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Effect of Micronutrients on the Biochemical Contents of Mulberry (*Morus Alba* L. Moraceae) Leaves

GRACE MARIN[1], PAULRAJ BLESSY[1], HELAN MARY[1], SUBRAMANIAN ARIVOLI[2] and SAMUEL TENNYSON[3]*

[1]Department of Zoology, Scott Christian College, Nagercoil Tamil Nadu, India.
[2]Department of Zoology, Thiruvalluvar University, Vellore, Tamil Nadu, India.
[3]Department of Zoology, Madras Christian College, Chennai, Tamil Nadu, India.

Abstract

The biochemical contents of mulberry leaf play a vital role in the field of moriculture and sericulture. Qualitative and quantitative production of silk depends upon the quality of mulberry leaves consumed by silkworm. The present study deals with the effect of micronutrients on the biochemical contents of the mulberry leaves. Field experiments were conducted with 12 treatments including a control (T_0 to T_{11}). Each treatment replicated thrice was supplemented with the desired quantity of micronutrients, *viz*., zinc, copper and iron in the form of their respective sulphates, either in single or in combination. The biochemical contents of the mulberry leaves *viz*., leaf moisture, leaf moisture retention, chlorophyll, carbohydrate, protein, amino acid and nitrogen were analysed. At the 45th and 60th day of mulberry leaf pruning, maximum leaf moisture content was recorded in T_8($CuSO_4$ 10Kg/ha + $ZnSO_4$ 10Kg/ha + $FeSO_4$ 20Kg/ha) which increased by 10.30% and 12.39% over control, and maximum leaf moisture retention percentage in T_8 which increased by 29.32% and 11.02% over control respectively. Maximum chlorophyll 'a', 'b' and total chlorophyll content was recorded in T_8, T_7($CuSO_4$ 5Kg/ha + $ZnSO_4$ 5Kg/ha + $FeSO_4$ 10Kg/ha) and T_8 with an increase of 18.42%, 48.71% and 21.87% over control, respectively at the 45th day of pruning, and in T_8, T_8 and T_9($CuSO_4$ 15Kg/ha + $ZnSO_4$ 15Kg/ha + $FeSO_4$ 30Kg/ha) with an increase of 29.05%, 18.52% and 26.25% over control, respectively at the 60th day of pruning. Maximum carbohydrate and protein content were noted in T_{10}($CuSO_4$ 20Kg/ha + $ZnSO_4$ 20Kg/ha + $FeSO_4$ 40Kg/ha) and T_9 with an increase of 22.25% and 60.56%, respectively over control at the 45th day of pruning and in T_9 and T_8 with

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CONTACT Samuel Tennyson samtennyson@gmail.com Department of Zoology, Madras Christian College, Chennai, Tamil Nadu, India.

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an increase of 24.99% and 70.69% over control at the 60^{th} day of pruning, respectively. Free amino acid and nitrogen content was observed maximum in T_8 and T_9 with an increase of 112.76% and 30.81% over control at the 45^{th} day of pruning and in T_4($CuSO_4$ 5Kg/ha + $ZnSO_4$ 5Kg/ha) and T_{10} with an increase of 123.21% and 11.93% over control at the 60^{th} day of pruning, respectively. The findings of the present study emphasized that supplementation of micronutrients was found to enhance the biochemical contents of the mulberry leaves.

Introduction

In moriculture, quality of mulberry leaves has been considered as a prime feature in good crop production.[1] Better the leaf quality, greater are the possibilities of obtaining good cocoons, resulting in qualitative and quantitative silk production. The biochemical composition of mulberry leaf, *viz*., carbohydrates, proteins, minerals, fatty acids, amino acids and vitamins satisfy the nutritional requirement of silkworm larvae ensuring their healthy growth, and development of quality silk gland.[2] Application of fertilizers and biofertilizers have been reported to increase the biochemical contents of the mulberry leaves with regard to its nutritional status.[3-5] Albeit, information on the nutritional status of the biochemical aspects of mulberry leaves through soil application of micronutrients is scanty. The quality and quantity of mulberry leaves can be increased by adopting physiological manipulations.[6] Micronutrients are involved in numerous metabolic events of mulberry plant *viz*., photosynthesis, chlorophyll establishment, cell wall development, water absorption and xylem permeability, plant growth and resistance to plant diseases, responsible for quality leaf production.[7] Further, micronutrients perform the role of cofactors in enzyme stimulation, partake in redox reactions, photosynthesis and respiration, besides playing an indispensable part in the metabolism of carbohydrates and translocation of sugars.[8-12] Therefore, in the present study, the impact of micronutrients on the biochemical contents of mulberry leaves was assessed.

Materials and Methods

Study Area

A three year old mulberry garden, free from other plants which received direct sunlight exposure with proper irrigation served for conducting field experiments. This experimental plot was situated at an altitude of 29 m above sea level at Poovancode village, Kanyakumari district, Tamil Nadu, India (8.3031° N, 77.2881° E).

Mulberry Cultivation

For the experimental study, MR_2 (Mildew Resistant Variety 2) mulberry plant (*Morus alba*) was selected. This was developed by the Sericulture Department, Govt. of Tamil Nadu experimental station, Coonoor, Tamil Nadu, India.The mulberry plants were pruned in the month of June, ploughed, FYM applied at 20t/ha/year, and a single dose of nitrogen, phosphorous and potash at 120:120:60 kg/ha/year was hoed in the soil uniformly. All of the above were done prior to the commencement of the experiment. Irrigation at an interval of five day was provided, depending upon the climatic conditions. After twenty days of pruning, the micronutrients were added to the soil. Care was taken to ensure that the experimental plot was protected from plant pests and also in periodical removal of diseased/affected parts of the plant.

Experimental Design and Treatments

A randomized block design with twelve treatments with spacing of 90x60cm between the plants was chosen for the field experiments. Recommended dose of fertilizers and the macronutrients, *viz*., nitrogen, phosphorous and potassium (NPK) in the form of ammonium sulphate, super phosphate, potash (120:120:60) was common for all treatments. Each treatment (except control) was supplemented with the required amount of micronutrients, *viz*., zinc, copper and iron in the form of zinc sulphate, copper sulphate and ferrous sulphate either individually or in combination (Table 1) and were given as soil application. Each treatment replicated thrice, with ten plants per replication was supplemented with the required amount of micronutrients.

Table 1: Treatments used for the present study

Treatment		Micronutrients (individual/combination)				
T_0	:	Control				
T_1	:	$FeSO_4$ 10Kg/ha				
T_2	:	Zn SO_4 5Kg/ha				
T_3	:	$CuSO_4$ 5Kg/ha				
T_4	:	$CuSO_4$ 5Kg/ha	+	$ZnSO_4$ 5Kg/ha		
T_5	:	$CuSO_4$ 5Kg/ha	+	$FeSO_4$ 10Kg/ha		
T_6	:	$FeSO_4$ 10Kg/ha	+	$ZnSO_4$ 5Kg/ha		
T_7	:	$CuSO_4$ 5Kg/ha	+	$ZnSO_4$ 5Kg/ha	+	$FeSO_4$ 10Kg/ha
T_8	:	$CuSO_4$ 10Kg/ha	+	$ZnSO_4$ 10Kg/ha	+	$FeSO_4$ 20Kg/ha
T_9	:	$CuSO_4$ 15Kg/ha	+	$ZnSO_4$ 15Kg/ha	+	$FeSO_4$ 30Kg/ha
T_{10}	:	$CuSO_4$ 20Kg/ha	+	$ZnSO_4$ 20Kg/ha	+	$FeSO_4$ 40Kg/ha
T_{11}	:	$CuSO_4$ 25Kg/ha	+	$ZnSO_4$ 25Kg/ha	+	$FeSO_4$ 25Kg/ha

Five plants in each replication were randomly selected and labelled for recording observations at the 45th and 60th day of pruning for its biochemical parameters *viz*., leaf moisture, leaf moisture retention, chlorophyll 'a', 'b', total chlorophyll, carbohydrates, proteins, free amino acids and nitrogen.

Biochemical Analysis of Mulberry Leaf

Leaf moisture and leaf moisture retention was estimated through gravimetric method on fresh weight basis.[13] Chlorophyll 'a', 'b', and total chlorophyll content of leaves[14-16] were estimated using spectrophotometer at a wavelength of 645 to 663nm, and computed via Arnon's formulae,[17] and expressed in mg/g of leaf on fresh weight basis. The harvested leaves (at 45th and 60th day of pruning) from the plant were oven dried at 70°C for one hour and powdered. The dried leaf samples were then analysed for carbohydrate,[18-21] protein,[22,23] l free amino acids[24] and nitrogen[25] content.

Statistical Analysis

Pooled data affirmed as Mean ± S.D, and subjected to Student's 't' test to determine significant difference between control and treatment groups.

Results

Leaf Moisture

The leaf moisture was found to be more in apical leaves, followed by middle and bottom leaves. Maximum mean leaf moisture content on the 45th and 60th day of pruning was noted in T_8($CuSO_4$ 10Kg/ha + $ZnSO_4$ 10Kg/ha + $FeSO_4$ 20Kg/ha) (80.72 ±1.44%) and (81.41 ±3.32%) which increased by10.30% and 12.39% over control, respectively and its minimum was recorded in T_6(FeSO4 10Kg/ha + $ZnSO_4$ 5Kg/ha) (74.48 ±1.31%) which increased by 1.77% over control, and in T_4($CuSO_4$ 5Kg/ha + $ZnSO_4$ 5Kg/ha) (74.8 ±4.44%) with an increase of 3.27% over control, respectively(Table 2; Figure 1).

Leaf Moisture Retention

The bottom leaves had higher moisture retention percentage when compared to top and middle leaves. Maximum mean leaf moisture retention percentage on the 45th and 60th day of pruning was noted in T_8($CuSO_4$ 10Kg/ha + $ZnSO_4$ 10Kg/ha + $FeSO_4$ 20Kg/ha) (70.70 ±7.86%) and (80.67 ±5.66%) which increased by 29.32% and 11.02% over control, respectively and its minimum was recorded in T_5($CuSO_4$ 5Kg/ha + $FeSO_4$ 10Kg/ha) (59.58 ±1.34%) with an increase of 8.98% over control, and in T_{11}($CuSO_4$ 25Kg/ha + $ZnSO_4$ 25Kg/ha + $FeSO_4$ 25Kg/ha) (77.12 ±2.26%) with an increase of 6.13% over control respectively (Table 3, Figure 1).

Chlorophyll

On the 45th day of pruning, maximum and minimum chlorophyll 'a' content was observed in T_8($CuSO_4$ 10Kg/ha + $ZnSO_4$ 10Kg/ha + $FeSO_4$ 20Kg/ha) (1.80 ±0.25mg/g) and T_3($CuSO_4$ 5Kg/ha) (1.61 ±0.08mg/g) which increased by18.42% and 5.92%, respectively over control, while for chlorophyll 'b' it was recorded in T^7(CuSO4 5Kg/ha + $ZnSO_4$ 5Kg/ha

+ $FeSO_4$ 10Kg/ha) (0.58 ±0.05mg/g) and T_{11}($CuSO_4$ 25Kg/ha + $ZnSO_4$ 25Kg/ha + $FeSO_4$ 25Kg/ha) (0.42 ±0.01mg/g) which increased by 48.71% and 7.69%, respectively over control. The total chlorophyll content was maximum in T_8 (2.34 ±0.34mg/g) which increased by 21.87% over control, and the minimum value was reported in T_1($FeSO_4$ 10Kg/ha) (2.05 ±0.12mg/g) with an increase of 6.77% over control (Table 4; Figure 1). Whereas, on the 60th day of pruning, maximum and minimum chlorophyll 'a' content was observed in T_8 and T_9($CuSO_4$ 15Kg/ha + $ZnSO_4$ 15Kg/ha + $FeSO_4$ 30Kg/ha) (1.91 ±0.10mg/g and 1.91 ±0.08mg/g) and T_1 (1.16 ±0.06mg/g) which increased by 29.05% and 2.03%, respectively over control, while for chlorophyll 'b' it was again recorded in T_8 and T_9 (0.64 ±0.12 and 0.64 ±0.13mg/g) with an increase of 18.52% over control and in T_1 and T_2($ZnSO_4$ 5Kg/ha) (0.54 ±0.16mg/g) which was on par with the control. Maximum total chlorophyll content was observed in T_9 (2.56 ±0.18mg/g) which increased by 26.25% when compared to control and the minimum value was reported in T_1 (2.05 ±0.18mg/g) with an increase of 1.48% over control (Table 4, Figure1).

Table 2: Effect of micronutrients on leaf moisture of mulberry leaf

Treatment	45th day of pruning				60th day of pruning			
	Top	Middle	Bottom	Mean	Top	Middle	Bottom	Mean
T_0	75.75±0.88	72.97±2.06	70.82±0.09	73.18±2.47	73.58±0.71	72.57±1.29	71.14±1.01	72.43±1.22
T_1	80.57±0.21	78.89±0.50	77.6±2.26	77.39±2.72	78.05±1.03	76.58±0.71	75.13±1.02	76.58±1.46
T_2	80.14±1.43	78.97±1.32	77.12±0.44	78.74±1.52	78.22±1.40	76.23±1.0	72.15±2.88	75.53±3.09
T_3	80.0±0.05	78.06±2.04	75.23±0.16	77.76±2.39	80.85±2.24	77.71±0.61	72.64±3.58	77.06±4.14
T_4	77.79±1.40	75.91±2.14	73.66±0.17	76.12±2.57	79.90±5.07	72.72±1.14	71.78±0.68	74.80±4.44
T_5	79.41±2.70	77.80±1.14	76.86±2.43	78.02±1.28	82.90±4.58*	76.42±0.79	74.47±1.37	77.93±4.41
T_6	75.86±1.43	74.34±1.11	73.24±2.38	74.48±1.31	79.22±1.74	76.75±0.12	73.42±2.59	76.46±2.91
T_7	79.17±3.10	77.88±1.27	76.14±1.43	77.73±1.52	83.23±3.22*	78.67±1.16	75.32±3.36	79.07±3.97
T_8	82.22±3.61*	80.62±1.19*	79.33±1.67*	80.72±1.44*	80.78±2.98	85.0 ±1.00*	78.45±4.63*	81.41±3.32*
T_9	81.48±2.64*	80.22±1.17*	78.66±2.83*	80.12±1.41*	81.12±1.02*	79.67±0.66	80.62±0.07*	80.47±0.73*
T_{10}	79.41±2.74	77.53±1.34	76.32±1.16	77.75±1.55	81.64±2.57*	78.0±1.00	76.59±0.92	78.74±2.60
T_{11}	77.14±3.20	75.37±1.51	73.45±0.76	75.32±1.84	82.56±2.10*	79.58±0.71	75.28±2.04	79.14±3.65

Values expressed in %; *Significant @ P<0.05 (t-test)

Table 3: Effect of micronutrients on leaf moisture retention of mulberry leaf

Treatment	45th day of pruning				60th day of pruning			
	Top	Middle	Bottom	Mean	Top	Middle	Bottom	Mean
T_0	54.16±1.86	56.52±2.25	53.33±2.12	54.67±2.91	78.78±1.71	76.36±4.86	62.84±4.32	72.66±8.59
T_1	60.14±5.85*	68.42±3.71*	73.68±4.43*	67.41±6.82*	80.23±1.19	78.54±1.96	72.65±2.18*	77.14±3.97
T_2	58.31±4.51	64.70±4.25*	70.58±4.27*	64.53±6.13*	81.89±2.51	78.34±0.86	74.43±2.59*	78.22±3.73
T_3	59.32±1.82	61.90±5.18*	69.23±4.06*	63.48±5.14*	80.50±1.61	78.21±1.42	73.38±2.21*	77.36±3.63
T_4	59.28±6.46	68.42±2.26*	65.22±0.64*	64.30±4.63*	82.75±0.85	81.54±3.30	72.63±2.67*	78.97±5.52
T_5	58.55±0.38	59.09±1.42	61.11±1.07*	59.58±1.34	80.65±2.21	77.52±0.49	74.82±2.11*	77.66±2.91
T_6	54.47±7.09	62.5±5.56*	70.37±6.07*	61.78±8.97*	81.36±0.58	80.53±3.39	71.63±2.48*	77.84±5.39
T_7	57.81±5.23	65.21±1.11*	63.64±1.04*	62.22±3.89*	79.35±1.43	77.32±0.18	79.36±0.47*	78.67±1.17
T_8	61.87±10.64*	76.92±2.53*	73.33±1.85*	70.70±7.86*	84.12±0.25*	83.76±3.82*	74.14±2.43*	80.67±5.66*
T_9	63.09±0.64*	64.0 ±5.25*	71.43±3.71*	66.17±4.57*	83.97±1.81	81.40±2.42	73.87±2.98*	79.74±5.24
T_{10}	59.79±3.83*	65.21±2.10*	68.18±2.67*	64.39±4.25*	82.97±2.25	79.78±2.32	72.18±3.29*	78.31±5.54
T_{11}	58.29±2.29	61.53±6.40	70.59±5.03*	63.47±6.37*	79.13±1.10	77.57±0.82	74.67±1.41*	77.12±2.26

Values expressed in %; *Significant @ P<0.05 (t-test)

Table 4: Effect of micronutrients on the chlorophyll content of mulberry leaf

Treatments	Chlorophyll 'a' (mg/g)				Chlorophyll 'b' (mg/g)				Total chlorophyll (mg/g)			
	Tender	Medium	Coarse	Mean	Tender	Medium	Coarse	Mean	Tender	Medium	Coarse	Mean
45th day of pruning												
T_0	1.58±0.21	1.59±0.22	1.41±0.02	1.52±0.10	0.42±0.03	0.41±0.01	0.35±0.03	0.39±0.03	2.0±0.04	2.0±0.02	1.76±0.50	1.92±0.13
T_1	1.71±0.31	1.68±0.12	1.49±0.20	1.62±0.11	0.43±0.05	0.44±0.05	0.42±0.17*	0.43±0.01*	2.14±0.01	2.12±0.01	1.91±0.70	2.05±0.12
T_2	1.69±0.20	1.71±0.15	1.67±0.19	1.69±0.02*	0.49±0.01*	0.51±0.12*	0.48±0.14*	0.49±0.01*	2.18±0.03	2.22±0.03*	2.15±0.20*	2.18±0.03*
T_3	1.67±0.41	1.65±0.28	1.51±0.14	1.61±0.08	0.51±0.04*	0.52±0.15*	0.49±0.24*	0.50±0.01*	2.18±0.07	2.17±0.05	2.0 ±0.40*	2.11±0.10
T_4	1.68±0.37	1.70±0.13	1.54±0.17	1.64±0.08	0.43±0.02	0.46±0.02*	0.41±0.13*	0.43±0.02*	2.11±0.01	2.16±0.06	1.95±0.10*	2.07±0.10
T_5	1.79±0.16*	1.74±0.09	1.69±0.20*	1.74±0.05*	0.50±0.09*	0.48±0.05*	0.39±0.27*	0.45±0.05*	2.29±0.07	2.22±0.01*	2.08±0.10*	2.19±0.10*
T_6	1.76±0.02*	1.79±0.23*	1.48±0.02	1.67±0.17	0.47±0.16*	0.45±0.07	0.39±0.19*	0.43±0.04*	2.23±0.06*	2.24±0.02*	1.87±0.30	2.11±0.21
T_7	1.85±0.56*	1.79±0.12*	1.59±0.01*	1.74±0.13*	0.53±0.12*	0.64±0.17*	0.58±0.20*	0.58±0.05*	2.38±0.03*	2.43±0.04*	2.17±0.20*	2.32±0.13*
T_8	1.95±0.43*	1.95±0.08*	1.51±0.17	1.80±0.25*	0.61±0.13*	0.58±0.21*	0.43±0.16*	0.54±0.09*	2.56±0.02*	2.53±0.05*	1.94±0.40*	2.34±0.34*
T_9	1.96±0.24*	1.98±0.21*	1.37±0.19	1.77±0.34*	0.48±0.02*	0.55±0.03*	0.32±0.11	0.45±0.11*	2.44±0.04*	2.53±0.02*	1.69±0.40	2.22±0.46*
T_{10}	1.91±0.12*	1.59±0.04	1.47±0.11	1.65±0.22	0.45±0.05	0.75±0.04*	0.37±0.18	0.52±0.20*	2.36±0.05*	2.34±0.07*	1.84±0.50	2.18±0.29*
T_{11}	1.84±0.15*	1.81±0.26*	1.53±0.04	1.72±0.17*	0.43±0.07	0.43±0.04	0.41±0.18	0.42±0.01	2.27±0.07*	2.24±0.07*	1.94±0.10*	2.15±0.18*
60th day of pruning												
T_0	1.48 ±0.20	1.49 ±0.19	1.47 ±0.13	1.48 ±0.01	0.49 ±0.02	0.77 ±0.07	0.38 ±0.02	0.54 ±0.16	1.97 ±0.19	2.26 ±0.20	1.85 ±0.19	2.02 ±0.17
T_1	1.52 ±0.13	1.53 ±0.06	1.48 ±0.14	1.51 ±0.02	0.47 ±0.06	0.78 ±0.06	0.39 ±0.01	0.54 ±0.16	1.99 ±0.16	2.31 ±0.23	1.87 ±0.27	2.05 ±0.18
T_2	1.74 ±0.14*	1.78 ±0.13*	1.69 ±0.15*	1.73 ±0.04*	0.45 ±0.05	0.77 ±0.03	0.40 ±0.03	0.54 ±0.16	2.19 ±0.18*	2.55 ±0.24*	2.09 ±0.25*	2.27 ±0.19*
T_3	1.82 ±0.12*	1.85 ±0.16*	1.78 ±0.11*	1.81 ±0.03*	0.47 ±0.07	0.78 ±0.05	0.42 ±0.03*	0.55 ±0.15	2.29 ±0.21*	2.63 ±0.25*	2.20±0.29*	2.37 ±0.18*
T_4	1.76 ±0.15*	1.80 ±0.15*	1.74 ±0.17*	1.76 ±0.03*	0.50 ±0.04	0.79 ±0.05	0.48 ±0.03*	0.59 ±0.14	2.26 ±0.23*	2.59 ±0.29*	2.22 ±0.26*	2.35 ±0.16*
T_5	1.67 ±0.14*	1.70 ±0.18*	1.58 ±0.16	1.16 ±0.06	0.51 ±0.01	0.81 ±0.06	0.47 ±0.04*	0.59 ±0.15	2.18 ±0.26*	2.51 ±0.15*	2.05 ±0.25*	2.24 ±0.19*
T_6	1.91 ±0.13*	1.92 ±0.13*	1.79 ±0.15*	1.87 ±0.07*	0.53 ±0.02	0.82 ±0.01	0.50 ±0.05*	0.61 ±0.14*	2.44 ±0.27*	2.74 ±0.13*	2.29 ±0.24*	2.49 ±0.18*
T_7	1.93 ±0.11*	1.93 ±0.15*	1.78 ±0.12*	1.88 ±0.08*	0.55 ±0.03*	0.84 ±0.03*	0.52 ±0.02*	0.63 ±0.14*	2.48 ±0.21*	2.77 ±0.16*	2.30±0.27*	2.51 ±0.19*
T_8	1.97 ±0.17*	1.98 ±0.11*	1.80 ±0.13*	1.91 ±0.10*	0.57 ±0.04*	0.81 ±0.03	0.54 ±0.04*	0.64 ±0.12*	2.54 ±0.22*	2.79 ±0.27*	2.34 ±0.28*	2.55 ±0.18*
T_9	1.97 ±0.09*	1.96 ±0.12*	1.81 ±0.14*	1.91 ±0.08*	0.56 ±0.08*	0.84 ±0.05*	0.54 ±0.01*	0.64 ±0.13*	2.53 ±0.24*	2.80±0.18*	2.35 ±0.24*	2.56 ±0.18*
T_{10}	1.96 ±0.11*	1.94 ±0.14*	1.77 ±0.15*	1.89 ±0.10*	0.54 ±0.02*	0.82 ±0.06	0.51 ±0.02*	0.62 ±0.13*	2.50±0.26*	2.76 ±0.23*	2.28 ±0.24*	2.51 ±0.19*
T_{11}	1.88 ±0.17*	1.89 ±0.12*	1.81 ±0.13*	1.86 ±0.04*	0.51 ±0.05	0.80 ±0.08	0.49 ±0.03*	0.60 ±0.14*	2.39 ±0.29*	2.69 ±0.27*	2.30±0.21*	2.46 ±0.16*

Values expressed in mg/g; *Significant @ P<0.05 (t-test)

Carbohydrate

The soil application of micronutrients increased the sugar content in middle leaves followed by apical and bottom leaves. On the 45th and 60th day of pruning, maximum carbohydrate content was recorded in T_{10}($CuSO_4$ 20Kg/ha + $ZnSO_4$ 20Kg/ha + $FeSO_4$ 40Kg/ha) (373.41 ±45.65µg/mg) and T_9($CuSO_4$ 15Kg/ha + $ZnSO_4$ 15Kg/ha + $FeSO_4$ 30Kg/ha) (236.66 ±62.13µg/mg) which increased by 22.25% and 24.99% respectively, over control, and its minimum was recorded in T_{11}($CuSO_4$ 25Kg/ha + $ZnSO_4$ 25Kg/ha + $FeSO_4$ 25Kg/ha) (317.53 ±60.47µg/mg) and T_7($CuSO_4$ 5Kg/ha + $ZnSO_4$ 5Kg/ha + $FeSO_4$ 10Kg/ha) (195.66 ±32.46µg/mg) which increased by 3.96% and 3.34% respectively, over control (Table 5; Figure 1).

Table 5: Effect of micronutrients on the carbohydrate content of mulberry leaf

Treatment	45th day of pruning				60th day of pruning			
	Top	Middle	Bottom	Mean	Top	Middle	Bottom	Mean
T_0	310.13±42.42	371.50±3.53	234.67±10.77	305.43±71.45	232.50±19.60	181.5±26.16	154.0 ±5.65	189.33±37.65
T_1	385.21±21.21*	355.86±63.63	267.57±15.09*	336.21±61.23*	263.50±10.61*	210.0 ±70.71*	158.50±2.12	210.66±52.50*
T_2	355.63±21.21*	325.45±21.23	280.93±16.99*	320.67±37.57	250.50±16.57	250.0 ±107.27*	160.50±0.70	220.33±51.81*
T_3	310.0±0.0	380.19±84.85	348.12±27.82*	346.10±35.13*	269.50±16.27*	223.0 ±80.61*	162.50±4.94	218.33±53.65*
T_4	385.72±16.0*	362.50±3.53	269.13±15.97*	339.11±61.71*	259.0 ±12.83*	231.50±101.01*	155.50±3.53	215.33±53.61*
T_5	390.81±14.14*	385.22±7.07	259.50±14.12*	345.17±74.25*	268.50±17.68*	270.50±96.85*	159.0 ±2.82	232.66±63.80*
T_6	360.37±28.28*	410.84±42.42*	264.45±16.34*	345.22±74.36*	264.50±13.34*	268.50±57.68*	153.0 ±5.65	228.66±65.55*
T_7	305.54±49.49	375.14±48.49	313.32±27.18*	331.33±38.13	218.50±72.83	210.0 ±84.85*	158.50±12.0	195.66±32.46
T_8	345.23±49.49*	420.32±56.56*	298.46±20.11*	354.67±61.47*	238.50±15.25	234.50±106.77*	158.0 ±1.41	210.33±45.36*
T_9	430.57±42.42*	375.25±7.07	278.53±17.83*	361.45±76.95*	269.50±16.27*	275.50±47.78*	165.0 ±8.48*	236.66±62.13*
T_{10}	410.0 ±62.63*	388.0 ±61.35	322.25±22.73*	373.41±45.65*	237.50±12.53	216.0 ±94.65*	163.50±2.12	205.66±38.06
T_{11}	270.47±41.42	385.75±21.21	296.39±20.93*	317.53±60.47	261.50±13.44*	219.0 ±72.12*	160.50±10.6	213.66±50.71*

Values expressed in µg/mg; *Significant @ P<0.05 (t-test)

Table 6: Effect of micronutrients on the protein content of mulberry leaf

Treatment	45th day of pruning				60th day of pruning			
	Top	Middle	Bottom	Mean	Top	Middle	Bottom	Mean
T_0	268.50±19.30	311.50±20.59	268.0 ±1.14	252.33±75.66	223.0 ±1.41	250.0 ±34.30	204.0 ±32.50	225.66±23.11
T_1	330.12±10.07*	367.50±25.50*	332.50±13.43*	343.37±20.9*	278.0 ±57.30	308.0 ±35.38	238.0 ±20.81	274.66±55.67*
T_2	321.0 ±57.98*	322.50±60.10	295.0 ±21.21*	312.83±15.46*	275.0 ±49.49	429.50±67.9	158.0 ±15.96	287.50±136.14*
T_3	328.50±19.09*	355.0 ±13.0*	325.12±14.31*	336.20±16.36*	278.0 ±28.56	282.50±29.03	178.0 ±14.27	246.16±59.07
T_4	334.50±16.26*	357.50±11.50*	331.62±12.19*	341.20±14.18*	317.0±4.42	333.0 ±21.0	316.0 ±5.65	322.0±9.53*
T_5	316.50±12.02*	338.0 ±13.0	314.37±9.01*	322.95±13.07*	308.0 ±23.24	321.50±48.19	298.0 ±29.10	309.83±7.01*
T_6	407.0 ±59.39*	414.50±48.79*	260.50±26.57	360.66±86.82*	311.50±10.60	341.50±17.50	314.0 ±14.11	322.33±16.60*
T_7	373.50±13.42*	381.0 ±17.0*	375.87±10.07*	376.79±3.83*	315.87±11.10	338.0 ±9.0	318.50±14.84	324.12±12.08*
T_8	400.50±6.36*	410.0 ±84.85*	375.0 ±91.92*	395.16±62.63*	384.12±6.89	389.0 ±13.0	382.50±9.19	385.20±3.38*
T_9	408.0 ±93.33*	408.50±8.48*	399.0 ±21.83*	405.16±83.51*	368.12±2.61	398.50±3.50	367.50±3.53	378.04±17.25*
T_{10}	396.50±45.30*	403.50±16.17*	329.50±40.75*	376.50±85.54*	356.87±2.65	386.50±6.50	377.50±3.53	373.62±15.19*
T_{11}	366.50±0.70*	383.50±23.33*	343.50±33.23*	364.50±20.07*	312.50±3.53	326.0 ±16.0	313.12±2.65	317.20±7.62*

Values expressed in µg/g; *Significant @ P<0.05 (t-test)

Table 7: Effect of micronutrients on the free amino acid content of mulberry leaf

Treatment	45th day of pruning				60th day of pruning			
	Top	Middle	Bottom	Mean	Top	Middlea	Bottom	Mean
T_0	19.50±12.02	22.0 ±5.65	17.50±6.36	19.66±4.25	8.66±3.01	2.0±1.19	1.0 ±0.01	8.66±3.88
T_1	29.0 ±18.38*	22.0 ±8.48	35.0 ±9.89*	28.66±6.50*	9.66±8.54	2.70±1.74*	2.90±0.82*	9.66±3.88
T_2	27.50±23.33*	33.50±14.84*	17.0 ±8.48	26.0 ±8.35*	10.0 ±5.29*	2.60±0.02	6.80±2.10*	10.0 ±5.08*
T_3	36.50±17.67*	40.50±12.02*	28.0 ±5.65*	35.0 ±6.38*	15.0 ±2.76*	9.0 ±1.63*	2.60±1.36*	15.0 ±6.46*
T_4	29.0 ±15.55*	35.0 ±7.07*	24.0 ±8.48*	29.33±5.50*	19.33±1.01*	5.0 ±2.47*	2.00±0.03*	19.33±8.86*
T_5	30.50±53.03*	20.0 ±5.65	27.50±47.37*	25.83±5.34*	13.66±1.18*	13.50±1.73*	2.10±0.06*	13.66±8.77*
T_6	27.50±9.19*	34.50±19.09*	41.0 ±9.89*	34.33±6.75*	14.33±3.50*	14.0 ±1.31*	2.0 ±0.02*	14.33±9.75*
T_7	25.0 ±1.41*	36.0 ±16.97*	37.0 ±15.55*	41.66±19.4*	12.66±3.31*	15.0 ±1.32*	1.90±1.25	12.66±8.18*
T_8	30.50±0.70*	47.50±24.74*	47.50±24.74*	41.83±9.81*	11.66±2.20*	10.0 ±2.73*	2.90±0.84*	11.60±7.26*
T_9	40.50±17.67*	41.50±16.26*	29.0 ±1.41*	37.0 ±6.94*	16.0 ±6.70*	4.0 ±0.14*	1.80±0.07	16.0 ±10.22*
T_{10}	21.0 ±7.07	22.0 ±5.65	17.0 ±1.41	20.0 ±2.64	17.0 ±3.89*	8.0 ±1.10*	10.0 ±0.47*	17.0 ±9.85*
T_{11}	24.50±16.26*	44.0 ±11.31*	32.50±27.57*	33.66±9.80*	13.66±9.60*	13.0 ±1.87*	4.0 ±1.12*	13.66±10.11*

Values expressed in µg/g; *Significant @ P<0.05 (t-test)

Table 8: Effect of micronutrients on the nitrogen content of mulberry leaf

Treatment	45th day of pruning				60th day of pruning			
	Top	Middle	Bottom	Mean	Top	Middle	Bottom	Mean
T_0	3.99±0.55	3.21±0.06	3.12±0.22	3.44±0.47	3.11±0.43	3.10±0.64	3.10±0.27	3.10±0.05
T_1	4.32±0.76	3.24±0.15	3.46±0.15*	3.67±0.57	3.19±0.75	3.12±0.47	3.17±0.84	3.16±0.03
T_2	4.29±0.48	3.61±0.21*	3.31±0.30	3.73±0.50	3.18±0.83	3.16±0.32	3.24±0.69	3.19±0.04*
T_3	4.23±0.48	3.54±0.19*	3.27±0.28	3.68±0.49	3.28±0.90	3.25±0.18	3.27±0.91	3.24±0.03*
T_4	4.53±0.72*	3.50±0.01	3.48±0.25*	3.83±0.60*	3.14±0.83	3.16±0.42	3.12±0.01	3.14±0.02
T_5	4.63±0.73*	3.59±0.02*	3.62±0.23*	3.94±0.59*	3.40±0.53	3.31±0.13	3.29±0.98	3.33±0.05*
T_6	4.01±0.01	3.99±0.28*	3.59±0.19*	3.86±0.23*	3.28±0.42	3.08±0.43	3.05±0.76	3.13±0.12
T_7	4.45±0.09*	4.32±0.28*	3.91±0.22*	4.22±0.28*	3.32±0.19	3.20±0.14	3.17±0.91	3.23±0.07
T_8	4.76±0.52*	4.02±0.16*	3.78±0.28*	4.18±0.51*	3.49±0.25*	3.18±0.06	3.16±0.57	3.27±0.18
T_9	5.20±0.67*	4.24±0.12*	4.06±0.31*	4.50±0.61*	3.51±0.23*	3.28±0.79	3.25±0.12	3.34±0.14
T_{10}	3.87±0.24	4.22±0.62*	3.34±0.33	3.81±0.44*	3.53±0.47*	3.46±0.54*	3.41±0.32*	3.46±0.06*
T_{11}	4.08±0.26	3.71±0.14*	3.50±0.18*	3.76±0.29	3.45±0.63*	3.38±0.93	3.32±0.25	3.38±0.06*

Values expressed in %; *Significant @ P<0.05 (t-test)

Protein

The soil application of micronutrients increased the protein content in middle leaves followed by apical and bottom leaves. On the 45th and 60th day of pruning, maximum protein content was recorded in T_9($CuSO_4$ 15Kg/ha + $ZnSO_4$ 15Kg/ha + $FeSO_4$ 30Kg/ha) (405.16 ±83.51µg/g) and T_8($CuSO_4$ 10Kg/ha + $ZnSO_4$ 10Kg/ha + $FeSO_4$ 20Kg/ha) (385.20 ±3.38µg/g) which increased by 60.56% and 70.69% respectively, over control, and its minimum was recorded in T_2($ZnSO_4$ 5Kg/ha) (312.83 ±15.46µg/g) and T_3($CuSO_4$ 5Kg/ha) (246.16 ±59.07µg/g) which increased by 23.97% and 9.08% respectively, over control (Table 6; Figure 1).

Free Amino Acid

In all the treatments, free amino acid content was higher in the apical leaves followed by middle and bottom leaves. On the 45th and 60th day of pruning, maximum free amino acid content was recorded in T_8($CuSO_4$ 10Kg/ha + $ZnSO_4$ 10Kg/ha + $FeSO_4$ 20Kg/ha) (41.83 ±9.81µg/g) and T_4($CuSO_4$ 5Kg/ha + $ZnSO_4$ 5Kg/ha) (19.33 ±8.86µg/g) which increased by 112.76% and 123.21% respectively, over control,

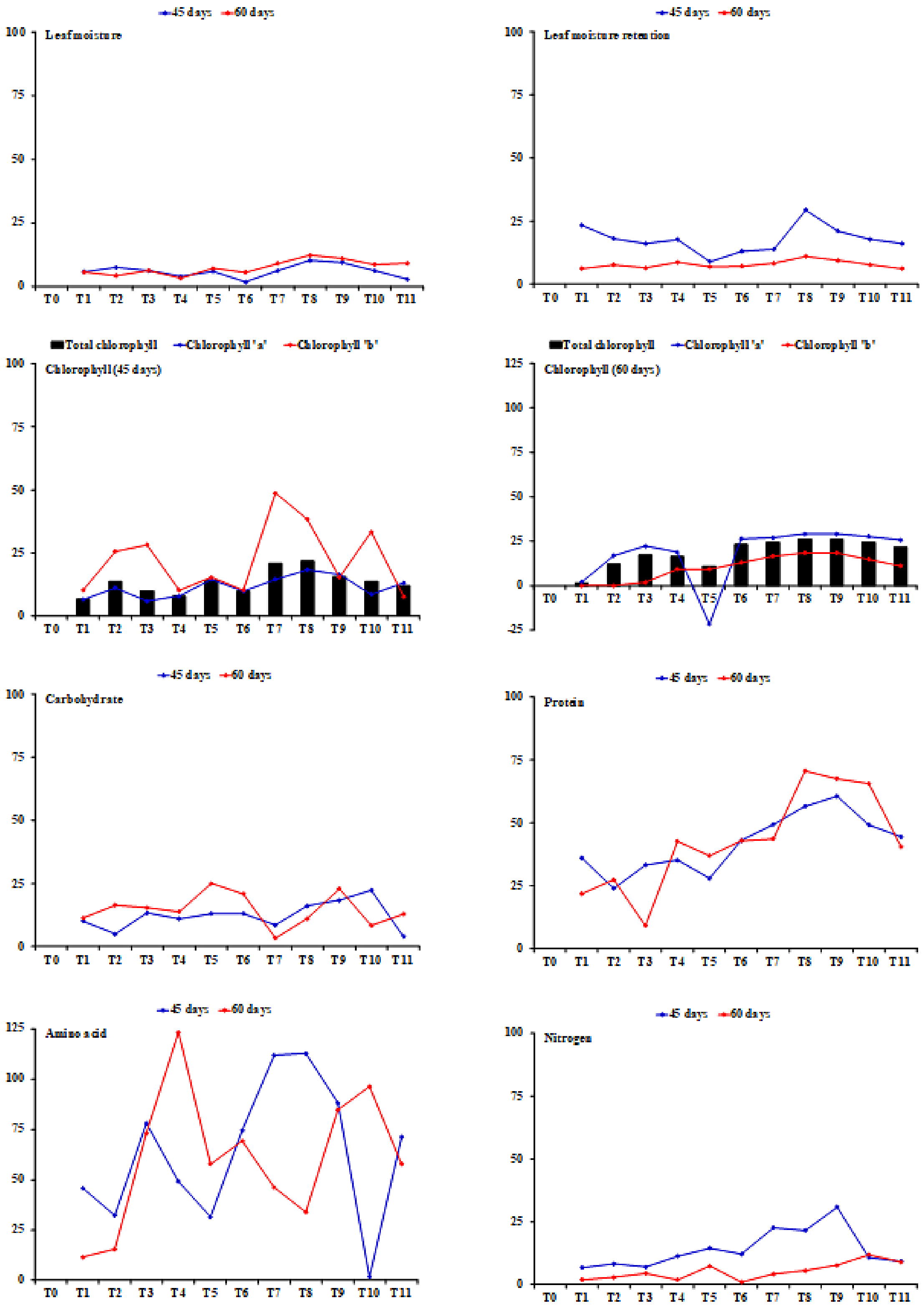

Fig. 1: Effect of micronutrients on biochemical contents of mulberry leaves

and its minimum was recorded in T_{10}($CuSO_4$ 20Kg/ha + $ZnSO_4$ 20Kg/ha + $FeSO_4$ 40Kg/ha) (20.0 ±2.64µg/g) and T_1($FeSO_4$ 10Kg/ha) (9.66 ±3.88µg/g) which increased by 1.72% and 11.54% respectively over control (Table 7; Figure 1).

Nitrogen

In all the treatments, nitrogen content was higher in the apical leaves followed by middle and bottom leaves. On the 45th and 60th day of pruning, maximum nitrogen content was recorded in T_9($CuSO_4$ 15Kg/ha + $ZnSO_4$ 15Kg/ha + $FeSO_4$ 30Kg/ha) (4.50 ±0.61%) and T_{10}($CuSO_4$ 20Kg/ha + $ZnSO_4$ 20Kg/ha + $FeSO_4$ 40Kg/ha) (3.46 ±0.06%) which increased by 30.81% and 11.93% respectively, over control, and its minimum was recorded in T_1($FeSO_4$ 10Kg/ha) (3.67 ±0.57%) and T_6($FeSO_4$ 10Kg/ha + $ZnSO_4$ 5Kg/ha) (3.13 ±0.12%) which increased by 6.68% and 0.96% respectively, over control (Table 8; Figure 1).

Discussion

Micronutrients have an impact on the mulberry plant in terms of quality, growth and yield,[26] and contribute to the foremost part in numerous metabolic actions accountable for synthesis of proteins, sugars and enzymes, which leads to superior production of quality mulberry leaf.[27]. Machii and Katagiri[28] and Suryanarayan and Shivashankar[29] reported that increased contents of nitrogen and amino acids in mulberry leaves are nutritionally greater and are positively linked to silkworm development and growth. The present results opined that soil application of micronutrients supplemented the betterment of yield and quality in terms of proteins and carbohydrates, as these two biochemical constituents determines the feeding value of silkworm which in turn reflects upon the silk production.

Leaf moisture

Moisture content in mulberry leaves is a very important factor which influences silkworm growth, as it has an intimate relation to an easy ignition of leaves to silkworm, as high moisture make it more palatable to them. Micronutrients treated mulberry leaves revealed significant variations in moisture and moisture retention capacity. Yokoyama[30] reported 64-83% moisture content in mulberry leaves. On the 45th and 60th day of pruning, the moisture capacity in the present study increased to 10.30% - 12.39% as the maximum in T_8 treatment ($CuSO_4$ 10Kg/ha + $ZnSO_4$ 10Kg/ha + $FeSO_4$ 20Kg/ha) when compared to control. Higher moisture content in T_8 treatment may be due to genetic character, and influence of balanced fertilizers combination which helped in uptake of available nutrients. During the study period, the tender leaves had more moisture content than middle and bottom leaves. Leaves possessing higher leaf moisture content are recognised as good quality leaves,[31,32] and are considerably related to the silkworm growth and nutritional parameters[33] The moisture content in mulberry leaves plays an important part in uplifting the nutritional levels, which in turn improves the scrumptiousness and digestibility of leaves by silkworms, feeding efficiency of silkworm larvae, increased growth rate, as well as quality development of cocoon.[34,35] An optimum level of leaf moisture content is directly related to the growth of silkworm, since low leaf moisture content influences the assimilation and conversion efficiency of food (energetic parameters) to decrease. Sinha *et al*[36] reported that corresponding increased leaf growth and maturity can lead to a gradual decreased in the moisture content of mulberry leaves. The present findings correlated with the above facts. The enhancement of leaf moisture due to application of micronutrients might be due to enhancement of organic matter and water holding capacity in the soil, thereby, increasing the water absorption by plant.[37] The current observations are related to the results of Babu *et al.*[38] too. Increased leaf moisture content, and fresh leaf weight are due to the water retention capacity which steadily supplies moisture to the leaf. Further, microbial inoculants in the soil rhizosphere may also facilitate in the moisture accessibility, towards water uptake and added metabolic activities for upholding normal plant growth.

Leaf Moisture Retention

Moisture retention capacity also play a vital role, because silkworms prefer fresh leaves with high moisture for longer time.[39] Micronutrients influences the moisture retention capacity of mulberry leaves due to water retention capacity of organic manures which steadily supplied the moisture.[40-42] Increased moisture retention capacity in T_8 ($CuSO_4$ 10Kg/ha + $ZnSO_4$ 10Kg/ha + $FeSO_4$ 20Kg/ha) was 11.02% - 29.32% higher when compared to control on the 45th and 60th day of pruning This may be due to increased stomata size due to uptake of water, and balanced mixture of zinc, copper and iron.

Chlorophyll

Chlorophyll content plays a vital role in computing the photosynthetic productivity of plant, and is a critical component in evaluating leaf quality. Total chlorophyll content was recorded high in T_8($CuSO_4$ 10Kg/ha + $ZnSO_4$ 10Kg/ha + $FeSO_4$ 20Kg/ha) and T_9($CuSO_4$ 15Kg/ha + $ZnSO_4$ 15Kg/ha + $FeSO_4$ 30Kg/ha) treated mulberry leaves on the 45th and 60th day of pruning, which increased to 21.87% and 26.25%, respectively when compared with control. Micronutrients significantly increased the levels of chlorophyll reflecting its impact on the increased qualitative parameters of leaf. The amplified volume of chlorophyll content in leaves specifies the photosynthetic effectiveness, the primary criterion for measuring photosynthetic proportion in mulberry.[32] The positive effect of micronutrients on chlorophyll content of mulberry leaves might be attributed to the fact that nitrogen, an essential constituent of chlorophyll helps in harnessing more solar energy, and plays a greater role in improving the chlorophyll synthesis, an essential constituent of photosynthesis.[43] Increased chlorophyll content of mulberry leaves be due to foliar nutrition.[37,44] In the present study, increased chlorophyll content was obviously due to zinc which acted as structural and catalytic components of protein, enzymes and as cofactors for normal development of pigment biosynthesis.[45]

Carbohydrate

Leaf quality determined by the presence of sugar, as the main source of energy, induces the silkworms to bite the leaves (biting factor) and to cherish it well, which in turn influences the healthy growth and development of silkworms. Carbohydrates, in mulberry leaves are present abundantly, and are stated to be the silkworm's primary source of energy.[46,47] High content of total sugar in mulberry leaf was accredited to the enhanced mineralization resulting in superior production of plant growth substances and enzyme activity due to soil application of micronutrients. The photosynthetic action aids in synthesis of carbohydrates in mulberry leaves, and principally the sugar content are in close relation with the silkworm health. The carbohydrate content significantly varied among the treatments and the highest carbohydrate content was found in the T_{10}($CuSO_4$ 20Kg/ha + $ZnSO_4$ 20Kg/ha + $FeSO_4$ 40Kg/ha) and T_5($CuSO_4$ 5Kg/ha + $FeSO_4$ 10Kg/ha) treated mulberry leaves which increased by 22.25% and 24.99% when compared to control on the 45th and 60th day of pruning respectively. This might be due to rapid conversion of starch to sugar. Further, optimum combination of micronutrients amplified nutrient uptake from soil, and caused the plant to drive additional sugars and extra exudates from its roots.

Protein

Leaf protein is a key element of silkworm nutrition, and approximately 70% of protein content of raw silk are biosynthesized directly from leaf protein, and 30% from the body tissue of silkworm.[48,49] Protein content in mulberry leaves have a direct bearing on silkworm, mostly on larval growth with special reference to development of silk gland and cocoon characters.[31] In the present study, the protein content was maximum in T_9($CuSO_4$ 15Kg/ha + $ZnSO_4$ 15Kg/ha + $FeSO_4$ 30Kg/ha) and T_8($CuSO_4$ 10Kg/ha + $ZnSO_4$10Kg/ha + $FeSO_4$ 20Kg/ha) treated mulberry leaves which increased by 60.56% and 70.69%, respectively when compared with control. This was due to zinc in combination with copper and iron. Zinc and copper are taken up in the form of their respective ions, by the plants, and iron in the form of ferrous or ferric ions, which are needed for protein synthesis, and are involved in the activation of several enzyme systems. Further, the protein content of middle leaves was more when compared with top and bottom leaves, which was contradictory to the results of Murthy *et al.*[50] but correlates with the findings of Singhvi *et al.*[51] Protein, the core constituent of mulberry leaf, contributes to silk synthesis,[52] and increase in the protein content is directly revealed on the leaf yield. Increased protein content might be due to the accessibility of adequate amount of nitrogen to the plants. Increase in crude protein was largely accredited to the soil application of micronutrients which were rapidly absorbed by the leaves, thereby activating plant metabolism, and thus leading to production of healthy green foliage, and the same was stated by Singhvi *et al.*[51] too. Increased protein content in mulberry leaves due to micronutrient application was strongly supported by Sterling,[53] through microorganisms, as it signifies increased nutritional status of mulberry leaves with regard to biochemical aspects.[5]

Free Amino Acid

Amino acids determine the leaf quality.[54] Mulberry leaves, the significant constituent for silkworm nutrition are rich in amino acids.[8,55] Amino acids help silkworm in food selection, and are utilised for the formation of haemolymph, development of silk glands and cocoon production. In the present study, the highest amino acid content was recorded in T_8($CuSO_4$ 10Kg/ha + $ZnSO_4$ 10Kg/ha + $FeSO_4$ 20Kg/ha) and T_4($CuSO_4$ 5Kg/ha + $ZnSO_4$ 5Kg/ha) treated apical mulberry leaves which may be due to the application of required amount and combined effect of zinc, copper and iron,and also by the age of the plant, growth stage and maturity.

Nitrogen

Nitrogen also determines leaf quality,[54] and is the vital component of protein, nucleic acids, chlorophyll and growth hormones.[56] The present study recorded maximum nitrogen content in T_9($CuSO_4$ 15Kg/ha + $ZnSO_4$ 15Kg/ha + $FeSO_4$ 30Kg/ha) and T_{10}($CuSO_4$ 20Kg/ha + $ZnSO_4$ 20Kg/ha + $FeSO_4$ 40Kg/ha) treated mulberry leaves, which corroborates the results of Rafiq *et al.*[57] Nitrogen uptake from soil, assimilation and consumption via proliferated mulberry roots directly improve numerous strategic roles, like, transfer of energy, photosynthesis level, conversion of sugars, movement of nutrients within the plant cell, metabolic constituents, several physiological process, chlorophyll, and protein matters. Consequently, nitrogen is involved in production of leaf area, and by net assimilation rate with regard to growth parameters like, leaf yield and leaf quality *viz*., moisture, chlorophyll ‘a’ and ‘b’, total sugar, soluble carbohydrate, reducing sugar and crude protein. Besides these, the nitrogen fixing microorganisms which increases the nitrogen accessibility might have increased the protein content in the leaves too in the present study.

Conclusion

The present investigation showed that the micronutrients improved the biochemical parameters of mulberry leaves, and indicated variations amongst the treatments due to the effect of micronutrients. Further, more research with more varied and different combination of treatments should be carried out to support the current findings in regard to the biochemical aspects of mulberry leaves so as to yield mulberry leaves of more quality and quantity.

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Conflict of Interest

The authors declare no conflict of interest.

References

1. Ravikumar C. Western ghats as a bivoltine region prospects, challenges and strategies for its development. *Indian Silk.* 1988; 26(9):39-54.
2. Qader M.A. Improvement of nutritional quality of mulberry leaves. *Bulletin of the Sericultural Experiment Station.* 1995; 26(1):14-19.
3. Sengupta K., Ray D., Singh B.D., Krishnaswami S. Studies on the effect of high dose nitrogen fertilization of soil, on the yield, chemical composition and nutritive value of mulberry leaf, as evaluated from rearing results. *Indian Journal of Sericulture.* 1972; 11(1):28-32.
4. Lokanath R., Shivashankar K., Kasi viswanathan K. Effect of foliar application of magnesium and micronutrients to mulberry on the quality and production of cocoons. *Indian Journal of Sericulture*. 1986; 24:40-45.
5. Subburathinam K.M., Sulochanachetty J. Effect of fortification of mulberry leaves with minerals to silkworm, *Bombyx mori* L. *Indian Journal of Sericulture.* 1991; 30(2):121-123.
6. Venkataraman P., Murthy N.B., Rao K.J.V., Kamble C.K. Efficacy of foliar sprays of vermi wash and cow dung wash on biochemical and yield attributes and yield of mulberry (*Morus alba* L.). *Karnataka Journal of Agricultural*

Sciences. 2009; 22(4):921-923.

7. Vitti A.M., Nuzzaci A., Scopa G., Tataranni I., Sofo A. Hormonal response and root architecture in *Arabidopsis thaliana* subjected to heavy metals. *International Journal of Plant Biology*. 2014; 5:5226-5232.
8. Bose P.C., Singhvi N.R., Dutta R.K. Effect of micronutrients on yield and yield attributes of mulberry (*Morus alba* L.). *Indian Journal of Agronomy*. 1994; 39(1):97-99.
9. Sinha U.S.P., Sinha A.K., Banerjee N.D., Prasad J., Chaudhary S.K., Suryanarayana N. Effect of micronutrients on the growth and leaf yield of *Terminalia arjuna. Sericologia.* 2006; 46(1):127-132.
10. Bose P.C., Bindroo B.B. Effect of micronutrients on yield of mulberry in sub-tropical region. *Journal of Crop and Weed.* 2009; 5(2):142-143.
11. Shilpashree K.G., Subbarayappa C.T. Effect of micronutrients on growth and yield of mulberry (*Morus alba* L.) and silkworm (*Bombyx mori* L.). *The Mysore Journal of Agricultural Sciences.* 2015; 49(2):167-170.
12. Sowmya P., Narayana swamy T.K. Influence of micronutrients on yield and yield attributing parameters of mulberry (*Morus* spp.) and silkworm (*Bombyx mori* L.). *The Mysore Journal of Agricultural Sciences.* 2015; 50(2):453-456.
13. Vijayan K., Tikader A., Das K.K., Roy B.N., Pavan K.T. Genotypic influence on leaf moisture content and moisture retention capacity in mulberry (*Morus* spp.). *Bulletin of Sericultural Research*. 1996; 7:95-98.
14. Shoof T.W., Lium B.W. Improved extraction of chlorophyll a and b from algae using dimethyl sulfoxide. *Limnology and Oceanography.* 1976; 21:926-927.
15. Hiscox J.D., Israelistam G.F. A method for extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany.* 1979; 57:1332-1334.
16. Barnes J.D., Balaguer L., Manrique E., Elvira S., Davison A.W. A reappraisal of the use of DMSO for the extraction and determination of chlorophylls a and b in lichens and higher plants. *Environmental and Experimental Botany.* 1992; 32:85-100.
17. Arnon D.I. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris. Plant Physiology.* 1949; 24:1-15.
18. Loomis E.W., Shull A.C. Methods in plant physiology. McGraw- Hill Book Company, New York. 1937.
19. Mccready R.M., Guggolz J., Silviera V., Owens H.S. Determination of starch and amylose in vegetables. *Analytical Chemistry.* 1950; 29:1156-1158.
20. Dubios M., Giles K.A., Hamilton T.K., Robeos R.A., Smith R. Calorimetric determination of sugars and related substances. *Analytical Chemistry.* 1956; 28:250-256.
21. Miller L.G. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry.* 1972; 31(3):426-428.
22. Wong S.Y. The use of persulfate in the estimation of nitrogen by the arnold-gunning modification of Kjeldahl's method. *Journal of Biological Chemistry.* 1923; 55:427.
23. Lowry O.H., Rosenbrough N.J., Farr A.L., Randall R.J. Protein measurement with folin phenol reagent. *Journal of Biological Chemistry*. 1951; 193:265-267.
24. Spies J.R. Colorimetric procedure for amino acids and phenol In: Methods in enzymology. Colonick SP. and Kaplan NPO (Eds). 1955, 461-477.
25. Jackson M.L. Nitrogen determination for soil and plant tissue In: Soil chemical analysis. Prentice-Hall of India, Pvt. (Ltd). New Delhi, India. 1973, 183-204.
26. Chikkaswamy B.K., Paramanik R.C., Gopinath S.M., Shivashankar M. Effect of foliar nutrients on the growth, quality and yield of mulberry. Proceeding of the National Seminar on Soil Health and Water Management for Sustainable Sericulture, Bangalore, India. 2006, 95.
27. Geetha T.K., Ramamoorthy K., Murugan N. Effect of foliar application of micronutrients on mulberry (*Morus alba* L.) leaf yield and silkworm (*Bombyx mori* L.) economic parameters. *Life Sciences International Research Journal.* 2016; 3(1):23-26.
28. Machii H., Katagiri K. Varietal differences in nutritive values of mulberry leaves for rearing silkworm. *Japan Agricultural Research Quarterly.* 1991; 25:202-208.

29. Suryanarayana N., Shivashankaramurthy T.C. Differences in amino acid contents in leaf blades of mulberry (*Morus* spp.) varieties. Advances in Plant Sciences. 2002; 15:475-481.
30. Yokoyama T. Text book of tropical sericulture. Japan Overseas Cooperation Volunteers, Tokyo. 1974, 444-537.
31. Bongale U.D., Chaluvachari. Evaluation of eight mulberry germplasm varieties by leaf biochemical and bioassay moulting studies. *Sericologia*. 1995; 35:83-94.
32. Sujathamma P., Dandin S.B. Leaf quality evaluation of mulberry (*Morus* spp.) through chemical analysis. *Indian Journal of Sericulture*. 2000; 39:117-121.
33. Rahmathulla V.K., Himanthraj M.T., Srinivasa G., Rajan R.K. Association of moisture content in mulberry leaf with nutritional parameters of bivoltine silkworm (*Bombyx mori* L.) Acta Entomologica Sinica. 2004; 47:701-704.
34. Sastry C.R., Jolly M.S., Subramanyam M.R., Rao M.Y.R. Studies on the varietal differences in the loss of moisture from harvested mulberry leaves. *Indian Journal of Sericulture.* 1988; 27(53):85-91.
35. Koul A., Darshan S., Gupta S.P. Seasonal fluctuations in leaf moisture characters and dry matter in mulberry in subtropics. *Journal of Sericultural Science.* 1996; 4:23-27.
36. Sinha U.S.P., Sinha A.K., Srivastava P.P., Brahmachari B.N. Variation of chemical constituents in relation to maturity of leaves in mulberry varieties S1 and K2 under the agro-climatic conditions of Ranchi district. *Indian Journal of Sericulture*. 1993; 32:196-200.
37. Rao D.M.R., Chikkanna, Vindhya G.S., Reddy M.M., Dasappa, Qadri S.M.H. Effect of green manure and seri-compost on soil health, leaf quality and quantity traits of mulberry under tropical conditions. *Green Farming.* 2011; 2(3):290-293.
38. Babu C.M., Dandin S.B., Thippeswamy T., Renukeswarappa J.P. Nutritional status of mulberry leaf produced through organic farming and its impact on cocoon production. *Indian Journal of Sericulture*. 2013; 52(1):14-18.
39. Pathak J.P.N., Iyer. Correlation between water contents of mulberry leaves, larvae and amount of urination in the spinning larvae of multivoltine race of *Bombyx mori* L. *Indian Journal of Sericulture*. 1988; 27:122-125.
40. Rashmi K. Effect of organic manures and inorganic fertilizers on growth, yield and quality of mulberry leaf. M.Sc. (Sericulture) Thesis, University of Agricultural Sciences, Bangalore, India. 2005.
41. Sunil S.H. Influence of organic based nutrient management on biochemical parameters of mulberry leaf and silkworm, *Bombyx mori* L. M.Sc. (Agricultural Biochemistry) Thesis, University of Agricultural Sciences, Bangalore, India. 2005.
42. Rashmi K., Shankar M.A., Shashidhar K.R., Narayanaswamy T.K. Growth and foliar constituents of mulberry (M5) cultivated under organic based nutrient management. *International Journal of Industrial Entomology.* 2009; 19(1):165-169.
43. Singhal B.K., Chakraborti S., Mala V.R., Sarkar A., Datta R.K. Photosynthesis for crop improvement in mulberry (*Morus* spp.) - A review. Sericologia. 2000; 40:27-55.
44. Vijaya D., Yeledhalli N.A., Ravi M.V., Nagangoud A., Nagalikar V.P. Effect of fertilizer levels and foliar nutrients on M-5 mulberry leaf nutrient content, quality and cocoon production. *Karnataka Journal of Agricultural Sciences.* 2009; 22(5):1006-1012.
45. Balashouri P., Prameeladevi Y. Effect of zinc on germination, growth, pigment content and phytomass of *Vigna radiata* and Sorghum *bicolor. Journal of Ecobiology.* 1995; 7:109-114.
46. Hiratsuka E. Researches on the nutrition of the silkworm. Shangai Shkenjo Hoko Ku Technical Bulletin. 1917; 2:353-412.
47. Horie Y. Quantitative requirement of nutrition for growth of silkworm *Bombyx mori* L. *Japan Agricultural Research Quarterly*. 1978; 12(4):211-217.
48. Ito T. An approach to nutritional control mechanisms in silkworm *Bombyx mori. Israel Journal of Entomology.* 1972; 7:1-6.
49. Sarker A.A., Haque M.R., Rab M.A., Absar N. Effect of feeding mulberry (*Morus* sp.) leaves supplemented with different nutrients of silkworm (*Bombyx mori* L.) *Current Science*.

1995; 69(2):185-188.

50. Murthy V.N.Y., Ramesh H.L., Lokesh G., Munirajappa, Yadav D.B.R. Leaf quality evaluation of ten mulberry (*Morus*) germplasm varieties through phytochemical analysis. *International Journal of Pharmaceutical Sciences Review and Research.* 2013; 21(1):182-189.
51. Singhvi N.R., Sarakar A., Datta R.K. Effect of seriboost on the mulberry leaf yield and some commercial characters of silkworm, *Bombyx mori* L. Central Sericultural Research and Training Institute, Mysore, India. 2000, 59.
52. Kawase S., Michihiro K. Small molecular weight DNA of the silkworm, *Bombyx mori. Insect Biochemistry.* 1976; 6:355-362.
53. Sterling T.M. Transpiration: water movement through plants. *Journal of Natural Resources and Life Sciences Education.* 2005; 34(1):123.
54. Machii H. Varietal differences of nitrogen and amino acid contents in mulberry leaves. *Acta Sericologica et Entomologic*a. 1989; 1:51-61.
55. Ito T, Arai N. Nutrition of the silkworm *Bombyx mori* VIII, amino acid requirements and nutritive effects of various proteins. *Bulletin of the Sericultural Experiment Station*. 1965; 19:345-373.
56. Barker A.V., Maynard D.N., Mills H.A. Variations in nitrate accumulation among spinach cultivars. *Journal of American Society for Horticultural Science.* 1974; 99:132-134.
57. Rafiq M.A., Ali A., Malik M.A., Hussain M. Effect of fertilizer levels and plant densities on yield and protein contents of autumn planted maize. P*akistan Journal of Agricultural Sciences*. 2010; 47:201-208.