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Effect of Nano Nitrogen Fertilizer on Mulberry and its Influence on Larval and Cocoon Traits of Silkworm, *Bombyx mori* L. (FC_1 x FC_2)

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Abstract

An experiment was carried out at Department of Sericulture, UAS, GKVK, Bengaluru to know the efficacy of nano nitrogen foliar application to mulberry on larval parameters of silkworm, *Bombyx mori* L. (FC_1 x FC_2). The study resulted in significant improvement in the larval traits of silkworm fed with mulberry leaves with foliar application of nitrogen nano-fertilizer. Significantly shorter larval duration (4.73, 8.69 and 24.42 days, fourth instar, fifth instar and total larval duration, respectively), highest fifth instar larval weight (36.66 g /10 larvae), better effective rearing rate (97.78 %) were noticed in silkworms fed with leaves of mulberry with foliar application of 0.4 per cent nano nitrogen fertilizer on 25th day after pruning. However, significantly lowest fifth instar larval weight (26.72 g / 10 larvae), effective rearing rate (67.78 %) and longest fourth instar larval duration, fifth instar larval duration and total larval duration (5.51, 9.76 and 26.27 days) were observed in the silkworm group fed with mulberry leaves of absolute control. Significantly highest cocoon yield by weight (26.36 kg / 10000 worms), cocoon yield by number (9777 / 10000 worms), single cocoon weight (2.69 g), single cocoon shell weight (0.63 g) and cocoon shell ratio (23.28 %) was noticed in the silkworms fed with leaves of mulberry plants with foliar application of 0.4 per cent nano nitrogen fertilizer. Similarly, reeling parameters like average filament length (1510.19 m) and filament weight (0.46 g) were also recorded maximum when silkworm groups fed with leaves harvested from mulberry plants raised with foliar application of 0.4 per cent nano nitrogen fertilizer on 25 DAP.

Keywords : Nano nitrogen, Mulberry, *B. mori,* Cocoon traits

The mulberry silkworm, *Bombyx mori* L. economically important insect, its economic significance is attributed to its silk secreting ability. The successful harvest of quality cocoons depends exclusively on the nutrition of the silkworm. Mulberry leaves serve as ideal food and provide various nutrients to carry out the physiological activities in *B. mori* L. Nutrition of silkworm is the sole factor which almost individually augments the quality and quantity of cocoon production (Kamala and Karthikeyan, 2019). Nutrition should include the essential trace elements for insect growth *viz.,* iron, nickel, copper, manganese, potassium, zinc and iodine. Generally, vitamins present in the mulberry leaves fulfill the minimum needs of silkworms but the amount of vitamins present in mulberry leaves diverges on the basis of environmental conditions, usage of fertilizers in field and mulberry varieties and other field practices. The mulberry leaf quality is influenced by variety of spacing's, irrigation levels, nitrogen levels, seasons and the extra foliates that are supplied exogenously through mulberry leaves. Mulberry as a foliage crop responds well to timely application of nutrients through foliar sprays (Geetha, 2019). Foliar nutrition plays important role in the better growth, yield and biochemical constituents of mulberry leaves and also in superior quality of silk (Dhiraj and Kumar, 2012).

Enrichment of the mulberry leaves is one of the strategies by which cocoon and silk productivity can be increased and the quality can be enhanced. Feeding of nutritionally enriched leaves *i.e.,* with ascorbic acid, folic acids, elements like Selenium and metal nanoparticles have showed better growth and development of silkworms as well as improve the economic value of cocoons. At recent, nanotechnology

is being extensively used in modern agriculture to support precision agriculture with nano-particles (Duhan *et al.,* 2017). Nano-particles are smaller in size, ranges from 1-100 nm and they have larger surface area, because of this unique property nano-particles might penetrate more effectively and efficiently when applied through foliar spray compared to chemical nutrients, so foliar application of these nano-nutrients can result in rapid absorption of nutrients by the plants (Qureshi *et al.,* 2018). Plant growth and yield parameters, and also benefit cost ratio was significantly highest by the foliar application of nano zinc oxide at 500 ppm compared to other treatments (Uma *et al.,* 2019).

Prabu *et al.* (2012) reported that larval growth and development of mulberry silkworm was better when fed with mulberry leaves treated with nanoparticles. Patil *et al*. (2016) concluded that, the larval traits of mulberry silkworm were superior when silkworms were fed with nanoparticles treated mulberry leaves. An appropriate technology that is suitable to social and economic conditions which is environment friendly and effective in practice with easy is to be evolved. Some researchers showed that nano-fertilizer released nutrients up to 1200 hrs while conventional fertilizer could support only for less than 500 hrs (Preetha and Balakrishnan, 2017). Keeping in view of the above aspects and importance of nano-fertilizers, the present work is planned to study the effect of foliar application of nano nitrogen fertilizer to mulberry on larval and cocoon traits of silkworm, *Bombyx mori* L. (FC_1 x FC_2).

Material and Methods

The experiment was laid out in a well established V-1 mulberry garden at Department of Sericulture, UAS, GKVK, Bengaluru following Randomized Complete Block Design (RCBD) with 10 treatments; each replicated thrice. The commercial bivoltine double hybrid silkworms (FC_1 x FC_2) were used for study.

Before the commencement of silkworm rearing, the appliances were sun dried and rearing room, rearing trays and other appliances were thoroughly cleaned and washed using two per cent bleaching powder solution. Then entire rearing room was disinfected by following standard procedures (Dandin and Giridhar, 2014). The larvae were provided with chopped mulberry leaves of required quantity and quality. After second moult, silkworms were separated and 90 silkworms were transferred to each experimental tray in three replications. In order to assess the efficacy of nitrogen nano nitrogen foliar application to mulberry on larval traits of silkworm, the silkworm rearing was carried out and fed with mulberry leaves as per the treatment details.

Observations such as fourth instar larval duration (days), fifth instar larval weight (g / 10 larvae), effective rearing rate (ERR) (%), cocoon yield by weight (kg / 10,000 worms), cocoon yield by number (No. / 10,000 worms), single cocoon weight (g), single cocoon shell weight (g), cocoon shell ratio (%), average filament length (m), filament weight (g) and denier were recorded. The data recorded on various parameters were subjected to fisher's method of Analysis of Variance (ANOVA) and interpreted according to Sundararaj *et al.* (1972). The level of significance used in F and t-tests was P = 0.05 for RCBD. The critical difference (CD) values were computed where the F test was found significant.

Treatment Details

T_1 : Foliar application of 0.2 per cent nano N at 18 DAP + 50 per cent N soil application

T_2 : Foliar application of 0.4 per cent nano N at 18 DAP + 50 per cent N soil application

T_3 : Foliar application of 0.6 per cent nano N at 18 DAP + 50 per cent N soil application

T_4 : Foliar application of 2 per cent Urea at 18 DAP + 50 per cent N soil application

T_5 : Foliar application of 0.2 per cent nano N at 25 DAP + 50 per cent N soil application

T_6 : Foliar application of 0.4 per cent nano N at 25 DAP + 50 per cent N soil application

T_7 : Foliar application of 0.6 per cent nano N at 25 DAP + 50 per cent N soil application

T_8 : Foliar application of two per cent Urea at 25 DAP + 50 per cent N soil application

T_9 : Absolute control

T_{10} : Control (Recommended POP)

Note : DAP - Days After Pruning; POP - Package of Practice

RESULTS AND DISCUSSION

Larval Duration (days)

Significantly shortest fourth instar larval durations (4.73 days) was recorded when silkworms were fed with V-1 mulberry leaves with foliar application of treatment T_6 which followed by T_7 (4.74 days), T_2 (4.75 days) and T_3 (4.76 days). The silkworms reared on mulberry leaves of absolute control (T_9) resulted in longest fourth instar larval duration (5.51 days) (Table 1; Fig. 1).

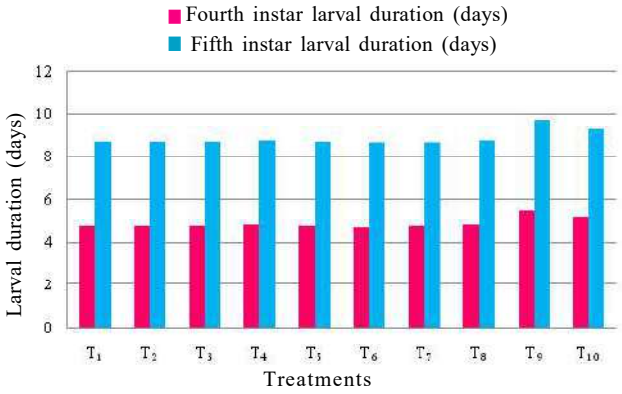

Fig. 1: Effect of foliar application of nano nitrogen fertilizer to mulberry on larval duration of silkworm, *B. mori* L. (FC_1 x FC_2)

TABLE 1

Effect of foliar application of nano nitrogen fertilizer to mulberry on larval traits of silkworm, *B.mori.* L (FC_1 x FC_2)

Treatments	Fourth instar larval duration (days)	Fifth instarlarval duration (days)	Total larval duration (days)	Fifth instar larval weight (g/10 larvae)	ERR (%)
T_1: Foliar application of 0.2 % nano N at 18 DAP + 50 % N soil application	4.77	8.76	24.53	33.93	94.44
T_2: Foliar application of 0.4 % nano N at 18 DAP + 50 % N soil application	4.75	8.74	24.49	34.97	94.44
T_3: Foliar application of 0.6 % nano N at 18 DAP + 50 % N soil application	4.76	8.75	24.51	35.31	92.78
T_4: Foliar application of 2 % Urea at 18 DAP + 50 % N soil application	4.81	8.83	24.64	32.19	76.67
T_5: Foliar application of 0.2 % nano N at 25 DAP + 50 % N soil application	4.76	8.73	24.48	35.67	96.67
T_6: Foliar application of 0.4 % nano N at 25 DAP + 50 % N soil application	4.73	8.69	24.42	36.66	97.78
T_7: Foliar application of 0.6 % nano N at 25 DAP + 50 % N soil application	4.74	8.69	24.43	36.08	95.56
T_8: Foliar application of 2 % Urea at 25 DAP + 50 % N soil application	4.81	8.82	24.63	32.29	73.33
T_9: Absolute control	5.51	9.76	26.27	26.72	67.78
T_{10}: Control (Recommended POP)	5.19	9.33	25.51	33.30	91.67
F-test	*	*	*	*	*
Mean ± SE	0.02	0.04	0.05	0.40	3.18
CD @ 5%	0.07	0.13	0.14	1.17	9.44

Note: * Significant at 5 %, DAP: Days after pruning, POP: Package of Practice

Significantly shorter fifth instar larval durations (8.69 days) was recorded when silkworms were fed with V-1 mulberry leaves with foliar application of treatment T_6 and T_7 followed by T_5 (8.73 days), T_2 (8.74 days) and T3 (8.75 days). The silkworms reared on mulberry leaves of absolute control (T_9) resulted in longest fifth instar larval duration (9.76 days).

Total duration of silkworm varied significantly, shorter total larval duration (24.42 days) was recorded when silkworms were fed with V-1 mulberry leaves with foliar application of treatment T_6 followed by T_7 (24.43 days) followed by T_5 (24.48 days) and T_2 (24.49 days). The silkworms reared on mulberry leaves of absolute control (T_9) resulted in longest total larval duration (26.27 days).

Fifth Instar Larval Weight (g / 10 Larvae)

Significantly highest fifth instar fifth day larval weight (36.66 g / 10 larvae) was recorded when silkworms were fed with mulberry leaves sprayed with T_6 followed by T_7 (36.08 g / 10 larvae), T_5 (35.67 g /10 larvae) and T_3 (35.31 g / 10 larvae). The lowest fifth instar larval weight was noticed in T_9 (26.72 g /10 larvae) (Table 1; Fig. 2).

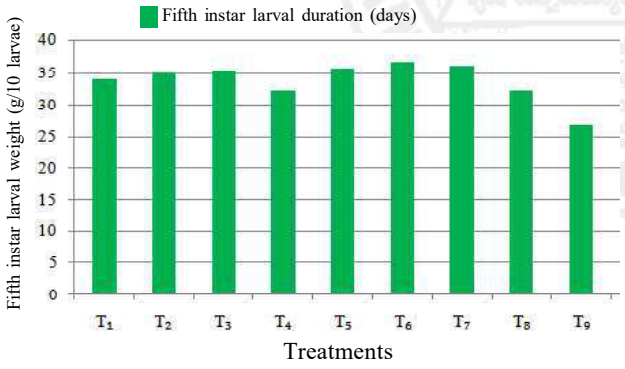

Fig. 2 : Effect of foliar application of nano nitrogen fertilizer to mulberry on larval weight of silkworm, *B. mori* L. (FC_1 x FC_2)

Effective Rate of Rearing (ERR) (%)

Significantly maximum ERR (97.78 %) was recorded in silkworm batch fed on mulberry leaves harvested from treatment T_6 followed by silkworm batches reared on mulberry leaves from T_5 (96.67 %), T_7 (95.56 %) and T_1 (94.44 %). The lowest ERR (67.78 %) was observed in silkworm groups reared on mulberry leaves from absolute control (T_9) (Table 1).

Mulberry silkworms being reared completely as domestic insect, besides other factors, feeding the quality leaves is important for growth of silkworm and production of silk. The nutritive feed especially with nitrogen would act as a stimulant for rapid growth of silkworms. Silkworm groups which were reared on mulberry leaves sprayed with nano nitrogen fertilizer showed significantly improved rearing parameters. Mulberry leaves that have been sprayed with nano nitrogen fertilizer have considerably enhanced leaf nutrient content, crude protein, carbohydrate and crude fibre content, resulting in better growth of silkworm.

Cocoon Yield by Number (No. / 10,000 Worms)

Significantly highest cocoon yield by number (9777 / 10,000 worms) was recorded in T_6 followed by T_5 (9666 / 10,000 worms), T_7 (9555 / 10,000 worms). However, minimum cocoon yield by number was recorded in silkworm groups reared on mulberry leaves of absolute control (T_9) (6777 / 10,000 worms) and T_8 (7333 / 10,000 worms) (Table 2).

Cocoon Yield by Weight (kg / 10,000 Worms)

Significantly highest cocoon yield by weight (26.36 kg /10,000 worms) was recorded from cocoons of silkworms reared on mulberry leaves of treatment T_6 followed by T_5 (26.10 kg / 10,000 worms) and T_2 (25.82 kg / 10,000 worms). However, the significantly lowest cocoon yield by weight was recorded in silkworm groups reared on mulberry leaves of absolute control (T_9) (17.80 kg / 10,000 worms) (Table 2).

Single Cocoon Weight (g)

Among different treatments, significantly highest single cocoon weight was recorded in the cocoons harvested from T_6 (2.69 g) followed by T_5 (2.63 g), T_2 (2.57 g), T_1 (2.56 g) and T_7 (2.53 g). Significantly lowest single cocoon weight was in cocoons harvested from T_9 (absolute control) (1.78 g) (Table 2; Fig. 3).

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Single Shell Weight (g)

The single shell weight was significant among different treatments, however T_6 (0.63 g) followed by T_5 (0.60 g), T_2 (0.58 g) and T_7 (0.57 g). Significantly lowest single cocoon weight was in cocoons harvested from T_9 (0.35 g) (Table 2; Fig. 3).

Cocoon Shell Ratio (%)

The cocoon shell ratio did not differ significantly amongst the treatments of the experiment. However, the per cent cocoon shell ratio was maximum with T_6 (23.28 %) followed by T_5 (22.69 %) and T_7 (22.43 %). The per cent cocoon shell ratio was least with T_9 (19.92 %) (Table 2; Fig. 3).

Cocoon parameters of silkworm fed with leaves from mulberry plants sprayed with nano nitrogen fertilizer varied significantly. The better larval growth and development with qualitatively enriched leaves of mulberry this can be attributed to the larvae spun good quality cocoons with highest cocoon weight, cocoon shell weight and shell ratio.

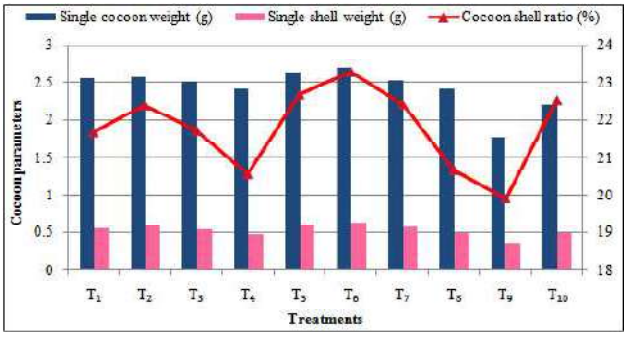

Fig. 3: Effect of foliar application of nano nitrogen fertilizer to mulberry on cocoon parameters of silkworm, *B. mori* L. (FC_1 x FC_2)

TABLE 2

Effect of foliar application of nano nitrogen fertilizer to mulberry on cocoon parameters of silkworm, *B.mori.* L (FC1 x FC2)

Treatments	Cocoon yield by weight (kg / 10,000 cocoons)	Cocoon yield by number (No. /10,000 worms)	Single cocoon weight (g)	Single cocoon shell weight (g)	Cocoon shell ratio (%)
T_1: Foliar application of 0.2 % nano N at 18 DAP + 50 % N soil application	25.67	9444	2.56	0.55	21.67
T_2: Foliar application of 0.4 % nano N at 18 DAP + 50 % N soil application	25.82	9444	2.57	0.58	22.39
T_3: Foliar application of 0.6 % nano N at 18 DAP + 50 % N soil application	25.14	9277	2.50	0.54	21.72
T_4: Foliar application of 2 % Urea at 18 DAP + 50 % N soil application	24.05	7666	2.41	0.48	20.56
T_5: Foliar application of 0.2 % nano N at 25 DAP + 50 % N soil application	26.10	9666	2.63	0.60	22.69
T_6: Foliar application of 0.4 % nano N at 25 DAP + 50 % N soil application	26.36	9777	2.69	0.63	23.28
T_7: Foliar application of 0.6 % nano N at 25 DAP + 50 % N soil application	25.44	9555	2.53	0.57	22.43
T_8: Foliar application of 2 % Urea at 25 DAP + 50 % N soil application	24.30	7333	2.42	0.50	20.67
T_9: Absolute control	17.80	6777	1.77	0.35	19.92
T_{10}: Control (Recommended POP)	22.91	9166	2.20	0.50	22.53
F-test	*	*	*	*	NS
Mean ± SE	0.66	317.63	0.07	0.02	1.32
CD @ 5%	1.97	943.74	0.22	0.06	-

Note: * Significant at 5 %, NS: Non-Significant, DAP: Days after pruning, POP: Package of Practice

Average Cocoon Filament Length (m)

Significantly longest filament length was recorded in the cocoons spun by the silkworms fed on leaves harvested from treatment T_6 (1510.19 m) followed by T_5 (1409.75 m), T_2 (1404.72 m) and T_7 (1395.86 m). However, significantly shortest filament length was noticed in the batches of cocoons obtained from the silkworms fed with absolute control leaves (T_9) (1060.79 m) (Table 3; Fig. 4).

Non-breakable Filament Length (m)

Significantly longest non-breakable filament length was recorded in the cocoons spun by the silkworms fed on leaves harvested from treatment T_6 (1369.34 m) followed by T_5 (1287.79 m), T_2 (1225.36 m) and T_7 (1213.23 m), and significantly shortest filament length was noticed in the batches of cocoons obtained from the silkworms fed with absolute control leaves (T9) (471.13 m) (Table 3; Fig. 4).

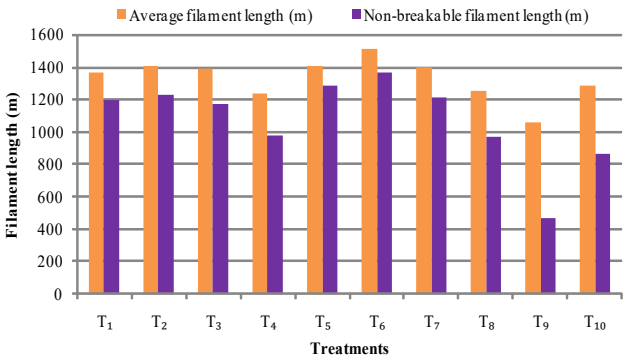

Fig. 4 : Effect of foliar application of nano nitrogen fertilizer to mulberry on filament length of silkworm, *B.mori* L. (FC_1 x FC_2)

TABLE 3

Effect of foliar application of nano nitrogen fertilizer to mulberry on reeling parameters of silkworm, *B.mori.* L (FC_1 x FC_2)

Treatments	Average filament length (m)	Non-breakable filament length (m)	Cocoon filament weight (g)	Denier
T_1: Foliar application of 0.2 % nano N at 18 DAP + 50 % N soil application	1362.69	1192.42	0.43	2.85
T_2: Foliar application of 0.4 % nano N at 18 DAP + 50 % N soil application	1404.72	1225.36	0.44	2.82
T_3: Foliar application of 0.6 % nano N at 18 DAP + 50 % N soil application	1388.83	1175.34	0.43	2.77
T_4: Foliar application of 2 % Urea at 18 DAP + 50 % N soil application	1234.50	980.06	0.42	3.07
T_5: Foliar application of 0.2 % nano N at 25 DAP + 50 % N soil application	1409.75	1287.79	0.44	2.82
T_6: Foliar application of 0.4 % nano N at 25 DAP + 50 % N soil application	1510.19	1369.34	0.46	2.74
T_7: Foliar application of 0.6 % nano N at 25 DAP + 50 % N soil application	1395.86	1213.23	0.45	2.88
T_8: Foliar application of 2 % Urea at 25 DAP + 50 % N soil application	1248.69	971.82	0.41	2.98
T_9: Absolute control	1060.79	471.13	0.36	3.01
T_{10}: Control (Recommended POP)	1287.40	861.11	0.41	2.84
F-test	*	*	*	NS
Mean ± SE	34.94	119.24	0.01	0.09
CD @ 5%	103.81	354.29	0.03	-

Note: * Significant at 5 %, NS: Non-Significant, DAP: Days after pruning, POP: Package of Practice

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Cocoon Filament Weight (g)

Significantly maximum filament weight was recorded in the cocoons spun by the silkworms fed on leaves harvested from treatment T_6 (0.46 g) followed by T_7 (0.45 g), T_2 (0.44 g), T_5 (0.44 g) and While the significantly least filament weight was obtained in cocoons of silkworms fed with leaves of T_9 (absolute control) (0.36 g) (Table 3).

Denier

The filament denier was non-significant among the different treatments. However, denier recorded was in the order of T_6 (2.74), T_3 (2.77), T_2 (2.82), T_{10} (2.84), T_1 (2.85), T_5 (2.85), T_7 (2.86), T_8 (2.98), T_9 (3.01) and T_4 (3.07) (Table 3).

Feeding the quality leaves is important for growth of silkworm and production of silk. However, leaves of nitrogen nano-fertilizer sprayed mulberry plants were nutritionally superior, the larvae fed with those leaves showed better growth and development and spun good quality cocoons. The nutritive feed especially with nitrogen would act as a stimulant for rapid growth of silkworm results in better silk production.

Present findings were in line with results of Manchashetty (1979) who found that foliar application of 0.5 per cent urea increased the larval weight, cocoon parameters and also average filament length, weight of silk filament and denier. Significantly highest larval weight, cocoon weight, shell weight and filament length were recorded when silkworms fed with nitrogen supplemented mulberry leaves (Mahmood, 2002). Correspondingly, Maqbool (1991) reported that feeding mulberry leaves treated with nitrogen increased the growth and development of silkworm and improvement in the silk yield.

Indistinguishably, Zaman (1995) noticed that silkworms fed with mulberry leaves treated with 0.2 per cent nitrogen + magnesium 0.15 per cent, consumed more food and were having significantly highest larval weights, cocoon and reeling parameters, compared to other treatments and untreated leaves. Rasool (1995) concluded that silkworms fed with mulberry leaves treated with 0.2 per cent nitrogen and 0.5 per cent manganese increased food consumption and significant increase in the larval parameters, cocoon quality and silk filament. Ashfaq *et al.* (1998) reported that silkworms fed with mulberry leaves treated with 0.2 per cent nitrogen and 0.05 per cent copper significantly improved larval weight, cocoon weight and filament length. Etebari *et al.* (2007) recorded significant improvement in the larval parameters, quality of cocoon and filament length, when silkworms fed with mulberry leaves treated with 0.1 per cent nitrogen when compared to other treatments.

The outcome of Prabu *et al.* (2011) and Patil *et al.* (2016) also showed that silkworms fed with mulberry leaves sprayed with nano-particles showed significantly highest larval weight other rearing parameters, cocoon weight, shell weight, cocoon shell ratio, filament length and filament weight. Nano particles which improves the feed efficacy of silkworms, Nano-particles, that might have stimulated the metabolic activity in silkworms leading to improved rearing performance, cocoon quality and reeling parameters.

According to Nithya (2018), adequate supply of zinc nanoparticles which accelerates the activity of enzymes and auxin metabolism in the plants, thereby increased the larval parameters, improvement in larval and cocoon parameters increased the reeling parameters of silkworm, *B. mori*. Pramila *et al.* (2019) observed better larval growth and development when silkworm fed with mulberry leaves with foliar application of nano micronutrients, produced good quality cocoon, resulting in better silk production. Shilpashree (2015) observed that silkworm cocoon weight was significantly and positively correlated with total soluble sugars (0.84) and total soluble protein (0.96) contents of mulberry leaf. These nano-particles can be used as fertilizer for efficient nutrient management which are more eco-friendly and reduce environmental pollution (Meena *et al.,* 2017).

All the larval and cocoon parameters of silkworm, *B. mori* performed better at 0.4 per cent nano nitrogen fertilizer foliar application on 25th day after pruning + 50 per cent N, 100 per cent P and K through soil

application + FYM. Hence, this could be the optimum dose of nitrogen nano-fertilizer for V-1 mulberry.

Nutrient loss caused by fertilizer application without assessing crop needs, resulting in nutrient waste and pollution of soil microorganisms and ground water, can be decreased by using nitrogenous nano-fertilizer. As a result, nitrogenous nano-fertilizers can be used efficiently in the field of sericulture when applied at ideal nitrogen levels.

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