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International Sericultural Commission (ISC)

Central Silk Board Complex, BTM Layout

Madiwala, Bengaluru - 560 068, INDIA

Tel: +91 80 26282191, 26680162, 26681663

Fax: +91 80 26681663

E-mail: sericologia@gmail.com

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PEBRINE DISEASE (*NOSEMA BOMBYCIS* N.) IN *BOMBYX MORI* L.: THE RESEARCH TRENDS

Satadal Chakrabarty^{1*}, B. Manna², A. K. Saha¹, B. B. Bindroo³ and K. Trivedy¹

¹Central Sericultural Research and Training Institute, Berhampore, West Bengal 742101, India.

²Parasitology Research Unit, Department of Zoology, The University of Calcutta,
Kolkata, West Bengal 700019, India.

³Central Sericultural Research and Training Institute, Mysuru, Karnataka 570008, India.

*E-mail: satadal.chak@gmail.com

ABSTRACT

Crop loss is a regular feature associated with Indian sericulture scenario for various reasons. It may be accounted for different sorts of diseases, parasites, weather conditions or even for cultural reasons. But none of them is as severe as the larval mortality owing to pebrine disease where the entire crop is lost. The causative agent for the disease is *Nosema bombycis* Nägeli, a spore forming eukaryotic, obligatory and intracellular protozoan. Detection and elimination of pebrine disease is essential for sustenance of cocoon crop from industrial point of view. The age old detection procedure, the only reliable and widely accepted technique for preparation of pebrine free eggs, is the one which involves examination of mother moth tissue smears under light microscope. The technique was developed by Louis Pasteur in 1870 and is still followed to detect the pebrine spore in the field of commercial seed production in all silk producing countries including India. Thereafter, though many novel techniques were developed to detect pebrine spore, the popularity of the mother moth examination has not been dwindled. Due to many practical reasons, the other detection procedures remained only as of theoretical interest for the researchers. This review accounts the exhaustive amount of research work detailing the characteristic features, developmental cycle and the many factors related to the pathogenicity of microsporidians affecting silkworm. It highlights all the scientific techniques formulated so far for detection of pebrine spores, as carried out by many technologists, pinpointing their merits and demerits, and attempts to throw light on the new strategies to be carried forward.

Key words: *Nosema bombycis*, pathogenicity, spore wall proteins, transovarial transmission.

INTRODUCTION

Nosema bombycis Nägeli, is the causative agent for pebrine disease (Nägeli, 1857), that had once emaciated the sericulture industry in Europe, especially in Italy and France during the mid 19th century (Becnel *et al.*, 1999). The *N. bombycis* spore detection technique using crushed mother moth examination under light microscope (600x) formed the basis for its identification and further studies on biology, pathogenicity, biochemistry, mode of transmission of disease and formulation of preventive measures for eradicating the deadliest disease to sustain

sericulture industry. Later on, in the late 20th century, advanced molecular methods *viz.*, fluorescence / monoclonal antibody technique, latex agglutination test and electron microscopical techniques were invented for the same purpose. Further, Random Amplification of Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR), Western blot analysis, Southern blot hybridization and cloning were developed. Patil (1993) and Kawarabata (2003) have provided very comprehensive reviews in the field. Extensive research has also been done to find out fungal-like eukaryotic origin of *N. bombycis* based on phylogenetic analysis (Thomarat *et al.*, 2004) and

evolution of mitosomes due to degeneration of microsporidia (Katinka *et al.*, 2001). All these have generated some special interest on the pathogen and its pathogenicity. Recently mitosome, has been discovered as the remnant of mitochondrial organelle which relates the pathogen phylogenetically with fungi on the basis of analyses of rDNA sequences and conserved proteins (Hirt *et al.*, 1999; Keeling *et al.*, 2000; Fabienne *et al.*, 2004; James *et al.*, 2006; Burri *et al.*, 2006; Goldberg *et al.*, 2008; Lee *et al.*, 2008). This review is an exhaustive account of the enormous amount of studies carried out in the field and novel strategies proposed to combat the disease.

Systematic position

N. bombycis Nägeli (1857) is a spore forming eukaryotic, obligatory, unicellular, intracellular protozoan with resemblance to fungus (Xu *et al.*, 2006 a). Presence of nucleus, nuclear envelope, small subunit ribosomal RNA (ssu - rRNA) genes, size less than those of typical eukaryote (Vossbrink *et al.*, 1993), absence of mitochondria, golgi apparatus and centriole are the unique features of primitive eukaryotic, *N. bombycis* with systematic position (Sprague, 1977) as follows:

Phylum	Microspora
Class	Microsporea
Order	Microsporidia
Sub-order	Apansporoblastina
Family	Nosematidae
Genus	<i>Nosema</i>
Species	<i>bombycis</i>

Nosema bombycis NIS - 001 had been maintained as the major reference strain in Japan for more than 80 years. The other strains that are reported to infect silkworm are *Nosema* sp. NIS-M11, *Nosema* sp. NIS-M14, *Varimorpha* sp. NIS-M12, *Microsporidium* sp. NIS-M25, *Pleistophora* sp. NIS-M27 and *Thelohania* sp. NIS-M32 (Kawarabata, 2003). In India, *N. bombycis*, NIK-1s, NIK-2r, NIK-4m, NIAP-6p, NIAP-7g, NIK-5d, and NIK-3h are the major referred strains maintained in various laboratories across the country.

Morphological variations

Fujiwara (1984), Hayasaka and Kawarabata (1990), Iwano (1993), Patil (1993) and Kawakami *et al.* (2001) examined spore morphology under light, phase contrast and electron microscope and they considered that the shape and size of the spores is one of the characters for identification of the genus, *Nosema*. All the species of this genus share a basic spore shape (Larsson, 1999). Sometimes, the shape and size are different for the same species when they are collected from different hosts. Iwano and Ishihara (1991) reported that the shape and size of *Nosema bombycis* collected from mulberry silkworm, *Bombyx mori* is different from that of *N. bombycis* collected from Lawn grass cutworm, *Spodoptera depravata*. Only in 80 % cases on light microscope, successful identification could be made (Ghosh and Weiss, 2009).

As highly specialized parasites, *N. bombycis* are characterized by a number of unusual adaptations, many of which are manifested as extreme reduction at the molecular, biochemical, and cellular levels. One interesting aspect of reduction is mitochondrion and there has been a recent discovery of mitosome, the remnant mitochondrial derived organelle (Burri *et al.*, 2006).

Histology and developmental stages as observed under light and electron microscopy

N. bombycis multiplies *in vivo* only and has no active stage outside their host cells. It produces environmentally resistant spores with a unique extrusion apparatus that contains a coiled polar tube ending in an anchoring disc at the apical part of the spore (Vavra and Larsson, 1999). Life cycle of *N. bombycis* was studied by examining smears of gut juice and cell line under light microscope, Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) by various workers. Lom and Vavra (1963), Ishihara (1969), Sato and Watanabe (1986 a), Iwano and Ishihara (1991) and Chakrabarty and Manna (2006 and 2009 a) have thrown much light on the mode of

sporoplasm extrusion, sporogonial sequence *etc.*

Iwano and Ishihara (1991) reported that surface wall of mature spore of *N. bombycis* is smooth and distinct under SEM. Chakrabarty and Manna (2006) reported that the spore of *N. mylitta* is having a prominent, distinct, rough and wrinkled surface with fibrous coating, that of *N. ricini* is with slight concavity or tapering at both ends with distinct and smooth double wall external membrane, and that of *N. assamensis* is having fibrous hard coating externally with slight concavity in the middle. They also reported the presence of mucocalyx, a flotation device on the spore surfaces of *N. mylitta* and *N. assamensis*. Mucocalyx has also been observed on the spore surface of *Gurleya elegans* (Vavra and Barker, 1980).

Under appropriate conditions inside a suitable host, the polar tube is discharged through the thin anterior end of the spore, penetrating a new host cell and inoculating the infective sporoplasm through the hollow tube into the new host cell. The developmental stages of the pathogen consist of stages such as sporoplasm, planont, meront and sporont. The process starts with the release of sporoplasm with nuclei into the host cells, proliferating thereafter to planont, meront/schizont, sporonts, and finally into a spore protected by a thick wall. The dimorphic spore completes its life cycle within 4 - 6 days.

N. bombycis exhibits dimorphism in spore production (Iwano and Ishihara, 1991). One group of spores isolated from moths of the lawn grass cutworm, *Spodoptera depravata* contained 3- 4 number of coils of polar tube characterized by 3.8 turns (ST = short tube or FC = few coils). Alternately, another group of spore isolated from cell culture of *B. mori* contained 12-14 number of coils of polar tube characterized by 11.8 turns (LT = long tube or MC = multiple coils). *Nosema* sp. is disporous (Cali, 1970) and the spores develop from binucleate sporoblasts after the binary fission of binucleate sporonts (Ishihara, 1969). Steinhaus and Hughes (1949) reported such type of dimorphism of spores in *N. bombycis*. Kawarabata and Ishihara (1984) confirmed that the two sporonts of *N. bombycis* usually produced two sporoblasts. The

appearance of two distinct spores within the same species has been reported by Loubes *et al.* (1979). Spore dimorphism and developmental cycle was also reported in *N. apis*. The first population of spores mainly spread the parasite in the epithelium and the second population of spores is formed later on and is adapted to survive outside the host (Graff *et al.*, 1994). Spore dimorphism in *N. apis* may be due to adaptation of the parasite to different needs during their life cycle. Spores, which appear during the early development of the parasite apparently, have the ability to germinate intracellular. Intracellular extrusion of the relatively short polar tube can serve as a means to deliver sporoplasm to neighbouring cells (Iwano and Ishihara, 1989). Early spores and environmental spores are immature and variants of the same spore type and normally occurs in different tissues in the host (Larsson, 1999). The number of coils varies within the *Nosema* collected from other three types of non-mulberry silkworms (Chakrabarty and Manna, 2006). The gender or sex of the host may also influence the dimorphism in *N. bombycis* (Chakrabarty *et al.*, 2013 b). The number of coils of polar filament varied from 3 - 4 coils in *N. cuniculi* to 44 in *N. apis*. The majority of species, however, had 11 coils out of 15 species listed by Milner (1972 a). Neither the number of coils, nor their arrangement within the spore, appears to be correlated with the genus (Milner, 1972 b). Dimorphism causes difficulties in accurate identification of species (Hazard and Weiser, 1968) that leads to unnecessary creation of a number of new species (Mercer and Wigley, 1987). Although, the number of coils of polar filament and the angle of tilt of the coils are considered as distinguishing characters of species (Burgess *et al.*, 1974), such study is unable to distinguish among closely related species with almost similar number of coils of polar filament and only a little difference in their angle of tilt (Sprague *et al.*, 1968).

Prior to the widespread application of molecular diagnostic methods, TEM was used to confirm existence of pebrine spore through polar filament study (Cali *et al.*, 1991; Orenstein, 2003). TEM being labour intensive, time consuming and expensive (Ghosh and Weiss, 2009), is hence unpopular for detection of pebrine spores. However, SEM and TEM are still required for identification of new

strain of *Nosema* spores isolated from insect hosts viz., silkworm, bee *etc.* as compared with existing strain of *Nosema*. TEM is still important for detecting the ultrastructural features along with newly-applied molecular biology approaches (Cali *et al.*, 1993; Larsson, 2005). Data on shape, size, external spore surface morphology, exospore, thickness of endospore, number of coils in polar filament and angle of tilt on the long axis of polar filament under light, SEM and TEM are all still used to differentiate *Nosema* spp. collected from similar hosts viz., different varieties of silkworm. This led to the identification of three new species, *N. mylitta*, *N. ricini* and *N. assamensis* collected from tasar, eri and muga silkworms, respectively quite different from that of *N. bombycis* (Chakrabarty and Manna, 2006).

Abe and Fujiwara (1979) and Fujiwara (1979) observed pathogenicity of *N. bombycis* in the midgut and in various tissues of silkworm, *B. mori*, three days after infection. Longevity of instars and the amount of spores influenced the disease manifestation (Ishihara and Fujiwara, 1965). The technique to detect pebrine spore from silkworm gut, is not popular as pathogen is available primarily in the different life cycle stages. Moreover, detection is difficult due to formation of very thin spore wall, which is not generally visible under light microscope (600x) and without proper histological preparation of gut tissues. Moreover, the histological study is not at all popular for detection of pebrine spore, as it is laborious, time consuming and requires technical skills.

It is reported that *N. assamensis* collected from muga silkworm, *Antheraea assamensis* completes its life cycle within 5-8 days, i.e., 1-2 days more than that of *N. bombycis* (4-7 days) and passes through planont, an extracellular stage, schizont/meront, an intracellular stage and sporont, the sporulation stage as observed under light, SEM and TEM study.

Incidence and development of pebrine disease in relation to environmental and biotic factors

Many scientists have paid their attention to the effect

of different environmental factors viz., temperature, humidity, rainfall, altitude and biotic factors viz., age of silkworm, spore concentration of *Nosema* *etc.* on manifestation of pebrine disease in silkworm. Kudo (1930), Dasgupta (1950), Maddox (1973), Kramer (1976), Brooks (1988), Samson *et al.* (1990) and Chakrabarty and Manna (2008 a; 2008 b) were a few who utilized the influence of the above factors as a basis to develop various techniques for easy detection of pebrine spore. The 'pebrine' disease flourishes under certain environmental and biotic factors in our country. Devaiah and Krishnaswami (1975) emphasized on the seasonal incidence of pebrine disease in the silkworm. Later on, Griyaghey and Sengupta (1989) found that larval mortality rate of tasar silkworm, *A. mylitta* due to different concentrations of *Nosema* sp. is directly proportional to the amount of spore administered. Patil (1993) opined that 3rd and 4th stage larvae of tasar silkworm are more susceptible to pebrine infection and maximum mortality was recorded during late age and spinning time. The percentages of progeny getting pebrine infection during transovarian transmission depend on the degree of infection that the mother moth suffers (Griyaghey and Krishnan, 1976). Mariswamy (1977) reported that temperature and humidity plays a vital role in development of pebrine disease in muga and eri silkworms. The incidence of pebrine is more during summer season followed by autumn and spring in temperate countries. Contrary to this, the incidence is low during summer and increases by winter in tropical countries. Higher temperature and lower humidity reduce pathogenicity and the intensity of disease during summer. Hence, temperature, humidity and biotic components viz., age of silkworm, concentration of the pathogen administered *etc.* influence the survival of the pathogen. Spore under humid conditions remain active for one year. High intensity bright sunlight inactivates the spores. There have been differences in opinion in relation to environmental factors with incidence and development of pebrine disease (Jamenson, 1922; Tanada, 1963; Nomani *et al.*, 1971; Kamble *et al.*, 2005; Singh *et al.*, 2007). Recently, it is observed that environmental factors viz., temperature and relative humidity influence the multiplication of pebrine spore (Chakrabarty and Manna,

2008 a). Many detection techniques have been developed utilizing the influence of above environmental factors. For a long time, the heat drying method had been applied in the regular pebrine inspection in Japan, in order to prevent further decomposition of moth bodies by microorganisms. Usually, the bodies of mother moths after oviposition were desiccated at 65-75 °C for 5 hours and stored individually in a paper container at room temperature until inspection under light microscope (600x) (Kawarabata, 2003). Delayed mother moth technique was invented by Singh *et al.* (2004). In this method, mother moths are collected in groups in perforated cardboard boxes / covers and preserved in an isolated, well-ventilated room at 25-30 °C for a period of 3-4 days after oviposition and then examined the crushed moths under microscope. But this technique invites enough bacterial and fungal contaminations and thereby makes the detection of pebrine spores somewhat difficult. Therefore, the technique has not been popularized. Recently, Chakrabarty *et al.* (2013 a) reported that addition of powdered formulation of Ambistryn-S (antibiotic preparation of streptomycin sulphate) and Transco Trichostar (a bio-fungicidal preparation of *Trichoderma viridae*) to the larval, pupal and crushed moth (after oviposition) tissue preparations arrests the growth of invading bacteria and fungi. This method helps the maturing pathogen to complete its life cycle utilizing available host nutrition. Such mature spores with long polar tubes and with highly developed coat of wall protein facilitate easy observation even under light microscope (600x) with minimum skill. This low-cost method is performed successfully in detecting the pebrine spore at very low intensity of the disease and in small populations. It involves minimum skill but offers satisfactory result in minimum time (Chakrabarty *et al.*, 2013 a).

Host specificity, cross-infectivity and alternate host

The existence of more than one host species in the same habitat and presence of disease in any one of them may lead to the chances of cross-infection. Cross-infection does occur in nature among closely related species. Infection of *Nosema* sp. to *A. mylitta* D. is

reported by Jolly and Sen (1972). Besides *N. bombycis*, other 4-6 microsporidian belonging to genus *Nosema*, *Pleistophora* and *Thelohania* were isolated from *B. mori* (Fujiwara, 1980). Patil *et al.* (1993) reported that *Nosema* spores from *A. mylitta* infected and caused disease in *B. mori*.

Tanaka *et al.* (1972), Kawarabata and Ishihara (1984), Fujiwara *et al.* (1985), Sato and Watanabe (1986 b) and Srikanto (1987) studied on host specificity, cross-infectivity and alternate host. More than 32 Lepidopterans are reported to be the host of *N. bombycis* (Kawarabata, 2003), while *Nosema*, *Varimorpha*, *Pleistophora* and *Thelohania* sp. are found in silkworm, *Bombyx mori*. Butterflies, *Eurena*, *Zizina*, a mulberry pest, *Spilosoma obliqua* and Bihar hairy caterpillar, *Diacrasia obliqua*, etc. are the natural hosts of *Nosema* (Samson *et al.*, 1990). *Bombyx mori* is also cross-infected by *N. mylitta* (Chakrabarty and Manna, 2009 b).

Cross-infection studies are necessary to understand the cumulative effect of infection which leads to extinction of small host population. It is reported that *N. mylitta* collected from tasar silkworm, *A. mylitta* cross-infects mulberry silkworm, *B. mori* L., but fails to cross-infect eri silkworm, *Philosamia ricini* B. *N. assamensis* collected from muga silkworm, *A. assamensis* Ww. and *N. bombycis*, can cross-infect tasar silkworm, *A. mylitta* D. Whereas, *N. ricini* collected from eri silkworm, *P. ricini* fails to cross-infect tasar silkworm. *N. bombycis*, *N. mylitta*, and *N. assamensis* cannot cross-infect eri silkworm, *P. ricini*. The cross-infection studies help to identify risky zones in Assam (Chakrabarty, 2014), particularly to prevent extinction of muga silkworm, as it is reared side by side with tasar and mulberry culture in Assam. *A. assamensis* is the most susceptible and is cross infected by the *Nosema* of tasar, eri and mulberry.

Biochemical/ Immunological studies

Presently, biochemical and immunological techniques are being used for the detection of pebrine, since they are more sensitive, specific and depend less on technical skill

of the observer (Procop, 2007).

Ishihara and Hayashiya (1968), Fowler and Reeves (1974) and Streett and Briggs (1982) studied the chemical composition of spore wall and attempted identification of pebrine spore using Polyacrylamide Gel Electrophoresis (PAGE) and Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis (SDS-PAGE). But the techniques proved less fruitful in detection and identification of closely related strains of *Nosema*. Kohler and Milston (1975) Knell and Zam (1976) Sato *et al.* (1981), Hayasaka and Ayuzawa (1987), Kawarabata and Hayasaka (1987) Langley *et al.* (1987) and Baig *et al.* (1992) used various techniques *viz.*, enzyme linked immuno-absorbant assay (ELISA), latex agglutination test, fluorescence antibody test, monoclonal antibody, double immuno-diffusion technique, 2- dimensional (2-D) PAGE protein A, Linked Latex Antisera Test (PALLAS) *etc.*, for early detection and identification of pebrine spore. *N. locustae*, *N. bombycis* and *Varimorpha nectar* were found different in their spore protein composition but PALLAS is reported unsuitable for their differentiation. Agglutination is clearly visible when host contain minimum of 10^6 spores / ml. Since no distinct difference could be observed between virulent and non-virulent spores through latex agglutination test, the technique is not adopted in practical field (Siromani, 1997).

Molecular studies

Compared to traditional microscopy or culture based methods, molecular methods can offer the following potential advantages: increased sensitivity, by virtue of amplification of signals; greater specificity, when appropriate detection probes are employed; faster result; and greater ease of interpretation by non-specialists (Procop, 2007). Additionally, sophisticated nucleotide based methods such as Real time PCR and oligonucleotide microarrays are intrinsically higher through put and quantitative, enabling simultaneous analysis of specimens for multiple pathogens as well as

assessment of infection intensity. Several workers have evolved various techniques based on molecular biology.

Moore *et al.* (1991), Schlotterer *et al.* (1991), Tompkins (1992), Beckmann and Weber (1992), Vossbrink *et al.* (1993), Barker *et al.* (1994), Wang *et al.* (1994) Hartskeeri *et al.* (1995), Malone and McIvor (1995), Cavalior – Smith and Chao (1996), Dugourd *et al.* (1996), Field and Wills (1996), Noman *et al.* (1996), Hung *et al.* (1998), Raynaud *et al.* (1998) Olive and Bean (1999), Elain *et al.* (2001), Garcia (2002), Tsai *et al.* (2003), Nageswara Rao *et al.* (2004, 2005) and Hatakeyama *et al.* (2008, 2009) have studied pebrine spore at molecular level using small subunit ribosomal – RNA sequence (ssu-rRNA) and ssu-rRNA gene sequence to facilitate early detection. Several *Nosema* species ssu-rRNA gene sequence is used to design specific primers for molecular diagnosis (Hatakeyama and Hayasaka, 2001, 2003). Kawakami *et al.* (1995, 2001) have published several PCR protocols based on the amplification of rRNA gene fragments for use in the diagnosis and species identification of *N. bombycis* and they observed that *N. bombycis* is not competitive in nature and have developed strategies for co-existence *in vivo* and *in vitro*. However, molecular biology technique has some of the demerits. Primer set is not effective for *N. bombycis* Sd-NU – 8401 and *N. bombycis* Y901, isolated from *Spodoptera depravata* and *S. exigna*, respectively. *N. bombycis* isolates could be divided into two distinct groups by PCR, reflecting high and low pathogenicity of *N. bombycis*. Highly specific primers have not been developed to distinguish between *Vairimorpha* sp. NIS-M12 from *V. necatrix* (Kawarabata, 2003). The technique has been estimated to be 89 % successful by Ghosh and Weiss (2009).

Sprague (1977), Brooks (1988), Larsson (1988), Sprague *et al.* (1992), Barker *et al.* (1994) and Malone and McIvor (1996) studied taxonomy and phylogeny of *Nosema* at molecular level. The study is based on spore morphology, ultra structure, and developmental cycle in a particular host. *Nosema* sp. is diplokaryotic and has two sporogonial sequences. The same is true for *Vairimorpha*. Phenotypical characters divide *Vairimorpha* and *Nosema*

in two distantly related taxa. However, ssu-rRNA phylogeny demonstrated that *Nosema* and *Vairimorpha* are genetically closely related groups. *N. bombycis* and *N. furnacalis* (host-Lepidoptera) belong to the first group, while Group 2 contains *N. apis* (host-Hymenoptera), *N. lymantriae* (host-Lepidoptera) and *V. necatrix* (Weiss and Vossbrinck, 1999). Insect evolution and its parasitic molecular structure establish a new correlation (Kawarabata, 2003). The *Nosema* sp. which parasitize only on Lepidoptera suggest such probable co-evolution over a period of time. However, several types of *N. bombycis* have been isolated and shown to be identical both on morphological and gene sequence analysis but differed in their virulence score asking for reconsideration of the phylogenetic relationship (Kawarabata, 2003; Hatakeyama *et al.*, 2009).

N. bombycis isolated from *Bombyx mori* consist of near about 1233 bps in 16S rRNA gene sequence. Several 16S rRNA gene sequences have been entered into Gene Bank for *N. bombycis* isolates, which differ by only up to 17 nucleotides (Canning, 1998). More than 1250 bps is required for sequence study for consideration of a new or separate species in the case of *Nosema*. But more than 1250 bps could not be recovered practically for sequence study. *N. bombycis* collected from different insect hosts consist up to 1233 bps and their sequence are almost same or alike. The sequence beyond 1250 bps can only lead to differentiation of a new species, which is practically impossible. Mere difference of just one or a few gene sequence is insufficient to define a new species of *Nosema* (Canning *et al.*, 1998). Sophisticated laboratory, costly equipment, chemicals, high technical skills *etc.*, are required for the techniques which are unavailable in many research Institutes also. Besides, multiple 'validated primers' are required for identification of different species / sub-species of genus *Nosema* for identification of spore, which makes it a tedious job and hence not popular in practical field.

Some workers *viz.*, Hatakeyama *et al.* (1997, 2009), Sambrook and Russel (2006) and Ghosh and Weiss (2009) have utilized RAPD-PCR, Inter simple sequence

repeat (ISSR)-PCR, Multiplex-PCR methods *etc.*, for detection of pebrine disease.

ISSR-PCR analysis is a powerful tool to detect polymorphism and identify different types of *Nosema* while ssu-rRNA gene sequence analyses is used to develop phylogenetic tree (Nageswara Rao *et al.*, 2004). RAPD-PCR is capable of detecting different types of *N. bombycis* under identical condition. Multiplex-PCR is capable of detecting the presence or absence of pathogen using multiple primers. In this technique, when two types of pathogenic microorganisms *viz.*, protozoa, virus *etc.* are predicted to be involved as the cause of infection, the two pathogens can be detected by mixing the specific primers at a time in one time operation of PCR machine, unless they compete (Chamberlin *et al.*, 1988; Hatakeyama and Hayasaka, 2003). However, the number and type of primers which will be mixed are limited and identification of infectious microorganisms requires repeating the PCR in many cases. Moreover, in this method, detection of novel pathogenic microorganisms is impossible unless specific primers are designed by interpretation of genomic information (Hatakeyama *et al.*, 2008). Therefore, use of multiplex-PCR is restricted only for detecting the presence or absence of pathogen using multiple primers (Chamberlin *et al.*, 1988). It is used for detecting targeted pathogenic microorganisms and virus only, and hence comparison of basically similar type of microorganisms is possible by using multiplex-PCR (Hatakeyama *et al.*, 2008). Recently, Seri-biotech Research Laboratory, Central Silk Board, Bangalore developed some multiple primers detecting pebrine incidence as well as Denso Nucleus Virus (DNV) and Nuclear Polyhedrosis Virus (NPV) at a time in Multiplex-PCR.

Genome profiling (GP) was developed using Temperature Gradient Gel Electrophoresis (TGGE) of random PCR (similarly to RAPD PCR) products for investigation of the relationship of the microorganisms by comparing the whole genome, but not for sequence comparison of DNA fragments (Nishigaki *et al.*, 1991). The genomic constitution of silkworm infected with pathogenic microorganisms is different from that of

healthy silkworm, because the DNA extracted from infected individual contains the genome of the infectious pathogen also and may alter the profile image of the infected silkworm from that of the healthy one. Utilizing the concept of GP, a new technique, Hyper Detection of infectious disease based on Genome Profiling (HDGP) has been developed, which is very simple and capable of detecting pathogenic microorganisms which have infected the silkworms at a high sensitivity. In such case, no specific DNA marker is necessary as the profile image of a healthy host is used as a marker for detection of the profile image of an infected one. Thus all microorganisms (including unknown pathogens) which have infected the silkworm are detected by identical procedure under identical conditions in a single operation (Hatakeyama *et al.*, 2008).

Representational difference analysis (RDA) is a very sensitive technique that detects minute differences in DNA (Lisitsyn and Wigler, 1995). Connectable sequences were searched in the DNA fragments and analyzed by RDA methods. Several types of *N. bombycis* have been isolated and shown to be identical on morphology and gene sequence analysis (Kawarabata, 2003), but the identity is in question from phylogenetic point of view. It is reported that there is no morphological difference between the two strains, *N. bombycis* NIS-001 and Sd-NU (Nihon University culture collection) and reports only a few base differences on a small subunit ribosomal DNA partial sequence alignment (Hatakeyama *et al.*, 1997). *N. bombycis* Sd-NU shows low level of virulence against silkworms despite having no morphological difference. This finding also suggests that all previously isolated strains of *N. bombycis* are not identical, but are mixed. RDA is used in the study, but investigation based on more specific strategy is still necessary (Hatakeyama *et al.*, 2009). Selection of spore that exhibit virulence against insect pests but non-virulent in silkworm is yet to be identified. That type of study has enough scope for detection of non-virulent pebrine spore.

Recent research on spore wall proteins of *N. bombycis* contributes useful information to discover the molecular

mechanism of pathogen development. Identification of spore wall protein is a major information tool for detection of pebrine spore (Wu *et al.*, 2008, 2009). Various workers *viz.*, Frixione *et al.* (1992), Vavra and Larrson (1999), Couzinet *et al.* (2000), Hayman *et al.* (2005), Franzen *et al.* (2005), Southern *et al.* (2006), Xu *et al.* (2006 b), Li *et al.* (2008), and Cai *et al.* (2011) studied different aspects of spore wall proteins *viz.*, molecular characteristics, pathogenesis, *etc.* Exospore wall protein (SWP 26) is a good prospective target for diagnostic research and drug design for controlling pebrine disease in *B. mori* (Li *et al.*, 2008.) Exospore wall proteins (SWP 32) play a role in early life cycle stage of *N. bombycis* at 36 hours post infection (meront stage), whereas endospore wall protein (SWP 25 and SWP 30) have role at 72 hours post infection (sporeblast to mature stage). But the little sequence similarity between the known spore wall proteins with other eukaryotic proteins, makes it difficult to differentiate a new spore wall protein. For that reason, only four spore wall proteins, SWP25, SWP30, SWP26 and SWP32 have been reported from *N. bombycis* (Wu *et al.*, 2008, 2009) and seven spore wall proteins from *Encephalitozoon cuniculi* (Bohne *et al.*, 2000; Brosseau *et al.*, 2005; Peuvrel-Fanget *et al.*, 2006; Xu *et al.*, 2006 b, Southern *et al.*, 2007) and *E. intestinalis* (Hayman *et al.*, 2001; Southern *et al.*, 2007). Sometimes, same protein is found in spore wall and in other body parts *e.g.*, SWP 5 is available in the exospore of *N. bombycis* as well as inside the spore in association with the coils of the polar tube. This protein is thought to interact with polar tube proteins (PTP 2 and 3) and can be detected immunologically in the extruded polar tube (Li *et al.*, 2012).

Research for identification and characterization of spore wall proteins responsible for formation and maturation of spore wall during life cycle stage of the pathogen may be a big step in controlling pebrine disease. Spore wall proteins are now drawing great interest for their important role in the infection mechanism. Immune response to *N. bombycis* is often directed at the polar tube and spore wall (Weiss and Vossbrinck, 1999). Studies of the spore wall protein and its composition can define a new antigen for immuno-protection and new diagnostic

reagents. It will also improve our understanding of the process of germination in these ubiquitous pathogens (Xu *et al.*, 2006 b). Considerable inter- and intra-specific variability of the spore wall proteins exists, and it has been suggested that these proteins might be helpful in diagnostics and in molecular epidemiology studies (Xiao *et al.*, 2001).

It is known that a number of pathogens are capable of taking up and utilizing the breakdown products of host. Pathogen is dependent upon the digestive enzymes of the host for their nutrition, growth and development. *N. bombycis* also depends on enzyme of host origin, *i. e.*, *B. mori*. This intracellular protozoan parasite do not have mitochondria in its cytoplasm, but, when examined under the electron microscope, a large number of host mitochondria could be seen clustered around each parasite (Cali, 1970). It has been suggested that *N. bombycis* is dependent upon the mitochondrial respiratory activities of the host, *B. mori* for its energy production and the mitochondrial enzymes of the host for its metabolic function (Cheng, 2011). Therefore, one of the new directions of research proposed utilising the modern biotechnological tools is to block the attachment of *N. bombycis* with the host mitochondria, so that the source of energy supply and metabolic function of the pathogen will be hindered. Practical technologies based on such scientific principles may turn out to be breakthroughs in combating the most dreadful disease encountered in the field of sericulture.

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REFERENCES

- Abe Y. and Fujiwara T. (1979) Mode of multiplication of a protozoan, *Pleistophora* sp. (Microsporidia – Nosematidae) in the midgut epithelium of the silkworm larvae. *J. Seric. Sci. Jpn.*, **48**: 19-23.
- Baig M., Dutta R. K., Nataraju B., Samson M. and Sivaprasad V. (1992) Protein-A linked latex Antisera test for the detection of *Nosema bombycis* Naegeli spores. *J. Invertebr. Pathol.*, **60**: 312-313.
- Barker M. D., Vossbrink C. R., Dider S., Maddox J. V. and Sidduck J. A. (1994) Phylogenetic relationships among *Varimorpha* and *Nosema* species (Microsporida) based on ribosomal RNA sequence data. *J. Invertebr. Pathol.*, **64**: 100-106.
- Beckmann J. S. and Weber J. C. (1992) Survey of human and rat microsatellites. *Genomics*, **12**: 627-631.
- Becnel J. J. and Andreadis T. G. (1999) Microsporidia in insects. In: *The Microsporidia and Microsporidiosis*, Wittner M. and Weiss L. M. (Eds.), ASM Press, Washington DC, pp. 447-501.
- Bohne W., Ferguson D. J., Kohler K. and Gross U. (2000) Developmental expression of a tandemly repeated, glycine- and serine-rich spore wall protein in the microsporidian pathogen *Encephalitozoon cuniculi*. *Infect. Immun.*, **68**: 2268-2275.
- Brooks W. M. (1988) Entomogenous protozoa. In: *Handbook of Natural Pesticides*, C. M. Ignoffo (Ed.), CRC Press, Inc. Boca Rota, Florida, Vol. 5, pp. 1-149.
- Brosson D., Kuhn L., Prensier G., Vivares C. P. and Texier C. (2005) The putative chitin deacetylase of *Encephalitozoon cuniculi*: a surface protein implicated in microsporidian spore-wall formation. *FEMS Microbiol. Lett.*, **247**: 81-90.
- Burges H. D., Canning E. U. and Hulls I. K. (1974) Ultrastructure of *Nosema oryzaephili* and the taxonomic value of polar filament. *J. Invertebr. Pathol.*, **23**: 135-139.
- Burri L., Williams B. A. P., Bursac D., Lithgow T. and Keeling J. P. (2006) Microsporidian mitochondria retain elements of the general mitochondrial targeting system. The National Academy of sciences of the USA. *PNAS*, **30**: 15916-15920 (www.pnas.org.doi 10.1073/pnas.0604109103).

- Cai S. F., Lu X. M., Qiu H. H., Li M. Q. and Feng Z. Z. (2011) Identification of a *Nosema bombycis* (Microsporidia) spore wall protein corresponding to spore phagocytosis. *Parasitology*, **138**: 1102-1109.
- Cali A. (1970) Morphogenesis in the genus *Nosema*. *Proc. Fourth Int. Colloq. Insect Pathol.*, Cleege Park, Maryland, pp. 431-438.
- Cali A., Kotler D. P. and Orenstein J. M. (1993) Septata intestinalis n. g., n. sp., an intestinal microsporidian associated with chronic diarrhoea and dissemination in AIDS patients. *J. Eukaryot. Microbiol.*, **40**: 101-112.
- Cali A., Orenstein J. M., Kotler D. P. and Owen R. (1991) A comparison of two microsporidian parasites in enterocytes of AIDS patients with chronic diarrhoea. *J. Protozool.*, **38**: 96S-98S.
- Canning E. U., Curry A., Cheney S. A., Lafrachi-Tristem N. J. and Ishihara R. (1998) What is *Nosema bombycis*? Observation based on *Nosema* spp. parasite in Cinnaber moth, *Tyria jacobaeae*. *The Society of Protozoologists*, 51 Annual Meeting, August 1-8, 1998, p.1.
- Cavalior - Smith E. and Chao T. E. (1996) Molecular phylogeny of the free living archeozoan *Trepomonas agilis* and the nature of the first eukaryotes. *J. Mol. Evol.*, **43**: 551-562.
- Chakrabarty S. (2014) *Pebrine Disease of Silk Moths in Assam*. LAP LAMBERT Academic Publishing [ISBN: 978-3-359-56571-7], D-66121, Saarbrücken, Deutschland, Germany.
- Chakrabarty S. and Manna B. (2006) Three new species of *Nosema* from non-mulberry silkworms in Assam: light, scanning and transmission electron microscopy studies. *J. Parasitic Dis.*, **30**: 125-133.
- Chakrabarty S. and Manna B. (2008 a) Influence of temperature and relative humidity in infection of *Nosema bombycis* (Microsporidia: Nosematidae) and cross-infection of *N. mylitta* on growth and development of mulberry silkworm, *Bombyx mori*. *Int. J. Indust. Entomol.*, **17**: 173-180.
- Chakrabarty S. and Manna B. (2008 b) Effect of microsporidian infection on reproductive potentiality on mulberry silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae) in different seasons. *Int. J. Indust. Entomol.*, **17**: 157-163.
- Chakrabarty S. and Manna B. (2009 a) Studies on ultrastructure and life cycle of *Nosema assamensis* (Protozoa: Microsporidia), a parasite of muga silkworm, *Antheraea assamensis* Ww. *Indian J. Seric.*, **48**: 60-67.
- Chakrabarty S. and Manna B. (2009 b) Studies on cross-infection of microsporidian spores of non-mulberry silkworms to mulberry silkworm, *Bombyx mori* L. and its impact on economic parameters. *Indian J. Seric.*, **48**: 162-168.
- Chakrabarty S., Saha A. K., Bindroo B. B., Manna B. and Nirmal Kumar S. (2013 a) An improved method for detection of pebrine (*Nosema bombycis* N.) in silkworm (*Bombyx mori*). *Appl. Biol. Res.*, **15**: 51-56.
- Chakrabarty S., Saha A. K., Manna B. and Bindroo B. B. (2013 b) Gender influenced dimorphism in *Nosema bombycis* Nageli, causing pebrine disease in silkworm, *Bombyx mori* L. *Walailak J. Sci. Technol., Thailand*, **10**: 103-111.
- Chamberlin J. S., Gibbs R. A., Ranier J. E., Nguyen P. N. and Caskey C. T. (1988) Detection screening of the Duchenne muscular dystrophy locus via multiplex DNA amplification. *Nucleic Acid Res.*, **16**: 11141-11156.
- Cheng T. C. (2011) *General Parasitology*. Academic Press, Indian Reprint by Elsevier, a division of Reed Elsevier India Private Limited, New Delhi, p. 61.
- Couzinet S., Cejas E., Schittny J., Deplazes P., Weber R. and Zimmerli S. (2000) Phagocytic uptake of *Encephalitozoon cuniculi* by nonprofessional phagocytes. *Infect. Immunol.*, **68**: 6939-6945.
- Dasgupta M. R. (1950) *Monograph on cottage Industries* No.1, Diseases of silkworm, Govt. Press, Calcutta, India, p. 9.

- Devaiah M. C. and Krishnaswamy S. (1975) Observation on the seasonal incidence of pebrine disease on silkworm *B. mori*, *L. Indian J. Seric.*, **14**: 27-30.
- Dugourd D., Jacques M., Bigras-Poulin M. and Harel J. (1996) Characterization of *Serpulina hyodysenterae* isolates of serotypes 8 and 9 by random amplification of polymorphic DNA analysis. *Veter. Microbiol.*, **48**: 305-314.
- Elain M. K., Peter M. T., Ann C., Herbert B., Murray W. and Louis W. M. (2001) Monoclonal antibodies to cytoplasmic antigens of *Nosema locustae* (Microsporidia: Nosematidae). *J. Invertebr. Pathol.*, **77**: 81-82.
- Fabienne T., Christian P. V. and Manolo G. (2004) Phylogenetic analysis of the complete genome sequence of *Encephalitozoon cuniculi* supports the fungal origin of microsporidia and reveals a high frequency of fast-evolving genes. *J. Mol. Evol.*, **59**: 780-791.
- Field D. and Wills C. (1996) Long polymorphic micro-satellites in simple organisms. *Proc. R. Soc. B*, **263**: 209-215.
- Fowler J. L., and Reeves E. L. (1974) Detection of relationships among microsporidian isolates by electrophoretic analysis, hydrophobic extracts. *J. invertebr. Pathol.*, **23**: 3-12.
- Franzen C., Muller A., Hartmann P. and Salzberger B. (2005) Cell invasion and intracellular fate of *Encephalitozoon cuniculi* (Microsporidia). *Parasitology*, **130**: 285-292.
- Frixione E., Ruiz L., Santillan M., De Vargas L. V., Tejero J. M. and Undeen A. H. (1992) Dynamics of polar filament discharge and sporoplasm expulsion by microsporidian spores. *Cell Motil. Cytoskel.*, **22**: 38-50.
- Fujiwara T. (1979) Infectivity and pathogenicity of *Nosema bombycis* to larvae of the silkworm. *J. Seric. Sci. Jpn.*, **48**: 376-380.
- Fujiwara T. (1980) Three Microsporidians (*Nosema* sp.) from silkworm *Bombyx mori*. *J. Seric. Sci. Jpn.*, **49**: 229-236.
- Fujiwara T. (1984) A *Pleistophora* like microsporidian isolated from the silkworm *Bombyx mori*. *J. Seric. Sci. Jpn.*, **53**: 459-460.
- Fujiwara T., Kagawa T. and Andou H. (1985) *Nosema bombycis* like microsporidians isolated from silkworm moths in egg-production. *J. Seric. Sci. Jpn.*, **54**: 117-121.
- Garcia L. S. (2002) Laboratory identification of the microsporidia. *J. Clin. Microbiol.*, **40**: 1892-1901.
- Ghosh K. and Weiss L. M. (2009) *Molecular diagnostic tests for microsporidia*. Hindawi Publishing Corporation, Interdisciplinary Perspectives on Infectious diseases, Vol. 2009, Article ID 926521, doi. 1155/2009/926521.
- Goldberg A. V., Molik S., Tsaousis A. D., Neumann K., Kuhnke G. and Delbac F. (2008) Localization and functionality of microsporidian iron-sulphur cluster assembly proteins. *Nature*, **452**: 624-628.
- Graff D. C. D., Raes H. and Jacobs F. J. (1994) Note: Spore dimorphism in *Nosema apis* (Microsporidia: Nosematidae) developmental cycle. *J. Invertebr. Pathol.*, **63**(1): 92-94.
- Griyaghey U. P. and Krishnan G. (1976) Transmission of *Nosema* through parasites and predators of Tasar silkworm. *Annual Report*, Central Tasar Research Station, Ranchi, India, 1976, pp. 89-91.
- Griyaghey U. P. and Sengupta K. (1989) Studies on the transmission of *Nosema* sp. in tropical tasar silkworm, *Antheraea mylitta*, *D. Sericologia*, **29**(3): 383-387.
- Griyaghay U. P. and Singh M. K. (1976) Extent of transovarian transmission of *Nosema* sp. in *A. mylitta*. *Annual Report*, Central Tasar Research Station, Ranchi, India, 1976, pp. 87-88.
- Hartskeeri R. A., Vangool. T., Sehuitema A. R., Didier E. S. and Terpstraw J. (1995) Genetic and immunological characterization of the microsporidians *Sepata intestinales* Cali, Kotter and ovenstein 1993; reclassification to

- Encephalitozoon intestinalis*. *Parasitol.*, **110**: 277-285.
- Hatakeyama Y., Hamango K. and Iwano H. (2008) An ultimate method for detection of infected pathogenic microorganism from silkworm using HDGP. *J. Insect Biotechnol. Sericol.*, **77**: 1-7.
- Hatakeyama Y. and Hayasaka S. (2001) Specific amplification of microsporidia DNA fragments using multiprimer PCR. *J. Seric. Sci. Jpn.*, **70**: 163-166.
- Hatakeyama Y. and Hayasaka S. (2003) A new method of pebrine inspection of silkworm eggs using multiprimer PCR. *J. Seric. Sci. Jpn.*, **82**: 148-151.
- Hatakeyama Y., Kawakami Y., Iwano H., Inoue T. and Ishihara R. (1997) Analysis and taxonomic inferences of small subunit ribosomal RNA sequences of five microsporidia pathogenic to silkworm, *Bombyx mori*. *J. Seric. Sci. Jpn.*, **66**: 242-252.
- Hatakeyama Y., Yukita S., Koichi M., Yukawa M. and Iwano H. (2009) Search for detection of specific DNA fragments in high- and low virulent strains of *Nosema bombycis* (Microsporidia: Nosematidae). *J. Insect Biotechnol. Sericol.*, **78**: 17-21.
- Hayasaka S. and Ayuzawa C. (1987) Diagnosis of microsporidians, *Nosema bombycis* and closely related species by antibody-sensitized latex. *J. Seric. Sci. Jpn.*, **56**: 169-170.
- Hayasaka S. and Kawarabata T. (1990) Cloning of a microsporidian, *Nosema bombycis* (Microsporidia: Nosematidae) in insect cell cultures by a limiting dilution method. *J. Invertebr. Pathol.*, **55**: 35-40.
- Hayman J. R., Hayes S. F., Amon J. and Nash T. E. (2001) Developmental expression of two spore wall proteins during maturation of the microsporidian *Encephalitozoon intestinalis*. *Infect. Immun.*, **69**: 7057-7066.
- Hayman J. R., Southern T. R. and Nash T. E. (2005) Role of sulfated glycans in adherence of the microsporidian, *Encephalitozoon intestinalis* to host cells *in vitro*. *Infect. Immunol.*, **73**: 841-848.
- Hazard E. I. and Weiser J. (1968) Spores of *Thelophania* in adult female Anopheles: development and transovarial transmission and redescription of *T. legeri* Hesse and *T. obesa* Kudo. *J. Protozool.*, **15**: 817-823.
- Hirt R. P., Logsdon J. M., Healy B., Dorey M. W., Doolittle W. F. and Embley T. M. (1999) Microsporidia are related to fungi: Evidence from the largest subunit of RNA polymerase and other proteins. *Proc. Natl. Acad. Sci. USA*, **96**: 580-585.
- Hung H. W., Loc F., Tseng C. C., Peng S. E., Chow C. M. and Kong G. H. (1998) The small subunit ribosomal RNA gene sequence of *Pleistophora anguillarum* and the use of PCR primers for diagnostic detection of the parasite. *J. Eukaryot. Microbiol.*, **45**: 556-560.
- Ishihara R. (1969) The life cycle of *Nosema bombycis* as revealed in tissue culture cell of *Bombyx mori*. *J. Invertebr. Pathol.*, **14**: 316-320.
- Ishihara R. and Fujiwara T. (1965) The spread of pebrine disease within a colony of the silkworm *Bombyx mori* (Linnaeus). *J. Invertebr. Pathol.*, **7**(2): 126-131.
- Ishihara R. and Hayashiya Y. (1968) Some properties of ribosome from the sporoplasm of *Nosema bombycis*. *J. Invertebr. Pathol.*, **11**: 377-385.
- Iwano H. (1993) Identification of insect microsporidians. In: *Manual for Microbial Control Agents*, Okada M., Iwahana H., Kunimi H. and Shimazu M. (Eds.), Plant Protection Association, Tokyo (in Japanese), pp. 81-90.
- Iwano H. and Ishihara R. (1989) Intracellular germination of spores of a *Nosema* sp. immediately after their formation in cultured cell. *J. Invertebr. Pathol.*, **54**: 125-127.
- Iwano H. and Ishihara R. (1991) Dimorphic development of *Nosema bombycis* spore in gut epithelium of larva of the

- silkworm *Bombyx mori*. *J. Seric. Sci. Jpn.*, **60**: 249-256.
- Jamenson P. A. (1922) *Report on the diseases of silkworms in India*, Govt. Printing Press, Calcutta, India.
- James T. Y., Kauff F., Schoch C. L. *et al.* (2006) Reconstructing the early evolution of fungi using a six-gene phylogeny. *Nature*, **443**: 818-822.
- Jolly M. S. and Sen S. K. (1972) Infection of *A. mylitta* Drury (Lepidoptera: Saturniidae) by microsporidia, *Nosema* sp. *Indian J. Seric.*, **11**(1): 52-54.
- Katinka M. D., Duprat S., Cornillot E., Metenier G., Thomarat F., Prensier G., Barbe V., Peyretailade E., Brottier P., Wincker P., Delbac F., El Alaoui H., Peyret P., Saurin W., Gouy M., Weiss-enbach J. and Vivares C. P. (2001) Genome sequence and gene compaction of the eukaryote parasite *Encephalitozoon cuniculi*. *Nature*, **414**: 450-453.
- Kamble C. K., Magadum S. B., Singh R. N., Daniel A. G. K. and Manjula A. (2005) Incidence of pebrine in *Bombyx mori* L. at seed multiplication levels in South India and its management-A review, 20th Congress of International Sericultural Commission, 15th - 18th December, 2005, Bangalore, India, pp. 241-244.
- Kawakami Y., Inoue T., Uchida T., Hatakeyama V., Iwano H. and Ishihara R. (1995) Specific amplification of DNA from reference strain of *Nosema bombycis*. *J. Seric. Sci. Jpn.*, **64**: 165-172.
- Kawakami Y., Iwano H., Hatakeyama Y., Inoue T., Canning E. U. and Ishihara R. (2001) Use of PCR with specific primers for discrimination of *Nosema bombycis*. *J. Seric. Sci. Jpn.*, **70**: 43-48.
- Kawarabata T. (2003) Review – Biology of microsporidians infecting silkworm, *Bombyx mori* in Japan. *J. Insect Biochem. Sericol.*, **72**: 1-32.
- Kawarabata T. and Hayasaka S. (1987) An enzyme linked immunoabsorbant assay to detect alkali soluble spore surface antigens of strains of *Nosema bombycis*. *J. Invertebr. Pathol.*, **50**(2): 118-123.
- Kawarabata T. and Ishihara R. (1984) Infection and development of *Nosema bombycis* (Microsporidia: Protozoa) in a cell line of *Antheraea eucalypti*. *J. Invertebr. Pathol.*, **50**: 118-123.
- Keeling P. J., Luker M. A. and Palmer J. D. (2000) Evidence from Beta-tubulin that microsporidia evolved from fungi discusses placement of microsporidians as fungal relatives rather than as basal amitochondriate protists. *Mol. Biol. Evol.*, **17**: 1-9.
- Knell J. D. and Zam S. G. (1976) A serological comparison of some species of microsporidia. *J. Invertebr. Pathol.*, **31**: 280-288.
- Kohler G. and Milston C. (1975) Continuous cultures of fused cell screening antibody of predefined specificity. *Nature*, **256**: 495-497.
- Kramer J. P. (1976) The extra corporeal ecology of Microsporidia. In: *Comparative Pathobiology*, Vol.1, Bulla Lee A. Jr. and Cheng T. C. (Eds.), Plenum Press, ISDA, Manhatta, Kanswa, USA, pp. 127-135.
- Kudo R. (1930) Microsporidia: In: *Problems and methods of Research in Protozoology*, Henger and Macmillan Co., New York, pp. 325-347.
- Langley R. C. Jr., Cali A. and Somberg E. W. (1987) Two dimensional electrophoresis analysis of spore proteins of the microsporidia. *J. Parasitol.*, **73**(5): 910-918.
- Larsson J. I. R. (1988) Identification of microsporidian genera: a guide with comments on the taxonomy. *Arch. Protistenkd.*, **136**: 1-37.
- Larsson J. I. R. (1999) Identification of microsporidia. *Acta Protozoologica*, **38**: 161-197.
- Larsson J. I. R. (2005) Molecular versus morphological approach to microsporidian classification. *Folia Parasitol.*

- (Praha), **52**: 143-144.
- Lee S. C., Corradi N., Byrnes III E. J., Torres-Martinez S., Dietrich F. S., Keeling P. J. and Heitman J. (2008) Microsporidia evolved from ancestral sexual fungi. *Curr. Biol.*, **18**: 1675-1679.
- Li Y., Wu Z., Pan G., He W., Zhang H., Hu J. and Zhou Z. (2008) Identification of novel spore wall protein (SWP26) from microsporidia *Nosema bombycis*. *Int. J. Parasitol.*, **39**(4): 391-398.
- Li Z., Pan G., Li T., Huang W., Chen J., Geng L., Yang D., Wang L. and Zhou Z. (2012) SWP5, a spore wall protein, interacts with polar tube proteins in the parasitic microsporidian, *Nosema bombycis*. *Eukaryot. Cell.*, **11**: 229-237.
- Lisitsyn N. and Wiggler M. (1995) Representational difference analysis in detection of genetic lesions in cancer. *Methods Enzymol.*, **254**: 291-304.
- Lom J. and Vavra J. (1963) The mode of sporoplasm extrusion in microsporidia spores. *Acta Protozool.*, **1**: 81-90.
- Loubes C., Maurand J. and Ormieres R. (1979) Etude ultrastructurale de *Spraguea lophii* (Doflein), Microsporidae parasite de Baudroie: Essai d'interprétation du dimorphisme sporae. *Parasitologica*, **15**: 43-54.
- Maddox J. V. (1973) The persistence of the microsporidians in the environment. *Entomol. Sci. Amer. Isc. Publ.*, **9**: 99-104.
- Malone L. A. and McIvor C. A. (1995) DNA probes for two microsporidia *Nosema bombycis* and *Nosema costelytrae*. *J. Invertebr. Pathol.*, **65**(3): 262-273.
- Malone L. A. and McIvor C. A. (1996) Use of nucleotide sequence data to identify a microsporidian pathogen of *Pieris rapae* (Lepidoptera, Pieridae). *J. Invertebr. Pathol.*, **68**: 231-238.
- Mariswamy N. (1977) Studies on pebrine disease of muga and eri silkworm. *Annual Report*, Central Muga and Eri Research Station, Titabar, Assam, 1977, pp. 43 - 44.
- Mercer C. F. and Wigley P. J. (1987) A microsporidian pathogen of the poporo stem borer, *Scelodes cordalis* (Dbl) (Lepidoptera: Pyralidae). *J. Invertebr. Pathol.*, **49**: 93-101.
- Milner R. J. (1972 a) *Nosema whitei*, a microsporidian pathogen of some species of *Tribolium*. *J. Invertebr. Pathol.*, **19**: 230-247.
- Milner R. J. (1972 b) *Nosema whitei*, a microsporidian pathogen of some species of *Tribolium* III. 1. Morphology, life-cycle, and generation time. *J. Invertebr. Pathol.*, **19**: 248-255.
- Moore S. S., Sargeant L. L., Kind T. J., Mattick J. S., Georges M. and Hetzel D. J. S. (1991) The conservation of dinucleotide microsatellites among mammalian genomes allow the use of heterologous PCR primer pairs in closely related species. *Genomics*, **10**: 654 - 660.
- Nageli C. (1857) Ueber die neue Krankheit der Seidenraupe und verwandte Organismen. *Botanischer Zeitschrift*, **15**: 760-761.
- Nageswara Rao S., Mithulakshmi M., Kanginakudru S. and Nagaraju J. (2004) Phylogenetic relationships of three new microsporidians isolated from the silkworm, *Bombyx mori*. *J. Invertebr. Pathol.*, **48**: 355-366.
- Nageswara Rao S., Surendra Nath B. and Saratchandra B. (2005) Characterization and phylogenetic relationships among microsporidia infecting silkworm, *Bombyx mori* using inter simple sequence repeat (ISSR) and small subunit rRNA (SSU-rRNA) sequence analysis. *Genome*, **48**: 355-366.
- Nishigaki K., Amano N., and Takasawa T. (1991) DNA profiling – an approach of systematic characterization, classification and comparison of genomic DNAs. *Chem. Lett.*, **20**: 1097-1100.
- Noman J. P., Alexandrea Silva J., Susan B. S., Govinda S., Visvesvarathimothy S. and Chisa Yasungara J. K. (1996)

- Nosema trichoplusia* is a synonym of *Nosema bombycis* based on the sequence of the small subunit Ribosomal RNA coding region. *J. Invertebr. Pathol.*, **67**: 316-317.
- Nomani M. K. R., Krishnaswami S. and Sahakundu A. K. (1971) Observation on the seasonal incidence and intensity of pebrine disease of the silkworm under West Bengal climate conditions. *Indian J. Seric.*, **9**: 101-104.
- Olive D. and Bean M. P. (1999) Principles and applications of methods for DNA based typing of microbial organisms. *J. Clin. Microbiol.*, **37**: 1661-1669.
- Orenstein J. M. (2003) Diagnostic pathology of microsporidiosis. *Ultrastruct. Pathol.*, **27**: 141-149.
- Pasteur L. (1870) *Etudes sur la maladie des vers a soie*. Gauthier-Villars, Imprimeur-Libraire, Paris, p. 322.
- Patil C. S. (1993) Review on pebrine, a microsporidian disease in the silkworm, *Bombyx mori* L. *Sericologia*, **33**(2): 201-210.
- Peuvel-Fanget I., Polonais V., Brosseau D., Texier C., Kuhn L., Peyret P., Vivares C. and Delbac F. (2006) EnP1 and EnP2, two proteins associated with the *Encephalitozoon cuniculi* endospore, the chitin-rich inner layer of the microsporidian spore wall. *Int. J. Parasitol.*, **136**: 309-318.
- Procop G. W. (2007) Molecular diagnosis for the detection and characterization of microbial pathogens. *Clinical infectious disease*, **45**(2): 99-111.
- Raynaud L., Delbace F., Broussolle V., Rabodonirina M., Girault V., Wallon M., Cozon G., Vivares C. P. and Peyron F. (1998) Identification of *Encephalitozoon intestinalis* in travelers with chronic diarrhoea by specific PCR amplification. *J. Clin. Microbiol.*, **36**: 37-40.
- Sambrook J. and Russel D. W. (2006) *The condensed protocols from molecular cloning: A laboratory manual*, Cold Spring Harbor Laboratory Press, New York, pp. 734-735.
- Samson M. V., Santha P. C., Singh R. N. and Sasidharan T. O. (1990) A new microsporidian infecting *Bombyx mori* L. *Indian Silk*, **37**(10): 10-12.
- Sato R., Kobayashi M., Watanabe H. and Fujiwara T. (1981) Serological discrimination of several kinds of microsporidian spores isolated from silkworm *Bombyx mori* by an indirect fluorescent antibody test. *J. Seric. Sci. Jpn.*, **50**: 180-184.
- Sato R. and Watanabe H. (1986 a) Sporogonial sequence of three microsporidia pathogenic to the silkworm, *Bombyx mori* L. *J. Seric. Sci. Jpn.*, **55**(1): 10-26.
- Sato R. and Watanabe H. (1986 b) Pathways of oral infection with four microsporidia in the silkworm *Bombyx mori* L. *J. Seric. Sci. Jpn.*, **55**(1): 28-32.
- Schlotterer C., Amos B., and Tautz D. (1991) Conservation of polymorphic simple sequence loci in certain species. *Nature*, **354**: 63-65.
- Singh R. N., Daniel A. G. K., Sindagi S. S. and Kamble C. K. (2007) Microsporidians infecting silkworm, *Bombyx mori* L. – A review. *Sericologia*, **47**(1): 1-6.
- Singh R. N., Santha P. C., Sasidharan T. O., Manjula A. and Kamble C. K. (2004) Delayed mother moth testing for effective detection of pebrine. *Indian Silk*, **43**: 7-8.
- Siromani A. (1997) Detection of *Nosema bombycis* infection in the silkworm, *Bombyx mori*, by Western Blot Analysis. *Sericologia*, **37**(2): 209-216.
- Southern T. R., Jolly C. E. and Hayman J. R. (2006) Augmentation of microsporidia adherence and host cell infection by divalent cations. *FEMS Microbiol. Lett.*, **260**: 143-149.
- Southern T. R., Jolly C. E., Lester M. E. and Hayman J. R. (2007) EnP1, a microsporidia spore wall protein that enables spores to adhere and infect host cells *in vitro*. *Eukaryotic Cell*, **6**: 1354-1362.

- Sprague V. (1977) Classification and phylogeny of microsporidia. In: *Comparative Pathobiology*, Bulla L. A. and Cheng T. C. (Eds.), Plenum Press, New York, Vol. 2, pp.1-30.
- Sprague V., Becnel J. J. and Hazard E. I. (1992) Taxonomy of phylum Microspora. *Crit. Rev. Microbiol.*, **18**: 285-395.
- Sprague V., Vernick S. H. and Lloyd B. J. (1968) The fine structure of *Nosema* sp. (Microsporidia, Nosematidae) with particular reference to stage in sporogony. *J. Invertebr. Pathol.*, **12**: 105-117.
- Srikanto H. K. (1987) Studies on the cross-infectivity and viability of *Nosema bombycis*. *M. Sc. Thesis*, University of Agricultural Sciences, Bangalore, India.
- Steinhaus E. A. and Huges K. M. (1949) Two newly described species of microsporidia from the potato tuberworm, *Gnorimoschema operculella*, Zellar (Lepidoptera: Gelechiidae). *J. Parasitol.*, **35**: 57-65.
- Streets D. A. and Briggs J. D. (1982) An evaluation of sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the identification of microsporidia. *J. Invertebr. Pathol.*, **40**(2): 159-165.
- Tanada Y. (1963) Epizootiology of infectious diseases. In: *Insect Pathology*, E. A. Steinhaus (Ed.), Academic Press, New York, Vol. II, pp. 423-475.
- Tanaka S., Shimizu T., Kobayashi M. and Ishihara R. (1972) A new microsporidian pathogenic to the silkworm *Bombyx mori*. *J. Seric. Sci. Jpn.*, **41**: 89-95.
- Thomarat F., Vivares C. P. and Gouv M. (2004) Phylogenetic analysis of the complete genome sequence of *Encephalitozoon cuniculi* supports the fungal origin of microsporidia and reveals a high frequency of fast-evolving genes. *J. Mol. Evol.*, **59**(6): 780-791.
- Tompkins L. S. (1992) The use molecular methods in infectious diseases. *New Engl. J. Med.*, **327**: 1290-1297.
- Tsai S. J., Lo C. F., Sochi Y. and Wang C. H. (2003) The characterization of microsporidian isolates (Nosematidae: *Nosema*) from five important lepidopteran pests in Taiwan. *J. Invertebr. Pathol.*, **83**: 51-59.
- Vavra J. and Barker R. J. (1980) The microsporidian mucocalyx as seen in Scanning Electron Microscope. *Folia Parasitologica* (Prague), **27**(1): 19-21.
- Vavra J. and Larson R. J. I. (1999) Structure of the microsporidia. In: *The Microsporidia and Microsporidiosis*, Wittner M. and Weiss L. M. (Eds.), ASM Press, Washington DC, pp. 7-84.
- Vossbrinck C. R., Baker M. D., Didier E. S., Debrunner - Vossbrinck B. A. and Shadduck J. A. (1993) Ribosomal DNA sequence of *Encephalitozoon hellem* and *Encephalitozoon cuniculi*: species identification and phylogenetic construction. *J. Eukaryot. Microbiol.*, **40**: 354-362.
- Wang Z., Weber J. L., Zhong G. and Tanksley S. D. (1994) Survey of plant short tandem DNA repeats. *Theor. Appl. Genet.*, **88**: 1-6.
- Weiss L. M. and Vossbrinck C. R. (1999) Molecular biology, molecular phylogeny, and molecular diagnostic approaches to the microsporidia. In: *The Microsporidia and Microsporidiosis*, Wittner M. and Weiss L. (Eds.), Amer. Soc. Microbiol., Washington D. C., pp. 129-171.
- Wu Z. L., Li Y. H., Pan G. Q., Tan X. X., Hu J. H., Zhou Z. Y. and Xiang Z. H. (2008) Proteomic analysis of spore wall proteins and identification of two spore wall proteins from *Nosema bombycis* (Microsporidia). *Proteomics*, **8**(12): 2447-2461.
- Wu Z. L., Li Y. H., Pan G. Q., Zhou Z. Y. and Xiang Z. H. (2009) SWP25, A novel protein associated with the *Nosema bombycis* endospore. *J. Eukaryot. Microbiol.*, **56**(2): 113-118.
- Xiao L., Li L., Visvesvara G. S., Moura H., Didier E. S. and Lal A. A. (2001) Genotyping *Encephalitozoon cuniculi* by multilocus analyses of genes with repetitive sequences. *J.*

Clin. Microbiol., **39**: 2248–2253.

1049-1056.

Xu J., Pan G., Fang L., Li J., Tian X., Li T., Zhou Z. and Xian Z. (2006 a) The varying microsporidian genome: Existence of long-terminal repeat retrotransposon in domesticated silkworm parasite *Nosema bombycis*. *Int. J. Parasitol.*, **36**:

Xu Y., Takvorian P., Cali A., Wang F., Zhang H., Orr G. and Weiss L. M. (2006 b) Identification of a new spore wall protein from *Encephalitozoon cuniculi*. *Infect. Immun.*, **74**(1): 239 -247.

Revue

LA PEBRINE (*NOSEMA BOMBYCIS* N.) CHEZ *BOMBYX MORI* L.: LES TENDANCES DE LA RECHERCHE

Satadal Chakrabarty^{1*}, B. Manna², A. K. Saha¹, B. B. Bindroo³ and K. Trivedy¹

¹Central Sericultural Research and Training Institute, Berhampore, West Bengal 742101, India.

²Parasitology Research Unit, Department of Zoology, The University of Calcutta, Kolkata, West Bengal 700019, India.

³Central Sericultural Research and Training Institute, Mysuru, Karnataka 570008, India.

*E-mail: satadal.chak@gmail.com

R É S U M É

La perte de récolte est un phénomène régulier dans la sériciculture indienne pour diverses raisons. Cela peut être du à différents types de maladies, à des parasites, à des conditions météorologiques ou pour des raisons culturelles. Mais aucune perte n'est aussi sévère que celle due à la maladie de la pébrine qui entraîne la perte totale de la récolte. L'agent causal est *Nosema bombycis* Nägeli une spore à l'origine d'un protozoaire intracellulaire obligatoire. La détection et l'élimination de la pébrine sont essentielles à la durabilité de la récolte de cocons d'un point de vue industriel. Le procédé ancien de détection, le seul fiable et largement accepté pour la préparation d'œufs exempts de pébrine, comprend l'examen de broyats de papillons femelles sous microscope optique. La technique a été développée par Louis Pasteur en 1870 et est toujours utilisée pour détecter les spores de pébrine pour la production d'œufs (graines) commerciale dans tous les pays producteurs dont l'Inde. Alors que beaucoup de nouvelles techniques ont été développées pour détecter les spores de pébrine, l'examen des papillons femelles demeure toujours incontournable et populaire. Pour plusieurs raisons pratiques, les autres procédés de détection restent intéressants seulement d'un point de vue théorique pour les chercheurs. Cette revue tente de mettre en lumière toutes les techniques proposées jusqu'à aujourd'hui pour détecter les spores de pébrine par divers technologues, en montrant les mérites et les défauts de chacune et en proposant des stratégies nouvelles à entreprendre.

Mots-clés: *Nosema bombycis*, pathogénie, protéines de la paroi des spores, transmission transovarienne.



COST BENEFIT ANALYSIS OF COMMERCIAL SEED PRODUCTION OF TASAR SILKWORM (*ANTHRAEA MYLITTA* D.) IN THE CENTRAL INDIAN TRIBAL BELT

Sathyanarayana K^{1*}, Soumen Nandi² and Mohd. Shamshad Alam³

¹Central Silk Board, Ministry of Textiles, Govt. of India, BTM Layout, Bengaluru 560016, Karnataka, India.

²Basic Seed Multiplication and Training Centre, BTSSO, Central Silk Board, Ministry of Textiles, Govt. of India, B. Deoghar 814112, Jharkhand, India.

³Professional Assistance for Development Action (PRADAN), B. Deoghar 814112, Jharkhand, India.

*E- mail: skutala@yahoo.com

ABSTRACT

In India, productivity, sustainability and profitability of tasar silk industry depends mainly on timely supply of quality tasar silkworm seed (egg). While the state sericulture departments have launched ambitious plans for tasar expansion, silkworm seed remained a major constraint, though the same is partly met by the Basic Seed Multiplication and Training Centres (BSM&TC) under Central Silk Board (CSB) and Pilot Project Centres (PPC) under State. Though, in recent times, states have opened doors for the private sector for commercial seed production, the same needs to be consolidated with effective backward and forward linkages, to nurture private graineurs as micro-entrepreneurs in the tribal areas, to cater to the needs of this important seri-input. CSB with help of some reputed NGOs through various development initiatives made efforts to bring in the business sense among tribals through the concept of private graineurs. This network was enlarged on scale under the Special Swarnajayanti Gram Swarajgar Yojana (SGSY) Projects in the States of Bihar and Jharkhand being implemented by Professional Assistance for Development Action (PRADAN), an NGO. Strategies followed in selection of the entrepreneurs, profile of the private graineurs, status before the intervention, assistance for carrying out the activity, capacity building, initial hand holding, organizing suitable and manageable activity groups with backward and forward linkages for sustenance of this economic activity. under the Projects are discussed besides the future strategies for replicating this activity in other tasar producing states. Production and productivity details and scale of operation over a period of time along with detailed economic analysis including Benefit : Cost Ratio and Internal Rate of Returns are detailed.

Key words: B:C ratio, IRR, micro-entrepreneur, private graineur, silkworm seed.

INTRODUCTION

Life cycle of tropical tasar silkworm, *Antheraea mylitta* D. consists of four stages viz., egg, larva, pupa and adult, of which egg stage in the developmental perspective, is the most critical input. The brood of eggs laid by one female silkworm moth is called a laying. The number of eggs per laying varies from 150 to 250. Each laying is subjected to microscopic testing and disinfection to produce disease free laying or DFL. In tasar sericulture,

parlance DFL is also called silkworm seed. High quality tasar silkworm seed (egg), an important seri-input which plays a vital role in productivity, sustainability and profitability of sericulture industry continues to be the main constraint. The paradigm shift took place during the last two five year plan periods of Govt. of India from practicing tasar culture as subsidiary occupation to replicable micro-enterprise with interventions across the value chain. Massive expansion plans by tasar producing states viz., Jharkhand, Chhattisgarh, Orissa, Madhya

Pradesh, Uttar Pradesh, West Bengal, Bihar, Maharashtra, Telengana and Andhra Pradesh in recent times, due to its unexplored potential, demand-supply gaps, its potential to create sustainable livelihoods and eco-friendly approach besides foreign earning would peg up demand for commercial seed in the years to come.

Seed multiplication system in tasar sector: Under the present seed multiplication system in tropical tasar, while Central Tasar Research and Training Institute (CTRТИ), Ranchi is expected to supply elite seed, further multiplication to nucleus and basic seed is taken up by Basic Tasar Silkworm Seed Organization (BTSSO) and its nested units, Central Tasar Silkworm Seed Station (CTSSS) and Basic Seed Multiplication and Training Centres (BSMTCs). BTSSO has stretched out to its maximum installed capacities and further scaling up may not be possible due to infrastructure and manpower constraints. In spite of support from Catalytic Development Programme (CDP) of CSB and also state plan schemes, Pilot Production Centres (PPC) in most of the tasar producing states are yet to gear up to the rising demands, due to inherent state specific issues. Major constraints in tasar seed multiplication include, poor infrastructure, lack of skilled staff and timely supply of required inputs, non-availability of revolving capital for purchase of seed cocoons and poor levels of technology adoption at PPCs leading to lower levels of multiplication, absence of demarcated seed zones, slow implementation of private graineurs scheme under CDP and its monitoring, subsidizing the commercial seed costs hampering entrepreneurial spirit of private grainage concept *etc.*

The above constraints coupled with thought process of tasar farmers, treating tasar culture just as livelihood opportunity and not as a commercial venture, have not attracted any entrepreneurial attention in commercial production on scale. However, requirement of huge quantum of commercial seed incidental to the thrust imposed by various projects on tasar silk industry, during the last decade warranted the entry of private sector to cater to the needs as no single source can meet the requirement (Amarnath *et al.*, 2007).

Private participation in tasar silkworm seed production: Central Silk Board with the help of some reputed NGOs through various development initiatives viz., United Nations Development Programme (UNDP) assisted Project and Catalytic Development Programme (CDP) schemes of CSB since X Plan period brought in the business sense among tribals, roping in their entrepreneurial abilities by developing other required capacities to build an economically replicable business model. The concept of private graineurs has proved to be very beneficial in this sector. Augmentation of commercial seed production through private graineurs in muga and eri sector was reported by Bhat *et al.*, 2006. Though, the performance of private graineurs under supervision of CSB units was found to be satisfactory, the same needs to be replicated on a large scale to meet the requirement of tasar commercial DFLs. In this backdrop, the critical node of tasar silk value chain viz., commercial seed production was taken up with major objectives of nurturing local tribal community to take up this as a rural micro-enterprise, maintain the required quality standards, and ensure timely supply and to reduce dependency on outside agencies.

MATERIALS AND METHODS

With the positive response under United National Development Programme (UNDP) Project, where the concept of private graineurs for production of commercial seed was well received by enterprising and educated tribal youth in tasar sector, as a viable rural enterprise, under the Special Swarnajayanti Gram Swaroggar Yojana (SGSY) Projects in Bihar and Jharkhand with the assistance from the Ministry of Rural Development, Govt. of India in the States of Bihar and Jharkhand, Central Silk Board with the help of an NGO, Professional Assistance for Development Action (PRADAN) achieved remarkable success in seed augmentation in general and commercial seed in particular, which was one of the major interventions.

Selection of private graineurs and profile: Selection of the private graineurs was done by PRADAN by following Participatory Rural Appraisal (PRA) techniques. Priority was given to unemployed educated rural youth/women,

progressive seed rearers, and enterprising commercial rearers. Of the 252 private graineurs, about 78 % belonged to Scheduled tribe while 11, 4 and 7 % belonged to Scheduled caste, Other Backward Castes and General category, respectively. Majority of graineurs (52 %) have less than 8 members in the family and 15 % have a family size of more than 10 members. In terms of land holding, private graineurs comprise of almost equal percentage of marginal, small and medium farmers and a small percentage (7 %) of large farmers as well. Major source of livelihood is agriculture (83 %) and Non-Timber Forest Produce (NTFP) (10 %).

other tasar producing states, it could not sustain on its own without constant monitoring and assistance, as the entrepreneurial capabilities were lacking in them. Under these Projects, PRADAN in addition to equipping the private graineurs with technical capabilities through technical training with the help of BSMTC's scientists in the Project areas, has also trained them in other important aspects like client servicing, labour management, motivational skills to instill risk taking behaviour, maintenance of accounts to keep track of inflow and outflow of various financial transaction and understand the cost-benefits and management of working capital and

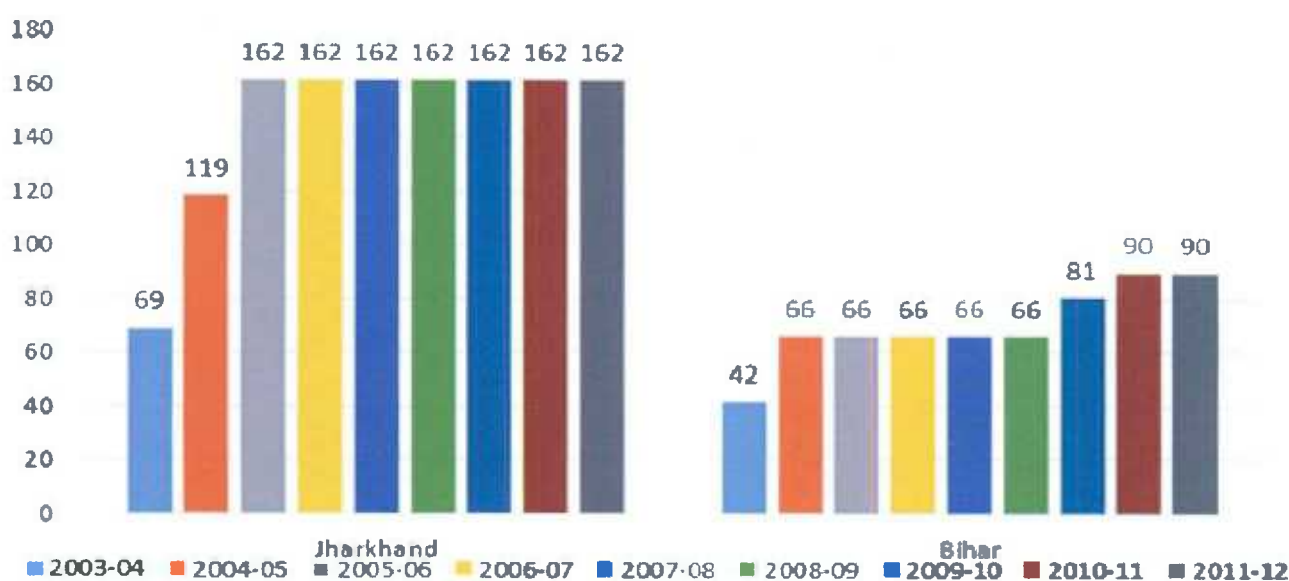


Figure 1: No. of private graineurs who operated in Bihar and Jharkhand during 2003-2012

Identified private graineurs were trained in the latest seed production technology, moth examination techniques for production and supply of quality disease free seed to commercial rearers. These grainages were equipped with all necessary equipment and provided with management inputs for successful running of the enterprise, in maintaining records *etc.* PRADAN organized these graineurs into suitable manageable groups and assisted them in establishing backward and forward linkages with the seed rearers for seed cocoon and commercial rearers, reelers and spinners for selling the end products.

Though private graineur concept was introduced in

credit recovery. This helped the private graineur to take up grainage operation as an enterprise so that it can sustain even after the completion of the projects. Further, a pool of resource persons in the villages *viz.*, microscopists, who are experts in carrying out microscopic examination in the grainages and input procurers, who procure critical inputs like basic seed *etc.*, from different places and deliver to remote villages located in forests, within the stipulated time, were nurtured to complement efforts of private graineurs.

Though each private graineur is envisaged to have capacity to process 20,000 seed cocoons and produce 5,000 DFLs per grainage operation, majority of them

ended up producing around 6000 DFLs toward the end of the project. Project assistance was extended towards grainage equipment (₹33000/-), grainage building (₹60000/-) and consumables (₹1000/-) as one time grant. The graineurs were linked with financial institutions for credit facilities for working capital requirement for purchase of seed cocoons from basic seed rearers and other consumables.

RESULTS AND DISCUSSION

Under the Special SGSY Projects in Bihar and Jharkhand, during the period starting from 2003-04, 252 private graineurs were promoted, 162 in Jharkhand and 90 in Bihar to cater to the needs of commercial seed requirements. Though 252 grainages were established, over a period of time, grainage operations conducted varied between 169 to 259. While some of the graineurs took up two grainage operations (one for bivoltine and one for trivoltine), a few of them could not carry out grainage operations due to various field problems. Even the seed cocoons processed and commercial DFLs produced varied between 22.02 to 56.76 lakhs and 3.83 to 12.03 lakhs, respectively during the period 2003-12. State-wise details of grainage operations, seed cocoons processed and commercial DFLs produced are indicated in Table 1.

operation steadily raised from around 12000 to around 27000. Similarly, against the originally envisaged 5000 DFLs production per grainage operation, it went up to 5700 with a cocoon: DFL ratio of 4.72 : 1, which is almost nearer to envisaged norms of 4 : 1, which are detailed in Table 2.

An independent study to analyse the socio-economic impact of the above intervention indicated that about 92 % graineurs earn between ₹22,000/- and ₹30,000/- as income from grainery. 4 % made loss and another 4 % earned more than ₹40,000/-. About 74 % of graineurs also participated in commercial rearing and 30 % in seed rearing, which might have resulted in an additional income of around ₹20000/-. Only 15 % graineurs did not participate in any other activity. For most of the graineurs (*i.e.*, about 67 %), the vocation has resulted into asset creation. In terms of debt, 93 % of the graineurs who were under debt before, have completely repaid their loan while 7 % have repaid partial loan. About 30 % of the graineurs had taken loan against land. All the families have freed their mortgaged land. The source of credit has changed from moneylenders to SHGs and not taking loan at all. There was, however, a decrease in percentage of graineurs taking loan from the Banks. All the graineurs were found to have stopped migrating for work. Earlier, only 33 % of the families did

Table 1: Commercial tasar seed production by private graineurs

Year	No. of grainage operations			Seed cocoons processed (Lakh nos.)			Commercial DFLs produced (Lakh DFLs)		
	Jharkhand	Bihar	Total	Jharkhand	Bihar	Total	Jharkhand	Bihar	Total
2003-04	117	97	214	14.99	11.05	26.04	3.08	3.36	6.44
2004-05	122	96	218	12.58	9.44	22.02	2.09	1.74	3.83
2005-06	166	86	252	26.53	12.71	39.24	6.14	2.9	9.04
2006-07	133	55	188	21.15	7.34	28.49	4.27	1.99	6.26
2007-08	163	91	254	33.14	17.7	50.84	7.57	4.24	11.81
2008-09	164	95	259	37.09	17.67	54.76	7.67	4.03	11.7
2009-10	90	79	169	19.79	14.65	34.44	4.6	3.72	8.32
2010-11	110	70	180	25.52	15.28	40.8	5.68	2.91	8.59
2011-12	137	73	210	37.18	19.58	56.76	7.95	4.08	12.03

While the commercial seed production by private graineurs was proved successful on scale over a period of more than a decade, even the seed cocoons processed per

not migrate. The remaining migrated for varied periods - 1-3 moths (41 %), 4-6 months (19 %) and 7-9 months (7 %). All the families are now food secure with 74 % families

reporting surplus. In the before situation, about 74 % families were food secure for up to 9 months. A considerable fraction of women (56 %) said that the workload on them has increased due to the vocation.

Economics of tasar private graineur: With the present

experience of the scale of operation and capacity utilisation, under ideal conditions, a private graineur can preserve 25000 cocoons and produce 6250 DFLs/operation. The cash outflow & inflow of the grainage operation are depicted in Table 3 with relevant assumptions.

Table 2: Comparative performance of private grainages in Bihar and Jharkhand (2003-12)

Year	Seed cocoons/ Operation			Cocoon : DFL ratio			DFL Production/ Operation		
	Jharkhand	Bihar	Average	Jharkhand	Bihar	Average	Jharkhand	Bihar	Average
2003-04	12812	11392	12168	4.86	3.28	4.04	2636	3473	3009
2004-05	10311	9833	10101	6.00	5.42	5.75	1719	1814	1757
2005-06	15982	14779	15571	4.32	4.38	4.34	3700	3374	3587
2006-07	15902	13345	15154	4.95	3.69	4.55	3213	3617	3330
2007-08	20331	19451	20016	4.37	4.17	4.30	4652	4664	4656
2008-09	22616	18600	21143	4.83	4.38	4.68	4682	4247	4517
2009-10	21989	18544	20379	4.3	3.93	4.14	5114	4719	4923
2010-11	23200	21829	22667	4.49	5.25	4.75	5167	4158	4772
2011-12	27139	26822	27029	4.67	4.79	4.72	5811	5600	5729

Table 3: Cost economics of tasar private grainage

Sl. No.	Particulars	Year-1	Year-2	Year-3	Year-4	Year-5	Year-6	Year-7
A	Cash outflow							
1	Cost of grainage building <1>	60000	0	0	0	0	0	0
2	Cost of grainage equipment <1>	33000	0	0	0	0	0	0
3	Production cost							
a.	Cost of seed cocoons <2>	31250	31250	31250	31250	31250	31250	31250
b.	Consumables	1000	1000	1000	1000	1000	1000	1000
c.	Hiring charges of microscopists <3>	1500	1500	1500	1500	1500	1500	1500
d.	Interest on working capital <4>	1013	1013	1013	1013	1013	1013	1013
	TOTAL	127763	34763	34763	34763	34763	34763	34763
B.	Cash inflow							
	Gross cash flow through sale of DFLs <5>	37500	37500	37500	37500	37500	37500	37500
	Gross cash flow through sale of pierced cocoons <6>	25000	25000	25000	25000	25000	25000	25000
		62500	62500	62500	62500	62500	62500	62500
C	Net cash flow	-65263	27737	27737	27737	27737	27737	27737

Internal Rate of Return = 30.55 %;

BCR at 15 % Discount rate = 1.15 : 1;

<1> Unit cost for Private Tasar Grainage;

<2> Seed cocoons rate: ₹1.25 per cocoon;

<3> Hiring charges of microscopists (one each for 10 days) @ ₹150/- per day;

<4> Interest @ 12 % p.a. on working capital for three months;

<5> Cocoon : DFL ratio - 4:1, Cost of commercial DFLs - ₹6/- per DFL;

<6> Cost of pierced cocoons - ₹1

Based on the above assumptions, Internal Rate of Return and Benefit : Cost ratio was worked out including the project grant following the standard statistical procedures, which works out to 30.55 % and 1.15:1 (at

mentioned below.

- Rearing of nucleus seed through adopted seed rearers in isolated patches in the tasar producing states on the

Table 4: Financial analysis of tasar private grange activity

i. Internal rate of return of private grainage

Year	Total project cost	Total returns	Net returns	D. F. 10 %	NPV at 10 % D. F.	D. F. 15 %	NPV at 15 %
1	127763	62500	-65263	0.909	-59324	0.800	-52210
2	34763	62500	27737	0.826	22911	0.640	17752
3	34763	62500	27737	0.751	20831	0.512	14202
4	34763	62500	27737	0.683	18945	0.410	11361
5	34763	62500	27737	0.621	17222	0.328	9090
6	34763	62500	27737	0.565	15658	0.262	7270
7	34763	62500	27737	0.467	12940	0.210	5817
Total	336341	437500	101163		49183		13281

IRR = 30.55 (D. F.: Degrees of freedom, NPV: Net present value)

ii. Benefit-Cost ratio (BCR) of grainage operation

Year	Total project cost	Total returns	D. F. 10 %	NPV of cost	NPV of return	D. F. 15 %	NPV of cost	NPV of return
1	127763	62500	0.909	116136	56812.5	0.870	111102	54350
2	34763	62500	0.826	28713.8	51625	0.756	26284	47256
3	34763	62500	0.751	26106.6	46937.5	0.658	22856	41094
4	34763	62500	0.683	23742.8	42687.5	0.572	19877	35738
5	34763	62500	0.621	21584	38806.3	0.497	17284	31075
6	34763	62500	0.565	19623.4	35281.3	0.432	15028	27019
7	34763	62500	0.467	16216.7	29156.3	0.376	13067	23494
Total	336341	437500		252124	301306		225499	260025

BCR = 1.20 (at 15 % DF); 1.15 (at 10 % DF)

15% Discount rate), respectively which is higher than the prevailing interest rates *i.e.*, opportunity cost. It would be very high excluding the project grant which is extended as one time assistance for this rural micro-enterprise.

Future strategies: Though remarkable achievements have been made under the Special SGSY Projects in Bihar and Jharkhand in roping private participation in tasar seed multiplication, this needs to be replicated in other tasar producing states for empowerment of tribals involved in this culture. Some of the strategies suggested are

lines of special SGSY project in Jharkhand and Bihar as a step towards privatization and increasing beneficiary participation.

- The Self Help Groups (SHG) attached to block plantations may be encouraged to take up production of commercial seed, making the activity as more remunerative and economically viable.
- Identification and demarcation of seed zones for production and procurement of seed cocoons.

- Development of a policy on pricing of seed cocoons and insurance for seed rearers to take up seed rearing during adverse seasons and to grainers to take care of risk in case of disease incidence.
- Periodic monitoring of seed multiplication related activities including disease assessment and certification of seed produced at various levels.
- Strengthening of PPCs to take care of basic seed requirement with required linkages between private grainers, seed rearers, commercial rearers and spinners.
- Systematic exposure of bankers at different levels and policy advocacy are required so that private grainers do not face difficulty in accessing credit from banks to meet their working capital requirements.
- Enactment of necessary legislation to maintain quality and disease freeness of the stock and also not to offer subsidy on commercial seed to encourage entrepreneurial opportunities in commercial seed production.

The success of the present initiative on scale and over a period of more than a decade in spite of several challenges, has proved private grainage activity a profitable venture beyond doubt, which need to be upscaled in tropical tasar seed multiplication which can sustain on its own with involvement of professional NGOs like PRADAN to provide opportunities to tribal entrepreneurs, locally.

REFERENCES

- Amarnath S., Sathyanarayana K. and Khanna R. P. (2007) *Tasar Silkworm Seed Production in Private Sector under Special SGSY Projects in Bihar and Jharkhand - A Breakthrough. Abstracts of International Conference "Sericulture Challenges in the 21st Century" (Serichal 2007) & the 3rd BACSA meeting, 18 -21 September 2007, Vratza, Bulgaria, pp. 23-24.*
- Bhat M. M., Das P. K., Choudury B. and Singh B. K. (2006) *Augmentation of Muga and Eri commercial seed by involving private grainers. Proceedings of the Regional Seminar on "Prospects & Problems of sericulture as an economic Enterprise in North-West India, Dehradun, India, 11 & 12th November 2006, pp. 345-348.*

Article scientifique

ANALYSE COUT/BENEFICE DE LA PRODUCTION COMMERCIALE D'OEUFS DE VER A SOIE TASAR (*ANTHRAEA MYLITTA* D.) DANS LA CEINTURE TRIBALE INDIENNE CENTRALE

Sathyanarayana K^{1*}, Soumen Nandi² and Mohd. Shamshad Alam³

¹Central Silk Board, Ministry of Textiles, Govt. of India, BTM Layout, Bengaluru 560016, Karnataka, India.

²Basic Seed Multiplication and Training Centre, BTSSO, Central Silk Board. Ministry of Textiles, Govt. of India, B. Deoghar 814112, Jharkhand, India.

³Professional Assistance for Development Action (PRADAN), B. Deoghar 814112, Jharkhand, India.

*E- mail: skutala@yahoo.com

R É S U M É

En Inde, la productivité, la durabilité et les profits de l'industrie de la soie tasar dépendent principalement de la fourniture en temps et en heure de graines (oeufs) de ver à soie tasar de qualité. Alors que les départements de

sériciculture des états ont lancé des plans ambitieux pour l'expansion du tasar, les graines de ver à soie restent la contrainte principale, bien que ceci soit partiellement traité par les Centres de formation et de multiplication des graines (BSM&TC) sous l'égide du Bureau central de la soie (CSB) et les Centres de projet pilote (PPC) de l'Etat. Récemment, les états ont ouvert la porte au secteur privé pour la production de graine commerciale, mais ceci doit être consolidé par des liens effectifs en amont et en aval, pour nourrir les graineurs privés comme micro-entrepreneurs dans les zones tribales, pour satisfaire les besoins de cet important apport séricicole. Le CSB avec l'aide de quelques ONGs réputées à travers diverses initiatives a fait des efforts pour porter du sens dans ce projet auprès des tribus à travers le concept de graineurs privés. Ce réseau a été élargi grâce aux Projets spéciaux Swarnajayanti Gram Swarojgar Yojana SGSY) dans les Etats du Bihar et de Jharkhand exécutés par l'Assistance professionnelle pour l'action de développement (PRADAN) et des ONG. Les stratégies suivies pour la sélection des entrepreneurs, le profil des graineurs privés, les statuts avant l'intervention, l'assistance pour engager l'activité, la capacité à construire, la prise en charge initiale, l'organisation de groupes d'activité adéquats et gérables avec des liens en amont et en aval pour la durabilité de cette activité économique, tout cela est discuté ainsi que les stratégies futures pour transposer l'activité dans d'autres états producteurs de tasar. La production et la productivité ainsi que l'étendue de l'opération sur une période de temps et l'analyse économique comprenant le rapport coût : bénéfice et le taux interne de retours sont détaillées.

Mots-clés: Taux bénéfice : coût, IRR, micro-entrepreneur, graineur privé, graine de ver à soie.



EVALUATION OF HOST PLANT SUITABILITY TO INTRODUCE COMMERCIAL REARING OF TROPICAL TASAR SILKWORM, *ANTHRAEA MYLITTA* IN UTTARAKHAND

Narendra Kumar Bhatia¹* and Mohammad Yousuf²

¹Regional Sericultural Research Station, Central Silk Board, Ministry of Textiles, Govt. of India,
Sahaspur 248197, Dehra Dun (Uttarakhand), India.

²Forest Entomology Division, Forest Research Institute, New Forest 248006, Dehra Dun (Uttarakhand), India.

*E-mail: nkb1123@rediffmail.com

ABSTRACT

Antheraea mylitta Drury is a commercially exploited silk producing forest insect thriving in nine tasar producing states of India, but remains still untapped of its potential in Uttarakhand. Experimental rearing of Daba ecorace (BV) of *A. mylitta* was conducted on seven forest tree species at Forest Research Institute, Dehra Dun (Uttarakhand) during 2012 to 2014 and data were collected on seven variables to assess the host plant suitability in view of introduction of forest based commercial rearing of *A. mylitta* in Uttarakhand. *Terminalia alata*, *T. tomentosa*, *Lagerstroemia speciosa* and *T. arjuna* were found the best suitable forestry host plants in their order of merit to have better growth and development of *A. mylitta*. Further, SRTM (Shuttle Radar Topography Mission) mapping of Uttarakhand indicated that 3217.9 km² of forest area distributed up to an altitudinal range of <610 meter in Dehra Dun, Haridwar, Nainital and U.S. Nagar districts of Uttarakhand can be explored for forest based rearing of *A. mylitta*. Accordingly, State Forest Department in association with Directorate of Sericulture, Uttarakhand may initiate systematic plantation of these forestry host plants through their various afforestation and plantation schemes to create a new forest insect industry in the state to improve livelihood delivery of the tropical forests.

Key words: Forest conservation, forest silkworm, livelihood improvement, poverty alleviation, vanya silk.

INTRODUCTION

Normally, any research activity in forestry sector carries two objectives: first, to put the generated knowledge on public domain; and second to use the research findings in improving the livelihood earnings of the forest dependent people. This study is aimed at introducing forest based rearing of tropical tasar silkworm, *Antheraea mylitta* (Lepidoptera: Saturniidae) as a new forest insect industry in tropical forest areas of Uttarakhand to improve livelihood earning of forest dependent people. Studies have indicated that such income generating activities of means of livelihood naturally link with forest conservation measures and so evolves a workable model of Collaborative Forest Management (Bhatia and Yousuf, 2013 a & b).

In this study, evaluation of *A. mylitta* on different forest tree species in respect of the traits that contribute to larval growth, development and cocoon yield potential was considered as primary research requisite to explore the feasibility of commercial rearing of *A. mylitta* in tropical forest areas of Uttarakhand, because studies on the effects of host plants on the biology of insects is important in understanding the host suitability under different environmental condition (Xue *et al.*, 2009) and secondly, cocoon crops of *A. mylitta* are known to be influenced by the season, host plants and their interactions (Venugopal and Krishnaswami, 1987).

There have been a number of studies on the biological parameters of different economic insects on different host

plants under different environmental conditions (Etman and Hooper, 1979; Bae *et al.*, 1997; Guan and Chen, 1999; Ahmad *et al.*, 2007), but *A. mylitta* has never been experimented in Uttarakhand (Bhatia and Yousuf, 2013 c). This study is the follow up of the recommendations of two national workshops viz., “Vanya silk culture and forestry” and “Potential, and strategies for sustainable development of Vanya silk in the Himalayan state” that were held in Dehra Dun during 2003 & 2004, specifically advising that *A. mylitta* should be tested on different forestry host plants in tropical forest areas of Uttarakhand.

MATERIALS AND METHODS

Lagerstroemia speciosa, *L. tomentosa*, *Terminalia alata*, *T. arjuna*, *T. bellirica*, *T. chebula* and *T. tomentosa* that are widely distributed in tropical forest areas of Uttarakhand (Thangavelu, 2004) were selected as the experimental host plants. The bivoltine (BV) Dabecorace of *A. mylitta* endowed with high exploitation potential and better ecological adaptability formed the insect of choice for the study. Effect of seven forest tree

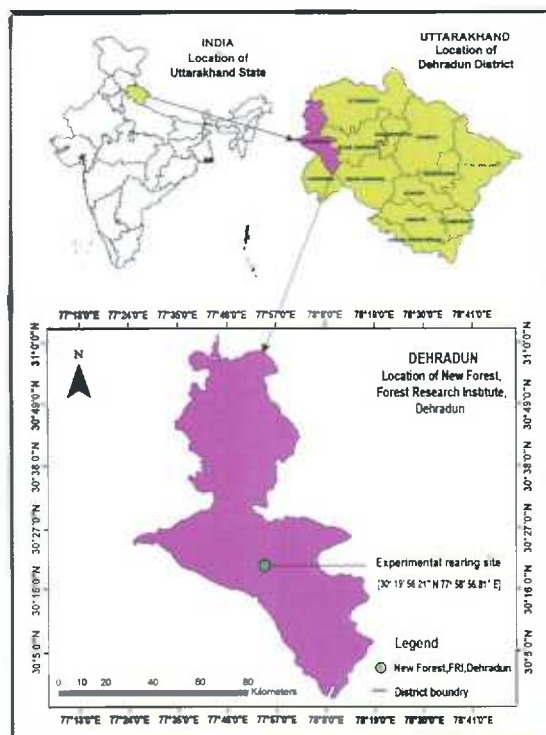


Figure 1: Map of the study area

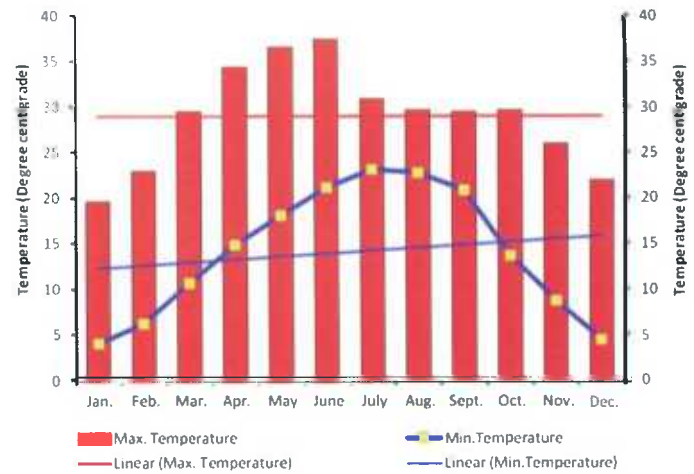


Figure 2: Maximum and minimum temperature (°C) at the study site (Year: 2012-14)

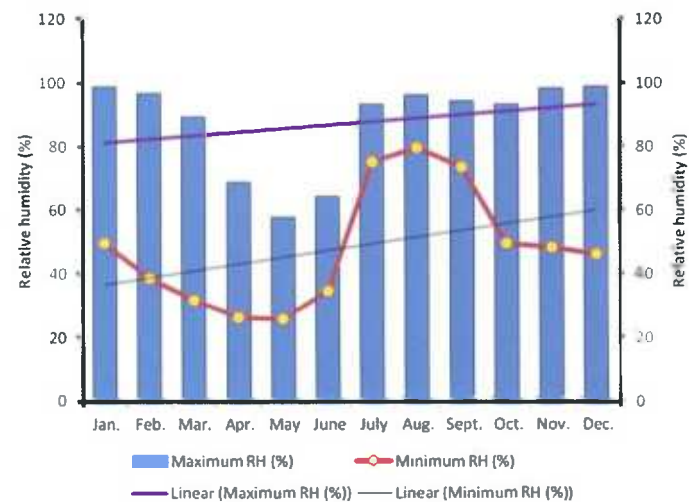


Figure 3: Maximum and minimum relative humidity (%) at the study site (Year: 2012-14)

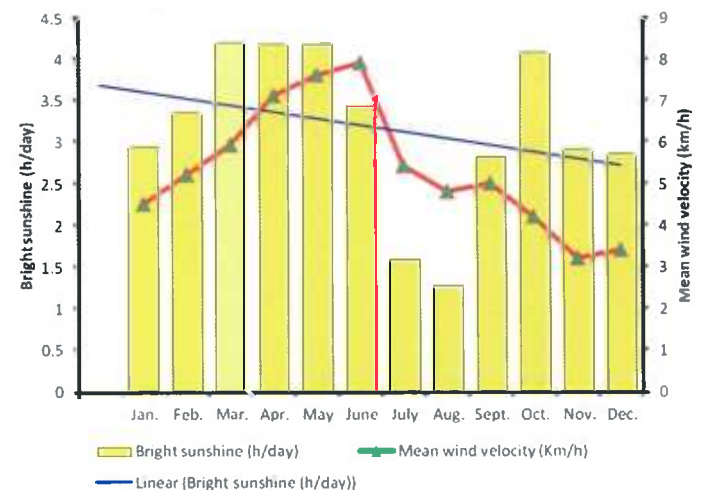


Figure 4: Bright Sunshine (h/day) & mean wind velocity (km/h) at the study site (Year: 2012-14)

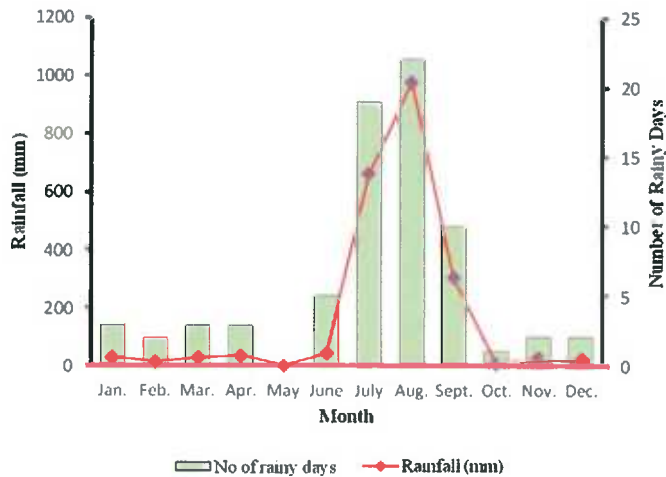


Figure 5: Rainfall and number of rainy days at study site (Year: 2012-14)

species, rearing seasons and their interactions was investigated on various economic traits of *A. mylitta* from 2012 to 2014 at New Forest, Forest Research Institute, Dehra Dun Uttarakhand (Figure 1). The climate of New forest, Dehra Dun, is moderate due to its location at the foothills of the Himalayas (WMD, 2011). Data on weather variables of the study period are presented in Figures 2 to 5.

Four outdoor experimental rearing of *A. mylitta* were conducted as per the improved rearing technology of Mathur *et al.* (1998) during July-August and September-November of 2012 and 2013. Similarly, four grainage operations were conducted on generated seed cocoons of all the replicates during Sept-Oct of 2012 & 2013 and June-July of 2013 & 2014 as per the improved grainage technology of Narain *et al.* (2001).

Variables measured

Effect of different forestry host plants, seasons of rearing and their interactions on following variables of *A. mylitta* were measured:

Mean weight (A) of the larvae

Mean weight of larva was calculated as the average of final weight (W_f) of larva before moult and the initial weight (W_i) of larva at the beginning of the instars. It was

calculated instar wise by the following formula as advocated by Gordon (1999):

$$\text{Mean weight of the larva (A)} = \frac{W_f + W_i}{2}$$

Larval weight gain (W_g)

Weight gain (W_g) by the larva was calculated by subtracting the value of initial larval weight (W_i) from the final larval weight (W_f) (Waldbauer, 1968).

Duration of the feeding (T)

Duration of larval feeding of each instar in every replicate of all the treatments was recorded in hours, and later, it was transformed into days, as it was required to compute the Relative Growth Rate (RGR) of the *A. mylitta* larvae reared on different host plant species.

Relative growth rate (RGR)

Relative Growth Rate (RGR) of larval biomass in different instars as well as for the total larval period was calculated by gravimetric methods of Waldbauer (1968) on fresh weight basis. RGR is similar to a compound-interest rate that, if sustained during the time interval T , would cause W_i to become W_f . RGR of *A. mylitta* larvae reared on different host plants of forestry importance was calculated by the following formula:

$$\text{Relative growth rate of larva (RGR)} = \frac{\text{Weight gain (} W_g \text{)}}{\text{Duration of feeding (T)}} \times \text{Mean weight of larva (A)}$$

Larval period

This variable is measured as the time taken from hatching until the commencement of spinning. The mean larval duration was recorded in days for each replicate from the time of brushing until the time of approximately 50 per cent of larvae of a particular replicate begin to spin.

Cocoon yield

After completion of the silkworm rearing, number of the

cocoons harvested from each replicate of 300 worms of every treatment was recorded.

Effective rate of rearing (ERR)

Effective rate of rearing indicates the efficacy of the forestry host plants and larvae of *A. mylitta* to produce maximum number of cocoons and this is represented in percentage. The variable was calculated using the following formula (Reddy *et al.*, 2010):

$$\text{Effective rate of rearing (\%)} = \frac{\text{Number of cocoons harvested}}{\text{Number of worms brushed}} \times 100$$

Statistical methods

The seven tested forest tree species were taken as treatments, each with six replications. Five samples were drawn from each replication to measure most of the variables, except the cocoon yield and ERR that was measured replication wise. Normality of data was checked prior to statistical analysis. Percentage data were transformed to their respective arcsine values as per the methods of Fisher and Yates (1963). Descriptive statistics was worked out using Microsoft Excel. Data of first and second rearing and grainage seasons were pooled and treatment wise descriptive statistics were calculated for all the variables and mean tables were prepared. Two-way completely randomized block factorial design was used to test the significance of difference in the means of variables. The factorial ANOVA package of advanced statistical software, STATISTICA 10 was adopted for the analysis. Rearing season, host plant and their interactions were treated as the main (fixed) effects and all the variables of the study served as dependent variables for block effect. The level of significance was fixed at $P=0.05$. Post HOC test was carried out by using Tukey's HSD test to compare the homogeneous pairs of means.

Evaluation Index (EI)

Evaluation Index (E.I.) shows an aggregate unit in respect of assessed variables of *A. mylitta* reared on a particular host plant in different rearing seasons, which

was calculated as per the procedures outlined by Mano *et al.* (1993, 1998).

$$\text{Evaluation index (E.I.)} = \frac{(A - B)}{C} \times 10 + 50$$

Where, A = Mean of a variable on a particular treatment; B = Overall mean of that variable on all the treatments; C = Overall standard deviation of that variable on all the treatments; 10 = Standard unit and 50 = Fixed value

SRTM mapping of Uttarakhand

An altitudinal map of Uttarakhand (Figure 11) was developed at Forest Informatics Division, FRI, Dehra Dun by using Shuttle Radar Topography Mission (SRTM) mapping technology. Digital elevation SRTM data developed by NASA were downloaded, and an equal area was extracted by using Arc GIS software to develop scaled map.

RESULTS AND DISCUSSION

Results on analysis of variance presented in Table 2 reveals that null hypothesis (H_0) for no difference on effect of rearing seasons, host plants and their interactions on all the five measured variables was rejected by ANOVA ($P>0.05$), except to the effect of interaction between rearing season and host plants on cocoon yield (number) / 300 larvae (DF=6, MS=115, $F=0.99$, $P<0.05$) and effective rate of rearing (DF=6, MS=8.3, $F=1.85$, $P<0.05$), which did not differ significantly between each other. The role of host plant is an important factor in regulating insect population (Umbarihowar and Hastings, 2002), because variation in host plant quality affects the life history parameters of herbivores (Awmack and Leather, 2002). It has been found that most of the economic traits of silkworms are quantitative in nature and seasonal variables like temperature, relative humidity, rainfall, and photoperiod influence them greatly (Legay, 1958; Yokoyama, 1963).

Table 1: Effect of rearing seasons, host plants and their interaction on various economic traits of *A. mylitta*

Variable	Rearing season	<i>T. alata</i>	<i>T. tomentosa</i>	<i>T. arjuna</i>	<i>L. speciosa</i>	<i>T. bellirica</i>	<i>L. tomentosa</i>	<i>T. chebula</i>
Mean weight (g) of larvae (1 st to 5 th instar)	I	17.555 ^a (±0.137)	16.251 ^m (±0.144)	15.113 ⁱ (±0.117)	14.544 ⁱ (±0.121)	11.904 ^a (±0.101)	8.813 ^b (±0.076)	10.210 ⁱ (±0.078)
	II	20.108 ^p (±0.119)	19.279 ^q (±0.142)	17.286 ^o (±0.125)	16.613 ⁿ (±0.127)	15.101 ⁱ (±0.136)	11.695 ⁱ (±0.136)	12.576 ^b (±0.131)
	III	18.831 ^q (±0.148)	17.765 ⁱ (±0.172)	16.199 ^q (±0.131)	15.578 ^q (±0.129)	13.502 ⁱ (±0.169)	10.254 ^a (±0.153)	11.393 ^b (±0.132)
Relative growth rate (RGR) of larvae (1 st to 5 th instar)	I	0.076 ⁱ (±0.000)	0.073 ^q (±0.000)	0.077 ^r (±0.000)	0.068 ^q (±0.000)	0.055 ^a (±0.000)	0.040 ⁱ (±0.000)	0.043 ^b (±0.000)
	II	0.058 ^a (±0.000)	0.053 ^m (±0.000)	0.054 ^{mm} (±0.000)	0.050 ⁱ (±0.000)	0.040 ⁱ (±0.000)	0.031 ^b (±0.000)	0.035 ⁱ (±0.000)
	III	0.067 ^a (±0.001)	0.063 ^q (±0.001)	0.066 ⁱ (±0.001)	0.059 ^q (±0.001)	0.047 ⁱ (±0.001)	0.035 ⁱ (±0.000)	0.039 ^b (±0.000)
Larval period (1 st to 5 th instar)	I	34.45 ^b (±0.10)	35.38 ⁱ (±0.12)	34.05 ^b (±0.12)	37.79 ⁱ (±0.10)	47.79 ^m (±0.18)	62.12 ^o (±0.23)	57.51 ^o (±0.19)
	II	42.42 ^b (±0.13)	45.63 ⁱ (±0.17)	44.83 ⁱ (±0.18)	48.31 ^m (±0.15)	62.13 ^o (±0.30)	75.24 ^q (±0.23)	68.77 ^q (±0.27)
	III	38.44 ^a (±0.37)	40.51 ⁱ (±0.48)	39.44 ^b (±0.51)	43.05 ^d (±0.49)	54.96 ⁱ (±0.68)	68.68 ^q (±0.62)	63.14 ⁱ (±0.54)
Cocoon yield (number) / 300 larvae	I	171.58 ^o (±4.06)	156.75 ^q (±4.65)	148.17 ^{mm} (±3.78)	133.17 ^{kl} (±3.46)	112.96 ⁱ (±3.09)	57.69 ^b (±1.19)	83.54 ⁱ (±2.04)
	II	156.64 ^q (±3.45)	141.03 ^{lm} (±2.81)	131.81 ^{kl} (±3.40)	125.77 ^{jk} (±3.49)	98.02 ⁱ (±2.90)	39.92 ^a (±1.04)	61.52 ^b (±1.58)
	III	164.11 ⁱ (±3.04)	148.89 ⁱ (±3.12)	139.99 ^e (±3.02)	129.47 ^d (±2.53)	105.49 ^c (±2.59)	48.81 ^b (±2.01)	72.53 ^b (±2.62)
Effective rate of rearing (ERR %)	I	49.15 ^p (±0.79)	46.30 ^{op} (±0.89)	44.65 ^{mm} (±0.72)	41.77 ^{lm} (±0.67)	37.83 ^b (±0.61)	26.00 ^b (±0.29)	31.83 ⁱ (±0.43)
	II	46.27 ^{op} (±0.66)	43.28 ^{mm} (±0.54)	41.51 ^{lm} (±0.66)	40.54 ^{kl} (±0.63)	34.84 ⁱ (±0.59)	21.38 ^a (±0.29)	26.91 ^b (±0.37)
	III	47.71 ⁱ (±0.58)	44.79 ^a (±0.60)	43.08 ^a (±0.58)	41.05 ⁱ (±0.49)	36.34 ^a (±0.52)	23.69 ^b (±0.52)	29.37 ⁱ (±0.58)

- Values represent combined mean ± standard error of thirty observations of five samples each from six replications for the year 2012 and 2013.
- Significant difference within every variable are indicated by different superscript; whereas same superscript within every variable represents homogeneous groups (Tukey's HSD test at alpha = 0.05).

I - I crop (Combined for 2012 & 2013); II - II crop (Combined for 2012 & 2013); III - Combined for I & II crop 2012 & 2013.

Mean weight of the larvae

There was significant effect of forestry host plants on larval weight that differed significantly ($P < 0.05$) among all the treatments. Mean weight of *A. mylitta* larvae fed on *T. arjuna* (15.11 g) and *L. speciosa* (14.54 g) in the first rearing season and on *T. bellirica* (15.10 g) in the second rearing season did not differ significantly and formed a homogeneous group. In the second rearing season, *T. alata* fed larvae attained the highest larval weight (20.11 g) followed by *T. tomentosa* (19.28 g) and *T. arjuna* (17.29 g) fed larvae that differed significantly with one another. However, the lowest larval weight was shown by *L. tomentosa* (11.70 g) with *T. bellirica* (11.90 g) registering at par with that.

Growth is defined as the change in size of individual organism or part of it, and development is change in form. Both phenomena are complex, their variables are interrelated (Gordon, 1999). Results of Tukey's HSD test

presented in Table 3 indicate that there was an increase of 19.35 % in overall mean larval weight of *A. mylitta* from first (13.48 g) to second rearing season (16.09 g). The warm (26.25-29.25 °C) and wet (84.58-88.78 % RH) weather conditions of first rearing season and comparatively cool (17.28-26.15 °C temperature) and low humid weather conditions (73.05-83.75% RH) of second rearing season might have been the decisive factors for the observed difference. The efficiency of an insect in relation to its food plants in different seasons depends on the efficiency of conversion of ingested food into body biomass (Bhattacharya and Pant, 1976). There are reports that forestry host plants reach to their maximum nutritional potentiality in second rearing season (Sinha and Jolly, 1971). Ponnuvel *et al.* (1996) have also reported that biochemical constituents of oak (*Quercus serrata*) leaves vary with season.

Results indicated that *T. alata* provided the best nourishment to *A. mylitta* that influenced better larval

Table 2: Analysis of variance for effects of rearing seasons, host plants and their interactions on growth and development of *A. mylitta* in Uttarakhand

Trait	Factor	DF	SS	MS	F value	P -value
Mean weight (g) of larvae (1 st to 5 th instar)	Rearing seasons	1	1430.4	1430.4	1589.3	0.000
	Host plants	6	7386.8	1231.1	1367.9	0.000
	Rearing season* host plant	6	34.2	5.7	6.3	0.000
	Error	826	743.4	0.9		
	Total	839	9594.8			
Relative growth rate (RGR) of larvae (1 st to 5 th instar)	Rearing seasons	1	0.0514	0.0514	14148.655	0.000
	Host plants	6	0.1240	0.0207	5690.957	0.000
	Rearing season* host plant	6	0.0055	0.0009	252.290	0.000
	Error	826	0.0030	0.0000		
	Total	839	0.1839			
Larval period (1 st to 5 th instar)	Rearing seasons	1	26233.24	26233.24	12582.43	0.000
	Host plants	6	111529.93	18588.32	8915.65	0.000
	Rearing season* host plant	6	765.67	127.61	61.21	0.000
	Error	826	1722.14	2.08		
	Total	839	140250.97			
Cocoon yield (number) / 300 larvae	Rearing seasons	1	10214	10214	88.24	0.000
	Host plants	6	256016	42669	368.63	0.000
	Rearing season* host plant	6	689	115	0.99	0.433*
	Error	154	17826	116		
	Total	167	284744			
Effective rate of rearing (ERR %)	Rearing seasons	1	454.0	454.0	101.74	0.000
	Host plants	6	10984.1	1830.7	410.21	0.000
	Rearing season* host plant	6	49.6	8.3	1.85	0.093*
	Error	154	687.3	4.5		
	Total	167	12175.0			

growth in both the rearing seasons, followed by *T. tomentosa*, *T. arjuna* and *L. speciosa*. In a comparative biochemical study of *T. tomentosa* and *T. alata*, Sinha *et al.* (2007) found that non-hairy leaves of *T. alata* are better than hairy leaves of *T. tomentosa*. The moisture level, total carbohydrate, protein, amino acid and mineral contents were also found higher in non-hairy leaves than the hairy leaves; however, it was *vice versa* in the case of total fibre content.

Results have shown that under the prevailing climatic conditions of New Forest, Dehra Dun, *A. mylitta* larvae grow well on *L. speciosa* (Figure 6), which is considered as a secondary host plant. Here, the performance of *A. mylitta* is almost equally well as that of the larvae fed on *T. arjuna* (Table 1). Our results are supported by the findings of Kumar *et al.* (2009), who also reported higher larval weight of fifth instar larvae of *A. mylitta* on *L. speciosa* (31.18 g) than on *T. arjuna* (29.76 g) during first crop rearing season in Ranchi, Jharkhand; whereas, in second

Table 3: Tukey's HSD test for effect of rearing seasons on various economic traits of *A. mylitta*

Parameter	Homogenous groups statistical descriptions	Rearing season	Mean*
Mean weight of larvae (g) (1 st to 5 th instar)	$\alpha = 0.05000$; Error MS = 0.90001, df = 826.00	I	13.484
		II	16.094
Relative growth rate (RGR) of larvae (first to fifth instar)	$\alpha = 0.05000$; Error MS = 0.00000, df = 826.00	II	0.046
		I	0.061
Larval period (first to fifth instar) (Days)	$\alpha = 0.05000$; Error MS = 2.0849, df = 826.00	I	44.160
		II	55.330
Cocoon yield (number) / 300 larvae	$\alpha = 0.05000$; Error MS = 115.75, df = 154.00	II	107.81
		I	123.41
Effective rate of rearing (ERR %)	$\alpha = 0.05000$; Error MS = 4.4628, df = 154.00	II	36.360
		I	39.650

* Combined mean of the variable on all the forestry host plants during first and second rearing season.

Table 4: Evaluation Index (E. I.) for variables of *A. mylitta*, reared on different forestry host plants in Uttarakhand

Dependent variable	Rearing season	<i>T. alata</i>	<i>T. tomentosa</i>	<i>T. arjuna</i>	<i>L. speciosa</i>	<i>T. bellirica</i>	<i>L. tomentosa</i>	<i>T. chebula</i>
Mean weight (g) of larvae (1 st to 5 th instar)	I	63.01	58.84	55.21	53.39	44.95	35.07	39.53
	II	62.89	60.22	53.83	51.67	46.81	35.88	38.71
	III	61.95	58.8	54.17	52.33	46.2	36.59	39.96
Relative growth rate (RGR) of larvae (1 st to 5 th instar)	I	59.42	57.75	60.22	54.15	45.55	35.35	37.55
	II	62.26	57.36	58.46	54.06	43.66	35.56	38.66
	III	58.94	56.37	58.06	53.47	45.63	37.65	39.88
Larval period (1 st to 5 th instar) (Days)	I	41.14	41.99	40.77	44.19	53.31	66.4	62.19
	II	39.53	42.13	41.49	44.31	55.51	66.14	60.9
	III	41.25	42.86	42.03	44.82	54.03	64.65	60.36
Cocoon yield (number) / 300 larvae	I	62.06	58.35	56.2	52.44	47.38	33.55	40.02
	II	61.8	58.03	55.8	54.34	47.63	33.59	38.81
	III	61.75	58.06	55.9	53.36	47.55	33.82	39.57
Effective rate of rearing (ERR %)	I	62.06	58.35	56.2	52.44	47.38	33.55	40.02
	II	61.8	58.03	55.8	54.34	47.63	33.59	38.81
	III	61.75	58.06	55.9	53.36	47.55	33.82	39.57

I - I crop (combined for 2012 & 2013); II - II crop (combined for 2012 & 2013); III - Combined for I & II crop 2012 & 2013.

crop rearing, *T. arjuna* performed better than *L. speciosa*. Less larval weight of *A. mylitta* on *L. tomentosa* and *T. chebula* might have been attributed to low availability of nutrients and higher contents of feeding deterrents; as the low biomass accumulation due to decreased food assimilation has been reported on less preferred host

plants by insects (Shiva Kumar, 1995; Shiva Kumar *et al.*, 1997).

In respect of larval weight, a higher value of E.I. is desirable. Evaluation index provided in Table 4 reveals that four host plants viz., *T. alata* (61.95), *T. tomentosa*

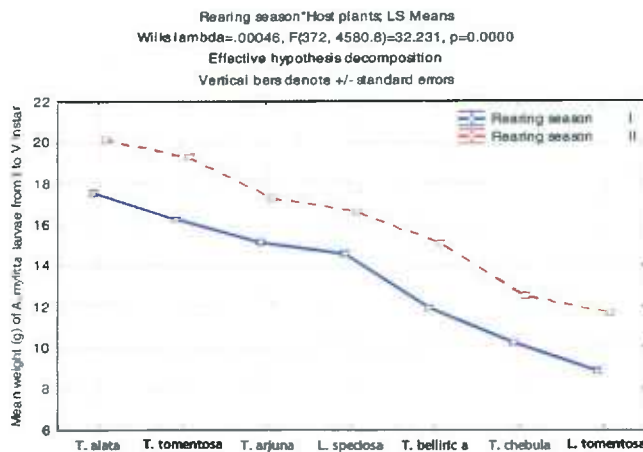


Figure 6: Effect of rearing seasons and host plants on mean weight (g) of *A. mylitta* larvae fed on different host plants

(58.80), *T. arjuna* (54.17) and *L. speciosa* (52.33) scored E.I. of more than 50 and were found superior in their order of merit. However, E.I. of *T. bellirica* (46.20), *T. chebula* (39.95) and *L. tomentosa* (36.59) fell below the benchmark E.I. value of 50 and therefore, were found inferior for supporting better larval weight of *A. mylitta*.

Relative growth rate (RGR)

Relative growth rate (RGR) refers to increase in body substance per unit of time with regard to the rate at which digested matter is available to the insect during experimental period (Bhattacharya and Pant, 1976). Results indicated significantly higher RGR in the first rearing season as compared to second one (Figure 7) and Tukey's HSD test (Table 3) evidenced it. Since larvae of first rearing season were exposed to higher temperature and relative humidity regime, their rate of feeding and corresponding physiological rate remained high that resulted into higher relative growth rate. Ojha *et al.* (2000) had also recorded similar trend of RGR of *A. mylitta*.

RGR of *A. mylitta* differed significantly on all the seven treatments. An interesting difference was found in RGR of *A. mylitta* larvae fed on different food plants (Table 1). *T. arjuna* provided the fastest growth rate during first rearing season (0.077); whereas, in second

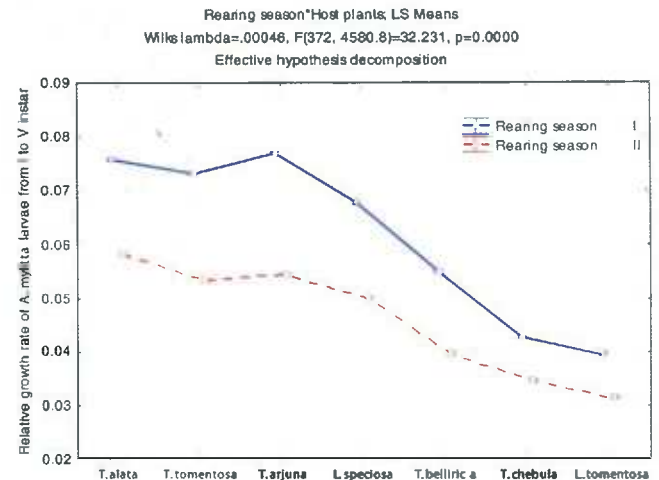


Figure 7: Effect of rearing seasons and host plants on Relative Growth Rate of *A. mylitta* larvae from first to fifth instar

rearing season, *T. alata* provided the best RGR (0.058). In both the rearing seasons, *L. tomentosa* fed larvae showed the lowest RGR followed by *T. chebula* and *T. bellirica*. *A. mylitta* larvae show differential preferences in selection of food plants, but selected plants may or may not support their growth due to nutritive deficiency or presence of toxic substances (Nayak *et al.*, 2000; Hansda *et al.*, 2008; Ojha *et al.*, 2009). Further, forestry host plants differ greatly in relation to their nutrient profile (Kohli *et al.*, 1969; Sinha and Jolly, 1971; Agarwal *et al.*, 1980; Sinha and Chaudhury, 1992; Puri, 1994) that has direct influence on the health, growth, and survival of *A. mylitta* (Sinha *et al.*, 1986). Better, the quality of leaves, greater are the possibilities for obtaining good cocoon crops. Higher RGR of *A. mylitta* on *T. alata* and *T. arjuna* leaves might have been attributed to the adapted feeding behaviour to maximize its feeding rate at which the nutrients are absorbed from the gut that eventually led to higher RGR.

For the first time, *L. tomentosa* was experimented as a host plant for *A. mylitta* and it was found that *A. mylitta* completed its life cycle on it, but the performance being the lowest among all host plants for all the studied parameters (Figure 7). Similarly, Zhu *et al.* (2005) found that though *Spodoptera litura* larvae did not prefer feeding on banana leaves and had lower relative growth rate, it was the extremely higher rate of efficiency of conversion of

digested food that led to completion of its life cycle. Similarly, *A. mylitta* did not prefer much to feed on *L. tomentosa* but completed its life cycle, which might be attributed to a correspondingly higher rate of efficiency of conversion of digested food.

Overall, E.I. of *T. alata* was found the highest (58.94) among all the treatments in respect of RGR, followed by *T. arjuna* (58.06) and *T. tomentosa* (56.37) as shown in Table 4. Slansky and Scriber (1985) outlined that in most of the cases, the combination of a certain growth rate and the attainment of a particular larval weight has a significant impact on biological success of an insect, because, RGR below the ideal value may reduce fitness due an extended larval period that increases vulnerability to predators and parasitoids (Price *et al.*, 1980).

Relative Growth Rate (RGR) of *A. mylitta* is a cumulative function of the influence of ambient temperature, relative humidity and the water and nutritional contents of the forestry host plants. Temperature influences everything that an insect does (Clarke, 2003), humidity affects embryonic development (Tamiru *et al.*, 2012), and rainfall affects both. In the first rearing season, temperature remains on the higher side leading to higher RGR, shorter larval period and lower larval weight gain; because, developmental time of an insect is inversely proportional to temperature (Tamiru *et al.*, 2012). It was reported by Singh *et al.* (2009) that with increase in temperature, larval growth and development is accelerated resulting into reduction in larval duration, cocoons of lower weight and quality. However, in second rearing season, RGR was found a bit lower due to reduced temperature that resulted in increased larval weight gain owing to extended feeding period that might be a cumulative function of improved feeding behaviour on account of better climatic conditions and improved nutritional efficiency of the forestry host plants, as reported by Sinha and Jolly (1971). Secondly, efficiency of conversion of ingested food into larval body mass might have been on the higher side during second rearing season that might contribute to higher larval weight gain.

Larval period

In silkworm, shorter larval period is a desirable character as it minimizes risk of losses due to parasitoids, predators, and diseases. A good diet always supports larval development in shorter periods (Bhattacharya and Pant, 1976). Table 3 indicates that overall mean larval duration of *A. mylitta* differed significantly between first (44.16 days) and second rearing seasons (55.33 days). Literature suggests that temperature followed by humidity has a direct effect on growth and physiological activity of insect. Therefore, higher temperature regime during first rearing season accelerated the larval growth and incidentally shortened the larval duration of *A. mylitta*.

T. arjuna and *T. alata* fed larvae registered shortest larval duration in first (34.05 days) and second rearing seasons (42.42 days), respectively; whereas, *L. tomentosa* fed larvae recorded the highest larval duration in both the rearing seasons, followed by *T. chebula* and *T. bellirica* (Figure 8). A variation to the extent of 30.24 days was observed in larval duration of *A. mylitta* larvae reared on different forestry host plants in Dehra Dun, Uttarakhand. Such variation in larval duration might have occurred due to comparatively lower nutrient level in the leaves of *L. tomentosa*, *T. chebula*, and *T. bellirica*, because, poor nutritional quality of host plants adversely affect the larval growth and development of herbivorous insects and hence

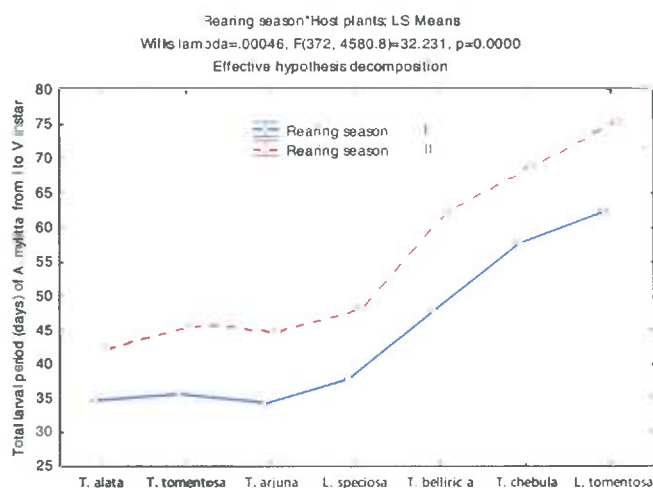


Figure 8: Effect of rearing seasons and host plants on total larval period of *A. mylitta* from first to fifth instar

need to feed more quantity for more days to reach the next stage of development (Clancy and Price, 1987; Shreedhara *et al.*, 1991). Benchamin and Jolly (1986) and Situmorang (2002) have reported that variations in larval span caused variations in the cocoon parameters.

Findings of many workers support the results of present study. Rath (2000) found significant differences in larval period of *A. mylitta* when reared on *T. tomentosa*, *T. arjuna* and *Z. jujuba*. Reddy *et al.* (2010) compared rearing performance of *A. mylitta* on two forestry host plants viz., *T. tomentosa* and *L. parviflora* and found significant difference in total larval period. Kumar *et al.* (2009), Reddy *et al.* (2012) and Deka and Kumari (2013) reported similar results with a bit of variation in larval period of *A. mylitta* on different host plants in different rearing seasons.

Further, prolongation of larval period when fed on *L. tomentosa* and *T. chebula* might become necessary to reach their intake and growth targets to attain functional optima as advocated by Raubenheimer and Simpson (1999). Kumar *et al.* (2009) had also reported that increase in larval duration of *A. mylitta* had adverse effects on cocoon weight, and in such cases, insects manage to accumulate required quantity of nutrients by extending the feeding period to maintain the critical level of growth (Slansky and Feeny, 1971).

From combined Evaluation Index, it is clear that four forestry host plants viz., *T. alata* (41.25), *T. tomentosa* (42.86), *T. arjuna* (42.03) and *L. speciosa* (44.82) scored E.I. of less than 50 and found better. For traits like larval period, preservation loss *etc.*, lower values are considered as desirable. Larval duration of *A. mylitta* is also correlated with the profitability of tropical tasar Seri-business, as the cost of labour engagement depends on larval duration. This study indicates that for Uttarakhand, *T. alata*, *T. tomentosa*, *T. arjuna* and *L. speciosa* are the best-suited host plants to realise maximum profitability from forest based commercial rearing of *A. mylitta*.

Cocoon yield

Results indicated that cocoon yield of *A. mylitta* differs significantly between rearing seasons and among host plants ($P < 0.05$). Cocoon yield in *A. mylitta* is a complex character that depends on interaction of various contributing traits (Sinha *et al.*, 1995). In the present study, higher cocoon yield was obtained in the first rearing season than the second one, which confirms that seasonal variations play a major role in the growth and development of *A. mylitta* larvae and weight of silk gland that directly contribute to cocoon yield (Ueda *et al.*, 1969; Takeshita *et al.*, 1975; Mathur and Mathur, 1996). Further, in the second rearing season, prolonged larval period due to low temperature (Tamiru *et al.*, 2012) increases caterpillar's exposure to natural enemies (Isenhour *et al.*, 1987).

It is found that *T. alata* fed larvae recorded the highest cocoon yield, followed by *T. tomentosa*, *T. arjuna* and *L. speciosa* fed larvae whereas, *L. tomentosa* fed larvae provided the lowest cocoon yield, followed by *T. chebula* (Figure 9). It was also found that cocoon yield of *T. tomentosa* and *T. arjuna* fed larvae did not differ significantly with each other. Our results are in conformity with the findings of Deka and Kumari (2013).

The success of an insect depends significantly upon an optimal diet in both quantity and quality (Hassell and

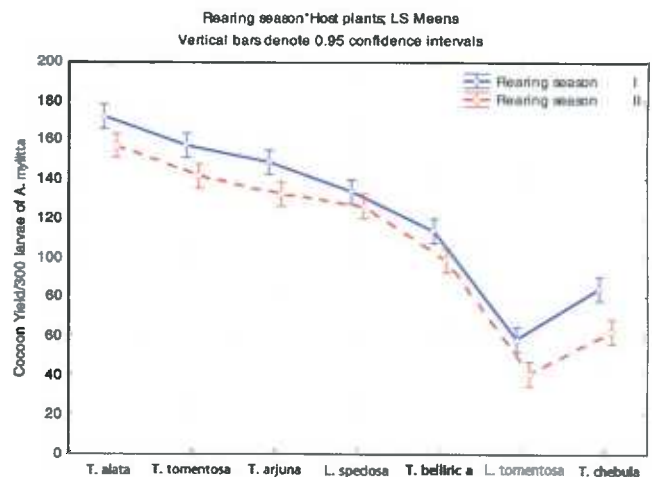


Figure 9: Effect of rearing seasons and host plants on cocoon yield (number) / 300 larvae of *A. mylitta*

Southwood, 1978), which provides energy, nutrients, and water to carry out life's activities (Slansky, 1993). Carbohydrates, proteins and lipids are the main sources of energy at the time of larval-larval, larval-pupal, pupal-adult transformation (Krishnaswami, 1978; Thangamani and Vivekanandan, 1984). Availability of these nutritional components has been reported in *T. alata*, *T. tomentosa*, *T. arjuna* and *L. speciosa* as higher than that in *T. bellirica*, *T. chebula* and *L. tomentosa* (Sinha and Jolly, 1971; NISCAIR, 1976; Agarwal *et al.*, 1980).

Reports are available that moisture content of leaves has a positive correlation with cocoon productivity of *A. mylitta* (Krishnaswami, 1978; Thangamani and Vivekanandan, 1984). Deka and Kumari (2013) stated higher leaf moisture content of 72.07 % in *T. tomentosa*, followed by that in *L. speciosa* (71.21 %) and *T. arjuna* (70.36 %) and recorded productivity of 86, 80 and 82 cocoons/DFL, respectively.

These results support the present findings. Further, some workers (Stride and Stratman, 1962; David and Gardiner, 1966; Jermy *et al.*, 1968; Fraenkel, 1969) have produced experimental evidences that insect becomes "conditioned" to a particular host plant, because food first eaten by a phytophagous insect becomes its subsequent feeding stimulus. In our experimental rearing also, this fact stands true for the larvae of *A. mylitta*, which adopted *L. tomentosa* host plants by the first time and completed its life cycle.

Effective rate of rearing

Effective rate of rearing (ERR) indicates the success rate of silkworm rearing based on larval survivability from brushing until completion of cocoon formation. Our study reveals that ERR of *A. mylitta* differs significantly between rearing seasons and among host plants ($P < 0.05$); but their interactions were found insignificant ($P > 0.05$). Siddiqui *et al.* (1989), Dash *et al.* (1994), Rath (2000) and Reddy *et al.* (2012) have also found significant difference in ERR of *A. mylitta* when reared on different forestry host plants.

ERR was found higher during first rearing season as compared to the second one (Figure 10). Devaiah and Dayashankar (1982) have also reported that the ERR largely depends on type of the host plants along with the rearing season. It was found that in both the rearing seasons, *T. alata* fed silkworm larvae showed the highest ERR, followed by *T. tomentosa*, *T. arjuna* and *L. speciosa*. However, *L. tomentosa* fed larvae exhibited the lowest ERR, followed by *T. chebula*. All the tested forestry host plants, differing in their nutritional contents, had their impact on the growth and development of *A. mylitta* and ultimately ERR differed significantly among host plants in both the rearing seasons.

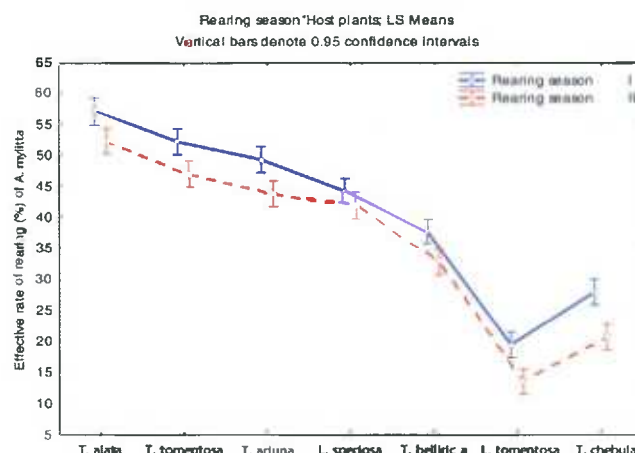


Figure 10: Effect of rearing seasons and host plants on effective rate of rearing (ERR %) of *A. mylitta*

The relationship between phytophagous insects and plants is essentially nutritional, because phytophagous insects visit the plant solely to procure food for themselves or for their progeny (Bhattacharya and Pant, 1976). The success of tasar silkworm rearing mainly depends on the accessibility of food plant with optimum nutritional status so that larval rearing could result in higher number of cocoons of superior quality in terms of pupation or silk content (Ojha and Panday, 2004; Kumar *et al.*, 2009; Reddy *et al.*, 2012). Jayaramaiah and Sannappa (1998) studied relationship between foliar constituents of castor genotypes and economic parameters of eri silkworm and found that the larval duration, weight, survivability and effective rate of rearing (ERR) were having significant

positive relationship with the moisture and nutritional contents of the host plant.

Our results are in close conformity with the findings of Srivastava *et al.* (1994), who used five forestry host plants for rearing of *A. mylitta* under the climatic conditions of Ranchi, and found that *T. tomentosa* excels in ERR, followed by *T. arjuna*, *Ziziphus jujuba*, *L. parviflora* and *Shorea robusta*. Our results indicate that the performance of *A. mylitta* rearing on *L. speciosa*, which is considered as a secondary host plant, was found very close to that on *T. arjuna*, a primary host plant for *A. mylitta*.

Prospects of tropical tasar silkworm rearing in Uttarakhand

Forestry host plants of *A. mylitta* are widely distributed up to an altitudinal range of 2000 feet (610 meter) in tropical moist deciduous and tropical dry deciduous forest areas of the Uttarakhand (Thangavelu, 2004). Further, different eco-races of *A. mylitta* are also reported to be thriving well up to an altitude range of 610 m (Rao and Yadav, 2004). Accordingly, 3217.9 km² of forests distributed up to the altitudinal range of <610 meter in Dehra Dun, Haridwar, Nainital and U.S. Nagar districts of Uttarakhand, can be explored for forest based rearing of *A. mylitta* in the state (Figure 12).

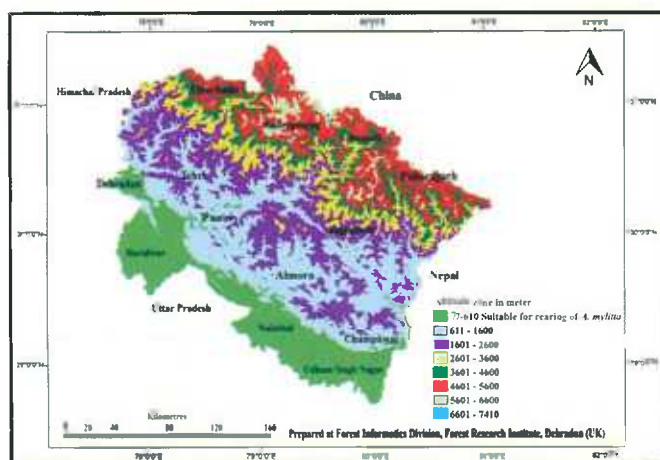


Figure 11: Altitudinal Zones of Uttarakhand state



Figure 12 : Tasar culture *in situ*

Further, in Uttarakhand, an area of 12,790.06 km², which is 23.91 % of its total geographical area, is wasteland (MoRD, 2013). Out of this, 989.77 km² area is distributed at the altitudinal range of < 610 meter having tropical moist deciduous and tropical dry deciduous forests conditions

Table 5: Prospect for introduction of forest based rearing of *A. mylitta* in Uttarakhand

District	Total forest cover (km ²)*	Percentage of forest area at altitudinal range of <1000 m**	Forest area for introduction of <i>A. mylitta</i> (km ²)
Dehra Dun	1607	56	899.92
Haridwar	618	67	414.06
Nainital	3093	44	1360.92
U.S. Nagar	543	100	543.00
Total	5861	54.90	3217.9

Source: * Forest Survey of India (2011) and ** Water Management Directorate (2011)

(FSI, 2011), which can also be utilised for the plantation of forestry tasar food plants to introduce forest based rearing of tropical tasar silkworm, *A. mylitta*.

Conclusion

From the present study, it can be concluded that *T. alata*, *T. tomentosa*, *L. speciosa* and *T. arjuna* are the best suitable forestry host plants in their order of merit to have better larval growth and development of Daba ecorace (Bivoltine) of *A. mylitta* under the climatic conditions of

Dehra Dun Uttarakhand. Accordingly, State Forest Department may initiate systematic plantation of these trees through their various afforestation programme and plantation schemes to create a new forest insect industry of *A. mylitta* in tropical forest area of Uttarakhand to promote its adoption by tribals and rural communities inhabiting in forest fringe areas to improve their economic condition. Since, the study was carried out in Dehra Dun, results need not necessarily represent entire Uttarakhand, as the climate, soil, topography *etc.*, vary widely within the state.

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REFERENCES

- Agarwal S. C., Jolly M. S. and Sinha A. K. (1980) Foliar constituents of secondary food plants of tasar silkworm, *Antheraea mylitta* D. *Indian For.*, **106**(12): 847-851.
- Ahmad M., Arif M. I. and Ahmad M. (2007) Occurrence of insecticide resistance in field populations of *Spodoptera litura* (Lepidoptera: Noctuidae) in Pakistan. *Crop Prot.*, **26**: 809-817.
- Awmack C. S. and Leather S. R. (2002) Host plant quality and fecundity in herbivorous insects. *Annu. Rev. Entomol.*, **47**: 817-844.
- Bae S. D., Park K. B. and Oh Y. J. (1997) Effects of temperature and food source on the egg and larval development of tobacco cutworm, *Spodoptera litura* Fabricius. *Korean J. Entomol.*, **36**: 48-54.
- Benchamin K. V. and Jolly M. S. (1986) Principles of silkworm rearing. In: *Proceedings of Seminar on Problems and Prospects of Sericulture*, Mahalingam S. (Ed.), Vellore, India, pp. 63-108.
- Bhatia N. K. and Yousuf M. (2013 a) Reassuring livelihood functions of the forests to their dependents: Adoption of collaborative forest management system over Joint forest management regime in India. *Ann. For. Res.*, **56**(2): 377-388.
- Bhatia N. K. and Yousuf M. (2013 b) Forest insect industry in collaborative forest management: an overview. *Int. J. Indust. Entomol.*, **27**(1): 166-179.
- Bhatia N. K. and Yousuf M. (2013 c) Assessment on climatic suitability of New Forest, FRI, Dehradun to introduce forest based rearing of tropical tasar silkworm, *Antheraea mylitta* Drury in Uttarakhand, India. *Ann. Entomol.*, **31**(1): 55-66.
- Bhattacharya A. K. and Pant N. C. (1976) Studies on the host plant relationships: Consumption and utilization profile in insects. *Proc. Natl. Acad. Sci. India*, **46**(B): 273-301.
- Clancy K. M. and Price P. W. (1987) Rapid herbivore growth enhances enemy attack: sublethal plant defences remain a paradox. *Ecology*, **68**: 733-736.
- Clarke A. (2003) Costs and consequences of evolutionary temperature adoption. *Trends. Ecol. Evolut.*, **18**(11): 573-581.
- Dash A. K., Patra P. C., Nayak B. K. and Dash M. C. (1994) Cocoon crop performances of the Indian wild tasar silkworm, *Antheraea paphia* (Lepidoptera: Saturniidae) reared on different food plants. *Int. J. Wild Silkmoth & Silk*, **1**: 72-74.
- David W. A. L. and Gardiner B. O. C. (1966) The effect of sinigrin on the feeding of *Pieris brassicae* L. larvae transferred from various diets. *Entomol. Exp. Appl.*, **9**(1): 95-98.
- Deka M. and Kumari M. (2013) Comparative study of the effect of different food plant species on cocoon crop performance of tropical tasar silkworm (*Antheraea mylitta* Drury). *Int. J. Res. Chem. Environ.*, **3**(1): 99-104.
- Devaiah M. C. and Dayashankar K. N. (1982) Effect of different host plants on the economic traits of eri silkworm, *Samia*

- cynthia ricini* Biosduval (Lepidoptera: Saturniidae). *Nat. Sem. Silk Res. Dev.*, March 10-13, Bangalore, pp. 25-32.
- Etman A. and Hooper G. H. S. (1979) Developmental and reproductive biology of *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). *J. Aust. Entomol. Soc.*, **18**: 363-372.
- Fisher R. A. and Yates F. (1963) *Statistical Tables for Biological, Agricultural and Medical Research*, 6th Edn., Edinburgh and London, Oliver & Boyd, p. 146.
- Fraenkel G. (1969) Evaluation of our thoughts on secondary plant substances. *Entomol. Exp. Appl.*, **12**(5): 473-486.
- FSI (2011) *State of forest report 2011*, Forest Survey of India, Ministry of Environment and Forest, Government of India, Dehradun, India, pp. 185-195.
- Gorden H. T. (1999) Growth and development of insects. In: *Ecological Entomology*, Huffaker C. B. and Gutierrez A. P. (Eds.), John Wiley & Sons, Inc., New York, pp. 55-82.
- Guan B. B. and Chen O. J. (1999) Biology and Ecology of *Spodoptera litura*. *Acta Entomol. China*, **8**: 57-61.
- Hansda G., Reddy R. M., Sinha M. K., Ojha N. G. and Prakash N. B. V. (2008) *Ex situ* stabilization and utility prospects of *Jata* ecorace of tropical tasar silkworm *Antheraea mylitta* Drury. *Int. J. Indust. Entomol.*, **17**: 169-172.
- Hassell M. P. and Southwood, T. R. E. (1978) Foraging strategies of insects. *Annu. Rev. Ecol. Evol. Syst.*, **9**: 75-98.
- Isenhour D. J., Wiseman B. R. and Layton R. C. (1987) Enhanced predation by *Orius insidiosus* (Hemiptera: Anthocoridae) on larvae of *Heliothis zea* and *Spodoptera frugiperda* (Lepidoptera: Noctuidae) caused by prey feeding on resistant corn genotypes. *Environ. Entomol.*, **18**: 418-422.
- Jayaramaiah M. and Sannappa B. (1998) Correlation coefficient between foliar constituent of castor genotypes and economic parameters of the eri silkworm, *Samia cynthia ricini* Boisduval (Lepidoptera: Saturniidae). *Third International Conference on Wild Silk Moths*, November 11-14, 1998, Bhubaneswar, Odisha, pp. 162-164.
- Jermy T., Hanson F. E. and Dethier V. G. (1968) Induction of specific food preferences in Lepidopterous larvae. *Entomol. Exp. Appl.*, **11**: 211-230.
- Kohli R. K., Jolly M. S. and Khan A. M. (1969) Foliar constituents of food plants of tasar silkworm *Antheraea mylitta* D. *Indian For.*, **95**(9): 614-617.
- Krishnaswami S. (1978) New technology of silkworm rearing. *Indian Silk*, **16**(12): 7-15.
- Kumar D., Mishra P. K., Singh B. M. K., Sharan S. K. and Suryanarayana N. (2009) Effect of feeding duration on some economical parameters of the tasar silkworm, *Antheraea mylitta* Drury. *Sericologia*, **49**(1): 85-90.
- Legay J. M. (1958) Recent advances in silkworm nutrition. *Annu. Rev. Entomol.*, **3**: 75-86.
- Mano Y., Nirmal Kumar S., Basavaraja H. K., Mal Reddy N. and Datta R. K. (1993) A new method to select promising silkworm breeds/combinations. *Indian Silk*, **31**(10): 53.
- Mano Y., Nirmal Kumar S., Basavaraja H. K., Mal Reddy N. and Datta R. K. (1998) Combined trait selection for silk yield improvement in *Bombyx mori* Linn. In: *Silkworm Breeding*, Reddy G. S. (Ed.), Oxford and IBH Publishing Company Private Limited, New Delhi, pp. 278-284.
- Mathur J. P. and Mathur A. P. (1996) Seasonal effect on survival and cocoon production of pure and hybrid races of *B. mori* L. under sub humid south plain zones of Rajasthan. *J. Parasitol. Appl. Anim. Biol.*, **5**(2): 75-79.
- Mathur S. K., Singh B. M. K., Sinha A. K. and Sinha B. R. R. P. (1998) Integrated package for rearing of *A. mylitta* D. *Indian Silk*, **37**(2): 15-18.
- MoRD (2013) *Wastelands Atlas of India 2011 (Uttarakhand)*. Department of Land Resources, Ministry of Rural

Development, Government of India.

Ecol. Syst., **11**: 41-65.

- Narain R., Rath S. S., Negi B. B. S., Nagendra S., Kulshrestha V., Prasad B. C., Sinha B. R. R. P. and Thangavelu K. (2001) Integrated package for seed cocoon preservation and seed production in *Antheraea mylitta* D. *Indian Silk*, **40**(6): 15-17.
- Nayak B. K., Dash A. K. and Patro K. B. G. (2000) Biodiversity conservation of wild tasar silk moth *Antheraea paphia* L. of Simnlipal biosphere reserve and strategy for its economic utilization. *Int. J. Wild Silkmoth & Silk*, **5**: 367-370.
- NISCAIR (1976) *The Wealth of India - An Encyclopedia of India's Raw Material Resources* Vol. X. Council of Scientific and Industrial Research - National Institute of Science Communication and Information Resources (CSIR-NISCAIR), New Delhi, India, pp. 157-177.
- Ojha N. G. and Panday P. N. (2004) *Silk Production*. A. B. H. Publishing Corporation, New Delhi, India, p. 278.
- Ojha N. G., Reddy R. M., Hansda G., Sinha M. K., Suryanarayana N. and Prakash N. B. K. (2009) Status and potential of Jata, a new race of Indian tropical tasar silkworm (*Antheraea mylitta* Drury). *Acad. J. Entomol.*, **2**(2): 80-84.
- Ojha N. G., Saran S. K., Rai S. and Pandey P. N. (2000) Studies on the sex wise consumption and utilization of the leaves of different food plants in different ecoraces of tropical tasar silkworm, *Antheraea mylitta* Drury during fifth instar of the first crop. *Int. J. Wild Silkmoth & Silk*, **5**: 241-245.
- Ponnuvel K. M., Noamani M. K. R., Luikham R. and Singh K. C. (1996) Seasonal variation in biochemical constituents of oak (*Quercus serrata*) leaf. *Proc. Natl. Acad. Sci. India*, **66**(B): 283-287.
- Price P. W., Bouton C. E., Gross P., Mcpherson B. A., Thompson J. N. and Weiss A. E. (1980) Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Ann. Rev. Ecol. Syst.*, **11**: 41-65.
- Puri G. S. (1994) The foliar constituents in some tree species of *Shorea robusta* forests of the Shivalik, U.P., India. *Indian For.*, **80**(11): 692-699.
- Rao J. V. K. and Yadav D. K. (2004) Utilization of Forest Flora for Tasar culture (tropical and temperate) and impact on Ecosystem. In: *Proceedings of National Workshop on Potential and Strategies for Sustainable Development of Vanya silk in the Himalayan States*, 8-9 November 2004, Directorate of Sericulture, Government of Uttarakhand, Dehradun, India, pp. 17-28.
- Rath S. S. (2000) Studies on growth and development of *Antheraea mylitta* Drury (Lepidoptera: Saturniidae) fed on three different natural host plants. *Int. J. Wild Silkmoth & Silk*, **5**: 99-103.
- Raubenheimer D. and Simpson S. J. (1999) Integrating nutrition: a geometrical approach. *Entomol. Exp. Appl.*, **91**: 67-82.
- Reddy R. M., Rupesh C., Mohan R. K., Angadi B. S. and Sivaprasad V. (2012) Commercial prospective of *Lagerstroemia parviflora* (Roxb.) as choice food plant of tropical tasar silkworm, *Antheraea mylitta* (Drury). *Int. J. Res. Chem. Environ.*, **2**(1): 70-73.
- Reddy R. M., Sinha A. K., Kumar R. and Prasad B. C. (2010) Parental combination and rearing season compatibility for silk yield in tropical tasar silkworm, *Antheraea mylitta* Drury. *World Appl. Sci. J.*, **9**(8): 855-859.
- Shiva Kumar C. (1995) Physiological and Biochemical studies on nutrition in silkworm, *Bombyx mori* L. *Ph.D. Thesis*, Bangalore University, Bangalore, India, p. 211.
- Shiva Kumar C., Sekharappa B. M. and Arangi S. K. (1997) Influence of temperature and leaf quality on rearing performance of silkworm, *Bombyx mori* L. *Indian J. Seric.*, **36**(2): 115-120.
- Shreedhara V. M., Shantikumari M. and Boraiah G. (1991)

- Preliminary studies on changes in levels of protein, sugar, chlorophyll and moisture content in the developmental leaves of mulberry, *Morus alba*. *Proceedings of International Congress on Tropical Sericulture Practices*, Bangalore, India, pp. 35-38.
- Siddiqui A. A., Sengupta A. K., Dasmahapatra D. P. and Sengupta K. (1989) Co - heritability and correlation of different quantitative characters in *Antheraea mylitta* D. *Sericologia*, **29**: 211-214.
- Singh T., Bhat M. M. and Khan M. A. (2009) Insect adaptations to changing environments - temperature and humidity. *Int. J. Indust. Entomol.*, **19**(1): 155-164.
- Sinha A. K. and Chaudhury A. (1992) Factors influencing phenology of different broods of tropical tasar silk moth, *Antheraea mylitta* Drury (Lepidoptera: Saturniidae) in relation to its emergence and post emergence behaviour. *Environ. Ecol.*, **10**(4): 952-958.
- Sinha A. K., Choudhary S. K., Brahmachari B. N. and Sengupta, K. (1986) Foliar constituents of the food plants of temperate tasar silkworm *Antheraea proylei*. *Indian J. Seric.*, **25**(1): 42-43.
- Sinha A. K. and Jolly M. S. (1971) Foliar constituents of the food plants of tasar silkworm *Antheraea mylitta* D. *Indian For.*, **97**(5): 261-263.
- Sinha A. K., Sengupta A. K., Sharma K. K., Sinha B. R. R. P. and Sinha S. S. (1995) Yield components and their implication to better genotype of *Antheraea mylitta* Drury (Lepidoptera: Saturniidae). In: *Perspective in Cytology and Genetics*, Manna G. K. and Roy S. C. (Eds.), Hindasia Publishers, Delhi, India, pp. 617-626.
- Sinha R. B., Pandey P. N., Suryanarayana N. and Srivastava A. K. (2007) Studies on ingestion, digestion and conversion efficiency in Sarihan larvae (*Antheraea mylitta* Drury) fed on leaves of *Terminalia tomentosa* during the commercial crop. *Proc. Zool. Soc. India*, **6**(2): 17-24.
- Situmorang J. (2002) Wild silk industry as new revenue to increase farmer's income and for environmental conservation. *Proceedings of International Conference on Wild silk Moths*, Yogyakarta, Indonesia, 23rd-26th April 2002, pp. 1-16.
- Slansky F. (1993) Nutritional Ecology: the fundamental quest for nutrition. In: *Caterpillars*, Stamp N. E. and Casey T. M. (Eds.), Chapman and Hall, London, pp. 29-91.
- Slansky F. and Feeny P. (1971) Stabilization of rate of nitrogen accumulation by the larvae of the cabbage butterfly on wild and cultivated food plants. *Ecol. Monograph*, **47**: 209-228.
- Slansky F. and Scriber J. M. (1985) Food consumption and utilisation. In: *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Vol. 4., Kerkut G. A. and Gilbert L. I. (Eds.), Pergamon Press, New York, pp. 88-163.
- Srivastava A. K., Sinha A. K., Bishwa Singh M. K., Bakshi R. R. P. and Sinha S. S. (1994) Efficiency of rearing on different host plants in three non mulberry silkworms. *Int. J. Wild Silkworm & Silk*, **1**(2): 241-244.
- Stride G. O. and Stratman R. (1962) The host plant relationship of an Australian swallow stain, *Papilio aegaeus* and its significance in the evolution of its host plant selection. *Proc. Linn. Soc. N.S.W.*, **87**: 69-78.
- Takeshita H., Suto M., Kumata K., Sakate S., Matsumura M. and Shigematsu H. (1975) Biological characteristic of factors concerning silk formation in the silkworm *Bombyx mori* Linn. *J. Seric. Sci. Jpn.*, **44**: 183-189.
- Tamiru A., Getu E., Jembere B. and Bruce T. (2012) Effect of temperature and relative humidity on the development and fecundity of *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae). *Bull. Entomol. Res.*, **102**: 9-15.
- Thangamani R. and Vivekanandan M. (1984) Physiological studies and leaf analysis in the evaluation of best mulberry varieties. *Sericologia*, **24**(3): 317-324.

- Thangavelu K. (2004) Host plant wealth of Vanya Silks in the Himalayan states and strategies for its utilization. In: *Proceedings of National Workshop on Potential and Strategies for Sustainable Development of Vanya silk in the Himalayan States*, 8-9 November 2004, Directorate of Sericulture, Government of Uttarakhand, Dehradun, India, pp. 1-8.
- Ueda S., Kimura R. and Suzuki K. (1969) Studies on the growth of the silkworm *B. mori* II. The influence of rearing condition upon the larval growth, productivity of silk substance and eggs and boil off loss in cocoon shell. *Bull. Seric. Exp. Stn. Jpn.*, **23**: 255-293.
- Umbarihwar J. and Hastings A. (2002) The impact of resource limitation and the phenology of parasitoid attack on the duration of insect herbivore outbreaks. *Theor. Popul. Biol.*, **62**: 259-269.
- Venugopal P. and Krishnaswami S. (1987) Adaptability of silkworm, *Bombyx mori* Linn. to tropical condition. *Indian J. Seric.*, **26(2)**: 63-71.
- Waldbauer G. P. (1968) Consumption and utilisation of food by insects. In: *Advances in Insect Physiology*, Beatment J. W. L., Trecherne J. W. and Wigglesworth V. B. (Eds.), Academic Press, London, pp. 229-288.
- WMD (2011) *Uttarakhand State Perspective and Strategic Plan 2009-2027*. Water Management Directorate (WMD), Government of Uttarakhand, Dehradun, India, p. 188.
- Xue M., Pang Y. H., Wang H. T., Li Q. L. and Liu T. X. (2009) Effects of four host plants on biology and food utilization of the cutworm, *Spodoptera litura*. *J. Insect. Sci.*, **10**: 1-14.
- Yokoyama T. (1963) Sericulture. *Annu. Rev. Entomol.*, **8**: 287-306.
- Zhu J. H., Zhang F. P. and Ren H. G. (2005) Development and nutrition of *Prodenia litura* on four food plants. *Chinese Bull. Entomol.*, **42**: 643-646.

Article scientifique

EVALUATION DE PLANTES HOTES CONVENABLES POUR INTRODUIRE UN ELEVAGE DE VER A SOIE TASAR TROPICAL, *ANTHERAEA MYLITTA*, DANS L'UTTARAKHAND

Narendra Kumar Bhatia^{1*} and Mohammad Yousuf²

¹Regional Sericultural Research Station, Central Silk Board, Ministry of Textiles, Govt. of India, Sahaspur 248197, Dehra Dun (Uttarakhand), India.

²Forest Entomology Division, Forest Research Institute, New Forest 248006, Dehra Dun (Uttarakhand), India.

*E-mail: nkb1123@rediffmail.com

RÉSUMÉ

Antheraea mylitta Drury est un insecte forestier produisant de la soie exploité commercialement dans neuf états producteurs de tassar en Inde mais restant inexploité en Uttarakhand. L'élevage expérimental de l'écorace Daba (BV) de *A. mylitta* a été conduit avec sept espèces d'arbres au Forest Research Institute, Dehra Dun (Uttarakhand) de 2012 à 2014 et les données sur sept variables ont été collectées pour évaluer leur aptitude comme plante hôte en vue d'introduire un élevage commercial forestier de *A. mylitta* en Uttarakhand. *Terminalia alata*, *T. tomentosa*, *Lagerstroemia speciosa* et *T. arjuna* se sont avérées les

plantes hôtes forestières les plus convenables dans cet ordre de mérite en ce sens qu'elles permettent la meilleure croissance et le meilleur développement de *A. mylitta*. De plus, la SRTM (Shuttle Radar Topography Mission) qui cartographie l' Uttarakhand a indiqué que 3217,9 km² de zone forestière comprise à une altitude inférieure à 610 mètres dans les districts de Dehra Dun, Haridwar, Nainital et U.S. Nagar en Uttarakhand peuvent être explorés pour un élevage de *A. mylitta*. En conséquence, le Département de la forêt de l'état associé avec le Directoire de la sériciculture en Uttarakhand peut engager des plantations systématiques de ces plantes hôtes forestières selon leurs différents schémas d'implantation pour créer dans l'état une nouvelle industrie basée sur les insectes forestiers afin d'améliorer le revenu fourni par les forêts tropicales.

Mots-clés: Conservation de la forêt, ver à soie forestier, amélioration du revenu, diminution de la pauvreté, soie vanya.



INFLUENCE OF GROWTH ATTRIBUTES AND THEIR ASSOCIATION WITH LEAF YIELD POTENTIALITY IN MULBERRY

M. S. Rahman

Bangladesh Sericulture Research and Training Institute, Rajshahi 6207, Bangladesh.

E-mail: msrahman57@yahoo.com

ABSTRACT

Correlation and path coefficient analyses were done to estimate the influence of growth attributes and their relationship with leaf yield in mulberry. Forty - five mulberry genotypes maintained in the germplasm bank of Bangladesh Sericulture Research and Training Institute were used in this study. Growth attributes were estimated following standard techniques of growth analysis. Correlation and path coefficient analysis were done following standard statistical method. It is revealed that all the growth attributes showed very strong and positive association with leaf dry weight / plant (LDW / plant) at both phenotypic and genotypic level except leaf area ratio (LAR). Among all the growth attributes taken under the study, only relative growth rate (RGR) and total dry weight (TDW) showed very high positive direct effect on LDW. Therefore, these two growth attributes might be considered for direct selection towards the improvement of leaf yield potentiality in mulberry.

Key words: Correlation, dry matter, growth attribute, leaf yield, mulberry, path analysis.

INTRODUCTION

The principal aim of modern agriculture is to improve and control the plant growth leading to high yield through maximum utilization of solar energy. The growth of the plant is a complex biological phenomenon influenced by several factors. Among these factors, photosynthesis might have been thought to be the most important metabolic process responsible for growth in green plant by consistent increment of dry weight. It is thought that the plant growth is related with the crop yield. But how these contributing factors influence the growth to determine the yield should be known to breeders. Therefore, the ultimate aim of plant physiologists as well as plant breeders from the practical view point is to determine these factors contributing to the growth and understand the mechanism by which they act on the growth phenomenon towards the improvement of yield. Among the growth attributes, Relative Growth Rate (RGR) is a complex and important physiological component of yield and it is reported that high biological

yield is associated with high RGR (Wallace *et al.*, 1972). An increase in NAR during the growing season is interpreted as a response of the photosynthetic apparatus to an increased demand for assimilates to afford rapid growth of the grain fraction (Valero *et al.*, 2005)

Yield, whether it is grain yield or leaf yield is considered as the output of interaction of a large number of physiological and morphological characters. It's possible that breeders may concern more on morphological characters for improving yield and neglect the role of physiological characters in breeding due to limited understanding on the role of physiological components in controlling the plant growth and determining the yield. In this context, Lupton (1968) has proved the usefulness of physiological characters in breeding for improving yield potentiality of crop plant.

Mulberry is a perennial woody plant and is the key plant of sericulture. Economic product of this plant is leaf. As

lodging is not a very important problem, unlike that in cereal crops, vigorous plant growth is directly associated with the leaf yield in mulberry. Therefore, physiological characters assume a great role for improving leaf yield in mulberry. But the information on growth attributes and their relationship with leaf yield is very scanty. Hence, the present work was undertaken to study the influence of growth attributes on leaf yield in mulberry and to find out the interrelationship if any in order to use the information for improving genetic pattern in mulberry under the joint venture of breeders and physiologists.

MATERIALS AND METHODS

Forty - five mulberry genotypes maintained in the germplasm bank of Bangladesh Sericulture Research and Training Institute were used in this study. The plants were raised under row to row and plant to plant spacing of 150 and 120 cm, respectively. Standard cultural practices were employed as and when needed. Data were taken for four seasons in a year covering three harvests in a particular season at the interval of thirty days. At each harvest, plants were separated into leaves, stems and petioles and dry weight was taken after drying in oven at 80°C for 72 hours. Dry weights of different plant parts were used to calculate the growth attributes viz., Relative Growth Rate (RGR), Relative Leaf Growth Rate (RLGR), Net assimilation rate (NAR), Leaf area ratio (LAR), Total dry weight/plant (TDW/P) and Leaf dry weight/plant (LDW/P). Leaf area (LA) was determined by disc method following Islam and Paul (1986) and growth attributes were calculated following the classical techniques of growth analysis as described by Radford (1967). Correlation coefficient was calculated from the variance and covariance components following Al-Jibouri *et al.* (1958) and path coefficient was done at genotypic level by using Wright's (1921, 1923) formula as was extended by Dewey and Lu (1959) taking leaf dry weight/plant as the end product.

RESULTS AND DISCUSSION

Correlation coefficients in all possible combinations were done both at phenotypic and genotypic level and the results are shown in Table 1 and path coefficients analysis in Table 2. Correlation study reveals a wide spectrum of relationship between the growth attributes and leaf dry

weight and among themselves both at phenotypic and genotypic levels. In every pair of association, genotypic correlation coefficients were found higher than the phenotypic values indicating that the expression of phenotypic values might be modified under the environmental influences.

Both at phenotypic and genotypic levels, leaf area/plant exhibited highly significant positive correlation with RGR, RLGR, NAR, TDW and LDW and non-significant but positive relationship with LAR. Leaf area is an important physiological component of growth as well as yield. Increase in leaf area means increase in photosynthetic surface and consequently increase in assimilation of food which is ultimately reflected on RGR, RLGR, NAR, LAR and dry matter accumulation. Bari (1989) also found the significant positive association of LA with TDW, LDW, RLGR and non-significant but positive relationship with RGR and LAR. Strong, positive association of TDW and LDW with LA was also reported in soybean by Shibles and Weber (1965) and Weber (1968) and Enji (1973). Path coefficient analysis revealed that leaf area/plant showed negative influence on leaf dry weight though it showed very high positive correlation with LDW. High positive indirect effect of RGR and TDW made the relation positive and strong.

Since both NAR and LAR are the two components of RGR, it is important to examine the relative contribution of the rate of assimilation (NAR) and the expansion of the photosynthetic surface (LAR) in determining dry matter production. At both the levels, RGR exhibited very high positive association with NAR and non-significant but positive correlation with LAR. The results suggest that any change in RGR is directly influenced by NAR and LAR in positive direction. At later stage of growth in mulberry, Bari (1989) also found more or less same association of RGR with NAR and LAR. The relative importance of these two components and their contribution to RGR was reported previously by a number of workers. Wilson and Cooper (1969), Thurling (1974), Tan *et al.* (1978) Pandey and Sinha (1979) and Paul (1980) also reported that NAR exerted stronger influence on RGR and LAR which is in

Table 1: Phenotypic (above the diagonal) and genotypic (below the diagonal) correlation coefficients of different growth attributes in mulberry

Character	Leaf area/plant (LA/P)	Relative growth rate (RGR)	Relative leaf growth rate (RLGR)	Net assimilation rate (NAR)	Leaf area ratio (LAR)	Total dry weight/ plant (TDW/P)	Leaf dry weight/ plant LDW/P
LA/P	-	0.9005**	0.8800**	0.3690*	0.2224	0.9434**	0.9805**
RGR	0.9319**	-	0.9315**	0.5429**	0.0978	0.8848**	0.9198**
RLGR	0.9270**	0.9790**	-	0.2822	0.3583*	0.8018**	0.8930**
NAR	0.4762**	0.5605**	0.3826*	-	-0.7398**	0.5612**	0.4218**
LAR	0.2829	0.2487	0.4425**	-0.6611**	-	-0.0057	0.1695
TDW/P	0.9813**	0.9183**	0.8780**	0.6239**	0.1053	-	0.9433**
LDW/P	0.9974**	0.9541**	0.9419**	0.5148**	0.2518	0.9790**	-

* Significant at 5 % level; ** Significant at 1 % level

Table 2: Direct (diagonally placed highlighted values) and indirect effects of different growth attributes with leaf dry weight in mulberry

Character	Leaf area (LA)	Relative growth rate (RGR)	Relative leaf growth rate (RLGR)	Net assimilation rate (NAR)	Leaf area ratio (LAR)	Total dry weight (TDW)	r_i with leaf dry weight
LA	-4.74188	10.20270	-8.51090	-1.53875	0.05753	5.52884	0.9974
RGR	-4.41900	10.94800	-8.98800	-1.81100	0.05060	5.17410	0.9541
RLGR	-4.39589	10.71804	-9.18077	-1.23650	0.08999	4.94718	0.9419
NAR	-2.25785	6.13598	-3.51276	-3.23165	-0.13445	3.51515	0.5148
LAR	-1.34151	2.72313	-4.06275	2.13653	0.20336	0.59327	0.2518
TDW	-4.65298	10.05316	-8.06089	-2.016113	0.02141	5.63448	0.9790

Residual effect = 0.16700

line with the present findings. RGR also exhibited strong positive correlation with RLGR, TDW and LDW. These results are also similar to the findings of Bari (1989). Strong positive association of RGR with TDW and LDW holds the view that if dry matter accumulation per unit plant dry weight in a unit time will increase, the total biomass as well as leaf dry weight will also be increased with the same speed and in the same direction. Wallace *et al.* (1972) also stated that high biological yield must be resulted from high RGR. From the path coefficient analysis, it is seen that the self contribution of RGR towards the leaf dry weight was highly positive which signifies that the attribute is remarkably responsive for direct selection towards the improvement of leaf yield in mulberry.

RLGR was observed to have a highly positive relationship with NAR, LAR, TDW and LDW at genotypic level. More or less similar trends were also observed at phenotypic level. Bari (1989) also found the positive association of RLGR with NAR, LAR, TDW and LDW. Wallace and Munger (1965) reported that the higher RLGR of pea bean was closely related to their much higher leaf area ratio. Elmore *et al.* (1967) reported the relation of biological yield with RLGR. Samsuddin (1983) also could record the positive association of total dry weight with RGR and RLGR in sweet potato. Direct effect of RLGR on LDW was though highly negative, high positive indirect effect of RGR and TDW made the association positive and significant.

NAR and LAR are the most important physiological components of growth and their outcome virtually controls the over all growth rate of plant. In the present study, NAR was found highly and positively related with TDW and LDW but strongly and negatively related with LAR. The present finding also corroborates with the report of Bari (1989) in mulberry. High positive association of RGR and NAR with dry matter accumulation (TDW and LDW) indicates that the dry matter accumulation is increased when the rate of growth and assimilation move in the same direction. Highly significant negative association between NAR and LAR indicates that the expansion of photosynthetic surface ultimately limits the assimilation rate in mulberry. Results in support of this are also reported by Laws and Trechare (1971) in oat and wheat, and Thurling (1974) and Islam and Paul (1986) in rape seed. Path coefficient analysis reveals that NAR exhibited negative direct effect on LDW though it had significant positive association with leaf dry weight at both the levels. NAR showed the strong positive relation with LDW through positive indirect effect of RGR and TDW.

LAR showed very low negative association with TDW at phenotypic level and positive but small in amount with LDW at genotypic level revealing that with the increase of dry matter accumulation, photosynthetic surface is decreased. Bari (1989) also observed the same association of LAR with TDW and LDW in mulberry at late stage of growth. This attribute showed low positive direct effect on LDW.

Total dry weight showed very high positive correlation with leaf dry weight at both the levels. Highly significant positive association of TDW with LDW indicates that with increase of total biomass or biological yield, leaf dry weight or economic yield is also increased with same speed and in the same direction. In fresh weight condition, Bari *et al.* (1989) and Rahman *et al.* (1994) also recorded highly positive and significant association between total stem weight and leaf yield in mulberry. This attribute also showed very high and positive direct effect on leaf dry weight which signifies that high positive

correlation of TDW with LDW is mainly due to its own contribution. High positive direct effect of TDW on LDW suggests that the attribute might be considered for direct selection towards further improvement of leaf yield potentiality in mulberry.

REFERENCES

- Al-Jibouri H. A., Miller P. A. and Robinson H. F. (1958) Genotypic and environmental variances in an upland cotton cross of inter-specific origin. *Agron. J.*, **50**: 633-637.
- Bari M. A. (1989) Studies on growth and yield of mulberry (*Morus alba* L.). *Ph. D. Thesis*, University of Rajshahi, Bangladesh.
- Dewey D. R. and Lu K. H. (1959) A correlation and path coefficient analysis of components of crested wheat grass seed production. *Agron. J.*, **51**: 511-518.
- Elmore C. D., Hesketh J. D. and Muramoto H. (1967) A survey of rates of leaf growth, leaf aging and leaf photosynthetic rates among and within species. *J. Ariz. Acad. Sci.*, **4**(4): 215-219.
- Enji B. A. C. (1973) Effect of plant population on growth and yield of soybean (*Glycine max*). *J. Agric. Sci. Camb.*, **80**: 131-138.
- Islam M. M. and Paul N. K. (1986) Comparative growth analysis of six cultivars of rape seed. (*Brassica campestris* L.). *Bangladesh J. Agric. Sci.*, **13**: 35-39.
- Laws D. A. and Trechare K. J. (1971) Variation in photosynthetic ability in cereals and its implications in a plant breeding programme. 1. Variation in seedling leaves and flag leaves. *Euphytica*, **20**: 86-92.
- Lupton F. C. H. (1968) The analysis of grain yield of wheat in terms of photosynthesis ability and efficiency of translocation. *Ann. Appl. Biol.*, **61**: 109-119.
- Pandey B. N. and Sinha R. P. (1979) Effect of temperature on growth of *Crotalaria juncea* L. and *C. sericea*. *Retz. Ann. Bot. N. S.*, **44**: 685-691.
- Paul N. K. (1980) Genetic analysis of seed yield and yield associated characters in rape seed (*Brassica campestris* L.). *Libyan J. Sci.*, **9**: 7-12.
- Radford P. J. (1967) Growth analysis formulae-their use and

- abuse. *Crop Sci.*, **7**: 171-175.
- Rahman M. S., Sarker A. and Chaturvedi H. K. (1994) Association of characters of leaf production in germplasm mulberry varieties. *Bull. Seric. Res.*, **5**: 9-13.
- Samsuddin S. A. K. (1983) Studies on the physiological factors affecting storage root formation in sweet potato (*Ipomoea batatas* L.) and its relationship with photosynthetic area. *M. Phil. Thesis*, University of Rajshahi, Bangladesh.
- Shibles R. M. and Weber C. R. (1965) Leaf area, solar radiation interception and dry matter production by soybeans. *Crop Sci.*, **5**: 575-577.
- Tan, Geok-Yong, Wai-Koon, Tan and Walton P. D. (1978) Seedling growth of smooth brome grass measured in five environments. *Crop Sci.*, **18**: 601-604.
- Thurling N. (1974) Morpho-physiological determinants of yield in rape seed (*Brassica campestris* and *B. napus*) 1. Growth and morphological characters. *Aust. J. Agric. Res.*, **25**: 697-710.
- Valero A., de Juan, Maturano M., Ramirez A. A., Tarjuelo
- Martin-Benito J. M. and Ortega Alvarez J. F. (2005) Growth and nitrogen use efficiency of irrigated maize in a semiarid region as affected by nitrogen fertilization. *Spanish J. Agric. Res.*, **3**(1): 134-144.
- Wallace D. H., Ozbun J. L. and Munger H. M. (1972) *Physiological genetics of crop yield. Advance in agronomy*, N. C. Brady (Ed.), Academic Press, Vol. **24**, pp. 97-146.
- Wallace D. M. and Munger H. M. (1965) Studies on the physiological basis for yield differences. 1. Growth analysis of six dry bean varieties. *Crop Sci.*, **5**: 343-348.
- Weber C. R. (1963) Physiological concepts for high soybean yields. *Field Crop Abst.*, **21**: 313-317.
- Wilson D. and Cooper J. P. (1969) Assimilation rate and growth of lolium populations in the glass house in contracting light intensities. *Ann. Bot. N. S.*, **33**: 951-965.
- Wright S. (1921) Correlation and causation. *J. Agric. Res.*, **20**: 557-585.
- Wright S. (1923) The theory of path coefficient, a reply to Niles criticism. *Genetics*, **8**: 239-255.

Article scientifique

INFLUENCE DES ATTRIBUTS DE CROISSANCE ET LEUR ASSOCIATION AVEC LE POTENTIEL DE RENDEMENT EN FEUIKES CHEZ LE MURIER

M. S. Rahman

Bangladesh Sericulture Research and Training Institute, Rajshahi 6207, Bangladesh.

E-mail: msrahman57@yahoo.com

RÉSUMÉ

Des analyses de coefficients de corrélation et de chemin ont été effectuées afin d'évaluer l'influence des attributs de croissance et leurs relations sur le rendement foliaire chez le mûrier. Quarante cinq génotypes de mûrier maintenus dans la banque génétique du Bangladesh Sericulture Research and Training Institute ont été utilisés dans cette étude. Les attributs de croissance ont été évalués en suivant les techniques standard d'analyse de croissance. Les analyses des coefficients de corrélation et de chemin ont été effectuées par les méthodes statistiques classiques. Nous montrons que tous les attributs de croissance sont fortement et positivement associés avec le poids sec de feuilles par plant (LDW/plant) aux niveaux phénotypique et génotypique sauf pour le taux de surface foliaire (LAR). Parmi tous les attributs de croissance, seuls le taux de croissance relative (RGR) et le poids sec total (TDW) montrent un effet positif direct sur le poids sec des feuilles. Aussi, ces deux attributs de croissance doivent être considérés pour une sélection directe en vue de l'amélioration du potentiel de rendement foliaire chez le mûrier.

Mots-clés: Corrélation, matière sèche, attributs de croissance, rendement foliaire, mûrier, analyse de path.



EXPRESSION OF PARALYTIC PEPTIDE BINDING PROTEIN (PP-BP) GENE DURING INDUCTION OF EGG DIAPAUSE AND ITS MULTI-GENE ORGANIZATION IN SILKWORM *BOMBYX MORI*

K. M. Ponnuvel*, S. Sasibhushan, E. Vijaya Gowri, Geetha N. Murthy and A. K. Awasthi

Genomics Division, Seribiotech Research Laboratory, Carmelaram Post, Kodathi, Bengaluru 560035, India.

*E-mail: kmpvel@yahoo.com

ABSTRACT

The gene expression levels of paralytic peptide (PP) and paralytic peptide binding proteins (PP-BP) were compared in diapausing and non-diapausing eggs of silkworm, *Bombyx mori* L. The role of PP as well as PP-BP in diapause induction in polyvoltine silkworms was also investigated and the multigene organization of PP-BP in the *B. mori* genome was analyzed. PP-BP are 30KP proteins with similarity to ENF binding proteins, the multifunctional insect cytokines. Microarray analysis was attempted to compare the gene expression at different time intervals. Results indicated up regulation of PP at 18 h and PP-BP at 12 and 18 h after oviposition along with a few other genes. The silkworm tissue specific expression analysis revealed high expression of PP-BP in fat body followed by egg and brain while no expression was observed in midgut. Realtime PCR results revealed that PP is highly expressed at 18 and 24 h while PP-BP expression is high at 12 and 18 h suggesting their possible role in diapause induction. The whole genome survey of the PP-BP paralogous sequences revealed a total of 46 *B. mori* PP-BP homologs classified into 3 categories viz., ENF-BP, Typical 30KPs and serine/threonine rich 30KPs. These paralogous sequences are distributed on chromosomes 7, 20, 22 and 24 with all 30KP and S/T rich 30KP proteins present in the same locus on chromosome 20.

Key words: *Bombyx mori*, diapause, gene expression, PP, PP-BP.

INTRODUCTION

The central nervous system is the major site that regulates growth, metamorphosis, diapause and reproduction during insect development, by secreting peptides. The insect hemolymph contains various proteinaceous components. Injection of the hemolymph from larvae of other *Manduca* species into that of *Manduca sexta* caused paralysis of the latter. This hemolymph factor was isolated and characterized as 23 amino acid peptide from several kinds of lepidopteran hemolymph and named as Paralytic peptide (PP) (Ha *et al.*, 1999).

In *Bombyx mori*, the diapause hormone (Bommo-DH), which is produced in the pupae from the suboesophageal

ganglion (SG), induces embryonic diapause. Injection of *Antheraea yamamai*-paralytic peptide (Antya-ParP) into non-diapausing pupae of *B. mori*, however, resulted in the production of diapausing eggs. Even extirpation of brain along with SG had no influence on the induction of diapausing egg production by Antya-ParP, which demonstrated that Antya-ParP does not act via endogenous *B. mori* diapause hormone (Bommo-DH) but mimics its action at the level of target tissues. The primary sequences of the Bommo-DH and ENF peptide families did not reveal any homology, but this does not exclude the possibility that one can mimic the action of the other (An *et al.*, 2007). Abundant level of 30KPs expression observed suggests their probable role as storage proteins (Izumi *et al.*, 1981; Wang *et al.*, 1989; Fujiwara and Yamashita, 1992). Studies on expression of PP and paralytic peptide binding protein

(PP-BP) in diapause induced eggs of multivoltine silkworm *B. mori* are yet to be attempted.

In this study, an attempt was done to analyze the expressions of PP-BP gene in diapause induced eggs of multivoltine silkworm *B. mori* and to correlate its possible role with egg diapause induction.

MATERIALS AND METHODS

Silkworm rearing

The *B. mori* multivoltine silkworm strain MW13 (Indian origin) was selected and the larvae were reared up to last instar as per standard rearing method to obtain non-diapausing eggs. However, a separate batch of the late stage (4th & 5th instars) were reared under low temperature (18 °C) and 6L: 18D regime of photoperiod up to cocooning stage and the resulting moths laid diapausing eggs under normal room temperature (25 °C) (Saravanakumar *et al.*, 2008).

RNA isolation, cDNA preparation

The diapause and non-diapause egg samples were collected from 0 to 48 h at every 6 h time interval, after oviposition. Other tissues of the silkworm *viz.*, fat body, brain and mid gut were collected from day 3 of 5th instar larvae. Total RNA was extracted from the diapause and non-diapause eggs using TRIzol reagent (Invitrogen, USA) and first strand cDNA synthesized as per standard protocol (Saravanakumar *et al.*, 2008).

Microarray experiment and data analysis

Investigations on the gene expression profiles of diapause induced and non-diapause eggs of multivoltine silkworm *B. mori* were carried out at 18 and 30 h after oviposition using a genome wide oligonucleotide microarray containing 24,924 probes. The complete raw and normalized data sets from this study have been deposited in the NCBI Gene Expression Omnibus (GEO) repository (accession number GSE35622). The

paralogous gene sequences in *B. mori* genome was identified through BLAST search of cDNA sequence with *Silkworm Genome Database* (<http://silkworm.genomics.org.cn>).

Real time PCR (qPCR) analysis

qPCR analysis was carried out in a 25 µl reaction mixture containing SYBR green mastermix (ABI, CA, USA) and specifically designed primers (STRATAGENE Mx 3005P qPCR) using 1µl of first strand cDNA synthesized from diapause and non-diapause eggs of 6 to 48 h after oviposition as template.

RESULTS

Analysis of the microarray gene expression data of diapause induced and non-diapause eggs of multivoltine silkworm *B. mori* after oviposition showed specific upregulation of a few genes in the diapause induced eggs, especially PP and PP-BP that were significantly up-regulated at 12 and 18 h after oviposition (Figure 1).

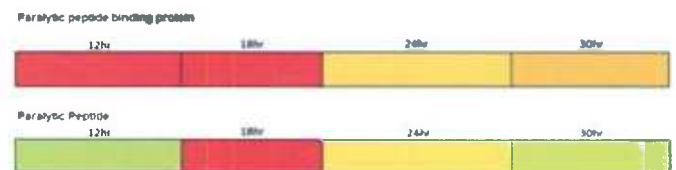


Figure 1: Microarray expression profile of PP and PP-BP in diapause induced eggs of silkworm *B. mori* at different time intervals

PP-BP gene expression in different tissues

RT-PCR analysis of different tissues *viz.*, fat body, brain and mid gut of larvae on the 3rd day of 5th instar as well as diapause induced eggs revealed tissue specific distribution of PP-BP transcripts. The results indicated high expression of PP-BP in fat body followed by brain and eggs, while no expression was observed in mid gut tissue (Figure 2). *B. mori* β -actin gene was used as an internal control.

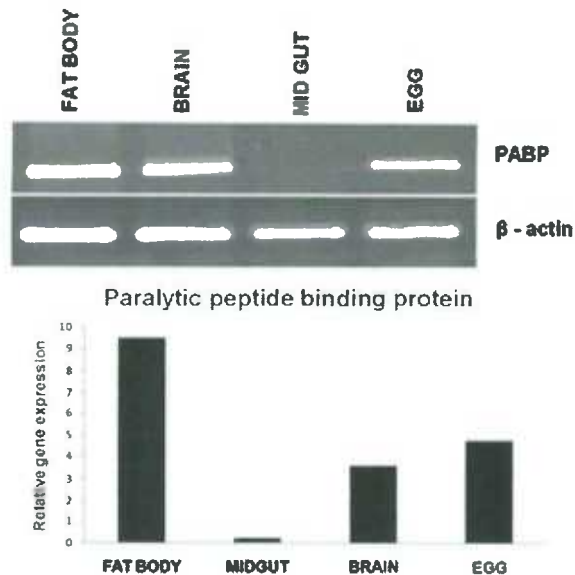


Figure 2: Expression profiles of PP - BP in different tissues analyzed using RT-PCR and qPCR

Differential expression of *B. mori* PP-BP and PP in diapause induced eggs

qPCR analysis revealed gradual increase in the expression level of PP-BP from 0 to 24 h, where, the expression was at maximum, followed by a very steep decrease from 30 to 48 h in diapause induced eggs. The gene expression in non-diapause eggs was very low from 6 to 48 h compared to diapause eggs. The expression levels of PP and PP-BP were almost similar in diapause eggs, while, in non-diapause eggs, PP expression was slightly higher compared to that of PP-BP (Figure 3).

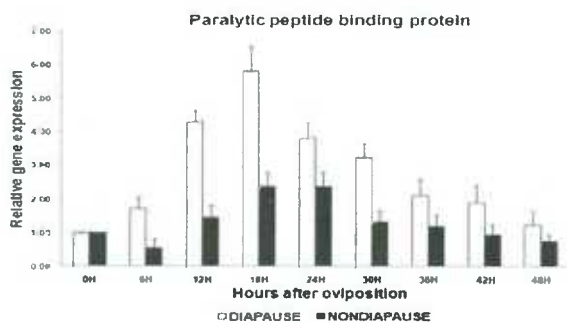


Figure 3: Relative gene expression patterns of PP-BP gene upregulated during diapause using qPCR

Genomic organization of *B. mori* PP-BP paralogous genes

Blast analysis of the cDNA sequence of *B. mori* PP-BP could identify several paralogous contigs totaling to 46 paralogous sequences distributed on chromosomes 7, 20, 22 and 24 (Figure 4). Based on structural characteristics, the above 46 sequences identified have been classified into ENF-BP, typical 30KP, and serine/threonine-rich 30KP. ClustalW analyses performed with the multiple sequence alignment using ClustalW through MEGA 4 revealed that these paralogous sequences formed three major clusters. Of the 46 sequences identified, 10 fall under the category of ENF-BP cluster, 12 under S/T-rich 30KP cluster and 24 under typical 30KP clusters.

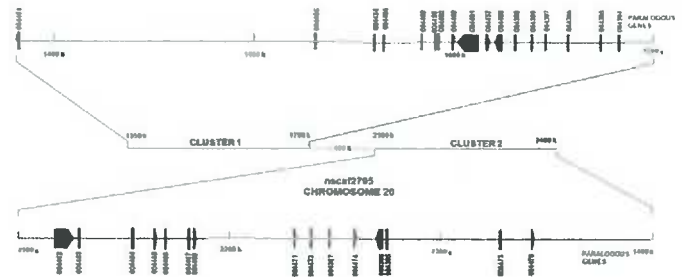


Figure 4: Paralogous gene clusters of 30KP and S/T rich 30KP genes in *B. mori*

DISCUSSION

In the present study, a comparison was made in the gene expression levels of paralytic peptide (PP) and paralytic peptide binding proteins (PP-BP) in diapause and non-diapause eggs after oviposition. Most of the reports in this line emphasize the role of ENF peptides in cellular immunity and cell proliferation. An *et al.* (2007) proved that *Antheraea yamamai* paralytic peptide induces egg diapause even in pupae where subesophageal ganglion had been extirpated and also causes a rapid and rigid larval paralysis in *B. mori*. It was also inferred that Antya-ParP interacts with other pathways to excite diapause rather than those involved in paralysis. It was also speculated that Antya-ParP and homologs act on the same channel in the cascade of diapause egg induction as the DH.

The structural and phylogenetic analyses of paralogous sequences revealed that they can be grouped into three categories *viz.*, ENF-BP, typical 30K proteins and S/T rich 30K proteins. The 30K proteins are involved in the diapause mechanism which they regulate through paralytic peptide action.

Analysis of a total of forty six *B. mori* PP-BP homologs retrieved from the silkworm genome database (<http://silkworm.genomics.org.cn>) indicated that these paralogous gene sequences, belonging to PP-BP gene family diverged into three different groups, *viz.*, ENF-BP, S/T rich and Typical 30KP. Comparative analysis of the molecular distance of the paralogous genes within the species offers the amount of coincidental evolution (Walsh, 1995). The S/T rich subgroup present in the 30KP group is indicative of the recent evolution of both the groups from the ancestral gene. The original gene would perhaps have originated from common ancestors by gene duplication, forming individuals within the multigene family later during the evolutionary process. The multigene cluster of ENF-BP was spread over different chromosomes *viz.*, chromosome number 7, 22 and 24 unlike the typical 30KP and ST rich 30KPs. Further exploitation of paralytic peptide and paralytic peptide binding protein interaction would throw light on whether it is an alternative mechanism of diapause induction and its potential to design novel growth regulators in other insects.

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REFERENCES

- An Y., Yamashita T., Seino A., Imai K. and Suzuki K. (2007) Functional Mimicry of the Silkworm Diapause Hormone by an Insect Paralytic Peptide. *J. Insect Biotechnol. Sericology*, **76**: 51-55.
- Fujiwara Y. and Yamashita O. (1992) Gene structure of *Bombyx mori* larval serum protein (BmLSP). *Insect Mol. Biol.*, **1**: 63-69.
- Ha S., Suzuki A., Nagata S., Tanaka M. and Kataoka H. (1999) Structure determination and cDNA cloning of paralytic peptide in *Bombyx mori*. In: *Peptide Science - Present and Future*. Y. Shimonishi (Ed.), Kluwer Academic Publishers, Great Britain, pp. 460-461.
- Izumi S., Fujie J., Yamada S. and Tomino S. (1981) Molecular properties and biosynthesis of major plasma proteins in *Bombyx mori*. *Biochem. Biophys. Acta*, **660**: 222-229.
- Saravanakumar R., Ponnuvel K. M. and Qadri S. M. H. (2008) Expression of metabolic enzyme genes and heat-shock protein genes during embryonic development in diapause and non-diapause egg of multivoltine silkworm *Bombyx mori*. *Biologia*, **63**: 737-744.
- Walsh J. B. (1995) How often do duplicated genes evolve new functions? *Genetics*, **139**: 421-428.
- Wang X. Y., Cole K. D. and Law J. H. (1989) The nucleotide sequence of a microvitellogenin encoding gene from the tobacco hornworm, *Manduca sexta*. *Gene*, **80**: 259-268.

Article scientifique

EXPRESSION DU GENE DE LA PROTEINE FIXATRICE DU PEPTIDE PARALYTIQUE (PP-BP) PENDANT LA DIAPAUSE EMBRYONNAIRE ET ORGANISATION MUTIGENIQUE CHEZ LE VER A SOIE *BOMBYX MORI*

K. M. Ponnuvel*, S. Sasibhushan, E. Vijaya Gowri, Geetha N. Murthy and A. K. Awasthi

Genomics Division, Seribiotech Research Laboratory, Carmelaram Post, Kodathi, Bengaluru 560035, India.

*E-mail: kmpvel@yahoo.com

RÉSUMÉ

Les niveaux d'expression des gènes du peptide paralytique (PP) et des protéines fixatrices du peptide paralytique (PP-BP) ont été comparés chez des œufs diapausant et non diapausant du ver à soie *Bombyx mori* L. Le rôle de PP et PP-BP dans l'induction de la diapause chez des vers à soie polyvoltins a également été exploré et l'organisation multigénique de PP-BP dans le génome de *B. mori* a été analysée. PP-BP sont des protéines 30KP présentant des similarités avec des protéines fixatrices ENF, les cytokines d'insecte multifonctionnelles. Une analyse a été tentée afin de comparer l'expression génique à différents intervalles de temps. Les résultats montrent une régulation positive de PP 18h et de PP-BP 12 et 18h après l'oviposition avec quelques autres gènes. L'analyse de l'expression dans les tissus du ver à soie révèle une expression élevée de PP-BP dans le corps adipeux puis dans l'œuf et dans le cerveau alors qu'aucune expression n'est observée dans l'intestin. Les résultats des PCR en temps réel montrent que PP est fortement exprimé à 18 et 24h alors que l'expression de PP-BP est forte à 12 et 18h ce qui suggère leur rôle possible dans l'induction de la diapause. L'exploration des séquences de PP-BP dans l'ensemble du génome révèle 46 homologues de PP-BP de *B. mori* classables en 3 catégories : ENF-BP, 30KPs typiques et 30KPs riches en sérine/thréonine. Ces séquences sont distribuées sur les chromosomes 7, 20, 22 et 24, toutes les séquences 30KP et les 30KP riches en s/t étant présentes dans le même locus du chromosome 20.

Mots-clés: *Bombyx mori*, diapause, expression des gènes, protéines fixant le peptide paralytique.



CHARACTERIZATION AND EVALUATION OF WILD SERICIGENOUS INSECTS OF MANIPUR: EXPLORATION FOR CONSERVATION

Reeta Luikham^{1*}, T. Shantibala², T. K. James¹ and L. Bidyapati Devi¹

¹Regional Tasar Research Station, Central Silk Board, Mantripukhri, Imphal, Manipur, India.

²Institute of Bioresources & Sustainable Development, Department of Biotechnology, Manipur, India.

*E-mail: reeta_luikham@rediffmail.com

ABSTRACT

While exploring the wild sericigenous insects from Manipur, we identified 17 species under nine genera (*Antheraea*, *Actias*, *Attacus*, *Bombyx*, *Samia*, *Cricula*, *Dendrolimus*, *Lebeda* and *Rhodinia*). The maximum species composition was noticed with Genus, *Antheraea*. Twenty host plants were noticed as fed by diverse sericigenous insects. Maximum rate of incidence was recorded on *Lithocarpus dealbata*, *Litsea polyantha* and *Ricinus communis*. Seasonal incidence showed maximum population with *Samia canningi* that occurred throughout the year. Alpha diversity (Shannon, Menhinic and Margalef index) were higher in the hilly region indicating more species richness and total individuals than valley region. *Attacus atlas* is the largest and *B. huttoni* is the smallest moth. The male cocoon weight (3.92-6.71 g) is less than that of female (6.20-9.19 g) but the male shell percentage was higher indicating more of silk content. *A. frithi* has the highest shell percentage. Among the reelable cocoons, the highest reelability was noticed for *A. frithi* (60.25 % $\pm$ 4.27) and the lowest with *A. roylei* (20.75 % $\pm$ 4.03). The ghicha yarn revealed the longest filament with *A. helferi* (1323.3 cm) that is ever more than those of the commercially exploited species, *A. proylei* (1278.67 cm). Characterization, evaluation and categorisation of wild sericigenous insects revealed that *A. frithi* and *S. canningi* would be the promising candidates for the region. The occurrence of *R. newara*, *D. grisea* and *A. helferi* were reported for the first time from Manipur. The population levels of a few species viz., *R. newara*, *A. helferi*, *A. roylei* and *A. compta* were low because of being rare and endemic. Therefore, it's high time that serious efforts are made for conservation and population enhancement of sericigenous species for wild silk production, primarily for the uplift of the tribals and to safeguard the rich biodiversity along with conservation of valuable genetic resources.

Key words: Characterisation, conservation, evaluation, Manipur, wild sericigenous insects.

INTRODUCTION

Sericigenous insects are wild or semi domesticated insects that produce lustrous silk. Globally, the wild silk moths belong to four families viz., Bombycidae, Saturniidae, Lasiocampidae and Notodontidae, comprising several thousands of described species. Majority of the wild silk moths belong to the family Saturniidae of the order Lepidoptera comprising 2010 described species in 176 genera and nine subfamilies (Lemaire and Minet, 1998; Nässig *et al.*, 1996). Of these,

47 species were reported from India (Singh and Suryanarayana, 2005). From Northeast India alone, 24 species were listed (Singh and Chakravorty, 2006). Being an Indo-Burma biodiversity hotspot region, Northeast India makes an ideal home for many wild sericigenous insects and is a centre of wild silk culture including muga, eri, tropical tasar, temperate tasar and fagara silks (Jolly *et al.*, 1975, 1976, 1979; Peigler, 1993), however, there remains a great scope for producing novel silk from *Actias selene*, *Antheraea roylei*, *Samia canningi* and *Cricula trifenestrata* (Kakati and Chutia, 2009). The forest fauna in

sub-Himalayan belt of India is the natural home of large variety of sericigenous insects (Singh *et al.*, 2000; Singh and Maheswari, 2003) which play an important role in the conservation and utilization of biodiversity (Frankel, 1982; Sih *et al.*, 2000; Sahu and Bindroo, 2007).

Several workers have attempted exploration of wild sericigenous or silk producing insects from Northeast India (Thangavelu *et al.*, 1987; Singh *et al.*, 2008). Despite this, detailed and descriptive information on these sericigenous insects occurring in Manipur is still meagre may be as their distribution is primarily restricted to highly inaccessible areas. Sericigenous genetic resources are facing major threats due to rapid transformation of original vegetation and intensive land use practices. Hence, it is essential to characterize, classify and document the status of these precious faunal species in the region. The conservative efforts make way for linking genetic diversity to utilization and protection of diverse gene pool, habitat or ecosystem for human socioeconomic needs (Metzler and Zebold, 1995). Therefore, an attempt was made to survey, collect, characterize and identify the sericigenous insects and their host plants of Manipur to link up their conservation with socioeconomic enhancement.

MATERIALS AND METHODS

Study area

Manipur, the mountainous state in the extreme border of Northeast India lies between 23°50'N to 25°42'N latitudes and 92°58'E to 94°45'E longitudes and constitutes a part of Indo-Burma biodiversity hotspot. The state has an area of 22,327 sqkm bounded by Nagaland in the north, Mizoram in the south, Myanmar in the east and Assam in the west. The topography of the state is characteristically hilly, displaying rugged terrain broken by deep gorges and steep slopes at various places. The altitude varies from 700 to 2000 m above mean sea level. Climate is humid subtropical to temperate. The mean annual rainfall is ca. 1436 mm, with a prolonged rainy season from May to October. The temperature ranges from 14 to 34 °C in summer and 4 to 24 °C in

winter and average relative humidity varies from 50 to 78 %.

Exploratory surveys for collection of wild sericigenous insects and their food plants were conducted at hill (Chandel, Churachanpur, Senapati, Tamenglong and Ukhrul) and valley (Bishnupur, Imphal east, Imphal west and Thoubal) districts of Manipur in different months/seasons during 2011-2014. The natural habitats and undisturbed forest areas were surveyed extensively to record the existence of wild silk moths, worms, cocoons and their host plants. During the survey, tribal folk, farmers and private rearers were interviewed for gathering information on wild sericigenous insects, their preferred host plants and attempts were made to rear the wild silk moths. The incidence pattern of all the wild silk species were recorded throughout the year.

The collected specimens of wild species including worms, cocoons and their host plants were preserved in the Regional Tasar Research Station, Central Silk Board, Imphal and Institute of Bioresources & Sustainable Development, Imphal. Identification and authentication of the insect species were done at University of the Incarnate Word, Texas, USA; Uljanovsk State Pegagogical University, Russia and by referring to authentic literature (Arora and Gupta, 1979; Nässig *et al.*, 1996; D'Abrera, 2012; Peigler and Naumann, 2003). Taxonomic observations were made on head, thorax, abdomen and wing span. Morphometric characterization was done following the method of Jolly *et al.*, 1979 and Nässig *et al.*, 1996.

The seasonal incidence pattern of all the wild silk species was recorded. The total population incidences of diverse silk moths of the hill and valley regions were analysed for alpha and beta diversity analysis. For Alpha biodiversity, the species richness and evenness/heterogeneity were calculated using Shannon-weaver diversity index H' , Simpson index D' , Berger-Parker Dominance index $p^{\max}$, Menhinic index M_h and Margalef index MI (Chima *et al.*, 2013). For Beta diversity, Sorensen's similarity index, SI (Pielou, 1969) was

calculated to measure the insect species' compositional similarity and variation between valley and hilly regions. The formulae used for the respective parameters are given below.

Diversity index	Formula	Indication
Shannon-Weaver, H	$= -\sum \left(\frac{n_i}{N} \cdot \ln \left(\frac{n_i}{N} \right) \right)$	N= total number of individual encountered n_i =number of individuals
Simpson, D	$= \frac{\sum n_i(n_i - 1)}{N(N - 1)}$	of i^{th} species enumerated
Menhinick index, Mh	$= \frac{S}{\sqrt{\sum n_i}}$	S=number of species n_{max} = the number of individuals in the most abundant species
Margalef richness index, MI	$= \frac{S - 1}{\ln N}$	a=number of species present in both region under consideration
Berger-Parker Dominance index, p^{max}	$= \frac{n_{\text{max}}}{N}$	b=number of species present in hill site but absent in valley site
Sorensen's similarity index, SI	$= \left[\frac{a}{a + b + c} \right]$	c= number of species present in valley site but absent in hill site

Morphometric characterization of wild silk moths were analysed by measuring the length and breadth of the wing span, antennae, thorax and abdomen. The economic parameters of the wild cocoons were recorded by measuring their length, breadth, cocoon weight, shell weight, shell percentage and peduncle length. The silk yarn productivity of the wild silk moths was determined by recording the filament denier, reelability and ghicha yarn.

RESULTS

In this study, 20 different species were collected from different parts of the state, of which 16 were identified and are listed in Table 1 and shown in Figure 1. They belong to three families viz., Bombycidae, Saturniidae and Lasiocampidae, under nine genera, *Antheraea*, *Actias*, *Attacus*, *Bombyx*, *Samia*, *Cricula*, *Dendrolimus*, *Lebeda* and *Rhodinia*. Of these, seven species belong to *Antheraea*, two to *Samia* and one each to *Bombyx*, *Actias*, *Attacus*, *Cricula*, *Dendrolimus*, *Lebeda* and *Rhodinia*. The distribution patterns of the species in nine districts of the hill (Chandel, Churachanpur, Senapati, Tamenglong and Ukhrul) and valley (Bishnupur, Imphal east, Imphal

west and Thoubal) regions are presented in Figure 2. More than 20 different host plants are exploited by diverse sericigenous insects (Table 1). Maximum number of species occurrence was recorded on *Lithocarpus dealbata*,

Litsea polyantha and *Ricinus communis*. The wild eri silk, *S. canningi* feeds on more than eight species of plants. *A. proylei*, *A. pernyi*, *A. assamensis*, *Bombyx* sp. (var. *Leimaren*) and *S. ricini* being commercially exploited species, were not included during population studies of the wild silk moth.

Seasonal incidence pattern of wild silkworm species observed during 2011-14 revealed that *S. canningi* and *A. atlas* occurred almost throughout the year except December and January (Figure 3). Incidences of other species were low and scattered over a few months only. However, *A. frithi* occurred in outbreak numbers (>1,00,000) in wild condition indicating the best adaptability to build up the population in the prevailing ecological conditions of Phaibung (1800-2000 m ASL) region of Senapati district. The population levels of a few species viz., *R. newara*, *A. helferi*, *A. roylei* and *A. compta* were low because they being rare and endemic species. The occurrence of *R. newara*, *D. grisea* and *A. helferi* were reported for the first time from Manipur. *D. grisea* is regarded as a pest of pine trees. The diapausing character of these wild silkworms in their natural habitat is an important

Table 1: Distribution of wild sericigenous insects and its host plants in Manipur

Scientific name	Host plant	Distribution (District-wise)
<i>Bombyx sp. (var. Leimaren)</i>	<i>Morus sp.</i>	1,2
<i>Bombyx huttoni</i>	<i>Morus sp.</i>	1,2,4,7
<i>Antheraea assamensis</i> Helfer	<i>Litsea polyantha</i> , <i>L. citrate</i> , <i>Persea bombycina</i>	1,2,6,9
<i>Antheraea compta</i> Roth	<i>L. polyantha</i> , <i>L. citrate</i> , <i>P. bombycina</i>	1,2,6,9
<i>Antheraea pernyi</i> Guérin -Menevelli	<i>Quercus serrata</i> , <i>Q. griffithii</i> , <i>Lithocarpus dealbata</i>	1,2,8
<i>Antheraea proylei</i> Jolly	<i>Q. serrata</i> , <i>Q. griffithii</i> , <i>L. dealbata</i>	1,2,3,4,5,6,7,8,9
<i>Antheraea roylei</i> Moore	<i>L. dealbata</i> , <i>Q. serrata</i>	7,8
<i>Antheraea frithi</i> Moore	<i>L. dealbata</i> , <i>Q. serrata</i>	5,7,9
* <i>Antheraea helferi</i> Moore	<i>L. dealbat</i> , <i>Q. serrata</i>	8
* <i>Rhodinia newara</i> Moore	<i>Syzygium cumini</i> (L)	8
<i>Actias selene</i> Hubner	<i>Rhus javanica</i> Murray, <i>Prunus cerasuoides</i> , <i>Eugenia praecox</i> / <i>S. praecox</i> , <i>S. cumini</i> (L), <i>Ricinus communis</i> ,	1,2,4,7,8
<i>Attacus atlas</i> Linnaeus	<i>Maesa indica</i> , <i>Psidium guajava</i> , <i>Delonix regia</i>	1,2,3,7,8
<i>Samia rizini</i> Donovan	<i>R. communis</i> , <i>Manihot esculenta</i> , <i>Heteropanax fragrans</i>	1,2,3,4,5,6,7,8,9
<i>Samia canningi</i> Hutton	<i>R. communis</i> , <i>H. fragrans</i> , <i>M. utilissima</i> , <i>Zanthoxylum armatum</i> , <i>Zizyphus jayube</i> , <i>L. polyantha</i> , <i>P. guajava</i> L., <i>Plumeria acutifolia</i>	1,2,3,4,5,6,7,8,9
* <i>Dendrolimus grisea</i> Moore	<i>Pinus roxburghii</i>	4,8
<i>Cricula trifenestrata</i> Helfer	<i>P. bombycina</i>	1,6
<i>Lebeda nobilis</i> Walker	<i>Q. serrata</i>	1,2

*Species reported for the first time from Manipur.

District code: Imphal east-1; Imphal west-2; Thoubal-3; Bishnupur-4; Chandel-5; Tamenlong-6; Ukhrul-7; Senapati-8; Chuchanpur-9

Table 2: Total number of species traced in the hill and valley regions

Species	Valley region (750 - 850 m ASL)	Hill region (950 - 2000 m ASL)
<i>Samia canningi</i>	4520	520
<i>Attacus atlas</i>	130	34
<i>Bombyx huttoni</i>	50	10
<i>Dendrolimus grisea</i>	0	92
<i>Antheraea roylei</i>	0	16
<i>Antheraea frithi</i>	22	10,000
<i>Actias selene</i>	18	52
<i>Rhodinia newara</i>	0	3
<i>Antheraea compta</i>	0	32
<i>Antheraea helferi</i>	0	13
Total	4,740	10,772

Table 3: Diversity indices of wild silk moth community of valley and hilly region of Manipur

Diversity index	Valley region (750 - 850 m ASL)	Hilly region (950-2000 m ASL)
Shannon-Weaver, H	0.2381	0.3438
Simpson, D	1.82	1.728
Menhinick index, Mh	0.07282	0.09635
Margalef richness index, MI	0.4728	0.9693
Berger-Parker Dominance index, $p^{\max}$	0.9538	0.9283
Richness, R	5	10
Sorensen's similarity index, SI	50 %	



Figure 1: Male and female wild silk moths: A - ♂ & B - ♀ *A. proylei*; C - ♂ & D - ♀ *A. frithi*; E - ♂ & F - ♀ *A. roylei*; G - ♂ & H - ♀ *A. assamensis*; I - ♂ & J - ♀ *A. helferi*; K - ♂ & L - ♀ *A. compta*; M - ♂ & N - ♀ *R. newara*; O - ♂ & P - ♀ *A. atlas*; Q - ♂ & R - ♀ *D. grisea*; S - ♂ & T - ♀ *S. canningi*; U - ♂ & V - ♀ *A. selene*; W - ♂ & X - ♀ *Bombyx* sp. (var. *Leimaren*); Y - ♂ & Z - ♀ *B. huttoni*; 1 - ♂ & 2 - ♀ *L. nobilis*.



Figure 2: Distribution of wild silkworms species in various regions of Manipur

feature and reflected in their seasonal incidence. The voltinism of the wild silk moths is varied, as *A. proylei* and *A. pernyi* are weakly bivoltine, *A. roylei* and *A. frithi* are strictly bivoltine, *A. selene* and *S. canningi* are trivoltine, while *B. sp.* (var. *Leimaren*), *S. ricini* and *A. assamensis* are purely multivoltine. The pupae of wild silk moths exhibited variations in colour (light brown, red and black metallic) and weight (ranges from 3.30–8.26 g).

Diversity analysis

15,512 individuals of wild silk moths belonging to 10 species were collected and identified. The higher value of Shannon, Menhinic and Margalef index for the hilly region indicated the species richness and more individuals than the valley region (Table 2). Simpson index and Berger-Parker Dominance index showed slightly higher values for valley than hilly region indicating lower diversity and dominance by some species within the community of silk moths (Table 3). The level of similarity of insect species composition between the hill and valley region was observed to the extent of 50 %.

Morphometric Characterization

The morphometric characterization of the wild adult moths revealed a wide variation in colour, size and shape (Table 4). Antennal character is one of the important parameters for distinguishing male and female individuals. The antennae of male moths have broad fringe and that of female have narrow fringe except *S. canningi*, wherein both the sexes possess broad antennae. *Attacus atlas* is the largest moth having average wing spans of 215.16 mm in males and 221.61 mm in females, while *B. huttoni* is the smallest averaging 35.47 mm in males and 53.22 mm in females.

Cocoon analysis indicated always lower weight in the male (1.38–8.82 g) than in the female (1.63–11.80 g) in all the wild silk moths (Table 5). However, male shell percentage (8.77–15.94 %) is higher than that of female (7.85–15.33 %). The highest average shell percentage is observed in *A. frithi* (15.21 % and 12.49 % in the male and

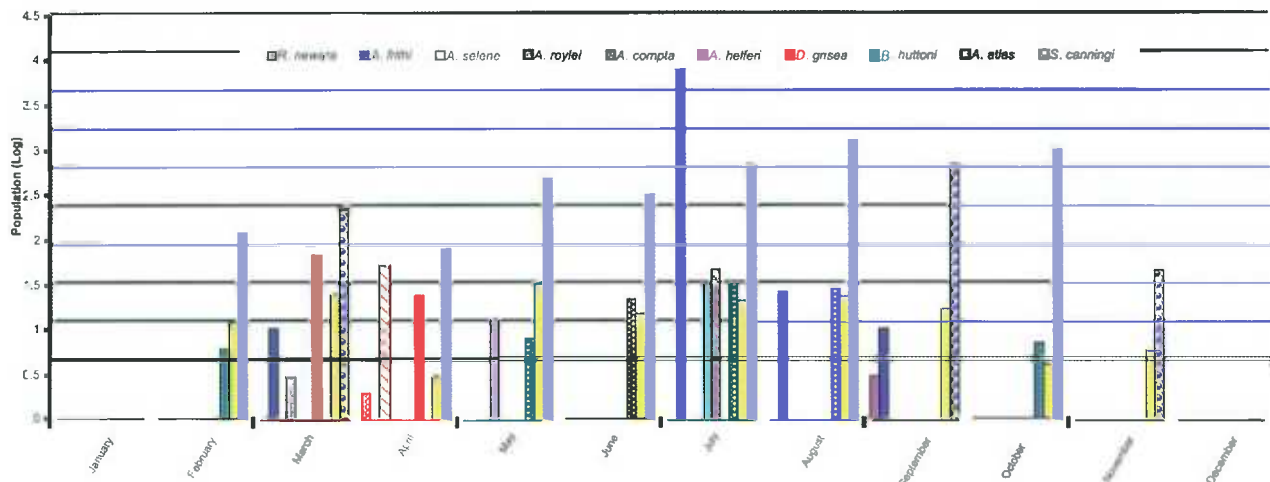


Figure 3: Seasonal incidence pattern of wild silk moth species in Manipur

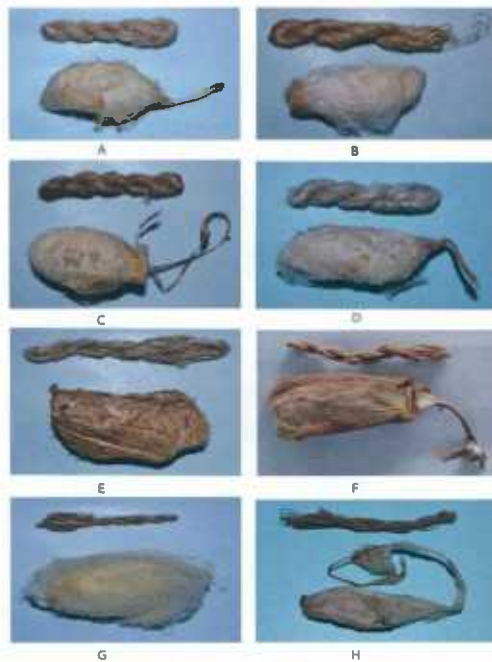


Figure 4: Cocoon and yarns of wild silk moths: (A) *A. proylei*; (B) *A. compta*; (C) *A. frithi*; (D) *A. helferi*; (E) *A. selene*; (F) *A. atlas*; (G) *B. huttoni*; (H) *S. canningi*.

female, respectively) indicating high silk content while the lowest shell percentage and thereby the least silk content was recorded in *A. assamensis* (8.77 % and 7.85 % for male and female, respectively). *A. atlas* was observed to possess the longest peduncle of 115.93 mm. Almost all the wild species spin the cocoons on their host plant, except *A. assamensis* which spins on the ground. All the wild silk cocoons are single layered, except for *A. roylei* that produces a double layered cocoon. *Cricula trifenestrata* and *Actias selene* are conspicuous by having

perforated cocoons while others make compact ones.

Post cocoon analysis was conducted for different wild silk moth species and compared with that of commercially exploited species, *A. proylei* (Tables 6 and 7). The cocoons of *A. proylei*, *A. pernyi*, *A. roylei* and *A. frithi* can be reeled into silk yarn, as the cocoons are compact without preformed exit holes. The raw silk content of *A. frithi* (41.25 %) is at par with that of the commercially important oak tasar silkworm, *A. proylei* (42.50 %). *A. roylei* cocoon contains minimum silk per cent (8.25 %) and *A. pernyi* (61.00 %) have the maximum silk content. The maximum filament length was observed in *A. pernyi* (697.50 m $\pm$ 38.62) and minimum in *A. roylei* (193.50 m $\pm$ 10.75). However, the highest reelability was recorded for *A. frithi* (60.25 % $\pm$ 4.27) and the lowest for *A. roylei* (20.75 % $\pm$ 4.03). Further, productivity of ghicha yarn was also tested (Figure 4). Traditionally, most of the sericulturist women produced raw silk manually using an inverted earthen pot or pedal operated machine called spinner. One of the main objectives for surveying the wild silk moths was to discover the potential species that can yield utilizable silk for fabric production. The pierced (emerged) cocoons were used for ghicha yarn production. Among the *Antheraea* species, *A. helferi* produced the longest thread (1323.30cm) and the shortest was by *A. proylei* (1278.67 cm). Apart from the *Antheraea* group, *Actias selene* recorded maximum yarn (1213.30 cm) and minimum was in *B. huttoni* (290.67 cm). The filament lengths stated here are within agreeable range as reported by Devi *et al.*, 2011.

Table 4: Morphometric characterization of wild adult silk moths (Mean $\pm$ SD)

Species	Colour	Moth wing span (mm)										
		Fore wing			Hind wing		Antenna		Thorax		Abdomen	
		L (mm)	B (mm)	Total wing span	L (mm)	B (mm)	L (mm)	B (mm)	L (mm)	B (mm)	L (mm)	B (mm)
<i>A. frithii</i>	Brick red &	39.65	70.10	140.20	23.09	39.34	10.60	3.72	10.04	8.54	20.33	10.04
	Light brown	± 2.62	± 3.05	± 6.01	± 2.56	± 2.63	± 0.51	± 0.26	± 1.15	± 0.47	± 1.36	± 1.32
<i>A. proylei</i>	Greenish brown	38.92	64.31	128.92	24.93	38.15	12.94	4.30	10.66	9.26	17.33	9.55
		± 2.54	± 3.49	± 19.69	± 3.64	± 3.12	± 0.52	± 0.46	± 1.11	± 0.87	± 1.20	± 1.66
<i>A. assamensis</i>	Deep brown &	29.27	53.35	106.70	21.56	32.91	11.91	4.79	11.04	8.83	22.21	8.36
	Light brown	± 1.68	± 3.61	± 7.23	± 2.84	± 2.53	± 0.19	± 0.22	± 1.17	± 1.23	± 3.04	± 1.21
<i>A. helferi</i>	Copper red &	36.75	72.01	144.01	29.89	42.89	12.11	4.23	10.26	9.11	20.29	9.43
	Yellow	± 1.15	± 1.20	± 2.41	± 1.99	± 2.43	± 0.75	± 0.52	± 0.76	± 0.11	± 1.56	± 1.15
<i>A. pernyi</i>	Camel brown	34.67	59.83	119.88	19.62	34.02	11.12	3.79	10.23	9.29	15.57	8.12
		± 0.84	± 2.27	± 3.10	± 0.54	± 1.46	± 0.63	± 0.32	± 1.15	± 0.55	± 1.23	± 0.62
<i>A. compta</i>	Light yellowish	36.52	63.16	126.33	34.42	39.54	13.39	3.62	10.40	8.48	22.25	10.63
	brown & Yellow	± 1.11	± 0.82	± 4.65	± 1.02	± 0.73	± 1.98	± 0.11	± 0.32	± 0.83	± 1.35	± 0.67
<i>A. roylei</i>	Greenish grey	46.22	72.90	145.80	24.98	36.27	10.69	4.84	9.65	8.58	16.32	9.29
		± 0.70	± 1.95	± 1.74	± 1.10	± 0.95	± 0.20	± 0.44	± 0.66	± 0.47	± 0.57	± 1.14
<i>Rhodinia newara</i>	Yellow & brown	35.00	60.33	120.66	26.85	36.90	12.44	4.36	9.3	6.38	20.54	9.72
		± 0.57	± 2.78	± 5.56	± 0.60	± 0.41	± 0.11	± 0.23	± 0.29	± 0.20	± 0.33	± 0.32
<i>A. atlas</i>	Reddish brown	56.93	109.19	218.39	58.18	57.39	17.51	6.27	13.60	10.94	21.31	9.94
		± 10.41	± 11.31	± 22.63	± 4.63	± 4.69	± 1.20	± 0.49	± 1.13	± 0.74	± 2.24	± 2.11
<i>A. selene</i>	Bluish green+pink	41.87	83.94	169.11	74.73	39.40	14.11	4.90	11.66	10.95	21.38	11.63
	& Bluish green	± 1.58	± 1.77	± 6.83	± 2.14	± 1.34	± 0.95	± 0.71	± 0.66	± 0.81	± 3.06	± 0.93
<i>S. canningi</i>	Greenish brown to	38.49	53.87	107.74	36.50	34.00	10.29	3.64	7.49	6.40	15.93	7.75
	pinkish white	± 3.11	± 3.78	± 7.57	± 5.76	± 4.27	± 0.74	± 0.28	± 0.28	± 0.88	± 1.58	± 0.73
<i>Bombyx huttoni</i>	Blackish brown	10.74	22.18	44.35	7.05	13.02	4.46	1.44	5.55	5.06	14.17	5.43
		± 1.30	± 1.06	± 2.11	± 1.04	± 0.90	± 0.47	± 0.58	± 1.02	± 0.75	± 1.76	± 1.08
<i>Bombyx sp. var. Leimaren.</i>	Dirty white &	8.67	17.71	35.42	6.12	11.03	8.12	1.71	6.07	4.94	12.30	5.54
	Dull white	± 2.04	± 0.79	± 1.61	± 0.77	± 1.11	± 1.30	± 0.15	± 0.49	± 0.06	± 0.04	± 0.55
<i>Dendrolimus grisea</i>	Brown	17.82	36.36	72.73	11.06	23.26	10.89	1.38	8.08	7.55	23.37	7.88
		± 0.47	± 0.90	± 1.80	± 0.30	± 0.82	± 1.18	± 0.12	± 0.18	± 0.25	± 0.51	± 0.48
<i>Lebeda nobilis</i>	Whitish with	10.76	23.33	46.66	7.39	14.77	3.99	0.48	3.09	2.47	10.64	3.67
	spotted black	± 0.40	± 1.00	± 1.99	± 0.22	± 0.26	± 0.12	± 0.04	± 0.17	± 0.90	± 0.20	± 0.24

L=Length; B=Breadth.

DISCUSSION

The Indian Sub-Himalayan belt extending from Jammu & Kashmir in the North West to Manipur in the Northeast is the natural abode of many wild sericigenous species that feeds on naturally grown plants. Saturniidae or wild silk moths of world was recorded (Regier *et al.*, 2008), approximately of 1861 species in 162 genera and nine subfamilies. In the present observations, 17 species

belonging to nine genera viz., *Antheraea*, *Actias*, *Attacus*, *Bombyx*, *Samia*, *Cricula*, *Dendrolimus*, *Lebeda* and *Rhodinia* were recorded from Manipur. Earlier, Thangavelu, (1991) reported nine species belonging to three genera viz., *Antheraea*, *Samia* and *Attacus* in the sub-Himalayan region and Northeast India. Kakati and Chutia (2009) reported 14 species of eight genera, such as *Antheraea*, *Attacus*, *Archaeoattacus*, *Actias*, *Cricula*, *Loepa*, *Samia* and *Sonthonnaxia* from the Nagaland. The

Table 5: Economic parameters of wild silk cocoon

Species	Cocoon character							
	Colour	Shape	Length (mm)	Breadth (mm)	Cocoon weight (g)	Shell weight (g)	Shell percentage	Peduncle (mm)
<i>Antheraea frithi</i>	Greenish yellow	Oval	39.92 ± 1.10	20.05 ± 0.63	5.62 ± 0.50	0.72 ± 0.15	13.52 ± 2.08	68.67 ± 26.02
<i>A. proylei</i>	Greenish brown	Oval	45.36 ± 3.22	24.18 ± 1.55	6.77 ± 0.94	0.67 ± 0.21	10.11 ± 2.34	68.51 ± 34.62
<i>A. assamensis</i>	Light brown	Oval	46.18 ± 2.11	21.05 ± 0.53	5.01 ± 0.38	0.40 ± 0.06	8.31 ± 1.36	32.68 ± 5.71
<i>A. helferi</i>	Light brown	Oval	46.95 ± 2.02	22.46 ± 0.71	7.75 ± 0.94	0.85 ± 0.15	11.15 ± 1.73	56.73 ± 18.79
<i>A. pernyi</i>	Camel brown	Oval	42.04 ± 3.31	23.64 ± 1.37	6.07 ± 1.30	0.72 ± 0.18	11.85 ± 1.15	50.52 ± 8.04
<i>A. roylei</i>	Creamish white	Oval	52.19 ± 3.97	36.59 ± 2.96	6.63 ± 0.56	0.69 ± 0.05	10.66 ± 0.77	83.21 ± 32.57
<i>A. compta</i>	Yellow	Oval	40.59 ± 2.58	17.92 ± 0.95	3.69 ± 0.49	0.33 ± 0.05	9.65 ± 0.60	68.06 ± 17.78
<i>Samia ricini</i>	Creamish white	Oval	48.25 ± 2.93	20.44 ± 1.93	3.68 ± 0.18	0.53 ± 0.05	14.49 ± 1.60	NA
<i>S. canningi</i>	Light brown	Oval	35.60 ± 3.16	14.38 ± 0.39	2.90 ± 0.35	0.39 ± 0.35	13.38 ± 1.31	97.12 ± 22.84
<i>Actias selene</i>	Light brown	Oval	58.65 ± 1.57	28.25 ± 1.04	7.02 ± 0.20	1.02 ± 0.18	14.56 ± 2.13	33.63 ± 1.22
<i>Attacus atlas</i>	Light brown	Oval	74.34 ± 3.49	29.99 ± 1.59	10.31 ± 0.41	1.26 ± 0.07	12.26 ± 0.44	95.37 ± 39.25
<i>Rhoanina newara</i>	Green	Dumb bell with a slid	45.47 ± 2.00	31.82 ± 0.98	5.49 ± 0.67	0.66 ± 1.15	11.91 ± 0.77	70.37 ± 24.49
<i>Cricula trifenestrata</i>	Light brown	Oval	34.84 ± 0.27	12.49 ± 0.46	2.05 ± 0.12	0.16 ± 0.04	7.89 ± 1.54	98.26 ± 11.24
<i>Bombyx huttoni</i>	Creamish yellow	Oval	27.81 ± 1.12	12.46 ± 0.70	2.01 ± 0.17	0.22 ± 0.04	12.78 ± 1.21	NA
<i>Bombyx sp.</i> (var. <i>Leimaren</i>)	Golden yellow	Oval	36.47 ± 1.65	14.37 ± 0.61	1.36 ± 0.19	0.21 ± 0.03	15.62 ± 2.49	NA
<i>Dendrolimus grisea</i>	Brown	Oval	61.29 ± 6.17	17.78 ± 1.11	3.43 ± 0.51	0.27 ± 0.03	7.96 ± 0.79	NA
<i>Lebeda nobilis</i>	Creamish white	Oval	19.13 ± 0.47	7.25 ± 0.39	0.209 ± 0.03	0.024 ± 0.01	11.57 ± 1.02	NA

total number of wild silk moth species collected during the present work is more than those reported from the other states of Northeast region. Among the silk moth species, the overall highest population was with *S. canningi* except during the extremely severe winter months, December and January. Consistent availability indicated that it has high adaptability in the region, offering scope as a promising candidate that can be introduced as a trade variety in the future. The natural population of a few species viz., *R. newara*, *A. helferi*, *A. roylei* and *A. compta* were low. These wild sericigenous insects were not given much attention due to their

unrecognised character and were even treated as pests of the forest and therefore, their population level declined over the years. There is apprehension that certain species may get wiped out from the region. Hence, this calls for initiation of immediate steps towards conservation and population multiplication of sericigenous species that can be exploited for production of silk.

The higher insect species richness and diversity in the hilly region than valley could be because of higher diversity of host plant availability, restrictions on human induced activities, existence of more dense forest areas and

Table 6: Post cocoon characters of economically important wild silkmoth species

Species	Boil-off loss (%)	Raw silk (%)	Denier	Reelability (%)
<i>A. proylei</i>	20.25±1.70	42.50±2.08	5.70±0.57	51.25±2.98
<i>A. pernyi</i>	20.00±2.94	61.00±2.58	5.30±0.21	42.75±2.21
<i>A. roylei</i>	19.50±2.08	8.25±1.70	5.97±0.25	20.75±4.03
<i>A. frithi</i>	16.75±2.21	41.25±2.21	5.45±0.42	60.25±4.27

Table 7: Ghicha yarn of wild sericigenous insects

Species	Ghicha yarn		Single filament	
	Length (cm)	Weight (g)	Length (cm)	Weight (g)
<i>A. proylei</i>	1277 ±52.47	0.52 ±0.08	635±48.05	0.47±0.11
<i>A. compta</i>	1251 ±46.14	0.38 ±0.14	288±37.12	0.20±0.13
<i>A. frithi</i>	1247 ±55.75	0.41 ±0.06	642±33.10	0.43±0.27
<i>A. helferi</i>	1323 ±48.58	0.47 ±0.07	639±49.14	0.40±0.18
<i>A. pernyi</i>	1174±44.23	0.41 ±0.12	612±41.76	0.45±0.26
<i>A. roylei</i>	876±36.44	0.32±0.25	223±36.09	0.18±0.22
<i>A. assamensis</i>	1223±41.85	0.39±0.17	296±43.12	0.21±0.17
<i>A. selene</i>	1214 ±36.16	0.43 ±0.11	273±52.15	0.21±0.21
<i>R. newara</i>	672 ±48.45	0.28±0.21	-	-
<i>A. atlas</i>	939 ±62.06	0.32 ±0.11	286±59.46	0.23±0.25
<i>B. huttoni</i>	291 ±51.53	0.10 ±0.02	214±47.28	0.13±0.31
<i>S. canningi</i>	339 ±29.85	0.15 ±0.23	357±71.81	0.25±0.19
<i>S. ricini</i>	375±43.76	0.20±0.19	-	-
<i>B. sp. (var. Leimaren)</i>	236±38.19	0.12±0.24	605±47.48	0.09±0.27
<i>D. grisea</i>	143±52.13	0.08±0.16	-	-

more favourable environmental conditions. Moreover, natural population of these species has become sparse and distribution is extremely restricted along the hilly areas of the interior forests. Maximum numbers of the host plants of these wild silk moths were forest dwelling trees. Positive relationship observed between food plant and animal species richness revealed that more plant species could provide more niches for the coexistence of animal species (Hutchinson, 1959). *S. canningi* was the most abundant species both in the hilly and valley region because of extensive host adaptability and availability. Fifty per cent of compositional similarity of species (high beta diversity) observed between hilly and valley regions is probably due to habitat fragmentations that blocks the

ecosystem corridors needed for insect dispersal. Ecosystem loss and separation has been noted as the main causes of current biodiversity problems (Sih *et al.*, 2000).

The wild silk moths are spectacular species, which produce lustrous silk and exhibits a great range of variation in life history from egg to adult moth with characteristically different physiological, morphological and feeding parameters. The varied species, showed their capacity to adjust with different ecological conditions that reveals the potential and genetic adaptability to struggle and sustain in varying ecological niches (Kakati and Chutia, 2009). Among the wild silk moths, *A. atlas* is the largest followed by *A. selene* and *A. frithi*. The saturniid

species exhibit genetic diversity and natural variations in the wild population indicative of specialized adaptations to specific niches (Thangavelu, 1991).

Economic parameters of wild cocoons revealed the highest shell percentage with *A. frithi* (13.85 %). The wild silk moths offer a promising future for novel silk with unique features and high economic value. The variability in qualitative and quantitative characters of the cocoon depends on the food plants (Sharma *et al.*, 1995). In terms of filament length and reelability, the wild species, *A. frithi* performed the best and deserves higher value than the commercially exploited oak tasar, *A. proylei*. The present observations indicate that *A. frithi* can come up to the status of commercial exploitation if serious efforts are undertaken and could open up a new venue in tasar silk production. These findings provide additional evidence to the claim that *A. frithi* is the progenitor of the tropical tasar silk moth (*A. paphia* = *A. mylitta*), which has been artificially selected for larger cocoons by humans for millennia (D'Abrera, 2012). The region has salubrious climatic conditions during spring and summer which assure better production of the cocoons. This condition is supported by the outbreak population build-up of *A. frithi* in the region. The diapausing character of these wild silk moths in their natural habitat indicated their adaptability during severe winter. The present scenario is that *B. mori* silks are flooding in the market resulting in low prices. India is the largest consumer of silks in the world, and the consumers are presently been increasingly interested in the wild silk fabrics, that have unique properties. Raw silk reeled from *A. yamamai* is expensive and 40 times costlier than *B. mori* silk, because of its characteristic greenshade and lustre (Akai, 1998). *Bombyx* raw silk is the most popular and the best known textile material for high class fabrics, and it is well-known for its unique sheen. However, the wild silks of *A. yamamai* and *A. assamensis* are even shinier than *B. mori* silk (Akai, 2005). *A. assamensis* produces golden silk and its fabric is unique and expensive. Golden cocoons produced by *C. trifenestrata* is appreciated for its natural colour, though considered a harmful insect. It has become a producer of high price silk in Indonesia (Akai, 2005). In West Africa,

wild silk moth fabric from various *Anaphe* and *Epanaphe* (Notodontidae) is highly valued for its special soft feeling and antibacterial function (Akai, 1998). Thus, the silk generated by wild silk cocoons have their unique identity and position in world commerce.

The habitation of the tribal people in the hilly areas of Manipur is sustained through shifting cultivation or jhum. Deforestation, unsustainable fuel wood extraction, unplanned agricultural practices, encroachment into forest land for agriculture and settlements, forest fire, over grazing by livestock, using oaks for firewood and fodder for livestock and acorns for human food are some important factors contributing to loss of wild sericigenous insects (Singh and Rawat, 2012). The tropical and subtropical biomes of Southeast Asia are increasingly suffering from the effects of deforestation, acid rain, climatic change, and pollution (Peigler, 2012). Increasing urbanization, lopping of trees and lack of knowledge on wild silkworms and their host plants contribute towards extinction of species. Moreover, wild sericigenous insects are often treated as a pest, for instance, *C. trifenestrata* for som tree, though it has immense potential for commercial exploration (Ahmed *et al.*, 1996; Kakati and Chutia 2009). Wild silk moth, *S. canningi* remains an important genetic resource for its cultivated strains *S. ricini*, and this eri silk is the second next to mulberry silk in world production and consumption of silk (Peigler, 2012). Several workers have noted the importance of conserving the natural populations and habitats of these wild sericigenous insects (Singh and Suryanarayana, 2005; Devi *et al.*, 2011; Peigler, 2012). Wild silk moth population comprising diverse gene pool hold great potential utility for mankind. However, species composition and population density appears to be declining due to change in environmental factors caused by extensive deforestation for jhum cultivation, timber and human colonization of forest (Thangavelu, 1991). Worldwide extinction of a species is as high as a species per hour and is the current toll because of the extensive habitat changes brought by mankind (Kothari, 1993). The prevailing scientific opinion is that the next few decades will be a period of maximum danger because possibly, 40 % of the world's present species are likely to

be extinct within 50 years.

Conservation of this precious genetic resource would be imperative for breeding of better adapted and more desired genotypes. Hence, initially, a conservation awareness program may be organized for the local communities in Manipur by introducing wild silkworm farming. This practice not only provides substantial economic gain to tribal people, but conserves forests and regional biodiversity. Conservation of wild population of sericigenous moths is much easier than that of domesticated ones provided it can be carried out *in-situ* (Frankel, 1982). This would ensure protection of forest wealth by utilizing the available food plants for rearing thereby, generating revenue for the inhabitants rather than using forest resources for fuel and timber only. It also improves the genetic variability of indigenous silk moth population by developing improved hybrids (Bhattacharya and Teotia, 2000). Encouragement of traditional skills such as maintenance of sacred groves close to villages, rewarding local conservators and organizing and management of ecotourism would further support these conservation programs (Sahu and Bindroo, 2007). Many important genetic resources of sericigenous insects may become extinct due to large-scale deforestation, threat from various pests and predators, soil and air pollution by chemical insecticides and adverse climatic change. Therefore, proper conservation and utilization of the wild silk moths are the need of the hour to boost the silk production, thereby helping in the conservation and utilization of biodiversity. The descriptive characterization of wild silk moths, cocoons and yarns will support in adopting conservation measures and selecting the prospective wild silk moth suited in the region.

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REFERENCES

- Ahmed S. A , Dutta L. C. and Khound J. N. (1996) Biology and feeding behavior of *Cricula trifenestrata* Helf (Lepidoptera: Saturniidae) a pest of som, *Machilus bombycina* King. *J. Appl. Zool. Res.*, **7**(2): 135-136.
- Akai H. (1998) Cocoon filament characters and post-cocoon technology. *The 3rd International Conference on Wild Silkmooths*, November 11-14, Bhubaneshwar, India, pp. 255-259.
- Akai H. (2005) The potential for non-mulberry silks (wild silks). The 20th Congress of the International Sericultural Commission, Bangalore, India, *Conference Papers*, **2**: 6-11.
- Arora G. S. and Gupta I. J. (1979) Taxonomic studies of some of the Indian non-mulberry silk moths (Lepidoptera: Saturniidae). *Memoirs of the Zoological Survey of India*, **16**:1-63.
- Bhattacharya A. and Teotia R. S.(2000) Conservation strategies of wild silk moths in the North-Eastern Region of India. *Int. J. Wild Silkmooth & Silk*, **5**: 311-313.
- Chima U. D., Omo~~kh~~uua G. E. and Iganibo-Beresibo E. (2013) Insect species diversity in fragmented habitats of the University of Port Harcourt, Nigeria. *ARPJ. Agric. Biol. Sci.*, **5**: 160-168.
- D'Abrera B. (2012) *Saturniidae mundi: saturniid moths of the world*, Part 2, Melbourne: (Hill House), p.174.
- Devi K. I., Singh L. S., Singh N. I., Dutta K. and Singh K. C. (2011) Biodiversity of sericigenous insects and their food plants in Manipur *The Ecoscan*, **5**: 65-68.

- Frankel O. H. (1982) Can genetic diversity survive? In: *Advances in Cytogenetics and Crop Improvement*, Singh R. B., Singh R. M. and Singh B. D. (Eds.), Kalyani Publications, New Delhi.
- Hutchinson G. E. (1959) Homage to Santa Rosalia, or why are there so many kinds of animals? *American Naturalist*, **93**: 145-159.
- Jolly M. S., Chowdhury S. N. and Sen S. K. (1975) *Non-Mulberry Sericulture in India*, Central Silk Board, Bombay.
- Jolly M. S., Sen S. K., Sonwalkar T. N. and Prasad G. K. (1979) Non-mulberry silks. *Agricultural Services Bulletin*, **29**: xvii + 178.
- Jolly M. S., Sonwalkar T., Sen S. K. and Prasad G. K. (1976) Non-mulberry silks. *Manual on Sericulture*, FAO Service Bulletin, United Nations, Rome, **4**: 1-178.
- Kakati L. N. and Chutia B. C. (2009) Diversity and ecology of wild sericigenous insects in Nagaland, India. *Tropical Ecology*, **50**(1): 137-146.
- Kothari A. (1993) Biodiversity convention for those vanishing species: survey of the environment, Ravi N. (Ed.), In: *The Hindu*, Madras, pp. 44-47.
- Lemaire C. and Minet J. (1998) The Bombycoidea and their relatives. In: *Lepidoptera, Moths and Butterflies*, Kristensen N. P. (Ed.), Volume 1: Evolution, Systematics and Biogeography, Berlin (Walter de Gruyter), Inc., Hawthorne, pp. 321-353.
- Metzler E. H. and Zebold R. A. (1995) Twenty eight species of moths new to Ohio from Huffman Prairie, Green County (Lepidoptera). *Ohio J. Sci.*, **95**: 240-242.
- Nässig W. A., Lampe R. E. J. and Kager S. (1996) The Saturniidae of Sumatra (Lepidoptera). *Heterocera Sumatrana*, **10**: 3-10.
- Peigler R. S. (1993) Wild silks of the world. *Am. Entomol.*, **39**: 151-163.
- Peigler R. S. (2012) Diverse evidence that *Antheraea pernyi* (Lepidoptera: Saturniidae) is entirely of sericultural origin. *Tropical Lepidoptera Research*, **22**(2): 93-99.
- Peigler R. S. and Naumann S. (2003) *A Revision of the Silkmoth Genus Samia*. University of the Incarnate Word, San Antonio, Texas.
- Pielou D. C. (1969) *An introduction to mathematical ecology*. New York (Wiley-Interscience), p. 294.
- Regier J. C., Grant M. C., Mitter C., Cook C. P., Peigler R. S. and Rougerie R. (2008) Phylogenetic relationships of wild silkmoths (Lepidoptera: Saturniidae) inferred from four protein-coding nuclear genes. *Systematic Entomology*, **33**: 1-10.
- Sahu A. K. and Bindroo B. B. (2007) Wild silk moth biodiversity in the north eastern region of India: need for conservation. *Indian Silk*, **46**: 16-19.
- Sharma K. K., Sinha A. K., Bansal A. K., Goel A. K. and Sinha B. R. R. P. (1995) Correlation and regression studies between cocoon weight and shell weight in two races of *Antheraea mylitta* Drury on two different food plants. *Sericologia*, **35**: 365-369.
- Sih A., Johnson B. G. and Luikart G. (2000) Habitat loss: ecological, evolutionary and genetic consequences. *Trends Ecol. Evol.*, **15**: 132-134.
- Singh G. and Rawat G. S. (2012) Depletion of oak (*Quercus* spp.) forests on the western Himalaya: grazing, fuelwood and fodder collection. In: *Global perspectives on sustainable forest management*, C. A. Okia (Ed.), In Tech, Shanghai, pp. 29-42.
- Singh K. C. and Chakravorty R. (2006) Seri-biodiversity of North Eastern India - an update. In: *Biodiversity conservation and future concern*, Handique J. P. and Kalita

- M. C. (Eds.), Gauhati University, Guwahati, pp. 8-19.
- Singh K. C. and Suryanarayana N. (2005) Wild silk moth wealth of India. In: *Advances in tropical sericulture*, Dandin S. B., Mishra R. K., Gupta V. P. and Reddy Y. S. (Eds.), National Academy of Sericultural Sciences, Bangalore, India, pp. 419-421.
- Singh N. I., Singh L. S., Singh N. I. and Suryanarayana N. (2008) Diversity of oak fed *Antheraea* spp. in North-east India. *Proceedings of the International Conference on Biodiversity and Conservation Management*, pp. 122-127.
- Singh N. I., Singh N. I., Keisa T. J., Singh Y. R and Singh K. C. (2000) Conservation and utilization of Indian oak fed *Antheraea* fauna. *Int. J. Wild Silkmoth & Silk*, **5**: 330-331.
- Singh R. N. and Maheswari M. (2003) Conservation and utilization of sericigenous insects in North East Region of India. *Sericologia*, **43**: 1-15.
- Thangavelu K. (1991) Wild sericigenous insects of India: A need for conservation. *Wild Silkmoths*, **91**:71-77.
- Thangavelu K., Bhagawati A. K. and Chakraborty A. K. (1987) Studies on some wild sericigenous insects of North-Eastern India. *Sericologia*, **27**:91-98.

Article scientifique

CARACTERISATION ET EVALUATION D'INSECTES SERICIGENES SAUVAGES DU MANIPUR: EXPLORER POUR CONSERVATION

Reeta Luikham¹, T. Shantibala², T. K. James¹ and L. Bidyapati Devi¹

¹Regional Tasar Research Station, Central Silk Board, Mantripukhri, Imphal, Manipur, India.

²Institute of Bioresources & Sustainable Development, Department of Biotechnology, Manipur, India.

E-mail: reeta_luikham@rediffmail.com

RÉSUMÉ

En explorant les insectes séricigènes sauvages du Manipur nous avons identifié 16 espèces appartenant à neuf genres (*Antheraea*, *Actias*, *Attacus*, *Bombyx*, *Samia*, *Cricula*, *Dendrolimus*, *Lebeda* et *Rhodinia*). Le plus grand nombre d'espèces concerne le genre *Antheraea*. Vingt plantes hôtes sont utilisées pour se nourrir par divers insectes séricigènes. Le taux de présence maximum a été observé sur *Lithocarpus dealbata*, *Litsea polyantha* et *Ricinus communis*. La présence saisonnière correspond à une population maximale avec *S. canningi*. La diversité alpha (indice Shannon, Menhinic et Margalef) est plus élevée dans les régions montagneuses ce qui indique une richesse en espèces et en nombre d'individus supérieure à celle des vallées. *Attacus atlas* est le plus grand papillon et *B. huttoni* le plus petit. Le poids du cocon mâle (3,92-6,71g) est plus faible que celui du cocon femelle (6,20-9,19g) mais la richesse soyeuse est supérieure. *A. frithi* a la plus forte richesse soyeuse. Parmi les cocons dévidables, on obtient la meilleure dévidabilité avec *A. frithi* (60,25 % ± 4,27) et la plus faible avec *A. roylei* (20,75 % ± 4,03). Le fil ghicha montre le plus long filament avec *A. helferi* (1323,3 cm), ce qui est même supérieur à celui de l'espèce exploitée commercialement *A. proylei* (1278,67 cm). La caractérisation, l'évaluation et la catégorisation des insectes séricigènes sauvages montrent que *A. frithi* et *S. canningi* sont des candidats prometteurs pour la région. La présence de *R. newara*, *D. grisea* et *A. helferi* est signalée pour la première fois au Manipur. Les niveaux de populations de quelques espèces comme *R. newara*, *A. helferi*, *A. roylei* et *A. compta* sont faibles car rares et endémiques. Ainsi, il est grand temps que des efforts sérieux soient faits pour la conservation et l'augmentation des populations d'espèces séricigènes afin de produire de la soie sauvage d'abord pour l'élévation des conditions tribales, la sauvegarde d'une riche biodiversité et de ressources génétiques de valeur.

Mots-clés: Caractérisation, conservation, évaluation, Manipur, insectes sauvages séricigènes.



SERICULTURE STATUS AND DEVELOPMENT STRATEGIES IN ARGENTINA

Pescio Francisco^{1*} and Marino Patricia²

¹Instituto Nacional de Tecnología Agropecuaria EEA AMBA, Av Udaondo 1695, 1714, Castelar, Buenos Aires.

²Instituto Nacional de Tecnología Industrial - Referente argentina RELASEDA.

*E-mail: pescio.francisco@inta.gob.ar

ABSTRACT

Sericulture in Argentina has had a considerable development until the 1940s but later, it retracted due to the prevalence of synthetic fibers. After a few decades, as a result of the deep economic crisis, sericulture production got revived again, with even the 1st National Sericulture Conference being held in 2002. The Argentine sericulture value chain is focused on the production and processing of silk on a small scale comprising of about 200 small farmers spread over the country. Mulberry plantations are mainly under tree cutting system. The cocoons are transformed by local artisans and entrepreneurs of design, but quality and sale prices vary widely. Gradually, there was an increase in the number of producers and volume of cocoons, influencing the quality of the final products as well as the development of appropriate technologies for silk processing. A novel proposal emerged was the implementation of sericulture in educative centers. Among institutional goals, it's worth to emphasize the integration of Argentina to RELASEDA, the enactment of a specific national law and the continuity in the management of the Sericulture National Conference. The revival of sericulture activities and its steady growth in Argentina as seen today promises to be a tool for development.

Key words: Chain value, sericulture, small farming.

INTRODUCTION

Sericulture and silk production in Argentina has been documented since the seventeenth century (Corcuera, 2006). By the 1930s, the industry was capable of processing more than 400,000 kg of raw silk when the national production was around 70,000 kg/fresh cocoons/annum (División de Sericicultura, 1948). In the early 1950s, the local silk industry became extinct because of the rise in synthetic fiber factories. This incidentally resulted in a major decrease in the number of silkworm rearers, the very few remaining ones being often oriented to non-productive purposes.

In 1993, when Argentina faced a deep economic crisis, new producers and technical agencies incorporated sericulture in their development strategies. Among the most conspicuous lacunae identified were the absolute

dearth of accurate technical information, difficulty in accessing to critical inputs (specially selected silkworm eggs and mulberry varieties), besides other technical problems. Under this general situation, it's appropriate to describe the Argentinian sericulture sector, as a case study to analyze the obstacles faced and advantages of sericulture in a non-traditional sericulture country.

MATERIALS AND METHODS

The methodological strategy used was based on methodological triangulation (Valles, 2007) using quantitative and qualitative methods. Primary sources were based on in-depth interviews with qualified informants, following the methodology proposed by Ander-Egg (2003) and database query of the National Register of Sericulture (MAGyP, 2013). As secondary sources, bibliographic records were used. The value chain

was analyzed by the approach as proposed by Ceverio *et al.* (2010) and Kaplinsky and Morris (2000). The sericulture phases were grouped in accordance to the productive stages as proposed by Soria *et al.* (2001): moriculture, silkworm rearing and processing stage. Each stage was characterized by a brief technology package, and in cases wherever it was possible, we analyzed the different stakeholders involved. Furthermore, we included chain related activities, such as the non-mulberry silkworm rearing, inclusion of sericulture in institutional and legal framework.

RESULTS AND DISCUSSION

Moriculture: The most common mulberry species in Argentina is *Morus alba* L., which is an endemic species and a major woody weed (Ghersa *et al.*, 2002). Original mulberry trees were brought from Italy and Spain (Lanus, 1943). Due to cross pollination, a lot of spontaneous hybrids were generated, which can be a reservoir of genes of interest in the long term.

A peculiarity of the country is that mulberry is widely used in multipurpose systems, as for urban forest, shelterbelts and as animal feed. Nowadays, mulberry fruits are used for preparation of fruit jellies and liquor production. Mulberry leaves are used for livestock feeding and silkworm rearing. In the case of livestock feed, it is intended especially for small farm animals *viz.*, goat and sheep (Brem *et al.*, 2012).

Mostly, the wild mulberry trees and old high stem trees are used for silkworm rearing as described by Grekov *et al.* (2005) and Lim (1990). The strategy consisted of harvesting branches of large size trees located near the rearing house. Although no food shortage was detected, the harvest technique was complex and pesticide contamination was a usual problem (Pescio, 2010 a). According to preliminary estimations, about 30 mulberry trees were necessary to support rearing of 10 grams of silkworm egg (Pescio, 2005). Wild mulberry feeding

strategy was usually cumbersome and generated poor cocoon harvest as well. However, to resolve this, mulberry breeding programs were drawn by identifying plants with characteristics of interest.

The most experienced rearers attempted a gradual implementation of mulberry plantation from hardwood cuttings. Each farm had a range of 0.5 to 3 ha with implanted mulberry, based on the requirement for cocoon production. In subtropical regions (near Brazil), bush type plants were predominant, with a planting density of 10,000 plants / ha, and a low stem pruning system (Grekov *et al.*, 2005). In temperate and semi-arid areas, the density was about 3,000 plants / ha, with medium and high stem pruning system. In several cases, planting schemes included intercropping techniques *viz.*, horticultural crops (lettuce, pumpkin) or green manures, as lucerne or *vicia* sp. (Pescio, 2010 a).

Silkworm rearing: Rearing is performed from the south of the Province of Buenos Aires to the north of the country. At higher latitudes, the combination of low temperature and dry weather due to scarce rainfall regime causes a very short rearing season.

The real quantity of rearing units and silkworms eggs reared is unclear, as the National Registry of Sericulture is voluntary. It was observed that silkworm rearing was spread in very small units, with a diffused pattern of distribution covering Central and Northern areas of the country (Table 1). Currently, some farmers' groups in coordination have been emerging as production clusters.

Table 1: Silkworm rearing in different regions of Argentina

Region	Rearing units (No.)	Egg boxes (No.)
Northwest	50	25
Northeast	130	50
Central	40	17
Total	220	92

The most representative silkworm rearers were those who used sericulture as diversification activity. Most of the production units were located in small towns or suburban areas of cities. A significant percentage of rearers were women, using sericulture as a direct source of cash income. In several cases, sericulture was used to create socialization spaces, since the groups of women who reared larvae used to organize group meetings as a platform to discuss issues. This point scored great appreciation during interviews.

The quantity of seed reared annually by each farmer ranged from 1 to 10 boxes (10 g egg/box), and was defined according to the availability of labor, since in most cases, it was provided exclusively by family members. Almost all farmers reared a complete cycle, from 1st up to 5th instar. Only those units near Demonstration Centers made cooperative rearing of young silkworms.

The predominant source of egg supply was the combination of self-production and purchase of hybrids. Since cocoon yield and quality obtained from self-produced eggs were not consistent and of poor quality, only isolated units could sustain its production over a period. The most experienced farmers mainly used hybrid eggs produced by two national research centers.

Average yields obtained were about 25 kg of fresh cocoons / 10 g egg box, which stands below that of the international average (Cifuentes and Sohn, 1998). The progressive farmers achieved rates of up to 70 % of first class-cocoons. This coincided with progressive additions of hybrid materials.

The most common technique used for young larvae rearing was shelf rearing. For late-age silkworms, either shoot or shelf rearing was used, depending on the size of the rearing house. Diverse approaches were adopted as mounting methods. Initially, rearers used wood branches, like paddy straw mountages (Grekov *et al.*, 2005). However, this method was abandoned on account of being very disadvantageous, producing a lot of 2nd class

cocoons. Later, it was replaced by frames made of recycled poultry egg containers, in a rotary mountage frame style (Grekov *et al.*, 2005). Of late, with the support of Red Latinoamericana de la Seda (RELASEDA), the National Institute of Industrial Technology has started manufacturing plastic mounting systems. This technology was quickly adopted by farmers and today, plastic mounting frames coexist with poultry-egg mounting frames.

Regarding sanitary issues, in subtropical areas, *Nomuraea rileyi* and *Beauveria bassiana* outbreaks were detected, especially during the rainy season. In agricultural regions, numerous cases of poisoning by agrochemicals have been identified, especially in those units where mulberry leaves were harvested outside the limits of farmers' land.

Silk production: The internal trade consists mainly of raw silk or other silk intermediates. The commercialization of fresh cocoons is very reduced. The principal causes which deserve mention are the extremely low volumes produced by each farmer, the lack of intermediate agents, long distances between producers and buyers and deficient knowledge about processing technologies by potential buyers (artisans, designers, companies, *etc.*).

The 2nd class cocoons, *i.e.*, discarded cocoons, and even a proportion of 1st class cocoons were converted into spun silk yarn and non-yarn products (*e.g.*, silk hankies). These products are appreciated because hand-spinning wool and its subsequent products are traditional crafts in Argentina. In fact, we detected a significant number of products made in combination of silk and cotton, wool, llama and vicuna fibers, among others.

Both, farmers and the different entrepreneurs who processed silk, offered a range of products with varying degrees of processing: dried cocoons, skeins of raw silk, silk yarn dyed with natural dyes, silk noil yarn, silk hankies, silk paper, *etc.* According to Acerbi *et al.* (2005) and Pescio (2005), this behaviour is completely rational, because in this way, they can capture a larger share of the

final value of the products (Watanabe and Yamaoka, 1998) and diversify income with products that have different capital turnover rates (Pescio, 2010 a).

The processing units surveyed, mostly of traditional textile artisans and entrepreneurs, required low volumes of silk (about an equivalent to 10 kg raw silk / purchaser / year). While there are some artisanal clusters scattered throughout the country, most of them are in the large cities viz., Buenos Aires, Rosario, Cordoba and Salta. The role of design entrepreneurs has great importance for Argentinian textile value chain, as these Small and Medium Enterprises (SMEs) are usually created by textile, fashion and industrial designers. They generate high value added products, either clothes, furniture or decorative accessories. In this situation, silk was used as raw material in combination with wool, cotton or other natural fibers. This allowed to obtain significant price differentials as well that the terms of trade were relatively equitable into the commercialization chain.

Prices fixation mechanism: Until 2010, information derived from international stock markets was used, as the *Camera di Commercio di Como* (CamCom; 2015). However, it was observed that there were local peculiarities that had distorted international prices taken as reference. During the annual meetings of the National Conference of Sericulture, prices and costs were shared among stakeholders which served as a common reference. Unlike other countries with sericulture tradition and strong industries, silk products in Argentina have not presented a classical behaviour of commodities, with quality systems adjusted and institutionalized commercialization channels. Its behaviour as a product is comparable to that of non-traditional agricultural products (Ceverio *et al.*, 2010). Thus, each farmer had to create his own channel to meet demand, and taking into account the lack of raw material replacement, there was a relative independence of the international prices. This meant that the absence of established channels involved a process of business development for rearers. This apparent lack of market information was a barrier to the new farmers' activity (Carlton, 2005).

This led to a reformulation of the strategy for pricing reference. Generally, transactions of cocoons, yarn and crafts were fixed to local scale (town or region). Furthermore, the production areas were not feasible for the supply of imported silk. In this way, an important proportion of farmers expressed that the cocoon prices did not cover their production costs (this is partly due to inefficient production). All this was manifested as "*the difficulty in acquiring cocoons*" as an expression of the imbalance of demand over supply. However, this local situation was not reflected in the price adjustment. From that moment, stakeholders decided to use international prices for general reference, but the local price fixation would arise from free interaction of supply and domestic demand. Thus, the prices grew up quickly, so the price of raw silk skeins craft to March 2014 was about USD 150/kg; while 1st class fresh cocoons were of around 30 USD/kg.

Support institutions: Analyzing the structure of value chain of sericulture, several authors emphasize the importance of institutions that could organize and promote the activity. In the case of Argentina, it was essential to have public support and international co-operation (ISC & ISA, 2001) to consolidate the sector.

Among the major achievements, the enactment of a Sericulture National Law can be highlighted. Considering the support of public agencies, the actions that were carried out by TEXTILES Center, belonging to National Institute for Industrial Technology (INTI), must be highlighted. Also, the collaboration of the National Institute of Agricultural Technology (INTA) was really valuable, through its Family Agriculture Programs.

An important element was the inclusion of Argentina in 2006 to the Project *Red Latinoamericana de la Seda (RELASEDA)* - Silk Latinamerican Network-, financed by the Instituto Italo Latino Americano (IILA) and with the support of SocioLario (Italy). Silkworm breeding and egg production and biomedical applications, among other areas were studied. In summary, they were able to train more than 15 farmers and professionals through more than 25 training events and scholarship schemes in the major

production and research centers of Latin America and Europe. Later, each of these professional and advanced farmers trained hundreds of local farmers. Selected silk handicraft producers also participated in Fiera Milano 2009.

Three Sericulture Demonstration Centers were established with financial support from RELASEDA. They are located in the Central Region, the city of Realicó (La Pampa), in collaboration with the Housewives' League; in the Northwest Region, in El Galpón in the province of Salta, in collaboration with INTA; and in the Northeast Region, in the Technology Park in Posadas, Misiones province. The last one was constituted as the Entomology Research Center, where research work on silkworm eggs and mulberry, among other species are carried out.

Finally, it is worth to mention that the National Conference on Sericulture has been performed regularly, with an annual frequency. These conferences have a double purpose. On one hand, it offers a venue for presentation of productive experiences and technical advances, and on the other, it is an instance of generating discussion and agreements between the various stakeholders involved. An important milestone was the realization in 2009 of the Latin American Silk Meeting. The leading specialists in the region attended to this meeting.

Educational approach of sericulture: One key element of sericulture in Argentina is its strong cultural component. It was observed that those persons who rear silkworms usually have a different logic than earning profit. It means that, although the usual volumes reared by these subjects are minimal, the activity is not odd for people.

This tradition allowed a great diffusion of sericulture for educational purposes. Educational institutions of different levels (kindergarten, primary, secondary, agro-technical, technological) have progressively incorporated silkworm rearing and silk handicrafts in their curricular

activities. Most of these schools are meant for people in situations of vulnerability (poor, handicapped, *etc.*). Many of them are located in rural areas. About 300 institutions that had incorporated sericulture in their educational strategy were recorded until 2014. Taking into account the experience of many teachers and researchers, a series of participatory meetings was conducted during 2009 which resulted in several educational materials used as methodological guidelines, as Pescio *et al.* (2010 b).

Experiences of non - mulberry silkworm: In Argentina, there are several native species of Lepidoptera belonging to *Rothschildia* genus (*R. schreiretiana*, *R. jacobaceae*, *R. maurus*, *etc.*) (Zapata and Ludueña-Almeida, 2005). Indigenous-peasant communities of northern region of the country have developed techniques and been collecting and processing these lepidopterous cocoons for thousands of years (Fernandez Distel, 2015). However, this knowledge has gradually become extinct. At present, there are some research groups, as Beccacece *et al.* (2011) and Zapata and Ludueña-Almeida (2005) making efforts to revive and systematize these techniques, studying local lepidopteran biological cycles, feeding and reproductive habits. The main goal is to establish a system that can take care of its sustainability. Although this is an incipient study, it would provide a thrust to the vast marginal rural areas so as to improve their economic situation with appropriate use of natural forest.

Conclusion

Sericulture has given a new impetus to the developmental programme of Argentina, although the results are still limited. While it has a historical tradition, its development in the last fifty years was minimal. The deep economic crisis that occurred in the 90s, has in turn shown the way forward to new producers but of course, it needed more of an organized system to sustain the industry. An intensive development strategy began to be implemented by technological supporting institutions, so that sericulture could mainly generate income for farm families, and also fulfill other social and productive functions. However, the learning process has taken several years.

The major lesson in the Argentinian case, as it is a non sericulture traditional country, was the process of construction of a common objective. Argentina couldn't be a cocoon or silk yarn provider, especially regarding high volumes and 1st quality silk. The productive orientation was focused on small scale rearing; enhancement of products with high value addition with short channels of commercialization and, finally, value distribution as fairly as possible between each link of the production chain. This objective required coordination and consensus among stakeholders. The other important issue was the prominent gap between local and international prices. This situation is certainly an opportunity for cocoon and silk imports. However, since this situation has not reached a considerable level, it only acted as an incentive to cocoon production; situation that eventually and hopefully will undoubtedly lead to a gradual decline in price.

REFERENCES

- Acerbi M., Vieites C. M. and Mozeris G. (2005) *Value system of silk products in Argentina*. EFA-FAUBA, Buenos Aires.
- Ander-Egg E. (2003) *Methods and techniques of social research*. Vol. IV: *Data recollection technics*. Lumen, Buenos Aires.
- Beccacece H. M., Zapata A. I., Villafañe N. A., Zarco A., Cherini M. and Drewniak M. E. (2011) Hawkmoths richness (Lepidoptera: Sphingidae) of the Serrano Forest in "La Quebrada Park" natural hydrologic reserve and its surroundings (Córdoba, Argentina). *Rev. Soc. Entomol. Argent.*, **70**: 137-140.
- Brem J. J., Ortiz M. L., Trulls H. E., Zach A., Brem J. C. and Rem J. J. (2012) Seasonal behaviour of mineral content in leaves of *Morus* sp. used in goat feeding. *Rev. Vet.*, **23**: 116-119.
- Camera di Comercio Como (2015) Prezzi seta greggia di qualità 20/22 grado 5A e 6A. www.co.camcom.gov.it.
- Access on 05/1/2015
- Carlton D. W. (2005) Barriers to entry. National Bureau of Economic Research, *Working paper series* No.1645.
- Ceverio R., Brieva S. and Iriarte L. (2010) *Market opportunities guide*, Ediciones INTA, Balcarce.
- Cifuentes C. and Sohn K. (1998) *Sericulture Handbook: Mulberry and silkworm rearing*, Fondo Editorial de Risaralda, Pereira
- Corcuera R. (2006) Land and silk womans, *Editorial Argentina*, Buenos Aires.
- División de Sericicultura (1948) *Silkworm rearing and mulberry crop Instructions*, Ministerio de Agricultura de la Nación, Buenos Aires.
- Fernandez Distel A. (2015) Identification of the material of hunter-gatherer rattles: Lepidoptera importance in Andean archeology. In: *Arqueometría argentina, metodologías científicas aplicadas al estudio de los bienes culturales: datación, caracterización, prospección y conservación*, Pifferetti A. and Dosztal E. (Eds.), Aspha Ediciones, Cordoba.
- Ghersa C., De la Fuente E., Suárez S. and León R. J. C. (2002) Woody species invasion in the Rolling Pampa grasslands, Argentina. *Agriculture, Ecosystems and Environment*, **88**: 271-278.
- Grekov D., Kipriotis E. and Tzenov P. (2005) *Sericulture Training Manual*, Komotini, Greece.
- International Silk Committee and International Silk Association (2001) Development problems in sericulture. In: *Forum "La Seda en los Países de la Comunidad Andina"*, IILA, Quito.
- Kaplinsky R. and Morris M. (2000) *A Handbook of value chain research*. Institute of Development Studies, Canada.

- Lanus E. (1943) *Silkworm: Sericulture principles*. Enciclopedia Agropecuaria Argentina, Buenos Aires.
- Pescio F. (2010 b) *Sericulture: Teachers handbook*. Ediciones INTI, Buenos Aires.
- Lim S. H. (1990) *Sericulture training manual*. Food and Agriculture Organization of the United Nations, Rome.
- Soria S., Salice G. and Avendaño F. (2001) *Sericulture: Practical Handbook*, IILA, Roma.
- Ministerio de Agricultura, Ganaderia y Pesca (2013) Sericulture National Registry. Ministerio de Agricultura, Ganaderia y Pesca de la Nacion, Buenos Aires. Access on 05/06/2014
- Valles M. S. (2007) *Qualitative social research techniques: methodological reflection and practice*. (1^o Edicion), Síntesis, Madrid.
- Pescio F. (2005) Financial evaluation of small scale silk production in Argentina. *Thesis degree*, FAUBA, Buenos Aires.
- Watanabe J. and Yamaoka R. J. (1998) Silk value chain and Paranaense Fórum of Sericulture industrial complex. IAPAR – EMATER, Rio Grande Do Sul.
- Pescio F. (2010 a) Evaluation of situated technologies for sericulture in small-scale systems In: *Memorias Del Congreso Argentino de Extensión Rural*, Potrero de Funes, San Luis, 7-8 Octubre, 2010.
- Zapata A. I. and Ludueña-Almeida F. (2005) Biological aspects and distribution of *Rothschildia schreiteriana* Breyer y Orfila, 1945 and *Rothschildia maurus* (Burmeister, 1879) (Lepidoptera: Saturniidae). *Rev. Soc. Entomol. Argent.*, **64**: 326-330.

Rapport technique

STATUT DE LA SERICICULTURE ET STRATEGIES DE DEVELOPPEMENT EN ARGENTINE

Pescio Francisco^{1*} and Marino Patricia²

¹Instituto Nacional de Tecnología Agropecuaria EEA AMBA, Av Udaondo 1695, 1714, Castelar, Buenos Aires.

²Instituto Nacional de Tecnología Industrial - Referente argentina RELASEDA.

*E-mail: pescio.francisco@inta.gob.ar

RÉSUMÉ

La sériciculture en Argentine a eu un développement considérable jusque dans les années 1940 mais elle s'est rétractée ensuite en raison de la prévalence des fibres synthétiques. Après quelques décennies, à la suite de la forte dépression économique, la production séricicole a de nouveau repris avec même la première Conférence Nationale de Sériciculture en 2002. La chaîne de production séricicole argentine est basée sur la production et la transformation de soie à petite échelle avec 200 petits fermiers environ dispersés dans le pays. Les plantations de mûriers sont principalement issues de bouturage. Les cocons sont transformés par des artisans locaux et des entrepreneurs de design, mais la qualité et le prix de vente varient énormément. Graduellement le nombre de producteurs et le volume de cocons se sont accrus ce qui a influencé la qualité des produits finis ainsi que le développement de technologies appropriées pour la transformation de la soie. Une nouvelle proposition d'implantation de la sériciculture dans des centres éducatifs a émergé. Parmi les objectifs institutionnels, il faut insister sur l'intégration de l'Argentine au RELASEDA, une loi nationale spécifique et la continuation de la gestion de la Conférence Nationale de Sériciculture. La revitalisation des activités séricicoles et leur croissance constatée aujourd'hui en Argentine promettent d'être un outil pour le développement.

Mots-clés: Valeur de la chaîne, sériciculture, petit fermage.

International Workshop "Potential of Sericulture and Silk Industry for Employment & Income Generation in AARDO Member Countries" held at Mysuru, India from 11-16 April, 2016



As part of ISC efforts to develop sericulture and silk industry across the countries, an international workshop on sericulture industry was held at Mysuru, India, during 11-16 April, 2016. This prestigious event was jointly organised by International Sericultural Commission (ISC), Bengaluru, African-Asian Rural Development Organisation (AARDO), New Delhi and Central Silk Board, Ministry of Textiles, Govt. of India, Bengaluru. The workshop was inaugurated by His Excellency Eng. Wassfi Hassan El-Sreihin, Secretary General, African-Asian Rural Development Organization. The event witnessed the participation of 19 delegates from 13 countries besides 25 scientists and technocrats of Central Silk Board, India.

The workshop was structured in a way to enable the country delegates to study the successful economic models of sericulture practice developed in India for replicating in their respective countries. The two days' workshop deliberations resulted in adopting major policy recommendations for future actions. The remaining four days were devoted for exposure visits to sericulture areas and facilities in and around Bengaluru and Mysuru. The field visits have provided an opportunity for the delegates to study on how the Indian silk industry has emerged as a commercially viable enterprise for employment generation and poverty alleviation.



Scholarship Programme commenced

A new initiative of ISC namely; "Scholarship Programme" commenced at Central Sericultural Research & Training Institute (CSRTI), Central Silk Board, Mysuru. The Scholarship Programme aims to inspire the youngsters in sericulturally developing ISC Member Countries to take up research on sericulture and related fields or specialised studies/assignments by providing them opportunity to further their interest.

Two researchers from Plan Protection Research Institute, Cairo, Egypt, namely Dr. Ibrahim Abdul Saad and Dr. Mahmoud Saad Ibrahim Saad have undergone the 1st batch of Scholarship Programme for a period of one month starting from 4th January 2016. They have undertaken research scholarship work in the areas of Silkworm Rearing, Physiology and Pathology. Apart from working in the respective laboratories, the researchers were also exposed to the various activities of sericulture industry in different areas in and around Bengaluru and Mysuru.



Updates on 24th ISC Congress, Bangkok, Thailand; 10-14 August, 2016

The Final Announcement on the 24th ISC Congress scheduled to be held at Bangkok, Thailand during 10-14 August, 2016 has been released by the organisers. For more details, please visit the website; <http://www.qsds.go.th/isccongress24th/>.

Dates to Remember:

- | | |
|-----------------------------------|----------------------------|
| 1) Submission of research papers: | 15 th May 2016 |
| 2) Registration with payment: | 10 th July 2016 |



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ANNOUNCEMENT

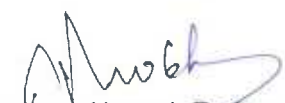
The International Sericultural Commission (ISC), Bangalore, India, an inter-governmental organization engaged in the development of sericulture and silk industry, has been conferring the prestigious Louis Pasteur Award on persons who have made outstanding contributions to the development of silk industry in their country or across the world. The award is given away once in three years for three persons selected from across the globe. The Louis Pasteur Award consists of a Citation and a Medal. The Award for 2016 shall be presented during the 24th ISC Congress on Sericulture and Silk Industry scheduled to be held at Bangkok, Thailand during 10-14 August, 2016.

International Sericultural Commission hereby invite nominations for the **Louis Pasteur Award-2016** from the ISC Member Countries (**Afghanistan, Bangladesh, Brazil, DPR Korea, Egypt, France, Greece, India, Iran, Indonesia, Japan, Kenya, Madagascar, Romania, Syria, Thailand and Tunisia**). The nominated candidates should have involved with the development of sericulture industry in their country or across the world. Each Member Country can nominate up-to two candidates for the Award. The candidates may be identified by an Expert Committee among the applications received from interested candidates through open advertisement. The nominations of each candidate should be submitted along with the following documents:-

- (a) Duly filled-in "Application Form for Louis Pasteur Award". The Application Form is attached.
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The nominations along with all relevant details from the Member Countries should be sent to the Secretary General, International Sericultural Commission, Bangalore, India, by **31st May 2016**, through E-mail: iscbangalore@inserco.in.

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


Dr. H. Nagesh Prabhu
Secretary General

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

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

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Sericologia

International Sericultural Commission

CSB Complex

BTM Layout, Madiwala, Bengaluru - 560068

Karnataka, India

Phone: +91-80-26282191

+91-80-26680162

+91-80-26681663

Fax: +91-80-26681663

Email: sericologia@gmail.com

Website: www.inserco.org