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Effect of Micronutrients Supplemented Mulberry Leaves on the Cocoon Parameters of Mulberry Silkworm, *Bombyx mori* Linnaeus 1758 (Lepidoptera: Bombycidae)

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A B S T R A C T

Nutritive value of mulberry leaf is a key factor besides environment and technology for better production of silkworm cocoons. The present study deals with the effect of micronutrient treated mulberry leaves on the economic characters of PM×CSR$_2$ hybrid variety of mulberry silkworm, *Bombyx mori*. The field experiment was laid out in a randomized block design with twelve treatments including a control (T_0 to T_{11}), and the treatments were replicated thrice. Each treatment was supplemented with the desired quantity of the respective micronutrient in single or in combination. Twenty early *Bombyx mori* fourth and fifth instar larvae were used for each replicate of the respective treatment, and were fed with 5-6 fully grown mature mulberry leaves (T_0 to T_{11}) twice a day (morning and evening). The parameters pertaining to the cocoon characteristics, viz., cocoon length, width, its ratio, cocoon and pupal weight, shell weight and ratio, sericin and fibroin, filament length and weight, ERR and silk productivity, and denier and renditta were calculated. The highest cocoon length, width and its ratio was recorded in T_{10} (3.93±0.04cm), T_{10} (1.90±2.70cm) and T_8 (2.12±0.05cm) with an increase of 20.55, 14.46 and 8.16% over control respectively. The maximum cocoon weight and pupal weight was recorded in T_{10} (16.43±1.02g) and T_8 (13.21±0.5g) which increased 28.56 and 26.29% over control respectively. Maximum shell weight was registered in T_9 (3.21±0.16g) with an increase of 42.66% over control. In the case of shell ratio, maximum value was noted in T_9 (19.77±1.28g) with an increase of 12.32% over control. The highest sericin and fibroin content was recorded in T_8 (27.29±4.80) and T_3 (79.50±4.76) with an increase of 28.66 and 6.31% over control. The longest filament length was noted in T_6 (765.1±14.62m) with an increase of 6.73% over control, while the maximum filament weight was observed in both T_9 and T_8 (0.25±0.04 and 0.25±0.02g) which increased by 25.0% over control. ERR was maximum in both T_8 and T_9 (97.32±18.34%) with an increase of 9.76% over control, and silk productivity was maximum in T_{10} (4.55±2cg/day) and its increase was by 46.77% over control. The maximum denier and renditta was recorded in T_9 (2.68±0.26) and T_9 (8.41±1.24) which increased by 11.66 and 12.28% respectively when compared with control. Overall results of the present study emphasized that supplementation of micronutrients were found to enhance the economic characteristics of *Bombyx mori* cocoons.

***Key words*:** Pupa, Shell, Sericin, Fibroin, Filament, ERR, Silk productivity, Denier, Renditta

Insects like vertebrates, require a variety of micronutrients for their growth and development. Silkworm fed with good quality mulberry leaves in abundant quantity yields successful cocoon production, thereby enhancing the economic traits of the *Bombyx mori* cocoons (length, width, weight, pupal weight, shell weight, shell ratio, silk characters like filament length and weight, sericin and fibroin, and denier and renditta) to get more quality and quantity of silk yield. Better response could be attributed to better nutritive value of leaves resulting in better silkworm cocoon yield and quality [1]. Nazar *et al*. [2] stated that current emphasis is being given to major nutrients with less focus on micronutrients. Cocoon production depends on the nutrition and health status of silkworm larvae, and supplementation of various micronutrients is vital to increase the economic parameters of the *Bombyx mori* cocoons and their quality [3-13]. In recent years, several attempts have been made to improve leaf quality with different micronutrients, to enhance the economic quality of cocoon. Employment of soil application of micronutrients can improve the quality and quantity of the mulberry leaves. Micronutrients in the leaves

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may improve the plant health, quality and yield and subsequently the healthy growth of silkworms, resulting in increased cocoon yield with quality. Recently, the effect of micronutrients supplemented mulberry leaves on the biochemical characteristics of *Bombyx mori* larvae was reported by Marin *et al*. [14]. Therefore, keeping in view the above facts, an attempt was made to find out the effect of mulberry leaves supplemented with micronutrients to assess the economic parameters of mulberry silkworm, *Bombyx mori* cocoons.

MATERIALS AND METHODS

The field experiment was conducted under irrigated conditions at Poovancode village, Kanyakumari district, Tamil Nadu, India (8.3031° N, 77.2881° E) on a three year old mulberry garden. The experimental plot was free from other plants and received direct sunlight exposure. MR2 (Mildew Resistant Variety –2) mulberry plant (*Morus alba*) developed by the Sericulture Department, Govt. of Tamil Nadu experimental station, Coonoor, Tamil Nadu, India was selected for the experiments, and were planted at 90x60cm spacing. Prior to the commencement of the experiment, mulberry plants were pruned, followed by ploughing. Farm yard manure was applied at the rate of 20t/ha/yr, and single dose of nitrogen, phosphorous and potash at 120:120:60kg/ha/yr was incorporated in the soil uniformly by hoeing. Irrigation was provided at five days interval depending upon the climatic conditions. 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 and the treatments were replicated thrice. Each treatment was supplemented with the desired quantity of the respective micronutrient in single or in combination which are as follows:

T_0: Control (mulberry plants which did not receive micronutrients supplementation)
T_1: $FeSO_4$ 10Kg/ha
T_2: Zn SO_4 5Kg/ha
T_3: Cu SO_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 + $FeSO_4$ 10 Kg/ha
T_8: $CuSO_4$ 10Kg/ha + $ZnSO_4$ 10Kg/ha + $FeSO_4$ 20 Kg/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

Twenty early *Bombyx mori* fourth and fifth instar larvae were used for each replicate of the respective treatment, and were fed with 5-6 fully grown mature mulberry leaves (T_0 to T_{11}) twice a day (morning and evening).

The present investigation was carried out on PM×CSR_2 hybrid variety of *Bombyx mori* procured from Government Sericulture Training Centre, Konam, Nagercoil, Kanyakumari, Tamil Nadu, India. The rearing of silkworm commenced 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 [15]. Mature larvae were handpicked and mounted on netrika for spinning at 30 larvae/30cm.sq. The cocoons were harvested manually on the fifth day after mounting. The fifth instar on completion of seventh day was picked up in time and mounted in netrika for cocooning to avoid silk wastage. During cocooning, moderate temperature, proper ventilation, light, and mounting density were maintained. Spinning was completed in 2/3 days. The larvae inside the cocoon turn into pupa on the fourth day, and the seed cocoons were harvested on the fifth day of spinning. The cocoon length, width, its ratio, cocoon and pupal weight, shell weight and ratio, sericin and fibroin, filament length and weight, Effective rate of rearing (ERR), silk productivity, denier and renditta were calculated.

All data were affirmed as Mean±S.D. Data obtained were subjected to student's 't' test to find out the significant difference between control and treatment groups.

RESULTS AND DISCUSSION

Cocoon morphometrics

The highest cocoon length, width and its ratio was recorded in T_{10} (3.93±0.04cm) with an increase of 20.55% over control, T_{10} (1.90±2.70cm) with an increase of 14.46% over control and T_8 (2.12±0.05cm) with an increase of 8.16% over control respectively, while their respective lowest values were noted in T_3 (3.30±0.21cm), T_2 (1.70±0.0cm) and T_3 (1.86±0.09) with an increase of 1.22, 2.41% and a decrease of -5.10% when compared to control (Table 1, Fig 1).

Table 1 Morphometrics of *Bombyx mori* cocoon

Treatment	Cocoon length	Cocoon width	Cocoon length width ratio
T_0:	3.26±0.09	1.66±0.05	1.96±0.10
T_1:	3.46±0.04	1.73±0.05	2.00±0.04
T_2:	3.43±0.04	1.70±0.00	2.01±0.02
T_3:	3.30±.021	1.76±0.05	1.86±0.09
T_4:	3.70±0.08*	1.76±0.05	2.09±0.02
T_5:	3.53±0.04	1.76±0.05	2.00±0.04
T_6:	3.66±0.09*	1.76±0.05	2.07±0.05
T_7:	3.83±0.09*	1.83±0.05*	2.09±0.05
T_8:	3.83±0.09*	1.80±0.00*	2.12±0.05
T_9:	3.80±0.08*	1.83±0.05*	2.07±0.06
T_{10}:	3.93±0.04*	1.90±2.70	2.07±0.02
T_{11}:	3.70±0.08*	1.86±0.05*	1.98±0.09

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

Table 2 Cocoon weight, pupal weight, shell weight and shell ratio of *Bombyx mori*

Treatment	Cocoon weight	Pupal weight	Shell weight	Shell ratio
T_0:	12.78±0.04	10.46±0.04	2.25±0.02	17.60±0.01
T_1:	12.25±0.22	9.64±0.23	2.23±0.15	18.78±1.25
T_2:	13.29±0.34	8.73±0.40	2.67±0.08*	19.64±0.12*
T_3:	13.82±0.89	8.79±0.28	2.66±0.14*	16.53±1.55
T_4:	15.55±1.33*	12.14±0.56*	2.94±0.12*	18.99±0.70
T_5:	13.8±0.31	11.02±0.25	2.61±0.01*	18.95±0.42
T_6:	15.03±0.45*	12.18±0.43*	2.78±0.01*	18.54±0.41
T_7:	14.46±0.69*	11.59±0.57*	2.87±0.18*	18.04±1.02
T_8:	15.45±0.26*	13.21±0.58*	2.95±0.01*	19.10±0.02
T_9:	14.12±0.80*	10.87±1.03	3.21±0.16*	19.77±1.28*
T_{10}:	16.43±1.02*	12.99±1.66*	3.17±0.46*	19.66±3.04*
T_{11}:	16.41±0.29*	13.11±0.74*	3.19±0.10*	19.67±1.53*

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

Cocoon weight, pupal weight, shell weight and shell ratio

The maximum cocoon weight and pupal weight was recorded in T_{10} (16.43±1.02g) which increased 28.56% over control and T_8 (13.21±0.5g) which increased 26.29% over control respectively, while their respective lowest weights were observed in T_1 (12.25±0.22g) with a decrease of -4.14% over control and T_2 (8.73±0.4g) with a decrease of -7.8% over control. Maximum and minimum shell weight registered in T_9

(3.21±0.16g) and T_1 (2.23±0.15g) with an increase of 42.66% and a decrease of -0.88% over control respectively. In the case of shell ratio, maximum value was noted in T_9 (19.77±1.28g) with an increase of 12.32% over control, while T_3 recorded the minimum value (16.53±1.55g) with a decrease of -6.07% when compared to control (Table 2, Fig 1).

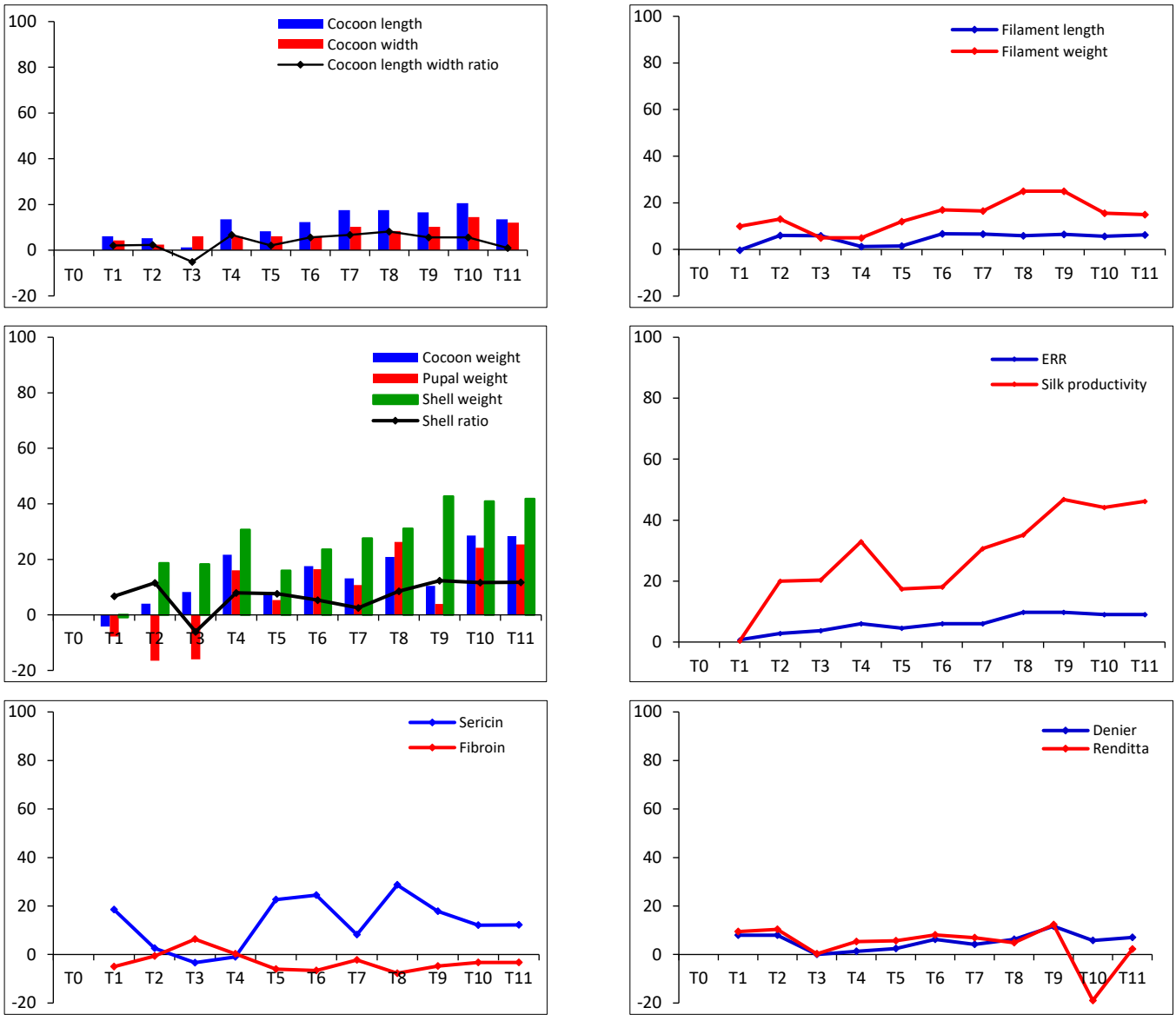

Fig 1 Percent deviation of treatments over control of *Bombyx mori* cocoon parameters

Table 3 Sericin and fibroin of *Bombyx mori*

Treatment	Sericin	Fibroin
T_0:	21.21±3.88	78.79±2.14
T_1:	25.13±2.63*	74.87±2.73
T_2:	21.75±4.12	78.25±4.10
T_3:	20.50±3.61	79.50±4.76
T_4:	21.40±4.37	79.00±2.93
T_5:	26.00±2.98*	74.00±2.45
T_6:	26.40±0.87*	73.60±3.87
T_7:	22.95±3.26	77.05±4.21
T_8:	27.29±4.80*	72.71±2.97
T_9:	25.00±1.42*	75.00±2.23
T_{10}:	23.77±5.87*	76.23±3.80
T_{11}:	23.80±2.64*	76.20±3.19

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

Sericin and fibroin

The highest sericin content was recorded in T_8 (27.29±4.80) and the lowest in T_3 (20.5±3.61) with an increase and decrease of 28.66 and -3.35% over control. For fibroin content the highest and lowest values were observed in T_3 (79.50±4.76) and T_8 (72.71±2.79) and it had an increase of 6.31% and a decrease of -7.72% over control (Table 3, Fig 1).

Table 4 Filament length and weight of *Bombyx mori*

Treatment	Single filament length (m)	Single filament weight (g)
T_0:	716.92±19.04	0.20±0.07
T_1:	714.86±18.26	0.22±0.04*
T_2:	759.74±10.14	0.23±0.02
T_3:	759.5±16.44	0.21±0.08
T_4:	726.05±21.87	0.21±0.03
T_5:	728.0±26.11	0.22±0.05*
T_6:	765.1±14.62	0.23±0.01*
T_7:	764.34±16.14	0.24±0.01*
T_8:	759.14±13.16	0.25±0.02*
T_9:	763.33±11.36	0.25±0.04*
T_{10}:	757.67±14.66	0.23±0.06*
T_{11}:	762.04±10.80	0.23±0.05*

*Significant @ P<0.05 (t-test)

Filament length and weight

The longest and shortest filament length was noted in T_6 (765.1±14.62m) and T_1 (714.86±18.26m) with increase of 6.73% and decrease of -0.28% over control respectively. The maximum and minimum filament weight was observed in both T_9 and T_8 (0.25±0.04 and 0.25±0.02g) which had an increase of 25.0% over control, and T_4 and T_3 (0.21±0.08 and 0.21±0.03g) which had a decrease of -5.0% over control. (Table 4, Fig 1).

Effective rate of rearing (ERR) and silk productivity

The maximum and minimum ERR was 97.32±18.34% (T_8 and T_9) and 89.32±1.77% (T_1) with an increase and decrease of 9.76 and -0.74% over control respectively. For silk productivity, their maximum and minimum values were recorded in T_{10} (4.55 ±0.02cg/day) and T_1 (3.11±0.01cg/day) and their increase and decrease were 44.19 and -0.32% respectively (Table 5, Fig 1).

Table 5 ERR and silk productivity of *Bombyx mori*

Treatment	ERR (%)	Silk productivity (cg/day)
T_0:	88.66±15.01	3.10±0.01
T_1:	89.32±21.77	3.11±0.01
T_2:	91.13±21.54	3.72±0.06*
T_3:	92.0±17.53	3.73±0.01*
T_4:	94.0±10.24	4.12±0.02*
T_5:	92.66±16.71	3.64±0.01*
T_6:	94.0±16.54	3.66±0.01*
T_7:	94.0±18.21	4.05±0.03*
T_8:	97.32±18.34*	4.19±0.03*
T_9:	97.32±18.34*	4.55±0.02*
T_{10}:	96.66±17.45*	4.47±0.01*
T_{11}:	96.66±17.45*	4.53±0.01*

*Significant @ P<0.05 (t-test)

Denier and renditta

The maximum and minimum denier was recorded in T_9 (2.68±0.26) which increased 11.66% when compared with control and T_1 (2.40±0.62) which was on par with the control. In the case of renditta, their respective values were noted in T_9 (8.41±1.24) and T_{10} (6.07±0.56) with an increase and decrease of 12.28 and -18.95% over control (Table 6, Fig 1).

Table 6 Denier and renditta of *Bombyx mori*

Treatment	Denier	Renditta
T_0:	2.40±0.01	7.49±1.12
T_1:	2.59±0.32	8.20±1.87*
T_2:	2.59±0.57	8.27±1.33*
T_3:	2.40±0.62	7.51±1.45
T_4:	2.43±0.11	7.89±1.89
T_5:	2.46±0.03	7.91±0.57
T_6:	2.55±0.18	8.09±0.66
T_7:	2.50±0.57	8.01±0.02
T_8:	2.55±0.42	7.85±1.33
T_9:	2.68±0.26*	8.41±1.24*
T_{10}:	2.54±0.18	6.07±1.56
T_{11}:	2.57±0.27	7.66±2.07

*Significant @ P<0.05 (t-test)

Cocoon, pupal and shell parameters

The role of micronutrients had a positive impact in the present study, and has enhanced the economic traits of cocoon. This has been well documented with reference to the nutritional role of several micronutrients, viz., calcium chloride, cobalt chloride, magnesium chloride, potassium iodide [3], [16-17], copper sulphate, nickel chloride, potassium iodide [18], phosphorus [19], nitrogen [20], ferrous sulphate, magnesium sulphate [6], potassium nitrate, nickel chloride [21], iron [22], potassium permanganate, potassium chloride, magnesium chloride [10-13], potassium bromide, nickel sulphate [23], potassium carbonate, magnesium carbonate [24], and zinc chloride [25]. The results of present investigation indicated that the zinc, copper and iron combination had significant effect on the economic parameters of *Bombyx mori* cocoons. Chamundeswari and Radhakrishnaiah [26] reported increased cocoon weight, when silkworm larvae were fed with zinc and nickel fortified mulberry leaves.

Cocoon weight and shell weight are the most important characteristics evaluated for silk productivity, and shell ratio depends upon the quantity of the silk produced from each cocoon [27]. Cocoon shell weight and its ratio are important parameters that denotes the available silk filament in a cocoon, based on which the cocoon price is fixed [28]. Increased cocoon weight was contributed by increased shell weight and pupal weight in the present study. Mulberry leaves supplemented with zinc chloride significantly increased cocoon weight, shell weight and shell ratio [9], [29-31]. Further, Sivaprasad *et al*. [25] reported that supplementation of zinc chloride at higher concentrations enhanced the cocoon yield and quality. These findings are in parity with the present observations.

Kochi and Kaliwal [23] reported that there was a significant increase in cocoon weight, cocoon shell weight and cocoon shell ratio in CSR_2, CSR_4 and $CSR_2 \times CSR_4$ cross breed races of silkworm treated with mixtures of potassium bromide and nickel sulphate. The results of the present study, seems to suggest that soil application of zinc sulphate, copper sulphate and ferrous sulphate were more effective in increasing cocoon weight when compared to control group. Amongst the treatments, T_9 showed a significant increase in cocoon weight and shell weight which may be due to the application of micronutrients as mixed dose which would have enhanced the silk content in the cocoon shell as reported by Nirwani and Kaliwal [7]. The results of the present study therefore seem to suggest that the soil application with a mixture of copper, zinc and iron was more effective in increasing the cocoon weight and shell weight when compared to other single and mixed doses of micronutrients.

Sericin and fibroin

Silk is secreted by the silk glands, which are a reservoir for two silk proteins, viz., sericin and fibroin [32], and the floss-silk ratio is a measure of relative proportions of sericin and fibroin contents of silk [33]. Fibroin is a fibrous protein secreted in the lumen of the posterior silk gland, while sericin, which coats fibroin, is a natural macromolecular glycoprotein protein serves as an adhesive to unite fibroin for making silk cocoons is secreted by the central silk gland [34]. The fibroin protein is transferred by peristalsis into the central silk gland where it is stored until required for spinning [35].

Filament length and weight

Silk, a 100% natural protein is an essential constituent of cocoon filament [36]. In the present study, filament length and weight in the treatment groups were more when compared to the control group, which might possibly be due to the stimulatory effect of micronutrients on the silk gland activity. Similar results have been reported after supplementing the feed with zinc salts in *Bombyx mori* [26], [37-38].

ERR and silk productivity

The effective rate of rearing is an important parameter for judging mulberry varieties in respect of rearing performance

[39]. In the present study, the ERR increased for all treatments which indicated increased number of harvested cocoons as reported by Radjabi [40] and Shifa *et al*. [41]. Higher ERR could also be attributed to the combined application of micronutrient to mulberry leaves when fed to silkworms give higher ERR with good quality cocoons [42]. Silk productivity in the present study increased in treatment groups when compared to control, and this was supported by the findings of Kamel [43]. Further, Hugar and Kaliwal [37] and Ashfaq *et al*. [30] reported that supplementation of zinc chloride led to high silk productivity, which validated the findings of the present study too, confirming the presence of zinc.

Denier and renditta

Denier is the measure of silk texture and thickness of silk fibre used to estimate the number of cocoons required to reel the silk [33], [44]. Renditta is the number of cocoons required for the production of a kilogram of raw silk [33]. There was significant improvement of denier and renditta among the parameters related to the quality of silk over the control by soil application of micronutrient treated mulberry leaves to *Bombyx mori* in this study. It is opined from the present study that combination of micronutrients accelerated the growth of silkworm through orientation of physiological activity, and thereby improving the quality of cocoon and silk.

Wani *et al*. [33] reported that all economic parameters of cocoon, viz., cocoon weight, shell weight, floss weight, shell protein, floss protein, shell-cocoon ratio, floss-shell ratio, silk-shell ratio, floss-silk ratio, raw silk weight, raw silk percentage, renditta and denier were enhanced after the silkworm larvae were fed with the mulberry leaves enriched predominantly with zinc salts like zinc chloride and zinc sulphate, since zinc has a positive impact at two levels. Firstly, it stimulates silk protein synthesis in the silk gland and enhances silk output, as reflected in higher shell cocoon ratios, silk-body ratio, raw silk percentage, denier and renditta. Secondly, it lowers the floss-shell ratio by decreasing the floss protein synthesis, which is removed as wastage at the time of silk reeling. Hence, all the economic parameters of the cocoon in the present study indicated that the quantity of micronutrients which also contained zinc supplemented to mulberry plants reflected on cocoon production, as they brought considerable improvement in increased quality cocoon production and silk productivity.

CONCLUSION

The cocoon parameters in the present study indicated variations among the treatments due to the effect of micronutrients. Further, more research should be carried out to support the current findings in regard to the economic traits of the cocoon parameters to obtain more quality and quantity of silk.

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