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MOLECULAR MECHANISM OF DENSOVIRUS RESISTANCE IN SILKWORM, *BOMBYX MORI*

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ABSTRACT

Sericulture farmers are often challenged with the situation of disease incidence during the process of silkworm rearing and the consequent cocoon crop losses. Among the known infectious diseases of silkworm, flacherie accounts for substantial crop loss in India due to fluctuating temperature and humidity conditions as well as inferior quality of mulberry leaf. Studies revealed that, *Bombyx mori* densovirus type 2 causes destructive damage during rearing and is reported to be a serious problem with the infected susceptible larvae becoming flaccid, developing diarrhoea and finally die within seven days. BmDNV2 was detected in Indian *B. mori* samples which included productive hybrids such as CSR2 x CSR4 and PM x CSR2 widely reared by sericulturists under Indian conditions, many of them being detected with BmNPV co-infection. The Indian BmDNV2 isolate revealed maximum homology with the Yamanashi DNV2 isolate from Japan and transmission electron microscopy studies on midgut tissues of the Indian isolate of *B. mori* silkworm infected with BmDNV-2 revealed hypertrophy of columnar cell nuclei. An unlinked mutation nsd-2 was discovered to control non-susceptibility to BmDNV2 in *B. mori*. Most of the Indian multivoltine as well as bivoltine silkworm germplasm races harboured either gene for susceptibility or genes for both resistance and susceptibility to DNV2 indicating a heterozygous susceptible condition. Selection of parental stock of silkworm breeds possessing the gene for resistance to BmDNV2 could be a prospective approach towards controlling flacherie disease in the field without compromising on cocoon yield. This review provides a consolidated account on the scientific findings of studies on BmDNV-2 and its molecular pathogenesis in *Bombyx mori*.

Key words: *Bombyx mori*, densovirus, disease resistance, pathogenesis.

INTRODUCTION

Densoviruses (DNVs) are small icosahedral, non-enveloped single strand DNA viruses mostly infecting invertebrates. They were first discovered during the early 1960s in diseased greater wax moth, *Galleria mellonella* (Meynadier *et al.*, 1964; Vago *et al.*, 1964). The histological sections of infected larval tissue displayed enlarged, strongly Feulgen-positive nuclei with heavy virus particles and from this originated the name, 'densonucleosis'. Thousands of small isometric viral

particles were produced in these nuclei (Amargier, 1966). The viral particles purified from the dead larvae were observed as 6 kb long single-stranded linear DNA molecule with the capsid made up of four proteins. Since the virus indicated sharing of the nuclear site of replication as well as the structural and biochemical features of vertebrate parvoviruses, it was accordingly classified as a new genus *viz.*, Densovirus within the Parvoviridae family. Later, several DNVs have been isolated from different insect orders *viz.*, Lepidoptera, Hemiptera, Orthoptera, Diptera and Dictyoptera as well as Crustacea.

As on date, 30 DNVs have been identified and 20 different DNV genomes cloned and completely sequenced revealing significant information on the biology of DNVs at the molecular level as well as the diversity of their organization and genomic structure. Both invertebrate and vertebrate parvoviruses recorded highly conserved sequence motifs indicating common ancestral origin.

Most DNVs are responsible for fatal diseases in insects, mostly infecting the larval stages, although pupal or imaginal stages are also infected in natural populations or due to experimental contamination (Boemare and Bres, 1969; Suto *et al.*, 1979). In most Lepidoptera, DNV infections cause symptoms like anorexia and lethargy followed by flaccidity and inhibition of moulting or metamorphosis with the larvae progressively getting paralyzed leading to death in 2-20 days depending on the type of isolate, larval stage, viral concentration and method of infection *i.e.*, natural or induced (Vago *et al.*, 1966; Kelly *et al.*, 1980; Chao *et al.*, 1985). Members of the Densovirus genus have a 6 kb long genome with about >500 nt long inverted terminal repeats (ITRs) (Dumas *et al.*, 1992; Tijssen *et al.*, 2003; Fediere *et al.*, 2004). Thus an ambisense genome organization is exhibited with the sequences encoding non-structural and viral proteins located on the complementary strands in the 5' half. During viral morphogenesis, both the strands are encapsidated separately in equimolecular ratio. Infection of parvovirus initiates through capsid-mediated binding to one or more glycosylated receptor molecule on the host midgut cell surface followed by virion uptake into the cell via receptor-mediated endocytosis. Then the virion is transferred across the delimiting lipid bilayer of the entry vesicle into the host cell cytoplasm by a capsid-borne phospholipase, followed by entry into the nucleus, where the viral genome is finally released from its protective shell. As in the case of most of the vertebrate parvoviruses, DNV virions are very resistant to extreme environmental conditions which included a wide range of pH and temperature, especially in an unpurified state (dead larvae). However, as a purified virus suspension or in dry state, the infectivity tends to decrease rapidly (Boemare *et al.*, 1970; Odier and Boemare, 1972).

The mulberry silkworm, *Bombyx mori* is of great value as a model for genetics as well as molecular biology research. *B. mori* is susceptible to a number of commercially important infectious diseases caused by protozoa, fungi, bacteria as well as viruses leading to crop loss, adversely affecting silk production. Research aimed at better understanding of the etiology, epizootiology, prevention and control aspects in respect of the various infectious silkworm diseases is the key for increasing silk productivity. Most cases of silkworm cocoon crop failures recorded at farmers' level are the direct result of disease outbreak during silkworm rearing. However, the silkworm has an efficient immune system to combat invading microbial pathogens including viruses. Among the known infectious diseases of silkworm, flacherie accounts for considerable degree of crop loss in India. Fluctuating temperature and humidity conditions as well as poor quality of mulberry leaf are the main predisposing factors of the disease. The symptoms of flacherie are atypical in the early stage and at the later stage of infection, larvae appear soft and flaccid with retarded growth and translucent cephalothoracic region. Flacherie is a syndrome caused by viruses as well as bacteria. The viruses associated with flacherie are cytoplasmic polyhedrosis virus (CPV), infectious flacherie virus (IFV) and denonucleosis virus (DNV). In general, resistance to viral diseases in silkworms is predominantly controlled by polygenes. IFV and CPV are RNA viruses, while, DNVs are single stranded DNA viruses. In CPV, the RNA is a segmented double strand, while, in IFV, it is a positive single strand, while, in DNV, the DNA is single stranded. This review is an attempt to consolidate the studies on BmDNV-2 and its molecular pathogenesis in *Bombyx mori*.

***Bombyx mori* densovirus (BmDNV)**

In 1968, disease caused by a parvovirus was discovered in the silkworm, *B. mori* from the sericultural farms in Ina City of Japan. The causative agent of this disease was determined to be a *Bombyx* Densovirus (BmDNV) based on the cytopathological, chemical and physical

characteristics. BmDNV has a diameter of roughly 22 nm and protein analysis showed that the virion possesses four structural proteins (VP1, VP2, VP3, and VP4). The DNA of BmDNV is composed of a linear, single-stranded molecule with a molecular weight of about 1.7×10^6 , and two complementary strands are contained in different particles of the DNV. DNA synthesis occurs predominantly in infected nuclei of columnar cells of the silkworm midgut, indicating that the virus multiplies in the nucleus. The silkworm larvae infected with the BmDNV usually die after seven days showing body flaccidity as a major sign. The alimentary canal of the diseased larvae appears pale yellow with little internal content. BmDNVs multiply only in the nuclei of the columnar cells of the larval midgut epithelium (Seki and Iwashita, 1983). To date, three major isolates of BmDNVs have been reported: BmDNV-1 (Ina isolate), BmDNV-2 (Saku isolate and Yamanashi isolate), and BmDNV-Z (Zhenjiang isolate) (Tijssen and Bergoin, 1995). Based on their genomic characteristics, BmDNVs are classified into two major types, type I (BmDNV-1) and type II [BmDNV-2]. The genome of BmDNV-1, contains single-stranded complementary DNA molecules that are about 5 kb in length (Bando *et al.*, 1990), while, that of BmDNV - 2 possesses a split genome comprising of two types of single-stranded linear DNA molecules. BmDNV-2 includes the Yamanashi and Zhenjiang isolates (BmDNV-Z) containing two ssDNA molecules, which are 6.0 and 6.5 kb in length (Bando *et al.*, 1992; 1995; Wang *et al.*, 2006) (Table 1).

Table 1: Details of different types of BmDNV

Character	BmDNV-1	BmDNV-2	BmDNV-Z
Virion size	20 nm	24 nm	24 nm
Genome topology	Linear	Linear	Linear
Genome molecule	ssDNA	Segmented ssDNA	Segmented ssDNA
Genome size	5.0 kb	6.0 kb, 6.5 kb	6.0 kb, 6.5 kb
Target tissue	Midgut columnar cell	Midgut columnar cell	Midgut columnar cell & cup shaped cells of midgut columnar cell during late stage of infection
No. of genes	4	6	7

According to the previous classification, BmDNV-1, -2, and -Z had been assigned into Densovirinae of the Parvoviridae. BmDNV-1 was classified as a genus Iteravirus, while BmDNV-2 and BmDNV -Z were classified into a genus Bidsenovirus. However, BmDNV-2 and Z were later excluded from the family Parvoviridae because of their genome organization and presence of a DNA polymerase motif in their own genomes (Tattersall *et al.*, 2005). The present article emphasizes a review on the work carried out with respect to BmDNV-2.

Bombyx mori densovirus type 2 (BmDNV-2)

Mechanism of infection

BmDNV-2 causes destructive damage to *B. mori* during rearing and is reported to be a serious problem. The infected susceptible larvae become flaccid, develop diarrhoea and finally die within seven days (Ito, 2008; Ito *et al.*, 2008). The DNA strands of the virus are of two different lengths (6.6 kb and 6.1 kb). BmDNV-2 does not replicate with the self priming and hairpin transfer mechanism as in the case of BmDNV-1 (Hayakawa *et al.*, 1997; Wang *et al.*, 2007). The two viral DNAs of BmDNV-2 have been reported to increase logarithmically with time reaching maximum level at 48 h post infection and maintaining it until 102 h. The level decreased during ecdysis indicating dropping out of the midgut epithelium. Since both DNAs were similar in multiplication, each of them can be used as template DNA for detection of BmDNV-2 by PCR. In general, the replication of 6.1 kb DNA was more compared to that of 6.6 kb suggesting independent replications (Nakagaki *et al.*, 1999). A mutual interference has been reported between DNV1 and DNV2 in the infection of *B. mori* at cellular as well as organismal levels on simultaneous infection (Abe and Watanabe, 1987).

Histopathological features

Histopathological studies on the midgut epithelium of the silkworm infected with BmDNV-2 showed that nuclei of the columnar cells hypertrophied and the degenerated columnar cells were liberated into midgut lumen (Figure 1) (Watanabe *et al.*, 1976; Seki and Iwashita, 1983; Wang

et al., 2007). Two non-viral polypeptides (45.5 and 44 K) were found to accumulate in the virus-infected degenerate cells of *B. mori* midgut epithelium which were first temporarily detected at 3 days post-infection (pi) and then persistently at around 8 days pi onwards, the amount being inversely correlated to that of BmDNV-2 structural polypeptides. TUNEL staining showed that the non-viral polypeptides are involved in the clearance of the cells from BmDNV-2-infected midgut epithelium damaged by virus multiplication through a mechanism different from apoptosis induction leading to restricted accumulation of viruses in the BmDNV-2-infected midgut epithelium (Hisaya *et al.*, 2005).

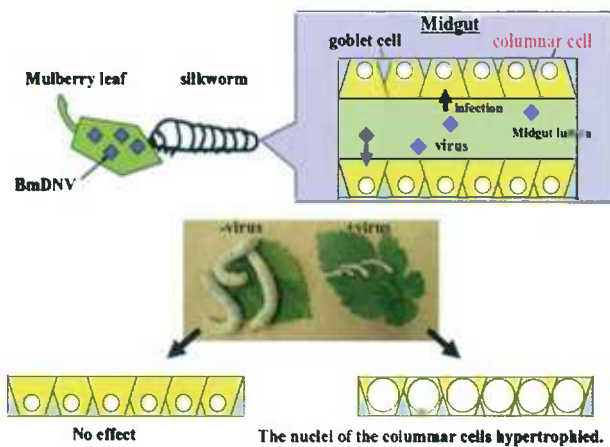


Figure 1: Pictorial representation of BmDNV-2 infected silkworm midgut epithelium (Diagram courtesy: Ito, 2008)

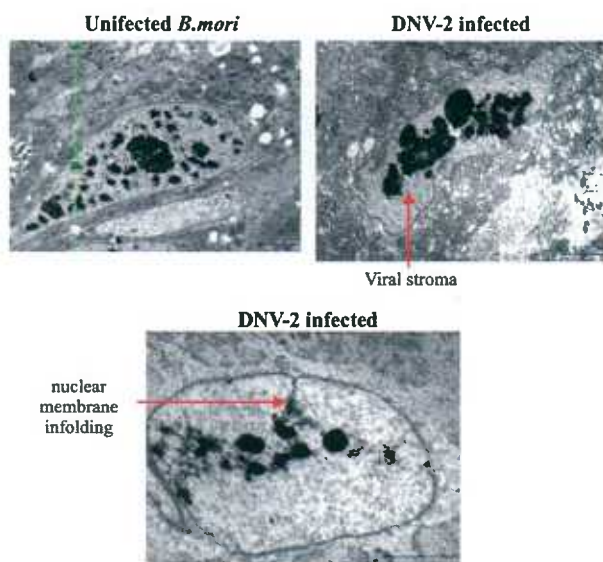


Figure 2: Transmission Electron microscopy indicating impact of BmDNV-2 infection in the nucleus of *B. mori* midgut epithelial cell

Transmission electron microscopy (TEM)

TEM studies on midgut tissues of the Indian isolate of *B. mori* silkworm infected with BmDNV-2 (Figure 2) revealed that, the nucleus of columnar cells hypertrophied as reported earlier (Watanabe and Kurihara, 1988; Murthy *et al.*, 2014). The epithelium of the infected cells revealed folding and the viral stroma also filled the nucleus reducing the chromatin content and pushing it to the periphery. Shallow infolding of nuclear membrane was also noticed.

Status of BmDNV-2 prevalence under Indian field conditions

Collection of flacherie diseased silkworms

For detection and isolation of BmDNV-2, flacherie diseased Indian silkworm races were collected from farmers' fields in the southern states of India viz., Karnataka, Tamil Nadu, Andhra Pradesh and Kerala. The diseased worms revealed typical flacherie symptoms like flaccidity, retarded growth and did not spin or produced only flimsy cocoons (Awasthi *et al.*, 2008; Ponnuvel *et al.*, 2010).

Semi-quantitative Polymerase Chain Reaction (PCR) analysis

Polymerase Chain Reaction (PCR) analysis of the flacherie affected midguts from silkworm samples was carried out using BmDNV-1, BmDNV-2 and BmNPV specific primers. BmDNV-2 was present in all the samples which included productive hybrids viz., CSR2 x CSR4 and PM x CSR2 widely reared by sericulturists under Indian conditions. However, many samples revealed presence of BmNPV along with BmDNV-2 indicating co-infection which was corroborated during discussions held with extension personnel working in various field units in India (Figure 3) (Ponnuvel *et al.*, 2010; Murthy *et al.*, 2014).

Semi-quantitative Reverse Transcriptase Polymerase Chain Reaction (PCR) analysis

The diseased worms were simultaneously subjected to Reverse Transcription PCR (RT-PCR) analysis with BmDNV-2, BmIFV and BmCPV specific primers using β actin as internal standard. RNA was extracted from the dissected midguts using Trizol reagent (Invitrogen) and quantified by measuring the UV absorbance at 260 or

280nm. After DNase treatment of the total RNA sample, the first strand cDNA was synthesized as per standard protocol using M-MLV Superscript III reverse transcriptase (Invitrogen) and utilized for further study. Results confirmed presence of BmDNV-2 in all the samples, but none of the samples revealed presence of BmIFV or BmCPV (Figure 3).

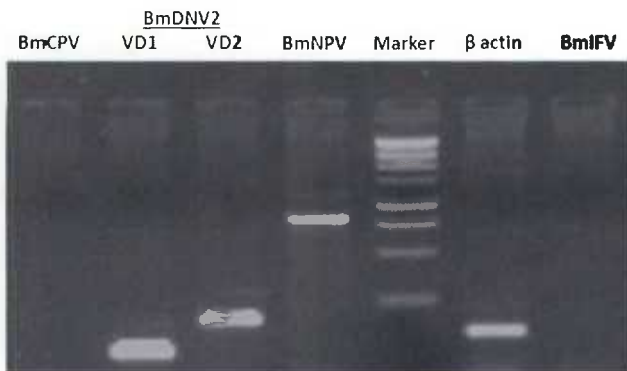


Figure 3: BmDNV-2 and BmNPV gene amplification in flacherie diseased *B. mori* DNA

Phylogenetic analysis

Sequencing of the amplified partial BmDNV-2 DNA fragment followed by phylogenetic analysis revealed maximum homology of the Indian BmDNV-2 isolate with the Yamanashi DNV-2 isolate from Japan (Figures 4 and 5).

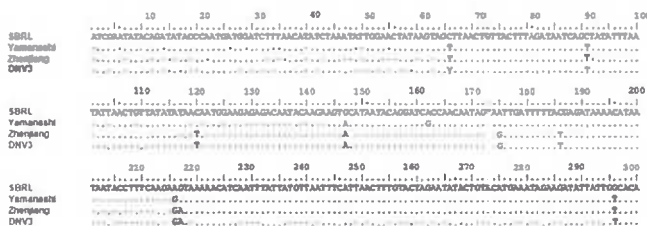


Figure 4: Comparison of DNA sequences of various BmDNV-2 isolates

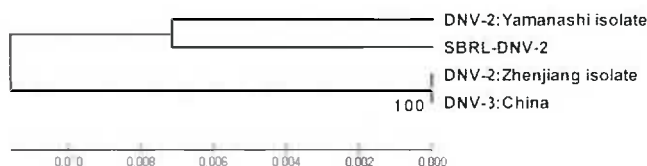


Figure 5: Phylogenetic relationship of various BmDNV-2 isolates

Realtime PCR analysis

Further, the collected diseased worms were also subjected to Realtime PCR analysis with BmDNV-2 specific primers to ascertain the population of the virus in the midguts of the diseased silkworms. The RNA was extracted and first strand cDNA synthesized as described for RT-PCR analysis. 1 µl of first strand cDNA was used in a 20 µl reaction mixture using primers specifically designed to amplify the DNA of BmDNV-2 with β actin as internal standard. The reactions were carried out on a STRATAGENE Mx 3005 P realtime PCR system with each sample tested in triplicate and the mean value ± SD were used for analysis of relative transcript levels. A non-template control NTC sample was also run to detect contamination if any. Results revealed high copy numbers of BmDNV-2 in the midguts of the flacherie diseased silkworms ranging from an average of 2.9×10^5 to 6.4×10^9 (Table 2 and Figure 6) at the end of the reaction. This confirmed the presence of viable BmDNV-2 in quite high numbers in the field and its widespread association with flacherie disease in *B. mori*.

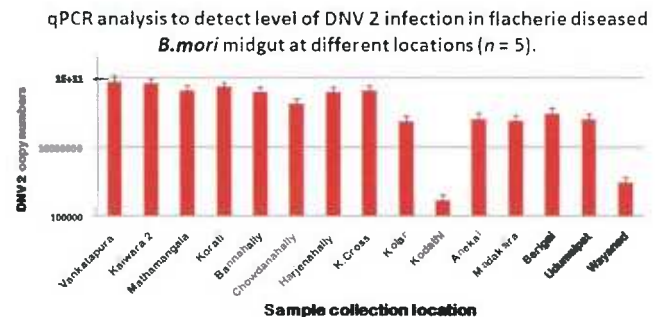


Figure 6: Realtime PCR quantification of BmDNV-2 infection in flacherie diseased, field samples of *B. mori*

Mechanism of resistance to BmDNV-2

There are ample evidences to state that, certain strains of *B. mori* are susceptible to BmDNV-2, while, some are resistant (Watanabe and Maeda, 1978; Watanabe and Maeda, 1981; Sek, 1984; Ponnuvel *et al.*, 2010, 2011). A supraoptimal temperature was observed to inhibit accumulation of BmDNV-2 polypeptides in the midgut (Kobayashi and Choi, 1990). Inhibition of DNV-2 proliferation in the midgut of *B. mori* races resistant to the

virus have been reported where 11 genes were upregulated (Bao *et al.*, 2008). Further, a total of 154 *B. mori* races have also been reported as resistant to DNV-2 (Furuta, 1995).

An unlinked mutation *nsd-2* was discovered to control non-susceptibility to BmDNV-2 in *B. mori*. Two RAPD markers were identified to be linked to the *nsd-2* gene 4.7cM away from the gene that controls non-susceptibility to BmDNV-2. Analysis of RFLP inheritance patterns using probes specific to each of the 28 linkage groups of *B. mori* indicated that the non-susceptibility gene was linked to linkage group 17 with a linkage map of 30.6 cM with *nsd-2* mapped at 24.5 cM and three closely linked cDNA markers were identified (Abe *et al.*, 2000; Ogoyi *et al.*, 2003). The *nsd-2*, a putative transporter gene was isolated for the first time by positional cloning using *Bombyx* genome information which indicated that a deletion of ~6kb and an insertion of 34 bp in the region corresponding to 9 of 12 predicted transmembrane domains conferred resistance to BmDNV-2. It was suggested that, the complete membrane protein functions as a receptor for BmDNV-2, and the site that the virus recognizes as a target is present in the deleted portion of the membrane protein, *nsd-2*. Thus the full length amino acid transporter gene functions as the BmDNV-2 susceptible gene and the truncated gene as the BmDNV-2 resistant gene. The analysis of full length cDNA as well as corresponding genomic DNA sequences in the candidate gene revealed that, the structure of *nsd-2* gene in the susceptible race had 14 exons, while, in the resistant race, there were only 5 exons. The ~6kb deletion in the resistant race corresponded to the region from exons 5-13 in susceptible race (Figure 7) (Ito, 2008; Ito *et al.*, 2008)

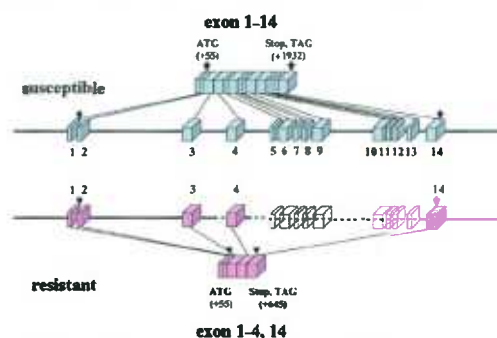


Figure 7: Genomic structure of *nsd-2* genes (Diagram courtesy: Ito, 2008)

Screening *B. mori* germplasm races for *nsd-2* genes

In India, the invaluable genetic stocks of silkworm, *B. mori* are maintained at the germplasm station of Central Silk Board *viz.*, the Central Sericultural Germplasm Resources Centre (CSGRC) at Hosur. None of the reports published till date on flacherie disease indicate studies on the availability of BmDNV-2 resistant / susceptible Indian *B. mori* races. In this backdrop, 70 multivoltine and 28 productive bivoltine silkworm germplasm resources were collected from CSGRC Hosur and subjected to PCR analysis using two sets of primers *viz.*, aa-trans1 to identify resistance to the BmDNV-2 and aa-trans3 to identify susceptibility to the virus. The aa-trans1 forward primer (5'-TCTACGTGCTTTCATACTACGTATC) had binding site within exon 4 and reverse primer (5'-TTCCTCACGTTTCTGAATTTCTCTTG) within exon 14. The aa-trans3 forward primer (5'-GGTAAGAGGTCCAACGCTGTAAAGTT) had binding site at exon 13, 3' flanking region and reverse primer (5'-TTCCTCACGTTTCTGAATTTCTCTTG) within exon 14.

These results emphasize that, most of the multivoltine as well as bivoltine silkworm germplasm races harboured either gene for susceptibility or genes for both resistance and susceptibility to DNV-2 indicating a heterozygous susceptible condition. Four multivoltine races revealed presence of gene for only resistance to BmDNV-2, while, six races revealed genes for resistance to BmDNV-2 as well as absence of genes for both resistance and susceptibility to DNV-2. The presence of gene for resistance to BmDNV-2 / absence of genes for both resistance and susceptibility to BmDNV-2 in the races indicate a homozygous resistant condition. With respect to bivoltine silkworm germplasm races, the races with genes for susceptibility / resistance as well as susceptibility included those most widely reared by sericulturists such as CSR2, CSR4 *etc.* This justifies the widespread prevalence and severity of infection by BmDNV-2 in the field. Among bivoltine silkworm germplasm races, race KA revealed presence of BmDNV-2 resistance gene, while, race M-III recorded absence of genes for both BmDNV-2 resistance as well as susceptibility.

The above studies have thus provided evidence for availability of Indian silkworm germplasm resources with probable resistance to BmDNV-2 (Ponnuvel *et al.*, 2010; Murthy *et al.*, 2014) (Tables 2a & 2b).

Table 2a: Multivoltine *B. mori* germplasm races revealing only nsd-2 gene for resistance to DNV-2

Germplasm Acc. No.	Race	Parentage
48	NISTID(W)	(Nistari.Italian race)
24	A23 [WB]	Nistari,M2,O,G
5	<i>C.nichi</i>	Original
9	KOLLEGAL JAWAN	(PM.NN6D) (Hosho.Shungetsu)

Table 2b: Multivoltine *B. mori* germplasm races revealing nsd-2 gene for resistance as well as absence of genes

Germplasm Acc. No.	Race	Parentage
54	DMR	(Nistari.Nistari M)
26	OVAL	N(X-ray),M2,KPG-B,CB-1
44	MHMP (W)	(PM.HM)MP
7	MYSORE PRINCESS	(PM.NN6D) (Hosho.Shungetsu)
42	MY23	(HN.Nistari)(PM.HM)PA11
30	GNM	Guangnong

CONCLUSION

Silkworm stocks resistant to BmDNV-1 are reported to protect themselves from BmIFV infection (Sen *et al.*, 2003). Development of silkworm breeds resistant to BmDNV-2 could provide scope for controlling flacherie disease in the field without compromising on cocoon yield. Since resistance to BmDNV-2 in *B. mori* involves a single gene, a well planned, systematic conventional breeding approach in combination with molecular biology approach can lead to promising inroads resulting in BmDNV-2 resistant *B. mori* line within a shorter period of time compared to a conventional breeding approach alone. Since using Indian silkworm races is a novel attempt and the gene for resistance is recessive, an indepth breeding programme has to be planned at laboratory level to realize expected results that can later be confirmed through large scale field level breeding. Analysis of F1 hybrids and backcrosses will reveal status of introgression of the gene. A conventional approach

depends on visual observations for flacherie symptom expressions after inoculation of the virus which would take long time. Considering the enormous size of segregating silkworm population that have to be tested under any breeding programme, a combination of conventional and molecular biology approaches will yield more fruitful, precise and faster results as molecular biology techniques are straightforward and less time consuming requiring lesser manpower. A study can be initiated by crossing a race harbouring gene for resistance to BmDNV-2 with an existing superior productive race widely used by sericulturists through conventional breeding followed by F1 hybrid production and backcrossing to introgress the gene. The introgression of the gene can be confirmed early through molecular biology techniques.

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MECANISME MOLECULAIRE DE LA RESISTANCE AU DENSOVIRUS CHEZ LE VER A SOIE *BOMBYX MORI*

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R E S U M E

La plupart des pertes de récoltes de cocons enregistrées chez les fermiers est le résultat direct du développement des maladies pendant l'élevage. Parmi les maladies infectieuses chez le ver à soie, la flacherie entraîne des pertes substantielles de récolte en Inde en raison des conditions de température et d'humidité fluctuantes ainsi que de la mauvaise qualité des feuilles de mûrier. Des études ont montré que le densovirus de type 2 de *Bombyx mori* entraîne des dommages destructeurs pendant l'élevage et est considéré comme un problème sérieux en rendant les larves sensibles flasques qui développent des diarrhées et meurent en sept jours. Le BmDNV2 a été détecté dans des lots de *B. mori* indiens comprenant des hybrides productifs comme CSR2 x CSR4 et PM x CSR2 largement utilisés par les sériciculteurs sous des conditions indiennes, beaucoup d'entre eux présentant une co-infection par BmNPV. L'isolat indien de BmDNV2 présente une homologie maximale avec l'isolat Yamanashi du Japon et les études par microscopie électronique de transmission sur des tissus intestinaux de l'isolat indien du ver à soie *B. mori* infecté par BmDNV-2 montrent une hypertrophie des noyaux des cellules colonnaires. Une mutation non cartographiée nsd-2 a été identifiée comme contrôlant la non-sensibilité à BmDNV2 chez *B. mori*. La plupart des races de vers à soie indiennes polyvoltines aussi bien que bivoltines présentent soit un gène de sensibilité soit des gènes de résistance et de sensibilité à la fois vis-à-vis de DNV2 ce qui indique une situation hétérozygote de la sensibilité. La sélection de lignées parentales possédant le gène de résistance à BmDNV2 représenterait une approche prospective vers le contrôle de la flacherie sur le terrain sans compromettre le rendement en cocons. Cette revue fournit un compte rendu sur les découvertes scientifiques des études sur BmDNV-2 et ses pathogénèses chez *Bombyx mori*.

Mots-clés: *Bombyx mori*, densovirus, résistance à la maladie, pathogénèse.



LIFE TABLE ANALYSIS OF BIVOLTINE FORM OF INDIAN WILD TASAR SILK MOTH, *ANTHRAEA PAPHIA* LINN. (LEPIDOPTERA: SATURNIIDAE)

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ABSTRACT

On an altitudinal gradient with differential photoperiod, humidity and temperature conditions, wild tasar silk moth, *Antheraea paphia* exhibits differences in voltinism, i.e., univoltine, bivoltine and trivoltine generations. Hence, life table studies in different seasons at different altitudes will provide valuable information on its population dynamics. The present study deals with the life table analysis of *A. paphia* both in rainy and autumn seasons at higher altitudes where it shows bivoltine life cycle. Considering various life table parameters, it was established that the autumn season is more favourable than the rainy for rearing the univoltine Modal ecotype of wild tasar silk moth at higher altitude where two crops are possible because the expectancy of life (e_x), net reproductive rate (R_0), mean length of generation (T) and potential fecundity (P) were maximum in this season (164.78, 108.60, 275.78, and 320, respectively) whereas, it was less in the rainy season (44.39, 100.33, 71.26 and 300, respectively).

Key words: *Antheraea paphia*, bivoltine, life table, Modal ecotype, tasar silk moth.

INTRODUCTION

Studies on life table of any commercially reared animal are of importance from management point of view to assess the role of different factors on its life cycle. Moreover, climatic factors such as photoperiod, temperature and relative humidity are important components of the environment which influence the chance of survival and multiplication by modifying fecundity, longevity and speed of development in tasar silk moth. Seasonal life table studies can predict the number of silk moths surviving at a particular time under field conditions and hence provides a direction towards framing control measures against predators and parasites.

The life cycle of tasar silk moth includes distinct developmental stages viz., egg, larva, pupa and imago, the adult which reproduces only once during its lifetime. In other words, adults die after laying eggs, which hatch to form the successive generation. On an altitudinal gradient with differential photoperiod, humidity and temperature conditions, *Antheraea paphia* Linn. exhibits one (univoltine), two (bivoltine) and three (trivoltine)

generations (Nayak and Dash, 1991). Hence, life table studies in different seasons at different altitudes will provide valuable information.

Even after having much economic importance, it is very surprising that no extensive efforts have been made to study quantitatively the life table and population dynamics of the tasar silk moth, *A. paphia*. Mishra and Joshi (1980), Joshi (1984) and Joshi and Mishra (1985) have studied population dynamics of eri silk insect *Phylosamia ricini* Hutt. only by taking season and varieties of food as the variables. Seasonal life table of Indian wild tasar silk moth *A. paphia* was studied in different rearing seasons at lower altitude (51-300 m ASL) where it shows trivoltine life cycle (Nayak and Dash, 1999). According to them, in trivoltine life cycle, the winter season is the best for rearing the tasar silk moth. The rainy and autumn seasons come in the second and third place, respectively. But from the commercial management point of view, it is highly essential to know the life table of the species at different altitudes in different rearing seasons. The present study deals with the life table analysis of *A. paphia* in different

rearing seasons of the year at altitudes 500-600 m ASL where it exhibits bivoltine life cycle.

MATERIALS AND METHODS

1. Maintenance of generation

From 1999 to 2010, two sets of experiments were carried out in 20 successive generations in two rearing seasons, *i.e.*, rainy and autumn in Kendumundi area at an altitude of 500-600 m ASL of Similipal Biosphere Reserve (SBR), Mayurbhanj, Odisha, India. One set of experiment consisted of 1500 healthy eggs laid on the same day, of *A. paphia* in five replications (Southwood, 1966), having 300 eggs (=1.5 DFL) in each replication. As per practice, rearing was conducted outdoors on Asan (*Terminalia alata*) food plant. Suitable rearing and grainage (emergence, mating and egg laying) procedures were followed as per the package of practices recommended by Jolly *et al.* (1979) and Nayak (1988).

2. Recording of life parameters

Mortality of *A. paphia* was recorded at every stage *viz.*, egg, larva, pupa and adult for each generation by simple counting of the live population. Survival rate at every stage of tasar silk moth was assessed accordingly. Number of fertile female moths and average fecundity were also recorded.

3. Environmental parameters

Environmental parameters were observed daily for five years from 1997 to 2001 regarding temperature, humidity, rainfall, duration of sunshine and day length. The observatory was installed at well-exposed level spots away from high hills, buildings, water channels, tall trees and dense forests (Venkiteswaran, 1956). The air temperature was measured by maximum-minimum thermometer placed inside a Stevenson's screen at 1.3 m above the ground level. The humidity was measured with the help of a hygrometer exposed in a Stevenson's screen. The rainfall was measured with rain gauge. The duration of the sunshine was recorded by using a sunshine recorder. The day length was calculated from the timing of sunset to sunrise, which was related to the visibility of the upper beam of the sun on the horizon. Season-wise values of the above parameters were arrived at from the

observations recorded during five years.

4. Construction of life table

A life table is primarily a means for portraying the survival experience of a group of individuals. The basic variables of a life table are time or age (x) and the life table function l_x . A cohort or horizontal life table is calculated by recording the number of survivors at successive times (ages) until all members of a cohort are dead. Based upon the above idea, mathematical models were developed by Deevey (1947). The procedures formulated by Southwood (1966) were used to represent the following derived variables of the life table of tasar silk moth.

1. The pivotal age for the age class in days = x .
2. The number of surviving individuals at the beginning of age class x which is the probability of surviving beyond $x = l_x$.
3. The unit survival rate l_x calculated as a fraction of one = l_{x1} .
4. The number of dying individuals (mortality) during the age interval $x = d_x = l_x - l_{x+1}$.
5. The probability of dying in the interval $(x, x+1)$, given that the individual survives at least to x , it is also known as the mortality rate = q_x .

$$q_x = \frac{d_x}{l_x}$$

6. Actually, all the individuals do not die suddenly at the end of the age class interval as it is shown in the life table for the purpose of calculation. The mortality continues in a random manner all through the interval. Thus the number of animals surviving at middle point of class interval between age x and $x+1 = L_x$.

$$L_x = \frac{l_x + l(x+1)}{2}$$

7. The total number of individuals who have lived all class interval is denoted as T_x . In other words, total life time remaining to individuals alive at x (L_x) is the sum of their individual life time.

$$T_x = \sum_{i=x}^W (L_i) \text{ or } T_x = L_x + L(x+1) + L(x+2) + \dots + L_W$$

Where, 'w' is the time (age) when all individuals were dead. This statistic was computed from the bottom of the L_x column upwards carrying the running cumulative total at the top of the T_x column which represents the population that has lived the entire lifespan from which the expectancy of life e_x was calculated.

8. The life expectancy of an individual at $x = e_x$. It is sometimes called the mean residual lifetime.

$$e_x = \frac{T_x}{L_x} \times 4$$

9. Life expectancy is simply how long an individual is expected to live given that it survived until x . Finally, the hazard function was the instantaneous rate of death at midpoint of $(x, x+1)$ (i.e., x') given that the individual survived until x' .

The hazard rate = h_x'

$$h_x' = \frac{2q_x}{2 - q_x}$$

Hazard rate can also be called the age specific death rate.

5. Construction of fertility table

Life tables are important summaries of population data but they lack information on the equally important attributes of reproduction. To incorporate reproduction, life tables are expanded into fecundity tables. Fertility can be used instead of fecundity as it refers to live births (Southwood, 1966).

A fertility table was constructed by preparing a life table as per Joshi (1984) and Joshi and Mishra (1985) with x and l_x as before, but the L_x column referred entirely to females alive during a given age interval as a fraction of an initial population of one.

1. Age specific fertility or natality rate = m_x

It is the number of eggs laid/offspring born in unit time to an individual in a population and is determined by dividing the number of eggs laid at x by the total number of females alive at x .

2. Total number of eggs laid by a unit female over her lifetime was the potential fecundity.

$$P_f = \sum m_x$$

3. The net replacement (reproduction) rate of the female population = R_0

It is the product of average number of eggs produced (natality rate) by the females surviving at the age l_{x1} .

$$R_0 = l_{x1} \cdot m_x$$

4. The total replacement made by an average female from each generation = R_0 .

It would be the average carrying capacity of the tasar silk moth under specified environmental conditions.

5. Innate capacity for increase or maximum intrinsic rate of increase = R_m

It would summarise the physiological qualities of the tasar silk moth with respect to its capacity to increase in number during one-generation time (T) which is mathematically determined by Dublin and Lotka (1925) as follows:

$$T = \frac{\sum (l_x \cdot m_x \cdot x)}{\sum (l_x \cdot m_x)}$$

Thus,

$$R_m = \frac{\log_e e_x \cdot R_0}{T}$$

6. The annual Biotic Potential = $BP = P \cdot Zn$ where, P is the potential fecundity and Zn is the number of generations in a year.

6. Comparison of mortality factors within a generation

a. Apparent mortality (AM) is the measured mortality, the number dying as a percentage of the numbers entering that stage, i.e., d_x as a percentage of l_x .

$$AM = \frac{d_x}{l_x} \times 100$$

b. Real mortality (RM) is calculated on the basis of the population density at the beginning of the generation.

$$RM = \frac{d_x}{l_c} \times 100$$

Where, l_x is the size of the cohort at the beginning of generation.

c. Mortality/survival (M/S) quotient represents the mortality factor that ranges between 0 and 1. If the population at the beginning of the succeeding generation is multiplied by the M/S quotient of that of the preceding generation, the resulting value will be equal to the mortality (d_x) of the preceding generation.

$$\frac{M}{S} = \frac{dx}{lx+1}$$

RESULTS

1. Survivorship (l_x)

The sex ratio, rates of survival, death and birth of wild tasar silk moth, *A. paphia* can reasonably be assumed to be the function of the environmental conditions, because the same food, i.e., Asan (*T. alata*) leaf was given in all the cases during the commercial rearing. The comparison of the Tables 1 and 2 showed that the survival of the species from egg to pupal stage was the highest (Hatching =270.00, I instar =252.00, II instar =237.00, III instar =217.00, IV instar =202.00, V instar =190.00, Prepupa =171.00, Pupa =166.00) in bivoltine form during autumn season. The number of survivors reaching the adult stage was more or less the same, i.e., 127 and 134 out of 300 in each case during rainy and autumn, respectively (Table 5).

2. Mortality factors

The mortality factors in wild tasar silk moth in different seasons are presented in Tables 3 and 4. The mortality factors of a species include mortality (dx), rate of mortality (qx), percentage of apparent mortality (AM), percentage of real mortality (RM), mortality: survivorship ratio (M:S) and hazard rate (hx). In this tropical wild tasar silk moth, *A. paphia*, the mortality factors were always comparatively higher in egg, hatching, first instar, fourth instar and prepupal stages. The survivorship of the species depended upon the hazard rate in different stages of life cycle. Generally, the hazard rate of wild tasar silk moth was more in early stages than the late stages (Hatching = 0.12, I instar = 0.09, II instar = 0.08, III instar = 0.08, IV instar = 0.13) in rainy season

than autumn. But this hazard rate was more in late stages (V instar = 0.11, Pupa = 0.20) of wild tasar silk moth in autumn than rainy. Hence, it is observed that the overall mortality factors were always the highest in rainy season in comparison to autumn (Table 5).

3. Life expectancy (e_x)

The life expectancy in different developmental stages of *A. paphia* showed that it was the highest at all stages (Hatching =174.42, I instar =182.77, II instar =190.18, III instar = 199.36, IV instar = 205.88, V instar = 210.63, Prepupa = 213.11, Pupa =207.16) in autumn except for the adult stage which was more or less same in both the

Table 1: Life table of wild tasar silk moth, *A. paphia* reared at altitude, 500-600 m ASL during rainy season in Similipal Biosphere Reserve

Life stage	Age in days (x)	No. of survivors (l_x)	Fraction survivors (l_x/l_0)	Mortality (d_x)	Mortality rate (q_x)	Average alive (L_x)	Total alive (T_x)	Life expectancy (e_x)	Hazard rate (h_x)
Egg	0	300	1.00	0	0.00	300.0	3329.0	44.39	0.00
Egg	4	300	1.00	36	0.12	282.0	3029.0	40.39	0.13
Hatching	8	264	0.88	28	0.11	250.0	2747.0	41.62	0.12
I instar	2	236	0.79	22	0.09	225.0	2497.0	42.32	0.09
II instar	16	214	0.71	17	0.08	205.5	2272.0	42.47	0.08
III instar	20	197	0.66	15	0.08	189.5	2066.5	41.96	0.08
IV instar	24	182	0.61	12	0.07	176.0	1877.0	41.25	0.07
IV instar	28	170	0.57	10	0.06	165.0	1710.0	40.42	0.06
V instar	32	160	0.53	7	0.04	156.5	1536.0	38.40	0.04
V instar	36	153	0.51	5	0.03	150.5	1379.5	36.07	0.03
Pre-pupa	40	148	0.49	0	0.00	148.0	1229.0	33.22	0.00
Pre-pupa	44	148	0.49	4	0.03	146.0	1081.0	29.22	0.03
Pupa	48	144	0.48	0	0.00	144.0	935.0	25.97	0.00
Pupa	52	144	0.48	0	0.00	144.0	791.0	21.97	0.00
Pupa	56	144	0.48	0	0.00	144.0	647.0	17.97	0.00
Pupa	60	144	0.48	0	0.00	144.0	503.0	13.97	0.00
Pupa	64	144	0.48	17	0.12	135.5	359.0	9.97	0.13
Adult	68	127	0.42	31	0.24	111.5	223.5	7.04	0.27
Adult	72	96	0.32	40	0.41	76.0	112.0	4.67	0.52
Adult	76	56	0.19	48	0.86	32.0	36.0	2.57	1.51
Adult	80	8	0.03	8	1.00	4.0	4.0	2.00	2.00

Table 2: Life table of wild tasar silk moth, *A. paphia* reared at altitude, 500-600 m ASL during autumn season in Similipal Biosphere Reserve

Life stage	Age in days (x)	No. of survivors (l_x)	Fraction survivors (l_x/l_0)	Mortality (d_x)	Mortality rate (q_x)	Average alive (L_x)	Total alive (T_x)	Life expectancy (e_x)	Hazard rate (h_x)
Egg	0	300	1.00	0	0.00	300	12359.0	164.78	0.00
Egg	4	300	1.00	30	0.10	285.0	12059.0	160.78	0.11
Hatching	8	270	0.90	18	0.07	261.0	11774.0	174.42	0.07
I instar	12	252	0.84	15	0.06	244.5	11513.0	182.77	0.06
II instar	16	237	0.79	11	0.05	231.5	11268.5	190.18	0.05
III instar	20	226	0.75	9	0.04	221.5	11037.0	195.34	0.04
IV instar	24	217	0.72	8	0.04	213.0	10815.5	199.36	0.04
V instar	28	209	0.69	7	0.03	205.5	10602.5	202.91	0.03
VI instar	32	202	0.67	6	0.03	199.0	10397.0	205.88	0.03
VII instar	36	196	0.65	6	0.03	193.0	10198.0	208.12	0.03
VIII instar	40	190	0.63	5	0.03	187.5	10005.0	210.63	0.03
IX instar	56	173	0.58	2	0.01	172.0	9282.5	214.62	0.01
Pre-pupa	60	171	0.57	0	0.00	171.0	9110.5	213.11	0.00
Pupa	68	171	0.57	5	0.03	171.0	8768.5	205.11	0.03
Pupa	72	166	0.55	0	0.00	168.5	8597.5	207.16	0.00
Pupa	108	166	0.55	0	0.00	166.0	7101.0	171.10	0.00
Pupa	144	166	0.55	0	0.00	166.0	5607.0	135.10	0.00
Pupa	180	166	0.55	0	0.00	166.0	4113.0	99.10	0.00
Pupa	216	166	0.55	0	0.00	166.0	2619.0	63.10	0.00
Pupa	268	166	0.55	32	0.19	150.0	461.0	11.10	0.20
Adult	272	134	0.45	29	0.21	119.5	311.0	9.28	0.23
Adult	276	105	0.35	18	0.17	96.0	191.5	7.29	0.19
Adult	280	87	0.29	35	0.40	69.5	95.5	4.39	0.50
Adult	284	52	0.17	52	1.00	26.0	26.0	2.00	2.00

Table 3: Comparison of mortality factors within a generation of wild tasar silk moth, *A. paphia* reared in rainy season at altitude, 500-600 m ASL of Similipal Biosphere Reserve

Life stage	Number of survivors (l_x)	Mortality (d_x)	Apparent mortality (AM %)	Real mortality (RM %)	Mortality/survival (M/S)
Egg	300	36	12.00	12.00	0.14
Hatching	264	28	10.61	9.33	0.12
I instar	236	22	9.32	7.33	0.10
II instar	214	17	7.94	5.67	0.09
III instar	197	15	7.61	5.00	0.08
IV instar	182	22	12.09	7.33	0.14
V instar	160	12	7.50	4.00	0.08
Pre-pupa	148	4	2.70	1.33	0.03
Pupa	144	17	11.81	5.67	0.13
Adult	127	127	100	42.33	0.00

Table 4: Comparison of mortality factors within a generation of wild tasar silk moth, *A. paphia* reared in autumn season at altitude, 500-600 m ASL of Similipal Biosphere Reserve

Life stage	Number of survivors (l_x)	Mortality (d_x)	Apparent mortality (AM %)	Real mortality (RM %)	Mortality/survival (M/S)
Egg	300	30	10.00	10.00	0.11
Hatching	270	18	6.67	6.00	0.07
I instar	252	15	5.95	5.00	0.06
II instar	237	20	8.43	6.66	0.09
III instar	217	15	6.91	5.00	0.07
IV instar	202	12	5.94	4.00	0.06
V instar	190	19	10.00	6.33	0.11
Pre-pupa	171	5	2.92	1.67	0.03
Pupa	166	32	19.28	10.67	0.24
Adult	134	134	100	44.67	0.00

seasons (Table 5). But during rainy season, it increased after hatching and gradually fell after second instar with the increase of age. During autumn season (Table 5), it increased after hatching and reached the highest at prepupal stage followed by a fall thereafter.

4. Net reproductive rate (R_0)

The net reproductive rate was maximum, *i.e.*, 108.60 in autumn and minimum, *i.e.*, 100.33 in rainy season. It was also seen that the R_0 was maximum on the first day of oviposition and the value was reduced thereafter in both

seasons. The maximum population turnover occurred during the autumn than the rainy season (Tables 6 and 7).

5. Innate capacity for increase (R_m)

The R_m value gives an instantaneous rate of population increase, which is mathematically computed as the mean

period elapsing from birth of parent to birth of offspring (T). The R_m value was the lowest, *i.e.*, 0.880 during autumn and the highest, *i.e.*, 2.319 in the rainy season. The lowest generation time was during the rainy (71.26) followed by the autumn season (275.78) (Table 8).

Table 5: Comparison of life table (mortality factors and life expectancy) of wild tasar silk moth, *A. paphia* reared in different seasons at altitude, 500-600 m ASL in Similipal Biosphere Reserve

Life table parameter	Season	Egg	Hatching	Instar					Prepupa	Pupa	Adult
				I	II	III	IV	V			
Survivorship (l_x)	Rainy	300.00	264.00	236.00	214.00	197.00	182.00	160.00	148.00	144.00	127.00
	Autumn	300.00	270.00	252.00	237.00	217.00	202.00	190.00	171.00	166.00	134.00
Mortality (d_x)	Rainy	36.00	28.00	22.00	17.00	15.00	22.00	12.00	04.00	17.00	127.00
	Autumn	30.00	18.00	15.00	20.00	15.00	12.00	19.00	05.00	32.00	134.00
Rate of mortality (q_x)	Rainy	0.12	0.11	0.09	0.08	0.08	0.13	0.07	0.03	0.12	1.00
	Autumn	0.10	0.07	0.06	0.09	0.07	0.06	0.11	0.03	0.19	1.00
% of apparent mortality (AM %)	Rainy	12.00	10.61	9.32	7.94	7.61	12.09	7.50	2.70	11.81	100.00
	Autumn	10.00	6.67	5.95	8.43	6.91	5.94	10.00	2.92	19.28	100.00
% of real mortality (RM %)	Rainy	12.00	9.33	7.33	5.67	5.00	7.33	4.00	1.33	5.67	42.33
	Autumn	10.00	6.00	5.00	6.66	5.00	4.00	6.33	1.67	10.67	44.67
Mortality/survival (M/S)	Rainy	0.14	0.12	0.10	0.09	0.08	0.14	0.08	0.03	0.13	0.00
	Autumn	0.11	0.07	0.06	0.09	0.07	0.06	0.11	0.03	0.24	0.00
Hazard rate (h_x)	Rainy	0.13	0.12	0.09	0.08	0.08	0.13	0.07	0.03	0.13	2.00
	Autumn	0.11	0.07	0.06	0.09	0.07	0.06	0.11	0.03	0.20	2.00
Life expectancy (e_x)	Rainy	44.39	41.62	42.32	42.47	41.96	41.25	38.40	33.22	25.97	7.04
	Autumn	164.78	174.42	182.77	190.18	199.36	205.88	210.63	213.11	207.16	9.28

Table 6: Net reproduction rate of wild tasar silk moth, *A. paphia* reared in rainy season at altitude, 500-600 m ASL of Similipal Biosphere Reserve

Actual age (days)	Pivotal age (x)	Age specific survival rate of female (l_{x1})	Natality rate (m_x)	Net productive rate ($R_0 = l_{x1} \cdot m_x$)	$l_{x1} \cdot m_x \cdot x$
70	1	0.37	98.00	36.26	36.26
71	2	0.35	73.00	25.55	51.10
72	3	0.32	61.00	19.52	58.56
73	4	0.29	50.00	14.50	58.00
74	5	0.25	18.00	4.50	22.50
75	6	0.22	0.00	0.00	0.00
76	7	0.19	0.00	0.00	0.00
77	8	0.15	0.00	0.00	0.00
78	9	0.11	0.00	0.00	0.00
79	10	0.07	0.00	0.00	0.00
80	11	0.03	0.00	0.00	0.00
Total	Σ		300.00	100.33	226.42

$$\frac{\sum l_{x1} \cdot m_x \cdot x}{\sum l_{x1} \cdot m_x} = \frac{226.42}{100.33} = 2.26$$

$$T = 69 + 2.26 = 71.26$$

Table 7: Net reproduction rate of wild tasar silk moth, *A. paphia* reared in autumn season at altitude, 500-600 m ASL of Similipal Biosphere Reserve

Actual age (days)	Pivotal age (x)	Age specific survival rate of female (l_{x1})	Natality rate (m_x)	Net productive rate ($R_0 = l_{x1} \cdot m_x$)	$l_{x1} \cdot m_x \cdot x$
274	1	0.40	83.00	33.20	33.20
275	2	0.38	65.00	24.70	49.40
276	3	0.35	51.00	17.85	53.55
277	4	0.34	40.00	13.60	54.40
278	5	0.32	28.00	8.96	44.8
279	6	0.31	21.00	6.51	39.06
280	7	0.29	13.00	3.77	26.39
281	8	0.26	0.00	0.00	0.00
282	9	0.23	0.00	0.00	0.00
283	10	0.20	0.00	0.00	0.00
284	11	0.17	0.00	0.00	0.00
Total	Σ		301.00	108.60	300.80

$$\frac{\sum l_{x1} \cdot m_x \cdot x}{\sum l_{x1} \cdot m_x} = \frac{300.80}{108.60} = 2.76$$

$$T = 273 + 2.76 = 275.76$$

DISCUSSION

1. Survivorship (L)

The survivorship of different stages of larvae, pupae and adults was the highest in autumn due to prevalence of conducive climate [mean maximum temperature-30.70°C, mean minimum temperature-20.45°C, mean average temperature-25.57°C, rainfall-200.00 mm, relative humidity-80.45 % and duration of sunshine - 118.70 hours/month (Tables 8 and 9)]. But in rainy season, the survivorship of larvae decreased due to high larval mortality, incidence of heavy rain, cyclonic wind and pests. Further, the rainy season was hot and more humid in comparison to autumn due to prevailing climatic factors of mean maximum temperature-33.60 °C, mean minimum temperature-20.85°C, mean average temperature-27.23°C, rainfall-283.70 mm, humidity,-84.50 % and duration of sunshine-93.55 hours/month (Tables 8 and 9). Moreover, the life expectancy and

survivorship of larvae and pupae were more in autumn season than the rainy season due to higher quantity of cocoons (111 cocoons per DFL) produced in autumn crop than in rainy crop (96 cocoons per DFL).

From the above analysis, the highest survivorship was observed in autumn crops, because the temperature regime, rainfall, humidity and photoperiod were conducive for them. The survivorship gradually decreased from autumn crops to rainy crops. The highest survivorship during the autumn crop appears to be destined to meet the adverse climatic stress occurred during diapause period. Generally, this period continues for about eight months covering winter, spring, summer and pre-rain season. Thus, the highest survivorship of silk moth during autumn rearing appeared to help the population pool of the species to be maintained at a level from generation to generation.

Table 8: Comparative performance of life expectancy (e_x), net reproduction rate (R_0), innate capacity for increase (R_m) and potential fecundity (P_f) of wild tasar silk moth, *A. paphia* reared in different seasons at altitude, 500-600 m ASL in Similipal Biosphere Reserve

Season	Life expectancy (days) (e_x)	Net reproduction rate (R_0)	Mean length of duration (T)	Innate capacity for increase (R_m)	Potential fecundity (P_f)	Annual biotic potential (B_r)
Rainy	44.39	100.33	71.26	2.319	300	600
Autumn	164.78	108.60	275.78	0.880	320	640

Table 9: Meteorological (climatological) parameters at study site in Similipal Biosphere Reserve during different seasons

Parameter/season	Summer	Rainy	Autumn	Dew	Winter	Spring
Maximum temp. (°C)	36.80 (0.55)	33.60 (1.00)	30.70 (0.20)	29.50 (1.20)	25.05 (1.55)	31.80 (1.24)
Minimum temp. (°C)	20.20 (1.09)	20.85 (0.15)	20.45 (0.05)	17.15 (1.15)	12.80 (1.80)	16.60 (0.09)
Average temp. (°C)	28.50 (0.42)	27.23 (0.55)	25.57 (1.00)	23.33 (1.15)	18.93 (0.15)	23.20 (0.66)
Relative humidity (%)	67.90 (5.73)	84.50 (4.05)	80.45 (2.10)	79.65 (1.45)	69.35 (1.15)	63.50 (3.28)
Rainfall (mm)	38.00 (13.61)	283.70 (12.10)	200.00 (25.40)	103.50 (63.00)	63.30 (0.10)	16.30 (2.25)
Duration of sunshine (h/month)	216.40 (21.51)	93.55 (8.15)	118.70 (6.70)	229.05 (6.45)	236.70 (5.40)	224.50 (8.67)

Figures in parentheses are $\pm$ SD values.

2. Mortality factors

The environmental condition during the rainy season was found hazardous to the larval stage than that of autumn season. The pupal mortality in the autumn crop was very less though it experienced a longer diapause period, because a good air conditioning medium prevailed inside the cocoon. The environmental condition during the rainy season was found hazardous to the larval stage mostly due to occurrence of cyclones and incidence of more predators, parasites and diseases. Higher pupal mortality was observed in the rainy season due to the higher temperature and higher humidity regime.

Moreover, mortality factor was more or less very uniform in adults of all seasons because the moths prefer to emerge at favourable conditions. So, the rate of mortality (q_x) was 1 and percentage of apparent mortality (AM %) was 100 in adult stages in all the rearing seasons (Table 5).

3. Life expectancy (e_x)

The life expectancy of wild tasar silk moth, *A. paphia* was not uniform throughout the rearing seasons due to irregular mortality rate. In rainy season, the mortality rate was higher during the early stages. However, after attainment of the longest life expectancy at a particular stage (*i.e.*, IV instar) of development, the life expectancy decreased continuously as the age of the silk moth increased which showed a uniform mortality rate in the later stage of life. However, this trend did not occur in autumn season. Rather, the life expectancy was increased continuously from larva to pupa due to favourable environmental conditions.

4. Net reproduction rate (R_0)

The decreasing pattern of net reproduction rate (R_0) was found in different rearing seasons due to advancement of age of the female silk moth, *A. paphia*. The value of R_0 was high in autumn crops than the rainy season. This type of decreasing pattern of R_0 is also observed in Eri silk moth, *Philosamia ricini* (Joshi, 1984) and trivoltine form of wild tasar silk moth, *A. paphia* (Nayak and Dash, 1999).

5. Innate capacity for increase (R_m)

At lower altitude, the lesser value of R_m was found in winter due to reduced metabolic rate of larvae. Moreover, the pupa remained in diapause during the rest of the seasons after winter, up to summer season (Nayak and Dash, 1999). As an almost winter climatic condition of lower altitude prevailed in the autumn season at higher altitude, the autumn was comparatively cooler than the rainy season in this altitude. Therefore, the value of R_0 was also more in autumn season than the rainy.

CONCLUSION

The earlier works on voltinism and life table of *A. paphia* in different altitudes revealed that at lower altitude (51-300 m ASL), it is trivoltine in nature and winter is the most favourable season for rearing (Nayak and Dash, 1999) whereas, at higher altitude (600-900 m ASL), it shows univoltine nature and the favourable season is rainy (Dey *et al.*, 2008). The present study on voltinism of this species at altitude 500-600 m ASL shows bivoltinism. Considering various life table parameters of this bivoltine form of *A. paphia*, it can be concluded that the autumn is the best season for rearing of the wild tasar silk moth where two crop systems are possible, because the expectancy of life (e_x), net reproductive rate (R_0) and mean length of generation (T) were maximum in autumn season.

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ANALYSE DU CYCLE D'UNE FORME BIVOLTINE DU VER A SOIE INDIEN TASAR SAUVAGE *ANTHERAEA PAPHIA* (LEPIDOPTERE: SATURNIDE)

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RESUME

Sur un gradient d'altitudes avec des photopériodes et des conditions d'humidité et de température différentes, le papillon sauvage tasar présente des différences de voltinisme en étant soit monovoltin, soit bivoltin ou soit trivoltin. L'étude des cycles de vie à différentes saisons et différentes altitudes doit permettre d'obtenir une information précieuse sur la dynamique de sa population. L'étude présente concerne l'analyse du cycle de *A. paphia* à la fois pendant la saison des pluies et en automne aux altitudes les plus élevées où son cycle de vie est bivoltin. Considérant des paramètres variés nous avons établi que l'automne est plus favorable que la saison des pluies pour élever l'écophage Modal du tasar sauvage à haute altitude où deux récoltes sont possibles, en raison de l'espérance de vie (e_x), du taux de reproduction net (R_0), de la longueur moyenne d'une génération (T) et de la fécondité potentielle (P_f) qui sont maximum durant cette saison (respectivement 164.78, 108.60, 275.78, et 320) alors qu'ils sont inférieurs pendant la saison des pluies (44.39, 100.33, 71.26 et 300).

Mots-clés: *Antheraea paphia*, bivoltin, cycle de vie, écophage Modal, papillon tasar.



SOCIO-ECONOMIC DETERMINANTS OF TRADITIONAL TASAR COCOON MARKETING AGENTS OF ANDHRA PRADESH, INDIA

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ABSTRACT

Influence of socio - economic factors on business volume, marketing cost and prize spread in traditional cocoon marketing system of Andhra Pradesh was studied. Primary data have been collected from 72 marketing agents with pre-tested schedule. Marketing agents as defined in the main existing marketing system included primary purchasers, wholesalers (reeler cum weaver) and silk processors (processor of silk yarn to fabrics). Variables such as education, initial capital, type of trader, business ownership, and mode of business acquisition and financial capacity of agents had a direct and significant relationship with cocoon volume handled in the open market. Tasar cocoon producer received the price in the range of ₹ 1100 ~ 1622 for 1000 cocoons and shared accordingly 67.81 - 91.02 % of consumer's price. Primary purchaser doubled his margin by ₹ 150.00 through incurring marketing cost of ₹ 75.00 per 1000 cocoons. The price spread in trading was ₹ 871.00 per 1000 cocoons and the major shares come from wholesaler (34.10 %) and primary purchaser (26.00 %) indicating their important roles. Marketing efficiency (4.46 - 10.14) was found highly efficient as the unit was more than one. Establishing more reeling units, on line futures trading system and consciousness about cocoon quality will be instrumental in speedy marketing of tasar cocoons.

Key words: Marketing agents, marketing costs and margins, price spread, socio- economic factors.

INTRODUCTION

Tropical tasar silkworm, *Antheraea mylitta* Drury, is one of the four non mulberry silkworms thriving on the foliage of primary host plants *Terminalia arjuna* (Arjun) and *T. tomentosa* (Asan). Globally, India ranks first in the production of Eri, Muga and tropical Tasar silks. Tasar silkworm rearing is concentrated mainly in tribal hamlets of Bihar, Jharkhand, West Bengal, Chhattisgarh, Madhya Pradesh, Andhra Pradesh, Maharashtra and Uttar Pradesh. It is a very remunerative crop providing lucrative returns (₹ 15,000 to 20,000 per crop) to the tribal farmers of Andhra Pradesh. Since 2007-08 to 2010-11, the marketing price of tasar reeling cocoons ranged from ₹ 1050 to ₹ 1200/1000 cocoons (Anonymous, 2010-11).

A substantial, 35.40 % growth in tasar raw silk

production from 428 MT in 2007-08 to 1166 MT during XI plan period (2007-11) clearly indicated the demand for raw silk which is a major source of livelihood for thousands of tribal farmers of the country. It is well documented that silk industry is a labor intensive activity and can be used as a tool for rural employment and poverty alleviation. Out of 7.25 million people employed in sericulture, 2.00 lakh people are engaged directly or indirectly with tasar sector (Roy, 2011).

Tasar cocoon production is recognized as a forest based industry and income generation venture with high employment potential. However, tasar silk industry has been facing a number of constraints of late, resulting in uncertain growth prospects. Income from tasar culture relies on cocoon price offered by the trader. The interest of

tasar silk rearers is at stake when they are bereft of fair cocoon price. The major factor affecting tasar industry, in general, is lack of organized market support. The poor market accessibility and alternative options force the tasar cocoon producers to sell their produce in traditional market which is virtually controlled by trader. This kind of situation eclipses producer's interest as long as trader's monopoly prevails in the market. However, in Andhra Pradesh, Raw Material Bank of Central Silk Board, Govt. of India, operates for purchase and disposal of cocoons and yarn (Jahagirdar, 1998).

The understanding of the traditional cocoon marketing and processing chains are helpful in evolving policies and developmental strategies for creating an efficient cocoon marketing system. In this backdrop, the present study was undertaken with the objectives of, i. identifying the socio-economic factors that determine the volume of business of tasar market agents, ii. examining the costs and returns in cocoon marketing and iii. assessing price spread and efficiency in the market.

METHODOLOGY

The data were collected from representative random samples of traditional tasar silk cocoon market agents of Andhra Pradesh by conducting a survey in all tasar cocoon producing districts viz., Warangal, Karimnagar, Adilabad, Mahaboobnagar and Khammam by interacting with pre-structured questionnaire. The agents as defined in this study included (I) primary purchaser (II) wholesaler (reeler-cum-weaver) and (III) silk processor (processor of silk yarn to fabric). The overall sample size of seventy - two market agents was comprised of 36 primary purchasers, 28 wholesalers and 8 silk processors. Twelve primary purchasers were from Karimnagar, Adilabad and Khammam districts. Group of 7 wholesalers and 2 silk processors were interacted evenly in Warangal, Karimnagar, Mahaboobnagar and Adilabad districts. Tasar cocoons produced at growers' level go to silk processor via primary purchaser and wholesaler where they are reeled to raw silk yarn and fabrics. The main marketing channel identified was as following :

Cocoon producer-primary purchaser-wholesaler-silk processor.

The other marketing channels are:

1. Producer-Primary purchaser - wholesaler
2. Producer-raw material bank (RMB) wholesaler - silk processor
3. Producer-wholesaler-silk processor

Tasar raw material bank (RMB) is a marketing functionary of Central Silk Board, Govt. of India and participates in the open auction market. RMB also fixes the floor price on the basis of cocoon quality and ensures remunerative price to the producer. It plays a vital role in tasar marketing and has contributed towards breaking the monopoly of traders.

In the present study, the cocoon selling price of producer, primary purchaser and wholesaler is considered as purchasing prices of primary purchaser, wholesaler and silk processor, respectively. The selling price of silk processor is accounted for purchasing price of consumer. The basic information on socio-economic profile of marketing agents was collected to understand the status of tasar cocoon marketing and assess the profitability of different agents in the traditional marketing system by computing cost and returns. The marketing efficiency of tasar cocoon was estimated by dividing the net price received by the cocoon producer with the marketing costs and margins. The marketing margins for different market functionaries were estimated to assess distribution of gains across the market chain using the following equation (Kumar *et al.*, 2010):

$$MM = Sp - Cp - Mc$$

Where, MM = Market margin

Sp = Sale price

Cp = Cost price

Mc = Marketing cost incurred by the trader

The producer's share (Ps) in consumer rupee for different marketing channels was calculated using the following equation:

$$Ps = \frac{PF}{PC} \times 100$$

Where, PF = Price received by tasar cocoon producer and
PC = price paid by consumer

Spearman's correlation technique and regression technique (Srinivasa *et al.*, 2004; Kumar *et al.*, 2010) were employed in the study to understand the relationship of socio-economic (independent variables) factors of the marketing agents with volume of business of tasar cocoons (no. of cocoons marketed by a trader), considered as dependent variable. The algebraic form of the Spearman's technique used is as follows:

$$r = \frac{\sum (xy) - N(\bar{x})(\bar{y})}{[\sum x^2 - N(\bar{x})^2] [\sum y^2 - N(\bar{y})^2]}$$

Where, r = Correlation co-efficient to be estimated

x = Independent variable

y = Dependent variable

$\bar{x}$ = Mean value for independent variable

$\bar{y}$ = Mean value for dependent variable

N = Number of pairs of observation

The data were analyzed using regression technique to identify the socio-economic factors influencing the volume of business of cocoon market agents.

$Y = a + b_1x_1 + b_2x_2 + b_3x_3 + \dots + b_{13}x_{13} + \mu$ where,

Y = Dependent variable (No. of cocoons handled by a trader)

a = Intercept

b_1 to b_{13} = parameters to be estimated

x_1 to x_{13} = Independent variables

x_1 = Age of trader (years)

x_2 = Education of trader (years of schooling)

x_3 = Initial capital (₹)

x_4 = Experience in tasar cocoon marketing (years)

x_5 = Occupation of trader (only cocoon marketing = 1; others = 0)

x_6 = Family participation in business (if yes = 1; otherwise = 0)

x_7 = Ownership of business (sole = 1; otherwise = 0)

x_8 = Type of trader (cocoon trader = 1; otherwise = 0)

x_9 = Distance traveled by trader (km) for business

x_{10} = Travel time (minutes)

x_{11} = Location of business (urban = 1; otherwise = 0)

x_{12} = Mode of business acquisition (self-started = 1; otherwise = 0)

x_{13} = Source of financing (formal credit = 1; otherwise = 0)

μ = Error term

RESULTS AND DISCUSSION

a. Socio-economic profile of cocoon market agents

The socio-economic profile of sampled tasar cocoon market agents is depicted in Table 1. Cocoon market was dominated by primary purchaser and the wholesaler. The

Table 1: Demographic characteristics and scale of business of informal tasar cocoon traders (N= 72)

Characteristic	Primary Purchaser	Wholesaler (reeler cum weaver)	Silk processor (raw silk to fabric maker)	All
1	2	3	4	5
Sample size	36	28	08	72
Average age (years)	39.55	47.60	43.80	43.71
Average education (years)	5.60	9.75	10.30	8.55
Average cocoons handled/year	162100	133387	216800	512287
Participation of family members				
Full time - 1	1	1	0.72	0.91
Part time - 0	0	0	0.28	0.09
Ownership of business				
Single proprietorship	33	22	07	62
Joint partnership	03	06	01	10
Experience in the business (years)	36.70	23.30	24.48	28.16
Mode of Business acquisition				
Self started	32	21	06	59
Inherited	4	7	2	13
Occupational profile				
Cocoon marketing only	26	13	4	43
Cocoon marketing and other activities	10	15	4	29
Contribution of cocoon marketing in household income (No.)				
0-50 %	4	1	0	5
51-75 %	11	3	5	19
76 % and above	31	11	6	48

wholesaler who process raw silk yarn holds a major portion of tasar cocoon market system. On an average, agents had 8 years of schooling out of average age of 44 years. The wholesalers and primary purchasers were found to spent 9.75 and 10.30 years of their age, respectively for education. This indicates that low level of education could be a form of barrier for entry into value added silk processing activities. Tasar silkworm cocoon production, reeling and processing activities were mostly attended by women folk in Andhra Pradesh, as amongst 72 cocoon market agents, only 27 were males.

On an average, traditional market agents handle 512287 cocoons per year, it being 162100, 133387 and 216800 cocoons per year for primary purchaser, wholesaler and silk processor, respectively. The aggregation of data has revealed that the traditional wholesaler (reeler cum weaver) handled 77.87 % of the

total cocoons marketed per year followed by 12.66 % and 9.46 % handled by silk processor and primary purchaser, respectively. Full time family involvement in cocoon marketing was significant as it consumed 0.72 man days/trader family on full time basis and 0.28 man days/trader family as a part time basis. A majority of tasar cocoon market agents (86.11 %) hold their business as single proprietor whereas the partnership pattern of business was operated by the remaining agents. Geetha (2011) inferred that women falling in the age group of 25-40 years with low education status had 20 years of vast sericulture experience and taken up sericulture as a main remunerative occupation in Karnataka state of India.

Tasar cocoon trading offers off farm employment to considerable number of people in the state, who derive not only livelihood but also substantial portion of their household income from cocoon marketing. Of the 72 cocoon market agents, 43 (60.00 %) relied fully on cocoon marketing as the source of their household income. The cocoon market agents had 28 years of experience on an average in market dealings, which denotes that the enterprise is an age old practice. It is pertinent to note that only 18 per cent had inherited the business from their ancestors while 82 per cent agents had started it on their own. More or less similar trends were also reported in traditional milk marketing system of Assam (Kumar *et al.*, 2010) state of India. The opportunities for income and employment generation might have prompted the large portion of agents to adopt this business themselves.

b. Costs and margins in cocoon marketing

The data presented in Table 2 reveals the average marketing costs and margins involved in marketing of tasar cocoon. Only variable cost was considered for estimating the market margin. The variable costs mainly consisted of transaction costs in purchasing and selling of cocoons and on the transportation in particular. No expenditure was incurred by tasar cocoon producers in marketing because they sell their produce to primary purchaser. Tasar cocoons produced in August, October and November months are usually marketed as reelable cocoons in the open auction markets. The quantity of each commercial cocoon sale was in the range of 20 to 25 lakh cocoons with sale turnover of ₹ 26.00 lakh in the state.

The primary purchaser received the highest margin of ₹150.00 by selling 1000 cocoons for ₹ 1325.00. Low margins were realized by wholesaler (₹ 100.00), silk processor (₹ 95.00) and RMB (₹ 67.00) after selling 1000 cocoons for ₹ 1622.00, 1782.00 and 1314.00, respectively. The highest marketing cost of ₹ 197.00 per 1000 cocoon sale was incurred by wholesaler when compared to RMB (₹ 122.00), primary purchaser (₹ 75.00) and silk processor (₹ 65.00). All cocoon marketing traders spent more on transportation of cocoons. Primary purchaser and wholesaler incurred considerable loss of ₹ 40.00 and 35.00, respectively caused by rodent attack to stored cocoons (Table 2). Generally, storage of cocoons and their

Table 2: Net price distribution and margins among different functionaries of cocoon market chain (₹/1000 cocoons)

Cocoon marketing trader	Cost of cocoons	Marketing cost				Price received	Marketing margin	Marketing efficiency
		Transportation cost	Storage charges	Misc. costs	Total			
Primary purchaser	1100.00	20.00	40.00	15.00	75.00	1325.00	150.00	4.89
Wholesaler	1325.00	140.00	35.00	22.00	197.00	1622.00	100.00	4.46
Silk processor	1622.00	65.00	-	-	65.00	1782.00	95.00	10.13
RMB (raw material bank)	1125.00	80.00	15.00	27.00	122.00	1314.00	67.00	5.95
Mean	1293.00	76.25	22.50	16.00	114.75	1510.75	103.00	6.36
All	5172.00	305.00	90.00	64.00	459.00	6043.00	412.00	-

sale to buyers depend mostly on the market demand and existing admissible rates. Thus, it is quite apparent that the wholesaler is a vital player in the marketing of cocoons in the study area.

The net price spread in trading was ₹ 871.00 that includes marketing costs and margins and the lion shares come from wholesaler (34.10 %) and primary purchaser (26 %) indicating their roles. The marketing efficiency of tasar cocoon can be estimated by dividing the net price received by cocoon producer with marketing costs and margins. Marketing efficiency is considered as inefficient when the unit value is less than one. The cocoon markets were found highly efficient as reflected in the higher unit values (4.46 to 10.14) because of lower marketing margins and costs. Better marketing efficiency is predictable by averting commission agents interfering in cocoon trading.

c. Producer's share in consumer price

Cocoon marketing system in Andhra Pradesh is fairly complex. A number of different marketing agents interact at various levels in carrying silk cocoons from producers to consumer. Out of four functional marketing channels in the state, the main chain *i.e.*, producer - primary purchaser - wholesaler - raw silk processor was found more successful.

In each marketing channel, the selling price of wholesaler/silk processor is considered as consumer's purchase price (Table 3). The net price received by the cocoon producer was in the range of ₹ 1.10-1.33 per cocoon in marketing by open auction which constitutes 67.82 – 74.35 % of consumer's price depending upon the marketing channels. The producer's share in consumer rupee (68 to 84 %) is an assessment of the relative bargaining position in the milk market of Assam state of India (Kumar *et al.*, 2010). It also reflects producer's access and integration with the market. Naidu *et al.*, 2003 in their study on price spread in Andhra Pradesh observed that farmers' share in consumer's purchase price was around 64 to 81 % and Varadaraj *et al.*, 2003 found it around 45 to 95 % in marketing of important minor forest produce (MFP) in Tamilnadu state of India. While computing net price spread in Lac market in Madhya

Pradesh, Govindpal *et al.*, 2009 reported that Lac grower's share in consumer's price was around 77 %. A perusal of Table 3 reveals that the producers got a higher share when they sold cocoons directly to wholesaler or silk processor and ensured a higher share in the consumer's rupee to the producer. The producers got the lowest share as well as the lowest absolute amount when they sold to the primary purchaser. The milk producer's share (CALPI, 2006) in the consumer's price of milk in different parts of the country has been reported to be 50 to 98 %.

Table 3: Producer's share in consumer's rupee in different marketing channels

Marketing channel	Selling/Purchase price at various levels (₹/1000 cocoons)				Producer's share in consumer price %
	I	II	III	IV	
Producer - primary purchaser - wholesaler	1100.00	1325.00	1622.00	-	67.82
Producer - primary purchaser - wholesaler - raw silk processor	1100.00	1325.00	1622.00	1782.00	67.81
Producer - RMB-wholesaler-raw silk processor	1125.00	1314.00	1622.00	1782.00	63.13
Producer - whole saler- raw silk processor	1622.00	1782.00	-	-	91.02

d. Determinants for volume of cocoons marketed by informal cocoon market agents

The association of marketing agent variables with business volume (cocoons handled by a trader) is presented in Table 4. The independent variables such as initial capital and source of finance were found to have positive and significant association with business volume supporting the fact that initial capital contributes more in marketing. The variables namely, age, experience, family participation, market location, ownership of business and type of trader depicted negative association with the cocoon market. The mode of business variable was positively and highly significantly correlated with dependent variable (Table 4). With respect to business volume, type of trader was found to have negative and significant association. The association of female family

members (Srinivasa *et al.*, 2004) was found negatively correlated with income and cocoon yield. This corroborated the fact that sericulture is not practiced solely for income, but also for keeping them employed.

Table 4: Association of factors of marketing agents on business volume in the study area (correlation values)

Variable	Business volume (Cocoons handled/trader)
Age	- 0.211
Education	0.302*
Initial capital	0.426*
Experience	- 0.004
Occupation	0.150
Family participation	- 0.016
Type of trader	- 0.684*
Distance travelled	0.244
Travel period	0.276
Ownership of business	- 0.070
Market location	- 0.040
Mode of business	0.492
Source of finance	0.162

* Significance at 5 % level

The relationship between business volume (no. of cocoons handled/trader) and socio-economic characters of tasar cocoon market agents (Table 5) viz., education, initial capital and mode of business acquisition was found significant and positively related whereas source of finance variable was significant with negative correlation. The type of trader and ownership of business had a direct and highly significant relationship with volume of cocoon handled in the market. The low level of education, initial capital, type of trader, ownership of business, source of finance and mode of business acquisition seemed to be hurdles in high volume of business. The financial capacity of tasar cocoon market agent also dictated the level of business. While studying potential, income and employment generation in milk marketing of Assam state, Kumar *et al.*, 2010 observed that level of education, financial capacity and distance travelled were the variables which determined the volume of milk handled by the marketing agents. The cocoon marketing agents who relied solely on income of cocoon trading for their livelihood tended to have a higher level of business. Association with a single ownership was

observed to have brought in more expertise and enhanced skill to increase the business volume. Variables such as distance, travel time and market location did not influence the dependent variable *i.e.*, volume of business as they exhibit non significant relationship with trading.

The cocoon market agents who travelled shorter distance by spending less time were intended to handle higher volume of cocoon trading at a stretch. Cocoon marketing mainly contributed to the household income of agent as major source of finance in Andhra Pradesh.

Table 5: Factors influencing the cocoon handling (trading) by traders (N=72)

Variable	Co-efficient (b)	Standard Error	t' value
Age	-1567.70	0.113	1.806
Education	1514.96*	0.108	2.652
Initial capital	1696.00*	0.096	3.698
Experience	3.33	0.117	0.033
Occupation	- 86.23	0.115	1.356
Family participation	102.50*	0.118	0.1835
Type of trader	88.60**	0.063	7.842
Distance travelled	0.8664	0.111	2.105
Travel period	0.1765	0.109	0.250
Ownership of business	-441.88**	0.118	6.780
Market location	-196.50	0.0116	0.335
Mode of business /acquisition	73.83*	0.089	4.736
Source of finance	-241.80*	0.105	2.988
Constant	7.526	-	-
R ²	11.028	-	-

*Significance at 5 % level; **Significance at 1 % level

CONCLUSION

It may be inferred that most of the tasar cocoon marketing agents in Andhra Pradesh are small traders who derive above 75 % of their household income from it. The marketing cost and margins were considered as main

indicators of marketing efficiency. The primary purchasers, wholesalers and silk processors were found to be highly efficient (4.46 to 10.14) in marketing because of lower marketing costs and margins. It is observed that the tasar cocoon producer received ₹ 1100.00, 1325.00 and 1622.00 per 1000 cocoon sale and shared 67.82, 74.35 and 91.02 % of respective consumer's price. In existing marketing channels, the selling price of silk processors' goods was considered as buying price of consumer (end user) for estimation of producer's share in consumer's price. Primary purchaser received the margin which was quite double of marketing cost. Wholesaler and primary purchaser preferred to procure entire cocoon lots at once in a year that led to storage of cocoons and subsequent losses by rodents attack. Setting up of more reeling units should be promoted locally by imparting training in post cocoon technology to farmers of Andhra Pradesh so that regular and speedy disposal of cocoons could be achieved for the benefit of farmers in the state.

Central Silk Board, Govt. of India has introduced on line futures marketing system in silk cocoon and garments producing areas in order to facilitate nationwide trading at efficient and transparent prices between cocoon producers and buyer. Rao (2009) and Elumalai (2010) reported that the futures marketing has potential role in minimizing the price risk faced by the farmer. It is an agreement between a buyer and a seller obligating the seller to deliver a specified asset of specified quality and quantity to the buyer on a specified date at a specified place and the buyer in turn is obligated to pay to the seller a renegotiated price in exchange of the delivery. Much awareness on the advantage of online future's trading is to be created among the cocoon producers and buyers for scaling up of volume of cocoon business at specified place and period.

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DETERMINANTS SOCIO-ECONOMIQUES DES AGENTS DU MARKETING TRADITIONNEL DES COCONS TASAR EN ANDHRA PRADESH, INDE

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R E S U M E

Nous avons étudié l'influence des facteurs socio-économiques sur le volume d'affaires, le coût du marketing et le prix dans le système traditionnel de marketing des cocons en Andhra Pradesh. Les données primaires ont été collectées chez 72 agents de marketing après un test du planning. Comme définis par les principaux systèmes de marketing existants, les agents du marché sont les acheteurs primaires, les grossistes (filateurs-tisseurs) et les transformateurs de soie (du moulinage aux tissus). Les variables comme l'éducation, le capital initial, le type de négociants, la propriété de l'affaire, et le mode d'acquisition de l'affaire et la capacité financière des agents sont en relation directe et significative avec le volume des cocons traités dans un marché ouvert. Les producteurs de cocons tasar perçoivent un prix compris entre 1100 et 1622 pour 1000 cocons correspondant à 67.81 – 91.02 % du prix payé par le consommateur. L'acheteur primaire double sa marge à 150.00 en subissant un coût de marketing de 75.00 pour 1000 cocons. Le prix répandu dans les échanges est de 871.00 pour 1000 cocons et la part majeure vient au grossiste (34.10 %) et à l'acheteur primaire (26.00 %) ce qui montre l'importance de ces deux segments. L'efficacité du marketing est élevée (4.46 – 10.14) puisque l'unité est supérieure à un. En établissant plus d'unités de filature, un système futur d'échanges en ligne et une connaissance de la qualité des cocons on peut obtenir les instruments pour un marketing rapide des cocons tasar.

Mots-clés: Agents du marketing, coûts du marketing et marges, prix courant, facteurs socio-économiques.



ISOLATION AND CHARACTERIZATION OF GUT-BACTERIA OF MUGA SILKWORM (*ANTHRAEA ASSAMENSIS* HELFER) COLLECTED FROM DIFFERENT LOCALITIES OF ASSAM

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ABSTRACT

Muga silkworm (*Antheraea assamensis* Helfer) producing golden-yellow silk is a semi-domesticated, multivoltine, sericigenous insect endemic to North-east India. *A. assamensis* primarily feeds on Som (*Persea bombycina* Kost) leaves, the major ingredients of which being pectins, cellulose, starch, xylan, lipid and fatty acids. Gut microflora of muga silkworm synthesize digestive enzymes and play a functionally significant role by degrading the leaf contents to provide better nutritional uptake by the silkworm and eventually improve the productivity. In the present study, bacteria were isolated from the gut of muga silkworm larvae collected from different localities of Assam by culture dependent technique using Nutrient Agar and Brain Heart Infusion Agar media. The 12 bacterial isolates comprised of eight gram positive rods, two gram negative rods, one gram positive coccus and one gram negative coccus. Further biochemical characterization of the gut-bacteria was carried out by using rapid identification kit (Himedia Ltd., Mumbai). On the basis of biochemical characteristics, the dominant gut-bacteria identified were *Bacillus* sp., *Proteus* sp., *Escherichia coli* etc. *In-vitro* screening of the isolates were done by amylase, pectinase, xylanase, lipase and cellulase test. The positive results revealed the possible influence of gut-bacteria on digestion and utilization of leaf carbohydrates, lipids and fatty acids for better productivity of Muga.

Key words: Amylase, *Antheraea assamensis*, cellulase, gut microflora, pectinase, xylanase.

INTRODUCTION

Muga silkworm (*Antheraea assamensis* Helfer) is a semi-domesticated, multivoltine, sericigenous insect endemic to North-east India. It produces the natural glistening golden-yellow silk of good economic value. Initially, Muga silk production was confined to the state of Assam and later, adopted by other North Eastern states and certain parts of West Bengal. Muga silkworm life cycle include four stages viz., egg, larval instars, pupa within cocoon and adult moth. The larvae primarily feed on the leaves of Som (*Persea bombycina*) and Soalu (*Litsea polyantha* Juss) plants belonging to the family, Lauraceae. The major components of *P. bombycina* leaves are carbohydrate, pectins, cellulose, starch, xylan, lipid and fatty acids (Sharma and Devi, 1997; Singh *et al.*, 2000; Yadava and Goswami, 1992; Dutta *et al.*, 1997;

Choudhury *et al.*, 1998).

The entire insect gut contains diverse groups of microorganisms that are potential source of digestive enzymes (Zhang and Brune, 2004) and metabolites (Wilkinson and Disloyalty, 2001). They play significant role in nutrition, immunity, growth and reproduction (Brand *et al.*, 1975, Brune *et al.*, 1995; Moran *et al.*, 2005). The bacterial community harboured by mid guts of Lepidopteran insects produces digestive enzymes and help in digestion of leaf constituents such as cellulose, xylan, pectin and starch (Dillon and Dillon, 2004). Different types of cellulose, xylan, pectin and starch degrading microflora have been isolated and characterized from 5th instar larvae of *B. mori* (Khayade and Marathe, 2012). *Streptomyces noursei*, is a potential gut bacteria

that can be used for eco-friendly management of silkworm diseases (Subramanian *et al.*, 2009). The *Bombyx mori* guts contain certain beneficial microflora and secrete lipase that might be related to BmNPV resistance in the silkworm (Wei Feng *et al.*, 2011).

However, there is very limited information on the microbial population in the gut of muga silkworm, *A. assamensis*. The knowledge of bacterial composition will facilitate to explore and exploit the function of the gut microflora. In this study, an effort has been made to isolate and characterize the gut microflora of muga silkworm collected from different localities of Assam.

MATERIALS AND METHODS

Collection of sample

The 4th and 5th instar larvae of *A. assamensis* were collected from different localities of Assam. The silkworm samples were collected aseptically and immediately brought to the laboratory for dissection. The relevant information on prevalent temperature, humidity, rainfall, host plant, date of brushing *etc.* were taken under consideration.

Isolation and enumeration of gut microflora

The 4th and 5th instar larvae of Muga silkworm were dissected and the gut-microflora was isolated as per the method described by Khyade and Marathe (2012) with a little modification, in the laboratory. The larval samples were kept under starvation for 24 h so that no faecal particle remains in the alimentary tract. The larvae were anesthetized with a chloroform soaked cotton pad and surface sterilized with 70 % ethanol. The entire alimentary canal of the larvae was dissected out aseptically in a UV laminar flow chamber (Labotech, India). The isolated digestive tract was washed with sterile, cold 0.9 % NaCl solution and cut into small pieces with a sterile scalpel, homogenized in 0.1 M Potassium Phosphate buffer and incubated at 37°C for 1 h. The sterile homogenate was centrifuged at 10,000 rpm (REMI, C24-BL) for 10 minutes and the supernatant was taken as stock for preparation of dilution up to 10⁻⁵.

The gut bacteria were isolated by pour plate method

using Nutrient agar (Hi-media Ltd., Mumbai) and Brain heart infusion agar (Hi-media Ltd., Mumbai) containing different substrate. The plates were incubated (OVFU, O-CIS-4D) at 32°C for 24 to 48 h. Enumeration was done by total viable colony count and expressed as the number of colony forming units (CFU) in 1 ml of homogenate sample.

Morphological and biochemical characterization

The isolates were tested in terms of Gram reaction and biochemical characteristics (Holt *et al.*, 1994) and further identified on the basis of pigment, colony form, elevation, margin, texture and opacity (Smibert and Krieg, 1981). Biochemical tests performed for both Gram positive and negative bacteria were Citrate utilization, Lysine utilization, Ornithine utilization, Urease, Phenylalanine deamination, Nitrate reduction, H₂S production, Glucose, Adonitol, Lactose, Arabinose, Sorbitol, Malonate, Voges proskaur, ONPG, Catalase, Arginine, Sucrose, Mannitol and Trehalose by using Biochemical Test Kit (Hi-media Ltd., Mumbai).

SCREENING FOR ENZYMATIC ACTIVITY

Amylase

Amylase test was carried out by inoculating/streaking the gut-bacteria into medium containing starch 20 g, (NH₄)₂SO₄ 1 g, glucose 1 g, NaCl 1g, MgSO₄.7H₂O 1 g, sodium thiosulphate 1 g, K₂HPO₄ 2 g, Agar 20 g and H₂O 1l. The plates were incubated at 32°C for 5 days and a few drops of iodine solution (iodine 1 g, KI 2 g and H₂O 300ml) poured over the colonies. Destaining of blue colour around the colonies indicated positive test for amylase. Qualitative estimation of amylase producing bacteria was calculated as hydrolysis capacity (HC) *i.e.*, the ratio of diameter of clearing zone and colony (Hendricks *et al.*, 1995).

Cellulase

Cellulose degrading ability of the isolated gut bacteria was performed by streaking on the Cellulose Congo-red agar media (KH₂PO₄ 0.5 g, MgSO₄ 0.25 g, CMC 2 g, agar 15 g, Congo-red 0.2 g, Gelatin 2 g and distilled water 1l; pH 6.8-7.2). Colonies showing discolouration of the

Congo-red were considered as positive cellulose-degrading bacteria. Hydrolysis capacity was calculated as per the method mentioned above.

Xylanase

The test for xylanase activity was carried out by streaking the bacteria on NA medium plate supplemented with 0.01 % xylan. The plates were incubated for 24 h and 10 ml of Congo-Red (1 mg/ml H₂O) was added followed by incubation at 32°C for 15 minutes. The Congo-red was poured off and added 10 ml of 1M NaCl solution and again incubated for 15 minutes. Appearance of clear zone around the colonies indicated positive test for xylanase.

Pectinase

Screening for pectinase was performed by using Pectinase Screening Agar Medium (PSAM; pH 4.5) with the composition of pectin 10 g, (NH₄)₂HPO₄ 3 g, KH₂PO₄ 2 g, K₂HPO₄ 3 g, MgSO₄ 0.1 g, Agar 15 g and H₂O 1 l. The cultures were incubated at 37°C for 24 h and then flooded with 50 mM iodine solution. After incubation for 15 min., appearance of clear zone around the growth of the bacteria confirmed positive pectinase test.

Lipase

Lipase test was carried out by inoculating the isolates in tributyrin agar medium (pH 6.0) containing peptone 5 g, yeast extract 3 g, tributyrin 10 ml, agar 15 g and H₂O 15 ml. The plates were inoculated at 32°C for 48 h. A clear zone developed around the colonies of fat-splitting organisms indicated lipase positive reaction. Lipase HC was calculated as the ratio of diameter of clearing zone and colony.

RESULTS

Muga silkworm larvae (100 nos.) of 4th and 5th instar were collected randomly from ten different farms of Assam and subjected to isolation of gut microflora. The average Muga silkworm gut-bacterial population enumerated as total viable count (CFU) was more in the sample collected from Charaideo farm (2.500 x 10⁶ CFU/ml). Variation in the cultivable bacterial population was observed in the gut-homogenates derived from the

larval samples collected from different localities (Table 1).

Altogether, 12 bacterial strains were isolated from the Muga silkworm gut homogenates prepared from the collected larval samples. Out of them, eight nos. were gram positive rods (Figure 1 A,B,C), two gram negative rods (Figure 1D), one gram positive coccus (Figure 1E) and one gram negative coccus (Figure 1F). They were found to be arranged in different orientations under microscope. The cultural and morphological characterization of the isolates is presented in Table 2.

All the bacterial isolates exhibited diverse biochemical properties during the analysis for the biochemical tests as per Bergey's Manual of Determinative Bacteriology and Biochemical Test Kit (Hi-media Ltd., Mumbai). The results of the biochemical characterization are shown in Table 2. Further, the results of the tests for amylase, cellulase, xylanase, pectinase and lipase are represented in Table 3. Isolate no. MGS-5 showed positive results for all the enzymatic tests, whereas isolate nos. MGB-10 and MGB-12 exhibited prominent cellulolytic activity.

The isolate no. MGS-5 has shown positive results for gram stain, motility, cellulase, xylanase, pectinase, amylase, catalase and sugar utilization test. On the basis of biochemical properties, the isolate no. MGS-5 was identified as genus *Bacillus*. The MGB-10 has been identified as *Escherichia coli*, as it is gram negative rod and showed positive test for methyl red, oxidase, indole, catalase, sugar utilization, whereas negative for cellulase, xylanase, pectinase, amylase, VP, urease and gelatin liquefaction. Isolate no. MGB-12 was gram negative rod shaped bacterium which exhibited positive biochemical tests for indole, methyl red, catalase, cellulase, glucose and negative for VP, complex sugar like starch, mannitol, lactose *etc.* and hence may be considered as belonging to the genus *Proteus* (Table 4).

DISCUSSION

The isolation and enumeration of gut bacteria revealed that the microbial population is more in the Muga silkworm larvae collected from Charaideo localities. The gut-microbial population in total viable count varies depending upon the environment of localities, silkworm

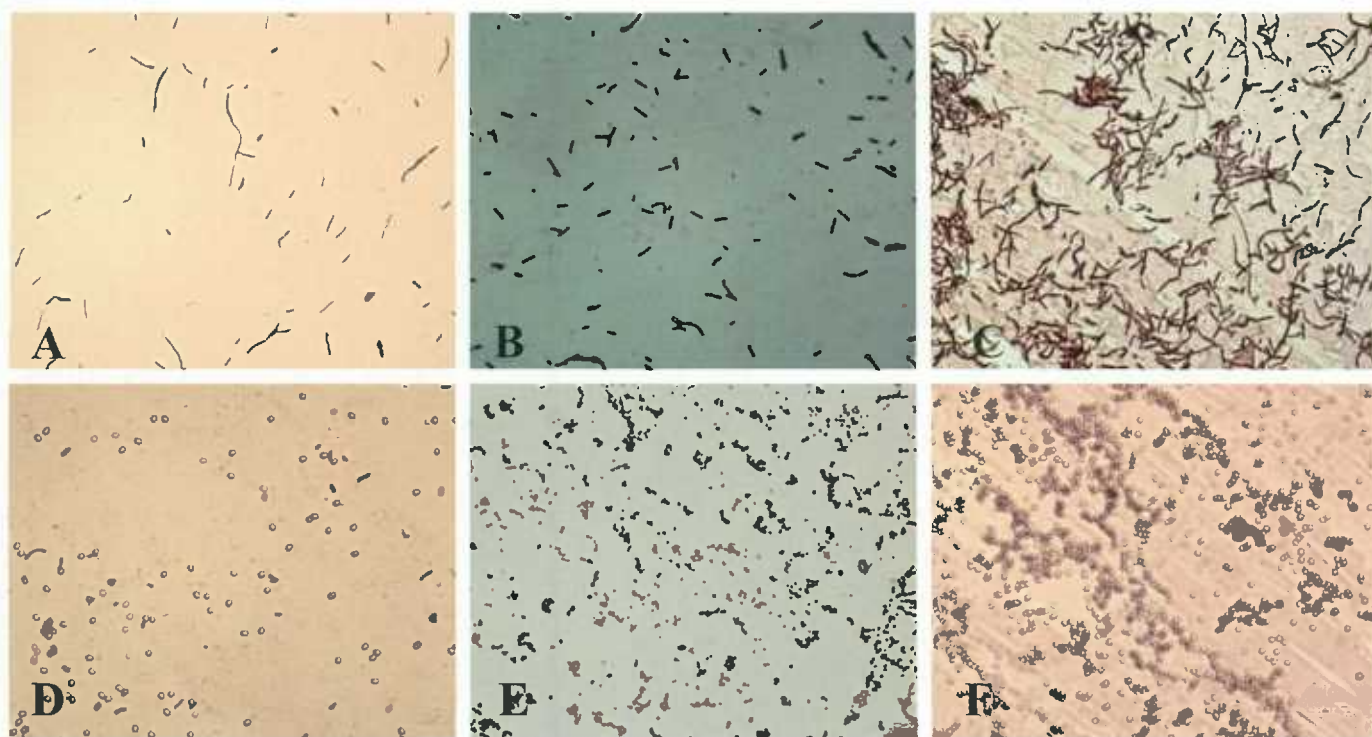


Figure 1: Muga silkworm gut-bacteria: A- isolate no. MGS5; B- isolate no. MGBS3; C- isolate no. MGS13; D- isolate no. MGB10; E- isolate no. MGB8; F- isolate no. MGB14.

Table 1: Gut-bacterial population of muga silkworm larvae collected from different farms

Locality/Farm	Bacterial population (CFU/ml)
Farm no. 1, CMERTI, Lahdoigarh	1.24×10^5
Farm no. 2, CMERTI, Lahdoigarh	9.80×10^5
Farm no. 3, CMERTI, Lahdoigarh	6.56×10^5
Titabor farm	2.350×10^5
Goleghat	2.200×10^6
Jagduar	2.150×10^6
Charaideo	2.500×10^5
Kazhiranga	1.850×10^6
Sivasagar	7.24×10^5
Bor-ahom gaon	1.670×10^6

health, feeding potential and eco-races or breeds (Nangia *et al.*, 1999; Subramanian *et al.*, 2010). The silkworm digestive tract is highly alkaline and very rich in culturabile as well as unculturable microbial diversity throughout the entire larval period (Steinhaus, 1963; Mohanraj *et al.*, 2009). In this study, as in the mulberry silkworm (*Bombyx mori*), the bacteria were found to be dominant microflora in the gut of *A. assamensis*. The

morphological and biochemical features of bacteria are important key for their identification. The dominant bacteria species in Muga silkworm gut were identified as per biochemical classification (Bergey's Manual of Systematic Bacteriology) as *Bacillus* sp., *Escherichia coli* and *Proteus* sp. Khayade and Marathe (2012) had reported certain common gut bacterial species viz., *Bacillus circulans*, *Citrobacter freundii*, *Serratia liquefaciens*, *Proteus vulgaris*, *Klebsiella pneumonia*, *Enterobacter* sp., *Escherichia coli* etc. in the mid gut of mulberry silkworm. The gut bacteria in tropical tasar silkworm, *A. mylitta* D. were isolated and characterized by Singh *et al.* (2011).

The functional role of gut microflora has been studied extensively in some economically important insects and their positive influence in enhancing the larval growth has been reported by many workers (Indiragandhi *et al.*, 2008; Visotto *et al.*, 2009).

Katiyar and Pant (1987) reported the presence of microorganisms in both the mid and hind gut regions of eri

Table 2: Morphological and biochemical characteristics of the gut-bacterial strains isolated from muga silkworm (*A. assamensis*)

Isolate	Gram stain	Shape	Biochemical test (Gram positive bacteria)													Sugar utilization test				
			MR	Indole	Citrate	Catal-ase	Urease	NR	VP	Malon	ONPG	SH	GL	Argini- ne	Glucose	Sucrose	Manni- tol	Arabi- nose	Trehal- ose	
																				-ate
MGBS2	'+'ve	Oval	+	+	+	+	+	+	-	+	+	+	-	+	-	+	+	-		
MGBS3	'+'ve	Rod	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
MGS4	'+'ve	Short rod	+	+	+	+	-	+	-	+	+	+	-	+	-	+	+	+		
MGS5	'+'ve	Rod	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
MGS6	'+'ve	Short rod	-	-	+	-	-	-	+	-	+	+	+	+	+	+	+	+		
MGB8	'+'ve	Coccus	+	+	+	+	-	-	+	+	+	-	-	+	+	+	+	-		
MGS9	'+'ve	Short rod	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+		
MGS13	'+'ve	Rod	+	+	+	+	+	+	+	-	+	+	-	+	-	-	+	-		
MGBS6	'+'ve	Oval	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Isolate	Gram stain	Shape	Biochemical test (Gram positive bacteria)													Sugar utilization test				
			MR	Indole	Citrate	Lysine	Urease	NR	VP	Phenyl	H ₂ S	SH	GL	Ornit- hine	Glucose	Lactose	Arabin- ose	Sorbitol	Adonitol	
																				alanine
MGB10	'-'ve	Rod	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+	-		
MGB12	'-'ve	Rod	+	-	+	+	+	+	+	+	+	+	+	+	-	+	-	+		
MGB14	'-'ve	Coccus	+	+	+	+	+	+	+	+	+	-	-	+	-	+	-	+		

MR: Methyl red; NR: Nitrate reductase; VP: Voges proskaur; SH: Starch hydrolysis; ONPG: β -galactosidase; GL: Gelatin liquefaction.**Table 3: Enzymatic activities of the gut-bacteria isolated from the digestive tract of *A. assamensis*.**

Isolate	Hydrolysis capacity				
	Amylase	Cellulase	Xylanase	Pectinase	Lipase
MGBS2	+ 1.125	- NA	- NA	+ 1.049	+ 1.013
MGBS3	+ 1.106	- NA	- NA	- NA	+ 1.087
MGS4	+ 1.023	+ 1.021	+ 1.018	NA	+ 1.048
MGS5	+ 1.217	+ 1.226	+ 1.261	+ 1.112	+ 1.067
MGS6	+ 1.184	- NA	- NA	- NA	+ 1.091
MGB8	- NA	- NA	+ 1.214	- NA	+ 1.214
MGS9	+ 1.321	NA	+ 1.118	+ 1.034	+ 1.143
MGS13	+ 1.162	+ 1.014	NA	- NA	+ 1.031
MGBS6	+ 1.084	NA	NA	- NA	- NA
MGB10	- NA	+ 1.012	- NA	NA	+ 1.333
MGB12	- NA	+ 1.157	+ 1.164	- NA	- NA
MGB14	+ 1.114	- NA	NA	- NA	- NA

Numericals: Hydrolysis capacity - the ratio of diameter of clearing zone and colony; NA: Not applicable.

silkworm, *Philosamia ricini* that secrete the enzyme cellulase and assists in the digestion of cellulose content of castor leaves. The present study showed that certain Muga silkworm gut-bacteria have got ability to hydrolyze the major leaf contents *viz.*, starch, cellulose, pectin, xylan, fat, lipid *etc.* This reflects the possibilities of their role in enhancement in the digestion process of Muga silkworm. The endogenous gut-bacteria that synthesize digestive enzymes can be utilized as probiotic consortium for improvement of productivity. Application of commercial *Lactobacillus plantarum* improves the larval weight, cocoon weight, shell weight and ERR of mulberry silkworm (Singh *et al.*, 2005). Gut microflora biosynthesize secondary metabolites like antibiotics, vitamins and growth hormones which can also inhibit or counteract the invading pathogens in silkworm and other insects (Watve *et al.*, 2001). Moreover, silkworm probiotic plays significant role in development of colonization resistance against pathogenic microorganisms.

Table 4: Identification of gut-bacteria isolated from the digestive tract of *A. assamensis*

Characteristic feature	Bacterial isolate		
	MGS5	MGB10	MGB12
Gram stain	Positive	Negative	Negative
Morphology	Rod	Rod	Rod
Methyl red test	+	-	+
Indole test	+	+	+
Citrate utilization	+	-	+
Catalase test	+	+	+
Urease test	+	+	+
Nitrate reductase	+	+	+
Voges proskaur	+	+	+
ONPG test	+	+	+
Gelatin liquefaction	+	+	-
Phenylalanine deaminase	+	+	+
H ₂ S production	-	+	+
Amylase test	+	+	+
Lipase test	+	+	+
Cellulose utilization	+	+	+
Pectin utilization	+	+	+
Xylan utilization	+	-	+
Arginine	+	+	-
Ornithine	+	+	+
Sugar utilization			
Glucose	+	+	+
Sucrose	+	-	+
Lactose	+	+	+
Mannitol	+	-	-
Arabinose	+	+	+
Trehalose	+	-	+
Sorbitol	+	+	-
Adonitol	+	+	+
Identification	<i>Bacillus</i> sp.	<i>Escherichia coli</i>	<i>Proteus</i> sp.

There are very sporadic reports on the characterization of the microbial diversity prevalent to muga silkworm digestive tract. Isolation, characterization, screening and partial identification of *A. assamensis* gut-bacteria will provide firsthand information to assess the beneficial microflora towards the formulation of probiotics consortia for better productivity in muga sector of sericulture industry.

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ISOLEMENT ET CARACTERISATION DE BACTERIES INTESTINALES DU VER A SOIE MUGA (*ANTHRAEA ASSAMENSIS* HELFER) COLLECTE DANS DIFFERENTES LOCALITES EN ASSAM

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R E S U M E

Le ver à soie Muga (*Antheraea assamensis* Helfer) qui produit de la soie jaune d'or est un insecte séricigène semi-domestiqué, polyvoltin et endémique du Nord-est de l'Inde. *A. assamensis* mange préférentiellement des feuilles de Som (*Persea bombycina* Kost), dont les composants majeurs sont des pectines, de la cellulose, de l'amidon, des xylanes, des lipides et des acides gras. La flore microbienne intestinale du ver à soie muga synthétise des enzymes digestives et joue un rôle fonctionnel significatif en dégradant les contenus foliaires pour fournir une meilleure absorption nutritionnelle par le ver à soie et améliorer la productivité. Dans cette étude, des bactéries ont été isolées de l'intestin de larves du ver à soie muga collectées dans différentes localités en Assam par culture sur des milieux Agar. Les 12 isolats bactériens comprennent huit gram-positifs en bâtonnets, deux gram-négatifs en bâtonnets, un gram-positif en coques et un gram-négatif en coques. Une caractérisation biochimique des bactéries intestinales a été effectuée en utilisant un kit d'identification rapide (Himedia Ltd., Mumbai). Sur cette base, les bactéries dominantes identifiées sont *Bacillus* sp., *Proteus* sp., *Escherichia coli* etc. Le criblage *in vitro* des isolats a été effectué par des tests amylase, pectinase, xylanase, lipase et cellulase. Les résultats positifs révèlent l'influence possible des bactéries intestinales sur la digestion et l'utilisation des hydrates de carbone, des lipides et des acides gras foliaires pour une meilleure productivité du Muga.

Mots-clés: Amylase, *Antheraea assamensis*, cellulase, microflore intestinale, pectinase, xylanase.



BIOLOGY AND BEHAVIOUR OF THE LEAF-ROLLER, *DIAPHANIA PULVERULENTALIS* (HAMPSON) (LEPIDOPTERA: PYRALIDAE) AT DIFFERENT TEMPERATURES

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ABSTRACT

Life cycle features of *Diaphania pulverulentalis*, a pest of mulberry, *Morus alba* L. (Moraceae) were studied at three different conditions of temperatures of $20 \pm 1^\circ\text{C}$, $25 \pm 1^\circ\text{C}$ and $30 \pm 1^\circ\text{C}$. The developmental cycle of this insect is characterized with distinct stages of egg, larva, pre-pupa and pupa. There was a clear trend of decrease in the duration of different stages viz., egg (5.93, 3.77, 2.47 days), caterpillar (11.61, 9.88, 6.50 days), pre-pupa (3, 2.38, 1.77 days) and pupa (12.70, 9.16, 6.46 days) corresponding to increase in temperature ($20 \pm 1^\circ\text{C}$, $25 \pm 1^\circ\text{C}$ and $30 \pm 1^\circ\text{C}$, respectively). The average duration of life cycle of *D. pulverulentalis* at 20, 25 and 30°C was 33.24, 25.19 and 17.2 days, respectively with the mean fecundity of 399.2, 373.2 and 250 eggs, respectively. A detailed account on the studies carried out on mating, oviposition, longevity and sex-ratio is provided in this paper.

Key words: Biology, *Diaphania pulverulentalis*, fecundity, life cycle, longevity, mating, oviposition, sex-ratio.

INTRODUCTION

The leaf-roller, *Diaphania* (*Glyphodes*, *Margaronia*) *pulverulentalis* (Hampson) (Lepidoptera: Pyralidae) was first recorded from Ngas, Tenasserim (Nagaland) and described (Hampson, 1896). This pest was reported to infest mulberry plantations in Malaysia (Sengupta *et al.*, 1990). Later, Siddegowda *et al.* (1995) have reported *Diaphania* sp. as a pest of mulberry in Karnataka, Andhra Pradesh and Tamil Nadu in South India.

The leaf-roller feeds on tender mulberry leaves, used for rearing young silkworms and also damages the terminal bud. As a result, the plant ceases to grow and the leaves at the lower portion mature fast. The quantity of mulberry leaf loss due to leaf-roller infestation during rainy and winter season was 16.17 and 12.46 %, respectively. Seasonal occurrence of the mulberry leaf-roller and its parasitoids was studied and reported (Geetha Bai and Marimadaiah, 2002). Age-specific fertility life-table of leaf-roller was also worked out at different temperatures (Marimadaiah and Geetha Bai, 2006). Adequate information on the biology and behaviour of

leaf roller, *D. pulverulentalis*, as generated in the present work assumes significance in view of effective management strategies, yet to be formulated for this insect pest.

MATERIALS AND METHODS

Life cycle

Freshly emerged male and female moths obtained from the laboratory colony were released on potted mulberry plants, enclosed by nylon net for oviposition. They were provided with 50 % aqueous honey solution. The moths were observed periodically and the number of eggs laid on the leaf were recorded. Subsequently, eggs were transferred to petridishes (10 cm diameter) individually. On hatching, the caterpillars were fed daily with tender and fresh mulberry leaves till pupation. The larval instars were determined by periodical observations on moulted cuticle. The incubation period of eggs, development durations of larvae, pre-pupae and pupae were recorded. Further, the length and breadth of eggs, larvae (first and last day of every instar), pre-pupae (first and last day) and pupae were recorded. This experiment was replicated 10 times.

Studies on the life cycle was conducted at three different temperatures and humidity conditions viz., $20 \pm 1^\circ\text{C}$, RH: 70-85 %; $25 \pm 1^\circ\text{C}$, RH: 70-85 % and $30 \pm 1^\circ\text{C}$, RH: 55-70 %. Analysis of variance was employed to find out the variations in the data obtained at three temperatures on different stages of the leaf-roller pest.

Oviposition behaviour

Four days old, honey fed, gravid, mated *D. pulverulentalis* female moth, was released on a potted mulberry plant, enclosed in a nylon net. Insect plant contact was ensured for oviposition from morning to sunset and the same moth was transferred to another plant from sunset till morning during dark hours and the results were recorded. These observations were replicated ten times. This study was conducted in the laboratory at $25 \pm 1^\circ\text{C}$ and 70-85 % RH. Visual observations on the activity of leaf-roller moths were recorded during day and night.

Mating, oviposition, fecundity, mortality, sex-ratio and longevity

Freshly emerged leaf-roller moths from the laboratory colony were used for this experiment. A mated female and a male were held in a conical flask (one litre capacity) with tender mulberry shootlets for oviposition and 50 % honey solution was provided as feed. The shoot-lets were examined daily for leaf-roller eggs and were replaced by fresh shootlets every day till death. The shootlets after oviposition were held in polythene bags (25 x 37 cm) and the emerged caterpillars were fed daily with fresh mulberry leaves till pupation. The total number of eggs laid were recorded. The pre-oviposition and oviposition periods, mortality during development, sex-ratio and longevity were recorded.

These studies were conducted at three different temperatures and humidity conditions viz., $20 \pm 1^\circ\text{C}$, RH 70-85 %; $25 \pm 1^\circ\text{C}$, RH 70-85 % and $30 \pm 1^\circ\text{C}$, RH 55-70 % in five replications. Analysis of variance was worked out to ascertain the statistical significance of the variations in the data recorded.

RESULTS AND DISCUSSION

Life cycle of *D. pulverulentalis* comprises of different

developmental stages viz., egg, five larval instars, pre-pupa and pupa.

Egg: Eggs are laid singly along the midrib and veins among trichomes on the under surface of mulberry leaves. Freshly laid eggs are soft, translucent, dorsally convex, ventrally flat and creamish-white in colour. On the second day, they turn pink (Figure 1). Eggs measured 0.43 to 0.60 mm long and 0.32 to 0.45 mm broad in the widest region (Table 1). The mean duration of egg stage decreased significantly with increase in temperature (5.93, 3.77 and 2.47 days at $20 \pm 1^\circ\text{C}$, $25 \pm 1^\circ\text{C}$ and $30 \pm 1^\circ\text{C}$, respectively) (Table 2). Srinivasa Gowda *et al.* (1999) have reported the duration of egg stage to be 4.0 days at 18.5 to 28.8°C and 53.6-83.8 % RH.

Table 1: Size of immature stages of *D. pulverulentalis* at $25 \pm 1^\circ\text{C}$, 70-85 % RH

Stage	Length (mm)		Width (mm)	
	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD
Egg	0.43-0.60	0.55 $\pm$ 0.06	0.32-0.45	0.4 $\pm$ 0.04
I Instar	1.22-1.77	1.46 $\pm$ 0.25	0.2-0.35	0.27 $\pm$ 0.07
II Instar	1.85-2.54	2.20 $\pm$ 0.33	0.37-0.48	0.43 $\pm$ 0.05
III Instar	3.54-5.69	4.61 $\pm$ 1.12	0.6-0.95	0.78 $\pm$ 0.17
IV Instar	5.62-10.85	7.86 $\pm$ 2.56	0.92-1.69	1.23 $\pm$ 0.34
V Instar	10.77-17.31	13.95 $\pm$ 3.30	1.54-2.69	2.11 $\pm$ 0.55
Pre-pupa	13.85-14.65	14.15 $\pm$ 0.36	2.89-2.94	2.92 $\pm$ 0.01
Pupa	12.77-13.85	13.01 $\pm$ 0.32	2.69-3.00	2.78 $\pm$ 0.32

Larva: Five larval instars were recorded as reported earlier by Srinivasa Gowda *et al.* (1999).

First instar: First instar larvae, soon after hatching from the egg are translucent. The head is semi-circular and brownish-black, whereas, the remaining portion of the body is yellowish pink. Subsequently, body colour changes to yellowish green. They inhabit the young mulberry buds or lower surface of the tender leaves. The caterpillars secrete white silky threads and construct tunnels along the side of mid-rib and veins and live between the leaf surface and the silken threads and scrape the soft green tissues and feed on this. First instar

caterpillars measured 1.22 to 1.77 mm long and 0.20 to 0.35 mm broad in the widest region (Table 1).

There are twelve body segments with black spots on dorsal and lateral regions of each segment with a bristle on each spot. Three pairs of pro-legs in the thoracic region and five pairs of pro-legs in the abdominal region were found. The average duration of first instar caterpillar was 2.52 days at $20 \pm 1^\circ\text{C}$, 2 days at $25 \pm 1^\circ\text{C}$ and 1.39 days at $30 \pm 1^\circ\text{C}$. The duration varied significantly with temperature (Table 2). Srinivasa Gowda *et al.* (1999) have reported the duration of first instar to be 2.78 ± 0.031 days at 18.5 to 28.8°C and 53.6 - 83.8% RH.

Second instar: Second instar caterpillars are yellowish green with black head. There are black spots on dorsal and lateral regions of each segment with a bristle on each spot. They feed on green tissues of tender leaves and inhabit the silken tunnels. Second instar caterpillars measured 1.85 to 2.54 mm long and 0.37 to 0.48 mm broad in the widest region (Table 1). The mean duration of second instar caterpillar was 1.84 days at $20 \pm 1^\circ\text{C}$, 1.57 days at $25 \pm 1^\circ\text{C}$ and 1.01 days at $30 \pm 1^\circ\text{C}$. The duration of the second instar varied significantly with temperature (Table 2). Srinivasa Gowda *et al.* (1999) have reported the duration of second instar to be 1.08 ± 0.023 days at 18.5 to

28.8°C and 53.6 - 83.8% RH.

Third instar: Third instar caterpillars are light brownish green with prominent black spots on each segment and black head. The bristles are situated singly on each black spot. They feed on green soft tissues of leaves and inhabit the silken tunnels. Third instar caterpillars measured 3.54 to 5.69 mm long and 0.60 to 0.95 mm broad in the widest region (Table 1). The mean duration of third instar caterpillar was 2.03 days at $20 \pm 1^\circ\text{C}$, 1.58 days at $25 \pm 1^\circ\text{C}$ and 1.07 days at $30 \pm 1^\circ\text{C}$. The duration of the third instar varied significantly with temperature (Table 2). Srinivasa Gowda *et al.* (1999) have reported the duration of third instar of *D. pulverulentalis* to be 2.14 ± 0.012 days at 18.5 to 28.8°C and 53.6 - 83.8% RH.

Fourth instar: Fourth instar caterpillars are brownish green with black head. The intersegmental region becomes prominent and black spots on body segments are larger than that of third instar caterpillars. A white band running from the meso-thoracic region to the last segment of the abdomen on the mid-dorsal surface of the caterpillar is seen. The caterpillar web the two sides of a leaf using white silky filaments, inhabit and feed inside. Fourth instar caterpillars measured 5.62 to 10.85 mm long and 0.92 to 1.69 mm broad in the widest region (Table 1). The mean duration of fourth instar caterpillar was 2.18 days at

Table 2: Life cycle of *D. pulverulentalis* at different temperatures and humidity

Temp./ RH (%)	Egg (days)		Larva (days)										Pre-pupa (days)		Pupa (days)	
	Range	Mean $\pm$ SD	I instar		II instar		III instar		IV instar		V instar		Range	Mean $\pm$ SD	Range	Mean $\pm$ SD
			Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD				
$20 \pm 1^\circ\text{C}$ 70-85%	5.81 -6.00	5.93 ± 0.06	2.42 -2.92	2.52 ± 0.16	1.73 -1.94	1.84 ± 0.06	1.96 -2.26	2.03 ± 0.09	2.05 -2.25	2.18 ± 0.07	2.9 -3.13	3.03 ± 0.07	2.96 -3.04	3.00 ± 0.03	12.00- 13.46	12.30 ± 0.62
$25 \pm 1^\circ\text{C}$ 70-85%	3.17 -3.92	3.77 ± 0.23	1.85 -2.08	2.00 ± 0.07	1.21 -1.96	1.57 ± 0.23	1.34 -2.04	1.58 ± 0.23	1.06 -2.08	1.92 ± 0.31	1.79 -3.38	2.81 ± 0.53	1.89 -2.85	2.38 ± 0.29	8.42- 9.92	9.16 ± 0.50
$30 \pm 1^\circ\text{C}$ 55-70%	2.46 -2.50	2.47 ± 0.01	1.33 -1.44	1.39 ± 0.03	0.92 -1.08	1.01 ± 0.08	1 -1.19	1.07 ± 0.07	1.23 -1.4	1.31 ± 0.08	1.35 -1.83	1.72 ± 0.15	1.56 -1.92	1.77 ± 0.15	5.94- 7.0	6.46 ± 0.48
F test		**		**		**		**		**		**		**		**
CD @ 5%		0.10		0.08		0.11		0.11		0.14		0.24		0.14		0.41
CD @ 1%		0.15		0.12		0.16		0.16		0.21		0.35		0.21		0.59

** - Highly significant ($P < 0.01$)

20 ± 1 °C, 1.92 days at 25 ± 1 °C and 1.31 days at 30 ± 1 °C. The duration of the fourth instar varied significantly with temperature (Table 2). Srinivasa Gowda *et al.* (1999) have reported the duration of fourth instar *D. pulverulentalis* to be 2.18 ± 0.021 days at 18.5 to 28.8 °C and 53.6-83.8 % RH.

Fifth instar: Fifth instar caterpillars are greenish-brown in body colour and has a bristle on each black spot (Figure 1). The head is black and semi-circular. The inter-segmental regions are prominent. The white band extends to the anterior region *i.e.*, from the meso-thorax to the pro-thorax. The caterpillars mostly remain inside the leaf fold and are sometimes seen outside too. Fifth instar caterpillars measured 10.77 to 17.31 mm long and 1.54 to 2.69 mm broad in the widest region (Table 1). The mean duration of fifth instar caterpillars was 3.03 days at 20 ± 1 °C, 2.81 days at 25 ± 1 °C and 1.72 days at 30 ± 1 °C. The duration of the fifth instar varied significantly with temperature (Table 2). Srinivasa Gowda *et al.* (1999) have reported the duration of fifth instar *D. pulverulentalis* to last for 2.11 ± 0.04 days at 18.5 to 28.8 °C and 53.6-83.8 % RH.

Pre-pupa: Fully grown fifth instar caterpillars stop feeding and secrete white silky filaments and enclose themselves in a leaf-fold. They become stout and inactive. The pre-pupae are light brownish-green in colour. Pre-pupae measured 13.85 to 14.65 mm long and 2.89 to 2.94 mm broad in the widest region (Table 1). The mean duration of pre-pupae was 3.0 days at 20°C, 2.38 days at 25°C and 1.77 days at 30°C. The duration of pre-pupae varied significantly with temperature (Table 2). The duration of pre-pupa of *D. pulverulentalis* was reported to be 3.45 ± 0.05 days at 18.5 to 28.8 °C and 53.6-83.8 % RH (Srinivasa Gowda *et al.*, 1999).

Pupa: Initially, pupae are brownish-green gradually turning to brown and later to dark brown. The anterior end is blunt and the posterior end is pointed and curved. The eyes are black and are situated dorso-laterally (Figure 1). The pupae were found within the folded leaves in the laboratory. The body of male pupae is narrow, the wing pads extend up to sixth abdominal segment. The female pupae are larger than the males and are broader at the middle portion. Pupae measured 12.77 to 13.85 mm long

and 2.69 to 3.00 mm broad in the widest region (Table 1). The mean duration of pupae was 12.70 days at 20 ± 1 °C, 9.16 days at 25 ± 1 °C and 6.46 days at 30 ± 1 °C. The duration of pupae varied significantly with temperature (Table 2). Srinivasa Gowda *et al.* (1999) have reported the duration of pupa of *D. pulverulentalis* to be 7.90 ± 0.08 days at 18.5 to 28.8 °C and 53.6-83.8 % RH.

The average duration of life cycle of *D. pulverulentalis* at 20, 25 and 30 °C was 33.24, 25.19 and 17.2 days, respectively, indicating that duration of development decreased with increase in temperature.

Adults: Moths are greyish-brown with oblique, lateral stripes on the abdomen and possess brown antennae. Wings have brownish wavy stripes. The moths are triangular in shape at rest. Male moths have sparse anal tufts whereas, female moths (Figure 1) have dense anal tufts on the last abdominal segment. The anal tufts are black on the sides, and brown in the middle. Male moths are more active than females. The length of male and female moths was 12.30 ± 0.67 and 12.50 ± 0.52 mm, respectively, and the width of male and female moths, when wings stretched was 20.19 ± 12.8 and 21.40 ± 0.96 mm, respectively. Srinivasa Gowda *et al.* (1999) have reported the width of adult male and female *D. pulverulentalis*, when wings were stretched, to be 19.87 ± 0.04 mm and 20.39 ± 0.09 mm, respectively at 18.5 to 28.8 °C and 53.6-83.8 % RH.

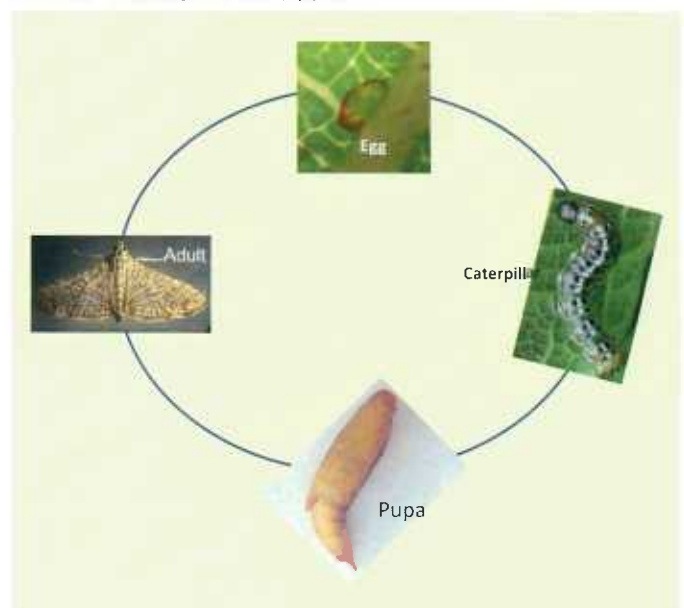


Figure 1: Life cycle of *Diaphania pulverulentalis*

Number of larval instars, position of egg laying and nature of feeding has been reported in *Margaronia pyloalis* (Lepidoptera: Pyralidae) (Sengupta *et al.*, 1990). Life-cycle of *Glyphodes pyloalis*, also a pest of mulberry in Kashmir, Formosa and Japan have been reported to have five larval instars (Dar, 1993).

Information on mating, oviposition, fecundity, mortality, sex-ratio and longevity of a pest gives insight into important reproductive attributes of the pest.

Mating: Leaf-roller moths mate for 13 days after emergence at 20°C, for 3-4 days at 25°C and for 1-2 days at 30°C. Dar (1993) has reported that, *G. pyloalis* females mate for 3-4 days after emergence.

Pre-oviposition period: The average pre-oviposition period of leaf-roller was 13 days at 20°C, 3.60 days at 25°C and 2.00 days at 30°C. Pre-oviposition period of leaf-roller reduced with increase in temperature. The differences in pre-oviposition period between 20 and 25°C and 25 and 30°C was significant (Table 3). Dar (1993) has reported in *G. pyloalis* that, oviposition commenced from 5 to 7 days after emergence at 22 to 25°C.

Oviposition behaviour: *D. pulverulentalis* moths are nocturnal. Gravid females oviposit only during night. The average number of eggs laid per moth in one night was 48.14 ± 18.24 . Rangaswami *et al.* (1976) have reported that *G. pyloalis* is also nocturnal.

Oviposition period: The average oviposition period was 4.80 days at 20°C, 4.40 days at 25°C and 2.80 days at 30°C (Figure 2 A-C). Oviposition period of leaf-roller decreased with increase in temperature. Dar (1993) has reported the oviposition period of *G. pyloalis* to be seven days at 22 to 25 °C.

Oviposition: The first batch of eggs were laid on 14th day (83) at 20 °C, the no. gradually increased on 15th day (102.8) and reached its peak on day 16th (133.8). Subsequently, oviposition capacity decreased as the age of adult advanced. It was 46.4 on 17th day, 29.0 on 18th day, 4.2 on 19th day and finally ceased on 20th day (Table 3 and Figure 2A).

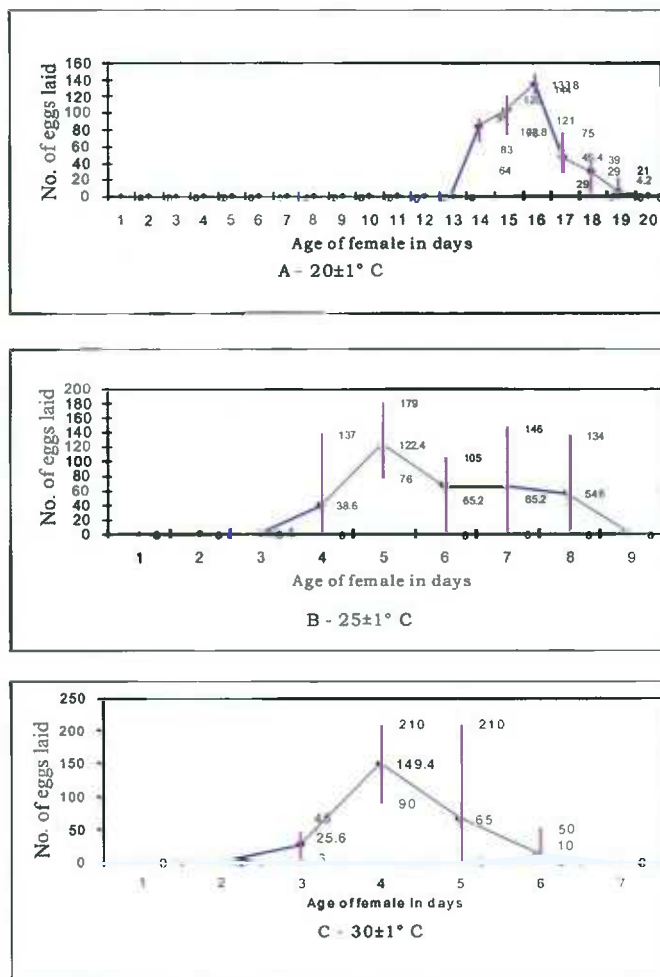


Figure 2: Oviposition pattern in *D. pulverulentalis* at different temperatures

The mean number of eggs laid at 25°C was 38.6 on 4th day, peak was observed on 5th day (122.4), 66.20 each was laid on 6th and 7th day, 54.60 on 8th day and no oviposition on 9th day (Table 3 and Figure 2 B).

Gravid females began ovipositing on the 3rd day at 30°C. On this day, average number of eggs laid was 25.6. The peak of 149.4 was observed on the 4th day. On 5th, 6th and 7th days, egg laying capacity declined to 65.0, 10.0 and no oviposition, respectively (Table 3 and Figure 2 C).

Fecundity: Fecundity in *D. pulverulentalis* decreased with increase in temperature. The mean fecundity at 20, 25 and 30°C was 399.2, 373.2 and 250 eggs, respectively. No significant difference in fecundity was observed at 20 and 25°C while between 20 and 30°C and 25 and 30°C, there was a significant difference in fecundity (Table 3).

Table 3: Oviposition, fecundity, mortality, sex-ratio and longevity of *D. pulverulentalis* at different temperatures

Temp (°C)	RH (%)	Pre-oviposition period (days)		Ovposition period (days)		Fecundity		Mortality (%)		Adult emergence %				Sex - ratio		Longevity (days)			
		Range	Mean	Range	Mean	Range	Mean	Range	Mean	Male	Female	Male-Female	Mated male	Mated female					
								%	%	Range	Mean	Range	Mean		Range	Mean	Range	Mean	
20±1	70-85	13-13	13.00	4-6	4.80	351 -422	399.20	49.87 -58.05	54.05	20.62 -24.94	22.8	21.33 -25.19	23.20	1.00:1.02	14-19	15.8± 2.17	17-20	18.00	
25±1	70-85	3-4	3.60	2-5	4.40	316 -461	373.20	42.6 -56.1	48.89	20.57 -27.72	24.3	20.86 -31.01	26.77	1.00:1.10	4-8	6.00± 1.58	5-9	8.20	
30±1	55-70	1-2	2.00	2-4	2.80	192 -306	250.00	79.2 -87.6	83.74	6.21 -10.42	8.12	6.21 -10.42	8.14	1.00:1.00	4-5	4.6± 0.55	4-7	5.40	
F test			**		*		**		**		**		**	NS		**		**	
CD @ 5 %			0.61		1.95		60.82		7.01		3.18		3.99	-		1.55		2.76	
CD @ 1 %			0.84		2.71		84.42		9.73		4.41		5.53			1.79		3.83	

** -Highly significant (P<0.01); * - Significant (P<0.05)

Srinivasa Gowda *et al.* (1999) have reported fecundity of *D. pulverulentalis* to be 202.43 ± 19.97 at 18.5 to 28.8°C when fed on 10 % honey solution. Dar (1993) has reported that *G. pyloalis* females lay 112 to 225 eggs with a mean of 157 eggs at 22 to 25°C in Kashmir, India. Maki (1920) in Formosa found that *G. pyloalis* laid 90 to 150 eggs (mean = 80 eggs). In Japan, Yokoyama (1995) reported the fecundity of *G. pyloalis* to vary from 159-298 eggs with an average of 216 eggs.

Mortality: Mortality of leaf-roller during development increased when temperature fluctuated below and above the optimum. The average mortality in leaf-roller at larval stage was 54.05 % at 20 °C, 48.89 % at 25 °C and 83.74 % at 30 °C (Table 3).

The difference in mortality between 20 and 25 °C was not significant but significant differences were observed between that of 30 and 25 °C and 30 and 20 °C (Table 3).

Adult emergence: Rate of emergence of moths was slightly higher at 25° C than 20° C and was low at 30° C.

Male: The average emergence of male moths was 22.75% at 20°C, 24.34 % at 25 °C and 8.12 % at 30 °C (Table 3). The difference in emergence rate of male moths between 25 and 20°C was not significant but significant differences were observed between that of 20 and 30 °C and 25 and 30°C.

Female: The average emergence of female moth was 23.20 % at 20 °C, 26.77 % at 25 °C and 8.14 % at 30 °C. The difference in female moth emergence was not significant between 25 and 20 °C but significant differences were observed between that of 20 and 30 °C and 25 and 30 °C (Table 3).

Sex-ratio: The sex-ratio between male and female moths was 1:1.02 at 20° C, 1:1.10 at 25°C and 1:1 at 30°C. The sex-ratio between male and female did not show significant differences between the three treatments.

Longevity

Male: Longevity of mated male leaf-roller moths decreased with increase in temperature. The average longevity of the mated male was 15.8 days at 20 °C, 6.0 days at 25 °C and 4.6 days at 30 °C. The differences in longevity of mated male moths were significant between 20 and 25 °C and 25 and 30 °C (Table 3). Srinivasa Gowda *et al.* (1999) have reported the longevity of male leaf-roller moths, when fed on water and 10 % honey solution to be 5.4 ± 0.03 and 7.9 ± 0.08 days, respectively at 18.5 to 28.8°C.

Female: The average longevity of the ovipositing females was 18 days at 20°C, 8.2 days at 25°C and 5.4 days at 30°C, indicating that longevity decreased with increase in temperature. The differences in longevity of leaf-roller ovipositing female moths were significant between 20 and

25°C and 25 and 30°C (Table 3). Srinivasa Gowda *et al.* (1999) have reported the longevity of female leaf-roller moths, when fed on water and 10 % honey solution to be 8.65 ± 0.02 and 11.45 ± 0.03 days, respectively, at 18.5 to 28.8°C.

CONCLUSION

The present study on leaf-roller pest, gives an insight into its biology, which could be useful in formulation of integrated management practices.

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BIOLOGIE ET COMPORTEMENT DE L'ENROULEUR DE LA FEUILLE, *DIAPHANIA PULVERULENTALIS* (HAMPSON) (LEPIDOPTERE: PYRALIDE) A DIFFERENTES TEMPERATURES

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RESUME

Nous avons étudié les données du cycle de *Diaphania pulverulentalis*, une peste du mûrier, *Morus alba* L. (Moracée) à trois températures différentes de 20±1°C, 25±1°C et 30±1°C. Le cycle de développement de cet insecte est caractérisé par des stades distincts d'œuf, de larve, de prépupe et de pupa. Il y a une tendance claire à la réduction de la durée des différents stades c'est-à-dire œuf (5.93 ; 3.77 ; 2.47 jours), chenille (11.61 ; 9.88 ; 6.50 jours), prépupe (3 ; 2.38 ; 1.77 jours) et pupa (12.70 ; 9.16 ; 6.46 jours) lorsque la température augmente (20±1°C, 25±1°C, et 30±1°C respectivement). La durée moyenne du cycle de *D. pulverulentalis* à 20, 25, et 30°C est de 33.24, 25.19 et 17.2 jours respectivement avec une fécondité moyenne de respectivement 399.2, 373.2, et 250 œufs. Cet article donne des éléments détaillés sur l'accouplement, l'oviposition, la longévité et le sexe-ratio.

Mots-clés: Biologie, fécondité de *Diaphania pulverulentalis*, cycle de vie, longévité, accouplement, oviposition, sexe-ratio.



PATHOGENICITY OF *EUPROCTIS PSEUDOCONSPERSA* NUCLEOPOLYHEDROVIRUS TO MULBERRY SILKWORM

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ABSTRACT

Euproctis pseudoconspersa is a major pest of tea plants in East Asia and *E. pseudoconspersa* nucleopolyhedrovirus (EpNPV) has been used to control the pest. So far, there are no reports available on pathogenicity of EpNPV against silkworms or on differentiation of EpNPV and *Bombyx mori* nucleopolyhedrovirus (BmNPV) from silkworm. Hence, a study was conducted in this direction. There was no mortality in silkworms when injected with budded viruses (EpNPV). However, when EpNPV was per orally inoculated, mortality was noticed up to three days after inoculation. EpNPV was detected from the dead silkworms by polymerase chain reaction method using multiplex primers, and differentiated from BmNPV. The percentage of EpNPV detected from survived silkworms decreased in proportion to days elapsed after the ingestion.

Key words: *Bombyx mori* nucleopolyhedrovirus, *Euproctis pseudoconspersa* nucleopolyhedrovirus, silkworm.

INTRODUCTION

Euproctis pseudoconspersa (tea tussock moth) is a major pest of tea plants and causes serious damage to tea industry in East Asia. Chemical and biological control agents have been used to control the pest. As biological control agents, second metabolic substances from *Bacillus thuringiensis* and *Euproctis pseudoconspersa* nucleopolyhedrovirus (EpNPV) have been used. EpNPV has also been used as a virus pesticide in China (Tang *et al.*, 2009). When EpNPV is used as a biological control agent, there is a threat to the neighbouring plantations as some species of NPV have broad host range. *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) can infect several species of lepidopteran larvae (Vail and Jay, 1973). In East Asia, since the sericultural industry is at the prospering side, any possibility of exposure of EpNPV to mulberry field may cause cross infection to silkworms reared in those areas. However, till now, there is no report available on any sort of damage caused to silkworms. Therefore, the present experiment was worked out to examine the pathogenicity of EpNPV to silkworm, *Bombyx mori*.

MATERIALS AND METHODS

EpNPV sample was procured from National Agriculture and Food Research Organization, Japan and inoculated to larvae of *E. pseudoconspersa*. Larvae infected with EpNPV were collected and kept at -20°C until used. The larvae were put in 20 mM phosphate buffer saline (PBS: pH7.2) containing 1 % of Tween 20 (PBT) and homogenized in a blender at 4°C. The homogenates were filtered by double gauzes, and the polyhedral inclusion bodies (PIBs) of EpNPV were washed with PBT at x2,300 g for 5 min for ten times. PIBs of EpNPV were purified with 40 - 65 % (w/v) sucrose density-gradient centrifugation at x8,000 g for 30 min. The banded polyhera of EpNPV was collected and washed with PBS.

Extraction of EpNPV: Extraction method of viral DNA was followed with slight modification (Mitsuhashi *et al.*, 2007). The purified PIBs of EpNPV were incubated in 0.2M carbonate buffer (pH 10.5) for 1 h and then neutralized with 1M-Tris HCl (pH 8.0). The solution was treated with 1 % (w/v) of protease K and 0.25 % (w/v) of SDS for 2 h at 37°C. Viral DNA was purified by phenol / chloroform extraction and then precipitated using ethanol.

DNA of polyhedral genes were amplified using primers for cloning of polyhedral gene of NPV (Nakai *et al.*, 2002) by polymerase chain reaction (PCR) method using PSF001, PSF002, PMF001 and PER001 primers. DNA was cloned using pGEM-T Easy Vector System I (Promega, Tokyo). Sequences of the polyhedral gene were performed using Big Dye Terminator ver. 3.1 Cycle Sequencing Kit (Applied Biosystems, Tokyo) and the ABI PRISM 3730xl DNA analyzer (Applied Biosystems). Sequences of the polyhedral genes from the EpNPV were compared with sequences of polyhedral gene from NPVs reported at National Center for Biotechnology Information (NCBI) and hit with EpNPV from China (Tang *et al.*, 2009) at 100 % match.

Bombyx mori NPV(BmNPV): Silkworms (J137 x C146 race) were infected with BmNPV sample obtained from Dr. Furuta (Furuta, 1994 a, b). They were reared on artificial food (Sirukumeito 2S, NOSAN, Yokohama) at 25°C for one week. Hemolymph was collected in PBS containing 1% of sodium thiosulfate at 4°C. The homogenate containing BmNPV was washed with PBT ten times and purified by the same method followed for

EpNPV.

Inoculation / Injection of EpNPV: EpNPV PIBs (10^8 PIBs / ml) prepared by the above mentioned method were treated with equal volume of 1 N NaOH to make the budded viruses (BVs) solution. Dissolution of PIBs was ensured by microscopic observation. The solution was neutralized (pH 7.0) and injected (10^8 PIBs / ml of EpNPV) with a syringe into 36 silkworm races (Table 1) at fifth age. These races were procured from NIAS Gene bank. Twenty larvae of fifth instar were used for each treatment to be reared on artificial food. The larvae were dissected one week after the inoculation and midgut tissues were examined for the presence of PIBs by microscopic method.

Peroral infection of EpNPV: Fifteen larvae each of five races at first stage were ingested with $4.5 \times 10^{7-9}$ PIBs EpNPV/ g cf artificial food for two days and then were fed on artificial food without EpNPV since third day after the ingestion.

Differentiation between BmNPV and EpNPV by multiplex PCR method: Silkworms were homogenized

Table 1: Silkworm races injected with *Euproctis pseudoconspersa* nucleopolyhedrovirus

Race	Group	Race	Group	Race	Group
Agunisan	Japanese native	C kyuhken	Chinese native	Gubbio	European native
Datenishiki	Japanese native	Bunrihaku	Chinese native	Asukoriohken	European native
Koishimaru	Japanese native	Daikojoh	Chinese native	Aziakouken	European native
Hinode	Japanese native	Ohha	Chinese native	Maiera-zebura	European native
Murasakiko	Japanese native	Nanko	Chinese native	Sebuennushiro	European native
Ookusa	Japanese native	SI	Chinese native	Sanjurian	European native
Ryuukyutasan (Ohkenayabe)	Japanese native	Sekkoh	Chinese native	Y4	European native
Ryuukyutasanken (Shizuka)	Japanese native	208AB	Chinese native	y35	European native
Akako	Japanese improved	C7	Chinese improved	Yuhaku	European native
Kiuki	Japanese improved	Hakuyouen	Chinese native	Oh 1	European improved
Yoshi N	Japanese improved	Seikyou	Chinese native	H5 takei	European improved
Wata 49	Japanese improved			Maisohru	Tropical
				Ringetsu	Tropical

with buffer (1 M TrisHCl and 0.5 M EDTA, pH 8.0), and viral DNA was purified by phenol-chloroform extraction and ethanol precipitation. PCR were performed using Takara La Taq™ (Takara Bio Inc., Japan) using 100 ng of the DNA and 10 pmol of 4 primers for detection of polyhedral genes from BmNPV and EpNPV in a total volume of 20 µl, and the cycling parameters for PCR were as follows: cycle 1, 5 min at 94°C, 35 cycles, 1 min at 94°C, 1 min at 50°C and 1 min at 72°C; 7 min at 72°C using a DNA Thermal Cycler (PerkinElmer, Tokyo). Five µl of the reaction solution was electrophoresed using 1.5 % of agarose at 100 V for 30 min and the agarose was stained with ethidium bromide.

RESULTS

Injection with EpNPV: When 1×10^8 BVs / ml of EpNPV was injected into bodies of 36 races of silkworms, all of them survived even on seventh day after inoculation. All the treated worms were dissected and examined microscopically but PIBs were not observed.

Peroral inoculation with EpNPV: When silkworms were perorally inoculated with 4.5×10^7 PIBs / g artificial diet, only Akajuku race died and PIBs were detected from midgut of the larvae under microscopic observation (Table 2). When silkworms were inoculated with 4.5×10^8 PIBs or 4.5×10^9 PIBs / g artificial diet, mortality was noticed in all the races and PIBs were detected from the dead larvae. Mortality of the races was high at high concentrations of PIBs.

To examine mortalities of silkworms when perorally inoculated with EpNPV at each stage, larvae of J137xC146 race were ingested with 4.5×10^8 PIBs/g artificial diet from first to fifth stage (Figure 1). When the race was ingested with EpNPV at first and second stages, cumulative mortality increased until third stage, and beyond, there was no change in the mortality. Further, the survived larvae did not reveal EpNPV under microscopic examination. When the race was ingested at fourth stage, mortalities at fifth stage did not change. PIBs were observed from midgut of dead larvae by microscopic method but was not observed from the survived larvae of the treated population.

Table 2: Mortality (%) of silkworm races by peroral inoculation with *Euprotis pseudoconsersa* nucleopolyhedrovirus

Race	Group	Concentration of EpNPV (occluded body viruses / g)			
		0	4.5×10^7	4.5×10^8	4.5×10^9
Akajuku	Japanese native	0	2.0 ± 0.5	5.0 ± 0.9	8.7 ± 0.7
Akako	Japanese native	0	0	7.0 ± 0.9	10.7 ± 1.2
J137xC146	Hybrid	0	0	2.7 ± 0.7	10.0 ± 0.9
Tenmon	Chinese native	0	0	6.0 ± 0.9	11.7 ± 1.2
C15	Chinese improved	0	0	4.7 ± 0.7	6.0 ± 0.5

Fifteen larvae were used for each test, Data are mean $\pm$ SE.

Table 3: Primers for identification of *Bombyx mori* NPV (BmNPV) and *Euprotis pseudoconsersa* NPV (EpNPV)

Primer	Sequence	Product (bp)
Bmpoly-f	GGCAGAGTCTGTGCCGATG	396
Bmpoly-r	GTTAATCGTCAACTGGAGCGGC	
Eppoly-f	CGCCGAATCGGTTCTCTACA	330
Eppoly-r	TTTTCCCGTTGTGAATGACCAGGAAATC	

Differentiation of BmNPV and EpNPV: Since BmNPV causes one of the most serious diseases in sericulture (Yao *et al.*, 2003), differentiation of PIBs as that of EpNPV and BmNPV is very important in view of disease management strategies. The multiplex PCR method (Figure 2) was relied on for this purpose. PCR products of EpNPV were detected from dead larvae infected with EpNPV (Figure 3). BmNPV or EpNPV infection to silkworm was confirmed when 396 bp or 330 bp of PCR products were found, respectively (Table 3).

Detection of EpNPV from silkworms: To determine the period up to which EpNPV could be detected from silkworms, once ingested with the virus, ten larvae of J137xC146 race at first stage were ingested with 4.5×10^8 PIBs/g artificial diet followed by feeding on artificial food without EpNPV from second day onwards after the ingestion.

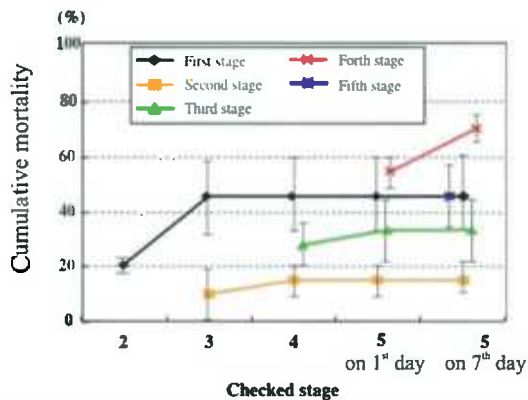


Figure 1: Cumulative mortality of J137xC146 race by peroral inoculation with 4.5×10^8 inclusion bodies of *Euproctis pseudoconspersa* nucleopolyhedrovirus /g of artificial diet.

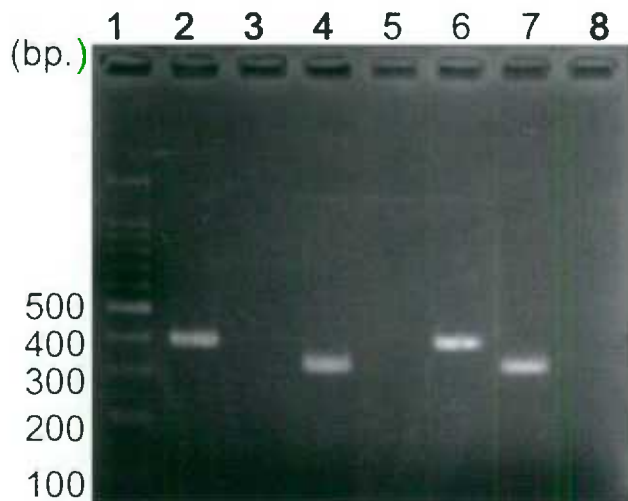


Figure 2: Multiplex polymerase chain reaction (PCR) amplified products of *Bombyx mori* nucleopolyhedrovirus (BmNPV) and *Euproctis pseudoconspersa* nucleopolyhedrovirus (EpNPV) using primers mentioned in Table 3.

1: 100 bp ladder marker (Takara), 2: PCR product of BmNPV using Bmpoly-f primer and Bmpoly-r primer, 3: PCR product of EpNPV using Bmpoly-f primer and Bmpoly-r primer, 4: PCR product of EpNPV using Eppoly-f primer and Eppoly-r primer, 5: PCR product of EpNPV using Bmpoly-f primer and Bmpoly-r primer, 6: multiplex PCR product of BmNPV using Bmpoly-f primer, Bmpoly-r primer, Eppoly-f primer and Eppoly-r primer, 7: multiplex PCR product of EpNPV using Bmpoly-f primer, Bmpoly-r primer, Eppoly-f primer and Eppoly-r primer, 8: Negative control (PCR amplification was done without sample).

The larvae were dissected on the first day of next stage after inoculation. When silkworms were inoculated on the first day of fifth stage, they were dissected on the seventh day of fifth stage for examination. Occlusion bodies in midgut were detected by microscopic method. DNA was extracted from each larva after first and up to the tenth day and tested for EpNPV by PCR method. EpNPV could be detected up to five days after the ingestion with the percentage of detection decreasing with progress of time (Figure 4).

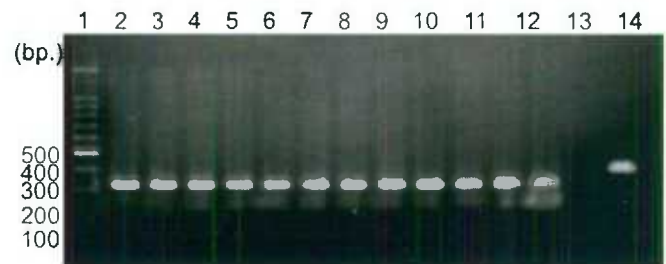


Figure 3: Multiplex polymerase chain reaction products of *Euproctis pseudoconspersa* nucleopolyhedrovirus (EpNPV) and *Bombyx mori* nucleopolyhedrovirus (BmNPV) from silkworms inoculated with EpNPV or BmNPV.

1: 100 bp ladder marker (Takara), 2 - 13: PCR products of silkworms infected by EpNPV using multiplex primers (Table 3), 14: PCR products of silkworms infected by BmNPV using multiplex primers.

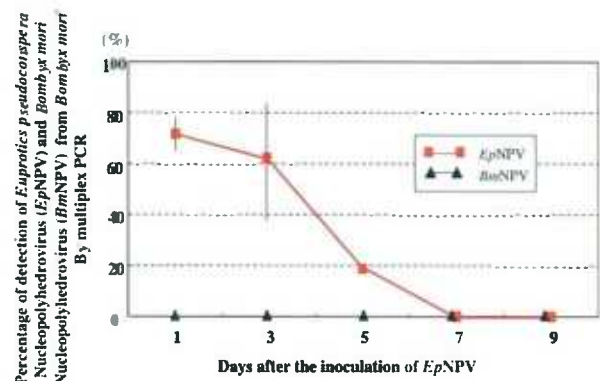


Figure 4: Fluctuation of percentage of detection of *Euproctis pseudoconspersa* nucleopolyhedrovirus (EpNPV) and *Bombyx mori* nucleopolyhedrovirus (BmNPV) by multiplex polymerase chain reaction (PCR) from ingested silkworms at first stage (J137 x C146 race).

DISCUSSION

Many species of entomopathogenic virus are employed as biological agents because the high degree of host specificity of virus reduces potential ecological hazard, and there are many environmental benefits including safety for other non-target organisms, reduced chemical pesticides contamination in foods and increased biodiversity in managed ecosystem. Among microbiocontrol agents, NPV is the most used in integrated pest management programs (Payne, 1982; Lacey *et al.*, 2001). EpNPV is usually used to control tea tussock moth in tea plants. All silkworms survived by injection with BVs of EpNPV, but there was larval mortality when peroral inoculation was done with PIBs of EpNPV. The percentage of EpNPV detected from larvae decreased in proportion to time from the point of inoculation with EpNPV. Hence, it is assumed that there are larvae sensitive to the infection with EpNPV which die within one to three days after the infection whereas less sensitive larvae continue to survive after three days. By PCR method, EpNPV was detected from all dead larvae and also survived larvae, initially. However, detection percentage of EpNPV decreased in the survived larvae. These results indicate that it is not possible to multiply EpNPV utilizing silkworm as host.

Genome sequence of EpNPV from China was mapped in 2009 (Tang *et al.*, 2009). We compared the nucleic acid sequence of polyhedral gene of EpNPV from China to that of EpNPV used in this experiment. As the results matched 100%, our data refer to EpNPV from China.

Of late, there are many primers used for identification of NPV by PCR method (Woo, 2001), but there were no reports on primers for detection of EpNPV. Therefore, this is the first report on detection primer of EpNPV for PCR method.

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PATHOGENICITE DU VIRUS DE LA POLYEDROSE NUCLEAIRE DE *EUPROCTIS PSEUDOCONSPERSA* VIS-A-VIS DU VER A SOIE DU MURIER

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RESUME

Euproctis pseudoconspersa est une peste majeure du théier en Asie de l'Est et le virus de la polyédrose nucléaire de *E. pseudoconspersa* (EpNPV) a été utilisé pour lutter contre cette peste. Jusqu'ici, aucun rapport n'est disponible concernant la pathogénicité de EpNPV vis-à-vis des vers à soie ou sur les différences entre EpNPV et le virus de la polyédrose nucléaire de *Bombyx mori* (BmNPV). Nous avons donc conduit notre étude dans cette direction. Il n'y a pas de mortalité induite par l'injection de virus (EpNPV) chez les vers à soie. Cependant, lorsque EpNPV est inoculé par voie orale, on note une mortalité jusqu'à trois jours après l'inoculation. EpNPV a été détecté chez les vers à soie morts par réaction en chaîne à la polymérase en utilisant des amorces multiples et ainsi différencié de BmNPV. Le pourcentage de EpNPV détectés chez les vers à soie survivants décroît proportionnellement au cours des jours suivants l'ingestion.

Mots-clés: Virus de la polyédrose nucléaire de *Bombyx mori*, virus de la polyédrose nucléaire de *Euproctis pseudoconspersa*, ver à soie.



COMPARATIVE ANALYSIS OF THE MORPHOLOGY, STRUCTURE AND THERMAL PROPERTIES OF SILK PROTEIN FILMS OBTAINED FROM THE MANGO LEAF WEBBER, *ORTHAGA EXVINACEA* AND *BOMBYX MORI*

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ABSTRACT

The silk protein from the silken web of *Orthaga exvinacea* and from the cocoon of *Bombyx mori* were isolated by solubilising degummed silk fibres in a saturated solution of lithium bromide and purified by dialysis. The purified silk protein of *O. exvinacea* was subjected to amino acid analysis using HPLC. The silk protein of pyralid moth was rich in Serine, Histidine and Alanine. After quantifying the protein in the purified sample, it was cast into films. The dried films were subjected to morphological, structural and thermal analysis using SEM, FTIR and TG-DTA, respectively. Comparative analysis of silk from *O. exvinacea* and *B. mori* showed that they differ in their β -sheet content. The strength of the film is dependent on its β -sheet which was more in *B. mori* than in *O. exvinacea*. But thermal stability of two protein films was almost equal. Anyhow, the temperatures of maximum degradation rate of both the protein films varied by a degree centigrade. This information suggests the potential of the silk of the mango leaf webber for use as a substitute for various biomedical and biotechnological applications.

Key words: *Bombyx mori*, FTIR, *Orthaga exvinacea*, silk fibroin, TG-DTA.

INTRODUCTION

Silk, a naturally occurring polymer is produced by a number of organisms including spiders, insects, mites, flies *etc.* (Kaplan *et al.*, 1992; 1994; 1998). It has various structural roles in spiders and insects such as cocoon formation, nest building, formation of traps, webbing of leaves, safety lines and egg protection (Kaplan *et al.*, 1998; Wong *et al.*, 2002). The commercial production of silks is centered towards the families of Saturniidae and Bombycidae of the insect order Lepidoptera. The different varieties of mulberry and non-mulberry silks are commercially exploited in recent years. The mulberry silk is produced by *Bombyx mori*. However, the wild varieties of silks such as tasar, eri and muga are produced by *Antheraea pernyi*, *Philosamia ricini* and *Antheraea assamensis*, respectively (Robson, 1998; Sen and Babu, 2004 a, b). The excellent lustre, texture, durability and mechanical strength make silk a fascinating biomaterial of this millennium.

Silk fibers are an economically important commodity in the textile industry even after the invention of nylon and other synthetic fibers and their application in textile industry. Silk fibers are composed of two proteins, fibroin and sericin, synthesized and secreted by the silk glands. The silkworm larvae move its head in a specific manner during which silk protein is subjected to various mechanical stress followed by exposure to external environment when it is passed through the spinneret. This help in the transformation of liquid silk into silk fibers (Vollrath and Knight, 2001). The fibroin protein forms the core of the silk fibre and is composed of two units, heavy (H) 325 kDa and light (L) 25 kDa chain that are linked together by a disulfide bond (Shimura *et al.*, 1976). Fibroin and sericin protein together make up 95 % of silk fiber and the remaining 5 % comprises of other proteins, waxes, salts and ashes. Sericin alone, accounts for 20-30 % (w/w) of *B. mori* cocoon fibers (Masahiro *et al.*, 2000; Wei *et al.*, 2005). But the molecular weight of spider silk

protein is different from that of *B. mori*, and ranges from 70 to 700 kDa. The lepidopteran and spider silks are composed primarily of Glycine, Alanine and Serine.

Generally, silk fibroin protein is insoluble in water. But the solubility of fibroin was found to be high in neutral salt solutions such as lithium bromide and lithium thiocyanate (Lizuka, 1985; Tsukada *et al.*, 1994). Because of its reproducibility, biocompatibility, biodegradability and non toxicity properties, silk fibroin was elevated beyond its use in textile industry to various other biotechnological and biomedical fields. For biomedical applications, silk fibroin has to be regenerated in various forms like gel, film, fiber, membrane *etc.* (Park *et al.*, 2004). There is a problem of conformational change such as a transition from random coil or α -helix to β -sheet or β -turn in the fibroin protein during its solubilization and recreation from liquid state into various forms like gel, scaffold, films, powder *etc.* In order to avoid such transformational changes, it is necessary to characterize the fibroin protein in its liquid state itself. The silk fibroin of *B. mori* exists in two crystalline modifications, silk I and silk II. The silk I structure is metastable, it can be obtained only when the content of silk gland are dried without mechanical disturbance (Shimizu, 2000). The silk I can be changed to silk II on heating or stretching. The structural, thermal and mechanical properties of regenerated fibroin depend on its conformational characteristics. Various factors such as the chemical composition of protein, temperature, pH, pressure, chemical used for its solubilization *etc.* influence the conformational features of regenerated silk fibroin.

Due to the increase in demand of silk as biomaterial for various purposes, the research has been extended into silk produced by various other insects. The present study focuses on the silk produced by the mango leaf webber, *Orthaga exvinacea*. The cost effectiveness with regard to rearing and resistance to diseases make *O. exvinacea*, a pyralid moth, a desirable option because the rearing of this pyralid moth is virtually effortless and cheaper because of the availability of mango leaves in plenty throughout the year. Another advantage is the resistance of the larvae to diseases due to infections. However,

thorough characterization and biocompatibility studies on the proteins are essential before we make any suggestions regarding its biotechnological and biomedical applications.

The present work was an attempt to compare the morphological, structural and thermal properties of the regenerated protein films of *B. mori* and *O. exvinacea* using SEM, TG-DTA and FTIR.

MATERIALS AND METHODS

Materials

The larvae of the mango leaf webber, *O. exvinacea* were collected from various places in Kozhikode and Malappuram districts of Kerala State and were transferred into wooden cages, maintained in the insectary, at temperature of about $27 \pm 2^\circ\text{C}$ and relative humidity of 70-80 %. They were reared by feeding on mango leaves. From the culture, silk produced by the insects was recovered and used for further analysis.

The cocoons of *B. mori* were collected from the cocoon market, Coimbatore, Tamil Nadu, India.

Methods

Purification of fibroin like protein from the web of *O. exvinacea*

Degumming

The silken web of *O. exvinacea*, which was secreted for holding the mango leaves together, was a network of discontinuous fibres. It was collected from the culture, cleaned off excreta, plant debris *etc.* and transferred into a 250 ml beaker containing 0.05 % (w/v) of sodium carbonate solution for degumming. The beaker was kept in a boiling water bath for 15 min. The web and the solution were maintained at a ratio of 1:150 (w/v) throughout the process of degumming. The web was then washed several times in boiling water followed by several rinsing in cold water until it became non-sticky. The web was then transferred onto a blotting paper to remove moisture and dried for 24 h at room temperature.

Dissolution of web

One gram of degummed and dried web was dissolved in 10 ml of a saturated solution of lithium bromide at $65 \pm 2^\circ\text{C}$ in a water bath shaker set at 200 rpm for 24 h. The solution was filtered and diluted to 100 ml.

Purification of protein by dialysis

Dialysis membranes of cut off size 12-14 kDa were used for purification of proteins. A piece of dialysis tube of 12 cm length was taken, transferred into a beaker containing lithium bromide (1 mM) and EDTA (1 mM) and boiled for 30 min. on a hot plate. While boiling, stirring was done using glass rod. The dialysis membrane was washed, taken out and washed thoroughly with distilled water. One end of the pretreated tube was tied with nylon thread. The filtered, diluted sample solution was poured into the pretreated membrane tube. Then the other end of the tube was also tied with a nylon thread. The dialysis bag was then transferred to a beaker for dialysis against distilled water. The ratio of the sample in the dialyzer bag to distilled water was kept at 1:100. The distilled water was changed every 12 h and dialysis continued for 4 days to remove lithium bromide and other impurities. After dialysis, the content of protein present in the sample was quantified using the method of Bracford (1976).

Extraction, purification and quantification of fibroin from the cocoon of *B. mori*

The cocoon shells were cut into small pieces, cleaned off from the debris of pupae and transferred into a beaker containing 0.5 % sodium carbonate solution. The solution was heated to boiling on a hot plate for about half an hour. During the process of degumming, the solution was frequently stirred using a glass rod and the ratio of cocoon shell to solution was kept at 1:100 (w/v). After treatment with sodium carbonate solution, the silk fibers were taken out and washed several times with boiling water until the sticky nature of the silk fibers was completely removed. The degummed fibers were then dried at room temperature.

The degummed and dried fibre (1 mg) was dissolved in 10 ml of a saturated solution of lithium bromide maintained at $60 \pm 2^\circ\text{C}$ in a water bath shaker, keeping it

for 12 h. The method used for purification and quantification of fibroin silk protein of *B. mori* was same as that used for *O. exvinacea*.

Casting films of protein extracted from silk of *O. exvinacea* and silk fibroin from the cocoon of *B. mori*

Pure solutions of web protein of *O. exvinacea* and silk fibroin of *B. mori* obtained after dialysis as described were used for casting films. Samples of 15 ml each of the solutions of both proteins at 1 % concentration were poured into separate petri dishes with circular area of about 40 cm^2 and kept it over the activated silica gel present in dessicator. The silica gels absorbed the moisture of the protein solution. Reactivation of silica gel was necessary for further absorption of moisture and it was done by keeping the silica gel in the hot air oven, followed by reintroducing into the desiccator. This was being performed on daily basis for a week. Subsequently, the silk protein was dried enough to peel off from the petri dishes.

High performance liquid chromatography (HPLC)

The amino acid analysis of the dialyzed protein obtained from the web of *O. exvinacea* was carried out on Agilent 1100 HP-HPLC system.

Characterization of pure protein films of *O. exvinacea* and *B. mori*

Surface morphology was studied by taking images of the films with Hitachi's SU6600 model SEM.

The secondary structure and conformational characteristics of the pure protein films (10 cm^2) were analysed using FTIR spectroscopy (JASCO-4100) by placing the thin films of protein directly on the holder of the instrument. The frequency of absorption was in the region of 4000 to 400 cm^{-1} at room temperature. Infrared spectra were recorded from 32 scans per sample with resolution of 4 cm^{-1} .

The thermogravimetric analyses of the samples were carried out using Perkin Elmer STA-6000 instrument (USA). Each sample (1.5 mg) was heated non-isothermally from 40 to 500°C at a heating rate of 10°C

/min. The inert atmosphere was provided by nitrogen at a flow rate of 200 ml/min.

RESULTS AND DISCUSSION

Characterization of amino acids present in the silk protein of *O. exvinacea*

Figure 1 depicts the chromatogram obtained for purified protein from the web of *O. exvinacea*. There were four major peaks in the chromatogram, which represents the amino acids Serine, Histidine, Alanine and Aspartic acid. It was noticed that the peaks of Glycine and Histidine joined together and appeared as a single peak. The silk fibroin of *B. mori* is rich in Glycine and Alanine (Sen and Babu, 2004 a) whereas the silk fibers of the lace wing insects *Chrysopa* sp. are rich in Serine, Alanine and Glycine (Spiegler, 1962). The composition of amino acids present in the silk of the pyralid moth, *O. exvinacea* was found to be different from that of *B. mori*. The properties of silk mainly depend on the composition of amino acids and it varies from silk to silk and is an important aspect in determining its biomedical applications.

Morphological studies using SEM

The films prepared out of purified protein samples were subjected to SEM. The micrographs of the two samples appear to be morphologically similar and exhibit a very smooth texture without granular appearance. The surface morphology of the samples is shown in Figure 2.

Previous studies conducted on *B. mori* silk fibroin film had also been shown to have smooth surface (Srihanan, 2011). It is therefore suggested that the protein from *B. mori* and *O. exvinacea* form films of uniform thickness.

Secondary structure analysis of protein films using FTIR

The spectrum of pure web protein film of *O. exvinacea* had shown several absorption bands among which those representing amide bands are the most sensitive in the case of proteins. The absorption band at 1650 cm^{-1} in the spectrum of pure web protein film of *O. exvinacea* (Figure 3), represented amide I band. The amide II, another

important absorption band in the case of protein, appeared at 1529 cm^{-1} in the above spectrum. The band at a frequency of 1243 cm^{-1} in the spectrum (Figure 3) was the amide III band. The band appeared at a frequency of 649 cm^{-1} corresponds to amide IV band. Amide A and amide B were visible at a frequency of 3282 cm^{-1} and 3081 cm^{-1} in the spectrum. The absorption bands other than amide bands also appeared in the above spectrum at various frequencies. They were at 900 cm^{-1} , 964 cm^{-1} , 1068 cm^{-1} , 1170 cm^{-1} , 1330 cm^{-1} , 1407 cm^{-1} , 1448 cm^{-1} , 2962 cm^{-1} , 2940 cm^{-1} and 2140 cm^{-1} .

The frequencies of absorption band in the spectrum of fibroin film (Figure 4) were slightly different from silk protein film of *O. exvinacea* (Figure 3).

The amide I band of fibroin film (Figure 4) prepared from *B. mori* appeared at 1621 cm^{-1} , which possessed lower wave number than the same band appeared in the spectrum of web protein (Figure 3). The absorption band at a frequency of 1525 cm^{-1} fell in the amide II region. The second amide band had also shown lower wave number to the corresponding band of web protein, where it appeared at 1529 cm^{-1} (Figure 3). The amide A and B were noticed at frequencies of 3297 cm^{-1} and 3079 cm^{-1} respectively. The amide A band of fibroin had shown higher wave number to that of web protein. But the wave number of amide B was slightly lower than that of web protein. The amide III band was noticed at a frequency of 1243 cm^{-1} . The frequency of the band was similar to the frequency of the same band seen in the spectrum of web protein. The amide IV appeared at a frequency of 680 cm^{-1} , which was higher in wave number than that of same band in the web protein. As in the case of web protein, some additional absorption bands were seen in the spectrum of fibroin from *B. mori* also. They were at 900 cm^{-1} , 956 cm^{-1} , 1060 cm^{-1} , 1170 cm^{-1} , 1332 cm^{-1} , 2981 cm^{-1} and 2937 cm^{-1} .

Generally, the most sensitive amide bands in the proteins are amide I and amide II. The amide I band fall in the range of $1700\text{--}1600\text{ cm}^{-1}$ (Elliott and Ambrose, 1950; Miyazawa *et al.*, 1956; Krimm and Bandekar, 1986; Banker, 1992). The amide I band arise from the vibration of C=O bond in the protein backbone and its frequency of

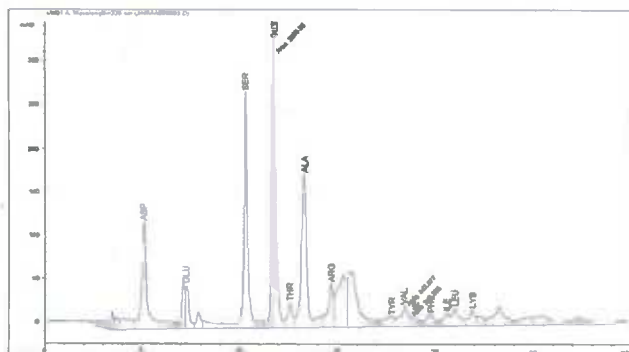


Figure 1: Separation of amino acids present in the purified silk protein of *O. exvinacea* using HPLC

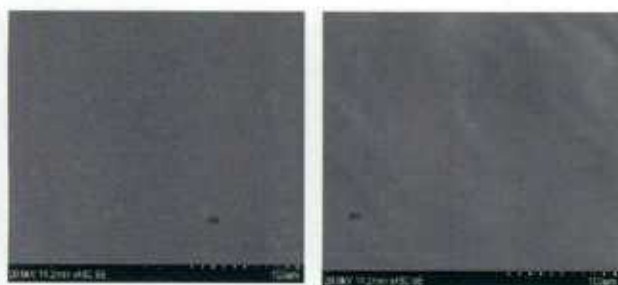


Figure 2: SEM images of protein film of *O. exvinacea* (a) and silk fibroin film of *B. mori* (b)

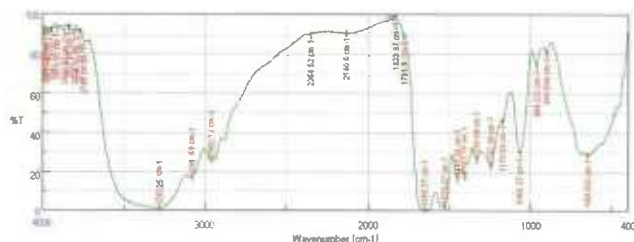


Figure 3: FTIR spectrum of silk protein film of *O. exvinacea*

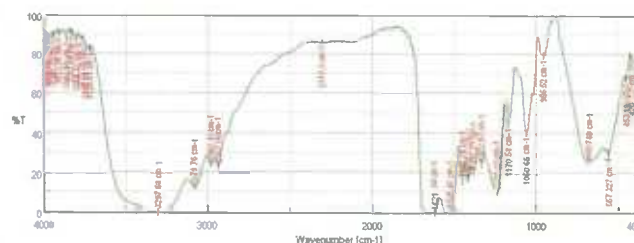


Figure 4: FTIR spectrum of silk protein film of *B. mori*

vibration changes due to the influence of H-bonds between N-H and C=O. The FTIR spectrum of the protein film of *O. exvinacea* showed amide I band at a frequency of 1550 cm^{-1} (Dong *et al.*, 1992 a, b), whereas the same band appeared at a wave number of 1621 cm^{-1} in the case of *B. mori*. The conformational characteristics assigned

to the band were different in both protein films because absorption peaks were seen at different frequencies. In *O. exvinacea*, this band is assigned to have a random coil structure (Dong *et al.*, 1992 a, b). On the other hand, to the band at 1621 cm^{-1} in the case of *B. mori* silk fibroin film, a β -sheet conformation has been assigned.

The absorption bands of protein appearing in between 1480 cm^{-1} and 1575 cm^{-1} region constitute amide II band. This band arises from C-N stretching coupled with N-H bending vibration (Elliott and Ambrose, 1950; Miyazawa *et al.*, 1956; Krimm and Bandekar, 1986; Banker, 1992). The amide II band of pure protein films of *O. exvinacea* and *B. mori* appeared at frequencies of 1529 cm^{-1} and 1525 cm^{-1} respectively. Goormaghtigh *et al.* (2006) found that amide II band of protein at a frequency of $1530 \pm 1\text{ cm}^{-1}$ provide β -sheet structure to the protein. Likewise, in *O. exvinacea* also, the same band might be responsible for β -sheet structure to the protein film and this may also increase its strength.

The third amide band resulted from C-N stretching and N-H bending vibrations of the protein (Elliott and Ambrose, 1950; Miyazawa *et al.*, 1956; Krimm and Bandekar, 1986; Banker, 1992). Protein films of *O. exvinacea* and *B. mori*, also gave amide III band both at a frequency of 1243 cm^{-1} , which is assigned to the presence of an unordered structure with β -turn conformation.

An absorption band is seen in the spectrum of *O. exvinacea* at frequency of 649 cm^{-1} , which may be an amide IV band and the same band appeared at a higher frequency of 680 cm^{-1} in *B. mori*. These may confer α -helix or random coil conformation to the protein film.

The spectrum of the pure protein film of *O. exvinacea* has an amide A band at 3282 cm^{-1} , whereas in the spectrum of the pure fibroin film from *B. mori*, it appeared at 3297 cm^{-1} , resulting from N-H stretching and O=C=N bending vibration. The frequency of amide B band of *O. exvinacea* and *B. mori* draw around 3080 cm^{-1} . Moreover, the absorption bands at various frequencies such as 900 cm^{-1} , 964 cm^{-1} , 1068 cm^{-1} , 1170 cm^{-1} , 1330 cm^{-1} , 1407 cm^{-1} , 1448 cm^{-1} , 2962 cm^{-1} and 2140 cm^{-1} have been noticed in the spectrum of *O. exvinacea*, which may be due to the

various intra and intermolecular bonds and its vibration. Additional absorption peaks were also found in the spectrum of *B. mori* fibroin film, which were at the frequencies of 900 cm^{-1} , 956 cm^{-1} , 1060 cm^{-1} , 1170 cm^{-1} , 1332 cm^{-1} , 2981 cm^{-1} and 2937 cm^{-1} .

The amide I band of protein film of *O. exvinacea* obtained in the present study was almost identical to that of silk fibroin of *B. mori* reported elsewhere, where it came at a frequency of 1649 cm^{-1} involved in the random coil conformation (Tretinnikov and Tamada, 2001). But the result of the present study on the silk fibroin of *B. mori* was different, where the amide I band provide β -sheet structure to the protein film. Studies conducted by Agostini de Moraes *et al.* (2010) on the regenerated silk fibroin of *B. mori* showed two peaks at frequencies, 1641 cm^{-1} and 1632 cm^{-1} , which were assigned β -sheet structure. The conformational characteristics of amide I band were same as seen in the spectrum of *B. mori* (Figure 4). In contrast to that of *O. exvinacea*, the amide I band of the pure eri silk fibroin film was assigned an α -helical conformation (Prasong, 2009). The conformational features provided by amide I band of *O. exvinacea* was exactly similar to that in the silk fibroin film of *A. pernyi* (Kweon and Park, 2001) but different from that of *B. mori*. In addition to the mulberry and non-mulberry silkworms, the spidroin film from iron spider also had an amide I band at 1660 cm^{-1} , which gave the aforementioned conformation of silk fibroin of *B. mori* (Bai *et al.*, 2006; Goormaghtigh *et al.*, 2006).

Although the frequencies of absorption bands seen in the amide II region were different, the structural characteristics provided by the protein films of *O. exvinacea* and *B. mori* were the same. Previous studies conducted on the tasar silk fibroin also revealed that its amide II band appearing at a frequency of 1520 cm^{-1} , have β -sheet conformation for its fibroin film (Kweon and Park, 2001). The amide II band of mulberry silk fibroin film at 1532 cm^{-1} has the same conformation as the amide II band of protein film of *O. exvinacea* (Chen *et al.*, 2001). The frequency and conformation of amide II band of spidroin film from iron spider was similar to that seen in mulberry silk fibroin film (Bai *et al.*, 2006).

However, Um *et al.* (2001) and Wang *et al.* (2008) found that the amide III band at 1235 cm^{-1} of the silk fibroin film of *B. mori*, could be assigned random coil conformation. Here the amide III band appeared at 1243 cm^{-1} and this is different from our studies conducted on the protein films of *O. exvinacea* and *B. mori*. Another report on the spectrum of tasar silk fibroin, states that the protein had an α -helical conformation at the amide III region (Kweon and Park, 2001). In iron spider, the amide III band obtained for the spidroin film has been assigned β -sheet structure (Bai *et al.*, 2006).

Thermal analyses of protein films

Thermogravimetric curve of web protein of *O. exvinacea* (Figure 5) provides information about its thermal stability. The weight loss brought to the film at 100°C was about 8 %. In between 100 and 250°C, the protein lost 20 % of its weight. About 17 % of the protein has been burned off at the temperature range from 250 to 300°C. Considerable weight losses of about 36 % have been noticed in between 300 and 350°C. As the temperature increased, the rate of decomposition and weight loss were also high. The protein exhibited 50 % of mass loss at 308°C and the maximum rate of thermal degradation was at 307°C (Figure 6). The film showed rapid degradation beyond 300°C and it lost about 95 % of weight at 500°C. However, the residual weight of the film at 500°C was about 4.2 %.

The pure fibroin protein of *B. mori* had an initial mass loss of about 8 % (Figure 5) at 100°C. A further mass loss of about 9 % was noticed between 100 and 200°C. The maximum rate of thermal degradation was seen at 306.75°C (Figure 7). But the film lost half of its weight at 311°C. Tremendous weight loss of about 41 % has been observed in the temperature range from 250 to 350°C. The weight loss continued as temperature increased. About 95 % of protein was burned off at 500°C. The remaining weight was found to be 5 % at 500°C.

Thermal analysis of the protein films were done by TG-DTA analysis. The initial weight loss encountered at a temperature below 100°C was due to the moisture evaporation (Kweon and Park, 2001). The range of temperature at which the protein films of *O. exvinacea* and

B. mori experienced maximum weight loss was almost similar. Furthermore, temperature required for maximum decomposition and the residual weight of protein film of *O. exvinacea* and silk fibroin film of *B. mori* were found to be the same. Even though the β -sheet content of protein was high in *B. mori*, the thermal stability of the two proteins were almost equal. A similar case was reported in *B. mori*, where, the film had gone through a maximum decomposition rate at a temperature of around 308°C (Prasong *et al.*, 2010). At temperature above 100°C, the weight loss experienced by the film was associated with the breakdown of side chains and peptide bonds between the amino acids (Freddi *et al.*, 1997). In the case of the film of eri and tasar silk fibroin, a maximum loss of weight occurred in range of 300 to 400°C (Kweon and Park, 2001; Li *et al.*, 2003; Prasong, 2009) unlike the temperature reported in our study.

Our study focussed on the application of pyralid silk in the biomedical and biotechnological fields rather than its application in textile field. Even though the amino acid composition of pyralid silk and mulberry silk were different, the spectra of both the protein films were composed of α -helix, random coil, β -sheet and β -turn structures. The content of β -sheet was higher in the protein film of *B. mori* than that of *O. exvinacea*. Here, the difference in the content of β -sheet did not influence the thermal stability of the protein films and they possessed almost similar thermal characteristics. The slight deviation observed in the structural and thermal data for *B. mori* silk fibroin film from the previously published data may be due to the difference in the processing conditions such as temperature, pH, salts used for solubilisation, casting conditions of the films *etc.* It thus becomes possible that we can consider the silk protein of *O. exvinacea* as a supplement or alternative to *B. mori* silk fibroin in various biotechnological and biomedical applications.

CONCLUSION

Silk protein from *O. exvinacea* was rich in Serine, Histidine and Alanine, whereas the silk fibroin of *B. mori* is composed of Glycine and Alanine. Comparative morphological, structural and thermal characteristics of

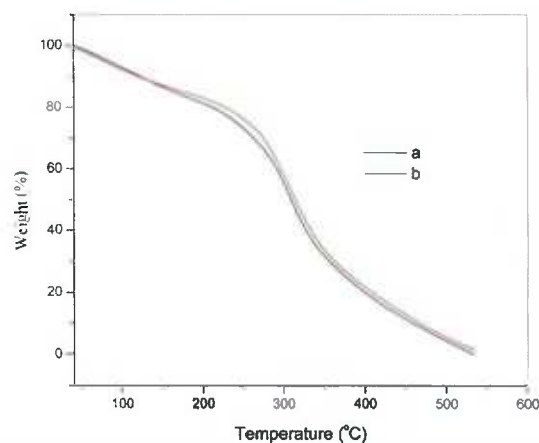


Figure 5: Thermogravimetric curves of protein film of *O. exvinacea* (a) and silk fibroin film of *B. mori* (b)

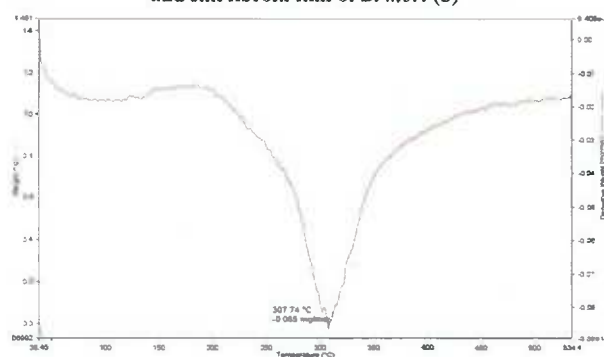


Figure 6: DTG curve of protein film of *O. exvinacea*

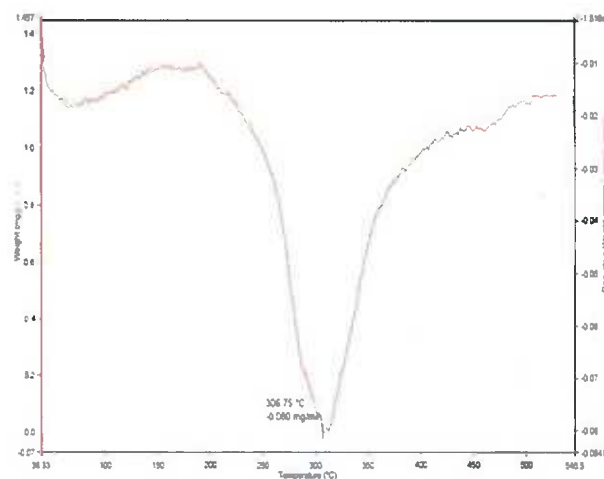


Figure 7: DTG curve of protein film of *B. mori*

protein films of *O. exvinacea* and *B. mori* were worked out using SEM, FTIR and TG-DTA. The structural characteristics of the spectrum of *O. exvinacea* revealed that the regenerated protein film has a mixture of random coil, β -sheet and unordered structure with β -turn. The protein film of *B. mori* was composed of β -sheet and unordered structure with β -turn, which was found to be more stable than conformational characteristics shown by

O. exvinacea. These changes did not have any major effect on their respective thermal properties. The similarity in structural and thermal properties of silk of *O. exvinacea* with that of *B. mori* give a ray of hope to utilize the pyralid silk in various biotechnological and biomedical fields. For application of this pyralid silk in diverse fields, more detailed analyses of structural and functional characteristics are required.

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ANALYSE COMPAREE DE LA MORPHOLOGIE, DE LA STRUCTURE ET DES PROPRIETES THERMIQUES DE FILMS DE SOIE OBTENUS A PARTIR DE LA PYRALE DU MANGUIER, *ORTHAGA EXVINACEA* ET DE *BOMBYX MORI*

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RESUME

La soie de la toile d'*Orthaga exvinacea* et celle du cocon de *Bombyx mori* ont été isolées par solubilisation de fibres décreusées dans une solution saturée de bromure de lithium puis purifiées par dialyse. La composition en acides aminés de la protéine de soie d'*O. exvinacea* a été analysée par HPLC. La soie de la pyrale est riche en Sérine, Histidine et Alanine. Après avoir quantifié la protéine dans l'échantillon purifié, nous avons formé des films. Les films séchés ont été soumis à des analyses morphologiques, structurales et thermiques en utilisant SEM, FTIR et TG-DTA. L'analyse comparée de la soie d'*O. exvinacea* et de celle de *B. mori* montre qu'elles diffèrent par leur contenu en feuillets β . La résistance du film dépend des feuillets β , qui sont plus abondants chez *B. mori* que chez *O. exvinacea*. Mais la stabilité thermique des deux types de films est à peu près égale. Les températures du taux de dégradation maximale des deux films protéiques diffèrent d'un degré centigrade. Cette information suggère que la soie de la pyrale du manguier peut être substituée à celle du ver à soie dans diverses applications biomédicales et biotechnologiques.

Mots-clés: *Bombyx mori*, FTIR, *Orthaga exvinacea*, fibroïne de soie, TG-DTA.



EVALUATION OF LOW-STRESS MECHANICAL PROPERTIES OF ERI / WOOL BLENDED FABRICS

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ABSTRACT

The blending of eri with wool fibre in spinning has proved to bring in more comfort, luster, thermal and shrinkage properties in the blended fabric. To determine the utility of the fabric for apparel applications, the effects of blend at six different levels of the low stress mechanical characteristics of eri / wool blended fabrics have been investigated. The KES-F system was used for this purpose and analysed for tensile, compression, shear, surface, bending and geometric roughness properties. The study exhibited increase in tensile energy, compression energy, shear properties, shrinkage and geometric roughness of the fabric and *vice versa*, in bending and frictional properties of the blended fabric with increase of eri component. The effects of the blends at various levels of blends are discussed.

Key words: Eri / wool blend, KES-F system, low stress mechanical properties.

INTRODUCTION

Eri is one of four kinds of silks produced in India. While eri silk is extruded from silkworm which feeds on castor / tapioca leaves, wool is an animal fiber that is sheared from the sheep but both are protein fibres.

The fineness of eri fiber (14-16 microns) is very close to that of wool so that they can be blended easily. As finer wool fibre is not available in India, it is imported from other countries. In place of finer wool of 14-16 microns, eri fibre may be an alternative. By blending eri with wool, fabric properties such as smoothness, luster, durability, dimensional stability, pilling properties, crease resistance *etc.* can be improved since eri has better properties than wool. However, thermal properties of eri are close to that of wool (Kariyappa *et al.*, 2006). Hence, to achieve the above properties, eri / wool blend 2/68 count spun yarn was produced with different proportions of wool: eri ratio *viz.*, 0:100, 30:70, 40:60, 50:50, 70:30 and 100:00 and analysed for their properties so as to select appropriately for end uses.

Blending of eri and wool fibres is a new attempt but prior to this, mulberry silk was blended with wool. This

combination reduces the cost of the blended yarn since the cost of 16 microns wool is about ₹ 800-900 per kg and the cost of eri cocoons is ₹ 280-350 per kg. The eri / wool mill spun yarn can be utilized for manufacturing of all the varieties of fabrics such as inner wear, dress material, ornamental fabric, thicker fabrics like chaddars and carpet, wall hangings and furnishings. This yarn is the best suited for hosiery fabric too. The mill spun yarn can be woven on hand loom, power looms and even on the latest shuttle looms. Hosiery items can also be produced in both flat knitting and circular knitting machines, hand operated as well as on the latest high speed ones. Thus the functionality of eri / wool blended fabric has proved excellent.

Generally, for any end use of textiles, the mechanical characteristics of fabrics play an important role and they are tensile, bending, shear, compression and surface roughness properties (Kavitha *et al.*, 2007). These elementary properties are generally related to important fabric characteristics such as drape handle, tailorability, wrinkling, creasing and shape retention.

The earlier works in this direction were conducted only on pure mulberry silk and woollen fabric. Hence, the

present study was carried out to have a general view of performance of eri / wool blended fabrics when subjected to a wide range of complex deformations.

MATERIALS AND METHODS

Preparation of material

- Six different combinations of eri / wool (2/68^s) blended yarn were produced by M/s Swan silk Ltd., Doddaballapur.
- Using these blended yarn, 10 meters of plain fabric was woven out of each combination at Lakshmi power loom, Doddaballapur with cloth particulars of Reed 60^s, PPI 45, plain weave, and same count of yarn used in warp and weft.

Assessment of low-stress mechanical properties

The fabric was tested at CIRCOT Mumbai for low stress mechanical properties (KES values).

The blended fabric was subjected to the low stress mechanical properties which were assessed at standard laboratory conditions of 20 °C and 65 % RH. The Kawabata fabric evaluation system (KES-F) was employed to measure the low stress properties, which included tensile, shear, bending, compression and surface properties. The KES-F measures deformation in the low stress region where apparel fabric deforms. The outline of the key parameters involved in the KES-F test is shown in Table 1.

Table 1: Principle of KES-F measurement

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

Fabric shrinkage has been measured as per IS 3561-1989

The dimensional changes in the eri / wool blended spun yarn fabrics were tested according to IS 3561-1989 method. The dimension of the sample used was 50 x 50 cm, square marked inside the fabric as reference point. Laid one test specimen flat, after removing all creases and wrinkles by hand for one hour in a water tight tray containing 5gpl of soap solution at 50 °C. The depth of the solution above specimen was ensured to be at least 25 mm. After one hour, poured off the liquid and washed the specimen first with water at 50 °C for 20 minutes and then with cold water for 5 minutes in flat condition. Removed the specimen without distortion from the tray and placed it flat on a towel. Ensured that the specimen is not stretched during handling. Laid the specimen on a smooth flat surface and allowed drying at room temperature and remeasured at corresponding reference points. For each variety of fabric, five replications were taken. The degree of shrinkage (expressed in %) was calculated using the following equation:

$$\text{Fabric shrinkage \%} = \frac{\text{Original area of fabric} - \text{area of fabric after treatment}}{\text{Original area of fabric}} \times 100$$

Thermal insulation value (TIV)

TIV of fabric was measured from Thermolabao-II as per Guarded hot plate method maintained at 37 °C and wind speed of one meter per second.

Data were analysed using SPSS 11.5 and Windo stat package.

RESULTS AND DISCUSSION

Tensile properties

The tensile properties of the eri / wool blended fabric were measured using tensile tester (KES-FBI). The average and CD values of LT, WT, RT and EMT of the samples are presented in Table 2.

LT represents the linearity of load extension curve. LT values below 1.0 indicate that the stress strain curve falls above a 45° straight line. It is also indicative of wear. Lower value indicates better comfort. The table shows decreasing LT values with increasing eri component. This shows that the linearity of stress strain curves

Table 2: Tensile properties

Fabric	Linearity load extension curve (LT)	Tensile energy (WT) gf.cm/cm ²	Tensile resiliency (RT) (%)	Extensibility EMT (%)
E 100 %	0.699 ± 0.017	8.14 ± 0.918	61.92 ± 3.602	4.41 ± 0.757
W:E (30:70)	0.705 ± 0.036	8.01 ± 3.685	57.29 ± 4.558	4.43 ± 1.616
W:E (40:60)	0.719 ± 0.049	7.96 ± 3.125	56.88 ± 6.242	4.24 ± 1.731
W:E (50:50)	0.743 ± 0.054	7.90 ± 3.195	56.76 ± 6.892	4.22 ± 1.622
W:E (70:30)	0.744 ± 0.045	7.60 ± 3.009	56.26 ± 8.891	4.16 ± 1.402
W 100 %	0.754 ± 0.017	7.03 ± 1.476	55.96 ± 5.977	4.02 ± 1.671
CD at 5 %	0.06 ^{NS}	4.38 ^{NS}	9.30 ^{NS}	2.23 ^{NS}

E - Eri, W - Wool

reduces with increase in eri component in the blended yarn because of the high elongation percentage of eri silk (Matsudaira and Kawabata, 1988).

WT represents the tensile energy per unit area *i.e.*, the area under load elongation curve. Higher value of WT corresponds to higher extensibility. The Table shows an increase in WT values with increase of eri component in the blended yarn. This implies that durability of fabric increases with increase in eri component in the blended yarn of the fabric. This is due to the fact that tenacity of eri silk is higher than wool.

Tensile resilience (RT %) is recovery from tensile deformation. A completely elastic fabric is characterized by 100 % RT while an inelastic fabric has 0 % RT. Increased proportion of eri component in the blended yarn has brought in a corresponding increase in RT (Table 2). It shows that the elasticity of the blended fabric increases with increase in eri component in blended yarn. This increase in resilience provides springness to the fabric.

EMT is extensibility under a load of 500 g/cm². A higher value of EMT indicates wear comfort. However, it creates problems in stitching and seam pressing. The values of EMT from the Table show an increase in extensibility of the fabric with increase in eri component. This is because of the elasticity rendered to the fabric by blending of eri with wool. This may be attributed to the flexibility of the fabrics due to eri silk which has got

an elongation percentage higher than that of wool.

From ANOVA (Table 8), it is inferred that there are no significant differences among all the six varieties of blended fabrics in respect of LT, WT, RT and EMT.

Shear properties

The shear properties of the eri / wool blended fabric measured using tensile tester (KES-FBI) are shown in Table 3.

Table 3: Shear properties

Fabric	Shearing stiffness (G) gf.cm.deg	Hysteresis of shear force at 0.5° of shear angle (2HG)	Hysteresis of shear force at 5° of shear angle (2HG5)
E 100 %	1.60 ± 0.161	8.00 ± 0.719	8.83 ± 0.268
W:E (30:70)	1.38 ± 0.209	6.34 ± 0.524	7.27 ± 0.521
W:E (40:60)	1.33 ± 0.048	5.86 ± 0.385	6.38 ± 0.189
W:E (50:50)	1.28 ± 0.037	4.98 ± 1.007	6.25 ± 0.924
W:E (70:30)	0.97 ± 0.100	4.72 ± 0.540	5.56 ± 0.459
W 100 %	0.85 ± 0.073	4.32 ± 0.396	5.16 ± 0.269
CD at 5 %	0.18**	0.94**	0.75**

The behavior of a fabric when subjected to shearing force is one of the factors that determines its performance when subjected to a wide variety of complex deformations during use. It is this property that enables the fabric to undergo more complex deformations than two-dimensional bending which conform to the contours of the body in clothing applications.

Shear rigidity is a measure of fabric's ability to deform in its plane in a trellising motion where the angle between warp and weft threads is changed from 90°. The data show that the shear rigidity (G) increases with increase in eri component in eri / wool blended yarn of the fabric. Fabric becomes resistant to the rotational movement of the warp and weft threads within it. Hysteresis of shear force at 0.5° shear angle (2HG) and of shear force at 5° shear angle (2HG5) shows the same trend. The dimensional stability of the fabric increases with increase of eri silk component in the blended yarn fabric (Behara, 2007).

ANOVA (Table 8) indicates significant differences

among all the six varieties of blended fabrics in respect of G, 2HG and 2HG5.

Bending properties

Fabric bending is affected by the inter fibre friction within and between the yarns as well as the bending properties. 2HB represents the hysteresis of bending moment, which is a measure of recovery from bending deformation. Bending rigidity is one of the important properties that influences the tailorability of the fabric. Table 4 shows the average and CD values of bending rigidity (B) and hysteresis of bending moment (2HB) measured using bending tester (KES-FB2). There is decrease in B and 2HB values with increase in eri component in the blended yarn of the fabric. This reduction implies increased flexibility of fabric with increase in eri component. This is because of higher elongation percentage of eri fibre compared to wool. Therefore, tailorability of fabric increases with increase in eri component.

Table 8 shows significant differences among all the six varieties of blended fabrics in terms of B and insignificant differences in respect of 2HB.

Table 4: Bending properties

Fabric	Bending rigidity (B) gf.cm ² /cm	Hysteresis of bending moment (2HB) gf.cm/cm
E 100 %	0.0773 ± 0.005	0.1288 ± 0.009
W:E (30:70)	0.1055 ± 0.015	0.1411 ± 0.010
W:E (40:60)	0.1096 ± 0.003	0.1901 ± 0.021
W:E (50:50)	0.1249 ± 0.018	0.1927 ± 0.022
W:E (70:30)	0.1373 ± 0.016	0.2000 ± 0.086
W 100 %	0.1388 ± 0.03	0.2151 ± 0.052
CD at 5 %	0.03**	0.06 ^{NS}

Compressional properties

Kawabata system (KEF-FB3) characterizes the compressional characteristics using four different parameters such as the compressional energy (WC), the

linearity of the compression/decompression curve (LC), the resilience (RC) and the thickness (To). Average and CD values for the samples tested are given in Table 5.

CD values are given in Table 6. MIU is a measure on the mean value of coefficient of friction between fabric

Table 5: Compression properties

Fabric	Linearity compression thickness curve (LC)	Compressional energy (WC) gf.cm ² /cm	Compression resilience (RC) %	Fabric weight (mg/cm ²)	Thickness mm (To)	Thickness mm (Tm)
E 100 %	0.267 ±0.072	0.273 ±0.024	43.27±5.157	13.82	0.767±0.058	0.419±0.004
W:E (30:70)	0.276 ±0.040	0.271 ±0.014	44.70±3.927	11.68	0.694±0.053	0.413±0.023
W:E (40:60)	0.327 ±0.036	0.255 ±0.022	48.38±3.132	14.82	0.725±0.037	0.404±0.009
W:E (50:50)	0.331 ±0.013	0.255 ±0.038	49.82±4.829	13.73	0.824±0.116	0.395±0.050
W:E (70:30)	0.340 ±0.025	0.248 ±0.022	50.13±2.006	13.38	0.726±0.036	0.429±0.004
W 100 %	0.345 ±0.053	0.231 ±0.049	59.28±2.323	14.77	0.721±0.040	0.419±0.005
CD at 5 %	0.07 ^{NS}	0.05 ^{NS}	5.58**		0.09 ^{NS}	0.03 ^{NS}

LC represents linearity of compression-thickness curve. Completely linear curve has 1 as its LC value while extremely non-linear curve has 0 value. Linearity shows a marginal reduction with increase in eri component in the blended yarn.

Compressional energy of the samples represented by WC shows marginal increase with increases of eri component in the blended yarn. Higher WC values correspond to higher compressibility of the fabric. As the thickness of the samples increases with increase in eri component, the work of compression increases due to which the fabric becomes soft.

Compressional resilience (RC %) indicates the springness of the fabrics. Thicker fabrics have low RC values in general and the data show decrease in RC values with increase in eri silk component in the blended yarn of the fabric. This is because of the higher bulkiness of wool fibre compared to eri silk fibre.

ANOVA (Table 8) indicates insignificant differences among all six varieties of blended fabrics in terms of LC and WC and significant differences in respect of RC.

Surface properties

Surface properties of the eri / wool blended fabric were measured using surface tester (KES-FB4). Average and

surface, metallic piano model surface and metallic piano model surface detector, whose surface is simulated to the finger surface. The data show that coefficient of friction of the fabric is reduced with increase in eri component in the blended yarn of the fabric. This reduction in MIU values implies that eri component in the fabric have reduced the friction between the yarns. Mean deviation of the coefficient of friction (MMD) reduced with increase of the eri component in the blended yarn. Geometric roughness (SMD) of the fabric increased with increase in the eri component of the blended yarn. This increase in geometric roughness in the fabric is due to the flat cross section of eri silk filaments in spun yarn fabric.

Significant differences among all the six varieties of blended fabrics were noticed in MIU, MMD and SMD (Table 8).

Thermal insulation value

Thermal insulation value (TIV) is a measure of thermal properties of the fabric. TIV value at its highest is observed for 50:50 eri / wool fabric, followed by 60:40 eri / wool fabric, 30:70 eri / wool fabric, 100 % wool fabric, 70:30 eri / wool fabric and 100 % eri fabric (Table 7). From ANOVA (Table 8), it is observed that there are significant differences among all varieties of blended fabrics.

Table 6: Surface properties

Fabric	Coefficient of friction (MIU)	Mean deviation coefficient of friction (MMD)	Geometric roughness (SMD) (microns)
E 100 %	0.152 ±0.004	0.0255 ±0.004	14.19 ±1.258
W:E (30:70)	0.166 ±0.009	0.0261 ±0.003	11.64 ±1.999
W:E (40:60)	0.171 ±0.009	0.0299 ±0.016	10.59 ±1.214
W:E (50:50)	0.170 ±0.002	0.0519 ±0.004	9.86 ±1.350
W:E (70:30)	0.170 ±0.001	0.0654 ±0.016	8.79 ±1.181
W 100 %	0.177 ±0.002	0.0677 ±0.004	8.58 ±0.418
CD at 5 %	0.01**	0.01**	1.96**

Table 7: Thermal insulation value

Fabric	Blend ratio (%)	TIV (%)
Eri	100	23.6±0.000
Eri/Wool	70/30	23.9±0.376
Eri/Wool	60/40	27.4±0.000
Eri/Wool	50/50	28.0±0.000
Eri/Wool	30/70	27.3±0.000
Wool	100	26.9±0.000
CD at 5 %		0.39636**

Fabric shrinkage

It is a measure of shrinkage of the fabric, the average and CD values of eri /wool blended spun yarn fabric shrinkage properties are depicted in Table 9. It is observed that fabric shrinkage percentage is the highest in the case of pure eri spun silk fabric in both the directions *i.e.*, warp way (5.16 %) and weft way (3.9 %). The fabric shrinkage percentage (warp way and weft way) decreases with increase in wool component in the blended yarn. The fabric shrinkage percentage is the lowest in the case of pure wool in warp way (2.33 %) and weft way (0.74 %). From the statistical analysis, it is observed that there are significant differences among six varieties of blended spun fabric for shrinkage percentage in warp and weft way. Fabric shrinkage in warp way in all six varieties of fabric is significantly higher than that in weft way.

Table 8: ANOVA

Character	Sum of Squares	Df	Mean squares	F	Significance
LT	.010	5	.002	1.337	0.294
WT	3.327	5	.665	.077	0.995
RT	97.598	5	19.520	.498	0.773
EMT	.484	5	.097	.043	0.999
G	1.537	5	.307	20.753	0.000
2HG	36.485	5	7.297	18.234	0.000
2HG5	35.068	5	7.014	27.760	0.000
B	.011	5	.002	7.573	0.001
2HB	.024	5	.005	2.577	0.063
LC	.023	5	.005	2.373	0.081
WC	.005	5	.001	1.048	0.420
RC	635.425	5	127.085	9.017	0.000
TO	.042	5	.008	2.127	0.109
TM	.004	5	.001	1.578	0.216
MIU	.002	5	.000	9.548	0.000
MMD	.008	5	.002	29.312	0.000
SMD	95.573	5	19.115	10.977	0.000
TIV	73.939	5	14.788	628.970	0.000

Table 9: Fabric shrinkage

Blend	Blend ratio (%)	Shrinkage %	
		Warp	Weft
Eri	100	5.16 ±0.27	3.92 ±0.22
Eri/Wool	70/30	4.66 ±0.42	3.42 ±0.22
Eri/Wool	60/40	4.24 ±0.27	2.00 ±0.00
Eri/Wool	50/50	2.83 ±0.27	1.74 ±0.27
Eri/Wool	30/70	2.66 ±0.27	1.51 ±0.22
Wool	100	2.33 ±0.27	0.74 ±0.27
CD at 5 %		0.39636**	0.2546**

CONCLUSION

From the above study, it is concluded that blending of eri fibre with wool fibre in spinning has affected the low-stress mechanical characteristics of eri / wool blended fabric. The effects were found to be dependent on the component of eri silk in the fabric. By blending of eri silk with wool in spinning, important fabric properties *viz.*, wear comfort, softness, smoothness thermal comfort, dimensional stability and tailorability of fabric could be improved.

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EVALUATION DES PROPRIETES MECANQUES EN TENSION FAIBLE DE TISSUS CONSTITUES D'UN MELANGE SOIE ERI / LAINE

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RESUME

Il est prouvé que le mélange de fibres eri et de laine au dévidage donne plus de confort, de lustre et de résistance au déchirement aux tissus mélangés. Afin de définir l'utilité du tissu pour des applications en habillement, nous avons étudié l'effet du mélange à six niveaux différents dans les tissus de mélange eri/laine en tension mécanique faible. Le système KES-F a été utilisé à cet effet et à l'analyse des propriétés d'élasticité, de compression, de cisaillement, de surface, de pliage et de rugosité géométrique. L'étude montre que l'énergie d'élasticité et de compression, les propriétés de cisaillement, le déchirement et la rugosité géométrique s'accroissent et inversement pour les propriétés de pliage et de friction des tissus mélangés lorsque la part eri augmente. Les effets du mélange à différents niveaux sont discutés dans cet article.

Mots-clés: Mélange eri/laine, système KES-F, propriétés mécaniques à faible tension.



A NEW ROBUST BIVOLTINE DOUBLE HYBRID (CSR50 x CSR52) x (CSR51 x CSR53) OF *BOMBYX MORI* L. FOR HIGHER EGG AND COCOON YIELD

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ABSTRACT

The present study was undertaken to assess the performance of newly evolved bivoltine double hybrid, (CSR50 x CSR52) x (CSR51 x CSR53) with reference to egg and cocoon yield parameters and also under field conditions. Four parental breeds viz., CSR50, CSR52 (oval type), CSR51 and CSR53 (dumbbell type) and two foundation crosses (FCs) viz., CSR50 x CSR52 (oval x oval) and CSR51 x CSR53 (dumbbell x dumbbell) were utilized for preparation of new double hybrid, (CSR50 x CSR52) x (CSR51 x CSR53) / (CSR51 x CSR53) x (CSR50 x CSR52) and studied along with control single hybrid CSR2 x CSR4/CSR4 x CSR2 and double hybrid (CSR6 x CSR26) x (CSR2 x CSR27) / (CSR2 x CSR27) x (CSR6 x CSR26). Statistical analysis indicated that the new double hybrid is significantly ($P < 0.05$) superior over single hybrids in respect of egg yield (34.23 %), disease free layings (dfls) recovery (18.46 %), eggs / disease free laying (20.11 %), less unfertilized and non-diapause eggs. Further, a marginal improvement in respect of egg yield (6.42 %) and disease free layings recovery (8.15 %) was recorded over control double hybrid. Field evaluation of new double hybrid (CSR50 x CSR52) x (CSR51 x CSR53) indicated that the average cocoon yield was 68.72 kg / 100 dfls as against 63.02 kg in CSR2 x CSR4 and 65.18 kg / 100 dfls in (CSR6 x CSR26) x (CSR2 x CSR27). The results indicate the suitability of the new double hybrid (CSR50 x CSR52) x (CSR51 x CSR53) for commercial rearings in southern states.

Key words: Bivoltine silkworm, *Bombyx mori* L., double hybrids, egg yield, field performance.

INTRODUCTION

The phenomenon of hybrid vigour was exploited in plants especially in maize much earlier to that of silkworm, but the extent of exploitation in silkworm is unparalleled to any of the commercial crops. Hybrid vigour in silkworm has received a considerable attention because of marked effect on the yield components (Harada, 1961; Sengupta *et al.*, 1974). In India, of late, much emphasis is being given for bivoltine silkworm rearing to boost up the quality silk production matching international standards. With the increase in demand for F1 hybrids, the double hybrids are popularized because of its obvious advantages viz., easy rearing of foundation crosses and higher vigour compared to parental breeds. The polygenic expression in double hybrids is more stable than that in single hybrids in unfavourable environments because of their flexibility in gene constitution within the population (Watanabe, 2002).

The primary goal of silkworm breeding is to develop breeds and select hybrids with better qualitative as well as quantitative traits. In this context, systematic evaluation of hybrids among the farmers plays an important role in achieving the goal. Therefore, the authorized hybrids are to be evaluated at field level in large scale. The F1 hybrids being more productive and robust can be easily reared by the farmers by adopting appropriate rearing technology. However, when it comes for F1 seed production, the parental breeds with high cocoon shell percentage show low viability and produce less number of eggs. Moreover, owing to the existence of negative correlation between cocoon shell percentage and survival percentage in pure races, the handling of these parental breeds needs more care and attention. To overcome this, the double hybrids with suitable foundation crosses (FCs) were developed with clear advantages such as easy rearing, better growth and vigour of FCs and superior economic characters over

single hybrids (Nirmal Kumar *et al.*, 1998). This helps not only in generation of quality seed cocoons but also high egg recovery. Due to its multifarious advantages, attempts have been made to develop and identify suitable double hybrids at Central Sericultural Research and Training Institute, Mysore. The quality of silkworm eggs, the basis for sustenance of sericulture, is commonly judged by characteristics such as freeness from disease, absence of unfertilized eggs, more number of eggs and effective hatching. Egg yield refers to number of eggs laid by individual female moth, number of eggs per gram and total egg yield per kilogram of cocoons which form the vital index for an egg producer for production of quality seed. The present investigation was undertaken to generate information on comparative performance of egg and cocoon yield parameters in newly developed double hybrid (CSR50 x CSR52) x (CSR51 x CSR53) and its field performance as well.

MATERIALS AND METHODS

Four parental breeds *viz.*, CSR50, CSR52 (oval type), CSR51 and CSR53 (dumbbell type) and two foundation crosses (FCs) *viz.*, CSR50 x CSR52 (oval x oval) and CSR51 x CSR53 (dumbbell x dumbbell) were utilized for preparation of double hybrid, (CSR50 x CSR52) x (CSR51 x CSR53) / (CSR51 x CSR53) x (CSR50 x CSR52) and studied along with control single hybrid CSR2 x CSR4/CSR4 x CSR2 and double hybrid (CSR6 x CSR26) x (CSR2 x CSR27)/(CSR2 x CSR27) x (CSR6 x CSR26). The required cocoons of parental breeds and FCs were generated as per standard rearing techniques (Datta, 1992). One kilogram of cocoons was taken at random from the harvested lot and the cocoons were cut open and pupae were sexed. The pupae of both sexes were kept separately (parents/foundation crosses) under optimum temperature ($25 \pm 1^\circ\text{C}$) and relative humidity (75–80 %). The pupae were preserved under dark condition for uniform emergence and covered with a perforated paper to facilitate easy picking of moths. The emerged moths were paired as per the respective parental combinations. After 3–4 hours, the females were depaired smoothly and placed individually on starch coated egg sheets and allowed for egg laying for 24 hours in an oviposition room under dark condition. The depaired males were preserved at 5–7 °C and were reused for coupling for a maximum of

two times only. Loose eggs were prepared according to the method suggested by Samson *et al.* (1995).

A total of 20200 dfls of new double hybrid (CSR50 x CSR52) x (CSR51 x CSR53) were distributed to different locations in three southern states, Karnataka (Srirangapatna and Koppal), Tamil Nadu (Gobichettipalyam, Hosur, Udumalpet and Salem) and Andhra Pradesh (Venkatagiri Kota, Penukonda and Vikarabad) through Regional Sericultural Research Stations/Research Extension Centres of Central Silk Board. The data pertaining to cocoon and yield parameters were recorded and compiled.

RESULTS AND DISCUSSION

The data on performance of parents, foundation crosses and hybrids are given in Tables 1, 2 and 3. Though there were no significant differences in shell percentage among the parental breeds and foundation crosses, significant differences ($P < 0.05$) were noticed for pupation rate, cocoon yield, cocoon weight, shell weight and cocoon melting percentage. Melting percentage of cocoon was less in FCs (1.2 to 1.6 %) as against parents (2.3 to 3.5 %). In all the breeds/foundation crosses, the percentage of male population was more than the female. The lowest (45.0 %) female and the highest male population (55.0 %) was recorded in CSR51 whereas the highest female (48.0 %) and the lowest male population (52.0 %) was recorded in CSR50 x CSR52. Statistical analysis indicated that the FCs have significant ($p < 0.05$) improvement over parental breeds in respect of pupation rate (3.94 %), cocoon yield (11.99 %), cocoon weight (8.48 %) and shell weight (9.48 %).

The egg yield per kilogram of cocoons is considered as another important parameter, which reflects the quality of cocoons. This ranged from 60.4 (CSR4 x CSR2) to 85.6 g (CSR50 x CSR52) x (CSR51 x CSR53). The highest egg yield was recorded in double hybrids which is significantly higher than the F1 hybrid. The differences observed in egg yield can be attributed to the quality of seed cocoons, sex ratio, number of pairs obtained and egg recovery which also decide the cost of egg production for egg producers (Benchamin and Krishna Reddy, 1981). Gupta *et al.* (1991) observed that the egg yield was higher in bivoltine breeds as compared to multivoltine pure

Table 1: Performance of pure breeds and foundation crosses (FC) of double hybrids

Breed/FC	Pupation (%)	Cocoon yield/ 10,000 larvae (kg)	Cocoon weight (g)	Shell weight (g)	Shell (%)	Melting (%)	Sex ratio (%)	
							Female	Male
CSR2	90.3	16.12	1.835	0.437	23.8	3.6	46	54
CSR4	89.0	15.10	1.732	0.383	22.1	5.2	45	55
CSR6	90.6	15.74	1.768	0.391	22.1	3.1	44	56
CSR26	93.2	15.81	1.726	0.380	22.0	2.9	44	55
CSR27	91.3	15.87	1.769	0.428	24.2	4.2	48	52
CSR50	91.2	16.63	1.854	0.439	23.7	3.2	46	54
CSR51	89.3	15.37	1.752	0.389	22.2	3.5	45	55
CSR52	93.5	16.97	1.815	0.421	23.2	2.3	46	54
CSR53	91.2	15.91	1.745	0.386	22.1	3.2	45	55
CSR6 x CSR26	94.6	17.38	1.867	0.413	22.1	1.8	46	54
CSR2 x CSR27	95.6	18.15	1.928	0.453	23.5	1.6	47	53
CSR50 x CSR52	95.2	18.42	1.964	0.464	23.6	1.2	48	52
CSR51 x CSR53	94.6	17.91	1.923	0.431	22.4	1.6	47	53
CD at 5 %	1.8	1.2	0.045	0.03	0.75	1.2		
% improvement -parents vs foundation crosses (new double hybrid)	3.94	11.99	8.48	9.48	0.88			
% improvement -parents vs foundation crosses (control)	2.85	9.95	6.9	5.87	-0.87			

Table 2: Egg yield in single and double hybrids

Hybrid	Egg yield / kg of cocoons (g)	Dfls recovery (%)	Unfertilized egg (%)	Non-diapause egg (%)	No. of eggs / g	No. of eggs / dfl
(CSR50 x 52)x(51 x 53)	85.6	39.5	2.1	1.5	1656	630
(CSR51 x 53)x(50 x 52)	83.4	38.8	1.9	1.3	1675	626
(CSR6 x 26)x(2 x 27) ©	80.4	35.9	2.4	1.3	1685	624
(CSR2 x 27)x(6 x 26) ©	78.4	36.5	1.8	1.7	1660	630
CSR2 x CSR4 ©	65.5	33.6	2.4	1.5	1786	542
CSR4 x CSR2 ©	60.4	32.5	2.9	2.4	1825	512
CD at 5 %	3.6	0.8	0.6	ns	ns	60.0
% improvement over control double hybrid	6.42	8.15	-4.75	-6.67	-0.42	0.16
% improvement over single hybrid	34.23	18.46	-24.53	-28.21	-7.75	19.17

© Control hybrid

Table 3: Performance of double hybrid, (CSR50 x CSR52) x (CSR51 x CSR53) vs control double and single hybrids

Hybrid	Pupation (%)	Cocoon yield/ 10,000 larvae (kg)	Cocoon weight (g)	Shell weight (g)	Shell (%)	Reelability (%)	Filament length (m)	Denier (d)	Raw silk %	Neatness (p)
(CSR50 x 52)x(51 x 53)	95.2	19.36	2.020	0.479	23.7	86	1150	2.89	18.6	94.5
(CSR51 x 53)x(50 x 52)	94.9	19.35	2.030	0.483	23.8	85	1160	2.92	17.8	95.2
(CSR6 x 26)x (2 x 27) ©	94.8	18.89	2.000	0.472	23.6	86	1145	2.98	16.9	94.5
(CSR2 x 27)x(6 x 26) ©	95.3	19.50	2.010	0.476	23.7	86	1175	3.01	17.9	94.6
CSR2 x CSR4 ©	94.8	18.25	1.973	0.462	23.4	85	1000	2.89	18.1	94.5
CSR4 x CSR2 ©	94.6	17.89	1.969	0.463	23.5	85	1020	2.93	17.9	94.5
CD at 5 %	ns	0.56	0.045	0.013	ns	ns	95.3	ns	ns	ns

© Control hybrid

breeds and multivoltine x bivoltine hybrids owing to higher weight of cocoons, pupae and moths. Heavier and number of eggs are considered as indicative of good quality seed (Benjamin *et al.*, 1998). The number of eggs / gram in double hybrid was less and ranged from 1656 (CSR50 x CSR52) x (CSR51 x CSR53) to 1685 (CSR6 x (CSR26) x (CSR2 x CSR27), whereas in single hybrid, it ranged from 1786 (CSR2 x CSR4) to 1825 (CSR4 x CSR2). The fecundity (No. of eggs laid /moth) in single hybrid ranged from 512 (CSR4 x CSR2) to 542 (CSR2 x CSR4) and in double hybrids, it varied from 624 (CSR6 x CSR26) x (CSR2 x CSR27) to 630 (CSR50 x CSR52) x (CSR51 x CSR53). The data clearly showed that the double hybrids are significantly superior over single hybrids in respect of egg yield (34.23 %), laying recovery (18.46 %), eggs / disease free laying (20.11 %) and less number of unfertilized and non-diapause eggs. However, marginal improvement in egg yield (6.42 %) and layngs recovery (8.15 %) was recorded in new double hybrid (CSR50 x CSR52) x (CSR51 x CSR53) / (CSR51x CSR53) x (CSR50 x CSR52) over control double hybrid (CSR6 x CSR26) x (CSR2 x CSR27) / (CSR2 x CSR27) x (CSR6 x CSR26). Significant differences ($P < 0.05$) were observed between single and double hybrids in respect of cocoon yield, cocoon weight, shell weight and filament length. However, there were no significant differences between single and double hybrids in other parameters such as pupation rate, shell percentage, reelability, denier, raw silk percentage and neatness.

The major improvement of FCs over that of pure strains are in pupation rate and egg yield which in turn reduce the cost of egg production. The improvement of egg yield recorded up to the extent of 34.23 % in new double hybrid over single hybrids parallels the observations of Nirmal Kumar *et al.* (2002) and Harada (1961). Gowda *et al.* (1989) reported that variation in fecundity is due to differential pupal weight, which is also true to the present study. In silkworm, it is well known that selection for one trait results in correlated changes in qualitative traits of economic importance (Kobari and Fujimoto, 1966). For example, with increase in qualitative traits beyond certain level, survival and fecundity are affected considerably. Although survival could be maintained in single hybrids, they are poor in egg number; unless the moth is a hybrid, the fecundity cannot be increased (Yokoyama, 1979). Moreover, the cost of maintaining these parental breeds especially strains with less fecundity and survival for the purpose of supply of parental stocks for preparation of single hybrid can be high. This has necessitated the development of FCs and in turn double hybrids containing genetic material from four parental strains to obtain sustainable cocoon yield for commercial exploitation.

The average cocoon yield per 100 disease free layings (dfls) for the hybrid, (CSR50 x CSR52) x (CSR51 x CSR53) at different locations of Karnataka, Tamil Nadu and Andhra Pradesh is presented in Table 4. A total of 5250 dfls were distributed to 24 farmers in Karnataka and the

Table 4: Performance of double hybrid (CSR50 x CSR52) x (CSR51 x CSR53) in field at different locations during 2010-2012

Sl. No	State/location	No. of farmers	No. of dfls	Actual yield (kg)	Yield/100 dfls (kg)	Cocoon weight (g)	Shell weight (g)	Shell (%)
Karnataka								
1	Srirangapatna	15	3250	2037	62.67	1.685	0.349	20.7
2	Koppal	9	2000	1199	59.97	1.820	0.383	21.0
	Total/average	24	5250	3236	61.64	1.753	0.366	20.9
Tamil Nadu								
3	Gobichettipalyam	7	1250	775	62.00	1.805	0.370	20.5
4	Hosur	6	1300	723	55.62	1.865	0.398	21.4
5	Udumalpet	48	8100	6262	77.31	1.870	0.399	21.3
6	Salem	4	670	533	79.55	1.799	0.392	21.8
	Total/average	65	11320	8293	73.26	1.835	0.390	21.2
Andhra Pradesh								
7	Venkatagiri Kota	7	1850	1131	61.15	1.888	0.425	22.5
8	Penukonda	2	500	329	65.80	1.825	0.380	20.8
9	Vikarabad	5	1100	768	69.82	1.818	0.408	22.4
	Total/average	14	3450	2228	64.59	1.844	0.404	21.9
	G. Total/average	103	20020	13757	68.72	1.820	0.389	21.4

Table 5: Performance of control hybrids in the field (Karnataka, Tamil Nadu and Andhra Pradesh)

Sl No	Hybrid	No. of farmers	No. of dfls	Actual yield (kg)	Yield/100 dfls (kg)	Cocoon weight (g)	Shell weight (g)	Shell (%)
1	CSR2 x CSR4	84	22200	13990.3	63.02	1.775	0.370	20.7
2	(CSR6 x CSR26) x (CSR2 x CSR27)	100	20720	13504.8	65.18	1.811	0.385	21.3

hybrid recorded an average cocoon yield of 61.64 kg/100 dfls. In Tamil Nadu, a total quantity of 11,320 dfls were distributed to 65 farmers and the hybrid recorded an average cocoon yield of 73.26 kg/100 dfls. In Andhra Pradesh, 14 farmers reared a total of 3450 dfls recording an average cocoon yield of 64.59 kg/100 dfls. Overall, the new double hybrid recorded an average cocoon yield of 68.72 kg/100 dfls. The control hybrids, CSR2 x CSR4 and (CSR6 x CSR26) x (CSR2 x CSR27) recorded cocoon yield of 63.02 and 65.18 kg/100 dfls, respectively (Table 5). Even though, the rearing of silkworm is conducted under indoor locations throughout the year, the cocoon yield contributing parameters are greatly affected in different seasons/locations due to rearing management and variations in leaf quality. The difference in the

cocoon yield parallels the earlier findings of Ueda *et al.* (1969), Gabriel and Rapusas (1976) and Thiagarajan *et al.* (1993) who opined that the same hybrid performs differently in different seasons and locations. The results obtained in the present study indicate that the new double hybrid (CSR50 x CSR52) x (CSR51 x CSR53) possesses stability in commercial rearings at different locations. In view of the above, the double hybrid can be exploited in large scale at different sericultural locations of southern states of India. Realising its potential, the new double hybrid (CSR50 x CSR52) x (CSR51 x CSR53) was selected for commercial exploitation by Central Silk Board, Bangalore, Government of India after conducting hybrid authorization test at different test centers situated in different zones of India.

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UN NOUVEL HYBRIDE DOUBLE BIVOLTIN ROBUSTE (CSR50 x CSR52) x (CSR51 x CSR53) DE *BOMBYX MORI* L. POUR UN MEILLEUR RENDEMENT EN ŒUFS ET EN COCONS

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R E S U M E

Nous avons entrepris cette étude pour évaluer les performances d'un nouveau double-hybride bivoltin, (CSR50xCSR52) x (CSR51xCSR53), en quantifiant les paramètres de rendement en oeufs et en cocons dans les conditions de terrain. Quatre lignées parentales, CSR50, CSR52 (cocons ovales), CSR51 et CSR53 (cocons cintrés) et deux croisements fondateurs (FCs), CSR50xCSR52 (oval x oval) et CSR51xCSR53 (cintré x cintré) ont été utilisés pour obtenir un double-hybride nouveau, (CSR50xCSR52) x (CSR51xCSR53) / (CSR51xCSR53) x (CSR50xCSR52) et étudiés par comparaison avec les hybrides simples témoins CSR2xCSR4/CSR4xCSR2 et les hybrides doubles (CSR6 xCSR26) x (CSR2 x CSR27) / (CSR2 x CSR27) x (CSR6 x CSR26). Les analyses statistiques indiquent que le nouvel hybride double est significativement supérieur ($P<0.05$) aux hybrides simples quant au rendement en œufs (34.23%), au nombre de pontes saines (dfls) obtenues (18.46%), au nombre d'œufs par ponte (20.11%) avec moins d'œufs non fécondés ou non diapausant. De plus, une amélioration marginale par rapport aux hybrides doubles témoins est observée en ce qui concerne le rendement en œufs (6.42%) et l'obtention de pontes saines (8.15). L'évaluation sur le terrain du nouvel hybride double (CSR50xCSR52) x (CSR51xCSR53) montre que le rendement moyen en cocons est de 68.72 kg/100 pontes saines alors qu'il n'est que de 63.02 chez CSR2xCSR4 et de 65.18 chez (CSR6xCSR26) x (CSR2xCSR27). Les résultats indiquent que l'utilisation de l'hybride double (CSR50xCSR52) x (CSR51xCSR53) pour l'élevage commercial dans les états du sud est pertinente.

Mots-clés: Ver à soie bivoltin, *Bombyx mori* L., double hybride, rendement en oeufs, performances de terrain.



EFFECT OF ESTRADIOL-17 β ON CERTAIN ECONOMIC TRAITS OF A MULTIVOLTINE SILKWORM, *BOMBYX MORI* (RACE: NISTARI)

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ABSTRACT

The presence of estradiol - 17 β in the ovaries and haemolymph of silkworms being an established fact, many an investigation on its role in *Bombyx mori* have been undertaken by several workers. The present study was carried out in this line, in order to understand the possibility of estrogen having any effect on its life span, cocoon shell weight, fecundity and hatchability. Although estrogen could not impose significant changes in economic traits of a multivoltine silkworm (*B. mori*) race Nistari, it strikingly shortened the fifth instar larval span in female by approximately two days. This phenomenon might turn out to have some positive effect on sericulture in the long run.

Key words: Cocoon, egg laying, estradiol - 17 β , hatchability, life span, silkworm.

INTRODUCTION

Estrogen has been detected in haemolymph, ovary and silk gland of silkworms and other insects (Ohnishi *et al.*, 1985; De Loof and De Clerck, 1986; Denlinger *et al.*, 1987; Keshan and Ray, 2001; Das and Ray, 2007;). Effects of ethanolic extracts of phytoestrogen-rich plant, white Kwao-Krua (*Peuraria mirifica*) on toxicity, larval maturation and cocoon character of Thai multivoltine silkworm, (*Bombyx mori*) have been studied (Chawna *et al.*, 2007). Our earlier studies have demonstrated profound metabolic effects of estradiol on different organs such as fatbody, ovary and silk gland of *B. mori* L. (Das and Ray, 1996; Das and Ray, 1997; Keshan and Ray, 2000; Keshan and Ray, 2001). Interestingly, specific binding sites for radioactive estradiol-17 β have also been demonstrated in the cytosol of posterior silk gland (Keshan and Ray, 2001). Recently, the estradiol-17 β induced alteration of female specific protein was demonstrated, which signifies possible involvement of this sex steroid in the biological processes of silkworm, *B. mori* (Das and Ray, 2013). The present investigation was carried out in order to explore the possibility of estrogen having an effect on some important physical parameters viz., V instar larval span, cocoon shell weight, fecundity and hatchability in a multivoltine silkworm (*B. mori*) race, Nistari.

MATERIALS AND METHODS

B. mori L. (race Nistari) larvae were reared on fresh mulberry leaves at 25 $\pm$ 1 $^{\circ}$ C and 80-90 % relative humidity under 12 L + 12 D photoperiod condition. The larvae were fed 4 times a day. For the treatment, fifth instar female larvae were separated and divided at random into different groups. The 3 and 5 day old larvae were injected with three different doses (0.5, 2.0 and 4.0 μ g/g body weight) of estradiol-17 β (E2, Sigma chemical company, U.S.A.) through the second abdominal leg. The control animals were injected with equal volume of groundnut oil. Data on the V instar larval span, cocoon shell weight, fecundity and hatchability of silkworms were recorded by following standard methods. The moths were allowed to complete the life cycle by emerging from the cocoons followed by mating and egg laying.

The data were analysed by Student's "t" test package. Data are expressed as Mean $\pm$ S.E. of 10 individual observations in each group.

RESULTS

V instar duration

Estradiol-17 β treatment with three different doses (0.5, 2.0 and 4.0 μ g/g) reduced the larval span of the silkworm (Figure 1). The initiation of spinning in the 5th instar larvae

was found to be earlier after estrogen treatment. The 2.0 $\mu\text{g/g}$ of E2 treated groups started spinning on 6.72 ± 0.093 days whereas, control and other two E2 treated groups (0.5 $\mu\text{g/g}$ and 4.0 $\mu\text{g/g}$) started it on 8.57 ± 0.088 , 7.24 ± 0.076 and 7.13 ± 0.077 days, respectively. The 2.0 $\mu\text{g/g}$ of E2 treatment could advance the spinning process by two days compared to control group ($p \leq 0.001$). However, there was no significant difference in the initiation of spinning time between 0.5 and 4.0 $\mu\text{g/g}$ treatments of E2 on silkworm.

Cocoon shell weight

The differences in cocoon shell weight remained insignificant among various E2-treated and control groups of silkworms (Figure 2).

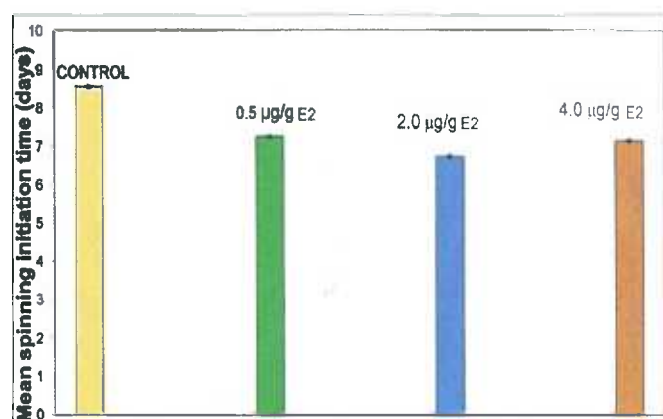


Figure 1: Effect of various doses of estradiol-17 β (E2) on mean spinning initiation time of *Bombyx mori*.

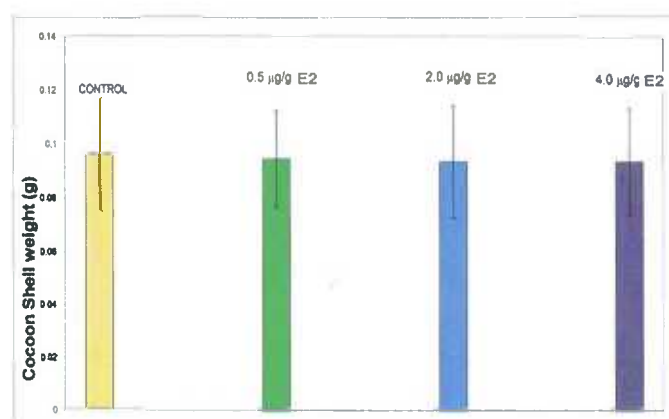


Figure 2: Effect of various doses of estradiol-17 β (E2) on cocoon shell weight of *Bombyx mori*.

The vertical bars on the columns denote standard error.

Fecundity

Total number of eggs laid by each female silk moth after mating was counted both in control and E2-treated silkworms (Figure 3). The differences in the number of eggs laid by control and E2-treated silkworms were not significant.

Hatchability of eggs

Hatchability was recorded by counting the number of viable eggs hatched out from each laying (Figure 4). Estradiol-17 β treatment in various doses did not impose any effective change on the hatchability of eggs in the silkworm.

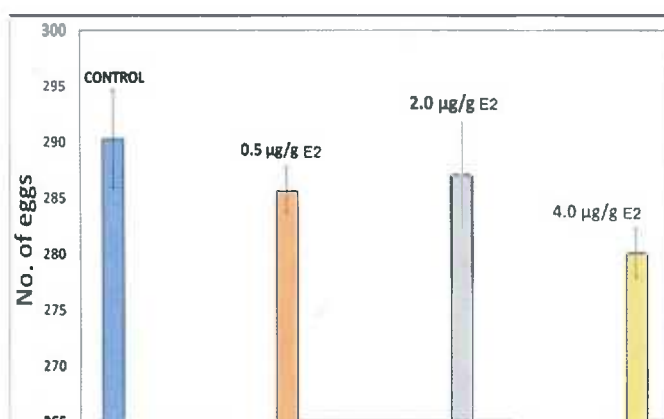


Figure 3: Effect of various doses of estradiol-17 β (E2) on fecundity of *Bombyx mori*.

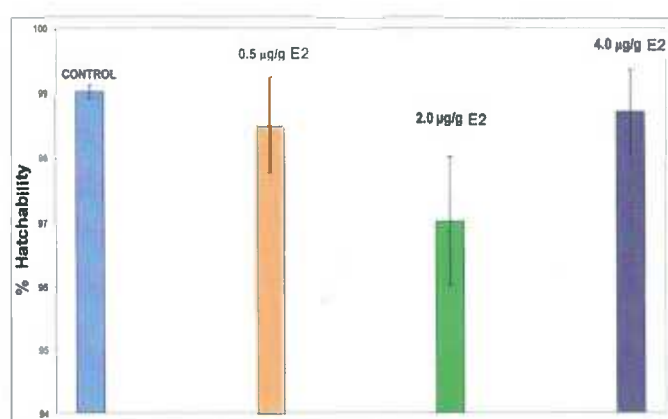


Figure 4: Effect of various doses of estradiol-17 β (E2) on hatchability of *Bombyx mori*.

DISCUSSION

In view of the commercial value of silkworms, some important economic parameters were taken into consideration for the study. Most interesting fact is that the E2 treatment augmented significantly the 5th instar larval span or more technically the initiation of mean spinning time. The maximum reduction of the initiation of spinning time by 2 days was effected by the 2.0 $\mu\text{g/g}$ of E2 treatment. The effect was very similar to that found with application of ecdysone or 20-hydroxyecdysone as reported earlier by other workers (Chou and Lu, 1980). Whether estrogen acts through its conversion to ecdysone or as gonadotropin is a matter of further investigation. It might also be possible that treatment of E2 stimulated brain which also regulates the synthesis and release of prothoracicotrophic hormone (PTTH) and thus stimulated the activity of prothoracic gland for secretion of ecdysone which in turn shortened the larval period. Chawna et al. (2007) demonstrated the shortening of larval duration after the application of phytoestrogen contained in *P. mirifica* in their study, though they couldn't obtain synchronized spinning of treated larvae. Whatever the mechanism of action of E2 in our experimental condition, the effect of hormone on the larval span proved to be a beneficial one. Other doses of E2 (0.5 and 4.0 $\mu\text{g/g}$) could not alter the normal pattern of life cycle. Silkworm larvae have been found to be highly sensitive to exogenous hormones, the effects of hormone applications being much dependent on the age of the larvae and the particular dose of the hormone (Keshan and Ray, 2000). However, reduction of larval life by 2 days in the life span (usually 42-45 days) of multivoltine Nistari race of *B. mori* is advantageous from economic point of view and of academic interest as well.

The insignificant differences in cocoon shell weight resulted after treatment with three different doses of E2 in comparison to the control values and the reduction of the 5th instar larval life span by 2 days clearly assures the possibilities of increased rate of silk production after E2 treatment. The fecundity and hatchability in the normal range after various doses of E2 treatment also ruled out any ill effect of E2 treatment. Further, development of farmers-friendly techniques for large scale application of E2 treatment in the field will create a condition for its use

by the sericulturists for enhancement of silk production.

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EFFET DU 17 β -ESTRADIOL SUR CERTAINS CARACTERES ECONOMIQUES D'UNE RACE POLYVOLTINE (NISTARI) DU VER A SOIE *BOMBYX MORI*

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RESUME

La présence de 17 β -estradiol dans les ovaires et l'hémolymph des vers à soie étant un fait établi, de nombreuses investigations sur son rôle chez *Bombyx mori* ont été entreprises par plusieurs auteurs. Notre étude a été effectuée dans cette optique afin de déterminer si les estrogènes ont un quelconque effet sur la durée de vie, le poids de la coque soyeuse, la ponte et l'éclosion. Bien que l'estrogène n'entraîne aucun effet significatif sur les caractères économiques de la race polyvoltine Nistari de *B. mori*, il conduit à un raccourcissement notable du cinquième âge larvaire de deux jours chez les femelles. Ce phénomène pourrait avoir un effet positif en sériciculture à long terme.

Mots-clés: Cocon, ponte des oeufs, 17 β -estradiol, éclosion, durée de vie, ver à soie.

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The International Sericultural Commission is organizing the **23rd International Congress on Sericulture & Silk Industry** with a focused theme, **“Silk for Inclusive Growth”**. The Congress is scheduled to be held at Hotel Leela Palace, Bangalore, India, from 24th to 27th November, 2014.

The Congress is open to all persons associated with the development of sericulture and silk industry. The composition of participants, limited to about 350, has been planned to be a diverse international group comprising policy planners, scientists, technocrats, resource persons, university faculty & students, NGOs, entrepreneurs, industrialists, silk exporters & traders, fashion designers, silk machinery manufacturers, rearing accessories & chemicals manufacturers, Government officials and a cross-section of the sericulture and silk fraternity.

The Congress encompasses eight sessions such as Mulberry, *Bombyx mori*, Non-mulberry silkworms, Bacoology of silkworms, Post-Cocoon Technology, Economy, Sericulture in non-textile industries and Silk processing, trading and marketing.

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