Review Article

Recent Developments in Endocrinology of Silkworm, *Bombyx Mori* L.

Abstract

Mulberry silkworm has a distinctive role in many of the discoveries which explained the role of the endocrine organs in insectgrowth and development. Hormones exert specific actions on the growth and development of silkworms, making them advantageous tools for manipulation in sericulture. By precisely controlling hormone levels or applying hormone analogs, sericulturists can conveniently regulate various aspects of silkworm development, such as larval growth, cocoon formation, and pupation. There have been several attempts to utilize hormones, including phytoecdysteroids, JHAs and AJHs, in commercial sericulture to enhance silk production. Phytoecdysteroids, natural compounds found in plants, have been studied for their potential to promote growth anddevelopment in silkworms. Similarly, JHAs and AJHs have been explored for their ability to manipulate hormone signaling pathways and influence developmental processes in silkworms.

Keywords: Endocrinology, Silkworm, Phytoecdysteroid, Hormone.

1. INTRODUCTION

Sericulture is an agro-based industry and have a vital role in the improvement of rural economy of India. In India, over three million people are employed in various field of sericulture. Silkworm rearing is mainly carried out for the production of silk. From past few decades, apart from silk production silkworms were also used as an important laboratory tool in the various fields of science. Scientists in the field of sericulture are busy in exploring new avenues for increasing the silk yield. Among many such approaches, one of the avenue is the possibility of increasing silk production by utilizing the benefit of insect endocrine system. Endocrine systemis an important link between the environment and various physiological and developmental events not only in insects but in all organisms.

Endocrinology is the study of hormones, their functions, and the disorders related to them. Hormones are chemical messengers secreted by various glands in the body, known as endocrine glands, and they regulate many physiological processes, including growth and development, metabolism, reproduction, and mood. A hormone is a chemical substance secreted by specializedcells of the body and it is used by insects to regulate physiological, developmental and behavioral activities. Hormones are produced in very small quantities and hormonal effects maybe stimulatory or inhibitory (Chapman, 2013).

2. ENDOCRINE GLANDS

Endocrine glands, consists of neurosecretory cells of the brain, suboesophageal ganglion (SG), corpora cardiaca (CC), corpora allata (CA) and prothoracic gland. Various types of neurosecretory cells are present in the brain and produce neurohormones. The main function of the endocrine system is metabolism, growth and development, sexual function and reproduction, appetite, heart rate and regulation of body temperature (Gullan and Cranston, 2014).

It is well known that prothoracicotropic hormone (PTTH), moulting hormone (MH), juvenile hormone (JH) and diapause hormone(DH) are synthesized in the brain, prothoracicgland, corpora allata and sub-oesophageal gland, respectively. When insects reach the appropriate size i.e., critical weight, PTTH is released from the brain and stimulates the secretion of ecdysone from prothoracic gland,which triggers moulting (Ishizaki and Suzuki,1994).

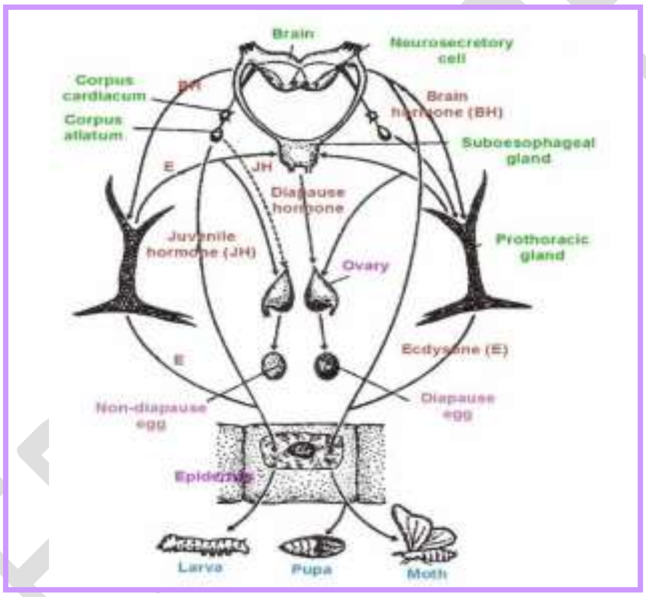


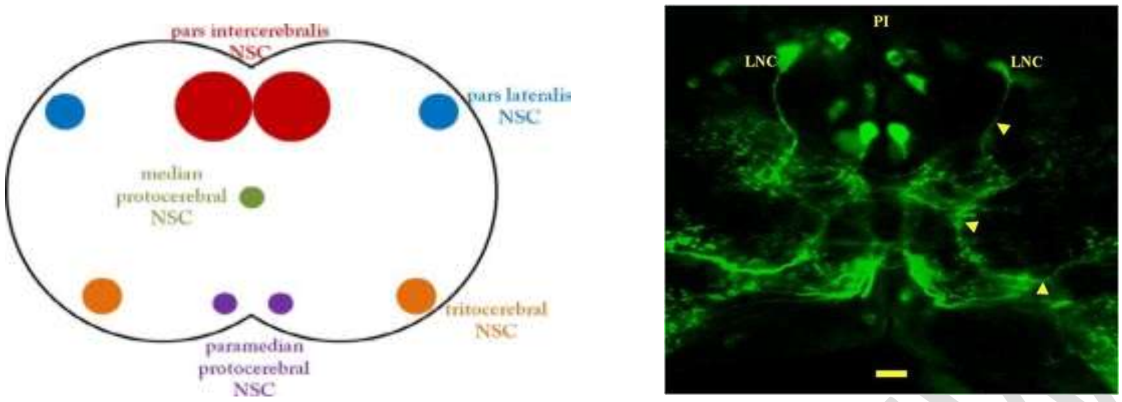
Fig. 1: Endocrine system of silkworm *Bombyx mori* L.

The endocrine system of *Bombyx mori* L. consists of five categories of glands.

a. Neurosecretory cells (NSCs) / Brain – Activation hormone (AH)
b. Corpora cardiaca (CC) – Storage and release of AH
c. Corpora allata (CA) – Juvenile hormone (JH)
d. Prothoracic gland (PG) – Moulting hormone (MH)
e. Sub-oesophageal gland – Diapause hormone (DH)

a. Neurosecretory cells (NSCs) / Brain

Neurosecretory cells (NSCs) are specialized cells found in the brain tissue of insects, playing a crucial role in the regulation of various physiological processes through the secretion of specific hormones. The activation hormone released by NSCs acts as a signaling molecule that triggers responses in target tissues and glands, coordinating the insect's overall physiological state.



(Bo *et al.*, 2012)

Fig. 2: Neuro Secretory Cells

b. Corpora Cardiaca (CC)

The corpora cardiaca serve as vital neuroendocrine organs in insects, integrating signals from the brain with local environmental cues to regulate physiological processes essential for survival and reproduction.

c. Corpora Allata (CA)

One of the primary hormones secreted by the corpora allata is juvenile hormone (JH), which plays a pivotal role in controlling insect metamorphosis, reproduction, and other physiological processes.

d. Prothoracic gland (PG)

The prothoracic gland is a crucial endocrine organ in insects responsible for synthesizing and releasing the hormone ecdysone, commonly referred to as the moulting hormone. It regulates the shedding of the old exoskeleton (ecdysis) and initiates the subsequent growth and development of the new exoskeleton during moulting cycles.

e. Sub-oesophageal gland

It plays a pivotal role in regulating various physiological processes, Secretion of Diapause hormone (DH), Activity in hibernating silkworm races, Determines voltinism and genetic control, Metabolic changes during diapause, particularly in response to environmental conditions and genetic factors.

3. TYPES OF HORMONES

It is well known that neurosecretory peptide hormone (PTTH and Eclosion), moulting hormone (MH), juvenile hormone (JH) and diapause hormone (DH) are synthesized in the brain, prothoracic gland, corpora allata and sub oesophageal gland, respectively.

i. Nuerosecretory Peptide Hormones

- Prothoracicotropic hormone (PTTH)

- Eclosion hormone (EH)

ii. Moulting Hormone (MH)

iii. Diapause Hormone (DH)

iv. Juvenile Hormone (JH)

i. NUEROSECRETORY PEPTIDE HORMONES

In the silkworm *Bombyx mori,* neurosecretory cells (NSCs) are present in the brain and neurosecretory cells are specialized to produce hormones. These NSCs are bluish white cells embedded in the brain tissue. It is divided into 4 groups, two medials in the intercerebalis and two laterals in protocerebrum (Akai, 1988). Generally, the medials neurosecretory cells are found at early larval stage though pupa to moth. As well as lateral neurosecretory cells are present in the fifth instar larvae to moth. Neurosecretory cells are large in size and it has large nuclei compare to other nerve cells (Kobayashi, 1957). On the basis of cytological feature, neurosecretory cells are of two types A-cells and B-cells. In A-cells, vacuole is present, ellipticalin shape. In B-cells, vacuole is absent, round in shape.

a. Prothoracicotropic hormone (PTTH)

The production and release of ecdysone is primarily controlled by a neuropeptide hormone, prothoracicotropic hormone (PTTH). PTTH is a homodimeric protein consist of subunits composed of 109 amino acid residues (Kataoka *et al*., 1991). PTTH is synthesized in two pairs of dorsolateral neurosecretory cells in the brain and transported to the corpora allata (CA), an endocrine organ that produces JH, by axons running through the contralateralhemisphere of the brain (Mizoguchi *et al*., 1990). PTTH is secreted into the hemolymph from arborized axon endings in the CA. In prothoracic gland (PG) cells, PTTH binds to its receptor, Torso (a receptor tyrosine kinase), and activates ecdysone synthesis via the MAPK/ERK (mitogen-activated protein kinase/ extracellular signal-regulated kinase) pathway (Lin and Gu 2007).

The activity of the PG is directly or indirectly suppressed by JH during the early stagesof the last larval instar. When larvae attain the critical size for metamorphosis, the synthesis of JH in the CA is shut-off and the JH declines to a very low level, allowing for the release of PTTH at the next photoperiodic gate. The release of PTTH induces the synthesis of ecdysone in the PG, thereby initiating the cessation of feeding and the onset of wandering behaviour. Thus, the regulation of the release of PTTH during the last larval instar is considered to be thekey physiological event that determines the timing of metamorphosis and final body size (Miwa *et al*., 2017).

b. Eclosion hormone (EH)

Eclosion hormone (EH) is a neuropeptide hormone that plays a crucial role in regulatingthe process of eclosion, which is the emergence of an adult insect from its pupal case. These are the chain of around 62 amino acids made of peptide bonds.

In the silkworm (*Bombyx mori*), eclosion hormone is known to be involved in coordinating the intricate physiological and behavioural changes required for successful emergence. Eclosion hormone in silkworms is primarily produced and secreted by a pair of neurosecretory cells in the suboesophageal ganglion. These cells release eclosion hormone intothe hemolymph (insect blood) shortly before eclosion, initiating a cascade of physiological events that culminate in the emergence of the adult moth.

ii. Moulting Hormone (MH)

Ecdysone is a crucial hormone involved in regulating various physiological processes, particularly moulting and metamorphosis. Moulting is the process by which insects shed their exoskeleton to accommodate growth, while metamorphosis involves the transformation from one life stage to another, such as from larva to pupa to adult. Ecdysone exists in twointerconvertible forms, α-ecdysone and β-ecdysone, with α-ecdysone being the prohormone andβ-ecdysone being the active form (20-OH-ecdysone). This means that α-ecdysone can be converted into β-ecdysone within the silkworm's body.

The activity of these forms of ecdysone can have significant implications for silkworm development. It's noted that α-ecdysone is twice as active as the β-form. This suggests that the conversion of α-ecdysone to β-ecdysone is an essential step in regulating the physiological effects of ecdysone. In the context of silkworms, this interconversion likely plays a critical role in coordinating moulting and metamorphosis. The precise timing and levels of ecdysone, particularly its active form, are crucial for orchestrating these developmental processes effectively.

For example, during the larval stage of a silkworm's life cycle, ecdysone levels rise to initiate moulting, allowing the silkworm to shed its old exoskeleton and grow larger. The balance between α-ecdysone and β-ecdysone, as well as their interconversion, helps regulate the timing and intensity of moulting events.

As the silkworm progresses through its life cycle and prepares for metamorphosis into a pupa and then an adult moth, ecdysone levels and activity patterns shift again. This transition likely involves complex regulatory mechanisms that rely on the interconversion between α- ecdysone and β-ecdysone to ensure proper timing and coordination of developmental events. The interconvertible forms of ecdysone, particularly the difference in activity between α- ecdysone and β-ecdysone, are essential for regulating moulting and metamorphosis in silkworms. This hormonal control is critical for ensuring proper growth and development throughout the silkworm's life cycle.

Activation of PG by the release of PTTH

PTTH is secreted into the hemolymph from arborized axon endings in the CA. In prothoracic gland (PG) cells, PTTH binds to its receptor, Torso (a receptor tyrosine kinase), and activates ecdysone synthesis via the MAPK/ERK (mitogen-activated protein kinase/extracellular signal-regulated kinase) pathway (Lin and Gu, 2007). Then, dietary cholesterol issequentially converted to ecdysone in PG cells by ecdysteroidogenic enzymes encoded by the “Halloween genes”, which include neverland (nvd), noppera-bo (nobo), shroud (sro), spook (spo), spookier (spok), phantom (phm), shadow (sad), and disembodied (dib) (Enya *et al.*, 2014). Synthesized ecdysone is released from the PG into the hemolymph and converted to themore active form, 20E, in the peripheral tissues by shade (shd), another member of the Halloween genes. In the cultured PG of Bombyx, PTTH stimulates the transcription of spo, phm, and dib but not sad, indicating that PTTH selectively upregulates Halloween genes at thetranscriptional level.

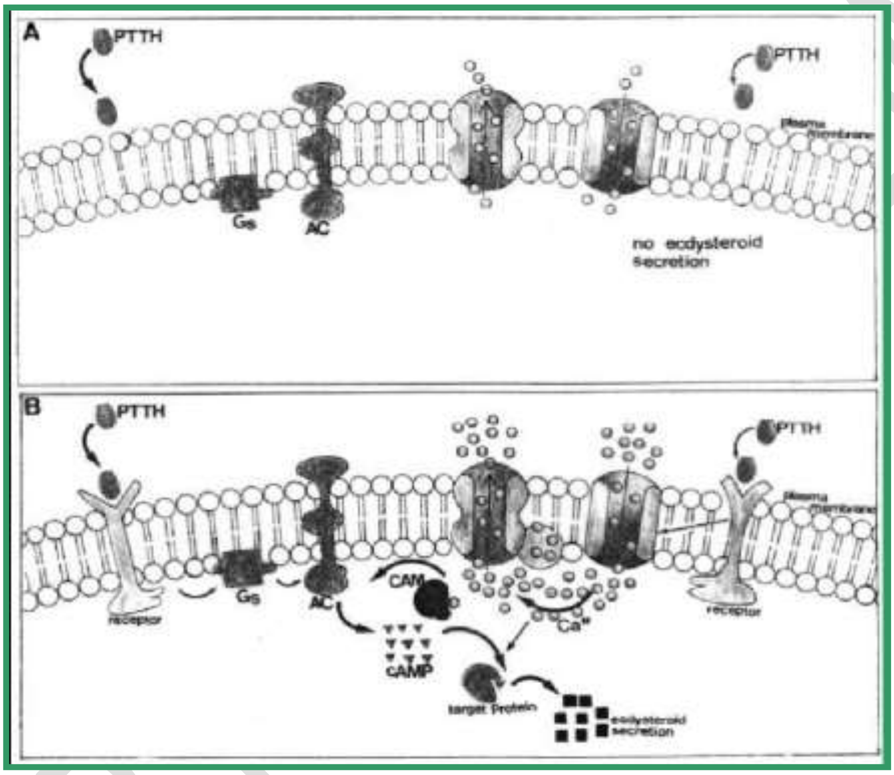


Fig. 3: Activation of PG by the release of PTTH **(Gu *et al.,* 1997)**

The process of ecdysone formation from cholesterol in the prothoracic gland.

Ecdysone is a crucial hormone in insects, playing a central role in the process of moulting and metamorphosis. The prothoracic gland, located in the thorax of insects, is primarily responsible for synthesizing ecdysone. The synthesis of ecdysone involves several steps, starting with the precursor cholesterol.

a. **Uptake of cholesterol**: The prothoracic gland cells take up cholesterol from the hemolymph (insect blood) or produce it internally via de novo synthesis.
b. **Conversion to 7-Dehydrocholesterol**: Cholesterol is converted into 7-dehydrocholesterolthrough enzymatic processes within the prothoracic gland. This step typically involves theremoval of a hydrogen atom from the 7th carbon atom of the cholesterol molecule.
c. **Formation of ecdysone**: 7-Dehydrocholesterol is then converted into ecdysone through aseries of

enzymatic reactions within the prothoracic gland. The specific enzymes involved in these conversions include cytochrome P-450 monooxygenases and other regulatory enzymes. These enzymes catalyze the modification of the steroid backbone of 7- dehydrocholesterol, leading to the formation of ecdysone.

d. **Release into hemolymph**: Once synthesized, ecdysone is released into the hemolymph, where it circulates throughout the insect's body.

e. **Conversion to active form (20-Hydroxyecdysone)**: Ecdysone is not the biologically active form responsible for moulting and metamorphosis. Instead, it is converted into its active form, 20-hydroxyecdysone, primarily in peripheral tissues such as the epidermis and the fat body. This conversion usually involves further enzymatic modifications, often by hydroxylation at the 20th carbon position.

f. **Binding to receptors and cellular response**: 20-hydroxyecdysone binds to specific receptors within target cells, triggering a signalling cascade that leads to the expression of genes involved in moulting and metamorphosis. This results in physiological changes such as the shedding of the old exoskeleton (ecdysis) and the formation of a new one.

The synthesis of ecdysone from cholesterol in the prothoracic gland is a complex process involving multiple enzymatic reactions and regulatory steps. It plays a vital role in regulating insect development and growth. Administration of moulting hormone when the silkworms are mature, it results in more or less simultaneous spinning with improved mounting efficiency and uniformity in pupation. The acceleration in spinning does not affect the cocoon characters (Chandrakala *et al.*, 1998).

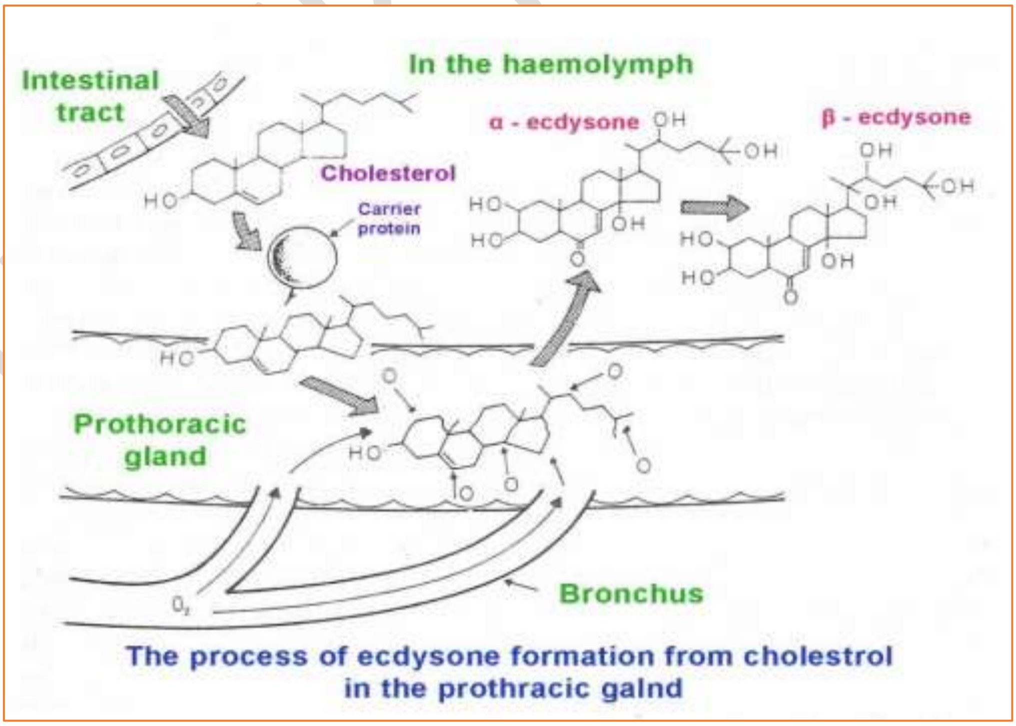


Fig. 4: The process of ecdysone formation from cholesterol in the prothoracic gland

Hormonal mechanism of moulting

The process of larval moulting, regulated by the release of ecdysone hormone, involves intricate changes in the structure of the insect's cuticle, the outer protective layer of the exoskeleton. The key events that occur within the 24-hour timeframe following ecdysone release:

- **Within 12 hours of ecdysone release - Digestion of endocuticle:** Endocuticle, the inner layer of the old cuticle, begins to undergo digestion. This process involves the breakdownof chitin, proteins, and other components by enzymes such as chitinases and proteases. Asthe endocuticle is digested, it gradually loses its structural integrity, preparing the way forthe shedding of the old cuticle.
- **Within 18 hours of ecdysone release- Formation of new epicuticle:** Simultaneously withthe digestion of the endocuticle, the synthesis of a new epicuticle begins. The epicuticle isthe outermost layer of the cuticle, providing waterproofing and protection against environmental stressors. It is primarily composed of lipids and waxes. Under the influenceof ecdysone, cells in the epidermis start producing and secreting the components necessaryfor epicuticle formation. This process progresses rapidly, ensuring that the newly formed cuticle is adequately protected.

Within 18 hours- Completion of exocuticle formation and shedding of old cuticle: Bythis stage, the synthesis of the new exocuticle, the middle layer of the cuticle, is completed.The exocuticle provides structural support and determines the overall strength of the cuticle. As the exocuticle matures, it becomes hardened and sclerotized, providing rigidityto the exoskeleton. Meanwhile, the digestion of the endocuticle continues, weakening the attachment between the old cuticle and the underlying epidermal cells. Eventually, the oldcuticle splits along predetermined lines of weakness, and the insect emerges through the process of ecdysis, leaving behind the old exoskeleton.

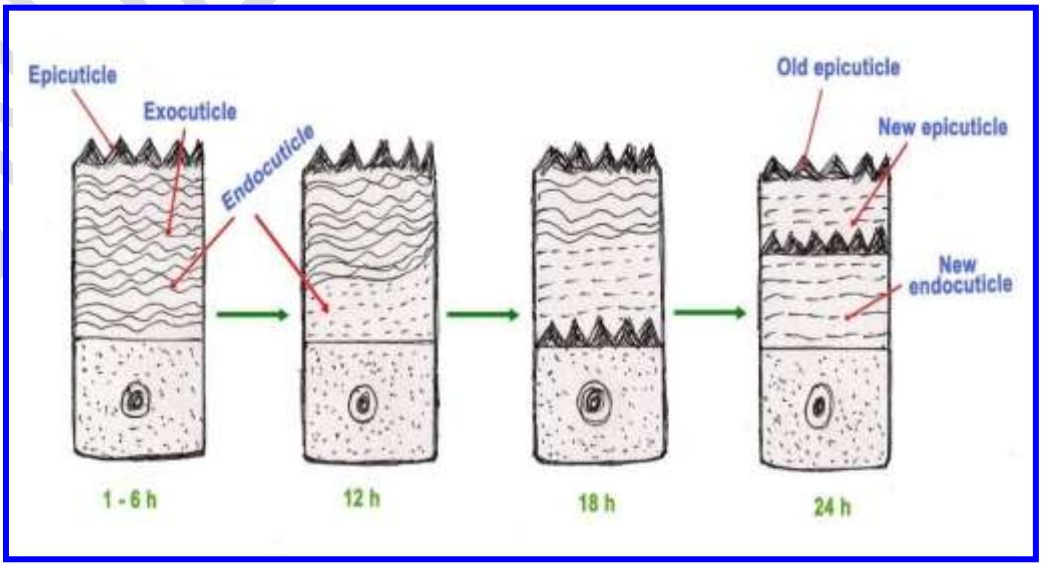


Fig. 5: Schematic representation of larval moulting

Phyecdysteroids and their uses in sericulture

Phytoecdysteroids are natural compounds found in plants that are structurally similar to ecdysteroids, which are hormones involved in the moulting and growth regulation of insects, including silkworms.

Table 1: List of some plants contain phytoecdysteroid compound.

Country	Plants	Author
China	*Pteridium aquilinum, Serratula* sp., *Cyanothis arachnoids*	Campus *et al.*, 1990;Costd *et al.*, 1993; Chou and Lu, 1980
India	*Achyranthus aspera, A. Fauriei, Trianthema portulacastrum, Gompherna eclsoides, Silene* spp *Sesuvium portulacastrum* and *Cassia tora*	Banerji *et al.*, 1997; Shivakumar *al., 1995*
Japan	*Pfaffia iresinoides* – approved asAgricultural chemical for sericulture – 1994	Ninagi and Maruyama,1996

Application of phytoecdysteroids in sericulture

a. To promote uniformity in spinning

By administering moulting hormone when the silkworms are about to spin, spinning will be more or less simultaneous, enabling improved efficiency in mounting and uniformity of pupation. Silkworms (Double hybrid silkworm) take 36-40 hours in normal course for completion of mounting completed the same in 18-24 hours when fed with mulberry leaves sprayed with phytoecdysteroids extracted from Spinach (Sameena *et al.* 2022).

b. To Shorten the Larval Duration

Application of phytoecdysteroids is mainly done during the shortage of mulberry leaf due to improper planning and might be applied in the unfavourable climatic conditions for thesilkworms to make the worms to ripen earlier than the normal. Administration of phytoecdysteroids at higher doses after middle stage of fifth instar results in shortening of larvalduration (Zhuang *et al.*, 1992). It will be a boon to the farmers as it shortens the larval durationby 2-3 days without affecting the survival, though the cocoon weight is slightly reduced.

c. To prevent or reduce non-cocooning silkworms

Non-cocooning silkworms exists because of high rearing temperature, feeding of tender leaves or by application of high dosage of JH. These ecdysteroids can be applied to the non- spinning silkworms which will promote spinning. By application of these ecdysteroids non- cocooning silkworm can be brought down to 9.6%.

d. To increase cocoon filament yield

The administration of moulting hormone at earlier stages of fifth instar, increases cocoon filament yield because of the increase in cocoon shell ratio (Zhuang *et al.,* 1992).

e. To reduce crop loss due to diseases

Application of phytoecdysteroids reduces the crop loss caused by diseases viz; grasserie, flacherie and muscardine. By application of phytoecdysteroids improves antimicrobial activity, enhances immune response, reduces mortality rate and improves growth and development (Barczak, 2007).

Application of phytoecdysteroid "Sampoorna" silkworm reduces the cocoon melting and disease incidence caused by *Bm*NPVand *Bm*IFV. However, applying sampoorna to healthy batches improved productivity and cocoon quality, suggesting benefits for uniform maturation and labour efficiency (Mithilesh *et al.* 2009).

Suryabhan and Anilkumar (2019) reported that the supplementation of phytoecdysteroid (2.0%) resulted increasedcocoon weight (2.178 g). The average silk filament length, fibroin and sericin and denier increased. Phytoecdysteroid of *Coix aquatica* have growth promoting effect and improved the cocoon characteristics of silk in *Bombyx mori*.

iii. Diapause hormone (DH)

Diapause is a fascinating phenomenon observed in many insect species, including the silkworm *Bombyx mori*, wherein growth and development are temporarily suspended to cope with unfavourable environmental conditions. In the case of *Bombyx mori*, diapause specificallyoccurs during the late gastrula stage of embryogenesis, a crucial phase in the development of the embryo.

The diapause hormone (DH) is a key regulator of the diapause process. DH is a proteinaceous substance, meaning it is composed of proteins. In *Bombyx mori*, there are two types of DH isolates viz., DH-A and DH-B. The diapause hormone (DH) in the silkworm Bombyx mori, designated DH-A and DH-B, also known as *Bom*DH-I. These hormones play a crucial role in regulating diapause, the temporary interruption of growth and development in response to adverse environmental conditions. Reddy *et al*. (2005) demonstrated that DH-B is notably more potent, being three times more active than DH-A. This indicates that DH-B likelyplays a more significant role in initiating and maintaining diapause in *Bombyx mori*.

The control of diapause in *Bombyx mori* involves the corpora allata and the suboesophageal gland. The corpora allata regulate protein synthesis (N) in the ovary of pupae, while the suboesophageal gland controls the amount of carbohydrate (C) present. Environmental factors such as temperature, photoperiod, and nutrition play significant roles in influencing diapause behaviour. For instance, specific temperature thresholds may trigger diapause or prevent it, depending on the species. Photoperiod, or the duration of light exposure, can also signal the onset of diapause in many insects. Additionally, the availability and quality of nutrition can impact the balance between N and C, thus affecting diapause tendencies.

Table 2: Environmental factors responsible for hibernating and non-hibernating eggs

Stage	Temperature	Photoperiod	Diapause
Egg	<15°C	<12 h	Non-hibernating eggs
Egg	>25°C	>16 h	Hibernating eggs
Late larval	<20°C	<12 h	Hibernating eggs
Late larval	>28°C	>16 h	Non-hibernating eggs

Sonobe and Ohnishi (1971) reported that a protein fraction of silkworm adult head showed diapauses hormone activity. Diapause hormone consist of 2 µg of DH-B per pupa induce diapauses in 50% of the eggs. The glycogen content in diapauses eggs increases from 0-24 h after oviposition, then decreases until the end of diapause. On the other hand, the amountof sorbitol and glycerin became large with decrease of glycogen content, suggesting the conversion of glycogen to sorbitol and glycerin through trehalose.

iv. Juvenile hormone (JH)

Juvenile hormones (JHs) are a group of acyclic sesquiterpenoids that regulate many aspects of insect physiology. The term juvenile hormone is derived from the fact that it blocks the developmental stages of nymphs into imagoes or the development of pupae into adult insects. JHs regulate development, reproduction and diapause. In insects,JH (formerly called neotenin) refers to a group of hormones, which ensure growth of the larva, while preventing metamorphosis. Because of their rigid exoskeleton, insects grow in their development by successively shedding their exoskeleton (a process known as moulting). JHs are also important for the production of eggs in female insects. JH was discovered in 1965 and the first of six molecular structures solved in 1967. The physiological balance of juvenile hormone (JH) in insects depends on its biosynthesis and degradation pathway (Jun *et al.*, 2011). There are several Indian studies on this subject like, study of juvenile hormone (JH) mimic R394 (ethyl 9-cyclohexyl-3, 7-dimethyl-2, 4- nonadienoate) applied topically on the abdominal tergum of silkworm (*Bombyx mori* L.,after the fourth ecdysis (Trivedy *et al*., 1997). One of the latest interesting studies is on using phyto-Juvenile hormone mimics for augmentation in cocoon yield in silkworm, Bombyx mori (Nair *et al.*, 2010).

Mousumi *et al.* (2018) have used juvenile hormone (JH) mimic of ω-formyl longifolene oxime propargyl ether (NL13), derived from longifolene (extracted from *Pinus longifolia*) and bakuchiol, isolated from the medicinal weed, *Psoralea coryllifolia*. These compounds were administered in various concentrations to the fifth instar larvae of bivoltine silkworm hybrid (KA×NB4D2). It was observed that cocoon weight, shell ratio, shell weight and SR% were more in winter seasonsthan summer and rainy seasons. The netincrease in profit in Serimore treated batches were Rs. 1835-2500 per 100 dfls.

JH analogues and Anti - JHs in sericulture

Juvenile hormone analogs (JHAs) and anti-juvenile hormones (anti-JH) play importantroles in silkworm rearing, particularly in sericulture, which is the cultivation of silkworms for the production of silk. HAs and anti-JHs can be used to manipulate the growth and developmentof silkworms. By mimicking or blocking the action of juvenile hormones, these compounds canalter the pace and timing of various developmental stages in silkworms. This control is crucial for synchronizing the development of silkworms with the desired production schedule and ensuring uniformity in silk production.

a. JH analogues

Several JH analogs such as methoprene (manta), hydoprene (Altozar / ZR-512), JH-I analogue (Farnesyl methyl ester-FME), R394, fenoxycarb (Roche / Maag), diofenolan (Ciba Geigy) and pyriproxyfen (Sumitomo Chemical Co.) etc., are some of the important JHAs whichare utilized for studies involving commercial application in sericulture.

In India, juveno-mimetic activity was found in some common plants like *Tamarindus indica, Manihot esculenta, Mangifera indica, Tabernaemontana dichatama, Tectana grandis, Pterocarpus marsupium, Terminalia paniculata* and *Lantana camera,* in varying degrees.

Applications of JH analogs

Commercial traits such as cocoon weight, cocoon shell weight and silk filament length were enhanced through administration of exogenous JH analogues in minutequantities (Mamatha and Rajeswara, 2008). 21% increment of silk production by the use of the SJ-42-F juvenile hormone (Chowdhary and Ogra, 1990). Hormone like methoprene JH analogue have long been utilized for the improvement of silkproduction in the silkworm *Bombyx mori* L. (Miranda and Takahashi, 2002). Application of the C18JH synthetic hormone increased 30% of silk ratio (Miranda and Takahashi, 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 comparedto untreated larvae (Magadum *et al.,* 1990). Studies with Manta and ZR-512 revealed that the treatment on third day of fifth instar will prolonging the larval period, increased the cocoon yield by 10-11 per cent.

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 betterresults while as juvenile hormone analogue application @ C3 (0.1µl/larva) was found to be the most effective concentration (Asif *et al*. 2023).

b. Anti-juvenile hormones

Anti-juvenile hormones (anti-JH) have several applications in sericulture, the cultivation of silkworms for silk production. These compounds, which inhibit the action of juvenile hormones (JHs), offer various benefits and can be used in different aspects of sericulture.

Anti-JHs can improve the quality of silk cocoons produced by silkworms. By delaying or inhibiting pupation, these compounds prolong the feeding phase of silkworm larvae, resultingin larger and stronger cocoons. Enhanced cocoon quality translates to higher silk yield and better silk fibre properties, such as strength, length, and luster, which are desirable traits for commercial silk production.

Furuta *et al*. (2006) tested a series of ethyl 4-(2 - aryloxyhexyloxy) - benzoates to induce precocious metamorphosis in silkworm. They found that ethyl 4-(2- aryloxyhexyloxy) - benzoates as a new class of potent anti-JH agents.

Some chemicals like such as SM-1, Jinlu trimolter inducer, and KK-42, KK-22, Kang-20 and YA20, SSP-11 and SD-III applied to induce trimolter silkworms. Zhiping *et al*. (2013) stated that, the induction and regulation of trimolter induced by anti-juvenile hormone have shown vast potential particularly with increased cocoon quality, super thin cocoon filaments would be very useful as new silk materials.

CONCLUSION

The endocrinology of silkworms, particularly regarding the application of hormones such as phytoecdysteroids, juvenile hormone analogs (JHA), and anti-juvenile hormones (AJH), holds significant potential for improving silk yield and quality in commercial sericulture. Studies have shown that the application of hormones, particularly JH, can lead to a net increase in profit in sericulture. This enables them to optimize production parameters and achieve desired outcomes in silk yield and quality. In summary, the endocrinology of silkworms presents exciting opportunities for improvingsilk yield and quality in commercial sericulture. While significant progress has been made in understanding hormone actions and their application, further research, development, and implementation efforts are needed to realize the full potential of hormonal manipulation in sericulture.

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