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Influence of brushing schedule on leaf biochemical analysis for commercial character expression of bivoltine silkworm hybrids (*Bombyx mori* L.)

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Abstract
In the present investigation, the biochemical analysis of mulberry varieties along with the effect of brushing schedule, hybrids and mulberry varieties for commercial cocoon character expression was studied. The treatments comprised of two brushing schedule (early i.e. 1st of September and late i.e. 20th of September), two hybrids ($PO_3 \times ND_5$ and $SH_6 \times NB_4D_2$) and two mulberry varieties (China white and Tr-10). The experiment was laid out in factorial-completely randomized block design with eight treatments and three replications and the analysis of treatments was worked out by analysis of variance technique. Biochemical analysis revealed that mulberry variety China white had significantly higher carbohydrate and biomass content while as, crude protein, ash content and amino acids were significantly higher in Tr-10. Observations made for commercially important traits indicated that, single shell weight, single cocoon weight, shell ratio percentage and fibroin content were significantly higher for early brushing schedule, $PO_3 \times ND_5$ and China white, whereas, effective rate of rearing (by weight and by number), sericin content and renditta was significantly higher for late brushing schedule, $SH_6 \times NB_4D_2$ and Tr-10. Thus, two commercial rearing's are possible with brushing schedule of early i.e. 1st of September and late i.e. 20th of September for hybrid $PO_3 \times ND_5$ fed on China white mulberry variety for commercial cocoon characters.

Keywords: Brushing schedule, Mulberry, *Bombyx mori*, Biochemical, Commercial characters

1. Introduction
Silkworm, *Bombyx mori* L. is an important economic insect, feeding exclusively on the mulberry leaves (*Morus* spp.). Mulberry leaf quality is one of the key factors influencing the growth and development of silkworm, as the insect derives all the nutrients from mulberry leaf itself. The production of quality cocoon crop is directly correlated with mulberry leaf and it contributes about 38.20 per cent towards successful rearing/quality cocoon production (Bothikar *et al.*, 2014) [2], besides environmental conditions which contributes about 37.00 per cent. Better the quality of mulberry leaves greater are the possibilities of obtaining good cocoon crops. Since the production of good quality cocoons depends on providing good quality leaves to silkworm, the development of superior quality leaf has become one of the prime objectives in mulberry breeding programme. Appropriate selection of the cultivars based on the plant morphology, disease resistance, biochemical properties and their effect on growth and cocoon yield parameters of *Bombyx mori* races/hybrids in different agro-climatic conditions is essential to select and exploit promising cultivars for better sericulture practices. However, a significant variation occurs in the nutritional value and composition of mulberry leaves depending upon the season, environmental conditions, agronomic practices, age of leaf etc. This variation impacts both qualitatively and quantitatively on the cocoon production (Gawade and Medhe, 2010) [11]. Under such conditions, the suitable mulberry variety needs to be developed for specific seasons and tested against the silkworm rearing for its quality in the form of worm growth, development and silk production as silkworm itself is the best evaluator of mulberry. Varieties having higher yield containing better nutritional elements are always desirable to feed silkworm. It is a confirmed fact that leaf quality differs among mulberry varieties in different seasons which are responsible for the difference in rearing performance of the silkworm. Silkworm breeds/hybrids differ in their nutritional requirements depending on the variety, rearing environment, season and quantum of nutrition. Thus, an experiment was conducted to study the effect of rearing schedules, mulberry varieties and silkworm hybrids on biochemical aspect of mulberry and commercial character expression of bivoltine silkworm hybrids.

Materials and Methods

The present investigation was conducted during autumn 2014-15 at Division of Sericulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu. The experimental research material for the proposed study comprised of two brushing schedules forearly autumn (1st of September) and late autumn season (20th of September). Two bivoltine silkworm hybrids, $PO_3 \times ND_5$ (evolved at Division of Sericulture, SKUAST-J, Udheywalla) and popular hybrid $SH_6 \times NB_4D_2$ were taken for the study. Two mulberry varieties (China white and Tr-10) leaves were used as food for rearing of worms. The experiment was laid out in Factorial-Completely Randomized Block Design (F-CRBD) with three replications. The data was recorded for various traits and subjected to angular transformation for statistical analysis.

Biochemical analysis

Leaves from mulberry varieties were harvested after 60 days of sprouting. The harvested leaves were oven dried and then utilized for biochemical estimations. Total carbohydrates were estimated by Phenol-Sulphuric Acid method as suggested by Dubios *et al*. (1965) [8]. Crude protein in different mulberry accessions was estimated by determining nitrogen content in 0.5 g sample by Micro-Kjeldhal method. Moisture content, ash content and biomass was determined gravimetrically according to standard methods of AOAC (1997) [1]. Total amino acid content was estimated by following ninhydrin method as described by Spies, 1955 [31].

Experimental rearing trial

Silkworm seed of two hybrid combinations ($PO_3 \times ND_5$ and $SH_6 \times NB_4D_2$), was released from cold storage after completion of chilling treatment of 90 days. Standard rearing techniques as suggested by Dandin *et al*. (2003) [6] were followed. After brushing, the silkworms were reared on two mulberry varieties viz., China white and Tr-10 separately. Three replications were maintained for each treatment. Each replication had a population of 200 larvae. Ripe worms were picked for seriposition and spinning was conducted on collapsible plastic mountages. The cocoons were harvested on fifth day during autumn season after mounting. Data was recorded replication-wise for all commercial traits viz., cocoon yield per 10,000 larvae (by weight and by number), single cocoon weight, single shell weight, shell ratio percentage, fibroin and sericin content, total filament length, non-breakable filament length and denier

Statistical analysis

To estimate significant differences among brushing schedules, silkworm hybrids and mulberry variety the mean data of each character were subjected to Analysis of Variance techniques. The results were tested for the treatments mean by applying F- test of significance (ANOVA) on the basis of null hypothesis as mentioned by Cochran and Cox (1957) [5].

Results and Discussion

Biochemical analysis of mulberry varieties

The chemical analysis of mulberry leaves is an indicator of its status for silkworm feed. It is violary to bioassay. The nutritional status of mulberry leaves which influences the silkworm growth depends upon the levels of moisture, crude protein, soluble carbohydrates, total sugars, less minerals, ash content and crude fiber and is best relished and utilized by silkworm larvae (Bose and Bindroo, 2001) [3].

Carbohydrates

For silkworms, carbohydrates are very essential constituent because it acts as chief source of energy (Horie, 1995) [15]. Silkworms fed with leaves having high content of carbohydrates, gain more energy and in turn enhances the synthesis of silk. In the present investigation, during autumn seasons the carbohydrate percentage was significantly higher in variety China white (21.14) than in Tr-10 (19.89) (Table 1 & Fig. 1). Hiratsuka (1971) [14] has reported that mulberry leaves contain plenty of carbohydrates which get stored in silkworm as glycogen and is subsequently utilized for physiological consumption as well as fat accumulation. The results obtained in present investigation corroborate with the findings of Manjula and Vijaya Kumari (2015) [20].

Crude protein

The protein content present in mulberry leaves has direct bearing on silkworm larval growth, silk gland development and cocoon characters. Silkworm needs high protein diet to synthesis large amount of silk. The varieties under study expressed significant differences. Mulberry variety Tr-10 (21.59) recorded higher crude protein value than China white (13.71) (Table 1 & Fig. 1). This may be due to the leaf quality variation between diploid (China white) and triploid (Tr-10) variety. Rama Rao *et al.* (2000) [28] also reported that triploid varieties recorded distinctly higher values of leaf protein, sugars and mineral contents associated with superior rearing performances and bioassay moulting test parameters.

Moisture content

In mulberry, high leaf protein content is closely associated with higher level of moisture and moisture retention capacity of leaves. Moisture in leaves plays an important role during feeding of silkworm by increasing the palatability factor of the worms. In the present study, moisture content (Table 1 & Fig. 1) was significantly higher in China white (76.93) than Tr-10 (75.95). Ramachandra *et al*. (2008) [27] also reported highly significant association of all nutritional parameters with moisture content of leaves

Ash content

Ash content being an important constituent is an indicator of inorganic salt accumulation in the leaf. These constituents are important for silkworm as a source of minerals especially during late age rearing. In the present investigation, no significant difference was found among the varieties studied (Table 1 & Fig. 1). However, Tr-10 (13.11) recorded numerically higher value as compared to China white (13.06).These results are in close conformity with those reported by Guven (2012) [13].

Amino acids

Amino acids are important for phytophagous insects as they help the insects in food selection. Mulberry leaves are generally rich in amino acid content and it forms an important constituent of silkworm nutrition. Ito and Arai (1965) [16] reported that considerable amount of amino acids is utilized for the formation of haemolymph, development of silk glands and cocoon production. In this investigation, total amino acid content was found to be significantly higher in Tr-10 (18.48) than China white (9.53) (Table 1 & Fig. 1). Varieties possessing high nitrogen and amino acids content in leaves are nutritively superior and promote growth and development of silkworm (Suryanarayanan and Shivashankar, 2002) [32]. These results corroborate with the findings of Jyothi *et al.* (2014) [17].

Biomass

Leaf biomass is an actual indicator of organic components of the leaves. The variation in biomass can itself act as an indicator of quantitative feeding potential. Less biomass in leaves attribute to overall thin leaves. This character was significantly higher in China white (109.39) in comparison with Tr-10 (86.00) (Table 1 & Fig. 1). Similar were the findings of Ghosh *et al.* (2011) [12].

Table: 1 Biochemical characters of leaf for different mulberry varieties

Mulberry Varieties	Carbohydrates (% dry weight)	Crude protein (% dry weight)	Moisture content (%)	Ash content (% dry weight)	Amino acid (mg/g dry weight)	Biomass (g/100 leaves)
China white	21.14	13.71	76.93	13.06	9.53	109.39
Tr-10	19.89	21.59	75.95	13.11	18.48	86.00
SEm (±)	0.33	0.42	0.13	0.12	0.21	0.18
LSD (p=0.05)	0.02	1.69	0.54	NS	0.841	0.73

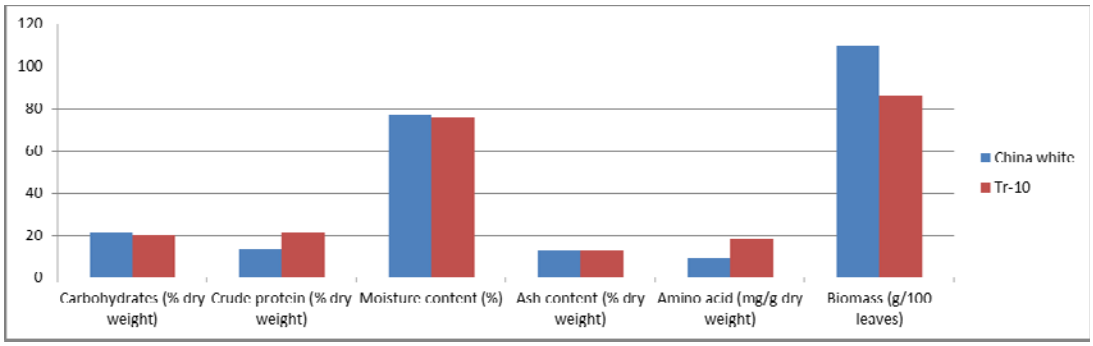

Fig 1: Chemical characters of leaf for different mulberry varieties

Commercial character expression of silkworms

Cocoon characters are commercially most important and do have close relation with mulberry leaves as food. Cocoon characters generally get influenced by brushing schedule, hybrids and varieties.

Effective rate of rearing (ERR)

Malik *et al.* (2006) [19] suggested that effective rate of rearing (by weight and by number), good cocoon percentage, pupation percentage, single cocoon weight, shell weight and shell ratio percentage are important parameter for quality cocoon crop for potential hybrid.

By weight

From different treatments studied, only brushing schedule was found to be significant for effective rate of rearing (by weight), while as, hybrids and varieties had no significant difference (Table 2). For brushing schedule, late brushing schedule recorded higher and significant results of 14.99 than early brushing schedule (11.81). This may be due to congenial temperature and relative humidity conditions prevailing during study period. The findings are in close conformity to that of Kumar *et al.* (2013) [18].

By number

ERR by number being an important parameter contributes towards viability. From different treatments studied, brushing schedule was found to be significant for effective rate of rearing (by number). Hybrids and varieties did not exhibit any significant difference (Table 2). For brushing schedule, late brushing schedule rearing recorded higher and significant results of 9450 than early brushing schedule (8392). Higher significant values for cocoon yield by number can be ascribed to the fact that cocoon yield by weight also exhibited significant differences for seasons and hybrids. It may be due to the negative correlation of fecundity (Ram, 2010) [25]. The present results corroborates with the findings of Gangwar (2012) [9].

Single cocoon weight

The cocoon weight, shell weight and shell ratio are the important commercial parameters for cocoon stage and reeling performance. Cocoon weight and shell weight are the most important characters evaluated for productivity (Gaviria *et al.*, 2006) [10]. Cocoon weight has a negative correlation with shell ratio but positive correlation with shell weight, whereas shell weight has a positive correlation with shell ratio. Data presented in Table 2 represents significant difference in different brushing schedule of rearing. Early brushing schedule exhibited significant increase in single cocoon weight of 1.59 than late brushing schedule (1.55). Hybrids and mulberry varieties depicted non-significance in single cocoon weight. This indicates the superiority of hybrids in different brushing schedule and is in support of the view that phenomenon of heterosis could be either due to additive gene action or due to dominance hypothesis as reported by Petkov (1989) [23].

Single shell weight

Shell weight content is a quality parameter for evaluation of mulberry and has a positive co-relation with cocoon shell ratio. Shell weight in the present study, exhibited significant superiority for brushing schedule, hybrids and varieties (Table 2). Early brushing schedule was significantly superior (0.33 g) to late brushing schedule having value of 0.31. Hybrids significantly influenced the shell weight during autumn season. $PO_3 \times ND_5$ resulted in significant increase in shell weight with corresponding value of 0.34 over $SH_6 \times NB_4D_2$ (0.29 g). Among cultivars, China white proved superior to Tr-10 and resulted in significantly higher shell weight of 0.33 g. This indicates that varieties studied are having potential for economic exploitation. Similar trend with respect to shell weight was observed by Maqbool *et al.* (2005) [21].

Shell ratio percentage

Shell ratio percentage indicates the amount of raw silk reeled from given quantity of fresh cocoons and shell percentage

varies according to silkworm breed/hybrids. Shell ratio percentage was found to be significant for hybrids only and data presented in Table 2 shows that brushing schedule and varieties do not have any significant difference. Hybrid, $PO_3 \times ND_5$ at 21.82 was significantly superior to hybrid $SH_6 \times NB_4D_2$ (18.65). This might be due to higher ingestion, digestion and conversion factors. These results corroborate with the findings of Chandraju *et al.* (2013) [4].

Fibroin and Sericin content

Silk fiber is almost a pure protein composed of fibroin and sericin. Fibroin is the main body of the thread whereas sericin is a gummy substance meant for holding the fibroin intact in the form of cocoon. Different brushing schedule and varieties had no significant difference in terms of fibroin and sericin content, while as significant increase in fibroin and sericin content was recorded for hybrids only (Table 2). $PO_3 \times ND_5$ recorded significantly higher fibroin content of 72.93 whereas, $SH_6 \times NB_4D_2$ recorded 68.99 only. Ram *et al.* (1998) [26] reported highly positive correlation between cocoon shell weight and fibroin. Hybrid $SH_6 \times NB_4D_2$ recorded significantly higher sericin content of 31.00 over $PO_3 \times ND_5$ which remained at 27.19 only for sericin content. Higher fibroin content could be due to higher shell ratio among the treatments studied. Fibroin content determines the amount of raw silk produced. It is desirable to have less sericin content as higher sericin content indirectly effects the raw silk production and as such is not a desirable character from commercial and breeding point of view. Similar findings were also recorded by Sabina *et al.* (2012) [29].

Table 2: Effect of different treatments on cocoon traits

Treatments	Effective rate of rearing (ERR)		Single cocoon weight (g)	Single shell weight (g)	Shell ratio percentage	Fibroin content (%)	Sericin content (%)
	By weight (kg)	By number					
BRUSHING SCHEDULE							
Early (1st of September)	11.81	8392	1.59	0.33	20.33	71.15	28.84
Late (20th of September)	14.99	9450	1.55	0.31	20.13	70.76	29.36
SEm (±)	0.18	75.69	0.01	0.004	0.16	0.24	0.25
LSD (p=0.05)	0.54	226.95	0.04	0.01	NS	NS	NS
HYBRIDS							
$PO_3 \times ND_5$	13.42	8975	1.57	0.34	21.82	72.93	27.19
$SH_6 \times NB_4D_2$	13.38	8867	1.59	0.29	18.65	68.99	31.00
SEm (±)	0.18	75.69	0.01	0.004	0.16	0.24	0.25
LSD (p=0.05)	NS	NS	NS	0.01	0.49	0.71	0.74
VARIETY							
China white	13.27	8892	1.59	0.33	20.44	70.96	29.03
Tr-10	13.53	8950	1.56	0.31	20.03	70.95	29.17
SEm (±)	0.18	75.69	0.01	0.004	0.16	0.24	0.25
LSD (p=0.05)	NS	NS	NS	0.01	NS	NS	NS

Comparative performance of post cocoon characters

Post cocoon/reeling characters may not be of much importance to rearers but have greater significance not only from reeler's point of view but also from industrial view.

Total filament length

Among post cocoon parameters, filament length and denier are considered as important characters from economic point of view and have direct bearing on the merit of a breed/hybrid. Increase or decrease in filament length is dependent on increase or decrease in the thickness of silk filament and cocoon shell weight of breeds and hybrids. Results of the present study did not revealed any significant difference for brushing schedule, hybrids and varieties for total filament length (Table 3). This finding can be attributed to longer V instar duration and higher larval weight (Satenahalli *et al.*, 1990) [30].

Non-breakable filament length

Rajalakshmi *et al.* (2000) [24] opines that the quality of a good hybrid is to have minimum or no breaks during the process of reeling. Different brushing schedule, hybrids and varieties again did not reveal any significant difference in autumn crop for non-breakable filament length (Table 3). Similar were the findings of Kumar *et al*. (2013) [18].

Denier

Denier represents the thinness or thickness of the filament and it was found to be non-significant for brushing schedule, hybrids and varieties during autumn. Denier being genetically controlled trait and as such it may not have significant co-relation with other parameters. The results are in accordance with the findings of Dayananda *et al*. (2011) [7].

Renditta

Renditta is the number of cocoons required to produce 1 kg of raw silk. It is better to have less value of renditta. The mean data (Table 3) reveals that brushing schedule, hybrids and varieties significantly increased renditta for autumn crop. Late brushing schedule rearing resulted in significant increase in renditta with corresponding value of 7.33 over early brushing schedule rearing with a value of 7.01. Hybrids were found to influence renditta significantly. Among hybrids, $SH_6 \times NB_4D_2$ recorded significantly higher renditta of 8.13, whereas $PO_3 \times ND_5$ recorded a value of 6.21 only. Mulberry variety Tr-10 produced significantly more renditta (7.34) than China white (7.00). The results are in close conformity with those reported by Murthy *et al*. (2013) [22].

Based on the investigation, it can be concluded that mulberry variety, Tr-10 is recommended for chawki rearing and China white for late age rearing. During autumn, two commercial rearing's are possible with brushing schedule of early i.e. 1st of September and late i.e. 20th September for hybrid $PO_3 \times ND_5$ fed on China white mulberry variety with respect to good crop production..

Table 3: Effect of different treatments on post-cocoon traits

Treatments	Total filament length (m)	Non-breakable filament length (m)	Denier (d)	Renditta
BRUSHING SCHEDULE				
Early (1st of September)	688.50	652.04	2.59	7.01
Late (20th of September)	688.33	641.29	2.45	7.33
SEm (±)	10.62	12.64	0.05	0.07
LSD (p=0.05)	NS	NS	NS	0.22
HYBRIDS				
$PO_3 \times ND_5$	684.45	646.25	2.49	6.21
$SH_6 \times NB_4D_2$	692.37	647.08	2.56	8.13
SEm (±)	10.62	12.64	0.05	0.07
LSD (p=0.05)	NS	NS	NS	0.22
VARIETY				
China white	674.16	638.04	2.57	7.00
Tr-10	702.66	655.29	2.48	7.34
SEm (±)	10.62	12.64	0.05	0.07
LSD (p=0.05)	NS	NS	NS	0.22

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