

## Effect of Methoprene on Larval and Cocoon Weight, Ovariole Length, Egg Number and Fecundity of *Bombyx mori* L.<sup>1</sup>

S. B. MAGADUM<sup>2</sup>, M. A. HOOLI and V.B. MAGDUM

Department of Zoology, Karnatak University, Dharwad-580 003, Karnatak, India

### ABSTRACT

The effect of Methoprene on larval and cocoon weight, ovariole length, egg number and fecundity of *Bombyx mori* (Polyvoltine Pure Mysore breed) was studied. Methoprene at 2.75, 4.0 and 8.0 µg/ml dilutions was topically applied/sprayed on silkworm larvae repeatedly at 36h in 3rd, 4th and 5th stadium, at 48h in 4th and 5th stadium and singly at 72h in 5th stadium. Of the various dosages used, the repeated applications of 2.75 µg/ml at 36h in 3rd, 4th and 5th stadium of silkworm larvae resulted in significant increase in larval weight, cocoon weight, ovariole length, ovariole egg number and fecundity when compared to untreated controls.

### Introduction

In recent years juvenile hormone (JH) and its analogues have been used in practical sericulture in Japan to improve the silk production of *Bombyx mori*. The administration of JH/JH analogues to silkworm larvae prolonged the larval stage and significantly increased the cocoon weight (Akai and Kobayashi 1971). There is a number of reports on the effect of single application of JH/JH analogues, but reports on the effect of repeated applications on a single stadium or on different stadia are very few. It is suggested that repeated applications of JH to 5th stadium larvae are more effective in increasing cocoon weight than a single application (Amori et al. 1977). Kobari and Akai (1978, 1979) reported that topical application of Methoprene (2.5 ppm/ml) at 48-60h increases the cocoon weight by 10%. Washida (1984) reported that administration of 1, 5, 10 and 25 µg/larva in 4th stadium larvae at 36, 48 and 60h resulted in an increase of larval and cocoon weight (1 and 25 µg/larva treated groups). Akai et al. (1985) have shown more in-

creased cocoon weight in repeated applications of Methoprene in 3rd, 4th and 5th stadium or in 4th and 5th stadium silkworm larvae in high yielding breeds of Japanese silkworms. These studies recorded only the larval and cocoon weight, while studies on the ovariole length, ovariole egg number and fecundity are lacking. Therefore this study was undertaken to study the effect or repeated and single applications on the above mentioned parameters of Pure Mysore breed of *Bombyx mori* L.

### Materials and Methods

Polyvoltine silkworms reared in the laboratory and maintained on fresh mulberry leaves (Krishnaswami 1978) were utilised in this study. The JH analogue Methoprene (Zoecon, Palo Alto, California, supplied by M/s Otsuka Pharmaceuticals, Osaka; 6.25 mg Methoprene in 5 ml acetone) was diluted to 2.75, 4.0 and 8.0 µg/ml by adding distilled water. The 3rd, 4th and 5th stadium larvae were grouped into three experimental groups. Each group consisted of 20 worms of five replications (20 × 5). Each group received 4 ml Methoprene solution in each application. Each concentration of methoprene was topically sprayed at 36h in 3rd, 4th and 5th stadium larvae (received 12 ml solution/group), at 48h in 4th and 5th stadium larvae (received 8 ml solu-

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<sup>2</sup> Present address: Central Sericultural Research and Training Institute, Mysore-570008, India.

tion/group) and at 72h in 5th stadium larvae (received 4 ml solution/group) of silkworm. The exact quantity of methoprene absorbed by the larva is not known. Distilled water treated and untreated controls were also included. The temperature and relative humidity were  $27 \pm 1$  °C and 65-70%, respectively. Larval weight was recorded after the completion of 6 days in 5th stadium. The weight of live cocoons was recorded on the 6th day after spinning. Five females emerged from the cocoons were sacrificed to measure and count the ovariole length and egg number, respectively (before oviposition). Only mean values for 8 ovarian tubules/moth of five females were recorded (Table 1). Five female moths were mated with males for 3-4h and then separated and allowed to lay eggs for 24h. The average eggs laid from five female moths in each group are recorded as fecundity. Means of five larval and cocoon weights for each group were recorded (Table 1). The data collected were subjected to one way analysis of variance test to study the significance between the treatment and control groups of each parameter (Raghava Rao 1983). This experiment was repeated twice to conclude the results.

### Results and Discussion

The data are given in Table 1. Larval weight

was significantly ( $p < 0.05$ ) increased in repeated applications of 2.75 µg Methoprene at 36h in 3rd, 4th and 5th stadium larvae when compared to the controls (Table 1). The repeated application of 2.75 µg and 4.0 µg at 36h in 3rd, 4th and 5th stadium larvae, repeated application of all the doses at 48h in 4th and 5th stadium larvae and single application of 4µg and 8 µg Methoprene in 5th stadium larvae improved the larval weight significantly when compared to the untreated controls. There was no improvement in repeated application of 8 µg at 36h in 3rd, 4th and 5th stadium larvae and single application of 2.75 µg at 72h in 5th stadium larvae. Of course the dose may be too little to induce improvement in a single application of 2.75 µg/group. The fact that no alteration was observed in the larval weight due to repeated applications (8µg at 36h) with high dose of juvenoid cannot be explained.

Cocoon weight was significantly improved in repeated applications of 4µg and 8 µg Methoprene at 36h in 3rd, 4th and 5th stadium larvae, 8µg at 48h in 4th and 5th stadium larvae and single application of 2.75 µg and 8 µg at 72h in 5th stadium larvae when compared to both controls. The repeated applications of 2.75 µg Methoprene at 36h in 3rd, 4th and 5th stadium

TABLE 1. Effect of Methoprene on larval and cocoon weight, ovariole length, ovariole egg number and fecundity in the silkworm, *Bombyx mori* L.

Group & Treatment		Mean larval weight (g)	Cocoon weight (g)	Length of ovariole (mm)	Ovariole egg number	Fecundity
I	A <sub>1</sub> (2.75 µg)	2.143 ab	1.014 b	87.6 ab	68.0 ab	421 ab
	A <sub>2</sub> (4.0 µg)	1.989 a	1.055 ab	79.0	58.8	462 ab
	A <sub>3</sub> (8.0 µg)	1.900 ab	1.033 ab	80.0	60.8	444 ab
II	B <sub>1</sub> (2.75 µg)	1.920 a	1.020 b	80.8	57.8	452 ab
	B <sub>2</sub> (4.0 µg)	1.993 a	0.973	82.4	59.8	455 ab
	B <sub>3</sub> (8.0 µg)	1.921 a	1.113 ab	84.8	60.8	411 ab
III	C <sub>1</sub> (2.75 µg)	1.756	1.028 ab	84.6 a	71.6 ab	317 ab
	C <sub>2</sub> (4.0 µg)	1.909 a	1.009 b	81.0	62.6	383 b
	C <sub>3</sub> (8.0 µg)	1.917 a	1.091 ab	88.6 ab	65.8 b	411 ab
IV	Distilled water treated control	1.807	0.931	77.5	56.8	346
V	Untreated control	1.719	0.974	74.0	61.2	357
SE ±		0.066	0.017	2.638	2.087	9.207
CD at 5%		0.190	0.049	7.542	5.966	26.316

a: Significant over the untreated control.

b: Significant over the distilled water treated control.

A<sub>1</sub>-A<sub>3</sub>: Repeated applications of Methoprene at 36h in 3rd, 4th and 5th stadium of silkworm larvae.

B<sub>1</sub>-B<sub>3</sub>: Repeated applications of Methoprene at 48h in 4rd and 5th stadium of silkworm larvae.

C<sub>1</sub>-C<sub>3</sub>: Single application of Methoprene at 72h in 5th stadium of silkworm larvae.

larvae, at 48h in 4th and 5th stadium larvae and single application of 4 µg Methoprene at 72h in 5th stadium larvae improved the cocoon weight when compared to distilled water treated control only. The reason for no change in cocoon weight due to repeated applications of 4 µg Methoprene at 48h in 4th and 5th stadium larvae is yet to be revealed.

The length of ovariole was increased significantly in repeated applications of 2.75 µg Methoprene at 36h in 3rd, 4th and 5th stadium larvae and single application of 8 µg Methoprene at 72h in 5th stadium larvae when compared to both controls. The repeated applications of 8 µg Methoprene at 48h in 4th and 5th stadium larvae and single application of 2.75 µg Methoprene at 72h in 5th stadium larvae improved significantly the length of ovariole when compared to untreated controls (Table 1). Although there was an increase in the length of ovariole in other treatments as well, the differences recorded were not significant.

The egg number per ovariole was significantly increased in repeated applications of 2.75 µg Methoprene at 36h in 3rd, 4th and 5th stadium larvae and in single application of 2.75 µg Methoprene at 72h in 5th stadium larvae when compared to both controls. The single application of 8 µg Methoprene at 72h in 5th stadium larvae significantly improved the egg number per ovariole when compared to that of distilled water treated control. The single application of 4µg Methoprene at 72h in 5th stadium larvae did not show any effect. Further investigation is needed to reveal the reason for no alteration in the egg number in this treatment.

Fecundity was significantly increased in all the doses of Methoprene in repeated applications at 36h in 3rd, 4th and 5th stadium larvae and at 48h in 4th and 5th stadium larvae and single application of 8 µg at 72h in 5th stadium larvae when compared to both controls. However there was a significant decrease in fecundity in single application of 2.75 µg Methoprene at 72h in 5th stadium larvae when compared to both controls. Single application of 4 µg Methoprene at 72h in 5th stadium larvae significantly increased the fecundity when compared to distilled water treated controls only.

Amori et al. (1977) administered JH II (0.5 µg/larva) to 5th stadium larvae from 2nd to 4th day and found an effective improvement in the cocoon weight of C9 × N9 breed of silkworm.

They also reported that larval weight was not altered when JH(I) was administered on the first day in the 5th instar (0.5µg/larva), but cocoon weight was improved by 7% in the same breed of silkworm. Kobari and Akai (1978, 1979) administered 2.5 ppm/ml Methoprene (topically) to silkworm breeds of N134 × C135 and N 132 × C132 at 60, 72, 84h and 48-60h in the 5th instar, respectively, and found an improvement in larval and cocoon weight by 8-17% and 10-19%, respectively. Washida (1984) applied 1, 5, 10, 25 µg/larva of Methoprene at 36, 48 and 60h after the 3rd larval ecdysis. The 1µg/larva and 25µg/larva Methoprene treated groups showed an improvement of larval weight and 26% improvement in cocoon weight when compared to controls. Akai et al. (1985) also showed that repeated applications of 2.5 ppm/ml Methoprene at 29,34 and 72h in 3rd, 4th and 5th instars improved the silk production in *B. mori*. The results obtained in the present study are in conformity with earlier workers (Amori et al. 1977, Kobari and Akai 1978, 1979, Washida 1984, Akai et al. 1985). The above reports indicate that an improvement or nonimprovement of larval and cocoon weights by the application of juvenoids maybe of specific responses such as application time, dose of juvenoid and breed response to juvenoid. The unalteration in the larval weight and cocoon weight during high dose treatment with Methoprene in the present study requires further investigation.

Juvenile hormone acts as gonadotrophic hormone on the ovaries of insects (Kerkut and Gilbert 1983). The improvement in length of ovariole, egg number per ovariole and fecundity might be attributed to gonadotrophic effect or stimulatory effect of Methoprene. It may be concluded that repeated applications of 2.75 µg/ml Methoprene at 36h in 3rd, 4th and 5th stadium larvae had better effect on improving larval and cocoon weight, ovariole length, egg number per ovariole and fecundity than the other treatments.

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## References

- Akai, H. and M. Kobayashi. 1971. Induction of prolonged larval instar by the juvenile hormone in *Bombyx mori* L. (Lepidoptera: Bombycidae). Appl. Entomol. Zool. 6: 138-139.
- Akai, H., K. Kimura, M. Kiuchi and A. Shibukawa. 1985. Increase of silk production by repeated treatment with a juvenile hormone analogue. J. Seric. Sci. Jpn. 54: 297-298.
- Amori, S., Y. Ozawa and M. Nishimura. 1977. Timely administration of synthetic compounds with juvenile hormone activity to silkworm larvae reared on artificial diet. J. Seric. Sci. Jpn. 46: 69-76.
- Kerkut, G.A. and L.I. Gilbert. 1983. Comprehensive Insect Physiology, Biochemistry and Pharmacology. Vol. VII, Chp. Juvenile Hormone. Pergamon Press, London.
- Kobari, Y. and H. Akai. 1978. Utilisation of Manta (synthetic compound with juvenile hormone activity) for the silkworm rearings. J. Seric. Sci. Jpn. 47: 315-317.
- Kobari, Y. and H. Akai. 1979. On quantitative increase of silk production by administration of juvenile hormone Manta. J. Seric. Sci. Jpn. 48: 37-42.
- Krishnaswami, S. 1978. New technology of silkworm rearing. Bulletin No. 2. CSRTI, Mysore.
- Raghava Rao, D. 1983. Statistical techniques in agricultural and biological research. Oxford and IBH Publishing Co. New Delhi.
- Washida, S. 1984. Influence of juvenile hormone analogue applied at fourth instar on larval growth and cocoon weight in *Bombyx mori*. J. Seric. Sci. Jpn. 53: 210-215.

KEY WORDS: *Bombyx mori*, Silkworm, Methoprene

## Επίδραση του Methoprene στο Βάρος Προνύμφης και Κουκουλιού, Μήκος Ωοσωλήνα, Αριθμό Αυγών και Γονιμότητα του Μεταξοσκώληκα

S. B. MAGADUM, M. A. HOOLI και V. B. MAGDUM

Τμήμα Ζωολογίας, Πανεπιστήμιο Karnatak,  
Dharwad - 580003, Karnatak, India

### ΠΕΡΙΛΗΨΗ

Μελετήθηκε η επίδραση του Methoprene στο βάρος προνύμφης και κουκουλιού, μήκος ωοσωλήνα, αριθμό αυγών και γονιμότητα του *Bombyx mori* L. (Polyvoltine Pure Mysore Breed). Από τις διάφορες δόσεις που χρησιμοποιήθηκαν, η επανειλημμένη εφαρμογή 2,75 µg/ml Methoprene στις 36 ώρες 3ου, 4ου και 5ου σταδίου προνύμφης είχε σαν αποτέλεσμα στατιστικά σημαντική αύξηση του: βάρους προνύμφης και κουκουλιού, μήκους ωοσωλήνα, αριθμού ωοκυττάρων ωοσωλήνα και γονιμότητας σε σύγκριση με το μάρτυρα.