Indian Journal of Entomology 85(4): 914-920 (2023) DoI. No.: 10.55446/IJE.2022.584

EFFECT OF MICRONUTRIENT SUPPLEMENTED MULBERRY LEAVES ON LARVAE OF SILK WORM *BOMBYX MORI*

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ABSTRACT

The present study explores the effect of micronutrient supplemented mulberry leaves on PM×CSR_2 hybrid variety of *Bombyx mori* L. larvae. The field experiment with twelve treatments including a control (T0 to T11), replicated thrice, was supplemented with the desired quantity of the respective micronutrient in individual or in combination. Twenty early *B. mori* fourth and fifth instar larvae/ replication were fed with the 5-6 fully mature mulberry leaves (T0 to T11) twice a day (morning and evening). The results showed that food consumption increased to a maximum of 31.56 and 22.98%, and assimilation increased by 51.38 and 34.56% over control (4th and 5th instar, respectively) with treatment T9 ($CuSO_4$ 15 kg/ ha + $ZnSO_4$ 15 kg/ ha + $FeSO_4$ 30 kg/ ha). With the same treatment, total growth increased by 94.95 and 65.67%, and approximate digestibility by 15.14 and 9.33%; tissue growth efficiency also increased by 65.35 and 49.61% with this treatment, while maximum ecological growth efficiency increased by 49.58% and 52.0% over control in T11 ($CuSO_4$ 25 kg/ ha + $ZnSO_4$ 25 kg/ ha + $FeSO_4$ 25 kg/ ha). Larval duration was maximum with T1 ($FeSO_4$ 10 kg/ ha alone) (7.63± 19.16 days) as against when fed with the control (T0). Larval length increased by 18.0 and 18.51% in T9, with the larval width increasing by 50.0% in more than one treatment. Maximum chawki larval weight and larval weight increased by 16.78 and 29.44%, while maximum silk gland weight was 2.14± 0.05g in T9.

Key words: *Bombyx mori*, micronutrients, mulberry, $CuSO_4$, $ZnSO_4$ food consumption, larval assimilation, total growth, digestibility, tissue growth efficiency, morphometrics, characteristics

Nutritional quality in mulberry leaves helps to accelerate the growth and metamorphosis in *B. mori* which are the physiological foundations for sericulture. The mulberry silkworm, *B. mori* feeds exclusively on the leaves of mulberry (*Morus alba* L). During the last two instars (i.e. 4th and 5th instars) of the silkworm life cycle, 97% of the total food intake and 80-85% of the feed utilization in the fifth instar larva of the total leaves consumed were metabolically active (Rahamathulla et al., 2003; Rahamathulla and Suresh, 2012). It is therefore essential to improve either food quality or appetite (or both) of larval instars of silkworm for better performance in silk production. Nutritional qualities of food, biochemical status of nutrients in the food, hormonal level in the body and environmental conditions all play a role in the growth, development and eventually physiology of insect bodies (Murugan and George, 1992; Singh et al., 2021). The silkworms respond in variety of way when fed with the mulberry leaves of different nutritional indicating efficient food utilization and conversion into silk substances (Yamamoto and Fujimaki, 1982). Furthermore, the rate of food consumption and leaf quality influence significantly on larval growth and weight. Micronutrients are useful in enhancing the energetic efficiency of the silkworm, as they are cofactors in the activity of several enzymes, increasing energy levels, which in turn reflects on the silk production in silkworms (Chamundeswari and Radhakrishnaiah, 1994). Additionally, foliar micronutrient supplementation has been shown to improve the yield and quality of mulberry leaves, and subsequently the healthy growth of silkworms, resulting in increased cocoon yield and quality (Geetha et al., 2016). The effect of micronutrients supplementation on the biochemical characteristics of *B. mori* larvae was recently reported by the present authors (Marin et al., 2021). Therefore, analysing the nutritional indices of the larvae as well as, morphometrics and their characteristics would be useful in understanding the larval parameters of the silkworm. In view the above facts, an attempt was made to determine the impact soil application of micronutrients in mulberry plants fed to *B. mori*.

MATERIALS AND METHODS

The field experiment was conducted on a three years old mulberry garden at Poovancode village, Kanyakumari district, Tamil Nadu, India (8.3031° N, 77.2881° E) at an elevation/ altitude of 29 m above sea level. The experimental plot was free from other plants and received direct sunlight exposure with proper irrigation. For the experiments, MR2 (Mildew Resistant Variety –2) mulberry plant (*M. alba*) developed by the Sericulture Department, Govt. of Tamil Nadu experimental station, Coonoor, Tamil Nadu, India was selected and the spacing between the plants was 90x60cm. Prior to the commencement of the experiment, mulberry plants were pruned in June, followed by ploughing, and farm yard manure was applied at the rate of 20 t/ ha/ year, and single dose of nitrogen, phosphorous and potash at 120:120:60 kg/ ha/ year was hoed in the soil uniformly. Depending upon the climatic conditions, irrigation was provided every five days of interval and micronutrients were added to the soil after twenty days of pruning. The experimental plot was protected from plant pests, and the diseased/ affected parts of the plant were removed periodically. The field experiment was laid out in a randomized block design with twelve treatments, each treatments were replicated thrice. Each treatment was supplemented with the required amount of each micronutrient, either individual or in combination as follows. T0 - Control (mulberry plants which did not receive micronutrients supplementation); T1 - $FeSO_4$ 10 kg/ ha; T2 - Zn SO_4 5 kg/ ha; T3 - Cu SO_4 5 kg/ ha; T4 - $CuSO_4$ 5 kg/ ha + $ZnSO_4$ 5 kg/ ha; T5 - $CuSO_4$ 5 kg/ ha + $FeSO_4$ 10kg/ ha; T6 - $FeSO_4$ 10 kg/ ha + $ZnSO_4$ 5 kg/ ha; T7 - $CuSO_4$ 5 kg/ ha + $ZnSO_4$ 5 kg/ ha + $FeSO_4$ 10 kg/ ha; T8 - $CuSO_4$ 10 kg/ ha + $ZnSO_4$ 10 kg/ ha + $FeSO_4$ 20 kg/ ha; T9 - $CuSO_4$ 15 kg/ ha + $ZnSO_4$ 15 kg/ ha + $FeSO_4$ 30 kg/ ha; T10 - $CuSO_4$ 20 kg/ ha + $ZnSO_4$ 20 kg/ ha + $FeSO_4$ 40 kg/ ha; and T11 - $CuSO_4$ 25 kg/ ha + $ZnSO_4$ 25 kg/ ha + $FeSO_4$ 25 kg/ ha. Twenty early *B. mori* fourth and fifth instar larvae were used as a replication, and were fed with the 5-6 fully grown mature mulberry leaves (T0 to T11) twice a day (morning and evening).

The PMxCSR$_2$ hybrid variety of *B. mori* used in the present study was procured from Government Sericulture Training Centre, Konam, Nagercoil, Kanyakumari, Tamil Nadu, India. Silkworm rearing began when the mulberry plants were 45 days old. Since the experiments required continuous maintenance of the test species, the silkworms were reared in a rearing room in accordance with the procedure of Krishnaswami (1978). *B. mori* larvae were fed with mulberry leaves and the remaining leaves and litter were weighed and recorded on a daily basis, and also the weight of the larvae was recorded in each treatment. The remaining leaves and the excreta were dried in a hot air oven at 70°C till constant weight and the values were recorded. To determine the larval growth, initial and final weights of the larvae as suggested by Waldbauer (1968) and Kaushal et al. (1988) food consumption, assimilation, total growth, approximate digestibility, tissue and ecological growth efficiency were calculated, besides other larval parameters. All data were affirmed as mean ± standard deviation (S.D.), and were subjected to Student's 't' test to find out the significant difference between control and treatment groups.

RESULTS AND DISCUSSION

On fourth and fifth instar *B. mori* larvae, several treatments had different effects. Among the treatments, T9 had a higher value in both the fourth and fifth instar. Highest food consumption by fourth and fifth instar was observed in T9 (1032.8± 48.3 and 2080.4± 164.6 mg/ larva/ day), with an increase of 31.56% and 22.98% over control, was observed in T9, and T1 had the lowest food consumption (848.3± 26.5 and 1819.2± 113.3 mg/ larva/ day) with an increase of 8.14% and 7.54% over control respectively. T9 (730.3± 21.4 and 1694.7± 110.7 mg/ larva/ day) had highest assimilation which increased by 51.38% and 34.56% over control, while T11 (504.3± 26.9 mg/ larva/ day) which increased by 4.50% over control and T1 (1291.8± 104.3 mg/ larva/ day) which increased by 3.41% over control had lowest. The maximum total growth of the fourth instar was recorded in T9 (522.1± 4.7 mg/ larva/ day) which increased by 94.95% over control, and minimum in T1 (296.3± 3.4 mg/ larva/ day) which increased by 10.64% over control. For fifth instar, it was also observed in T9 (954.8± 14.7 mg/ larva/ day) and T1 (612.7± 8.5 mg/ larva/ day) with an increase of 65.67% and 6.31% over control respectively (Table 1; Fig. 1). Food consumption has direct influence on larvae weight, and also depends on the types of nutrition (Shivakumar, 1995) as well as silkworm breeds (Remadevi et al., 1992). T9 showed considerably greater rate of food consumption (31.56% and 22.98%) in the fourth and fifth instar over control, which may be assumed due to their palatability, nutritional superiority and water retention capacity of leaves for longer duration. Further, the rate of food consumption would have also been influenced by the physical and chemical nature of food and also the physiological state of the insect too (Waldbauer, 1968).

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

Treat-ment	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*

Morphometrics

Treat-ment	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	Fourth instar	Fifth instar	Fourth instar	Fifth instar			
T0	7.79± 17.12	25.22± 55.11	5.0± 0.12	5.4± 0.17	0.4± 0.9	1.0± 0.22	1.43± 0.59	33.89± 4.8	1.44± 0.02
T1	7.63± 19.16	25.08± 56.44	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± 23.22	25.06± 58.14	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± 34.45	25.06± 59.17	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± 34.23	24.46± 61.23	5.1± 0.15	5.5± 0.18	0.5± 0.4*	1.5± 0.23*	1.43± 0.83	38.67± 2.9*	1.62± 0.01
T5	7.20± 25.45	24.87± 63.23	5.2± 0.11	5.6± 0.11	0.5± 2.0*	1.5± 0.21	1.51± 1.20	40.05± 4.22*	1.83± 0.02
T6	7.20± 36.21	24.73± 63.34	5.3± 0.19	5.7± 0.08	0.5± 2.0*	1.4± 0.26	1.48± 1.04	40.72± 3.54*	1.87± 0.04
T7	7.01± 39.26	24.68± 67.56	5.4± 0.21	5.7± 0.23	0.5± 0.5*	1.5± 0.11*	1.56± 0.73*	42.11± 1.11*	1.71± 0.07
T8	6.86± 43.35	23.81± 69.12	5.7± 0.10*	6.2± 0.08*	0.6± 0.8*	1.6± 0.10*	1.62± 1.42*	42.87± 5.30*	2.00± 0.04
T9	6.94± 42.21	23.96± 70.13	5.9± 0.13*	6.4± 0.10*	0.6± 0.1*	1.6± 0.43*	1.67± 1.52*	43.87± 3.29*	2.14± 0.05
T10	7.14± 35.45	23.03± 70.16	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± 45.32	24.04± 73.18	5.5± 0.14*	5.9± 0.12*	0.6± 0.6*	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)

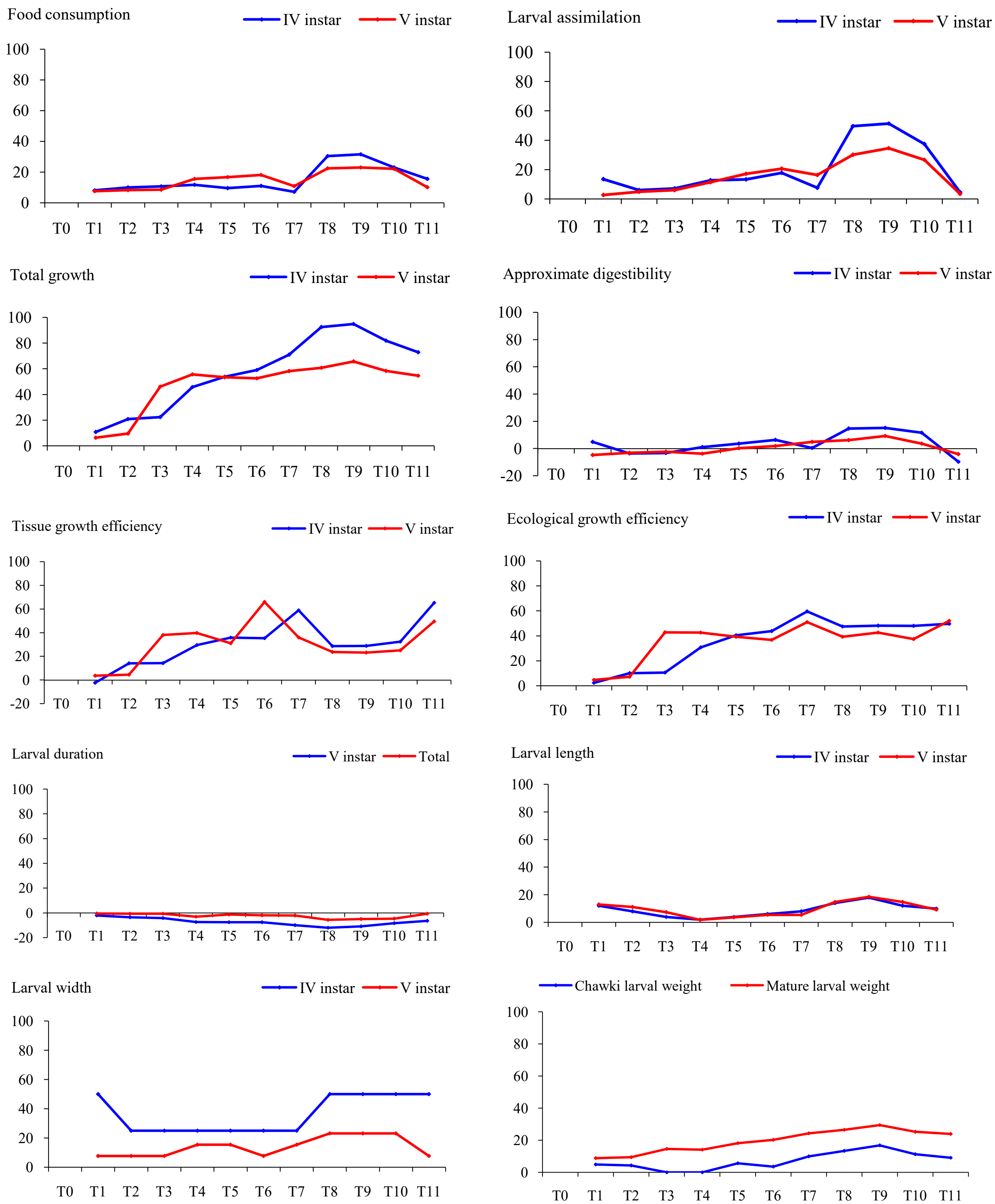

Fig. 1. *B. mori* larval parameters - effects of treatments

Similar to food consumption, assimilation of fourth and fifth instar was also significantly higher in all the treatments when compared to control. Increased digestion and assimilation results in improved assimilation rate efficiency (Thrived et al., 2003), and the larval performance in the present study was evident by the digestion and assimilation of the nutritional materials present in mulberry leaves as reported by Lalfelpuii et al. (2014 a,b). Food assimilation depends on various factors like ingested food and leaf moisture content, and enhanced assimilation of silkworm may be due to easy digestibility, and higher enzyme synthesis corresponding to enhanced food intake. The larvae growth is determined by the amount of food they consume and how well they utilised it. Nearly 80% of the food is consumed in its late stage of life cycle. Micronutrients have been shown to accelerate larval growth (Ito and Niminura, 1966; Horie et al., 1967), and this was observed in the present findings too, wherein, total larval growth was found to be higher in T9, due to the increase in food intake, and the ability of the larva to convert the food into biomass, besides, rate of metabolism, and hormonal mechanisms which directly/indirectly favours growth.

Maximum approximate digestibility was noticed in T9 for fourth (70.74± 4.9%) and fifth (81.44± 6.7%) instars with an increase of 15.14% and 9.33% over control, while minimum approximate digestibility was observed in T11 (55.56± 5.8%) and T1 (70.97± 7.9%) with a decrease of -9.60% and -4.70% over control respectively. The fourth and fifth instars showed maximum tissue growth efficiency in T11, with values of 91.77± 4.2% and 68.45± 4.6% which increased by 65.35% and 49.61% over control, while their minimum values were 54.18± 4.6% and 45.45± 6.4% in T1, which decreased and increased by -2.37% and 3.71% over control respectively. For ecological growth efficiency, the fourth instar larvae registered its maximum and minimum value in T11 (51.07± 3.3%) and T1 (34.93± 5.2%) with an increase of 49.58% and 2.31% over control respectively, and the respective values for fifth instar were 48.9± 1.5% and 33.67± 2.6% which was also observed in T11 and T1 with an increase of 52.0% and 4.66% over control (Table 1; Fig. 1). Approximate digestibility is the ratio of the amount of food digested to the amount of food ingested in percentage. The variation among the different treatments in this study was due to the general difference in the leaf quality. The passage of food through gut facilitates increased digestion and assimilation which ultimately result in improved approximate digestibility. According to Muniandy et al. (1995), multivitamins and mineral compounds increase food intake, growth and conversion efficiency of silkworm. The present findings clearly indicated that the leaves supplemented with micronutrients showed high conversion efficiencies which may reduce the larval life span. The amount of digested food metabolized for energy decreases tissue growth efficiency but increases the maintenance of physical activities (Waldbauer, 1968), and this study correlated with the above facts. The decreasing trend of tissue growth efficiency and ecological growth efficiency with the increasing age of larvae was attributed to their differential response of the physical and chemical constitution of food (Delvi and Pandian, 1972; Mehrotra et al., 1972).

T1 had a long larval duration (in days) (7.63± 19.16), and total duration of 25.08± 56.44. This was reported to be short when compared to the control group (T0). On the other hand, the shortest duration was noticed in T8 (6.86± 42.21) with total duration of 23.81± 69.12. The maximum length of fourth instar was reported in T9 (5.9± 0.13 cm), and its minimum value was reported in T4 (5.1± 0.15 cm) which increased by 18.0% and 2.0% over control. For the fifth instar it was 6.4± 0.10 cm in T9 and 5.5± 0.18 cm in T4 which increased by 18.51% and 1.85% over control respectively. With respect to the larval width, significantly maximum values were recorded in T8 (0.6± 0.8 cm), followed by T11 (0.6± 0.6 cm), T1 (0.6± 0.3 cm), T10 (0.6± 0.14 cm) and T9 (0.6± 0.1 cm) all of which increased by 50.0% over control, while the remaining treatments (i.e. T2, T3, T4, T5, T6, T7 and T8) reported significantly minimum values of 0.5 cm. In the case of fifth instar, maximum width was observed in T9 (1.6± 0.43 cm), T10 (1.6± 0.13 cm) and T8 (1.6± 0.10 cm) which increased by 23.07% over control, while a minimum value of 1.0cm was observed in the other treatments (Table 2; Fig. 1). Maximum chawki larval weight was recorded in T9 (1.67± 1.52 g/ 10), with an increase of 16.78% over control, and minimum value in T3 and T4 (1.43± 0.47 g/ 10 and 1.43± 0.83 g/ 10) which was similar with the control (1.43± 0.59 g/ 10). For mature larval weight, maximum and minimum values were 43.87± 5.30 g/ 10 in T9 and 36.86± 5.2 g/ 10 in T1 which increased by 29.44% and 8.76% over control. In the case of silk gland weight, the maximum value was noted in T9 (2.14± 0.05 g), and minimum in T2 (1.53± 0.1 g) (Table 2; Fig. 1).

The morphometrics of fourth and fifth instar larvae significantly increased in the present study. Increased larval weight could be attributed to high quality of leaves containing micronutrients, which makes the larvae healthier with better utilization and assimilation of micronutrients (Bose et al., 1994; Ramarethinam and Chandra, 2007), and other larval characteristics (Sarker et al., 1995; Nirwani and Kaliwal, 1995, 1996; Etebari et al., 2004; Balasundaram et al., 2008). The higher larval weights lead to higher silk gland weight. Silk gland attain maximum growth towards the end of the fifth instar owing to fibroin synthesis, and it is clear that the silk gland weight is one of the vital parameters for assessing the silk production potential of the larvae (Legay, 1958). Micronutrients show positive growth trends with reference to the growth of silk gland and larval body (Chakraborti and Medda, 1978; Bhattacharya and Kaliwal, 2005a,b), and the same was reported in the present study too. Amongst all treatments, T9 showed the maximum value as compared to other treatments. The aadministration of zinc to silkworm larvae through mulberry leaves significantly increase larval weight (Chamundeswari and Radhakrishnaiah, 1994; Balamani et al., 1995; Hugar and Kaliwal, 1999, 2002; Ashfaq et al., 2010). Further, zinc has a stimulating effect on the growth of silk gland and larval body, and with this effect, it shifts the balance towards higher gland-body ratio during fifth instar development (Kavitha et al., 2012).

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(Manuscript Received: March, 2022; Revised: May, 2022;
Accepted: May, 2022; Online Published: June, 2022)
Online First in www.entosocindia.org and indianentomology.org Ref. No. e22186