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[Article]

Influence of Moisture Content of Mulberry Leaf on Growth and Silk Production in *Bombyx mori* L.

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Abstract
The influence of moisture content of mulberry leaf on the growth, development and moisture build up in the body of silkworm was studied by feeding with different maturity leaves to late age of silkworm larvae. Significantly higher larval moisture (79.78%), larval weight (65.65 g), pupal moisture (73.81%) was recorded in top tender leaf (high moisture) fed batches and least was recorded in the coarse leaf (lower moisture) fed batches. Significantly positive correlation between moisture content of leaf and larva to different variables like growth rate, larval weight, single cocoon weight, single shell weight and average filament length were recorded.

Key words: *Bombyx mori*, Development, Growth, Larval weight, Larval moisture, Leaf moisture

Introduction
Nutritional status of mulberry leaves largely influence the growth and development of *Bombyx mori* and subsequently affects the rearing and grainage parameters. The economics and ultimate success in sericulture depends on increased productivity in terms of good quality cocoon. The quality of mulberry leaves was determined mainly based on moisture content (Kumar *et al.,* 1996). Higher moisture content of mulberry leaves is one of the important factors and it has a direct effect on growth and development of silkworm (Paul *et al.,* 1992). Higher moisture content in mulberry leaves favorably affects their ingestion, digestion and also the assimilation of nutrients.

The role of moisture in determining sericultural productivity is generally recognised but doesn't appear to have been critically evaluated and properly appreciated. Hence, improvement in water /moisture content has to be made in soil as well as in mulberry leaf for obtaining maximum productivity in sericulture. Dietary water intake of silkworm is directly related to the moisture content of mulberry leaf and the amount of food intake (Beegum, 1992). The method and extent of dietary water intake and loss are complex, interrelated and continuously exchanging phenomenon (Delvi *et al.*, 1988).

Moisture content in mulberry leaves is considered as one of the criteria in estimating the leaf quality. Recently for labour saving in sericulture shoot feeding system was introduced for last instar of larva in which there is no selection of leaves for each instar and entire shoot can harvested and fed to larva.

Further sericulturists have a belief that tender leaf with high leaf moisture feeding during last instar of rearing causes grasserie diseases. Hence,the present study was undertaken to evaluate the effect of feeding different moisture content leaves (Different maturity leaves) on the growth and subsequent moisture accumulation in silkworm larval body (4^{th} and 5^{th} instar) and its influence on the silk production of popular Indian bivoltine hybrid silkworm.

Materials and Methods
The newly evolved bivoltine silkworm hybrid ($CSR_3 \times CSR_6$) was used as an experimental material by feeding with leaves of victory (V_1) mulberry variety. In the present study a mass experimental rearing was conducted up to third moult by standard rearing procedure developed for tropical sericulture especially for Indian bivoltine

hybrids and three feedings schedule (7 a.m., 3 p.m. and 10 p.m) in a day was followed (Krishnaswami, 1978; Rajan *et al.*, 2001). The temperature (27- 28°C and 23- 25°C) and humidity (85- 90% and 65- 70%) during young age and late age silkworm rearing respectively was maintained.

Late age silkworm larvae (4th and 5th instar) was selected for this experiment because the bulk food consumption (about 90%) takes place during these instars, which will be affected by moisture content in the leaves and in turn affect growth, development and silk production. More over, generally shoot feeding methods start from fourth instar onwards and in that method there is no selection and feeding of different maturity leaves for each instar. After third moult, the silkworm larvae were divided into six groups and each group into three replicate(Table 1). For each replicate 250 larvae were maintained and was fed with same quantity leaves (different maturity leaves) required for each instar and days as per the standard feeding schedule (Rajan. et al, 2001). Before feeding the moisture content of leaves was recorded replication wise for different treatments by using infrared moisture determination balance. Mulberry branches were divided into three regions, namely top tender (high moisture 75- 80%), middle medium (moisture content 65- 75%) and bottom coarse leaves (low moisture 60- 65%).

Observation on weight of larva, initial and final moisture percentage of larva was recorded for each replication and treatments at the end of each instar. The leaf moisture, larval moisture, cocoon shell moisture and pupal moisture was calculated by using infrared moisture determination balance.

The growth rate was calculated for both fourth and fifth instar larva by using following formulae: Growth rate= Final weight of larvae (g)/ Initial weight of larvae (g) × 100.

Table 1. The type of leaf fed for each feed during experimentation is given below.

Treatment	Type of leaves
T1	Tender leaf only
T2	Medium leaf only
T3	Coarse leaf feeding only
T4	First feeding tender; second feeding coarse and third feeding tender leaf in a day
T5	First feeding tender; second feeding medium and third feeding coarse leaf in day
T6	Mixer of tender, medium and coarse leaf for each feeding (1:1:1)

The matured silkworm larvae were mounted separately in a plastic collapsible mountage for spinning. After sixth day, cocoon harvesting was done and subsequently cocoon assessment was carried out to determine the quality of cocoon and calculated single cocoon weight and single shell weight. The sample of cocoon from each treatment and replication were subjected for reeling test to determine post cocoon characters and find out average filament length. The experiment was repeated three times and pooled data were statistically analyzed by ANOVA test. Correlation and partial regression analysis was also carried out to establish relation between moisture content in the mulberry leaves with silkworm larval weight and cocoon parameters.

Results and Discussions

Highly significant difference in larval duration was observed between the various treatments at the end of the IV instar. The larval duration for T1 was recorded significantly least (Table 2). While the larvae of T3 showed longest larval duration. Similar trend was recorded in 5th instar also. Larvae fed on tender leaves recorded reduced larval duration followed by larvae fed on mixed leaves. There was no significant difference observed between T5 and T6 (Table 3). Earlier studies proved that tender leaves are nutritionally richer than the coarse leaves (Krishnaswami *et al.*, 1970; Bongale *et al.*, 1991).

Significant difference in larval moisture was observed between T1, T2 and T3. Tender leaf fed larvae recorded maximum moisture content followed by medium leaf and least in coarse leaf fed batch. However, T5 and T6 did not show any significant difference with each other (Table 2). In fifth instar also tender leaves fed larvae had significantly higher moisture content than other treatments. Significant difference was found between T1 and T2 and T3 treatments respectively (Table 3). Krishnaswami *et al.* (1970) reported that tender leaves were not harmful for the silkworm. However, higher water content in mulberry leaves enhanced the metabolic activity and transportation of the nutrients in the whole silkworm body (Anonymous, 1980), which favoured the

Table 2. Effect of different moisture content leaves on 4th instar larval parameters of silkworm.

Treatments	Larval duration (hrs.)	Initial larval moisture (%)	Final larval moisture (%)	Initial larval weight (10 no.) (g)	Final larval weight (10 no.) (g)	Growth rate
T1 (79.24)	97	87.58	89.06	2.84	13.42	472.66
T2 (69.64)	104	87.58	86.57	2.84	11.07	389.69
T3 (61.82)	106	87.68	85.13	2.77	10.24	362.13
T4 (76.07)	99	87.52	88.33	2.85	12.46	436.97
T5 (71.26)	101	87.67	86.47	2.84	11.39	401.08
T6 (71.43)	101	87.59	86.72	2.84	11.56	406.50
± SE	0.318	-	0.574	-	0.070	8.371
F-Test	**	NS	**	NS	**	**

** Significant at 1% level; NS –Non significant

Note: Value in parentheses indicates average moisture content (%) of leaves.

growth and development of the larvae (Veda *et al.*, 1997). From the above results it is clear that higher leaf moisture leads to higher larval moisture in different stages. Pathak and Ashish (1998) also recorded a direct correlation between the moisture content of leaves, body water content of larvae and amount of urination. The sericulturists are of opinion that feeding silkworms with tender leaves in the late larval periods causes the grasserie disease (Vage & Ashoka, 1999). However, we found that feeding the larvae with tender leaves did not show any trace of diseases during late age silkworm rearing. Higher survibality percentage was recorded for tender leaf fed batches (94.48%) and earlier workers like Krishnaswami *et al.* (1970) made the same observation. Barman (1992) also reported that disease incidences were very low in case of tender leaf feeding batches.

Significantly highest larval weight at the end of 4th instar was recorded for T1 followed by T4 (Table 2). T2 and T3 showed significantly less larval weight when compared with others while T5 and T6 did not show any significant difference in larval weight. In fifth instar also it was found that tender leaf fed larvae had significantly higher weight than other treatments. While coarse leaf fed larvae showed significantly least weight. Comparison of T5 and T6 did not show any significant difference (Table 3). Vage and Ashoka (1999) observed higher larval weight and lower grasserie incidences during all the season when 5th instar larvae were fed with tender shoots up to three days, followed by matured shoots. Diwedi (1992) reported that feeding the silkworms with tender leaves in general gives better performance as compared to the other leaf qualities. Thus our results are in agreement with results reported by Sudo and Okajima. (1981), Basu *et al.* (1995) and Rashid *et al.* (1993). The soluble protein, sugars and moisture content are higher in top tender leaves and it might have contributed for increased larval weight in T1. The larvae, fed on the tender leaves, showed significantly highest larval weight at the ripening stage followed by T4.

Growth rate was calculated for the 4th instar larva and it was found that T1 treatment recorded maximum growth index followed by T4 treatment. While least growth rate was found T3. This might be due the reason

Table 3. Effect of different moisture content leaves on 5th instar larval parameters of silkworm.

Treat.	Larval duration (hrs.)	Initial larval moisture (%)	Final larval moisture (%)	Initial larval weight (g)(10 nos.)	Final larval weight (g)(10 nos.)	Growth rate
T1 (78.23)	166	87.37	79.78	13.13	65.65	499.95
T2 (68.49)	182	85.98	78.39	10.87	54.86	504.69
T3 (61.28)	192	84.63	76.54	10.00	47.93	479.23
T4 (75.58)	174	86.84	79.02	12.23	62.70	512.60
T5 (69.57)	180	86.58	78.45	11.01	56.50	513.09
T6 (68.57)	179	86.65	78.41	11.13	56.97	511.46
SE ±	0.279	0.140	0.171	0.141	0.259	4.095
F-Test	**	**	**	**	**	**

** Significant at 1% level; NS –Non significant

Note: Value in parentheses indicates average moisture content (%) of leaves.

Table 4. Effect of different moisture content leaves on Post cocoon parameters of silkworm.

Treatments	Cocoon shell Moisture	Pupal moisture	Survibality
T1 (78.23)	7.54	73.81	94.48
T2 (68.49)	7.13	71.82	92.29
T3 (61.28)	6.80	71.15	91.14
T4 (75.58)	7.16	72.97	94.87
T5 (69.57)	6.96	72.77	93.64
T6 (68.57)	7.59	72.39	91.54
SE ±	-	0.301	0.380
CD @ 5%	-	0.866	0.891
F-Test	NS	**	**

** Significant at 1% level; NS –Non significant
Note: Value in parentheses indicates average moisture content (%) of leaves.

that higher water and protein content have favoured the growth of the silkworms. Paul et al. (1992) also found that the growth of larvae increased with increase of leaf moisture and there was a positive correlation between growth of larvae and leaf moisture. When growth rate was calculated for the 5th instar silkworm body it was found that maximum weight was attained by T5 followed T4. While least growth index was recorded in T3 treatments (Table 3). It was noted that the larvae fed with tender (T1) leaves had shown less growth rate in 5th instar when compared with other treatments except T3 and T8 because tender leaf fed larva attained maximum growth in fourth instar itself.

Generally, it is expected that cocoons built up by the worms fed on tender leaves should have higher moisture content in cocoon shell. However there was no significant difference of shell moisture content observed among different treatments (Table 4). These results show that leaf or larval moisture content do not have any relation with moisture content of the cocoon shell.

Pupal moisture content indicated that tender leaves treated larvae had significantly highest moisture content than other treatments (Table 4). While least moisture content was recorded in T3.

Our results showed that there was a positive correlation between leaf moisture and larval moisture, growth index and final larval weight in 4th instar larvae. However, a negative correlation was observed between larval moisture and leaf moisture with larval duration in 4th instar (Table 5). Significantly negative correlation of larval duration and growth index was recorded in 5th instar. However, in case of other parameters significantly positive correlation was recorded (Table 6).

There was a significant partial regression coefficient of leaf moisture, larval moisture, larval weight and growth index and had influence on the cocoon weight, shell weight and average filament length (Table 7). Which indicated that these three variables exerted their influence on quality cocoon productivity parameters.

Our results showed that mulberry silkworm feeding with higher moisture content of leaves lead to more moisture build up in the body of silkworm and a higher productivity in sericulture. The above results obtained in the present study and discussion it is clear that tender leaves are not harmful to late age silkworm and sericulturist must

Table 5. Correlation of moisture content of leaves and larva (4th instar) with different larval parameters

Parameters	Leaf moisture	Larval duration	Initial larval moisture	Final moisture	Initial larval weight	Final larval weight	Growth rate
Leaf moisture	-	-0.760**	NS	0.904**	NS	0.777**	0.637**
Final larval moisture	0.904**	-0.753**	NS	-	NS	0.810**	0.638**

** Significant at 1% level

Table 6.Correlation of moisture content of leaves and larva (5th instar) with different larval and cocoon parameters

Parameters	Leaf moisture	Larval duration	ILM	FLM	ILW	FLW	Growth rate	SCW	Shell weight	AFL
Leaf moisture	-	-0.674**	0.810**	0.846**	0.882**	0.922**	-0.584**	0.784**	0.863**	0.809**
ILM	0.810**	-0.471**	-	0.807**	0.872**	0.875**	-0.675**	0.867**	0.911**	0.776**
FLM	0.846**	-0.469**	0.807**	-	0.826**	0.853**	-0.597**	0.806**	0.833**	0.730

** Significant at 1%level
ILM: Initial larval Moisture; FLM: Final Larval Moisture;
ILW: Initial larval weight; FLW: Final larval weight

Table 7. Standard regression coefficients of cocoon characters with independent variables

Parameters	Leaf moisture		Larval moisture		Larval weight		Growth rate	
	Coefficient	t' value	Coefficient	t' value	Coefficient	t' value	Coefficient	t' value
Cocoon weight	1.311 (0.009)	2.236**	1.245 (0.032)	2.347**	1.298 (0.031)	2.456**	2.378 (0.004)	2.345**
Shell weight	1.007 (0.002)	2.252**	1.892 (0.007)	3.831**	1.567 (0.007)	2.398**	1.981 (0.001)	2.234**
Average filament length	1.567 (4.792)	2.345**	1.231 (17.389)	2.652**	2.234 (17.334)	3.230**	1.651 (2.336)	3.979**

(Values in parenthesis are standard error) ** Significant at 1% level

have try to produce as much as succulent and tender leaves with higher moisture content. It was clear from the above study that in case of shoot feeding method, if the shoots are not given exchanging the tip and bottom direction, the growth and development of larvae in the same bed may differ and this can lead to create un size worms because of difference in nutritional quality of top and bottom leaves.

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