African Journal of Biotechnology Vol. 5 (15), pp. 1422-1426, 3 August 2006
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2006 Academic Journals

Full Length Research Paper

Studies on the quantitative and qualitative characters of cocoons and silk from methoprene and fenoxycarb treated *Bombyx mori* (L) larvae

D. M. Mamatha[1]*, H. P. P. Cohly[2], A. H. H. Raju[1] and M. Rajeswara Rao[1]

[1]Division of Sericulture, Department of Zoology, S.V. University, Tirupati-517 502, A.P., India.
[2]Department of Surgery, University Medical Centre, Jackson M.S. 39216, U.S.A.

Accepted 10 July, 2006

Juvenile hormone analogues (JHA s) are known to prolong larval life in insects, and these have been tried for the improvement of silk production in the silkworm, *Bombyx mori* (L). In the cocoons and silk from 5th instar *B. mori* treated with selected doses of methoprene and fenoxycarb, quantitative parameters like cocoon weight, shell weight, shell percentage, filament length and denier followed by qualitative characters of the silk like non-breakable filament length, reelability, winding capacity, tenacity, elongation percentage, cohesiveness, sericin and fibroin contents were determined. The cocoon from 1.0 µg methoprene and 3.0 fg/larvae treated on days one and two showed improved quantitative characters of cocoons followed by qualitative characters of the silk over the control. The use of juvenile hormone like methprene and fenoxycarb during summer season will help to get improved cocoon yield.

Key words: Silkworm, methoprene, fenoxycarb, cocoons and silk.

INTRODUCTION

Juvenile hormone analogues (JHA) which prolong the larval life in insects have been long employed for the improvement of silk production in the silkworm *Bombyx mori* (L). Akai et al. (1971) have demonstrated increased accumulation of silk protein accompanying the prolongation of larval growth in *B. mori* by treatment with synthetic JHA. Similar reports were also available from the work of Ratnasen (1988), Rani and Bharathi (1998), Nair et al. (1998), Kamimura and Kuchi (1998), and Chengamma et al. (2000).

Summer is the season where the sericulturists face odd environmental conditions that directly reduce the silk yield. During this season, the use of JHA compounds has been shown to enhance cocoon yield (Akai et al., 1971, 1985). In view of this, the effect of two JHA compounds namely methoprene and fenoxycarb on the cocoon characters from *B. mori* were investigated in the presented study.

*Corresponding authors E-mail: drdmmamatha@gmail.com.

MATERIALS AND METHODS

Bombyx mori breed of PM X NB_4D_2 was selected for the study and only the 5th instar larvae were used. The JHAs selected for the study were methoprene (isopropyl (2E, 4E)-11-methoxy-3,7,11-trimethyl-2,4-dodecadinoate) and fenoxycarb (6-ethyl-N-[2-(4-Phenoxy phenoxy) ethyl] carbamate) (Fisher et al., 1979).

Based on a pilot study, a dose of 1.0 µg/larvae of methoprene and 3 fg/larvae of fenoxycarb (each in 5 µl of acetone) were topically applied to the individual 5th instar larvae (40 h after the fourth ecdysis) (n=100 each time) with the help of a microsyringe on days one and two along the dorsal midline. Experimental control group received 5 µl/larvae of acetone and normal controls were maintained without any treatment. Methoprene at the selected dose increased the larval growth period by 15 h and that of fenoxycarb by 10 h. The control and JHA treated group of larvae were reared and allowed to pupate. In the control and experimental groups of cocoons, the quantitative parameters such as cocoon weight, shell weight, shell percentage, filament length and denier were measured by the procedures given by Sonwalkar (1993). The qualitative profiles of the silk from the control and JHA treated *B. mori* like non-breakable filament length, reelability, winding capacity, tenacity and elongation, cohesiveness, sericin and fibroin contents were also measured by adopting the procedures given by Sonwalkar (1993).

For each parameter, the mean of individual observations (for both

Table 1. Methoprene induced changes of quantitative parameters of cocoons and silk.

Quantitative parameters	Control	Experimental control	Experimental
Cocoon weight (g)	1.59±0.02	1.66±0.05 (4.40)^{y}	2.96±0.04 (78.31)^{y}
Shell weight (g)	0.29±0.04	0.294±0.01 (1.03)^{NS}	0.62±0.15 (110.54)^{y}
Shell percentage (%)	18.23±1.05	17.71±0.82 (-2.85)^{NS}	20.94±1.01 (18.06) ^{NS}
Filament length (m)	803.14±2.31	806.05±2.66 (0.36)^{x}	925.59±4.83 (14.83)^{y}
Denier (d)	2.41±0.03	2.39±0.055 (-0.83)^{NS}	0.935±1.98 (-60.87)^{y}

Each value is the mean ± SD of 20 samples.
Values in bracket are percent changes over normal control.
^{x}Significant at P<0.05.
^{y}Significant at P<0.001.
^{NS}Not significant.

Table 2. Fenoxycarb induced changes of quantitative parameters of cocoon and silk

Name of the quantitative parameters	Control	Experimental Control	Experimental
Cocoon Weight (gms)	1.64	1.63^{a}	2.41^{b}
SD	±0.05	±0.036	±0.031
PC		(0.609)	(47.852)
t		NS	P<0.001
Shell weight (gms)	0.274	0.268^{a}	0.415^{b}
SD	±0.013	±0.032	±0.126
PC		(-2.189)	(54.85)
t		NS	P<0.001
Shell percentage (%)	16.70	16.44^{a}	17.21^{b}
SD	±0.56	±0.62	±0.85
PC		(-1.55)	(4.68)
t		NS	P<0.001
Filament length (mts)	805.46	806.05^{a}	890.50^{b}
SD	±3.89	± 2.91	±4.62
PC		(0.07)	(10.48)
t		NS	P<0.001
Denier (d)	2.35	2.32^{a}	1.38^{b}
SD	±0.04	±0.02	±0.621
PC		(-1.27)	(-40.51)
t		P<0.001	P<0.001

Each value is the mean ± SD of 20 samples, PC: Percent change.
NS: Not Significant
a: Percent change over normal control
b: Percent change over experimental control

control and experimental groups) were taken into consideration. Statistical significance of the data was analysed through two way ANOVA (analysis of variance); SNK (Student Newman-Keuls) test and regression analysis (Zar, 1984).

RESULTS AND DISCUSSION

The cocoons from methoprene and fenoxycarb treated *B. mori* larvae showed increased cocoon weight, shell weight, shell percentage, filament length followed by decreased levels of their denier compared to either untreated/acetone treated control group of cocoons (p<0.001) (Tables 1 and 2). Except the winding capacity, the remaining qualitative parameters like non-breakable filament length, reelability tenacity, elongation and cohesiveness showed statistically significant (p<0.001) increase (Tables 3 and 4). Similar trends were also observed for methoprene and fenoxycarb treated silk fibrion and sericin content (Tables 5 and 6).

From time to time, many JHAs are being introduced into the market. Their mode of action and efficacy in promoting the quality and quantity of cocoons from JHA treated silkworm larvae have been worked out by many investiga-

Table 3. Methoprene induced changes of qualitative parameters of silk

Name of the qualitative parameters	Control	Experimental Control	Experimental
Non-breakable filament length (mts)	711.22	712.54	885.27
SD	±4.75	±1.22	±5.64
PC		(0.18)[a]	(24.24)[b]
T		NS	P<0.001
Reelability (%)	88.55	88.39	95.64
SD	±2.08	± 0.96	±1.39
PC		(-0.18)[a]	(8.20)[b]
T		NS	P<0.001
Winding Capacity (breaks/hr)	4.35	4.02	3.36
SD	±0.06	±0.07	±0.264
PC		(-5.28)[a]	(-18.44)[b]
t		P<0.001	P<0.001
Tenacity (gms/denier)	3.79	3.16	5.10
SD	±0.03	±0.04	±0.31
PC		(-16.62)[a]	(61.39)[b]
t		P<0.001	P<0.001
Elongation (%)	23.15	24.42	29.30
SD	±1.39	±0.08	±1.50
PC		(5.48)[a]	(19.98)[b]
t		P<0.001	P<0.001
Cohesiveness (strokes)	146.77	148.77	170.44
SD	±3.60	±2.11	±0.905
PC		(1.36)[a]	(14.57)[b]
t		P<0.05	P<0.001

Each value is the mean ± SD of100 samples, PC: Percent change.
NS: Not Significant
a: Percent change over normal control
b: Percent change over experimental control

tors (Asano et al., 1986; Kamimura and Kiuchi, 1998; Bharathi and Yungen, 2000; Nair et al., 2000; Yungen and Bharathi, 2001). The present observed trends for methoprene and fenoxycarb treated cocoons are in agreement with reports of the earlier authors for various JHA compounds (Kobari and Akai, 1979; Akai et al., 1985; Trivedi et al., 1993; 1997; Thapa and Tuan, 1989; Chengamma et al., 2000).

The results on reeling parameters (Tables 3 and 4) suggest that the quality of silk is superior which has much economic importance in the reeling industry. Increases in filament length, non-breakable filament length, and reelability percentage of silk produced are the most important commercial characters in the improvement of silk quality and yield (Kajiura et al., 1989; Kamimura, 1998).

In the present investigation, methoprene and fenoxycarb have significantly enhanced the fibroin and sericin contents of the silk over the experimental control (Tables 5 and 6) and this could be attributed to the stimulatory affect on the silkgland to synthesize more DNA and RNA as earlier reported (Mamatha et al., 2000; 2002).

It is a well known fact that the insect larval moulting is determined by the interaction of JH secreted by corpora allata and MH secreted by prothorasic gland activated by the brain hormone PTTH. The larvae, pupae and adult transformations are determined mainly by the functions of MH (Bharathi and Yungen, 2000). MH has been shown to regulate many important functions such as morphogenesis (Kajiura and Yamashita, 1992; Tanka and Takeda, 1993), the metabolism of nucleic acids (Lagan et al., 1975) and protein synthesis (Chen, 1985; Dai et al., 1985). JH and ecdysone decide the pattern of development in the silkworm as in the case of any other insect (Nair, 1998). JH mimics have direct stimulators effect of silkgland metabolism (Kajiura and Yamashita, 1989). The stimulating capacity of both the JHA hormones on various characters of the silkworms contributing to quality silk yield in the present study may be attributed to the synthesis of proteins and nucleic acids. Methoprene and fenoxycarb act similarly by prolonging the larval duration. Similar reasons have been reported for various JHA compounds for improved quality and quantity of cocoons and silk (Nair et al., 1998; Bharathi and Yungen, 2001; Chengamma et al., 2000). From the overall experimental data of the present study,

Table 4. Fenoxycarb induced changes of qualitative parameters of silk

Name of the qualitative parameters	Control	Experimental Control	Experimental
Non-breakable filament length (mts)	709.53	708.65	801.96
SD	± 2.67	±4.10	±2.60
PC		(-0.12)	(13.17)
t		NS	P<0.001
Reelability (%)	88.08	87.91	90.050
SD	±0.84	±0.951	±3.53
PC		(-0.19)	(2.43)
t		NS	P<0.001
Winding Capacity (breaks/hr)	4.05	4.13	4.01
SD	±0.05	±0.03	±0.37
PC		(1.97)	(-2.90)
t		NS	P<0.001
Tenacity (gms/denier)	3.75	3.68	4.88
SD	±0.03	±0.0411	±0.20
PC		(-1.87)	(32.61)
t		NS	P<0.001
Elongation (%)	21.92	20.96	26.74
SD	±0.62	±0.82	±1.69
PC		(-4.38)	(27.57)
t		P<0.05	P<0.001
Cohesiveness (strokes)	147.57	148.56	156.79
SD	±2.41	±1.08	±3.54
PC		(0.67)	(5.58)
t		NS	P<0.001

Each value is the mean ± SD of 100 samples, PC: Percent change
NS : Not Significant
a: Percent change over normal control
b: Percent change over experimental control

Table 5: Methoprene induced changes in the levels of fibroin and sericin in the silk gland of silkworm *Bombyx mori.* L.

Constituents of silk gland	Control	Experimental control	Experimental
Fibroin (%)	61.66	62.74	74.08
SD	±2.80	±1.06	±2.60
PC		(1.75)[a]	(18.07)[b]
t		NS	P<0.001
Sericin (%)	23.80	23.15	25.17
SD	±0.842	±0.841	±1.35
PC		(-2.73)[a]	(8.72)[b]
t		P<0.005	P<0.001

Each value is the mean ± SD of 20 samples,
PC: Percent change NS: Not Significant.
a: Percent change over normal control
b: Percent change over experimental control
P<0.001

we have established that methoprene and fenoxycarb extends the 5th instar *B. mori* larvae feeding in summer. Both the JHA compounds improve the overall biomass of *B. mori* larva and these events might be responsible for improved silk quality and quantity.

Table 6: Fenoxycarb induced changes in the levels of fibroin and sericin in the silk gland of silkworm *Bombyx mori.* L.

Constituents of silk gland	Control	Experimental control	Experimental
Fibroin (%)	60.36	62.16	71.41
SD	±2.63	±0.869	±1.24
PC		(2.98)[a]	(14.88)[b]
t		P<0.05	P<0.001
Sericin (%)	24.65	23.06	25.57
SD	±1.04	±0.924	±0.762
PC		(-6.45)[a]	(10.88)[b]
t		NS	P<0.001

Each value is the mean ± SD of 20 samples,
PC: Percent change NS: Not Significant
a: Percent change over normal control
b: Percent change over experimental control
P<0.001

REFERENCES

Akai H, Kobayashi M (1971). Induction of prolonged Larval instar by the Juvenile Hormone in *Bombyx mori.* L. (Lepidoptera: Bombycidae). Appl. Entomol**.** 2001 (6): 1938-1939.

Akai H, Kimur K, Kiuchi M, Shibukawa A (1985). Increase in silk production by repeated treatments with a Juvenile Hormone Analogue. J. Seric. Sci. Jpn. 54 (4): 297-299.

Asano S, Kuwano E, Eto M (1986). Anti-juvenile hormone activity of Imidazole compound (KK-22) and its dimunition by Methoprene in the 4th instar silkworm, *Bombyx mori*. L. Applied Entomology Zoology. 21 (1) : 63-69.

Bharathi D, Miao Yungen (2000). The impact of juvenile and moulting hormone analogues on silk quality of silkworm, *Bombyx mori* L. Fed on mulberry leaf and artificial diet. Philippine J. Sci. 129 (2): 125-130.

Chengamma C, Rajesekhar R, Govindappa S (2000). Influence of methoprene on *Bombyx mori*. L. Indian. J. Seric. 39: 135-138.

Chen PS (1985). Amino acid and protein metabolism. In : Comparative inext physiology, Biochemistry. Pharmacology. (Eds. Kerkut. G.A. and Gilbert) Pergamon press. England. 19: 177-216.

Dai YJ, Li R, Zhang YO (1985). Moulting Hormone and and silk protein synthesis : Effect of ecydysone on nucleic acid metabolism in the posterior portion of silk gland in *B. mori*. Acta. Entomol. Sinicia. 28: 8-14.

Kajiura Z, Yamashita O (1992). Induction of metamorphosis of the methoprene induced dauer larvae of the silkworm, *Bombyx mori,* by ecdysteroids. Comp. Biochem. Physiol. 101A : 277-280.

Kajiura Z, Yamashita O (1989). Super growth of silk glands in the dauer larvae of silkworm, *Bombyx mori* induced by a juvenile hormone analogues. J. Seri. Sci. Jpn. 58 : 39-46.

Kamimura M, Kiuchi M (1998). Effect of Juvenile Hormone Analog Fenoxycarb on 5th stadium larvae of the silkworm, *Bombyx mori.* L. Applied. Entomology and Zoology. 33 (2): 333-338.

Kobari Y, Akai H (1979). On quantitative increase of silk production by the administration of juvenile hormone (manta). J. Seric. Sci. Jpn. Sonwalker TN (1993). Hand book of Silk Technology, Wiley Eastern Limited, New Delhi, pp.14-25.

Tanka Y, Takeda S (1993). Ultra neurmary larval ecdysis of the silkworm, *Bombyx mori* induced ecdysone. Nature Wissens Chatten. 80 : 131-133.

Trivedi K, Ramadevi OK, Magadum SB, Datta RK (1993). Effect of Juvenile
hormone analogue, Labomin on the growth and economic characters of silkworm, *Bombyx mori* L. Indian. J. Seric. 32 (2) : 162-168.

Trivedi K, Nair KS, Absan MM, Datta RK (1997). A juvenile normal mimic modulated enhancement in silk productivity in silkworm. *Bombyx mori*. L Indian J. Seric. 36 (1): 42-44.

Yungen M, Bharathi D (2001). Effect of methoprene and MH alone and in combination on the ultrastructural changes in the posterior silkgland of the silkworm, *Bombyx mori* L. Sociologia. 41 (2): 169-175.

Zar JH (1984). Bio statistical Analysis. 2nd ed. Prentice Hall , EngleWood Diffe, NJ.