Chapter – VII

Post cocoon characterization of silkworm after peptide elicitation

7.1 INTRODUCTION

Silk known as "Queen of textile", is the only natural animal fiber obtained from mulberry as well as non mulberry silkworm (Chandra *et al.,* 1997). Silk is widely used in textile industry and biomedicine due to its mechanical properties, stability, biocompatibility and degradability (Ning *et al.,* 1999; Altman *et al.,* 2003). Mainly silk is composed of two types of silk protein: fibroin, a core structure (72-81%) and sericin (19-28%) which is peripheral glue protein (Dutta *et al.,* 2013). Fibroin has a protein group with molecular mass of 370 kDa and sericin, a soluble glycoprotein has proteins ranging between 20-400 kDa (Dutta *et al.,* 2013).

The quality of mulberry leaves determine the larval growth and development as well as cocoon production. The superiority of the cocoon and post cocoon attributes of *B. mori* is influenced by dietary nutritional management (Murugan *et al.,* 1998). In recent years, many attempts have made to enhance post cocoon quality of silk. Recently in sericulture research, a technique based on the supplementation and fortification of mulberry leaves is evolved to improve silk production and fiber quality (Murugan *et al.,* 1998). Supplemented protein had an essential role on silk production. Narayanan *et al.* (1967) stated that almost 70% silk proteins produced by silkworm are directly derived from the mulberry leaf protein. Horie and Watanabe (1983) found soybean meal as a protein source in silkworm diet that can increase the weight of silkworm larvae and fresh silk glands. El-Sayed (1999) found the effects of dietary protein on larval growth and silk production.

In view of this, present investigation was designed to find out the effects of low molecular weight peptide (0.5-3 kDa) on post cocoon attributes of *Bombyx mori*. SEM analysis was done for finding the effects of peptide on silk fiber. Previous studies (mention at chapter V and VI) revealed that peptides isolated from S1 mulberry variety had significant effects on larval growth and cocoon parameters. Therefore, present study was conducted in large scale silkworm rearing under S1 peptide treatment and post cocoon characterization also was evaluated.

7.2 MATERIAL AND METHODS

7.2.1 Isolation and purification of low molecular weight peptide(s)

7.2.2 Rearing of silkworm larvae

Mention in section 2.2.2.1

7.2.2.1 Rearing room

The present experiment was performed in the farmer's rearing room at Amriti village of Malda district through the well established method (Krishnaswami *et al.,* 1978). The rearing room have adequate number of windows and also have cross ventilation for sufficient aeration. The roof has sufficient height (10′) and the straw was placed over the roof to avoid heat reflection. Figure 7.1 showed the outline of rearing room.

7.2.2.2 Leaf treatment

Low molecular weight peptide(s) isolated from S1 mulberry leaves was 20 times diluted by distilled water. Leaves were soaked in peptide(s) for 30 min before feeding them to the larvae and air-dried for 15 min and given to silkworm. Separate rearing trays were maintained for treatment as well as control by feeding peptide(s) treated or untreated leaves.

Rearing in large scale with 1000 larvae also was conducted to analyze the post cocoon attributes in economic aspect.

7.2.2.3 Rearing bed maintenance procedure

Mention in section 2.2.2.1

7.2.3 Scanning electron microscopy

Raw silk fibers were collected from green cocoons of control as well as peptide treated set and threads were collected on stubs for gold coating (Edwards S 150 Ion Sputter Coater – 4 mA/10^{-1} torr 2 cycle repeat/ min). The ion coated stubs were collected and photographs were taken under a Scanning Electron Microscope (Quanta 200, FEI Company) at same physical parameters and magnification (3000 ×).

7.3 RESULTS AND DISCUSSION

Average silk filament length was higher by application of peptides isolated from S1 young leaves (PY) treatment followed by peptides isolated from S1 mature and senescence leaves (PM and PS respectively) and control. But filament weight was enhanced by PM treatment when compared with other counterparts (Table 7.1). Maximum filament size (d) was recognized in control among all peptide treatments which proportionately negative with average filament length. Non-breakable filament length was also measured (Table 7.1). For determining the relationship among various attributes, correlation matrix analysis was performed in between different economical parameters and post cocoon yields of silkworm rearing under different peptides treatment (Table 7.2). Weight of single shell (WSS) was significantly correlated with final growth rate (FGR) of larvae, shell ratio% and average filament length (AFL). Likewise AFL exhibits high affinity with shell ratio% and non-breakable filament length (NBFL).

Raw silk fiber was isolated from cocoon of each set. Morphology of silk fiber in terms of structure was established by SEM analysis. From Figure 7.2(a-f), it is evidenced that the silk produced by silkworm nourished with peptide treated mulberry leaves of different maturity stages were remarkably improved than their respective untreated counterparts. Visible increase in the diameter of the raw silk was observed under peptide treatment which was consistent with the cocoon morphology. The peptide(s) maintained the structural integrity of sericin during the extension of the fiber due to peptide treatment, as evidenced from Figure 7.3 (a-c), representing Scanning Electron Photomicrograph (SEM) of side view of fractured silk fiber after forcefully detached and Figure 7.3 (c), showing the surface of silk fiber after peptide (PM) treatment.

SEM analysis of raw silk fiber and other cocoon and post cocoon parameters were significantly improved by PY treatment followed by PM and PS. Therefore, rearing with only S1 peptide (PY) was conducted again in large scale. Figure 7.4-7.9 showed few moments of silkworm rearing in large scale at farmer house. Post cocoon attributes in economical aspect was analyzed with the help of Silk Conditioning and Testing House (SCTH), Central Silk Technological Research Institute, Central Silk Board, Malda, West Bengal. Post cocoon attributes were differentiated into three major parts:

i. Physical characteristics of cocoon
ii. Single cocoon reeling characteristics
iii. Commercial reeling characteristics

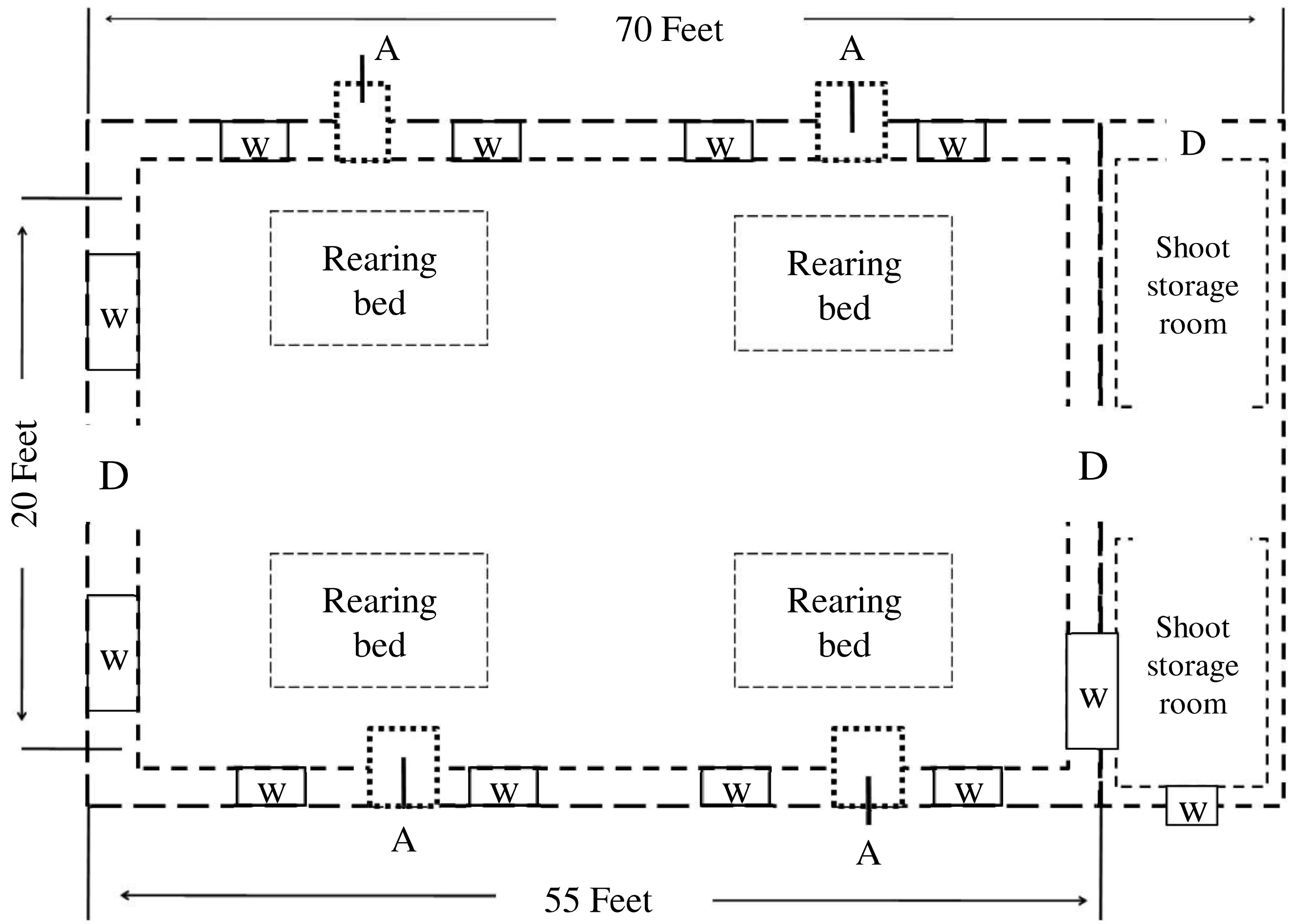

Figure 7.1: Outline of rearing room for large scale silkworm rearing

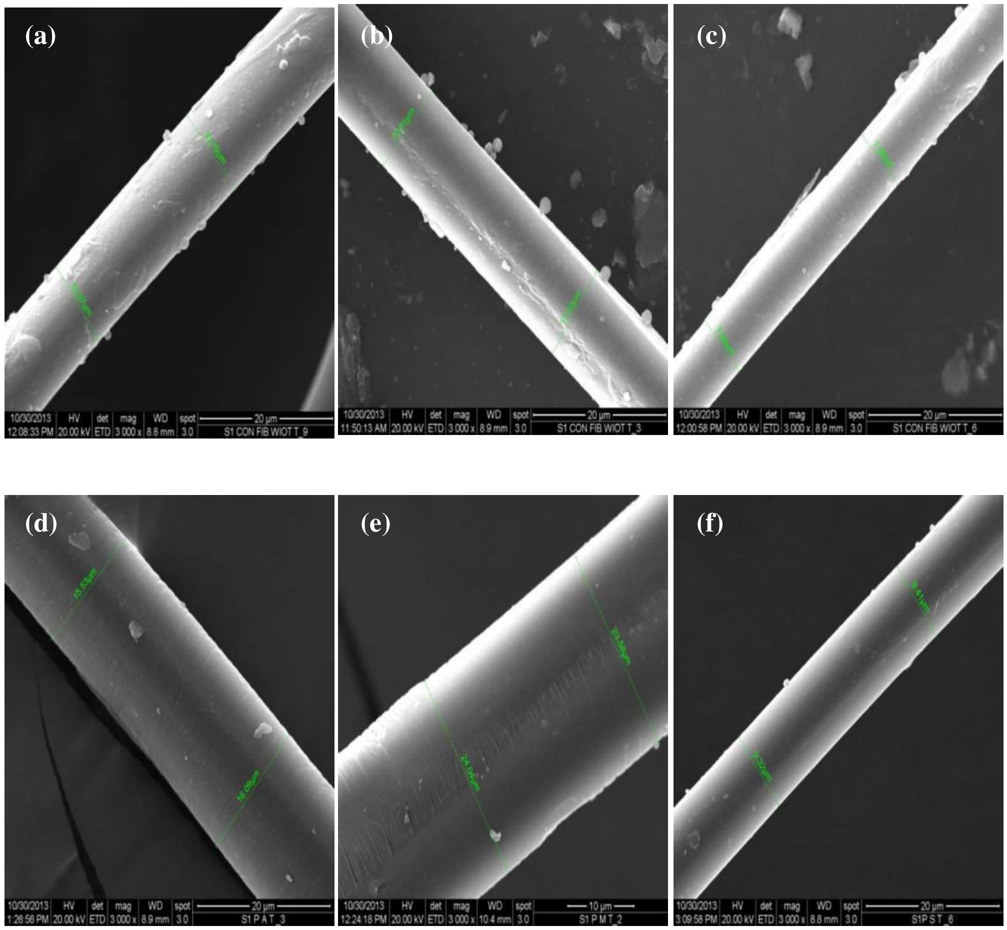

Figure 7.2(a-f): Representation of Scanning Electron Photomicrograph (SEM) of silk fibers obtained from cocoon after feeding S1 mulberry young (a), mature (b) and senescence (c) leaves as control; whereas (d), (e) and (f) demonstrated the SEM analysis of silk fibers acquired after treatment with peptide (0.5-3 kDa) isolated from S1 young, mature and senescence leaves respectively.

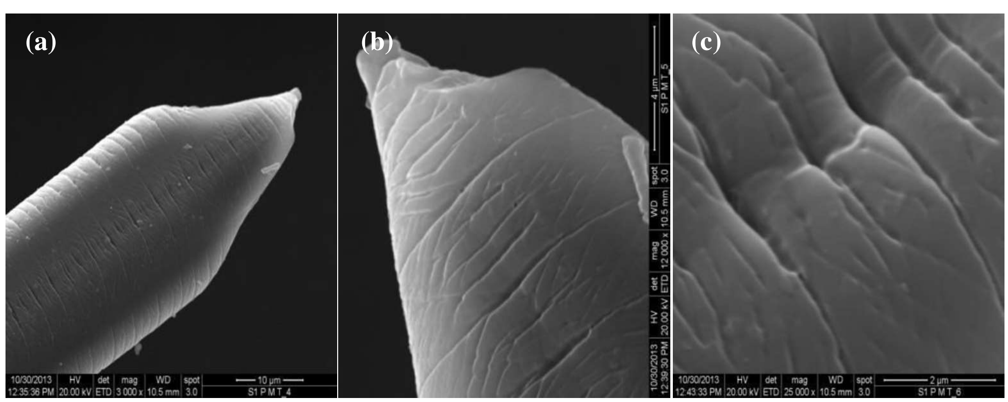

Figure 7.3(a-c): (a) and (b): Representation of Scanning Electron Photomicrograph (SEM) of side view of silk fiber of fracture point after forcefully detached. (c): Showing the surface of silk fiber after peptide treatment.

Figure 7.4: Silkworm rearing bed (a); large scale silkworm rearing after peptides treatment with the help of local farmers

Figure 7.5: Silkworm rearing after peptides treatment at the rearing house

Figure 7.6: Cocoon harvested within chandraki

Figure 7.7: Cocoon obtained from silkworm rearing under peptides (isolated from S1 mulberry leaves) treatment as well as control set.

Abb. Used: PY, PM and PS: peptides isolated from young, mature and senescence S1 mulberry leaves respectively.

Figure 7.8: Cocoon obtained after large scale rearing under peptides treatment in the farmer rearing house.

Figure 7.9: Silk fibre obtained from cocoon under peptide treatment and control set.

Table 7.1: Cocoon and post cocoon attributes of silkworm rearing system under peptide treatment.

Parameters	Control (S1 leaves)	Peptide treatment (0.5-3 kDa)		
		PY	PM	PS
Final growth rate of larvae	41.55 ± 0. 52^{d}	69.25 ± 0.42^{a}	60.93 ± 0.48^{b}	48.45 ± 0.51^{c}
Weight of single cocoon	0.66 ± 0 .027^{b}	0.79 ± 0 .017^{a}	0.78 ± 0.025^{a}	0.78 ± 0.020^{a}
Weight of single shell	0.1 ± 0.019^{b}	0.16 ± 0.024^{a}	0.13 ± 0.016^{ab}	0.11 ± 0.018^{b}
Shell ratio (%)	15.15	19.75	16.26	14.65
ERR (%)	90	100	100	90
Average filament length (m)	399.375	590.062	446.785	407.32
Filament weight (cg)	0.172	0.325	0.67	0.341
Non-breakable filament length (m)	399.375	590.062	390.937	392.16
Filament size (d)	3.87	2.24	2.93	2.67

Results are represented as Mean ± SEM, n = 25. Values with different letters (a, b, c & d) are significantly ($P < 0.05$) different from each other by Duncan's Multiple Range Test (DMRT).

Table 7.2: Correlation between different pre-cocoon and post cocoon parameters of silkworm rearing system

	FGR	WSC	WSS	Shell ratio%	ERR%	AFL	FW	NBFL
WSC	0.758							
WSS	**0.978***[*]	0.682						
Shell ratio%	0.881	0.444	**0.954**[*]					
ERR%	0.938	0.607	0.873	0.780				
AFL	0.891	0.528	**0.966**[*]	**0.991**[**]	0.750			
FW	0.510	0.619	0.319	0.072	0.664	0.066		
NBFL	0.743	0.370	0.866	**0.952**[*]	0.558	**0.965**[*]	-0.197	
FS	-0.802	-0.937	-0.799	-0.637	-0.573	-0.726	-0.340	-0.637

[*]Correlation is significant at the 0.05 level (2-tailed)

[**]Correlation is significant at the 0.01 level (2-tailed)

Table 7.3: Post cocoon attributes of silkworm under peptide treatment as well as control

A.	General		
1	Lot Name	Control	Peptide
2	Race	N×Bi	N×Bi
3	Crop	Falguni 16	Falguni 16
4	Qnty. of cocoon (Kg)	1.5	2
B.	Physical Characteristics		
1	Avg. single cocoon wt.	1.4	1.53
2	Avg. Single shell wt.	0.21	0.28
3	SR%	15.02	18.1
4	Chit/Kg	718	663
5	Defective %	6.11	5.98
C.	Single Cocoon Reeling Characteristics		
1	Avg single Filament length (mt)	525.6	587.62
2	Avg single Filament Denior (d)	2.97	2.76
3	Avg. Non-breakable filament length (mt)	525.6	578.836
D.	Commercial Reeling Characteristics		
1	Type of Reeling machine used	Cottage Basin reeling m/c	Cottage Basin reeling m/c
2	Wt. of cocoon reeled (green wt. in kg.)	1.4	1.8
3	Wt. of raw silk obtained (kg).	0.13	0.19
4	Wt. of silk waste (kg)	0.06	0.04
5	Renditta		
i.	On good cocoon	10.76	9.47
ii.	Overall	11.53	10.22
6	Raw silk %		
i.	On good cocoon	9.28	10.55
ii.	Overall	8.66	9.5
7	Waste % on silk weight	31.57	30.76
8	Raw silk Recovery %	58.63	60.27
9	Reelability %	80.73	84.22

Table 7.3 exhibited all tested post cocoon attributes after peptide treatment as well as control set. Shell ratio (SR %) gives a satisfactory indication of the raw silk amount from fresh cocoons. The SR% assists to estimate raw silk yield and helps to determine the appropriate price for the cocoon. Approximately, peptides treatment enhanced 20.51% shell ratio over control. As like SR %, length of the bave also was an important post cocoon attributes. Peptide treatment increased the average single filament length (mt) in comparison with control. The size of thread was expressed in denier. Finer filament with 2.79 denier was obtained from peptide treatment.

$$\text{The Denier (d)} = \left(\frac{Fibre\ weight\ (g)}{Fibre\ length\ (m)}\right) \times 9000$$

A sequence of minor defects may be found in silk fiber like loops, fuzziness, split ends, hairiness etc (Figure 7.10). The defective percentage directly affects the raw silk quality. Peptides application reduced 2.13% defects on silk filaments. Reelability is considered as the fitness of cocoons in commercial aspect. Poor reliability causes various problems in production. It halts the production due to filament breakage and huge amount of waste products. 84.72 % of reelability was recorded under peptide treatment which is 4.94% greater than control.

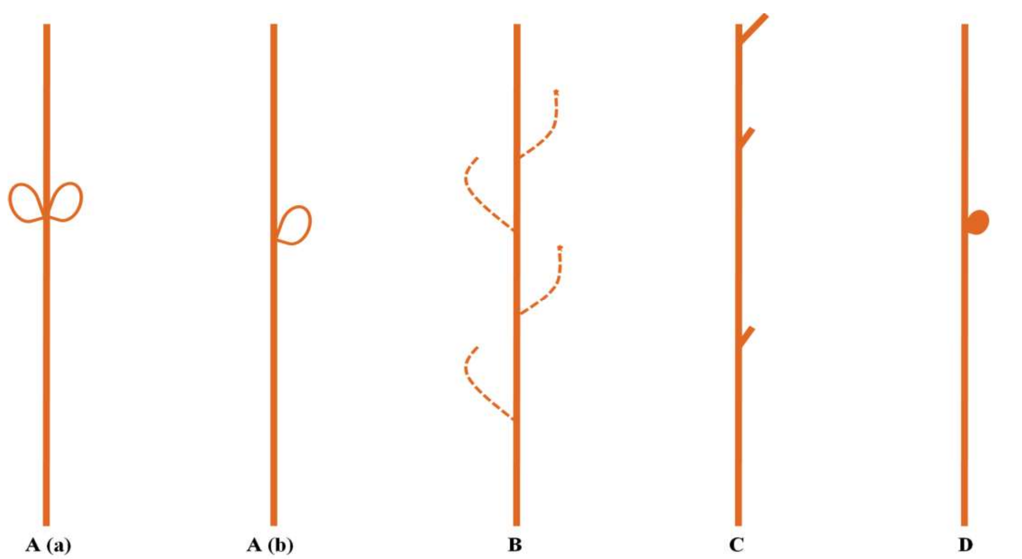

Figure 7.10: Nature of silk filament defects. A(a and b): Loop; B: Hairiness; C: Split ends; D: Nibs (Lee, 1999).

Renditta is another important commercial reeling characteristic of silkworm rearing. Low renditta reflects the high quality of cocoons. Peptides treatment lowered the renditta of cocoon in comparison with control. Waste percent is the percentage of raw silk amount which is unable to wind in silk production. Waste is the floss of upper most layer of thread comes out during reeling and expressed as waste %. High waste % was recorded under control set.

Similar kind of post cocoon attributes was evaluated by Shingh (2010) and Bhuyan *et al.* (2017). Bhuyan *et al.* (2017) measured the variation of post cocoon parameters of eri silkworm at different season. Significant variation in reelability and waste % of muga silk under different seasonal variation was established by Barah (2010). Narayanan *et al.* (1966) reported that application of nitrogen to mulberry significantly increased different cocoon parameters. According to Narayanan *et al.* (1966), nitrogen promotes foliar protein content which had great influence on cocoon production. Trivedy *et al.* (1996) could able to increase the cocoon and shell weight significantly under administration of Juvenile hormone analogue. Hiware (2006) reported positive effects of *Nux vomica* on shell weight and filament length of silkworm. Cocoon and post cocoon attributes of *B. mori* were influenced by mulberry leaves fortified with Kenyan royal jelly (Nguku *et al.,* 2007). Shashidhar *et al.* (2009) worked out on different cocoon attributes of silkworm after organic based nutrient management of mulberry leaves. Chen *et al.* (2012) evaluated different structural and physiological properties of silkworm cocoons. Post cocoon attributes of eco-races of Tasar silkworm also was evaluated by Renuka and Shamitha (2015).

7.4 CONCLUSION

Mulberry leaves fortified with low molecular weight peptides significantly increased the cocoon production, shell weight, shell ratio and other post cocoon attributes. Peptides isolated from young leaves had better response on cocoon production and thread quality than peptides isolated from mature and senescence one. When rearing was conducted in laboratory in small scale, the peptides increased approximately by 30.36% shell ratio whereas, large scale rearing in rearing house under natural condition reduced the effects of peptides. Even though the peptides enhanced 20.51% shell ratio in large scale rearing, the phenomenon which was significantly effective for industrial production. Not only peptides increased productivity but also it improved thread quality by reducing denier of fiber, waste of silk, defective percent and renditta. Therefore, mulberry leaves treated with naturally occurring peptides could be recommended for silkworm rearing to enhance quality and quantity of the silk in industrial scale.