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Effect of Methyl Testosterone on the Economic Parameters of the Silkworm, *Bombyx mori* L.

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The effect of topical application with methyl testosterone on economic parameters was analysed following treatment of last larval stadium. The treated larvae showed significantly increased silk gland weight at 15 µg/ml treated group along with other enhanced larval, cocoon and adult parameters. The larval duration was significantly decreased in all the treated groups with significantly increased cocooning percentage, female coon weight at 15 µg/ml, female cocoon shell weight, filament length, weight, denier and moth emergence percentage in all the treated groups. However length of the ovariole, eggs per ovariole and fecundity were significantly decreased in all the treated groups and hatching percentage was significantly decreased at 10 and 15 µg/ml treated groups when compared with the corresponding parameters of the carrier control.

Key words : Methyl testosterone, Economic parameters, Cocoon, Silkworm, *Bombyx mori*

Introduction

Hormones are molecules that carry messages and their structures have been quite conserved during evolution: this means that identical or at least very similar molecules (both for messages and their receptors) can be found in vertebrates and invertebrates. But this does not mean that the messages they carry are equally conserved, either between vertebrates and nvertebrates or even within invertebrates (Lafont, 1991). The presence and activity of various mammalian hormones have been demonstrated in life system of many insects (De Loof and De Clerk, 1986; Dehlinger, 1987; Novak and Lambert, 1989; Bradrook *et al.*, 1990; Hugar *et al.*, 1997; Goudar and Kaliwal, 1999).

The metabolic significance of vertebrate type steroids in the silkworm, *B. mori* L. has been reviewed (Venkataramiraddi, 1994). However, the treatment with methyl testosterone is not unusual as insects and their relatives are phylogenetically closer to mammals than are plants. These finding and studies may indicated a wider potential use of vertebrate steroid hormones outside the mammalian systems specially in the insects. It has been reported that testosterone like immunoreactive substances was identified in the haemolymph of larval and pupal stages of silkworm by radio immunoassay (RIA) (Nagashima *et al.*, 1983). Ogiso and Onishi (1986) have reported that the treatment with testosterone decreases the rate of oviposition and egg hatchability. Magadum and Magadum (1993) have reported that testosterone propionate improves the fecundity and decrease the cocooning percentage and moth emergence percentage in pure Mysore breed of the silkworm, *B. mori.* Recently it has been reported that testosterone propionate increase larval weight, silkgland weight, denier, moth emergence percentage fecundity and hatching percentage in bivoltine silkworm *B. mori* (Hugar *et al.*, 1997).

Perusal of these literatures promted this examination of the possible effects of methyl testosterone to explain the physiological function on the multivoltine silkworm, *B. mori* by studying its effect on the economic parameters.

Materials and Methods

The eggs of multivoltine cross breed ($PM \times NB_{18}$) silkworm were obtained from Grainage center Rayapur, Dharwad, Karnataka and reared in the laboratory by improved methods of silkworm rearing (Krishnaswami, 1978). The larvae were maintained on fresh mulberry leaves (K_2). The V stadium larvae were divided into five experimental groups including controls and every group

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consists of uniformly weighed larvae in five replications of 20 worms. The methyl testosterone procured from M/s Sigma Laboratory Pvt. Ltd., Bombay.

It was dissolved in small quantity of acetone and diluted to form 5, 10 and 15 µg/ml by adding acetone. Each larva was topically applied with one of the three doses of methyl testosterone at alternate days. In each larvae application 5 ml of solution was used to treat 100 larvae.

The larval, cocoon and adult parameters were recorded separately. The larvalduration was recorded from the day of hatching till the completion of spinning. The larval and silk gland weights were measured before commencement of spinning. The cocoon parameters such as female and male cocoon weights and their shell weights were measured on 5 day after the completion of the spinning. The filament length was measured with epprovette by reeling a single cocoon. The reeled silk was dried in hot-air oven and weight was taken in an electrical balance. The shell ratio and denier of the filament were calculated. The adult parameters such as moth emergence percentage, length of the ovariole, eggs per ovariole, fecundity and hatching percentage were recorded in the adult after mating. The cocooning percentage, moth emergence percentage and hatching percentage were also calculated by the formulas shown in the tables. Each mean value, a record of ten worms is shown in Tables 1, 2 and 3. The data collected were subjected to analysis of variance tests to find out the significance between the treated and control group (Raghava Rao, 1983). The percent values of cocoon shell ratio, cocooning percentage, moth emergence percentage and hatching percentage were transformed to sine angular values for statistical analysis.

Results and Discussion

The data on the effect of methyl testosterone on larval, cocoon and adult parameters of the silkworm *B. mori* are summarized in Tables 1, 2 and 3.

Larval weight

Larval body weight did not show any significant change in

Table 1. Effect of methyl testosterone on the larval parameters of the silkworm, *B. mori*

Treatment	Dose µg/ml	Larval Weight (g)	Silkgland Weight (g)	Larval Duration (g)	Cocooning Percentage (%)
		2.679	1.248	672.4*	96.70
Methyl testosterone	5				79.53**
		(96)	(105)	(96)	(99)
		2.860	1.225	680.4*	97.36*
Methyl testosterone	10				80.54**
		(102)	(106)	(97)	(101)
		2.939	1.314*	687.2*	97.87*
Methyl testosterone	15				81.47**
		(105)	(111)	(98)	(101)
		2.779	1.180	695.6	96.77
Carrier Control	Acetone				79.53**
		(100)	(100)	(100)	(100)
		2.693	1.190	689.8*	95.45*
Normal Control	-				77.61**
		(96)	(100)	(99)	(98)
		NS	S	S	S
S.Em±		0.128	0.052	1.072	0.051
C.D.at 5%		0.252	0.102	2.199	0.105

* -Significant increase/decrease at 5%

** -Angular transformed values

S.Em± -Standard error mean

CD -Critical difference

NS -Non significant

S -Significant

Percentage increase/decrease over that of the carrier controls in paranthesis.

$$\text{Cocooning percentage} = \frac{\text{Number of cocoons formed}}{\text{Total number of cocoons kept}} \times 100$$

Table 2. Effect of methyl testosterone on the cocoon parameters of the silkworm, *B. mori*

Treatment	Dose µg/ml	Female			Male			Felament Length (mts)	Filament Weight (g)	Denier
		Cocoon Weight (g)	Cocoon Shell weight (g)	Cocoon Shell ratio (g)	Cocoon Weight (g)	Cocoon Shell weight (g)	Cocoon Shell ratio (g)			
Methyl testosterone	5	1.632	0.258*	15.91	1.566	0.254	16.36	650.00*	0.260*	3.598*
				23.50**			23.81**			
		(109)	(113)	(104)	(96)	(102)	(105)	(104)	(115)	(110)
Methyl testosterone	10	1.669	0.263*	15.87	1.569	0.256	16.35	680.00*	0.300*	3.969*
				23.42**			23.81**			
		(111)	(115)	(104)	(96)	(102)	(105)	(108)	(132)	(121)
Methyl testosterone	15	1.753*	0.280*	16.12	1.619	0.263	16.31	710.00*	0.330*	4.182*
				23.66**			23.81**			
		(117)	(123)	(105)	(99)	(105)	(105)	(113)	(146)	(128)
Carrier control	Acetone	1.497	0.227	15.24	1.621	0.249	15.51	625.00	0.226	3.263
				22.95**			23.19**			
		(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)
Normal Control	-	1.561	0.178*	11.47*	1.570	0.211*	13.72*	623.33	0.225	3.245
				19.73**			21.72**			
		(104)	(78)	(75)	(96)	(84)	(88)	(99)	(99)	(99)
		S	S	NS	NS	NS	NS	S	S	S
S.Em±		0.089	0.014	0.909	0.092	0.012	0.846	4.114	0.003	0.031
C.D.at 5%		0.176	0.027	1.782	0.181	0.027	1.659	8.845	0.008	0.067

* - Significant increase/decrease at 5%
** -Angular transformed values
S.Em± -Standard error mean
CD -Critical difference
NS -Non significant
S -Significant
Percentage increase/decrease over that of the carrier controls in paranthesis

$$\text{Female/male cocooning shell ratio} = \frac{\text{Cocoon shell weight (g)}}{\text{Cocoon weight (g)}} \times 100$$

$$\text{Denier} = \frac{\text{Single cocoon shell weight (g)}}{\text{Single cocoon filament length (mt)}} \times 100$$

all the doses of methyl testosterone when compared with that of carrier control (Table 1). Similar results were obtained by treatment with testosterone propionate in the polyvoltine pure Mysore breed (Magadum and Magadum, 1993). However, testosterone propionate has shown to increase in larval weight in case of bivoltine (Hugar *et al.*, 1997). Therefore the non-response of methyl testosterone on the larval weight may be either due to the change in breed or hormone specificity.

Silkgland weight

The weight of the silkgland was significantly increased in the group treated with 15 µg/ml when compared with that of the carrier control (Table 1). Similar results have been reported at high doses in the bivoltine silkworm, *B. mori* (Hugar *et al.*, 1997). The significant increase in the silkgland weight might be due to the response of the hormone at high concentration.

Larval duration

The larval duration was significantly decreased in all the treated groups of methyl testosterone when compared with that of the carrier control (Table 1). The significant decrease in larval duration suggested that the concentration applied to the larvae, may have some effect on he hormonal level, juvenile hormone and moulting hormone which control moulting and metamorphosis in insects. On the contrary, it has been reported that the topical application of testosterone propionate did not show any significant change in larval duration in polyvoltine pure Mysore breed of *B. mori* (Magadum and Magadum, 1993) and in bivoltine of *B. mori* (Hugar *et al.*, 1997). However it needs further investigation.

Cocooning percentage

The cocooning percentage was significantly increased in the 10 and 15 µg/ml treated groups of methyl testosterone when compared with that of the carrier control (Table 1). This shows the larvae did not show any mortality at their larval stage and used concentrations are safe and have not

Table 3. Effect of methyl testosterone on the adult parameters of the silkworm, *B. mori*

Treatment	Dose µg/ml	Moth emergence percentage (%)	Length of the ovariole (mm)	Eggs per ovariole (No)	Fecundity (No)	Hatching percentage (%)
		94.50*	130.1*	67.75*	540.0*	99.14
Methyl testosterone	5	76.44**				84.68**
		(101)	(97)	(93)	(93)	(100)
		94.71*	126.0*	64.62*	520.0*	97.11*
Methyl testosterone	10	76.69**				80.19**
		(101)	(94)	(89)	(89)	(98)
		94.61	123.7*	62.50*	462.5*	97.00*
Methyl testosterone	15	76.56**				80.02**
		(101)	(92)	(86)	(80)	(98)
		93.54	133.3	72.25	578.0	98.27
Carrier Control	Acetone	75.23**				82.29**
		(100)	(100)	(100)	(100)	(100)
		93.18	137.8*	80.50*	675.0*	97.80*
Normal Control	-	74.77**				81.47**
		(99)	(103)	(111)	(116)	(99)
		S	S	S	S	S
S.Em±		0.041	0.728	0.580	18.936	0.047
C.D.at 5%		0.085	1.426	1.137	37.115	0.101

* -Significant increase/decrease at 5%
** -Angular transformed values
S.Em± -Standard error mean
CD -Critical difference
NS -Non significant
S -Significant
Percentage increase/decrease over that of the carrier controls in paranthesis.

$$\text{Moth emergence percentage} = \frac{\text{Number of moths emerged}}{\text{Number of cocoons kept}} \times 100$$

$$\text{Hatching percentage} = \frac{\text{Total number of eggs hatched}}{\text{Total number of eggs laid}} \times 100$$

adversely affected the cocooning percentage. Similar results have been reported in the in bivoltine breed of silkworm *B. mori* where cocooning percentage was significantly increased (Hugar *et al.*, 1997).

Cocoon weight and its shell weight

A significant increase in the female cocoon weight in 15 µg/ml and shell weight were obtained in all the treated groups when compared with that of carrier control (Table 2). There was no significant change in the male cocoon weight and its shell weight in all the treated groups when compared with that of the carrier control (Table 2). The reason for significant increase in female cocoon weight, shell weight and not in male cocoon weight and its shell weight is not known. On the contrary, it has been reported that the topical application of testosterone did not show any significant change in female/male cocoon weight, its shell weight and shell ratio in bivoltine silkworm *B. mori* (Hugar *et al.*, 1997). However, it needs further investigation. The increase/decrease in male and female cocoon shell ratio is given in Table 2.

Filament length, weight and denier

A significant increase in the filament length, weight and denier were obtained in all the treated groups of methyl testosterone when compared with that of the carrier control (Table 2). A maximum filament length, weight and denier were obtained with 15 µg/ml methyl testosterone treated group.

Moth emergence percentage

There was a significant increase in moth emergence in all the treated groups except 15 µg/ml when compared with that of carrier control (Table 3). The increase in moth

emergence may be attributed to non increased mortality I pupa/moth stage. However, testosterone propionate increases in moth emergence in bivoltine silkworm *B. mori* (Hugar *et al*., 1997) not in polyvoltine pure Mysore breed of silkworm, *B. mori* (Magadum and Magadum, 1993), this led to conflicting results and needs further investigation.

Ovariole length, eggs per ovariole and fecundity

There was significant decrease in ovariole length, eggs per ovariole and fecundity when compared with that of the carrier control (Table 3). These parameters are dose dependent. On the contrary, it has been reported that the testosterone propionate increases egg productivity in both polyvoltine and bivoltine of the silkworm *B. mori* (Magadum and Magadum, 1993; Hugar *et al.*, 1997). However, it needs further investigation.

Hatching percentage

There was significant decrease in hatching percentage in higher doses of 10 µg/ml and 15 µg/ml treated groups when compared with that of the carrier control (Table 3). Similar results have been reported in the silkworm *B. mori* after treatment with testosterone were decreased egg hatchability was shown (Ogiso and Onishi, 1986).

There was significant increase/decrease in the larval duration, cocooning percentage, female and male cocoon shell ratio, moth emergence percentage, length of the ovariole, eggs per ovariole, fecundity and hatching percentage of the normal controls when compared with the corresponding parameters of the acetone treated controls. This significant difference might be due to the effects of acetone since the acetone has considerable effects on the larval body weight and food utilization in silkworm (Padaki, 1991; Radhakrishna and Delvi, 1992).

The effect of testosterone (either added to the diet injected or topically applied) have been investigated in silkworm, but this led to conflicting reports. Testosterone decreases the rate of oviposition and egg hatchability in silkworm, *B. mori* (Ogiso and Onishi, 1986). Magadum and Magadum (1993) have reported that testosterone propionate improves fecundity and decrease in the cocooning percentage and moth emergence in pure Mysore breed of *B. mori.* Recently it has been reported that testosterone propionate increase larval weight, silkgland weight, denier moth emergence percentage in bivoltine silkworm, *B. mori* (Hugar *et al.*, 1997). But paradoxical thing is that the results due to the effect of these steroids on physiological process and economic parameters do not reveal similar in silkworm *B. mori* since the physiological processes and economic parameters are dependent on the dose, way of administration, duration of treatment and race of the silk worm. In our study it is interesting to know that the methyl testosterone enhance silk yield like silk gland weight and shell weights, filament length, weight, denier and moth emergence percentage. However, larval duration, length of the ovariole, eggs per ovariole, fecundity and hatching percentage was significantly decreased.

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