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Development and silk production by silkworm larvae after topical application of fenoxycarb

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Abstract
Juvenile hormone analogues have been tested as insect growth regulators in silkworm (*Bombyx mori*), seeking an increment of silk production. These chemical products, when applied in small or moderate rates, promote the extension of the last larval instar. To understand the physiologic consequences on silk production by the silkworm strain CSR2 × CSR4.The application of fenoxycarb, a juvenile hormone analogue was performed to evaluate its effects on larval development and silk production. Fenoxycarb was topically applied 48h after the fourth larval ecdysis, on the dorsal integument of the 2nd thoracic segment of the insects,between 1 and 1.5 µg fenoxycarb influenced positively the duration of the fifth instar andthe weight gain of the insects. The application of 1µg fenoxycarb resulted in the heaviest silkglands, cocoons, shell cocoons and pupae weights. Comparatively to the control, the increment on silk production by the use of 1µg fenoxycarb was more accentuated than the corresponding negative effects on the cocooning rate.

Keywords: silkworm, *bombyx mori,* juvenile hormone analogues, temperature, R.H%, pupa, larvae

Introduction
Sericulture is one of the agro based industries in India. India occupies second place of Mulberry raw silk production in the world. Mulberry silk comes from the cocoons of *Bombyx mori (L).* The fact that the Silkworm, *Bombyx mori* is domesticated for nearly four thousand years ago is well established. It is well documented that all insects require proper environmental conditions for normal life [1]. The environment influences the activities of the organism directly or indirectly. This is true for domesticated insects like *Bombyx mori.* Due to continuous domestication for many years, the silkworm has lost many of its natural activities as sense of smell, flight etc. and it is completely under the protection of the silkworm growers [2]. Though the number of generations in a year is controlled genetically in the silkworm, environmental conditions like photoperiod, temperature, humidity etc., are known to influence during the entire life cycle of the silkworm [3]. Juvenile hormone analogues have been tested in *Bombyx mori* as insect growth regulators in order to increase silk production [4]. These chemical products, when applied in diminute and appropriate rates, promote the extension of the larval period, when the insect feeds. The first studies with juvenile hormone analogues in *B. mori* were accomplished through topical applications on the insect [5]. Later, researchers looked for the practical application of these hormones in the sericulture, through spraying or immersion of the leaves into the products [6]. The physiological effects on insects caused by the application of juvenile hormone analogues vary according to the product, rate [7], insect strain [8], application method and time [9]. Therefore, the present study was taken to determine the effects of the topical application of rates of fenoxycarb on the larval development and silk production of CSR2 × CSR4 silkworm larvae at different temperature range (20, 25, 30 and 35°C) and constant humidity(75-80%).

Material and Methods
Study Area
Present work was carried out at Government Resham Kendra, Indore region (Sanwer and Sonkatch) (M.P.)

Experimental Species
Productive bivoltine silkworm hybrid (CSR2 × CSR4) is obtained fromCentral Sericulture Research and Training Institute (Mysore, India) was used as an experimental animal. This hybrid is suitable to rear during favourable season (August–February).

Rearing method
Bombyx mori larvae (CSR2 × CSR4 strain) were reared in laboratory conditions under different temperature (20, 25, 30 and 35°C) and constant relative humidity (75-80%). For larvae feeding, mulberry leaves were supplied, five times a day.

Temperature and Humidity conditions
Four different types of temperature, viz. 20, 25, 30, 35°C were maintained in the laboratory throughout the experimentation, using an Environmental chamber. Constant relative humidity (R.H. %) 75-80 % were induced for the study on the experimental silkworm breeds/hybrids, all through the experimentation.

Drug concentration and treatment
Four different drug concentrations, viz. 0.5, 1, 1.5 and 2µg were applied to the larvae throughout experimentation. *Bombyx mori* larvae were reared in laboratory conditions under controlled range of temperature (20, 25,30 and 35°C) and constant relative humidity (75-80%). 48 hours after beginning of the fifth instar, the silkworm larvae were

topically treated with fenoxycarb at the rates of 0.5, 1, 1.5 and 2µg diluted in 5 mL ethanol solvent. Larvae of the control were only treated with the solvent. The applications were on the larval thoracic tergum (dorsal portion of larvae segment other than the head), ideally on the second thoracical by using 100 mL micro-syringe.

Results

Cocoon production

Cocoon production is summarized in table-1. 50 larvae were taken for the experiment and treated with juvenile hormone analogue (fenoxycarb).

At 20ºC temperature 31, 33, 32 and 29 larvae were converted into cocoons at 0.5, 1, 1.5 and 2µg drug concentration respectively.

At 25ºC temperature 38, 41, 35 and 32 larvae were converted into cocoonsat 0.5, 1, 1.5 and 2µg drug concentration respectively.

At 30ºC temperature 30, 33, 28 and 24 larvae were converted into cocoons at 0.5, 1, 1.5 and 2µg drug concentration respectively.

At 35ºC temperature 25, 30, 28 and 21 larvae were converted into cocoons at 0.5, 1, 1.5 and 2µg drug concentration respectively.

The maximum no. of cocoon obtained was 41 at 1µg on 25ºC whereas minimum cocoon obtained was 21 at 2µg on 35ºC.

Table 1: Effect of Juvenile hormone analogue Fenoxycarb on pupation

Temperature (°C)	Larvae	Humidity	Drug Concentration	No. of Cocoon produced	Percentage (%)
20°C	50	75-80%	0.5µg	31	62%
			1µg	33	66%
			1.5µg	32	64%
			2µg	29	58%
25°C	50	75-80%	0.5µg	38	76%
			1µg	41	82%
			1.5µg	35	70%
			2µg	32	64%
30°C	50	75-80%	0.5µg	30	60%
			1µg	33	66%
			1.5µg	28	56%
			2µg	24	48%
35°C	50	75-80%	0.5µg	25	50%
			1µg	30	60%
			1.5µg	28	56%
			2µg	21	42%

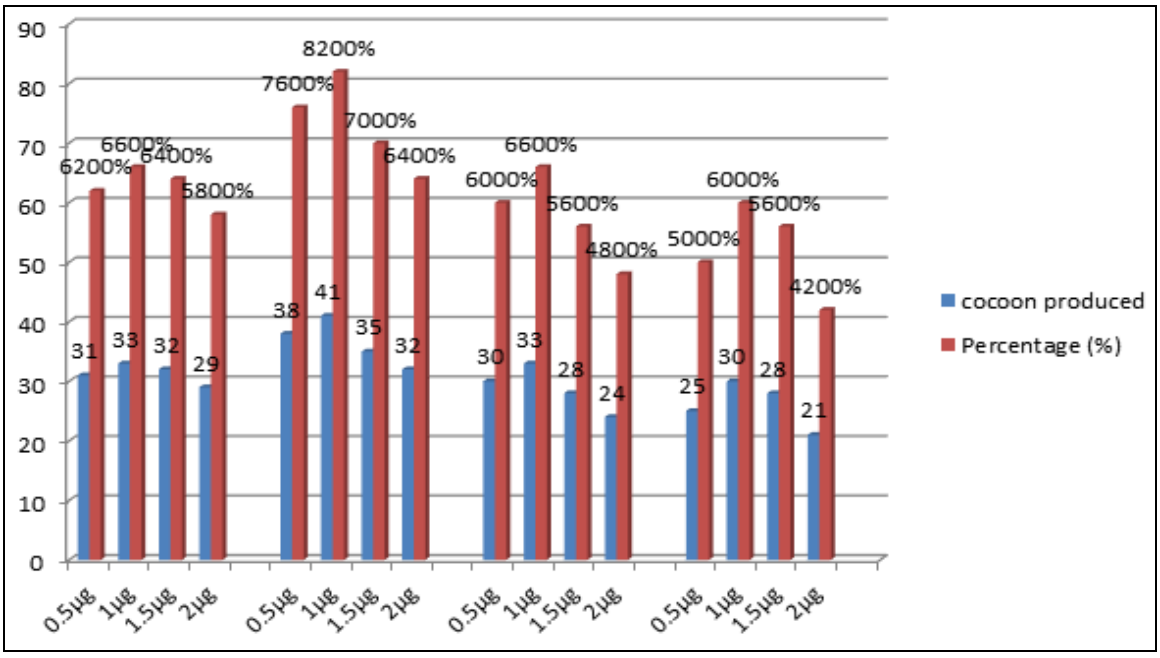

Fig 1: shows the effect of juvenile hormone analogue fenoxycarb on pupation

Weight of silkworm and productivity of silk

Larvae weight, Cocoon weight and length of silk produced by the topical application of fenoxycarb of CSR2 × CSR4 bi-voltine breed is summarized in table 2.

Larval weight was 2.1, 2.89, 2.01 and 1.90gms at 0.5,1, 1.5 and 2 µg fenoxycarb respectively. Cocoon weight was 3.10, 3.29, 3.00 and 2.69gms at 0.5, 1, 1.5 and 2 µg fenoxycarb respectively. Length of silk obtained 900-1100, 1000-1200,

900-1200 and 800-1000 meters at 0.5, 1, 1.5 and 2 µg fenoxycarb, respectively.

The maximum larvae weight, Cocoon weight and length of silk obtained was 2.89 gms, 3.29gms and 1000-1200 meters at 1 µg drug concentration respectively whereas minimum larvae weight, Cocoon weight and length of silk obtained was 1.90 gms, 2.69 gms and 800-1000 meters at 2µg drug concentration respectively. In the control group the larvae weight, Cocoon weight and length of silk obtained were 2.5 gms, 2.97 gms and 800-1000 meters. In the present investigation, more larvae weight, Cocoon weight and length of silk obtained in experimental group as compared to control group.

Table 2: Effect of Juvenile hormone analogue Fenoxycarb on weight of silkworm and productivity of silk (at temperature 25°C and humidity 75-80%)

S.NO.	Concentration	Weight of larvae	Weight of cocoon	Length of silk produced
	0.50µg	2.1gm	3.10gm	900-1100m
2.	1µg	2.89gm	3.29gm	1000-1200m
3.	1.5µg	2.01gm	3.00gm	900-1200m
4.	2µg	1.90gm	2.69gm	900-1000m
5.	Control (untreated)	2.5gm	2.97gm	800-1000m

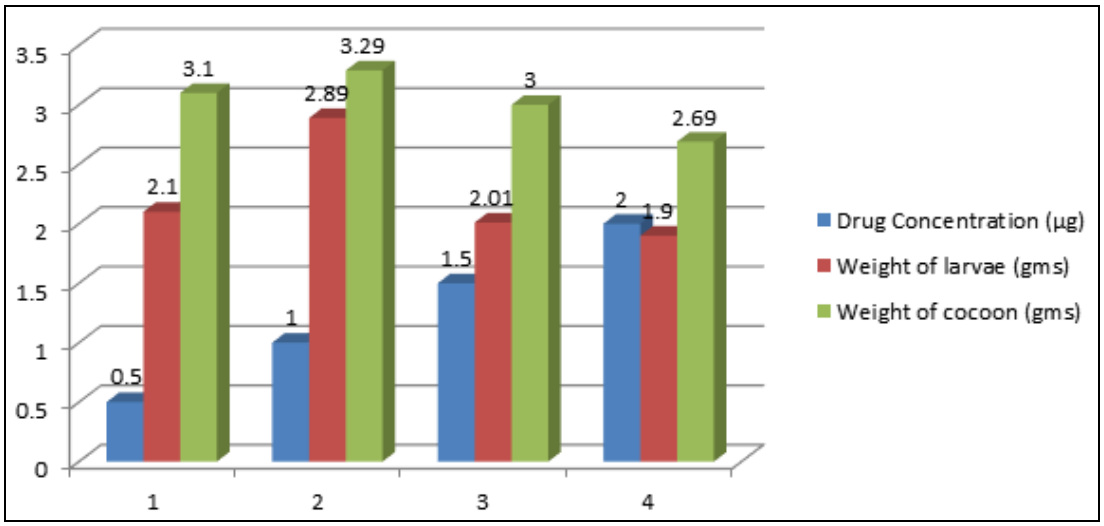

Fig 2: show the effect of Juvenile hormone analogue Fenoxycarb on weight of larvae and cocoon.

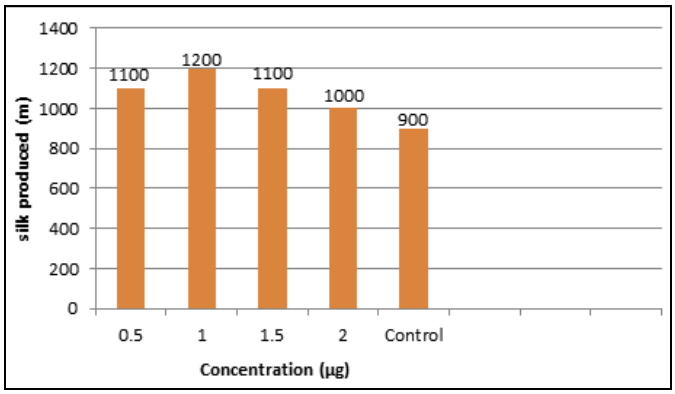

Fig 3: Show the productivity of silk at different concentration of Fenoxycarb.

Discussion

It is a well-known fact that many factors such as weather conditions, feeding habit, diet, chemicals may affect the enzymatic and non-enzymatic compound of insect body [10]. Fenoxycarb is a Juvenile Hormone analogue, which has been used extensively in insect endocrinology to investigate Juvenile Hormone regulation of morphology, reproduction, development, and metabolism [11]. This study demonstrates that juvenile hormone analogue, fenoxycarb (JHA) is proved to be very active at low concentrations and induces physiological changes when applied to fifth instar larvae of silkworm, *Bombyx mori L.* The physiological effects on insects caused by the application of juvenile hormone analogues vary according to the product rate [12], insect strain [13], application method and time [14]. This proves that Juvenile Hormone alters the biochemical pathway in insects. The present study demonstrates that the application of fenoxycarb causes increase in cocoon weight, shell weight, and extension of the fifth instar larval period. The extension is highly related to the quantity, i.e., increase in the quantity resulted in linear increase of the larval period of the treated larvae. Moreover, the effect of the juvenile hormone analogue on insects is through the influence on their morphogenesis, i.e., causing an increment in weight gain, and a portion of this biomass weight gain is directed to the growth of silk gland and its metabolism resulting in increased cocoon weight and shell weight [15]. The results of present study indicate that stimulating capacity of the Juvenile Hormone Analogue on various characteristic of the silkworm contributing to the quality silk yield. Results of present study prove that the appropriate application of Juvenile Hormone fenoxycarb brings about significant improvement in silk production.

Conclusion

The application of 1 µg fenoxycarb to fifth instar larvae of *B. mori* increases their development and silk production, compensating the corresponding reduction of cocooning rate. However, field studies using practical methods of application of the product on the insects, are needed to confirm the benefit of such technique in sericulture.

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