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# Improvement of Silkworm (*Bombyx mori* L.) Growth and Cocoon Quality Using Nickel Sulfate Supplementation

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Authors SH and ARR did the conceptualization. Author MV did the data curation and resources. Authors VM, SH, SD and ARR did the formal analysis. Author SD did the investigation, supervision, visualization and project administration. Authors MV and DS did the methodology. Authors MV and SH did the software and validation. Author VM did the writing original draft. Authors SD, SH and RRA writing review and editing. All authors read and approved the final manuscript.*

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## ABSTRACT

The silkworm belongs to the order Lepidoptera and the family Bombycidae (*Bombyx mori L.*). The silkworm industry plays a crucial role in providing employment opportunities in India. This study investigated the effects of nickel sulfate supplementation on the growth and cocoon quality of silkworm (*Bombyx mori* L.). The experiment was conducted at a silkworm-rearing center in Palayamkottai, India, from December 2022 to January 2023. Silkworms were fed Victory-1 (V1)

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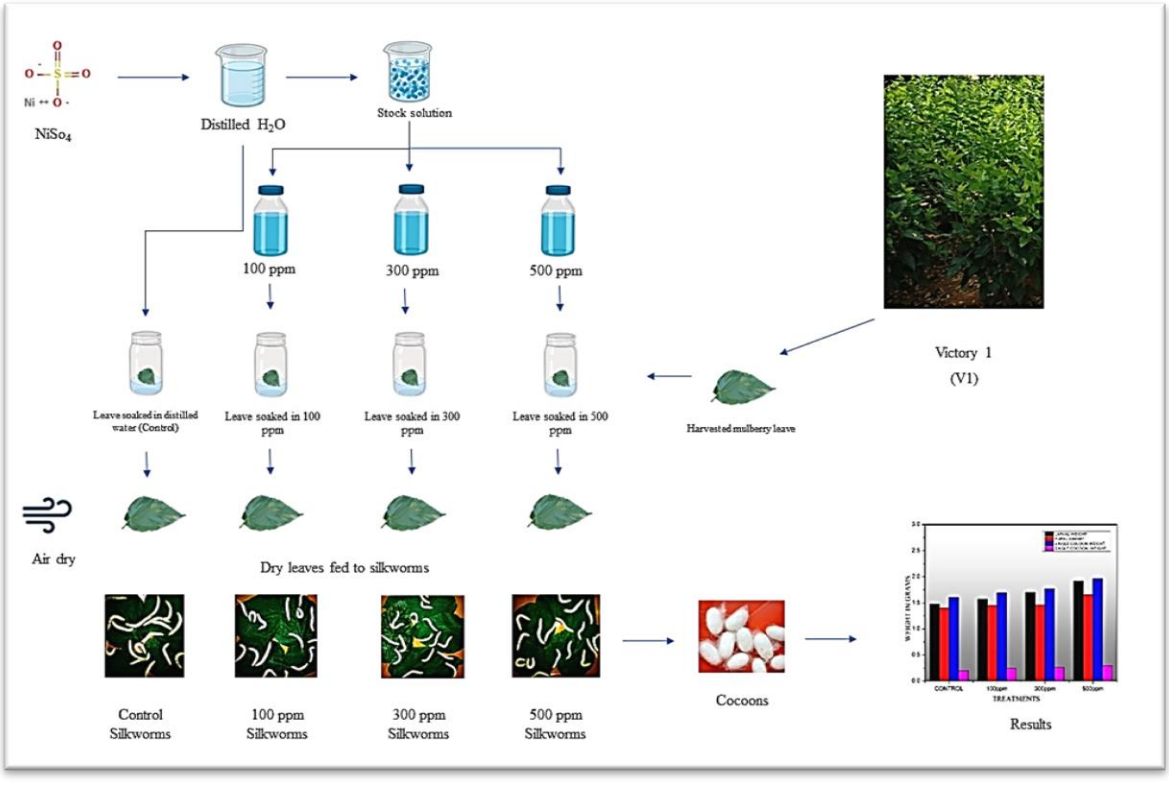
mulberry leaves soaked in different nickel sulfate solutions (100 ppm, 300 ppm, and 500 ppm). Larval weight, duration, cocoon weight, shell weight, shell ratio, and pupal weight were measured for 10 healthy silkworms with good quality cocoons selected from each treatment group. Compared to the control group, supplementation with 500-ppm nickel sulfate solution significantly improved silkworm growth performance (weight) and economic traits (cocoon weight and quality).

**GRAPHICAL ABSTRACT**




*Keywords: Bombyx mori; feed supplementation; mulberry and life cycle.*

## 1. INTRODUCTION

Silkworms (*Bombyx mori* L.) are the cornerstone of the silk industry, providing significant employment opportunities in countries like India. These insects rely solely on mulberry leaves (*Morus* L.) for their growth and cocoon production. While various mineral supplements have been explored to enhance silkworm performance, the impact of nickel sulfate supplementation, particularly at different concentrations, remains under-investigated. This study explores how supplementing the diet of silkworms fed on Victory-1 (V1) mulberry leaves with nickel sulfate can further enhance their growth performance and cocoon quality.

*Morus L*. is an economically important plant being cultivated for its leaves to feed the silkworm (*Bombyx mori L.*) [1]. The CSR2 breed larvae fed V1 mulberry leaves with four (04) feedings a day clearly recorded higher values for all the economic traits. The biochemical parameters of mulberry V1 plant leaves, such as Chlorophyll a (1.83 mg/g) and Chlorophyll b (0.79 mg/g) [2,3]. Positive seasonal impact and increased photoperiod impacts in improving physiological parameters like enzyme activity in silkworms using V1 mulberry plants were observed by Kadam *et al.,* [4]. The comparison of M5 and V1 mulberry plant leaves fed to silkworms (*B. mori*) showed that higher cocoon yield, shell weight, shell ratio, and cocoon thread length were obtained with V1 leaves Babu *et al.,* [5]. Vitamin B-complex at 0.5% was found to be the best nutritional supplement for silkworm's larval length, larval breadth, larval weight, larval duration, pupal length, pupal breadth, pupal weight, and pupal duration when using V1 mulberry plant leaves Chavan *et al.,* [6]. In this study, analyzing the growth performance of the silkworm and cocoon weight by feed supplement

using mulberry V1 leaves, nickel sulfate improved the economic characters and growth parameters of silkworms.

## 2. MATERIALS AND METHODS

The study was carried form December 2022 - January 2023 at the silkworm rearing center, Department of Zoology, St. John's college, Palayamkottai, Tamil Nadu (8°.4315 N, and 77°.4439 E). Disease-free larvae of the *Bombyx mori* (Hybrid CSR2 x CSR4) double hybrid were brought from the local farmhouse, V.M. Chatram, and acclimatized to the laboratory conditions. They were reared on bamboo trays. Later, the fourth instar larvae were separated into four groups, each containing 25 larvae. These larvae were fed with V1 variety mulberry leaves.

### 2.1 Feed Supplementation Using V1 Mulberry

The mulberry leaves were soaked in solutions of Nickel Sulphate (Nice Chemicals, India (99% of $NiSO_4$ of Purity) at different concentrations. Such as one hundred, three hundred, and five hundred ppm. The dipped mulberry leaves were dried at room temperature until the wetness was removed and were used to feed three groups of experimental larvae of the V instar worms until spinning. The sub-lethal toxicity effect was evaluated by analyzing the percentage of larval mortality. Every day, the tray (15-inch Length and 12-inch width) were cleaned to remove the fecal matter and unfed leaves and to maintain them in hygienic conditions.

### 2.2 Larval Weight (LW)

The larval weight was taken using a digital weighing balance in the fourth instar.

### 2.3 Larval Duration (LD)

In the fourth instar, the larval duration was calculated from the third moulting to the spinning stage. The total number of days was noted.

### 2.4 Pupal Weight (PW)

After removing the floss, the cocoons were cut open and the pupae were taken out without causing any damages to them. Then the pupae were weighed using an electronic balance and their weights were expressed in mg.

### 2.5 Effective Rate of Rearing (ERR)

$$\text{ERR by number} = \frac{\text{TOTAL NO.OF GOOD COCCONS HARVESTED}}{\text{TOTAL NO.OF LARVAE RETAINED AFTER MOULT}} \times 100$$

### 2.6 Cocoon Weight (CW)

The average weight in grams of 10 cocoons taken at random was measured on the 5$^{th}$day after the onset of spinning.

### 2.7 Cocoon Shell Weight (CSW)

The average weight in grams of 10 cocoon shells selected randomly was recorded from the same cocoons used for recording cocoon weight.

### 2.8 Shell Ratio (SR)

The shell ratio was computed on the basis of the cocoons selected for the cocoon weight and shell weight. The cocoon shell ratio was expressed as a percentage. Masthan *et al.,* [7].

$$S.R\% = \frac{\text{WEIGHT OF THE SHELL}}{\text{WEIGHT OF THE WHOLE COCOON}} \times 100$$

### 2.9 Statistical Analysis

The fifth (V) instar silkworm's weight parameters were analysed by One-way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) using a commercially available statistics software package (SPSS® for Windows, V. 16.0, Chicago, USA). Results were presented as Mean ± Standard Deviation [8].

## 3. RESULTS

### 3.1 Larval Weight (LW)

The larvae that consumed leaves treated with 500ppm of NiSO4 exhibited the highest average weight of 1.918 gm. This was followed by the larvae fed 300 ppm NiSO4 treated leaves, which had an average weight of 1.698 gm. The larvae that consumed 100 ppm NiSO4 treated mulberry leaves had an average weight of 1.567 gm. Lastly, the control group of larvae fed untreated mulberry leaves had the lowest average weight at 1.472 gm Fig. 1.

### 3.2 Larval Duration (LD)

Silkworm fed 500ppm,300ppm and 100ppm nickel sulphate- treated V1 mulberry leaves has larval duration were decreased in 7 days

respectively, when compared to the control (8 days).

### 3.3 Effective Rate of Rearing (ERR)

The effective rate of rearing increased as the concentration of nickel sulphate increased. However, the use of nickel sulphate at a dose of 500 ppm reduced the effective rate of rearing Fig. 2 shows the differences.

### 3.4 Cocoon Weight (CW)

Among the larvae reared on nickel sulphate-treated mulberry leaves, there were differences in the cocoon weight. The larvae fed with 500 ppm nickel sulphate-treated mulberry leaves recorded the highest cocoon weight (1.96 grams), followed by those fed with 300 ppm (1.77 grams),100 ppm (1.69 grams), and then the larvae fed with control mulberry leaves (1.60 grams) Fig. 4a- 4d.

### 3.5 Cocoon Shell Weight (CSW)

The values computed for this trait for the control and experimental groups exhibited variability ranging from 0.20 gram to 0.30 gram.

### 3.6 Shell Ratio (SR)

The larvae fed with 500 ppm nickel sulphate recorded the maximum shell ratio (15.3%), and the lowest shell ratio was recorded in the larvae reared on control mulberry leaves (12.5%). The Table. 1 shows the morphometric growth rate of V instar larvae was compared to control and NiSO4 treated mulberry leaves -fed *B. mori* Fig. 3a – 3d. The morphometric growth rate, weight parameter of *B. mori* fed with control and NiSo4 treated mulberry leaves the mean weight of V instar larvae in the 500 ppm were (1.8 ± 0.07) respectively. The mean weight of V instar larvae in the 300 ppm were (1.6 ± 0.05), respectively. The mean weight of V instar larvae in the 100 ppm were (1.5 ± 0.03) respectively. The mean weight of V instar larvae in the control were (1.3 ± 0.06) respectively. Among the four observations, the V instar 500 ppm weight was significantly increased Compared to the control.

## 4. DISCUSSION

Kochi [9] reports that the silk-gland showed a good response to a mineral mixture of nickel sulfate and potassium bromide. Wani *et al.,* [10] analyzed that zinc-stimulated mulberry leaves increased the fecundity after feeding to silkworms and gave good economic parameters and silk threads. Banu [11] reports that the larval, pupal, and adult growth in *Bombyx mori L*. were enhanced at a concentration of 800 ppm, and the economic characters (cocoons) of the mulberry were enhanced in 800 ppm of nickel chloride. Bora *et al.,* [12] reported that all concentrations had a positive impact on all parameters, but a lower concentration (2 µg/ml) of zinc chloride was more effective and played a promontory role than other dose in improved larval growth and cocoon parameters of eri silkworm. Riaz *et al.,* [13] reported that inorganic feed supplementation, such as zinc sulfide, sodium sulfide, potassium, sodium, calcium, and calcium, increased the economic parameters of the silkworm. Murugesh *et al.,* [14] analyzed that zinc sulfate, magnesium sulfate, and potassium chloride at 100 ppm, 200 ppm, respectively, during the first, third & fifth days of the fifth instar of the silkworm, *B. mori,* significantly improved the biological traits of larvae as well as economic traits of cocoons. Kavitha *et al.,* [15] report that the nutritive value of zinc chloride was higher than that of zinc sulfate, and it increased silk production.

**Table 1. The weight of V instar larvae of *B. mori* fed different concentration of $NiSO_4$ using V1 Mulberry leaves**

| Treatment | Larvae weight (gm) Mean ± SD |
|---|---|
| Control | $1.3 \pm 0.06^{a}$ |
| 100 ppm | $1.5 \pm 0.03^{b}$ |
| 300 ppm | $1.6 \pm 0.05^{c}$ |
| 500 ppm | $1.8 \pm 0.07^{d}$ |

*Value are Mean ± Standard Deviation of four observation. Values in the same column with different superscript letters (a, b, c and d) are significant at P<0.001.*

Sivanesh *et al.,* [16] analyzed that the larval duration was significantly decreased in the 5th instars when using MR2 mulberry leaves, lasting 7 days, respectively, compared to the G4-fed mulberry leaves lasting 8 days. Sarkar [17] reports suggest that it is better to feed silkworm larvae with tender shoots and medium-sized leaves during the late instar stage in wet summer. Feeding mature and over-mature leaves to silkworms results in inferior performance in terms of all characters due to their poor nutritional composition. Kadam *et al.,* [18] analyzed positive seasonal impact and

increased photoperiod have an impact in improving the physiological parameters of silkworms. The V1 mulberry variety, winter season, all these factors showed improved enzymatic activities. Ji *et al.,* [19] reports the concentration of Rhodamine B (RhB) in feeding (FM) silk is less than in dyeing (DM) silk, and the interaction between RhB and fibroin is much stronger. Kadam *et al.,* [20] analyzed positive seasonal impact and increased photoperiod have an impact in improving the physiological parameters of silkworms. The V1 mulberry variety, winter season, all these factors showed improved enzymatic activities. Ji *et al.,* [21] reported that the concentration of Rhodamine B (RhB) in feeding (FM) silk is less than in dyeing (DM) silk, and the interaction between RhB and fibroin is much stronger. Cui *et al.,* [22] analyzed the AgNPs-B group, which promoted a cocoon ratio of 20.16%, the largest value compared to the control groups, and it improved the growth rate of *B. mori*. Several studies show that various mineral supplements like zinc sulfate, nickel chloride, and nickel sulfate can improve silkworm growth, cocoon quality, and silk production.

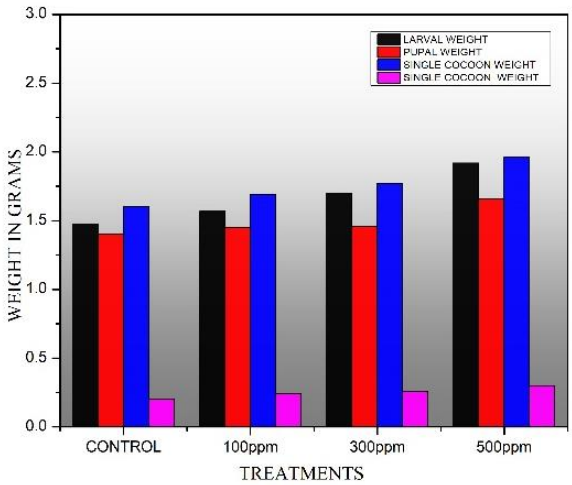



**Fig. 1. Growth performances of *B. mori* in larval, pupal and cocoons different concentration of $NiSO_4$ - with control**

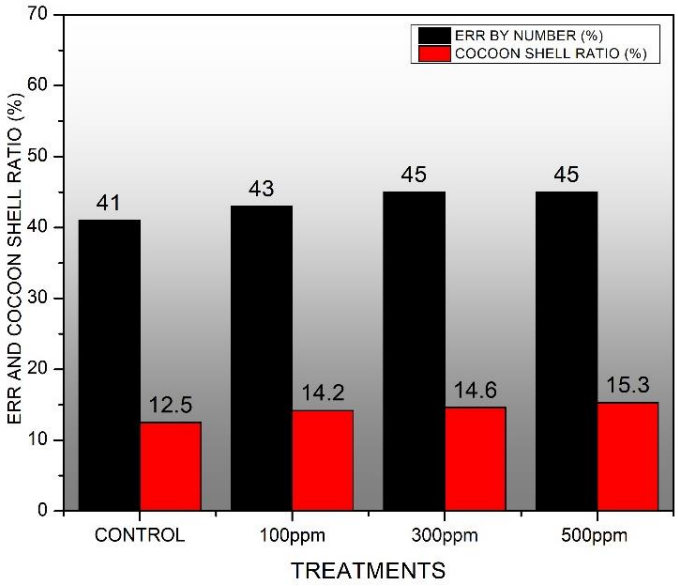



**Fig. 2. Effective rate of rearing and Cocoon shell ratio of *B. mori* in different concentration of $NiSO_4$ with control**

**Fig. 3. (a) Control group of V instar *B. mori* larvae**

**Fig. 3. (b) 100ppm group of V instar *B. mori* larvae**

**Fig. 3. (c) 300 ppm group of V instar *B. mori* larvae**

**Fig. 3. (d) 500ppm group of V instar *B. mori* larvae**

**Fig. 4. (a) Control *B. mori* Cocoons**

**Fig. 4. (b) 100ppm *B. mori* Cocoons**

**Fig. 4. (c) 300ppm *B. mori* Cocoons**

**Fig. 4. (d) 500ppm *B. mori* Cocoons**

## 5. CONCLUSION

The study demonstrated that supplementing the feed of silkworms (*Bombyx mori* L.) with 500 ppm nickel sulphate significantly improved their growth performance and economic parameters like larval weight, pupal weight, and cocoon traits compared to the control. This finding highlights the potential benefits of incorporating nickel sulfate into silkworm diets

to boost productivity in the Indian silk industry.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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