Role of Hormones In Silkworm For Enhancing Silk Production

Saranya. M[1], G. Umapathy [2], K. A. Murugesh[3]
Department of Sericulture
[1,2,3] Forest College and Research Institute, Tamil Nadu Agricultural University Mettupalayam- 641 301, Tamil Nadu.

Abstract*- Sericulture, which is an agro-based cottage industry in India, needs increased production of silk along with the raw silk. Insect growth regulators (IGRs) such as juvenile and moulting hormones or their analogs (juvenoids and ecdysoids) when used judiciously, have been found to be useful in sericulture industry. The ecdysone is known to influence the reproductive potential and silk producing potential of Bombyx mori L. Hormone like methoprene juvenile hormone (JH) analogue have long been utilized for the improvement of silk production in the silkworm Bombyx mori L. JH analogues or the mimics have been celebrated option for sericulturists can control silk gland function and indirectly cause an increase in silk production. In recent years, several technologies have been developed to improve the quality of silk production, labour saving devices linked with improvement in quality and saving the feed for the larvae through the effective use of hormones for better silk production.*

Keywords- silkworm, juvenile hormones, growth promoters, silk production

I. INTRODUCTION

Sericulture plays an important role in transformation of the rural economy as it assures regular employment, income resource and provides return round the year. To increase the silk production efforts have been made to study the effect of ecological factors (Upadhyay & Prasad, 2011). The ecdysone is known to influence the reproductive potential and silk producing potential of Bombyx mori (Parlak et al., 1992). Insect growth regulators (IGRs) such as juvenile and moulting hormones or their analogs (juvenoids and ecdysoids) when used judiciously, have been found to be useful in sericulture industry. In addition, ecdysoids also show a variety of other uses such as insecticidal, as biochemical tool in gene expression studies, as wound healing and anabolic agents (body building agents with enhancing protein synthesis), as nutraceuticals and cosmetics (hair growth) IGRs occur in insects in very small amounts and are not practical source for these phytochemicals. However, with the discovery of their occurrence in significant quantities in some plants, IGRs and their analogs beca.me easily available in substantial amounts. As a result, many new bioactivities of ecdysoids and juvenoids were discovered. Besides use in sericulture, they have found applications in apiculture and aquaculture (prawns). Ecdysoids show remarkable anabolic activities in human and are very much in demand as nutraceuticals (food supplements) including body building agent.

II. IGRS IN SERICULTURE

India is one of the largest silk producing countries. Mulberry silk accounts for most of the silk produced in India. Three other species namely, Eri, Tasar and Muga are mainly grown in the north-eastern India. India is also one of the major consumers of silk. Sericulture, the agro-industry of cocoon and silk production is the source of income of several marginal farmers of India. However there is a shortfall of 7000 metric tonnes of silk fibre within the country. The silk production in India needs improvement in quantity as well as quality compared to other silk producing countries.

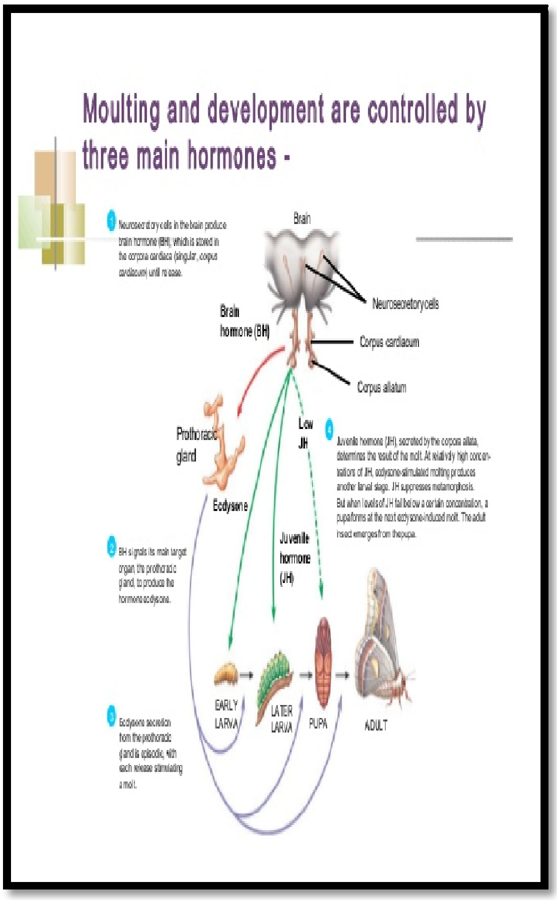
Central Sericulture Research and Training Institute (CSRTI), Mysore has been involved in the improvement of the mulberry seri-technology in a holistic way. However there is considerable scope of improving the varieties of silk produced in north eastern India.

Sericulture is a labour intensive agro-based industry which provides additional income to marginal farmers. Substantial part of labour in sericulture management goes in the picking up of the mature larvae for mounting. The duration of mounting period is quite variable. Duration of the moulting from beginning to end may vary between 48-72 hours. This implies that labour must be available continuously all through the period (day and night) of moulting. Application of exogenous ecdysoids helps in the synchronization and reducing the time for spinning. Its application hastens the maturation without affecting the quality and yield of silk. . Use of juvenoids on the other hand, tend to keep the fifth instar silkworm young by extending the larval period and increasing the silk secretion. It postpones the spinning stage by few hours. Thus when applied judiciously, IGRs can contribute significantly in increasing the silk production.

Insect growth regulators(IGRs)

- Juvenile hormones (Juvenoids)

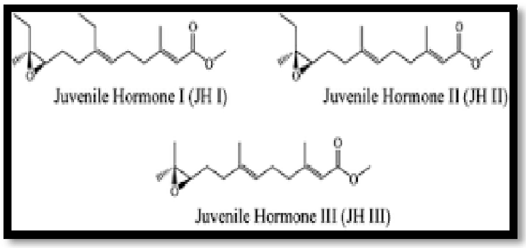
- Moulting hormones (Ecdysoids)
- Phytoecdysterone (Phytoecdysoids)



Juvenile hormones (JHs)

Juvenile hormones (JHs) are a group of acyclic sesquiterpenoids that regulate many aspects of insect physiology. JHs regulate development, reproduction, diapause, and polyphenisms. 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 molting). Juvenile hormones are secreted by a pair of endocrine glands behind the brain called the corpora allata. 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.Most insect species contain only juvenile hormone (JH) III. To date JH 0, JH I, and JH II have been identified only in the Lepidoptera (butterflies and moths). The form JHB_3 (JH III bisepoxide) appears to be the most important JH in the Diptera, or flies. Certain species of crustaceans have been shown to produce and secrete methyl farnesoate, which is juvenile hormone III lacking the epoxide group. Methyl farnesoate is believed to play a role similar to that of JH in crustaceans.

Types of JH:



JH I and II - Produced during 3 and 4^{th} larval instar

JH III - Produced during 5^{th} larval instar

Mechanism of action of juvenile hormone

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. JH controls the switches between alternative pathways of development at various points in the life cycle of insects .In support of these roles of JHs, many researchers have assumed that different concentrations of the hormone are responsible for specifying the different pathways.

Most of the work on the hormonal control of moulting and metamorphosis gave results that are consistent with the hypothesis that metamorphosis in holometabolous insects is caused by gradual lowering of JH titer. Holometabolous insects continue as larvae only at high concentrations of JH, but pupation occurs when JH gradually declines to an intermediate or low level, and adult insects are formed in absence of JH.

The synthesis of JH-III, the homolog lacking branched side chains are now known to follow the path similar to the initial steps in cholestrol synthesis. The precursors for the carbon skeleton are two-carbon (C-2) units that are products of metabolism of glucose, leucine, isoleucine and threonine and the precursor of acetate.The 3 units of C-2 in the form of acetyl CoA undergo enzymatic condensation to yield C-6 intermediate 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). The HMG-CoA is then reduced to mevalonate by HMG-CoA reductase which utilise NADPH as an electron donor. The next step in JH biosynthesis is the conversion of mevalonate to 3-isopentenyl pyrophosphate (IPP) that isomerizes to 3,3-dimethylallyl pyrophosphate (DMAPP).

The last step is the condensation of two units of IPP and one unit of DMAPP to form the basic farnesyl pyrophosphate unit. Farnesyl pyrophosphate is then converted to farnesol. Farnesol is oxidized to farnesal by a dehydrogenase that requires NAD, which is then oxidized to farnesoic acid by another dehydrogenase that also requires NAD. The terminal steps in biosynthesis are methyl ester formation at C-1 and epoxidation at the C-10/C-11 position.

The sequence of the terminal two steps in JH biosynthesis is reversed in the Lepidoptera (i.e., farnesoic acid to epoxyfarnesoic acid to JH-III) when compared to the order in other situations **(farnesoic acid → methyl farnesoate → JH-III).**

The branched precursors of JH0, JH-I and JH-II originate from propionyl-CoA. The branched chain of homoisoprenoid units are formed from condensation of 1 propionyl-CoA and 2 acetyl-CoA units and results in the synthesis of a homomevalonate intermediate, whose composition varies in the different forms of JH. The JH0 is composed of 3 homomevalonate units, JH-I of 2 homomevalonate and 1 mevalonate units, and JH-II consists of 1 homomevalonate and 2 mevalonate units.

A. Biosynthesis of juvenile hormones

The juvenile hormones (JH) play unique roles in almost all aspects of insect development and reproduction that includes embryogenesis, larval moulting, metamorphosis, caste determination in the social life of insects, vitellogenin synthesis and ovarian development, phase determination in locusts and aphids, larval and adult diapause regulation, colour, polymorphism and various aspects of metabolism associated with these functions. The hormone (JH-I, C_{18}JH) showed that it is an unusual sesquiterpenoid with epoxide group near one end and a methyl ester on the other end.

The hormone is one of a series of naturally occuring juvenile hormones, all having similar chemical structures Juvenile hormone III is the principal form of JH found in the Orthopteroidea, Coleoptera, Hemiptera and Hymenoptera. The Lepidoptera is unique in possessing a mixture of JH-I and JH-II. However, the corpora allata (CA) of the tobacco hornworm moth, Manduca sexta secretes JH-III in addition to JH-II and JH-I. The male of certain Lepidoptera secretes JH acids (JHA-I, JHA-II, JHA-III, iso-JHA-II) from the CA that are later methylated in the accessory gland. Methyl farnesoate (MF), an acyclic sesquiterpenoid ester that is closely related to JH-III in structure is also tentatively added to the group of JH acids. The MF was detected in high levels from the embryos of cockroach, Nauphoeta cinerea produced in vitro by CA.

III. EFFECT OF HORMONES ON SILK GLANDS

In most Lepidoptera and Tricoptera, the posterior gland region secretes fibroin and one to several small proteins. In preparation for hatching, Lepidopteran silk glands become secretory. And during each stage of moulting the insects increase their secretory potential by growing in size and ploidy. This is characterized by fluctuation in function in each instar according to a larval pattern which is manifested by initiation of RNA transcription, by a high rate of proteosynthesis during feeding and by absence of these activities with the advancement of the next moult.

In the last larval instar the silk glands develop according to a metamorphic pattern that differs from the larval one by enhanced function and programming of silk glands for histolysis. Development of silk glands is altered with hormonal treatment of the larvae. JH inhibits silk gland function, prevents their degeneration and indirectly cause an increase in silk production. Low doses of ecdysteroids stimulate silk gland development to increase the function, while high doses cause regression and degeneration. The JH anologues and anti-JH compounds are utilized in sericulture to control the yield and quality of silk.

The changes from larval to the metamorphic developmental pattern is caused by a drop in JH titre. Trace amounts of JH in the last larval instars affect silk glands via regulation of feeding and moulting time. The function of the silk glands depends on nutrient supply which is stimulated by a brain neurohormone. Slight elevation of ecdysteroid titre that is associated with the termination of feeding and initiation of cocoon spinning may be implicated in the culmination of proteolysis and in the initiation of functional regression in the silk glands. The moult-inducing surge of ecdysteroids induces regression in the silk glands. Juvenile hormone, Juvenile hormone analogue (Methoprene), Juvenile hormone activating peptide (ATANA) applied to freshly ecdysed Vth instar larvae of Anthereae assama causes better larval growth, larval body weight, cocoon weight and silk weight.

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 JH analogues in minute quantities (Mamatha et al., 2008). 21% increment of silk production by the use

of the SJ-42-F juvenile hormone .Chowdhary et al.,(1986)

- ✓ Hormone like methoprene JH analogue have long been utilized for the improvement of silk production in the silkworm Bombyx mori (L). Miranda et al., (2002)
- ✓ 30% increase on silk ratio over the control, after the application of the C18JH synthetic hormone Akai et al., (1985)

Specific effects:

- ✓ R 394: Application on 1st day of V instar, increases shell weight by 8%
- ✓ ZR 512: Increases the larval duration by one day,filament length is increased by 5-10%
- ✓ Methoprene @ 5 µg / larva prolonged the larval duration and 50% increase in yield.

Synthetic JH compounds :

- ✓ Farnesyl methyl ether (FME)
- ✓ Methylene dioxyphenyl (MDD) derivatives No.1 or No.2

Effect of synthetic JH on 4th instar of silkworm larvae:

- ✓ Effect of JH analog MDD-2 was the strongest followed by MDD- 2 and FME. MDD injection @ 10 µg resulted in higher appearance of non-diapausing egg producing moths.

Synthetic JH injection during second day of 5th instar:

- ✓ Application of JH compounds during initial stage of 5th instar larvae of silkworm prolonged the larval duration, (24 -36h), increase in cocoon and shell weight. There is no increase in shell %. Pupal duration is also prolonged. Total number of eggs laid / moth also increases by 10% and 50% of moths were of non diopausing egg producing type.
- ✓ When JH is applied to pupa, no change was observed regarding diopausing states of the moth.
- ✓ Studies conducted in N 106 x Daizo, (a diapause egg producing strain) with JH compounds revealed that JH application resulted in transformation of diapause to non-diapause state.

Effect of spray of JH compounds on silkworm larva

- ✓ Spraying of JH compounds was taken up on second day of 5th instar larvae of silkworm. JH application increases the larval duration by 24 to 36 hours with higher cocoon and shell weight and no difference in cocoon shell percentage.
- ✓ Spraying of JH compounds on 1st day of 4th instar resulted in no significant change in economic parameters. However, excess ecdysis was reported when the concentrations of JH compounds were increased beyond a limit. However, the limitation is that application of JH compounds increase the larval duration consequently consuming more feed materials.

<u>Anti Juvenile Hormone :</u>

Removal of corpora allata leads to precocious metamorphosis in larvae. Anti JH activity was noticed in plant extracts of Aegeratum sp. Bowers, 1985 reported that application of these extracts will bring precocious pupation and named them as Precocenes.

Anti JH analogues:

- ✓ Topical application of Imidazole during III and IV instar induced pupation. KK 42 is a potent anti ecdysteroid agent. ETB [ethyl -4t -2 tertiary butyl carbonyl-oxy-butoxy benzoate], EMD [Ethyl methyl dodecenoate] and J – 2710 are some of the proven anti JH compounds. Among these anti JH compounds, J2710 is safer to silkworm larvae while others are found to be toxic.
- ✓ KK 42 is moderately toxic to newly moulted II and II instar where as toxicity was degraded easily during fourth instar. Application during III instar induced precocious pupal development. It is generally used for induction of Trimoulters, as shorter larval duration with high fecundity is a favourable trait for grainages.

Prothoracic gland (PG)

- ✓ PG in silkworm consists of many NSC enveloped with a common membrane. It is divided into 4 parts, viz., trunk or basal part, anterior branch, median branch and posterior branch. Secretions of PG are dispersed into blood during 4th and 5th instars. The hormone plays a major role in moulting. Several moulting hormones have been identified.
- ✓ Ecdysone is a lipid steroid hormone. Ecdysone originally refers to α - ecdysone and now it refers to all steroid hormones associated with moulting in insects. Ecdysone involves in many functions during embryonic and adult stages. Ecdysone influences the synthesis of enzyme and blood proteins.

Phyto ecdysteroids:

- ✓ Phyto ecdysteroids extracted from Achyranthus aspera @ 40 to 6 mg / litre in aqueous and ethanol extracts were sprayed on mulberry leaves and fed to V age worms, which recorded more than 50% maturation and increased shell ratio by 22%.

- CSR & TI have isolated extracts from Caryophyllaceae plants and branded it as 'Sampoorna'. Spraying of 'Sampoorna' @ 20 mg / lit on 3rd day of 5th instar reduces the larval duration by one day and ensures uniform spinning of larval. Similarly, KSSR&DI have developed a formulation called 'Chetna' with ecdysteroids activity.
- Commercial ecdysone formulations @ 1% from NISES, Tokyo, Japan when applied at 12 h and 18 h before maturation ensures 80% larval maturity at 12 h after spraying.

Role of phytojevenoids in improving silk yield:

Several plant products have been reported to have juvenoid activity on silkworm, which include

- Psoralea corylifolia ,
- Parthenium hysterophorus,
- Lanatana camera,
- Tribulus terrestris and
- Vetivera zizanoides

Application of phytojuvenoids is reported to have increased the larval duaration and as well the silk yield.

Studies conducted at TNAU have revealed that application of Psoralea corylifolia or Lantana camera extracts @ 800ppm to the third instar larvae of B.mori has increased the larval duration by one day and improved the cocoon yield and economic parameters.

Advantages : Sampoorna

- Sampoorna is a plant-based steroid with moulting hormone activity.
- Early and uniform maturation (20 mg / 1 lit)
- Saves labour in picking the matured worms.
- Reduces moulting period by 18 - 40 hours from the onset of spinning.
- Early maturation can save the crop from out break of diseases in the fifth instar.
- It can save the crop from unforeseen shortage of mulberry leaf in final instar
- The cocoon characters are not affected.
- Dosage for 100 dfls: 4 liters of diluted hormone.

Mode of action

- Ecdysone is a potent growth hormone either in the presence or absence of juvenile hormone.
- Juvenile hormone is active only in the presence of ecdysone.
- When juvenile hormone is absent, ecdysone promotes, not only the synthetic acts prerequisite for growth, but also the new synthetic acts that are necessary for metamorphosis.

Moulting and endocrine hormones

- JH is traditionally known as larval stage maintaining hormone. Genotype determines the number of moulting (moultinism). Moultinism is controlled by a major gene which affects the function of corpus allatum. The presence or absence of or high / low activity of JH during larval stages determines the duration of larval stage and extra moulting. Spinning activities can be advanced or synchronized by administering ecdysone (One set of spinning) Chang et al.,1972.
- Uniform spinning (Li et al., 1992).Ecdysone is known to influence the reproductive potential and silk producing potential of Bombyx mori L. Parlak et al.,1992

- JH has a role in protein metabolism. It controls the progress of growth and has some role in metabolism of carbohydrates. JH suppresses the protein synthesis in Sg and its enlargement. JH activity declines during 5th instar. Timing and periodicity of JH determines the moultinism.
- Trimoulters have abundant JH activity in early stage so each instar duration is long and last moulting is skipped as Corpora allatum activity is weak.
- In Penta moulters, there is lower secretion of JH and as such duration of each instar is short and hence there is an additional moult.

Effect of ecdysone

- While JH is secreted from early stage of instar, ecdysone is secreted from mid stage of each instar. Ecdysone has a definite role in growth and development. Prothoracic gland is activated by brain hormone, releasing ecdysone promoting faster growth and development. Ecdysone role is related to ecdysis - larval, pupal ecdysis and adult emergence.
- Ecdysone is transported into haemolymph to target tissues to exert action. Ecdysone acts directly on epidermal structures and triggers moult cycle which involves, apolysis, moulting fluid production, initiation of cuticle formation, moulting fluid

activation, exocuticle secretion, ecdysis, tanning and endocuticle deposition.

- ✓ Endocrine secretions are governed by genes and environment. Environment acts on brain and their action directly affects corpora allatum, prothoracic gland and Suboesophagal gland. Environment controls the growth. Complex interaction of genes, environment and endocrine glands determine the growth and development.

Methoprene:

- Long chain hydrocarbon ester (1, isopropyl 2E, 4E-11 methoxy-3,7,11-trimethyl-2, 4 dodecadienoates)
- Insect growth regulator and effective against dipteran insect
- Methoprene application during II to IV instar increased larval weight, cocoon weight, ovariole length, egg no and fecundity.

IV. CONCLUSION

Silk production in India needs improvement in quality and quantity for the better production of silk yield. Juvenile hormones and ecdysterrids play an important role in the regulation of growth and reproduction of Insects. The vertebrate hormones like prolactin (PRC), thyroxin (THY).Insulin (INS) and other pituitary extracts caused shortening of larval duration, increased the larval, silk gland weights and fecundity of the silk worm.It is reported that thyroxine works more efficiently when applied to 2nd instar larvae causing the enhancement of haemolymph pralines and ecdysteroid levels in the silkworm However, the precise mechanism of action of the vertebrate hormone in invertebrates remains to be understood. It may be a direct Labour intensive method and proper use of hormones at optimum dose and time of application. Hormones will boost the sericulture industry and leads to result in increased silk production.

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