Advances in Bioresearch
Adv. Biores., Vol 14 (5) September 2023: 01-09

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.14.5.19

Advances in Bioresearch

ORIGINAL ARTICLE

Biochemical Profiling of Local Mulberry Variety: An Approach for Determination of Its Nutrient Value

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ABSTRACT
Mulberry leaf being the only preferred food material by silkworm Bombyx mori should necessarily accounts for good qualitative and quantitative aspects. The current study was formulated to enumerate information about the morphological and nutritional status of the local mulberry leaf supplied to silkworm during different instars, so as to draw conclusions about variation in morphology of mulberry and its relation with the biochemical constituents of the leaf. Morphology of leaf shape was recorded to vary from ovate to wide ovate and palamipatritate for different collections. Also, wide range of diversity was recorded for size and fresh and dry weight of the mulberry leaf. Biochemical analysis of studied mulberry leaves revealed maximum concentration of protein as 37.36mg and minimum as 28.29mg, carbohydrates as 37.36mg and 20.02mg respectively, lipids as 54%, crude fibre as 13.50% and ash content as 18%. From the current investigation it can be concluded that if silkworm larvae were supplied with mulberry leaf rich in appropriate nutrients, satisfactory yield results in the form of quality cocoon could be obtained. The study also serves as an initiating point towards the improvement of local mulberry varieties with the help of new breeding techniques.
Key words: *Carbohydrates, Lipids, Mulberry, Protein, Qualitative, Quantitative.*

Received 24.05.2023 Revised 01.07.2023 Accepted 11.08.2023

How to cite this article:
Suraksha Chanotra, Muzafar Ahmad Bhat and Avleen Kour. Biochemical Profiling Of Local Mulberry Variety: An Approach for Determination of Its Nutrient Value . Adv. Biores., Vol 14 (5) September 2023: 01-09.

INTRODUCTION

Sericulture is one of the most important economic sectors of the agriculture field involves a continuous series of integral activities including moriculture, rearing of lepidopteron larvae and various industrial aspects of manufacturing of fabric from fibres. Mulberry leaf being the most important and integral part of successful silkworm rearing contributes about 9.7% of the overall attributes [1&2]. Thus, holds unique position and interest among the mulberry breeders. Unique nutritional and biochemical characteristics of mulberry leaf which makes the silkworm to feed on it particularly the Morin pigment for consumption by lepidopteron larvae of silkworm *Bombyx mori* L. The optimum composition of mulberry leaf required for good rearing performance relies on the amount of available ash and moisture content of leaves that should be in the range of 8.12 to 12.6% and 72.16 to 79.35% [3].

Mulberry leaves supplied to the silkworm larvae is the only nutritional source providing all essential major and minor components to the silkworm and the ideal range constitutes crude protein about 18.41 to 24.63%, total carbohydrates about 47.27 to 56.42% and crude fat about 4.24 to 6.57% . Fiber, cyanide and tannin in mulberry leaf were reported to be in the range of 8.74 to 13.70%, 1.01 to 2.14mg/kg and 3.54 to 5.32 mg/kg, respectively by Anonymous [4 & 5]. *Morus* leaves contain various organic and biochemical constituents including sugars, and polysaccharides which stimulates the synthesis of glycosidase enzymes in mammalian cells. 1-deoxynojirimycin (DNJ) is one of the most significant bio constituent derived from *Morus* species. Dried mulberry leaves have been reported to contain DNJ in concentrations of 1.389 to 3.483mg/g and 0.1341 to 1.472mg/g [6]. A number of anti-oxidative constituents have also been reported in chemical analysis of mulberry leaf [7]. Studies on qualitative parameters revealed mulberry leaf to contain total phenols and total flavonoids in the range of 16.21 to 24.37 mg. in addition, gallic acid equivalent /g and 26.41 to 31.28 mg rutin equivalent/g respectively [8].

Hence, current experiment was undertaken to draw picture of biochemical profile of mulberry leaf for making effective selection for silkworm rearing.

MATERIAL AND METHODS

I. Morphological analysis of the leaf

For morphological analysis various parameters such as leaf Shape, size, fresh and dry leaf weight, moisture percentage and moisture retention capacity of the selected leaf was determined by visual examination method [9].

II. Biochemical analysis of the leaf

a) Total Protein Content (%)

The total content of available protein in the studied leaf sample was estimated by Biuret method [10] with slight modification as given below:

PROCOTOL

With the help of pipette take out 0.0, 0.2, 0.4, 0.6, 0.8 and 1ml of working standard protein solution in fresh test tubes.

Take out 1ml of mulberry leaf extract sample in fresh test tube.

⇩

Make the final volume to 1ml in all the labeled test tubes with distilled water. Keep a test tube as blank containing 1ml of distilled water.

⇩

Add 3ml of Biuret reagent to the series of all test tubes including blank having distilled water.

⇩

Shake well the mixture by vortexing the test tubes and place them in water bath for 10 mins at 37°C.
Let the content to cool down and records the values of absorbance at 540 nm against blank.

⇩

Make a standard curve by plotting values of protein concentration along X-axis and values of absorbance at 549nm along Y-axis. Then

⇩

With the help of the standard curve thus formed, calculate the concentration of available protein content in the studied leaf sample.

⇩

b) Determination of total carbohydrates: The total carbohydrates was estimated by Anthrone method [11] with slight modification as given below:

⇩

PROTOCOL:

Pipette out few drops of stock solution (200µg /ml) of glucose in a series of test tubes and add distilled water to make up the final volume of 1 ml.

⇩

Consider test tube no. 1 as blank and tubes 2 through 9 will be used for preparation of standard curve. Test tubes from serial 10-15 are meant for experiment purpose only for some other samples.

⇩

To each tube put 5 ml of anthrone reagent (supplied) and let the solution to mix well by vortexing the testes tubes for 5 mins. Let the test tubes to cool down at room temperature.

⇩

Incubate the sealed test tubes at 90ºC for 17 minutes in warm water bath for 10 minutes.

⇩

Let the test tubes to cool at room temp. and determine the optical density at 620nm against blank for plotting standard curve for values of absorbance vs. µg glucose.

c) Lipids: lipids will be extracted from Soxhlet method [12] with slight modification as given below:

PROTOCOL

1) Wipe up or clean the test tubes and other glassware with petroleum spirit, oven dry them for 30 mins at 120ºC followed by cooling at room temp.
2) Seal the bottom of 100ml beaker with cotton plug, similarly put cotton plug on the bottom of extraction thimble and stand the thimble in centre of the beaker.
3) Put 5 g of sample into the thimble, pour 1-1.5g of sand and mix the two by continuous stirring with the help of glass rod and place cotton wool in the top of the thimble. Oven dry the sample for 5 hours at 102ºC and let the sample to cool in a desiccator.
4) Take out the cotton plug from bottom of the beaker and place it on top of the thimble and insert the thimble in a Soxhlet liquid/solid extractor.
5) Pour 90 ml of petroleum spirit into 150 ml round bottom flask.
6) Place the extraction unit in warm water bath with temp. 60 ºC.
7) Boil the solution to vapour formation and collect the drops in a container. Make the adjustment of the condenser so as to release 6 drops of sample per second from the outlet of the condenser.
8) Carry out the process for 6 hours. Take out the thimble from extractor unit and transfer the collected sample to 100 ml beaker.
9) Stir the sample with glass rod and again place the sample to thimble in the extractor. Clean the beaker with petroleum spirit and put few drops of rinsing solution into the extract and continues the process for 2 hour more.
10) Stop the heat and remove the extractor and condenser. Again put the flask on heating burner till solvent evaporates off.
11) Oven dry the contents in the flask by placing it in oven at 120ºC for 1-2 hours till a constant weight is achieved.
12) Let the flask to cool in a desiccator and weigh the sample for total lipid contents.

d) Crude fibre content: Crude fibre in the studied leaf sample was estimated by Titrimetric method of analysis with slight modification as given below:

PROTOCOL:

Treat 2g of the powdered leaf sample with ether or petroleum ether for fat removal by initial boiling of the sample at 35-38ºC and final heating at 52ºC.

⇩

Boil 2g of treated sample with 200 ml of sulphuric acid for 30 mins with bumping chips.

⇩

Filter the solution by using muslin cloth and wash with boiling water to remove the acidic content from it. Heat the sample solution with 200 ml of sodium hydroxide solution for 30 mins.

⇩

Again filter the solution by using muslin cloth and rinse with 25 ml of boiling 1.25%H_2SO_4,50 ml of water and 25 ml of alcohol.

⇩

Place the residual content in ashing dish (pre weighed dish W_1)

⇩

Oven dry the residue at 130± 2ºC for 2 hours and let the dish to cool in a desiccator (W_2).

⇩

Ignite the sample at 600 ± 15°C for 30 mins and let it cool in desiccator and weigh again for W_3

⇩

$$\text{Crude fibre content} = \frac{(W_2 - W_1) - (W_3 - W_1)}{W_1} \times 100$$

Where,

W_1= Pre-ignition weight of the leaf sample
W_2= Oven dry weight of the leaf sample
W_3= Post-ignition weight of the leaf sample

e) Ash Content: The ash content in the studied sample was determined by placing the samples in furnace for initial and final weight.

$$\frac{\text{Initial weight- Final (Dry) weight}}{\text{Initial weight}} \times 100$$

RESULTS

The results recorded for various morpho-physiological parameters of mulberry leaf are as given below:

I. Morpho-physiological studies of mulberry leaf:

Leaf shape and size: During the feeding period, mulberry leaves supplied to the silkworm have been recorded to possess variable shapes and size. The most frequently utilised leaves were recorded to be of oval, ovate, wide-ovate and palami-petrate shapes (Fig.-01) measuring about 15cm^2 to 93cm^2 size (Table-01).

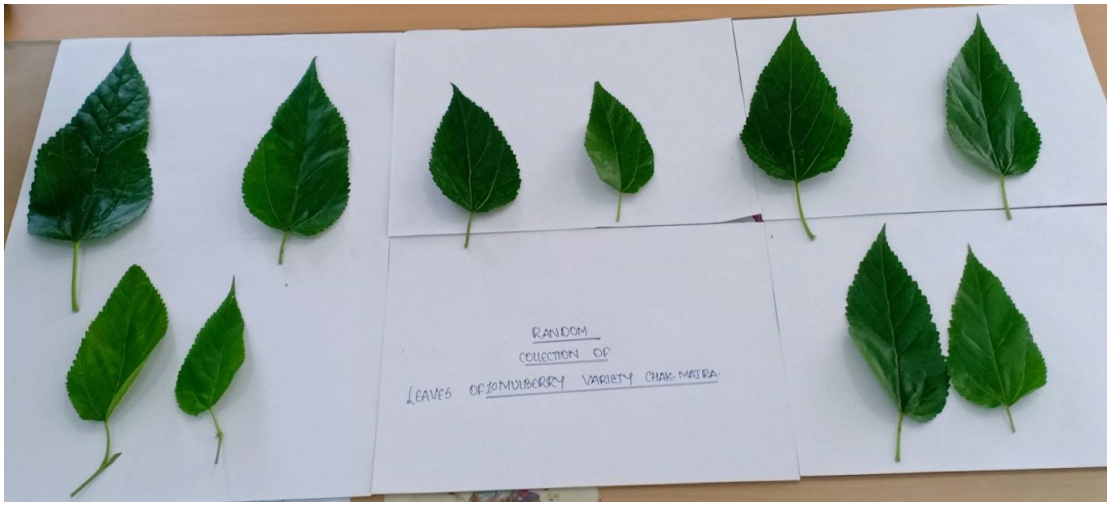

Fig-01:Random Collection of Mulberry Leaves selected for Feeding the Chawki Worms.

Table-01: Readings of the values indicating the leaf size of different mulberry varieties used in different instars.

SIZE OF MULBERRY LEAF USED IN DIFFERENT INSTARS (cm^2)					
S.NO.	**1st Instar (cm^2)**	**2nd Instar (cm^2)**	**3rd Instar (cm^2)**	**4th Instar (cm^2)**	**5th Instar (cm^2)**
Day 01	32±3.06b	67±18.78a	42±6.65b	93±17.61b	37±14.38a
Day 02	21±3.06b	73±18.78b	55±6.65c	82±17.61c	55±14.38b
Day 03	40±3.06b	79±18.78bc**	51±6.65c	49±17.61c	64±14.38b
Day 04	-	78±18.78c	-	83±17.61c	71±14.38bc**
Day 05	-	66±18.78c	-	90±17.61c	63±14.38bc**
Day 06	-	15±18.78c	-	-	83±14.38bc**
Day 07	-	42±18.78c	-	-	69±14.38c
Values are Means ± SE					
Means within a column followed by different letters are significantly different at P<0.01 ** Highly Significant data at p=0.05					

Fresh and dry weight of the leaf: Fresh mulberry leaves are considered to be the best food for silkworm larvae and the fresh weight of the leaf is directly proportional to the content of available nutrient and moisture present in it. Therefore, fresh and dry weight of mulberry leaf is important for determining the moisture percentage and moisture retention capacity of the mulberry variety. Maximum fresh leaf weight was recorded during the 7th day of 5th instar as 19.51g and minimum as 1.03g during 3rd day of 1st instar. Similarly maximum dry weight was recorded as 1.86g during 5th day of 5th instar and minimum as 0.11g during 7th day of 2nd instar (Table-02).

Table-02: Readings of the values indicating the fresh and dry weight of different mulberry varieties used in different instars.

S.NO.	1st Instar		2nd Instar		3th Instar		4th Instar		5th Instar	
	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry Weight (g)	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
Day 01	1.64a	0.54b	2.50a	1.09a	4.46a	1.05b	2.31a	0.65b	2.68a	0.17a
Day 02	1.08a	0.42c	1.98a	0.56b	2.51a	0.56b	3.61ab**	1.02bc**	4.77b	0.56ab**
Day 03	1.03b	0.11c	2.36b	1.12b	2.40b	0.34c	3.91ab**	1.01c	5.01b	0.59ab**
Day 04	-	-	2.19b	1.02b	-	-	2.86bc**	0.23c	10.51b	1.84bc**
Day 05	-	-	2.15bc**	1.07b	-	-	3.62c	0.95c	15.60c	1.86bcd***
Day 06	-	-	1.43cd**	0.86c	-	-	-	-	12.36c	1.29cd**
Day 07	-	-	1.82d	0.15c	-	-	-	-	19.51d	1.98c
P=0.01	*	*	*	*	*	*	*	*	*	*
Means ± SE	1.06	0.26	0.66	0.30	0.29	0.68	2.14	0.16	8.11	0.23

Means within a column followed by different letters are significantly different at P<0.01**, *** Significant data at p=0.05

Moisture percentage and Moisture retention capacity (MRC) of the mulberry leaf: Moisture percentage of different mulberry leaves supplied to the worms of different instars during the current study have been recorded as maximum 93.51 per cent (2nd day 5th instar) and minimum as 14.81 per cent (2nd day 1st instar) as given in Table-03 and maximum MRC percentage was recorded as 91.24 per cent in day 2nd of 5th instar and minimum as 18.71 per cent in day 1st of 2nd instar (Table-04).

Table – 03: Readings of the values indicating the moisture percentage of different mulberry varieties used in different instars.

S.NO.	1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar
Day 01	48.17b	32.41a	42.60b	22.71b	68.28a
Day 02	14.81c	80.80a	35.52b	65.47c	93.51a
Day 03	59.22d	35.16b	62.68c	63.63c	69.66b
Day 04	-	65.75b	-	67.95c	86.60b
Day 05	-	69.30bc**	-	70.89c	88.07b
Day 06	-	55.24bc**	-	-	89.78b
Day 07	-	62.08c	-	-	89.85b
P=0.01	*	*	*	*	*
Means ± SE	±5.04	±5.23	±6.47	±9.06	±3.44

Means within a column followed by different letters are significantly different at P<0.01
, * Significant data at p=0.05

Table-04: Values indicating the moisture retention capacity of different mulberry varieties used in different instars.

S.NO.	1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar
Day 01	36.77b	18.71a	37.07b	57.05b	51.05a
Day 02	21.14c	87.41a	23.38c	66.93bc**	91.24a
Day 03	57.22d	33.23b	59.26d	61.82bc**	71.83bc**
Day 04	-	79.08bc**	-	62.95cd*8	79.54bc**
Day 05	-	67.15bc**	-	77.91d	77.92bc**
Day 06	-	69.89bc**	-	-	80.16bc**
Day 07	-	75.65c	-	-	78.38c
P=0.01	*	*	*	*	*
Means ± SE	±6.04	±8.23	±10.47	±11.06	±13.74

Means within a column followed by different letters are significantly different at P<0.01
** Highly Significant data at p=0.05

II. Biochemical analysis of mulberry leaf

The results recorded for biochemical analysis of mulberry leaf supplied to the silkworm under study are as given below:

a) PROTIEN ESTIMATION IN MULBERRY LEAF

For the studied mulberry leaf sample, notable protein content been estimated by Biuret method (Fig. 02). The mulberry leaf sample extracts are subjected to protein estimation in the form of absorbance values at photo calorimeter at 540 absorbance (A_{540}) and the values recorded for the studied samples varied from 28 to 36nm (Table-05) and in the form of graph (Fig-03) depicting standard curve for protein by Biuret method.

Fig.-02: A-J (a:preparation of reagents, b: weighing of leaf tissue, c: grinding of leaf sample, d: Poeredere leaf tissue, e: adding the leaf sample into reagent solution, f: seiving through musslen cloth, g: washing under tap water, h: air dringy the sample, i: tranfering to the burret soultion anf j: sample analysis on photocalori meter) Steps carried out during protein estimation by Biuret Method.

Table- 05: Observations recorded for absorbance of protein values in mulberry leaf at A_{540}

Volume of BSA Solution(ml)	Volume of Distilled water(ml)	Concentration of standard protein (mg)	Volume of Biuret reagent(ml)	Incubate at 37°C for 10 min and cool to room temperature	A_{540}
0.0	1.0	0	3		0.00
0.2	0.8	1	3		28.29
0.4	0.6	2	3		31.50
0.6	0.4	3	3		33.75
0.8	0.2	4	3		34.19
1.0	0.0	5	3		36.42

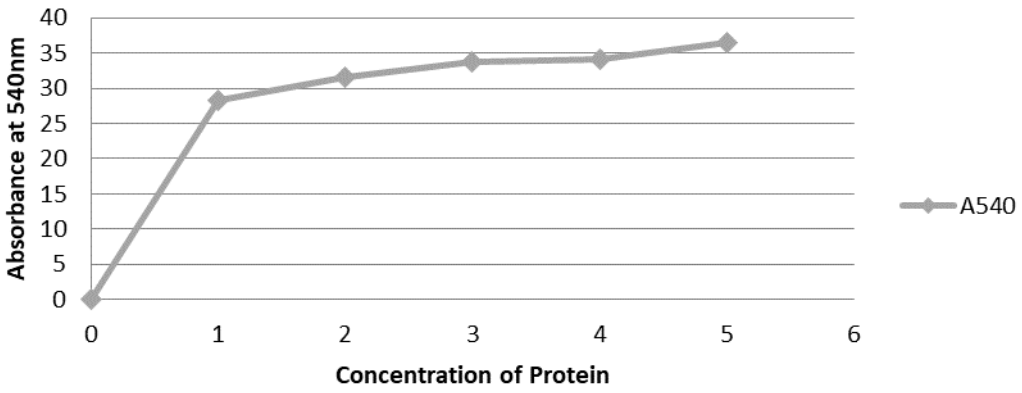

Fig.-03: Graph depicting standard curve for protein by Biuret method in mulberry leaf.

b) ESTIMATION OF CARBOHYDRATE IN MULBERRY LEAF

Carbohydrate content in the studied mulberry leaf sample have been isolated and estimated by Anthrone method (Table-06). The extract of leaf sample was subjected to data recorded at absorbance range of A_{620} and following values have been recorded for the current sample (Fig. 04)

Table-06: Observations recorded for absorbance of carbohydrate values in mulberry leaf at A_{540}

S.NO	Glucose		DH_2O (µL)	Anthrone reagent (mL)	Incubate at 90°C for 17 mins or 100°C for 10 mins	A_{620}
	(µL)	(µg)				
1	00	00	00	00		00
2	-	-	1000	5		20.02
3	50	10	950	5		24.00
4	100	20	900	5		25.02
5	300	60	800	5		27.02
6	300	60	700	5		27.64
7	400	80	600	5		27.00
8	500	100	500	5		28.78
9	750	150	250	5		26.47
10	1000	200	200	5		23.44

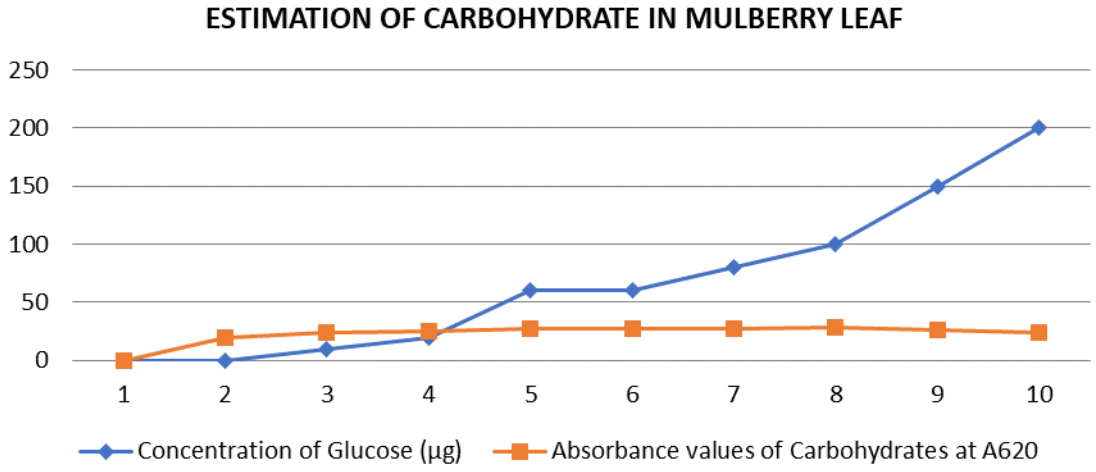

Fig.04: Graph depicting standard curve for carbohydrate content by Anthrone method in mulberry leaf.

c) Lipid content (%): The mulberry leaves utilised in the experimental silkworm rearing, the lipid content was detected by Soxhlet method and the studied sample revealed 54% availability of lipid content in it.

d) Crude fibre content (%): Crude fibre constitutes the very important part of mulberry leaf as the biochemical nature of leaf is attribute per cent by crude fibre alone. In the studied sample the content of crude fibre was recorded to be 13.5%.

a) Ash content (%): Ash content of mulberry leaf depicts the suitability of leaf for the worm and a critical percentage of 25±3% depicts the most appropriate range of ash content in the mulberry leaf. In the studied leaf sample the ash content was recorded to be 18% showing the suitability of leaves for silkworm larvae.

DISCUSSION

Morpho-physiological studies revealed variable types of leaf shapes and size ranging from ovate to wide-ovate and maximum size of about 90cm^2 and minimum of about 15cm^2. Thus, revealing significant variability in mulberry genotypes which shows close conformity to the earlier results of Chanotra *et al.,* [14 & 15]. Maximum values recorded for fresh leaf weight as 15.60g and minimum as 0.11g which showed close conformity to the earlier results of Chikkalingaiah *et al.,*[16] and Craiciu, A. [17] who had recorded values as fresh weight leaf (63.7g) followed for actual leaf area (30cm^2) and dry leaf weight (17.8g).

In current study, the maximum values recorded for moisture retention capacity of different mulberry leaves were recorded to be 79.54%. Earlier, Mallikarjunappa *et al.,* [18] evaluated the moisture retention capacity of five mulberry varieties and recorded relatively higher values in Viswa (77.74%) and S-36 (77.24%), leaf moisture loss at 6 hours after harvest was significantly less in S-36 and S-30 (13.46 and 13.92%) respectively. Sujathamma *et al.,* [19] too recorded presence of huge variability in values of moisture content of fresh leaves ranging from 64.41 to 76.94%.

The studied leaf sample revealed the available protein content in the range of 28.29 to 36.42 at A540 absorbance on 1 and 5mg of protein concentration which lies in close conformity to the results presented earlier by Thangamani *et al.,* [20]. The lipid content in mulberry leaf was recorded as 54% and 13.5% of crude fibre content. The current results are strongly in agreement with that of the Vijayashekara *et al.,*[21] and Viswanath, S. [22] who reported that increase in lipid content attributes in mulberry leaf, enhance silk production. The ash content was recorded to be 18% which lies in agreement with earlier reports of Absar *et al.*, [23].

CONCLUSION

The morphological parameters of mulberry leaf revealed the leaf shape to vary from ovate to wide ovate and palamipatritate for different verities. Also, wide range of diversity was recorded for size and fresh and dry weight of the mulberry leaf. In biochemical analysis of mulberry leaves protein content was recorded with maximum concentration as 37.36mg and minimum as 28.29mg, carbohydrates with maximum concentration as 37.36mg and minimum as 20.02mg, lipids as 54%, crude fibre as 13.50% and ash content as 18% in the studied mulberry leaf.

Conflict of Interest: None.

ACKNOWLEDGEMENTS:

The authors are highly thankful to Prof. Dipankar Sengupta, Director Poonch campus, university of Jammu for providing lab facilities for undertaking this experiment.

REFERENCES

1. Gupta, S.K. and Dubey, R.K. (2021). Environmental factors and rearing techniques affecting the rearing of silkworm and cocoon production of *Bombyx mori* Linn. *Acta Entomology and Zoology.* 2021; 2(2): 62-67.
2. Chanotra, S. and Angotra, J. (2022). Implications of Meteorological Forecasting for Accelerating the Success Rate in Sericulture: New Avenues in Seri-industry: A Review. *Agricultural Reviews.* DOI: 10.18805/ag.R-2513.
3. Anonymous. 2013. Studies on positional mineral constituents of mulberry leaves. *Central Sericultural Research & Training Institute, Mysore, India*. 81-82.
4. Sastry, D. H. and Edwards, C. A. (2018). Studies on varietal differences with regard to the degree of moisture loss from the leaves. *International Journal of Sericulture*. 4(**67**): 327-354.
5. Matei, Y. T. and House, H. L. 2019. Studies on mulberry leaf protein value of five mulberry varieties. *International Journal of Sericulture*. 2(**43**): 29-39.
6. Susheelamma, R. T., Folch, J., Lees, M. and Stanley, S. G. H. (2019). Biochemical analysis of mulberry leaf. *International Journal of Sericulture*. 3(**226**): 497-509.
7. Patil, R.M., Alagumalai, K., Hepshyba, C. S. S. and Ramaraj, P. (2015). Studies on quanitative parameters of the mulberry leaf. *Journal of Sericulture*. 30(**6**): 10–11.

8. Pathak, P., Mahalingam, C.A. and shashidhar, K.R. (2017). Biochemical constituent of mulberry leaf. *Indian. J. Sericulture*. 4(**35**): 3-5.
9. Lawrence, G. H. M. (1967). Taxonomy of Vascular Plants, The Mac Millan Company, *New York, U. S. A*. pp. 742-744.
10. Kalaydzhiev, H., Ivanova, H., Uzunova, G., Manolov, I. and Chalova, V. (2018). Biuret and bradford methods suitability for protein quantification in rapeseed meal. The Serbian Journal of Agricultural Sciences. 67 (1): 87-92.
11. Hedge, J.E. and Hofreiter, B.T. (1962) Carbohydrate chemistry 17. Whistler, R.L. and Be Miller, J. N., Eds., *Academic Press, New York.*
12. Ingle KP, Deshmukh AG, Padole DA, Dudhare MS, Moharil MP, Khelurkar VC. (2017). Phytochemicals: Extraction methods, identification, and detection of bioactive compounds from plant extracts. *J Pharmacogn Phytochem.* 6: 32–6.
13. Kim, Gyo-Nam; Jang and Hae-Dong. (2011). Flavonol content in the water extract of the mulberry (*Morus alba* L.) leaf and their antioxidant capacities. *PubMed.* 2011-08-01.
14. Chanotra, S., Bali, K. R. and Bali, K. 2019a. Morpho-physiological characterization of mulberry genotypes (*Morus spp*.) for inheritance in future breeding programme. *Journal of Pharmacognosy and Phytochemistry.* 8(**1**): 2695-2701.
15. Chanotra, S., Bali. K. R., Salgotra, K. R., Bali, K. and Rai, K. G. (2019b). Evaluation of Genetic Diversity in Selected Morus Genotypes Using Molecular Markers. *Journal of Pharmacognosy and Phytochemistry.* 8(**1**): 2617-2622.
16. Chikkalingaiah, W. K., Centhilnayaki, N., Selvisabhanayakam and Mathivanan, V. 2015. Development of issue on Mulberry cultivation in relation of silk production. *Indian Journal of Environment and Ecoplan*. 8(**1**): 111-117.
17. Craiciu, A. 2011. Evaluation of different hybrids and local mulberry varieties for their yield and quality attributes. *Journal of Genetics*. 1(**7**):27-33.
18. Mallikarjunappa, E. T. and Etebari, K. (2019). Effect of enrichment mulberry leaves (*Morus alba*) with some vitamins and nitrogenous compounds on some economic traits and physiological characters of silkworm, *Bombyx mori L. International Journal of Entomology*. 3(**8**): 81-87.
19. Sujathamma, S. F., Fathima, P. S., Das, P. K., katiyar, R. S., Himanthraj, M. T. and Pallavi, S. N. (2019). Studies on 23 elite mulberry genotypes. *Indian Journal of Sericulture.* 2(**35**): 99-103.
20. Thangamani, F. J.., Ghosh M., Chottopadhya, G. N., Baral, K. and Munsi, P. S. (2020). Biochemical analysis of mulberry leaf. *International Journal of Sericulture*. 3(**67**): 64-68.
21. Vijayashekara, S. H. and Chaluvachari. (2016). Leaf quality evaluations of few selected mulberry varieties. *Indian Journal of Sericulture.* 5(**220**): 9-13.
22. Viswanath, S. (2018). Nutritional quality of mulberry leaf. *Sericulture Scientific Publishers*. 5(**67**): 127-130.
23. Absar, G.K. and Kale, R. D. (2015). Studies on Micronutrient effect on mulberry varieties. *Indian Journal of Sericulture.* 4(**24**): 1317- 1320.