PERFORMANCE WISE EVALUATION OF THE MORPHOLOGICAL TRAITS OF BIVOLTINE SILKWORM (*BOMBYX MORI* L.) BREEDS IN SUB-TROPICAL CONDITIONS

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ABSTRACT
The morphological characterization among domesticated insect like silkworm (*Bombyx mori* L.) is an important aspect for selection of suitable parents for breeding programme. The present study was conducted on twenty bivoltine silkworm breeds to estimate the extent of relationship by morphological characterization. Eighteen morphological traits both quantitative and qualitative viz., Egg shape, Chorion colour, Colour of the newly hatched larva, Larval marking, Cocoon colour, Cocoon shape, Pupa colour and Shape, Colour of male and female moth, Fecundity, Hatching percentage, Larval weight 5th day, Vth instar duration, Total larval period, Percentage of malformed cocoons, Pupation rate, Single cocoon weight, Single shell weight and Shell percent were studied and data revealed significant variability among the breeds. Promising breeds were short listed and identified by using multiple trait Evaluation Index method for qualitative and quantitative characters. On the basis of highest cumulative evaluation values (> 50) for ten quantitative economically commercial characters, ten breeds; ND_3, ND_5, CSR_{19}, PO_1, SPO, UDHEY-3, PO_3, ND_2, CSR_{18} and NSP were short listed. These breeds scored higher cumulative evaluation values (> 50) and have been identified as promising breeds. They are recommended for further breeding to boost bivoltine silk production; and could also be used for developing high yielding, sustainable and productive breeds.

KEYWORDS: Silkworm, morphological characterization, bivoltine breeds, Coefficient Correlation, Evaluation Index, Yield.

Introduction

Silkworm (*Bombyx mori* L.) originated in China in the southern district of Yangtze. Owing to its commercial value; the insect was brought to Korea, Japan, and Europe and subsequently to India through the Silk Road. In due course of time, the species adapted to respective local conditions and got differentiated into Chinese, Japanese, European, and Korean varieties; and thereafter is being domesticated for over 5000 years. It has its use in textile as well as non- textile industries; and is a model organism for studying microbiology, physiology and genetics. Silkworm comprises of large number of geographical races and inbred lines; distributed in temperate and tropical countries; and showing substantial variation in their qualitative and quantitative traits. Currently, it is the major economic resource for nearly 30 million families in countries such as China, India, Japan, Korea, Vietnam and Thailand (Rathore *et al.*, 2011; 2012). The tropical genotypes (non diapausing) are hardy and withstand adverse eco-climatic conditions but produce small quantum of poor quality silk, whereas genotypes of temperate (dia-pausing) origin produce higher quantity of international quality silk. The conventional breeding approaches, although have increased the silk productivity, but it has not integrated the high yielding traits of temperate genotypes with the low yielding disease resistant tropical genotypes. Moreover, the environmental factors interact specifically with the phenotypic and conventional breeding and, thereby limit the yield improvement of the strains of silkworm. The genetic resources are the basic material for crop improvement; and the success of a breeding

programme depends on the initial selection of parents and their effective utilization in desirable combinations. Further, it also depends on the ability of breeds to assemble and recombine the genetic variability; and extraction of the potential gene combinations from the gene pool based on phenotypic expression; leading to genetic fixation of the traits over generations. Therefore, silkworm genetic resources need to be properly evaluated and screened for identifying the potential of promising parental lines. In light of above background, the present study has been designed to evaluate the indigenous silkworm bivoltine breeds on the basis of their morphological traits.

RESEARCH METHODOLOGY

The research work was conducted at SKUAST-J on twenty different races of Indigenous silkworm bivoltine breeds namely; PO_1, PO_3, SPO, ND_2, ND_3, ND_5, NSP, UDHEY-1, UDHEY-2, UDHEY-3, UDHEY-4, UDHEY-5, UDHEY-6, UDHEY-7, UDHEY-8, CC_1, CSR_{18}, CSR_{19}, NB_4D_2, and SH_6. The rearing of silkworm was conducted for a good harvest of the quality cocoon crop at the Division of Sericulture Udheywala, SKUAST-J, with standard rearing techniques suggested by Dandin (2003). To prevent diseases and to maintain good sanitation, the rearing room and other rearing appliances were disinfected with a 5% formaldehyde solution following Ullah and Narasimhanna (1978) procedure. Then the eggs were incubated at 25±1°C with humidity 80%±5% at normal daylight and dark in the night. A black pin-like spot appeared on the egg two days before hatching and on the following day it turned completely blue called body pigmentation of the egg. The eggs at this stage were disinfected with 2% formaldehyde solution for five minutes recommended by Jolly (1983) and transferred to the rearing house, where the newly hatched silkworms were transferred to the rearing tray with a feather following the brushing technique of Ullah and Narashimhanna (1978). Cocoons were harvested after 4 to 5 days of mounting. After that, male and female pupae were segregated and the fresh moths were allowed to mate after emergence. When the egg-laying was completed the females were subjected to microscopic examination. The eggs produced were called disease free layings (dfls) and they were utilized for experiment. After the formation and maturation of cocoons, data of different characters were collected intensively, wherever necessary. The characters studied and observational procedures adopted are given under the following heading:

Qualitative Characterization of bivoltine silkworm breeds

The experimental bivoltine silkworm breeds were characterized for morphology of egg, larva, cocoon, pupa and adult. The characterization was done by collecting the samples at various stages of development.

EGG STAGE

For recording different morphological characters at egg stage five layings from each of the 20 breeds were observed.

1. **Egg shape:** External morphology of the eggs was observed under microscope.
2. **Chorion colour:** The colour of the egg shell after ecolosion was microscopically observed under natural light.

LARVAL STAGE

3. **Colour of the newly hatched larva:** The body colour of the neonate was visually observed through microscope under natural light immediately after ecolosion and was recorded for all the twenty breeds.
4. **Larval marking:** The larval marking was observed during second and third day of fifth instar larva. Larval marking as eye spot on second thoracic segment, crescent on second abdominal segment and star marks on fifth abdominal segments were recorded as either absent, faint or present among the breeds studied. Among sex limited breeds (SL) markings were present in female and absent in males.

COCOON STAGE

5. **Cocoon colour:** The colour of the cocoons was visually observed under natural light as white, creamy white and dull white.
6. **Cocoon shape:** The cocoon shapes were recognized visually as oval and elongated with faint constriction.

PUPAL STAGE

7. **Pupal colour and shape:** The external morphology of pupa was visually observed.

ADULT STAGE

8. **Colour of the male and female moth**

The body colour of moths was observed visually and recorded as creamish white, dull white, dirty white and dirty. Within studied breeds difference in body colour for male and female moths was recorded.

Quantitative Characterization of bivoltine silkworm breeds

The quantitative characters for commercial production performance viz. fecundity, hatching percentage, larval weight of 10 mature larvae, duration of fifth age larval life, total larval duration, malformed cocoons, pupation percentage, single cocoon weight, single shell weight and shell ratio percentage were recorded and evaluated.

EGG STAGE

9. **Fecundity:** It is the total number of eggs laid by a single mother moth and was calculated by counting the total number of eggs laid by the female moth. Average of three layings in each replicate was recorded for analysis.

10. **Hatching percentage:** It is the number of larvae hatched out from the total eggs laid by a mother moth and was recorded as an average of three layings in each replicate and was calculated by the following formula:

$$\frac{\text{Number of eggs hatched}}{\text{Total number of eggs per laying}} \times 100$$

LARVAL STAGE

Following observations were recorded for different parameters of larval stages:-

Larval duration of each instar (Days and hours): It was recorded as the duration of larval age of each instar (I, II, III, IV, and V).

I age larval duration (D: H): It was recorded as the larval duration from the day of brushing to I moult and was recorded in days and hours for each breed.

II age larval duration (D: H): It was recorded as the larval duration from I moult out to initiation of II moult and was recorded in days and hours for each breed.

III age larval duration (D: H): It was recorded as the larval duration from II moult out to initiation of III moult and was calculated in days and hours for each breed.

IV age larval duration (D: H): It was recorded as the larval duration from III moult out to initiation of IV moult and was recorded in days and hours for each breed.

11. V^{th} age larval duration (D: H): It was recorded as the larval duration from IV moult out to pre-spinning stage and was recorded in days and hours for each breed.

12. Total larval duration (D: H): It was recorded as an average of total larval span in days and hours from brushing to pre- spinning stage including the moulting period in each instar.

13. Weight of 10 mature larvae (g): Ten mature larvae were picked randomly from each replicate of each breed from 4^{th} to 6^{th} day of fifth instar and weighted on digital balance. Maximum larval weight was recorded in each breed.

COCOON STAGE

14. Pupation percentage: This parameter represents the average number of live pupae obtained and is represented in percentage. It was calculated by using the following formula:

$$\frac{\text{Number of live pupa in cocoons harvested}}{\text{Total number of larvae retained after III moult}} \times 100$$

15. Malformed cocoon percentage

a) Double cocoon percentage

This represented an average number of double cocoons sorted out in each replicate and was determined by using the following formula:

$$\frac{\text{Number of double cocoons harvested}}{\text{Total number of larvae retained after III moult}} \times 100$$

b) Flimsy cocoon percentage

This parameter was worked out as average number counted in each replicate and was computed by using the following formula:

$$\frac{\text{Number of flimsy cocoons harvested}}{\text{Total number of larvae retained after III moult}} \times 100$$

c) Dead cocoon percentage

This was calculated as an average number of cocoons with dead pupae obtained in each replicate and was determined by using the following formula:

$$\frac{\text{Number of dead cocoons harvested with dead pupae}}{\text{Total number of larvae retained after III moult}} \times 100$$

16. Single cocoon weight (g): Twenty five male and twenty five female cocoons were randomly selected and weighed on digital balance to determine the average cocoon weight by using the following formula:

$$\frac{\text{Weight of 25 male + 25 female cocoons}}{50} \times 100$$

17. Single shell weight (g): Same twenty five male and twenty five female cocoon shells from each replicate were weighed on digital balance to determine average single shell weight. The formula applied was as under:

$$\frac{\text{Weight of 25 male + 25 female cocoon shells}}{50} \times 100$$

18. Shell ratio percentage: It was calculated as an average of twenty five male and twenty five female cocoon shells to that of average cocoon weight of same cocoons per replicate and was calculated by using the following formula:

$$\frac{\text{Average weight of 25 cocoon shells of each sex}}{\text{Average weight of same cocoons of each sex}} \times 100$$

Data analysis: The collected data were analyzed successfully by following statistical technique:

1. The average mean value and standard deviation were calculated separately for each character.
2. The coefficient of correlation of each character with yield was computed separately and were performed using following formulae:

$$r = \frac{\sum xy - \frac{\sum x . \sum y}{n}}{\sqrt{\sum x^2 - \frac{(\sum x)^2}{n}} \cdot \sqrt{\sum y^2 - \frac{(\sum y)^2}{n}}}$$

Where, in this formula, N is equal to the number of pairs of scores and ∑xy is called the sum of the cross.

3. Further based on Evaluation Index Method, the breeds were ranked according to their Evaluation Index (E.I.) (Mano *et.al* 1993) by using the following formula:

$$\textbf{E.I.} = \frac{A - B}{C} \times 10 + 50$$

Where, A = Value obtained for a particular traits of the breed.
B = Mean value of a particular trait of all breed.
C = Standard deviation of a particular trait.
10 = Standard unit.
50 = Fixed value.

RESULTS AND DISCUSSION

Qualitative Characterization of bivoltine silkworm breeds

The silkworm rearing trial of twenty bivoltine silkworm breeds was carried out in 2017 spring rearing season. The observations were recorded at egg, larval, and cocoon stages for eighteen morphological and commercial parameters. The results obtained are presented under the following headings:

EGG STAGE

1. **Egg shape:** The shape of the eggs of all silkworm breeds studied, were ellipsoidal type.
2. **Chorion colour:** Twelve breeds viz. PO_1, PO_3, SPO, UDHEY-1, UDHEY-2, UDHEY-3, UDHEY-4, UDHEY-5, UDHEY-6, UDHEY-7, UDHEY-8 and SH_6 recorded creamish white chorion colour. Five breeds namely ND_2, ND_3, ND_5, NSP, and NB_4D_2 were light yellow in colour. Remaining three breeds of CSR&TI Mysore origin viz. CSR_{18}, CSR_{19} were having pale yellow colouration while as breed CC_1 had white chorion (Table 1 and Fig. 1).

Table 1: Frequency distribution of chorion colour

Colour	Number of breeds
White	01
Creamish white	12
Light yellow	05
Pale yellow	02

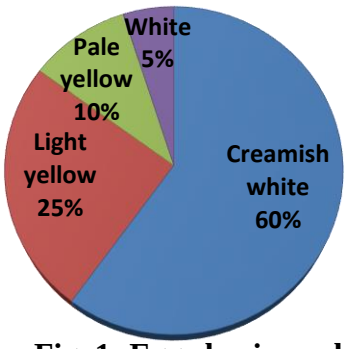


Fig. 1: Egg chorion colour

LARVALSTAGE

3. Colour of newly hatched larva: Out of twenty breeds, ten breeds viz. UDHEY-1, UDHEY-2, UDHEY-4, UDHEY-5, UDHEY-6, UDHEY-8, CSR_{18}, CSR_{19}, NB_4D_2, and SH_6 recorded dark brown colour. Four breeds PO_1, PO_3, SPO, and CC_1 were brown in colour while as remaining six breeds viz. ND2, ND3, ND5, NSP, UDHEY-3 and UDHEY-7 were having black neonatal larval colouration (Table 2 and Fig. 2).

Table 2: Frequency distribution on colour of newly hatched larvae.

Colour	Number of breeds
Dark brown	10
Brown	04
Black	06

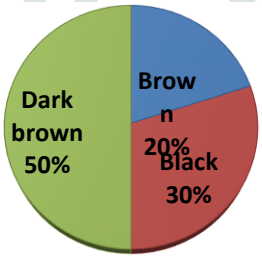


Fig. 2: Colour of newly hatched larvae

4. **Larval marking:** The larval marking in nine breeds; PO_1, PO_3, SPO, UDHEY-2, UDHEY-4, UDHEY-6, UDHEY-8, CC_1 and NB_4D_2 were plain and breeds viz. ND_2, ND_3, ND_5, NSP, UDHEY-1, UDHEY-3, UDHEY-5, UDHEY-7 and SH_6 had crescent marked larvae. Two breeds, CSR_{18} and CSR_{19} were sex limited having marking on female larvae only while as male larvae were plain with no markings (Table 3 and Fig. 3).

Table 3: Frequency distribution on larval marking

Colour	Number of breeds
Crescent	09
Sex limited	02
Plain	09

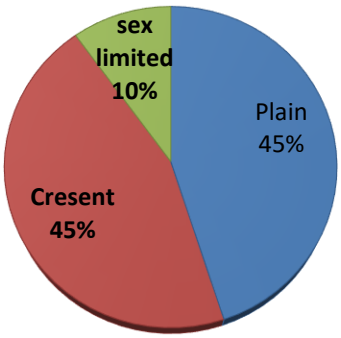


Fig. 3: Marking on larva

COCOON STAGE

5. **Cocoon Colour:** Among twenty breeds, eight breeds viz. PO_1, PO_3, ND_5, UDHEY-3, UDHEY-4, UDHEY-5, UDHEY-6 and UDHEY-8 recorded creamish white colour while as eleven breeds; SPO, ND_2, ND_3, UDHEY-1, UDHEY-2, UDHEY-7, CC_1, CSR_{18}, CSR_{19}, NB_4D_2 and SH_6 were white in colour. Breeds NSP recorded dull white coloured cocoons (Table 4 and Fig. 4).

Table 4: Frequency distribution on cocoon colour

Colour	Number of breeds
White	11
Creamish white	08
Dull white	01

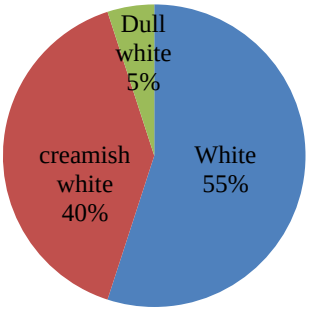


Fig. 4: Cocoon colour

6. **Cocoon shape**: Among twenty breeds studied, nine breeds viz. PO_1, PO_3, SPO, UDHEY-2, UDHEY-4, UDHEY-8, CC_1, CSR_{18} and SH_6 had oval cocoon shape. Seven breeds viz. ND_2, ND_3, UDHEY-1, UDHEY-3, UDHEY-5, CSR_{19} and NB_4D_2 had elongated faint constriction and breed UDHEY-6 recorded oval faint constriction. Three breeds, viz. ND_5, NSP and UDHEY-7 were elongated constricted (Table 5 and Fig. 5).

Table 5: Frequency distribution on cocoon shape.

Cocoon shape	Number of breeds
Oval	09
Oval faint constriction	01
Elongated faint constriction	07
Elongated constricted	03

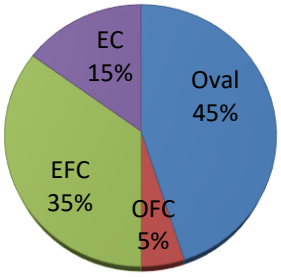


Fig. 5: Cocoon Shape

PUPAL STAGE

7. Pupa colour: Ten plain larva viz. breeds PO_1, PO_3, SPO, UDHEY-2, UDHEY-4, UDHEY-6, UDHEY-8, CC_1, CSR_{18}, CSR_{19} (male) and NB_4D_2 recorded yellow colour while as ten marked larvae ND_2, ND_3, ND_5, NSP, UDHEY-1, UDHEY-3, UDHEY-5, UDHEY-7, CSR_{18} CSR_{19} (female) and SH6 were of yellowish brown colour. The phenotypic pupal colouration for plain and marked larvae was in 1:1 ratio (Table 6 and Fig. 6).

Table 6: Frequency distribution on pupal colour

Colour	Number of breeds
Yellow colour	10
Yellowish brown colour	10

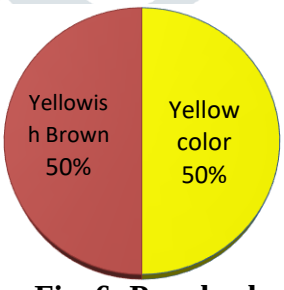


Fig. 6: Pupal colour

7. Pupa shape: No variation in pupa shape was observed and all the pupae of all twenty breeds were of elliptical shape.

MOTH STAGE

8. Colour of male and female moths: No variation in male moth colour was observed. All the twenty breeds studied had creamish white coloured male moths. Limited variation in female moth colour was observed. Female moth colour in breeds ND_2, ND_3, ND_5, NSP, UDHEY-1, UDHEY-2, UDHEY-3, UDHEY-4,, UDHEY-5, UDHEY-6, UDHEY-7, UDHEY-8, NB_4D_2 and SH_6 were dirty white while as

breeds PO_1, PO_3, SPO, and CC_1 had dull white female moths. Sex limited breeds, CSR_{18}, and CSR_{19} had dirty colour female moths (Table 7 and Fig. 7).

Table 7: Frequency distribution on female moth colour.

Female moth colour	Number of breeds
Dull white	04
Dirty white	14
Dirty	02

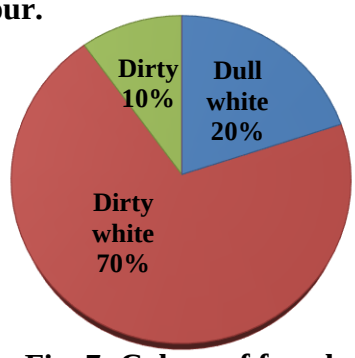


Fig. 7: Colour of female moth

Quantitative Characterization of bivoltine silkworm breeds

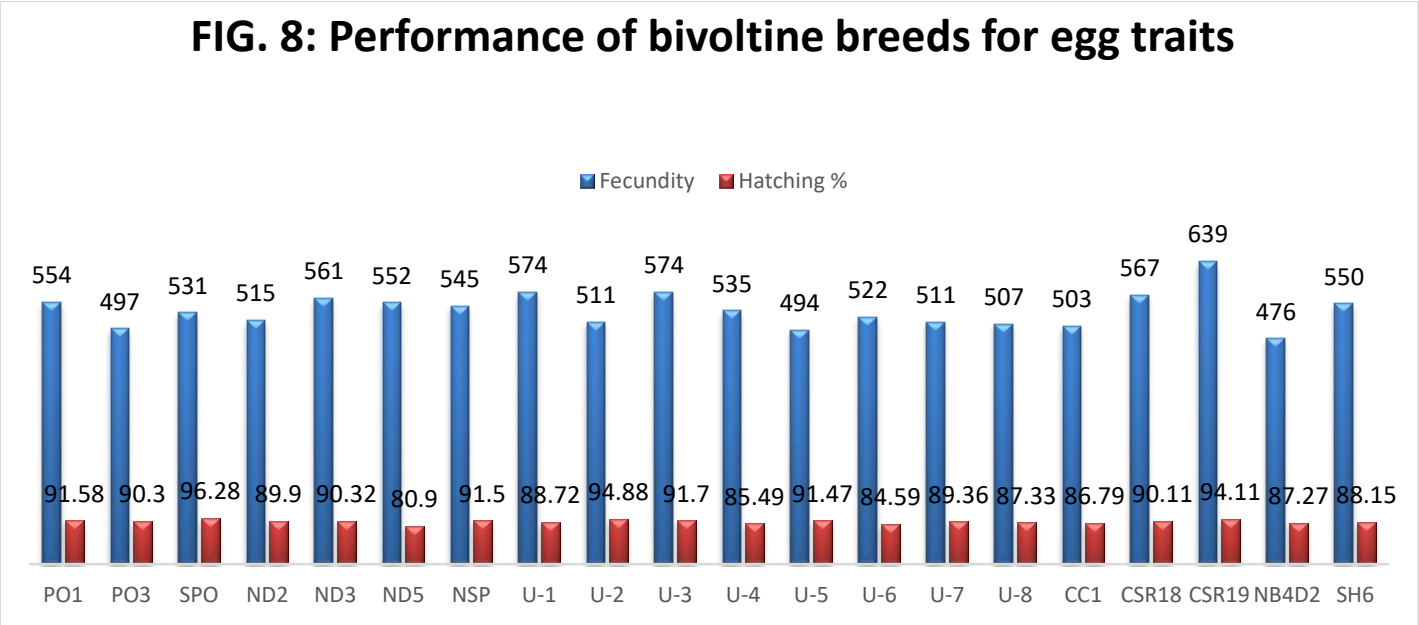
The performance of twenty breeds for nine commercial characters was recorded for following parameters at egg stages (Table 8 and Fig. 8).

9. Fecundity: Among all the twenty breeds highest number of eggs were found in breed CSR_{19} (639) followed by UDHEY-1, UDHEY -3 (574) and lowest in breed NB_4D_2 (476) which stood at below average.

10. Hatching percentage: The hatching percentage among studied breeds was maximum in SPO (96.28) followed by UDHEY-2 (94.88) and minimum in breed ND_2 (80.90) which was below average.

Table 8: Performance of bivoltine silkworm breeds for egg traits.

Breeds	Fecundity (no.)	Hatching percentage
PO_1	554	91.58
PO_3	497	90.30
SPO	531	96.28
ND_2	515	89.90
ND_3	561	90.32
ND_5	552	91.50
NSP	545	89.73
UDHEY -1	574	88.72
UDHEY -2	511	94.88
UDHEY -3	574	91.70
UDHEY -4	535	85.49
UDHEY -5	494	91.47
UDHEY -6	522	84.59
UDHEY -7	511	89.36
UDHEY -8	507	87.33
CC_1	503	86.79
CSR_{18}	567	90.11
CSR_{19}	639	94.11
NB_4D_2	476	87.27
SH_6	550	88.15
MEAN	**535.9**	**89.97**
SD	**37.46**	**02.99**



LARVAL STAGE

At larval stage following observations were recorded for different parameters and data presented in Table 9 and Fig. 9.

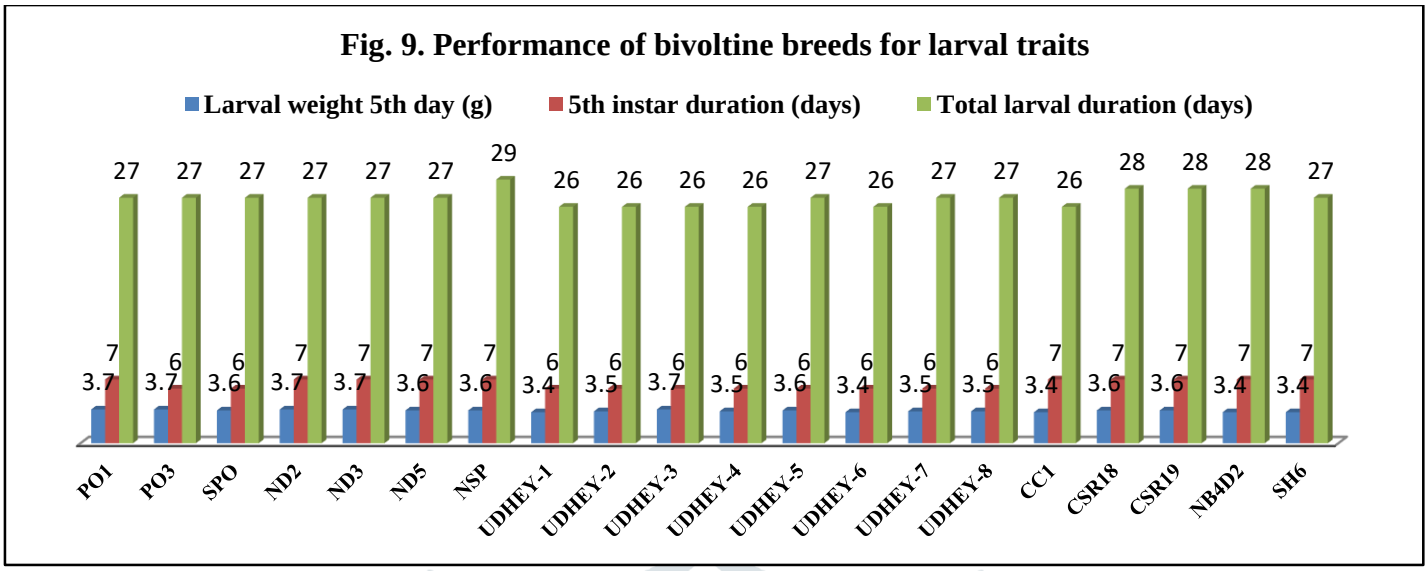
11. Duration of 5th instar (Days): Among breeds, fifth age larval duration was longer in PO_1, ND_2, ND_3, ND_5, NSP, CC_1, CSR_{18}, CSR_{19}, $NB4D_2$, and SH_6 (7:0) and shorter in PO_3, SPO, UDHEY -1, UDHEY-2, UDHEY-3, UDHEY-4, UDHEY-5, UDHEY-6, UDHEY-7 and UDHEY-8 (6:0).

12. Total larval duration (Days): The longest and above average total larval life was recorded in breed NSP (29.00) followed by CSR_{18}, CSR_{19} and NB_4D_2 (28.00). Shortest total larval duration was observed in breeds UDHEY-1, UDHEY-2, UDHEY-3, UDHEY-4, UDHEY-6 and CC_1 (26.00).

13. Larva weight 5th day (g): Maximum single mature larval weight was randomly recorded in breeds; PO_1, PO_3, ND_2, ND_3, and UDHEY-3, (3.7) followed by SPO, ND_5, NSP, UDHEY-5, CSR_{18}, and CSR_{19} (3.6) whereas, it was minimum in UDHEY-1, UDHEY-6, CC_1, NB_4D_2 and SH_6 (3.4).

Table 9: Performance of bivoltine silkworm breeds for larval traits

Breeds	Larva weight 5th day (g)	5th instar duration (days)	Total larval duration (days)
PO_1	3.7	7.00	27.00
PO_3	3.7	6.00	27.00
SPO	3.6	6.00	27.00
ND_2	3.7	7.00	27.00
ND_3	3.7	7.00	27.00
ND_5	3.6	7.00	27.00
NSP	3.6	7.00	29.00
UDHEY-1	3.4	6.00	26.00
UDHEY-2	3.5	6.00	26.00
UDHEY-3	3.7	6.00	26.00
UDHEY-4	3.5	6.00	26.00
UDHEY-5	3.6	6.00	27.00
UDHEY-6	3.4	6.00	26.00
UDHEY-7	3.5	6.00	27.00
UDHEY-8	3.5	6.00	27.00
CC_1	3.4	7.00	26.00
CSR_{18}	3.6	7.00	28.00
CSR_{19}	3.6	7.00	28.00
NB_4D_2	3.4	7.00	28.00
SH_6	3.4	7.00	27.00
MEAN	**3.55**	**6.50**	**26.95**
S D	**0.11**	**0.51**	**00.82**



COCOON STAGE

The following performance observations of parental breeds were recorded for different cocoon parameters and data presented in Table 10 and Fig. 10.

14. Malformed cocoon percentage: The percentage of malformed cocoons was maximum in breed PO_1 (3.98) followed by SH_6 (3.79) and least in breed UDHEY-6 (2.20). The malformed cocoon comprises of flimsy, thin end, and deformed cocoons.

15. Pupation rate percentage: Highest pupation percentage was recorded in breed ND_5 (92.00) followed by ND_3 and NSP (90.00). It was lowest in breeds UDHEY-6 and NB_4D_2 (82.00).

16. Single cocoon weight (g): It is an important parameter and was maximum and above average in breed UDHEY-3 (1.800) followed by ND_3 (1.726) and PO_3 (1.725). Breed UDHEY-6 recorded minimum and below average cocoon weight of 1.425g.

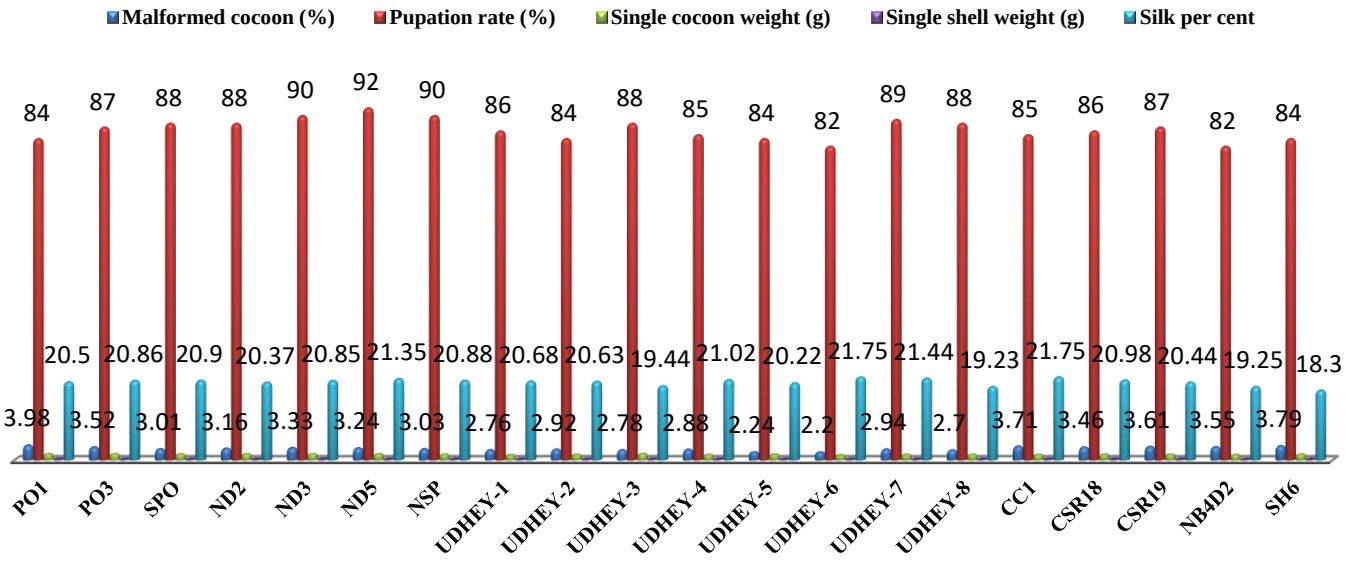
17. Single shell weight (g): Three breeds PO_3, ND_3 and ND_5 recorded higher single shell weight of 0.360 followed by breeds PO_1, SPO, ND_2, and UDHEY-3 (0.350). Lowest value of 0.290 shell weight was recorded in breed NB_4D_2.

Table 10: Mean performance of bivoltine silkworm breeds for cocoon traits.

Breeds	Malformed cocoon (%)	Pupation (%)	Single cocoon weight (g)	Single shell weight (g)	Silk per cent
PO_1	3.98	84	1.707	0.350	20.50
PO_3	3.52	87	1.725	0.360	20.86
SPO	3.01	88	1.674	0.350	20.90
ND_2	3.16	88	1.718	0.350	20.37
ND_3	3.33	90	1.726	0.360	20.85
ND_5	3.24	92	1.686	0.360	21.35
NSP	3.03	90	1.628	0.340	20.88
UDHEY-1	2.76	86	1.450	0.300	20.68
UDHEY-2	2.92	84	1.575	0.325	20.63
UDHEY-3	2.78	88	1.800	0.350	19.44
UDHEY-4	2.88	85	1.522	0.320	21.02
UDHEY-5	2.24	84	1.632	0.330	20.22
UDHEY-6	2.20	82	1.425	0.310	21.75
UDHEY-7	2.94	89	1.590	0.341	21.44
UDHEY-8	2.70	88	1.570	0.302	19.23
CC_1	3.71	85	1.563	0.340	21.75
CSR_{18}	3.46	86	1.620	0.340	20.98
CSR_{19}	3.61	87	1.614	0.330	20.44
NB_4D_2	3.55	82	1.506	0.290	19.25
SH_6	3.79	84	1.639	0.300	18.30
MEAN	**3.14**	**86.45**	**1.618**	**0.332**	**20.54**
SD	**0.49**	**02.72**	**0.09**	**0.022**	**00.88**

18. Silk percent: Highest and above average silk percent was observed in breed UDHEY-6 and CC_1 (21.75) followed by UDHEY-7 (21.44) ND_5 (21.35) UDHEY-4 (21.02). Lowest silk ratio of 18.30 was recorded in breed SH_6.

Fig. 10. Mean performance of bivoltine breeds for cocoon traits



Statistical Analysis by Evaluation Index method

The evaluation values for fecundity, hatching percentage, larval weight for 10 mature larvae, malformed cocoon, pupation percentage, single shell weight, single cocoon weight and silk percent are presented in Table 11 and 12.

EGG STAGE

1. **Fecundity:** Among breeds, maximum Evaluation Index value of 77.52 was recorded in breed CSR_{19} followed by UDHEY-I and UDHEY-3 (60.17) whereas minimum E.I. value was recorded in NB_4D_2 (34.00).
2. **Hatching Percentage**: Out of twenty breeds, E.I. value was maximum in SPO (71.10) followed by UDHEY-2 (66.42) and CSR_{19} (63.84). Breed UDHEY-6 recorded a minimum value of 32.00.

LARVAL STAGE

3. **Larva weight of 10 mature larvae 5^{th} (D):** Among twenty breeds, maximum E.I. value of 63.63 was recorded in PO_1, PO_3, ND_2, ND_3, and UDHEY-3, for weight of 10 mature larvae and a minimum value of 36.36 in breeds UDHEY-1, UDHEY-6, CC_1, NB_4D_2, and SH_6.
4. **V^{th} Instar larval duration (D):** V^{th} age larval duration contributes maximum for silk attributing character. Among breeds, highest E.I. value of 59.80 was recorded in PO_1, ND_2, ND_3, ND_5, NSP, CC_1, CSR_{18}, CSR_{19}, NB_4D_2, and SH_6 indicating longer fifth age. Lower E.I. value of 40.19 was obtained in breeds PO_3, SPO, UDHEY-1, UDHEY-2, UDHEY-3, UDHEY-4, UDHEY-5, UDHEY-6, UDHEY-7, and UDHEY-8 was recorded in ten breeds reflecting shorter larval span in fifth age.
5. **Total larval duration (D):** Among twenty breeds, maximum E.I. value of 75.00 was obtained in breed NSP followed by CSR_{18}, CSR_{19}, and NB_4D_2 with E I value of 62.80. Minimum E.I. value of 38.41 was recorded in six breeds viz. UDHEY-1, UDHEY-2, UDHEY-3, UDHEY-4, UDHEY-6, and CC_1.
6. **Malformed cocoon percentage:** Among twenty breeds, maximum E.I. value of 67.14 was obtained in breed PO_1 followed by SH_6, (63.26) and minimum E.I. value of 30.81 was recorded in breed UDHEY-6.
7. **Pupation rate percentage:** Among breeds, maximum E.I. value of 70.40 was recorded in ND_5 followed by ND_3 and NSP with E.I. value 63.05. Breeds UDHEY-6 and NB_4D_2 recorded lowest E.I. value of 33.63 for pupation character.
8. **Single cocoon weight (g):** For single cocoon weight maximum E.I. value of 70.22 was observed in UDHEY-3 followed by ND_3 with E.I. value of (62.00) whereas lowest E.I. value of (28.55) was observed in breed UDHEY-6.
9. **Single shell weight (g):** Out of twenty breeds, maximum E.I. value of 64.00 was recorded in PO_3, ND_3 and ND_5 followed by PO_1, SPO, ND_2, and UDHEY-3 (59.00) whereas breed NB_4D_2 recorded lowest E.I. value of 29.00.

10. Silk percent: Breeds, UDHEY-6 and CC_1 scored maximum E.I. value of 63.59 followed by UDHEY-7 at 60.11 whereas the breed SH_6 scored minimum E.I. value of (24.83).

Identification and evaluation of superior breeds

The important commercial traits of silkworm on which silk industry sustains are fecundity, hatching, larva weight 5th day, V^{th} instar duration, total larval period, percentage of malformed cocoons, pupation rate, single cocoon weight, single shell weight and silk percent. For assessing superior breeds on cumulative basis Evaluation Index method was adopted and results are presented in Table 12.

Among studied breeds, the cumulative E.I. value for V^{th} instar and total larval life was maximum in NSP with EI value of 67.40 followed by $CSR_{18,}$ CSR_{19} and NB_4D_2 at 61.30. Breed PO_1, ND_2, ND_3, ND_5 and SH_6 recorded cumulative EI values of 55.20 whereas breeds UDHEY-1, UDHEY-2, UDHEY-3, UDHEY-4 and UDHEY-6 recorded minimum E.I. value of 39.30 (Table 4.15). Among twenty breeds, the cumulative EI value for eight commercial parameters recorded maximum EI value of 58.48 by ND_3 followed by ND_5 (58.37) and CSR_{19} (56.61) whereas breed NB_4D_2 scored minimum value of (38.17) (Table 11).

Table 11: Evaluation Index values of bivoltine silkworm breeds for larval life characters

Breeds	V^{th} instar duration (days)	Total larval duration (days)	Cumulative EI values	Rank
PO_1	59.80	50.60	55.20	III
PO_3	40.19	50.60	45.39	V
SPO	40.19	50.60	45.39	V
ND_2	59.80	50.60	55.20	III
ND_3	59.80	50.60	55.20	III
ND_5	59.80	50.60	55.20	III
NSP	59.80	75.00	67.40	I
UDHEY-1	40.19	38.41	39.30	VI
UDHEY-2	40.19	38.41	39.30	VI
UDHEY-3	40.19	38.41	39.30	VI
UDHEY-4	40.19	38.41	39.30	VI
UDHEY-5	40.19	50.60	45.39	V
UDHEY-6	40.19	38.41	39.30	VI
UDHEY-7	40.19	50.60	45.39	V
UDHEY-8	40.19	50.60	45.39	V
CC_1	59.80	38.41	49.10	IV
CSR_{18}	59.80	62.80	61.30	II
CSR_{19}	59.80	62.80	61.30	II
NB_4D_2	59.80	62.80	61.30	II
SH_6	59.80	50.60	55.20	III

Table 12: Evaluation Index values of bivoltine silkworm breeds for commercial characters

Breeds	Fecundity (no.)	Hatching (%)	Larva weight 5th day (g)	Malformed cocoon (%)	Pupation (%)	Single cocoon weight (g)	Single shell weight (g)	Silk per cent	Cumulative EI of eight character	Rank
PO_1	54.83	55.38	63.63	67.14	40.99	59.88	59.00	49.55	56.30	IV
PO_3	39.61	51.10	63.63	57.75	50.02	61.88	64.00	53.59	55.19	VII
SPO	48.69	71.10	54.54	47.34	55.69	56.22	59.00	54.04	55.82	V
ND_2	44.42	49.76	63.63	50.40	55.69	61.11	59.00	48.08	54.01	IX
ND_3	56.70	51.17	63.63	53.87	63.05	62.00	64.00	53.48	58.48	I
ND_5	54.29	55.11	54.54	52.04	70.40	57.55	64.00	59.10	58.37	II
NSP	52.42	49.19	54.54	47.75	63.05	51.11	54.00	53.82	53.23	X
UDHEY-1	60.17	45.81	36.36	42.24	48.34	31.33	34.00	51.57	39.18	XVIII
UDHEY-2	43.35	66.42	45.45	45.51	40.99	45.22	46.50	51.01	48.05	XIII
UDHEY-3	60.17	55.78	63.63	42.65	55.69	70.22	59.00	37.64	55.59	VI
UDHEY-4	49.75	35.01	45.45	44.69	44.66	39.33	44.00	55.39	44.78	XV
UDHEY-5	38.81	55.01	54.54	31.63	40.99	51.55	49.00	46.40	45.99	XIV
UDHEY-6	46.28	32.00	36.36	30.81	33.63	28.55	39.00	63.59	38.77	XIX
UDHEY-7	43.35	47.95	45.45	45.91	59.37	46.88	54.00	60.11	49.87	XI
UDHEY-8	42.28	41.17	45.45	41.02	55.69	44.66	35.00	35.28	42.56	XVII
CC_1	41.21	39.36	36.36	61.63	44.66	43.88	54.00	63.59	48.08	XII
CSR_{18}	58.30	50.46	54.54	56.53	48.34	50.22	54.00	54.94	53.41	VIII
CSR_{19}	77.52	63.84	54.54	59.59	50.02	49.55	49.00	48.87	56.61	III
NB_4D_2	34.00	40.96	36.36	58.36	33.63	37.55	29.00	35.50	38.17	XX
SH_6	53.76	43.91	36.36	63.26	40.99	52.33	34.00	24.83	43.67	XVI

Linear Pearson Correlation analysis between parameters

Phenotypic data of ten parameters was analyzed by Pearson correlation coefficients (Table 13). Highly significant and positive correlations were observed between hatching percentage and 5th day larval weight (0.545). Single cocoon and shell weight with hatching percentage (0.551) and (0.492) respectively. Pupation rate with 5th day larval weight (0.524), single cocoon weight with 5th day larval weight (0.864). Single cocoon weight with 5th day larval weight (0.816).Total larval duration with Vth instar duration (0.559). Malformed cocoon percentages with duration of Vth instar (days) (0.730). Single cocoon weight with pupation rate percentage (0.536), single shell weight with pupation rate percentage (0.624). Single shell weight with Single cocoon weight (0.775). Silk percentage with single shell weight (0.470).

Fecundity: While studying the correlations among ten economic parameters fecundity parameter showed positive correlation (P<0.01) while as with silk per cent the correlation was negative and non-significant.

Hatching percentage: Fifth day larva weight (0.545) had significant positive correlation with single cocoon weight (0.551) and shell weight (0.492).

Duration of Vth Instar: This character correlated positively with total larval duration (0.559), and malformed cocoon percentage (0.730).

Total larval duration: The total larval duration parameter was non-significant.

Matured larval weight: Mature larval weight had significant and positive correlation with pupation rate (0.524), single cocoon (0.865), and single shell weight (0.816).

Malformed cocoon percentage: This character was also non- significant and had no correlation with any parameters.

Pupation rate percentage: Pupation rate depicted significant correlation with single cocoon (0.536) and single shell weight (0.624).

Single cocoon weight: Single cocoon weight was positively correlated with single shell weight (0.775).

Single shell weight: Single shell weight had significant correlation with silk percentage (0.470; P<0.01).

Table 13: Linear Pearson Correlation analysis on morphological traits

Character	Fecundity	Hatching	5th day larva weight	Vth instar Duration	Total larval duration	Malformed cocoon	Pupation rate	Single cocoon weight	Single shell weight	Silk per cent
Fecundity										
Hatching	0.326									
5th day larva weight	0.258	**0.545***								
Vth instar Duration	0.282	-0.011	0.134							
Total larval duration	0.151	0.187	0.253	**0.559***						
Malformed cocoon	0.221	0.135	0.149	**0.730***	0.337					
Pupation rate	0.303	0.326	**0.524***	0.132	0.268	0.017				
Single cocoon weight	0.193	**0.551***	**0.864***	0.235	0.184	0.336	**0.536***			
Single shell weight	0.160	**0.492***	**0.816***	0.167	0.079	0.212	**0.624***	**0.775***		
Silk per cent	-0.009	-0.018	0.058	-0.086	-0.165	-0.160	0.208	-0.193	**0.470***	

*** Correlation significant at p < 0.01 level.**

Morphological characters are predominant descriptors for assessment of breeds for distinctiveness, uniformity and stability; but are always influenced by prevailing environment. In present study, the morphological characters recorded wide phenotypic variation in Chorion colour, Colour of newly hatched larvae, Larval marking, Cocoon colour, Cocoon shape, Pupa colour and moth colour except egg and pupa shape. The observations recorded are in line with earlier workers, Basavaraja *et al*. (2005); Ram *et al*. (2006) and Sajgotra *et al*. (2016) and two patterns of larval marking, plain and crescent were observed. Plain larvae did not have any markings, whereas crescent shaped marking had different characteristic spots. In literature, different type of larval markings have been reported, these include zebra, speckled, quail, multistar and others (Tazima 1978). Larval marking is useful variable as it helps to prevent the mixing of larvae of different strains. Cocoon shape is an extremely relevant variable in commercialization since automated wiring admits elliptical cocoons only into the machines (Basavaraja et al. 2005). However, the breeds evaluated in the present study were oval, elongated with faint constriction and elongated constricted. All strains produced white cocoons that varied in brightness indicative of qualitative distinction. The egg laying potential of *Bombyx mori* L. has been noticed to be a heritable character expressed within the genotypic limitations and the results revealed maximum fecundity in breed CSR_{19} followed by UDHEY-3. The superior fecundity indicates their genetic constitution. Hatching percentage is an important component reflecting variability of the eggs and higher hatching percentage in breed SPO indicates the genetic and physiological state of the female moth. The results fall in line with results of earlier workers Ram *et al*., (2003 and 2006). Larval duration is an important attribute of economic value and shorter larval duration minimizes the quantum of total food consumption and labour requirement. In the present study, the total larval duration was longer in higher metric trait breeds in comparison to UDHEY breeds. The results are in accordance with Ram *et al*. (2003, 2006). Daniel *et al*. (2015) reported that rate of development depends on both genetic and environmental factors. Larval weight is one of the important parameters which determine not only the health of the larvae but also the quality of cocoons spun. Non-significant variation in larval weight of studied breeds was recorded. The present findings confirm the observation made by Masarat *et al*. (2014). Cocoon weight, shell ratio and filament length are highly heritable traits determining the quality, quantity and efficiency of the reeling. The observations made for five important parameters of cocoon stage revealed significant variations among breeds. Higher cocoon weight was recorded in breeds UDHEY-3, ND3, and PO3 indicating clear difference in maximum nutrient utilization by the breeds in 5^{th} instar. The present findings are in agreement with Masarat *et al*. (2014). Present findings are also in conformity with the report of Singh *et al*. (2010) who concluded that environmental factors influence the physiology of the insect and also have deleterious effect on the economic traits. Cocoon shell weight is an important character in determining the silk content. Present study results are in conformity with the findings of Basavaraja *et al*. (1998) and Sajgotra *et al*. (2017) who have reported that cocoon shell weight shows variability under varying environmental conditions. The variations in the present finding in the shell weight and silk percent may also be due to racial character. To establish the superiority among the breeds several breeders (Ramesh Babu *et al*. 2005b, Vidyumala *et al*. 1998, Lakshmi 2008) followed Multiple Trait Evaluation Index method where in each character is given equal weightage. The degree of association or relationship between two variables is measured by correlation coefficient. In present study, the correlation coefficient is highly significant and positive correlations were recorded between single cocoon weight and 5^{th} day larva weight (0.864) followed by single shell weight and 5^{th} day larval weight (0.816), single shell weight and single cocoon weight (0.775), malformed cocoon and 5^{th} instar duration (0.730), single shell weight and pupation rate (0.624), total larval duration and 5^{th} instar duration (0.559), single cocoon weight and hatching percentage (0.551), larval weight 5^{th} day and hatching percentage (0.545), single cocoon weight and pupation rate (0.536), pupation rate and larval weight 5^{th} day (0.524), single shell weight and hatching percentage (0492) and silk percent and single shell weight (0.470). These findings are in accordance with the results of Chatterjee *et al.* (1993 a), Kamili (1996), Kumaresan *et al.* (2000); Sonwalkar (2001); Chanda *et al*. (2013) and Chandrakanth *et al*. (2016).

CONCLUSION

On the basis of highest cumulative evaluation values (> 50) for ten economic commercial characters in ten breeds; ND_3, ND_5, CSR_{19}, PO_1, SPO, UDHEY-3, PO_3, ND_2, CSR_{18} and NSP were short listed. These breeds scored higher cumulative evaluation values (> 50) and have been identified as promising breeds and are recommended for further breeding material to boost bivoltine silk production.

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