



Nanoparticles supplementation through foliar feed contributed to the growth and cocoon yield of silkworm (*Bombyx mori*)

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Received: 09 January 2024; Accepted: 24 July 2024

ABSTRACT

An experiment was conducted during 2021–22 and 2022–23 at University of Agricultural Sciences, Dharwad, Karnataka to study the effect of metal nanoparticles on silkworm (*Bombyx mori*) growth and cocoon yield. The experiment was laid out in completely randomized design (CRD) comprised of 11 treatments with 3 replications. The various concentrations (50, 100 and 200 ppm) of nanoparticles, viz. nano silver, nano zinc and nano copper were extrafoliated to mulberry leaves and were administered to silkworms daily once at morning (first feed) from second day of fifth instar. The study revealed that, silver nanoparticles at 200 ppm concentration emerged as the best supplement with improved larval, cocoon and silk parameters by recording shorter larval duration (616.42 h), fifth instar duration (166.12 h), higher mature larval weight (40.626 g/10 larvae), effective rate of rearing (97.58%), silk productivity (6.225 cg/day), silk gland weight (1.885 g/larva), pupal weight (13.757 g/10 pupae), cocoon yield (794.85 g/df), cocoon weight (18.102 g/10 cocoons), shell weight (4.307 g/10 shells), cocoon shell ratio (23.79%), cocoon filament length (948.52 m), finer denier (2.75 d), highest fibroin (80.23%) and lowest sericin protein (19.78%).

Keywords: Growth and cocoon yield, Nanoparticles, Nano silver, Silkworm

Silk holds a significant place in Indian life and culture, deeply rooted in the country's history of silk manufacturing dating back to the 15th century. The sericulture sector in India employs approximately 8.7 million people, many of whom are from economically disadvantaged backgrounds, including women in rural and semi-urban areas. India's domestic silk market is rich with cultural diversity, featuring a wide array of silk garments that showcase regional distinctiveness. This diversity has contributed to India becoming a global leader in the silk industry, producing all four major commercial silks: Mulberry, Tasar, Eri, and Muga. India is the world's second-largest producer of silk after China. In 2022, Mulberry silk accounted for 74.03% of India's total raw silk production of 34,923 MT, followed by Eri silk (21.07%), Tasar (4.17%) and Muga (0.73%). The export value of Indian silk reached 1848.96 crores, reflecting its economic importance (Anonymous 2022).

Nanoparticles supplementation might be an economical with significantly lower quantity because of vast surface area and smaller size which makes it to penetrate more efficiently to the leaf surface. Balasundaram *et al.* (2012) revealed that

silver nanoparticles (AgNPs) can be utilized as an ancillary complex to boost up the growth and development of the larvae and also the quality and quantity of the cocoons. Moreover, research on silkworm clearly demonstrates that nanoparticles could stimulate more production of fibroin protein (Bhattacharya and Mukherjee 2008). Cu NPs and Ag NPs are highly conductive and are also thought to have significant bioactivity, such as antimicrobial activity against fungi, bacteria and viruses (Bondarenko *et al.* 2013, Ingle *et al.* 2014). The inclusion of nanomaterials in sericulture is new therefore, it is imperative to exploit their effects on silkworms (*Bombyx mori*) and on silk regarding the improvement of larval body growth and on the development of silkworms. Hence, the present study was aimed to know the impact of nanoparticles supplementation on the performance of silkworms so as to spot out the most effective nanoparticle to improve silk productivity.

MATERIALS AND METHODS

The present experiment was conducted during 2021–22 and 2022–23 at University of Agricultural Sciences, Dharwad (15.49 °N, 74.98 °E), Karnataka. Leaves from 12 years old mulberry variety V1 were used for feeding the larvae. Mulberry plants were pruned before the study and raised by using recommended fertilizers and other management practices (Dandin *et al.* 2000). Experiment

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was laid out in completely randomized design (CRD) with 11 treatments. Each treatment was replicated three times with one disease free laying (dfl) for chawki larva rearing and 200 worms for late age rearing. Disease free layings of FC1 × FC2 hybrid were procured from National Silkworm Seed Organization (NSSO), Central Silk Board, Bengaluru, Karnataka. The chawki worms were reared in a wooden tray (2.5 feet × 2.5 feet) by feeding four times a day (8.00 am, 2.00 pm, 6.30 pm and 9.00 pm) with chopped leaves. Whereas, during late age (after IV moult), 200 healthy worms were randomly picked and reared by providing cut shoots (10–15 cm length) and fed daily thrice (8:00 am, 2:00 pm and 9:00 pm). Silkworms were reared till fifth instar without extrafoliation of nanoparticles.

Nano silver (Ag), nano zinc (Zn) and nano copper (Cu) particles were procured from Sisco Research Laboratories Pvt. Ltd. The various concentrations (50, 100 and 200 ppm) of nanoparticles as per the treatments were prepared by dispersing nano powder in distilled water. The obtained solutions were uniformly spread onto freshly harvested mulberry leaves and were administered to silkworms daily once at morning (first feed) from second day of fifth instar and two remaining feeds in a day were normal without any extrafoliation. Observations were recorded on larval, cocoon and silk parameters.

Statistical analysis: All the data were analysed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using a commercially available statistics software package, Web Agri Stat Package 2.0.

RESULTS AND DISCUSSION

Data from two years is displayed in Table 1 and 2. Table 3 presents the pooled analysis, which shows no variations between seasons. The analysis of pooled data revealed shorter larval duration in nano Ag 200 ppm (616.42 h), followed by nano Ag 100 ppm (621.67 h), nano Ag 50 ppm (624.04 h) and Zn 200 ppm (624.38 h) and were on par with nano Ag 200 ppm. Conversely, larval duration was significantly longer in silkworms fed with water sprayed (639.96 h) and untreated leaves (639.96 h). Similar trends were observed for fifth instar duration, where nano Ag 200 ppm resulted in the shortest duration (166.12 h), followed by nano Ag 100 ppm (171.37 h) and nano Ag 50 ppm (173.75 h). With respect to larval weight, nano Ag 200 ppm supplementation led to the highest weight (40.626 g/10 larvae), followed by nano Ag 100 ppm (38.431 g/10 larvae). Nano Ag 200 ppm also enhanced the effective rate of rearing significantly (97.58%), followed by nano Ag 100 ppm (96.33%) and nano Zn 200 ppm (95.92%), all outperforming untreated leaves (87.50%). In terms of silk productivity, nano Ag 200 ppm found superior with the highest rate (6.225 cg/day), followed by nano Ag 100 ppm (5.734 cg/day) and nano Zn 200 ppm (5.478 cg/day). The analysis of silk gland weight revealed that nano Ag 200 ppm produced the heaviest glands (1.885 g/larva), followed by nano Ag 100 ppm (1.719 g/larva) and nano Zn 200 ppm (1.684 g/larva). While, water sprayed (1.037 g/larva) and

untreated leaves (1.029 g/larva) yielded significantly lighter glands. Pupal weight was highest with nano Ag 200 ppm (13.757 g/10 pupae) and nano Ag 100 ppm (13.136 g/10 pupae) and were at par (Table 3).

Feeding silkworms with nano Ag 200 ppm supplemented leaves yielded the highest cocoon weight/10 cocoons (18.102 g), followed closely by nano Ag 100 ppm (17.288 g) and nano Zn 200 ppm (16.901 g), which were on par. In contrast, the lowest cocoon weights were observed with untreated (14.151 g) and water sprayed leaves (14.017 g). The shell weight was significantly higher in silkworms fed on nano Ag 200 ppm (4.307 g/10 shells), followed by nano Ag 100 ppm (4.096 g/10 shells), nano Zn 200 ppm (3.972 g/10 shells) and nano Ag 50 ppm (3.888 g/10 shells), all of which showed comparable results. Nano Ag 200 ppm resulted in the highest cocoon shell ratio (23.79%), followed closely by nano Ag 100 ppm (23.69%) and nano Zn 200 ppm (23.47%), with untreated control having the lowest shell ratio (22.06%). Cocoon yield was significantly higher with nano Ag 200 ppm (794.85 g/dfl), followed by nano Ag 100 ppm (749.42 g/dfl), nano Zn 200 ppm (729.69 g/dfl) and nano Ag 50 ppm (710.18 g/dfl) and were on par. Longest cocoon filament was registered in nano Ag 200 ppm (948.52 m), followed by nano Ag 100 ppm (907.63 m) and both were at par. Thickness of silk filament was finer in nano Ag 200 ppm (2.75 d), nano Ag 100 ppm (2.77) and were on par. While, untreated (3.27) and water sprayed (3.28) treatments produced coarser silk. Supplementation of nano Ag 200 ppm enhanced fibroin protein upto 80.23% over rest of the treatments and unsprayed (67.90%) and water sprayed treatment (67.53%). Significantly lower sericin was observed in nano Ag 200 ppm (19.78%) against untreated (32.10%) and water sprayed mulberry leaves (32.47%) (Table 3).

Pooled data of two seasons revealed that, supplementation of nanoparticles had a positive impact on growth and cocoon yield of silkworm. Rao *et al.* (2019) opined supplementation of nanoparticles can improve metabolism of both carbohydrates and protein resulting in increased cocoon yield. In present study, supplementation of nano Ag 200 ppm was found superior. It might be due to stimulant activity of Ag nanoparticles that might have increased the carbohydrates and lipids metabolism in larvae leading to better larval growth and development resulting in superior cocoon and silk traits. Prabhu *et al.* (2011) reported that Ag nanoparticles act as vitamins and stimulate silkworm to feed more amount of nutrients intake than the control. Wherein, Meng *et al.* (2017) postulated silver nanoparticles at lower doses (less than 400 µg/ml) improved larval growth. 70% of protein in silkworm is derived from mulberry leaves and 96% of protein ingested during fifth instar is used for synthesis of silk protein. Hence, nutrition of leaves both in terms of quality and quantity have significant impact on silkworm growth and development (Fukuda and Higuchi 1963). Sangamithirai *et al.* (2013) suggested use of silver nanoparticles as ancillary products to boost the growth and development of silkworms as well as quantity and quality of cocoons. Similar reports on improved economic traits of

Table 1 Effect of nanoparticles supplementation on growth and cocoon yield of silkworm during 2021–22

Treatment	Larval duration (h)	Fifth instar duration (h)	Mature larval weight (g/10 larvae)	Effective rearing rate (%)	Silk pro-ductivity (cg/day)	Silk gland weight (g/larva)	Pupal weight (g/10 pupae)	Cocoon weight (g/10 cocoons)	Cocoon shell weight (g/10 shells)	Cocoon shell ratio (%)	Cocoon yield (g/df)	Cocoon filament length (m)	Denier	Silk protein	
														Fibroin (%)	Sericin (%)
Nano Ag @50 ppm	624.33 ^{abcd}	174.04 ^{bc}	35.267 ^{cd}	94.17 (76.08) ^{bc}	5.145 ^{cd}	1.629 ^{cd}	12.140 ^{bcd}	15.893 ^{cd}	3.732 ^{cd}	23.47 (28.98) ^{bed}	673.48 ^{cd}	872.19 ^{bc}	2.88 ^{ab}	75.54 (60.39) ^{abcd}	24.46 (29.64) ^c
Nano Ag @100 ppm	621.42 ^{ab}	171.12 ^{ab}	37.532 ^{ab}	96.50 (79.60) ^{ab}	5.694 ^b	1.728 ^b	12.781 ^{ab}	16.897 ^{ab}	4.063 ^b	24.03 (29.35) ^{ab}	733.74 ^b	910.34 ^{ab}	2.76 ^a	77.52 (61.74) ^{ab}	22.48 (28.30) ^b
Nano Ag @200 ppm	615.25 ^a	164.95 ^a	39.463 ^a	97.33 (81.31) ^a	6.250 ^a	1.869 ^a	13.443 ^a	17.770 ^a	4.293 ^a	24.14 (29.43) ^a	778.33 ^a	941.26 ^a	2.77 ^a	79.81 (63.34) ^a	20.19 (26.70) ^a
Nano Zn @50 ppm	631.33 ^{bcd}	181.04 ^{cdef}	32.310 ^{ef}	92.83 (74.60) ^{bcd}	4.469 ^{fg}	1.391 ^{fg}	11.157 ^{ef}	14.552 ^{efg}	3.370 ^{ef}	23.16 (28.77) ^{cde}	607.83 ^{ef}	789.27 ^{def}	3.07 ^c	72.64 (58.48) ^{cdef}	27.36 (31.54) ^{ef}
Nano Zn @100 ppm	626.92 ^{abcd}	176.62 ^{bcd}	34.315 ^{cde}	94.33 (76.91) ^{abc}	4.905 ^{de}	1.552 ^{de}	11.833 ^{cde}	15.465 ^{cde}	3.611 ^d	23.35 (28.90) ^{cde}	656.28 ^d	840.41 ^{cd}	2.97 ^{bc}	74.43 (59.65) ^{bcd}	25.57 (30.37) ^{cd}
Nano Zn @200 ppm	623.67 ^{abc}	173.37 ^{abc}	36.333 ^{bc}	95.50 (77.79) ^{ab}	5.375 ^c	1.675 ^{bc}	12.478 ^{bc}	16.369 ^{bc}	3.884 ^{bc}	23.69 (29.13) ^{abc}	703.79 ^{bc}	906.55 ^{ab}	2.87 ^{ab}	77.18 (61.49) ^{abc}	22.82 (28.53) ^b
Nano Cu @50 ppm	636.08 ^{de}	185.79 ^{ef}	31.155 ^{fg}	90.33 (71.99) ^{cde}	4.183 ^{gh}	1.210 ^h	10.769 ^f	14.031 ^{gh}	3.221 ^{fg}	22.94 (28.62) ^{def}	570.39 ^{fg}	745.59 ^{fg}	3.10 ^{cd}	70.46 (57.09) ^{ef}	29.54 (32.92) ^g
Nano Cu @100 ppm	634.50 ^{cde}	184.20 ^{def}	32.190 ^{efg}	93.00 (74.90) ^{bcd}	4.369 ^g	1.323 ^g	11.122 ^{ef}	14.504 ^{fgh}	3.337 ^{ef}	23.03 (28.68) ^{def}	606.83 ^{ef}	776.31 ^{ef}	3.11 ^{cde}	71.28 (57.61) ^{def}	28.72 (32.40) ^{fg}
Nano Cu @200 ppm	628.17 ^{bcd}	177.87 ^{bcd}	33.642 ^{de}	93.67 (75.61) ^{bc}	4.766 ^{ef}	1.462 ^{ef}	11.582 ^{de}	15.163 ^{def}	3.529 ^{de}	23.28 (28.85) ^{cde}	639.13 ^{de}	804.69 ^{de}	3.02 ^{bc}	73.66 (59.14) ^{bcd}	26.34 (30.88) ^{de}
Absolute control	639.67 ^e	189.37 ^f	30.121 ^g	88.17 (69.90) ^{de}	3.944 ^h	1.043 ⁱ	10.423 ^f	13.561 ^h	3.101 ^g	22.86 (28.56) ^{ef}	538.13 ^g	704.28 ^g	3.29 ^e	67.71 (55.38) ^f	32.29 (34.63) ^h
Untreated control	639.33 ^e	188.70 ^f	30.352 ^{fg}	87.83 (69.62) ^e	3.931 ^h	1.020 ⁱ	10.521 ^f	13.672 ^{gh}	3.090 ^g	22.60 (28.38) ^f	540.77 ^g	708.49 ^g	3.27 ^{de}	68.19 (55.68) ^f	31.81 (34.33) ^h
SEm (±)	4.17	2.940	0.712	1.74	0.104	0.031	0.249	0.315	0.077	0.13	13.36	18.13	0.06	1.04	0.36
CV (%)	1.15	2.913	3.734	4.05	3.829	3.778	3.784	3.663	3.822	1.42	3.58	3.80	3.548	3.09	2.08

Figures in parenthesis are arc sine transformed values.

Figures in the column followed by same letters are not-significant at $P=0.05$ by Duncan's multiple range test.

Table 2 Effect of nanoparticles supplementation on growth and cocoon yield of silkworm during 2022–23

Treatment	Larval duration (h)	Fifth instar duration (h)	Mature larval weight (g/10 larvae)	Effective rearing rate (%)	Silk productivity (cg/day)	Silk gland weight (g/larva)	Pupal weight (g/10 pupae)	Cocoon weight (g/10 cocoons)	Cocoon shell weight (g/10 shells)	Cocoon shell ratio (%)	Cocoon yield (g/dfi)	Cocoon filament length (m)	Denier	Silk protein	
														Fibroin (%)	Sericin (%)
Nano Ag @50 ppm	623.75 ^{ab}	173.45 ^{ab}	38.54 ^{3b}	95.33 (77.53) ^{abc}	5.59 ^{6b}	1.61 ^{2bc}	13.33 ^{4b}	17.41 ^{1b}	4.04 ^{3b}	23.23 (28.81) ^{abc}	746.89 ^b	865.74 ^{cd}	2.91 ^{abc}	77.26 (61.56) ^{abc}	22.74 (28.48) ^{cd}
Nano Ag @100 ppm	621.92 ^{ab}	171.62 ^{ab}	39.33 ^{0b}	96.17 (79.22) ^{ab}	5.77 ^{5b}	1.71 ^{0b}	13.49 ^{0ab}	17.67 ^{9ab}	4.12 ^{9ab}	23.36 (28.90) ^{ab}	765.10 ^b	904.92 ^b	2.78 ^a	78.43 (62.37) ^{abc}	21.57 (27.67) ^{bc}
Nano Ag @200 ppm	617.58 ^a	167.29 ^a	41.78 ^{8a}	97.83 (82.02) ^a	6.20 ^{0a}	1.90 ^{2a}	14.07 ^{2a}	18.43 ^{3a}	4.32 ^{2a}	23.44 (28.96) ^a	811.38 ^a	955.78 ^a	2.73 ^a	80.64 (63.94) ^a	19.36 (26.10) ^a
Nano Zn @50 ppm	633.67 ^{def}	183.37 ^{def}	35.57 ^{2cd}	94.50 (76.95) ^{bc}	4.79 ^{4de}	1.42 ^{1de}	12.31 ^{1c}	16.03 ^{0cd}	3.65 ^{8cde}	22.83 (28.54) ^{bc}	681.68 ^{cd}	795.44 ^{ef}	3.05 ^{bc}	74.13 (59.45) ^{cde}	25.87 (30.57) ^f
Nano Zn @100 ppm	625.92 ^{bc}	175.62 ^{bcd}	36.20 ^{1c}	95.17 (77.45) ^{abc}	5.15 ^{9c}	1.59 ^{3c}	12.52 ^{9c}	16.31 ^{2c}	3.77 ^{0c}	23.11 (28.73) ^{abc}	698.48 ^c	832.26 ^{de}	3.00 ^{bc}	76.17 (60.81) ^{abcd}	23.83 (29.22) ^{de}
Nano Zn @200 ppm	625.08 ^{abc}	174.79 ^{abc}	38.65 ^{7b}	96.33 (79.24) ^{ab}	5.58 ^{1b}	1.69 ^{3b}	13.34 ^{3ab}	17.43 ^{2b}	4.06 ^{1b}	23.26 (28.83) ^{abc}	755.59 ^b	898.63 ^{bc}	2.90 ^{ab}	78.91 (62.70) ^{ab}	21.09 (27.34) ^b
Nano Cu @50 ppm	638.75 ^{ef}	188.45 ^{ef}	34.35 ^{0cd}	92.00 (73.59) ^{cd}	4.47 ^{3f}	1.25 ^{5f}	11.92 ^{2cd}	15.47 ^{5cde}	3.50 ^{8de}	22.69 (28.45) ^c	640.46 ^d	752.25 ^g	3.07 ^{bc}	69.81 (56.68) ^{efg}	30.19 (33.33) ^h
Nano Cu @100 ppm	632.33 ^{cde}	182.04 ^{cde}	34.05 ^{3de}	92.83 (74.60) ^{bc}	4.60 ^{3ef}	1.33 ^{0ef}	11.83 ^{0cd}	15.34 ^{4def}	3.49 ^{2e}	22.75 (28.49) ^c	640.83 ^d	781.22 ^{fg}	3.09 ^{cd}	72.44 (58.35) ^{def}	27.56 (31.66) ^g
Nano Cu @200 ppm	629.17 ^{bcd}	178.87 ^{bcd}	35.57 ^{4cd}	94.67 (76.84) ^{bc}	4.96 ^{6cd}	1.49 ^{2d}	12.38 ^{5c}	16.12 ^{0cd}	3.70 ^{4cd}	22.95 (28.62) ^{abc}	686.71 ^c	810.11 ^{ef}	2.99 ^{bc}	75.09 (60.08) ^{bcd}	24.91 (29.94) ^{ef}
Absolute control	640.25 ^f	189.95 ^f	32.05 ^{8e}	87.33 (69.22) ^d	3.99 ^{2g}	1.03 ^{1g}	11.30 ^{6d}	14.47 ^{3f}	3.15 ^{0f}	21.77 (27.81) ^d	568.67 ^e	707.64 ^h	3.28 ^d	67.35 (55.16) ^g	32.65 (34.85) ⁱ
Untreated control	640.58 ^f	190.29 ^f	32.20 ^{5e}	87.17 (69.02) ^d	3.95 ^{0g}	1.03 ^{8g}	11.46 ^{3d}	14.63 ^{1ef}	3.13 ^{1f}	21.52 (27.64) ^d	573.92 ^e	710.57 ^h	3.26 ^d	67.61 (55.32) ^{fg}	32.39 (34.69) ^j
SEm (±)	2.62	2.630	0.693	1.60	0.103	0.033	0.242	0.298	0.069	0.13	15.07	13.11	0.064	1.08	0.34
CV (%)	0.72	2.577	3.395	3.74	3.638	4.018	3.436	3.241	3.269	1.83	3.84	2.81	3.744	3.15	1.96

Figures in parenthesis are arc sine transformed values.

Figures in the column followed by same letters are not-significant at $P=0.05$ by Duncan's multiple range test.

Table 3 Effect of nanoparticles supplementation on growth and cocoon yield of silkworm (Pooled data of 2021–22 and 2022–23)

Treatment	Larval duration (h)	Fifth instar duration (h)	Mature larval weight (g/10 larvae)	Effective rearing rate (%)	Silk productivity (cg/day)	Silk gland weight (g/larva)	Pupal weight (g/10 pupae)	Cocoon weight (g/10 cocoons)	Cocoon shell weight (g/10 shells)	Cocoon shell ratio (%)	Cocoon yield (g/df)	Cocoon filament length (m)	Denier	Silk protein	
														Fibroin (%)	Sericin (%)
Nano Ag @50 ppm	624.04 ^{abc}	173.75 ^{bc}	36.905 ^{bc}	94.75 (76.78) ^{bcd}	5.371 ^c	1.620 ^{cd}	12.737 ^{bc}	16.652 ^{bc}	3.888 ^{bc}	23.35 (28.90) ^{bcd}	710.18 ^{bc}	868.97 ^{bc}	2.90 ^{ab}	76.40 (60.97) ^{abc}	23.60 (29.06) ^c
Nano Ag @100 ppm	621.67 ^{ab}	171.37 ^b	38.431 ^b	96.33 (79.41) ^{ab}	5.734 ^b	1.719 ^b	13.136 ^{ab}	17.288 ^{ab}	4.096 ^b	23.69 (29.13) ^{ab}	749.42 ^b	907.63 ^{ab}	2.77 ^a	77.98 (62.05) ^{ab}	22.03 (27.99) ^b
Nano Ag @200 ppm	616.42 ^a	166.12 ^a	40.626 ^a	97.58 (81.65) ^a	6.225 ^a	1.885 ^a	13.757 ^a	18.102 ^a	4.307 ^a	23.79 (29.19) ^a	794.85 ^a	948.52 ^a	2.75 ^a	80.23 (63.64) ^a	19.78 (26.40) ^a
Nano Zn @50 ppm	632.50 ^{cdef}	182.20 ^{ef}	33.941 ^{def}	93.67 (75.60) ^{bcd}	4.631 ^{ef}	1.406 ^{ef}	11.734 ^{de}	15.291 ^{de}	3.514 ^{def}	23.00 (28.66) ^{def}	644.75 ^{def}	792.36 ^e	3.06 ^{bc}	73.39 (58.96) ^{bcd}	26.62 (31.06) ^{ef}
Nano Zn @100 ppm	626.42 ^{bcd}	176.12 ^{cd}	35.258 ^{cd}	94.75 (76.95) ^{bcd}	5.032 ^d	1.572 ^d	12.181 ^{cd}	15.888 ^{cd}	3.691 ^{cd}	23.23 (28.81) ^{cde}	677.38 ^{cd}	836.34 ^{cd}	2.99 ^{bc}	75.30 (60.22) ^{bc}	24.70 (29.80) ^{cd}
Nano Zn @200 ppm	624.38 ^{abcd}	174.08 ^{bcd}	37.495 ^b	95.92 (78.38) ^{abc}	5.478 ^{bc}	1.684 ^{bc}	12.911 ^b	16.901 ^b	3.972 ^b	23.47 (28.98) ^{abc}	729.69 ^b	902.59 ^b	2.89 ^{ab}	78.05 (62.09) ^{ab}	21.96 (27.94) ^b
Nano Cu @50 ppm	637.42 ^{ef}	187.12 ^{gh}	32.753 ^{igh}	91.17 (72.75) ^{de}	4.328 ^f	1.232 ^g	11.346 ^{ef}	14.753 ^{efg}	3.365 ^f	22.81 (28.53) ^f	605.43 ^f	748.92 ^{fg}	3.08 ^c	70.14 (56.89) ^{de}	29.87 (33.13) ^g
Nano Cu @100 ppm	633.42 ^{def}	183.12 ^{fg}	33.122 ^{efg}	92.92 (74.74) ^{cd}	4.486 ^f	1.327 ^f	11.476 ^{def}	14.924 ^{ef}	3.414 ^{ef}	22.89 (28.58) ^{ef}	623.83 ^{ef}	778.77 ^{ef}	3.10 ^{cd}	71.86 (57.98) ^{cde}	28.14 (32.04) ^f
Nano Cu @200 ppm	628.67 ^{bcd}	178.37 ^{de}	34.608 ^{de}	94.17 (76.18) ^{bcd}	4.866 ^{de}	1.477 ^e	11.984 ^{de}	15.642 ^{de}	3.616 ^{de}	23.12 (28.74) ^{cdef}	662.92 ^{de}	807.40 ^{de}	3.00 ^{bc}	74.38 (59.61) ^{bcd}	25.63 (30.41) ^{de}
Absolute control	639.96 ^f	189.66 ^h	31.090 ^h	87.75 (69.53) ^e	3.968 ^g	1.037 ^h	10.865 ^f	14.017 ^g	3.126 ^g	22.31 (28.19) ^g	553.40 ^g	705.96 ^h	3.28 ^d	67.53 (55.27) ^e	32.47 (34.74) ^h
Untreated control	639.96 ^f	189.49 ^h	31.278 ^{gh}	87.50 (69.32) ^e	3.940 ^g	1.029 ^h	10.992 ^f	14.151 ^{fg}	3.111 ^g	22.06 (28.02) ^g	557.35 ^g	709.53 ^{gh}	3.27 ^d	67.90 (55.50) ^e	32.10 (34.51) ^h
SEM (±)	3.15	0.501	0.618	1.46	0.103	0.032	0.240	0.295	0.070	0.09	13.82	14.38	0.061	1.05	0.34
CV (%)	0.87	4.492	3.125	3.36	3.675	3.808	3.518	3.340	3.396	1.56	3.62	3.07	3.599	3.12	1.93

Figures in parenthesis are arc sine transformed values.

Figures in the column followed by same letters are not-significant at $P=0.05$ by Duncan's multiple range test.

silkworms due to diet supplementation made by Etebari *et al.* (2004) and Rajabi *et al.* (2006) support present findings.

In the present study, it has been observed that total larval duration, fifth instar duration and mature larval weight varied significantly and better values were obtained in nano Ag 200 ppm, followed by nano Ag 100 ppm and nano Zn 200 ppm. It could be attributed to growth stimulant activity of nanoparticles that might have enhanced metabolism of carbohydrates and lipids which accelerated the growth resulting in reduced larval duration and increased larval weight. Ashfaq *et al.* (2000) and Mihai Bentea *et al.* (2012) recorded improved larval weight and reduced larval duration upon zinc supplementation. Further, higher effective rate of rearing in silkworms fed on nano Ag 200 ppm supplemented leaves due to antimicrobial property of silver nanoparticles indicated lesser susceptibility of the worms to the diseases that increased larval survival achieving highest silk productivity and cocoon yield. The Ag nanoparticles entering the silkworm gut arrest the growth and multiplication of bacteria preventing the bacterial diseases and enhancing the feed efficacy, growth rate of larva, synthesis of silk protein and synergistic silk production (Thangapandiyan and Dharanipriya 2019). Prabhu *et al.* (2011) obtained significantly higher larval weight (3.55 g) in the larvae fed on MR 2 mulberry leaves supplemented with silver nanoparticles at 25%. Increased silk gland weight could be attributed to nanoparticles mediated activation of tissue metabolism that might have promoted the biological parameters of silk gland in the larvae.

The enrichment of mulberry leaves with silver nanoparticles enhanced cocoon and silk traits. This is due to nanoparticles enhanced growth and development of larvae as health and vigour of silkworm larvae is directly related to cocoon and silk traits. Sangamithirai *et al.* (2013) recorded significantly higher cocoon weight (2.02 g), shell weight (0.44 g), shell ratio (17.17%), pupal weight (1.57 g) and filament length (886.58 m) in the treatment where the larvae were fed on mulberry leaves treated with 25% Ag nanoparticles. Application of minerals led to excess amino group availability of fatty tissue in the pupal body and resulted in the heavier pupa (Muruges *et al.* 2021). Stimulation of posterior part of the silk gland due to nanoparticles might have increased silk fibroin. Nanoparticles could stimulate more production of fibroin protein (Bhattacharya *et al.* 2008). Patil *et al.* (2016) found that treatment with gold nanoparticle at a dose of 300 ppm exhibited 78.07% of fibroin with improved cocoon and silk traits. Present findings are in full agreement with Thangapandiyan and Dharanipriya (2019) who reported that cocoon weight (1.84 g), cocoon shell ratio (41.3%), silk gland weight (0.87 g), sericin (0.62 g) and fibroin (0.95 g) content, filament length (1336 m) and weight (0.32 g) were significantly increased in silver nanoparticles and spirulina treated groups when compared to those in the control and other groups. Earlier reports by Prabhu *et al.* (2012), Indrakumar Naik (2016) and Soliman and Gad (2020) are also in close conformity with present findings.

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