDA), Centro de Desarrollo Humano en Cultura y Economía Solidaria (CEBYCAM-CES), Fundación Progresamos, Asociación de Artesanos de la Seda del Eje Cafetero	DPF, PCP, PFF	High
Dominican Republic	Mama D.O.C.	DFF, EMV, ESH, PMA	Low
Ethiopia	Japan International Cooperation Agency (JICA)	CBF, CBT, DPF, PCP	Low
	Korea International Cooperation Agency (KOICA)	CBT, DPF	Low
Ghana	Food and Agriculture Organization (FAO), Sericulture Promotion and Development Association Ghana (SPDAG)	CBF, CBT, DFF, DPF, ISH, PCP, PIF, PMA	Low
India	Bharatiya Agro Industries Foundation (BAIF), International Development Research Centre (IDRC), European Commission (EC)	CBF, DFF, DPF, ESH, IMV, PCP, PIF, PMA	High
	Japan International Cooperation Agency (JICA)	CBF, CBT, DFF, DPF, IMV, ISH, PCP, PIF	High***
	World Bank, Swiss Agency for Development and Cooperation (SDC), Local NGOs	CBF, CBT, DFF, DPF, IMV, ISH, PCP, PFF, PMA	Low
Indonesia	Yayasan Sosial Bina Sejahtera (YSBS), Misereor	AFO, CBF, DFF, IMV, ISH, PIF	Low
Kenya	International Centre of Insect Physiology and Ecology (ICIPE), International Fund for Agricultural Development (IFAD), United Nations Development Programme (UNDP), Global Environment Facility (GEF), Biovision Foundation	CBF, CBT, DFF, IMV, ISH, PCP, PIF, PMA	High
Kyrgyzstan	Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ)	DFF, DPF, EMV, ESH, PIF	Low
	Japan International Cooperation Agency (JICA)	CBT, DPF, PCP	Low
	Participatory Development and Training Center (PADETC), Oxfam Novib	CBF, DFF, DPF, ISH, PCP, PIF, PFF, PMA	Low
Laos	Service Fraternel d'Entraide (SFE), Agence Française de Développement (AFD), Swiss Agency for Development and Cooperation (SDC), Lao Women's Union (LWU)	CBF, DFF, IMV, ISH, PCP, PIF, PMA	High
	United Nations Industrial Development Organization (UNIDO), Centre d'Information Technique et Économique (CITE), FAFIALA	CBT, DPF, ISH, PCP, PIF	Low
Madagascar	United Nations Industrial Development Organization (UNIDO), Centre d'Information Technique et Économique (CITE), FAFIALA	CBT, DPF, ISH	Low
Mexico	Japan International Cooperation Agency (JICA)	CBT, DPF, ISH	Low
Nepal	Japan International Cooperation Agency (JICA), Silk Association of Nepal (SAN), United Nations Development Programme (UNDP)	CBF, CBT, DFF, DPF, IMV, ISH, PCP, PMA	High
Peru	Instituto Italo-Latinoamericano (IILA), Red Latinoamericana de la Seda (RELA SEDA), Centro de Desarrollo Humano en Cultura y Economía Solidaria (CEBYCAM-CES)	CBF, DPF, ISH, PCP, PFF	Low
Philippines	Korea International Cooperation Agency (KOICA), Food and Agriculture Organization (FAO), United Nations Development Programme (UNDP)	CBF, CBT, DPF, PCP, PMA	High
	Organization for Industrial, Spiritual and Cultural Advancement (OISCA), Japan International Cooperation Agency (JICA)	AFO, CBF, DFF, DPF, PIF	High
Timor-Leste	Loron Aban Hahu Ohin (LAHO), Australian Agency for International Development (AusAID), Others****	AFO, CBF, CBT, DFF, DPF, IMV, ISH, PIF	Low
Uganda	Maendeleo Agricultural Technology Fund (MATF), Food and Agricultural Research Management Africa (FARM Africa), Kyembogo District Agricultural Training and Information Centre (Kyembogo DATIC), The Gatsby Charitable Foundation, The Rockefeller Foundation, Kilimo Trust, Uganda Women's Effort to Save Orphans (UWESO), Kabarole Silk Farmers Association (KASIFA)	CBF, DFF, DPF, IMV, ISH, PFF, PIF, PMA	Low
	United States African Development Fund (USADF), Japan International Cooperation Agency (JICA)	CBF, DFF, DPF, IMV, ISH, PCP, PFF, PMA	Low
	Local Silk Farmers Associations*****		
Uzbekistan	Japan International Cooperation Agency (JICA), Business Women Association of Uzbekistan (BWA)	CBF, DFF, DPF, ISH, PCP, PMA	High*****

Sources: Hanumappa and Erappa (1985), Kiyokawa (1993), Datta (1996), World Bank (1997, 2003, 2006, 2012), Esim (2001), JICA (2002, 2003, 2004, 2005, 2006, 2009, 2013, 2014 a, 2014 b), Kumaresan (2002), MG (2002, 2004), Lewis (2003), NABCONS (2004), Aguilar (2005), Cifuentes (2005), Dandin *et al.* (2005), SAN (2005), Fenomanantsoa (2006), Lewis and Siddiqi (2006), ATA (2007, 2010), CSB (2007, 2014), Gebert *et al.* (2007), JAICAF (2007), MATF (2007), UNIDO (2007), Armitage (2008), Ramanoelina *et al.* (2008), Soto and Tamayo (2008), Vestalys and Andriatovina (2008), CITE and BOSS Corporation (2009), Hilson and Banchingah (2009), IRIN (2009), Kramer *et al.* (2009), MolC (2009), Patil *et al.* (2009), Raina *et al.* (2009), URN (2009, 2010 a, 2010 b, 2012), USADF (2003 a, 2003 b), AILA (2010), ECOSORN (2010, 2014), GTZ (2010), Kaushiki (2010), Mey *et al.* (2010), PADETC (2010, 2012), Vicites *et al.* (2010), Anitha (2011), Asaoka (2011), Dillmann (2011), Kilimo Trust (2011), Sohn and Kim (2011), USAID (2011 a, 2011 b, 2012, 2013, 2014), Martínez and Barraza (2012), Nisar *et al.* (2012), Thomas (2012), Yamada *et al.* (2012), Yambay (2012), FAO (2013), ICIPE (2013), Ntuanu (2013), ACDI/VOCA (2014 a, 2014 b), Biovision Foundation (2014), Iikubo *et al.* (2014), KSV (2014), LAHO (2014 a, 2014 b), Pravda Vostoka (2014).

Notes: * This table has benefited from information provided, through interviews, by more than thirty organizations, including donors, NGOs, as well as other, public and private, organizations involved in sericulture. It should also be emphasized that some of the interventions in the table are constituted by several projects due to implementing the interventions in different phases. * The intervention types are: acquisition of silk farmers' output (AFO); capacity building for silk farmers (CBF); capacity building for technical personnel, including researchers, extension workers and managers (CBT); development of silk farming facilities (DFF); development of public goods provision facilities, i.e. facilities for research, extension, as well as input provision, viz. for silkworm eggs, mulberry saplings etc. (DPF); evaluation of mulberry varieties (EMV); evaluation of silkworm breeds and/or hybrids (ESH); introduction or diffusion of one or more mulberry varieties (IMV); introduction or diffusion of one or more silkworm breeds and/or hybrids (ISH); support to post-cocoon processing (PCP); provision of financial aid for silk farmers through promoting banking services (PFF); provision of inputs for silk farmers (PIF); promotion of market access for silk farmers (PMA). ** Explicit information concerning the income increase was unavailable in a few of the interventions. Thus, in the context of these interventions, the study assumes that a significant increase in quality and/or quantity of silk farmers' output, jointly with market access, imply significant improvements in silk farmers' incomes, and vice versa. *** The projects considered here are: Bivoltine Sericulture Technology Development Project, Project for Promotion of Popularizing the Practical Bivoltine Sericulture Technology, and Project for Strengthening of Extension System for Bivoltine Sericulture. Thus, the Manipur Sericulture Project has been excluded. **** Some of the other donors & NGO involved were: World Food Programme (WFP), Food and Agriculture Organization (FAO), Japan International Cooperation Agency (JICA), International Labor Organization (ILO), United Nations Development Programme (UNDP), Life Love and Health (LL&H), Alternative Technology Association (ATA), Aloia Foundation, Australian Volunteers International (AVI), World Vision Timor-Leste (WVT), and Centro de Desenvolvimento Comunitário (CDC). ***** Among the associations involved were those of Bushenyi, Kihhi, and Kabaga. ***** This intervention has so far had two phases. In the first phase, only silk farmers in the Fergana Valley of Uzbekistan were involved, and this phase has already finished. In the second phase, the goal is to replicate the success of the first phase elsewhere in the country. As the second phase recently has begun and still is ongoing, the degree of success here merely refers to the first phase of the associated intervention.

Uganda, silk farmers lacked market access, a problem that none of the associated interventions managed to appropriately address. Similarly, may be argued concerning, for instance: mulberry sapling provision in Madagascar; silkworm egg provision in Indonesia and Uganda; the provision of both of the aforementioned inputs and extension in Laos with respect to JICA's intervention; capacity building for silk farmers in Uganda; the introduction of mulberry varieties, the support to post-cocoon processing, and the development of silk farming facilities in Ghana; and the development of public goods provision facilities in Ethiopia, as well as in India and Cambodia in reference to the interventions implemented by the World Bank and others, and by AFD, respectively. Furthermore, some of the interventions were hampered by cooperation failures. In the interventions spearheaded by the World Bank in Bangladesh and India, there appeared to be disagreements between the World Bank and the respective governments regarding to what extent silkworm egg provision needed to be liberalized, in turn impeding practical action. In Mexico and Peru, the respective governments desired to prioritize other activities than sericulture, whereby the same governments only made a limited amount of resources available for the associated interventions. A comparable case can be found in the Dominican Republic where the associated intervention ultimately was terminated due to expropriation. In Timor-Leste, LAHO has attempted to cooperate with numerous donors & NGOs. Yet, the resulting amount of resources made available for LAHO has been limited, and so has the governmental support. This, latter, problem also characterized the intervention of PADETC and Oxfam Novib in Laos, an intervention that further exhibited cooperation issues with a local cocoon buyer; although the buyer, to which the silk farmers involved were connected, allegedly had a corporate social responsibility orientation, the silk farmers were not sufficiently paid for their cocoons. Moreover, a number of interventions were featured by dependency and commitment problems. In Cambodia and Madagascar, silk farmers were dependent on KSV for market access, and on UNIDO for the provision of imported silkworm eggs, respectively. Therefore, when ECOSORN ceased to

fund KSV, in turn causing KSV's exit, and when UNIDO exited, silk farmers in both countries were adversely affected. Likewise was the case in Kyrgyzstan, where the intervention was on the verge of having established a lucrative activity when GIZ, untimely, chose to exit. In addition, the commitment of FAO in Ghana as well as of MATF and others in Uganda was short-lived. On top of that, since MATF and others also had implemented a micro-credit scheme for the silk farmers involved in the associated intervention, the same silk farmers lost their only safety net while still being considerably indebted. Finally, despite that FAO's intervention in Cambodia established public goods provision facilities, these facilities were hardly used; commitment was not sufficient for silk farmers to become assured that the facilities would fulfill their role. Thus, to summarize, given the issues relating to the factors emphasized in the conceptual framework, sericulture did frequently not become a viable option for silk farmers in this category. As a consequence, many silk farmers in this category chose to opt out and diversify into other activities.

In the second category, the degree of success can be considered as high because significant increases in silk farmers' incomes have been realized due to the factors to which the conceptual framework refers. All interventions in this category have, to a great extent, sought and succeeded to address context-based issues with regard to supply and demand factors. This assertion is especially true for Colombia where the intervention of IILA and others ensured that a local university could overtake the responsibility of public goods provision after the former public goods provider, due to economic difficulties, was liquidated. Additionally, cooperation has generally been effective between donors & NGOs, and with stakeholders in recipient countries. A noteworthy intervention, in this regard, was that of JICA, SAN and UNDP in Nepal. This intervention not only displayed responsibility sharing between JICA, SAN and UNDP, but also cooperation with the government, in turn making substantial resources available for investments. Resembling arguments could be raised regarding, *inter alia*, the interventions of USAID and others in Afghanistan, BAIF and others in India,

KOICA and others in the Philippines, ICIPE and others in Kenya, and SFE and others in Laos. The intervention spearheaded by SFE also found a way of combating dependency as the involved silk farmers, at the end of the intervention, were connected to a local cocoon buyer with a strong corporate social responsibility orientation. Furthermore, there appear to only have been three other interventions in this category that exhibited dependency, namely, the interventions spearheaded by ICIPE in Kenya, by OISCA in the Philippines, and by BAIF in India. Yet, all three of these, still ongoing, interventions have been featured by long-term commitment and are thus likely to encourage self-sufficiency in the future. Commitment, to a high degree, also characterized the rest of the interventions in this category. Hence, it can, in short, be argued that the interventions in this category have fostered the development of a favorable institutional infrastructure in recipient countries owing to the benefits derived from the factors stressed in the conceptual framework.

In sum, it can be inferred that the empirical analysis confirms what was argued in the conceptual framework. While silk farmers in the first category largely were not provided appropriate incentives, the opposite holds for the second category. The promotion of empowerment has thus been greater in the second category since more opportunities, of higher income and to independently undertake long-term planning, have been created. As the factors in the conceptual framework can be regarded as determinants in this respect, a few important policy implications can be generated, all of which being presented in the conclusion.

CONCLUSION

This study has sought to unravel whether and how development cooperation can promote inclusive development in sericulture by investigating the role of donors & NGOs in promoting the empowerment of silk farmers. In this regard, it may be argued that donors & NGOs indeed can lend a helping hand. Notably, the analyzed interventions not only demonstrated that donors

& NGOs have a possibility of playing a vital role as facilitators, but also that the factors highlighted in the conceptual framework have been crucial for the degree of success in the interventions. The study thus concludes that development cooperation can promote inclusive development in sericulture if donors & NGOs: i) have an adequate focus on supply and demand factors affecting silk farmers' incomes; ii) cooperate with each other, and with stakeholders in recipient countries; iii) avoid to impose dependency; and iv) commit before exiting.

ACKNOWLEDGEMENT

I would like to extend my deepest gratitude to the organizations that I interviewed for providing complementary information concerning the activities of donors & NGOs in the analyzed interventions. Additionally, I would like to thank my teachers on the Bachelor of Science Programme in Development Studies (BIDS) and at the Department of Economics at Lund University School of Economics and Management for providing the theoretical tools required for the empirical analysis. Lastly, I would like to thank everybody else who in any way has contributed to the realization of this study.

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DONNEURS et ONGs EN SERICICULTURE: INSTITUTIONS, EMANCIPATION ET DEPENDANCE

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R E S U M E

Que devraient faire les donateurs et les organisations non-gouvernementales (donneurs et ONGs) afin de promouvoir avec succès le développement global de la sériciculture ? Bien que la réponse à cette question puisse avoir des implications importantes pour savoir comment le développement de la coopération peut contribuer à la réduction de la pauvreté en sériciculture, la question a, jusqu'à maintenant, reçu peu d'attention dans la littérature disponible. Aussi, cette étude vise à répondre à cette question en utilisant des concepts d'institutions, d'émancipation et de dépendance dans le but d'analyser l'information collectée dans un grand nombre de publications aussi bien qu'à partir d'entretiens avec plus de trente organisations impliquées dans la sériciculture. Au total, l'étude analyse vingt neuf interventions de donateurs et d'ONG qui ont œuvré depuis plus de vingt cinq ans dans les secteurs de la soie mûrier de vingt pays. A la lumière de cette analyse, l'étude conclut que la coopération pour le développement peut promouvoir un développement global de la sériciculture si les donateurs et les ONG : i) ont une mise au point adéquate sur les facteurs de l'offre et de la demande qui affecte les revenus des fermiers ; ii) coopèrent entre eux et avec les dépositaires dans les pays récepteurs ; iii) évitent d'imposer une dépendance ; et iv) transmettent avant de partir.

Mots-clés: Dépendance, donateurs et ONGs, émancipation, institutions, soie mûrier.



GENESIS AND PERFORMANCE EVALUATION OF BACULOVIRUS RESISTANT TRANSGENIC SILKWORM HYBRIDS

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ABSTRACT

Bombyx mori nucleopolyhedrovirus (*BmNPV*) causes major loss to the silk industry in Asia by hampering cocoon production. No effective treatment has so far been formulated against the virus. In a previous study, we have generated transgenic silkworm strains expressing multiple essential viral genes (*ie1*, *lef1*, *lef3* and *p74*) in an inverted repeat manner under *Actin* promoter, using a technique of *piggyBac* mediated germline transgenesis. The transgenic lines generated under Nistari genetic background showed stable resistance against the virus and this antiviral property was then transferred to a high yielding, commercial baculovirus susceptible, CSR2 silkworm strain through marker assisted backcross breeding technique. In the present study, we report generation of several transgenic hybrids by crossing Nistari and CSR2 transgenic lines with various commercial breeds. The best performing hybrids were identified for multilocation field trials based on their survival rate upon baculoviral infection and cocoon quality traits. As the silkworm is the first genetically modified insect being considered for commercial scale rearing in India, potential risk factors have been considered before contained trials. In future, application of this transgenic technology would provide opportunities for alleviating one of the major constraints to silk productivity.

Key words: Antiviral, *BmNPV*, *Bombyx mori* L., contained trials, transgenic silkworms.

INTRODUCTION

Bombyx mori nucleopolyhedrovirus (*BmNPV*) which is a natural pathogen of domesticated silkworm, causes on an average, 30% crop loss to the silk industry. The life cycle of virus consists of two types of virions; budded virions (BVs) that spread systemic infection within the host, and the occlusion-derived virions (ODV/OBs) that spread the baculovirus from host to host through *per os* infection (Rohrmann, 1992). No effective treatment is available against the virus. Since, the biology of the virus is well studied, and its entire genome sequence is available (Gomi *et al.*, 1999), attempts have been made in the past by providing immunity against the virus using RNA interference (RNAi) technique, where delivery of dsRNA or of constructs encoding dsRNA of virus into the host would combat viral infection. Subsequently, the

availability of established method of transgenesis in *Bombyx mori* (Tamura *et al.*, 2000; Tomita 2011) has led to the construction of transgenic silkworm lines harboring a dsRNA-encoding transgene targeting either single essential baculoviral gene *lef-1* (Isobe *et al.*, 2004) or *ie-1* (Kanginakudru *et al.*, 2007) or both *ie-1* and *lef-1* genes (Zhang *et al.*, 2014). With the aim of installing a strong antiviral trait, Subbaiah *et al.* (2013) assayed the properties of transgenic lines with transgene carrying tandem sequences of four baculoviral genes *ie1*, *lef1*, *lef3* and *p74* necessary for viral replication and *per os* infectivity (Subbaiah *et al.*, 2013). When these transgenic lines were infected with baculovirus, they provided high resistance compared to nontransgenic control lines. The antiviral property in the Nistari genetic background was then transferred to a high yielding, baculovirus susceptible

commercial silkworm strain, CSR2 through marker-assisted screening and repeated backcrossing. In the present study, to test transgenic lines at multilocations, transgenic hybrids were generated by crossing Nistari and CSR2 transgenic lines with various commercial breeds and their performance was tested against *BmNPV* infection.

MATERIALS AND METHODS

The *Bombyx mori* strains namely, Nistari and CSR2 used in the present study were same as previously reported (Subbaiah *et al.*, 2013). For testing transgenic silkworms at multiple locations, hybrids were generated by crossing Nistari and CSR2 transgenic lines with the existing commercial high yielding non-transgenic bivoltine and polyvoltine breeds *viz.*, NB4D2, CSR4, SK6 x SK7 (bivoltine) and Pure Mysore (polyvoltine). All the lines were maintained at Andhra Pradesh State Sericulture Research and Development Institute, Hindupur, Andhra Pradesh, India, under appropriate biosafety regulations.

The wild type *BmNPV* which is prevalent in farmers' silkworm rearing house was used for infection studies. The OBs used for *per os* inoculation of the silkworms were collected from the larvae with symptoms of NPV infection following the protocol as described by Subbaiah *et al.* (2013). From each of the selected silkworm hybrids, 300 newly molted 3rd instar healthy larvae were starved for 6 h and placed in separate trays. The larvae were then infected with *BmNPV* @ 40,000 OBs/larva and reared simultaneously in separate rearing houses with respective controls. Mortality was recorded up to moth emergence. The hybrids were tested for cocoon quality traits as well as reeling characteristics following standard procedures. Quantitative PCR analysis of *BmNPV* using *lef3* as a target gene was used to determine virus accumulation level in the transgenic hybrids versus their controls as reported previously (Subbaiah *et al.*, 2013). Data gathered were computed and significant differences wherever

applicable were determined using Student's *t* test.

In order to carry out multilocation contained trials of the transgenic silkworms, the Centre for DNA Fingerprinting and Diagnostics (CDFD) in co-ordination with Biotech Consortium India Limited (BCIL) approached Review Committee on Genetic Manipulation (RCGM) by providing a road map for field trials, seeking biosafety regulatory approvals. Since silkworm is the first genetically engineered insect being considered for commercial scale production in India, regulatory norms were framed for contained field testing. RCGM after detailed deliberation, has approved the conduct of multilocation trials in contained facilities at institutional and farmers' levels in 4 locations. The key biosafety issues include evaluation of performance of transgenic lines and the trait efficacy, adaptation of guidelines and Standard Operating Procedures, inclusion of appropriate controls, and appropriate trial design.

RESULTS

The transgenic hybrids were generated by crossing Nistari and CSR2 transgenic lines as females with the existing nontransgenic bivoltine and polyvoltine breeds as represented in Figure 1. The performance of the transgenic hybrids was tested against baculovirus infection and the data on their survival (pupation %) are presented as histograms (Figure 2). All the transgenic hybrids showed increased resistance over their nontransgenic control hybrids upon *BmNPV* infection ($P < 0.05$). Results showed a pupation rate of greater than 70 % in the transgenic hybrids upon infection as compared to only ~ 40 % in the nontransgenic hybrids. Overall, hybrids resulted from cross involving bivoltine transgenic lines showed higher survival rate as compared to polyvoltine transgenic lines. The best performing hybrids were selected for multilocation contained trials.

As shown in Figure 3, the data on various cocoon and silk characters of transgenic hybrids were studied and compared with the control hybrids. The silk characters of

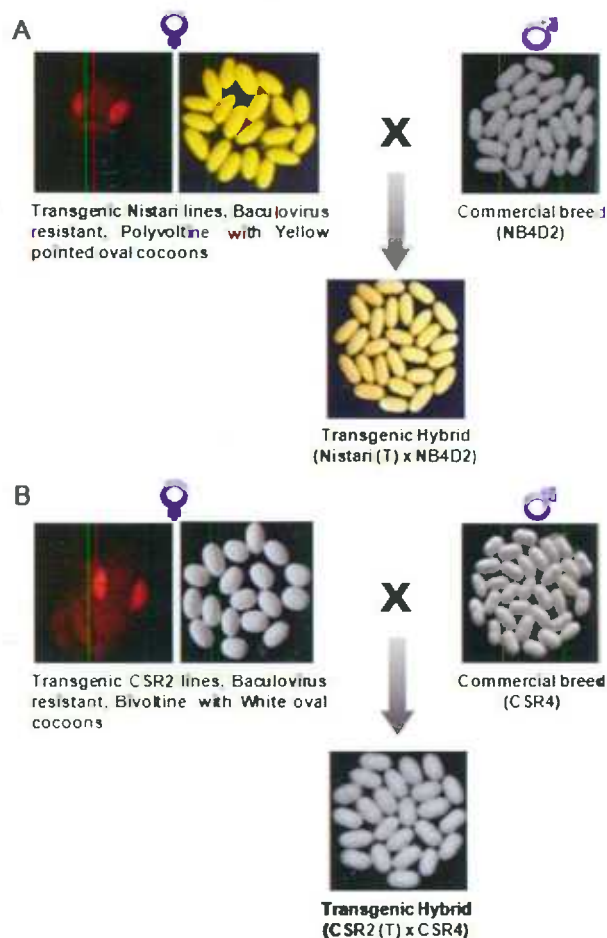


Figure 1: Schematic diagram representing cross between transgenic Nistari and CSR2 line with local commercial bivoltine breeds, A) NB4D2 and B) CSR4

the transgenic hybrids are on par with that of their respective control hybrids ($P > 0.05$). Among various hybrids, 717 x CSR4 hybrid showed higher shell weight, shell percentage, filament length and raw silk % and the lowest value for renditta.

The baculovirus accumulation in the transgenics was measured as the hemolymph concentration of OBs in the infected larvae after baculoviral infection. The viral load, as determined by the viral transcript level using qPCR, reduced approximately 6 folds in PM x 727 and 170B x (SK6 x SK7) hybrids ($P < 0.05$) and 16 folds in 727 x CSR4 hybrid ($P < 0.01$) as compared to their respective controls (Figure 4). Our results thus confirm that the virus load is significantly reduced in the transgenic hybrids in comparison to control lines.

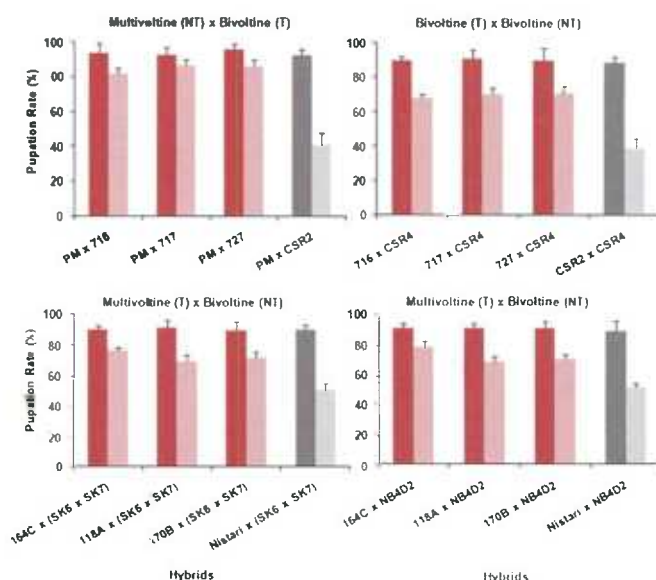


Figure 2: Performance of silkworm hybrids under normal and BmNPV inoculated conditions: Silkworm hybrids were generated for multilocal contained trials by crossing transgenic CSR2 lines (716, 717, 727) and transgenic Nistari lines (164C, 118A, 170B) with various local commercial multivoltine (Pure Mysore - PM) and bivoltine (CSR4, NB4D2, SK6 x SK7) strains. (Bars in dark and light brick colour represent uninfected and baculovirus infected transgenic lines, respectively; bars in dark and light grey shade represent uninfected and baculovirus infected control lines, respectively. T stands for transgenic and NT stands for nontransgenic lines). The y-axis represents pupation rate (%).

DISCUSSION

In sericulture, stable cocoon yield, quality silk and higher returns are the ultimate goals which are obtained through commercial rearing of sustainable silkworm hybrids. Improving silkworm production capacity beyond a certain level is difficult by relying only on traditional breeding methods. In this context, the technique of generation of transgenic silkworms deserves a mention as the strains remain unchanged in economic characteristics but are equipped with significantly increased viral resistance (Jiang and Xia, 2014). RNA interference-based anti-pathogen strategies are efficient to block the activity of genes crucial to the accomplishment of the viral proliferation (Davidson and McCray, 2011; Jiang *et al.*, 2012). Subbaiah *et al.* (2013) generated transgenic silkworms with baculoviral resistance through multigene

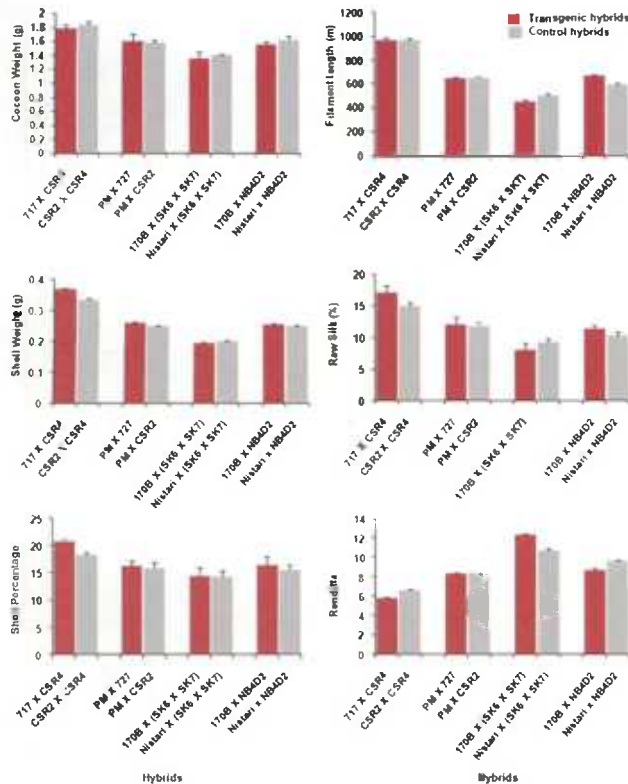


Figure 3: The cocoon and silk characteristics of the transgenic hybrids and the corresponding control hybrid: Transgenic CSR2 lines (717, 727) and transgenic Nistari line (170B) were crossed with multivoltine (Pure Mysore - PM) and bivoltine (CSR4, NB4D2, SK6 x SK7) breeds. The characters studied were cocoon weight (g), shell weight (g), shell percentage, filament length (m), raw silk (%) and renditta (Bars in black and grey represent transgenic hybrids and control hybrids, respectively).

RNA interference. The present work was an attempt to commercially exploit the benefit of transgene-mediated baculoviral suppression through varicous transgenic hybrids developed for the purpose. The hybrids displayed different levels of resistance against *BmNPV* infection, accounted for by the transgenic line as well as the breed used. The variable magnitude of resistance in the transgenic hybrids clearly indicates their superiority over the controls. The reduced viral load in the hemolymph of the infected transgenic larvae further demonstrated that the constraints on DNA replication strongly impaired virus replication. The resistance measured was mostly the consequence of the coincident inhibition of *ie1*, *lef1*, *lef3* and *p74* accumulation which impacted viral replication. It was recognized that the present application pertains to

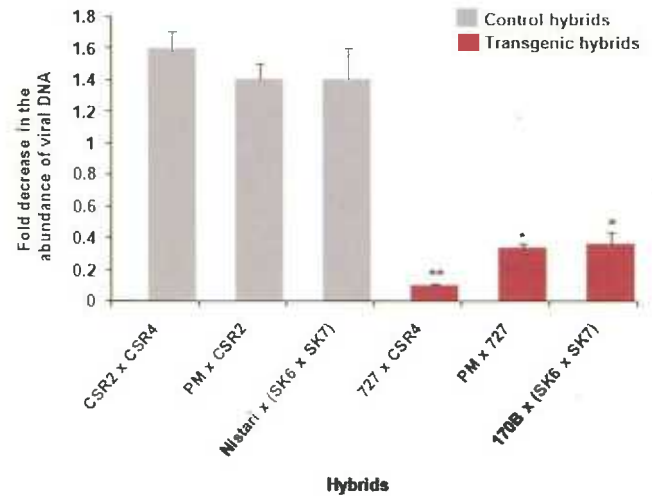


Figure 4: Quantitative PCR analysis of *BmNPV* using *lef3* as a target gene to determine the viral load in the transgenic and control hybrids infected with *BmNPV*: The results were normalized against endogenous 18S rRNA gene. Values shown are means $\pm$ S.E. * Significantly different ($P < 0.05$) between control and transgenic hybrids. ** Significant difference ($P < 0.01$) between control and transgenic hybrids.

testing of silkworms in contained conditions and does not require any open field testing. Moreover, silkworm rearing does not pose any environmental or health safety issues in view of the following: i) silkworms are always reared under contained conditions since they can't survive outdoor, ii) silkworm can't fly and it is a non-feeding stage, iii) F_1 hybrids are not reproduced further, the cocoons of which are killed to prevent emergence of moths, iv) the genetically engineered silkworms are easily identifiable with eye marker which enables easy monitoring, v) baculovirus is highly host-specific, and vi) mammals are unaffected even at a high level of baculovirus load.

This is a major achievement in silkworm improvement research where cutting edge modern biology technique clubbed with breeding strategy was used. Transgenic hybrids which exhibited their success in inhibiting viral proliferation under laboratory trials need to be further subjected to multilocalational contained trials.

ACKNOWLEDGEMENT

We dedicate this piece of work to Late Dr. Javaregowda

Nagaraju who had initiated it. This work was supported by the Centre of Excellence (CoE) grant by the Department of Biotechnology (DBT), Government of India, New Delhi. We are grateful to Dr. J. Gowrishankar, Director, CDFD, for his support. We are thankful to Dr. Vibha Ahuja, (BCIL) for providing a road map for contained multilocal, genetically engineered silkworm trials. We would like to thank the project personnel Mr. S. Subba Rao, Ms. S. Nageena and Ms. K. Usha Prasamsi for their help in the rearing and maintenance of transgenic strains.

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GENESE ET EVALUATION DES PERFORMANCES D'HYBRIDES DE VERS A SOIE TRANSGENIQUES RESISTANT AU BACULOVIRUS

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R E S U M E

Le virus de la polyèdrose nucléaire chez *Bombyx mori* (*BmNPV*) cause des pertes importantes à l'industrie de la soie entravant la production de cocons en Asie. Aucun traitement réel n'est disponible contre le virus. Dans une étude précédente, nous avons obtenu des lignées de vers à soie transgéniques qui expriment plusieurs gènes viraux essentiels (*ie1*, *lef1*, *lef3* et *p74*) de façon répétée inversée sous un promoteur d'*Actine* utilisant la technique de transformation de la lignée germinale par *piggyBac*. Les lignées transgéniques générées dans le contexte génétique Nistari montrent une résistance stable contre le virus et cette propriété antivirale a été transférée à une lignée de vers à soie commerciale à fort rendement sensible au virus, la souche CSR2, par la stratégie du croisement de retour assisté par marqueur. Dans cette étude, nous présentons l'obtention de plusieurs hybrides transgéniques par croisements des lignées transgéniques Nistari et CSR2 avec des lignées commerciales variées. Les hybrides les plus performants ont été identifiés par des essais sur le terrain dans diverses localités en mesurant le taux de survie et les caractères qualitatifs des cocons sous infection par le baculovirus. Comme le ver à soie est le premier insecte génétiquement modifié considéré pour une production à échelle commerciale en Inde, les facteurs de risque potentiel ont été pris en compte avant les essais. Dans le futur, l'application de cette technologie de transgénèse pourrait fournir des opportunités afin de réduire l'une des contraintes majeures à la productivité de la soie.

Mots-clés: Antiviral, *BmNPV*, *Bombyx mori* L., essais confinés, vers à soie transgéniques.



DEVELOPMENT OF FACIAL SERUM PRODUCT CONTAINING LIPOSOME-ENCAPSULATED SERICIN

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ABSTRACT

Sericin is a water-soluble protein obtained from the silk cocoons (*Bombyx mori*). Since sericin is reported to possess properties such as antioxidation, tyrosinase-activity inhibition and skin moisture absorption, it has been used as a cosmetic ingredient. At present, nanotechnology has extensively been applied in the field of cosmetic industry for cosmetic processing utilizing liposomes and niosomes. Generally, the liposome has been used for drug delivery system because of increasing drug efficacy and its stability. It is also to safeguard the active ingredients of the product. This study was aimed to develop a facial serum product containing liposome-encapsulated sericin. The product had gel-like texture, pale yellow color with L* a* b* values of 27.80, 0.14 and 1.29, respectively, and viscosity of 378 mP. The stability of the product was also studied using a heating-cooling cycle (45°C, 24 h; 4°C, 24 h) for 7 cycles. No change in appearance, color, viscosity and odor was observed. The clinical efficacy for increasing skin moisture and reducing skin redness and skin pigmentation was studied in 30 volunteers who were made to apply sericin serum and base serum on left or right of face (double-blind method) for 4 weeks. The use of the product did not cause any skin irritation but reduced skin inflammation, to some degree. There was no indication of increased skin moisture and reduced skin pigmentation. All the volunteers expressed satisfaction over the new product at good level of acceptance. Since the results are of a preliminary trial, it needs further investigation on quality.

Key words: Liposome-encapsulated sericin, skin moisture, skin pigmentation, skin redness.

INTRODUCTION

The use of natural ingredients for health care products and cosmetics has gained popularity over the past few years, driven by growing consumer interest in health and environment. Since consumers believe that natural products are safe for health, natural products' market has been continuously progressing. In the field of skin products development, the efficiency and stability of active ingredients are important issues. To date, the use of

some plant extracts as cosmetic substances has been limited because of low stability to air and light. To prevent these constraints, active ingredients should be encapsulated in vesicles such as liposome. They are widely known as efficient delivery system for drugs, vitamins and cosmetic substances because of properties such as biodegradability, biocompatibility, low toxicity and ability to target and slow release. In addition, liposomes can protect the encapsulated compounds from the external destructive conditions such as light, pH and

enzymes (Ruysschaert *et al.*, 2004).

Liposomes are microscopic vesicles composed of one or more concentric spheres of lipid bilayers separated by aqueous compartments. These spherical structures can be prepared in diameters ranging from 80 to 100 nm. The lipid vesicles are formed spontaneously when phospholipids are hydrated in aqueous medium. The hydrophilic substances are embedded in aqueous part of the vesicles while hydrophobic substances are intercalated into the vesicular membrane. Moreover, due to its structural similarity to phospholipid bilayer of cell membranes, liposome can penetrate into the deep layer of the skin and provide controlled release of drugs (Nii and Ishii, 2005). In Thailand, the utilization of liposomes for delivery of ingredients in cosmetics has been reported. A few examples to cite in this area are liposomes containing active ingredients such as tetrahydrocurcuminoids (GPO, 2010), lycopene (Suda, 2005), Indian gooseberry (*Phyllanthus emblica* L.) extracts (Homklob *et al.*, 2006), clove oil (Akrachalanont, 2008) and nicotinamide (Lenglah, 2012).

Silk is a fibrous protein produced by silkworms, spiders, scorpions and mites, to form their cocoons or webs. Mulberry silk (*Bombyx mori*) is one of the most widely studied, due to impressive mechanical property, good moisture absorption, softness, warmth and excellent wearability which are the advantages for manufacturing textile products. Silk fiber from *B. mori* contains two main proteins; fibroin and sericin which constitute ~70 and ~30% of the cocoon, respectively. Silk Fibroin is an insoluble fibrous protein, while sericin is a glue-like protein which holds two fibroin fibers together to form the environmentally stable cocoon structure. Sericin can be dissolved in hot water and easily degraded when dissolved in acid or alkaline solution. In presence of sericin, the fibres are hard and tough but become soft, lustrous and exhibit increased absorbance after its removal by the degumming process. After degumming, the fibres reach a ready to dye state. The sericin thus removed is usually discarded as waste in silk processing industry

Sericin consists of 18 kinds of amino acids most of which have strong polar side groups such as hydroxyl,

carboxyl and amino groups (Wei *et al.*, 2005). Sericins are known to have several excellent properties: moisturizing properties (Padamwar *et al.*, 2005) antibacterial properties, protection against UV exposure and oxidation (Dash *et al.*, 2008). These properties are highly valuable in the cosmetic and pharmaceutical products. Utilization of silk waste products from household silk industry is the potential way to increase the value of waste products and a means for extra income to farmers.

Therefore, this research was emphasized to develop a facial serum product containing liposome-encapsulated sericin. The characteristics, stability and clinical testing of the products were also investigated. This study can also serve as a guideline to develop other products from sericin silk protein.

MATERIALS AND METHODS

Preparation of liposome-encapsulated sericin

Liposomal formulation was prepared by a thin film hydration method (Prasartpornsirichoke, 2012). Firstly, the mixture of soybean lecithin and Montanov 82 in the weight ratio of 8:1 was dissolved in 30 ml diethyl ether and was put into a 1000 ml round-bottom flask. The solvent was removed by rotary evaporator under N₂ atmosphere at room temperature. After evaporation, the resulting thin film on the inner wall of flask was obtained. The flask was kept under vacuum overnight using a freeze dryer to completely remove all the traces of solvent.

For the preparation of liposome-encapsulated sericin formulation, the dried lipid film was hydrated with sericin solution followed by rotating the flask at about 300 rpm until the lipid film was completely hydrated. The concentration of sericin in water was 0.5 mg/ml. Subsequently, the liposomes were passed through an extruder using a polycarbonate membrane filter with a pore size of 200 nm.

Development of serum base for liposome-encapsulated sericin and evaluation of physical properties

The liposome-encapsulated sericin was used as an active ingredient. The other ingredients were Isononyl isononanoate, C12 alkyl benzoate, glyceryl stearate, PEG-40 stearate, PEG-100 stearate, cetostearyl alcohol, cetyl

alcohol, rice bran oil, aqua, EDTA, 1-3 butylene glycol, O-cymen-5-ol, proteoglycan, trehalose, dipotassium glycyrrhizinate, methyl paraben, propyl paraben and fragrance. The serum was then evaluated for its physical properties (color, odor, and viscosity).

Stability study of serum containing liposome-encapsulated sericin

Serum containing liposome-encapsulated sericin and serum base were kept in clear airtight glass containers and subjected to accelerated test: heating-cooling cycling method characterized by alternation of storage conditions from 45° C for 24 hours to 4° C for 24 hours (1 cycle) for 7 cycles. The physical properties *viz.*, color, odor and viscosity were monitored in every cycle.

Clinical testing in human volunteers

The clinical test in human volunteer of this study was approved by the Committee on Human Rights Related to Human Experimentation of Chulalongkorn University. Before participating in the clinical study, each subject received the information of this study and signed a written informed consent that contained all the basic elements outlined.

Facial skin test by the clinical method was performed in volunteers before and after the application of the serum. Thirty subjects were recruited from healthy volunteer groups of both the sexes of 20 years and above. Application of any moisturizer product was prohibited 1 week before starting the test. One side of the face was applied with serum containing liposome-encapsulated sericin and the other with serum base which contains the same ingredients without liposome-encapsulated sericin. Before using these serums, the testing area was cleaned with cleanser and dried with towel. The test serums were applied twice daily (once in the morning, once in the evening) for 4 weeks. The facial skin test was evaluated by Cutometer MPA 580 before and after the treatment for 2 and 4 weeks. The efficacy of test serums were assessed by clinical grading of characteristics such as skin moisture, skin pigmentation and redness. Side effects and complications if any were also assessed. Volunteers' satisfaction questionnaires were evaluated at the end of the study.

Product packaging design for commercialization

The packaging design for facial serum product

containing liposome-encapsulated sericin was developed. The design development process comprised documentary research, focus group discussion, brainstorming and field survey research. The survey research was conducted by using questionnaire as a data collecting tool. A feedback on consumers' attitude was collected from 100 consumers. Collected data were computed and analyzed in order to create appropriate packaging prototypes.

RESULTS AND DISCUSSION

Physical properties

The physical properties of serum are shown in Table 1. The serum containing liposome-encapsulated sericin had good appearance and no phase separation was observed. It was pale yellow in color with L* a* b* values of 27.80, 0.14 and 1.29, respectively. The product had gel-like texture with fragrant odor.

Table 1: Physical properties of serum containing liposome-encapsulated sericin

Physical property	Serum containing liposome-encapsulated sericin
Viscosity	378 mPa
Color	Pale yellow color L* = 27.80 a* = 0.14 b* = 1.29
Texture	Smooth and homogeneous
Odor	Fragrant

Stability

Stability test was performed to ensure that the product meets the intended physical, chemical and performance characteristics when stored under various conditions.

Figures 1-2 and Table 2 show the physical properties of serum base and serum containing liposome-encapsulated sericin before and after stability test. Freshly prepared, serum base and serum containing liposome-encapsulated sericin had good appearance with white or and light pale yellow color, respectively. After accelerated stability test, the physical properties suffered minimum alteration. The viscosity tended to increase but there was no phase separation observed. This may be due to the oxidation of

the products due to the environmental conditions such as, light, temperature, heat and quality of containers (Banks and Muir, 1985). The color of the serum was measured by colorimeter. The results were recorded in CIELAB or L* a* b* values. The color intensity was slightly increased, the degree of which was not been able to differentiate by visual examination. In addition, the variation in odor of the products was less significant. From this, it can be concluded that the serum containing liposome-encapsulated sericin had good stability.

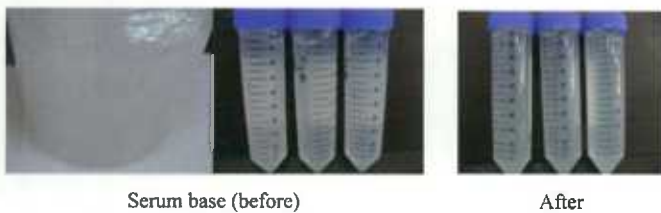


Figure 1: Serum base before and after heating-cooling cycle

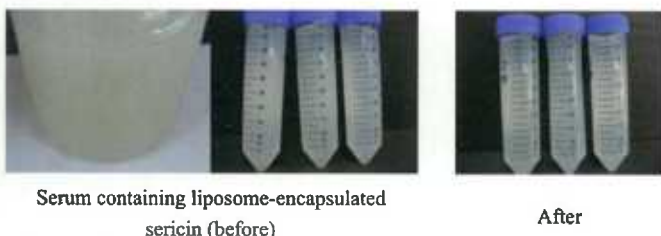


Figure 2: Serum containing liposome-encapsulated sericin before and after heating-cooling cycle

Table 2: Physical properties of the serum base and serum containing liposome-encapsulated sericin before and after heating-cooling cycle

Heating-cooling cycle	Serum base		Serum containing liposome-encapsulated sericin	
	Viscosity (mPa)	Color (L*, a*, b*)	Viscosity (mPa)	Color (L*, a*, b*)
Before testing	490.00	30.09, 0.09, 2.65	378.03	27.80, 0.14, 1.29
1 cycle	507.33	30.53, 0.12, 2.57	551.03	27.52, 0.08, 1.21
2 cycles	577.00	30.87, 0.15, 2.50	538.67	27.62, 0.10, 1.20
3 cycles	574.67	30.79, 0.08, 2.67	544.33	27.57, 0.07, 1.37
4 cycles	564.00	30.84, 0.10, 2.51	529.67	27.76, 0.10, 1.29
5 cycles	561.33	30.80, 0.07, 2.71	544.67	27.69, 0.09, 1.44
6 cycles	579.33	30.92, 0.09, 2.59	570.03	27.84, 0.12, 1.34
7 cycles	584.67	30.77, 0.02, 2.68	569.67	27.82, 0.08, 1.48

Clinical testing in human volunteers

Thirty healthy volunteers consisting of 6 males (20 %) and 24 females (80 %) with the average age of 36 were recruited. After 4 weeks of treatment, application of serum containing liposome-encapsulated sericin exhibited reduction of facial skin inflammation associated with a consequent decrease in redness value (Figure 3). As for skin moisture and skin pigmentation evaluation, the result showed no difference between the test serums before and after treatment (Figures 4-5). Hence, to compare the skin moisture and skin pigmentation properties of serum containing liposome-encapsulated sericin and serum base, the treatments should be imposed for a longer duration as well as with more number of subjects tested. No side effects such as skin irritation was reported for any of the treatment.

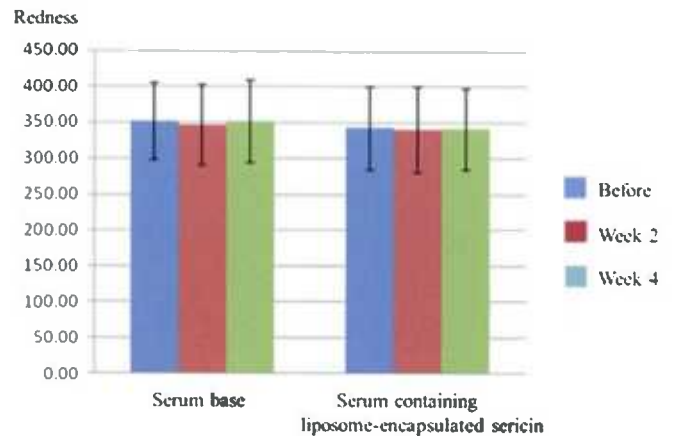


Figure 3: The redness value on the facial skin before and after 4 weeks of treatment

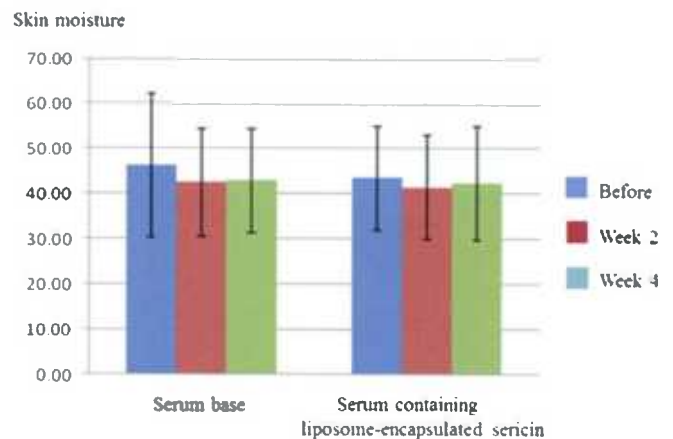


Figure 4: The skin moisture value on the facial skin before and after 4 weeks of treatment

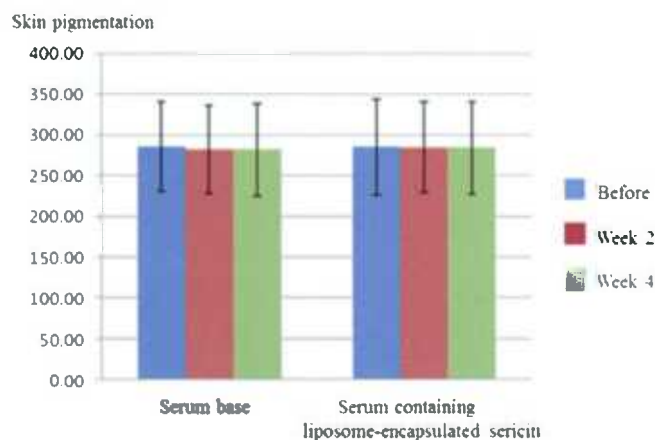


Figure 5: The skin pigmentation value on the facial skin before and after 4 weeks of treatment

The level of volunteers' satisfaction was evaluated through rating of the test serums, using a five-point 'like' scale (Table 3). The satisfaction level of test serums ranged from 'medium like' to 'like extremely' as exhibited by more than 80% for all properties such as serum texture, odor, skin absorbency, oiliness after massaging, skin moisturizing, skin firmness, skin elasticity, skin softness, skin rejuvenation and skin brightening. On the other hand, the rating of 'dislike extremely' was reported by less than 5% of consumers for all evaluated topics. Moreover, most of the volunteers expressed willingness to recommend this product to their friends.

Table 3: The rating on the use of serum containing liposome-encapsulated sericin

Property	Rating (%)				
	Like extremely	Like very much	Medium like	Like slightly	Dislike extremely
1. Serum texture	33.3	30.0	23.3	13.3	0
2. Odor	30.0	46.7	13.3	10.0	0
3. Skin absorbency	20.0	63.3	10	3.3	3.3
4. Oiliness after massaging	13.3	63.3	20.0	3.3	0
5. Skin moisturizing	30.0	56.7	6.7	6.7	0
6. Skin firmness	26.7	50.0	20.0	3.3	0
7. Skin elasticity	16.7	63.3	16.7	3.3	0
8. Skin softness	30.0	63.3	3.3	3.3	0
9. Skin rejuvenation	10.0	63.3	23.3	3.3	0
10. Skin brightening	20.0	50.0	23.3	6.7	0

Product packaging design for commercialization

Data collected from 100 consumers from the Queen Sirikit Department of Sericulture showed that consumers were mostly female, aged between 30-45 years, hold bachelor degree, work as government officer and had average income in the range of 10,001 – 20,000 Baht per month.

The major packaging factors of high impact were product labeling, unique packaging design and protective packaging measures. Evaluation of packaging style revealed satisfaction with the Japanese, the Korean and the European style.

The concept of design for packaging of facial serum product containing liposome-encapsulated sericin was derived out of the data collected from questionnaire and marketing surveys of cosmetic product packaging in the local markets. The container used for packing serum products were vacuum pump bottles with golden color. This container was not only easy and fast to use but also proved effective in product protection. With the concept of simplicity in design, the prototype packaging had light yellow color printed on white paper with the "silk cocoon" logo representing Thai silk (Figure 6). The label on the packaging composed of the ingredients and directions for use of the product. The development of an attractive packaging of cosmetics which facilitate sericin silk protein enhanced its value addition in the cosmetic market.



Figure 6: Packaging prototype for facial serum product

The results of this study supports value addition to silk production in the north-eastern regions of Thailand. However, further study should be undertaken to exclusively differentiate between the efficacies of serum containing liposome-encapsulated sericin and that of the serum base.

ACKNOWLEDGEMENT

Authors are grateful to the Queen Sirikit Department of Sericulture, Ministry of Agriculture and Cooperatives for financial support.

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DEVELOPPEMENT D'UN SERUM FACIAL CONTENANT DE LA SERICINE ENCAPSULEE DANS DES LIPOSOMES

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RESUME

La séricine est une protéine hydrosoluble obtenue des cocons de soie (*Bombyx mori*). Comme la séricine est réputée posséder des propriétés telles que anti-oxydante, inhibitrice de la tyrosinase et d'absorption de l'eau de la peau, elle a été utilisée comme ingrédient cosmétique. Aujourd'hui, la nanotechnologie a été largement appliquée dans l'industrie cosmétique en utilisant des liposomes et des néosomes. Généralement, les liposomes ont été utilisés pour la délivrance de médicaments car cela augmente l'efficacité et la stabilité du médicament. Ceci permet également de sauvegarder les principes actifs du produit. Notre étude a eu pour but de développer un sérum facial contenant de la séricine encapsulée dans des liposomes. Le produit a la consistance d'un gel, de couleur jaune pâle avec des valeurs $L^* a^* b^*$ de 27.80, 0.14 et 1.29, respectivement, et une viscosité de 378 mP. La stabilité du produit a été étudiée en utilisant un cycle chaud-froid (45°C, 24h ; 4°C, 24h) pendant 7 cycles. Aucun changement d'apparence, de couleur, de viscosité et d'odeur n'a été observé. L'efficacité clinique pour augmenter l'hydratation de la peau et réduire la rougeur et la pigmentation de la peau a été mesurée chez 30 volontaires qui ont appliqué le sérum séricine et le sérum de base sur la joue droite ou gauche (test en double aveugle) pendant 4 semaines. L'usage du produit ne cause aucune irritation de la peau mais réduit un peu l'inflammation. Nous n'avons pas constaté une augmentation de l'hydratation ni une réduction de la pigmentation de la peau. Tous les volontaires ont exprimé leur satisfaction pour le nouveau produit avec un bon niveau d'acceptation. Ces résultats étant préliminaires, des investigations supplémentaires sur sa qualité sont nécessaires.

Mots-clés: Séricine encapsulée dans des liposomes, hydratation de la peau, pigmentation de la peau, rougeur de la peau.



CHEMICAL MODIFICATION OF SILK USING METHACRYLIC ACID (MAA) AS THE MODIFYING AGENT UNDER THE INFLUENCE OF NON-PHOSPHOROUS ESTERIFICATION CATALYST AND FREE-RADICAL POLYMERIZATION CATALYST

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ABSTRACT

Studies embodied in the present work relate to chemical modification/finishing of silk fabrics using polycarboxylic acids as cross-linking agents and to replace the conventional formaldehyde based reagents and phosphorous based catalysts. The silk fabrics finished with Methacrylic acid (MAA), a non-polymerizable acid as an esterifying and cross-linking agent, were selectively compared with the untreated fabric systems in respect of functional group pattern (IR analysis) and mechanical properties viz., breaking strength, elongation at-break, tear strength, stiffness, abrasion resistance, moisture regain, wash cycle and wrinkle recovery angle. Treatment with 4.5 % MAA in presence of 0.5 % $K_2S_2O_8$ free-radical polymerization catalyst and 3 % citric acid as an esterification catalyst, followed by drying of the padded fabric at 95°C for 5 min and curing of the dried fabric at 140°C for 5 min produced the most balanced improvements in the properties such as tear strength, flexibility, tensile strength, elongation at-break, moisture regain, abrasion resistance, wash cycle and wrinkle recovery. IR analysis indicated that non-phosphorus citric acid finishing catalyst allowed esterification of MAA with tyrosine and serine amino acids of silk and the said process ultimately led to some degree of cross-linking of the chain polymers of silk.

Key words: Cross-linking of silk, esterification, finishes on silk, Methacrylic acid (MAA).

INTRODUCTION

Silk being an important environment friendly biodegradable protein fibre, is considered to be essential raw material for sustainable textiles for the future but suffers commonly from dimensional stability and wrinkle recovery which make them unsuitable and inconvenient for use in many textile applications. Technologies based on such fibre has passed through many stages of refinements and sophistications to suit newer demands and to eliminate or to improve upon some known deficiencies and additionally, to infuse efficiency in processing, enhance prospects of diverse applications and durability of such processed fibre, investigate various end-uses and also to make them more acceptable,

comfortable and attractive (Tuskda *et al.*, 2001; Cheng *et al.*, 2002; Leksophee *et al.*, 2004; Das *et al.*, 2006). For achieving wrinkle resistance, dimensional stability and other related advantages even at the cost of some strength, extensibility, abrasion resistance and moisture absorption characteristics, the finishing of silk textile is conventionally done using chiefly dimethylol dihydroxy ethylene urea (DMDHEU) resin that found applications in this kind of modification under suitable catalytic activities. Such methylolated resin finishes are associated with the disadvantages of formaldehyde splitting during processing and use, endangering the health of processors and users (Ghosh *et al.*, 2000; Ibrahim *et al.*, 2008). Use of poly(carboxylic acid) such as butane tetracarboxylic acid

(BTCA) and cyclopentane tetracarboxylic acid (CPTA) *etc.* as substitute finishing agents appears to be much more prospective in this respect in view of their environment friendly and nontoxic character. But finishes based on such non-polymeric poly(carboxylic acid) cannot retain and/or improve much the tear strength, tensile strength and moisture regain characteristics of silk fabric (Welch *et al.*, 1992; Choi *et al.*, 1994; Yang *et al.*, 1996; Shank *et al.*, 2002). Therefore, it is considered that chemical modification of proteinous fibres through a combination of (a) esterification, (b) chain polymerization and (c) ultimate cross-linking; consequent to application of a monomeric and readily polymerizable acid (Yang *et al.*, 1993; Weilian *et al.*, 2000; Choi *et al.*, 2005; Das *et al.*, 2005; Munshi *et al.*, 2014) such as Methacrylic acid (MAA) under the influence of phosphorous-free esterification catalyst and free radical polymerization catalyst by a pad-dry-cure technique over a temperature range of 95°C - 140°C may turn out to be more prospective in respect of all-round property improvement or improved property balance. Results of related studies are reported in this article.

MATERIALS AND METHODS

Materials

Fabric

Raw silk fabric with 364 end dm⁻¹ and 322 picks dm⁻¹, 50 denier warp and 50 denier weft having average area density 42 g m⁻² was used for the present study.

Chemicals

Textile grade 1, 3-dimethylol, 4, 5-dihydroxy ethylene urea (DMDHEU) obtained from Ahura Chemicals Ltd. (Ahuramine – YX), India, was used. Commercial grade Methacrylic acid (MAA) obtained from Macromols Polymer India Pvt. Ltd. was used without any treatment or purification. All other chemicals (E. Merck, India) used were of laboratory reagent grade.

Methods

Degumming of silk

To remove silk gum from the raw silk fabric, the latter was degummed at 90°C for 1.5 h in an aqueous solution

containing 6 g l⁻¹ olive oil soap and 2 g l⁻¹ sodium carbonate at fabric to liquor ratio 1: 20. Degummed fabric was washed using water at 70°C for 10 min, cold washed and finally dried in air.

Application of MAA on silk

Pre-soaking of degummed silk fabric with potassium peroxodisulphate (K₂S₂O₈) solution of concentration 0.5 % (w/v) and subsequent application of aqueous methacrylic acid (MAA) monomer formulation on the pre-soaked silk fabric were performed separately by padding technique in a laboratory two-bowl padding mangle. After two successive dipping in the aqueous MAA formulation, the fabric was passed through a pair of squeezing rollers. The rollers were adjusted to enable an overall pick-up of 100 %. The pH of the monomer solution was adjusted at different specified levels with the use of required dose soda ash and caustic soda and aqueous formulations of MAA as described above prepared using CO₂ - free distilled water. The aqueous monomer solution of MAA contained different dose levels of MAA as specified and also contained specific doses of esterification catalyst. The padded, squeezed fabrics were subjected to drying in an oven at 90°C for 5 min. The dried fabrics were then oven cured at 140°C for 5 min., soap washed following ISO-I wash method, washed further with water and dried in air. They were then analyzed for weight gain and some textile related properties.

Determination of weight gain

For the determination of weight gain upon finishing treatment using MAA, the finished fabric samples were first soap washed and then extracted under reflux in water bath for 8-10 h successively using water to ensure removal of traces of un-reacted MAA monomer along with polymeric MAA that remains unbound or un-grafted to the chain molecules of silk fabric samples. The extracted fabric samples were then oven dried to a constant weight (W₁) at 100°C. The weight gain (%) was then calculated on the basis of initial dry weight of the degummed silk (W₂), using the following relationship: Weight gain (%) = $(W_1 - W_2)/W_2 \times 100$.

Measurement of tensile properties

Breaking load and elongation at-break of some selected

fabric samples were measured in a Zwick 1445 CRT Universal Tensile Testing Machine, according to a method prescribed by IS: 1969-1968. Results obtained were based on an average of 10 tests in the warp direction of each sample. The test strip specimens were ravelled to a size of 50 mm x 20 mm between the jaws of the machine and the tests were performed with a traverse speed of 100 mm/min at a pretension of 0.5 N.

Determination of tearing strength

Tear strength measurement of unmodified/modified silk samples were done according to a method prescribed by IS: 6489-1971 in an Elmendorf Tearing Tester with a sample size of 100 mm x 63 mm. Relevant data reported in each case is an average of 10 tests.

Measurement of bending length

Fabric stiffness, as expressed by bending length, was measured as per IS: 6490-1971 in a SASMIRA Cantilever Stiffness Tester with a specimen size of 200 mm x 25 mm. Bending length measurement was done in the warp direction of the fabric and the result obtained in each case was the average of 10 tests.

Measurement of wrinkle recovery angle

The wrinkle recovery angle (warp + weft) of a selected fabric sample was measured using a SASMIRA Crease Recovery Tester in accordance with ASTM-D-1295-67 (1972) with a specimen size of 50 mm x 25 mm.

IR Spectroscopy

IR spectra of unmodified and selectively modified silk samples were obtained following KBr pellet technique as detailed elsewhere (Ghosh *et al.*, 2000) using Shimadzu IR 440 spectrophotometer. The dried fibre samples were crushed to a size finer than 20 mesh before pelletizing with KBr (each 0.3 g). Each of such test KBr pellets contained 1 % of powdered fibres.

RESULTS AND DISCUSSION

Effect of different phosphorous free esterification catalyst and pH

In view of recently reported suspected adverse effect of phosphorus based catalyst on environment,

particularly in respect of eutrophication (Choi *et al.*, 1994) caused by such phosphorus based catalyst when they are discharged into stream, application of MAA on silk was performed in presence of apparently innocuous mono, di and tri carboxylic acid in combination with $K_2S_2O_8$ with the aim to use such carboxylic acid compounds as esterification catalyst and the results are presented in Table 1. Batching after padding for such applications was done at 30°C and pH of the finished formulations containing different doses of MAA and different carboxylic compounds as the esterification catalysts mentioned above was adjusted to 7 by addition of sodium carbonate. Among the different types of esterification catalysts used in this study in combination with $K_2S_2O_8$ for MAA curing of silk, citric acid performed better commonly in terms of attainable weight gain, wrinkle recovery and retention of strength for silk fabrics. Whiteness index of silk fabrics finished in presence of $K_2S_2O_8$ and citric acid however is found somewhat less for silk fabrics for all the pH considered in this study, under otherwise comparable conditions. As a catalyst to bring about esterification reaction between free and/or silk bound MAA and -OH group of silk, efficacy of butyric acid is observed to be the least. Adipic acid however occupies an intermediate position in promoting wrinkle recovery of silk when used in combination with $K_2S_2O_8$ for MAA finishing of the fabrics. Among the different application pH studied for use of citric acid in the above system, pH 7 apparently provided the best effect in respect of attainable weight gain, wrinkle recovery, breaking load, whiteness index and retention of tear strength. Acidic pH caused substantial lowering of strength for silk commonly for all the catalysts employed. Lowering of such properties of silk fabrics (Table 1) are due apparently to hydrolysis of silk fabric under the acidic conditions employed for such studies.

Improvements in wrinkle recovery of silk fabrics finished with methacrylic acid in presence of phosphorous free esterification catalyst and free-radical polymerization catalyst are found to be low for all the types phosphorous free esterification catalyst used in the study even though such alkaline condition promoted substantial weight gain

Table 1: Methacrylic acid (MAA) finishing of degummed silk fabric: Effect of different phosphorous-free esterification catalyst and pH

Finishing catalyst		Application pH	Weight gain (%)	Wrinkle recovery angle (W+F)°		Breaking load (N/cm)	Tear strength retention (%)	Whiteness index (Hunter lab Scale)
K ₂ S ₂ O ₈	Phosphorous Free Esterification Catalyst			Dry	Wet			
+	Citric acid	6	5.84	234	252	30.23	63	70.52
+	Citric acid	7	6.72	253	276	32.14	73	71.34
+	Citric acid	8	6.31	222	238	35.36	78	71.58
+	Adipic acid	6	4.62	217	248	34.69	74	72.12
+	Adipic acid	7	5.33	235	257	38.38	82	71.97
+	Adipic acid	8	5.26	212	228	40.21	88	71.89
+	Butyric acid	6	4.21	196	211	36.23	79	72.84
+	Butyric acid	7	5.16	218	239	39.67	90	72.36
+	Butyric acid	8	5.03	208	226	42.15	94	72.09
Only Degummed Silk		-	-	175	183	44.09	100	73.45

Methacrylic acid: 4.5%, K₂S₂O₈: 0.5%, Esterification catalyst: 3%, Drying: 95°C, Curing: 140°C, '+' signs indicate presence of K₂S₂O₈ during application of MAA.

Table 2: Effect of variation of doses of Methacrylic acid (MAA) and Citric acid at pH 7 on silk fabric

Degummed silk treated with MAA (%)	Citric acid application (%)	Weight gain (%)	Wrinkle recovery angle (W+F)°		Breaking load (N/cm)	Whiteness index (Hunter lab scale)
			Dry	Wet		
0	0	-	175	183	44.09	73.45
1.5	1	2.32	198	210	41.17	72.86
3	2	3.96	222	248	37.64	72.47
4.5	3	6.72	253	276	32.14	71.34
6	4	7.88	262	278	31.33	66.39
7.5	5	8.92	264	278	30.56	61.44

Methacrylic Acid (MAA) and Citric Acid doses given are in weight per cent of silk

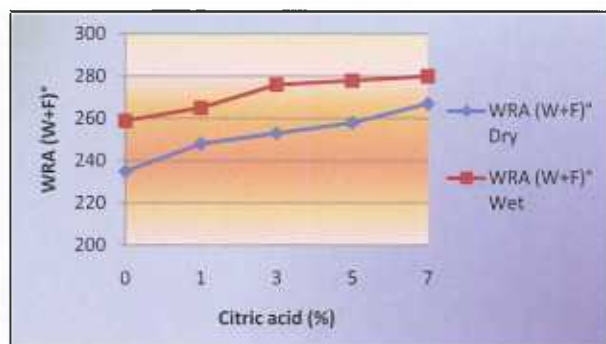
and retention of strength of the finished silk fabrics. Alkaline condition apparently could not provide favourable condition for optimal degree of polymerization and esterification which would otherwise lead to high improvement in wrinkle recovery of the finished fabrics. Whiteness index of silk on MAA treatment in presence of citric acid as the esterification catalyst however commonly suffer adversely for curing the fabrics at 140°C. The fall in whiteness index and a visual yellowing or darkening of the fabrics at marginal level is most likely to be related to commencement of fast thermal degradation of citric acid (dehydration of citric acid to the unsaturated aconitic acid [$\text{CH}(\text{COOH})=\text{C}(\text{COOH})\text{CH}_2(\text{COOH})$]).

Effect of variation of phosphorous free esterification catalyst dose level

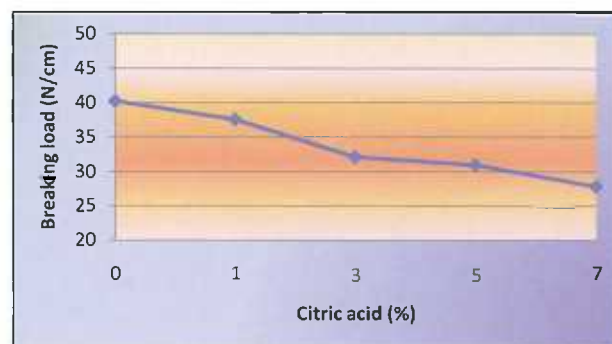
Data for 4.5 % methacrylic acid treatment on silk with the use of 0.5 % potassium peroxodisulphate as the free-radical polymerization catalyst for silk fabric and also with different doses of citric acid as the esterification catalyst are depicted in Figure 1. Higher dose of citric acid produced higher wrinkle recovery angle (Figure 1a) in view of consequent higher degree of esterification of silk fabric with carboxylic acid groups of methacrylic acid. Such improvement in wrinkle recovery angle however is observed to be marginal or low beyond a citric acid application level of 3 % for silk. A common falling trend in

Table 3: Comparison of properties of silk fabrics finished with MAA, BTCA and conventional DMDHEU resin

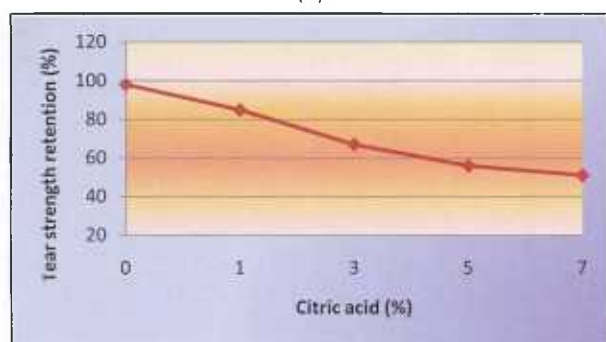
Degummed silk finished with	Wrinkle recovery angle (W+F) ^o		Breaking load retention (%)	Elongation at-break (%)	Tear strength retention (%)	Bending length (cm)	Moisture regain (%)	Whiteness index (Hunter lab scale)	Abrasion resistance (% weight loss in Martindale tester after 50 cycles)
	Dry	Wet							
None	175	183	100	16.2	100	1.4	12.19	73.45	0.51
4.5 % BTCA	250	262	70	12.4	67	1.4	13.22	71.11	0.59
4.5 % MAA	253	276	73	13.2	73	1.4	14.01	71.34	0.45
4.5 % DMDHEU	268	281	61	9.2	62	1.5	9.68	67.88	0.72



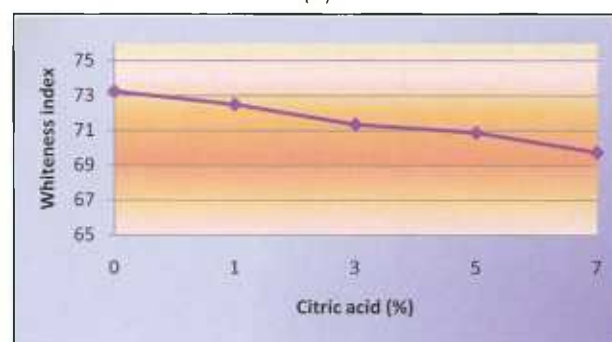
(a)



(b)



(c)



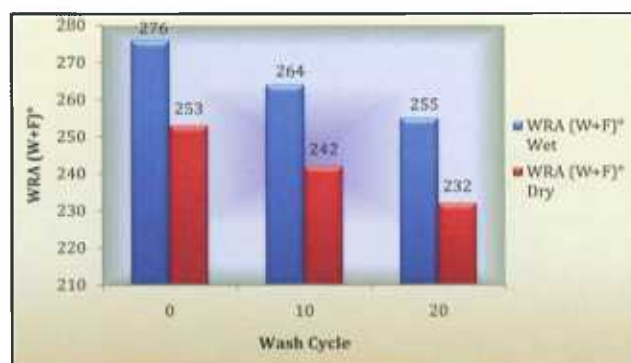
(d)

Figure 1: Effect of catalyst dose on silk fabric treated with 4.5 % MAA in presence of 0.5 % $K_2S_2O_8$

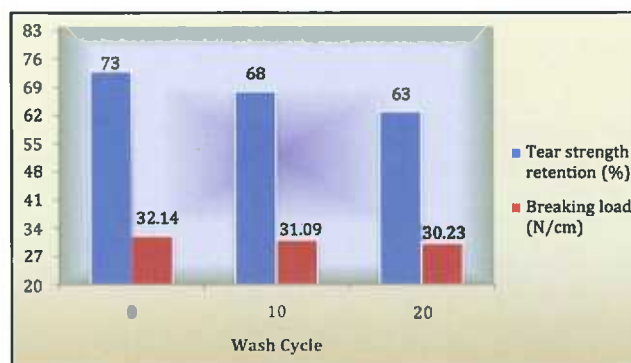
breaking load and tear strength retention (Figure 1b, c) for silk is observed with increase in dose level of citric acid. Whiteness index of silk suffers adversely (Figure 1d) for all the application levels of citric acid although the lowering of whiteness index being more beyond 3 % application level of citric acid for silk. Considering all data for the treated silk fabric (Figure 1), application of 4.5% methacrylic acid with 3 % citric acid in presence of 0.5% potassium peroxodisulphate produced optimum or the most balanced improvements in the properties.

Effect of variation of dose of MAA and citric acid at pH 7 on silk fabric

Relevant data are shown in Table 2. For this study, application dose ratio of methacrylic acid and citric acid was maintained at 1.5:1 with common use of 0.5 % potassium persulphate for silk fabric. With increasing dose level of methacrylic acid as well as citric acid by maintaining their dose ratio fixed, weight gain and property such as wrinkle recovery followed a common



(a)



(b)

Figure 2: Effect of variation of wash cycles (laundrying) on silk fabric treated with 4.5 % MAA and 3 % Citric acid in presence of 0.5 % $K_2S_2O_8$

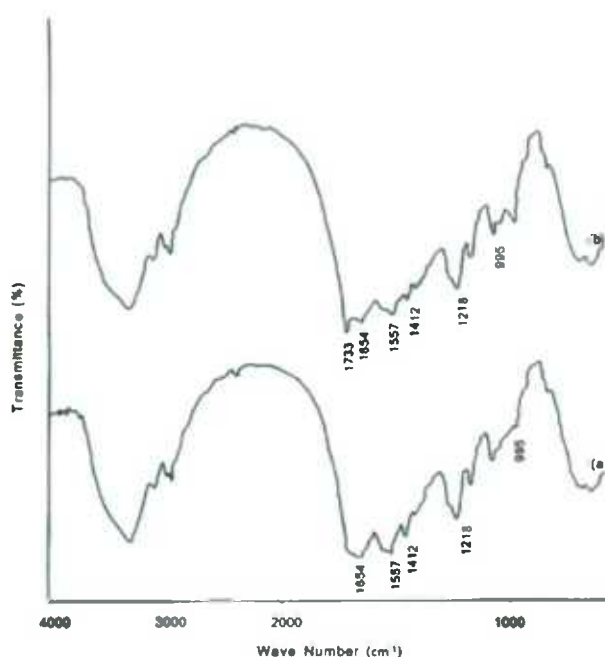


Figure 3: IR spectra of (a) unmodified silk fibre and (b) silk fibre modified with MAA using $K_2S_2O_8$ free-radical polymerization catalyst and citric acid esterification catalyst

increasing trend with a final leveling off trend in most cases at 4.5% application level of MAA for silk. Breaking load and whiteness index however follow dropping trends with increase in dose level of MAA commonly for silk fabrics. On overall assessment, 4.5 % MAA application for silk produced the most balanced effect in improving

properties of the fabrics when application of such MAA was accomplished without using phosphorous containing esterification catalyst.

Effect of progressive wash on silk

MAA finished silk fabrics were washed in launder-o-meter following ISO – I washing method and effect of such wash on wrinkle recovery and strength of finished fabrics are given in Figure 2. Figure 2a shows that the wrinkle recovery of finished fabrics suffer commonly a monotonic fall along with the increases in wash cycle. However, the difference in wrinkle recovery angle of wet/dry silk sample for 0 and 20 nos. wash cycles is not more than 21°. Tear strength retention (Figure 2b) also shows a falling trend with increase in wash cycle, however, the strength retention values of finished fabrics after 20 cycles of wash for silk appear to be $\geq 63\%$.

Comparison of properties of silk fabrics finished with MAA, BTCA and conventional DMDHEU resin

Table 3 gives data about textile related properties for i) unfinished, ii) BTCA treated, ii) MAA finished and iii) DMDHEU finished fabrics based on silk. It clearly indicates that MAA finished fabric produces much overall improvements in the fabric quality; MAA specifically and

commonly imparts higher retention of tear strength, abrasion resistance, breaking load and elongation at-break. Much higher retention of tear strength and breaking load makes the silk fabric much more durable and in this context, the conventional DMDHEU resin finish is far too inferior in comparison whereas, BTCA finished fabric occupy an intermediate position, as related results in Table 3 reveal. A good cohesive and somewhat hydrophilic film forming capacity of MAA, its polar nature and ductility of its polymer film as against relatively hard and stiff brittle nature of cross-linked DMDHEU resin make all the differences in the properties of the correspondingly treated and finished fabrics covering mechanical strength, elongation, wrinkle recovery and bending length.

IR Analysis

Infrared spectra of unmodified silk fibre [spectrum (a)] and of silk fibre modified with 4.5 % MAA using 3 % citric acid [spectrum (b)] are shown in Figure 3. A broad absorption band over $3400 - 3000\text{ cm}^{-1}$ characteristic of hydrogen bonded (N - H) stretching vibration and an absorption band at 1654 cm^{-1} characteristic of amide stretching are common to both the spectra. Two notable absorption bands at 1557 cm^{-1} and at 1412 cm^{-1} appearing in different intensities in the spectrum of unmodified silk [spectrum (a)] are characteristic of carboxylate anion stretching and phenolic (-OH) bending, respectively. Carboxylate anion stretching accounts for presence of free carboxylic acid group at the end of polypeptide chains and phenolic (-OH) accounts for presence of residues of tyrosine and serine fractions of amino acids in the unmodified silk. Strong absorption band at 1218 cm^{-1} appears also in the spectrum of unmodified silk and is attributed to (C - N) vibration of amine groups present at the end of polypeptide chains of silk. In the spectrum of unmodified silk, absorption band at 1733 cm^{-1} (ester stretching) and 995 cm^{-1} (vinyl un-saturation) are practically not existent.

MAA finish on silk under the influence of dual catalyst

(esterification catalyst + free-radical polymerization catalyst) system [spectrum (b)] results in the appearance of a strong absorption band at 1733 cm^{-1} characteristic of ester stretching and with weakening of the band corresponds to vinyl un-saturation at 995 cm^{-1} . Substantial weakening/disappearance of band at 1412 cm^{-1} corresponds to phenolic (-OH) bending due to significant disappearance of phenolic (-OH) groups. Thus it can possibly be assumed that an interaction has taken place between the carboxylate anion of MAA and phenolic (-OH) of tyrosine and serine of silk fibre. Intensification of absorption bands at 1557 cm^{-1} and 1218 cm^{-1} in the MAA modified silk as expected, are also due to the additional presence of un-reacted carboxylate (anion) groups and amino groups of MAA moieties incorporated in the silk during final stage curing induced by heat and catalytic action of citric acid.

CONCLUSIONS

Establishment of ester linkages and relevant cross-linkages in the silk fabric by pad-dry-cure technique was studied using MAA in presence of free radical polymerization catalyst potassium peroxy di-sulphate and citric acid as an esterification catalyst to produce the most balanced effects. Treatment with 4.5 % MAA and 0.5 % $\text{K}_2\text{S}_2\text{O}_8$ and 3 % citric acid at 30°C followed by drying of the treated fabric at 95°C for 5 min and curing of dried fabric at 140°C for 5 min produced optimum improvements in the properties such as wrinkle recovery and weight gain with retention of 73 % of original strength. MAA cure of silk was found to be superior to conventional DMDHEU resin cure considering improvements in resistance of breaking strength, extensibility, tear strength and flexibility for a comparable improvement in wrinkle recovery property whereas, BTCA treated silk fabric occupies an intermediate position in respect of wrinkle recovery and other property parameters. IR analysis indicated that citric acid allowed esterification of MAA with tyrosine and serine amino acids of silk; the said process ultimately led to some degree of cross-linking of chain polymers of silk.

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MODIFICATION CHIMIQUE DE LA SOIE PAR L'ACIDE METHACRYLIQUE (MAA) COMME AGENT DE MODIFICATION SOUS L'INFLUENCE D'UN CATALYSEUR D'ESTERIFICATION NON-PHOSPHORIQUE ET D'UN CATALYSEUR DE POLYMERISATION DE RADICAUX LIBRES

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R E S U M E

Les études présentées dans ce travail concernent des modifications/finitions de tissus de soie par séchage-remplissage sans utiliser une méthode conventionnelle de résine générée par méthyle ou formaldéhyde et un catalyseur non-phosphorique avec pour objectif d'atteindre un équilibre amélioré des propriétés du textile le rendant plus performant et augmentant les possibilités d'applications de diverses applications. Les tissus de soie finis avec l'acide méthacrylique (MAA), un acide non-polymérisable, comme simple agent d'estérification et de liaison, ont été comparés aux tissus non traités quant au profil de groupes fonctionnels (analyse IR), aux propriétés mécaniques comme la force de rupture, l'allongement à la rupture, la force de déchirure, la rigidité et l'angle de restauration du pli. Le traitement avec MAA à 4,5% en présence de $K_2S_2O_8$ à 0,5% catalyseur de polymérisation de radicaux libres et d'acide citrique à 3% comme catalyseur d'estérification, suivi par le séchage du tissu à 95°C pendant 5 minutes et purge du tissu sec à 140°C pendant 5 minutes produit les améliorations les plus importantes des propriétés telles que la force de déchirure, la flexibilité, l'élasticité, l'allongement à la rupture et la restauration du pli. Les analyses IR indiquent que le catalyseur de finition à l'acide citrique non phosphorique permet l'estérification de MAA avec la tyrosine et la sérine de la soie et que ce process conduit à un certain degré de liaison entre les chaînes de polymères de la soie.

Mots-clés: Liaisons de la soie, estérification, finitions sur la soie, acid acétique méthacrylique (MAA).



PILLING RESISTANCE OF ERI SILK KNITS DEVELOPED WITH ERI MILL SPUN YARN

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ABSTRACT

Pilling is a common problem mainly in the case of knitted fabrics produced from both natural, man-made and synthetic fibres and their blends. It affects fabric touch, handle and appearance. The factors which affect the pilling tendency of a fabric are the fibre properties, fabric construction parameters, dyeing and finishing parameters. The present study is aimed at the production of Eri silk knits from mill spun Eri yarn and studying the relationship between yarn properties such as count, twist, ply and pilling of these knits. Eri mill spun yarn of two counts with two twist levels was produced from Eri cocoons. Eri knits were developed with both degummed and dyed yarns on a flat bed knitting machine. It is observed that Knits produced from finer Eri spun yarns have better resistance to pilling compared to knits produced from coarser yarns. Knits produced from finer Eri spun yarns with higher twist levels have better resistance to pilling compared to knits produced from yarns with lower twist levels. Knits produced from dyed Eri spun yarns have better resistance to pilling compared to knits produced from undyed Eri spun yarns and knits dyed in fabric form. From the results, it can be inferred that Eri knits with excellent pilling resistance rating of 5 can be produced using mill spun dyed Eri yarns. Eri knits with good pilling resistance rating of 4-5 can be produced using degummed Eri yarns and dyeing the knits in fabric form. This study is an effort to build up a data base of Eri silk knits developed from mill spun Eri silk yarn and is intended to aid the silk knit designers and manufacturers in designing the right kind of knits starting from the selection of the raw material specifications and fabric structural parameters that would meet the technological as well as the economic requirements of the fabric in use.

Key words: Eri silk, flat bed knits, pilling resistance.

INTRODUCTION

Knitted fabrics are preferred for their adaptability for all types of end uses viz., casuals, sportswear, winter wear as well as formal wear. There has been a growing inclination towards natural and eco-friendly fibers and there cannot be a better answer than silk in harnessing the potential for knits to combine the luxury and comfort of silk. Silk knits provide smooth surface, pleasant feeling against the skin and suppleness owing to its light weight. The awareness about silk knitted goods has been quite less among the manufacturers and consumers in the Indian market.

Knitted fabrics produced from both natural, man-made and synthetic fibres and their blends have a common problem of fabric pilling. Pilling affects fabric touch, handle and appearance. The pilling tendency of a fabric is affected by many factors such as fibre properties, fabric construction parameters and dyeing and finishing

parameters (Elshankarkery, 2010).

Many research workers have worked on the pilling of knits produced from both natural, man-made, synthetic fibers and blends [1-13], though Eri silk knits is a new area. Senthil Kumar and Jambagi (2008) have investigated the physical and comfort properties of mulberry spun silk knitted fabrics. They observed that the spun silk knitted fabrics show fuzziness and less pilling resistance than the filament silk knitted fabrics irrespective of the knit structure. The effect of yarn properties on the pilling rates of cashmere knitted fabrics has been investigated by Long Li and Wei Zhou (2006). They observed that the dye colour is of primary importance for the pilling rates of cashmere knitted fabrics followed by yarn twist, yarn strength, fabric density etc. The relationship between the pilling rates and yarn properties has also been obtained using optimal scale regression analysis. They have also compared the pilling rates of different coloured fabrics. The multi linear relationship between fibre, yarn and fabric properties and

their effect on pilling propensity of pure wool knitted fabrics was modelled using ANN by Rafael Beltran *et al.* (2005). In another study, they have ranked the contribution of various fibre, yarn and fabric attributes to the pilling of wool knitwear. The ranking shows that fabric cover factor has the maximum effect on pilling followed by yarn count, thin places, fiber length, yarn twist *etc.* (Rafael Beltran *et al.*, 2006). The dimensional, pilling and abrasion properties of a series of plain single jersey, lacoste and fleece fabrics made from cotton ring and open end spun yarns as well as blended yarns was studied by Candan and Onal (2002). They observed that fabrics knitted with open end spun yarns have a lower propensity to pilling. The degree of pilling increases with the increase in the number of laundering cycles. The effect of washing and drying treatments on fibrillation, fuzzy and pile formation of lyocell knitted fabrics was investigated by Okubayashi and Bechtold (2005). They observed that fiber to fiber friction and decreasing water accessibility after certain number of washing and drying treatments lowers the pilling tendency. The present work is aimed at the production of Eri silk knits from mill spun Eri yarn and studying the relationship between yarn properties such as count, twist and pilling of these knits.

MATERIALS AND METHODS

Eri mill spun yarn of two counts with two twist levels was produced from Eri cocoons. The details of the yarn properties are given in Table 1.

The yarn count was 2/80^s Nm and 2/120^s Nm, the twist factor was kept at two levels and the twist levels were 360 and 460TPM for 2/80^s Nm and 440 and 560 TPM for 2/120^s Nm. The yarn evenness (Um%) varied between 10.57 to 11.35 and hairiness (H) was between 2.53 to 4.22. These yarns were dyed and Eri knits with single jersey structure were developed with both degummed and dyed yarns on a flat bed knitting machine. The fabric stitch

density and weight of fabrics produced from 2/80^s Nm and 2/120^s Nm Eri yarn are presented in Figures 1 and 2, respectively.

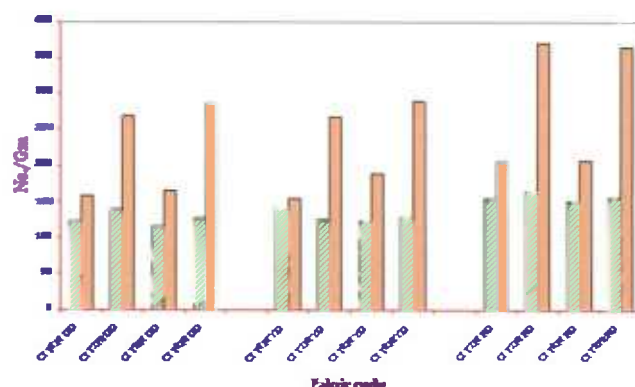


Figure 1: Fabric parameters of knits with 2/80^s yarn

C: count of the yarn, T: twist level, P: yarn ply, UD: undyed, YD: yarn dyed, FD: fabric dyed.

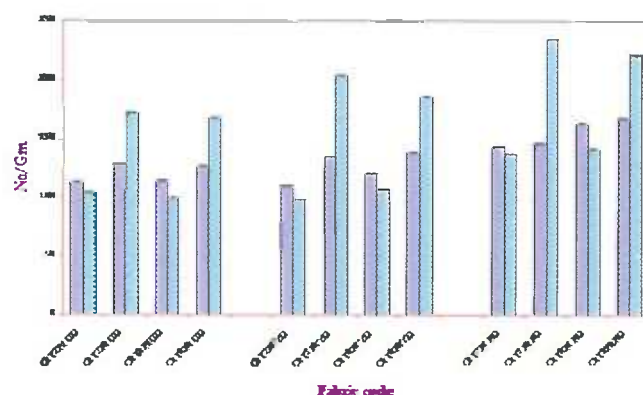


Figure 2: Fabric parameters of knits with 2/120^s yarn

C: count of the yarn, T: twist level, P: yarn ply, UD: undyed, YD: yarn dyed, FD: fabric dyed.

The stitch density varied from 114 to 164 loops/cm² in the case of knits produced from 2/80^s Nm and from 113 to 167 loops/cm² in the case of knits produced from 2/120^s Nm. The fabric weight (GSM) varied from 154 to 365 in

Table 1: Yarn properties

Yarn count (Nm)	Yarn count CV (Nm)	Twist (TPM)	Twist - CV (TPM)	Load (gf)	Elongation (%)	Um (%)	Thin places (-50%)	Thick Places (+50%)	Neps (+200%)	Hairiness (H)
2/30 ^s	4.26	360	3.47	448.67	16.31	10.57	0	9	18	4.22
2/30 ^s	6.10	460	6.06	530.45	16.15	11.18	1	23	60	2.94
2/120 ^s	3.36	440	2.67	356.47	15.63	11.35	3	10	30	3.24
2/120 ^s	2.44	560	2.67	357.27	15.25	11.29	3	9	37	2.53

the case of knits produced from $2/80^s$ Nm and from: 99 to 236 in the case of knits produced from $2/120^s$ Nm. The fabrics were subjected to pilling using the ICI Pillbox tester as per ISO 12945; Part-I test method. All the samples were subjected to pilling for standard 18,000 revolutions and the pilling rating was given after assessment of the pilled samples in comparison with the standard photographs. The pilling standard used for rating the fabrics had the following scale: 5 – no pilling, 4 – slight pilling, 3 – moderate pilling, 2 – severe pilling, 1 – very severe pilling and H- hairiness on the surface of the fabric.

RESULTS AND DISCUSSION

I. Pilling performance: The pilling performance rating for different fabric samples produced from $2/80^s$ Nm and $2/120^s$ Nm Eri yarn are shown in Figures 3 and 4, respectively.

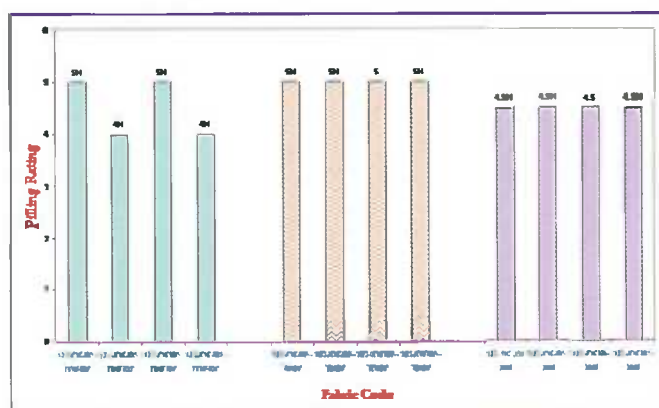


Figure 3: Pilling rating of coarse denier ($2/80^s$) undyed yarn (UDYF), yarn dyed (YDF) & fabric dyed (FD) Eri silk knits

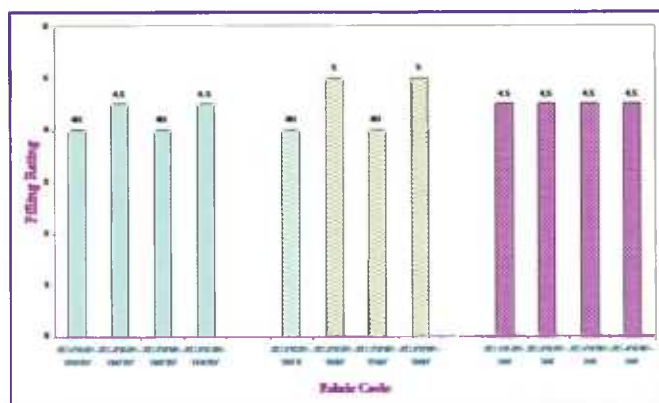
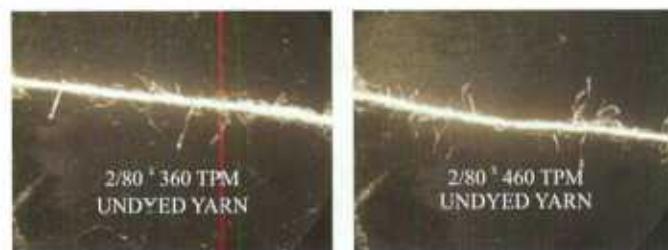


Figure 4: Pilling rating of finer denier ($2/120^s$) undyed yarn (UDYF), yarn dyed (YDF) & fabric dyed (FD) Eri silk knits

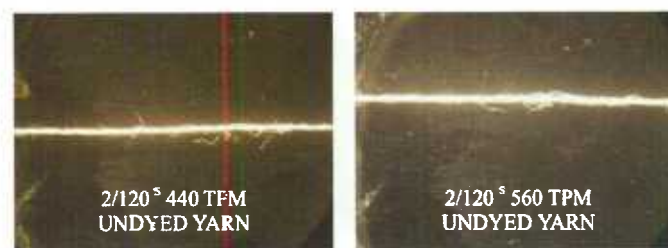
II. Relationship between yarn count, twist and ply with fabric pilling

A - Eri silk knits produced from degummed Eri yarns

Knits produced from Low twist yarns have better pilling resistance rating of 5H than high twist yarns 4H though the hairiness is lower in the case of yarn with higher twist level. This can be attributed to the higher number of neps, thick places and higher unevenness in high twist yarns compared to the low twist yarns. There is hairiness on the surface of the fabric which indicates that the yarn hairiness is reflected on the fabric surface too. Yarn ply has no influence on the pilling resistance of the knits.



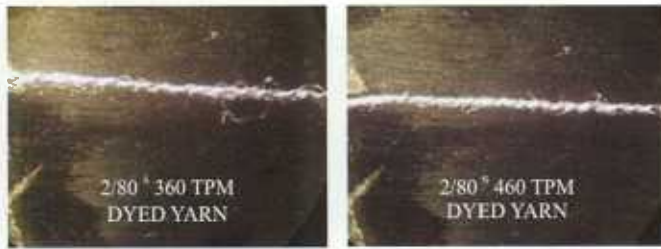
In the case of Eri silk knits produced from fine denier degummed Eri yarns, increase in twist improves the pilling resistance rating and no hairiness is observed on the surface of the fabric though it was observed on the yarn surface. Yarn ply has no influence on pilling resistance.



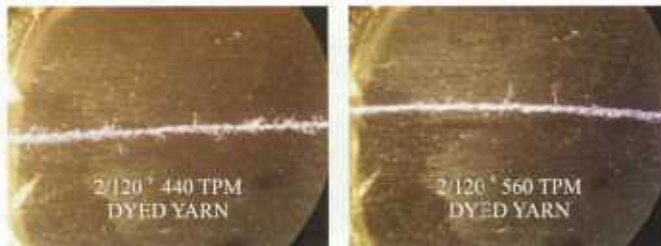
B - Eri silk knits produced from dyed yarns

In the case of Eri silk knits produced from coarse denier dyed Eri yarns, all the fabrics have good pilling resistance with a rating of 5 but there is hairiness on the fabric surface which indicates that the yarn hairiness is reflected on the fabric surface too except in the case of knits with higher twist level and with higher yarn ply, where the hairs could not be seen on the fabric surface.

Increase of Twist level and ply improves the pilling resistance of these fabrics.



In the case of Eri silk knits produced from fine denier dyed yarn, increase in twist level improves the pilling resistance from a rating of 4 with hairs on the surface of the fabrics to a rating of 5 without any visible hairs on the fabric surface. Yarn Ply has no influence on pilling resistance.



C - Fabric dyed sorts

In the case of Eri silk knits produced from coarse denier and dyed in fabric form, all the fabrics have good pilling resistance rating of 4-5 but there is hairiness on the fabric surface except in the case of knits with higher twist level and ply. Increase in Twist and ply improves the pilling resistance of these knits.

In the case of Eri silk knits produced from fine denier and dyed in fabric form, all the fabrics have good pilling resistance rating of 4-5 but there is no hairiness on the fabric surface. Twist and ply has no influence on the pilling resistance of these knits.

Conclusion

The best pilling resistance rating of 5 is exhibited by yarn dyed Eri silk knits produced from finer count with higher twist level in different plies and coarser count with higher twist level and higher ply. The rest of the knits produced from coarser denier using dyed yarn also exhibited a pilling resistance rating of 5 with different twist levels and plies but there is hairiness on the fabric surface which might lead to fabric pilling in further use.

Therefore, it can be inferred that Eri knits with excellent pilling resistance rating of 5 can be produced using mill spun dyed Eri yarns of 2/120^s Nm count with a twist level of 560 TPM in both two and three ply and 2/80^s Nm with a twist level of 460 TPM in three ply. Eri knits with good pilling resistance rating of 4-5 can be produced using degummed Eri yarns of 2/120^s count with a twist level of 440 and 560 TPM and dyeing the knits in fabric form.

This study is an effort to build up a data base of Eri silk knits developed from mill spun Eri silk yarn and is intended to aid the silk knit designers and manufacturers in designing the right kind of knits starting from the selection of the raw material specifications and fabric structural parameters that would meet the technological as well as the economic requirements of the fabric in use.

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RESISTANCE A L'EFFILOCHAGE DE MAILLES DE SOIE ERI REALISEES AVEC UN FIL DE SOIE ERI FILE AU MOULIN

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RESUME

L'effilochage est un problème courant notamment dans le cas d'étoffes tricotées produites à partir de fibres naturelles, artificielles et synthétiques et leurs mélanges. Il affecte la main, le toucher et l'apparence de l'étoffe. Les facteurs qui affectent cette tendance sont les propriétés des fibres, les paramètres de construction et les paramètres de teinture et de finition du tricot. Notre étude est consacrée à la production de mailles de soie Eri à partir de fils filés et à la relation entre les propriétés du fil comme le compte, la torsion, le pliage et l'effilochage de ces mailles. Le fil Eri filé au moulin de deux coups avec deux niveaux de torsion a été obtenu par filage de cocons Eri. Les tricots Eri ont été réalisés à partir de fils décreusés et teints sur une machine à tricoter à lit plat. Nous observons que les mailles obtenues avec le fil le plus fin montrent une meilleure résistance à l'effilochage que celles réalisées avec un fil plus gros. Les mailles obtenues avec un fil fin et la plus grande torsion ont une plus grande résistance que celles obtenues avec une torsion moindre. Les tricotages obtenus à partir de fils Eri teints montrent une meilleure résistance que ceux obtenus avec des fils non teints et les tricots teints en pièce. On peut déduire de nos résultats que des tricots de soie Eri avec une excellente résistance au pilling (5) peuvent être fabriqués en utilisant du fil de soie Eri filé au moulin décreusé et teint. On peut également obtenir de la maille avec un bon taux de résistance (4-5) en utilisant du fil décreusé et en faisant la teinture en pièce. Cette étude représente un effort pour construire une base de données des mailles de soie Eri obtenues avec du fil de soie filé à la quenouille et doit aider les stylistes du tricot et les manufacturiers en choisissant le bon type de mailles en partant de la sélection du matériel brut et des paramètres structurels du tissu qui pourrait associer les nécessités technologiques et économiques.

Mots-clés: Soie Eri, tricots à plat, résistance au pilling.



INFLUENCE OF MEDICINAL PLANT EXTRACTS ON THE GROWTH AND ECONOMIC PARAMETERS OF MULBERRY SILKWORM, *BOMBYX MORI* L.

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ABSTRACT

The *Bombyx mori* larvae (CSR 210R x CSR 280R) were fed on mulberry leaves fortified with botanicals (*Lantana camara*, *Hibiscus rosasinensis*, *Mentha spicata*, *Aloe vera*, *Centella asiatica*) and examined for their influence in silk production in relation to improvement in the larval, cocoon, shell and pupal weights, shell percentage, filament length and protein content. The mean larval weight, relative growth rate and larval consumption index of *B. mori* increased with the supplementation of *A. vera*, *H. rosasinensis*, *C. asiatica* and *M. spicata*. Pupal and cocoon weight also increased as a result of this supplementation. Maximum shell weight and shell percentage were noticed in the experimental sets treated with *A. vera*. In addition, the filament length of the cocoon shell showed significant increase over the control in response to the dietary supplementation of *A. vera*. Positive correlation was observed in growth parameters of silkworm and filament length against protein content of V instar larva. This is further confirmed through multiple regression analysis [Protein = -17756 + 21823 (cocoon weight) - 19583 (pupal weight) - 24397 (shell weight) + 1.422E-16 (shell percentage) + 12.126 (SFL) - 269.94 (larval weight)]. *A. vera*, *H. rosasinensis*, *C. asiatica* and *M. spicata* extract fortification enhanced the overall performance of *B. mori* in terms of growth and economic parameters.

Key words: *Bombyx mori*, botanicals, economic performance, fortification, growth.

INTRODUCTION

The silkworm, *Bombyx mori* L. is a monophagous insect feeding on mulberry leaves and any deficit in the quality and quantity of the feed causes imbalance in silkworm nutrition, which not only affects the silkworm physiology but also makes the larvae prone to most of the infectious diseases (Vidyasagar and Kotresha, 2003). Information on nutritional aspects of insects is a prerequisite for a better understanding of their ethnobiology and physiology. The quality of leaves provided to the worms for feeding has been considered as the prime factor governing the production of good cocoon crop (Ravikumar, 1988).

Plants are the richest source of organic chemicals on earth and phytochemicals have been reported to influence the growth and performance of different insects (Rajasekaragouda *et al.*, 1997). The prime role of silkworm nutrition on determining the silk production inspired the sericulturists to initiate studies on the fortification of mulberry leaves with some botanicals to boost the productivity of the silkworm. Administration of botanicals to silkworm help in supplementation of various biochemical constituents (amino acid, proteins, alkaloids, glucoside, phyllanthine, psoralen and betasitosterol) which have the property of phagostimulants and attractants (Manoharan, 1996). Extracts of medicinal plants have been tested as

supplement in the silkworm diet and was found to influence the body weight, silk gland weight and the silk filament length in *B. mori* (Murugan *et al.*, 1998). Leaf extracts of *Adhatoda vasica*, *Phyllanthus niruri* and *Psoralea corylifolia* when administered to silkworm exhibited phagostimulant activity and antifungal properties (Prajapathi *et al.*, 2003). Further, topical administration of *Tridax procumbens*, *Parthenium hysterophorus* and *Tribulus terrestris* increased the larval weight, effective rate of rearing (ERR), silk productivity and cocoon parameters of *B. mori* (Murugesk and Bhaskar, 2007).

Many of the earlier studies have established that fortification of mulberry with botanicals which possess variety of biochemical constituents can enrich the silkworm nutrition which in turn increase silk production, thereby boosting economic gains. In the present study, a few medicinal plants were selected to evaluate the potential of their leaf extracts on enhancing the economic parameters of the silkworm, *B. mori*.

MATERIALS AND METHODS

Sampling and preparation of extract

Disease free layings (DFL) of bivoltine mulberry silkworm (CSR 210R x CSR 280R) were collected from the Department of Sericulture, Aizawl, Mizoram, India. Leaves of *Lantana camara*, *Hibiscus rosasinensis*, *Mentha spicata*, *Aloe vera* and *Centella asiatica* were collected from Aizawl, Mizoram, India (23.7272° N, 92.7178° E) and washed under running water, shade-dried and crushed using an electrically operated grinder. One gram of ground leaf material dissolved in one litre of distilled water was filtered through linen cloth and again through Whatman No.1 filter paper. The filtrate was used as a stock solution (1 %).

Mass culture

Freshly moulted third instar silkworm larvae were

divided into six batches, including five for individual plant extract treatment and one for untreated control. The plant extracts were coated over the leaves of *Morus alba* using a brush and air-dried. Fifty IIIrd instar larvae were reared per treatment (50 larvae x 5 treatments = 250 larvae). Simultaneously, control larvae (50 larvae) were maintained without plant extract treatment.

Quantitative food utilization efficiency measures

A gravimetric technique was used to determine weight gain, food consumption, utilization efficiencies and amount of faeces produced. The weight gain of the insects, the weight of food consumed and the weight of faeces produced were determined using a monopan balance accurate to 0.1 g. After initial weighing, the larvae were introduced separately into separate containers and allowed to feed on weighed quantity of different botanical treated mulberry leaves for a period of 24 h. Larvae were again weighed. The difference in weight of the larvae gives the fresh weight gained during the period of study. Sample larvae were weighed, oven dried and reweighed to establish a percentage dry conversion value which was used to estimate the dry weight of the experimental caterpillars. The leaves remaining at the end of each day were oven dried and weighed. Aliquots of the different botanical treated mulberry leaves were weighed, oven dried and reweighed to establish per cent dry weight conversion values to allow estimation of the dry weight of the diet given to the larvae. The quantity of the food ingested was estimated by subtracting the (measured) dry weight of diet remaining at the end of each experiment from the (calculated) total dry weight of diet initially provided. Faeces were collected, weighed and then oven dried and reweighed daily to estimate the dry weight of excreta. Food consumption, relative growth rate, and post-ingestive food utilization efficiencies (all based on dry weight) were calculated in the traditional manner (Waldbauer, 1968; Slansky and Scriber, 1985). Consumption index (CI) = E/TA; relative growth rate (RGR) = P/TA; approximate digestibility (AD) = 100(E - F)/E; efficiency of conversion of ingested food (ECI) =

100 F/E; efficiency of conversion of digested food (ECD) = $100 P/(E - F)$, where A is the mean dry weight of animal during T, E is the dry weight of food eaten, F is the dry weight of faeces produced, P is the dry weight gain of insect, and T is the duration of experiment.

Economic parameters

Weight of mature larva, cocoon, pupa and shell were measured (in g) by electronic balance (Metler, Germany). Silk threads from the corresponding cocoon were carefully reeled by employing reeling apparatus and the total length was measured in meters. The insect legs were carefully cut using a sterile scissors to collect the haemolymph for determination of protein ($\mu\text{g/ml}$) by spectro-photometric method (Lowry *et al.*, 1951). Shell percentage was estimated using the formula: shell weight (g)/cocoon weight (g) x 100.

Statistical Analysis

Laboratory characteristics of studied parameters were expressed as mean $\pm$ standard error (SEM) by using Instat statistical software. All the data were analyzed with analysis of variance and the differences between the treatments were determined by Tukey's multiple range test ($P \leq 0.05$) (SAS Institute, 1988). In Tukey's test, we used the order in relation to the significance of the treatment, detected by statistical testing, and are marked with letter "a," the next lower with "b," *etc.* (Snedecor *et al.*, 1989). Correlation and regression and multiple regression analyses were performed for larval weight and larval protein content with growth and economic parameters by using SPSS software ver. 12.0.

RESULTS

Larval weight increased significantly when *B. mori* were fed with botanical extract treated mulberry leaves (Table 1). The mean weight of fifth instar larvae in respect

Table 1: Effect of leaf extracts treatment on larval weight of *Bombyx mori*

Treatment	Mean larval weight (g)	
	IV instar	V instar
<i>L. camara</i>	0.657 $\pm$ 0.05a	3.709 $\pm$ 0.21 a
<i>H. rosasinensis</i>	0.615 $\pm$ 0.01 b	3.959 $\pm$ 0.28 b
<i>M. spicata</i>	0.657 $\pm$ 0.02 a	3.869 $\pm$ 0.25 c
<i>A. vera</i>	0.664 $\pm$ 0.03 a	4.136 $\pm$ 0.32 d
<i>C. asiatica</i>	0.572 $\pm$ 0.02 c	3.660 $\pm$ 0.35 e
Untreated control	0.667 $\pm$ 0.05 a	3.279 $\pm$ 0.18 f
F _{5,17} value	21.297	2490.2
P value	< 0.0001	< 0.0001

Values show mean $\pm$ SE (N= 50) and three observations. In each column, values with the same letter are not significantly different at $P < 0.05$ level by Tukey's test.

of *A. vera* treatment was recorded the highest with 4.136 g followed by *H. rosasinensis* (3.959 g), *M. spicata* (3.869 g) and *L. camara* (3.709 g). The lowest value was recorded in *C. asiatica* (3.660 g). All the treatments were found to be superior over the untreated control.

Table 2 provides nutritional efficiency measures of *B. mori*. Higher CI (1.802, 1.793, 1.736) and RGR (0.25, 0.24, 0.24) were observed for *A. vera*, *H. rosasinensis* and *M. spicata* treatments, respectively. Higher ECI and ECD were observed for *H. rosasinensis*, *M. spicata* and *A. vera* compared to untreated control.

The cocoon weight (CW), pupal weight (PW), shell weight (SW), shell percentage, filament length (FL) and larval protein ranged between 1.362-1.510 g, 1.116-1.129g, 0.243-0.367 g, 18.01-24.59 %, 1393-1435 m and 338.33-524.77 $\mu\text{g/ml}$, respectively. It was observed that shell percentage, filament length and larval protein content increased by treatment with *A. vera*, *H. rosasinensis*, *M. spicata*, *L. camara* and *C. asiatica* when compared to untreated control (Table 3). The batch of silkworm fed on mulberry leaf treated with *A. vera* showed the highest shell weight (0.367 g), shell percentage (24.59 %), filament length (1435 m) and protein content (524.77 $\mu\text{g/ml}$). The highest cocoon weight (1.51 g) and pupal weight (1.2 g) were recorded against *L. camara* treatment. ANOVA revealed significant ($p < 0.0001$) increase in larval weight, cocoon weight, pupal weight, shell weight, shell percentage, filament length and protein content due to

Table 2: Nutritional efficiency measures of *Bombyx mori* fed on different plant extract treated mulberry leaves

Treatment	IV Instar					V Instar				
	CI (g/day)	RGR (g/day)	AD (%)	ECI (%)	ECD (%)	CI (g/day)	RGR (g/day)	AD (%)	ECI (%)	ECD (%)
<i>L. camara</i>	1.47 ± 0.47 ^b	0.22 ± 0.06 ^a	99.81 ± 1.93 ^a	14.12 ± 0.78 ^b	17.67 ± 1.56 ^b	1.626 ± 0.45 ^b	0.19 ± 0.02 ^b	99.72 ± 1.65 ^a	11.23 ± 1.25 ^b	41.63 ± 1.62 ^a
<i>H. rosasinensis</i>	1.39 ± 0.56 ^c	0.13 ± 0.04 ^b	99.80 ± 1.85 ^a	13.00 ± 0.66 ^c	17.89 ± 1.75 ^b	1.793 ± 0.62 ^a	0.24 ± 0.05 ^a	99.69 ± 1.26 ^a	12.11 ± 1.66 ^a	47.33 ± 1.65 ^a
<i>M. spicata</i>	1.47 ± 0.85 ^b	0.26 ± 0.05 ^a	99.80 ± 1.65 ^a	13.23 ± 0.58 ^c	14.34 ± 1.85 ^d	1.736 ± 0.52 ^a	0.24 ± 0.08 ^a	99.70 ± 1.75 ^a	12.56 ± 1.75 ^a	46.00 ± 1.82 ^b
<i>A. vera</i>	1.49 ± 0.26 ^b	0.23 ± 0.02 ^a	99.81 ± 1.49 ^a	15.18 ± 0.95 ^a	17.98 ± 1.46 ^b	1.802 ± 0.36 ^a	0.25 ± 0.02 ^a	99.71 ± 1.48 ^a	13.34 ± 1.55 ^a	43.93 ± 1.65 ^c
<i>C. asiatica</i>	1.22 ± 0.38 ^d	0.17 ± 0.06 ^b	99.82 ± 1.88 ^a	13.21 ± 0.75 ^c	19.35 ± 1.55 ^a	1.437 ± 0.28 ^d	0.15 ± 0.04 ^c	99.70 ± 1.35 ^a	10.23 ± 1.62 ^c	42.26 ± 1.47 ^d
Untreated control	1.71 ± 0.46 ^a	0.25 ± 0.05 ^a	99.81 ± 1.75 ^a	14.36 ± 0.48 ^b	16.37 ± 1.38 ^c	1.597 ± 0.27 ^c	0.13 ± 0.02 ^c	99.72 ± 1.44 ^a	10.89 ± 1.85 ^{bc}	42.00 ± 1.58 ^d
F value	1.85	0.59	58.16	57.30	91.35	1.820	7.81	9.061	33.16	91.46

CI = Consumption index, RGR = Relative growth rate, AD = Approximate digestibility, ECI = Efficiency of conversion of ingested food, ECD = Efficiency of conversion of digested food.

Table 3: Effects of different plant extract treatment on the economic parameters of *Bombyx mori*

Treatment	CW (g)	PW (g)	SW (g)	Shell percentage	FL (m)	Protein (µg/ml)
<i>L. camara</i>	1.510 ± 0.01 ^a	1.200 ± 0.05 ^a	0.310 ± 0.01 ^{ab}	20.54 ± 0.05 ^a	1420 ± 2.00 ^a	352.78 ± 2.54 ^a
<i>H. rosasinensis</i>	1.343 ± 0.03 ^b	1.090 ± 0.04 ^b	0.243 ± 0.03 ^{ac}	18.13 ± 0.15 ^b	1424 ± 3.60 ^a	485.87 ± 3.55 ^b
<i>M. spicata</i>	1.450 ± 0.03 ^a	1.129 ± 0.01 ^{ab}	0.323 ± 0.02 ^{abc}	22.27 ± 0.02 ^c	1433 ± 2.00 ^b	480.54 ± 2.50 ^b
<i>A. vera</i>	1.491 ± 0.01 ^a	1.119 ± 0.01 ^{bc}	0.367 ± 0.01 ^{ab}	24.59 ± 0.02 ^d	1435 ± 3.60 ^b	524.77 ± 3.02 ^a
<i>C. asiatica</i>	1.375 ± 0.02 ^b	1.074 ± 0.01 ^{bd}	0.297 ± 0.02 ^{abc}	21.60 ± 0.05 ^e	1421 ± 3.0 ^a	354.27 ± 3.03 ^c
Untreated control	1.362 ± 0.02 ^b	1.116 ± 0.01 ^{bc}	0.243 ± 0.02 ^{abd}	18.01 ± 0.18 ^b	1393 ± 3.05 ^c	338.33 ± 3.51 ^d
F Value _{5,17}	27.448	6.466	7.230	1851.7	75.003	2209.5
P Value	< 0.0001	< 0.0039	< 0.0025	< 0.0001	< 0.0001	< 0.0001

CW- cocoon weight, PW- pupal weight, SW- shell weight, FL- filament length.

Values show mean ± SE (N= 50) and three observations. In each column, values with the same letter are not significantly different at P< 0.05 level by Tukey's test.

botanical treatments (Tables 1 and 3).

Correlation and regression analysis was performed for larval protein content as independent variable (x) with dependent variables (y – cocoon weight, pupal weight, shell weight, shell percentage, filament length and larval weight). Positive correlation was obtained with cocoon weight ($y = 1.351 + 0.0001683x$; $r=0.19$; $r^2=0.03$), shell weight ($y = 0.1881 + 0.0002579x$; $r=0.44$; $r^2=0.19$), shell percentage ($y = 14.695 + 0.01457x$; $r=0.47$; $r^2=0.22$), filament length ($y = 1362.8 + 0.1378x$; $r=0.75$; $r^2=0.57$) and larval weight ($y = 2.440 + 0.003143x$; $r=0.88$; $r^2=0.77$), while negative correlation was observed for pupal weight ($y = 1.165 - 0.0001041x$; $r= -0.19$; $r^2=0.03$) (Table 4). Multiple regression analysis showed a strong correlation between botanical treatment and growth / economic

parameters. The derived multiple regression equation value for protein was $-17756 + 21823$ (cocoon weight) – 19583 (pupal weight) – 24397 (shell weight) + $1.422E-16$ (shell percentage) + 12.126 (SFL) – 269.94 (larval weight) (Table 4).

DISCUSSION

Balanced nutrition is the key for growth and development of *B. mori* and there has been significant improvement when mulberry leaves were supplemented with different nutrients (Sarker, 1993). In the present study, treatment of leaf extracts on the mulberry leaves revealed positive effects on the final instar larval weight and other economic parameters. The increase in larval weight may be due to the presence of phagostimulants of

Table 4: Correlation and multiple regression of larval weight, protein content with growth and economic parameters

Correlation and regression analysis								
Dependent variable (y)	Independent variable (x)	Regression	r	r ²	SD of residuals	SS	F _{1,5}	P-value
Cocoon weight (g)	Protein	y = 1.351 + 0.0001683x	0.196	0.0385	0.078	0.025	0.1602	< 0.0709
Pupal weight (g)		y = 1.165 - 0.0001041x	-0.198	0.0393	0.4778	0.009130	0.1635	< 0.706
Shell weight (g)		y = 0.1881 + 0.0002579x	0.445	0.198	0.04818	0.009285	0.9872	< 0.3767
Shell percentage		y = 14.695 + 0.01457x	0.477	0.227	2.490	24.810	1.180	< 0.3385
Filament length (m)		y = 1362.8 + 0.1378x	0.759	0.576	10.959	480.00	5.443	< 0.0800
Larval weight (g)		y = 2.440 + 0.003143	0.883	0.779	0.155	0.096	14.141	< 0.019
Multiple regression analysis								
Protein = -17756 + 21823 (CW) – 19583 (PW) – 24397 (SW) + 1.422E-16 (Shell percentage) + 12.126 (FL) – 269.94 (Larval weight)								
Variable	Coefficient SE	95% Confidence interval		P value	Significance			
		Lower	Higher					
Constant	-17756	2.741E-11	-17756	6.478E+14	<0.0001			
Cocoon weight	21823	2.720E-11	21823	8.024E+14	<0.0001			
Pupal weight	-19583	2.438E-11	-19583	8.032E+14	<0.0001			
Shell weight	-24397	3.026E-11	-24397	8.062E+14	<0.0001			
Shell percentage	1.422E-16	1.021E-14	-2.611E-14	0.9894	Not Significant			
Filament length	12.126	1.990E-14	12.126	6.093E+14	<0.0001			
Larval weight	-269.94	8.635E-13	-269.94	3.126E+14	<0.0001			

r = simple (multiple) linear correlation coefficient (Pearson's), r² = simple (multiple) coefficient of determination, SD = Standard deviation, SS = Sum of squares, F = value of test, P = probability).

plant extracts which might have stimulated the food intake and enhanced the digestibility. Hipparagi *et al.* (2001) recorded higher larval weight when the larvae were fed with mulberry leaves treated with *L. camara* (30%) and *Tridax procumbens* (40 %). Datta and Gupta (2002) reported that supplementation of 'Amlaki rasayan' and 'Dasmularishta' medicine resulted in the highest larval weight. The results obtained by Murugesh (2002) revealed that treatments of aqueous extracts of *Tridax procumbens*, *Tribulus terrestris* and *Parthenium hysterophorous* resulted in maximum larval weight compared to control. The economic characters of the silk cocoon were reported to be improved by feeding the silkworm with mulberry leaves treated with amino acids (Sridhar and Radha, 1986). *Phyllanthus niruri* treatment registered the longest filament followed by *Adhatoda vasica* and *Terminalia arjuna* and the shortest was registered in control (Sarithakumari *et al.*, 2011). Sridevi (2003) reported that administration of mulberry leaves fortified with *Withania somnifera*, *T. arjuna* and *Tinospora cordifolia* to silkworm recorded higher silk

filament length than control. The enrichment of mulberry leaves with calcium chloride led to increase in the cocoon characters *viz.*, cocoon weight, shell weight and shell percentage and larval protein as observed by Subburathinam and Krishna (1998). Similar results were also observed by Maribashetty *et al.* (2008) and Murari *et al.* (2008). In the present study, cocoon weight, pupal weight, shell weight, cocoon shell percentage, filament length and larval proteins were found to increase significantly over control by treatment with *L. camara*, *H. rosasinensis*, *M. spicata*, *A. vera* and *C. asiatica*. This indicates that the botanicals are also capable of influencing the economic parameters. Dietary supplementation of extracts of *Moringa oleifera* (Rajeswari and Isaiarasu, 2004) and chitosan solution (Bin Li *et al.*, 2010) elicited varied responses in final instar larvae of *B. mori*. Quantitative analysis of proteins clearly indicated that there is positive correlation between haemolymph proteins with commercial characters. Nagata and Yashitake (1989) demonstrated that haemolymph proteins which function as a specific transport media play a vital role in the growth and

development of larvae and it is variable among different breeds of silkworm *B. mori*.

Our results support the fact that the amount of soluble protein content in the haemolymph is positively correlated with growth and economic parameters of the silkworm. This fact was further confirmed with multiple regression analysis. The fortification of mulberry leaves with botanicals such as *A. vera*, *H. rosasinensis*, *M. spicata*, *L. camara* and *C. asiatica* had positive effects on silkworm (CSR 210R x CSR 280R) and may contribute to improve the economic parameters towards the benefit of silk industry.

ACKNOWLEDGEMENT

This study was supported by DBT-State Biotech Hub sponsored by Department of Biotechnology, Ministry of Science and Technology, New Delhi. The authors are thankful to Mizoram Sericulture Department, Aizawl, Mizoram and Central Silk Board, Aizawl, Mizoram for providing the larvae and rearing facilities.

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INFLUENCE D'EXTRAITS DE PLANTES MEDICINALES SUR LA CROISSANCE ET LES PARAMETRES ECONOMIQUES DU VER A SOIE DU MURIER, *BOMBYX MORI* L.

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RESUME

Les larves de *Bombyx mori* (CSR 210R X CSR 280R) ont été nourries avec des feuilles de mûrier enrichies avec des extraits de plantes (*Lantana camara*, *Hibiscus rosasinensis*, *Mentha spicata*, *Aloe vera*, *Centella asiatica*) et nous avons étudié leur influence sur la production de soie en relation avec une amélioration des poids larvaire, du cocon et de la chrysalide ainsi que de la richesse soyeuse, de la longueur du filament et du contenu en protéines. Le poids larvaire moyen, le taux de croissance relative et l'indice de consommation larvaire de *B. mori* augmentent avec l'ajout de *A. vera*, *H. rosasinensis*, *C. asiatica* and *M. spicata*. Les poids de la pupe et du cocon croissent également en raison de ce supplément. Le poids maximal de la coque et la richesse soyeuse ont été obtenus dans les lots expérimentaux traités avec *A. vera*. De plus, la longueur du filament montre un accroissement significatif par rapport au témoin chez les lots supplémentés avec *A. vera*. Une corrélation positive est observée chez les paramètres de croissance et la longueur du filament vis-à-vis du contenu en protéines des larves du Vème âge. Ceci est confirmé par l'analyse de régression multiple [Protéine = -17756 + 21823 (poids du cocon) - 19583 (poids de la pupe) - 24397 (poids de la coque) + 1,422E-16 (richesse soyeuse) + 12,126 (SFL) - 269,94 (poids larvaire)]. La fortification par des extraits de *A. vera*, *H. roseus*, *C. asiatica* et *M. spicata* augmente les performances de *B. mori* en terme de paramètres de croissance et économiques.

Mots-clés: *Bombyx mori*, extraits de plantes, performances économiques, fortification, croissance.



Research Paper

ROLE OF BACTERIA (*SERRATIA MARCESCENS* & *BACILLUS THURINGIENSIS*), VIRUSES (BmIFV & BmDNV1) AND THEIR COMBINED INFECTION IN CAUSATION OF FLACHERIE UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

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ABSTRACT

Flacherie disease in silkworm is caused by different genres of bacteria, viruses and their combined infection. The flacherie causing pathogens prevail in the entire silkworm rearing environment and premises. In the present study, the isolated bacteria (*Serratia marcescens* and *Bacillus thuringiensis*) and viruses (BmIFV and BmDNV1) were tested for their pathogenicity and combined infection in silkworm under different environmental conditions viz., optimum temperature and humidity (T1), high temperature and low humidity (T2) and fluctuating temperature and humidity (T3) for 12 h a day. It was observed that different environmental conditions influence the pathogenicity of *S. marcescens* and viruses viz., BmIFV & BmDNV1 and also combined infection of *S. marcescens* and viruses (BmIFV / BmDNV1). It caused high level of mortality under fluctuating temperature and humidity conditions (T3: 22.00 – 56.33 %) than high temperature and low humidity (T2: 3.33 – 16.33 %) and optimum temperature and humidity (T1: 0.67 – 3.33 %) conditions and also significantly higher than that due to the single infection of *S. marcescens* (T3: 18.67 – 19.67 %; T2: 3.33 – 5.33 %; T1: 0.33 %) or viruses (0.00 %) because of their synergistic association. But the influence on mortality due to *B. thuringiensis* (T1: 5.00 – 5.33 %; T2: 5.33 – 6.00 % & T3: 6.33 – 7.00 %) and combined infections with BmIFV / BmDNV1 (T1: 3.67 – 6.33 %; T2: 4.67 – 9.00 % & T3: 5.33 – 11.33 %) was not observed under the tested environmental conditions and it was due to toxicity only and not by the infection of *B. thuringiensis* or BmIFV / BmDNV1.

Key words: *Bacillus thuringiensis*, combined infection, environmental conditions, flacherie, *Serratia marcescens*.

INTRODUCTION

The success of silkworm rearing depends upon the protection of crops from diseases and parasitoids. The diseases in silkworm account for an estimated crop loss of 27 – 35 % with cocoon yield loss of 11 – 15 kg / 100 dfls (Selvakumar *et al.*, 2002). It is reported that flacherie is caused by different species of bacteria viz., *Streptococcus*, *Staphylococcus*, *Serratia*, *Micrococcus*, *Pseudomonas* and *Bacillus* (Nagae, 1977; Sam Devadas, 1991; Selvakumar *et al.*, 1999; Selvakumar, 2013), viruses viz., BmIFV and BmDNV (Shimizu, 1975; Watanabe, 1994; Selvakumar, 2013) and their mixed infection (Matsumoto *et al.*, 1985; Sivaprasad *et al.*, 2000; Selvakumar *et al.*, 2009; Selvakumar and Datta, 2013).

Under different conditions of physiological stress, the high rate of multiplication of bacteria and viruses enhances the development of flacherie. Adverse environmental conditions such as high temperature and humidity (Inoue, 1972; Miyajima, 1978), polluted air (Kanke *et al.*, 1987) and starvation (Samson *et al.*, 1981) are considered as important predisposing factors for development of flacherie and cocoon crop loss. Vijaya Kumari *et al.* (2001) reported that fourth and fifth instar silkworms are known to be sensitive to temperature and with the rise in temperature, the metabolic activities are accelerated while they slacken, along with drop in temperature. Krishnaswamy (1994) observed that late age silkworms prefer relatively lower temperature than the young age. Temperature higher or lower than 25°C acts as

a stress factor and increases the susceptibility of silkworm to viral infections (Steinhaus, 1958). If temperature and humidity are extremely high, the susceptibility of silkworm larvae increases to viral infections (Venugopala Pillai and Krishnaswamy, 1980). Kato *et al.* (1989) reported that exposure of fifth instar larvae to high temperature causes decline in their survival rate.

In the present study, the role of environmental factors on flacherie disease development by the individual and combined infection of pathogenic bacteria and viruses isolated from flacherie diseased silkworms are investigated and discussed.

MATERIALS AND METHODS

Influence of environmental factors on infection by individual pathogen

The silkworm larvae were reared till the beginning of second instar following the standard method. To study the influence of environmental factors on individual pathogen's infection, the bacteria such as *S. marcescens*, *B. thuringiensis*, viruses viz., BmIFV and BmDNV1 isolated from flacherie diseased silkworm were used as the inoculum. Different groups of 100 larvae each in three replications were inoculated *per os* individually with specific inoculum dose of specific pathogens. Based on the earlier study, the concentration (1×10^7 and 2.5×10^5 cells/ml) that caused the lowest mortality was selected with respect to *S. marcescens* and *B. thuringiensis*, respectively while for the viruses (BmIFV and BmDNV1), the infective concentration (dilutions of inoculum) that caused IC_{10} (10^{-9} & 10^{-7}), IC_{30} (10^{-8} & 10^{-6}) and IC_{50} (10^{-7} & 10^{-5}), respectively were selected.

Influence of environmental factors on infection by group of pathogens

Under combined infection, different groups of silkworm larvae were inoculated with specific bacteria (*S. marcescens* / *B. thuringiensis*) / virus (BmIFV / BmDNV1) on 1st day of second instar followed by second inoculation with specific bacteria / virus after 24 hours. The pathogens and their inoculum concentrations utilized are as following (Table 1):

Table 1: Pathogens and their inoculum concentrations

Pathogen		Inoculum concentration	
Individual infection			
<i>S. marcescens</i>		1 × 10 ⁷ cells/ml	
<i>B. thuringiensis</i>		2.5 × 10 ⁵ cells/ml	
BmIFV		Ic ₁₀ , IC ₃₀ , IC ₅₀ dosages	
BmDNV1		Ic ₁₀ , IC ₃₀ , IC ₅₀ dosages	
Combined infection			
1st day	2nd day		
<i>S. marcescens</i>	BmIFV	1 × 10 ⁷ & IC ₁₀ , 1 × 10 ⁷ & IC ₅₀	1 × 10 ⁷ & IC ₃₀
BmIFV	<i>S. marcescens</i>	IC ₁₀ & 1 × 10 ⁷ , IC ₅₀ & 1 × 10 ⁷	IC ₃₀ & 1 × 10 ⁷
<i>S. marcescens</i>	BmDNV1	1 × 10 ⁷ & IC ₁₀ , 1 × 10 ⁷ & IC ₅₀	1 × 10 ⁷ & IC ₃₀
BmDNV1	<i>S. marcescens</i>	Ic ₁₀ & 1 × 10 ⁷ , IC ₅₀ & 1 × 10 ⁷	IC ₃₀ & 1 × 10 ⁷
<i>B. thuringiensis</i>	BmIFV	2.5 × 10 ⁵ & IC ₁₀ , 2.5 × 10 ⁵ & IC ₅₀	2.5 × 10 ⁵ & IC ₃₀
BmIFV	<i>B. thuringiensis</i>	Ic ₁₀ & 2.5 × 10 ⁵ , IC ₅₀ & 2.5 × 10 ⁵	IC ₃₀ & 2.5 × 10 ⁵
<i>B. thuringiensis</i>	BmDNV1	2.5 × 10 ⁵ & IC ₁₀ , 2.5 × 10 ⁵ & IC ₅₀	2.5 × 10 ⁵ & IC ₃₀
BmDNV1	<i>B. thuringiensis</i>	Ic ₁₀ & 2.5 × 10 ⁵ , IC ₅₀ & 2.5 × 10 ⁵	IC ₃₀ & 2.5 × 10 ⁵

IC: Infective concentration

Treatments

Each silkworm group inoculated with specific concentration of specific bacteria, virus or both was subjected to the experimental treatments immediately after inoculation. To study the effect of treatment on the combined infection, first inoculation with specific concentration of bacteria / virus was followed by second inoculation (*vice versa*) after 24 hours. The experimental procedure involved the rearing of silkworm for 15 days PI (post inoculation) at different environmental conditions as treatments viz., T1 [$25 \pm 1^\circ\text{C}$ and $75 \pm 5\%$ RH (optimum temperature & humidity)], T2 [$28 \pm 1^\circ\text{C}$ and $60 \pm 5\%$ RH (high temperature & low humidity)] and T3 [25 & $28 \pm 1^\circ\text{C}$ and 75 & $60 \pm 5\%$ (fluctuating temperature and humidity) for 12 h/day].

Each treatment had three replications of 100 larvae. Normal control group was maintained separately. The larvae under each treatment and control group were

observed for a period of 15 days for disease development and the mortality due to respective pathogen (*S. marcescens* and *B. thuringiensis*) was recorded after ascertaining through Koch's postulates. In the case of BmIFV and BmDNV1, the midgut homogenate of respective dead silkworm larvae were subjected to textile dye-based dipstick immunoassay for detection of BmIFV (Nataraju and Datta, 1999) and agar gel immunodiffusion test for detection of BmDNV1 (Nataraju *et al.*, 1996). The data were compiled, analyzed (Scheffler, 1980) and discussed.

RESULTS

Influence of environmental factors on infection by individual pathogen

The mortality in silkworm due to *S. marcescens* was remarkably influenced by the fluctuating temperature and humidity conditions (T3: 18.67 - 19.67 %) when compared to constant high temperature and low humidity (T2: 3.33 - 5.33 %) and optimum temperature and humidity (T1: 0.33 %) conditions (Table 2). But the environmental influence on mortality was not observed in the case of *B.*

Table 2: Per cent mortality due to *S. marcescens*, BmIFV and BmDNV1 and by their combined infection in silkworm under different environmental conditions

Treatment	% mortality under different environmental conditions		
	25°C with 75±5% RH (T1)	28°C with 60±5% RH (T2)	25 & 28°C with 75 & 60±5% RH (T3)
1st day <i>S. marcescens</i> & 2nd day BmIFV		<i>S. marcescens</i> and BmIFV	
1×10 ⁷ + IC ₁₀	01.33 (07.42)	05.33 (13.90)	22.67 (28.76)
1×10 ⁷ + IC ₃₀	02.67 (10.15)	06.67 (15.43)	42.00 (40.68)
1×10 ⁷ + IC ₅₀	03.00 (10.71)	12.00 (20.67)	54.00 (47.58)
1st day BmIFV & 2nd day <i>S. marcescens</i>			
IC ₁₀ + 1 × 10 ⁷	00.67 (05.74)	03.33 (11.15)	22.00 (28.29)
IC ₃₀ + 1 × 10 ⁷	02.33 (09.66)	05.67 (14.30)	39.33 (39.13)
IC ₅₀ + 1 × 10 ⁷	02.67 (10.22)	11.67 (20.37)	52.33 (46.63)
<i>S. marcescens</i> 1 × 10⁷	00.33 (05.05)	03.33 (11.15)	18.67 (25.94)
BmIFV			
IC ₁₀	00.00 (04.06)	00.00 (04.06)	00.00 (04.06)
IC ₃₀	00.00 (04.06)	00.00 (04.06)	00.00 (04.06)
IC ₅₀	00.00 (04.06)	00.00 (04.06)	00.00 (04.06)
1st day <i>S. marcescens</i> & 2nd day BmDNV1		<i>S. marcescens</i> and BmIFV	
1×10 ⁷ + IC ₁₀	01.00 (06.30)	07.00 (15.83)	26.33 (31.19)
1×10 ⁷ + IC ₃₀	02.67 (10.22)	11.67 (20.37)	46.67 (43.38)
1×10 ⁷ + IC ₅₀	03.33 (11.15)	16.33 (24.21)	56.33 (48.93)
1st day BmDNV1 & 2nd day <i>S. marcescens</i>			
IC ₁₀ + 1×10 ⁷	01.33 (07.29)	06.67 (15.43)	23.67 (29.43)
IC ₃₀ + 1×10 ⁷	03.33 (11.15)	07.33 (16.16)	40.33 (39.72)
IC ₅₀ + 1×10 ⁷	03.33 (11.27)	14.33 (22.65)	54.67 (47.97)
<i>S. marcescens</i> 1 × 10⁷	00.33 (05.05)	05.33 (13.90)	19.67 (26.67)
BmDNV1			
IC ₁₀	00.00 (04.06)	00.00 (04.06)	00.00 (04.06)
IC ₃₀	00.00 (04.06)	00.00 (04.06)	00.00 (04.06)
IC ₅₀	00.00 (04.06)	00.00 (04.06)	00.00 (04.06)
F Test		S.E C.D (5%)	
Pathogens (with different viruses)		** 0.177 0.490	
Between environment		** 0.177 0.490	
Between treatment		** 0.323 0.895	
Pathogen × Environment		** 0.306 0.849	
Pathogen × Treatment		** 0.559 1.550	
Environment × Treatment		** 0.559 1.550	
Pathogen × Environment × Treatment		** 0.968 2.684	

Values in the parentheses are angular transformed (X + 0.5); ** - Significant at 1%.

Table 3: Per cent mortality due to *B. thuringiensis*, BmIFV, BmDNV1 and their combined infection in silkworm under different environmental conditions

Treatment	% mortality under different environmental conditions		
	25°C with 75±5% RH (T1)	28°C with 60±5% RH (T2)	25 & 28°C with 75 & 60±5% RH (T3)
1st day <i>B. thuringiensis</i> & 2nd day BmIFV		<i>B. thuringiensis</i> and BmIFV	
2.5 × 10 ⁵ + IC ₁₀	04.00 (12.20)	05.33 (13.90)	05.33 (13.82)
2.5 × 10 ⁵ + IC ₃₀	03.67 (11.76)	05.67 (14.30)	07.33 (16.22)
2.5 × 10 ⁵ + IC ₅₀	05.67 (14.30)	09.00 (17.94)	10.67 (19.51)
1st day BmIFV & 2nd day <i>B. thuringiensis</i>			
IC ₁₀ + 2.5 × 10 ⁵	04.33 (12.67)	05.67 (14.25)	07.33 (16.21)
IC ₃₀ + 2.5 × 10 ⁵	05.33 (13.90)	06.33 (15.03)	09.33 (17.95)
IC ₅₀ + 2.5 × 10 ⁵	06.33 (15.03)	08.33 (17.18)	11.33 (20.10)
<i>B. thuringiensis</i> 2.5 × 10⁵	05.00 (13.33)	05.33 (13.90)	07.00 (15.72)
BmIFV IC ₁₀	00.00 (04.06)	00.00 (04.06)	00.00 (04.06)
IC ₃₀	00.00 (04.06)	00.00 (04.06)	00.00 (04.06)
IC ₅₀	00.00 (04.06)	00.00 (04.06)	00.00 (04.06)
1st day <i>B. thuringiensis</i> & 2nd day BmDNV1		<i>B. thuringiensis</i> and BmDNV1	
2.5 × 10 ⁵ + IC ₁₀	03.67 (11.75)	05.33 (13.90)	07.33 (16.21)
2.5 × 10 ⁵ + IC ₃₀	06.00 (14.59)	07.67 (16.56)	09.33 (17.97)
2.5 × 10 ⁵ + IC ₅₀	06.33 (15.10)	08.00 (16.89)	09.67 (18.54)
1st day BmDNV1 & 2nd day <i>B. thuringiensis</i>			
IC ₁₀ + 2.5 × 10 ⁵	03.67 (11.76)	04.67 (13.09)	07.67 (16.56)
IC ₃₀ + 2.5 × 10 ⁵	04.33 (12.69)	07.33 (16.21)	09.33 (17.98)
IC ₅₀ + 2.5 × 10 ⁵	06.00 (14.59)	07.33 (16.21)	10.33 (19.14)
<i>B. thuringiensis</i> 2.5 × 10⁵	05.33 (13.90)	06.00 (14.59)	06.33 (15.03)
BmDNV1 IC ₁₀	00.00 (04.06)	00.00 (04.06)	00.00 (04.06)
IC ₃₀	00.00 (04.06)	00.00 (04.06)	00.00 (04.06)
IC ₅₀	00.00 (04.06)	00.00 (04.06)	00.00 (04.06)
F Test		S.E C.D (5%)	
Pathogens (with different viruses)		** 0.204 0.565	
Between environment		** 0.204 0.565	
Between treatment		** 0.372 1.356	
Pathogen × Environment		NS 0.353 0.979	
Pathogen×Treatment		** 0.645 1.787	
Environment×Treatment		NS 0.645 1.787	
Pathogen×Environment×Treatment		NS 1.117 3.095	

Values in the parentheses are angular transformed (X + 0.5); ** - Significant at 1%; NS - Non Significant

thuringiensis (T1: 5.00 – 5.33 %; T2: 5.33 – 6.00 % & T3: 6.33 – 7.00 %). Here, the mortality was reported to be due to the toxicity of *B. thuringiensis* but not due to any infection (Table 3).

The results also indicate that, the viruses, BmIFV and BmDNV1 do not cause mortality at IC₁₀, IC₃₀ & IC₅₀ dosages but infections were noticed and confirmed through immunological tests (Table 2 & 3).

Influence of environmental factors on infection by combination of pathogens

Combined infection of *S. marcescens* with BmIFV / BmDNV1

The different environmental conditions viz., T1, T2 and T3 influenced the combined infection of *S. marcescens* with BmIFV / BmDNV1 and vice versa resulting in higher level of mortality [0.67 - 3.33 % (T1), 3.33 - 16.33 % (T2) and 22.00 - 56.33 % (T3)] compared to individual infection by *S. marcescens* [0.33 % (T1), 3.33 - 5.33 % (T2) and 18.67 - 19.67 % (T3)] or BmIFV / BmDNV1 (0.00 %). The per cent mortality was also high under fluctuating temperature and humidity conditions (T3) than high temperature and low humidity (T2) and

optimum temperature and humidity (T1) conditions (Table 2).

Combined infection by *B. thuringiensis* and BmIFV / BmDNV1

The results of combined infection of *B. thuringiensis* and BmIFV / BmDNV1 are mentioned in Table 3. The different environmental conditions (T1, T2 & T3) did not influence the combined infection of *B. thuringiensis* with BmIFV / BmDNV1 and *vice versa* resulting in similar range of mortality under all the environmental conditions (T1: 3.67–6.33 %; T2: 4.67–9.00 %; T3: 5.33–11.33 %) which remained almost same as that by the individual infection by *B. thuringiensis* under different environmental conditions (T1: 5.00–5.33 %; T2: 5.33–6.00 % and T3: 6.33–7.00 %) (Table 3).

DISCUSSION

It is evident from the study that the pathogens *S. marcescens*, *B. thuringiensis*, BmIFV and BmDNV1 cause the disease individually and in combination under all the environmental conditions. The infectivity of individual pathogen viz., *S. marcescens*, BmIFV and BmDNV1 (0.00–19.67 %) as well as combined infection (*S. marcescens* with BmIFV / BmDNV1) was comparatively higher at fluctuating temperature and humidity (T3: 22.00–56.33 %) than high temperature & low humidity (T2: 3.33–16.33 %) and optimum temperature & humidity (T1: 0.67–3.33 %) conditions. Similar observation of low pathogenicity of *S. faecalis* (Selvakumar and Datta, 2013; Nataraju *et al.*, 1999), *S. aureus* (Nataraju *et al.*, 1999; Selvakumar *et al.*, 2009) and *S. marcescens* (Vasantharajan and Munirathnamma, 1978) under optimum environmental conditions and increased pathogenicity of *Streptococcus*, *Staphylococcus* sp. and *S. marcescens* under fluctuating temperature and humidity (Sivaprasad *et al.*, 2000) have been reported. The mortality was also comparatively higher at constant high temperature and low humidity (T2) than at optimum temperature and humidity (T1). But in the case of *B. thuringiensis*, the larval mortality in silkworm caused by individual or combined infection along with BmIFV / BmDNV1 was only due to toxicity of endotoxin (δ) and not due to infection. The variations in

mortality was also not significant under different environmental conditions tested (T1, T2 & T3).

The results also indicate the influence of environmental factors on the combined infection of bacteria (*S. marcescens*) and viruses (BmIFV / BmDNV1). Among the combined infections, the influence of fluctuating temperature and humidity (T3) was comparatively higher than constant high temperature & low humidity (T2) and optimum temperature & humidity (T1) conditions and also the combination involving *S. marcescens* and BmIFV had more impact than BmDNV1. It may be possible as reported by the earlier workers that high temperature may weaken the larvae resulting in their high susceptibility (Miyajima, 1978; Venugopala Pillai and Krishnaswamy, 1980; Kobayashi *et al.*, 1981). Inoue (1972) also reported that the multiplication of flacherie virus was less at 21°C and high at 31°C in the silkworm, *B. mori*. Vijaya Kumari *et al.* (2001) reported that minimum incidence of flacherie (0.00–3.31 %) was observed under the optimum temperature of 25°C & humidity of 70±5% whereas, higher incidence of flacherie of about 19.70 & 31.80 % was noticed under high temperature of 30°C & 8±5 % RH and 35°C & 50±5 % RH, respectively.

Under the fluctuating temperature and humidity (T3) conditions, the mortality caused by the combined infection of bacteria and viruses was more than twice (22.00–56.33%) than that of individual infection (18.67–19.67 %) by bacteria under similar conditions due to their synergistic association. The viruses, BmIFV and BmDNV1 at their infective concentrations did not cause mortality during the observation period of 15 days post inoculation. Ayuzawa *et al.* (1968) have also reported that *S. faecalis* alone caused the mortality of 0.00–20.00 % but with BmIFV, the mortality was increased and it ranged from 60.00–100.00 %. Govindan *et al.* (1990) reported simultaneous *per os* infection in fourth instar larvae of silkworm with kenchu virus (BmDNV2) and *S. aureus* resulting in higher mortality as well as deterioration in the quantitative traits of cocoon as compared to the infection with kenchu virus or bacterium alone. Sivaprasad *et al.* (2000) reported that *Streptococcus* and *Staphylococcus* sp. caused the mortality of 44.67 and 9.83 %, respectively and along with BmIFV, the mortality was increased and ranged from 50.00–84.67 % and 44.66–65.66 %, respectively under the fluctuating temperature and humidity

conditions. Since the bacteria and viruses (*S. marcescens*, *B. thuringiensis*, BmIFV and BmDNV1) cause mortality by their individual / combined infections, it is suggested that silkworm rearing environment should not be subjected to fluctuating temperature and humidity and high temperature and low humidity conditions.

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ROLE DE BACTERIES (*SERRATIA MARCESCENS* ET *BACILLUS THURINGIENSIS*) ET DE VIRUS (BmIFV et BmDNV1) AINSI QUE DE LEUR INFECTION COMBINEE DANS L'APPARITION DE LA FLACHERIE DANS DIFFERENTES CONDITIONS ENVIRONNEMENTALES

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RESUME

La flacherie chez le ver à soie est causée par différentes bactéries ou virus et par leur action combinée. Les pathogènes responsables de la flacherie sont présents dans tous les environnements et locaux des élevages de vers à soie. Dans cette étude, nous avons testé la capacité pathogène de bactéries (*Serratia marcescens* et *Bacillus thuringiensis*) et de virus (BmIFV et BmDNV1) ainsi que leur infection combinée chez le ver à soie dans différentes conditions environnementales comme une température et une humidité optimales (T1), une forte température et une faible humidité (T2) et une température et une humidité variables (T3) 12 heures par jour. Nous avons observé que les conditions environnementales influencent le pouvoir pathogène de *S. marcescens* et des virus BmIFV et BmDNV1 ainsi que celui de l'infection combinée de *S. marcescens* et des virus (BmIFV/BmDNV1). On observe un niveau de mortalité plus élevé dans les conditions fluctuantes (T3 : 22,00 – 56,33 % ; que sous T2 (3,33 – 16,33 %) et T1 (0,67 – 3,33 %) et significativement plus élevée que celle due à l'infection par *S. marcescens* seule (T3 : 18,67 – 19,67 % ; T2 : 3,33 – 5,33 % ; T1 : 0,33 %) ou les virus (0,00 %) en raison d'un effet synergétique. L'influence de *B. thuringiensis* (T1 : 5,00 – 5,33 % ; T2 : 5,33 – 6,00 % et T3 : 6,33 – 7,00 %) et de l'infection combinée avec BmIFV/BmDNV1 (T1 : 3,67 – 6,33 % ; T2 : 4,67 – 9,00 % et T3 : 5,33 – 11,33 %) sur la mortalité n'a pas été observée dans les différentes conditions ceci étant dû à la toxicité et non à l'infection par *B. thuringiensis* ou par BmIFV/BmDNV1.

Mots-clés: *Bacillus thuringiensis*, infection combinée, conditions environnementales, flacherie, *Serratia marcescens*.

VISITS OF DELEGATIONS



A two member delegation from South Korea headed by Ms. Hee Kyoung oh, The elderly, Silk, Fashion and Pattern CAD / Researcher, The Research Institute of Nursing Science, Seoul National University,

Nc. 313, 2nd Building College of Nursing, 103 Daehak-ro, Jongno-Gu, Seoul 110799, Korea visited the ISC HQs on 23rd January, 2015. The delegation had a detailed discussion with the ISC regarding the status of sericulture industry in India.



A three member delegation from University Technology of Malaysia comprising Dr. Arieff Salleh bin

Rosman. Director, Dr. Farahwahida bt Mohd Yusof, Assistant Director from Center of Research for Fiqh Science & Technology (CFIRST), University Technology of Malaysia and Dr. Dzaraini Kamarun, Associate Professor, Faculty of Applied Science, University Technology of MARA visited ISC HQs on 19th January, 2015. The delegation visited India to study on the development of silk and silk industry in India.



A four member delegation from Japan headed by Mr. Ejima, Head, JICA India office, New Delhi visited the office of International Sericultural Commission, Bangalore on 14th January, 2015. The other three Japanese members are

assigned to work in India as volunteers for a two year term. They

would help the sericulture farmers in Ramanagara (Karnataka), Hosur (Tamilnadu) and Hindupur (Andhra Pradesh).

SPECIAL TRAINING ON SERICULTURE AND SILK INDUSTRY

A special training on sericulture and silk industry organised for the candidates nominated from ISC Member Countries is scheduled to be held at Central Sericultural Research & Training Institute, Mysore from 9th March to 22nd April, 2015. This programme is sponsored by the Ministry of External Affairs, Govt. of India under the Indian Technical Economic Cooperation (ITEC). For more details of the ITEC programme, please visit; www.itec.mea.gov.in

Under the programme, the sponsored candidate shall be provided with return air journey in economy/excursion class, living allowance, accommodation in the place of training in Hotel/Hostel, project allowance, study tour, etc. The cost of organizing the training shall also be met under the programme.

The programme will be of 45 days (6 weeks) duration and the batch strength will be 20 persons from 7 countries. The training programmes will be conducted by covering following subjects.

- Silkworm rearing (Young age and Late age silkworm rearing),
- Mulberry cultivation technologies,
- Silkworm seed production technology,
- Extension management and transfer of technology, and
- Post cocoon technology.

The above subjects will be covered by undertaking theoretical classes in class rooms and practical orientation classes in the institute facilities and among the sericulture farmers. One silkworm rearing lasting one month will be conducted wherein, the trainees themselves will take part in rearing the silkworm to acquaint with the practical aspects of silkworm rearing. Practical sessions will also be arranged on other different disciplines of sericulture in the respective facilities of the Institute. The trainees will also be exposed to the commercial aspects of the sericulture enterprise practiced in the country through regular field visits. This would enable the trainees to replicate the successful economic model of sericulture enterprise practiced in India in their respective countries. Special focus would be given for highlighting the merits of silk industry like; poverty alleviation, employment generation, women empowerment and rural development.



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