**A review on effect of juvenile hormone analogues on growth and development of silkworm (*Bombyx mori* L.)**

**ABSTRACT**

The silkworm, *Bombyx mori* L., is a holometabolous insect of immense economic importance in sericulture. Its growth and development are closely regulated by hormonal control, particularly by the Juvenile Hormone (JH), which plays a key role in maintaining larval status and regulating metamorphosis. Juvenile Hormone Analogues (JHAs), which mimic the action of natural JH, have been widely studied for their influence on insect physiology and development. This review explores the impact of various JHAs on the growth and development of *B. mori*, focusing on their effects during the larval, pupal and adult stages. Application of JHAs has been found to alter the duration of larval instars, delay or induce premature pupation and affect cocoon parameters such as shell weight and filament length. While some analogues show promise in improving silk yield or synchronizing larval development, others may induce abnormalities or disrupt normal metamorphosis.

Keywords: Silkworm; Juvenile hormone analogues; Metamorphosis; Methoprene; Growth regulation

1. **INTRODUCTION**

The silkworm, *Bombyx mori* L., is a monophagous insect domesticated for silk production and serves as the backbone of the sericulture industry (Rahmathulla, 2012). Its economic importance lies in its ability to spin silk cocoons, which form the raw material for natural silk (Yazawa et al., 2023; Barcelos et al., 2021). The success of sericulture largely depends on the health, growth and development of silkworms, which in turn governed by both external environmental factors and internal physiological regulators. Among the internal factors, the insect endocrine system plays a pivotal role in controlling the progression through developmental stages such as larval moulting, pupation and adult emergence (Riddiford, 2012).

 Endocrine system acts as an important link between the environment and various physiological (Niimi and Sakurai, 1997) and developmental events in insects. Endocrinology is the study of hormones, their functions and the disorders related to them. A hormone is a chemical substance secreted by specialized cells of the body and it is used by insects to regulate physiological, developmental and behavioural activities. Hormones are produced in very small quantities and hormonal effects may be stimulatory or inhibitory (Chapman, 2013).

Among these hormones, juvenile hormone (JH), secreted by the corpora allata, is one of the key hormones that regulate insect growth and metamorphosis. It functions by maintaining larval characteristics and preventing premature transformation into pupal or adult forms. In *B. mori*, the appropriate balance of JH and ecdysteroids is essential for normal development (Riddiford, 2012). Manipulating this balance can alter the insect’s developmental trajectory, a concept that has been explored through the use of synthetic compounds known as Juvenile Hormone Analogues (JHAs) (Doshi et al., 2016). However, the timing, dosage, and method of JHA application are critical, as inappropriate use can lead to adverse effects such as prolonged larval duration, malformed pupae, or reduced cocoon quality and silk yield (Hua-Jun et al., 2011).

This review aims to consolidate current knowledge on the impact of JHAs on the growth and development of *Bombyx mori* by focusing on morphological, physiological and economic parameters. It further highlights the type of analogues used, methods of application, stage-specific responses and their implications in silkworm rearing practices.

1. **TYPES OF HORMONES**

In insects, several hormones regulate development and physiology:

1. **Neurosecretory peptide hormones**
* Prothoracicotropic hormone **(PPTH)** from the prothoracic gland
* Eclosion hormone **(EH)** from the brain
1. **Moulting hormone (MH)** from the prothoracic gland
2. **Juvenile hormone (JH)** from the corpora allata
3. **Diapause hormone (DH)** from the suboesophageal gland

These hormones regulate moulting, metamorphosis, reproduction, diapause and other physiological functions.

 (Steel and Davey, 2013).

1. **JUVENILE HORMONE IN INSECT PHYSIOLOGY**

Juvenile hormones (JHs) are acyclic sesquiterpenoids secreted by the corpora allatum located behind the insect brain of most insects involved in several aspects such as development, reproduction, metamorphosis (Riddiford, 2012; Jindra et al., 2013), caste determination in the social insects (Wheeler and Nijhout, 1981), diapause regulation (Yin and Chippendale,1973) and ovarian development (Bloch et al*.,* 2000). JHs maintain larval characteristics by preventing premature metamorphosis and are essential for female reproductive processes.

**3.1 Structures of juvenile hormone**

Corpora allata produces JH at different fractions in lepidoptera, those are JH 0, JH I, JH II, JH III and 4 methyl JHI (Roller et al*.,* 1967; Juddy et al*.,*1973; Hua-Jun et al*.,* 2011). The main difference between these JH lies in the number and type of alkyl group (ethyl and methyl) attached to their carbon atom. JH 0 contains three ethyl and one ketone group, JH I contain two ethyl, one methyl and one ketone group, JH II contains one ethyl, one methyl and one ketone group and JH III contains three methyl and one ketone group (Riddiford, 2020). These structural variations can influence their biological activity and role of insect physiology.





**Fig. 1: Structures of juvenile hormone** (Jindra et al*.,* 2013)

**3.2 Location and connection**

In insects, including lepidoptera, the corpora allata are typically found in pairs, with one gland on each side of the oesophagus. Each carpora allatum is connected to the corpus cardiacum, another endocrine structure, usually on the same side. Nerve fibres and neurosecretory cells (NSCs) facilitate communication between the corpora allata and the corpus cardiacum. The size of corpora allata tends to increase as the insect progresses in age and undergoes developmental stages. For example, in the silkworm, the corpora allata grow larger as the larva matures into a pupa and eventually into an adult moth. (Gilbert and King, 1973)

**3.3 Types of cells**

 Corpora allata consist of three main types of cells:

1. Undifferentiated cells: These cells have the potential to differentiate into other cell types.
2. Normal secretory cells: These cells are specialized for producing and secreting juvenile hormone.
3. Polyploid giant cells: These larger cells may contain multiple sets of chromosomes (polyploidy) and likely have specific functions in hormone production or regulation. Corpora allata typically contain 20 to 30 gland cells responsible for synthesizing and secreting juvenile hormone. These gland cells are essential for maintaining hormonal balance and coordinating various physiological processes in insects. (Cassier, 1979)

**3.4 Functions of Juvenile hormone (Noriega, 2014)**

* The JH inhibits metamorphosis
* It influences growth and development of male and female accessory glands
* It accelerates the lipid biosynthesis in ovaries and hence termed as yolk forming hormone, but it influences the utilization of lipids in the fat body for general metabolism
* It reduces the citric acid synthesis and increases RNA synthesis
* It regulates the production of sex pheromones which controls the sexual maturation and mating behaviour

1. **JUVENILE HORMONE ANALOGUES (JHAs)**

JHAs are synthetic or natural compounds that mimic the activity of natural JH. They interact with JH receptors and pathways, influencing growth, development and reproduction. Their application in sericulture aims to enhance enhance the silk production by promoting continuous larval growth, delaying metamorphosis and improving the overall quality and quantity of silk produce (Akai,1979).



 **Fig. 2: Structures of juvenile hormone analogues (Dhadialla et al., 1998)**

**4.3 Modes of Juvenile Hormone Analogue Administration in *Bombyx mori* L.**

**1. Topical Application**

Topical administration involves the direct application of JHA solution onto the cuticle of the larva, typically on the dorsal thoracic or abdominal region. The compound is usually dissolved in volatile organic solvents such as acetone, ethanol or dimethyl sulfoxide (DMSO). This method is widely used due to its simplicity, rapid effect and ease of dosage control. It effectively delays metamorphosis and can induce supernumerary instars, depending on the concentration and timing of application.

**2. Injection**

This method entails the direct injection of JHAs into the hemocoel using a fine syringe or microinjection apparatus. Though more technically demanding, it allows for precise control of dosage and ensures systemic distribution of the analogue through the haemolymph. Injection is particularly useful in studies requiring accurate pharmacological or physiological assessments, although it carries the risk of physical injury or infection to the larvae.

**3. Oral Administration (Feeding Method)**

Oral administration involves feeding larvae with mulberry leaves treated with JHA solutions or incorporating JHAs into artificial diets. This non-invasive method is suitable for large-scale experiments or commercial applications. However, the effectiveness of this route may vary due to differences in absorption, compound stability and metabolic degradation in the digestive tract.

**4. Environmental Exposure**

Though rarely employed, environmental exposure techniques involve placing larvae in enclosures containing JHA vapours or surfaces treated with the compounds. These methods are mainly experimental, used to investigate the volatility and persistence of JHA formulations. They are not commonly used in practical sericulture due to difficulties in dose regulation. (Jindra et al., 2013)

**4.4 Applications of JH analogues**

* Regulation of central nervous system which finally controls the ecdysteroids.
* Direct action on epidermis to retain their larval characters.
* When commercial traits such as cocoon weight, cocoon shell weight and silk filament length were enhanced through administration of exogenous JHAs in minute quantities (Mamatha et al., 2008). 21% increment of silk production by the use of the SJ-42-F juvenile hormone (Chowdhary and Mathu, 1986).
* Hormone like methoprene JHA have long been utilized for the improvement of silk production in the silkworm *Bombyx mori* L. (Miranda et al., 2002).
* Application of methoprene from II to V instar resulted in significant increases in larval weight, cocoon weight, ovariole length, ovariole egg number and fecundity when compared to untreated larvae (Magadum et al., 1990).
1. **Impact of juvenile hormone analogues on larval, cocoon, pupal traits, silk synthesis and reproductive performance of silkworm, *B. mori***
	1. **Effect of JHAs on larval, cocoon and pupal characters of silkworm**

The juvenile hormone analogue methoprene was tested for its effects on larval development and silk production in the silkworm strain C115×N108. Methoprene was topically applied 48 h after the fourth ecdysis at doses ranging from 0 to 20 ng. At a dose of 1 ng, methoprene extended the fifth instar larval duration, increased weight of larvae and significantly improved silkgland develpoment, cocoon weight, shell weight and pupal weight. Silk yield increased by 24% compared to 12% in the control group (Miranda et al*.,* 2002).

A study was conducted by Sisodia and Gaherwal (2017) to evaluate the effect of application of fenoxycarb on larval development and silk production. Topical administration of fenoxycarb at the concentration of 1 and 1.5 µg, 48 hours after the fourth larval ecdysis, showed positive influence on the fifth instar larval duration and weight of the silkworm. But, the heaviest silk gland, maximum cocoon and shell weight and increment in silk production were recorded in 1 µg fenoxycarb treated group.

The impact of Methoprene on trehalose, glycogen and economic characters of silkworm were studied in CSR2×CSR4 and PM×CSR2 hybrids. Methoprene significantly increased the trehalose and glycogen levels after 72 hours of treatments in both hybrids. In the fat body, a 25 % and 26 % increase in trehalose content was observed after 96 hours of treatment in CSR2×CSR4 and PM×CSR2, respectively. Additionally, Methoprene extended fifth instar larval period by 24 hours in PM×CSR2 and 30 hours in CSR2×CSR4 and also notable increase in cocoon weight, shell weight and cocoon shell ratio were observed compare to control (Moorthy *et al*., 2002).

Brindha *et al.* (2012) investigated the effect of 24 juvenile hormone (JH) mimic compounds on *B. mori* to enhance silk production. The compounds were topically applied in six concentrations at 24, 48, 72 and 96 hours of the fifth instar, among them five compounds *viz.*, NL-13, NL-24, BPE, BK and R394 significantly improved cocoon traits, with shell weight increased by 20 %. Specifically, R394 at a dose of 0.031 µl applied on day one of the fifth instar enhanced shell weight by 8% without prolonging larval duration.

Nair *et al.* (2012) studies the effect of JHA, SB-515 (Isopropyl (2E,4E)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate) on cocoon yield in the bivoltine silkworm hybrid CSR2 × CSR4 and its reciprocal. The JHA was administered at 24, 48 and 72 h of the fifth instar at concentrations of 1.25, 2.5 and 5 ppm. SB-515 extended the larval feeding period by 6 to 48 h depending on dose and timing and significantly improved cocoon traits such as cocoon weight, shell weight and shell percentage. The maximum yield increase was observed at 2.5 ppm administered at 48 h in CSR2 × CSR4 and at 72 h in CSR4 × CSR2.

Ishita *et al*. (2022) investigated the influence of certain juvenile hormone mimics on economic parameters of silkworm. Their study revealed that the expression of economic traits like cocoon weight, shell weight, shell ratio, filament length and denier were found to be increased with increase in number of larval treatments which is double administrated at the rate of 20 % and 30 % concentration for pinus and tapioca treated larvae. In case of custard apple treated larvae, double treatment at 20 % concentration showed best results. Single treatment at 5 µl and 10 µl concentration of pyriproxyfen gave similar outcome with respect to all economic parameters.

Saad *et al*. (2019) investigated the effects of the growth regulator pyriproxyfen (1, 10, and 100 μg/larva) and 1% glycine alone or in combination on *B. mori* during spring 2018. Feeding fifth instar larvae on mulberry leaves dipped in 1% glycine significantly increased larval weight (5.12 g) and reduced larval duration (9.00 days). The highest pupal weight (1.277 g) and improved economic traits including cocoon weight, shell weight, shell ratio and filament weight was observed when larvae were topically treated with 10 μg/larva pyriproxyfen combined with 1% glycine on the fifth day.

* 1. **Effect of JHAs on reproductive traits of silkworm**

Qian *et al*. (2020) assessed the impact of pyriproxyfen on the reproductive performance and ovarian development of *B. mori*. The result of their study revealed that, in control group, silk moths laid an average of 459 eggs with a hatching rate of 98.47 %. At a concentration of 0.001 μg/L, the average number of eggs decreased to 408 eggs/ moth and the hatching rate declined to 92.44%. Treatment with 1 μg/L pyriproxyfen further reduced egg production to 370 eggs/moth and the hatching rate to 89.43%. At the highest concentration (100 μg/L) egg production dropped sharply to an average of 142 eggs /moth with a hatching rate of just 5.20%.

* 1. **Effect of JHAs on biochemical traits of silkworm**

Mamatha *et al.* (2008) studied the impact of juvenile hormone analogue methoprene on selected key enzymatic activities of silkworm, *B. mori*. Topical application of methoprene at a dose of 1.0 µg/larva, 48 hours after the onset of the fifth instar, resulted in enhanced activity of protease, aspartate aminotransferase, alanine aminotransferase, adenosine triphosphate synthase and cytochrome c oxidase in the larval muscle and silk glands. These findings indicate an upsurge in the overall oxidative metabolism of *B. mori* larval tissues.

Santhy (2015) investigated the effects of methoprene on the feeding and nutritional physiology of fifth instar larvae of *B. mori*. The study evaluated larvae treated with different concentrations (0.1–1.0 µg) and measured parameters such as consumption, assimilation, production and egestion. The results showed that higher doses of methoprene significantly increases consumption, egestion, production and the efficiency of conversion of digested food (ECD). The most effective doses were 0.75 µg and 1.0 µg. However, assimilation rates and efficiencies declined with higher doses, likely due to hormonal effects on digestion.

Rahman *et al.,* (2024) evaluated the impact of serimore, a plant based growth promoter on haemolymph protein content, protease activity and economic traits in silkworm. Serimore at the rate of 0.2 % was topically administered as a single dose over the fifth instar larvae at 48 hours and 72 hours after ecdysis. The result showed that protein content in haemolymph was significantly enhanced at 48 hours compared to 72 hours of treatment and control. At 48 hours of treatment, there was a significant improvement in cocoon weight (2,64 g), shell weight (0.57 g), filament length (1268.06 m) and filament weight (0.53 g) were found over to that of 72 hours of treatment and control.

**Conclusion**

The application of juvenile hormone analogues significantly influences the growth and development of silkworm, *Bombyx mori*. By mimicking the action of natural juvenile hormone, these analogues prolong the larval stage, increase body size and enhance the accumulation of silk proteins, resulting in maximized silk production. The effects of juvenile hormone analogues are dose-dependent and improper usage can disrupt normal development and metamorphosis, leading to suboptimal outcomes. Hence, in sericulture the strategic use of juvenile hormone analogues holds promise for improving silk yield and quality, but requires careful management to avoid adverse effects on the silkworm life cycle. Among all the JHAs Methoprene appears to be the most promising option for commercialization in sericulture. Further, extensive research is essential to optimize the application protocols of juvenile hormone analogues and understand their long-term impact on silkworm health and productivity on a large scale.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE):**

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

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