OPEN ACCESS JOURNALS
Indiana Journal of Agriculture and Life Sciences
Abbriviate Tittle- Ind J Agri Life Sci
ISSN *(Online)*- 2583-1623
Journal Homepage Link- https://indianapublications.com/journal/IJALS
DOI- https://doi.org/10.5281/zenodo.14940170

INDIANA PUBLICATIONS
PRODUCTIVE AND QUALITY RESEARCH

Research Article

Volume-05|Issue-01|2025

Estimation of evaluation index and heritability for some economic parameters in the popular bivoltine hybrids of silkworm *Bombyx Mori* L.

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Article History
Received: 23.01.2025
Accepted: 24.02.2025
Published: 27.02.2025

Citation
Abhilash, H. K., Nayak, P. M., & Subramanya, G. (2025). Estimation of evaluation index and heritability for some economic parameters in the popular bivoltine hybrids of silkworm *Bombyx Mori* L. *Indiana Journal of Agriculture and Life Sciences, 5*(1), 18-33.

Abstract: Sericulture is an agro-based rural oriented industry harnessed and nurtured by several sericulturiest throughout the world. The Double hybrids concept in silkworm was introduced by Japanese breeders during 2008-09. The popular bivoltine foundation crosses, single hybrids and double hybrids FC1, FC2 and FC1 × FC2, FC2 × FC1 were reared by the sericulturist throughout the country in different seasons, but the rearers observed differential performance of these hybrids throughout India in different seasons. Hence, the popular single and double hybrids were selected in order to assess the performance in a uniform rearing condition. The analysis to identify the superior hybrids based on their performance through analysis of economic parameters, evaluation index, narrow sense heritability (h2) for selected quantitative traits and protein estimation as well as degumming loss the present work executed and results are interpreted. Thus, the investigation disseminates double hybrid performed well over single hybrid with respect to evaluation index of economic traits and uniform expression with respect to narrow sense heritability. Along with expression of protein concentration and low degumming loss showed more in silk filament of double hybrids.

Keywords: Foundation cross, Evaluation index, Narrow sense heritability, single hybrid, double hybrid.

INTRODUCTION

The domesticated silkworm, *Bombyx mori* L., is a lepidopteran insect known for the production of silk "queen of natural fibres" and extensive genetic studies have been carried out utilizing this insect (Doira *et al.*, 1978). Sericulture originated from China and spread mainly in temperate regions of the world where exclusively the bivoltine silkworms are reared. However, in the course of time, it spread to more than fifty countries in four continents (Lin *et al.*, 1994).

India, being world leader in tropical sericulture is gifted with salubrious tropical climatic condition (Datta, 1997). Hence the silkworm rearing and mulberry cultivation is practiced throughout the year. India largest producer and consumer of silk, second in global silk production by producing 36,582 MT of silk (CSB Annual report, 2022-23) has the unique distinction of producing all four major types of silk namely mulberry, tasar, muga and eri. Among these mulberry silk contributes bulk production (27.654 MT of silk).

In India, indigenous polyvoltine breeds like Pure Mysore, C. nichi in south India, Chotapolu, Borapolu, Nistari, Nistid, Nismo, Itan and Ichatin in West Bengal, Sarupat and Moria in the north east, Kashmiri race in Jammu and Kashmir were reared and multiplied at field level for the production of silk during 1950- 60 (Ghosh, 1949) and many of them being reared even today. Bivoltine rearing was restricted only to maintenance and multiplication of exotic races such as J112, J122, C108, CPP1, CC1 and CA2 up to 1950. As a result there is a rich collection of multivoltine and bivoltine silkworm races / breeds in several germplasm stations of our country. The first trial of rearing of silkworm hybrids was attempted in 1923 in Karnataka by crossing the multivoltine Pure Mysore race with exotic bivoltine Chinese/Japanese races and it was commercially exploited in 1925 (Anonymous).

A critical analysis of Indian sericulture reveals that majority of the silk in India is contributed by multi × bi hybrids (Himanthraj *et al.*, 1996; Roy *et al.*, 1997; Rao *et al.*,2001; Dandin *et al.*, 2003 and Roy, 2011), where females of multivoltine breeds / races of silkworms are crossed to males of bivoltine breeds/races. It is important that in south Indian states the practice of utilizing multivoltine Pure Mysore race as one of the female parent for the preparation of hybrid layings dominated the sericulture industry.

From 2000 onwards numerous productive CSR breeds from CSR&TI, Mysore, KSO_1 and NP series from KSSR&DI, Pam101 and Pam111 from CSR&TI Pampore, APS series by APSSR&DI, thermo tolerant breeds like CSR18 and CSR19, (Rao *et al.*, 2001), AHT, BHT, FHT, GHT (Kumar *et*

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al.,2002), sex limited breeds like Nistari (SL), PM (SL), CSR8 and CSR2 (SL) CSR18 and CSR19 were developed (Datta *et al*., 2000; Kumar *et al*.,2004). Since breeds like ATR series, SR2, SR5, B63, B65, CSR46, CSR47, CSR48, CSR50 CSR51 breeds were evolved (Maribashetty & Aftab Ahamed, 2002; Siddiqui *et al.*,2003; Basavaraja *et al.*,2004; Basavaraja *et al.*, 2005 and Dandin *et al.*, 2006). At the same time University of Mysore evolved some hardy bivoltine breeds like MG408 and MU854 (Ramesh *et al.*, 1996).

Hybrid vigor is so powerful that scientists began "double-crossing". Two inbred parents, A and B, are crossed at the same time that two other inbred parents, C and D, are crossed. The resulting hybrids, AB and CD are then crossed together to produce a hybrid with the characteristics of all four parents, ABCD. In addition, the new ABCD variety has two generations of hybrid vigour bred into it. This method of double crossing hybrids was used almost exclusively from 1926 until the 1960s. Compared to single-cross hybrid production, production of double-cross requires an extra step. During the early history of the hybrid seed industry in the United States, this extra step was necessary because the inbreeds available at that time produced so little grain that making commercial quantities of seed of single-cross hybrids was difficult. Even though the inbreeds of each pair of a double-cross hybrid were related, the resulting single-cross hybrids exhibited sufficient vigour to allow those single crosses to be used successfully as parents in mass production of commercial seed. As breeders gradually improved the performance of inbreeds through selection, it became possible to commercially produce the more desirable single-cross hybrids.

In the three-way crossing type it is difficult to make a choice between (Japanese × Japanese) × Chinese and (Chinese × Chinese) × Japanese hybrids. But considering the silk yarn quality the latter has been found to be better. The double crossing type (Japanese × Chinese) × (Japanese × Chinese) produces non uniform cocoons with variable cocoon shapes and filament length, as compared to the (Japanese × Japanese) × (Chinese × Chinese) type (Yokoyama, 1973). The crossing effect is expressed in different ways in case of different crossing order according to the quantitative characters, especially cocoon shell weight in (Japanese × Japanese) × (Chinese × Chinese) crosses. The crossing effect has been found to be highest when the parental strains are crossed in the order of (1 × 2) × (3 × 4), with 4 > 3> 2 > I for cocoon shell weight. Presently, improved silkworm strains which yield longer silk filament tend to lay fewer eggs (Ohi *et al*., 1970). The usage of double cross hybrids is to an extent of more than 40% in Japan, while such a use is yet to gain initiative in India. A model has been proposed by Minagawa and Ohtsuka (1975), which can predict the performance of three-way and double cross hybrids from the performance of single cross hybrids of the constituent lines.

Breeders in Japan and Thailand have demonstrated the superiority of single, three way and double hybrids over their parental races (Harada, 1961; Pannengpet and Jaroonchai, 1975). By hybridization of two closely valued inbred lines, the egg productivity in the F_1 can be increased and the double hybrid resulting from further crossing of these F1's, facilitate a gain in egg productivity without reducing the cocoon shell percentage (Gamo, 1976).

Realizing the need for productive hybrids with high silk content and quality, breeding experiment were initiated at Central Sericultural Research and Training Institute, Mysore; consequentially, quite a good number of productive bivoltine breeds (CSR2, CSR4, CSR5, CSR6, CSR16, CSR17, CSR21, CSR26, CSR27 etc.) were evolved by using Japanese commercial hybrids as breeding resource materials at Central Sericultural Research and Training Institute, Mysore. Among them one double hybrid including the four bivoltine parents were popularized at field level. Since double hybrid is derived from two heterozygous F1 hybrids their performance at field level bound to differ. In other to understand their performance under uniform laboratory condition and to understand the level of Genetic difference the present research work is being undertaken in the department.

In India, the concept of double hybrid was introduced during 2010 by Central Silk Board authorities after a successful completion of JICA project. The double hybrids which were consider as a high yielding variety is derived by utilizing four parental CSR breeds. In order to produce quality double hybrids the selected seed rearers were identified throughout the country to rare FC1 and FC2 combinations.

A comparative analysis of rearing performance of FC1, FC2, FC1 × FC2 and FC2 × FC1 it is clear from various report throughout the country indicates that among the rearers of Tamil Nadu and Andhra Pradesh double hybrids are popular where in certain regions of Karnataka FC1 and FC2 hybrid combination become popular thus the variable performance of the above four hybrids made the seed rearers to select hybrid of their choice. The rearing report from the seed rearers of Karnataka indicates

***Corresponding Author:** Abhilash H. K

differential performance of the hybrids. Among some selected seed farmers FC1 hybrid exhibited higher cocoon yield, whereas among the other rearers FC2 and double hybrids are superior. In order to understand the performance of these four hybrids in uniform laboratory condition and to adjudicate the best combination the present work undertaken.

MATERIALS AND METHODS

Characteristic Features of The Hybrids

FC1 Hybrid Larvae

FC1 Hybrid Cocoon

Hybrid	Parentage	Voltinism	Larval pattern	Cocoon colour	Cocoon shape	Origin
FC1	CSR2 × CSR27	Bivoltine	Marked	White	Dumb bell	India

FC2 Hybrid Larvae

FC2 Hybrid Cocoon

Hybrid	Parentage	Voltinism	Larval pattern	Cocoon colour	Cocoon shape	Origin
FC2	CSR6 × CSR26	Bivoltine	Plain	White	Oval	India

FC1 × FC2 Hybrid Larvae

FC1 × FC2 Hybrid Cocoon

Hybrid	Parentage	Voltinism	Larval pattern	Cocoon colour	Cocoon shape	Origin
FC1 × FC2	(CSR2 × CSR27) × (CSR6 × CSR26)	Bivoltine	Marked	White	Slightly constricted	India

***Corresponding Author:** Abhilash H. K

FC2 × FC1 Hybrid Larvae

FC2 × FC1 Hybrid Cocoon

Hybrid	Parentage	Voltinism	Larval pattern	Cocoon colour	Cocoon shape	Origin
FC2 × FC1	(CSR6 × CSR26) × (CSR2 × CSR27)	Bivoltine	Marked	White	Slightly constricted	India

Rearing Performance

In the present study breeds *viz*., CSR2, CSR7, CSR6, CSR26, FC1, FC2 and Double hybrid procured from the NSSO grainage, Central Silk Board, Mysore and the disease free layings (DFLs) were incubated and exposed to light on the expected date of hatching. I-III instars were reared at 27-28ºC with 85-90% relative humidity and the late age larvae (IV-V instars) at 24-26ºC with 70-80% humidity. At III instar, 1st day, the base number is fixed for each breed/race by retaining 300 larvae. Each breed/race was maintained by feeding suitable quality mulberry leaves harvested from the irrigated mulberry garden of the Department of Studies in Sericulture Science, Manasagangotri, Mysore. The rearing was conducted by following standard rearing methods (Yokoyama, 1963; Doira, 1978 and Krishnaswami, 1986). During the rearing, the performance of all the breeds/race were analysed by assessing the eight quantitative traits namely, hatching percentage, weight of single fifth instar larva (g), larval duration (h), single cocoon weight (g), single shell weight (g), shell percentage, filament length (m), denier (d) following the standard procedures.

Description of Quantitative Traits

Fecundity: It is the total numbers of eggs laid by a moth.

Hatching Percentage: This character denotes the number of larvae hatched from the disease free laying. The hatching percentage is arrived at, after deducting the unhatched, unfertilized and dead eggs from the total number of eggs laid by a moth.

Weight of single V instar larva (g): This trait reflects the healthiness and robustness of the larva. It is the measure of randomly selected larva weighed one day before spinning.

Single cocoon weight (g): This is the average weight of a cocoon in grams derived by calculating the weight of a random sample of 10 cocoons.

Single shell weight (g): This trait represents the total quantity of silk in a cocoon. It is the average individual shell weight in grams of 10 cocoons selected for assessment of "cocoon weight".

Single pupal weight (g): It is the average individual pupal weight in grams taken from 10 cocoons.

Shell percentage (Shell ratio): It is the ratio between the shell weight and the cocoon weight. It is calculated in percentage from a random sample of 10 cocoons.

$$\text{Shell percentage} = \frac{\text{Shell weight}}{\text{Cocoon weight}} x\ 100$$

Filament length (m): It is the length of the silk filament in meters reeled from a single cocoon. The mean value of the filament length is obtained by reeling 10 cocoons.

Filament weight (g): It is the average weight of non-breakable filament length reeled from 5 cocoons.

Denier (d): It is a measure of the thickness or size of the silk filament in a cocoon , represented as 'd' and calculated by using the following formula,

$$\text{Denier (d)} = \frac{\text{Weight of the reeled silk}}{\text{Length of the reeled silk}} x\ 9000$$

Where, 9000 is the length of the fibre in meters used as standard, represented in gram units.

Renditta: It is the number of kilograms of cocoons required to produce one kilogram of raw silk.

***Corresponding Author:** Abhilash H. K

Narrow Sense Heritability

Ten each of female and male larvae were randomly picked during fifth instar, a day before spinning from the rearing tray. The larval weight was recorded individually and left on mountages separately for spinning and the cocoons were harvested.

The harvested cocoons were analyzed for Cocoon weight, Shell weight, Shell percentage and pupal weight.

Narrow sense heritability is calculated with the following equation using Genstat version 2012.

$$h^2 = \frac{V_A}{V_P} = \frac{V_A}{V_G + V_E} = \frac{V_A}{V_D + V_I + V_E}$$

Where,

h^2 = Narrow sense heritability
V_A = Additive variance
V_P = Phenotypic variance
V_G = Genetic variance
V_E = Environmental variance
V_D = Dominance variance
V_I = Epistatic interaction

Haemolymph Protein Analysis

The haemolymph was extracted from 5th day of V instar larvae by puncturing abdominal legs and the haemolymph was collected in pre-chilled eppendorf tubes containing 1mM thiourea- crystals to prevent melanization.

The protein was estimated through standard procedure suggested by Lowry *et al.* (1951). The sample protein is compared with a known amount of a standard protein using Bovine Serum Albumin (BSA).

Estimation of protein through Lowry's assay

The Lowry's reaction for protein determination is an extension of the biuret procedure. The first step involves formation of a copper protein complex in alkaline solution. This complex then reduces a phosphomolybdic phosphor tungstate reagent to yield an intense blue colour. This assay is much more sensitive than the biuret method but is also more time consuming. The precaution to be observed when performing this assay concerns addition of the Folin's reagent. This reagent is stable only at acidic pH; however, the reduction indicated above, occurs only at pH 10. Therefore, when the Folin's reagent is added to the alkaline copper-protein solution, mixing must occur immediately so that the reduction can occur before the phosphomolybdic- phosphotungstate (Folin's) reagent breaks down. Phenols are capable of reducing molybdenum in a complex of phosphomolybdotungstic acid (Folin-Ciocalteu reagent). The tyrosine residues of protein provide phenolic groups and cupric ions enhance the sensitivity. When treated with Folin-Ciocalteu reagent, proteins produce colour (blue) in varying degrees depending on their tyrosine content. Hence different proteins give different colour values. Generally, the method can be used to determine 20 to 200 µg of protein per millilitre.

Reagents required

Bovine Serum Albumin (BSA) solutions (0.2, 0.4, 0.8, 1.0 mg/ml) Folin-Ciocalteu reagent: dilute 1:1 with distilled water so that its final concentration is 1N. Glass test tubes (12 × 75 mm) Micropipettor.

Reagent A: 1.56 g of $CuSO_4$, 5 H_2O/100 ml of distilled H_2O
Reagent B: 2.37 g of sodium tartrate/100 ml of dstilled H_2O.
Reagent C: 2.0 g of NaOH + 10 g of Na_2 CO_3/500 ml of distilled H_2O
Reagent D: Combine and mix 100 ml of reagent C + 1 ml of reagent
A + 1 ml of reagent B, in this order solution of protein of unknown concentration (between 100 and 2000 µg/ml).

Flow chart of the Experimentation

50 µl samples added
⇩
5ml of protein reagent added
⇩
Incubated at room temperature (10 min)
⇩
0.5 ml Folin-Ciocalteau reagent added
⇩
Incubated at room temperature for 30 min
⇩
OD is taken at 660 nm using spectrophotometer

Equipment: Spectrophotometer

Procedure for protein estimation:

- 1 ml of reagent D was placed in appropriately labelled tubes.
- 100 µl of each sample of BSA was added to a tube containing reagent D, various amounts of protein solution of unknown concentration was added to tubes of reagent D and vortexed immediately. A tube containing no BSA was taken as blank and incubated for 10 minutes at room temperature.
- After the 10 minutes of incubation, 100 µl of Folin-Ciocalteu reagent was added to the samples and vortexed immediately. It was then incubated for 30 minutes at room temperature.
- The samples were read at OD660 on the spectrophotometer using reagent D as blank.
- A curve of the OD660 was plotted (subtract the blank OD660 reading from each OD660 for the BSA solutions) against BSA concentration in milligrams per millilitre. This standard curve was then used to determine the protein concentration of the unknown.

***Corresponding Author:** Abhilash H. K

Evaluation Index (EI)

The evaluation index is defined as aggregate unit of the component traits that determines the silk yield. The pooled data was analysed statistically for eleven quantitative traits by adopting the multiple trait evaluation index (EI) method as described by Mano *et al.*, 1993 & 1994.

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

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

For the three traits such as larval duration, denier and renditta the following formula of Mano *et al.*, (1993) modified by Talebi & Subramanya, (2009) was adopted since lower values are preferred by breeders.

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

The index obtained as described above was estimated for each of the trait analysed. Further, the indices obtained for all the traits were combined to get a single value, the average of which is evaluation index value.

Cumulative Evaluation Index

It is as an important method to understand cumulative performance of a breed/ hybrid by taking averages of the evaluation index

Degumming Loss

Degumming loss following the procedure of Choe *et al.*, (1981) printed by College of Agriculture, Seoul National University, Suwon, South Korea.

Flow chart of the process

Weight of the silk skein (W1 g)

⇩

The silk skein is Immersed in the degumming bath (M: L ratio – 1: 40) containing soap (7g/l) and soda ash (1g/l).

⇩

Oiled the silk skein for 1 hour

⇩

Skein is dipped in hot water for 20 min and then washed in running water

⇩

The silk skein is dried and weighed (W2 g)

The degumming loss percentage is calculated by using the formulae,

$$\text{Degumming loss (\%)} = \frac{W1 - W2}{W1} \times 100$$

Where
W1= Initial weight of the silk skein.
W2= Weight of the degummed silk skein.

RESULTS

The mean values of the eleven economic traits namely fecundity, hatching percentage, larval weight, cocoon weight, shell weight, pupal weight, shell percentage, filament length, filament weight, denier and renditta in all the four hybrids are presented in Table 1 and depicted in figures 1 & 2. Perusal of the data in table 1 for eleven economic traits revealed the following results.

With regard to trait fecundity FC2 hybrid exhibited a highest fecundity of 681.66± 6.56, where as a lowest of 585±5.56 in FC2 × FC1 hybrid. The CD value for the same is 20.25 @ 5% level. For the trait hatching percentage FC2 revealed significant results (96.33±0.94) compare to other hybrids. For the trait larval weight FC2 × FC1 exhibited a highest weight of 4.96±0.035 per single larva compare to other three hybrids. For the traits of cocoon weight, shell weight, pupal weight, shell ratio, filament length and weight FC1 × FC2 revealed highest values which are statistically significant compared to other hybrids. However the table clearly indicates a highest denier of 2.48 in the FC1 × FC2 hybrid. For the trait renditta it is interesting to note that FC1 × FC2 exhibited a lowest renditta of 6.88± 0.226 compared to other three hybrids (Table 1 & Figure 1, 2)

The data pertaining to narrow sense heritability percentage (h^2) for five quantitative traits namely larval weight, cocoon weight, shell weight, pupal weight and shell ratio. The data clearly indicates for the trait larval weight that h^2 percentage value ranges from the lowest of 44.0 % in the FC1 hybrid and a highest of 49.0% in the FC1 × FC2 breed. Similarly for cocoon weight FC1 × FC2 revealed a highest heritability percentage of 39.1%, whereas a lowest of 36.3% is evident in FC2 × FC1. For the trait shell weight FC2 × FC1 revealed a highest heritability value (46.1%) compared to a lowest of 40.0% in FC1 hybrid. For the trait pupal weight the heritability value ranges from a lowest of 38.6% in the FC2 × FC1 hybrid compare to a highest of 43.0% in FC1 hybrid. The heritability percentage for the trait shell percentage revealed a highest of 43.8% in FC2 × FC1 hybrid than those of the other hybrids. The overall picture that emerges out from the narrow sense heritability studies is that all the four hybrids indicated uniform expression of economic traits for all the traits under study indicating the stability of the four hybrids (Table 2 & Figure 3).

The evaluation index and cumulative evaluation index in the four hybrids for eleven economic traits. The overall picture emerges out from the data clearly demonstrates that among the two foundation crosses FC1 revealed 52.43% for only one trait namely hatching % and an cumulative evaluation index value of 40.73%. However, in FC2 for five traits of fecundity, hatching %, shell %, denier and renditta the respective evaluation index value is 64.21%, 63.08%, 55.82%, 51.69 and 52.90. The overall evaluation index value is 51.40 (Table 3).

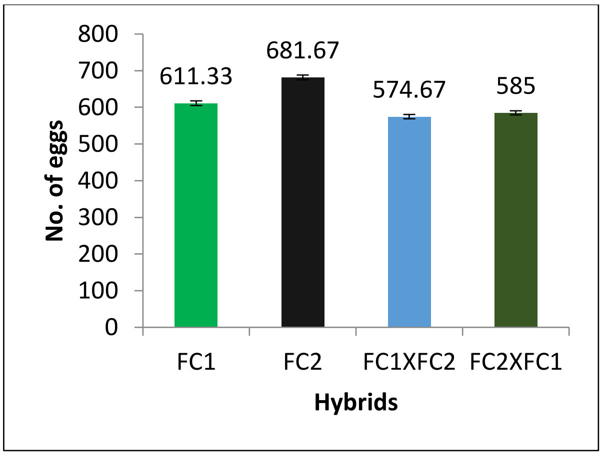
Among the two double hybrids FC1 × FC2 revealed evaluation index of >50 for larval weight (52.75), cocoon weight (58.99), shell weight (60.59), shell % (60.59), pupal weight (58.51), filament weight (59.41), filament length (59), Denier (61.18) and Renditta (51.65). In $FC_2 \times FC_1$, highest evaluation index of >50 was noticed for the traits larval weight (61.30), cocoon weight (57.11), shell weight (54.30), pupal weight (57.70), filament length (57), filament weight (50.24) and Renditta (59.52). The cumulative evaluation index for FC1 × FC2 is 55.05 where as it is 52.81 for FC2 × FC1 hybrids (Table 3 & Figure 4, 5).

The quantitative estimation of proteins in the haemolymph of fifth instar day 5 larva through the standard Lowry's method. Perusal of the data clearly indicates that among the two foundation crosses FC1 revealed 42.66 mg/ml protein compared to 39.86 mg/ml in the FC_2 hybrid. On the other hand, among the two double hybrids FC1 × FC2 recorded a highest protein concentration of 38.14mg/ml compared to 37.58mg/ml in the reciprocal double hybrid. A comparative study of all the four hybrids clearly revealed a highest protein concentration in FC1 hybrid (42.66mg/ml) compared to a lowest of 37.58mg/ml in FC2 × FC1 (Table 4 & Figure 6).

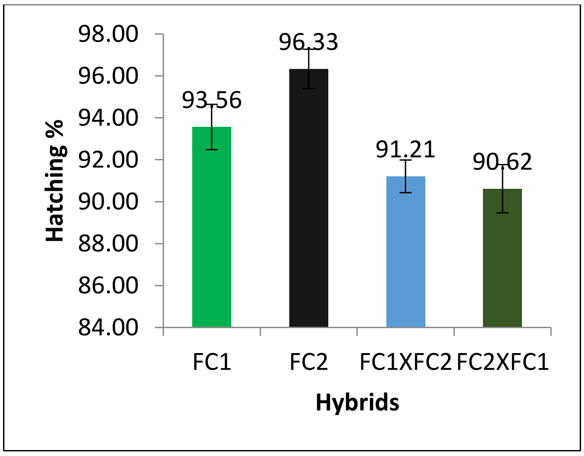
Degumming of the silk is to separate sericin from fibroin without no harm to the chemical, physical and mechanical properties of silk materials. The degumming property of the foundation crosses and double hybrids silk filament was examined and the degumming loss percentage was recorded. The data clearly indicates that the highest degumming loss of 21.86 is observed in the FC1 followed by FC1 × FC2 (21.33), FC2 (20.46) and FC1 × FC2 (20.42). Thus, FC1 × FC2 has exhibited superiority by revealing low degumming loss (Table 5 & Figure 8).

Table 1: Mean values of the eleven quantitative traits in four bivoltine hybrids.

Quantitative Traits / Hybrids	Fecundity (No.)	Hatching (%)	Weight of single V instar larva (g)	Single cocoon weight (g)	Single shell weight (g)	Single pupal weight (g)	Shell percentage (%)	Filament length (m)	Filament weight (g)	Denier (d)	Renditta (kg)
FC1	611.333 ± 6.36	93.563 ± 1.08	4.113 ± 0.213	1.597 ± 0.04	0.334 ± 0.007	1.26 ± 0.046	20.96 ± 0.949	924 ± 7.572	0.23 ± 0.002	2.377 ± 0.003	7.107 ± 0.158
FC2	681.667 ± 6.56	96.333 ± 0.94	4.673 ± 0.118	1.923 ± 0.107	0.41 ± 0.008	1.513 ± 0.1	21.42 ± 0.795	1,045.6 ± 24.74	0.27 ± 0.01	2.44 ± 0.012	7.113 ± 0.127
FC1 × FC2	574.667 ± 5.92	91.21 ± 0.78	4.87 ± 0.119	2.393 ± 0.018	0.518 ± 0.02	1.875 ± 0.021	21.63 ± 0.77	1,284.64 ± 15.07	0.337 ± 0.006	2.48 ± 0.012	6.88 ± 0.226
FC2 × FC1	585 ± 5.56	90.62 ± 1.15	4.96 ± 0.035	2.323 ± 0.076	0.472 ± 0.021	1.851 ± 0.064	20.307 ± 0.671	1,249.67 ± 13.38	0.322 ± 0.003	2.46 ± 0.012	7.22 ± 0.182
CD @ 5%	20.25	1.00	0.453	0.229	0.05	0.213	N/A	54.309	0.021	0.034	N/A
CV @ 5%	1.72	1.87	5.088	5.819	6.058	6.853	6.592	2.522	3.725	0.72	4.334

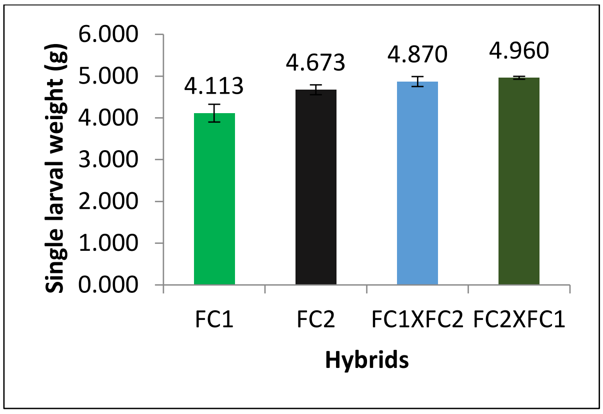


Fecundity (no.)

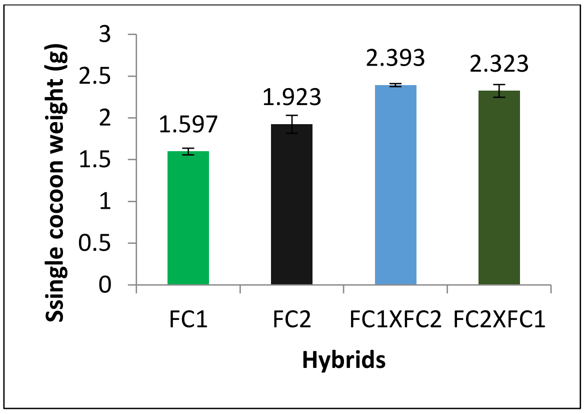


Hatching percentage

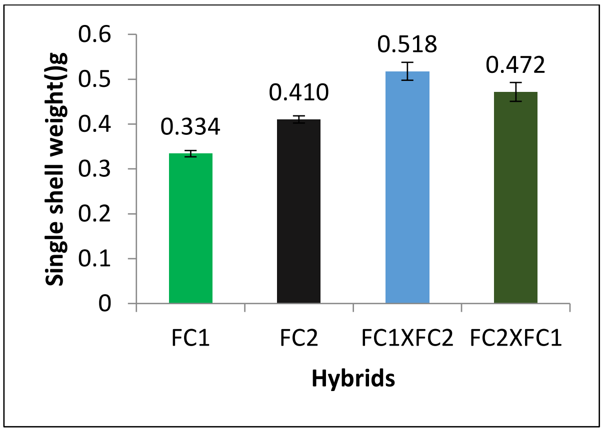
***Corresponding Author:** Abhilash H. K



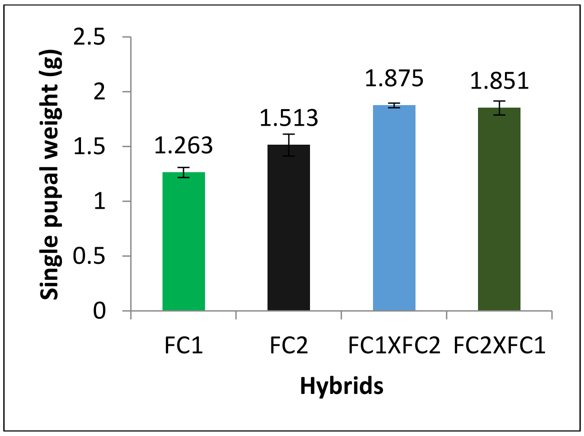
Larval weight (g)



Cocoon weight (g)

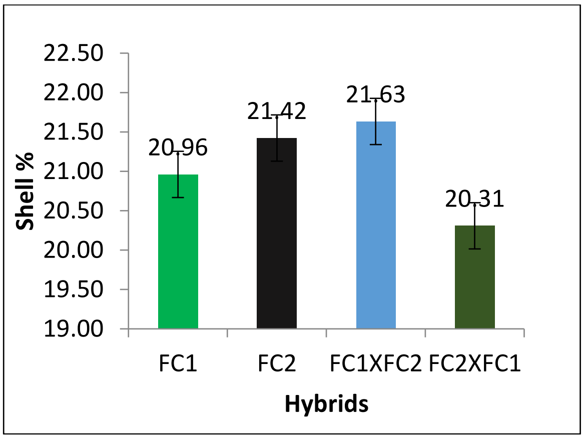


Shell weight (g)

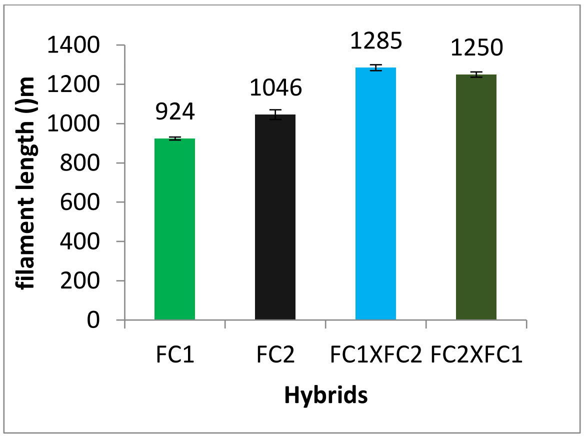


Pupal weight (g)

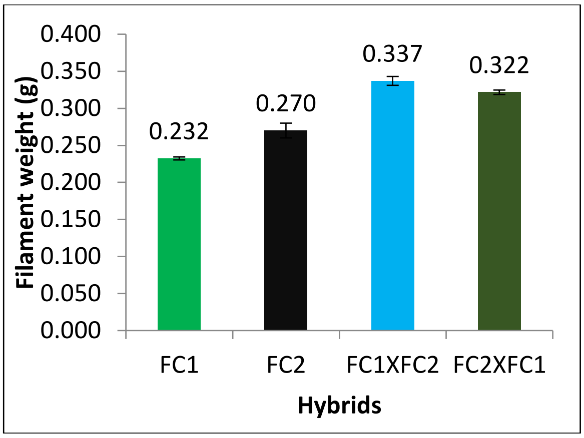
Figure 1. Histogram depicting mean values of fecundity, hatching percentage, larval weight, cocoon weight, shell weight and pupal weight of the four hybrids.



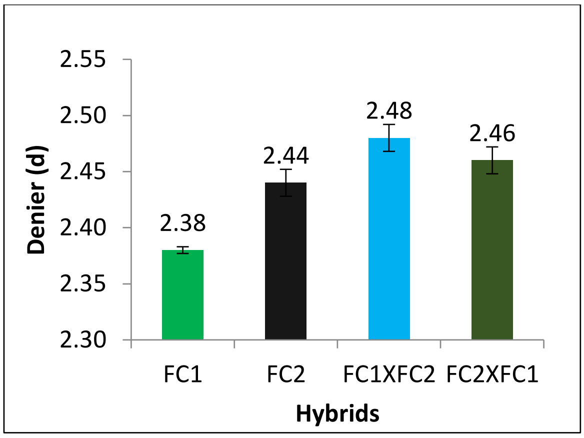
Shell ratio (%)



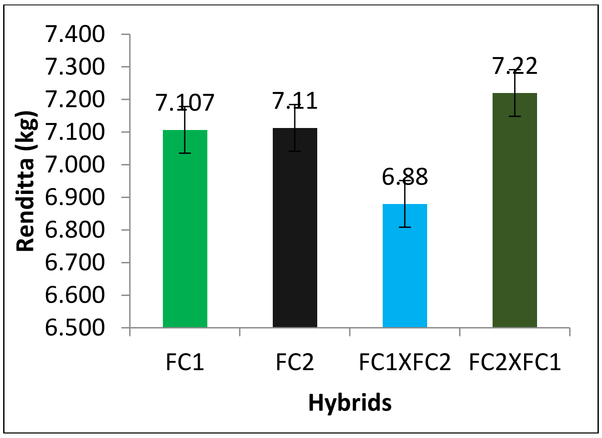
Filament length (m)



Filament weight (g)



Denier (d)

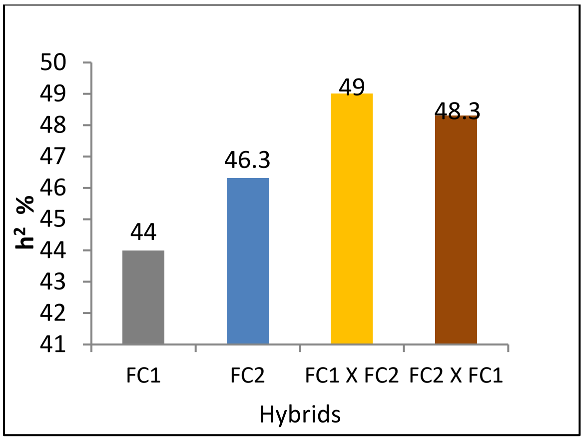


Renditta (kg)

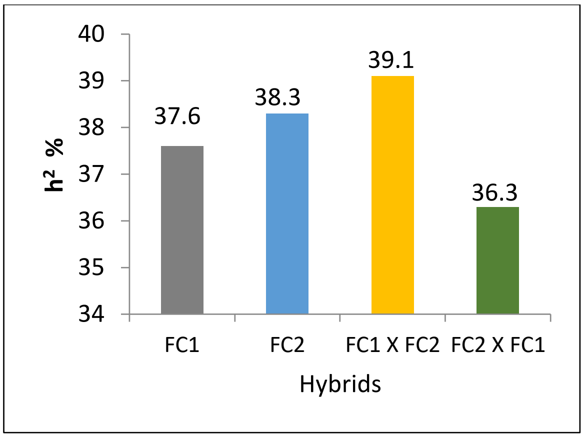
Figure 2. Histogram depicting mean values of shell ratio, filament length, filament weight, denier and renditta of the four hybrids.

Table 2: Narrow sense heritability percentage (h^2) for five quantitative traits of the bivoltine hybrids.

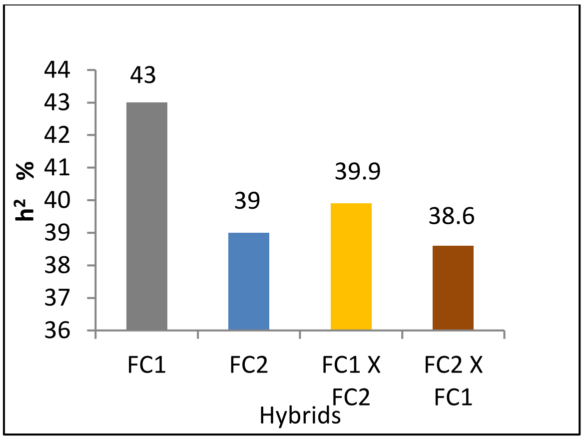
Quantitative traits / Hybrids	Weight of single V instar larva (g)		Single cocoon weight (g)		Single shell weight (g)		Pupal weight(g)		Shell percentage (%)	
	Mean ± SE	h^2 (%)	Mean ± SE	h^2 (%)	Mean ± SE	h^2 (%)	Mean ± SE	h^2 (%)	Mean ± SE	h^2 (%)
FC1	3.519 ± 0.259	44.0	1.61 ± 0.138	37.6	0.339 ± 0.028	40.0	1.275 ± 0.119	43.0	21.06 ± 1.32	42.3
FC2	4.237 ± 0.389	46.3	1.86 ± 0.138	38.3	0.4134 ± 0.017	41.0	1.407 ± 0.142	39.0	22.21 ± 1.12	42.9
FC1 × FC2	4.368 ± 0.381	49.0	2.07 ± 0.196	39.1	0.441 ± 0.023	43.8	1.628 ± 0.184	39.9	21.45 ± 1.73	43.1
FC2 × FC1	4.248 ± 0.386	48.3	1.92 ± 0.234	36.3	0.430 ± 0.033	46.1	1.496 ± 0.215	38.6	22.53 ± 2.09	43.8



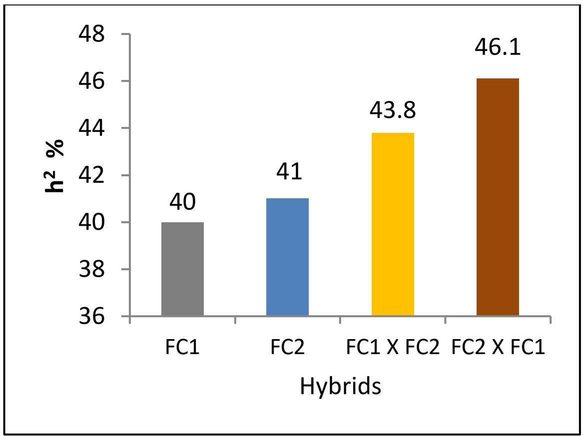
Larval weight



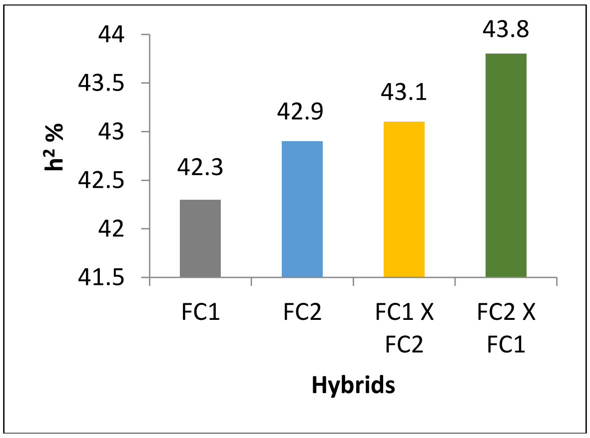
Cocoon weight



Pupal weight



Shell weight



Shell ratio

Figure 3. Histogram depicting heritability percentage of larval weight, cocoon weight, Shell weight, pupal weight and shell ratio of the four hybrids.

Table 3: Evaluation index of eleven quantitative traits of four hybrids.

Quantitative traits / Hybrids	Fecundity	Hatcing %	Larval weight	Cocoon weight	Shell weight	Pupal weight	Shell %	Filament length	Filament weight	Denier	Renditta	Cumulative evaluation index
FC1	49.62	52.43	37.230	37.547	37.500	37.620	47.95	38	37.95	36.89	35.92	40.73
FC2	64.21	63.08	48.718	46.343	47.098	46.156	55.82	45	45.78	51.69	52.90	51.40
FC1 × FC2	42.01	43.37	52.752	58.994	60.593	58.516	59.41	59	59.72	61.18	51.65	55.05
FC2 × FC1	44.15	41.1	61.300	57.116	54.809	57.708	36.83	57	56.53	50.24	59.52	52.81

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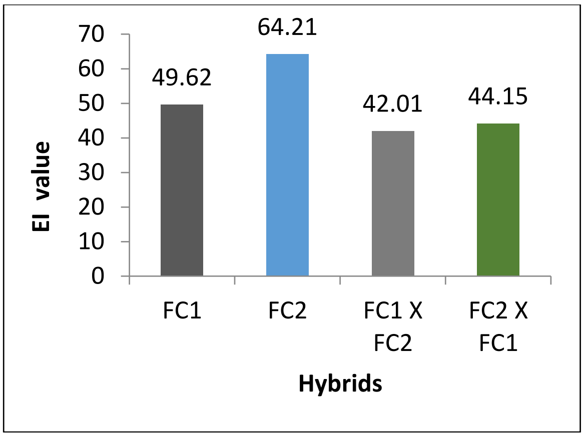
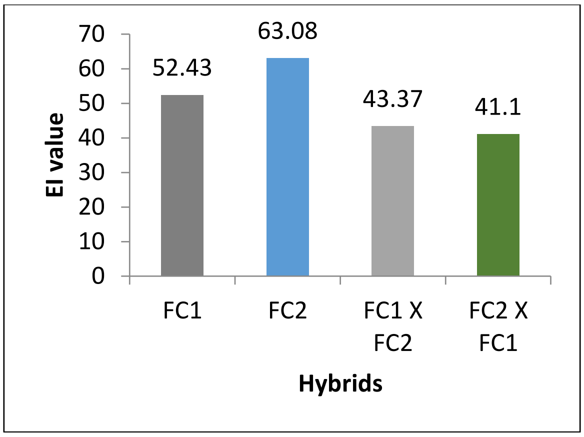
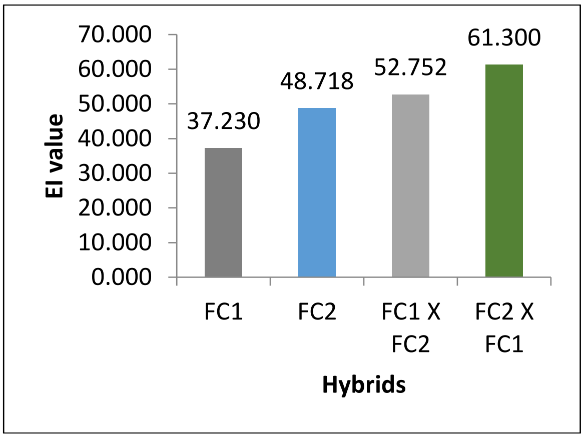


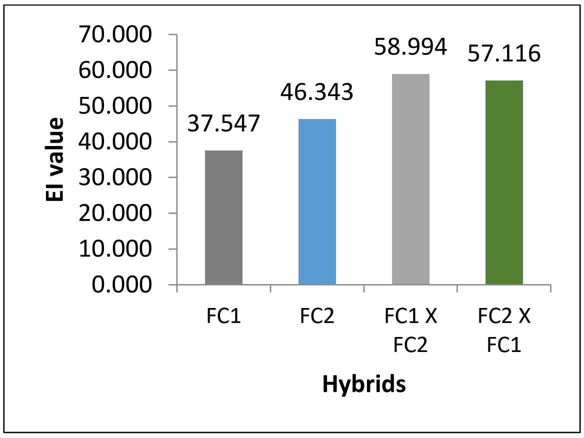
Evaluation index (EI) for fecundity



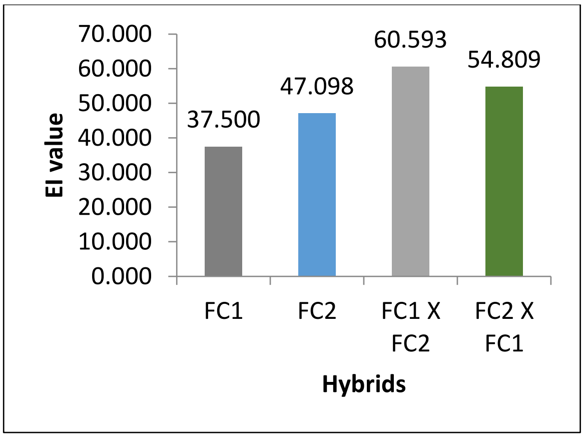
Evaluation index (EI) for Hatching %



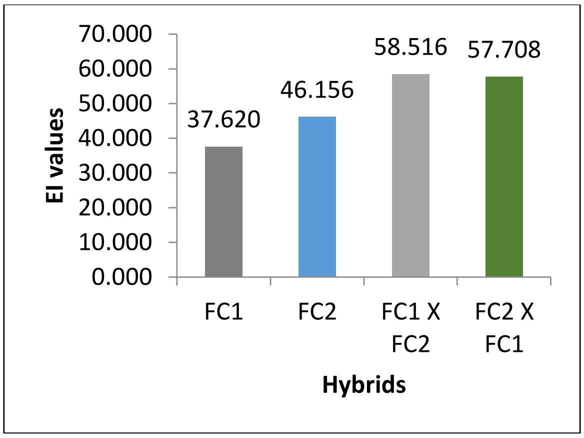
Evaluation index (EI) for larval weight



Evaluation index (EI) for cocoon weight

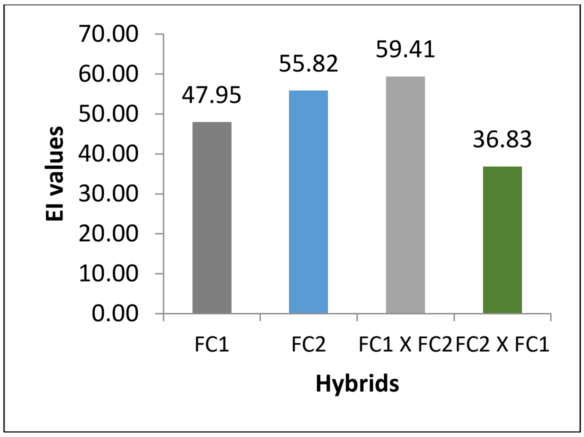


Evaluation index (EI) for shell weight

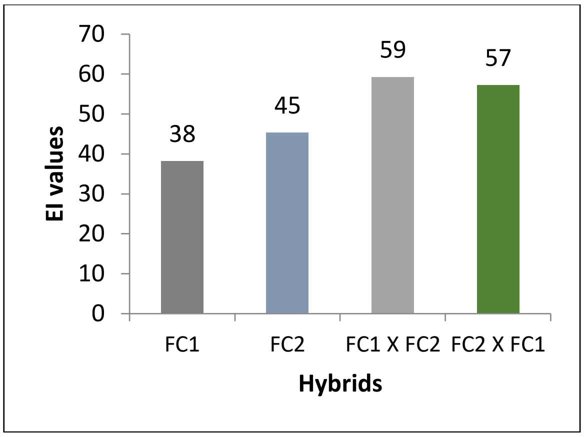


Evaluation index (EI) for pupal weight

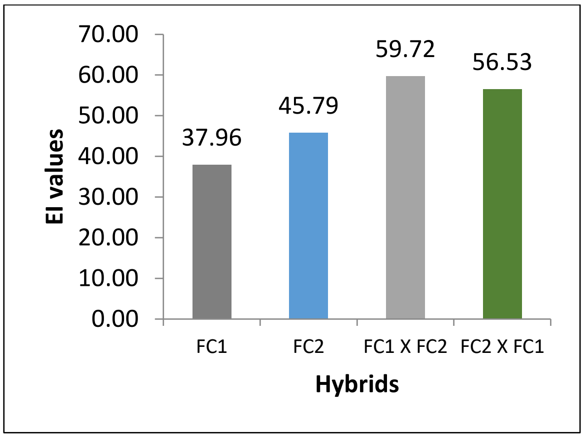
Figure 4. Histogram depicting Evaluation index of Fecundity, hatching %, larval weight, cocoon weight, Shell weight and pupal weight of the four hybrids.



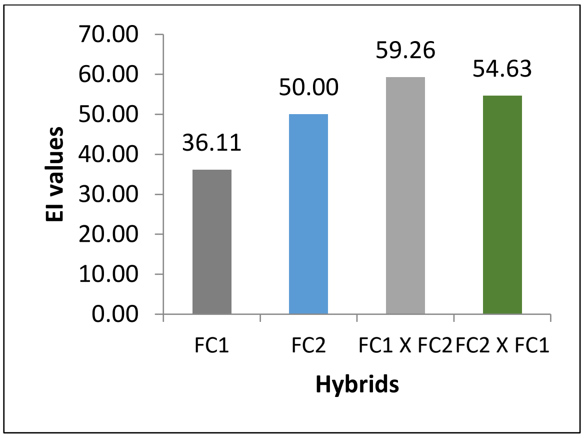
Evaluation index (EI) for shell ratio



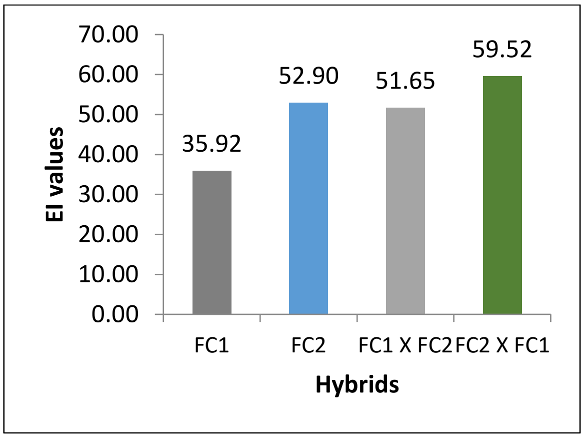
Evaluation index (EI) for filament length



Evaluation index (EI) for filament weight



Evaluation index (EI) for denier



Evaluation index (EI) for renditta

Figure 5. Histogram depicting Evaluation index of shell ratio, filament length, filament weight, Denier and Renditta of the four hybrids.

Table 4: Quantitative estimation of protein in the haemolymph of V instar 5th Day larvae through Lowry's method

Hybrids	Protein concentration (mg/ml)
FC1	42.66±1.37
FC2	39.86±1.29
FC1 × FC2	38.14±1.64
FC2 × FC1	37.58±0.88

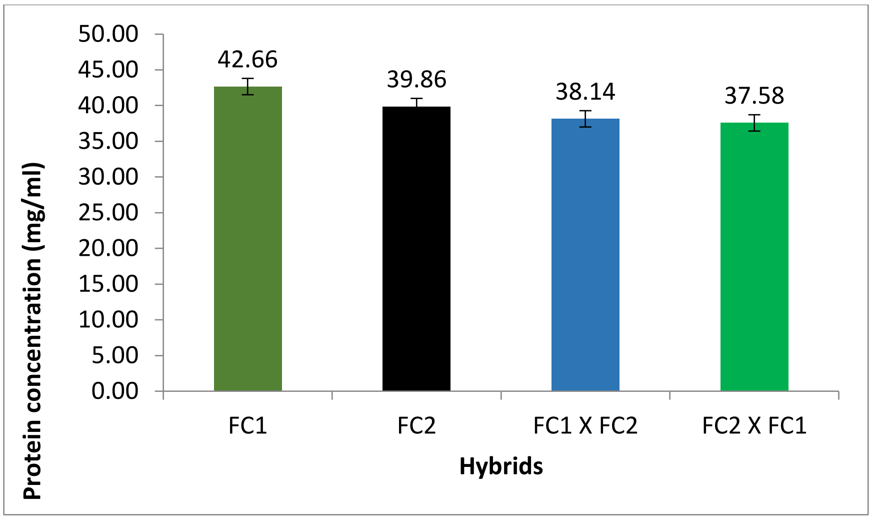


Figure 6. Graph depicting protein concentrations (mg/ml) in haemolymph of V instar 5th day larva of four races.

Table 5: Degumming loss percentage of silk filament.

Hybrids	Degumming loss (%)
FC1	21.86 ± 0.33
FC2	20.46 ± 0.51
FC1 × FC2	20.42 ± 0.45
FC2 × FC1	21.33 ± 0.46

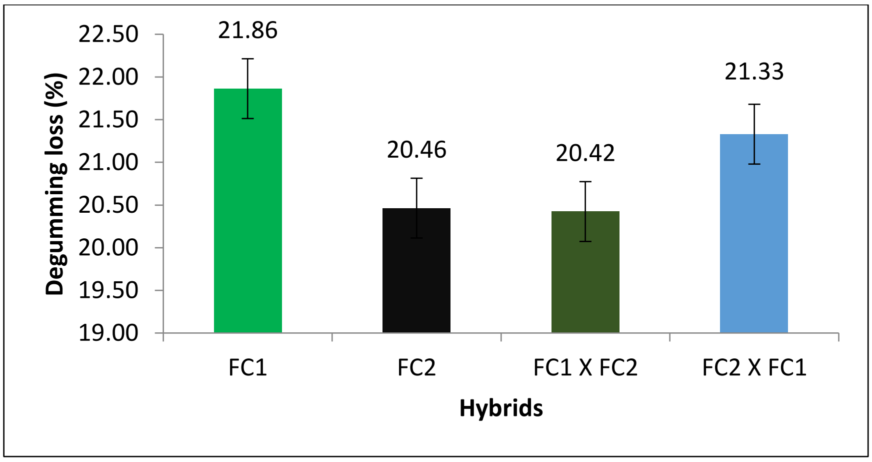


Figure 7. Graph depicting the degumming loss percentage in four hybrids.

DISCUSSION

Sericulture is an agrobased rural oriented industry providing employment to several lakhs of sericulturist in our country. We have observed a great progress in the total cocoon production and also raw silk yield since 1940. Perusal of literature in regard to the rearing of silkworm races/breeds/hybrids, upto1995 crossing of multivoltines Pure Mysore with evolved bivoltine is commonly practiced. Though word bank aided projects and national sericulture projects helped in the evolving of new productive bivoltine breeds, it is evident that crossing pure Mysore with productive bivoltine became popular in south India and crossing of Nistari with selected bivoltine was popular in West Bengal.

In the sericulturally advanced countries, it is observed that in the three-way crossing type it is difficult to make a choice between (Japanese × Japanese) × Chinese and (Chinese × Chinese) × Japanese hybrids. But considering the silk yarn quality the latter has been found to be better. The double crossing type (Japanese × Chinese) × (Japanese × Chinese) produces non uniform cocoons with variable cocoon shapes and filament length, as compared to the (Japanese × Japanese) × (Chinese × Chinese) type (Yokoyama, 1973). The crossing effect is expressed in different ways in case of different crossing order according to the quantitative characters, especially cocoon shell weight in (Japanese × Japanese) × (Chinese × Chinese) crosses. The crossing effect has been found to be highest when the parental strains are crossed in the order of (1 × 2) × (3 × 4), with 4 > 3> 2 >1 for cocoon shell weight. Presently, improved silkworm strains which yield longer silk filament tend to lay fewer eggs (Ohi *et al*., 1970). The usage of double cross hybrids is to an extent of more than 40% in Japan, while such a use is yet to gain initiative in India. A model has been proposed by Minagawa and Ohtsuka (1975), which can predict the performance of three-way and double cross hybrids from the performance of single cross hybrids of the constituent lines.

Breeders in Japan and Thailand have demonstrated the superiority of single, three way and double hybrids over their parental races (Harada, 1961; Pannengpet and Jaroonchai, 1975). By hybridization of two closely valued inbred lines, the egg productivity in the F1 can be increased and the double hybrid resulting from further crossing of these F1s, facilitate a gain in egg productivity without reducing the cocoon shell percentage (Gamo, 1976).

From 2000 onwards numerous productive CSR breeds from CSR&TI, Mysore, KSO_1 and NP series from KSSR&DI, Pam_{101} and Pam_{111} from CSR&TI, Pampore, APS series by APSSR&DI, thermo tolerant breeds like CSR_{18} and CSR_{19} (Rao *et al*., 2001), AHT, BHT, FHT, GHT, sex limited breeds like Nistari (SL), PM (SL), CSR8 and CSR2 (SL) CSR18 and CSR19 were developed (Datta *et al*., 2000; Kumar *et al*., 2004). Since breeds like ATR series, SR2, SR5, B63, B65, CSR46, CSR47, CSR48, CSR50 CSR51 breeds were evolved (Maribashetty & Aftab Ahamed, 2002; Siddiqui *et al.,*2003; Suresh Kumar *et al.,* 2004; Kalpana *et al.,* 2005 and Dandin *et al.,* 2006). At the same time University of Mysore evolved some hardy bivoltine breeds like MG408 and MU854 (Ramesh *et al.,* 1996).

From 2010 onwards the concept of double hybrids and rearing them among the Indian farmers was popularized through JICA project. Several foundation crosses were derived by selecting CSR breeds and by crossing foundation crosses many double hybrids are introduced. Among the many hybrids four hybrid combinations became so popular namely FC1, FC2, FC1 × FC2 and FC2 × FC1 (Figure 1). But the differential performance of these four hybrids is very common in sericulturally practicing states. In Jammu and Kashmir the laboratory rearing indicated superior performance of FC1 × FC2 hybrid (Nirmal singh *et al.,* 2016). In a detailed study, Dayananda *et al.,* 2015 revealed superior performance of FC2 than FC1. So in the light of the above the present project work is undertaken to adjudicate the superior among the above four hybrids by rearing them under uniform laboratory conditions and through biochemical approaches and correlation studies.

For almost one decade CSR hybrids were most popular among the rearers in addition to multi-bi hybrids. Heritability in the narrow genetic sense is defined by lush as the ratio of genic variance to the total variance and this method is of great importance to breed improvement. It primarily indicates to what extent genetic transmission of performance differences between animals can be expected and therefore, how large the progress by selection will be. A distinction is usually made between heritability in the broad and narrow sense, reflecting the components of variation. Narrow sense heritability is used exclusively by silkworm breeders. It is defined as the ratio of additive genetic variance to total phenotypic variance.

The data in table 2, with regard to the narrow sense heritability indicated uniform expression of the economic traits and their studies such a study utilizing bivoltine and multivoltuine races for breeds is well documented (Murthy, 2007; Thalebi, 2010; Jamuna, 2012) based on the results in studies on heritability FC1 × FC2 exhibited highest narrow sense heritability and it is possible to conclude that this hybrid is most suitable compare to others.

Utilization of evaluation index method is one of the most popular method proposed by Mano *et al.,* (1993) developed by taking advantage of “variation index value method” which is being used in japan education system to determine student’s merit. The evaluation index may be identified as an index of multiple traits or as a performance of a population. Selection based on the multiple traits is useful in judging the superiority of silkworms impartially and to evaluate desirable silkworm hybrids (Narayanaswamy *et al.,* 1991). Therefore it becomes obvious to devise the selection index so that it can be used to maximize the genetic importance of multiple traits simultaneously. The selected traits may vary from breeder to breeder. The indices could be obtained for each of the selected trait such as larval weight, cocoon weight, shell weight, pupal weight, and shell percentage. Further, the indices obtained for all the traits are combined to get a single value, which is actually Evaluation Index. The average index value fixed for the selection of a combinations is >50. The character showed relatively higher value of 50 only is considered to have greater economic traits, so that selection index used to maximize the genetic importance of multiple traits simultaneously (Table 3). Such, an attempt was also made on silkworms by Bhargava *et al.,* (1992); Nirmal kumar, (1995); Ramesh (1996); Talebi and Subramanya (2009) using Evaluation Index. Based on the index value unit of 50, FC1 × FC2 (9 traits), FC2 × FC1 (8 traits), FC2 (5 traits) and FC1 (1 traits) scored >50 index for this individual traits and therefore by Evaluation index and cumulative evaluation index it is concluded that FC1 × FC2 is better performer compared to other three hybrids.

The data pertaining to degumming loss which is presented in table 5 clearly shows that the percentage of degumming loss is different in four hybrids. Utilization of degumming loss to adjudicate superiority of breeds/hybrids are well documented (Huang Gue Rui, 1998; Sonwalkar, 1988; Somashekar 1985) it is possible to conclude that FC1 × FC2 hybrid revealed lower degumming loss thereby indicating FC1 × FC2 is

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superior breed than other hybrids with respect to silk quality.

CONCLUSION

The main aim of this work was to analyse the economic traits of single hybrids and double hybrids following standard rearing procedure. The results indicated that the double hybrid FC1 × FC2 is superior in its performance whereas among the single hybrid FC2 is superior to FC1.

The two popular biometrical procedures namely Evaluation Index (EI) and Narrow sense heritability (h^2) are used in the investigation. Based on the results of evaluation index, it is evident that FC1 × FC2 is the best performing double hybrid by showing high EI for 9 out of eleven traits analyzed. The heritability estimation clearly demonstrated uniformity in the expression of five traits analyzed.

From the results of the degumming loss it is clear that FC1 × FC2 exhibited low degumming loss in silk filament compared to others indicating low sericin content to other hybrids.

CONFLICT OF INTEREST

There is no such conflict of interest was reported by authors with respect to this manuscript.

AUTHOR CONTRIBUTION

All authors provided critical feedback and helped to shape the research, analysis and approval of final manuscript.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude and appreciation towards all the co-authors and Department of Studies in Sericulture, University of Mysore, who have supported to conduct experiment and complete this manuscript ultimately.

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