Research Article

COMPARATIVE ANALYSIS OF POST COCOON CHARACTERS ASSOCIATED WITH FILAMENT LENGTH BETWEEN MULTIVOLTINE RACES AND MULTI X BIVOLTINE CROSS BREEDS OF THE SILKWORM *BOMBYX MORI*

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KEYWORDS:
Bombyx mori, Cross breeds, Fibre quality, Filament characters, Gradable silk

ARTICLE INFO
Received on: 13.07.2019
Revised on: 08.08.2019
Accepted on: 10.08.2019

ABSTRACT

India is a country with tropical climate and multivoltine silkworm strains play important role in the production of silk, however low productivity and poor fibre quality continued to impair increase in silk production. In this context development of cross breeds (multivoltine x Bivoltine) has gained importance over the years owing to field constraints, fluctuations in the environmental conditions as well as socio economic compulsion. Due to the improved technologies and machinery, there is vast improvement in the post cocoon characters. In this study, we compared important post cocoon characters/parameters, shell ratio, filament length, reelability, renditta, raw silk percent, raw silk recovery, neatness and waste of popular cross breed varieties PM x CSR2 and Nistari x CSR2 with that of pure mutivotine races to establish improved post cocoon parameters of the cross breeds. The significance in improvement was analyzed using Students *t*-test to show variation in the post cocoon characters between the cross breeds and multivoltine races. Some characters like neatness and reelability were almost equal to that of bivoltine races. The Pearson correlation was employed to analyse the relation between different traits. The relation was significant and showed positive correlation between eight characters, except waste and renditta showed negative correlation with other characters. The quality of silk produced from theses cross breeds were highly improved and showed 2A gradable silk. Therefore, the results indicate the scope for developing potential cross breeds with improved yield characters, particularly filament characters to support the Indian sericulture industry.

INTRODUCTION

The silkworm, *Bombyx mori*, is one of the best-characterized silk producing model organisms because its silk has great economic value. In India, sericulture is not only a tradition but a living culture. It provides income and employment to the rural poor especially farmers with small-land holdings and the marginalized and weaker sections of the society. Several socio-economic studies have affirmed that the benefit-cost ratio in sericulture is highest among comparable agricultural crop (Gangopadhyay, 2008; Bharati, 2016). Bivoltine breeds although produce better quality silk under temperate condition it shows lower tolerance and low adaptability in tropical situation (Kariappa and Rajan, 2004). Though India is the second largest producer of silk in the world, twin problems of low productivity and poor fibre quality continue to impair increase in production. Indian silk is not of international grade (4A-6A), as a result, Indian raw silk cannot be exported leading to import of large quantities of silk from China to use in domestic market. Previously, different silkworm breeders tried to develop new multivoltine breeds by crossing multivoltine with exotic bivoltine breeds which were rejected due to poor survivability, occasional production of hibernating eggs and deterioration of heterosis (Mukhopadhyay *et al*., 2013). Hence, it is highly relevant to improve yield characters, particularly filament characters of the existing multivoltine races to support Indian sericulture industry.

Quantitative characters of economic importance determining silk productivity is of paramount importance to the breeders, cocoon and egg producers, reelers, weavers, twisters and buyers (Singh and Saratchandra, 2004). The egg producers are interested in breeds that can produce more number of eggs, cocoon growers are interested in the genotype having healthy larvae and producing high cocoon yield. Textile industry favours better filament length without any breakages, good reelability, high percentage of raw silk and superior quality of cocoons and weaving industry needs better neatness, denier, high tenacity and elongation(Singh *et al.*, 2010). Lustre, dye uptake and stiffness of the silk thread of cross breed is superior than bivoltine silk and largely preferred by the reelers (Dayananda *et al.,* 2015) and therefore development of multi x bivoltine cocoons for the production of quality raw silk is warranted. Longer filament length is preferred during reeling, because it contributes low workload, increase in production as well as quality of raw silk (Sing *et al.,* 2010; Bivoltine manual, 2003).

In the above context, this study was undertaken to compare important post cocoon characters viz., shell ratio, filament length, reelability, renditta, raw silk percent, raw silk recovery, neatness and silk waste of the cross breeds with that of pure mutivotine races.

MATERIALS AND METHODS

Silkworm Accessions

Fifth instar larvae of different races (BMI001, BMI002, BMI003, BMI005, BMI009, BMI034, BMI043, BMI059, BMI063, BMI064, BMI017 and were collected from CSGRC, Hosur and reared at Seri Biotech Research Laboratory. PM x CSR2 and Nistari x CSR2 cross breeds showed better yield traits and hence were synthesized by crossing at SBRL, Bangalore for experimentation (Table 1).

Table 1. Silk worm accessions collected from Central Silkworm Germplasm Center, Hosur

SL. No	Accession No	Accession Name
1	BMI 001	Pure Mysore
2	BMI 002	Sarupat
3	BMI 003	Moria
4	BMI 005	C'Nichi
5	BMI 009	Kollegal Jawan
6	BMI 017	Nistari
7	BMI 034	AP12
8	BMI 043	MW13
9	BMI 059	Bl24
10	BMI 063	TWxSK6xSK1
11	BMI 064	SK1x SK6xTW
12	BBE 290	CSR2

Green cocoon analysis

Initially the different lots cocoons were analysed for One kilogram assessment. From the given cocoon lot one kilogram of cocoons were taken randomly for assessment. Good and defective cocoons present in one kilogram are counted and weighed separately. The defective cocoons are sorted and defective cocoon percentage calculated based on its weight. The sorted good cocoons are counted and weighed. Based on the number and weight, the single cocoon weight of the lot calculated. Based on the single cocoon weight about 20 good cocoons were picked to get the same weight as per one kg assessment and weighed. These cocoons are cut open and pupae removed and the 20 shells are weighed, to determine the shell ratio percentage. The Shell ratio is the ratio of shell weight to the cocoon weight expressed in percentage.

Stifling

Each cocoon lots were stifled as per standard conditions in the stifling chamber. CSTRI batch type hot air drier is used for cocoon stifling. In stifling chamber the temperature is maintained at 110˚C for 1 hour. After 1 hour the temperature is reduced by 15˚C every hour to reach 55˚C so that the temperature profile of 110˚C - 100˚C - 85˚C - 70˚C - 55˚C is followed. The dried cocoons are stored after ascertain the driage percentage. The dried cocoons were stored in the cocoon racks at 27 °C and 65 % relative humidity maintained.

Sorting

The dried cocoons before taken for reeling are sorted for defective cocoons in the lot.

Cocoon Cooking and Brushing

Cocoon cooking is carried out using two pan cooking method. The cocoon cooking method has a significant influence on reelability of cocoons. Cooking stages viz., retting, high temperature permeation, low temperature water permeation, cooking and adjustment and its temperature 65°c, 90°c, 65°c, 94°c and 90°c and its duration in each stages 30 seconds, 30 seconds, 40 seconds, 45 seconds and 60 seconds accordingly. Cooked cocoons were brushed in hot water at 80°C in the cooking pan.

Reeling conditions: The brushed cocoons were reeled on CSTRI multi end reeling machine with following reeling conditions.

Reeling Speed	:	160 rpm
Basin Temperature	:	40° c
Croissure length	:	8 cm
Number of cocoons per end	:	8

Reelability test was conducted for each lot to determine the average filament length, non-broken filament length, single cocoon filament denier and reelability of each lot.

Reelability Test

For this study sorted good 300 cocoons were taken separately from each lot. The cocoons were weighed on the day of reeling and the weight was noted for each lot separately. The length of the cocoons reeled from the 300 cocoons are measured using length measuring meters fixed to each end in multi end reeling machine. Whenever a cocoon is fed to the reeling end, the stroke counter is activated to record the number of castings made during reeling. After cooking and reeling, the dropped cocoons in cooking and reeling are counted for calculating groping end percentage. The experiment is stopped when the remaining dropped cocoons are about 30. The unreelable cocoons i.e., cocoons which burst open during cooking and cocoons which have not yielded any thread even after repeated cooking are segregated as new cocoons, thick cocoons(1/4 reeled), middle(1/2 reeled), thin(3/4 reeled). Similarly, carryover cocoons i.e., the cocoons that are partially reeled and leftover cocoons are also segregated as thick, middle and thin. Then the reelability percentage, average filament length, non-broken filament length, single cocoon filament denier, raw silk recovery percentage and waste percentage on silk weight are calculated.

Reelability (%), average filament length (metre), non-broken filament length (metre), single cocoon filament denier were calculated using following formulas

$$\text{Reelability (\%)} = \frac{\text{Number of reeling cocoons}}{\text{Number of feeding ends}} \times 100$$

Whereas,

No. of reeling cocoons = No. of cocoons taken for testing – No. of new unreelable cocoons - No. of converted carry over cocoons.

No. of feeding ends = No. of castings + No. of carry over cocoons – No. of converted carry over cocoons.

$$\text{Average Filament Length} = \frac{\text{Length of raw silk reeled X No. of cocoons maintained per end}}{\text{No. of reeling cocoons}}$$

Length of the silk reeled

$$\text{Non Broken Filament Length} = \frac{\text{No. of cocoons maintained per end}}{\text{No of feeding ends}}$$

$$\text{Single Cocoon Filament Denier} = \frac{\text{Weight of raw silk reeled} \times 9000}{\text{Length of the silkreeled x No. of cocoons maintained per end}}$$

Mass reeling is conducted for to find the raw silk percentage, raw silk recovery and waste percentage on raw silk weight using following formulas.

$$\text{Raw silk (\%)} = \frac{\text{Weight of raw silk}}{\text{Weight of cocoons taken for reeling}} \times 100$$

$$\text{Waste (\%)} = \frac{\text{Total cooking and reeling waste weight (grams)}}{\text{Weight of raw silk reeled (grams)}} \times 100$$

Silk Testing: Five silk skeins were taken for testing purpose. The skeins were wound on to bobbins in winding process. The bobbins were taken to the seriplane winding machine and 10 panels were prepared on the black board. The seriplane inspection boards were assessed for neatness characteristics in illumination apparatus by visual inspection by comparing the standard boards.

Table 2. Post cocoon traits of multivoltine races and cross breeds compared with bivoltine CSR2

ocoon Rac	Shell Ratio		AFL		NBFL		SCFD		REEL		REND		RS		RSR		Waste		Neatness	
	Average	S.D.	Average	S.D.	Average	S.D.	Average	S.D.	Average	S.D.	Average	S.D.	Average	S.D.	Average	S.D.	Average	S.D.	Average	S.D.
CSR2	21.88	0.51	793	84.92	524	61.78	2.7	0.21	85.1	3.72	6.7	0.14	14.9	0.31	68.2	2.95	21.41	1.00	97.60	0.46
PM	15.06	0.20	513	13.10	337	18.08	2.45	0.25	65.7	2.19	14.5	0.24	6.9	0.11	45.7	1.34	62.4	3.93	85.5	0.29
TW	10.2	0.4	110	4.1	83	8.6	1.9	0.1	75.6	5.6	21.9	1.6	4.6	0.3	44.9	4.0	63.0	6.2	93.0	0.3
Sarupat	8.8	0.1	351	17.0	192	5.9	2.2	0.0	54.7	1.0	14.4	0.3	7.0	0.1	79.2	2.1	26.0	1.6	85.0	0.4
Moria	9.4	0.4	368	15.4	246	24.8	1.8	0.1	66.6	4.0	15.4	0.3	6.5	0.1	69.1	2.5	22.3	1.2	89.0	0.5
Nistari	11.1	0.2	166	6.5	66	6.0	2.6	0.2	39.6	2.1	21.7	1.2	4.6	0.3	41.9	2.2	48.3	1.2	83.5	0.6
C.Nichi	12.37	0.66	346	33.74	290	29.68	1.74	0.20	83.8	0.89	16.0	1.33	6.3	0.50	51.0	3.35	33.3	4.73	70.0	0.54
BL 24	11.07	0.48	286	19.43	210	8.49	2.06	0.14	73.65	5.57	15.07	0.82	6.66	0.37	60.11	1.54	44.10	3.00	85.00	0.37
TSS	9.73	0.34	342	18.83	252	38.48	1.65	0.06	69.29	3.73	19.60	7.14	5.71	1.66	58.18	15.59	41.7	2.34	75.0	0.41
SST	10.44	0.82	316	4.99	260	11.15	1.87	0.11	82.50	3.94	16.03	1.44	6.29	0.56	60.56	6.73	34.5	0.98	75.0	0.37
Kollegal Ja	16.11	1.77	357	21.25	287	17.46	1.69	0.06	80.26	2.88	16.33	2.54	6.75	0.43	39.97	9.89	41.70	3.58	85.00	0.41
AP12/A4e	13.44	1.06	286	11.56	178	14.01	1.74	0.09	62.13	5.16	15.00	0.82	6.69	0.36	50.10	5.20	40.77	3.90	82.00	0.41
MW13	13.14	2.22	303	4.71	227	15.51	1.94	0.11	77.17	1.06	12.63	0.25	7.92	0.16	62.50	13.30	34.47	3.98	83.50	0.22
PM X CSR	19.13	0.53	675	21.48	631	21.28	2.52	0.03	83.44	1.59	9.48	0.22	10.55	0.25	55.18	1.54	18.45	0.20	93.00	0.29
NISTRI X C	18.93	0.31	681	17.25	525	22.13	2.55	0.02	77.08	1.58	8.67	0.26	11.55	0.36	61.05	2.91	18.15	0.84	90.00	0.57

AFL: Average filament length, NBFL-Non broken filament length, SCFD-single cocoon fiament denier, Reel- reelability, RS Raw Silk and RSR- Raw silk recovery

RESULTS AND DISCUSSION

Comparative analysis of post cocoon traits

Quantity of silk produced from each cocoon depends upon weight of the cocoon shell. The shell ratio shows amount of raw silk that can be reeled from a given quantity of fresh cocoons under transaction (Sonwalker *et al.,* 2001,). Multivoltine races and cross breeds were compared for the filament characters with that of bivoltine races. Shell ratio was 22 % in CSR2 and between 12-14 % for the multivoltine races Kollegal jawan, AP12, MW13. In TW and BL24 the shell ratio ranged between 10-13% but in the cross breeds PM x CSR2 as well as Nistari x CSR2 SR% increased to 19.1% and 18.9% respectively indicating that shell ratio of the cross breeds increased possibly due to the genetic influence of bivoltine genome (Seshagiri *et al.,* 2009; Singh and Suresh Kumar, 2010).

NBFL of reeling cocoons is used to predict achievable filament production speed as reeling speed is directly proportional to NBFL (Lee, 1999). In our study we found that the average filament length (AFL) of CSR2 was approximately 800m and the multivoltine races Kollegal Jawan, AP12 and MW13 showed higher average filament length of 695 m, 632 m, 605 m respectively. C'nichi showed lower average filament length of 264m. AFL of PM and Nistari is noted as 513m and 166m respectively.

In the cross breeds, AFL was improved to 671 m in PM x CSR2 and 678m in Nistari x CSR2. Similarly the NBFL ranged between 200 – 300 m in multivoltine races and in cross breeds the NBFL was improved and ranged between 500 - 600 m. The single cocoon filament denier ranged between 1.7- 2.0 among the multivoltines and in the cross breeds the SCFD was 2.4 - 2.6 nearing to the estimates of CSR2 (= 2.8). The correlation between filament denier and length are positive in bivoltines and multivoltine cross breeds. Multivoltine cross breeds showed lower physical and mechanical properties, however the multivoltine races did not show significant change in denier from outer layer to inner layer facilitating better control during reeling (Vasumathi *et al.,* 1999; Chatopadhya *et al.,* 2018). Further the cross breeds of multi x bivoltine races showed better denier, AFL as well as NBFL indicative of better reeling ability in comparison to multivoltines.

Table 3. Pearson Correlation among the post cocoon traits of multivoltine races, cross breeds and CSR2

		SR	REEL	RS	RSR	WASTE	AFL	NBFL	SCFD	REND
SR	Pearson Cor	1								
	Sig. (2-tailed	.								
REEL	Pearson Cor	**0.3179	1.000							
	Sig. (2-tailed	0.008	.							
RS	Pearson Cor	**0.8298	0.233	1.000						
	Sig. (2-tailed	0.000	0.056	.						
RSR	Pearson Cor	-0.092	-0.026	**0.4600	1.000					
	Sig. (2-tailed	0.456	0.830	0.000	.					
WASTE	Pearson Cor	**-0.409	*-0.2823	**-0.666	**-0.600	1.000				
	Sig. (2-tailed	0.001	0.020	0.000	0.000	.				
AFL	Pearson Cor	**0.8361	**0.3197	**0.8923	*0.3077	**-0.6525	1.000			
	Sig. (2-tailed	0.000	0.008	0.000	0.011	0.000	.			
NBFL	Pearson Cor	**0.8174	**0.5500	**0.8129	0.216	**-0.6630	**0.9520	1.000		
	Sig. (2-tailed	0.000	0.000	0.000	0.076	0.000	0.000	.		
SCFD	Pearson Cor	**0.5783	-0.152	**0.5913	0.139	**-0.3223	**0.6431	**0.5585	1.000	
	Sig. (2-tailed	0.000	0.215	0.000	0.257	0.007	0.000	0.000	.	
REND	Pearson Cor	**-0.712	**-0.328	**-0.912	**-0.564	**0.6944	**-0.833	**-0.792	**-0.4964	1
	Sig. (2-tailed	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	.

(significance level ** 0.01, * 0.05)

Silk reelability was 85% in CSR2 and among the multivoltine races, highest reelability found in Kollegal jawan (83.5%), Ap12 (82.4%) and MW13 (82.6%) and lowest was found in Sarupat and BL24 (66%). The cross breeds PMxCSR2 and Ni xCSR2 showed reelability upto 79 - 83% indicating a significant (P<0.01) increase in reelability compared to other multivoltines.

Renditta is defined as a No of kgs of cocoons required to produce 1kg of raw silk, therefore a negative co relation exists between renditta and raw silk, in this study the CSR2 showed an average renditta of about 6.2-6.6 and Raw silk percent of about 15% and raw silk recovery approximately 70%, while among the multivoltines it was observed that the renditta was very high in races like TW and Nistari approximately 21.4 and raw silk percent was 4.6% and raw silk recovery was about 42-44% , lowest renditta of 12.6 among the multivoltines was observed in MW13, with raw silk percent of 7.9%, and raw silk recovery of 63.5% in cross breeds. The highest neatness of the yarn in multivoltines was observed in the range of 82-89 and the lowest was about 63 in BL24 while in the cross breeds the neatness points improved to 91% while in bivoltine the highest neatness point of 97 was observed. In the cross breeds the neatness points improved to 91% while in bivoltine it was 97 (Table-2). Neatness is a racial character and is greatly influenced by the silkworm race, origin and heterosis (Datta and Nagaraju, 1987; Datta *et al.*, 2001).

The above data showed that in the cross breeds post cocoon traits showed higher values than multivoltine races and there is scope for improving the polyvoltines to achieve qualitative traits closer to bivoltines.

Statistical analysis and correlation studies of the post cocoon characters of multivoltine races and cross breeds

Euclidean Distance model

Ten post cocoon traits (PCT) of the multivoltine races, the cross breeds and CSR2 (bivoltine race) were analysed by ALSCAL multidimensional scaling (SPSS) using squared Euclidean distance model. The analysis showed significant variation between multivoltine races and cross breeds. The Euclidean distance model based on the PCT showed distinct grouping of CSR2(C2.1-C2.4), Nistari x CSR2 (NxC) and PM x CSR2 (PxC) indicating that in cross breeds the post cocoon traits were improved and was nearing to the CSR2 (bivoltine) characters (Fig. 1). Suggestively greater scope for improvement in the cross breeds and Pure Mysore (PM) was observed as it formed subgroup within the cluster but was slightly distanced from the cross breeds. It show that some of the characters in the cross breeds are similar to that of their multivoltine parents. Another major group was formed by all the multivoltine races indicating that there is no significant variation among the post cocoon characters in the multivoltine races irrespective of their high or low yielding characters (Fig. 1). For synthesizing the potential polyvoltine cross breeds, superior bivoltine races are crossed with multivoltine parents so that the resultant cross breeds have better qualitative as well as quantitative traits (Rao *et al.*, 2006).

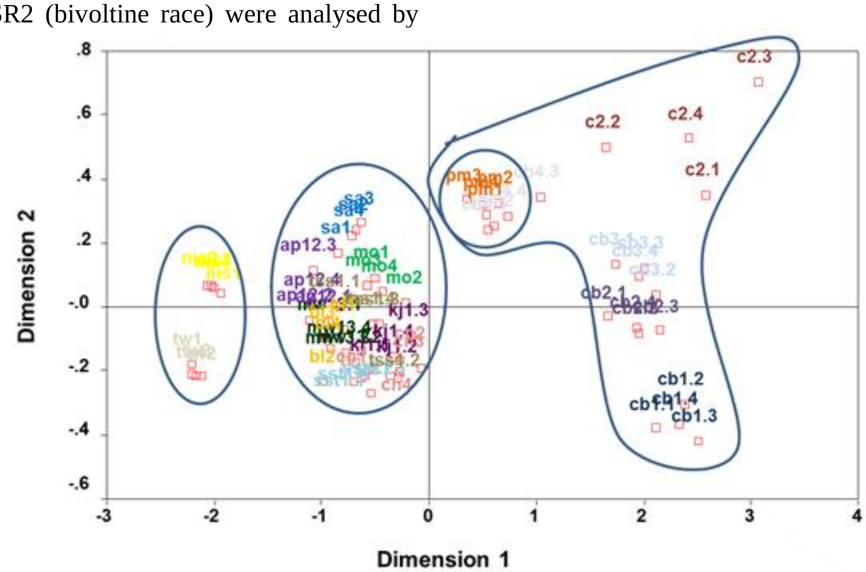

Fig. 1. ALSCAL multidimensional scaling using squared Eucledian distance model showed grouping of cross breeds with PM and CSR2 and another cluster of multivoltine races based on ten post cocoon characters. Isolation of Nistari and Tamil Nadu White is due to its lower traits.

Pearson Correlation studies

Pearson correlation analysis was performed to reveal correlation pattern between post cocoon traits and the influence of each post cocoon traits over the other. Among the traits, shell ratio (SR) showed significant (P < 0.01) positive correlation with raw silk percent, reelability and filament length (AFL & NBFL) (Table 3) revealing that shell ratio directly influences filament length and reelability. The relationship between cocoon and shell weight is considered to calculate the shell percentage which is linked to the ultimate raw silk yield (sericulture manual-silk reeling industry, http://bieap.gov.in). Similarly filament length showed significant (P <0.01) positive correlation with raw silk recovery, raw silk and negative correlation with denier, renditta, waste. The raw silk showed positive correlation with filament length, denier, silk recovery and negative correlation with waste and renditta. The renditta is negatively co related with filament length, denier, Raw silk recovery, raw silk and positively correlated with waste. These variables as selection markers to control the expression of other correlated variables (Kumaresan *et al.*, 2007). Similarly with increase in the silk content (raw silk, raw silk recovery) it is obvious that waste decreases while reelability and filament length increases (Chattopadhya *et al,* 2018; Sericulture manual-silk reeling industry, http://bieap.gov.in). Better quality of raw silk attributed to better quality cocoons, optimum degree of drying of cocoons, uniform cooking of cocoons to the required level using cooking machine and reeling with required denier and silk reeling machine (Mahadevaiah *et al.,* 2015).

CONCLUSION

It was shown that cross breeds showed improved shell ratio, raw silk recovery, reelability, and length of the filament than multivoltines. It was observed that all the multivoltine races irrespective of their yield (low or high) showed decrease in the Shell ratio (SR), Average filament length (AFL), non-broken filament length (NBFL), single cocoon filament denier (SCND), reelability, Raw silk percent (RS), raw silk recovery (RSR) and simultaneous increase in the Renditta compared to CSR2 (Bivoltine) race. The cross breeds showed comparatively higher yield than their respective multivoltine parents as well other multivoltines taken in the study exhibiting heritability of post cocoon characters in the cross breeds (Islam *et al.,* 2003, Kumaresan *et al.,* 2007). This indicates that multivoltine races can be improved by crossing with the bivoltine races that has better qualitative traits and adaptability than their multivoltine counter parts.

ACKNOWLEDGEMENT

The funding of this research project was provided by Central Silk Board, Bangalore, Karnataka.

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How to cite this article?
Aravind, S., Sahar Ismail, Hariraj, K.S. Tulsi Naik, A.R. Pradeep, R.K Mishra, Subhash. V. Naik. 2019. Comparative analysis of post cocoon characters associated with filament length between multivoltine races and multi x bivoltine cross breeds of the silkworm *Bombyx mori*. *Innovative Farming*, **4**(3): 123-128.