



EFFECT OF JUVENILE HORMONE MIMIC R394 ON SILKWORM (*Bombyx mori* L.) GROWTH AND DEVELOPMENT OF SILK GLAND

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ABSTRACT

The effect of a 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 has been studied. JH R394 ranging from 1.0, 0.1, 0.01 μ l / larva was applied at 48, 72 and 96 hours of V instar larva that was improved the cocoon weight, shell weight and shell ratio etc. The most effective dose observed 0.01 μ l / larva, when applied at 48 and 72 hours after resumption of the last moult. It was observed that the development of the silk gland takes place up to a particular period, after that the larval development increases due to the increase of the feeding period without any significant increase in silk and silk ratio.

Keywords: silkworm, juvenile hormone, cocoon, larva, pupa, growth.

INTRODUCTION

Application of high doses of juvenile hormone (JH) mimics induces prolongation of last instar larval duration of *Bombyx mori* L. [1-4]. Several workers [5-10] have reported that juvenile hormone (JH) and their derivative can be used as growth regulators of the commercial silkworm to increase silk yield. Akai [6] reported 30 % increase in shell weight on application of synthetic hormone CI8. Chang [7] observed a 20 % increase in cocoon and pupal weight with big cocoon formation when the larvae were treated with methylenedioxy phenyl derivatives. Chowdhary [11] reported that 21 % increase in silk yield and more than 40 % increase in silk ratio on application of JH, SJ-42-F. Use of R394 at 10-100 μ g to 7th instar larvae of *Galleria mellonella* on 3rd - 6th day led to imperfect super larvae [12] But with less dosage (0.1 - 1 μ g / larva), the larval growth was prolonged by 2 or 3 days and silk protein biosynthesis was increased with corresponding increase in cocoon and shell weights [5, 2, 13, 14]. The present study was conducted to observe the effect of exogenous JH mimic R394 (ethyl 9-cyclohexyl-3, 7-dimethyl-2, 4--nonadienoate), a strong juvenoid which acts on the growth and silk yield of *Bombyx mori* L. [15].

MATERIALS AND METHODS

An experimental study was carried out in Babasaheb Bhimrao Ambedkar University, Lucknow. The larvae of the breed KA x NB₄D₂ were reared on mulberry leaves in the laboratory at 23 $\pm$ 1°C and 70 $\pm$ 5% relative humidity [16]. Silkworms of equal size and weight were selected after the fourth ecdysis. The JH hormone compound was dissolved in alcohol and prepares three concentrations, i.e. 1.0 μ l/ 10 μ l; 0.1 μ l/10 μ l and 0.01 μ l/ 10 μ l was prepared and applied on the abdominal tergum of the larva using a 100 μ l micro-syringe controlled by a micro-applicator. Silkworms were taken in batch consisted of three replicates. Each replicate had 50 silkworms and treated with R394 ranging from 1.0 μ l/10 μ l; 0.1 μ l/10 μ l and

0.01 μ l/10 μ l at 48, 72 and 96 hours, respectively after resuming from the fourth ecdysis. The control larvae were treated with 10 μ l / larva and observed fifth instar larval duration and cocoon and shell weights were recorded.

RESULTS

The applications of juvenile hormone (JH) mimic R394 (ethyl 9-cyclohexyl-3, 7-dimethyl 1-2, 4-nonadienoate) on silkworm 5th instar larva is presented in Table-1. These data shows that the time of application i.e. 48, 72 96 hours and the test doses i.e. 1.0 μ l/10 μ l; 0.1 μ l/10 μ l, 0.01 μ l/10 μ l were highly significant with respect to the larval period, larval prolongation, survival percentage, cocoon weight, pupal weight, shell weight and shell percentage as shown in Table-1. The larval prolongation is directly proportional to the concentration of JH analogue for *Bombyx mori* L., to prolong the larval period over the control by 2 day and 2 hours at the concentration of 0.01 μ l/ larva at 48 hours of treatment and on the other hand 0.1 μ l/ larva prolonged the larval period by 7 days when applied at 48 hours. Concentration of 1.0 μ l/ larvae appeared to be too high and no cocoon formation occurred. The non-seriposed larvae with the treatment of JH were quite healthy and strong and were feeding well even after 10 days of the seriposition of the control larvae. The highest average cocoon weight of 3.48 g was recorded when treated with 0.1 μ l/ larva at 72 hours and that was a 32. 69 % increase in cocoon weight. The maximum silk weight of 0.52 g was recorded after the larvae were treated with 0.01 μ l /larva at 48 hours. The highest pupal weight of 3.10 g was recorded 0.1 μ l /larva at 72 hours treatment, which was 37.96 % higher than the control. The most significant silk ratio, i.e. 18.29 % was produced at 0.01 μ l /larva at 72 hours which was 2.60 % higher with a 2-day-increase in the feeding period. A maximum survival of 95 % was observed 0.01 μ l / at 48hours of treatment with the test compound R394. At a higher concentration i.e. 1.0 μ l /larva and no cocoon formation occurred.

**Table-1.** Effect of juvenile hormone mimic R394 on silkworm breed KA x NB₄D₂.

#	Dose µl / larva	Time of application (hours)	Larval period (D: H)	Larval prolongation (D: H)	Survival %	Cocoon weight (gm)	Pupal weight (gm)	Shell weight (gm)	Shell (%)	Percent increase over control			
										Cocoon weight	Pupal weight	Shell weight	Shell (%)
1	1.0	48		Did not Seri pose, survived up to 39 days of larval period									
2	0.1		33:16	7:00	38	3.24	2.78	0.46	13.50	+28.36	+33.78	-1.28	-29.25
3	0.01		30:32	2:02	95	2.98	2.48	0.52	16.45	+22.32	+25.24	+11.81	-13.98
4	1.0	72		Survived up to 42 days and no cocoon formation occurs									
5	0.1		36:00	7:00	58	3.48	3.10	0.42	12.82	+ 32.69	+37.96	-6.86	-60.08
6	0.01		31:10	2:10	62	2.61	2.05	0.48	18.29	+ 5.36	+4.68	+7.21	+2.60
7	1.0	96		Survived up to 42 days and no cocoon formation occurs									
8	0.1			Survived up to 42 days and no cocoon formation occurs									
9	0.01		36:00	7:00	30	3.14	2.74	0.45	17.85	+26.42	+30.23	+3.85	-26.94
Control			30:00	7:00	94	2.32	1.94	0.43					

DISCUSSIONS

The present study on the effect of JH analogue R394 (ethyl 9-cyclohexyl-3, 7-dimethyl-2, 4-nonadienoate) on the silkworm, *Bombyx mori* L., was showed the effective application time of JH analogue, it ranges from 48 hours to 96 hours. The most effective time of application was 48 hours. To get a significant increase in silk weight; it gave 11.81 % increase of silk, whereas at 72 hours. And 96 hours gives 7.21 % and 3.85 %, respectively. These findings was not reported till now, though Chang [7] found that effect of JH was highest on silkworm larvae when applied on 2nd and 3rd days of 5th instar larva. Chowdhary [11] found that one of the JH (SJ-42-F) gave the highest silk increase when applied on the 3rd day i.e. at 72 hours.

Prolongation of the larval period is directly proportional to the concentration of JH. The larval period was prolonged from 1-10 days with treatment of JH analogues in 5th instar larvae at different concentrations ranging from 1.0 µl/ larva; 0.1µl/ larva and 0.01µl/ larva. It was clear that the concentration of JH, when increased from 0.01µl/ larva; 0.1µl/ larva and 1.0 µl/ larva increase the larval period, But this prolongation of larval period was beneficial only at a low concentration (0.01µl/ larva), when treated at 48 hours, 72 hours and 96 hours as a positive increase in shell weight was obtained in this dose only.

Increase in the pupal weight was observed negative value as increases the weight of the cocoon and pupa but not increases the silk layers which is an economic character of silk reeling. These pupae was good to increase in fecundity but the prolongation of the larval period with a high dose was so long and It becomes uneconomical to get larger pupae while feeding 3-6 days and get little increase in fecundity. On the other hand, it was reported by several scientist [7, 17, 11] that on application of higher dose of JH, larval period increased and larvae were malformed and died.

It was reported that cocoon weight and pupal weight were directly proportional to the concentration of JH and the feeding period [5, 11] whereas, in the present study with a higher concentration of R394 (ethyl 9-cyclohexyl-3, 7-dimethyl-2, 4-nonadienoate) larvae did not seripose even after the control. Thus it is clear that the effective doses have to be found in each and every JH analogues. Chowdhary [11] pointed out that with the prolongation of the larval period increases in silk weight continued only up to 1-3 days, beyond which silk ratio is found to be negative as is evident from in the present study with R394 (ethyl 9-cyclohexyl-3, 7-dimethyl-2, 4-nonadienoate). Thus while working with JH analogues for silkworms, the main consideration should be to produce more silk. Secondly, the increase in feeding periods exceeding more than 1-2 days is uneconomical. As is evident from the treatment of R394 (ethyl 9-cyclohexyl-3, 7-dimethyl-2, 4-nonadienoate) juvenile hormone with the dose of 0.01 µl/ larva, there is an average increase of about 1 kg of silk/ounce of reared seed.

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