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

Abstract

The silkworm, *Bombyx mori* L., plays a vital role in silk production, making it a species of significant economic importance. Enhancing the nutritional intake of silkworms during their larval stages is key to improving their survival rates, growth, and silk yield. This study aims to assess how supplementing mulberry leaves with spermidine influences the survival of *Bombyx mori* L. larvae. In this experiment, the larvae were divided into control and experimental groups, with the experimental groups receiving mulberry leaves treated with varying concentrations of spermidine i.e., Spd 25, 50, 75 and 100 µM. The results showed that larvae fed spermidine-supplemented leaves exhibited greater survival rates compared to the control group. However, there was recorded decline in mortality per cent in the treated group as compared to the control. 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). Mulberry leaves, as suitable food for silkworms, must contain key chemical components such as water (80%), proteins (27%), carbohydrates (11%), along with essential minerals and vitamins (Koul, 1989).

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, ascorbic acid, another vitamin, 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 2023-24. 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 ± 1°C temperature and 80 ± 5% relative humidity until hatching. After hatching, the larvae were transferred to rearing trays and reared following the standard protocols described by Krishnaswami (1978).

This study was conducted on four concentrations of spermidine i.e., 25, 50, 75 and 100 µM and the mulberry leaves were dipped in solutions for 1 minute and left to dry for 15 minutes. Silkworms were fed on mulberry leaves supplemented with spermidine 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:

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

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:

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

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* (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) motality was recorded in control batch (Table 1 and Fig. 1). Additionally, results of larval mortality corelate 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 leav 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}

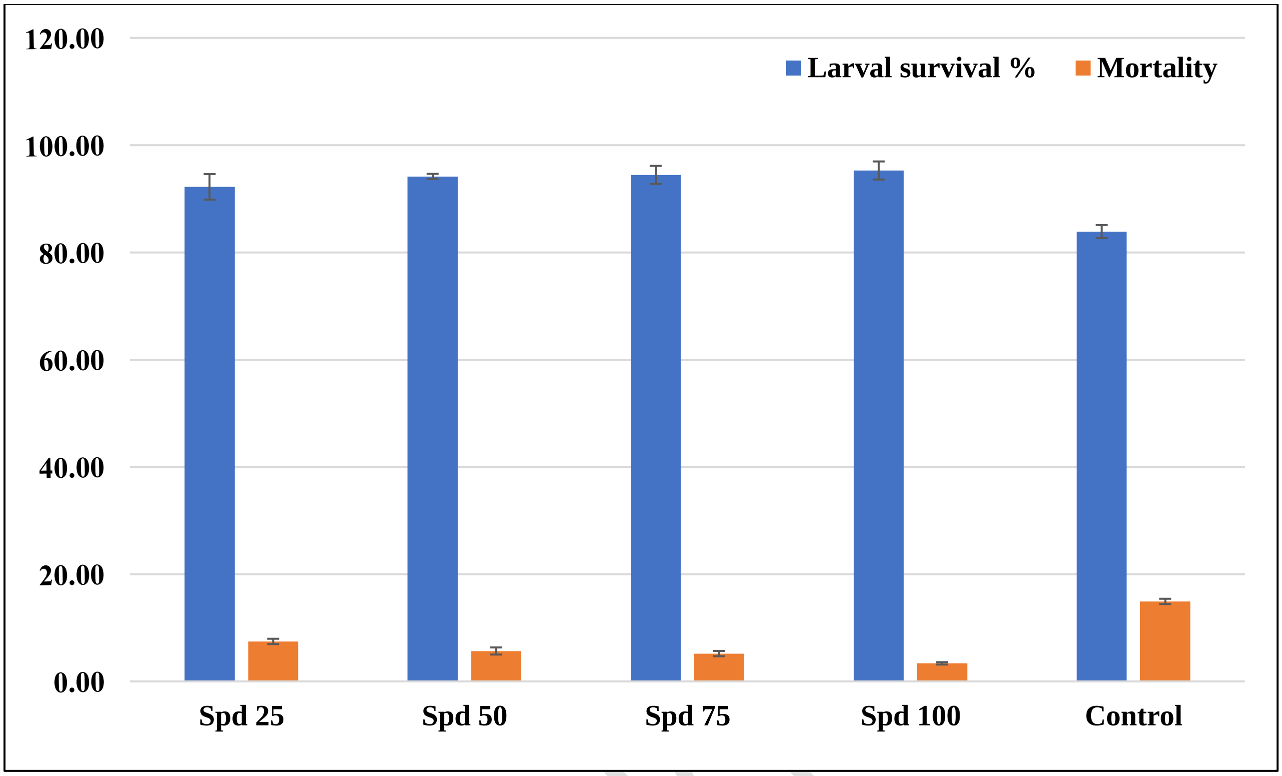

Figure 1: Impact of mulberry leaves supplemented with different concentrations of spermidine on larval survival (%) silkworm larvae

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*.

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