

Enhancing Silkworm (*Bombyx mori* L.) Larval Survivability Through Spermidine Supplementation

Abstract

The silkworm, *Bombyx mori* L., is a species of great economic significance since it is essential to the manufacture of silk. Improving silkworms' growth, survival rates, and silk yield throughout their larval stages requires increasing their food intake. The purpose of this study is to evaluate the effects of adding spermidine to mulberry leaves on *Bombyx mori* L. larvae survival. The experimental groups in this experiment were given mulberry leaves treated with different doses of spermidine, such as Spd 25, 50, 75, and 100 μ M. The larvae were separated into control and experimental groups. The findings demonstrated that larvae fed leaves laced with spermidine had higher survival rates than the control group. However, compared to the control group, the treated group's mortality rate was decreased. These findings indicate that spermidine could be a useful nutritional enhancement in sericulture, promoting better larval health and increased silk production.

Keywords: Silkworm, Survival, Spermidine, *Bombyx mori*, Mulberry

Introduction

The mulberry silkworm *Bombyx mori*, is a key species in the silk industry due to its ability to produce silk. Silk production heavily depends on the nutrition of larvae provided by mulberry leaves, which are crucial for the robust growth and development of the silkworm (Legay, 1958). The quality of cocoons is directly influenced by the nutrition of the silkworm, which requires specific amino acids, proteins, vitamins and sugars to thrive and enhance silk production. As a monophagous insect, *B. mori* relies entirely on mulberry leaves for its nutritional needs (Ito, 1978) (Savithri *et al.*, 2013). In addition to having favorable physical characteristics like the right amount of tenderness, thickness, and tightness, mulberry leaves that are suitable for silkworm consumption must also contain a variety of chemical constituents, including water (80%), protein (27%), carbohydrates (11%), minerals, and vitamins (Rajan & Himantharaj 2005).

Nutrition is a vital and essential element for the survival and well-being of all living organisms. Without adequate nutrition, no living being can survive or achieve healthy, disease-free growth. Like other organisms, silkworms are highly sensitive to nutritional factors. It is widely recognized that the

quantity and quality of the leaves they consume significantly impact their growth rate, developmental duration, body weight, and survival, as well as their future fecundity, movement, lifespan and competitive abilities (Parra, 1991). Silkworm nutrition encompasses the essential substances needed for growth and metabolism, which are derived from the mulberry leaves or artificial diets. Any remaining nutrients are synthesized through biochemical pathways (Takano and Arai, 1978; Hamano *et al.*, 1986; Zhang *et al.*, 2002).

The mulberry silkworm, *Bombyx mori*, is a domesticated, monophagous insect that relies exclusively on mulberry leaves for nutrition. These leaves primarily provide proteins, vitamins, carbohydrates, phagostimulants, sterols and minerals. The nutritional composition of its diet significantly influences various genetic traits, including larval and cocoon weight, silk yield, pupation, and reproductive characteristics (Ramesha *et al.*, 2010). When the required nutrients are lacking in the leaves, silkworms become susceptible to diseases, and the effective rearing rate declines, as the mortality rate is higher in silkworms fed on poor-quality food. Among different supplementation experiments with vitamins, antibiotics, amino acids, sugars, trace elements, etc., the fortification of mulberry leaves with amino acids was found to be more beneficial since it improved the leaf quality and the silk yield by 40-60 per cent (Senguptha *et al.*, 1972). Supplementing leaves with vitamin B derivatives have been shown to boost disease resistance, increase body weight, and improve silk yield in silkworms (Zah and Marghitas, 2011). Additionally, another vitamin *i.e.*, ascorbic acid has been found to enhance larval survival rates (Etebari and Fazilati, 2003).

Putrescine, spermidine (Spd), and spermine are polyamines present in all living species (Bachrach, 2010) and serve as key regulators of mammalian growth, gene expression, and ribosome-mediated translation (Childs *et al.*, 2003; Seiler *et al.*, 1996). Spd, a naturally occurring polyamine, has been shown to extend lifespan and enhance health in model organisms such as mice and non-human primates (Hofer *et al.*, 2021; Madeo *et al.*, 2019). Dietary spermidine is quickly absorbed and efficiently transported throughout the body. Polyamines have also been found to play an essential role in the development of *Bombyx mori* (Rajan *et al.*, 2022).

This study proposed that adding spermidine to the mulberry diet during the fourth and fifth instar stages could serve as a beneficial and nutritionally efficient supplement. To assess this, we analyzed the effect of spermidine on the survival rate of silkworm larvae.

Material and methods

The research was carried out at the Sericulture Research Laboratory, part of the Division of Sericulture at Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu, during the spring season of 2023 and 2024. The study utilized a bivoltine double hybrid of the silkworm *Bombyx mori* L., specifically FC1 × FC2. Eggs of this hybrid were sourced from the Sericulture Development Department of the Jammu and Kashmir Union Territory. These eggs were incubated under controlled conditions of $25 \pm 1^\circ\text{C}$ temperature and $80 \pm 5\%$ relative humidity until hatching. After hatching, the larvae were transferred to rearing trays and reared following the standard protocols described by Krishnaswami (1978).

Spermidine was collected from Sisco research laboratories Pvt. Ltd. For the stock solution of 15 mM was prepared by dissolving 0.02g of spermidine in 10 ml of distilled water. For this study four concentrations of spermidine i.e., 25, 50, 75 and 100 μM were prepared by dissolving 33, 66, 100 and 133 μl respectively in 20 ml of distilled water. Mulberry leaves dipped in different concentrations separately were fed to silkworms from first day of fourth instar till spinning stage and control group were fed with normal leaves. The data on the larval survival per cent and mortality per cent were recorded:

Larval survival percentage: Larval survival percentage depicts the number of worms survived during rearing up to the pre-spinning stage and was calculated by using the following formula:

$$\text{Larval survival (\%)} = \frac{\text{(Number of larvae survived till pre – spinning stage)}}{\text{(Total number of larvae retained after 3rd moult)}} \times 100$$

Mortality percentage: Larval mortality percentage depicts the number of larvae died during rearing up to the pre-spinning stage and was calculated by using the following formula:

$$\text{Larval mortality (\%)} = \frac{\text{(Number of dead larvae till pre – spinning stage)}}{\text{(Total number of larvae retained after 3rd moult)}} \times 100$$

Results and Discussion

Silkworm larvae pertaining to the spermidine treatment recorded maximum ($F= 76.35$; $df= 14$; $P= 0.00$) larval survival per cent for Spd 100 (96.39 ± 0.28) followed by Spd 75 (94.44 ± 0.56), SPd 50 (93.89 ± 0.74) and Spd 25 (91.94 ± 0.56). Whereas, significantly lower larval survivability was recorded in control group 83.89 ± 0.56 per cent (Table 1 and Fig. 1). The results on larval survival per cent were found to be in line with the study conducted on application of arginine, histidine and their

mixture on the economic characteristics of silkworm. It was observed in that study that the arginine, histidine as well as their mixture increased the survival per cent. Which indicated that the oral supplementation with arginine, histidine and their mixtures with used concentrations have stimulatory effect on survival rate of the silkworm, *B. mori* and it was (66 - 67 % approx.) (Chakrabarty and Kaliwal, 2012).

The observations for impact of different concentrations of spermidine supplementation from first day of 4th instar till spinning showed significant decrease in larval mortality. Significantly lowest (F= 82.12; df= 14; P= 0.00) larval mortality was recorded in Spd 100 (3.37 ± 0.22) followed by Spd 75 (5.20 ± 0.50), Spd 50 (5.67 ± 0.66) and Spd 25 (7.46 ± 0.50). However, significantly higher (14.92 ± 0.50) mortality was recorded in control batch (Table 1 and Fig. 1). Similar findings were reported by Hamzah *et al.* (2016), who noted that feeding larvae with leaves enriched with pollen, royal jelly, amino acids, and honey lowered mortality rates. Additionally, results of larval mortality correlate with the study conducted on Impact of glycine fortification of cassava leaves on the late instar larvae of eri silkworm (*Samia cynthia ricini* D.), in which they have observed no mortality (Prihatin *et al.*, 2023). Also, the study conducted by Nicodemo *et al.* (2014) to study the effect of threonine and valine on the silk performance, observed that the larval mortality was reduced in the threonine 2 % concentration (6.86) and control (7.71).

Table 1: Impact of mulberry leaves supplemented with different concentrations of spermidine on larval survival (%) silkworm larvae

Supplementation of mulberry leaves with Spermidine	Larval Survival (%)	Mortality (%)
Spd 25	91.94 ± 0.56^b	7.46 ± 0.50^c
Spd 50	93.89 ± 0.74^{bc}	5.67 ± 0.66^{bc}
Spd 75	94.44 ± 0.56^{cd}	5.20 ± 0.50^{ab}
Spd 100	96.39 ± 0.28^d	3.37 ± 0.22^a
Control	83.89 ± 0.56^a	14.92 ± 0.50^d

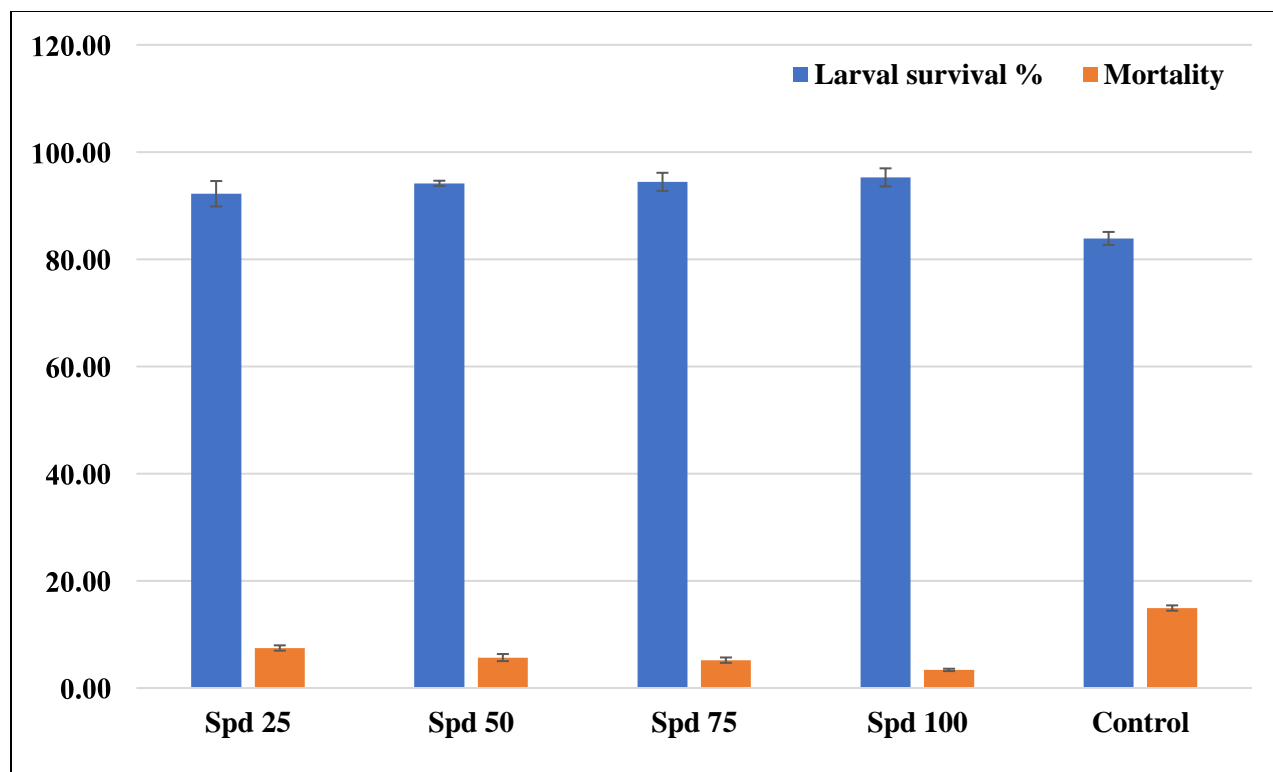


Figure 1: Impact of mulberry leaves supplemented with different concentrations of spermidine on larval survival (%) silkworm larvae

Conclusion

This study conducted on the supplementation of spermidine on mulberry leaves and their effect on the larval survival per cent of the silkworm, it was observed that spermidine effects the larval survival per cent and mortality and also overall growth of the silkworm is affected. It can be concluded from this work that spermidine can be included in the silkworm diet as it influences the survivability and lowers the mortality which can influence the cocoon productivity of the silkworm *B. mori*. Future research could focus on determining the ideal dosage and evaluating the prolonged effects of spermidine supplementation on the growth and productivity of silkworms. Moreover, assessing its influence on cocoon quality and cost-effectiveness under varying environmental conditions could offer valuable insights.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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