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QUANTIFICATION OF BOIL-OFF LOSS IN THE COCOON SHELLS OF THE SILKWORM (*BOMBYX MORI* L.) BREEDS AND THEIR HYBRIDS.

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Abstract:
The silkworm, *Bombyx mori* L. has been reared since time immemorial due to its economic importance. The silk bave is made up of two principle proteins namely, fibroin and sericin. The removal of sericin is known as boil-off loss ratio (B.O.R). It is considered as one of the important qualitative trait during development of breeds, race authorization and even manufacture of silk fabrics.

An attempt has been made in the present investigation to record cocoon parameters and boil-off loss ratio in multivoltine races *viz.*, Pure Mysore, Hosa Mysore, Nistari, MU303 and MU1, bivoltine breeds *viz.*, NB4, CSR4, JROP, JRMD and P31. The hybrids (multi bihybrids) of Pure Mysore x NB7, Hosa Mysore x CSR4, Nistari x JROP, MU303 x JRMD and MU1 x P31 were also recorded. The results of the study revealed that, the multivoltine breed MU303 and bivoltine breed CSR2 excelled over other breeds for cocoon weight, shell weight, shell ratio, filament length and denier. Further, significantly lower boil-off loss was registred in MU1, NB7 and Nistari x JROP. All the hybrids expressed better heterosis over mid parental value (MPV) for boil-off loss ratio. Whereas, MU1 x P31 performed well for this trait over better parental value (BPV).

Keywords: Bivoltine breeds, Boil-off loss, *Bombyx mori*, Heterosis, Multivoltine breeds.

INTRODUCTION:

The silkworm, *Bombyx mori* L. spins a protective cocoon shell by extruding silk bave at the end of its larval period in order to get protection from the adverse environment condition. The silk bave is made up of two principle proteins *viz.*, fibroin and sericin. The fibroin forms inner core of the silk filament representing 70-80% of cocoon weight, which is surrounded by sericin accounting for 20-30% of the weight. In addition to this, little quantity of fat, wax, colouring and mineral matters of the silk bave not exceeding 2-3% (Carboni, 1952).

The main silk substance fibroin is insoluble in alkaline hot water, whereas the sericin is easily soluble in boiling alkaline soap solution (Sadov *et al.*, 1978). Without degumming, the silk cannot be called as "Queen of Textile". Degumming is the process of removal of sericin. The cocoon shell has more boil-off loss percentage when compared to the raw silk. The percentage of boil-off loss has got paramount importance in reeling and weaving activities (Kannan, 1986).

In sericulturally advanced countries, the silkworm breeders have successfully bred productive hybrids with high quality raw silk. During the course of breeding, boil-off loss is considered as one of the important qualitative traits. The boil-off ratio for bivoltine is found to be 24% (optimum) and it is genetically differing among the silkworm strains (Sinha *et al.*,1992). The degumming loss percentage was higher in multivoltines than bivoltines due to genetic constitution (Sidhu and Sonwalker, 1969). The contribution of sericin is highest for mulberry silk (23 to 30 %) when compared to non mulberry silk (Venugopal, 1991).

Low boil-off ratio content improved cocoon reeling qualities and is manifested by dominant genes, while recessive genes act towards the opposite direction (Gamo and Hirabayashi, 1984). The boil-off ratio varies according to seasons and also influenced by diverse environment (Sonwalker, 1969).

The silk degumming is necessary to give the silk lustrous, soft feel and to enable the penetration of chemicals and other dye stuff substances easily. The silk without sericin is used in weaving process. In India, farmers rear multivoltine x bivoltine hybrids than bivoltine hybrids for the commercial cocoon production. Hence, it is necessary to study in detail the boil-off loss ratio in the cocoon shell of multivoltine x bivoltine hybrids. In this context, the present study was undertaken to record the boil-off loss ratio in few selected parental breeds (multivoltine and bivoltine breeds) and their hybrids (multivoltine x bivoltine hybrids). In addition to this heterosis was analyzed in respect of boil-off loss ratio.

Materials and Methods

In the present study, five multivoltine races/breeds *viz.*, Nistari, Hosa Mysore, Pure Mysore, MU$_1$ and MU$_{303}$, and five bivoltine breeds *viz.*, JROP, CSR$_4$, NB$_7$, P$_{31}$ and JRMD were drawn from the Department of Studies in

Sericulture Science, University of Mysore, Manasagangotri, Mysore.

Hybrids of Nistari x JROP, Hosa Mysore x CSR_4, Pure Mysore x NB_7, MU_1 x P_{31} and MU_{303} x JRMD were prepared and reared by following the standard rearing techniques have advocated by Dandin *et al.* (2010). After rearing 10 cocoons (4 replications each) from selected races/breeds and hybrids were subjected to record cocoon parameters which includes cocoon weight, shell weight, shell ratio, filament length and denier. In addition to this, degumming of cocoon shell was carried out by adopting modified

procedure outlined by Sonwalker (1969). The parameters namely, shell ratio, filament length and denier were calculated by using following formulae.

$$\text{Shell ratio (\%)} = \frac{\text{Shell weight (g)}}{\text{Cocoon weight (g)}} X\ 100$$

Filament length (m)

L= R×1.125

R = Number of revolutions recorded by epprouvette.

1.125 = Circumference of epprouvette in meter.

$$\text{Denier} = \frac{\text{Weight of the filament}}{\text{Length of the filament}} \times 9000$$

Degumming

The initial weight of 10 cocoon shell of multivoltine races/breeds, bivoltine breeds and multivoltine x bivoltine hybrids were recorded before degumming process. Further, cocoon shells were subjected to liquor bath consisting mixture of neutral soap and sodium carbonate. The amount of soap and sodium carbonate required for degumming for 1gram of cocoon shell are as below:

70 ml of liquor solution (Water)

Soap 7 gram per liter

Soda ash 1 gram per 1 liter

The cocoon shells of respective races/breeds and hybrids was immersed in the beaker containing liquor bath where the temperature was maintained at 90 to 95^{O} C. for about 1 hour. The material was turned up and down for uniform and effective degumming. Afterwards the material was boiled in distilled water for about half an hour. Finally, the material was washed in cold tap water and then dried. The final weight of the material was recorded.

The boil-off loss percentage was calculated by using the formula:

$$\text{B.O.R} = \frac{\text{Initial dry weight-Final weight after degumming}}{\text{Initial dry weight}} X\ 100$$

The heterosis and heterobeltiosis were calculated by using formulae.

$$\text{Heterosis \% [Over mid parental value (MPV)]} = \frac{F1 - MPV}{MPV} X\ 100$$

$$\text{Heterobeltiosis (Over dominance)}$$

$$\text{[(Over better parental value(BPV)]} = \frac{F1 - BPV}{BPV} X\ 100$$

The data obtained were statistically analyzed by employing one-way factorial complete randomized design at 5% level of significance, **OPSTAT** online statistical package developed by O.P. Sheoran, Programmer, Computer section Chaudhury Charan Singh, Hisar Agriculture University, Hisar, Haryana State, India.

RESULTS AND DISCUSSION

Cocoon weight

Cocoon weight is an important attribute for the yield as this character mainly depends on the race/breed and its ability to convert mulberry leaf into cocoon. Significant differences was observed in respect of cocoon weight in multivoltine races ranging from 0.702 to 0.817g with highest being in MU_{303} (0.817g) and lowest in MU_1 (0.702g) (Table.1). These results are in conformity with those of Anil Kumar (2009) who has reported that the multivoltine races are characterized with low cocoon weight as their potentiality is poor and its poor efficiency to build a better cocoon even in favourable climatic conditions. Similar trend was also observed by Datta (1984) and Sudha and Neelu Nangia (2000).

Among the bivoltine breeds, P_{31}, CSR_4 and NB_7 exhibited higher cocoon weight than JROP and JRMD showing their genetic constitution for the expression of cocoon weight. The present findings are in conformity with the observations of Anil Kumar (2009), Anantha and Subramanya (2010), Kumaresan *et al.* (2007) and Rayar *et al.* (2000).

Among the multivoltine × bivoltine hybrids, MU_1 x P_{31}, Nistari x JROP and Hosa Mysore x CSR_4 registered higher cocoon weight of 1.092, 1.056 and 1.029g (Table.1), respectively as against Pure Mysore x NB7 (0.92g) (Table.1). Increase in the cocoon weight in hybrids in due to additive gene action with maximum contribution of bivoltine parent. These results are in line with the earlier observations of Radhakrisha *et al.* (2001); Ravindra Singh *et al.* (2005) and Umadevi *et al.* (2005).

Shell weight

Shell weight is an important economic character which indicates the silk content. It varies from rearing condition and quality of mulberry leaf. Significantly notable variations was observed in respect of shell weight in multivoltine races with highest being in MU_{303} (0.127g), while it was lowest in Pure Mysore (0.091g) (Table.1). It clearly indicating that, genetic potentiality varies with in multivoltine races / breeds. These results are in agreement with earlier findings of Sudha and Neelu Nangia (2000) who have opined that increased shell weight of 1.648g in P_2D_1 and 1.710g in KA over control batch 1.613g (Pure Mysore). Similar results were also documented by Anantha and Subramanya (2010) and Rayar *et al.* (2000).

Among the bivoltine breeds, maximum shell weight of 0.231g recorded in CSR_4 and it was minimum in JROP (0.201g) (Table.1). The CSR_4 excel over other bivoltine breeds for this trait indicating its genetic superiority. These results are supported by the observations of Anil Kumar (2009). Similar trend was also noticed by Anantha and Subramanya (2010).

Among the hybrids, highest shell weight was recorded in Hosa Mysore × CSR_4 (0.163g) and it was lowest in Pure Mysore x NB_7 (0.139) (Table.1). Increase in shell weight in hybrid Hosa Mysore × CSR_4 might be contribution of productive breed CSR_4. These results are in conformity with the earlier results of Roy *et al.* (1997) who have opined that most of the quantitative traits in multivoltine x bivoltine were superior than bivoltine x multivoltine hybrids except for fecundity and larval duration. Further, the multivoltine x bivoltine hybrids PM x C_{104} and PM x C110 performed better in respect of shell weight when compared to PM x C. Nichi. These results clearly showing that, higher shell weight was noticed in multivoltine x bivoltine than the multivoltine x multivoltine hybrids (Raju *et al.*, 2003).

Shell ratio

Cocoon shell percentage is one of the important parameter contributing for silk productivity. These traits also vary significantly in different breeds. Highest shell percentage was expressed in MU_{303}(15.55%), while it was lowest in Hosa Mysore (12.05 %) (Table.1). The variations within multivoltine races is due to genetic variability for this trait. These results are in conformity with those of Anil Kumar (2009). Similar trend was also reported in other multivoltine breeds by Vijayaraghavan and Das (1992), Anantha and Subramanya (2010), Rayer *et al.* (2000) and Sudha and Neelu Nangia (2000).

Among the bivoltine breeds, highest shell percentage was registered in CSR_4 (18.20%) and lowest was recorded in NB_7 (16.41%) (Table.1) indicating genetic superiority of CSR4 over remaining bivoltine breeds. The present findings are in conformity with the observations of Anil Kumar (2009) and Anantha and Subramanya (2010).

The cocoon shell percentage also differ among multivoltine × bivoltine hybrids with being maximum in Hosa Mysore x CSR_4 (16.23%) and minimum was registered in Pure Mysore × NB_7 (15.10%) (Table.1). The superiority for this trait in Hosa Mysore × CSR_4 indicating maximum contribution by the productive breed CSR_4. Similar observations were also reported in some other multivoltine hybrids by Datta (1984), Chatterjee (1993), Mano (1988) and Raju *et al.* (2003).

Filament length

Filament length is one of the major contributory quantitative traits in silkworm (Miyahara, 1978; Yokoyama 1979). This trait differs significantly among the breeds and hybrids. Among the multivoltine races, MU_1 expressed maximum filament length of 480m, whereas Pure Mysore expressed minimum filament length of 356m (Table.1) indicating that this trait is racial. These results are in agreement with the earlier observations of Anil Kumar (2009) and Rayar *et al.* (2000) who have noticed that the variation in the filament length in some other multivoltine races.

Among the bivoltine breeds, longest filament length was registerd in NB_7 (753m) and shortest was observed in JROP (601m) (Table.1). The present findings on par with the observation of Anil Kumar (2009) and Anantha and Subramanya (2010).

The filament length also differs significantly among multivoltine × bivoltine hybrids with being maximum in Hosa Mysore x CSR_4 (590m) and minimum in MU_1 x P_{31} (535m) (Table.1). The superiority for this trait in Hosa Mysore × CSR_4 indicating maximum contribution by the productive breed CSR_4. Further, intermediate filament length for other multivoltine hybrids was also observed by Raju and Krishnamurthy (1995) and Doddaswamy *et al.* (2009).

Denier

Denier is an important trait identifying silk quality which denotes thickness of the filament. For this trait significant differences observed in multivoltine races. Out of five multivoltine races, lowest denier is expressed by MU_1 (1.0d), MU_{303} (1.5d) and Nistari (1.5d) (Table.1). The variations for this trait is due to differing in larval duration within multivoltine races. This corroborates the earlier worker (Kalpana *et al.,* 2002) who have reported that shorter the larval duration lesser will be the denier.

Both the bivoltine breeds and hybrids expressed non-significant variation for denier. It indicates that no differences for this trait within bivoltine breeds and hybrids.

Boil-off loss percentage

The cocoon shell has more boil-off loss percentage when compared to the raw silk. This trait differs significantly among the breeds and hybrids. Among multivoltine races, lowest boil-off loss ratio (B.O.R) was recorded in MU_1 (25.32%). While it was highest in Pure Mysore (26.60%) (Table.1). These results are also supported by the observations of Sidhu and Sonwalker (1969) who have reported that the degumming loss percentage was higher in multivoltine than bivoltine breeds. The increased (B.O.R) in multivoltine races is might be due to genetic constitution differing in two voltinism groups.

The boil-off loss ratio also differs among bivoltine breeds with being lowest in NB_7 (22.58%) and highest in P_{31} (23.62%) (Table.1). These results are in agreement with the earlier observations of Sinha *et al.* (1992) who have reported that the boil-off loss ratio in bivoltine breeds is found to be 24% (optimum) and it is genetically differing among the silkworm strains.

Among the hybrids, lowest boil-off loss ratio was recorded in MU_1 x P_{31} (22.88%) and highest was observed in Nistari × JROP (24.37%) (Table.1). Further, all the hybrids registered intermediate values for this traits when compared to their parents. These results are corroborate with the earlier findings of Sidhu and Sonwalker (1969), Sinha *et al. (1992)* and Basavaraja *et al.* (2000).

Heterosis and over dominance for boil-off loss ratio

The phenomenon of heterosis and over dominance in conjunction with the expression of boil-off loss ratio analyzed in the hybrids under present study facilitated procedures to identify the promising hybrids. Further, the more uniformity in the expression of this trait in hybrids than the parents is one of the desirable features to understand the genetic constitution of the hybrids for their commercial exploitation. For the trait boil-off loss ratio, negative heterosis is desirable. For instance, high magnitude of MU_1 × P_{31} (-6.49%), Pure Mysore × NB_7 (-4.63) and MU_{303} × JRMD (-3.17) (Table.2) which could be attributed to the higher mid parental values. Similarly, the negative heterosis over better parental value is recorded in MU_1 x P_{31} (-3.13%). These results are in conformation with the findings of Gamo and Hirabayashi (1983). Further, Basavaraja *et al.* (2000) reported that in hybrids heterosis was intermediate between parents. However, the manifestation of heterosis was noticed in different hybrids combinations for this trait.

CONCLUSION

The results of the presents study inferred that, the multivoltine x bivoltine hybrid (MU1 x P31) expressed heterosis over better parental value for boil-off loss ratio and this character can be exploited in silk weaving sector.

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Table1. Cocoon and post-cocoon parameters of multivoltine breeds, bivoltine breeds and their hybrids of *Bombyx mori*.

Multivoltine races/breed	**Cocoon weight (g)**	**Shell weight (g)**	**Shell ratio (%)**	**Filament length (m)**	**Denier (d)**	**Boil off loss (%)**
Pure Mysore	0.725± 0.007	0.091 ± 0.003	12.56±0.208 (20.75±0.180)	356 ± 8.446	1.8 ± 0.063	26.60 ± 0.409 (31.04±0.265)
Hosa Mysore	0.751± 0.013	0.113 ± 0.006	12.05±0.200 (20.30±0.176)	392 ± 3.488	1.6 ± 0.041	26.26 ±0.134 (30.81±0.087)
Nistari	0.726± 0.008	0.104 ± 0.002	14.33±0.200 (22.23±0.164)	380 ± 4.768	1.5 ± 0.103	25.50 ±0.154 (30.32±0.101)
MU303	0.817± 0.005	0.127 ± 0.002	15.55±0.208 (23.21±0.165)	451 ± 4.516	1.5 ± 0.103	25.66 ±0.043 (30.42±0.029)
MU1	0.702± 0.001	0.107 ± 0.001	15.25±0.200 (22.98±0.160)	480 ± 2.398	1.0 ± 0.063	25.32 ±0.203 (30.20±0.134)
F-test	*	*	*	*	*	*
SE(m)±	0.008	0.003	0.169	5.145	0.079	0.146
SE(d)±	0.011	0.004	0.239	7.277	0.111	0.207
C.D. at 5%	0.024	0.009	0.514	15.651	0.239	0.445
C.V.%	2.1	5.712	1.544	2.498	10.27	0.957
Bivoltine breeds						
NB$_7$	1.249 ±0.050	0.205 ±0.005	16.41 ±0.208 (23.89±0.161)	753±1.443	2.7±0.041	22.58 ±0.070 (28.36±0.048)
CSR$_4$	1.269 ±0.001	0.231 ±0.002	18.20 ±0.200 (25.24±0.149)	647±2.056	2.8±0.065	22.63 ±0.119 (28.39±0.081)
JROP	1.123 ±0.003	0.201 ±0.001	17.90 ±0.200 (25.02±0.150)	601±8.179	2.8±0.041	23.21 ±0.014 (28.79±0.010)
JRMD	1.257 ±0.034	0.213 ±0.003	16.95 ±0.208 (24.30±0.159)	629±5.10	2.9±0.024	23.54 ±0.024 (29.01±0.016)
P$_{31}$	1.284 ±0.005	0.213 ±0	16.59 ±0.208 (24.03±0.160)	538±2.097	2.7±0.103	23.62 ±0.009 (29.07±0.006)
F-test	*	*	*	*	NS	*
SE(m)±	0.027	0.003	0.156	4.553	0.061	0.043
SE(d)±	0.038	0.004	0.220	6.439	0.086	0.061
C.D. at 5%	0.083	0.009	0.474	13.851	--	0.131
C.V.%	4.391	2.740	1.272	1.438	4.35	0.301
Multi × bi hybrids						
Pure Mysore X NB7	0.92±0.035	0.139±0.005	15.10±0.208 (22.86±0.167)	569±6.762	2.6±0.065	23.45 ± 0.324 (28.94±0.219)
Hosa Mysore X CSR$_4$	1.029±0.015	0.167±0.003	16.23±0.200 (23.75±0.156)	590±7.192	2.5±0.111	24.27 ±0.564 (29.50±0.377)
Nistari X JROP	1.056±0.016	0.160±0.008	15.15±0.208 (22.90±0.166)	573±6.442	2.6±0.144	24.37 ±0.225 (29.57±0.150)
MU303 X JRMD	1.023±0.01	0.165±0.006	16.13±0.208 (23.67±0.16)	560±19.155	2.5±0.075	23.82 ±0.176 (29.19±0.118)
MU$_1$ X P$_{31}$	1.092±0.021	0.163±0.004	15.18±0.325 (22.92±0.260)	535±5.105	2.6±0.104	22.88 ±0.255 (28.56±0.174)
F-test	*	*	*	*	NS	*
SE(m)±	0.021	0.017	0.186	10.314	0.103	0.227
SE(d)±	0.03	0.006	0.263	14.587	0.146	0.320
C.D. at 5%	0.064	0.008	0.566	31.374	--	0.689
C.V.%	4.11	6.945	1.604	3.647	8.069	1.554

( ): Angular transformed values   *: Significant at 5% level   ±: Standard error values

Table 2: Heterosis over mid parental value (MPV) and better parental value (BPV) for boil-off loss ratio in hybrids

Hybrids	Heterosis % (Over MPV)	Heterosis % (Over BPV)
Pure Mysore x NB_7	-4.63	3.85
Hosa Mysore x CSR_4	-0.73	7.24
Nistari x JROP	-0.082	4.99
MU_{303} x JRMD	-3.17	1.19
MU_1 x P_{31}	-6.49	-3.13