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PURIFICATION AND IDENTIFICATION OF PHENOLICS FROM MULBERRY FRUIT

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ABSTRACT

Mulberry fruit is an excellent source of polyphenols, which contribute potential antioxidants that promote health. In this study, we have worked out a procedure for purification and identification of the polyphenol compounds extracted from mulberry fruit. Results showed that 30 % ethanol as solvent could efficiently extract the phenolic content. The amount of phenolics obtained (403.55 ± 0.02 mg GAE/g DW) using 30 % ethanol was 2.56 folds higher than that extracted with distilled water. After purification by high-pressure liquid chromatography, LC-MS/MS was performed to identify these compounds. The major compounds of the phenolic extracts from mulberry fruit were identified as belonging to the anthocyanins family of cyanidin-3-O-glucoside (MW. 287.59) and aglycone cyanidin (MW. 448.94).

Key words: Identification, mulberry fruit, polyphenol, purification.

INTRODUCTION

Mulberry (*Morus alba*) is a deciduous, fast growing, medium size tree, which has been historically used in sericulture for thousands of years. All parts of mulberry plant such as leaves, fruits, bark and branches have long been used in Chinese medicine. Mulberry fruit is known as traditional edible fruit in China, which contains many bioactive components, such as flavonoids (e.g. anthocyanins), alkaloids (e.g. 1-deoxynojirimycin), phenolic etc. (Kobus-Cisowska *et al.*, 2013; Sivakumar *et al.*, 2015). Those bioactive compounds show many functional characteristics, against cancer as well as in delaying aging process, fighting neurological diseases, inflammation, diabetes and bacterial infections (Wang *et al.*, 2013). Nowadays, in China, many mulberry fruit products are in commercial market, such as jams, juices, wine, vinegars, ice-creams, marmalades and other food related products. Due to increased awareness on the functional quality characteristics, good taste, and

nutritional value, the production and consumption of mulberry fruit have increased rapidly in recent years (Hu *et al.*, 2014).

Previously, we have evaluated the main chemical composition of anthocyanin, 1-deoxynojirimycin (DNJ), flavonoid, polysaccharides, amino acids, and protein contents from nine mulberry fruit varieties (Sivakumar *et al.*, 2015). We have also studied the ethanol flavonoid extract from mulberry fruit, it possessing a strong antioxidant activity both *in vivo* and *in vitro* (Sivakumar *et al.*, 2016). Mulberry fruit extracts contain high amounts of phenolic compounds, and have shown strong antioxidant activities (Kang *et al.*, 2006; Ajaykrishna *et al.*, 2017). In one of our earlier attempts, we could optimize the process conditions for the extraction of polyphenols from mulberry fruit phenols by response surface methodology (RSM) (Ajaykrishna *et al.*, 2016). Phenols are aromatic compounds containing one or several hydroxyl groups directly attached to the benzene ring. According to the

number of hydroxyl groups, phenols are classified as dihydric, trihydric or polyhydric. By the year 2005, thousands of polyphenolic compounds were isolated from different plants (Keskin-Šašić *et al.*, 2012).

While attending to the growing demand on mulberry fruit, in view of its health promoting features, the identification of phenolic substances in it is inevitable for assessing their biological and nutritional values. In this direction, here, we report an effective procedure for the purification and identification of phenolic compounds extracted from mulberry fruit by HPLC and LC- MS/MS analysis.

MATERIALS AND METHODS

Materials

Samples

Mulberry fruits were harvested from *Morus* J33 (*Morus alba* L.) variety grown in the plantation of National Mulberry Orchard (Zhenjiang, PR China). The fruits were then freeze-dried (EYELA FDU-2100, Japan) and ground to powder.

Chemicals

Standards of Gallic acid, HP-20 resin were purchased from Sigma Chemical Co. (St. Louis, MO, USA) whereas, n-hexane, ethanol and methanol were procured from Sinopharm chemical reagent co. Ltd., Shanghai. All other reagents used were of analytical grade.

Instruments

Constant-flow pump (BT1-100E, Shanghai), Spectrophotometer (UNICO UV-2100), UV/Vis spectrophotometer (Shimadzu UV-2450), L-7100Pump/HPLC (HITACHI) and Mass spectrometer (Finnigan LCQ Deca XP Plus, America) were utilized.

METHODS

Separation of phenolic compounds from mulberry fruit

The phenolic compounds from mulberry fruit were

extracted according to the previously reported method (Ajaykrishna *et al.*, 2016). The extracts were evenly dissolved in deionized water and this concentrated solution was chromatographed via a 40 cm x 2.5 cm Diaion HP-20 column and eluted with ethanol (Dugo *et al.*, 2001).

At first, the resin was activated by adding 95 % ethanol, for one hour and washed completely with distilled water. Then the extracts were packed into the column by dissolving in 3 ml of 95 % ethanol and covered with sea sand to avoid the resin from floating (Chen *et al.*, 2006). In total, 50 fractions were collected for further processes.

UV spectrophotometer

The dionex collected from the separated samples through fractionation process were subjected to UV spectrophotometer to determine the visible spectra of samples by scanning the absorbance between 200 to 750nm (Fazaeli *et al.*, 2013). The absorbance readings were made against distilled water as blank. All the measurements were carried out at room temperature.

Purification of phenolic compounds by HPLC

After evaporation of the organic solvents, the resultant extracts were dissolved in deionized water. Shortly, the extract was subjected to high performance liquid chromatography (HPLC) analysis.

The fraction was filtered through 0.22 µm filter disk and 10 µl of the filtrate was injected into a 5µm RP-18 column (4.5 x 250 mm). Chromatography was monitored at 280 nm with a flow rate of 0.8 ml/min at 35 °C (Tang *et al.*, 2013).

Identification of the phenolic compounds by LC-MS/MS analysis

Gradient elution was used with methanol and water as mobile phases (methanol and aq. 0.1 % formic acid,1:1) with the run time of 60 minutes. The flow rate was regulated at 0.8 ml/min at a maximum pressure of 431 bar by using the wavelength at 280 nm. The dionex C18 column (150 mm x 4.6 mm) was used in this analysis.

Subsequently, the injection volume was 10 μ l and column oven temperature was maintained at 30 °C. The MS detector setup was fixed with acquire time of 60 minutes, collision energy of 15.0 (v) and mass range between 50.00-2000.00 using both positive and negative modes (Koolen *et al.*, 2013).

Statistical analysis

Design-expert software 8.06 (Statease Inc., Minneapolis, USA) and MINITAB® software 16.2.0 (Inc., USA) were used for the experimental design and subsequent regression analysis of the experimental data. All data are presented as mean $\pm$ SD.

RESULTS

Isolation of phenolic compounds from mulberry fruit

The phenolic compounds were obtained from mulberry fruit by hot water extraction and ethanol precipitation. Figure 1 shows the elution profile of the phenolic compounds on the non-ion exchange column, during the purification process. Ethanol at 30 % concentration showed more efficiency in the purification of phenolic compounds *i.e.*, the highest of 403.55 ± 0.02 mg GAE/g DW of total phenolic content in the extracted elute. The phenolic content has shown a decreasing trend with increasing ethanol concentrations (Table 1). The UV absorption spectra (Figure 2) of phenolic compounds showed absorption at 280 nm. The spectral information provided through the UV spectra were utilized for further purification by HPLC technique and for identification of phenolic compounds.

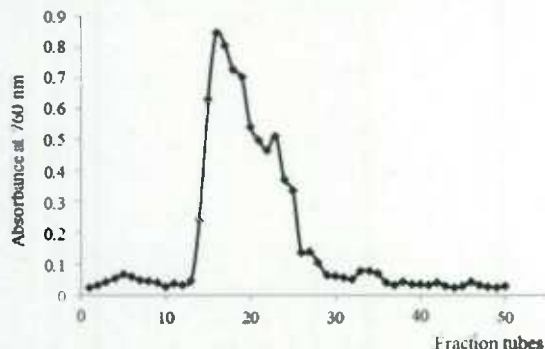


Figure 1: Elution profiles of phenolics extracted from mulberry fruit by non-ion column chromatography

Fraction tube: 1-10, dis. H₂O; 11-20, 30 % ethanol; 21-30, 50 % ethanol; 31-40, 70 % ethanol and 41-50, 90 % ethanol.

Table 1: Total phenolic content of purified samples with different ethanol concentrations

Polar solvent	Total phenolic content (mg GAE/g DW)
Distilled water	157.55 $\pm$ 0.02
30 % ethanol	403.55 $\pm$ 0.02
50 % ethanol	266.75 $\pm$ 0.04
70 % ethanol	167.98 $\pm$ 0.07
90 % ethanol	103.73 $\pm$ 0.02

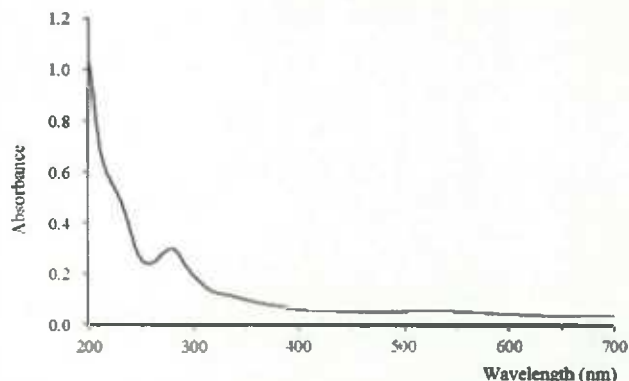


Figure 2: Ultraviolet absorption curve of extracted phenolic compounds from mulberry fruit

Purification of the extracted phenolic compounds by HPLC

To understand the components of the particular compound, the phenolic elute extracted by 30 % ethanol was subjected to high-pressure liquid chromatography. As shown in Figure 3, the phenolic extract was completely separated into its components. The peaks merged at 3.648 min. The purified compounds were finally identified by LC-MS/MS.

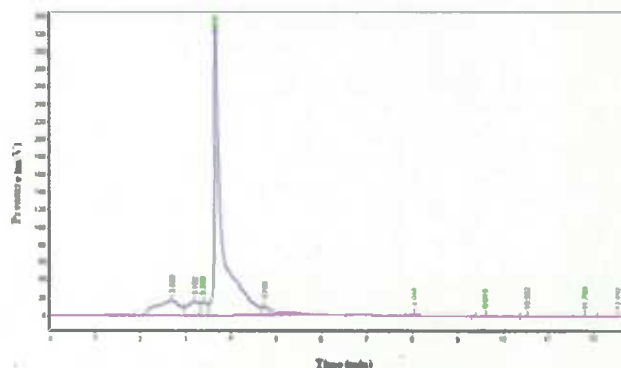


Figure 3: Phenolic compounds extracted using high-pressure liquid chromatography

Identification of the extracted phenolic compounds by LC-MS/MS

The separated phenolic compound was analyzed by LC-MS/MS. The 1st mass spectrum (MS) showed that *m/z* compounds are of 287.59, 449.48, 595.38 and 649.35 in molecular weight (Figure 4A). The 2nd mass spectrum (MS/MS) showed that the compounds are of 287.59 and 448.94 in molecular weight (Figure 4B). A reference to the database (Fornasaro *et al.*, 2016), revealed that the fragment ion at 449 *m/z* correspond to cyanidin-3-O-glucoside, while the fragment ion at 287 *m/z* correspond to aglycone cyanidin.

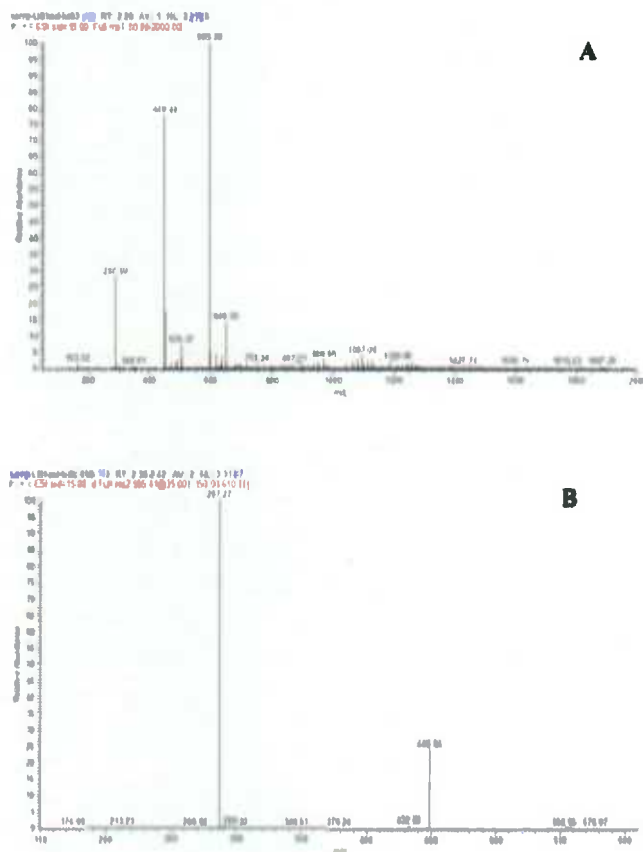


Figure 4: Identification of phenolic compounds by LC-MS/MS
A: 1st mass spectrum; B: 2nd mass spectrum.

DISCUSSION

Polyphenols are known as advantageous antioxidant agents. The ethanol extract from mulberry fruit (*M. alba*)

and that from five Brazilian plants (*Hyptis martiusii*, *Mentha arvensis*, *Momordica charantia*, *Eugenia jambolana*, and *E. uniflora*) have revealed the presence of phenolics with high antioxidant activity (Imran *et al.*, 2010; Santos *et al.*, 2010).

Our results indicated that the total polyphenol content of mulberry fruits ranged from 230.05 ± 0.05 to 23.59 ± 0.06 mg GAE/g dried weight. Polyphenol content in mulberry fruit is much higher than that of other berries including gooseberry (5.2 mg/g), elderberry (19.5 mg/g), chokeberry (20.1 mg/g), red currant (5.4 mg/g) and black currant (13.3 mg/g) (Wu *et al.*, 2004), and even higher than that in grapes (0.7 mg/g) (Kallithraka *et al.*, 2005). There have been observations that the polyphenol content of fruits of *Morus* species vary with varieties and plant locations.

The polarity of the extracting solvent and solubility of the compound in the solvent are key issues in recovery of phenolic compounds (Abozed *et al.*, 2014). In this study, we used ethanol as a solvent since it has been proven efficient for polyphenol extraction (Do *et al.*, 2014). Our results indicated that non-ion exchange chromatography could efficiently increase the phenolic content of the extract, and ethanol at 30 % brought out the best results. The phenolic content was 403.55 ± 0.02 mg GAE/g DW in 30 % ethanol extracted elute, which is 2.56 folds higher than that of the distilled water extracted elute. In order to purify and identify the polyphenol compounds, high-pressure liquid chromatography followed by LC-MS/MS was performed. The main compounds of the phenolic extracts were detected as cyanidin-3-O-glucoside and aglycone cyanidin. This is the first report citing these compounds as the major phenolics in mulberry fruit by MS/MS.

Cyanidin-3-O-glucoside and aglycone cyanidin belong to anthocyanins family. The antioxidant functions of anthocyanins have been ascribed to the aglycone moiety, cyanidin and glycosides. But the number of sugar residues at the 3-position, the oxidation state of the C ring, the hydroxylation and methylation pattern, as well as the acylation by phenolic acids are considered crucial factors for the expression of antioxidant effects (Bae and Suh, 2007).

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MODULATION OF CYTOTOXIC MOLECULES AND ENZYME ACTIVITIES DURING MOULTING CYCLE OF TASAR SILKWORM, *ANTHERAEA MYLITTA*

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ABSTRACT

Moulting is a stressful event in insect development, particularly in silkworm. As four incidences of moulting occur during larval stages of tasar silkworm, *Antheraea mylitta*, a baseline study of physiological responses during moulting period is necessary for a better management of tasar crop. The information would serve as a base to form strategies to counteract the high oxidative pressure encountered during the moulting stages so that a well-regulated larval phase could lead to higher productivity. With this aim, the study focussed on the analysis of physiological events, such as oxygen consumption and estimation of biochemical components viz., superoxide radicals, nitric oxide, total hydroperoxide, phenol oxidase, glutathione-S-transferase, reduced glutathione and ascorbic acid in haemolymph of *A. mylitta* during pre-moult, inter-moult, ecdysis and post-moult conditions. Superoxide radical level enhanced during inter-moult and ecdysis. Besides, levels of oxygen consumption, nitric oxide, and hydrogen peroxide also significantly increased during the period of ecdysis. Similarly, enhanced phenol oxidase, glutathione-S-transferase activities and ascorbic acid content were also observed during ecdysis. However, lower, reduced glutathione content was noticed during ecdysis, which may be interpreted as a strategy to prevent post-hyperoxic oxidative damage in tasar silkworm during ecdysis. The understanding of such physiological strategies of tasar silkworm may provide insight on how the insect combats the ill effects of oxidation through its innate defence mechanism in order to maintain the functional homeostasis.

Key words: Antioxidant defences, moulting, oxidative stress, tasar silkworm.

INTRODUCTION

Insect increases its body size through molting. Molt sequence includes apolysis (the separation of the cuticle from the epidermis), synthesis of a new cuticle and ecdysis (the shedding of the cuticle of the preceding instar, or stage). In addition to changing of structures and morphology, several biochemical changes also occur during molting cycle, such as the level of glucose, N-acetylglucosamine, glucosamine (Salaenoi *et al.*, 2006), glycogen, trace elements, proteinase (Salaenoi, 2006) organic and inorganic compounds in hemolymph, epidermal tissue and cuticle (Pratoomchat *et al.*, 2002).

Tasar silkworm is a holometabolous insect with four molting episodes and larva reaches the fifth instar without major phenotypic changes, except for a remarkable increase in body size.

In aerobic organisms, a variety of biochemical reactions induce the formation of reactive oxygen species (ROS). ROS are molecules derived from oxygen, such as the superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\cdot}$), among which, $OH^{\cdot}$ is highly cytotoxic and produce deleterious effects on biomolecules (Pannunzio and Storey, 1998). In order to counteract the ROS, organisms have evolved antioxidant

defense mechanisms, which consist of both enzymatic and non-enzymatic components. Enzymatic antioxidants consist of superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione-S-transferase (GST), and non-enzymatic counterparts include reduced glutathione (GSH) and ascorbic acid (ASA) (Halliwell and Gutteridge, 2001). In the enzymatic cascade, SOD is the first candidate, which deals with oxyradicals *i.e.*, dismutation reaction of O_2^- to H_2O_2 and O_2 . The H_2O_2 is in turn converted to water and oxygen by CAT. Other than this, reduced glutathione (GSH) and ascorbic acid (ASA) can directly bind with ROS and neutralize them.

Moulting is highly disruptive to respiratory physiology (Camp *et al.*, 2014), and the concentration of cellular oxygen is a vital component of many physio-biochemical processes. Hence, the hypothesis of this study is that disrupting the moulting cycle may alter the respiratory physiology and physio-biochemical status of *A. mylitta*. Information on the responses of antioxidant defense in the moulting cycle of *A. mylitta* has not been reported till date. Therefore, the present study was undertaken in order to investigate the status of oxidative stress and antioxidant defense in molting cycle of *A. mylitta*.

MATERIALS AND METHODS

Study animal

The bivoltine Daba ecorace of *A. mylitta* was selected for the study. Silkworm rearing was conducted on *Terminalia tomentosa* food plants in the field laboratory of the Central Tasar Research and Training Institute, Ranchi, India. Moulting stages (fourth molt) of *A. mylitta* such as pre-moult, inter-moult, ecdysis and post-moult (Figure 1) were identified according to their morphological characteristics.

Measurement of volumetric oxygen consumption

A respirometer was designed (Jena *et al.*, 2013) on the principle of Warburg's apparatus. For absorption of CO_2 , KOH (15 %) solution soaked in blotting paper was kept inside the flask with live larvae. The control was simultaneously run without larvae. The displacement of

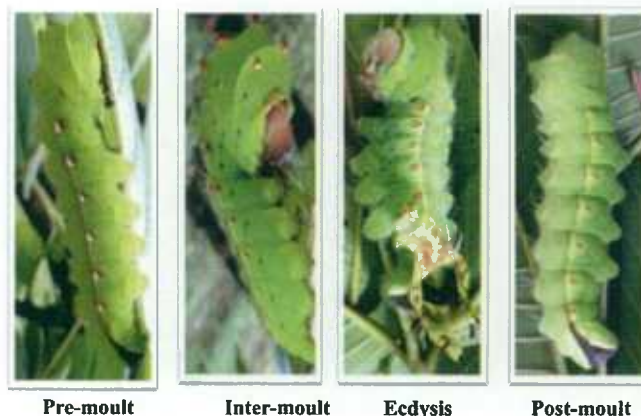


Figure 1: Morphological features of tasar silkworm *A. mylitta* during different stages of the moulting cycle

colour solution in the capillary was recorded. This was equivalent to the oxygen intake. The rate of oxygen intake was calculated as ml/g live tissue/h.

Estimation of biochemical constituents

Superoxide anion

Generation of intra-haemocyte superoxide anion in haemolymph was estimated spectrophotometrically by nitroblue tetrazolium (NBT) reduction reaction modified after Song and Hsieh (1994). Haemocytes were sedimented by cold (at 4 °C) centrifugation at 3000 g for 5 minutes and washed with PBS. It was then stained with NBT solution (0.03 %, 1 ml), vortexed and incubated for 30 min at 37 °C. Haemocyte suspension was sedimented for removal of NBT solution and reaction was stopped by adding 1 ml absolute methanol for 1 min. The formazan products were sedimented and solubilised by using mixture of KOH (4M) and dimethyl sulfoxide (DMSO). The dissolved formazan was measured at 630 nm and level of superoxide anion was expressed as OD /ml haemolymph.

Nitric oxide

Generation of intra-haemocyte nitric oxide (NO) level in haemolymph was estimated spectrophotometrically by Griess reaction (Green *et al.*, 1982). Haemocytes were sedimented by cold centrifugation at 3000 rpm for 5 min and was made to react with Griess reagent (1:1 v/v ratio of

0.1 % N-naphthyl-ethylene-diamine dissolved in 2.5 % phosphoric acid and 1 % sulphanilamide in water) and incubated for 30 min at 37 °C. NO was determined from the molar concentration of nitrite in the sample which was determined by using sodium nitrite as standard. The level of NO generation was expressed as μM nitrite / ml haemolymph.

Hydrogen peroxide

Hydroperoxides (water soluble form) were determined spectrophotometrically according to the method of ferrous oxidation with xylenol orange (FOX1) (Wolff, 1994). Hydroperoxides oxidize ferrous to ferric ions selectively in dilute acid and the resultant ferric ions can be determined by using ferric sensitive dyes as an indirect measure of hydroperoxide concentration. Xylenol orange binds ferric ions with high selectivity to produce a colour (purple-blue) complex. The absorbance was read at 560 nm after removal of any flocculated material by centrifugation at 4000 g for 10 min. The signal was read against H_2O_2 standard curve and expressed as nmol/mg protein.

Ascorbic acid

Samples (haemolymph) were precipitated in 5 % (w/v) TCA in an ice bath and were centrifuged at 5000 g for 10 min. The supernatant was used for the estimation of ASA and GSH. Ascorbic acid content was determined according to the method of Mitusi and Ohata (1961). The reaction mixture contained 2 % sodium molybdate, 0.15 NH_4SO_4 , 1.5 mM Na_2HPO_4 and sample. The mixture was boiled at 90 °C for 45 min and then centrifuged at 3000 g for 10 min. The absorbance of the supernatant was measured at 660 nm. ASA was used as standard and results were expressed as $\mu\text{g ASA/mg protein}$.

Reduced glutathione

The supernatant obtained as described above was used to assay GSH level (Ellman, 1959). Aliquots of supernatant (0.5 ml) were mixed with 0.6 mM DTNB and were incubated for 30 min at room temperature in dark. The sample was finally centrifuged at 3000 g for 10 min at room temperature and the absorbance of the supernatant

was measured at 412 nm. GSH was used as standard and results expressed as nmol GSH/mg protein. The protein content was estimated by the Bradford (1976) method using bovine serum albumin as standard.

Phenol oxidase (PO) activity

The PO activity was measured according to Asokan *et al.* (1997) by using L-DOPA as a substrate. The reaction mixture contained 850 μl of 100 mM phosphate buffer (pH 7.4), 100 μl of L-DOPA (10 mM) and 50 μl haemolymph sample. The reaction mixture was incubated for 30 min at 37 °C. The change in absorbance was recorded at 490 nm and enzyme activity was expressed as nmol dopachrome/mg protein which was calculated from the molar extinction coefficient of dopachrome as $3.6 \text{ mM}^{-1} \text{ cm}^{-1}$.

Glutathione-S-transferase activity

Glutathione-S-transferase activity was measured according to Habig *et al.* (1974) using 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate. Assay mixture contained 2.7 ml of 100 mM phosphate buffer (pH 7), 0.1 ml of 30 mM GSH, 0.1 ml of 15 mM CDNB and 0.1 ml of sample (haemolymph) containing 100 μg proteins. The change in absorbance of the above mixture was recorded at 340 nm and enzyme activity was expressed as nmol CDNB conjugate formed/ min/ mg protein using a molar extinction coefficient of CDNB-GSH conjugate as $9.6 \text{ mM}^{-1} \text{ cm}^{-1}$.

Statistical analysis

Significant variations in physio-biochemical parameters were investigated by one-way ANOVA, and post hoc tests were used to discriminate between values. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Tropical tasar silkworm, *A. mylitta* is a commercially important insect. Moulting in silkworms is a process closely associated with their growth and development. Tasar silkworm is a tetramoulter and larval body mass increases from a mere 10 mg while hatching to as many as

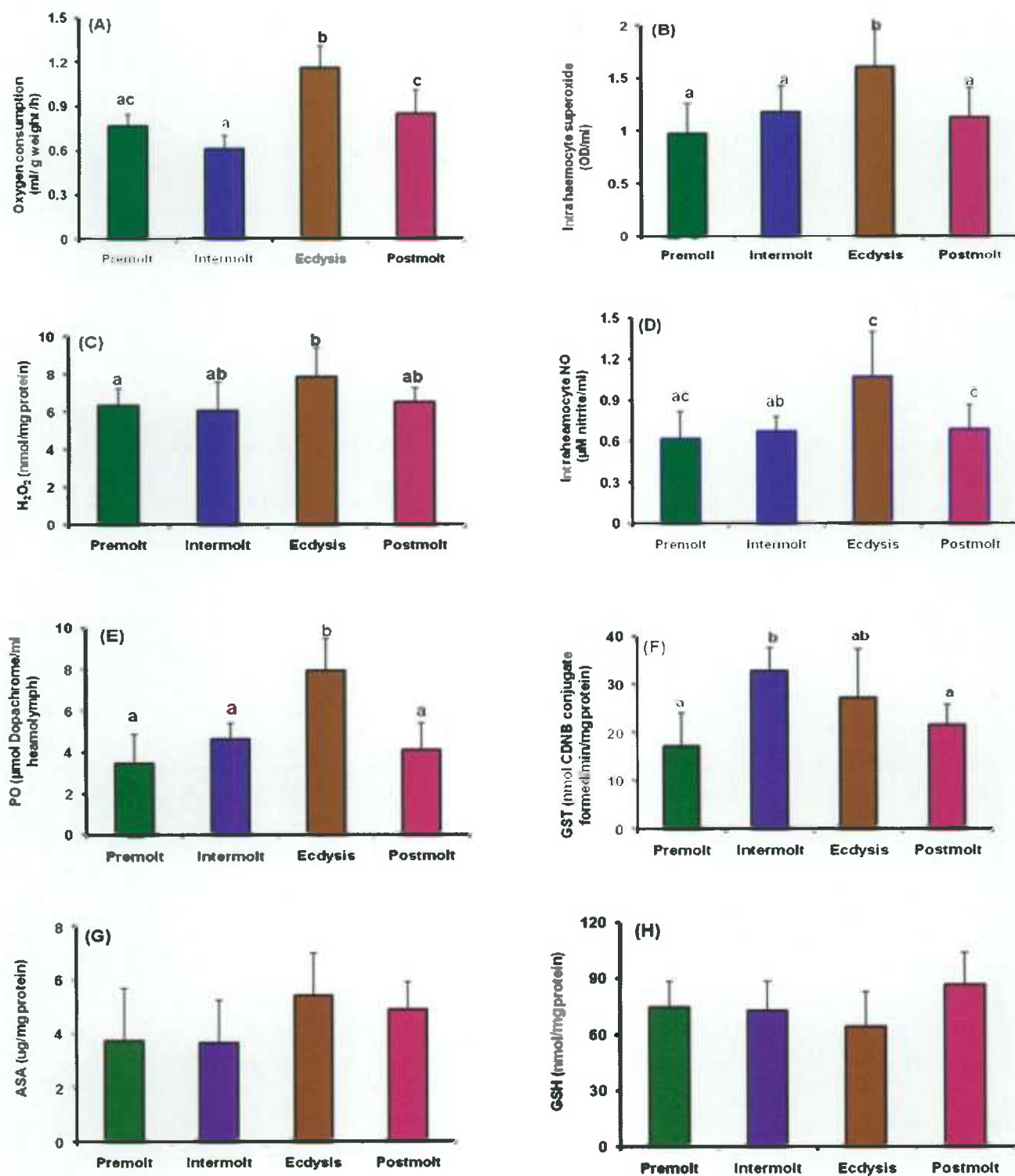


Figure 2: Physio-biochemical parameters, (A) oxygen consumption, (B) superoxide anion, (C) total hydroperoxide, (D) nitric oxide (NO), (E) phenol oxidase (PO), (F) glutathione-S-transferase (GST), (G) ascorbic acid (ASA) and (H) reduced glutathione (GSH) during moulting cycle of *A. mylitta*. Data are expressed as mean $\pm$ SD (n=5). Values depicted with superscripts of different letters are significantly different from each other at P < 0.05.

40 g at final instar, before spinning. Hence, every moult is very critical in terms of metabolic activities. Moulting is required for growth and development and occurs across a number of animal taxa. Moulting has discontinuous energetic costs because of associated events such as hormone production, apolysis, digestion of old cuticles, and production of new cuticle (Chapman, 1998). In addition, this process must occur rapidly for the organism to avoid both predation and desiccation. The moulting process is associated with several physio-biological changes such as an increase in the metabolic rate and oxygen consumption (Chang, 1995), variation in free sugar, glycogen and chitinase activity (Zou and Fingerman, 1999; Sugumar *et al.*, 2013) and of hyperglycaemic hormone (Chung *et al.*, 1999). In the present study, during moulting cycle (ecdysis period), oxygen consumption was increased (1.16 ± 0.145 ml/g/h, Figure 2A) followed by increase in free radicals such as superoxide anion (1.61 ± 0.42 OD/ml, Figure 2B), hydroperoxide (7.85 ± 1.55 nmol/mg protein, Figure 2C) and nitric oxide (1.07 ± 0.33 μ M nitrite/ml, Figure 2D). Ecdysis is particularly challenging to insects because the process includes the shedding of exoskeleton. In tasar silkworm, the respiratory organ comprises of tracheal based system. These tracheal linings are derived from ectodermal tissue and are discarded with the exoskeleton with every moult. Sudden shifting of hypoxia (inter-moult period) to hyperoxia during ecdysis might be responsible for production of more superoxide anions. Loft *et al.* (1994) reported that higher respiration is linked with higher production of reactive oxygen species. According to Dean *et al.* (2009), both hypoxia and hyperoxia condition lead to production of more superoxides. In tasar silkworm, high oxygen consumption (Rath *et al.*, 2005) and total hydroperoxides (Jena *et al.*, 2013) were observed in non-diapausing generation when compared to the diapausing generation. In the present study, higher level of the total hydroperoxide was also detected during ecdysis period (Figure 2C), which can be explained by the fact that oxygen consumption is higher during ecdysis than other phases of the moulting cycle, which is responsible for production of more ROS.

PO is an important humoral defense component in insects. Its activation results in induction of a number of potent bioactive molecules which assist various defensive

mechanisms (Soderhall and Cerenius, 1998). Generally, PO is present in silkworm plasma in an inactive prophenoloxidase (proPO) state. In the present study, higher level of PO activity was measured during ecdysis period (7.9 ± 1.6 μ mol dopachrome/ml, Figure 2E). It has been reported that PO is one of the key enzymes in moulting, which is closely related with the role of ecdysone in the regulation of biological moulting processes. Wang *et al.* (2013) also reported a peak expression of PO during molting stage of mulberry silkworm, subsequently followed by a decrease in its titre.

GST catalyze the nucleophilic addition of the thiol group of glutathione to toxic compounds to form a glutathione-conjugate. These conjugates are then transformed to a more water soluble form for easy elimination/excretion through urine and faeces (Al-Ghais and Ali, 1995; Gadagbui and James, 2000; Huang *et al.*, 2008). Increased GST activity during moulting suggests that higher metabolic stress may have induced GST expression in order to protect tissues from xenobiotic damage (Figure 2F). Similarly, elevated GST activity has been reported by Raja-aho *et al.* (2012) during moulting in the barn swallow (*Hirundo rustica* L.).

ASA is a crucial micronutrient that directly scavenge ROS (Halliwell and Gutteridge, 2001). It is required for normal collagen synthesis (Hunter *et al.*, 1979) and therefore, its deficiency leads to mortality associated with incomplete moulting in crustaceans (He and Lawrence, 1993). It also plays an important role in disease resistance (Felix and Sivakumar, 2003). Physiological consequences of ASA enhancement in haemolymph could be related to its role in protecting tissues during moulting to combat pro-oxidants (Figure 2G). The availability may be through mobilization from storage organs to haemolymph or through recycling of dehydroascorbic acid to ascorbate. Further investigation is needed to confirm the hypothesis.

GSH, the major non-protein thiol in animals, shows antioxidant properties. Under oxidative stress conditions, ROS are reduced by GSH, with the concomitant formation of the oxidized glutathione (GSSG). In addition, GSH also acts as a cofactor for GST and glutathione peroxidase. In the present study, depletion of GSH observed during ecdysis (63.47 ± 18.47 nmol/mg protein, Figure 2H), may

indicate higher utilization than synthesis or recycle of GSH, which plays an important role in scavenging of free radicals or reduction of dehydroascorbate to ascorbate (Winkler *et al.*, 1994). Further studies may provide additional information on this important conversion of GSH and ASA cycle during moulting period of silkworm.

Moulting being a very critical process, not only do new cells proliferate from stem cells but also the existing cells shrink. In *Manduca sexta*, epithelial cells are approximately the same size at the beginning of each instar, despite an estimated 200-fold increase in cell number and epithelial surface area from first to fifth instar (Baldwin and Hakim, 1991). Significant induction of $O_2^{\cdot-}$, NO and total hydroperoxides in response to moulting period is indicative of cytotoxic challenges faced by native *A. mylitta*. Modulation of immune responses and antioxidants such as GST, ASA and GSH indicate the strong potential of animal to protect against the oxidative stress generated during moulting period. It is clear that moulting is highly disruptive to respiratory physiology and is a more challenging process. These biochemical processes will help to understand the larval transformation from hypoxia to hyperoxia induced physiological changes in size-sensing (growth) mechanism. Understanding of normal regulations of growth and development could help us for better management of tasar crop.

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UTILIZATION OF AM FUNGI IN *EX-SITU* CULTIVATION OF MICROPROPAGATED MULBERRY PLANTS FOR ENHANCED GROWTH AND QUALITATIVE IMPROVEMENT

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ABSTRACT

Mulberry leaf is the sole food source of silkworm, *Bombyx mori* L., and hence, any step towards improvement in leaf quality and quantity will have a direct bearing on the cocoon productivity and quality. In an attempt towards this direction, conventionally raised and micropropagated mulberry plants (*Morus indica* var. Mysore local) were separately inoculated with arbuscular mycorrhizal fungi viz., *Glomus mosseae* and *G. fasciculatum*. Morphological and yield parameters were studied in normal and micropropagated plants with or without (control) AMF inoculation. Micropropagated plants inoculated with *G. fasciculatum* performed better in all the parameters studied. Phytochemical and phytoelementary studies have revealed that the leaves of micropropagated plants inoculated with *G. fasciculatum* had the highest content of primary metabolites and phytoelements indicating better quality of leaves for feeding the silkworm larvae, compared to other treatments. Moisture content and moisture retention capacity of leaves which play a vital role in improving nutrition levels of leaves and the palatability for silkworm, were estimated. The leaves of micropropagated plants inoculated with *G. fasciculatum* showed significantly more moisture content and moisture retention capacity compared to that of other samples. Rearing, cocoon silk productivity and grainage parameters analysed for Pure Mysore breed silkworms fed with the leaves of control and AMF treated normal and micropropagated plants substantiate the results of phytochemical and phytoelementary studies.

Key words: AM fungi, cocoon, growth performance, micropropagation, silk.

INTRODUCTION

Mulberry is mainly propagated by cuttings. From economic and commercial point of view, it is a laborious and lengthy process to evolve a desirable clone in vegetatively propagated plants through conventional hybridization. Further, many elite varieties of mulberry have poor rooting ability and propagation through cutting is restricted to only certain months of the year (Singh *et al.*, 2015). Moreover, longer juvenile period, high inbreeding depression and dioecious nature of the plant have restricted the development of inbred lines in mulberry (Vijayan *et al.*, 2012). Propagation through seeds is also not feasible commercially, because of heterozygosity of the parents (Vijayan *et al.*, 2011). Under

this backdrop, micropropagation has been employed as a viable tool for mass propagation, enhancement of growth and improvement of crop (Kapoor *et al.*, 2008; Tejavathi *et al.*, 2011, 2012).

Arbuscular mycorrhiza (AM) is the most wide spread type of symbiotic association that exists in the ecosystem. The beneficial role played by arbuscular fungi when they are inoculated to the roots of micropropagated plants have been reviewed (Lovato *et al.*, 1996; Rai, 2001). AM fungi (AMF), acting as bio fertilizer, play a significant role in increasing the rate of growth of the host by enhancing the uptake of nutrients in general and phosphorus in particular (Gianinazzi and Gianinazzi-Pearson, 1988; Krishna *et al.*, 2005). The interaction due to AMF association is largely

based on the transfer of carbon from the plant to the fungus and on the transfer of mineral nutrients via fungus to the host plant (Smith and Read, 2008).

Morus indica var. Mysore local, is comparatively a low yielding variety. However, its adaptability to low agronomic inputs and poor management practices, both under rain-fed and irrigated condition make this variety sustainable even though several high yielding varieties including hybrids have been released in the past few decades. Micropropagation studies in *Morus indica* and its cultivars are limited though such studies have been carried out in other species and cultivars of *Morus* (Sandhya *et al.*, 2009; Tejavathi *et al.*, 2009; Vijayan *et al.*, 2011). The earlier studies on AM fungal association in mulberry were mainly concentrated on the requirement of phosphorus fertilizer in presence of AM fungi. Ram Rao *et al.* (2007) and Bharadwaj and Sharma (2006) have reported that lesser quantity of phosphate fertilizers are required when mulberry plants were associated with AM fungi. Growth of the seedlings of *Morus alba* was promoted when they were inoculated with AM fungi (Lu *et al.*, 2015). However, studies on micropropagation coupled with mycorrhization are lacking in *M. indica*. Hence, employing alternate strategies, such as *in vitro* culture coupled with utilization of AM fungi were thought of as essential to enhance / improve the growth and qualitative output of the var. Mysore local.

MATERIALS AND METHODS

Plant source and preparation of cuttings

(i) Micropropagated plants – Shoots raised on MS + BAP (4.44 μ M) from nodal culture were transferred to MS + BAP (4.44 μ M) + GA3 (2.88 μ M) for elongation and to soilrite fed with liquid $\frac{1}{4}$ MS + IBA (4.90 μ M) for *in vitro* rooting. Plantlets thus obtained were transferred to pots containing sterilized soil (Tejavathi *et al.*, 2009). After one year of growth and maintenance in pots, cuttings were prepared for plantation in the experimental field.

(ii) Normal plants – Cuttings from one year old normal plants maintained in pots were used for planting in the field.

The basal portion of the cuttings was kept dipped in aqueous solution of IBA (4.90 μ M) for 24 h to induce optimum growth of roots before planting.

Plot preparation, planting and AMF treatment

Raised beds of 1½ m length, 1 m width and 15 cm height were made in the field. Farm yard manure was applied at the rate of 2 kg per bed, two weeks before planting the cuttings.

The experiment was laid out in a randomized complete block design with five replications. Treated cuttings were planted in slanting position on the bed with spacing of 3 ft x 3 ft between them with a total number of 72 cuttings. *Glomus mosseae* and *G. fasciculatum* were applied @ 20 g of inoculum (containing about 90-100 spores / gram) per planting point. Irrigation was provided at regular intervals of three to four days depending on the soil condition.

Experiment included six treatments as following:

1. Normal plants: control -1;
2. Normal plants treated with *G. mosseae*;
3. Normal plants treated with *G. fasciculatum*;
4. Micropropagated plants: control - 2;
5. Micropropagated plants treated with *G. mosseae* and
6. Micropropagated plants treated with *G. fasciculatum*

Mycorrhizal colonization

The colonization by AM fungi in both normal and micropropagated plants in each treatment and control were estimated according to Philips and Heyman method (1970) as follows:

$$\text{Per cent mycorrhizal colonization} = \frac{\text{Number of root bits colonized by AMF}}{\text{Total number of root segments taken for observation}} \times 100$$

Estimation of mycorrhizal spores

Extrametrical chlamydospores produced by the mycorrhizal fungi were estimated by wet sieving and decanting method as laid out by Gerdemann and Nicolson

(1963).

Per cent colonization and spore count were estimated periodically *i.e.*, 3, 6 and 9 months after inoculation, in all the above treatments. Data were recorded for five replications.

Morphological traits

The following morphological characters were studied in normal, micropropagated and AMF treated plants, 14 months after treatment following standard and customary methods. Five plants were randomly selected for recording the following characters and average values were derived.

1. Height of the plant; 2. Number of leaves; 3. Surface area of leaf; 4. Number of flowers and catkins per plant and 5 Fresh and dry weight of the plant.

Estimation of moisture content in leaves

Moisture content of leaf was estimated through gravimetric method by taking fresh and dry weight of leaf and expressed in percentage (A.O.A.C., 1995).

$$\text{Moisture percentage} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

Moisture retention capacity

Moisture retention capacity of leaf was estimated through gravimetric method (A.O.A.C., 1995).

Leaf moisture retention capacity (%) at a particular hour = $100 - X$

Where,

$$X = \frac{\text{Fresh weight} - \text{weight of leaves at a particular hour}}{\text{Fresh weight}} \times \text{Moisture content}$$

Phytochemicals

Healthy leaves from the 3rd to 6th node of 3, 6 and 9

months old field grown normal and micropropagated plants with or without AMF treatment were used for phytochemical analysis. Standard methods were adopted for estimation of chlorophyll (Arnon, 1949), total carbohydrates (Yemm and Willis, 1954), reducing sugars (Miller, 1959), total proteins (Lowry *et al.*, 1951), amino acids (Moore and Stein, 1948) and phenolics (Singleton and Rossi, 1965).

Phytoelements

Healthy leaves from the 3rd to 6th node of 3, 6 and 9 months old field grown normal and micropropagated plants with or without AMF treatment were used for the estimation of phytoelements. The macro and micro nutrients *viz.* potassium (Yoshida *et al.*, 1976), phosphorus (Yoshida *et al.*, 1976), nitrogen (Kjeldahl, 1883), manganese (Sandell, 1959), iron (Sandell, 1959), zinc (Kushizaki, 1968) and copper (Kushizaki, 1968) were estimated following standard procedures.

Bioassay

The Pure Mysore breed of silkworm selected for the study was procured from Grainage Centre, Magadi, Bangalore District, Karnataka State. For each treatment, 120 of healthy, second instar completed larvae were selected. Silkworms were reared as per standard package of practices (Dandin *et al.*, 2003)

Cocoon parameters

The cocoon, post cocoon and grainage parameters selected for assessing the crop performance *viz.*, cocoon weight, shell weight, cocoon yield, pupa weight, pupation rate, silk productivity, filament length and denier were recorded / estimated as per standard procedures (Dandin *et al.*, 2003).

Data analysis

The data on 6 treatments were tabulated. Standard error was calculated for the mean and subjected to TWO WAY ANOVA. Significant F ratios between group mean were further subjected to least significant differences (LSD) tests and values were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

The present study deals with the micropropagation and mycorrhization in *Morus indica* var. Mysore local to evaluate the beneficial effects of both *in vitro* technique and mycorrhizal inoculation on the growth performance of the host plant and the silkworm (*Bombyx mori*) which were fed with the leaves of normal and those of micropropagated plants inoculated with or without AM fungi. The conventional method of mulberry propagation through either stem cuttings or grafting has several limitations. Survival per cent of cuttings depends on the time gap between pruning and transplantation in addition to the rooting potentiality of the cuttings. Successful rooting of the cuttings generally depends on environmental factors and physiological status of the stem cuttings (Ohyama and Oka, 1976). Further, the possibility to lose vigour in the case of plants grown from stem cuttings as compared to those from seedlings cannot be ruled out (Zaman *et al.*, 1997). In this context, micropropagation technique acts as an alternative tool for the rapid and cost – effective multiplication of mulberry (Vijayan *et al.*, 2011).

AM fungi have shown a very broad host range with varieties / cultivars / the species that can be colonized to different extent (Bagyaraj, 1991). In the present work, the colonization of AM fungi was recorded every three months after inoculation. Three readings were recorded *i.e.*, 3, 6 and 9 months after inoculation with *G. mosseae* and *G. fasciculatum* (Table 1). The per cent of colonization was more significant in both normal and micropropagated plants colonized with *G. fasciculatum*. The maximum rate of colonization of $44.00 \pm 0.90\%$ was recorded 9 months after inoculation with *G. fasciculatum* in micropropagated plants, whereas, the minimum of $08.00 \pm 0.31\%$ was noted for normal plants without AMF inoculation. The next best colonization was recorded in *G. mosseae* inoculated plants. Mycorrhizal colonization of $30.00 \pm 0.54\%$ and $35.00 \pm 1.00\%$, respectively were recorded in normal and micropropagated plants inoculated with *G. mosseae*. Variation in the degree of colonization with plant age and appearance of vesicles with reference to the age have been demonstrated by Padma and Sullia (1991). The colonization in both normal and micropropagated plants stimulated more sporulation

in the soil as was observed in the present study. Number of vesicles, arbuscles in the root and spore count in the rhizosphere soil were significantly more in the micropropagated plants inoculated with *G. fasciculatum* that has a direct correlation with per cent of colonization (Table 1). Similar observations were made in *Mentha arvensis* (Gupta *et al.*, 2002), *Andrographis paniculata* (Tejavathi *et al.*, 2011) and *Agave vera cruz* (Tejavathi *et al.*, 2012). Contrary to the present study, Sowmya *et al.* (2004) have reported high intensity of colonization and spore count in *G. mosseae* treated micropropagated *Bacopa monnieri* plants than the *G. fasciculatum* treated ones. In our study, the number of spores counted in 100 g of soil collected from the rhizosphere of micropropagated plants treated with *G. fasciculatum* was 94.00 ± 0.63 compared to the normal plants where, only 9.60 ± 0.24 spores were recorded as per Gerdemann and Nicolson method (1963). At the same time, normal plants inoculated with *G. mosseae* and *G. fasciculatum* registered 72.60 ± 0.40 and 86.80 ± 0.30 spores per 100 g of the rhizospheric soil, respectively.

Morphological traits

Normal and micropropagated plants treated with AM fungi showed significant increase in height, surface area of leaves, number of leaves, number of flowers / catkins, number of fruits and biomass than uninoculated plants (Table 1, Figure 1). Beneficial effects of AM inoculation on morphological traits in normal and micropropagated plants have been reported in other species including mulberry (Katiyar *et al.*, 1989, 1995; Yano-Melo *et al.*, 1999; Rai, 2001; El Meskaoui *et al.*, 2008; Kapoor *et al.*, 2008). Katiyar *et al.* (1995) have reported the enhancement in the leaf yield in *Morus alba* due to AMF inoculation with lower dosage of phosphorus application. They also noted that *Morus alba* responded better to *G. fasciculatum* than *G. mosseae*. However, significant increase in biomass yield was observed in both normal and micropropagated plants of *Bacopa monnieri* in association with *G. mosseae* (Sowmya *et al.*, 2004). In the present investigation, increase in height, number of leaves, surface area of leaves and biomass were recorded in micropropagated plants treated with *G. fasciculatum* which is in agreement with the earlier observations in *Andrographis paniculata* (Tejavathi *et al.*, 2011) and

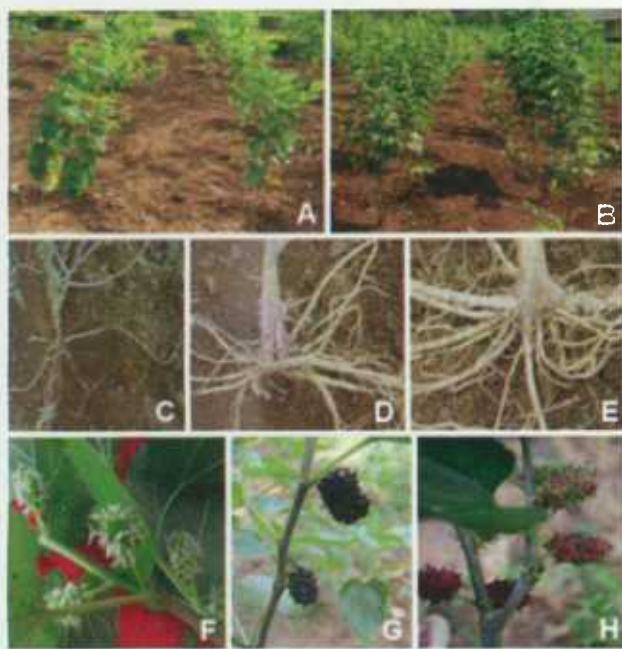


Figure 1: Morphological traits of *Morus indica* var. Mysore
Local with/without AMF association

A - Normal plant - Control; B - Micropropagated plant treated with *G. fasciculatum*; C - Root system of normal Plant-Control; D - Root system of normal plant treated with *G. fasciculatum*; E - Root system of Micropropagated plant treated with *G. fasciculatum*; F - Micropropagated plant treated with *G. fasciculatum* with catkins; G - Normal control plant with fruits; H - Micropropagated plant treated with *G. fasciculatum* with fruits

Agave vera-cruz (Tejavathi *et al.*, 2012). The micropropagated plants inoculated with *G. fasciculatum* exhibited maximum height of 197.60 ± 1.72 cm, 53.20 ± 0.58 leaves and leaf surface area of 147.40 ± 0.40 sq. mm, 9 months after inoculation in contrast to the normal un-inoculated plants where the corresponding figures were 158.60 ± 0.15 cm, 30.60 ± 0.50 leaves and 112.80 ± 0.86 sq. mm. Normal plants inoculated with *G. fasciculatum* also have shown significant improvement in the morphological characters with the height of 191.60 ± 1.01 m, 46.60 ± 0.81 leaves and 125.60 ± 1.12 sq. mm of leaf surface area. Increase in the plant height, leaf number and surface area, due to AMF inoculation in other varieties of mulberry were reported by Katiyar *et al.* (1995), Mamatha *et al.* (2002), Bharadwaj and Sharma (2006), Singh *et al.* (2012) and Lu *et al.* (2015). In the present study, un-inoculated normal, *G. mosseae* and *G. fasciculatum* treated plants produced 16.50 ± 0.41 , 19.00 ± 0.35 and 30.40 ± 0.12 g of fresh weight, respectively and correspondingly, 6.85 ± 0.29 , 8.05 ± 0.31 and 12.34 ± 0.11 g of dry weight. There is a significant increase in biomass in micropropagated plants with or without AMF inoculation. The fresh weight recorded for un-inoculated micropropagated, *G. mosseae* and *G. fasciculatum* treated

Table 1: AM fungal colonization and its impact on morphological traits of *Morus indica*

Treatment	Colonization (%)	No. of spores	Plant height (cm)	No. of leaves	Fresh weight (g)	Dry weight (g)	No. of catkins	No. of fruits	Leaf surface area (cm ²)	Leaf moisture content (%)	Moisture retention capacity (%)
Normal Plant	8.00 ± 0.31 ^e	9.60 ± 0.24 ^e	158.60 ± 0.15 ^f	30.60 ± 0.50 ^f	16.50 ± 0.41 ^f	06.85 ± 0.29 ^f	16.00 ± 0.01 ^f	13.00 ± 0.21 ^f	112.80 ± 0.86 ^f	61.42 ± 0.34 ^f	62.26 ± 0.01 ^f
Normal with <i>G. mosseae</i>	30.00 ± 0.54 ^d	72.60 ± 0.40 ^d	181.70 ± 3.86 ^d	42.80 ± 0.58 ^d	19.00 ± 0.35 ^e	08.05 ± 0.31 ^e	38.00 ± 0.21 ^d	33.00 ± 0.24 ^d	119.40 ± 0.92 ^e	64.90 ± 0.45 ^d	66.88 ± 0.32 ^e
Normal with <i>G. fasciculatum</i>	36.00 ± 0.70b ^c	86.80 ± 0.30 ^{bc}	191.60 ± 1.01 ^c	46.60 ± 0.81 ^{bc}	30.40 ± 0.12 ^b	12.34 ± 0.11 ^{ab}	122.00 ± 0.26 ^b	45.00 ± 0.11 ^c	125.60 ± 1.12 ^c	65.58 ± 0.54 ^{bc}	68.26 ± 0.22 ^d
Micropropagated Plant	07.60 ± 0.50 ^{ef}	07.60 ± 1.01 ^f	164.80 ± 1.63 ^e	37.60 ± 0.50 ^e	21.00 ± 0.14 ^{cd}	08.90 ± 0.16 ^d	35.00 ± 0.63 ^a	28.00 ± 0.12 ^e	124.60 ± 0.97 ^{cd}	63.46 ± 0.34 ^e	73.46 ± 0.12 ^c
Micropropagated with <i>G. mosseae</i>	35.00 ± 1.00 ^b	87.00 ± 0.37 ^b	193.40 ± 2.02 ^b	47.40 ± 0.67 ^b	23.28 ± 1.00 ^c	09.85 ± 0.20 ^c	149.00 ± 0.31 ^c	107.00 ± 0.31 ^b	135.20 ± 0.20 ^d	66.62 ± 0.43 ^b	81.01 ± 0.13 ^{ab}
Micropropagated with <i>G. fasciculatum</i>	44.00 ± 0.90 ^a	94.00 ± 0.63 ^a	197.60 ± 1.72 ^a	53.20 ± 0.58 ^a	33.26 ± 0.22 ^a	13.50 ± 0.31 ^a	282.00 ± 0.11 ^a	177.00 ± 0.26 ^a	147.40 ± 0.40 ^d	68.71 ± 0.32 ^a	83.36 ± 0.16 ^a

Data are represented by mean ± SE of three independent experiments. Mean values followed by different letters within columns are significantly different ($p < 0.05$) using Duncan's multiple range test. Data on per cent colonization and spore count were recorded 9 months after AMF inoculation and that on the plant traits after 14 months.

plants were 21.00 ± 0.14 , 23.28 ± 1.00 and 33.26 ± 0.22 g, respectively and the corresponding dry weights were 8.90 ± 0.16 , 9.85 ± 0.20 and 13.50 ± 0.31 g. The biomass produced by micropropagated plants inoculated with *G. fasciculatum* was significantly more than that of the un-inoculated micropropagated plants. Initiation of inflorescences was observed 13 months after planting in AMF treated plants while control plants flowered 14 months after planting. The un-inoculated normal, *G. mosseae* and *G. fasciculatum* treated plants produced 16.0 ± 0.01 , 38.00 ± 0.21 and 122.00 ± 0.26 catkins, respectively. On the other hand, the un-inoculated micropropagated, *G. mosseae* and *G. fasciculatum* treated plants produced 35.00 ± 0.63 , 149.00 ± 0.31 and 282.00 ± 0.11 catkins, respectively indicating a highly prominent influence in *G. fasciculatum* plants. Similarly, the number of fruits produced by un-inoculated normal, *G. mosseae* and *G. fasciculatum* treated plants was 13.00 ± 0.21 , 33.00 ± 0.24 and 45.00 ± 0.11 per plant, respectively, whereas, the un-inoculated micropropagated, *G. mosseae* and *G. fasciculatum* treated plants produced 28.00 ± 0.12 , 107.00 ± 0.31 , 177.00 ± 0.26 fruits, respectively. It has been reported that AMF symbiosis helps in increasing the rate of photosynthesis, storage of photosynthates and its mobilization at the same time (Auge, 2001).

Effect of AMF symbiotic association on leaf quality

The prime nutritional parameters of mulberry leaves which has a bearing on the economic characters of silkworm are the moisture content, moisture retention capacity, total protein, carbohydrates and total minerals (Bongale *et al.*, 1991; Sujathamma and Dandin, 2000; Sujathamma *et al.*, 2001; Kumar and Rao, 2008). The leaf quality is influenced by a number of factors such as variety, cultivation practices, incidence of pests and diseases, method of harvesting and preservation of leaves (Kumar and Rao, 2008). Leaves possessing higher leaf moisture content (LMC) and moisture retention capacity (MRC) are identified as of good quality and highly palatable (Bongale and Chaluvachari, 1995; Sujathamma and Dandin, 2000). In the present study, the leaves of micropropagated plants inoculated with *G. fasciculatum* revealed 68.71 ± 0.32 % of moisture content followed by *G. mosseae* treated plants with 66.62 ± 0.43 % and un-inoculated micropropagated plants with 63.46 ± 0.34 %.

AM fungus treated normal plants also showed a considerable increase in moisture content compared to un-inoculated normal plants with 65.58 ± 0.54 % in *G. fasciculatum* treated plants, 64.90 ± 0.45 % in *G. mosseae* treated and 61.42 ± 0.34 % in un-inoculated normal plants. Correspondingly, moisture retention capacity also followed the same trend with the highest record in micropropagated *G. fasciculatum* treated plants with 83.60 ± 0.16 % and the lowest in control normal plants with 62.26 ± 0.01 % (Table 1). However, Bongale and Chaluvachari (1995) had reported very low moisture content and moisture retention capacity in var. Mysore local compared to other varieties of mulberry. Thus, from our results, it is evident that mycorrhization enhances both these parameters which are considered as important determinants of the quality of leaf.

Phytochemicals

Primary and secondary metabolites were estimated in the leaves of normal and micropropagated plants with or without AMF treatment. The contents of chl a, chl b and total chlorophyll were significantly more in the leaves of micropropagated plants treated with *G. fasciculatum* followed by those treated with *G. mosseae* (Table 2). Untreated micropropagated plants registered 186.00 ± 0.44 $\mu\text{g/g}$ of chl a, 129.20 ± 0.37 $\mu\text{g/g}$ of chl b and 289.00 ± 0.44 $\mu\text{g/g}$ of total chlorophyll as against the corresponding values of 160.40 ± 0.67 , 112.00 ± 0.54 and 261.00 ± 0.44 $\mu\text{g/g}$ in un-inoculated normal plants. The maximum content of 193.60 ± 0.61 $\mu\text{g/g}$ of chl a, 135.60 ± 0.24 $\mu\text{g/g}$ of chl b and 328.00 ± 0.31 $\mu\text{g/g}$ of total chlorophyll were recorded in micropropagated plants inoculated with *G. fasciculatum* when estimated 9 months after inoculation, whereas, the normal plants inoculated with *G. fasciculatum* have shown 180.40 ± 0.24 , 121.60 ± 0.24 and 292.40 ± 0.40 $\mu\text{g/g}$ of respective values during the same phase. Increase in chlorophyll contents in AM fungal treated plants may be due to the improved N and K uptake as suggested by Zhao *et al.* (2001) and Menendez *et al.* (2002). It was shown earlier that chlorophyll concentration in AMF treated plants is higher than that of non-mycorrhizal plants (Giri *et al.*, 2003; Kapoor and Bhatnagar, 2007). However, on the contrary, Katiyar *et al.* (1995) have found no significant variations in the contents of chlorophyll due to VAM inoculation in the varieties of

Table 2: Effect of AM fungal association on the biochemical constituents in leaf of *Morus indica*

Treatment	Total chlorophyll ($\mu\text{g/g}$)	Carbohydrates ($\mu\text{g/g}$)	Reducing sugar ($\mu\text{g/g}$)	Proteins ($\mu\text{g/g}$)	Amino acids ($\mu\text{g/g}$)	N (%)	K ₂ O (%)	P ₂ O ₅ (%)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)
Normal Plant	261.00 $\pm$ 0.44 ^f	240.40 $\pm$ 0.50 ^b	160.80 $\pm$ 0.58 ^a	620.20 $\pm$ 0.37 ^f	525.00 $\pm$ 0.31 ^f	02.35 $\pm$ 0.01 ^f	01.71 $\pm$ 0.05 ^f	0.41 $\pm$ 0.01 ^f	33.20 $\pm$ 0.58 ^{bc}	539.00 $\pm$ 1.04 ^f	69.80 $\pm$ 0.58 ^f	36.00 $\pm$ 0.40 ^f
Normal with <i>G. mosseae</i>	279.60 $\pm$ 0.40 ^e	146.60 $\pm$ 0.40 ^f	112.20 $\pm$ 0.48 ^{ac}	680.80 $\pm$ 0.67 ^{cd}	559.80 $\pm$ 0.48 ^e	02.79 $\pm$ 0.02 ^d	01.90 $\pm$ 0.05 ^d	0.57 $\pm$ 0.02 ^d	19.80 $\pm$ 0.73 ^f	650.60 $\pm$.74 ^{cd}	78.00 $\pm$ 0.70 ^d	44.40 $\pm$ 0.40 ^d
Normal with <i>G. fasciculatum</i>	292.40 $\pm$ 0.40 ^{bc}	151.60 $\pm$ 0.24 ^e	88.40 $\pm$ 0.60 ^f	699.00 $\pm$ 0.54 ^a	575.40 $\pm$ 0.24 ^{cd}	02.90 $\pm$ 0.01 ^c	01.95 $\pm$ 0.17 ^e	0.62 $\pm$ 0.01 ^c	23.00 $\pm$.54 ^{ac}	654.20 $\pm$ 0.08 ^e	84.60 $\pm$ 0.74 ^{ab}	48.20 $\pm$ 1.01 ^e
Micropropagated Plant	289.00 $\pm$ 0.44 ^d	296.00 $\pm$ 0.54 ^a	147.80 $\pm$ 0.37 ^b	660.00 $\pm$ 0.54 ^c	571.00 $\pm$ 0.44 ^c	02.73 $\pm$.02 ^{ac}	01.88 $\pm$ 0.05 ^{ac}	0.48 $\pm$ 0.01 ^c	46.20 $\pm$ 0.58 ^a	624.60 $\pm$ 0.03 ^a	73.40 $\pm$ 0.50 ^e	39.00 $\pm$ 0.70 ^e
Micropropagated with <i>G. mosseae</i>	293.30 $\pm$ 0.31 ^b	156.60 $\pm$ 0.40 ^d	114.20 $\pm$ 0.66 ^d	680.60 $\pm$ 0.48 ^c	584.80 $\pm$ 0.48 ^b	03.45 $\pm$ 0.11 ^b	02.02 $\pm$ 0.09 ^{ab}	0.66 $\pm$ 0.01 ^{ab}	34.60 $\pm$ 0.40 ^b	703.80 $\pm$ 0.80 ^b	82.60 $\pm$ 0.60 ^c	52.60 $\pm$ 0.77 ^b
Micropropagated with <i>G. fasciculatum</i>	328.00 $\pm$ 0.31 ^a	181.00 $\pm$ 0.31 ^c	119.80 $\pm$ 0.58 ^c	696.40 $\pm$ 0.89 ^{ab}	596.80 $\pm$ 0.48 ^a	03.75 $\pm$ 0.03 ^a	02.04 $\pm$ 0.06 ^a	0.67 $\pm$ 0.01 ^a	24.40 $\pm$ 0.50 ^d	725.40 $\pm$ 0.81 ^a	85.00 $\pm$ 0.54 ^a	53.80 $\pm$ 0.76 ^a

Data are represented by mean $\pm$ SE of three independent experiments. Mean values followed by different letters within columns are significantly different ($p < 0.05$) using Duncan's multiple range test.

mulberry. They have attributed this observation to the application of nitrogen and potash as basal nutrients to all other nutrients.

Proteins and amino acids are of particular importance for the larvae because of their active utilization for the synthesis of silk protein. Hence, the varieties containing more proteins and amino acids in their leaves are considered as superior. In the present study, the leaves of micropropagated plants treated with *G. fasciculatum* contained 696.40 ± 0.89 $\mu\text{g/g}$ of protein and 596.80 ± 0.43 $\mu\text{g/g}$ of amino acids as against 620.20 ± 0.37 $\mu\text{g/g}$ of protein and 525.00 ± 0.31 of amino acids in un-inoculated control plants (Table 2). Normal plants inoculated with *G. fasciculatum* recorded the highest level of protein (699.00 ± 0.54 $\mu\text{g/g}$). Significant improvement in the contents of proteins and amino acids was noticed in un-inoculated micropropagated plants with 660.00 ± 0.54 $\mu\text{g/g}$ of proteins and 571.00 ± 0.44 $\mu\text{g/g}$ of amino acids. In general, AMF treated both normal and micropropagated plants contain considerably higher contents of protein and amino acids than the un-inoculated plants. Contrary to other phytochemicals, the carbohydrate content in the leaves of micropropagated and normal plants treated with

AM fungi was observed comparatively less than the un-inoculated plants because of carbohydrate drain for the sustainability of AM fungi (Smith and Read, 2008). However, the contents of carbohydrates in the AM treated micropropagated plants are significantly more than AM treated normal plants. *G. fasciculatum* treated micropropagated plants recorded 181 ± 0.31 $\mu\text{g/g}$ of carbohydrate compared to 151.60 ± 0.24 $\mu\text{g/g}$ in the same treatment with the normal plants. At the same time, *G. mosseae* treated, micropropagated plants have shown 156.60 ± 0.40 $\mu\text{g/g}$ of carbohydrates as against 146.60 ± 0.40 $\mu\text{g/g}$ in normal plants treated with *G. mosseae*. The maximum of 296.00 ± 6.54 $\mu\text{g/g}$ of carbohydrate was recorded in micropropagated un-inoculated plants followed by normal un-inoculated plants with 240.40 ± 0.50 $\mu\text{g/g}$. Kashyap *et al.* (2004) however, reported the enhancement of amino acids and carbohydrate contents in the leaves of AM fungal associated *Morus alba*.

Phytoelements

Nitrogen, phosphorus and potassium (NPK) are the major plant nutrients required for normal growth of the mulberry plants. It is now well established that AM fungi is

capable of solubilising the colloid form of phosphates in the soil in addition to enhancement of uptake of phosphorus in soluble form in the soil (Setua *et al.*, 1999). In the leaves of S1 Mulberry variety, the phosphorus content was slightly more in the samples treated with *G. fasciculatum* compared to other treatments (Setua *et al.*, 1999). Lu *et al.* (2015) have found more contents of nitrogen, phosphorus and potassium in *G. intraradices* treated plants. In the current study, the micropropagated plants inoculated with *G. fasciculatum* resulted in the most efficient nutrient absorption with 3.75 ± 0.03 % of nitrogen, 2.04 ± 0.06 % of K_2O and 0.67 ± 0.01 % of P_2O_5 , compared to un-inoculated micropropagated plant which possessed 2.73 ± 0.02 % of nitrogen, 1.88 ± 0.05 % of K_2O and 0.48 ± 0.01 % of P_2O_5 . But in normal un-inoculated plants, comparatively less amounts of nitrogen (2.35 ± 0.01 %), K_2O (1.71 ± 0.05 %) and P_2O_5 (0.41 ± 0.01 %) were recorded (Table 2). Inoculation with AMF has been recommended to enhance the uptake of P and other nutrients such as nitrogen, zinc, copper, manganese and iron (Smith *et al.*, 1994, Krishna *et al.*, 2005). Mycorrhizal roots are more efficient to take up all the three forms of phosphorus *viz.*, inorganic soluble, inorganic insoluble and organic than non-mycorrhizal

roots which can utilize only inorganic soluble phosphorus that is available in much smaller quantities in the rhizospheric soil (Jefferies *et al.*, 2003). Hence, AM fungal colonization can drastically reduce the quantity of P fertilizer application without reduction in the yield (Mamatha *et al.*, 2002; Bharadwaj and Sharma, 2006). In our study, micropropagated plants inoculated with *G. fasciculatum* have shown higher contents of Fe (725.40 ± 0.81 ppm), Mn (85.00 ± 0.54 ppm) and Zn (53.80 ± 0.76 ppm) compared to un-inoculated normal plant which contained 539 ± 1.04 ppm of Fe, 69.80 ± 0.58 ppm of Mn and 36.00 ± 0.40 ppm of Zn. Micropropagated un-inoculated plants possessed better nutrient contents than un-inoculated normal plants with 46.20 ± 0.58 ppm of Cu, 624.60 ± 0.03 ppm of Fe, 73.40 ± 0.50 ppm of Mn and 39.00 ± 0.70 ppm of Zn.

Bioassay

Third instar larvae of Pure Mysore breed of silkworm were fed with the leaves of normal and micropropagated plants and their performance was assessed with respect to cocoon, grainage and reeling parameters. Our results reveal significant variations in cocoon yield, shell weight

Table 3: Influence of AM fungi colonization in mulberry on silkworm growth and cocoon characters

Treatment	Larval weight (g)	No. of cocoons yielded / treatment	Cocoon weight (g)	Pupa weight (g)	Shell weight (g)	Filament length (m)	Filament weight (g)	Denier
Normal Plant	2.265 ± 0.05 ^{cd}	72.32 ± 0.12 ^f	1.068 ± 0.09 ^f	0.974 ± 0.07 ^f	0.108 ± 0.08 ^f	540 ± 0.65 ^f	0.128 ± 0.34 ^f	2.10 ± 0.33 ^f
Normal with <i>G. mosseae</i>	2.288 ± 0.02 ^{cd}	81.58 ± 0.11 ^{cd}	1.192 ± 0.01 ^c	1.207 ± 0.03 ^{cd}	0.143 ± 0.08 ^{cd}	546 ± 0.56 ^c	0.136 ± 0.45 ^{cd}	2.22 ± 0.24 ^d
Normal with <i>G. fasciculatum</i>	2.291 ± 0.05 ^c	83.75 ± 0.02 ^c	1.240 ± 0.08 ^b	1.245 ± 0.01 ^c	0.148 ± 0.07 ^c	550 ± 0.67 ^c	0.137 ± 0.56 ^c	2.24 ± 0.45 ^b
Micropropagated Plant	2.274 ± 0.02 ^c	76.52 ± 0.11 ^c	1.170 ± 0.01 ^{cd}	1.112 ± 0.01 ^c	0.126 ± 0.04 ^c	548 ± 0.78 ^{cd}	0.134 ± 0.66 ^c	2.20 ± 0.54 ^c
Micropropagated with <i>G. mosseae</i>	2.400 ± 0.04 ^{ab}	104.54 ± 0.12 ^{ab}	1.136 ± 0.06 ^c	1.306 ± 0.05 ^{ab}	0.166 ± 0.07 ^b	553 ± 0.76 ^{ab}	0.134 ± 0.66 ^c	2.24 ± 0.35 ^{bc}
Micropropagated with <i>G. fasciculatum</i>	2.448 ± 0.11 ^a	105.56 ± 0.17 ^a	1.354 ± 0.11 ^a	1.341 ± 0.01 ^a	0.166 ± 0.07 ^b	555 ± 0.45 ^a	0.134 ± 0.66 ^c	2.27 ± 0.55 ^a

Data are represented by mean $\pm$ SE of three independent experiments. Mean values followed by different letters within columns are significantly different ($p < 0.05$) using Duncan's multiple range test.

and silk filament length among the different treatments (Table 3). Plants treated with *G. fasciculatum* exhibited superiority in respect of rearing, grainage and reeling parameters. Larval and cocoon parameters substantiated the superiority of micropropagated plants treated with AMF as stated in the above sections (2.448 ± 0.11 g larval weight for micropropagated plants treated with AMF as against 2.265 ± 0.05 g in un-inoculated normal plants). The same treatment revealed superiority in terms of cocoon weight (1.354 ± 0.11 g), pupa weight (1.341 ± 0.01 g) and shell weight (1.73 ± 0.02 g). Cocoon yield was also drastically enhanced in AMF treatments as 105.56 ± 0.17 cocoons were recorded in treatment where larvae were fed with the leaves of micropropagated plants treated with *G. fasciculatum* compared to the control where larvae were fed with the leaves of normal plant (72.32 ± 0.12 cocoons). Even *G. mosseae* treatment with micropropagated plants has enhanced the cocoon yield by number (104.54 ± 0.12). The other important parameters viz., length, width and denier of silk thread have shown significant improvement when the larvae were fed with the leaves of micropropagated plants treated with *G. fasciculatum*. This observation corroborates the studies on the quality of leaves since the leaves of micropropagated plants treated with *G. fasciculatum* were found to be highly nutritious and contained more moisture content besides having high moisture retention capacity. Mixed inoculation of *G. mosseae* and *G. fasciculatum* with low dosage of fertilizers in V1 variety of mulberry had improved the length of the silk filament (Baqual, 2013). Ram Rao *et al.* (2007) have reported significant improvement in silkworm growth and cocoon characters when the larvae were fed with the leaves of var. S-13 which was inoculated with AMF and *Azotobacter chroococcum*. The length, weight and denier of the silk thread recorded in the case of micropropagated plants treated with *G. fasciculatum* were 555 ± 0.45 m, 0.140 ± 0.77 g and 2.270 ± 0.55 , whereas, for un-inoculated normal plants, they were 540 ± 0.65 m, 0.128 ± 0.34 g and 2.10 ± 0.33 , respectively.

From the aforesaid data, it can be concluded that micropropagated plants associated with *G. fasciculatum* are superior to other treatments in all the larval, pre and post-cocoon parameters studied. Further, *Morus indica* var. Mysore local, in spite of being an indigenous variety

with high adaptation to low agronomic inputs is not favoured for cultivation because of its low moisture content, low moisture retention capacity and poor yield of leaves compared to the hybrids (Bongale and Chaluvachari, 1995). However, the present work has revealed that both AMF inoculated normal as well as the micropropagated plants have better qualities to support silkworm growth and development, than the control normal plants. Since var. Mysore local is known for its adaptation to low agronomic inputs and poor management practices, it can be improved with mycorrhizal technology and brought into cultivation in the arid and semi arid regions.

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DEVELOPMENT OF IMPROVED LINE OF BREEDER STOCK DABA TRIVOLTINE OF TROPICAL TASAR SILKWORM, *ANTHRAEA MYLITTA* D. THROUGH RECURRENT SELECTION

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ABSTRACT

Daba trivoltine race of *Antheraea mylitta* D. was segregated from the Daba bivoltine population, for utilization in the hotter zones of the country to enable an additional crop to the farmers. The exploitation of this race for seed production over the years, to meet the demand of commercial rearers, resulted in a considerable decline in its vigour and quantitative traits of commercial importance. Hence, the breeder stock of the Daba trivoltine race was considered for improvement through the recurrent selection method. Initially, a base population was prepared by pooling the Daba TV populations collected from different geo-climatic regions of Chhattisgarh. Subsequently, recurrent selection strategy was resorted to for six generations, keeping the selection criteria of higher fecundity (215-240) and higher average shell weight (> 1.25 g). Due to repeated selection, an overall improvement was recorded in the quantitative characters of breeder stock in comparison with the control population. The improved line developed through recurrent selection, is being evaluated through field trials.

Key words: *Antheraea mylitta* D., breeder stock, Daba TV, recurrent selection.

INTRODUCTION

In India, Daba Bi and trivoltine races of tasar silkworm, *Antheraea mylitta* D. are the major source for commercial production of tasar silk. Due to the semi domestic nature of Daba, it is widely exploited for commercial seed production and outdoor silkworm rearing on the host plants, mainly *Terminalia tomentosa* and *T. Arjuna*. Of late, the tasar cocoon production has become an important livelihood activity for many people of Central India (Hansda *et al.*, 2008; Ojha *et al.*, 2009). Daba trivoltine (DTV) race of *A. mylitta* was isolated from the Daba bivoltine population, targetting the hotter zones of the country so that the farmers can harvest an additional crop (Srivastava *et al.*, 2003). Over the years, due to the continuous exploitation of Daba TV for the supply of

seed, there was a progressive decline in its quantitative characters as well as cocoon yield. In view of this aspect, the P4 tasar Breeding Station, Kargi Kota, Chhattisgarh has taken up an experiment to improve the breeder stock of Daba TV through recurrent selection method. The selection of a breeding method in sericulture is ultimately aimed to develop a breed with stability, productivity and higher quality of silk filament and for such goals, the right technique adopted is repeated breeding followed by selection (Tazima, 1984). Through recurrent selection, the mean performance of a population can be improved with minimal loss of genetic variation and also, there are many more opportunities for recombination (Comstock *et al.*, 1949). The selection of parental lines should be precise as per commercial requirement and productivity (Nagaraju *et al.*, 1996; Munoz *et al.*, 2004). The diverse mating system

can balance the traits in selected line for higher quantitative values (Yamaguchi, 2001; Manohar *et al.*, 2009) and specifically, the selection of higher-weight female pupa improves fecundity (Badhera, 1992; Singh *et al.*, 1994; Ghosh *et al.*, 1996; Miller, 2005). Keeping these points in view, the DTV breeder stock maintained for many generations at the P4 breeding station was considered for the stock improvement through recurrent selection.

MATERIALS AND METHODS

The present work was carried out at P4 Tasar Silkworm Breeding Station, Kargi Kota, Chhattisgarh. Seed cocoons of DTV race were collected from four different locations of Chhattisgarh state viz., Pali, Diodel, Boridadar and Kota for the development of source population. Cocoons collected were pooled and prepared a base population for the composite grainage. Cocoon selection was made based on the female cocoon weight and male shell weight to obtain desired cocoon characters for breeding. Grainage operation was conducted during June 2014 at P4 station Kargikota and the subsequent rearing was conducted on the natural plantation of Arjun (*T. arjuna*) plants.

Based on the criteria, high female pupal weight (>10.5 g) and high male shell weight (>1.4 g), crossing was established among the population for improvement. Further selection was made based on the higher fecundity with range, 215-240. In parallel, the control batch (Daba TV without any selection) DFLs were also prepared and selected randomly. Rearing of selected lines with high fecundity, and control (DTV) batches were conducted with 20 DFLs each in three replications in selected patches of natural plantation of Arjun.

Recurrent selection and maintenance

Rearing was carried out for selected lots for further evaluation of the performance in terms of larval weight, cocoon yield, shell weight and shell percentage. Family selection for higher fecundity and shell weight was carried out. The process of selection was repeated for six generations till the desired results were obtained.

Performance evaluation

The selection line was evaluated for pre and post-cocoon parameters in subsequent generations and compared with those of the ruling Daba (TV) race. Samples of 500 cocoons each from selection line and DTV control batches were randomly selected and assessed for the parameters, such as cocoon weight, shell weight, shell percentage, filament length, non-breakable filament length (NBFL), denier, reelability and raw silk percentage, for each crop harvest. Three replications were maintained in each generation and the data collected were analysed and used for further selection.

Statistical analysis: The data were subjected to ANOVA to assess the variations among generations and t-Test was conducted using INDOSTAT 9.2 to compare the performance between selection line and control.

RESULTS

The results presented in Table 1 substantiate the breeding strategy of recurrent selection adopted for silkworm rearing so that the evaluated population and isolated lots based on higher fecundity and shell weight could achieve enhancement in cocoon quality parameters and fecundity. The pooled mean value of the selected lines showed significant ($P<0.05$) improvement over the generations and comparatively better performance than ruling Daba TV populations in all the parameters considered for the evaluation. As a result of selection, the fecundity in the isolated line witnessed an improvement of over 20.51 % (DTV: 195 & isolated line: 235) in the first generation and this trend prevailed even up to the sixth generation. The improvement of fecundity in selection lines due to selection pressure is evident from the values recorded. Fecundity was 195 ± 4.5 in the control batch while it was recorded 235 ± 2 in the selected line in the 1st generation which consistently increased over the generations and recorded 247 ± 5 in the 6th generation. The highest average shell weight of 1.52 g was recorded in isolated line in 6th generation with a maximum value of 1.93 g in the range, which is even at par to that of Daba bivoltine. Average cocoon shell weight of 1.25 g, 1.32 g and 1.53 g were recorded in 1st 2nd and 3rd crop season, respectively during 2014-15. Similarly, 1.26 g, 1.33 g and

Table 1: Quantitative characters of DTV selection line and control, over six generations

Parameter	Generation / Group	1 st	2 nd	3 rd	4 th	5 th	6 th	f value	SED	CD 5 %	t-Test
Fecundity (No.)	Selection Line	235±2	240±5	241±9	237±8	244±4	247±5	1.65	4.86	10.83	147.24
	DTV Control	195±4.5	205±7	200±10	205±5	200±4.5	190±2	13.64	2.22	4.95	P:0.000** SED: 1.53
Hatching (%)	Selection Line	91.3±5.65	92.9±1.05	93.5±2.0	92.5±2.2	93±3.0	94±2.0*	0.57	1.75	3.91	6.39
	DTV Control	89.2±1.0	88.9±1.0	85.3±2.0	89±2.0	88±2.0	85±1.53	4.25	1.37	3.07	P:0.00** SED:0.83
Larval weight (g)	Selection Line	34.5±1.0	39.3±1.0	38.3±2.0	35.5±1.0	38±1.0	38.3±1.0*	5.57	1.08	2.41	2.26
	DTV Control	33.5±1.0	37.2±1.0	37.6±2.0	34.5±2.0	37±2.0	37.2±1.0	4.09	1.20	2.67	P:0.0029** SED:0.68
Cocoon yield (No./DFL)	Selection Line	22±3.0	45.5±1.61	45±1.0	38±3.0	32±1.0	36.5±3.0***	35.67	2.04	4.56	3.107
	DTV Control	18.5±1.53	40±1.0	34±1.0	25±3.0	23±3.0	28.5±2.08	78.54	1.23	2.75	P:0.00** SED:2.64
Cocoon weight (g)	Selection Line	10.02±2.0	11.05±1.0	11.12±1.0	10.08±1.0	11.2±1.0	11.28±1.0	0.67	1.00	2.23	1.390
	DTV Control	9.92±2.0	10.5±1.0	10.21±1.0	9.90±1.0	10.95±1.0	10.13±1.0	0.27	1.08	2.42	P:0.138 SED:0.376
Shell weight (g)	Selection Line	1.25±0.05	1.32±0.20	1.53±0.50	1.26±0.20	1.33±0.03	1.52±0.20*	1.44	0.14	0.33	1.201
	DTV Control	1.22±0.00	1.29±0.10	1.38±0.30	1.23±0.10	1.29±0.01	1.50±0.30	0.55	0.11	0.24	P:0.0106* SED:0.0684
Shell percentage	Selection Line	12.47±1.0	11.94±2.0	13.75±2.0	12.50±1.0	11.87±1.0	13.49±1.0	0.55	1.22	2.73	0.1096
	DTV Control	12.29±1.0	11.56±1.0	13.51±1.0	12.31±0.00	11.78±1.0	12.83±1.0	2.16	0.68	1.52	P:0.9116 SED:0.395

Data are mean ± SD; *Significant@ p<0.05; ** highly Significant@ p<0.01, ***Significant@ p<0.001

Table 2: Post-cocoon characters of DTV selection line and control, over six generations

Parameter	Generation / Group	1 st	2 nd	3 rd	4 th	5 th	6 th	f value	SED	CD 5 %	t-Test
Filament length (m)	Selection Line	495.1±4.3	470.5±2.0	560.03±2.1	495.9±1.8	442.8±2.1	577.80±7.0	498.99	3.294	7.340	4.288
	DTV Control	504.35±1.0	380.13±5.0	362.7±3.0	410.68±5.0	407.23±7.3	510.8±5.0	605.64	3.631	8.090	P:0.0001** SED:18.16
NBFL (m)	Selection Line	201.4±1.1	172.76±2.2	129.91±2.0	201.98±1.1	143.52±0.8	161.83±0.6	694.04	1.1653	2.596	2.194
	DTV Control	144.56±4.0	195.6±5.0	163.75±3.0	174.15±1.0	158.03±2.4	151.12±7.8	2.69	2.610	5.817	P:0.0180* SED:10.39
Denier	Selection Line	9.77±0.015	11.27±0.068	9.45±0.050	9.79±0.100	9.070±0.061	9.25±0.051	456.94	0.0523	0.116	0.435
	DTV Control	10.03±0.04	11.1±0.10	8.28±0.053	8.54±0.36	11.027±0.04	10.52±0.040	200.13	0.123	0.275	P:0.629 SED:0.326
Reelability (%)	Selection Line	40.71±1.12	35.49±1.00	31.42±1.43	40.21±0.26	34.52±1.00	32.63±1.03	35.82	0.9120	2.032	0.5679
	DTV Control	48.41±0.72	43.14±0.85	26.71±0.90	41.71±0.95	33.08±0.27	28.99±0.18	19.85	0.307	0.684	P:0.3419 SED:2.114
Raw silk (%)	Selection Line	57.15±2.00	61.32±1.68	54.98±0.97	57.05±1.50	52.42±0.78	51.70±1.19	16.78	1.223	2.725	3.420
	DTV Control	55.42±0.52	59.62±0.96	46.97±1.85	46.07±2.12	45.33±0.48	51.46±1.21	49.16	1.167	2.602	P:0.000** SED:1.552

Data are mean ± SD; *Significant@ p<0.05; ** highly Significant@ p<0.01, ***Significant@ p<0.001

1.52 g were recorded in three crops, respectively during 2015-16 which is comparatively higher than that of the control DTV. As a result of selection pressure, reasonably higher cocoon yield was recorded in the isolated lines

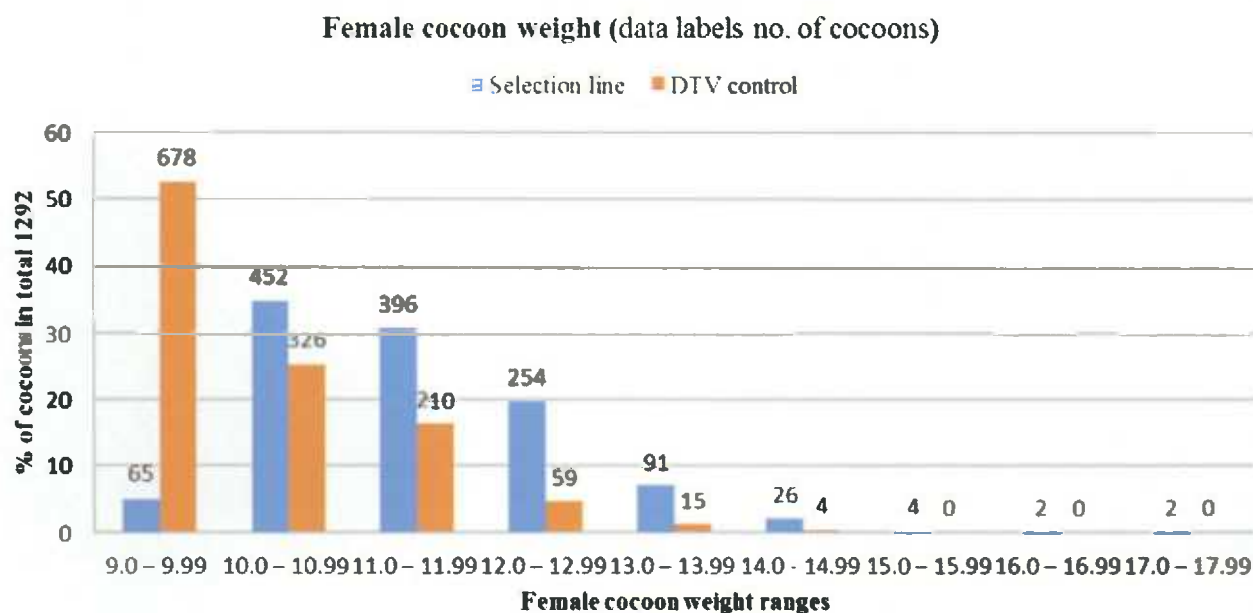


Figure 1: Improvement of female cocoon weight of selection lines over DTV control in 6th generation

irrespective of crop seasons and successive selection in the population. Isolated lines recorded the yield of 36.5 ± 3 cocoons per DFL against the control DTV with 28.5 ± 2.08 cocoons. Post cocoon characters in selection line (500 cocoons) showed highly significant ($p < 0.01$) differences between generations. Improvement was also recorded over control DTV population. Filament length of selection line (577 m) also revealed superiority over DTV control (510 m) in the 6th generation. Similarly, the other post cocoon parameters studied too recorded superiority in the selection lines (Table 2). The cocoons when assessed for the different weight ranges in the selection lines revealed more number of cocoons with higher values, that has contributed for the average improvement in the cocoon weight in the population. Out of 1292 cocoons each of selection line and control populations assessed, female cocoon weight ranging from 10 to 10.99 g recorded an incidence of 34 %, followed by the range of 11-11.99 g with 30 % and 12-12.99 g with 20% in selection line as against DTV recording 52 % in 9-9.99 g, 25 % in 10-10.99 g and 16 % in 11-11.99 g (Figure 1).

DISCUSSION

Various breeding techniques have since long been

aimed towards evolving superior and hardy silkworm breeds either by means of selection alone or by outcrossing or backcrossing followed by selection in the subsequent generations with an aim to stabilize crop or to improve the quantity and quality of silk (Tazima, 1984). Success in any breeding programme for evolving superior breeds depends on the selection of parental material on the basis of their genetic variability, geographical distribution and per se performances and some physio-morphological characters (Akmida *et al.*, 1998). But the breeder should always bear it in mind that selection for one trait has correlation with genetic change of other characters. The correlation between traits can either be negative or positive (Tsuchiya and Kurashima, 1959; Ohi *et al.*, 1970; Gamo and Ichiba, 1971; Gamo, 1976). The performance of silkworm strain in a particular environment decides the superiority (potential) of the strain for that environment (Allard and Bradshaw, 1964). It is reported by Singh *et al.* (2010) that fecundity has been found increasing from lower to higher female pupal weight revealing a positive correlation between the traits. Similar observation was also reported earlier by Singh and Saratchandra (2004) and Jayaswal *et al.* (1990). Hence, selection based on female pupal weight for fecundity improvement will have positive results (Basavaraja *et al.*, 1998). The improvement in commercial traits clearly indicate the role

of selection for desired traits (Reddy *et al.*, 2010 b) as the present observations record improvement of traits such as fecundity and hatching in the selected lines. The higher fecundity along with higher hatching are essential parameters to ensure higher yield in tasar rearing (Reddy *et al.*, 2010 a). The overall performance of the silkworm will ultimately be reflected in the quality and quantity of cocoon or otherwise, the rawsilk, the final out-put of the silk industry. Various factors such as genetic background of silkworm, food quality, environment *etc.*, influence the economic traits of the silkworm (Lokesh *et al.*, 2011). The recurrent selection in the present case has shown improved traits, higher values for the quantitative characters such as fecundity, hatching, cocoon weight and male shell percentage. Moreover, better post-cocoon performance was also recorded in the selection lines. Since the tasar silkworms are semi-domestic in nature, they are subjected to greater influence of the Gene x Environment interactions (Allard and Bradshaw, 1964), which finally decides the productivity. Reiterating earlier reports (Lokesh *et al.*, 2016), it is proven here that the recurrent selection is a crucial method for improvement of tasar silkworm in qualitative as well as productive characters. Further, tasar silkworm breeder stocks need to be maintained for longer period adopting suitable methods like recurrent selection to avoid the effects of inbreeding depression and to exploit maximum benefits of the breeds. The selection lines isolated are required to be test verified judiciously, before being disseminated in the field.

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GRAINAGE PERFORMANCE OF ERI SILKWORM, *SAMIA RICINI* (DONOVAN) DURING DIFFERENT SEASONS IN ASSAM

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ABSTRACT

India is considered as the hot spot of seri-biodiversity particularly in the case of eri and muga silkworm. The eri silkworm, *Samia ricini* (Donovan) is a multivoltine, domesticated insect that can be reared on diverse host plants. Eri silk is considered the pride of Assam and eri silkworm rearing is a heritage and prerogative for the tribals in north-east India, since time immemorial. Due to the implementation of various schemes viz., CDP, NERTPS etc., the demand for eri DFLs has increased manifold in native and non-traditional states as well. To ensure supply of quality eri DFLs as per their scheduled dates, stringent planning of grainage operations becomes essential. For effective programming of grainage schedules, one should have the vital information of seasonal influence on grainage performance. In the present study, eri silkworm grainage performance was studied in different seasons as experienced in Assam viz., winter (December - March), summer (April- June), early monsoon (July- August), late monsoon (September) and autumn (October - November) at ambient room temperature during the year 2015-16. The grainage performance during summer revealed significantly higher per cent of unemerged cocoons (27.2 ± 8.76) as compared to other seasons. The percentage of valid moth emergence with effective coupling was significantly high in autumn season (93.87 ± 1.43) followed by late monsoon (87.37 ± 5.35) and winter (71.10 ± 3.74). The DFL to cocoon ratio was observed to be ideal during autumn (1:4) and late monsoon (1:4.6). The DFL recovery was recorded the least (1:8) during early monsoon followed by summer (1:7) with the characteristic high temperature of 30 to 32°C. Temperature and humidity prevailing during seed cocoon rearing, host leaf nutrition and adoption of standard rearing practices were identified earlier as critical factors determining the grainage performance. The study indicated that eri silkworm grainage operations find it the most favourable in Assam during September to November coinciding with late monsoon and autumn seasons.

Key words: Eri silkworm, *Samia ricini*, seed production.

INTRODUCTION

The north-eastern India is the home tract for a number of wild sericigenous insects. Silk moths have been bred continuously for silk for over five thousand years and their wild counterparts gradually became extinct. The domesticated eri silk moth, *Samia ricini*, was derived from *Samia canningi* (Hutton), thousands of years ago in north-east India (Peigler, 1993). Eri silk has been corroborated in some literature with silks of *Samia cynthia* (Drury) from north-eastern China and *Samia pryeri* (Butler) from Japan. *Samia cynthia* was introduced in

Europe during 1850 and in the United States, in 1861, but is presently extinct in the United States (Peigler and Naumann, 2003). In order to conserve the existing eri silkworm eco races, grainage/seed production sector needs to be strengthened and proper gene bank maintenance is to be ensured.

Eri silk is considered the pride of Assam and rearing of eri silkworm is a heritage and prerogative for the tribals in north-east India since time immemorial. Eri culture is a small scale remunerative enterprise of Assam, often practiced without disturbing the natural ecosystem by

availing of the castor grown on roadsides. Apart from silk, eri pupa is also relished as a delicacy due to its high nutritional value and enriched protein.

Castor, *Ricinus communis* is the primary host of eri silkworm and the other hosts are kesseru (*Heteropanax fragrans*), tapioca (*Manihot utilissima*), jatropa (*Jatropha curcus*), papaya (*Carica papaya*), borpat (*Ailanthus grandis*), borkesseru (*Ailanthus excelsa*) and payam (*Evodia fraxinifolia*) (Kakati and Chutia, 2009). Eri silk is produced in almost all parts of Assam especially in the districts of Kamrup, Udalguri, Goalpara, Nagaon, Golaghat, Dhubri, Kokrajhar, N. C. Hills, Karbi Anglong, Cachar, Darrang, Morigaon, Dhemaji, Lakhimpur, Jorhat, Sivasagar, Dibrugarh and Tinsukia. In recent years, the eri culture is becoming popular in non-traditional states viz., Andhra Pradesh, Tamil Nadu, Orissa, West Bengal, Sikkim, Jharkhand, Bihar, Punjab and Uttar Pradesh (Sarmah *et al.*, 2012).

Due to the implementation of various schemes, the demand for eri silkworm seed has increased manifold in native and non-traditional states as well. To ensure supply of quality eri silkworm disease free layings (DFLs) as per their scheduled dates, stringent planning of grainage operations becomes essential. For effective programming of grainage schedules, seasonal influence on grainage need to be studied.

One of the major factors influencing eri culture is the ambient environment for rearing and grainage. This study is of particular interest to the impending climate change due to global warming and its impact on sericulture. In the present study, the effect of season on eri silkworm seed production / grainage performance was investigated and discussed.

MATERIALS AND METHODS

The study was conducted at Eri Silkworm Seed Production Centre, Central Silk Board, located at Azara, Kamrup district, Assam during the year 2015-16. Eri silkworm grainage was conducted in different seasons viz., winter (December - March), summer (April - June), early monsoon (July- August), late monsoon (September) and autumn (October - November) at ambient room

temperature. In each season, four batches of grainage was conducted on approximately 20,000 each of disease free seed cocoons of eri silkworms of local race which were reared only on castor, the primary host plant, in order to eliminate the variability due to different host plants. The seed cocoons were examined for diseases and culling of undesirable cocoons were done as per norms (Singh, 2015). Proper disinfection practices were undertaken in the grainage hall to ensure hygiene and pathogen free environment. Seed cocoons were kept for emergence in plastic trays in a single layer.

The data on different grainage parameters, such as moth emergence, number of males and females, percentage of healthy / valid and invalid moth emergence and non-emergence were recorded. After emergence, the adults were allowed to expand and harden their wings. Emerged valid male and female moths were kept in plastic crates for coupling. Natural mating occurred within 1-2 hours after emergence. The coupling duration has a direct impact on the fecundity (Behura and Panda, 1978). Only after completion of 6 hours of coupling, the fertilized female moths were placed in "Kharika" (traditional oviposition device) and allowed for egg laying. The total percentage of coupling was recorded and defective ones were noted as rejections. The egg laying was allowed for two days and mother moths were subsequently subjected to examination (Fujiwara *et al.*, 1966) for pebrine incidence. One DFL refers to the eggs laid by a single, disease free, fertilized moth. The DFLs of the entire batch were harvested after individual mother moth examination and surface sterilized. The cocoon: DFL ratio was calculated by finding the ratio of number of seed cocoons processed to the number of DFLs harvested. Fecundity and hatching percentage were also tabulated for analysis.

Data were subjected to analysis of variance (ANOVA) to test the significance of the variations in grainage parameters across the seasons. The pooled data of 4 batches of grainage trials conducted during the specific season is expressed as mean $\pm$ standard error. Multiple comparison of mean values were made depending on F-ratio and the critical difference (CD) values based on student t criteria at 5 and 1% levels of significance, utilizing the standard methods (Gomez and Gomez, 1984). The weather parameters were correlated with the moth emergence data and regression equations were computed.

RESULTS AND DISCUSSION

The pooled mean of grainage parameters of the four batches of eri silkworm seed production conducted in every season as observed in Kamrup district, Assam viz., winter, summer, early monsoon, late monsoon and autumn are presented in Table 1. The scatter plot graphs along with correlation coefficients and regression equations of various weather parameters versus emergence of eri silk moth are displayed in Figure 1.

Moth emergence

The percentage of valid moth emergence with effective coupling was significantly high in autumn season (93.87 ± 1.43) followed by late monsoon (87.37 ± 5.35) and winter (71.10 ± 3.74). During autumn, significantly higher per cent emergence of male, female and total valid moths were observed as compared to summer, winter and early monsoon. However, the male moth, valid male moth and total valid moth emergences were statistically on par for autumn and late monsoon seasons. Significantly higher number of female and valid female moth emergences were also recorded during autumn as compared to late monsoon.

During summer, higher per cent of non-emerged cocoons (27.2 ± 8.76) were recorded, besides higher emergence of crippled moths. Non-emergence of seed

cocoons is one of the vital parameter that can hamper the success of eri silkworm seed production. The present results revealed significantly less non-emergence of seed cocoons during autumn and late monsoon than all other seasons.

Coupling

The total coupling percentage was found significantly better in autumn, winter and late monsoon as compared to summer and early monsoon. However, differences in rejections due to the defective coupling across the seasons were statistically insignificant.

DFL recovery: Seed cocoon

The DFL to cocoon ratio is the ratio of DFLs harvested to seed cocoons processed in every batch. This ratio determines the economic feasibility and acts as an indicator of success or failure of the grainage operations. The DFL to cocoon ratio seemed to be ideal during autumn (1:4) and late monsoon (1:4.6). The DFL recovery was recorded the lowest (1:8) during early monsoon followed by summer (1:7).

Fecundity

Fecundity refers to the number of eggs in a disease free

Table 1: Eri silkworm grainage parameters with respect to different seasons in Kamrup district, Assam

Seasons (Months)	Moth Emergence (%)						Coupling (%)		Cocoon to DFL recovery	Fecundity	Hatching percentage
	Male	Valid male	Female	Valid female	Total valid moths	Non- emergence	Total	Rejected			
Winter (December- March)	53.53±6.37	50.18±4.79	22.62±1.16	20.92±2.03	71.10±3.74	23.85±5.65	28.06±4.29	1.78±1.01	5.54±0.37	325.50±9.63	79.25±1.55
Summer (April- June)	54.47±6.20	48.32±6.61	18.33±2.57	16.51±2.05	64.83±8.66	27.20±8.76	22.67±0.31	1.20±0.37	7.05±1.12	288.25±6.75	72.25±3.12
Early monsoon (July- August)	61.01±4.91	51.82±6.15	19.61±2.65	15.60±2.91	67.42±8.63	19.38±6.61	19.12±2.34	1.09±0.45	7.77±1.05	292.50±7.50	83.25±1.38
Late monsoon (September)	69.42±2.22	64.31±1.74	25.22±0.91	23.06±0.95	87.37±2.68	5.36±3.09	24.38±0.77	1.41±0.97	4.67±0.18	312.50±5.95	89.50±0.96
Autumn October-November)	68.99±1.59	65.81±1.00	29.07±1.97	28.06±2.15	93.87±1.43	1.94±0.64	28.59±2.08	1.45±0.35	3.90±0.33	345.50±7.71	88.25±2.22
CD value	8.19*	8.33*	3.80**	4.12**	10.80**	9.99**	4.44*	NS	1.32**	16.22**	3.83**

Each value represents pooled data of 4 batches of grainage trials conducted during the specific season as Mean ± Standard Error;

NS-Non-significant; ** Significant at 1 % level ; * Significant at 5 % level

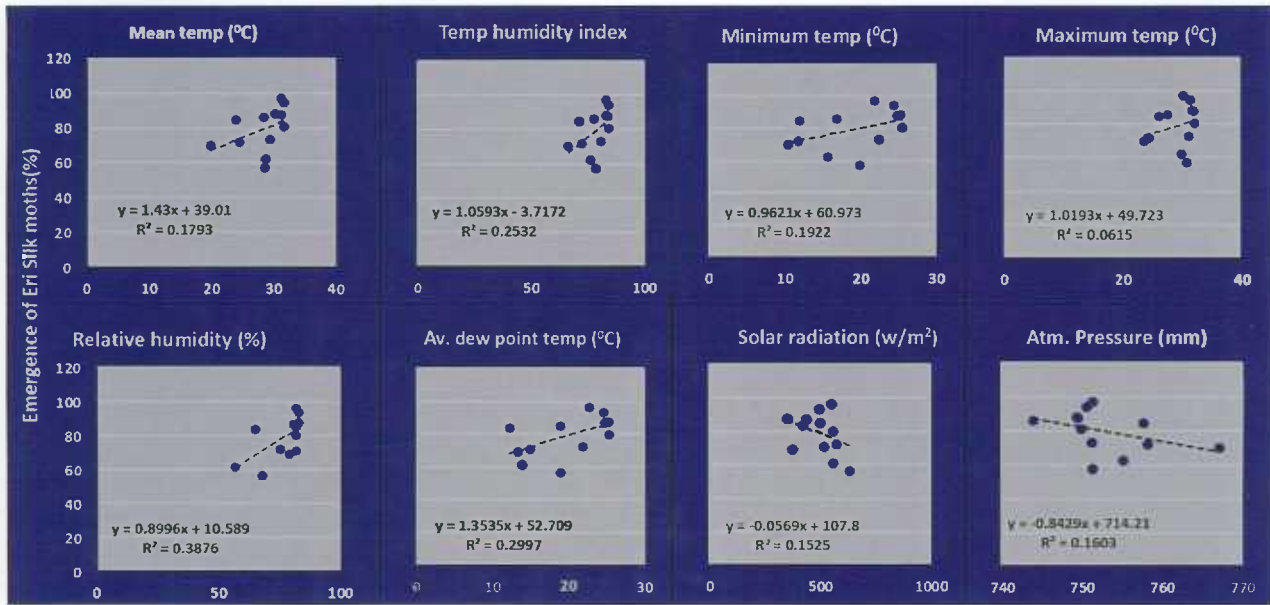


Figure 1: The scatter plot graphs of various weather parameters versus emergence of eri silk moths from seed cocoons during 2015-16 in Kamrup district, Assam

laying. The results revealed significantly higher fecundity during autumn (345.50 ± 7.71) followed by winter (325.50 ± 9.63) and late monsoon (312.50 ± 5.95).

Hatching

Low hatching percentage was recorded during summer when compared to all other seasons. This is due to the higher tendency of deposition of unfertilized eggs and low coupling efficacy encountered during summer because of the prevailing high temperature, ranging from $32\text{--}39^{\circ}\text{C}$. Higher coupling realization was achieved with the highest hatching percentage during the period of September to November.

Weather parameters vs moth emergence

The results (Figure 1) showed relative influence of various weather factors on the eri silk moth emergence from the seed cocoons. Simple correlation and linear regression equation were computed to depict the influence of weather parameters on moth emergence. The analysis revealed that maximum impact on moth emergence was exerted by relative humidity (Figure 1E) ($r=0.62$; $R^2=0.3876$) followed by average dew point temperature (Figure 1F) ($r=0.55$; $R^2=0.2997$) and temperature humidity index (Figure 1B) ($r=0.50$;

$R^2=0.2532$). The mean temperature (Figure 1A), minimum (Figure 1C) and maximum temperature (Figure 1D) showed positive correlation while the solar radiation (Figure 1G) ($r=-0.39$) and atmospheric pressure (Figure 1H) ($r=-0.40$) showed negative correlation with moth emergence.

Based on the results of the current study, it is inferred that the period from September to November, coinciding with the late monsoon and autumn seasons could be the most favourable period for eri silkworm seed production in Assam. Relative humidity, dew point temperature and temperature humidity index exhibits a positive correlation with moth emergence. The results are in agreement with the findings made in the study of grainage character of eri silkworm in different seasons by Sarkar *et al.*, 2010 and studies on fecundity by Debaraj *et al.*, 2003. Temperature during seed cocoon rearing, relative humidity, host leaf nutrition (Sarkar *et al.*, 2015; Sarmah *et al.*, 2015) and adoption of standard rearing practices (Basumatary *et al.*, 2003; Singh, 2015) were identified as other critical factors determining the grainage performance.

During winter, due to the extended larval period and scarcity of host plant leaves, eri silkworm seed cocoon rearing is severely hampered. The life cycle duration of the eri silkworm usually increases due to the lesser day

threshold temperature in winter (Singh and Benchamin, 2002). Due to these practical problems experienced during winter, only the autumn and late monsoon seasons spanning from September to November can be utilized effectively for eri silkworm seed production. However, the early monsoon season could be successful for eri silkworm seed production only if the seed cocoon rearing and spinning was not hampered by high temperature. Summer months are unsuitable for eri silkworm seed production due to the inferior performance characterised by the least moth emergence, low coupling recovery, low fecundity, low hatchability of eggs and higher emergence of deformed moths. Hence, large scale eri silkworm grainage should be avoided during summer.

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Research Paper

ASSESSMENT OF HOST PLANT SUITABILITY FOR BEST REPRODUCTIVE TRAITS OF *ANTHERAEA MYLITTA* IN UTTARAKHAND, INDIA

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ABSTRACT

Antheraea mylitta Drury being a lepidopteran insect, meet its entire nutritional requirement during larval stage to accumulate sufficient food energy to tide over non-feeding adult to egg laying stage. In this study, effects of host plants, rearing season and their interactions on reproductive traits of *A. mylitta* were taken as primary research requisites to assess the suitability of different forest tree species on grainage behaviour and seed multiplication pattern of *A. mylitta* with the primary aim to introduce tropical tasar culture in Uttarakhand. This is the first attempt of its kind on *A. mylitta* 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 grainage activities were carried out on the generated cocoons of all the treatments and data on thirteen reproductive variables of *A. mylitta* were collected. Results indicated that *Terminalia alata*, *T. tomentosa*, *Lagerstroemia speciosa* and *T. arjuna* are the best suitable forestry host plants in their order of merit for most of the examined variables of *A. mylitta*. Interestingly, overall reproductive performance of *A. mylitta* was found better on *L. speciosa* than *T. arjuna* and in Uttarakhand, *L. speciosa* grow luxuriously, not preferred by browsing animals, has larger leaf area than *T. arjuna* and can easily be propagated. 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 introduction of tropical tasar culture in the state.

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

INTRODUCTION

This study is aimed for 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.

Tasar culture is an environment-friendly endeavour and plays a significant role in generating employment and income for forest dependent people. Introduction of *A. mylitta* in Uttarakhand may be helpful in two ways; first, it would create a new livelihood opportunity for the poor families in forest fringe villages and second, this forestry activity may improve the efficacy of forest conservation efforts of the Government. Studies have indicated that

when forest dependent people are associated with forest insect related industry like rearing of *A. mylitta*, it links livelihood activity with initiatives on forest conservation (Bhatia and Yousuf, 2013 a & b). However, in Uttarakhand, *A. mylitta* has never been tried in spite of huge availability of its forestry host plants; hence, an attempt on evaluation of tropical tasar silkworm, *A. mylitta* Drury on different host plants of forestry importance in Uttarakhand was carried out.

The foundation for study of insect-host plant relationships was first laid by Charles T. Brues in 1920's (Brues, 1920, 1924) and after that, there have been numerous studies on different economic insects versus host plants under various 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 any of the Himalayan states of India, including Uttarakhand (Bhatia and Yousuf, 2013 c), which is bestowed with a huge forest and tree cover of 24993 km² (46.73 % of geographical area) providing livelihood support to 6.207 lakh BPL families residing in and around the forests (DES, 2013). It has been proven that host plant influence larval weight gain, survival percentage, growth index, pupal weight, adult emergence and fecundity of different lepidopterans (Basu, 1944; Srivastava, 1959; Thobbi, 1961; Pandey *et al.*, 1968; Singh and Byas, 1975; Dubey *et al.*, 1981), but relatively little attention has been paid for evaluating different forestry host plants for various reproductive traits of *A. mylitta*.

In this study, assessment of different forestry host plants on reproductive potential of *A. mylitta* has been taken up as a primary research requisite to assess the suitability of different forest tree species on grainage behaviour and seed multiplication traits of *A. mylitta* to introduce tropical tasar culture in Uttarakhand, because studies on host plant interaction of sericigenous insects is

suitability under different environmental conditions (Xue *et al.*, 2009) and third, cocoon crops of *A. mylitta* are known to be influenced by the season, host plants and their interactions (Venugopal and Krishnaswami, 1987).

MATERIALS AND METHODS

Study area and climate

Study was conducted between April 2012 and July 2014 at New Forest, Forest Research Institute (FRI), Dehra Dun, Uttarakhand, situated at 30°19' 56.21" N to 30°21' 5.35" N and 77°58' 56.81" E to 78°0' 59.73" E at 640.08 AMSL. The climate of New Forest, Dehra Dun is moderate due to its location at foot of the Himalayas (WMD, 2011). In the foothills, altitude is the main factor for variation in temperature and vegetation. Data on weather variables of the study period are presented in Table 1.

Forestry host plants

Seven forest tree species viz., *Terminalia alata* Heyne

Table 1: Data on weather variables of the study site (New Forest, FRI, Dehra Dun) for 2012-14

Month	Temperature (°C)		Relative humidity (%)		Rainfall	Bright sunshine		Wind velocity
	Maximum	Minimum	Maximum	Minimum	mm	Days	h/day	km/h
January	19.50	3.95	98.50	49.50	19.00	2.5	5.85	2.25
February	22.85	6.10	96.50	38.50	36.80	3	6.7	2.6
March	29.40	10.60	89.00	31.50	13.75	1.5	8.4	2.95
April	34.25	14.80	68.50	26.00	17.65	1.5	8.35	3.55
May	36.50	18.00	57.50	25.50	12.65	1.5	8.4	3.8
June	37.35	21.05	64.00	34.50	66.15	7	6.9	3.95
July	30.80	23.05	93.00	75.00	660.25	18	3.2	2.7
August	29.55	22.75	96.00	79.50	964.60	22	2.55	2.4
September	29.40	20.75	94.00	73.50	579.00	14.5	5.65	2.5
October	29.55	13.65	93.00	49.50	5.60	1	8.15	2.1
November	25.90	8.65	98.00	48.00	19.05	1.5	5.8	1.6
December	21.85	4.35	98.50	46.00	26.05	1.5	5.7	1.7

necessary for better management of sericulture industry in new climatic zones of India (Sinha *et al.*, 2000); secondly, studies on the effects of host plants on the biology of insects is important in understanding the host

ex Roth. (Combretaceae); *T. tomentosa* Weight et Arn. (Combretaceae); *T. arjuna* Bedd. (Combretaceae); *T. bellirica* Roxb. (Combretaceae); *T. chebula* Retz. (Combretaceae); *Lagerstroemia speciosa* (Linn.) Pers.

(Lythraceae) and *L. tomentosa* Presl. (Lythraceae) that are widely distributed in tropical forests of Uttarakhand (Thangavelu, 2004) were chosen. Identification of forest tree species was carried out at Systematic Botany Division, FRI, Dehra Dun and voucher specimens were deposited.

Test insect

Bivoltine (BV), Daba ecorace of *A. mylitta* that is endowed with high exploitation potential and better ecological adaptability formed the insect of choice for the study. Seventy five disease free layings (DFLs) of the race were procured from Basic Seed Multiplication and Training Centre (BSMTC), Central Silk Board, Ambikapur, District, Surguja, Chhattisgarh, on 8th July 2012. Microscopic examination of the layings was carried out as per methods described by Sharan *et al.*, 1992.

Equipment and chemicals

Denver Electronic Precision Balances SI-234, Olympus SXZ16 stereomicroscope, waterproof digital Mahr Digital Vernier Calliper 16 EX, microscope, Canon A3300 IS digital camera, BOD incubator, oven, hygrometer, thermometer, nylon net, insect collection net, spreading board, secateurs, plastic trays, egg laying nylon bags, egg boxes, insect cage, ethyl acetate, 80 % alcohol, ISI marked bleaching powder, formaldehyde *etc.* were used in this study.

Rearing and grainage operation

Four trials of 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 with the seed cocoons generated from all the replicates during September-October of 2012 & 2013 and June-July of 2013 & 2014 as per the improved grainage technology of Narain *et al.* (2001). Properly cleaned and disinfected insect cages, each of 21" L x 21" W x 30" H with wire mesh on top were used for preservation of seed cocoons and nylon bags of 8" x 4" were used for oviposition by the single moth.

Variables measured

Effect of different forestry host plants, rearing seasons and their interactions were measured on thirteen reproductive variables of *A. mylitta* and standard formulae as advocated by Reddy *et al.* (2010) and Ojha and Panday (2004) were used.

Statistical methods

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 to the moth emergence, copulation and hatching percentage. 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). Two-way completely randomized block factorial design was used to test the significance of differences in the mean 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 mean.

Evaluation index (EI)

Evaluation index (E.I.) shows an aggregate unit of traits under examination of *A. mylitta* reared on a particular host plant in different rearing seasons that was calculated as per the procedures outlined by Mano *et al.* (1993, 1998).

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

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 2) 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

The role of host plant is an important factor in regulating reproductive behaviour and thereby insect population (Umbarihowar and Hastings, 2002), because variation in host plant quality affects life history parameters of herbivores (Awmack and Leather, 2002). Results on analysis of variance presented in Table 3 reveal that null hypothesis (H_0) for no difference on effect of rearing seasons, host plants and their interactions on most of the measured reproductive variables of *A. mylitta* was rejected by ANOVA ($p < 0.05$), except to the effect of interaction between rearing season and host plants on emergence percentage of ♀ moths ($p > 0.05$), coupling percentage ($p > 0.05$) and hatching percentage ($p > 0.05$) that did not differ significantly with each other. Further, coefficient of egg laying of the mother moth differed significantly only between host plants ($p < 0.05$); however, between rearing seasons ($p > 0.05$) and their interactions, it was found insignificant ($p > 0.05$).

Results also showed that mean weight of ♂ and ♀ pupae, copulation percentage, mean weight of ♂ and ♀ moths, longevity of ♂ and ♀ moths, fecundity, hatching percentage and mean weight of hatched out larvae were significantly higher in first grainage season as compared to second grainage season, but mean coefficient of egg laying did not follow any definite trend in any of the grainage seasons as evidenced by Tukey's HSD test (Table 4). Further, emergence percentage of ♂ and ♀ moths was found significantly higher in second grainage season as compared to first grainage season.

Larvae of non-feeding adult insects like *A. mylitta* are voracious feeders; hence, their growth rate, survival and reproduction traits are influenced by food quality (Rath et

al. 2006), because most of the economic traits of silkworms are quantitative in nature and seasonal variables viz., temperature, relative humidity, rainfall and photoperiod influence them greatly (Legay, 1958; Yokoyama, 1963, Dash et al., 1992, Chakravorty and Pandey, 2005). Reproduction potential of an insect closely relates with the nutritional factors of the host plant (Rath, 2000). Adult emergence, adult weight, fecundity and fertility of *A. mylitta* are influenced by host plant species (Rath et al., 1997) and environmental conditions (Zhu et al., 2000; Chen et al., 2002).

Weight of male and female pupae

Pupal stage is the most crucial stage for adult life of *A. mylitta* (Dash et al., 2012), which is a quiescent and non-feeding stage that is adapted to survive the unfavourable environmental conditions (Gordon, 1999). Pupal weight resulting on a host plant is one of the major criteria for evaluating the quality of the food material (Bhattacharya and Pant, 1976). Quality of *A. mylitta* progeny largely depends on the quality of pupae that governs grainage behaviour and reproductive potential of parent stock (Gowda et al., 1988; Govindan et al., 1990).

Sengupta and Barah (1991) had reported that the pupal weight is the best and genuine estimator for fecundity in non-mulberry silkworms. The previous works of Ahsan and Khannajain (1976) on *A. mylitta*, Sengupta and Barah (1991) on *A. assama*, Nagalakshamma (1987) on *P. ricini*, Mukerjee et al. (1983) on *B. mori* had shown positive correlation between weight of the ♀ pupae and their total fecundity.

Irrespective of the host plants and rearing seasons, mean weight of the ♂ pupae of *A. mylitta* was always found lower than that of the ♀ pupae (Table 2). Panda (1972) had also reported same trend in the case of *A. paphia* and Xue et al. (2009) in the case of *Spodoptera litura*. Mean weight of ♂ and ♀ pupae differed significantly between rearing seasons, among host plants and their interactions ($p < 0.05$). Dash et al. (1994), Nayak and Guru (1998), Rath (2000) and Reddy et al. (2012) had also found a significant difference in average weight of ♂ and ♀ pupae of *A. mylitta* reared on different forestry host plants in different agro-climatic conditions. Devaiah and

Table 2: Effect of rearing seasons, host plants and their interaction on reproductive traits of *A. mylitta* in Uttarakhand

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>
Weight (g) of ♂ pupae	II*	10.28 ^l (±0.17)	8.34 ^k (±0.11)	7.65 ^{hi} (±0.11)	7.21 ^h (±0.08)	6.65 ^g (±0.09)	6.04 ^f (±0.06)	6.29 ^g (±0.06)
	I*	11.15 ^m (±0.17)	10.43 ^l (±0.12)	8.78 ^h (±0.11)	8.73 ^h (±0.08)	7.94 ^h (±0.09)	6.59 ^g (±0.06)	6.67 ^g (±0.06)
	III*	10.71 ^l (±0.12)	9.39 ^d (±0.13)	8.22 ^c (±0.10)	7.97 ^g (±0.09)	7.29 ^h (±0.08)	6.31 ^h (±0.05)	6.48 ^h (±0.06)
Weight (g) of ♀ pupae	II*	11.07 ^g (±0.11)	9.551 ^m (±0.11)	9.14 ^l (±0.10)	8.46 ^h (±0.11)	7.92 ^h (±0.11)	6.98 ^g (±0.07)	7.32 ^h (±0.08)
	I*	12.14 ^g (±0.11)	11.65 ^g (±0.12)	10.13 ^g (±0.10)	10.63 ^g (±0.11)	9.72 ^{mm} (±0.11)	7.61 ^h (±0.07)	8.09 ^h (±0.08)
	III*	11.61 ^f (±0.09)	10.60 ^g (±0.13)	9.64 ^g (±0.08)	9.55 ^g (±0.13)	8.82 ^g (±0.11)	7.30 ^h (±0.06)	7.71 ^h (±0.07)
Emergence percentage of ♂ moths#	II*	50.53 ^l (±0.33)	47.97 ^h (±0.27)	49.45 ^h (±0.27)	46.73 ^h (±0.23)	48.58 ^h (±0.28)	48.16 ^h (±0.37)	47.10 ^h (±0.3)
	I*	46.41 ^h (±0.36)	44.97 ^h (±0.35)	45.99 ^h (±0.32)	41.75 ^g (±0.28)	42.76 ^g (±0.28)	42.33 ^g (±0.49)	43.74 ^g (±0.33)
	III*	48.47 ^g (±0.49)	46.47 ^g (±0.38)	47.72 ^g (±0.42)	44.24 ^g (±0.55)	45.67 ^g (±0.64)	45.24 ^g (±0.68)	45.42 ^g (±0.41)
Emergence percentage of ♀ moths#	II*	37.66 ^g (±0.3)	39.25 ^h (±0.22)	37.31 ^g (±0.28)	39.9 ^h (±0.28)	38.08 ^g (±0.29)	37.24 ^g (±0.27)	37.03 ^g (±0.35)
	I*	35.33 ^g (±0.26)	35.89 ^h (±0.33)	34.66 ^g (±0.34)	37.18 ^g (±0.37)	34.25 ^g (±0.45)	34.28 ^g (±0.50)	34.62 ^g (±0.39)
	III*	36.50 ^g (±0.31)	37.57 ^g (±0.40)	35.98 ^g (±0.35)	38.54 ^g (±0.36)	36.17 ^g (±0.48)	35.76 ^g (±0.41)	35.83 ^g (±0.36)
Coupling percentage#	II*	75.29 ^g (±0.64)	74.59 ^g (±1.70)	73.54 ^g (±1.58)	74.69 ^g (±0.72)	68.17 ^g (±0.56)	64.20 ^g (±0.48)	65.32 ^g (±0.48)
	I*	77.45 ^g (±1.08)	75.97 ^g (±1.81)	74.43 ^g (±1.41)	76.4 ^g (±1.12)	68.90 ^g (±0.83)	64.39 ^g (±0.47)	67.37 ^g (±0.63)
	III*	76.37 ^g (±0.65)	75.28 ^g (±1.22)	73.98 ^g (±1.04)	75.55 ^g (±0.68)	68.54 ^g (±0.50)	64.3 ^h (±0.33)	66.35 ^h (±0.44)
Weight (g) of ♂ moths	II*	2.241 ^m (±0.02)	1.93 ^b (±0.03)	1.69 ^g (±0.03)	1.73 ^g (±0.02)	1.50 ^h (±0.02)	1.24 ^g (±0.02)	1.31 ^h (±0.02)
	I*	2.42 ^m (±0.03)	2.23 ^l (±0.04)	1.89 ^h (±0.03)	1.96 ^h (±0.02)	1.67 ^g (±0.02)	1.29 ^g (±0.02)	1.44 ^h (±0.02)
	III*	2.33 ^l (±0.02)	2.08 ^g (±0.03)	1.79 ^g (±0.02)	1.84 ^g (±0.02)	1.58 ^g (±0.02)	1.26 ^g (±0.01)	1.37 ^g (±0.02)
Weight (g) of ♀ moths	II*	5.19 ^g (±0.06)	4.70 ^g (±0.05)	4.32 ^g (±0.05)	4.41 ^m (±0.06)	3.45 ^h (±0.05)	2.80 ^h (±0.03)	3.15 ^h (±0.04)
	I*	5.78 ^g (±0.06)	5.23 ^g (±0.07)	4.191 ^m (±0.09)	5.02 ^g (±0.05)	3.94 ^g (±0.06)	2.95 ^h (±0.03)	3.33 ^h (±0.03)
	III*	5.48 ^g (±0.05)	4.97 ^g (±0.05)	4.25 ^g (±0.05)	4.71 ^g (±0.05)	3.69 ^g (±0.05)	2.88 ^g (±0.02)	3.24 ^h (±0.03)
Mean longevity (days) of ♂ moths	II*	8.20 ^m (±0.10)	6.93 ^h (±0.09)	4.96 ^g (±0.06)	5.65 ^g (±0.07)	3.46 ^g (±0.05)	3.53 ^g (±0.04)	3.55 ^g (±0.06)
	I*	8.83 ^g (±0.12)	7.60 ^g (±0.10)	5.79 ^g (±0.08)	6.18 ^g (±0.1)	3.84 ^g (±0.05)	3.62 ^g (±0.05)	3.66 ^g (±0.06)
	III*	8.52 ^g (±0.08)	7.26 ^g (±0.08)	5.37 ^g (±0.06)	5.92 ^g (±0.06)	3.65 ^h (±0.04)	3.57 ^g (±0.03)	3.60 ^h (±0.04)
Mean longevity (days) of ♀ moths	II*	7.05 ^m (±0.1)	5.73 ^h (±0.08)	4.37 ^g (±0.05)	4.63 ^g (±0.07)	3.01 ^g (±0.04)	2.92 ^g (±0.04)	3.10 ^h (±0.05)
	I*	7.67 ^g (±0.08)	6.35 ^g (±0.09)	5.08 ^g (±0.08)	5.47 ^g (±0.09)	3.36 ^g (±0.05)	3.12 ^h (±0.04)	3.27 ^h (±0.03)
	III*	7.36 ^g (±0.07)	6.04 ^g (±0.07)	4.73 ^h (±0.06)	5.05 ^g (±0.07)	3.18 ^g (±0.03)	3.02 ^g (±0.03)	3.19 ^g (±0.03)
Average fecundity	II*	224.7 ^m (±2.5)	192.5k ^l (±3.20)	185.7 ^h (±2.10)	199.4 ^l (±3.10)	135.8 ^g (±1.80)	71.2 ^h (±1.00)	97.2 ^l (±1.40)
	I*	244.1 ^g (±3.3)	220.8 ^m (±3.60)	196.6 ^h (±3.00)	229.1 ^m (±2.60)	146.9 ^g (±1.80)	75.4 ^h (±0.80)	106.8 ^g (±2.10)
	III*	234.4 ^g (±2.2)	206.6 ^g (±2.70)	191.1 ^g (±1.90)	214.2 ^g (±2.40)	141.4 ^g (±1.40)	73.3 ^g (±0.70)	102.0 ^h (±1.30)
Coefficient of egg laying (%)	II*	95.53 ^g (±0.12)	93.32 ^g (±0.14)	91.89 ^g (±0.06)	94.93 ^m (±0.08)	90.38 ^g (±0.12)	88.71 ^h (±0.09)	90.51 ^h (±0.08)
	I*	95.51 ^g (±0.11)	93.031 ^g (±0.14)	91.92 ^h (±0.09)	94.75 ^m (±0.07)	90.37 ^g (±0.13)	88.83 ^h (±0.08)	90.91 ^h (±0.17)
	III*	95.52 ^g (±0.08)	93.17e ^g (±0.10)	91.9 ^g (±0.05)	94.84 ^g (±0.05)	90.38 ^g (±0.09)	88.77 ^g (±0.06)	90.71 ^h (±0.09)
Hatching percentage#	II*	89.06 ^g (±0.74)	87.11 ^g (±0.80)	87.59 ^g (±0.76)	87.20 ^g (±0.70)	79.83 ^g (±0.98)	74.54 ^g (±0.90)	78.01 ^h (±1.04)
	I*	89.49 ^g (±0.63)	89.26 ^g (±0.95)	89.18 ^g (±0.74)	88.50 ^g (±0.77)	84.32 ^g (±1.02)	76.78 ^g (±0.99)	78.23 ^g (±1.02)
	III*	89.27 ^g (±0.48)	88.19 ^g (±0.63)	88.39 ^g (±0.53)	87.85 ^g (±0.52)	82.07 ^g (±0.73)	75.66 ^h (±0.68)	78.12 ^h (±0.73)
Weight (g) of hatched larvae	II*	0.012 ^h (±0.00)	0.011 ^h (±0.00)	0.013 ^h (±0.00)	0.011 ^g (±0.00)	0.011 ^g (±0.00)	0.011 ^g (±0.00)	0.011 ^g (±0.00)
	I*	0.015 ^l (±0.00)	0.014 ^g (±0.00)	0.013 ^h (±0.00)	0.012 ^h (±0.00)	0.014 ^g (±0.00)	0.013 ^h (±0.00)	0.013 ^h (±0.00)
	III*	0.013 ^g (±0.00)	0.013 ^g (±0.00)	0.013 ^g (±0.00)	0.012 ^g (±0.00)	0.013 ^h (±0.00)	0.012 ^h (±0.00)	0.012 ^h (±0.00)

#Data represent angular transformed values of mean and their descriptive statistics; *Values represent combined mean of six replications ± standard error for second crop grainage of 2012 and 2013 (II) and first crop grainage of 2013 and 2014 (I) and that combined for II and I (III); Each replication consisted of 5 samples, thus having a total of 30 observations; Significant differences within every variable are indicated by different superscripts; whereas, same superscript within every variable represents a homogeneous groups (Tukey's HSD test at alpha = 0.05).

Table 3: Analysis of variance for effects of rearing seasons, host plants and their interactions on reproductive traits of *A. mylitta* in Uttarakhand

Trait	Factor	DF	SS	MS	F value	P-value
Weight (g) of ♂ pupae	Rearing seasons	1	263.71	263.71	411.51	0.000
	Host plants	6	1798.02	299.67	467.62	0.000
	Rearing season* host plant	6	61.96	10.33	16.12	0.000
	Error	826	529.33	0.64		
	Total	839	2653.02			
Weight (g) of ♀ pupae	Rearing seasons	1	389.68	389.68	631.92	0.000
	Host plants	6	1677.08	279.51	453.27	0.000
	Rearing season* host plant	6	74.64	12.44	20.17	0.000
	Error	826	509.36	0.62		
	Total	839	2650.76			
Emergence percentage of ♂ moths	Rearing seasons	1	801.6	801.6	636.6	0.000
	Host plants	6	316.5	52.7	41.9	0.000
	Rearing season* host plant	6	50.5	8.4	6.7	0.000
	Error	154	193.9	1.3		
	Total	167	1362.5			
Emergence percentage of ♀ moths	Rearing seasons	1	351.6	351.6	255.4	0.000
	Host plants	6	157.9	26.3	19.1	0.000
	Rearing season* host plant	6	10.5	1.7	1.3	0.275*
	Error	154	212	1.4		
	Total	167	732			
Coupling percentage	Rearing seasons	1	71.3	71.3	5.17	0.024
	Host plants	6	3545.4	590.9	42.85	0.000
	Rearing season* host plant	6	19.2	3.2	0.23	0.966*
	Error	154	2123.5	13.8		
	Total	167	5759.4			
Weight (g) of ♂ moths	Rearing seasons	1	6.915	6.915	186.72	0.000
	Host plants	6	104.593	17.432	470.71	0.000
	Rearing season* host plant	6	1.081	0.18	4.87	0.000
	Error	826	30.59	0.037		
	Total	839	143.179			
Weight (g) of ♀ moths	Rearing seasons	1	25.44	25.44	148.4	0.000
	Host plants	6	651.8	108.63	633.67	0.000
	Rearing season* host plant	6	14.12	2.35	13.73	0.000
	Error	826	141.6	0.17		
	Total	839	832.96			
Mean longevity (days) of ♂ moths	Rearing seasons	1	45.21	45.21	126.47	0.000
	Host plants	6	2768.63	461.44	1290.82	0.000
	Rearing season* host plant	6	14.31	2.38	6.67	0.000
	Error	826	295.28	0.36		
	Total	839	3123.42			
Mean longevity (days) of ♀ moths	Rearing seasons	1	53.07	53.07	190.54	0.000
	Host plants	6	1962.42	327.07	1174.19	0.000
	Rearing season* host plant	6	12.44	2.07	7.44	0.000
	Error	826	230.08	0.28		
	Total	839	2258.01			
Average fecundity	Rearing seasons	1	55131	55131	151.53	0.000
	Host plants	6	2709963	451661	1241.45	0.000
	Rearing season* host plant	6	17464	2911	8	0.000
	Error	826	300514	364		
	Total	839	3083077			
Coefficient of egg laying (%)	Rearing seasons	1	428	310	19	0.895*
	Host plants	6	4358	726	1029	0.000
	Rearing season* host plant	6	9	1	2	0.052*
	Error	826	583	1		
	Total	839	4950			
Hatching percentage	Rearing seasons	1	661	661	14.5	0.000
	Host plants	6	22424	3737	82.2	0.000
	Rearing season* host plant	6	367	61	1.3	0.235*
	Error	826	37576	45		
	Total	839	61027			
Weight (g) of hatched larvae	Rearing seasons	1	0.0006	0.00056	363.44	0.000
	Host plants	6	0.0003	0.00004	28	0.000
	Rearing season* host plant	6	0.0001	0.00002	11.77	0.000
	Error	826	0.0013	0.00000		
	Total	839	0.0022			

Table 4: Tukey's HSD test for effect of rearing seasons on various reproductive traits of *A. mylitta*

Parameter	Homogenous groups statistical descriptions	Grainage season	Mean*
Weight (g) of ♂ pupae	alpha = 0.05000; Error MS = 0.64084, df = 826.00	II I	7.49 8.61
Weight (g) of ♀ pupae	alpha = 0.05000; Error MS = 0.61666, df = 826.00	II I	8.63 10.00
Emergence percentage of ♂ moths	alpha = 0.05000; Error MS = 1.2593, df = 154.00	II I	43.99 48.36
Emergence percentage of ♀ moths	alpha = 0.05000; Error MS = 1.3765, df = 154.00	II I	35.17 38.07
Coupling percentage	alpha = 0.05000; Error MS = 13.789, df = 154.00	II I	70.83 72.13
Weight (g) of ♂ moths	alpha = 0.05000; Error MS = 0.03703, df = 826.00	II I	1.66 1.84
Weight (g) of ♀ moths	alpha = 0.05000; Error MS = 0.17143, df = 826.00	II I	4.00 4.35
Mean longevity (days) of ♂ moths	alpha = 0.05000; Error MS = 0.35748, df = 826.00	II I	5.18 5.65
Mean longevity (days) of ♀ moths	alpha = 0.05000; Error MS = 0.27855, df = 826.00	II I	4.40 4.90
Average fecundity	alpha = 0.05000; Error MS = 363.82, df = 826.00	II I	158.06 174.26
Coefficient of egg laying (%)	alpha = 0.05000; Error MS = 0.70608, df = 826.00	II I	92.18 92.19
Hatching percentage	alpha = 0.05000; Error MS = 45.491, df = 826.00	II I	83.33 85.11
Weight (g) of hatched larvae	alpha = 0.05000; Error MS = 0.00000, df = 826.00	II I	0.012 0.013

Data are combined mean of the variable on all the forestry host plants during first and second rearing season.

Dayashankar (1982) had also reported same results in the case of *Samia cynthia ricini*.

In the present study, overall highest pupal weight was recorded by *T. alata* fed larvae (♂= 10.71 g; ♀= 11.61 g), followed by *T. tomentosa* (♂= 9.39 g; ♀= 10.60 g), *T. arjuna* (♂= 8.22 g; ♀= 9.64 g) and *L. speciosa* (♂= 7.97 g; ♀= 9.55 g) fed larvae. However, the lowest pupal weight was observed in the cocoons of *L. tomentosa* (♂= 6.31 g; ♀= 7.30 g), followed by *T. chebula* (♂= 6.48 g; ♀= 7.71 g) fed larvae. Results indicated that *T. alata* provided the best nourishment to *A. mylitta* that influenced better larval 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* were more preferred 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. Less pupal 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 pupal weight, a higher value of Evaluation Index is considered effective to have better copulation percentage and fecundity. Table 4 shows that *T. alata*, *T. tomentosa*, *T. arjuna* and *L. speciosa* scored higher indices (E.I. >50) in both the rearing seasons and therefore, these host plants were rated superior to *T. bellirica*, *L. tomentosa* and *T. chebula*. Dash *et al.* (2012) had also conducted biometric study of *A. mylitta* pupae raised on different forestry host plants at lower, medium, and higher altitudes in Odisha. They stated that *A. mylitta* larvae reared on *S. robusta* showed significantly the highest values on all the pupal growth parameters. The gradation of eight food plants in respect of the pupal growth performance of *A. mylitta* was found as *S. robusta* > *T. alata* > *T. arjuna* > *Ziziphus jujuba* > *L. parviflora* > *Anogeissus latifolia* > *T. bellirica* > *Syzygium cumini*.

Emergence percentage of male and female moths

Results showed that cocoons of *T. alata* fed larvae had overall, the highest emergence percentage of ♂ moths (48.47 %), followed by the cocoons raised on *T. arjuna* (47.72 %) and *T. tomentosa* (46.47 %). However, the highest ♀ moth emergence (38.54 %) was recorded for the

Table 5: Combined Evaluation Index (E.I.) for reproductive 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>
Weight (g) of ♂ pupae	II	67.72	55.40	51.01	48.18	44.63	40.75	42.31
	I	64.12	60.12	50.94	50.65	46.25	38.75	39.18
	III	64.97	57.50	50.92	49.52	45.73	40.22	41.14
Weight (g) of ♀ pupae	II	65.98	56.02	53.29	48.86	45.31	39.18	41.37
	I	62.26	59.42	50.79	55.63	48.41	36.37	39.14
	III	62.89	57.22	51.80	51.30	47.20	38.64	40.94
Emergence percentage of ♂ moths	II	63.72	47.55	56.96	39.58	51.45	48.73	42.01
	I	61.7	54.73	59.65	39.19	44.02	41.94	48.77
	III	58.01	51.05	55.41	43.22	48.22	46.73	47.36
Emergence percentage of ♀ moths	II	47.06	58.45	44.61	63.17	50.05	44.06	42.59
	I	50.91	54.35	46.79	62.43	44.36	44.55	46.61
	III	49.35	54.53	46.94	59.23	47.86	45.91	46.19
Coupling percentage	II	58.65	56.33	54.86	57.67	45.86	37.00	39.62
	I	58.82	55.87	54.28	57.43	45.31	35.94	42.36
	III	58.71	56.07	54.55	57.54	45.60	36.49	41.03
Weight (g) of ♂ moths	II	65.68	57.37	50.72	51.8	45.56	38.47	40.39
	I	63.4	59.03	51.17	52.62	45.90	37.23	40.64
	III	64.06	58.04	50.94	52.18	45.86	38.14	40.78
Weight (g) of ♀ moths	II	63.22	57.82	53.52	54.55	43.81	36.6	40.48
	I	63.51	58.31	48.51	56.33	46.14	36.83	40.36
	III	63.13	57.93	50.8	55.41	45.17	36.97	40.59
Mean longevity (days) of ♂ moths	II	66.72	59.65	48.78	52.57	40.48	40.86	40.95
	I	65.76	59.68	50.7	52.67	41.05	39.96	40.17
	III	66.08	59.58	49.8	52.6	40.85	40.47	40.62
Mean longevity (days) of ♀ moths	II	67.26	58.65	49.8	51.49	40.92	40.34	41.54
	I	66.19	58.47	51.04	53.35	40.96	39.57	40.44
	III	66.49	58.45	50.44	52.44	41.06	40.06	41.07
Average fecundity	II	61.88	56.13	54.92	57.36	46.03	34.53	39.16
	I	60.94	57.30	53.50	58.59	45.72	34.52	39.44
	III	61.26	56.68	54.12	57.93	45.91	34.68	39.42
Coefficient of egg laying (%)	II	63.6	54.62	48.82	61.16	42.7	35.88	43.21
	I	63.84	53.51	48.86	60.66	42.44	36.00	44.68
	III	63.73	54.08	48.84	60.92	42.57	35.93	43.94
Hatching percentage	II	56.77	54.46	55.04	54.57	45.85	39.6	43.71
	I	55.14	54.88	54.78	53.98	49.07	40.22	41.93
	III	55.92	54.65	54.89	54.25	47.48	39.96	42.85
Weight (g) of hatched larvae	II	53.95	46.32	59.67	45.37	49.18	47.27	48.23
	I	58.74	53.39	50.42	43.89	51.02	46.86	45.67
	III	55.46	50.53	53.61	45.60	49.91	47.45	47.45
Average		60.77	55.87	51.77	53.24	45.65	40.13	42.57

II- Second crop grainage (combined for 2012 & 2013); I - First crop grainage (combined for 2013 & 2014); III - Combined for II & I crop grainage

cocoons formed by *L. speciosa* fed larvae, followed by that by *T. tomentosa* (37.57 %) and *T. alata* (37.57 %) fed larvae (Table 2). Nayak *et al.* (1993) and Mishra *et al.* (1997) support the findings of the present study, that there exists significant differences in emergence percentage of ♂ and ♀ moths of *A. mylitta*, reared on different host plants during different rearing seasons. Further, Rath (2000) had also reported significantly higher adult emergence of *A. mylitta* from the cocoons of *T. tomentosa* and *T. arjuna* fed larvae than other host plants species.

Higher moth emergence was observed during second grainage season as compared to first season. Mishra *et al.* (1997) have also recorded higher moth emergence in second grainage season. Optimal temperature and relative humidity regime might have stimulated the reproductive physiology of the diapausing pupae. Further, Nayak *et al.* (1993) had established a positive correlation between temperature and relative humidity and adult emergence percentage of *A. mylitta*. Jolly *et al.* (1974) have also demonstrated significant effect of environmental variables on moth emergence and coupling percentage of *A. mylitta*. On the other hand, seed cocoons utilised during first grainage season were subjected to preservation period of more than six months that inflicted higher preservation losses and therefore, moth emergence percentage of both the sexes were adversely affected.

Copulation percentage

Copulation percentage is one of the important reproductive traits of *A. mylitta* that influences multiplication pattern and production of disease free layings, because mating and oviposition are two important behavioural aspects of insect reproduction. Overall, the highest copulation percentage was shown by the moths of *T. alata* fed larvae (76.37%), followed by the moths of *L. speciosa* (75.55 %) and *T. tomentosa* (75.28 %) fed larvae. Unlike that of moth emergence percentage, copulation percentage was recorded higher in the first grainage season, as compared to second; which, might have been attributed to the optimal temperature and relative humidity regime. The lower coupling percentage recorded during second grainage season might be the result of lower temperature and lower relative humidity during winter. Life history features of *A. mylitta* such as

moth emergence, coupling percentage, fecundity and hatching percentage are variable between seasons (Jolly *et al.*, 1974).

Nayak and Dash (1989) had demonstrated the influence of weather variables on reproduction in *A. mylitta*. Nayak *et al.* (1993) had also found significant differences between coupling percentage of *A. mylitta* during different rearing seasons. Present results also corroborate the findings of Mishra *et al.* (1997), who reported that the rearing seasons and forestry host plants of *A. mylitta* have significant effect on coupling percentage. However, unlike findings of the present study, they had recorded maximum coupling percentage during second grainage season, when larvae were reared on *T. tomentosa*; however, it was found the lowest on *S. robusta* during winter season.

Tukey's HSD test for the effect of host plants on copulation percentage suggests that moths emerged from the cocoons raised on *T. arjuna*, *T. tomentosa*, *L. speciosa* and *T. alata* did not differ significantly and formed a homogeneous group. However, Reddy *et al.* (2010) found significantly higher copulation percentage of *A. mylitta* in the moths of *T. tomentosa* fed larvae than *L. parviflora* fed larvae. Findings of the present study confirm that, for the best copulation percentage of *A. mylitta* under the climatic conditions of Uttarakhand, four forest tree species viz., *T. alata*, *L. speciosa*, *T. tomentosa* and *T. arjuna* in their order of merit, are the best suitable host plants (Tables 2 and 5).

Weight of male and female moths

Results of Tukey's HSD test presented in Table 4 indicate that overall weight of ♂ and ♀ moths were higher during the first grainage season. 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 these 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. Table 2 reveals that weight of ♂ moths was always lower than that of ♀ moths in all the replicates during both the grainage seasons. It is

reported that functional roles of male and female differs in the adult stage, which is also reflected due to differences in the consumption and utilization of food by male and female larvae. Females tend to be heavier than males (Scriber and Slansky, 1981), indicating greater nutrient accumulation, associated with their role as egg-producers that generally results from increased food consumption by female larvae because of a longer duration of the larval stage than their male counterpart (Slansky and Scriber, 1985).

Overall, the highest weight of ♂ moths (2.33 g) was recorded by *T. alata* fed larvae, followed by *T. tomentosa* (2.08 g), *L. speciosa* (1.84 g) and *T. arjuna* (1.79 g) fed ones. Similarly, moths of *T. alata* fed larvae have shown overall, the highest weight of ♀ moths (5.48 g), followed by the moths of *T. tomentosa* (4.97 g) and *L. speciosa* (4.71 g) fed larvae. Larvae of *A. mylitta* 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). There are reports that size of the moths of *A. mylitta* varies with nutritional contents of different food plants at different altitudes (Miller, 1991; Nayak, 1997). Rath (1998) had also found significantly higher weight of ♂ moths, whose larvae were reared on *T. tomentosa* than that of *T. arjuna*; but, female weight did not differ significantly. Farrell (1973, 1975) reported that host plant influences the weight of female that affects fecundity of individual female. Female of *A. mylitta* with higher weight contributes more towards fecundity (Yadav *et al.*, 2001).

Longevity of male and female moths

The longevity of ♂ and ♀ moths influences grainage performance of *A. mylitta* (Rath *et al.*, 1997). In general, moths of *T. alata* fed larvae showed the highest mean longevity in the case of ♂ and ♀ (8.52 & 7.36 days), followed by the moths of *T. tomentosa* (7.26 & 6.04 days), and *L. speciosa* (5.92 & 5.05 days) fed larvae; however, the lowest mean longevity of ♂ and ♀ moths (3.57 & 3.02 days) was shown by *L. tomentosa* fed larvae. These findings reveal that due to change in food quality, parameters on quantitative nutrition jointly influence adult characters and reproductive fitness of *A. mylitta*,

which are in accordance with the findings of Sinha *et al.* (2000); Scriber and Slansky (1981); Scriber and Feeny (1979); Slansky and Scriber (1982); Periasamy and Radhakrishnan (1985). Naik *et al.* (2010) had also recorded significant variations with regard to longevity of male and female moths of *S. cynthia ricini* reared on different host plants, these variations were due to biochemical composition of leaves that varies from season to season and from locality to locality. It is clear that because of higher nutrition in the leaves of *T. alata* (Sinha *et al.*, 2007), the grainage performance was quite better on *T. alata* than that on *T. tomentosa*.

Results indicated that ♂ moths lived longer than ♀ moths and mean longevity of ♂ and ♀ moths had shown significantly longer durations in the first grainage season. Similarly, Ravishankar *et al.* (2000) had also observed that ♂ moths of eri silkworm lived longer (9.60 days) than ♀ moths (7.35 days). Xue *et al.* (2009) had also reported that ♂ adults of *S. litura* lived longer than females, but differed on different host plants. Bae and Park (1999) suggested that adult longevity became shorter as temperature increased.

Average fecundity and coefficient of egg laying

Fecundity is a crucial element in reproductive behaviour of *A. mylitta*. In the present study, mean fecundity and mean coefficient of egg laying differed significantly between rearing seasons, host plants and their interactions ($P < 0.05$). Nayak *et al.* (1993) and Mishra *et al.* (1997) had also observed significant differences in fecundity of *A. mylitta* during different seasons.

The highest fecundity of *A. mylitta* was shown by the mother moths of *T. alata* fed larvae (234.40), followed by *L. speciosa* (214.20), *T. tomentosa* (206.60), and *T. arjuna* (191.10) fed larvae, whereas, the lowest overall fecundity was realised from the mother moths of *L. tomentosa* (73.30) and *T. chebula* fed larvae (102.0). Mother moths of *T. alata* fed larvae recorded the highest coefficient of egg laying (95.52 %), followed by those of *L. speciosa* (94.84 %) and *T. tomentosa* (93.17 %) fed larvae. In this study, the first grainage season recorded comparatively higher fecundity. Deka *et al.* (2011) had also reported higher fecundity of *A. mylitta* in the first grainage season. Host

plant quality is the key determinant factor for the fecundity of herbivorous insects (Awmack and Leather, 2002). Rath (2000) had also demonstrated significant differences in fecundity and coefficient of egg laying of *A. mylitta* reared on different forestry host plants. Chaudhuri (2003) and Radjabi *et al.* (2009) reported that quality of food plants have significant impact on genital development, fecundity and egg fertility in the mother moths of *A. mylitta*. It can be concluded that reduced fecundity of mother moths whose larvae were fed on *L. tomentosa* and *T. chebula* might be due to lack of proper nutrition or proportionally higher diversion of assimilated food for maintenance of other life activities instead of production of eggs. There are many reports that food deprivation during larval stage had a negative impact on the fecundity in many insects including sericigenous species (McGinnis and Kasting, 1959; Bhanot and Kapil, 1973; Muthukrishnan *et al.*, 1978; Srivastava *et al.*, 1982; Nath *et al.*, 1990).

Present findings indicated that *L. speciosa* is a potential host plant to have better fecundity and coefficient of egg laying of *A. mylitta* in comparison to *T. tomentosa* and *T. arjuna* under the climatic condition of Uttarakhand. This revelation deserves significance in any progressive planning for introduction of *L. speciosa* based commercial rearing of *A. mylitta* in tropical forest areas of Uttarakhand.

Hatching percentage

Time between egg laying and emergence of the first instar larva measures the development rate of insect eggs (Tear *et al.*, 1988). Hatching percentage is an important variable that indicates vitality and healthiness of the eggs that determines initial population of larvae at the time of brushing. In forestry tasar sector, less incubation period and higher hatching percentage is preferable. Hatching percentage was found higher in the first grainage season, as the temperature and relative humidity played a vital role in incubation of silkworm egg. Nayak *et al.* (1993) had also noticed significant differences in hatching percentage of *A. mylitta* during different rearing seasons.

Present study indicates that higher hatching percentage was recorded in those eggs, whose mother

moths were developed from *T. alata* (89.27 %) fed larvae, followed by *T. arjuna* (88.39 %), *T. tomentosa* (88.19 %) and *L. speciosa* (87.85) fed larvae. Observed difference in incubation period might have been attributed to differences in allocation of assimilated nutrients by the larvae fed on different host plants. Reddy *et al.* (2010) had also reported that forestry host plants affect fertility percentage of *A. mylitta*.

Weight of hatched out larvae

The mean weight of hatched out larvae was found higher on all the forestry host plants in the first grainage season as compared to second. Lack of pertinent literature pervades critical comparison on effect of forestry host plants on weight of the hatched out larvae of *A. mylitta*. However, it can be inferred that feeding behaviour and varying nutritional contents in the leaves of tested forestry host plants along with seasonal variations might have corroborated to variations in weight of the hatched out larvae of *A. mylitta* in the present study.

Combined multiple traits evaluation index analysis on reproductive traits of *A. mylitta*

Combined multiple evaluation index analysis for all the 13 reproductive traits of *A. mylitta* indicated that four forestry host plants viz., *T. alata* (60.77), *T. tomentosa* (55.87), *L. speciosa* (53.24) and *T. arjuna* (51.77) have performed well and scored higher indices (E.I. > 50) and were found more suitable in their order of merit for forest based commercial rearing of *A. mylitta* in Uttarakhand (Table 5, Figure 1). However, *T. bellirica* (45.65), *T. chebula* (42.57), and *L. tomentosa* (40.13) fell short of the benchmark value of E.I. (<50).

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) and 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); therefore, 217.9 km² of forests distributed up to the

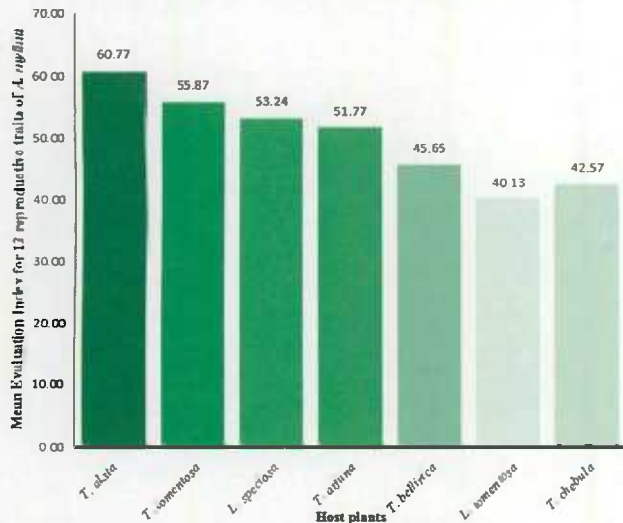
Best suitable host plants for *A. mylitta*

Figure 1: Combined multiple traits evaluation index analysis on reproductive traits of *A. mylitta*

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 (Bhatia and Yousuf, 2016). Further, in Uttarakhand, 12,790.06 km² is wasteland (MoRD, 2013), out of which, 989.77 km² area is distributed at the altitudinal range of <610 meter having tropical moist deciduous and tropical dry deciduous forests conditions (Anonymous, 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* in the state.

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 reproductive traits of 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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Research Paper

REELING PERFORMANCE AND QUALITY CHARACTERISTICS OF RAW SILK REELED USING SLUG CATCHER UNDER VARIOUS COOKING CONDITIONS

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ABSTRACT

Suitable slug catcher for arresting the slugs during the process of reeling was developed at Central Silk Technological Research Institute (CSTRI), Central Silk Board, Bengaluru. The slug catcher is a replacement for porcelain buttons used in silk reeling machine. It is developed to facilitate easy threading by reelers and to improve the efficiency of reeling operations. Earlier studies conducted on the comparison between porcelain buttons and slug catcher indicated no significant difference between reeling performance and quality characteristics of raw silk. Hence, in the present study, the suitability of slug catcher under different cocoon cooking conditions viz., under cooking, normal cooking and over cooking has been experimented and the reeling performance and raw silk quality characteristics have been studied. The results indicate that the reeling characteristics viz., reelability and raw silk percentage and the quality characteristics viz., cleanness, neatness, low neatness, elongation and cohesion were significantly influenced by the cooking conditions. It was further revealed that compared to under and over cooking conditions, normal cooking of bivoltine cocoons have yielded superior quality of raw silk of international grade. Further, the slug catcher was observed as capable of arresting the slugs under different cooking conditions.

Key words: Bivoltine cocoon, button, cleanness, neatness, raw silk percentage, reelability, slug catcher, thread breakage.

INTRODUCTION

The silk industry in India is in the de-centralized sector. In the reeling industry, around 10 ~ 15 kg of raw silk is produced per day by the small-scale reeling filatures whereas, 100 ~ 120 kg of raw silk is produced per day in large scale organized reeling filatures. The capacity of the reelers to purchase sophisticated and costly equipment is very much limited. In this regard, the Central Silk Technological Research Institute (CSTRI), Central Silk Board, Bengaluru, India has been working to evolve better techniques, equipment, machineries and practices on scientific base to achieve better reeling performance and international grade raw silk. Already many research findings have been successfully accepted by the industry. Some of them are hot air drying technology, Ushnakoti technology, cocoon cooking with cage using two pan

cooking methods, pressurized cocoon cooking technology, vacuum permeation cooking method and multiend reeling technology (Lakshmipathaiiah *et al.*, 2000).

Efforts have been underway at CSTRI, to incorporate various simple gadgets in silk reeling industry viz., tail end knot cutter, panel winder *etc.*, which have their share of improvement in the quality of raw silk. In the present study, a suitable gadget, namely slug catcher was developed to replace the buttons in the silk reeling machine to enable the reelers to improve the quality of raw silk produced in the small-scale silk reeling sector.

Development of slug catcher

Presently, porcelain buttons are used in the silk reeling

machine to arrest the slugs entering along with the raw silk during the process of reeling. However, in the small-scale silk reeling industry in India, the reelers have a practice of widening the buttonhole size to avoid frequent stoppage of reeling ends and hence, sometimes, the quality of raw silk is affected. Further the average age of the workers in the silk reeling industry is increasing and passing the silk thread through the buttonhole is becoming a difficult task. To avoid such problems, CSTRI has come out with an alternative facility *i.e.*, slug catcher, through which one can simply pass the thread through a slit and also arrest the slugs during the process of reeling. The line diagram of the button and slug catcher is shown in Figure 1. The slug catcher is made of acrylic material and the two pieces are fitted with a film in between, which can be changed depending upon the denier of raw silk to be reeled.

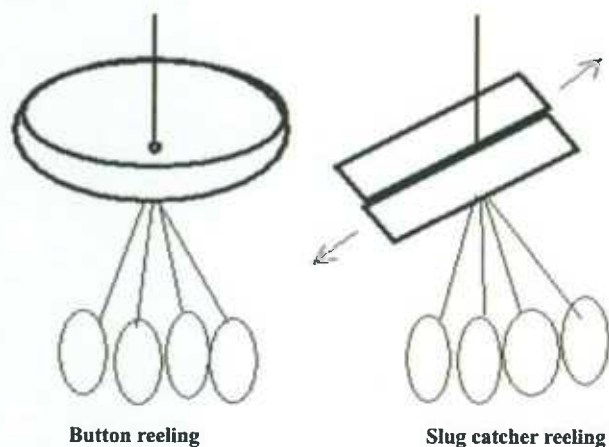


Figure 1: Line diagram of the button and slug catcher used in silk reeling



Figure 2: New model slug catcher developed at CSTRI



Figure 3: Field trials of slug catcher fitted to multiend reeling machine at Kolar

The field trials conducted by CSTRI in various reeling clusters *viz.*, Kollegal, Kolar, Siddlaghatta and other places indicate that the slug catcher works satisfactorily and it is very easy for threading the silk during thread breakages. Figures 2 and 3 display the photographs of the slug catcher and its field trials, respectively.

Further, in order to assess the effectiveness of slug catcher in silk reeling process, a study was conducted to investigate the influence of varied cooking conditions *viz.*, under cooking, normal cooking and over cooking on the reeling performance and quality of raw silk produced.

MATERIALS AND METHODS

Raw material: The commercial cocoons of bivoltine hybrid (CSR2 x CSR4) reared in Karnataka, during November 2015 were used in four replications.

Drying conditions: The cocoons were dried in Batch type hot air drier following the temperature pattern of 115-100-85-70-55 °C for bivoltine hybrid cocoons for a period of 5 hours. The degree of drying achieved was 39–41 % for the cocoons.

Cooking conditions: The hot air dried cocoons were cooked using two-pan following the temperature profiles and durations as given below.

Cooking conditions	Under cooking		Normal cooking		Over cooking	
	Temperature (°C)	Time (sec)	Temperature (°C)	Time (sec)	Temperature (°C)	Time (sec)
Retting	70	30	70	30	70	60
High temperature permeation	96	90	96	120	96	240
Low temperature permeation	70	20	70	60	70	90
Cooking	98	90	98	120	98	180
Adjustment	98 ~ 70	45	98 ~ 70	120	98 ~ 70	300

Brushing conditions: The cooked cocoons were brushed manually at 80 °C using paddy husk brush.

Reeling conditions: Three hundred good cocoons picked from the brushed cocoons were selected for reelability test developed by CSTRI and then reeled on 3 ends of multiend reeling machine, maintaining fixed number of cocoons to a reeling end. The temperature of reeling bath was maintained at 45 °C and reeling speed at 120 m/min.

Silk quality testing: The raw silk after reeling was assessed for quality characteristics. The testing was conducted as per the International Silk Association (ISA) standards (Book of Standards 1952-68). Among the eleven characters used for grading of raw silk, five characters, viz., neatness, cleanness, tenacity, elongation and cohesion, were determined. The testing equipment, viz., seriplane tester (Okamoto Kasakusho, Toyo Sangyo Consulting Inc., Japan), tensile tester (Instron Company Ltd, U.K) and Duplan cohesion tester (Toyo Sangyo Consulting Inc., Japan), were used for the study.

Data: The reeling characteristics viz., reelability percentage, renditta, raw silk percentage, raw silk recovery percentage and waste % on silk weight were recorded while reeling the cocoons subjected to different cooking conditions. The data on quality parameters viz., neatness, cleanness, tenacity, elongation and cohesion were registered after testing raw silk reeled under different cooking conditions. The experimental data were analyzed statistically using SPSS package.

RESULTS AND DISCUSSION

The analysis of variance results and mean values of reeling characteristics of bivoltine cocoons cooked using different cooking methods viz., under cooking, normal /

appropriate cooking method and over cooking method are presented in Tables 1 and 2. Similarly, the analysis of variance results and mean values of quality characteristics of the bivoltine cocoons treated with different cooking methods are presented in Tables 3 and 4.

Table 1: Analysis of variance results of influence of cooking conditions on reeling characteristics

Source	Mean sum of square			
	Degree of freedom	Reelability (%)	Raw silk (Dry) (%)	Waste % on silk weight
Cooking conditions	2	193.8**	7.278**	2.903 ^{NS}
Error	9	1.211	0.629	1.144

** = Significant at 1 % level, ^{NS} – Not significant

Table 2: Influence of different cooking conditions on reeling characteristics

Cooking conditions	Reelability (%)	Raw silk (Dry) (%)	Waste % on silk weight
Under cooking	72.53	35.79	18.97
Normal cooking	75.06	38.05	18.33
Over cooking	85.65	38.20	20.02

Effect of cooking conditions on reeling characteristics Reelability percentage

Reelability percentage is significantly influenced by cooking conditions at 1 % level (Table 1). It could be observed from Table 2 that as the cooking conditions is altered from under to over, the reelability showed an improvement. This is because of the increased sericin softening while over cooking of bivoltine cocoons.

Raw silk percentage

Our results (Table 1) reveal a significant (1 %) impact of the various cooking conditions under study, on raw silk

Table 3: Analysis of variance results of influence of cooking conditions on quality characteristics

Source	Degree of freedom	Mean sum of square					
		Cleanness (%)	Neatness (%)	Low neatness (%)	Tenacity (g/d)	Elongation (%)	Cohesion (Strokes)
Cooking conditions	2	8.083**	4.00**	18.08**	0.011 NS	1.75*	204.75**
Error	9	0.611	0.333	2.083	0.033	0.306	14.722

** - Significant at 1 % level, * - Significant at 5 % level, ^{NS} - Not significant

Table 4: Impact of different cooking conditions on silk quality characteristics

Cooking conditions	Cleanness (%)	Neatness (%)	Low neatness (%)	Tenacity (g/d)	Elongation (%)	Cohesion (Strokes)
Under cooking	98.3	96.5	90.0	3.63	18.5	66.8
Normal cooking	99.0	97.5	94.0	3.73	18.8	72.8
Over cooking	96.3	95.5	90.8	3.65	17.5	58.5

yield. When compared to under cooking conditions, the normal and over cooking conditions could improve the raw silk percentage (Table 2). This is because of the smooth unwinding of filament from the cross over points due to effective cooking of cocoons.

Waste per cent on silk weight

Table 1 shows that waste (%) on silk weight is not significantly influenced by the cooking conditions tested in the present work. However, it is evident that over cooking of cocoons increase the cooking waste generation due to over softening of outer cocoon layers.

Effect of cooking conditions on quality characteristics

Cleanness

ANOVA results presented in Table 3 reveal the highly significant (1 %) influence imposed by cooking conditions on cleanness of raw silk. The cleanness percentage suffered as the conditions changed from under cooking to over cooking (Table 4). This must be because over cooking softens the sericin more and results in slugs and hence reduction in cleanness percentage compared to under and normal cooking.

Neatness and low neatness

Table 3 discloses that neatness and low neatness percentage of raw silk are significantly influenced by cooking conditions at 1 % level. Though the neatness is a

racial character to the extent of 80 %, as described by Shimazaki (1964), the study revealed that different cooking methods could either improve or reduce the neatness character of bivoltine cocoons to some degree (Table 4).

Tenacity

Tenacity did not show significant differences in respect of different cooking conditions (Table 3). The tenacity of bivoltine raw silk remained almost stable *i.e.*, between 3.6 and 3.7 g/d for different cooking conditions in the present study (Table 4).

Elongation

In the case of elongation of raw silk, the cooking conditions exerted a significant impact at 5 % level (Table 3). The elongation of bivoltine raw silk ranged between 17.5 and 18.8 % for different cooking conditions tried in the present study (Table 4).

Cohesion of raw silk

A highly significant effect (at 1 % level) employed by cooking conditions (Table 3) was observed on cohesion of raw silk. Effective cooking of bivoltine cocoons had shown cohesion strokes of 73 whereas, under cooking and over cooking reduced the cohesion characteristics due to repeated brushing and over softening of sericin during cooking.

Better raw silk quality in the case of normal cooking method is attributed to better swelling and softening of sericin of all the filament layers and filament crossover points. Better cooking condition improved the reelability of cocoons and uniformity characteristic of raw silk. However, under cooking method resulted in slightly lower quality of raw silk, which may be attributed to repeated brushing of cocoons due to less softening of sericin in different layers. Similarly, over cooking methods softened the sericin more and reduced the cohesion characteristics. By adopting normal cooking method and reeling on CSTR Multiend reeling machine, high quality raw silk of international grade can be produced from bivoltine cocoons.

Conclusion

The study reveals that slug catcher can effectively be used for reeling purpose as it arrests the slugs under different types of cooking also. The slug catcher facilitates easy threading during the reeling process and

hence improves the reeler's efficiency. Further, the quality of slug catcher is fine and can be used effectively for a period of even one year without replacement. Moreover, it is highly affordable as the cost is very nominal (only Rs. 20/-). The bivoltine cocoons subjected to normal / appropriate cooking method facilitates production of quality raw silk with the use of slug catcher.

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Nepal enrolled as 19th Member Country of ISC

The Federal Democratic Republic of Nepal has enrolled as the 19th member Country of International Sericultural Commission with effect from 8th June, 2017. ISC has taken necessary action for notifying the Membership among all its Member Countries.

With this enrollment, ISC would now take up a Joint Sericulture Project involving Bangladesh, Nepal and India with financial assistance from ESCAP.



ISC Volunteer Experts visit Nepal

Based on the MoU signed between International Sericultural Commission and SAARC Agriculture Centre (SAC), Dhaka, Bangladesh, a Joint Sericulture Development Project would be taken up for the development of Sericulture and Silk Industry in Nepal, Bangladesh and India. In order to develop the Sericulture Project, ISC and SAC have decided to undertake evaluation studies in these countries by utilizing the services of three ISC volunteer experts.

A three member ISC Volunteer Experts Team has undertaken the evaluation study on the status of sericulture and silk Industry in Bangladesh during January 2017. The Team has already submitted the Evaluation Report which would help in formulating the project. After the enrollment of Nepal as a Member Country, ISC has deputed the three member Volunteer Expert Team to the sericulture areas and facilities of Nepal from 13th to 18th of July, 2017. The Team has now successfully completed the Evaluation Study and is in the process of preparing the report.

A similar Evaluation Study is currently undergoing in the sericulture areas of India.

MoU signed between China and India in Sericulture Sector

ISC facilitated the Governments of China and India to develop an MoU for cooperation in sericulture sector and its signing during the Textile India-2017, the mega trade event on textiles, held at Ahmadabad, Gujarat during 30th June to 2nd July, 2017. The event was inaugurated by the Hon'ble Prime Minister of India and attended by many Ministers of Govt. of India. The Govt. of China has deputed a six member delegation headed by Mr. Xie Dong, Deputy Director General, Guangxi Agriculture Department, Guangxi Province, PR China to sign the MoU.

The Chinese delegation had also visited the office of the International Sericultural Commission, Bengaluru and discussed on the various procedures related to the enrollment of China as a Member Country of International Sericultural Commission.

NEWS CORNER



8th International Conference on Wild Silkmoths, 22nd to 24th January, 2018

International Society for Wild Silkmoths and Central Silk Board, Ministry of Textiles have decided to organise the 8th International Conference on Wild Silkmoths at Guwahati, India during 22nd to 24th January, 2018. The following are the scientific sections of the Conference:

Section	Theme
Tasar silkworm	All aspects and areas concerning Tasar, including food plants, by- product and other utilities of tasar silkworm and silk.
Eri and Muga silkworm	All aspects and areas concerning Eri and Muga, including food plants, by-product and other utilities of Eri and Muga silkworm and silk.
Other wild silkworms & domesticated silkworms	All aspects and areas concerning other wild silkworms and silk, its use and application in various areas and all domesticated silkworms and its related activities.
Post-cocoon Technology	Reeling, cocoon preservation, silk quality, gradation, weaving, product diversification, marketing, export, etc.
Gene Technology	Silkworm as a biological model, genetics, physiology, biochemistry, genetic-engineering, molecular biology, developmental biology and genomics.

Interested persons may send their research paper abstracts to [email: wildsilkmothconference2018@gmail.com](mailto:wildsilkmothconference2018@gmail.com)

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- | | | | |
|----|--|---|--------------------------------|
| 1) | Contributions (submission of Abstract Research Papers) | - | 15 th November 2017 |
| 2) | Completed Registration Forms with Registration Fee | - | 15 th November 2017 |
| 3) | Late Registration with late registration fee | - | 15 th December 2017 |

For detailed information on the Conference, please contact:

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Central Silk Board- Ministry of Textiles, Govt. of India,
Silk Board Complex, BTM Layout, Madiwala, Bangalore-560 068, INDIA
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