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EFFECT OF THYROXINE ON GROWTH AND SILK PRODUCTION OF MULBERRY SILKWORM, *Bombyx mori* L. (BOMBYCIDAE: LEPIDOPTERA).

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ABSTRACT

Effect of feeding mulberry silkworm on leaves treated with Thyroxine on the mean weights of mature larva, silk gland, pupa, fresh cocoon, and cocoon shell of mulberry silkworm, *B. mori* was studied under laboratory laboratory hygrothermic conditions of 25±5^{0}C and 65-85% R.H. The last instar larvae which fed on mulberry leaves was dipped in Thyroxine solution at concentrations of 0.025, 0.125, 0.250, 1.250, 2.500, 5.000, and 10.000 ppm. Results indicated that Thyroxine revealed high values of the mean weight of mature larva ranged from 10.0% up to 18.9% more than untreated at concentrations of 10 and 0.025 ppm. Silk gland showed an increase of the mean weight ranged from 3.8% up to 24% at concentrations of 10.0 and 2.5 ppm. Mean weight of pupa increased by range from 3.0% up to 20.2% at concentrations of 0.025 and 2.5 ppm in comparison to untreated one. Fresh cocoon showed an increase ranged from 4% up to 19.8% at concentrations of 10 and 2.5 ppm. Cocoon shell presented also an increase ranged from 2.8% up to 17.9% at concentrations of 1.250 and 2.5 ppm in comparison to untreated one. Results indicated that the highly and significant values of the mean weight of mature larva, silk gland, pupa, fresh cocoon, and cocoon shell was gained by larvae fed on mulberry leaves treated with Thyroxine at the concentration of 2.5 ppm.

Keywords: *Bombyx mori*, Thyroxine, Vertebrate hormone, Silk production

INTRODUCTION

Sericulture is a labor intensive industry with its agricultural part of mulberry cultivation (Devaiah and Reddy, 1999). Recently, much research has been done on the diet supplementation of mulberry leaves fed to silkworms. These supplementations include hormones such as Thyroxine. It has been known that application of hormones to *Bombyx mori* L. could be used to improve the quality of silk (Akai *et al.*, 1985; and Ahmad *et al.*, 2007). Application of hormones to *B. mori* to improve the quality of silk has been suggested by many reports for example, Narasimha Murthy *et.al.* 1987; Thyagaraja *et al.*, 1991; Bharathi and Miao Yun-gen, 2002; Ahmad *et al.* 2007 and Ahmad *et al.*, 2009. Vertebrate hormones stimulate growth, lipid metabolism, sugar uptake and cellular utilization in insects and other invertebrates (Karmer, 1983; and Magadum and Hooli, 1988). It is well known that vertebrate hormones accelerate vitellogenesis in insects which is due to increase function of topocytes in the germanium (Landa, 1970). Application of hormones to mulberry silkworm, *B. mori* could be used to improve the quality of silk (Akai *et al.*, 1985). Thyagaraja, *et al.* (1991) reported that Thyroxine could be used to shorten larval period and at the same time increase cocoon and silk weight up to 150% as compared to controls. Ahmad, *et al.* (2007)

reported that adding of Thyroxine to *B. mori* larvae increased the ecdysteroid titer 33.34% higher than control; higher titer of ecdysteroid presumably would promote larval growth, as well as sericin and fibroin protein synthesis. Mansour, *et al.* (2009) added the vertebrate hormone Thyroxine and Melatonin and their mixture to the sugar syrup of honeybee *Apis mellifera*. The data proved that Thyroxine + Melatonin treatments gave the highest activities and honey yield. The influence of feeding mulberry silkworm on leaves treated with Thyroxine on the growth of the silk gland in the silkworm was studied by Ahmad, *et al.* (2009). They reported that silk glands from Thyroxine treated *B. mori* larvae weighed heavier than control. The present study was developed to observe the effect of Thyroxine on the larval growth and silk production of mulberry silkworm *B. mori* under laboratory conditions.

MATERIALS AND METHODS

A: The tested insect:

The mulberry silkworm used for the present study was the local hybrid (A422). The larvae were reared under the laboratory hygrothermic conditions of 25±5^{0}C and 65-85% R.H., according to the conventional method in trays and were provided with suitable amounts of fresh mulberry leaves (Gomaa, 1973). The experimental study was performed on the last larval instar. All larvae which molted to the last instar at the same time were grouped and used in the experiments. The determination of the last larval instar was determined by zero hour from the last larval ecdysis (Peferoen and Deloof, 1980).

B: Thyroxine and series of concentrations:

Thyroxine or 3:5, 3:5 tetra iodothronine (T4)

Thyroxine was obtained from SIGMA company as l – THYROXINE (3 – {4 –(4 –Hydroxy – 3,5 –diiodophenoxy) -3 ,5 –diiodophenyl} –l-alanine-.T4). Stock Solution of 25 ppm Thyroxine was prepared by dissolving 25 mg.Thyroxine in 100 ml sodium hydroxide 0.1 N and 900 ml distilled water. 200 ml of each concentration of Thyroxine was prepared by the stock solution 25 ppm Throxine and the distilled water.

C: Methods of test parameters:

The clean mulberry leaves were dipped in distilled water for untreated and in 200 ml of each concentration solution for the seven different treatments for 10 minutes and left to dry. The treated leaves were introduced to 20 larvae for check and 20 larvae for each of the seven concentrations. The daily mean weight of larvae, silk glands, fresh pupae, fresh cocoons and cocoon cortex's mean weight were recorded.

D: Data analysis:

Data of above parameters were subjected to one way analysis of variance (ANOVA), and the means were separated using LSD Test as described by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

1- Effect of Thyroxine on the larval weight.

Data in Table (1) show that larvae fed on mulberry leaves dipped in Thyroxine solutions revealed an increase than untreated in the mean weights of mature larvae at all tested concentrations. These increases ranged from 4.50% at 1.250 ppm up to 19.62% at 2.5 ppm. Data also showed that there are ideal hormone concentrate (2.5 ppm) for maximum increase of larval weight which participates in increase of silk production. The present results are in agreement with the findings of Narasimha Murthy *et.al.* (1987) who reported that larvae which received Thyroxine hormone showed an increase of 10.9% in the larval weight compared with the control. Thyagaraja *et.al.* (1991) found that larvae treated with Thyroxine during the second instar showed maximal growth along with other enhanced cocoon characters.

Thyroxine was reported to affect ecdysteroid metabolism and haemolymph protein in the silkworm, *B. mori* (Thyagaraja, *et. al.* 1991). The fat body adenosine triphosphatase is influenced by Thyroxine in all the stages of silkworm (Damodar Reddy, *et. al.* 1996, and Damodar Reddy and Chaudhuri, 1998).

Table (1): Effect of Thyroxine on the mean weight of mature larvae.

Concentration (p.p.m)	Mean Weight (g)±SD	% Increase
Untreated	3.089 ± 0.566 d	
0.025	3.674 ± 0.169 a	18.94
0.125	3.605 ± 0.482 ab	16.70
0.250	3.528 ± 0.386 abc	14.21
1.250	3.228 ± 0.416 cd	4.50
2.500	3.695 ± 0.405 a	19.62
5.000	3.633 ± 0.637 ab	17.61
10.000	3.397 ± 0.434 bc	9.97

*** Means followed by the same letter in a column are not significantly different at the 5% level of probability (LSD Test).**

2- Effect of Thyroxine on the silk gland.

Larvae fed on mulberry leaves dipped in Thyroxine solutions revealed an increase in the mean weight of silk gland at all tested concentrations in comparison to untreated as shown in Table (2). Data also showed that the relation between concentrations of Thyroxine hormone and the mean weight of fresh silk gland was positively proportional with series of concentrations between 0.025 ppm and 2.500 ppm and negative proportional with concentrations 5.0 and 10.0 ppm. The highest significant increase of fresh silk gland (23.75%) was done with the concentration of 2.5 ppm. Also the increases of fresh silk gland participate in increase silk production. A similar finding was reported by Ahmad *et. al.* (2009) who reported that silk gland of *B. mori* larvae treated with Thyroxine showed a heavier weight than untreated.

There is one possible explanation for this finding that is, application of Thyroxine may increase the ecdysteroid titre in haemolymph, stimulates

protein synthesis, including sericin and fibroin synthesis in the silk gland (Thyagaraja, *et. al.* 1991 and Ahmad, *et. al.* 2007)

Table (2): Effect of Thyroxine on the mean weight of fresh silk gland.

Concentration (p.p.m)	MeanWeight (g)±SD	% Increase
Untreated	0.758 ± 0.042 c	
0.025	0.800 ± 0.030 abc	5.54
0.125	0.815 ± 0.065 abc	7.52
0.250	0.905 ± 0.087 ab	19.39
1.250	0.917 ± 0.116 ab	20.98
2.500	0.938 ± 0.093 a	23.75
5.000	0.850 ± 0.122 abc	12.14
10.000	0.787 ± 0.051 bc	3.83

*** Means followed by the same letter in a column are not significantly different at the 5% level of probability (LSD Test).**

3- Effect of Thyroxine on the pupal weight.

As shown in Table (3), larvae treated with Thyroxine at concentrations of 0.125, 2.5, 5.0 and 10.0 ppm presented highly and significant increase in the pupal mean weights 12.93, 20.16, 13.65 and 9.27%, respectively in comparison with untreated one. While insignificant changes were observed with the concentrations of 0.025, 0.250, 1.250 and 10.0 ppm, respectively.

The injection of Thyroxine during the fifth instar larvae of silkworm, *B. mori* significantly elevated all ATPase activities in the larval and pupal stages, and the fat body ATPase were influenced by Thyroxine in all the stages of silkworm in a dose-dependent manner (Damodar Reddy, *et. al.* 1996 and Damodar Reddy, and Chaudhuri, 1998).

Table (3): Effect of Thyroxine on the mean weight of pupae.

Concentration (p.p.m)	Mean Weight (g)±SD	% Response
Untreated	0.982 ± 0.160 cd	
0.025	1.007 ± 0.183 bcd	+2.55
0.125	1.109 ± 0.082 ab	+12.93
0.250	0.974 ± 0.200 cd	-0.81
1.250	0.912 ± 0.230 d	-7.13
2.500	1.180 ± 0.256 a	+20.16
5.000	1.116 ±0.113 ab	+13.65
10.000	1.073 ± 0.255 abc	+9.27

*** Means followed by the same letter in a column are not significantly different at the 5% level of probability (LSD Test).**

4- Effect of Thyroxine on the cocoons and silk production.

Data in Table (4) showed that larvae treated with Thyroxine at concentrations of 0.125, 2.5 and 5.0 ppm presented highly and significant increase in the fresh cocoon mean weights of 11.81, 19.77 and 13.74%, respectively in comparison with untreated, while insignificant changes were observed with concentrations of 0.025, 0.250, 1.250 and 10.0 ppm, respectively. The highest value of the mean weights of fresh cocoons was

achieved with the concentration of 2.5 ppm, while the lowest was obtained with the concentration of 1.250 ppm.

Larvae treated with Thyroxine at all concentrations gained an increases in the mean weights of cocoon shell but the highly and significant increases were 17.92, 16.98 and 11.32% at concentrations of 2.5, 5.0 and 0.025 ppm, respectively. Cocoon shell weight to cocoon weight (cocoon shell ratio) showed high ratio in larvae treated with concentrations of 1.250, 0.025, 0.250 and 5 ppm. Meanwhile, these ratios were less at concentrations of 0.125, 2.5 and 10.0 ppm. The general mean of cocoon shell ratio was 18.6. The treatment of silk worm larvae *B. mori* with different concentrations of Thyroxine did not alter the ratio.

Table (4): Effect of Thyroxine on the mean weight of fresh cocoon and cocoon shell.

Concentration (p.p.m)	Fresh Cocoon		Cocoon shell		% Cocoon Shell Ratio
	Mean Weight (g)±SD	% Response	Mean Weight (mg)±SD	% Increase	
Untreated	1.194 ± 0.164 cd		212 ±27 c		17.76
0.025	1.242 ± 0.185 bcd	+4.02	236 ± 07 ab	11.32	19.00
0.125	1.335 ± 0.088 ab	+11.81	226 ± 24 bc	6.60	16.93
0.250	1.200 ± 0.203 cd	+0.50	226 ± 15 bc	6.60	18.83
1.250	1.129 ± 0.226 d	-5.44	218 ± 20 c	2.83	19.31
2.500	1.430 ± 0.265 a	+19.77	250 ± 34 a	17.92	17.48
5.000	1.358 ± 0.131 ab	+13.74	248 ± 56 a	16.98	18.26
10.000	1.302 ± 0.265 bc	+9.05	229 ± 21 bc	8.02	17.59

*** Means followed by the same letter in a column are not significantly different at the 5% level of probability (LSD Test).**

The increase in the cocoon weight, shell weight and shell percentage may be due to the stimulatory effect of Thyroxine, the hormone has been reported to be involved in the synthesis of proteins, lipids and nucleic acids in vertebrates (Ghosh and Medda, 1969; Tata, 1970; and Singh, 1972). The hormone also stimulates lipid metabolism, sugar uptake and cellular utilization in insects (Karmer, 1983 and Magadum and Hooli, 1988).

From these results, it could be reported that Thyroxine increase the economic traits (silk) and improve the biological parameters of silkworm *B. mori.*

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تأثير هرمون الثيروكسين على نمو وإنتاج الحرير لدودة الحرير التوتية

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تمت دراسة تأثير التغذية بأوراق التوت المعاملة بهرمون الثيروكسين على متوسط أوزان اليرقات الكاملة النمو، غدة الحرير، العذارى، الشرانق الطازجة وقشرة الحرير لدودة الحرير التوتية حيث تمت تغذية يرقات العمر الأخير على ورق توت معامل بالثيروكسين بطريقه الغمر بتركيزات ٠,٠٢٥ ، ٠,١٢٥ ، ٠,٢٥٠ ، ١,٢٥٠ ، ٢,٥٠٠ ، ٥,٠٠٠ ، ١٠,٠٠٠ جزء بالمليون.

وقد أوضحت النتائج فعالية الثيروكسين حيث تراوح متوسط الزيادة في وزن اليرقات من ٩,٩٧ إلى١٨,٩٤ ٪ عند التركيزين ١٠ ، ٠,٠٢٥ جزء في المليون على التوالي مقارنة بالكنترول وقد أظهرت غدة الحرير زيادة في متوسط الوزن تراوح من ٣,٨٣ إلى ٢٣,٧٥ ٪ عند التركيزان ١٠ ، ٢,٥ جزء في المليون على التوالي وقد زاد وزن العذارى بمتوسط تراوح من ٢,٥٥ إلى ٢٠,١٦ ٪ عند التركيزان ٠,٠٢٥ ، ٢,٥٠ جزء في المليون مقارنة بالعذارى الغير معامله وأظهر متوسط أوزان الشرانق الطازجة زيادة تراوح من ٤,٠٢ إلى ١٩,٧٧٪ عند التركيزان ١٠ ، ٢,٥ جزء في المليون على التوالي وقد زاد أيضا متوسط وزن قشرة الحرير بزيادة تراوحت من ٢,٨٣ إلى ١٧,٩٢ ٪ عند التركيزان ١,٢٥٠ ، ٢,٥٠ جزء في المليون على التوالي مقارنة بالكنترول.

وقد أوضحت النتائج أن التركيز ٢,٥٠ جزء في المليون أعطى أفضل النتائج بالنسبة لمتوسط أوزان اليرقات وغدة الحرير والعذارى والشرانق الطازجة وقشرة الحرير.

قام بتحكيم البحث

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