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ORIGINAL ARTICLE

Evaluation of Mulberry Genotypes for Chawki Rearing of Silkworm, *Bombyx mori* L. under Temperate Climatic Conditions

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ABSTRACT

The silkworm, *Bombyx mori* L., is a domesticated insect that feeds only on mulberry leaf (*Morus* spp.). The larval phase has five instars and undergoes moulting four times. The rearing of first two larval stages is known as chawki rearing and that of the other three stages as late age rearing. Chawki rearing though involves less feed, has great bearing on the growth of the larva during the late age and ultimately the economic parameters of silkworm. Further, there are different food requirement of the larva as far as its age is concerned. In order to evaluate different mulberry genotypes for their suitability to chawki rearing, a study was conducted on five different mulberry genotypes *viz.,* Ichinose, KNG, Goshoerami, Koksu-21 and Tr-10 wherein various nutritive parameters of the genotypes used in chawki rearing of silkworm and its consequent effect on the chawki larval characteristics was assessed which resulted in the identification of KNG as the best suitable mulberry genotype for chawki rearing of silkworms under Kashmir conditions.

Keywords: Silkworm, Chawki, Rearing, Mulberry.

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INTRODUCTION

Silkworm, *Bombyx mori* L. is a monophagous insect that feeds exclusively on mulberry leaves (*Morus* spp.) and the insect extracts all the nutrients from the leaf [1]. Since the nutritional status of leaf has a vital role in influencing the performance of different stages of silkworm [2], the selection of mulberry genotypes is an important aspect for better growth and development of silkworm and its economic parameters. The major determinants for the quality of leaf are generally the moisture content, nitrogen and carbohydrates.

The quantitative and qualitative feed requirements of the silkworm (*Bombyx mori* L.) are specific to the larval stage [16, 17]. The young age silkworms require soft and succulent leaf rich in moisture, protein and sugar content [3] whereas the late age worms require coarser leaf rich in protein having comparatively less moisture content [4-6]. Though, the leaf consumption is very less during young stage (0.33%), the increase in body weight, body size and silk gland weight is 400-500 times [1]. The success of chawki rearing depends on understanding of the requirements of silkworm at this stage and the selection of suitable leaf plays a major role in safeguarding health and robustness of chawki worms [50]. Varietal difference in terms of moisture content, crude protein, total minerals and total sugars are very common in mulberry [1, 29-234]. It is imperative to evaluate the mulberry genotypes available with respect to the silkworm age and the silkworm seems to be the best evaluator. Thus, the bioassay of mulberry genotypes using silkworm is the most effective way of evaluating the mulberry genotypes. Keeping this in mind, the present study was undertaken to evaluate five mulberry genotypes *viz.,* Ichinose, KNG, Goshoerami, Koksu-21 and Tr-10 for their suitability to chawki rearing of silkworm.

MATERIAL AND METHODS

The experiment was undertaken at College of Temperate Sericulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Mirgund during spring season on established plantation of five mulberry genotypes – Ichinose, KNG, Goshoerami, Koksu- 21 and Tr-10. The plantation was maintained as per the recommended package of practices [2]. The experiment started by brushing disease free layings of silkworm race APS-8. The chawki worms were reared as per the set procedure [12] under temperature 27±1ºC and relative humidity of 85±5 per cent. The worms were fed with the leaves of the above genotypes from brushing till second moult. Four feeds of mulberry leaves were given to the silkworm at 6 am, 10 am, 4 pm and 9 pm as recommended under four feed schedule. This experiment was laid in completely randomized block design with four replications for each treatment.

Simultaneously, samples of leaf used for rearing the chawki worms were also collected during the study. Tender leaves below the largest glossy leaf from the top of the shoot from three plants of each genotype of each replication. The samples were then shade-dried, packed in paper bags and dried in oven at 70ºC till constant weight was obtained. The samples were separately homogenised in a stainless steel blender to pass through 2 mm mesh sieve and were stored in airtight polythene bags for chemical analysis. The biochemical analysis of chawki leaf was conducted following standard procedures. The experimental was laid in randomized block design with four replications for each treatment. The observations on following parameters were recorded:-

a) Young age rearing parameters

Larval duration of first instar (hrs)

Larval duration of first instar was calculated by recording the total hours taken by the worms from brushing up to when the worms started settling for first moult.

Larval duration of second instar (hrs)

Larval duration of second instar was calculated by recording the total hours taken by the worms from initiation of second instar till they started settling for the second moult.

Weight of larvae (1st and 2nd instar) (g)

The weight of larvae was taken as the worms started settling for first and second moult by taking four samples of twenty randomly selected silkworms from each replicate of each treatment and weighed on a digital balance to determine average larval weight.

b) Chemo-assay of mulberry leaf:

Moisture content (%)

The moisture content (%) was calculated by the following formula:

$$\times 100 \quad \frac{\text{Weight of fresh leaves} - \text{Weight of dry leaves}}{\text{Weight of fresh leaves}}$$

Moisture retention capacity (%) after 6 hours

Moisture retention capacity (%) after 6 hours of harvest was recorded for all the selected genotypes under rearing conditions. The leaves were kept in the rearing trays, temperature of 27±1ºC and relative humidity of 85± 5 per cent was maintained in the rearing room.

The moisture retention capacity (%) after 6 hours was calculated by the following formula:

Moisture retention capacity (%) after 6 hours =

$$\frac{\text{Weight of leaves (after 6 hrs of harvest)} - \text{dry weight}}{\text{Weight of fresh leaves} - \text{Weight of dry leaves}} \times 100$$

Nitrogen (%)

It was determined by Microkjaldal's method as described by Jackson [20].

Phosphorus (%)

It was determined Vandomolybedate phosphoric acid yellow colour method [20].

Potassium (%)

It was determined by Flame photometer method as per the procedure outlined by Jackson [20].

Crude protein (%)

The crude protein was determined by multiplying the total nitrogen content by a factor of 6.25.

Carbohydrate (%)

The carbohydrate was determined by Anthrone method as per the procedure outline by Hedge and Hofreiter [16].

RESULTS AND DISCUSSION

Effect of mulberry genotypes on chawki rearing of silkworm, *Bombyx mori* L.

Larval duration (1st and 2nd instar)

The mulberry genotypes had a significant influence on first and second instar duration. Shortest first instar duration (77hrs) was recorded in the chawki batches fed with Ichinose, KNG and Koksu-21 and the longest instar duration (82hrs) in the batches fed with Goshoerami and Tr-10. Following the same trend, shortest second instar duration (73hrs) was recorded in case of larvae fed with KNG and Koksu-21 during chawki and the longest instar duration (77hrs) with Goshoerami and Tr-10 leaves (Table-1). In the present investigation, reduced larval duration in batches fed with Ichinose, KNG and Koksu-21 can be attributed to higher moisture and protein content in these genotypes which might have increased growth and development of silkworms. This is in agreement with the findings of Prakash *et al.* [39] who opined that leaf quality is a guiding factor for reducing larval period. The disparity in larval duration could be attributed to the feeding of nutritionally different mulberry genotypes to silkworms which is in agreement with the reports of Venkataramana *et al.* [50] who reported difference in larval duration of silkworm fed on different mulberry varieties. The richness of crude protein content might have also contributed towards shortening of larval duration which receives support from the findings of Hamzah *et al.* [15] who reported that protein supplemented mulberry leaves cause highly significant decreases in the larval duration besides enhancing the growth and development of larva.

Larval weight (1st and 2nd instar)

The larval weight during first two instars showed significant differences on feeding with different mulberry genotypes. First instar larval weight was maximum (0.09g) in silkworms fed with Koksu-21 and KNG where as it was minimum (0.06g) when reared on Ichinose, Tr-10 and Goshoerami. Similarly, maximum second instar larval weight (1.60g) was obtained when silkworms were reared on KNG and minimum (1.34g) when reared on Ichinose (Table-1). The increase in larval weight could be attributed to feeding of nutritionally superior mulberry genotype with higher levels of moisture, nitrogen, protein and carbohydrate content which might have improved the health and growth of silkworm. This can also be due to increased rates of digestive and oxidizing enzymes which help in utilizing the food and increasing food consumption. This receives support from the findings of Kasiviswanathan *et al.* [23], Mahmoud [30] and Ashour [4]. Chaluvachari and Bongale [11] reported that high values of moisture content in the leaves favoured increase in larval weight in case of the second instar with respect to ten tropical varieties. Similar results have been reported by Ravinder [43] and Murthy *et al.* [31] who concluded that first and second instar larval weight varies substantially with feeding different mulberry varieties. Chakrovorty *et al*. [10] have also reported higher weight of first and second instar larvae fed on soalu than that of the larvae fed on som and Digloti (*Litsaea salicifolia*) affirming the effect of differential nutrient content of feed on larval weight.

Babu *et al.* [5] has also reported that leaf protein content is positively correlated with larval weight. Further, nutritionally deficient diets directly influence the primary biochemical and physiological metabolism of insects which in turn disturb the detoxification mechanism [27] and hence the growth of insects.

Table 1: Effect of feeding leaf of different mulberry genotypes on larval parameters of silkworm *Bombyx mori* L. during chawki rearing

*Figures superscripted with the same letter in the column do not differ significantly

Parameter / Treatment	Instar Duration (hrs)		Larval weight (g/20 larvae)	
	I Instar	II Instar	I Instar	II Instar
G1 : Ichinose	77^{a}	74^{a}	0.06^{b}	1.34^{c}
G2 : KNG	77^{a}	73^{a}	0.09^{a}	1.60^{a}
G3 : Goshoerami	82^{b}	77^{b}	0.06^{b}	1.56^{b}
G4 : Koksu-21	77^{a}	73^{a}	0.09^{a}	1.56^{b}
G5 : Tr-10	82^{b}	77^{b}	0.06^{b}	1.56^{b}
C.D ($p \leq 0.05$)	**2.22**	**2.48**	**0.01**	**0.01**

* NS: Non Significant

Leaf quality variables of mulberry genotypes

Leaf moisture content and moisture retention capacity

Mulberry leaf moisture content and moisture retention capacity are key constituents determining the quality of the feed. They are documented to have positive impact on the growth of silkworm larvae especially during the of young age rearing [41] as they improve the palatability and digestibility of leaves by silkworm [23, 52] and have gustatory and olfactory stimulant effects [39]. Moisture content in among the different mulberry genotypes was significantly highest (78.89%) in KNG and lowest (76.11%).

However, Koksu-21 (77.03%), Goshoerami (76.91%), Tr-10 (76.15%) and Ichinose (76.11%) did not show any significant difference and occupied second rank. Similarly, the moisture retention capacity after six hours was highest (84.55%) in Goshoerami which was statistically at par with Ichinose (82.44%) and KNG (76.27%), Koksu-21(74.38%) and Tr-10 (72.85%) together occupied second rank (Table-2).

The variation in moisture content and moisture retention capacity of the genotypes under study can be attributed to the texture and anatomical features of the leaf. The results are in conformity with the finding of Kumar *et al*. (2018) who reported significant variation in the moisture content of ten mulberry genotypes under study. Moisture content is a genetic character and is influenced by available soil moisture and root proliferation character of the variety [40]. Similar findings were reported by [40,43, 45].

Since the water content is very low in newly hatched larvae and larva at this stage is less resistant to dryness, it requires mulberry leaves with good moisture content as ingestion rate of mulberry is slow but digestion is more [38]. Therefore, in order to feed chawki worms very succulent and nutritious leaves with high moisture and moisture retention capacity are needed during first two instars as this is a period of maximum water accumulation, failing which larvae will start desiccating and become undernourished. Among the genotypes, maximum moisture content was recorded in KNG and is thus suited for chawki rearing of silkworms. Moisture content of the leaves affects the energetic parameters such as assimilation and conversion efficiency of food which decrease with decreasing dietary moisture content of the leaves. Moisture content of the leaves decreases with corresponding increase in leaf growth and varieties [21]. Moisture retention capacity of a genotype not only depends upon the initial moisture content as reported by Khan (2006) but also on the thickness of upper cuticle and dimensions of stomata [49].

Table 2: Moisture content (%) and moisture retention capacity (%) as recorded in different mulberry genotypes

Parameter / Treatment	Moisture content (%)	Moisture retention capacity (%) after 6 hrs
G1 : Ichinose	76.11^{b}	82.44^{a} (9.13)
G2 : KNG	78.89^{a}	76.27^{b} (8.79)
G3 : Goshoerami	76.91^{b}	84.55^{a} (9.25)
G4 : Koksu-21	77.03^{b}	74.38^{b} (8.68)
G5 : Tr-10	76.15^{b}	72.10^{b} (8.55)
C.D ($p \leq 0.05$)	**1.70**	**0.40**

* Figures superscripted with the same letter/s in the column do not differ significantly

*Values in parenthesis are square root transformed values

Major nutrients

Nitrogen content of different mulberry genotypes ranged from 3.49 per cent in Goshoerami to 3.90 per cent in KNG which was followed by Koksu-21(3.67%). Further, Ichinose (3.56%) and Tr-10 (3.55%) were statistically at par with Koksu-21 and Goshoerami. There was also a significant difference amongst the five mulberry genotypes in the expression of phosphorus element in their leaves. The phosphorus content ranged from 0.20 per cent in Ichinose to 0.35 per cent in Tr-10 which was at par with Koksu-21 (0.34%). KNG with phosphorus content of 0.27 per cent occupied second rank. Potassium content recorded was lowest in Ichinose (1.20%) and highest in KNG (1.58%) followed by Koksu-21 (1.50%) Further, Tr-10 was statistically at par with Koksu-21(Table-3).

The variability in the leaf nutrient content of different genotypes indicates that the genotypes differ in their nutrient uptake ability from the soil which can be attributed to the difference in their root proliferation nature, profuseness of root system and availability of nutrients in the soil. The findings of the present study are in agreement with the findings[7-12, 34, 32].

Crude protein

The protein content of mulberry leaves is the paramount nutritional factor in determining the life cycle performance of silkworm [25-28]. It is one of the important constituent of cell for maintaining the metabolic activities of silkworm body and for silk and egg production [53].

Crude protein content differed significantly in various mulberry genotypes being lowest (21.81%) in Goshoerami and highest (24.38%) in KNG followed by Koksu-21 (22.94%). Further Ichinose (22.25%) and Tr-10 (22.19%) were statistically at par with Goshoerami and Koksu-21 (Table-3). The results are in line with observations of Rao *et al*. [42], Liu *et al.* [28], Ty *et al.* [49] who also reported existence of varietal differences in protein content of mulberry leaf.

Carbohydrate

Carbohydrates are derived on the basis of the amount of sugar and starch content available in leaves [6]. They are known to be the key source of energy for silkworm [17] and greater part of the it is used for physiological combustion and for making fat of the silkworm body [18].

The mulberry genotypes exhibited significant differences in terms of total carbohydrate content. Lowest carbohydrate content (18.55%) was reported in Ichinose and highest (21.48%) in Goshoerami followed by KNG (20.70%) and Koksu-21 (20.54%) (Table-3). Existence of varietal differences in total carbohydrates has also been reported by [35-53].

Table 3: Biochemical components in mulberry leaf of different genotypes

Parameter / Treatment	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Crude Proteins (%)	Carbohydrates (Dry matter %)
G1 : Ichinose	3.56^{bc}	0.20^{c}	1.20^{c} (1.48)	22.25^{bc}	18.55^{e}
G2 : KNG	3.90^{a}	0.27^{b}	1.58^{a} (1.61)	24.38^{a}	20.70^{b}
G3 : Goshoerami	3.49^{c}	0.23^{bc}	1.43^{b} (1.56)	21.81^{c}	21.48^{a}
G4 : Koksu-21	3.67^{b}	0.34^{a}	1.50^{b} (1.58)	22.94^{b}	20.54^{c}
G5 : Tr-10	3.55^{bc}	0.35^{a}	1.53^{a} (1.59)	22.19^{bc}	19.66^{d}
C.D ($p \leq 0.05$)	**0.15**	**0.04**	**0.02**	**0.86**	**0.04**

*Figures superscripted with the same letter/s in the column do not differ significantly

*Values in parenthesis are square root transformed values

CONCLUSION

Among the five mulberry genotypes *viz.,* Ichinose, KNG, Goshoerami, Koksu-21 and Tr-10 that were assessed using chemo and bio-assay for their impact on chawki rearing of silkworms, KNG demonstrated superior performance for most of the chawki rearing parameters *viz.,* shorter first instar duration (77hrs), shorter second instar duration (73hrs) and higher second instar larval weight (1.6g/20 larvae). The findings were substantiated by nutritional supremacy of KNG over the other mulberry genotype. In light of these results, it could be concluded that mulberry genotype KNG may be exploited for chawki rearing of silkworm (*Bombyx mori* L.).

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COMPETING INTEREST

The authors declare no conflict of interest in the publication of this manuscript.

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