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Biochemical and Behavioral Changes in Mulberry Silkworm Larvae Fed Micronutrient-Enhanced Mulberry Leaves

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Abstract: Larvae from a PMxCSR hybrid of the insect pest Bombyx mori L. are being fed mulberry leaves and micronutrients to see how they fare. We conducted a field experiment with twelve different treatments (T0-T11) including control (T0) group that received different amounts of each micronutrient. Twenty B. mori early instar larvae/replication stages were given mulberry leaves, twice a day (morning and evening) throughout their fourth and fifth instars. Compared to the control group (4th and 5th instars), treatment T9 (4 kg/ha of $ZnSO_4$ + 3 kg/ha of $FeSO_4$ + 3 kg/ha of $CuSO_4$) increased food intake and absorption by up to 31.56 and 22.98%, respectively (4th and 5th instar, respectively). In comparison to T10 (4 kg/ha of $CuSO_4$ + 3 kg/ha of $FeSO_4$ + 3 kg/ha of $ZnSO_4$), T11 (1:1:1 kg/ha of $CuSO_4$, $FeSO_4$ and $ZnSO_4$) exhibited a growth of 94.95 and a tissue growth efficiency of 65.67. A longer larval period (7.93±1.16 days) was seen in the T1 ($FeSO_4$ 10 kg/ha alone) group compared to the control group (T0). In T9, the larvae grew by 50.0% in length and breadth and 18.0 and 18.51% in length and width, respectively. Maximum chawki larval weight increased by 16.78 and 29.44%, and T9 had the largest silk gland at 2.14±0.05 g.

Keywords: *Bombyx mori*, Micronutrients, Mulberry, Larval assimilation, Digestibility, Morphometrics

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Introduction

Fast development and transformation of *Bombyx mori*, made possible by the nutritional content of mulberry leaves, constitute the physiological basis for sericulture. *Bombyx mori* silkworms specifically feed only on mulberry leaves (*Morus alba* L). Ninety-seven per cent of the silkworm's total food intake and eighty-five per cent to eighty-five per cent of feed utilization in the fifth instar larva came from the metabolically active leaves ingested during the last two instars (i.e., the fourth and fifth instars). Either improving the quality of the food or making the silkworm larvae instars hungrier might significantly boost silk production performance. Nutritional characteristics of food, biochemical state of nutrients in food, amount of hormones in body, and condition of environment all have a role in the growth, development, and eventually the physiology of insect bodies. When fed various mulberry leaves, silkworms efficiently metabolise their diet and transform it into silk (Yamamoto and Fujimaki, 1982). The quantity and quality of the leaves are directly related to the pace of growth and the weight of the larvae. Micronutrients, which function as cofactors in the activity of numerous enzymes, increase the silkworm's energy efficiency and, by extension, its silk production. Foliar vitamin supplementation has also been shown to improve mulberry leaf production and quality, which in turn promotes the healthy growth of silkworms and, in turn, boosts cocoon output and quality. Recently, it has been reported how supplementation with vitamins might alter the metabolic features of *B. mori* larvae (Marin *et al*., 2021). Analyzing the larvae's nutritional indicators in addition to its morphometrics and characteristics would help us better grasp the silkworm's larval parameters. As a result of the above, scientists investigated whether or not micronutrient addition to the soil influenced the development of *B. mori* in mulberry plants. We conducted a field experiment with twelve different treatments (T0-T11) including a control (T0) that received the optimal amount of each micronutrient.

Materials and Methods

The experiment was conducted at an altitude of 29 metres above sea level in Poovancode village, Kanyakumari district, Tamil Nadu, India (8.3031° N, 77.2881° E), where the mulberry garden was three years old. The test plot was planted in a completely bare, well-watered, and sun-drenched environment. A mulberry plant (*Morus alba*) of the MR2 (Mildew Resistant Variety -2) strain was provided by the Sericulture Department of the Government of Tamil Nadu's experimental station. A 90x60 cm gap was left between each plant. In June, before the trial began, the mulberry trees were felled. This was followed by ploughing, an annual application of 20 metric tonnes of farmyard manure, and a uniform single dose of 120 metric tonnes of nitrogen, phosphorous, and potassium (kg/ha). Every five days (or so, depending on the weather), water was applied, and twenty days after pruning, micronutrients were tilled into the soil. The experimental plants were protected from pests and any diseased or damaged sections were removed and discarded. The field experiment included 12 different treatments, and each treatment was replicated 3 times, for a grand total of 36 potential results. Every treatment received the appropriate quantity of each micronutrient, either alone or in different combinations. To clarify, Treatment 0 (No nutrients), Treatment 1 (10 kg/ha of $FeSO_4$), Treatment 2 (10 kg/ha of $ZnSO_4$), Treatment 3 (10 kg/ha of $CuSO_4$), Treatment 4 (5 kg/ha of $CuSO_4$ + 5 kg/ha of $ZnSO_4$), Treatment 5 (5 kg/ha of $CuSO_4$ + 5 kg/ha of $FeSO_4$), Treatment 6 (5 kg/ha of $ZnSO_4$ + 5 kg/ha of $FeSO_4$), Treatment 7 (5 kg/ha of $ZnSO_4$ + 5 kg/ha of $CuSO_4$), Treatment 8 (4 kg/ha of $FeSO_4$ + 3 kg/ha of $ZnSO_4$ + 3 kg/ha of $CuSO_4$), Treatment 9 (4 kg/ha of $ZnSO_4$ + 3 kg/ha of $FeSO_4$ + 3 kg/ha of $CuSO_4$), Treatment 10 (4 kg/ha of $CuSO_4$ + 3 kg/ha of $FeSO_4$ + 3 kg/ha of $ZnSO_4$) and Treatment 11 (1:1:1 kg/ha of $CuSO_4$, $FeSO_4$ and $ZnSO_4$). Each set of 20 B. mori fourth- and fifth-instar larvae was fed a meal of 5-6 completely grown mulberry leaves twice a day (morning and evening) to all the 12 groups (T0-T11). In order to conduct this research, we acquired B. mori PMxCSR2 hybrids from the Government Sericulture Training Centre in Konam, Nagercoil, Kanyakumari, Tamil Nadu, India. The procedure of rearing silkworms began when the mulberry trees were 45 days old. Because of the constant attention they required, the silkworms used in the trials were reared in a rearing chamber according to Krishnaswami's (1978) technique. Each day, the weight of the *B.*

mori larvae, the discarded mulberry leaves, and the litter was recorded. We dried the faeces and the leftover leaves in a hot air oven at 70°C until they were both the same weight. As suggested by Waldbauer (1968) we recorded the larvae's initial and final weights, as well as their food intake, assimilation, total growth, approximate digestibility, tissue development efficiency, and ecological growth efficiency. To check for statistical significance between the test and control groups, the student's t-test was used. Standard deviation and mean were confirmed for all data (S.D.).

Results and Discussion

The fourth and fifth instar *B. mori* larvae were subjected to several treatments, and the results were mixed. On both the fourth and fifth stages, T9 was the most beneficial treatment. Fourth and fifth instar T9 had the highest food consumption (31.56% and 22.98% higher, respectively; 1032.8 48.3 and 2080.4 164.6 mg/larva/day). In comparison to the control group (T0), T1 had the lowest food consumption throughout the fourth and fifth instars (848.3 26.5 and 1819.2 113.3 mg/ larva/day, respectively). The largest increase in absorption was seen in T9 (730.3 21.4 and 1694.7 110.7 mg/ larva/day; 51.38% and 34.56% above control, respectively). The worst absorption was found in the T11 strain (504.326.9 mg/larva/day, an increase of 4.50% over the control; the best assimilation was found in the T1 strain, an increase of 3.41% over the control). As a whole, the fourth instar grew by 522.1 mg/larva/day in T9, an increase of 94.95% over control, and by 296.3 mg/larva/day in T1, an increase of 10.64% over control. We also observed significant increases in the frequency of the fifth instar in the T9 population (954.8 14.7 mg/larva/day) and the T1 population (612.7 8.5 mg/larva/day), both compared to the control group by a factor of 65.67% and 6.31%, respectively. The quantity and composition of the food eaten have a significant bearing on the size of the silkworm larvae (Shivakumar, 1995). Because of their enhanced flavour, increased nutritional content, and ability to keep water in the leaves for longer periods of time, T9 showed significantly higher rates of food intake in the fourth and fifth instars (31.56% and 22.98%) as compared to controls. In addition, the insect's physiological state and the meal's physical and chemical makeup would have affected its rate of digestion and digestion.

All treatments, as compared to the control, greatly increased assimilation in the fourth and fifth instars. According to Thrived *et al*. (2003), a higher absorption rate is the result of better digestion and assimilation. Researchers Lalfelpuii *et al*. (2014 a, b) claim that larval performance may be explained by the larvae's ability to digest and absorb the nutritional components contained in mulberry leaves. The amount of food eaten and the humidity of the leaves are two elements that affect digestion. It is possible that silkworms' digestion and enzyme production improve as they consume more food. The larvae's growth depends on the quality and quantity of the food they ingest. The majority of food is eaten towards the end of its life cycle. As previously established, micronutrients have been proven to speed up larval development, and the present research verified this by showing that overall larval growth was higher in T9. Growth-promoting hormonal processes and an uptick in food intake and the larvae's capacity to turn nutrients into biomass were also implicated.

In comparison to the control, T9 exhibited an increased estimated digestibility of 15.14% in the fourth instar (70.74±4.9%) and 9.33% in the fifth instar (81.44± 6.7%). The lowest approximative digestibility was found in T11 (55.56x5.8%) and T1 (70.97±7.9%), both of which were decreased by -9.60% and -4.70%, respectively, from the control. Tissue growth efficiency was best in the fourth and fifth instars at T11, with values of 91.77± 4.2% and 68.45± 4.6%, respectively; these values were increased by 65.35% and 49.61% above the control. Their smallest T1 values, in comparison, were 54.18±4.6% and 45.45x6.4%, decreases of -2.37% and increases of 3.71% from the control. Maximum and minimum ecological

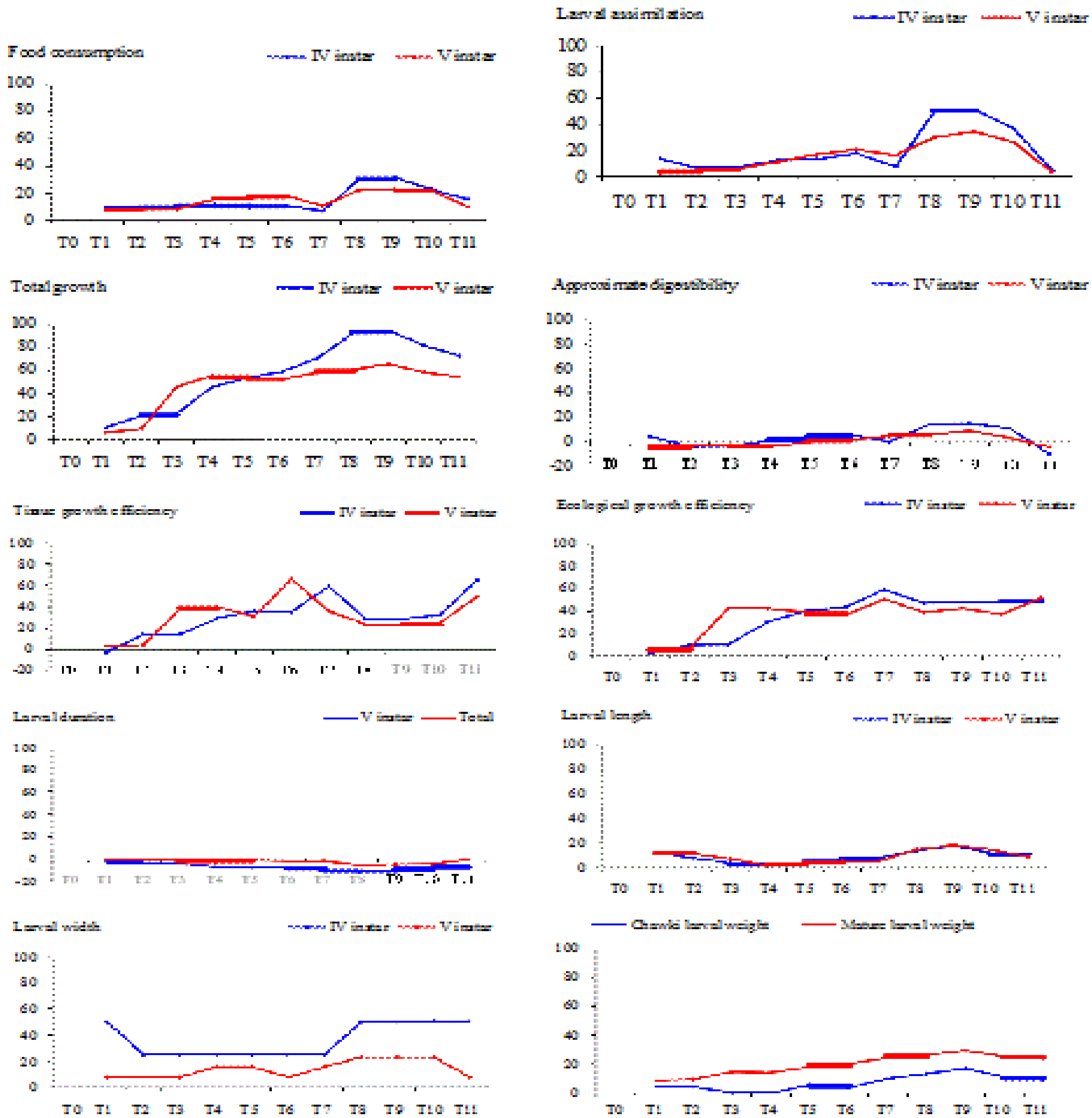

Fig. 1: *B. mori* larval parameters-effects of treatments.

growth efficiency values were recorded by the fourth instar larvae in T11 (51.07± 3.3%) and T1 (34.93± 5.2%), representing increases of 49.58% and 2.31%, respectively, over the control, while the fifth instar recorded 48.9±1.5% and 33.67±2.6%, representing increases of 52.0% and 4.66%, respectively, over the control (Table 1; Fig. 1). Food's approximative digestibility is measured as the percentage of food that is absorbed in comparison to how much is consumed. In this research, differences in leaf quality were the primary source of variance across treatments. Food's estimated digestibility increases as it moves through the stomach and is digested and assimilated more thoroughly. It was previously believed that larvae's variable reactions to the physical and chemical composition of food were to blame for the age-related decline in tissue development efficiency and ecological growth efficiency.

Both the adult and larval stages of T1 lived for a very long time (7.93 ± 1.16 days). When

Table 1: Effect of treatments on the nutritional characteristics of *B. mori* larvae

Treatment	Food consumption (mg/ larva/ day)		Larval assimilation (mg/ larva/ day)		Total growth (mg/ larva/ day)		Approximate digestibility (%)		Tissue growth efficiency (%)		Ecological growth efficiency (%)	
	Fourth instar	Fifth instar	Fourth instar	Fifth instar	Fourth instar	Fifth instar	Fourth instar	Fifth instar	Fourth instar	Fifth instar	Fourth instar	Fifth instar
T0	784.4± 31.4	1691.6± 123.5	482.4± 25.3	1259.4± 93.8	267.8± 3.6	576.3± 4.7	61.49± 6.2	74.45± 8.6	55.5± 6.8	45.75± 7.9	34.14± 4.1	32.17± 4.3
T1	848.3± 26.5	1819.2± 113.3	546.8± 18.4*	1291.8± 104.3	296.3± 3.4*	612.7± 8.5	64.45± 3.7	70.97± 7.9	54.18± 4.6	47.45± 6.4	34.93± 5.2	33.67± 2.6
T2	862.4± 49.2*	1830.4± 122.7	510.9± 19.4	1320.6± 95.3	323.7± 7.5*	631.0± 6.4*	59.24± 4.5	72.13± 6.4	63.35± 5.2*	47.80± 4.2	37.53± 3.7*	34.47± 2.3
T3	868.5± 21.6*	1834.6± 142.9	516.3± 22.4	1334.1± 104.8	327.8± 9.8*	842.3± 7.5*	59.44± 3.1	72.74± 5.7	63.49± 4.4*	63.14± 3.7*	37.74± 3.6*	45.91± 3.2*
T4	875.6± 14.3*	1954.7± 124.7*	543.2± 38.6*	1402.7± 129.5*	390.4± 5.9*	897.2± 8.2*	62.03± 3.6	71.75± 8.3	71.87± 5.1*	63.96± 3.2*	44.58± 3.4*	45.89± 5.2*
T5	858.7± 48.8*	1974.3± 134.7*	546.2± 24.3*	1474.8± 113.4*	411.8± 3.4*	884.1± 10.4*	63.60± 4.1	74.71± 8.9	75.42± 5.7*	59.94± 3.6*	47.95± 3.2*	44.78± 2.2*
T6	870.3± 32.4*	1998.5± 152.6*	568.1± 31.2*	1518.2± 108.5*	426.9± 6.4*	879.4± 7.2*	65.28± 4.7	75.97± 5.6	75.14± 5.6*	57.92± 3.8*	49.05± 3.6*	44.0± 2.3*
T7	840.4± 24.5	1875.4± 117.8*	518.7± 21.6	1465.1± 104.6*	457.8± 4.1*	911.3± 12.4*	61.72± 5.8	78.13± 7.8	88.25± 3.4*	62.20± 3.1*	54.47± 3.3*	48.59± 2.7*
T8	1023.3± 31.6*	2067.9± 103.7*	721.9± 33.7*	1637.6± 112.5*	515.5± 9.5*	926.7± 13.4*	70.48± 2.9*	79.19± 6.3	71.40± 3.2*	56.58± 5.7*	50.37± 4.1*	44.81± 3.4*
T9	1032.8± 48.3*	2080.4± 164.6*	730.3± 21.4*	1694.7± 110.7*	522.1± 4.7*	954.8± 14.7*	70.74± 4.9*	81.44± 6.7*	71.49± 4.1*	56.34± 4.4*	50.55± 4.2*	45.89± 3.8*
T10	964.2± 36.1*	2064.7± 142.5*	662.5± 25.2*	1594.9± 97.9*	487.2± 3.6*	912.4± 10.3*	68.65± 6.4*	77.24± 7.8	73.53± 5.3*	57.20± 5.1*	50.52± 3.7*	44.19± 4.1*
T11	906.1± 46.8*	1862.5± 136.3*	504.3± 26.9	1302.4± 97.2	462.8± 4.6*	891.3± 12.6*	55.56± 5.8	71.48± 6.5	91.77± 4.2*	68.45± 4.6*	51.07± 4.2*	48.90± 1.5*

Values mean± S.D. *Significant P≤0.05 (t-test)

Table 2: Effect of treatments on the morphometrics of *B. mori* larvae

Treatment	Larval duration (days)		Larval length (cm)		Larval width (cm)		Chawki larval weight (g/ 10)	Mature larval weight (g/ 10)	Silk gland weight (g)
	Fifth instar	Total	Fifth instar	Total	Fifth instar	Total			
T0	6.79± 1.12	19.22± 5.11	5.0± 0.12	5.4± 0.17	0.4± 0.09	1.0± 0.22	1.43± 0.59	33.89± 4.8	1.44± 0.02
T1	7.93± 1.16	25.08± 5.44	5.6± 0.15*	6.1± 0.26*	0.6± 0.3*	1.4± 0.3	1.50± 0.87	36.86± 5.2	1.58± 0.05
T2	7.51± 2.22	25.06± 5.14	5.4± 0.17	6.0± 0.19*	0.5± 0.1*	1.4± 0.14	1.49± 0.51	37.07± 3.9*	1.53± 0.1
T3	7.46± 3.45	25.06± 5.17	5.2± 0.14	5.8± 0.19	0.5± 0.2*	1.4± 0.3	1.43± 0.47	38.83± 1.6*	1.67± 0.03
T4	7.22± 3.23	24.46± 6.23	5.1± 0.15	5.5± 0.18	0.5± 0.1*	1.5± 0.23*	1.43± 0.83	38.67± 2.9*	1.62± 0.01
T5	7.20± 2.45	24.87± 6.23	5.2± 0.11	5.6± 0.11	0.5± 0.2*	1.5± 0.21	1.51± 0.20	40.05± 4.22*	1.83± 0.02
T6	7.20± 3.21	24.73± 6.34	5.3± 0.19	5.7± 0.08	0.5± 0.2*	1.4± 0.26	1.48± 0.14	40.72± 3.54*	1.87± 0.04
T7	7.01± 3.26	24.68± 6.56	5.4± 0.21	5.7± 0.23	0.5± 0.15*	1.5± 0.11*	1.56± 0.13*	42.11± 1.11*	1.71± 0.07
T8	6.86± 3.35	23.81± 6.12	5.7± 0.10*	6.2± 0.08*	0.6± 0.18*	1.6± 0.10*	1.62± 0.14*	42.87± 5.30*	2.00± 0.04
T9	6.94± 2.21	23.96± 7.13	5.9± 0.13*	6.4± 0.10*	0.6± 0.1*	1.6± 0.43*	1.67± 0.15*	43.87± 3.29*	2.14± 0.05
T10	7.14± 3.45	23.03± 7.16	5.6± 0.16*	6.2± 0.15*	0.6± 0.1*	1.6± 0.13*	1.58± 0.94*	42.45± 3.69*	1.98± 0.02
T11	7.29± 4.32	24.04± 7.18	5.5± 0.14*	5.9± 0.12*	0.6± 0.16*	1.4± 0.19	1.56± 0.39*	41.97± 4.62*	1.84± 0.01

Values mean± S.D. *Significant P≤0.05 (t-test) compared to the control group (T0), its duration was notably high. As a whole, T8 (6.86± 3.35) lasted only 23.81± 6.12. Compared to the control group, T9 showed an increase of 18.0% in the maximum and 2.0% in the minimum length of the fourth instar (5.9± 0.13 cm and 5.1± 0.15 cm, respectively). The fifth instar grew by 18.51% in T9, or 6.4 ± 0.10 cm, and by 1.85%, or 5.5 ± 0.18 cm, over the control period. Maximum values for T8 (0.6 ± 0.18 cm) were significantly higher than those for T11 (0.6 ± 0.1 cm), T1 (0.6 ± 0.3 cm), T10 (0.6 ± 0.1 cm), and T9 (0.6 ± 0.1 cm), all of which increased by 50.0% relative to the control, while minimum values for the other treatments (i.e. T2, T3, T4, T5, T6, T7, and T8) were significantly lower than 0.5 cm. The widest treatments were T9 (1.6± 0.43 cm), T10 (1.6± 0.13 cm), and T8 (1.6± 0.10 cm), all of which were increases of 23.07% over the control, while the narrowest were 1.0 cm or less (Table 2; Fig. 1). In T9, the chawki larval weight reached its maximum point (1.67± 0.15 g/ 10), a 16.78% increase over the control, and its lowest point (1.43± 0.47 g/ 10 and 1.43± 0.83 g/ 10), which was equivalent to the control (1.43± 0.59 g/ 10). Maximum and lowest mature larval weight values were 43.87 ± 3.29 g/ 10 in T9 and 36.86 ± 5.2 g/ 10 in T1, up 29.44% and 8.76% from control, respectively. T9 had the heaviest silk glands (2.14 ± 0.05 g), while T2 had the lightest (1.53 ± 0.1 g) (Table 2; Fig. 1).

This research significantly enhanced the morphometrics of late-stage larvae (stages four and five). The increased larval weight could be attributed to high-quality leaves with vitamin content.

Other characteristics of larvae include healthier larvae with improved micronutrient use and absorption (Bose *et al*., 1994; Sarker *et al*.,1995; Nirwani and Kaliwal, 1995, 1996; Etebari *et al*., 2004; Ramarethinam and Chandra, 2007; Balasundaram *et al*., 2008). Higher larval weights result in higher silk gland weights. Silk glands develop at their fastest rate around the end of the fifth instar due to fibroin synthesis, and it is obvious that the weight of the silk glands is one of the most important factors to consider when evaluating the larvae's capacity to produce silk. According to studies on micronutrients and the development of the silk gland and larval body (Bhattacharya and Kaliwal, 2005a, b), the latter finding was also observed in the current investigation. When compared to other treatments, T9 had the highest value across all therapies. When zinc is administered through mulberry leaves, the weight of silkworm larvae increases significantly (Chamundeswari and Radhakrishnaiah, 1994; Hugar and Kaliwal, 1999, 2002; Ashfaq *et al*., 2010). Furthermore, zinc promotes the development of the silk gland and the larval body, shifting the balance toward a higher gland-body ratio during the 5th instar (Kavitha *et al*., 2012).

Conclusion

This study clearly showed that the supplementation of the different micronutrients on different ratio have notable impact on the nutritional and morphometric characteristic features of the larvae. The positive impacts of the micronutrients on the total growth, food consumption, larval assimilation, digestibility, tissue growth and ecological growth characteristics were clearly observed. The notable effect of micronutrients on the larval length, larval width, larval weight, larval duration and silk gland weights were also clearly understood.

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