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EFFECT OF JUVENILE HORMONE ON ENHANCED SILK PRODUCTION IN *BOMBYX MORI*

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ABSTRACT
Twenty four Juvenile hormone mimic compounds procured from various sources were screened through silkworm to determine their effect on Silkworm in enhancing the silk production, along with duration of application, concentration of Juvenile hormone and its methods. They were applied to the local silkworm variety for about 24, 48, 72 and 96 hours of 5th instar as a single dose topically and bioassay in 5 batches. Six different concentration of each of the compounds were tried medium control and absolute control was maintained in parallel of the 24 compounds, R77 and W328 were not found to be suitable for sericulture industry. Other compounds were found to be bioactive in exogenous administration, five compounds *viz*., NL-13 (ω-formyl longifolene oxiome propargyl ether) NL 24 (ω-formyl longifolene oxime citronellyl ether), BPE (BPE epoxide) BK (non polar fraction of *Psoralea corylifolia* and R394 (Ethyl 9-cyclohexyl – 3,7-dimethyl-2,4-non adienoate) had been found to influence silkworm growth and economic traits to a significant extent. The cocoon shell weight was enhanced by over 20%. The utility of the bioactive juvenoid compounds in mass scale application and their physiological and economical importance are discussed. 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 µl 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 µl 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.

KEY WORDS: Juvenile hormone, instar, cocoon weight, shell weight, *Bombyx mori* 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.,* 1983).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, Trivedy *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 phyto-juvenoids 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.

MATERIALS AND METHODS

Topical Application, Substrate Treatment, Food Treatment, Systemic Application, Spray Applications, Scoring and Evaluation, Graded Scoring and Quantal Scoring were done according to Bhuvaneswari and Annadurai (2001)

Statistical Procedures

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

TABLE 1. Effect of juvenile hormone analogue (ZR 512) in silkworm (Mean ± SD)

Races		Vage larval duration (hrs)	Larval wt (g)	E.R.R. (%)	Cocoon weight (g)	Shell wt (g)	Absolute silk yield (g)
Pure	C	232 ±16	1.406±0.021	82.00	0.840±0.002	0.230±0.002	1016±75
Mysore	T	260±14	1.750±0.020	72.00	0.860±0.010	0.160±0.002	1168±52
Hosa	C	186±3	2.763 ± 0.025	76.66	1.152±0.007	0.122±0.003	1024±49
Mysore	T	230±4	2.893 ± 0.031	67.33	1.180±0.019	0.238±0.007	2142±76
NB4D2	C	155±8	2.797±0.015	66.00	1.380±0.002	0.228±0.003	2046±33
	T	220±9	2.693±0.035	82.00	1.299±0.005	0.248±0.002	2182±45
NB7	C	166±5	3.587±0.042	85.33	1.306±0.005	0.286±0.004	2436±49
	T	181±2	3.797±0.095	78.00	1.423±0.008	0.288±0.002	2322±77
NB18	C	171±6	3.700±0.070	78.00	1.325±0.002	0.225±0.005	2218±104
	T	198±8	3.657±0.040	84.00	1.508±0.008	0.280±0.010	2612±136
PMXNB4D2	C	134±2	3.180±100	96.46	1.320±0.007	0.240±0.007	2312±38
	T	162±2	3.265±100	94.10	1.460±0.010	0.260±0.005	2541±19

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

Table 1. Shows the effect of application of juvenile hormone analogue (ZR 512) in silkworm. From this, Vage larval period-larval weight, effective rate of rearing cocoon weight, shell weight and absolute silk yield was observed. In the Vage larval period, the maturity delayed was observed in all the breeds and the hybrids treated with juvenile hormone. It ranged from 15 to 30 hours. It was 24 hours in Hosa Mysore, 26 hours in NB_{18}, 28 hours in pure Mysore. The difference in the larval duration between the control and treated batches was very significant. Corresponding to the prolongation to the larval duration and increasing in growth indicated by the body weight was observed in all the breeds and hybrids. Maximum increasing was observed in NB_7, followed by pure meagre, Hosa Mysore NB_4D_2, in percentage, In effective rate of rearing significant differences between the control and treatment were observed. The treatment affected the survival rate adversely in most of the breeds and hybrids. The survival rate did not differ much in races of pure Mysore and Hosa Mysore. In NB_{18} treated batches survived better than control. The disadvantage in the survival rate due to treatment was comparatively less in hybrids than in pure races in general.

In the cocoon weight irrespective of the breeds and hybrid combination, the hormone treatment resulted in heavy cocoon weight. The difference among breeds and between treatment and control are highly significant. The improvement in cocoon weight ranged from 2.43% to 10.66% over the control. The treatment was effective in the races of Hosa Mysore, NB_4D_2, NB_7 and the hybrids. In pure races, the cocoon weight and 6.35% whereas in hybrids it was 10.25%

The shell weight was highly significant between treatment and control. In all the cases of breeds and hybrids. Among the pure breeds treatment was most effective in Hosa Mysore (16.21%) followed by NB7 of (12.03%) NB_4D_2 (10.68%) pure Mysore (7.69%). In the absolute silk yield the treatment improved the silk yield by 8.89% . It was nearly 12% increase in NBT; 5% increase in pure Mysore and NB_4D_2. Maximum amount of silk is observed in NB_{18} (17.40%).

Table 2 reveals the bioassay of juvenile hormone mimics on silkworm. The compounds are bioactive when administrated to silkworm. The response of the silkworm is different for different compounds and percentage as the medium control did not show any significant differences in performances from that of absolute control; the same was not included in the Table. In Table 2, seven compounds namely NL1, NL2, NL4, NL6, NL8, NL10, NL22 was applied and the improvement of cocoon weight ranged from 2 to 14%, cocoon shell weight.

Table 3 indicates the second batch of six compounds namely NL12, NL13, NL15, NL19, NL21, NL24. The major and most important economic trades improved quite remarkably in the case of NL13, and NL24.When treated with 5 ppm of these compounds at 48 hours, of 5^{th} instar, the increase in the cocoon weight and shell weight in case of NL24,it is 13.22% and 23.70.Likewise,there is an increase of larval weight and shell ratio was noticed in NL 12 and NL 13.

Table 4 present bioassay of screening of Juvenile hormme mimics on silkworm *Bombyx mori* L. of III batch. Here ATI, BK and BPE compounds induced high bioactivity in silkworm enhancing the cocoon shell weight by 8 to 22% of this 3 compounds BK and BPE performed well facilitating the choice of these compounds for mass scale trial on silkworm. BK was most active of the concentration of 1.25 ppm at 48 hours. Whereas BPE was most effective at 0.25 µl/ml at 24 hours at 5^{th} instar. These two compounds enhanced all characters except the shell ratio. The tune of increase of larval weight was 17.21% that of survival was 16.18%. Cocoon weight 16 to 17% and that of the shell weight was 22% when the silkworm was treated with BPE, the 5^{th} instar larval period was prolonged by 24 hours.

TABLE 2. Bioassay (screening of juvenile hormone mimics on silkworm) *Bombyx mori* L.

Name of the compound	Treatment (h) V instar	concn (ppm)	Larval Wt (g)	% difference	Survival (%)	% Difference	Cocoon Wt (g)	% Difference	Shell wt (g)	% difference	Shell ratio	% differenc e
Ibatch												
NL1	48	5	40.26	2.17	79.50	-2.62	2.125	7.06	0.389	10.73	20.28	2.80
NL2	24	20	40.69	8.76	82.50	-2.62	1.879	5.14	0.380	8.43	20.12	2.99
NL4	48	1.25	42.37	11.29	91.00	3.16	2.123	10.37	0.416	10.48	19.80	-0.06
NL6	48	5	40.45	1.18	90.50	-0.34	1.796	4.46	0.413	9.19	20.76	3.84
NL8	48	20	40.06	-2.05	86.00	-4.15	2.128	11.85	0.422	11.49	19.75	-0.85
NL10	48	20	40.67	3.24	90.50	-0.44	1.828	1.65	0.424	5.02	19.98	2.80
NL22	48	1.25	41.03	3.98	89.00	-2.07	2.187	12.48	0.442	14.37	20.16	0.63
Control	-	-	40.19	-	91.70	-	1.795	-	0.386		19.95	
	SE ±		0.348		0.591		0.032		0.004		0.282	
	CD at 5%		1.064		2.107		0.065		0.012		0.728	
	CD at 1%		1.701		2.922		0.086		0.018		0.924	

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

TABLE 3. Bioassay (screening of juvenile hormone mimics on silkworm) *Bombyx mori* L.

Name of the compound	Treatment (h) V instar	Concn (ppm)	Larval Wt (g)	% difference	Survival (%)	% Difference	Cocoon Wt (g)	% Difference	Shell wt (g)	% difference	Shell ratio (g)	% difference
II Batch												
NL12	48	1.25	63.29	5.25	93.50	13.86	2.204	9.64	0.450	20.42	1.34	
NL13	48	5.00	52.97	12.98	92.80	12.05	2.319	15.36	0.502	21.65	7.45	
NL15	48	1.25	58.47	7.96	87.80	8.43	2.188	8.84	0.456	20.82	3.33	
NL19	72	20.0	54.78	10.84	76.80	-7.23	2.089	3.92	0.439	21.02	4.31	
NL21	72	1.25	55.89	11.25	81.50	0.60	2.173	8.12	0.464	21.33	5.85	
NL24	48	5.0	56.80	13.04	83.80	1.20	2.278	13.32	0.501	21.99	9.13	
Control	-	-	48.67		78.00		1.980		0.395	19.95		
	SE ±		0.345		0.529		0.012		0.003		0.132	
	CD at 5%		1.062		1.632		0.037		0.008		0.408	
	CD at 1%		1.489		2.288		0.053		0.011		0.572	

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

TABLE 4. Bioassay (screening of juvenile hormone mimics on silkworm) *Bombyx mori* L.

Name of the compound	Treatment (h) V instar	conc. (ppm)	Larval Wt (g)	% difference	Survival (%)	% Difference	Cocoon Wt (g)	% Difference	Shell wt (g)	% difference	Shell ratio (g)	% difference
III Batch												
AT1	24	10	46.85	12.75	85.00	6.75	1.970	7.85	0.435	8.97	22.86	1.87
BK	48	48	54.85	25.15	98.50	18.75	2.125	17.75	0.469	22.06	23.65	1.95
BPE	24	0.25µl/ml	49.75	18.60	88.45	15.65	2.215	16.95	0.487	21.75	22.95	5.43
Control	-		42.45		84.50		1.850		0.356		20.65	
	SE ±		0.316		2.668		0.0085		0.087		0.540	
	CD at 5%		1.264		8.765		0.165		0.097		1.265	
	CD at 1%		1.854		12.452		0.245		0.065		1.780	

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

TABLE 5. Bioassay (screening of juvenile hormone mimics on silkworm) *Bombyx mori* L.

Name of the compound	Treatment (h) V instar	concn (ppm)	Larval Wt (g)	% difference	Survival (%)	% Difference	Cocoon Wt (g)	% Difference	Shell wt (g)	% difference	Shell ratio (g)	% difference
IV Batch												
AT4	24	10	47.05	10.98	82.75	6.24	1.765	7.84	0.516	12.96	23.15	5.65
AT6	24	10	45.67	9.74	78.97	8.65	1.659	7.56	0.526	13.87	22.96	6.76
AT7	24	10	45.82	8.87	84.75	10.92	1.786	7.98	0.512	14.25	23.08	6.74
AT8	24	10	45.25	9.65	79.65	9.75	1.987	5.78	0.498	11.89	23.06	5.95
AT9	48	10	45.90	4.85	76.84	4.95	1.957	5.95	0.412	10.96	23.74	4.75
Control	-	-	42.75		76.24		1.875		0.365		21.08	
	SE ±		0.564		1.265		0.098		0.004		0.265	
	CD at 5%		1.468		3.455		0.078		0.007		0.765	
	CD at 1%		1.897		5.165		0.086		0.017		1.154	

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

TABLE 6. Bioassay (screening of juvenile hormone mimics on silkworm) *Bombyx mori* L.

Name of the compound	Treatment (h) V instar	conc. (ppm)	Larval Wt (g)	% difference	Survival (%)	% Difference	Cocoon Wt (g)	% Difference	Shell wt (g)	% difference	Shell ratio (g)	% difference
V Batch												
R394	72	0.3125µl/	48.98	14.65	95.75	8.75	2.542	19.85	0.486	21.35	21.96	1.97
Control	-	ml	43.15		85.65		1.896		0.432		20.98	
	SE ±		0.312		0.985		0.007		0.003		0.132	
	CD at 5%		0.875		2.646		0.032		0.006		0.338	
	CD at 1%		1.234		3.675		0.045		0.007		0.465	

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

Table 5 explains the bioassay of screening of Juvenile hormone hormone mimics on silkworm is *Bombyx mori*. Here the 4th batch of five compounds namely AT4, AT6, AT7, AT8 and AT9 elicited moderate inducement in silkworm. they are active at 10 ppm concentration at 24 hours. The maximum response was observed in improvement in shell weight upto 14%.

Table 6 indicates the bioassay of screening of Juvenile hormone mimics on silkworm in *Bombyx mori*. Here three Juvenoids namely, R77, W328 and R394 were screened. These Juvenoids appeared at be very strong and continuous effects to bring down the concentration of R77 and W 328 which the silkworm with lands failed even with a concentration as low as $2X10^{-8}$ mg/µl. The larval period on administration of these compounds continued upto 12 days without any sign of spining and ultimately the larva died, but R394 was proved to be affordable to silkworm on exogenous administration at a very low dose. The dest result accured when a concentration of 0.3125 µl of this compounds was applied at 72 h of 5th instar. By this an improvement of about 18% of cocoon weight and 20% in cocoon shell weight was observed.

From the five batches of application the following five compounds can be short listed for further trials on a mass scale.

1. NL13 - ω - Formyl longifolene oxime propargyl ether.
2. NL24 - ω - Formyl longifolene oxime citronellyl either
3. BPE - ω- BPE epodide
4. BK – Nm. Palar fraction of Psoralea corylifolia
5. R394 – Ethyl of cyclo Redyl – 3, 7-dimethyl -2, 4 – non adienoate

TABLE. 7. Effect of JH mimic (R394) (0.031 nl/larva) on 5th instar larvae of *Bombyx mori*.

Hours of treatment (V instar)	V instar larval duration (h)	Cocoon wt (g)	Cocoon shell wt (g)	Shell ratio (%)
24	168	2.526	0.617	21.24
48	192	2.437	0.621	22.54
72	192	2.412	0.489	23.65
96	192	2.328	0.476	21.43
120	168	2.287	0.468	21.74
control	168	2.198	0.461	21.23
SE	1.854	0.045	0.006	0.24
CD at 1%	7.463	0.036	0.075	0.62

A total of 30 cocoons were assesed for each treatment

d.f. = degrees of freedom = n-1, Significance ++ = $p < 0.001$, + = $p < 0.05$, NS = Not significant

Table7 shows the effect of JH mimic (R394) on 5th instar larvae of *Bombyx mori*. The application of 0.1nl of R394 for 24 hours resulted in the improvement of cocoon weight by 13% and cocoon shell weight by 8% without prolonging larval period. When the same dose was applied for 48, 72 and 96 hours cort prolongation by one day with 5 to 8% improvement in cocoon weight and 3.10% in shell weight. When the same was treated for 120 hours, no effect on shell weight and larval duration.

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), 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. 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 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 mechanism of action of the vertebrate hormone in invertebrates remains 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 chang *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 Sashindran *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 Akai and Kobayashi, 1971. 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 dauer 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 convertion 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.,*1973; 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 otherhand, 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. 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 effect of Juvenoids is 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 ecdystervoid 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.,* 1983).

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 period for both the effects was slightly different. It could be concluded that the effects of JH mimic on spinning were not totally dependent on the effects of ecdysteroid production.

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