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Full Length Research Paper

Muga silkworm (*Antheraea assamensis* helfer) indoor rearing by integrated "leaf freshness technology"- A new technology

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Integrated Muga silkworm indoor rearing technology includes "leaf freshness technology" (LFT), "Nutrient Supplemented Phago-stimulant" and bacterial disease control by streptomycin sulfate. All these techniques were applied in complete indoor conditions up to cocoon formation and, very high positive significant results were achieved in respect of larval survivability, larval biomass growth and error (ERR) percent in comparison to outdoor conventional rearing. Muga silkworm, being wild in nature, is very difficult to rear in indoor condition. Moreover, the detached food plant leafs goes on wilting within few hours. LFT made it possible to rear Muga silkworm completely in indoor conditions and minimized early stage loss of larvae from nature's vagaries, like pest and predators, souring temperature, hailstorm, wind, etc. The use of Nutrient Supplemented Phago-stimulant resulted to increased leaf nutrient quality, arrestancy of larval movement and increased feeding rate which reflected in the higher biomass growth in comparison to treatment without it use. Spraying of 0.5% streptomycin sulfate solution in the indoor reared Muga silkworm, minimized the bacterial infection thereby contributing to larval survivability. Normal cocoon qualities such as silk ratio, denier, sex ratio of male and female cocoon, were not affected by the use of Nutrient Supplemented Phago-stimulant, chemically treated host plant leafs used in LFT and spray of streptomycin sulfate solution. This new integrated LFT may help in adopting indoor rearing of naturally wild Muga silkworm for more production of Muga silk yean.

Key words: Leaf freshness, phago-stimulant, nutrient, biomass, denier, integrated, streptomycin sulfate, indoor.

INTRODUCTION

Antheraea assamensis Helfer, the golden-yellow silk producer silk moth, is semi-domesticated sericigenous insect species endemic to northeast India particularly Brahmaputra valley of Assam. This silk moth is semi-domesticated owing to the fact that only cocooning and grainage operations are conducted indoor and, reared on outdoor host plant. Thus, Muga silkworm culture is a traditional outdoor rearing practice adopted by people of north eastern States of India mainly Assam. It is polyphagia's, multivoltine reared in six different seasons throughout the year. Out of these six seasons two seasons namely, May-June and October-November are

commercial crop season, whereas other seasons are seed crop season. Again, the seed crops during December-January and June –July are called pre-seed crop. Thus, each commercial crop is preceded by one pre-seed crop and one seed crop. Since this pattern of Muga silkworm cultivation has been an age old practice, it is obviously environment controlled and the rearing performance is quite different in each season. Muga silkworm belongs to Lepidoptera of Saturniidae family and, geographically isolated only to NE region of India. Geographical isolation of this silkworm is indicative of its special requirements for geo-climatic conditions that prevail in this region, that is, high humid temperate climate and forest vegetation of primary and secondary host plants. Thus this species is phylogenetically less adaptive reaching its ecological isolation that is indicative of being on verse of extinction. Although, Muga silkworm since time immemorial has been reared for Muga silk still it is purely an outdoor culture on host plant under natural conditions. Only cultural specificity is being managed and took care by Muga rearer. Being exposed to natural environment, Muga culture practices encounter lots of problem right from brushing of worms to spinning of cocoons. Outdoor silkworm larvae are invariably expose to nature's vagaries such as seasonal climate change, rainfall, strong wind, soaring temperature, besides pests, predators and pathogens inflecting heavy loss particularly in early three instars (Choudhury, 1981; Samson, 1987 and Thangavelu et al. 1988). Prophylactic measures adopted for pest and disease in outdoor rearing became fruitless due to cross infestation by both pests and pathogens are common in open conditions. In an average in all seasons more than 50% larval loss has been reported by many scientists. Sengupta et al. (1992) reported that during summer more than 50% loss was due to abiotic factors and 80% of the total loss of Muga silkworm occurred in second/third instar only. Several workers experimentally practiced indoor rearing of Muga silkworm applying different types of rearing devices and, some of them reported effective over outdoor rearing (Barman and Rana, 2011). Singh and Barah (1994) conducted partial indoor rearing up to third stages with Som and Soalu twigs in bottle, iron tray and wooden and, reported larval mortality could be reduced marginally as compared to outdoor rearing. Cellular rearing technique developed by Thangavelu and Sahu (1986) for indoor rearing of muga silkworm was found suitable during different seasons for improvement in error (ERR) on Soalu plant, but female cocoon weight and fecundity were found significantly higher on 'Som' plant. Similarly Bhuyan et al. (1991) reported that indoor rearing in iron tray (3" x 4" x 4") with water and sand bed covered with slotted cover containing 'Som' twigs showed better ERR (58.8%) as compared to control (51.3%). But all scientists so far tried indoor rearing of Muga silkworm, did not get desired success because of incidence of diseases and difficulties in keeping the leaves of detached twigs of food plant fresh used in indoor rearing.

Leaf of a plant is the most important organ that supports very vital metabolic activities of plant life system. Leaf anatomy and physio-chemical mechanisms are supportive to vital plant life activities like photo-synthesis, transpiration, respiration, chlorophyll synthesis, stomata movement, senescence etc. To make active of all these physio-chemical processes the leaf require standard level of water content 65 to 80% continuously throughout its life period. Depending upon structural and anatomical architecture of the plant as found in different ecological types, the standard level of water content in leaf shows variation. Further in the same plant itself the leaf water content vary subjecting to availability of absorbable soil water in root zone and atmospheric RH that change diurnally and seasonally. Since water is continuously used in the metabolic processes occurring in leaf, uninterrupted supply of water to leaf is very important to maintain standard level of water. Plant with the help of rhizoids in root system absorbs available root zone water in soil and transport to leaf through the xylem tissue. Any damage or blockage in this tissue system interrupt water supply to the leaf. As a result of insufficient water supply the metabolic activities in leafs are greatly hampered and started to wilt. Similarly in the cut twigs leafs start wilting immediately after their detachment from mother plant due to transpiration and non-availability of continuous supply of water through xylem vessels. If the physiological activities including transpiration process are retarded or stopped, leaf of the cut twigs will remain fresh for considerable period.

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Abbreviations: SR, Silk ratio; **CW,** cocoon weight; **SW,** shell weight; **ASR**, average silk ratio; **FL,** filament length; **FW,** filament weight; **D,** denier; **AD,** average denier; **M,** male; **F,** female; **R,** ratio; **ERR,** error; **LFT,** leaf freshness technology.

Otherwise continuous supply of water through xylem vessels to leaf has to be maintained in order to keep leafs in fresh condition. Thus by preventing transpiration to reduce water loss by leafs it was possible to keep leaves for several days without significant changes in either their total water potential or their osmotic relations as determined by the pressure-volume technique. Thus, maintenance of leaf moisture content to keep leaf fresh is an unsolved problem in different fields of its application.

Among other constrains in indoor rearing, silkworm diseases being the most important that inflect heavy loss to the crop. The 'flacherie' disease caused by bacteria is most important, causing serious damage to the Muga silkworm (Chakravorty et al., 2007). The chief disease affecting silkworm is flacherie caused by bacillus bacteria. One casual bacillus of silkworm flacherie is *B. thuringiensis* which is widely distributed facultative entomogenous bacterium with as many as 34 varieties. It is a gram-positive spore forming bacterium widely distributed in the soils of various regions of the world. The endotoxin of *B. thuringiensis* is known to destroy the gut lining, causing paralysis and death in many insect species belonging to orders, Diptera and Lepidoptera including economically important insects such as silkworm, *Bombyxmori* (Aizawa, 1971; Nataraju et al., 1991). *B. thuringiensis* infected larvae lost appetite and became sluggish from 5 to 6 h of infection, larvae vomited the green fluid, excreta were soft and stick to the rearing bed. During moulting, skin was not shed properly. The infection also led to diarrhoea. As the disease advanced, the larvae became extremely sluggish, showed irritability to touch as if in pain. Later the colour of the larvae started to change into dark colour and larvae became almost inactive and unable to spin cocoons. The larval body started to shrink and the larvae became completely paralyzed. Finally, larvae completely turned to brown colour. Within 30 min after the larvae ingested the spores of *B. thuringiensis*, the mid gut epithelial cells became disorganized compared to healthy larvae. Some of the epithelial cells became detached from the wall of the mid gut. There exist 34 different varieties of *B. thuringiensis*. Among the 23 varieties of *B. thuringiensis* tested, only eight were reported to be pathogenic to silkworms and the rest as non-pathogenic (Selvakumar et al., 1999). Disease incidence may be due to the lowering of pH by the introduction of bacteria, which provides congeniality and could lead to degeneration of peritropic membrane, which blocks the absorption of nutrients that is reflected by cessation of feeding. From flacherie infected mulberry silkworm, Sridhar et al. (2000) isolated bacteria belonging to genus *Streptococcus.* The biological defence against pathogens in insects included the innate physical barriers namely, integument and intestinal wall and humeral responses such as the activation of prophenol oxidase cascade and induction of immune proteins namely, lysozymes, lectins, antibacterial proteins and antifungal proteins primarily by the fat bodies. Intestine harbour a great diversity of native microbes which promote gut maturation, and integrity, antagonism against pathogens by producing antimicrobial proteins and immune modulation (Girishkumar et al., 2005). A countable number of researchers made effort to control 'flacherie' in Muga silkworm by various biological and non-biological agents as spray, including streptomycin, an antibacterial drug, and reported as effective. Streptomycin sulfate is a bactericidal antibiotic and is a water-soluble amino glycoside derived from *Streptomyces griseus*. It is marketed as the sulfate salt of Streptomycin. The chemical name of Streptomycin sulfate is D-Strep amine, O - 2 - dioxin - 2 - (methyl amino) - α - L - glucopyranosyl - (1→2) - O - 5 - dioxin - 3 - C - formyl - α - L - lyxofuranosyl - (1→4) - N,N1-bis(aminoiminomethyl)-,sulfate (2:3) (salt). The molecular formula for Streptomycin Sulfate is $(C_{21}H_{39}N_7O_{12})_2 - 3H_2SO_4$ and the molecular weight is 1457.41. It acts by interfering with normal protein synthesis. Streptomycin is considered a second-line agent for the treatment of Gram-negative bacillary bacteria. Barman (2011) used *in vivo* antibacterial drugs like streptomycin sulphate successfully through leaf freshness technology (LFT) useable in indoor rearing of Muga culture and succeeded in achieving 42% more survivability up to fifth instars over 92% mortality in control rearing.

Further, in indoor rearing generally detached twigs of food plant are used to feed the larvae. For better growth and development of larvae, leaf nutrient status greatly influences. Feeding highly nutritious leafs results robust larvae that increase physical strength to resist adverse and disease conditions. Barman et al. (2011) found more survivability of Muga larvae and higher cocoon production by using Nutrient Supplemented Phago-stimulant spray on leafs of potted Som plant in indoor rearing of Muga silkworm. In this spray formulation to increase the nutrient status of detach twigs of Som plant used and arrestance of Muga larvae on food, phago-stimulant chemicals and insect nutrient chemicals were used together in one formulation. Ascorbic acid and sodium cyclamate were used in this formulation.

So, keeping in view of the present constrains faced by Muga silkworm cultivation in outdoor conditions, the present study was undertaken with the attempt to integrate technology of leaf freshness, flacherie disease control and spraying 'nutrient supplemented phago-stimulant' in indoor rearing of Muga silkworm during late Chotua Crop.

METHODOLOGY

The experiment was carried out with late 'Chotua' crop during last part of March, 2010. In the first treatment with device No. 1, complete indoor rearing was done while with device No. 2, indoor rearing was done up to 2nd instar then reared outdoor on Som tree. To compare the treatment data complete outdoor rearing on Som tree as usual was done taking equal numbers of larvae. Tender twigs of 1' to 1½' long were collected from Som plant in a plastic bucket with clean water. Collected twigs were treated with solution of certain chemicals as per the method of Barman (2011). Treatment with the chemicals specified for leaf freshness purpose develops properties of the twigs to keep leafs fresh for 8 to 10 days. These twigs were used to feed Muga larvae in indoor condition. In two iron devices (1 and 2) filter water was kept so that the cut end of twigs remain fully deep while inserted upward position. After one hour, the twigs of device No. 1, were sprayed with 'Nutrient Supplemented Phago-stimulant' solution (Barmanand and Rajan, 2011) and kept overnight. This spray solution is a mixture of well balance phago-stimulant chemicals with insect nutrient chemicals. Spray of this solution increases nutrient as well as phago-stimulant quality of leafs use in indoor rearing of Muga silkworm. This was done prior to one day before brushing of larvae. On 24th March, 2010, newly hatched out Muga silkworm larvae were brushed at 500 Nos. in each device and on an outside Som plant of 9' tall with nylon net cover as control. In every alternative day from second day of brushing, all the twigs in these devices were sprayed with 0.5% solution of streptomycin sulfate. Similarly, Nutrient Supplemented Phago-stimulant solution was sprayed on the twigs of No. 1 device every day at regular time. Both the sprays were avoided during the time of molting. Tissue growth measuredas larval weight in gram after molt in 10 Nos. was recorded for all treatments including control. Larval survivability in each instar was recorded by counting the dead larvae collected for each treatment. After second molt, 3rd instars larvae were released from device No. 2 on another Som plant with nylon net cover nearby to Control plant. Ripen larvae of each treatment were collected separately in jali in nylon net bags for spinning. Cocoons were separated from jali and recorded their number for each treatment.

RESULTS AND DISCUSSION

Quantitative and qualitative data were tabulated separately in Tables 1 and 2, respectively for discussion. The quantitative data of the experiment included larval tissue growth measured as larval weight, nos. of living larvae in each instar as larval survivability and, nos. of cocoon harvested in each experiment calculated as ERR. On other hand, qualitative data represent the qualitative criteria of cocoon like silk ratio (SR), sex ratio and denier of filament. Table 1 revels that the weight difference of 10 Nos. larvae at each instars were different markedly among the treatments. In the first instar itself a difference in larval weight was observed among the treatments. In treatment No. 1, 10 larvae weighed 0.288 g whereas it was 0.218 218 g and 0.228228 g in treatment No. 2 and control, respectively. This difference increased gradually in subsequent instars. And finally in 5th instar 10 larvae weighed 118.200 g in treatment No.1. In treatment No. 2 weight of equal number of larvae of 5th instar was 94.300 g whereas it was 93.560 g in outdoor control. So, the larvae of treatment No. 1 were 2.39 g more heavier in 5th instar than their counterpart of treatment No. 2 which was attributed by the spray solution of "Nutrient Supplemented Phago-stimulant". Again 5th instar larvae of outdoor control were less heavier by 0.074 g than that of treatment No. 2. That implies body tissue growth of Muga silkworm reared indoor did not affected by chemically treated leafs of Som plant used in LFT. Larval weight of treatment-1 (device 1) with "Nutrient Supplemented Phago-stimulant" spray was always higher in each instar than the control and treatment 2 (device 2) without spray. Difference in larval weight in all the instars between treatment -1 and control has been found significant in ANOVA test. However, up to 2nd instars larval weight between treatment-1 and treatment-2 do not show significant difference both being reared by "LFT" in indoor room. After releasing on outdoor tree the differences in larval weight between treatments went on increasing and at 5th instar it became significantly different. Differences become prominently visible in graphical representation (Figure 1). So, "LFT" has positive effect on the tissue growth of larvae. The biomass increases in the larvae of indoor rearing by 'LFT" is due to increase in leaf feeding rate. Further, biomass increase in the larvae of all instars in treatment-1 sprayed with "Nutrient Supplemented Phago-stimulant" was higher. The nutrient supplement compositions in the phago-stimulant formulation increases nutrient quality of leafs used that in turn help in more biomass increase in treating Muga larvae.The spray solutions containing nutrients like ascorbic acid, proline, enhanced nutritional qualities of the host Som plant leafs (Barman et al., 2011). Farrar et al. (1994) conducted experiments with

Table 1. Quantitative rearing performance of integrated leaf freshness technology in device No. 1 and No. 2 in comparison to outside control.

Rearing type	Initial larvae brushed	Body tissue growth of larvae in gram weight (10 Nos.) and survivability in %										Cocoon harvested (Nos.)	ERR (%)
		1st Instar		2nd Instar		3rd Instar		4th Instar		5th Instar			
		Larval wt. of 10 nos. (g)	Larval survivability (%)	Larval wt. of 10 nos. (g)	Larval survivability (%)	Larval wt. of 10 nos. (g)	Larval survivability (%)	Larval wt. of 10 nos. (g)	Larval survivability (%)	Larval wt. of 10 nos. (g)	Larval survivability (%)		
Device No. 1 sprayed with 'nutrient supplemented phago-stimulant'	500	0.288	99.40	1.500	96.80	5.740	95.40	28.200	85.8	118.200	55.4*	121	24.20
Device No. 2 without 'nutrient supplemented phago-stimulant'	500	0.218	97.40	1.285	93.40	4.910	71.40	22.010	58.4	94.300	15.00	67	13.40
Control	500	0.228	87.33	1.294	75.00	4.907	69.00	21.253	35.9	93.560	11.00	46	9.20

Table 2. Qualitative rearing performance of integrated leaf freshness technology in device No.1 and No.2 in comparison to outside control.

Rearing type	Cocoon quality										Filament quality						
	Sex ratio			CW		SW		SR		ASR	FL (mtr.)		FW (g)		D		AD
	M	F	R	M	F	M	F	M	F		M	F	M	F	M	F	
Device No. 1 sprayed with 'nutrient supplemented phago-stimulant'	80	41	2:1.03	3.45	5.86	0.31	0.44	8.99	7.51	8.1	246.6	237.6	0.15	0.17	5.5	6.4	5.95
Device No. 2 without 'nutrient supplemented phago-stimulant'	44	23	2:1.05	3.92	6.08	0.31	0.50	7.91	8.22	8.1	279.4	342.6	0.16	0.21	5.2	5.5	5.4
Control	30	16	2:1.07	3.96	6.10	0.32	0.51	8.08	8.36	8.3	285	345.3	0.17	0.23	5.4	6.0	5.7

CW, cocoon weight; SW, shell weight; SR, silk ratio; ASR, average silk ratio; FL, filament length; FW, filament weight; D, denier; AD, average denier; M, male; F, female; R, ratio.

five different nutrient based phago-stimulants on the feeding behaviour of six lepidopteron insects and found great response of larvae in terms of attraction and arrestancy on feed used phago-stimulants in comparison to feed without phago-stimulants. Barman et al. (2011) established that the phago-stimulant formulation used in these spray solutions must have attracting and arresting effect on feeding leafs that ultimately increased feeding rate of Muga larvae.

Although equal number of newly hatched out larvae were brushed separately in each treatment, number of living larvae at each instar showed great variation among treatments. Control recorded lowest survivability in each instar in comparison to treatment-1 and treatment-2. At the end of 1st instar 99.40% larvae survived in treatment No. 1 whereas it was 97.40% in treatment No. 2 and 87.33% in outdoor control. High survivability of larvae in treatment No. 1 was achieved through all instars and in 5th instar 55.4% larvae survived. At the end of 2nd instar 3.4% more larvae survived in treatment no. 1 than treatment No. 2 which is attributed by "Nutrient

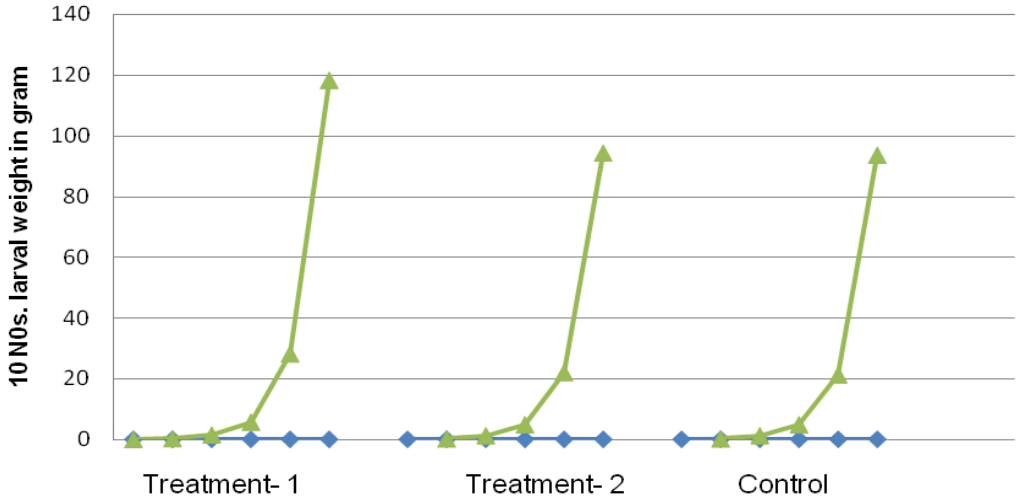

Figure 1. Graphical representation of larval tissue growth measured as gram weight of treatments and control.

Supplemented Phago-stimulant" spray (Table 1). Further there was 18.4% more survivability in treatment No. 2 over outdoor control implies that indoor rearing by "LFT" reduced loss of larvae as generally happen in outdoor conventional rearing of Muga silkworm. Thus, in treatment number, Muga larvae reared complete indoor by integrated technology meaning applying together "LFT", "nutrient supplemented phago-stimulant" and 0.5% Streptomycin spray, resulted 44.4% gain of 5th instar larvae over outdoor control rearing (Table 1). Being outdoor larvae in control suffered from natural vagaries like pest-predator, sourer temperature and strong wind. Whereas in indoor rearing by integrated "Leaf Freshness Technology" larval loss in 1st and 2nd instar were remarkably low. Moreover, the indoor rearing both the treatments 1 and 2 were treated with 0.5% solution of streptomycin sulfate that reduced bacterial infection. Differences in survivability of 1st and 2nd instar larvae in treatments 1 and 2, although non-significant contributed by the spray of "Nutrient Supplemented Phago-stimulant" that enriched the leaf nutrient quality and feeding rate of larvae in treatment- 1. Barman (2011) in his experiment with *in vivo* use of streptomycin sulphate through "LFT" found 42% more survivability up to fifth instars of Muga larvae over 92% mortality in control rearing. Dutta et al. (2010) also reported *in vitro* use of streptomycin sulfate at 1000 ppm and successfully controlled bacterial diseases in Muga silkworm up to 52.37%. After releasing 3rd instar larvae of treatment-2 on tree outside, larval mortality increased like control and at 5th instar only 15% larvae survived resulting 13.4% ERR (% of cocoon harvested). So, there was 4.2% gain of harvested cocoon over 9.2% total cocoon harvest in control. This 4.2 gain over harvested cocoon is obviously the result of indoor rearing up to 2nd instar that resulted significantly higher survivability at early instars. Barman et al. (2011) found from his experiment that indoor rearing of Muga silkworm on detached twigs of Som and Soalu in wooden tray does not differ significantly from outdoor rearing in trees. However, the result of this Muga silkworm rearing experiment with integrated "LFT" show that complete indoor rearing gave very high significant larval survivability up to 5th instar that recorded 55.4% against 11% survivability in control. This resulting 24.2% ERR (being wild in nature, some larvae escaped from rearing room) in complete indoor rearing with these technologies against 9.2% ERR achieved an unconventional control rearing on outdoor of some tree (Table 1). A graphical representation of larval survivability data of the treatment clearly reveals the differences (Figures 2 and 3).

In Table 2, it is recorded that the ratio of male and female cocoon in these treatments were 2: 1.03, 2 : 1.05 and 2 : 1.07, respectively in treatment 1, treatment 2 and control. So, Muga silkworm in harvested crops has

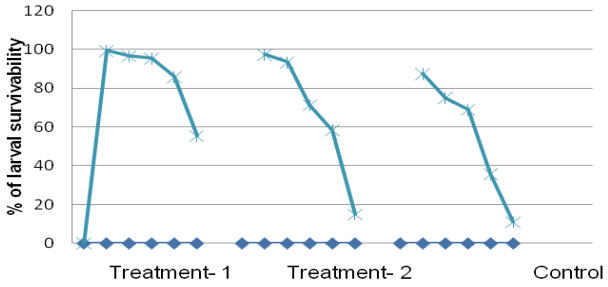

Figure 2. Graphical representation of larval survivability in treatments and control rearing.

Figure 3. Some of the photographs of complete indoor rearing of Muga silkworm *(A. assamensis)* by integrated "LFT".

almost an uniform sex ratio of two males against one female. There were variations in cocoon and shell weights of both male and female sex among the treatments. As a result the silk ratio of both male and female found different among treatments. But treatment wise average silk ratio were almost similar that is 8.1 in treatment 1 and treatment 2, 8.3 in control. The slightly higher silk ratio by 0.2 is statistically non-significant. So "LFT" with the spray of "Nutrient Supplemented Phago-stimulant" and streptomycin sulphate did not effect on cocoon's normal silk ratio. On other hand in filament length a significant difference exist between complete indoor rearing by integrated "LFT" and outdoor control rearing in both male and female cocoons. But the ultimate average denier of filaments of the treatments and control were found almost similar. Like silk ratio all these spray and the leaf freshness technique had not affect the denier of silk filaments that normally found in Muga silk. Natural golden colour of the yean also did not change thus produced in indoor rearing of Muga silkworm by this technology.

From the previous discussion it can be hoped that this new integrated "LFT" may be commercially exploited for Muga silk production by indoor rearing practice.

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