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EFFECT OF R394 ON ECDYSTEROID TITRE OF HAEMOLYMPH AFTER FOURTH MOULT OF SILKWORM BOMBYX MORI

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ABSTRACT
The effect of the Juvenile hormone (JH) mimic R 394 (ethyl 9-cyclohexyl-3, 7, dimethyl-2,4-non adienoate) on silkworm *Bombyx mori* was studied. JH mimic ranging from 500 nl to 0.0078 /5µl acetone/larva applied topically at 24, 48, 72, 96 and 120 h of V instar revealed that application of 0.031 nl on one day old V instar improved shell weight by 8% and without prolonging the V instar duration the same dose at 48, 72 and 96h prolonged larval period by one day noticed the improvement of 3 to 10% in cocoon and shell weight. The daily changes in haemolymph ecdysteroid titres of treated larvae seldom followed the pattern of control larvae and differences existed between the variation pattern of larvae treated at 24h and that of others. The possible reasons for non-prolongation of larval period in early treatment and prolongation in late treatments are discussed.

KEYWORDS: JH, haemolymph, titre, *Bombyx mori,* silk production *etc.*

INTRODUCTION

The process of moulting and metamorphosis characteristic to growth and development in insects is regulated by circulating hormones like prothoracicotropic hormone (PTTH), juvenile hormone (JH) and ecdysterone. The set pattern of the insect development can be altered to a certain extent by exogenous administration of the mimics or analogues of these circulating hormones (Sakurai, *et al.,* 1989).This principle has been exploited in the sericulture industry in which the silkworm rearing and the production of cocoons can be managed effectively or manipulated positively depending on the requirement by administering bioactive compounds mimicking the circulating hormone regardless of the source. JH analogues or the mimics have been a celebrated option for sericulturists as these can control silk gland function and indirectly cause an increase in silk production (Sehnal and Akai, 1990). Earlier it was made clear that exogenous JH delays the silkworm larval maturation and the increase in silk yield was mainly because of this phenomenon (Akai and Kobayashi, 1971, Akai *et al.,* 1988). Some of the synthetic JH analogues/ mimics popular elsewhere were later on experimented sparingly in India to see their effects on silk worm and reported improvement in their economic traits (Magadum and Hooli, 1991, Trivedi *et al.,* 1993, 1997). But use of such compounds is yet to become popular in the commercial scale either due to non availability or the difficulty to import such compounds regularly to India in mass scale. Keeping this in view, an effort was made to screen some of the indigenous bioactive phytojuvenoids extracted by some research institutes in India along with certain strong JH mimics which are used as pest control agents, on silkworm to see their effects on silkworm growth and silk yield. The primary objective of this screening was to short list the compounds based on its capacity to induce enhanced silk production so that these short listed compounds can be used for mass scale repetitive trials. Juvenile hormone activity from the adult male *Hyalophora cecropia* the immediate requirement was a rapid assay method to facilitate the isolation of the active substance. Among a wide range of insects studied at that time, the most consistent results were obtained on *Rhodnius;* but most time pest, giving the most rapid results, was that employing the pupa of Tenebrio. This *Tenebrio* test was used in the key experiments in which he identified that the material with juvenile hormone activity present in yeast, and in the excreta of Tenebrio, as a mixure of farnesol and farnesal. The same test slight modified was used by Rooler and Bjerke, 1965 in their isolation of the active substance from *Hyalophora.*

The purpose of the *Tenebrio* test, like the comparable 'wax' test applied to *Hyalophora* or to *Galleria*, was to compare concentrations of active substance in the various fractions of an extract. Even after the modifications it is not a good quantitative test. On the other hand, injection into the body cavity of Tenebrio has been used as a quantitative assay by *Roller* and *Dahm*, (1970) have made estimates on various insects by measuring the quantity of an given substance that is equivalent to a 'Tenebrio unit', and then observed the number of such unit that will give a standard effect on the insect under study. Now that the composition of the natural juvenile hormone from *Hyalophora* is known (Roller *et al.,* 1965), and many synthetic derivatives of farnesol are available, it is desirable to learn the relative amounts of these substances that are needed in different insects (table-1).

TABLE -1 Different Juvinile compounds for enhance silk production

No. 1.	(Farnesol), 3, 7, 11-Trimethyl-2,6,10-dodecatrien-1-ol (Roche)
No. 2.	(Nerolidol) 3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol (Roche)
No. 3.	3,7,11-Trimethyl-2-*cis*/*trans*-dodecene-1,11-diol (Roche)
No. 4.	(Farnesyl methyl ether) 1- Methoxy – 3,7,11-trimethyl-2-*cis*/*trans*,6-*trans*, 10-dodecatrience (Roche)
No. 5.	(Farnesyl methyl ether) 1- Methoxy – 3,7,11-trimethyl-2-*cis*/*trans*,6-*trans*, 10-dodecatrience (Roche)
No. 6.	Mixure of 6,7-epoxy-1-methoxy-3,78,11-trimethyl-6,7-*trans*-dodeca-2-*cis*/*trans*,10-diene and 10, 11-epoxy-1-methoxy-3,7,11-trimethyl-2-*cis*/*trans*,6-*trans*-dodecadience (Roche)
No. 7.	Mixure of 6,7-epoxy-1-methoxy-3,78,11-trimethyl-6,7-*cis*-dodeca-2-*trans*,10-diene and10,11-epoxy-1-methoxy-3,7,11-trimethyl-2-*trans*,6-*cis*-dodecadience (Roche)
No. 8.	6,7 : 10,11-Diepoxy-1-methoxy-3,7,11-trimethyl-trimethyl-6,7-*cis*-dodec-2-*trans*-ene (Roche)
No. 9.	(Farnesenic acid) 3,7,11-Trimethyl-2,6,10-dodecatrienoic acid (Roche)
No. 10.	Mixed farnesentic acid derivative (Law *et al.,* 1966)
No. 11.	Methyl 7,11-dichloro-3,7,11-trimethyl1-2dodecenoate (Dr. K. Slama)
No. 12.	Ethyl *trans*-7,11-dichloro-3,7,11-trimethyl-2-dodecenoate (Roche)
No. 13.	Ethyl 11-chloro-3,7,11-trimethyl-2-*cis*/*trans*-dodecenoate (Roche)
No. 14.	Ethyl all-*trans*-9-(2,2-dichloro-3,3-dimethylcyclopropyl)-3,7-dimethyl-2, 6-non adienoate (Roche)
No. 15.	Ethyl10,11-epoxy-3,7,11-trimethyl-2-*cis*/*trans*,6-*trans*-dodecadienoate(Roche)
No. 16.	Ethyl 10,11-epoxy-3,7,11-trimethyl-2-*cis*/*trans*-dodecadienoate (Roche)
No. 17.	Ethyl 10,11-epoxy-3,7,11-trimethyl-2-*cis*/*trans*-dodecenoate (Roche)
No. 18.	Ethyl 6,7:10,11-diepoxy –3,7,11-trimethyl-6,7-*trans*-dodec-2-*cis*/*trans*-enoate (Roche)
No. 19.	N,N-Diethyl-3,7,11-trimethyl-2-*cis*/*trans*,6-*trans*,10-dodecatrienylamine (Roche)
No. 20.	10,11-Epoxy-N,N-diethyl-3,7,11-trimethyl-2-*cis*/*trans*-dodecenamide (Roche)
No. 21.	10,11-Epoxy-N,N-diethyl-3,7,11-trimethyl-2-*cis*/*trans*-dodecenamide (Roche)
No. 22.	(Juvabione)1-Methoxycarbonyl-4-[1,5-dimethyl-3oxohexyl]cyclohexebe (Dr.K.Slama)
No. 23.	Doidecyl methl ether (Roche)
No. 24.	(Geranyl methl ether) 3,7-dimethyl-1-methoxy-2-octadiene (Roche)
No. 25.	As No. 26, mixed with other isomers.
No. 26.	Methl rac-all-*trans*-10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoiate (Roche)
No. 27.	As No.26 (Dr. H. Roeller, No.304)
No. 28.	Methl rac-6,7-epoxy-7-ethyl-3,11-dimethyl-2,6-*trans*-trideca-2-*trans*,10-*trans* dienote (Roche)
No. 29.	Methyl rac-10,11-epoxy-7-ethyl-3,11-dimethyl-10,11-*trans*-trideca-2-*cis*,6-*trans*-dienoate (Roche)
No. 30.	Methyl rac-6,7-epoxy-7-ethyl-3,11-dimethyl-6,7-*trans*-trideca-2-*cis*,10-*trans*- dienoate (Roche)
No. 31.	Methyl rac-6,7-epoxy-7-ethyl-3,11-dimethyl-10,11-*trans*-trideca-2-*trans*,6-*cis*- dienoate (Roche)
No. 32.	Methyl rac-10,11-epoxy-7-ethyl-3,11-dimethyl-10,11-*trans*-trideca-2-*cis*,6-*cis*-dienoate (Roche)
No. 33.	Methyl rac-10,11-epoxy-7-ethyl-3,11-dimethyl-10,11-*cis*-trideca-2-*trans*, 6-*trans*-dienoate (Dr. H. Roeller, No.440)
No. 34.	As No.33, contains about 8 per cent of 6-cus-compound, i.e. No.40
No. 35.	Ethyl rac-10,11-epoxy-7-ethyl-3,11-dimethyl-10,11-*cis*-trideca-2-*trans*,6-*trans*-dienoate (Dr. H. Roeller, No.409)
No. 36.	Methyl rac-6,7-epoxy-7-ethyl-3,11-dimethyl-6,7-*trans*-trideca-2- *trans* 10-*cis*-dienoate (Roche)
No. 37.	Methyl -7-ethyl-3,11-dimethyl-2-*trans*-6-*trans* 10-*cis*-tridecatrienoate (Dr. H. Roeller, No.390)
No. 38.	Ethyl rac-10,11-epoxy-7-ethyl-3,11-dimethyl-10,11-*cis*-trideca-2-*cis,6-trans*-dienoate (Roche)
No. 39.	Ethyl rac-6,7-epoxy-7-ethyl-3,11-dimethyl-6,7-*trans*- trideca-2-*cis* 10- *cis* -dienoate (Roche)
No. 40.	Ethyl rac-10,11-epoxy-7-ethyl-3,11-dimethyl-10,11-*cis*-trideca-2- *trans,6-cis*-dienoate (Roche)
No. 41.	Ethyl rac-6,7-epoxy-7-ethyl-3,11-dimethyl-6,7-*cis*-trideca-2- *trans,10-cis*-dienoate (Roche)
No. 42.	Ethyl rac-10,11-epoxy-7-ethyl-3,11-dimethyl-10,11-*cis*-trideca-2- *cis,6-cis*-dienoate (Roche)

MATERIALS AND METHODS

Materials and methods were applied according Porkodi *et al*., 2000 & Bhuvaneswari *et al.,* 2000. The methods are described below.

Topical Application

Topically applied compounds will not only affect the exoskeleton, but also act internally, as can be demonstrated by the delay of the metamorphic molt and the female "sterilization," particularly when fecundity is involved. Topical application can be used on all insect stages, including eggs. Sticking the eggs to tape, prior to application, can be a great help. Surface tensions in the solvent may create great practical difficulties with very small insect larvae, for which special techniques have to be worked out.

Substrate Treatment

Small insects can often be conveniently treated by allowing them to contact a surface treated with the compound. In aquatic larvae, water treatment expressed as parts per million is customary. Introduction in acetone solution even when larvae are present is acceptable provided that the ultimate acetone concentration does not exceed 1%. Many JHA are not soluble at high doses and may exert their effect on the surface layer, which will be frequented by many aquatic larvae and pupae for respiration. This may obscure the dose response curve for JHA with low activity.

Systemic Application

Certain compounds have been shown to have systemic activity when painted on stems and leaves of plants. Very careful elimination of contaminating factors (more specifically vapors) is an absolute requirement for this type of work.

Assays of Vapor Effects

Experiments set up to demonstrate vapor activity, have often been successful. Particularly in cases of very short sensitive periods, good evidence for a true vapor effect was obtained.

Spray Applications

The spraying of food plants with or without insects usually requires a water formulation in order to achieve a reproducible "run off" dose, to avoid phytotoxicity and adverse effects on the insects. Different compounds may require different formulations for optimal results but for reasons of comparison, standardization of the formulation is desirable. We found 1% of T. ween 20 and 1% acetone a practical formulation for most JHA.

Scoring and Evaluation

Every insect species and every stage will require a special scoring system unlike the evaluation of insecticides. Fortunately, often a test can be devised that uses a simple positive or negative score, for instance, where non emergence is a possible result.

Two types of scoring can generally be distinguished.

a. Graded Scoring

When different steps of intermediates can be recognized. Some of these scoring methods are published, new ones are devised frequently and they usually reflect the personal bias of the investigator. Rarely is such a scoring system immediately final after its inception. After more experience, modifications may be necessary. An unaffected animal should receive a zero score; a maximum observed response and not the maximum theoretical response should set the highest number, with the intermediates arbitrarily fixed in between. The total score can then be calculated as a percentage of the observed maximum and plotted on semi-logarithmic paper as log dose response curve. The intersection of this curve with the 50% response line, yields ID 50 response figure. If controls are positive (up to 20%), correction of all the values with Abbott's formula is desirable. With more than 20% positive response in the control, revising the assay will be necessary.

b. Quantal Scoring

If the results can be evaluated as clear cut positive or negative, the response percentage can be easily calculated and plotted. As attractive as this may seem, it has some inherent disadvantages compared with graded scoring. Graded scoring reveals more information and therefore requires fewer animals for the same variance in the end result. Unfortunately also, the result is the less specific it usually is for JH action. (*e.g.* the housefly pupal assay). Certain assays like the topical pupal assay in Galleria allow for both scoring systems simultaneously and experience teaches that they are usually reasonably well correlated. Yet we have to attach more significance to the graded score for an equal number of animals. Quantal scoring results lend themselves very well to prohibit conversion. Egg assays can usually be scored in both ways too, especially for those eggs in which the chorion can be cleared in Chlorox to allow for internal inspection. Half developed embryos may then be detected and unfertile eggs can be discounted. A bioassay scoring system should be restricted to one subsequent instar if possible. For instance, when treated larvae only partly develop into pupae, the assay should be scored on the pupae, however attractive it may be to wait until the present pupae have developed into adults. The effect on the adult stage should be the subject of a new and specially designed assay. The results are best expressed as the ID-50, IC-50, IC-95, *etc*., when applied to direct morphogenetic effects. For an evaluation of reproductive potential a comparison with a control group of animals will yield a percentage reduction in reproductive potential.

Statistical Procedures

The mean and the standard deviations were calculated from the determined values by using the standard procedures (Bailey, 1984). The standard deviations were calculated by using the formula.

$$S = \frac{(X_1 - \bar{X})^2}{n - 1}$$

Where X_1 = value of individuals
X = mean value of the sample
n = number of samples
In order to examine whether the difference in results obtained was significance or not the following formula (Student's `t' test) was employed.

$$t = \frac{X_1 - \bar{X}_2}{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}$$

Where $\bar{X}_1$ = mean value of one sample
X_2 = mean value of other sample
S_1 and S_2 = are corresponding standard deviations
n_1 and n_2 are the number of tests for each sample
The level of significance (P-value) between X_1 and X_2 was determined by using the students `t' distribution table of Fractiles and critical values (Radhakrishna Rao *et al.,* 1985).

RESULTS

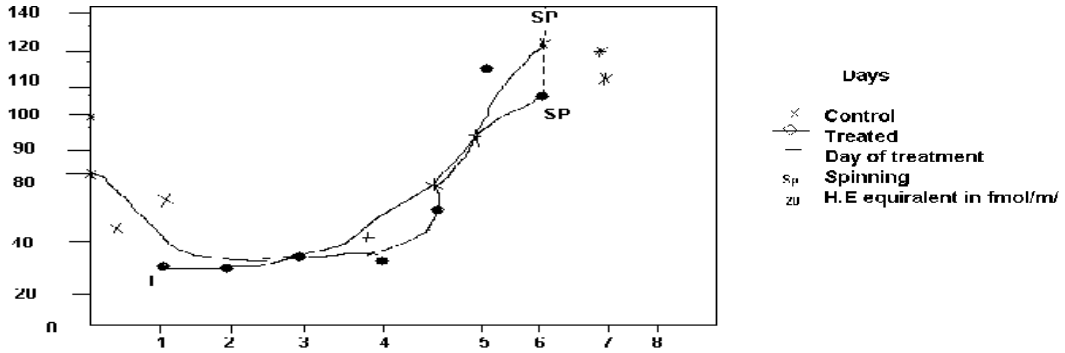

FIGURE 1. Effects of R394 (0.031 nl per larva) on ecdysteroid titre of haemolymph after fourth moult of silkworm, *Bombyx mori*, at 24 hours.
Values given are the mean value (X) of 4 data's
d.f. = degrees of freedom = n-1
Significance ++ = $p < 0.001$, + = $p < 0.05$, NS = Not significant

Figure 1 to 5 indicates the effect of R394 on ecdysteroid titre of haemolpmph after fourth moult of silkworm, *Bombyx mori* at 24, 48, 72, 96 and 120 hours. The hormone assay reveals in control 5th instar larvae ecdysteriod titre decreased significantly f mol/ml on day 0 to 32 f mol/ml on day 1 after 4th ecdysin. From the 6th day it increases.

Treatment of 24 hours: - Ecdysteriod titres of control and treated larvae didi not differe significantly fill day 6 significantly low titres were observed on day 7 in treated larvae (96 f mol /ml). The larvae started spinnig and formed cocoons without prolonging larval duration. Treatement at 48-96 hours: - The haemolyroph ecdysteroid lervels in R394 treatment at 48, 72 and 96h were significantly low on each day when compared to control (Fig. 1, 2, 3). Despite these low levels, the treated larvae stacted spinning on 8th day and formed cocoons. The cocoon shell weights were 9, 70, 3 and 3% higher in 48, 72 and 96h respectively then that in the control Table 9. Thus the ecdysteriod pead was delayed by about 1 day and hence the feeding period was lengthered by one day.

Treatment at 120 hours :- Treatment at 120 h induced no significant difference in ecdysteroid titre from control fig. 14 cocoon shell weight (0.457g) was not significantly altered and there was no prolongation in larval duration.

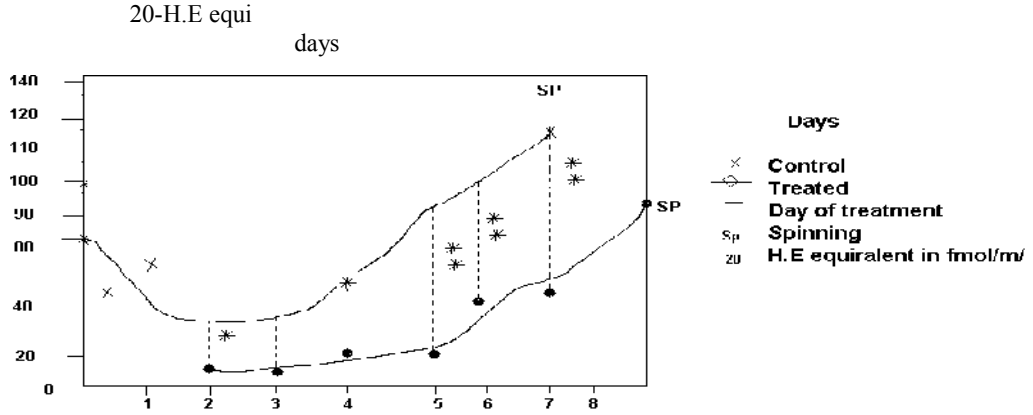

FIGURE 2. Effects of R394 (0.031 nl per larva) on ecdysteroid titre of haemolymph after fourth moult of silkworm, *Bombyx mori*, at 48 hours.

Values given are the mean value (X) of 4 datas
d.f. = degrees of freedom = n-1
Significance ++ = $p < 0.001$
+ = $p < 0.05$
NS = Not significant

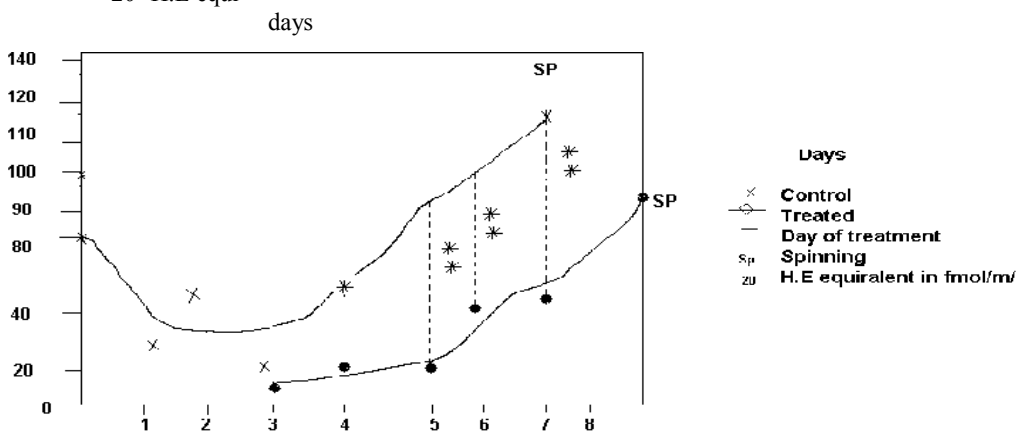

FIGURE 3. Effects of R394 (0.031 nl per larva) on ecdysteroid titre of haemolymph after fourth moult of silkworm, *Bombyx mori*, at 72 hours.

Values given are the mean value ($\bar{X}$) of 4 data's
d.f. = degrees of freedom = n-1
Significance ++ = $p < 0.001$
+ = $p < 0.05$
NS = Not significant

20-H.E equi

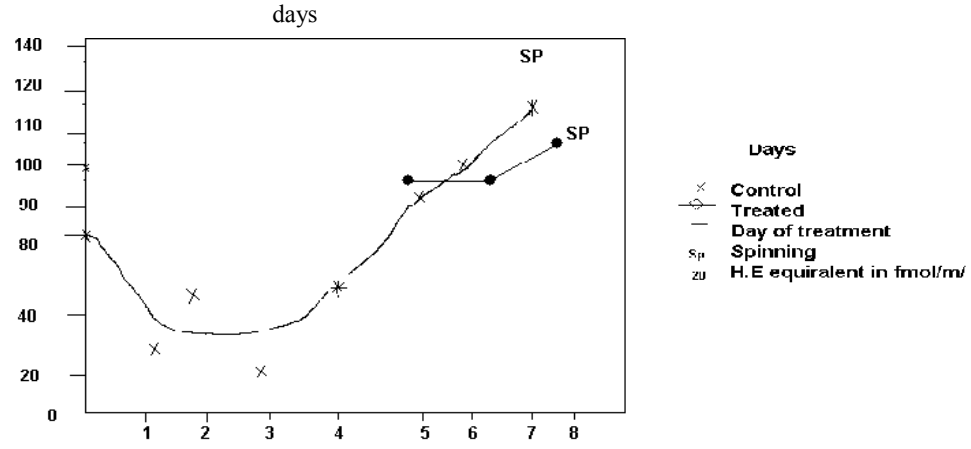

FIGURE 4. Effects of R394 (0.031 nl per larva) on ecdysteroid titre of haemolymph after fourth moult of silkworm, *Bombyx mori*, at 96 hours.

Values given are the mean value ($\bar{X}$) of 4 datas
d.f. = degrees of freedom = n-1
Significance ++ = $p < 0.001$
+ = $p < 0.05$
NS = Not significant

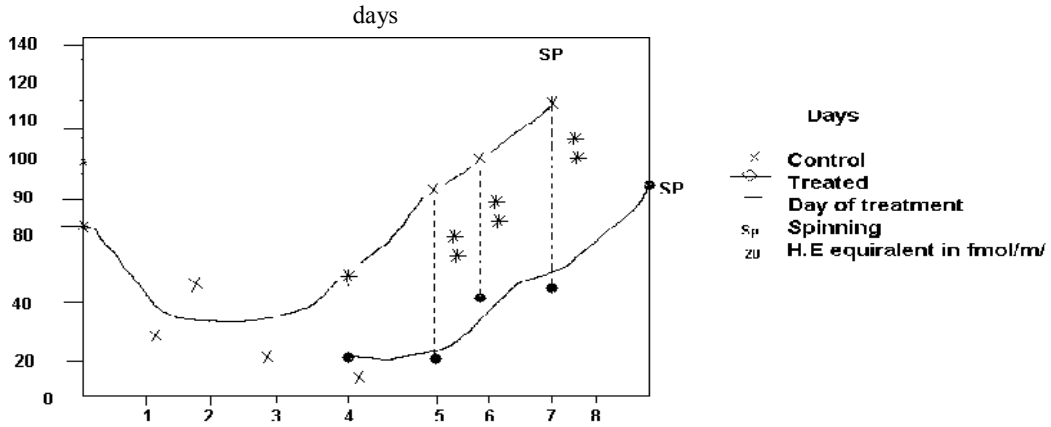

FIGURE 5. Effects of R394 (0.031 nl per larva) on ecdysteroid titre of haemolymph after fourth moult of silkworm, *Bombyx mori*, at 120 hours.

Values given are the mean value ($\bar{X}$) of 4 datas
d.f. = degrees of freedom = n-1
Significance ++ = $p < 0.001$
+ = $p < 0.05$
NS = Not significant

DISCUSSION

The interaction between effects of juvenile hormone and moulting hormone at the molecular level poses a particular challenge in insect endocrinology. Each hormone does not necessarily have a single primary site of action, and may function by effects at several levels of organization. However, current evidence indicates that most steroid hormones apparently exert their main action by controlling transcription of specific genes, thus inducing protein synthesis. The mechanism has been explained by several models. The mechanism of controlling gene expression in eukaryotic organisms has proved recalcitrant, since recent findings have led to re-appraisal of the structural organization of chromatin itself. These findings will undoubtedly influence substantially future ideas regarding the molecular mechanism of gene switching by hormones. Juvenile hormones and ecdysterrids play an important role in the regulation of growth and reproduction of Insects. The vertebrate hormones like prolactin (PRC), thyroxine (THY). Insulin (INS) and other pituitary entracts caused shortening of larval duration, increased the larval, silk gland weights and fecundity of the silk worm. Is reported that thyroxine works more efferinely when applied to 2nd instar larvae causing the enhancement of haemolymph pralines and ecdysteroid levels in the silkworm Charlet *et al.,* (1979) reported that vertebrate gonadotrophins such as follicular stimulating hormone (FSH), Leutinizing hormone (LH), and human chorionic gonadotrophic hormone (HCG) induce steroid biosynthesis in the gonads of insets. However, the precise mechanisms of action of

the vertebrate hormone in invertebrates remain to be understood. It may be a direct effect up on body cells or regulations of endocrine gland secretions or by both.Hence, presence of JH or haemolymph appears to be obligatory to bring about acceleration of tissue protein levels. The in vitro studies clearly demonstrate that vertebrate hormones either individually or collectively do not seem to be directly acting at the tissue but act indirectly through the mediation of circulating hormones of the insects.

The effect of Juvenile hormone was studied in order to develop a practical method for increasing silk output. The time (developmental stage) the dose and the method of application of hormone in the present experiment was based on results of previous reports. In the present experiments it ranged from 15 hours to 30 hours in different breeds and it should be thought that the sensitivity is races specific. Improvement in cocoon weight ranged from 2.43% to 10.66% in different breeds. This is lower than that reported earlier (Ching-fun chang *et al.,* 1972). Apart from the differences in the breed, dose and mode of application also might have caused this difference, improvement in cocoon layer also varied in different breeds. The larval prolongation was a common feature further increase in body weight, cocoon weight; shell weight was not proportional in breeds as a whole. Thus in pure Mysore, with the maximum increase in larval period by 30 hrs, the gain was 93 mg, 40 mg and 10 mg in body cocoon and shell weight respectively. The wide differences suggested that the sensitivity to the treatment is specific to the silkworm breed. While observing the survival capacity, the pure Mysore and Hosa Mysore were found to be on power with the control. In all other breeds, the survival rate was very low. When compared to control continued growth resulting in malformation and death without cocoon spinning, have been reported by (Ching-fun change *et al.,* 1972) either due to higher dosage or repeated application of Juvenile hormone.

Results of absolute silk yield show, a few races only maintain positive effect throughout. Productive races and hybrids responds better to the treatments screening of silkworm races or hybrids for sensitivity to the treatment is necessary to get optimum results in improvement of silk yield commercial application of the technique should prove quite viable. To exploit commercially the application of Juvenile hormone further experiment on selection of breeds. Improvement of viability, effective method for mass application is necessary. The results of the present investigation experimented by Table 3 to 8 reveals clearly that, the natural and synthetic bioactive compounds mimicking the Juvenile hormone activity can be judiciously explored for the benefit of the sericulture industry of the 24 compounds tested nearly 21 compounds show good responds. They are reported similarly to have bioactivity in silkworm (Sehnal and Akai, 1990 and Saslindran *et al.,* 1999). The present results let as to the conclusion that the JH mimicking compounds influence the silk production positively the results based on the dose dependency and the period of time application (Akai *et al.,* 1988; Trivedy *et al.,* 1993).When the compounds are applied in silkworm, wherever, the improvement in cocoon weight and shell weight was noticed, the larval weight also improved. It was there was understand that if the ingested food is properly converted to body matter under the influence of the exogenous JH compounds the body weight may go upto a certain extent and get further converted to the pupa and the shell this may be because, all the individual worm in each batch may not be in a compatible physiological status to make use of the exogenous the JH dose, instead, might have resulted in some mortality. Similar mortality reports was observed by Magadum and Hooli (1991), when Plant growth regulators are applied to silkworm. From the experiments it is clear the application of R394 and BPE extended the larval feeding which indirectly increases the silk production (Akai and Kobayashi, 1971).This increase of feeding may have stimulatory effect on protein synthesis in silk gland. This opinion falls in line with the observation of Brindha *et al.,* 2012. This suggest that the possibility of converting the ingested food for the silk synthesis, due to the changes in the physiological or molecular level alteration in the ratio of the circulating hormone from this it may be concluded that the Juvenile hormone analogues or mimics regardless of the source can be used in the sericulture industry for the improvement in yield of 24 hours compounds tested, five compounds namely NL13, NL24, BK, BPE and R394 were effective in increasing the silk of the silkworm R 394 is a very strong Juvenoid and the response of the silkworm to this compound is largely dose dependent. The stronger concentration either resulted in the formation of larvae or in pupal mortality. This may be due to the total disturbance in the endogenous hormone titres and concomitant derangement in the tissue metabolic activities. But lighter concentration though had its effects on the metamorphic rhythm and economic traits, did not altogether effect the spinning activity. It is vivid from the data that the improvement in cocoon traits in the larval treated at 24h was not a result of extended feeding period as in 48, 72 and 96 h treated larvae. The might be due to direct stimulatory effect of R394 on protein synthesis in silk gland. Another possibility is the increased efficiency of treated batches in the conversion of ingested food so that more silk is synthesized from unit quantum of food under the influence of Juvenoid. This result is to be considered remarkable because the increase in the silk synthesis is without prolonging the larval duration or without any other side effect on larval development. However, this result does not corroborate earlier reports where improvement of cocoon weight always occurs with prolongation of larval duration (Akai *et al.,* 1971; Shimada *et al.,* 1979). Presently the larvae became larger and silk production was enhanced as reflected by improvement in cocoon shell weight. But the treatment at 120 h proved ineffective. The changes in the levels of ecdysteroid in control larvae follow the normal pattern as observed by Calvez *et al.,* (1976) Akai *et al.,* (1988).

The present study shows that the response of silkworm to R 394 with regard to haemolymph ecdysteroid titres is also dependent on the time of application as the pattern of daily variation in the hormone titre was not alike in all the treated batches. JH on one hand is known to exert an inhibitory action on prothoracicotropic harmone (PTTH) release from brain and ecdysone synthesis by the prothoracic gland (PG) in early 5^{th} instar larvae of Bombyx *mori* which causes prolongation of larval duration (Sakurai *et al.,* 1989) On the other hand, JH is claimed to inhibit protein synthesis in early treated larvae which later on regain protein synthesis resulting in bigger

silk gland. This results in improvement of cocoon shell weight (Gangwar, 2009). The present result with R394 could be explained by the balance of these two effects on ecdysteroid titre and silk gland. This balance is different according to the time of R394 application as the effects of Juvenoids were different with physiological age. The above facts are epitomized in the present experiment. Treatment at 24 h of V instar though did not stimulate can extension in the feeding period, increased silk protein synthesis. Here, the threshold ecdysteroid peak required to elicit spinning process was not delayed by the exogenous JH as in the other batches. It was earlier reported that JH application to early last instar larvae lowers the threshold of PG sensitivity to PTTH while treatment later in the instar stimulates it (Cymborowski and Stolarz, 1979) following allatectomy, precocious ecdysteriod peak was observed. This confirms the role of endogenous JH to delay the appearance of ecdysteroid peak in *Bombyx mori* (Sakurai *et al.,* 1989).

The present results confirm the existence of a critical period for JH mimic treatment from one day to fourth day after fourth moult. Low dose of R 394 reduced ecdysteroid levels and improved cocoon shell weight. The fluctuation pattern of haemolymph. Ecdysteroid in the larvae treated at 24h exhibited a modest deviation from that of the rest and the cocoon shell weight improvement was accompanied by only a slight decrease in the basal ecdysteroid level. Therefore, the critical periods for both the effects were slightly different. It could be concluded that the effects of JH mimic on spinning were not totally dependent on the effects of ecdysteroid production.

REFERENCES

Akai, H., Takabayashi, K., and Kiuch, M. (1988) Induction of spinning from JH treated eternal larvae of *Bombyx mori* by ecdysteroid administraton. J. Seric. Sci. Jpn, 57, 341-344.

Akai, H., and Kobayashi, M. (1971) Induction of prolonged larval instar by the juvenile hormone in *Bombyx mori* L (Lepidoptera: Bombycidea) Appl. Entomol. Zool, 6, 1938-1939.

Bhuvaneswari, S.D., Mahalingam, S. and Annadurai, B. (2000) Mechanism of Regulation of Juvenile hormone biosynthesis in *Bombyx mori*,Thesis submitted to University of Madras,Chennai

Brindha, S., Maragathavalli, S., Gangwar, S. K. & Annadurai, B. (2012) Effect of juvenile hormone on enhanced silk production in *Bombyx mori. IJABR., Vol.*2(3):

Calvez, B., Hirn, M., and De Reggi, M. (1976) Ecdysone changes in the hemolymph of two silkworm (*Bombyx mori* and *Philosamia cynthia*) during larval and pupal development Febs. Lett., 71,57-61.

Charlet, M., Goltzene, F., and Hoffman, A. (1982). J. Insect. Physiol., 25, 463-466.

Ching-Fun-Chang, Shigeo murakorshi and Saburo Tamura (1972) Giant cocoon formation in the silkworm *Bombyx mori* L., Topically treated with Methylenedioxy phenyl derivatives. Agr. Biol. Chem, 36, 692-694.

Cymborowski, B. and Stolarz, G. (1979) The role of juvenile hormone during larval pupal transformation of spodoptera littoralis. Switch over in the sensitivity of the prothoracic gland to juvenile hormone. J. insect. Physiol, 25, 939-942.

Gangwar, S. K. (2009) Effect of juvenile hormone mimic R394 on silkworm, *Bombyx mori* L., growth and development of silk gland. *ARPN Journal of Agricultural and Biological Science*. Vol. 4(6), pp .65-67.

Magadum, S. B. and Hooli, M.A. (1991) Effect of Giberrellic acid on the economic parameters of the Silkworm, the pure Mysore breed of *Bombyx mori* L, Sericologia, 31, 667-676.

Porkodi, S. Mahalingam,, S. Annadurai, B. (2000) Studies on the physiology of internal fertilization of *Bombyx mori*, Thesis, University of Madras (Vellore Extension).

Roller, H., Bjerke, J.S. and W. H. MCS Han (1965) The Juvenile hormone – I Methods of purification and isolation. J. Insect. Physiol, 11, 1185-1197.

Roller, H., Dahm, K.H. (1970) Naturwiss, 57, 458.

Sakurai, S., Okuda, M. and Ohtaki,T. (1989) Juvenile hormone inhibits ecdysone secretion and responsiveness to prothoraciccotropic hormone in prothoracic glands of *Bombyx mori*, Gen. Comp., endocrinol,75,220-230.

Sashindran Nair, K., Vijayan, V. A., Jula, S, Trivedi, K. (1999) Juvenilomimic compounds for enhanced productivity in silkworm, *Bombyx mori* L. A screening.Indian J.Seric, 38,119-124.

Sehnal, F, and Akai, H. (19900 Insect silk glands: Their types, development and function and effects of environmental factors and morphogenetic hormones on them. Int. J. insect morphol. Embryo., 19,79-132.

Shimada, S., Kamada, A. and Asano, S. (1979) Studies on the utilization of methoprene a synthetic compound with juvenile hormone activity, for the silkworm rearing.II an aspect of silk formation in the silkworm, *Bombyx mori*, applied with methoprene examined by the vital staining with thione. J. seric. sci., Jpn, 48, 282-286.

Trivedi, K., Remadevi, O. K., Magadum, S. B. and Dutta, R. K. (1993) Effect of juvenile hormone analogue on the growth and economic character of silkworm *Bombyx mori L.*, Indian.J.seric, 32,618-624.

Trivedi, K., Sashindran Nair, K., Ahsan, M. M., Datta, R. K. (1997) A juvenile hormone mimic modulated enhancement of silk productivity in silkworm, *Bombyx mori* L, Indian. J. Seric, 36, 35-38.