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# Impact of Nano Nitrogen Sprayed Mulberry Leaves on *Bm*NPV Infected Larval and Cocoon Traits of Silkworm, *Bombyx mori* L.

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**Abstract**

Foliar application of nano nitrogen 0.4 per cent @ 200 L/acre after 25 DAP + without inoculation ($T_3$) has recorded lowest total larval duration (473.33 h), zero per cent disease incidence, zero per cent larval mortality, highest fifth instar larval progression (100.00%), ERR (100.00%), larval weight (35.71 g/10 larvae), cocoon weight (2.13g), pupal weight (1.67g), shell weight (0.45g), floss weight (23.09mg), cocoon shell ratio (21.35%) and denier (2.79) under healthy conditions. Among fourth instar *Bm*NPV inoculated batch, foliar application of nano nitrogen 0.4 per cent @ 200 L/acre after 25 DAP ($T_1$) has recorded lowest total larval duration (518.16 h), disease incidence (38.23%), larval mortality (29.31%), highest fifth instar larval progression (76.00%), ERR (62.98%), larval weight (29.38g), cocoon weight (1.73g), pupal weight (1.44g), shell weight (0.30g), floss weight (19.15mg), cocoon shell ratio (17.17%) and denier (2.53). Whereas, fifth instar *Bm*NPV inoculated batch foliar application of nano nitrogen at 0.4 per cent at 200 L/acre after 25 DAP ($T_2$) has recorded lowest total larval duration (484.16 h), disease incidence (27.02%), larval mortality (23.29%), highest fifth instar larval progression (84.33%), ERR (78.14%), larval weight (32.98g/10 larvae), single cocoon weight (1.90g), pupal weight (1.55g), shell weight (0.37g), floss weight (20.17mg), cocoon shell ratio (19.26%) and denier (2.65). The study evidenced that foliar application of nano nitrogen exhibited positive effects on growth and development of silkworm, *Bombyx mori* L. and also in managing the *Bm*NPV infection.

*Keywords* : Nano nitrogen, Mulberry, Silkworm, *Bm*NPV, Cocoon

Silkworm is a monophagous insect obtain its nutrients only from the mulberry leaves. It is well known that good nutrition strengthens the immune system of all living organisms. The mulberry leaf provide silkworm with all the nutrients it needs for growth, development and productivity. Hence, profit for the farmers and silk productivity primarily depends upon quality and quantity of mulberry leaves fed to the silkworm. Thus, proper nutrient management for growth and development of mulberry is highly essential for better performance of silkworm. Vegetative part is the main component of mulberry, which is stimulated by application of nitrogen. Although plants typically absorb nutrients through their roots, deep-rooted plants like mulberry may not be able to do so efficiently. The applied nitrogen is not entirely accessible to plants due to leaching. The primary component of a plant where food is prepared is leaf and timely application of fertilizer solutions directly to the leaves improves their ability to absorb nutrients (Geetha, 2019). Nano-sized particles applied directly to the leaves of mulberry plants exhibit unique properties, such as increased surface area and improved solubility, facilitating enhanced absorption by the plant tissues. The targeted delivery system ensures more efficient nutrient utilization, as the nano

nitrogen penetrates the leaf surface and enters the plant metabolic processes with greater ease (Qureshi *et al*., 2018). According to Uma *et al.* (2019), the foliar application of nano zinc oxide at 500 ppm considerably outperformed other treatments in terms of plant growth and yield characteristics as well as benefit-cost ratio. Apart from leaf nutrition, nitrogen provides required nutrition for silkworms too. Since, it is an integral component of many important structural, genetic and metabolic compounds in plant and animal cells *viz.,* DNA and energy-transfer compounds (ATP), component of chlorophyll, amino acids and also acts as enzymes. In plants the disease resistance is known to increase by nano-particles (NPs) through activation of plant defence mechanism. Antiviral assay of different NPs had shown excellent viricidal properties against different plant viruses (Mishra *et al.,* 2022). Silkworm has been domesticated for many years and is susceptible to many infections even with minor change in rearing practices. Among many viral diseases grasserie causing by *B. mori* Nuclear Polyhedrosis Virus (*Bm*NPV) accounts for 20-30 per cent crop loss across the country which belongs to the Baculoviridae family having *ds*DNA as genetic material (Selvakumar *et al.,* 2013). The NPV infection interferes with the host gene expression in silkworm to defend itself from invasive diseases silkworm uses a variety of innate immunological reactions which increased by feeding the silkworm with quality leaves. Keeping in view of the above aspects and importance of nano nitrogen fertilizer, the present work is planned to study the impact of nano nitrogen sprayed mulberry leaves on *Bm*NPV infected larval and cocoon traits of silkworm.

## Material and Methods

The experiment was executed during 2022-2023 at Department of Sericulture, College of Agriculture, GKVK, Bengaluru. A well-established paired row garden with popular mulberry variety, Victory 1 (V1) was selected and maintained as per Dandin and Giridhar (2014).

A popular bivoltine silkworm hybrid ($FC_1$ x $FC_2$) was used for the study. Experimental design was in CRD with 9 treatments, 3 replications and subjected 50 worms each per replications. Using Neubaure's hemocytometer, the concentration of polyhedral occlusion bodies (POBs) of *Bm*NPV was measured from NPV-infected silkworm that were collected from the field and the silkworm were infected per oral by

Treatment Details

| Treatments | Description |
|---|---|
| $T_1$ | Foliar application of nano nitrogen 0.4 % @ 200 L/acre after 25 DAP +50 % N soil application + Inoculated at the beginning of 4$^{th}$ instar |
| $T_2$ | Foliar application of nano nitrogen 0.4 % @ 200 L/acre after 25 DAP +50 % N soil application + Inoculated at the beginning of 5$^{th}$ instar |
| $T_3$ | Foliar application of nano nitrogen 0.4 % @ 200 L/acre after 25 DAP +50 % N soil application + without inoculation |
| $T_4$ | Foliar application of 2 % urea @ 200 L/acre after 25 DAP +50 % N soil application + Inoculated at the beginning of 4$^{th}$ instar |
| $T_5$ | Foliar application of 2 % urea @ 200 L/acre after 25 DAP +50 % N soil application+ Inoculated at the beginning of 5$^{th}$ instar |
| $T_6$ | Foliar application of 2 % urea @ 200 L/acre after 25 DAP +50 % N soil application + without inoculation |
| $T_7$ | RDF + Inoculated at the beginning of 4$^{th}$ instar |
| $T_8$ | RDF + Inoculated at the beginning of 5$^{th}$ instar |
| $T_9$ | RDF + Without inoculation |

feeding them on mulberry leaves smeared with viral suspension 40,000 POB's per viral suspension (Lekha *et al*., 2015). After moulting, the larvae were starved for three hours before feeding on the inoculated leaves and subsequently continued the feeding with leaves as per the treatment, respectively. The control batches were reared separately under disease free conditions. The leaves harvested from nano nitrogen sprayed mulberry plants were fed to silkworms thrice a day and different parameters such as larval mortality (%), disease incidence (%), larval progression (%), total larval duration (h), larval weight (g/10 larvae), effective rate of rearing (%), cocoon yield by number (No./10000 silkworm), cocoon yield by weight (kg/ 10000 silkworm), cocoon weight (g), pupal weight (g), shell weight (g), floss weight (g), cocoon shell ratio (%), average filament length (m), Non-breakable filament length (m), cocoon filament weight (g) and denier were recorded. The Fisher's method of Analysis of Variance (ANOVA) was used for data analysis and interpretation was according to Sundararaj *et al*. (1972). P = 0.05 is the level of significance used for F and t-tests in CRD. The critical difference (CD) values were computed where the F test was found significant.

## Results and Discussion

The data related to larval growth and development parameters *viz*., larval progression, larval mortality, larval duration, larval weight and effective rate of rearing were given in Table 1. Whereas, Fig. 1 showing the general symptoms of *Bm*NPV infected silkworm.

### Fourth Instar Larval Duration (h)

Among fourth instar *Bm*NPV inoculated batch, the fourth instar larval duration was significantly shortest in $T_1$ (Foliar application of nano nitrogen at 0.4 per cent at 200 L/acre after 25$^{th}$ DAP + 50 per cent N soil application + 4$^{th}$ instar *Bm*NPV inoculation) (118.66 h) followed $T_4$ (121.50 h). The larval duration was significantly longest in $T_7$ (RDF + Inoculated at the beginning of 4$^{th}$ instar) (127.83 h). In fourth instar healthy batch, fourth instar larval duration was significantly shortest in $T_3$ (Foliar application of nano

Table 1

Effect of nano nitrogen sprayed mulberry leaves on fourth and fifth instar larval parameters of *Bm*NPV inoculated silkworm

| Treatments | 4$^{th}$ instar larval duration (h) | 5$^{th}$ instar larval duration (h) | 4$^{th}$ instar larval progression (%) | 5$^{th}$ instar larval progression (%) | 4$^{th}$ instar larval weight (g/10 larvae) | 5$^{th}$ instar larval weight (g/10 larvae) |
|---|---|---|---|---|---|---|
| $T_1$ | 118.66 | 231.50 | 100.00 | 76.00 | 9.92 | 29.38 |
| $T_2$ | 108.25 | 207.91 | 100.00 | 84.33 | 10.15 | 32.98 |
| $T_3$ | 107.66 | 197.66 | 100.00 | 100.00 | 10.36 | 35.71 |
| $T_4$ | 121.50 | 239.16 | 99.33 | 71.33 | 9.38 | 27.99 |
| $T_5$ | 111.41 | 216.16 | 100.00 | 80.33 | 10.01 | 30.65 |
| $T_6$ | 110.25 | 202.50 | 100.00 | 100.00 | 10.02 | 33.85 |
| $T_7$ | 127.83 | 245.33 | 98.66 | 66.33 | 8.37 | 25.73 |
| $T_8$ | 117.66 | 232.08 | 100.00 | 78.33 | 9.01 | 29.32 |
| $T_9$ | 114.16 | 211.00 | 100.00 | 100.00 | 9.00 | 31.93 |
| F-test* | * | NS | * | * | * | |
| S. Em ± | 0.69 | 1.23 | - | 0.68 | 0.15 | 1.26 |
| $CD_{0.05}$ | 2.06 | 3.68 | - | 2.05 | 0.45 | 3.77 |
| CV | 1.04 | 0.97 | - | 1.41 | 2.72 | 7.06 |

*Note* : * Significant at the 0.05 level; NS – Non-significant

(a) Improper moulting (b) Inter segmental swelling (c) Milky white haemolymph

Fig. 1 Symptoms of *Bm*NPV infected silkworm

nitrogen at 0.4 per cent at 200 L/acre after 25 DAP + 50 per cent N soil application + without *Bm*NPV inoculation) (107.66 h) followed by $T_2$ (108.25 h) and longest larval duration was recorded in $T_8$ (RDF + Inoculated at the beginning of 5$^{th}$ instar) (117.66 h) (Table 1).

**Fifth Instar Larval Duration (h)**

Among fourth instar *Bm*NPV inoculated batch, the fifth instar larval duration was significantly shortest in $T_1$ (231.50 h) followed by $T_4$ (239.16 h) and longest larval duration was recorded in $T_8$ (245.33 h). Among fifth instar *Bm*NPV inoculated batch fifth instar larval duration was significantly shortest in $T_2$ (207.91 h) followed by $T_5$ (216.16 h) and longest larval duration was recorded in $T_8$ (232.08 h). In fifth instar healthy batch, significantly shortest fifth instar larval duration was recorded in $T_3$ (197.66 h) followed by $T_6$ (202.50 h) and longest larval duration was recorded in $T_9$ (211.00 h) (Table 1).

**Larval Progression (%)**

**Fourth Instar Larval Progression (%)**

The larval progression of fourth instar larvae was non-significant among the different treatments. Cent per cent larval progression of fourth instar larvae were recorded in all the treatments except $T_7$ (98.66%) and $T_4$ (99.33 %) (Table 1).

**Fifth Instar Larval Progression (%)**

The batches of silkworms without *Bm*NPV inoculation recorded 100 per cent fifth instar larval progression whereas, batches that were inoculated with *Bm*NPV exhibited were not. Among fourth instar *Bm*NPV inoculate batch, fifth instar larval progression was significantly maximum in $T_1$ (76.00%) followed by $T_4$ (71.33%) whereas, larval progression recorded significantly minimum in $T_7$ (66.33%). In fifth instar *Bm*NPV inoculated batch, significantly maximum fifth instar larval progression was recorded in treatment $T_2$ (84.33%) followed by $T_5$ (80.33%). Minimum fifth instar larval progression was recorded in $T_8$ (78.33 %) (Table 1).

**Larval Weight (g/10 larvae)**

**Fourth Instar Larval Weight (g/10 larvae)**

Among the healthy worms, significantly maximum fourth instar larval weight was recorded in $T_3$ (10.36g) followed by $T_6$ (10.02g) whereas, fourth instar larval weight was significantly minimum in $T_9$ (9.00g). Among fourth instar *Bm*NPV inoculated batch, significantly maximum fourth instar larval weight was recorded in $T_1$ (9.92g) followed by $T_4$ (9.31g) whereas, fourth instar larval weight was significantly minimum in $T_7$ (8.37 g) (Table 1).

**Fifth Instar Larval Weight (g)**

Among the healthy worms, significantly maximum fifth instar larval weight was recorded in $T_3$ (35.71g) followed by $T_6$ (33.85g). Whereas, fifth instar larval weight was significantly minimum recorded in $T_9$ (31.93g). Among fourth instar *Bm*NPV inoculated batch fifth instar larval weight was significantly

maximum in $T_1$ (29.38g) followed by $T_4$ (27.99g). Significantly minimum fifth instar larval weight was recorded in $T_7$ (25.73g). Among 5th instar *Bm*NPV inoculated batch, the maximum fifth instar larval weight was recorded in $T_2$ (32.98g) followed by $T_5$ (30.65g). The fifth instar larval weight was significantly minimum in $T_8$ (29.32g) (Table 1).

Mahmood *et al*. (2002) recorded better larval weight and size after feeding with nitrogen supplemented mulberry leaves. Silkworm being completely reared as domestic insect, besides of all other factors, feeding them with good quality leaves is important for increasing the growth and productivity of silkworm. Mulberry leaves are supplied with foliar application of nano nitrogen fertilizer have considerably increased leaf nutrient content, crude protein, carbohydrate and crude fiber content that might have resulted in better growth and development of silkworm.

**Total Larval Duration (h)**

Among fourth instar *Bm*NPV inoculated batch, significantly shortest larval duration was recorded in $T_1$ (518.16h) followed by $T_4$ (528.66h). However, significantly longest larval duration was recorded in $T_7$ (541.16h). Among fifth instar *Bm*NPV inoculated batch, significantly shortest larval duration (484.16h) was recorded in $T_2$ followed by $T_5$ (495.58h). The longest larval duration was recorded in $T_8$ (517.75h). In healthy batch, significantly shortest larval duration was recorded in $T_3$ (473.33 h) followed by $T_6$ (480.75 h). The longest larval duration was recorded in $T_9$ (493.16 h) (Table 2).

The infection of *Bm*NPV disrupts metabolic activity in silkworm body leading to slow growth and development, which is evident during the present study. Among the treatments, the *Bm*NPV infected silkworm fed with 0.4 per cent nano nitrogen sprayed leaves during both fourth and fifth instar inoculated batches showed relatively lesser larval duration that could be because of the feed quality. Pooja *et al*. (2022) also reported that larval duration was reduced when silkworm fed with leaves of mulberry plants with foliar application of 0.4 per cent nano nitrogen fertilizer on 25 DAP + 50 per cent soil application of nitrogen.

**Disease Incidence (%)**

Among fourth instar *Bm*NPV inoculated batch, disease incidence was significantly lowest in $T_1$ (38.23%) followed by $T_4$ (40.57%), whereas significantly highest disease incidence was recorded in $T_7$ (43.45 %). Among fifth instar *Bm*NPV inoculated batches significantly lowest disease incidence was recorded in $T_2$ (27.02%) followed by $T_5$ (29.54%). The disease incidence was significantly highest in $T_8$ (31.93%) (Table 2) (Fig. 2).

**Larval Mortality (%)**

Among the fourth instar *Bm*NPV inoculated batch, significantly lowest larval mortality was noticed in $T_1$ (29.31%) followed by $T_4$ (32.35%). The larval

TABLE 2

Effect of nano nitrogen sprayed mulberry leaves on total larval duration, disease incidence, larval mortality and ERR of *Bm*NPV inoculated silkworm

| Treatments | Total larval duration (h) | Disease incidence (%) | Larval mortality (%) | ERR (%) |
|---|---|---|---|---|
| $T_1$ | 518.16 | 38.23 | 29.31 | 62.98 |
| $T_2$ | 484.16 | 27.02 | 23.29 | 78.14 |
| $T_3$ | 473.33 | 0.00 | 0.00 | 100.00 |
| $T_4$ | 528.66 | 40.57 | 32.35 | 54.07 |
| $T_5$ | 495.58 | 29.54 | 26.30 | 69.87 |
| $T_6$ | 480.75 | 0.00 | 0.00 | 100.00 |
| $T_7$ | 541.16 | 43.45 | 35.45 | 46.75 |
| $T_8$ | 517.75 | 31.93 | 27.72 | 57.95 |
| $T_9$ | 493.16 | 0.00 | 0.00 | 100.00 |
| F-test | * | * | * | * |
| S. Em ± | 1.23 | 0.67 | 0.46 | 1.08 |
| $CD_{0.05}$ | 3.68 | 2.01 | 1.37 | 3.25 |
| CV | 0.97 | 4.97 | 4.11 | 2.53 |

*Note* : *Significance at 5%

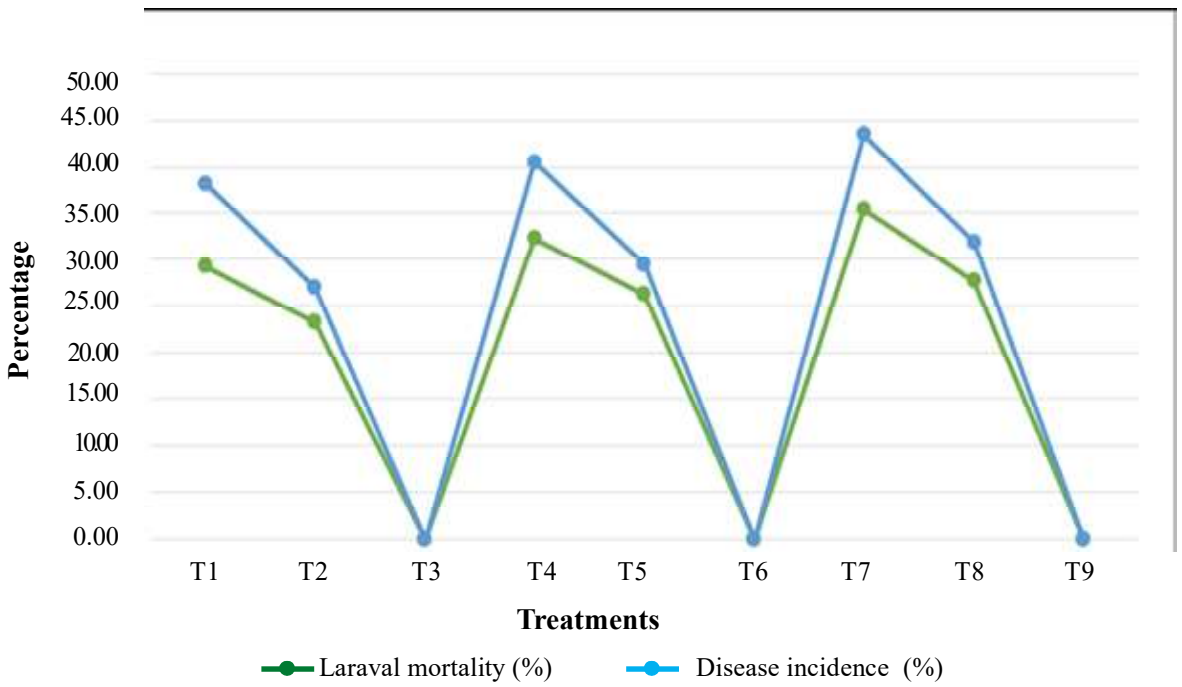


Fig. 2 Effect of nano nitrogen sprayed mulberry leaves on disease incidence and larval mortality in *Bm*NPV inoculated silkworm

mortality was significantly highest in treatment $T_7$ (35.45%). In fifth instar *Bm*NPV inoculated batch, significantly lowest larval mortality was recorded in $T_2$ (23.29%) followed by $T_5$ (26.30%). Larval mortality was significantly highest in $T_8$ (27.72%) (Table 2) (Fig. 2).

Similar reports were observed in a study conducted by Ruyi *et al.* (2012) where they investigated the effectiveness of silver nano particle colloids (AgNPs) in reducing the infection of *Bm*NPV in silkworms. The interaction between virus and AgNPs resulted in the production of free radicals that penetrated and disintegrated the virus capsids, proteins and DNA of *Bm*NPV, thereby reducing the pathogenicity. AgNPs was capable of decreasing the disease incidence of the silkworm from 67 to 22 per cent when compared to the untreated control group.

**Effective Rate of Rearing [ERR] (%)**

Cent per cent ERR was recorded in the healthy batches of silkworm which were fed with healthy mulberry leaves. The inoculated batches exhibited significantly different ERR when they were fed with *Bm*NPV treated mulberry leaves. Among fourth instar *Bm*NPV inoculated batch, significantly maximum ERR was noticed in $T_1$ (62.98%) followed by $T_4$ (54.07%). The ERR was significantly lowest in $T_7$ (46.75%). Among fifth instar *Bm*NPV inoculated batch, significantly maximum ERR was noticed in $T_2$ (78.14%) followed by $T_5$ (69.87%). Significantly minimum ERR was recorded in $T_8$ (57.95%) (Table 2).

The increased ERR might be attributed to the production of good quality foliage, which in turn provided the nutrients required to boost up the immunity in silkworm. Mulberry leaves sprayed with nano nitrogen fertilizer possess considerably higher leaf nutrient content, crude protein, carbohydrate and crude fibre that resulted in better larval growth and progression. Increase in the leaves quality which enhances the physiological activity in silkworm that might be the reason for increasing larval progression and ERR. Etebari *et al*. (2007) recorded a significant increase in the larval parameters when silkworms were fed with mulberry leaves treated with 0.1 per cent nitrogen compared to other treatments.

TABLE 3

Effect of nano nitrogen sprayed mulberry leaves on cocoon parameters of *Bm*NPV inoculated silkworm

| Treatments | Cocoon yield by weight (kg/ 10000 silkworm) | Cocoon yield by number (kg/ 10000 silkworm) | Cocoon weight (g) | Pupal weight (g) | Floss weight (mg) | Cocoon shell weight (g) | Cocoon shell ratio (%) |
|---|---|---|---|---|---|---|---|
| $T_1$ | 10.89 | 6298.44 | 1.73 | 1.44 | 19.15 | 0.30 | 17.17 |
| $T_2$ | 14.85 | 7814.33 | 1.90 | 1.55 | 20.17 | 0.37 | 19.26 |
| $T_3$ | 21.30 | 10000.00 | 2.13 | 1.67 | 23.09 | 0.45 | 21.35 |
| $T_4$ | 8.43 | 5406.96 | 1.56 | 1.31 | 18.05 | 0.25 | 15.85 |
| $T_5$ | 12.44 | 69 87.45 | 1.78 | 1.46 | 19.46 | 0.33 | 18.28 |
| $T_6$ | 20.10 | 10000.00 | 2.01 | 1.61 | 21.56 | 0.41 | 20.31 |
| $T_7$ | 6.92 | 4675.63 | 1.48 | 1.25 | 16.25 | 0.22 | 15.15 |
| $T_8$ | 9.74 | 5795.73 | 1.68 | 1.39 | 18.21 | 0.29 | 17.11 |
| $T_9$ | 19.00 | 10000.00 | 1.90 | 1.54 | 20.11 | 0.37 | 19.22 |
| F-test | * | * | * | * | * | * | * |
| S. Em ± | 0.43 | 108.68 | 0.06 | 0.06 | 0.13 | 0.01 | 0.80 |
| $CD_{0.05}$ | 1.30 | 325.42 | 0.19 | 0.18 | 0.41 | 0.03 | 2.39 |
| CV | 5.54 | 2.53 | 6.10 | 7.27 | 1.19 | 4.98 | 7.61 |

*Note* : *- Significant at 5 %

**Cocoon Yield by Number (No./ 10000 silkworm)**

The cocoon yield by number did not vary significantly in healthy batches. Among fourth instar *Bm*NPV inoculated batch, significantly highest cocoon yield by number was recorded in $T_1$ (6298.44 No.) followed by $T_4$ (5406.96 No.) whereas, cocoon yield by number significantly lowest in $T_7$ (4675.63 No.). Among fifth instar *Bm*NPV inoculated batch, the maximum cocoon yield by number was recorded in $T_2$ (7814.33 No.) followed by $T_5$ (6987.45 No.) whereas, cocoon yield by number significantly lowest in $T_8$ (5795.73 No.) (Table 3).

**Cocoon Yield by Weight (kg/10000 Silkworm)**

In the healthy batches of silkworms, significantly highest cocoon yield by weight was recorded in $T_3$ (21.30kg) followed by $T_6$ (20.10kg) whereas, significantly lowest cocoon yield by weight was recorded in $T_9$ (19.00kg). Among the fourth instar *Bm*NPV inoculated batches, significantly highest cocoon yield by weight was recorded in $T_1$ (10.89kg) followed by $T_4$ (8.43kg) whereas, significantly lowest cocoon yield by weight was recorded in $T_7$ (6.92kg). Among fifth instar *Bm*NPV inoculated batches, cocoon yield by weight was significantly highest in $T_2$ (14.85kg) followed by $T_5$ (12.44kg). Significantly lowest cocoon yield by weight was recorded in $T_8$ (9.74kg) (Table 3).

Nano particles enhance the feeding efficacy of silkworms leading to increased larval growth and development. This might have stimulated the metabolic activity in silkworm ultimately improving the quality of cocoon they produce. Prabu *et al*. (2011) and Patil *et al*. (2016) have reported that silkworms fed with mulberry leaves treated with foliar spray of nano particles exhibited significantly highest cocoon weight.

**Cocoon Weight (g)**

In the healthy batches of silkworms, single cocoon weight was significantly highest in $T_3$ (2.13g) followed

by $T_6$ (2.01g) and significantly lowest single cocoon weight was recorded in $T_9$ (1.90g). Among the fourth instar *Bm*NPV inoculated batch, the highest cocoon weight was recorded in the $T_1$ (1.73g) followed by $T_4$ (1.56g) and significantly lowest single cocoon weight was recorded in $T_7$ (1.48g). Among fifth instar *Bm*NPV inoculated batch, significantly highest cocoon weight was recorded in $T_2$ (1.90g) followed by $T_5$ (1.78g). Whereas, significantly lowest single cocoon weight was recorded in $T_8$ (1.68g) (Table 3). Pooja *et al*. (2022) reported that cocoon weight (2.69g) was maximum in the silkworms fed with leaves of mulberry plants with foliar application of 0.4 per cent nano nitrogen fertilizer on 25$^{th}$ DAP + 50 per cent soil application of nitrogen.

**Pupal Weight (g)**

In healthy batches of silkworms, significantly highest single pupal weight was recorded in $T_3$ (1.67 g) followed by $T_6$ (1.61 g) and significantly lowest single pupal weight was recorded in $T_9$ (1.54g). Among the fourth instar *Bm*NPV inoculated batch, the single pupal weight was significantly highest in $T_1$ (1.44 g) followed by $T_4$ (1.31 g) and significantly lowest single pupal weight was recorded in $T_7$ (1.25 g). Among fifth instar *Bm*NPV inoculated batch, significantly highest pupal weight was recorded in $T_2$ (1.55 g) followed by $T_5$ (1.46 g) whereas, significantly lowest single pupal weight was noticed in $T_8$ (1.39 g) (Table 3).

The better larval growth and development by feeding them with qualitatively enriched leaves of mulberry can be attributed to the good quality cocoons resulting with maximum cocoon weight and pupal weight. In the present study, foliar application of nano nitrogen has recorded maximum single cocoon weight (g) and single pupal weight (g) both under control and *Bm*NPV infection.

**Floss Weight (mg)**

In the healthy batches of silkworm floss weight was significantly highest in $T_3$ (23.09mg) followed by $T_6$ (21.56mg) whereas, floss weight was significantly lowest in $T_9$ (20.11mg). Among the fourth instar *Bm*NPV inoculated batch, significantly highest floss weight was recorded in $T_1$ (19.15mg) followed by $T_4$ (18.05mg), whereas floss weight was significantly lowest in $T_7$ (16.25mg). Among fifth instar *Bm*NPV inoculated batch, significantly highest floss weight was recorded in $T_2$ (20.17mg) followed by $T_5$ (19.46mg). Significantly least floss weight was recorded in $T_8$ (18.21mg) (Table 3).

**Cocoon Shell Weight (g)**

Among the healthy batches of silkworms, cocoon shell weight was significantly highest in treatment $T_3$ (0.45g) followed by $T_6$ (0.41g) and cocoon shell weight was significantly lowest in $T_9$ (0.37g). Among the fourth instar *Bm*NPV inoculated batch, significantly highest cocoon shell weight was recorded in $T_1$ (0.30g) followed by $T_4$ (0.25g). Cocoon shell weight was significantly least in $T_7$ (0.22g). Among fifth instar *Bm*NPV inoculated batch, significantly highest cocoon shell weight was recorded in $T_2$ (0.37g) followed by $T_5$ (0.33g). The cocoon shell weight was significantly least in $T_8$ (0.29g) (Table 3). According to Nithya (2018), adequate supply of zinc nanoparticles which accelerates the activity of enzymes and auxin metabolism in the plants that increased the larval parameters, thereby cocoon parameters of silkworms.

**Cocoon Shell Ratio [CSR] (%)**

In the healthy batches of silkworms, CSR was significantly highest in $T_3$ (21.35%) followed by $T_6$ (20.31%) and lowest CSR was recorded in $T_9$ (19.22%). Among the fourth instar *Bm*NPV inoculated batch, significantly highest CSR was recorded in $T_1$ (17.17%) followed by $T_4$ (15.85%) and CSR was significantly lowest in $T_7$ (15.15%). Among fifth instar *Bm*NPV inoculated batch, significantly highest CSR was recorded in $T_2$ (19.26%) followed by $T_5$ (18.28%) whereas, significantly lowest CSR was recorded in $T_8$ (17.11%) (Table 3). Pooja *et al*. (2022) reported that cocoon shell ratio (23.28%) maximum in the silkworms fed with leaves of mulberry plants with foliar application of 0.4 per cent nano nitrogen fertilizer on 25$^{th}$ DAP + 50 per cent soil application of nitrogen.

**Average Filament Length (m)**

Statistically significant difference in average filament length was noticed in both healthy and *Bm*NPV inoculated batches. In the healthy batches of silkworms, significantly longest average filament length was recorded in $T_3$ (1187.69m) followed by $T_6$ (1125.24m) and average filament length was significantly shortest in $T_9$ (1072.90m). Among the fourth instar *Bm*NPV inoculated batch, significantly longest average filament length was recorded in $T_1$ (846.56 m) followed by $T_4$ (751.75 m) and average filament length was significantly shortest in $T_7$ (693.31m). Among fifth instar *Bm*NPV inoculated batch, significantly longest average filament length was recorded in $T_2$ (1066.60 m) followed by $T_5$ (948.78 m) whereas, significantly shortest average filament length was recorded in $T_8$ (856.39 m) (Table 4) (Fig. 3).

**Non-breakable Filament Length (m)**

In the healthy batches of silkworms, non-breakable filament length was significantly longest in $T_3$

TABLE 4

Effect of nano nitrogen sprayed mulberry leaves on reeling parameters of *Bm*NPV inoculated silkworm

| Treatments | Average filament length (m) | Non-breakable filament length (m) | Filament weight (g) | Denier |
|---|---|---|---|---|
| $T_1$ | 846.56 | 619.28 | 0.24 | 2.53 |
| $T_2$ | 1066.60 | 803.45 | 0.31 | 2.65 |
| $T_3$ | 1187.69 | 1065.91 | 0.37 | 2.79 |
| $T_4$ | 751.75 | 546.78 | 0.19 | 2.31 |
| $T_5$ | 948.78 | 735.86 | 0.25 | 2.38 |
| $T_6$ | 1125.24 | 915.28 | 0.33 | 2.60 |
| $T_7$ | 693.31 | 445.64 | 0.18 | 2.30 |
| $T_8$ | 856.39 | 651.89 | 0.22 | 2.30 |
| $T_9$ | 1072.90 | 728.91 | 0.27 | 2.48 |
| F-test | * | * | * | * |
| S. Em ± | 35.02 | 28.27 | 0.01 | 0.11 |
| $CD_{0.05}$ | 105.00 | 84.75 | 0.02 | 0.34 |
| CV | 6.46 | 6.76 | 4.75 | 7.93 |

*Note* : *- Significant @ 5 %

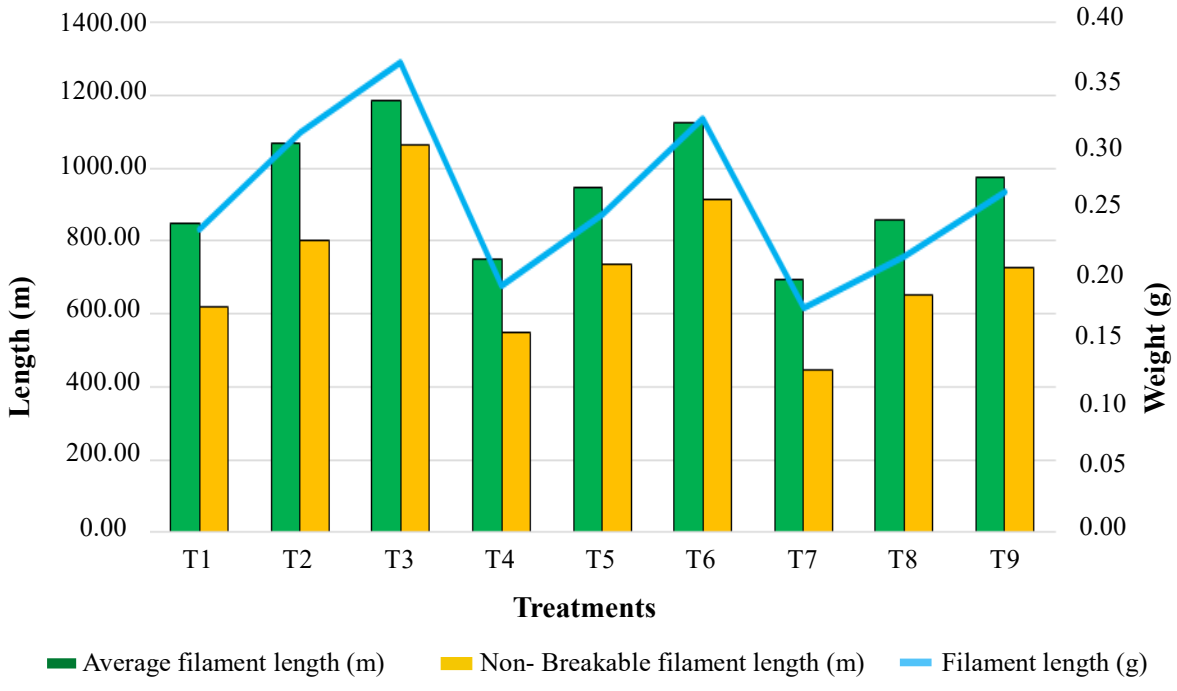


Fig. 3 : Effect of nano nitrogen sprayed mulberry leaves on average filament length, non-breakable filament length and filament weight in *Bm*NPV inoculated silkworm

(1065.91 m) followed by $T_6$ (915.28 m), whereas, significantly shortest non-breakable filament length was recorded in $T_9$ (728.91 m). Among the fourth instar *Bm*NPV inoculated batch, significantly longest non-breakable filament length was recorded in $T_1$ (619.28 m) followed by $T_4$ (546.78 m) and non-breakable filament length was significantly shortest in $T_7$ (445.64 m). Among fifth instar *Bm*NPV inoculated batch, significantly longest non-breakable filament length was recorded in $T_2$ (803.45m) followed by $T_5$ (735.86 m). Significantly the shortest non-breakable filament length was recorded in $T_8$ (445.64 m) (Table 4) (Fig. 3).

The quality of the leaves is very crucial for silkworm growth and production of silk. However, nano nitrogen sprayed mulberry leaves were found superior in nutritional content. Larvae that are fed with these leaves showed better larval growth and development and also spun good quality cocoons. In the current study, the foliar application of nano nitrogen has recorded highest average filament length and non-breakable filament (m) under both control and *Bm*NPV infected condition. Pooja *et al*. (2022) who reported that average filament length (1510.19m) recorded maximum when silkworm groups were fed with leaves harvested from mulberry plants raised with foliar application of 0.4 per cent nano nitrogen fertilizer on 25$^{th}$ DAP.

**Filament Weight (g)**

In the healthy batches of silkworms, filament weight was significantly maximum in $T_3$ (0.37g) followed by $T_6$ (0.33g) and significantly minimum filament weight was recorded in $T_9$ (0.27g). Among the fourth instar *Bm*NPV inoculated batch filament weight was significantly maximum in $T_1$(Foliar application of nano nitrogen 0.4 per cent @ 200 L/acre after 25$^{th}$ DAP + 50 per cent N soil application + Inoculated at the beginning of 4$^{th}$ instar) (0.24g) followed by $T_4$ (0.19g) and significantly minimum filament weight was recorded in $T_7$ (RDF + Inoculated at the beginning of 4$^{th}$ instar) (0.18g). Among fifth instar *Bm*NPV inoculated batch, significantly maximum filament weight was recorded in the $T_2$ (Foliar application of nano nitrogen 0.4 per cent @ 200 L/acre after 25$^{th}$ DAP + 50 per cent N soil application + 5$^{th}$ instar *Bm*NPV inoculation) (0.31g) followed by $T_5$ (0.25g) whereas filament weight was significantly minimum in $T_8$ (RDF + Inoculated at the beginning of 5$^{th}$ instar) (0.22g) (Table 4) (Fig. 3).

**Denier**

In the healthy batches of silkworms, denier was significantly maximum in $T_3$ (2.79) followed by $T_6$ (2.60) and significantly minimum denier was recorded in $T_9$ (2.48). Among the fourth instar *Bm*NPV inoculated batch, significantly maximum denier was recorded in the treatment $T_1$ (2.53) followed by $T_4$ (2.31) whereas, minimum denier was $T_7$ (2.30). Among fifth instar *Bm*NPV inoculated batch, significantly maximum denier was recorded in $T_2$ (2.65) followed by $T_5$ (2.38) whereas, minimum denier was noticed in $T_8$ (2.30) (Table 4).

Among the different treatments, foliar application of nano nitrogen at 0.4 per cent at 200 L/acre on 25$^{th}$ day after pruning has recorded maximum larval and cocoon parameters were found to be improved for both healthy and *Bm*NPV inoculation conditions. To conclude foliar application of 0.4 per cent nano nitrogen fertilizer on 25$^{th}$ DAP (@ 200 L/acre) + 50 per cent N through soil application would improve the quantity and quality of leaf in mulberry that in turn might be useful in *Bm*NPV disease management.

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