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ORIGINAL ARTICLES

Action of lettuce seed oil on the silk gland protein profile of the mulberry silk worm *Bombyx mori* L. (Lepidoptera, Bombycidae).

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ABSTRACT

Larvae of the mulberry silkworm *Bombyx mori* L. were fed on mulberry leaves treated with 0.01, 0.05, 0.10, 0.50 and 1.00% lettuce seed oil (LSO). The silk gland total protein was estimated from the 2nd day to the 10th day (spinning day) of the 5th larval instar. The highest value of total silk gland protein was obtained after feeding larvae on 0.10% LSO. The electrophoretic patterns of silk gland proteins showed no distinguished differences in protein patterns between the control and LSO treatments.

Key words: *Bombyx mori* L. lettuce seed oil, silk gland, total protein

Introduction

The mulberry silkworm is the major economic resource for more than 30 million families in China, India, Vietnam and Thailand (Li *et al.,* 2006). Recently, developing countries including Egypt have been given more attention to the production of natural silk for many socio-economic reasons. Silkworms produce a delicate twin thread of silk fibroin, which is coated by a protective cover of sericin (Komatsu, 1975). The silk fiber protein is synthesized by silk gland cells and stored in the lumen of the silk glands. Subsequently, it is converted into silk fibers. Silk fibroin secreted in the lumen of posterior silk gland of *B. mori* consists of three protein components, High (H) chain 350 kDa, Low (L) chain 26 kDa and glycoprotein (P_{25}) 30 kDa. (Mondal *et al.*, 2007), while three layers of sericin secreted from the middle silk gland in normal larvae (Akai *et al.*, 2005).

One of the most important objectives of scientists concerned sericulture problems is to find out practical and applicable methods to increase silk yield. Lettuce is an annual plant native to the ancient Egypt initially for the edible oil extracted from its seeds. LSO contains folic acid, which regulates digestion, strengthens the nervous system, and is necessary for normal functioning for female hormonal system and body metabolism. LSO is also rich in vitamins A, C, K, B group, beta-carotene, riboflavin, calcium, magnesium, iron and iodine. The present study was carried out to evaluate LSO impact on the silkworm silk gland proteins profiles during the fifth larval instar.

Materials and Methods

Insect:

Silkworm eggs of local hybrid were obtained from the Sericulture Research Department of Plant Protection Research Institute, Agricultural Research Centre, Giza, Egypt. Larvae were reared under the laboratory conditions of 25±2 ºC and RH 75±5 %. Fresh mulberry leaves were freely offered to *B. mori* larvae 4 times per a day.

Lettuce seed oil:

Lettuce seed oil of *Lactuca sativa* var. *longifolia* was purchased from EL Captain Company (Cap Farm) for extracting nutriments oil and natural herbs and cosmetics, Al Obour city, Cairo, Egypt.

Treatment and experimental design:

Lettuce seed oil (LSO) was dissolved in 0.5 – 1.0 % Triton X-100 and diluted to the experimental concentrations (0.01, 0.05, 0.10, 0.50, and 1.00%) using distilled water. The concentrations of LSO were chosen

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on the basis of preliminary experiments which were formerly carried out. Treated leaves were fed to the 4th and 5th instar larvae 4 times per day, while the control larvae were offered mulberry leaves dipped in a diluted Triton X-100 solution. Each treatment was replicated three times, and each replicate contained 500 larvae. Data were recorded during the 5th instar larvae, after 2, 4, 6, 8 and 10 days (spinning day), respectively.

Sample preparation:

Larvae were dissected and silk glands were collected. Both middle silk gland (MSG) and posterior silk gland (PSG) were homogenized in 0.01 *M* Tris-HCl (pH 8.8) buffer. The homogenates were filtered through cheese cloth and the filtrates were centrifuged at 10000 x*g* for 10 min. The supernatant was collected and stored at -20°C till being used. Total silk gland protein was estimated spectrophotometrically according the method of Bradford (1976) using bovine serum albumin as a standard.

Electrophoresis:

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed in a discontinuous buffer system according to (Laemmli, 1970). An appropriate volume of the haemolymph sample was mixed with an equal volume of the sample buffer (0.0625*M* Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 0.002% bromophenol blue, with 5% β-mercaptoethanol) and submitted to a moderate heat treatment at 50^{o}C for 5 min, then centrifuged at 10000 x*g*. Gels used were 10% T for separating and 4.5% T for stacking gel. After electrophoresis, gels were stained with 0.1% Coomassie blue R-250 (Bio-Rad). To determine the molecular weights (MW) of separated proteins, standard protein marker (Bio-Rad) was used according to the method described by Weber & Osborn (1969).

Statistical analysis:

The data were subjected to analysis of variance (ANOVA) of Duncan's multiple range function in SAS.

Quantitative determination of the resolved protein bands was carried out using the Total Lab Software Analysis (Microsoft version 11.0).

Results:

I. Effect of Lettuce seed oil administration on total silk gland protein concentrations:

An increase in silk gland total protein content during the last larval instar was recorded. The estimated total protein content at the 5th larval instar was 30.8 mg/g silk gland during day 2 to 72.92 mg/g silk gland at the end of the 5th instar (Fig. 1).

Generally, the level of total protein showed an increasing trend in the silk gland till the end of the 5th larval instar under both control conditions and LSO treatments (Fig. 1). Significant differences at 0.05 and 0.10 % LSO during the mature larval stage (spinning stage), the recorded means of total silk gland protein contents were 100.42 and 105.42 mg/g, respectively.

II. Electrophoretic patterns of silk gland proteins:

The SDS-PAGE electrophoretic profiles of the silk gland proteins during grown larval instars revealed 17 – 22 bands. At the 2nd day of the 5th larval instar, 17 bands with molecular masses of 58-338 kDa were detected. Four major bands were presented in both control and LSO treatments. Their corresponding molecular masses were 61, 80, 116 and 204 kDa, respectively. No distinguished differences in patterns of both control and LSO treatments were observed (Fig. 2).

As shown in Fig. 3, a slight decrease in the number of protein bands was noticed at the 4th day. No obvious difference between control and LSO treated samples in the number of bands (16 bands ranged from 58 to 338 kDa and four major protein subunit bands of 61, 80, 116 and 204 kDa). However, a marked difference in bands intensities, especially major bands was noticed. It seems that, the LSO treatments at concentrations of 1.00 & 0.05 % enhanced the synthesis of major bands.

An increase in the number of protein bands was observed at the 6th day of *B. mori* last larval instar (18 bands). Their molecular masses ranged between 72 to 338 kDa. These results clearly indicated that the most intensive protein bands were those which were synthesized and accumulated during the last days of the 5th instar (Fig 4).

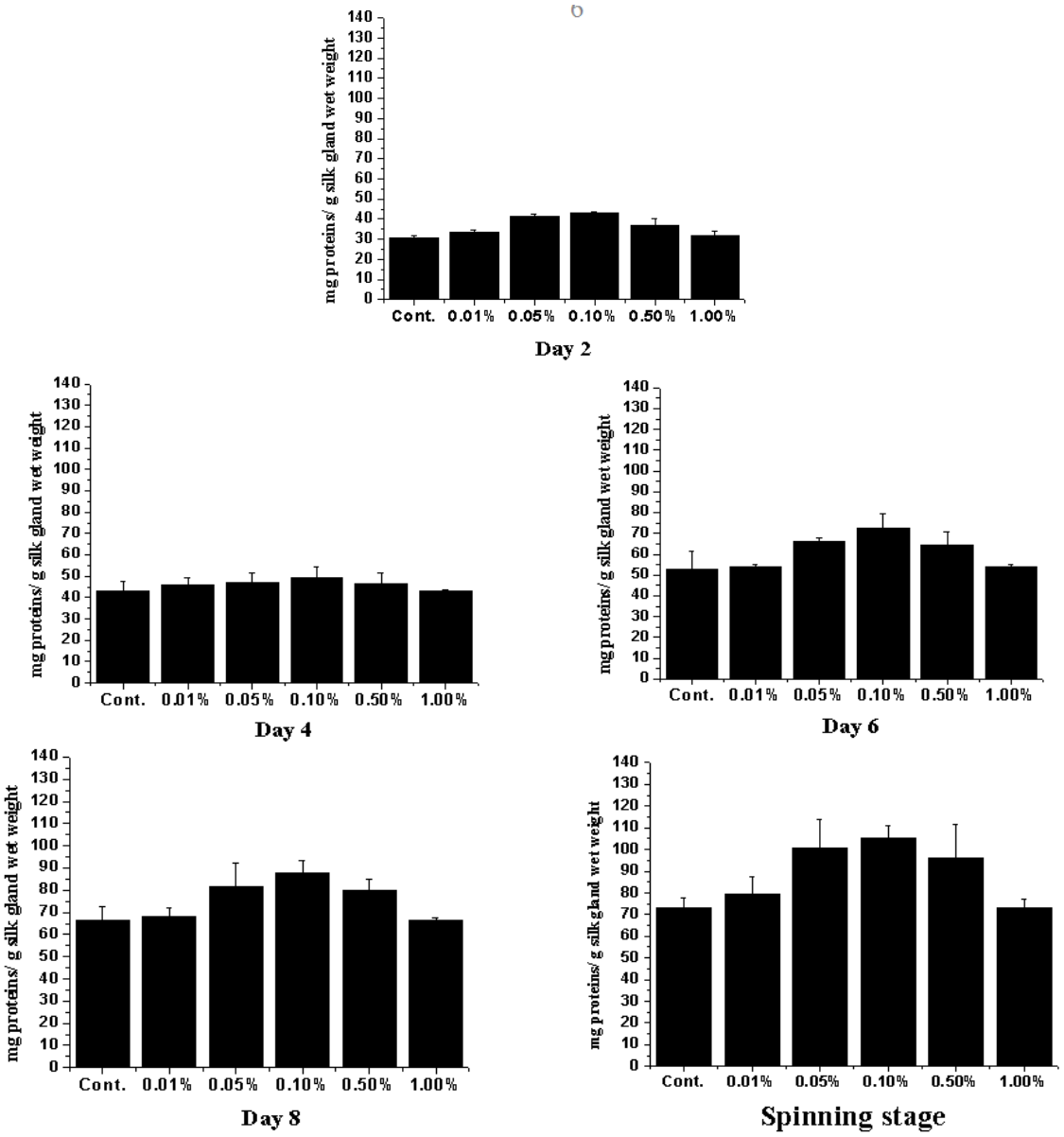

Fig. 1: The effect of lettuce seed oil on the total silk gland proteins of *B. mori* at 2, 4, 6, 8 and 10 days (spinning stage).

- Each column represents the mean of three replicates (each replicate consists of 10 records) and the vertical line is the standard error.

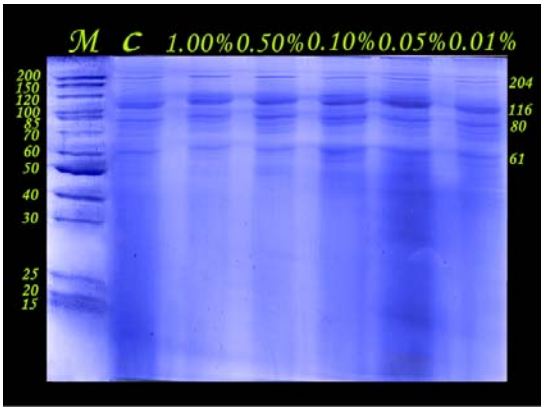

Fig. 2: SDS-PAGE patterns of the silk gland protein of *B. mori* 5th instar, Day 2 M: Standard protein marker, Lane C: Control, Lanes 2-6: Treatments with LSO; 1.00, 0.50, 0.10, 0.05 and 0.01 %, respectively.

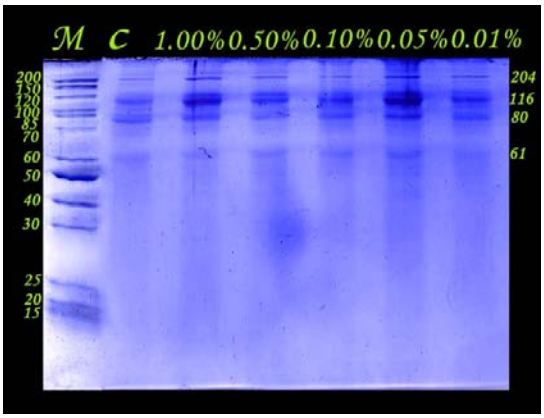

Fig. 3: SDS-PAGE patterns of the silk gland protein of *B. mori* 5th instar, Day 4, M: Standard protein marker, Lane C: Control, Lanes 2-6: Treatments with LSO; 1.00, 0.50, 0.10, 0.05 and 0.01 %, respectively.

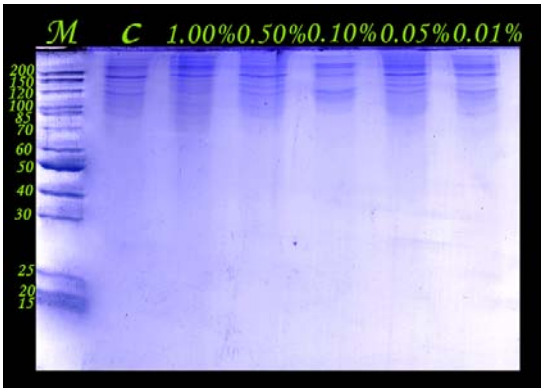

Fig. 4: SDS-PAGE patterns of the silk gland protein of *B. mori* 5th instar, Day 6, M: Standard protein marker, Lane C: Control, Lanes 2-6: Treatments with LSO; 1.00, 0.50, 0.10, 0.05 and 0.01 %, respectively.

A steady increase in the number of silk gland subunit bands was recorded at the 8th day of *B. mori* 5th larval instar. The number of subunit bands produced for silk gland samples was 20 subunit bands with molecular masses ranged between 80 to 338 kDa. Distinguished differences in intensity of bands among different LSO treatments were observed, (Fig. 5).

The maximum increase in the number of protein bands was detected at the 10th day, a total of 22 bands with molecular masses of 72 to 338 kDa. The three major protein bands were 105, 144 and 204 kDa (Fig. 6).

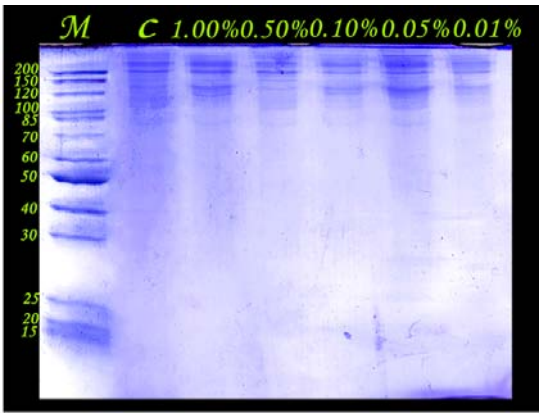

Fig. 5: SDS-PAGE patterns of the silk gland protein of *B. mori* 5th instar, Day 8, M: Standard protein marker, Lane C: Control, Lanes 2-6: Treatments with LSO; 1.00, 0.50, 0.10, 0.05 and 0.01 %, respectively.

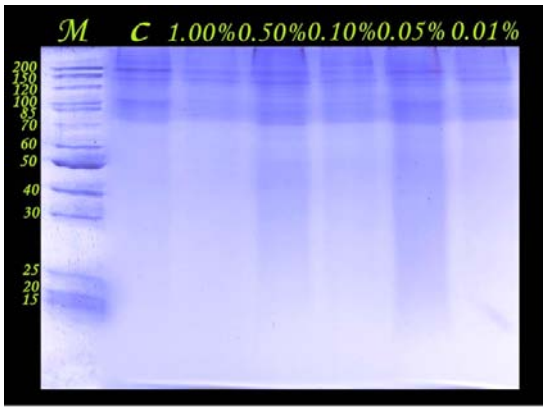

Fig. 6: SDS-PAGE patterns of the silk gland protein of *B. mori* 5th instar, Day 10, M: Standard protein marker, Lane C: Control, Lanes 2-6: Treatments with LSO; 1.00, 0.50, 0.10, 0.05 and 0.01 %, respectively.

Discussion:

I. Effect of Lettuce seed oil administration on total silk gland protein concentrations:

An increase in silk gland total protein content during the last larval instar was recorded during the present study. These results are supported by Singh *et al.* (1994); Mahmoud (1988) Passant & Szafranski (1970). They mentioned that the silk gland of *B. mori* and *Ph. ricini* silkworms formed the silk gradually during the 5th larval instar and reached its maximum level just before spinning commences. Maximum level of the P_{25} protein accumulation during the 5th instar can be correlated with the maximum feeding (Waldbauer, 1968). Similar findings were reported by Nagajyothi *et al.* (2010) who observed that the level of total and soluble proteins recorded an increasing trend in the silk gland tissue from day one to day 6 during the development of 5th instar being 64.54 mg/wet weight on day 6 of total protein comparing to 43.48 mg/wet weight on day 2 of the 5th larval instar. Sasaki & Noda (1973) reported that silkworms produce a large quantity of silk proteins for making the cocoons. The rate of feeding increases at the 4th and 5th larval instars of *B. mori*, the highest amount of silk proteins is synthesized at these stages as reported by Islam *et al.* (1977). Accumulation of the P_{25} protein/pair of silk glands/day was at the minimum level on the first day of the 5th larval instar. The level increased from first day to last day and reached its maximum level (Mathavan *et al.*, 1984).

II. Electrophoretic separation of silk gland proteins:

The protein components of the silk gland during grown larval instars were detected as about 17 – 22 bands by SDS-PAGE in the present study. No distinguished differences in patterns of both control and LSO treatments were observed except increasing in bands intensity toward of the 5th larval instars during day4 and 8. The present data confirm the findings obtained by Sarangi (1985), who found that the rate of protein synthesis during development have markedly increased until the 3rd day, while it showed a slight increase between the 3rd day and the 5th day of *B. mori* last larval instar. In the posterior area of the silk gland, proteins with various molecular weights were observed in all stages and increased with the growth of the silk gland (Okazaki *et al.*, 2005).

The observed gradual enrichment of protein spectrum was probably a result of the consecutive expression of protein in the period of gland growth. Also, these results clearly indicated that the most intensive were the protein bands synthesized and accumulated during the last days of the 5th instar which was probably due to the increased synthesis and accumulation of silk. These results may be supported by the findings of Mathavan *et al.* (1984) who reported that close to the end of the 5th instar. The level of P_{25} protein increased through the feeding period and reached the maximum on the final day of the 5th instar. Zhong *et al.* (2005) found that maximum level accumulation of P_{25} protein during 5th instar was similar to that observed for fibroin proteins.

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