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Response of silkworm (*Bombyx mori* L.) hybrid (CSR₂ x CSR₄) to topical application of juvenile hormone analogue (Methoprene)

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Abstract

Juvenile hormone analogues (JHA) are recognized for their ability to extend the larval stage in insects. These compounds have been observed to enhance both the qualitative and quantitative characteristics of the silkworm *Bombyx mori* L. In our current investigation, we applied the juvenile hormone analogue known as methoprene topically to a popular bivoltine hybrid of silkworms (CSR₂ x CSR₄) at different concentrations (0.01 µl, 0.1 µl, 1 µl per larva) at various time points (24, 48, 72, and 96 hours after the start of the fifth instar stage). This treatment combination resulted in a significant increase in the weight of individual cocoons, the weight of the cocoon shells and shell percentage. The most substantial improvement was observed in the silkworm groups treated with 0.1 µl of methoprene per larva 48 hours into the fifth instar. The incorporation of juvenile hormone analogue (Methoprene) into Sericultural practices holds promise for achieving higher quality and quantity of silk cocoons.

Keywords: Juvenile hormone, silkworm, methoprene, larva, cocoons

Introduction

Sericulture stands as one of India's agro-based industries, offering lucrative income within relatively short timeframes. The silkworm, *Bombyx mori* L., plays a vital role in this industry, being a key lepidopteran insect used for the commercial production of natural silk fibers. Sericulture contributes significantly to job creation, economic growth, and the enhancement of living standards in rural areas. As a result, it plays a crucial role in anti-poverty initiatives and helps prevent the migration of rural residents to urban centers in search of employment opportunities. This industry is highly labor-intensive and holds a central position in terms of providing employment and additional income to marginalized sections of society (Geetha and Indira, 2011) [7].

In sericulture, juvenile hormone analogues have been employed in various countries to enhance cocoon production. Insects primarily rely on two major circulating hormones, ecdysone and juvenile hormone, which regulate the majority of their growth and developmental activities. Juvenile hormone analogues, known for their ability to prolong the larval stage in insects, have been extensively used to improve silk yield in the *Bombyx mori* L. silkworm. Akai *et al.* (1971) [1] documented that the treatment of synthetic juvenile hormone analogues resulted in both increased silk protein accumulation and the extension of larval growth in *Bombyx mori*. Hormones such as thyroid (Thyagaraja *et al.*, 1991) [11] and methoprene, a juvenile hormone analogue (Akai *et al.*, 1985; Miranda *et al.*, 2002) [2, 9], have long been employed to enhance silk production in the silkworm *Bombyx mori* L. Over time, numerous juvenile hormone analogues have been introduced to the market. Researchers have extensively investigated their mechanisms of action and their effectiveness in promoting the quality and quantity of cocoons produced by *Bombyx mori* larvae treated with these analogues (Asano *et al.*, 1986; Kamimura & Kiuchi, 1998; Bharathi and Yungen, 2000; Nair *et al.*, 2000; Yungen & Bharathi, 2001) [3, 8, 5, 12, 14].

Taking into account the significant role of juvenile hormone analogue compounds in silk production, this study was initiated to explore the impact of a specific juvenile hormone analogue compound (Methoprene) on the economic characteristics of a bivoltine hybrid silkworm (CSR₂ x CSR₄).

Materials and Methods

Disease free laying of popular silkworm bivoltine hybrid (CSR₂ x CSR₄) were taken as a target material in the present study. Larvae were reared as per recommended conditions at a temperature and humidity of (27±1 to 23±1 & 85 ±5 to 65±5). The larvae were treated with Methoprene at 24, 48, 72 & 96 hours after 5th instar. Experimental control batches received same doses of alcohol and normal control batches were reared without any treatment. In the experimental and control batches, the quantitative parameters viz., cocoon weight, shell weight and shell percentage were measured.

Results and Discussion

The study found that cocoons harvested from silkworm larvae treated with the juvenile hormone analogue Methoprene displayed increased cocoon weight, shell weight, and shell percentage compared to both normal/untreated and alcohol-treated control batches of cocoons. The data on cocoon characteristics suggest that Methoprene had a positive impact on silk production. While many of the treatments seemed to have a significant effect on the silkworm hybrid CSR₂ x CSR₄, the highest cocoon weight of 2.35 grams was achieved with Methoprene applied at a dosage of 0.1 µl/10 µl per larvae after 48 hours during the spring season. Cocoon shell weight also saw a substantial increase following the application of Methoprene at the same dosage and timing. The pattern of improvement in shell weight paralleled that of cocoon weight with the highest cocoon shell weight of 0.56 grams in CSR₂ x CSR₄ recorded with Methoprene at 0.1µl/10µl per larvae after 48 hours during the spring season (Table-1). Shell percentage also demonstrated a significant increase over the control, reaching a maximum of 23.82 percent with Methoprene applied at 0.1 µl/10 µl per larvae after 48 hours during the fifth instar stage in the spring season.

Table 1: Effect of juvenile hormone analogue (Methoprene) on economic characters of mulberry silkworm, *Bombyx mori* L.

Time of administration (After hrs.)	Concentration (µl/10 µl/larvae)	Cocoon weight	Shell weight	Shell percentage
24	0.01	2.03	0.46	22.66
	0.1	2.07	0.48	23.18
	1.0	2.01	0.41	20.39
48	0.01	2.29	0.54	23.58
	0.1	2.35	0.56	23.82
	1.0	2.10	0.48	22.85
72	0.01	2.23	0.52	23.31
	0.1	2.30	0.54	23.47
	1.0	2.04	0.43	21.07
96	0.01	2.08	0.43	20.67
	0.1	2.09	0.44	21.05
	1.0	1.97	0.39	19.79
Control-I (Normal/Untreated)		1.98	0.39	19.69
Control-II (Alcohol)		2.00	0.41	20.53
C.D ($p \leq 0.05$)		0.04	0.002	0.21

These findings align with previous studies by Murakoshi *et al.* (1972) [10] and Chang *et al.* (1972) [6] which reported improvements in economic traits following the application of juvenile hormone analogues. Begum *et al.* (2011) [4] also revealed that the application of Methoprene had a notable impact on lengthening the fifth instar larval stage by an additional 24 hours in the PM x CSR2 hybrid of silkworms.

This prolongation of the larval phase was found to lead to an increase in the weight of the cocoons, the weight of the cocoon shells, and shell percentage. These findings further support the positive influence of Methoprene on cocoon characteristics and silk production in the studied silkworm hybrids. Additionally, Nair *et al.* (2000) [12] noted that silkworms were more responsive to juvenile hormone analogue application at the 48-hour mark during the fifth instar stage in terms of enhancing cocoon characteristics. These findings support the present study's conclusions.

In conclusion, the application of Methoprene resulted in significant alterations in the larval period, cocoon weight, and shell weight of the silkworm *Bombyx mori* L. This suggests that Methoprene can be effectively used to enhance silk productivity when applied at the appropriate dosage and timing.

Conclusion

During the course of the present study, juvenile hormone analogue application was studied to know their impact on the economic characters of silkworm, *Bombyx mori* L. Methoprene was found to exert a positive impact on cocoon weight, shell weight and shell percentage of silkworm *Bombyx mori* L. Among the four tested timings, juvenile hormone analogue application after 48 hours of commencement of 5th instar showed significantly better results while as juvenile hormone analogue application @ C3 (0.1µl/larva) was found to be the most effective concentration. It is concluded that application of methoprene brought out significant changes in larval period, cocoon weight and shell weight of silkworm *Bombyx mori* L. and can, therefore, be used for bringing about improvement in silk productivity when applied at proper dose and time.

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Conflicts of interest

There are no conflicts of interest to report and nothing to reveal on the part of the authors.

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