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Evaluation of promising mulberry genotypes through bioassay of silkworm *Bombyx mori* L.

Ananda Kumar MD and Sreeroopa

Karnataka State Sericulture Research & Development Institute, Thalagattapura, Bangalore, Karnataka, India

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Corresponding Author: Ananda Kumar MD

Abstract

The silkworms are monophagous insect which feeds only on mulberry leaves. It takes necessary nutrients for growth and development only from the mulberry leaves. The nutritive value of mulberry depends on many factors. The biomolecules like carbohydrates and protein play a major role in its growth, development and silk productivity. Enzymes along with other factors like hormones have a crucial role in the growth and metamorphosis of silkworm. The availability of biomolecules as nutrients has an important role in conversion efficiency of it to silk by silkworm. The plant parameters were found to be better in Suvarna3 and Vishala mulberry variety among the varieties studied. Biochemical work was carried out for that different variety of mulberry to check the nutrition value of that leaves. The IMH × CSR2 (Bangalore Belli) improved crossbreed (ICB) silkworm larvae were fed with different varieties of mulberry leaves. Finally, the economic parameters of cocoons fed on different mulberry varieties were analyzed. The present study depicts Suvarna3 and Vishala has a better performance among the mulberry varieties studied.

Keywords: Mulberry varieties, Bangalore belli, plant parameters, bioassay, economic parameters

1. Introduction

The *Bombyx mori* is essentially monophagous and survives solely on mulberry leaves (*Morus* sp.) which play an important role in the nutrition of the silkworms, and in turn cocoon and silk production (Nagaraju, 2002) [12]. About 82.20% of the silk produced in the world is obtained from mulberry silkworm (*Bombyx mori* L.) reared solely on mulberry leaves. Mulberry tree is grown under varied climatic conditions, ranging from temperate to tropical; hence, the growth and development as well the quality and quantity of cocoons produced are largely influenced by leaf quality. It is well-established fact that in sericulture, more than 60% of the total cost of cocoon production goes towards mulberry production alone.

The mulberry leaf consumption, digestion and utilization will have a greater impact on the silkworm growth and development and in-turn will reflect on the quality of the cocoon and silk produced. Hence, in recent years maximum attention has been given for the improvement of mulberry in terms of both quality and quantity. Leaf quality is an important parameter used for evaluation of varieties aimed at selection of superior varieties for rearing performance (Bongale *et al*., 1991) [1]. The nutritional elements of mulberry leaves determine the growth and development of the larvae and cocoon production (Seidavi *et al*., 2005) [21].

Modern methods of mulberry cultivation based on improved varieties and intensive cultivation practices have resulted in a manifold increase in the leaf yield. In order to harvest good mulberry foliage per unit area, it is necessary to create favorable conditions during the establishment of the mulberry garden. Hence, the proper use of manures and fertilizers through appropriate soil amendments to increase the foliage production is of utmost importance. Thus, manures and fertilizers, application are essential to improve the quality of mulberry leaves (Bongale, 1994) [2].

Many scientists have observed that the growth and development of silkworm as well as the economic character of cocoons were greatly influenced by the nutritional content of the mulberry leaf (Krishnaswami *et al.*, 1970) [8]. Generally, nitrogen, phosphorous and potassium are required among the essential elements for quality mulberry leaf production and hence application of organic manure and chemical fertilizers containing these elements are necessary. Recently, many mulberry varieties are introduced to improve the cocoon yield but the leaf quality is governed by several factors such as the variety (Chakravorty *et al.*, 2005) [3], agronomical conditions (Purohit and Pavankumar, 1996; Dandin *et al.*, 2005; Raghunath *et al.*, 2005; Singhal *et al.*, 2005) [15, 4, 16, 23] and agro-climatic conditions (Kaul and Bhagat, 1991; Chakravorty *et al.*, 2005) [7, 3]. Leaf is a key factor besides environment and technology adoption for better growth and development of the silkworms and cocoon production (Purohit and Pavankumar, 1996) [15]. It is a confirmed fact that, leaf quality differs among mulberry varieties which in turn responsible for the difference in silkworm rearing performances. Leaves of superior quality enhance the chances of good cocoon crop (Ravikumar, 1988) [20]. In the present study an attempt has been made to evaluate the elite mulberry varieties developed at KSSRDI with V1 as check, through bio assay studies.

2. Materials and Methods

In the present study mulberry varieties *viz.*, Suvarna-2, Suvarna-3, vishala were evaluated along with V1 as check variety for the comparison purpose. Mulberry plots of these varieties with spacing of $6^{l}x6^{l}$ were maintained (RBD) with recommended package of practices, at the campus of KSSRDI, Bengaluru. Disease free crossbreed (Bengaluru Belli) layings of silkworm were procured from SWB&G section, KSSRDI, Thalghattapura, Bangalore, Karnataka. Experiment was conducted in Silkworm Crop Improvement Section of KSSRDI, Thalaghattapura with four replications/variety. Two years old plants were used for silkworm rearing in three seasons of a year. Mulberry genotypes/Varieties of Suvarna-2, Suvarna-3, vishala and V1 (check) with a spacing of 6^1 x 6^1 were maintained. Hundred plants per replication were maintained for each variety. The cultural practices were maintained as per the recommendation. The leaf harvest method was followed for the harvesting. Bengaluru belli, (IMHxCSR2) the improved cross breed (ICB) silkworm race was used for the bioassay studies.

2.1 Plant parameters

Growth parameters such as total shoot, Length (cm), number of shoots and yield parameters such as leaf and shoot (kg/ha) were assessed. Leaf moisture%, sugars and protein content and bio-assay test were also conduced as suggested by Dandin and Jolly (1986) [4].

2.2 Biochemical analysis: The tender, medium and coarse leaves were collected from healthy mulberry plants maintained in plots. The leaves were dried at 65-70ºC, powdered and preserved. A 10% homogenate was prepared, centrifuged for 10 mins at 3000rpm and the supernatant was collected and stored at 4°C till further use.

2.3 Estimation of proteins: The quantitative estimation of proteins was done with leaf extract by the method of Lowry, *et al.*, (1951) [9] using bovine serum albumin (BSA) as standard. Appropriately diluted leaf extracts were mixed with 5 ml of protein reagent and incubated at room temperature for 10 min. After incubation, 0.5ml of Folin's phenol reagent was added and incubated at room temperature for 30 min and the absorbance was measured at 660 nm.

2.4 Estimation of reducing sugar and total soluble sugar

Reducing sugar was estimated according to the procedure of Koehler *et al*., (1952). 1ml of leaf extract was mixed with 0.5ml of DNS, the mixture was incubation on boiling water bath for 15 minutes, cooled and made up to 10ml using distilled water and the absorbance was measured at 540nm using glucose as standard. Estimation of total sugar was done according to Miller *et al*., (1959). 4ml of Anthrone reagent was added to 1 ml of leaf extract. The content was well mixed and kept for incubation on boiling water bath for 15 minutes. After cooling the absorbance was measured at 630nm using glucose as standard.

2.5 Silkworm Rearing

The new ICB (Bengaluru Belli) race developed by KSSRDI was used for this study. Silkworm rearing experiments were conducted at different seasons. For each mulberry variety, five dfls were reared with 4 replications. After III moult, 300 larvae/replication were maintained. Appropriate cellular rearing techniques were adopted and separate rearing trials were conducted for different varieties (Dandin & Giridhar, 2014) [4]. Larvae were fed three times daily (7am, 2pm, 10pm) with healthy, fresh leaves. Young age larvae were fed with tender, succulent and nutritious leaves known to favour growth and development of chawki silkworms, while mature and coarse leaves were fed to late age silkworms till ripening. Cocoons were harvested on 6^{th}day of mounting to assess the commercial parameters *viz.*, cocoon weight, shell weight, shell percentage, filament length, denier and rendita as per the methods of Sonwalkar.

3. Results and Discussion

The data of the plant parameters such as total shoot/plant, total shoot length/ plant, leaf yield/h/y and leaf moisture are presented in table-1. The total shoots/plant were found to be 7.22, 7.91, 7.45 and 7.34 for Suvarna2, Suvarna3, Vishala and V1 respectively. Among the plant varieties, Suvarna3 (7.91) showed highest total shoots/ plants and Suvarna2 (7.22) showed the lowest total shoot/plant. The total shoot length/ plant were found to be 1320, 1370, 1360 and 1355 (m) for Suvarna2, Suvarna3, Vishala and V1 respectively. Among the plant varieties, Suvarna3 showed highest total shoot length/plants (1370m) and Suvarna2 showed the lowest total shoot length / plant (1320m). A total of 61300, 64500, 61345 and 61000kg of leaf yield /h/y were found for Suvarna2, Suvarna3, Vishala and V1 respectively. Among the plant varieties, Suvarna3 showed highest Leaf yield/h/y (64500 kg) andV1 showed the lowest Leaf yield /hectare/year (61000 kg). Leaf moisture were found to be 76.10, 79.90, 76.90 and 76.35% for Suvarna2, Suvarna3, Vishala and V1 respectively. Among the plant varieties, Suvarna3 showed highest Leaf moisture (79.90%) and Suvarna2 showed the lowest leaf moisture (76.10%). Compared to both Suvarna mulberry varieties studied vishala variety grown with a spacing 6^1X6^1 ft was recorded to have highest fresh leaf weight on par with V-1 mulberry variety (Ramakanth *et al*., 2001) [18]. During spring moisture content in fresh leaf was maximum (75.34%) in V1 which was significantly higher than the remaining genotypes, whereas, it ranged from (71.66%) in Mysore local and (72.46%) in S34. Shivashankar *et al.*, (2015) [22] also reported that the moisture content in fresh leaf was highest in the V1 compared to other varieties studied. In Suvarna varieties such as S-1, S-13, S-34 the quality of leaves were evident through moisture, protein and sugar contents (Machii *et al*., 1997) [10]. The numbers of shoots per plant were also recorded highest in paired row system with vishala mulberry variety, when compared to row system with suvarna mulberry variety (Murthy *et al*., 2013) [11]. The mulberry varieties S1, S2 and S36 were evaluated for growth and yield parameters and reported that, all the varieties differ significantly indifferent conditions (Gnanaraj *et al*., 2011) [6].

Table 1: Biometric parameters of mulberry genotypes (Suvarna 2, Suvarna 3, Vishala & V1)

Mulberry Varieties	Total (average) (shoots/plant (No)	Total Shoot length/plant (cm, @ 65th day of pruning)	Leaf yield/ha/yr, under irrigated condition (kg)	Leaf moisture (%)
Suvarna-2	7.22	1320	61300	76.10
Suvarna-3	7.91	1370	64500	79.90
Vishala	7.45	1360	61345	76.90
V1	7.34	1355	61000	76.35
Mean	7.48	1351	62036	77.31
SD±	0.30	21.74	1649.62	1.75
Fvalue	1.36	4.48	3.53	2.76
CD@5%	5.87	42.44	2751.73	11.63
CD@1%	18.23	131.70	8538.74	36.08
Significance	NS	NS	HS**	HS**

The total protein content of tender, medium and coarse leaves are presented in Table-2. The protein content of Suvarna3 plant leaves were found to be higher in tender leaves compared to that of medium and coarse leaves. Whereas, in Vishala and V1 the protein content was found to be highest in tender leaves than that of medium and coarse leaves. Among the plant varieties Suvarna3 showed the highest, while Suvarna2 showed the lowest percentage of total protein content.

Table 2: Biochemical analysis of Total proteins in mulberry genotypes (Suvarna 2, Suvarna 3, Vishala& V1) Unit: mg/g dry wt.

Parameters	Suvarna-2	Suvarna-3	Vishala	V1
Tender	170.32	190.42	175.28	172.53
Medium	161.78	179.29	163.57	161.57
Coarse	154.39	165.43	157.43	156.26
Mean	162.12	178.97	165.85	163.74
SD±	6.91	10.79	7.86	7.24
F value	254.83	830.57	234.88	321.64
CD@5%	7.84	6.81	9.29	7.33
CD@1%	40.30	35.00	47.72	37.64
Significance	HS**	HS**	HS**	HS**

The amount of reducing sugar in tender, medium and coarse leaves are presented in Table-3. The reducing sugar content in leaves of Suvarna2 variety was found to be 92.39, 89.57 and 86.29 mg/g in tender, medium and coarse leaves respectively. Whereas, the reducing sugar content of Suvarna3 was found to be 98.12, 92.32, 88.22 mg/g and in Vishala it was 95.64, 89.46, 85.94 mg/g in tender, medium and coarse leaves respectively. Similarly, in V1 the reducing sugar content was found to be 95.12, 88.49 and 84.46mg/g in tender medium and coarse leaves respectively. Among the plant varieties Suvarna3 showed the highest while Suvarna2 exhibited the lowest content of reducing sugar. In our study, varietal variation of protein, total sugar and reducing sugar was observed. A similar varietal variation of protein content and sugar content was observed by the studies conducted by Rao *et al.*, (2000).

Table 3: Biochemical analysis of Reducing sugars in mulberry genotypes (Suvarna 2, Suvarna 3, Vishala& V1) Unit: mg/g dry wt.

Parameters	Suvarna-2	Suvarna-3	Vishala	V1
Tender	92.39	98.12	95.64	95.12
Medium	89.57	92.32	89.46	88.49
Coarse	86.29	88.22	85.94	84.46
Mean	89.84	93.48	90.24	89.95
SD±	2.83	4.42	4.36	4.49
F value	50.12	81.49	78.06	50.00
CD@5%	7.08	8.76	8.82	11.25
CD@1%	36.40	45.02	45.33	57.81
Significance	NS	NS	NS	NS

The total sugar content of tender, medium and coarse is presented in Table-4. The total sugar content of Suvarna2 variety leaves was found to be 5.74, 5.61 and 5.26mg/g and a similar trend was observed in Suvarna3 variety, 5.88, 5.74 and 5.66 mg/g tender, medium and coarse leaves respectively. However, in Vishala the total sugar content was found to be 5.78 mg/g in tender, 5.71mg/g in medium and 5.59 mg/gin coarse with the highest in tender leaves. In V1 variety, the concentration of total sugar was found to be 5.75 mg/g in tender, 5.64 mg/g medium and 5.48 mg/g in coarse leaves. Among the plant varieties Suvarna3 showed the highest while Suvarna2 showed the lowest total sugar concentration.

Table 4: Biochemical analysis of Total sugars in mulberry genotypes (Suvarna 2, Suvarna 3, Vishala& V1) Unit: mg/g dry wt.

Parameters	Suvarna-2	Suvarna-3	Vishala	V1
Tender	5.74	5.88	5.78	5.75
Medium	5.61	5.74	5.71	5.64
Coarse	5.26	5.66	5.59	5.48
Mean	5.45	5.77	5.73	5.70
SD±	0.25	0.09	0.09	0.13
F value	0.98	1.76	2.90	4.75
CD@5%	2.28	0.72	0.67	0.85
CD@1%	11.72	3.70	3.46	4.39
Significance	NS	NS	NS	NS

The data of the cocoon parameters studies such as cocoon weight, shell weight, shell percentage is depicted in table 5. The weight of ten cocoons obtained from silkworms fed on Suvarna2, Suvarna3, Vishala and V1 was found to be 18.88, 19.76, 19.28 and 19.21 g respectively. Among the plant varieties, Suvarna3 showed highest cocoon weight (19.76g)

and Suvarna2 showed the lowest cocoon weight (18.88 g). Shell weight for the same was found to be 3.32, 3.52, 3.42 and 3.40 (g). Among the plant varieties, S3 showed highest shell weight (3.52 g) and Suvarna2 showed the lowest shell weight (3.32g). Shell percentage which is an important cocoon parameter was found to be 17.58, 17.81, 17.73 and 17.69% for cocoons obtained from silkworms fed on Suvarna2, Suvarna3, Vishala and V1 respectively. Among the plant varieties, Suvarna3 showed highest shell percentage (17.81%) and Suvarna2 showed the lowest Shell percentage (17.58%). The results of post cocoon parameters shows that the filament length of cocoons obtained from silkworm fed on Suvarna2, Suvarna3, Vishala and V1 was 1175, 1233, 1212 and1191meters respectively. Among the plant varieties, Suvarna3 showed highest filament length (1233 m) and Suvarna2 showed the lowest filament length (1175 m). Renditta for the same was found to be 7.76, 7.21, 7.41 and 7.45 (kg) for Suvarna2, Suvarna3, Vishala and V1 varieties respectively. Among the plant varieties, Suvarna2 showed highest renditta (7.76kg) and S3 showed the lowest renditta (7.21 kg). The denier of 2.70, 2.75, 2.74 and 2.72 was observed for the silk filament obtained from silkworm fed on Suvarna2, Suvarna3, Vishala and V1 mulberry varieties respectively. Among the plant varieties, Suvarna3 showed highest denier (2.75) and Suvarna2 showed the lowest denier (2.70) value. The highest single cocoon weight was observed in the larvae fed on leaves of V-1 followed by BER-1, S-54, S-36 and S-30 which were at par with each other. In a similar study the lowest single cocoon weight was recorded in larvae fed on BER-779 variety of mulberry (Patil *et al.,* 2004) [13]. Other researchers have emphasized the direct effect of mulberry leaf quality on food intake (Rahmathulla *et al*., 2004) [17]. Single shell weight of silkworm *B mori* L. fed on V-1 and S-36 were at par with each other. Rearing performance and economic traits of silkworm in the present study was found to have similar trend as that of the work done by Gawade *et al.*, (2006) [5].

Table 5: Commercial parameters of Bengaluru belli cocoons (IMH x CSR2) fed with different types of mulberry genotypes (Suvarna-2, Suvarna-3, Vishala& V1)

Parameters	10 Cocoon wt. (g)	10 Shell wt. (g)	10 Shell ratio (%)	Single Fil. length (m)	Renditta (Kg)	Denier
Suvarna2	18.88	3.32	17.58	1175	7.76	2.70
Suvarna3	19.76	3.52	17.81	1233	7.21	2.75
Vishala	19.28	3.42	17.73	1212	7.41	2.74
V1	19.21	3.40	17.69	1191	7.45	2.72
Mean	19.28	3.41	17.70	1202	7.45	2.72
SD±	0.80	0.15	0.55	37.27	0.22	0.02
F-value	1.52	0.49	1.61	3.09	30.41	156.9
CD@5%	2.60	0.54	1.76	104.00	0.26	0.01
CD@1%	8.08	1.67	5.45	322.72	0.81	0.03
Significance	NS	NS	NS	NS	HS**	HS**

4. Conclusion

The nutritive value of mulberry depends on many factors. The biomolecules like carbohydrates and protein play a major role in its growth, development and silk productivity. Enzymes along with other factors like hormones have a crucial role in the growth and metamorphosis of silkworm. The availability of biomolecules as nutrients has an important role in their conversion efficiency to silk. The plant parameters were found to be better in Suvarna3 and Vishala mulberry variety among the varieties studied. Biochemical studies were carried out for different varieties of mulberry to juxtapose the nutrition value in leaves. The IMH × CSR2 (Bangalore Belli) crossbreed silkworm larvae were fed with different varieties of mulberry leaves. Finally, the economic parameters of cocoons fed on different mulberry varieties were analyzed. Suvarna3 and Vishala have superior values among the mulberry varieties studied. Hence, based on the present study, it can be recommended that, Suvarna-3 and Vishala followed by V1 variety are having better quality parameters for silkworm rearing.

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