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Effect of *Stevia Rebauidana* Extract of the Physiological and Economical Aspects of the Mulberry Silkworm, *Bombyx mori* L.

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ABSTRACT
The present work was conducted to study the influence of stevia leaf extract on the biological and economic characteristics of the mulberry silkworm, *Bombyx mori* L. as well as its influence on the physiological characteristics of the fifth instar larvae. The *B. mori* larvae, fifth instar were fed mulberry leaves supplemented with stevia leaf extract in various doses, since the last instar consumed more than 80% of mulberry leaves during its lifetime. The larva, silk gland, pupal cocoon shell weights, and quantity of laid eggs were all considerably increased in the treatment of 0.05 followed by 0.1% compared to the control. The highest total hemolymph protein contents were recorded at 0.05% stevia leaves treatment, the same trend with protease activity were noticed. The use of stevia leaves appears to have a positive impact on raising the quantity of silk produced by improving the digestibility, which in turn increases the production of protein and fertility. Due to the fact that the larval stage was prolonged by two days over the control, it was determined that stevia leaves may have the same effect as juvenile hormone.

Keywords: mulberry silkworm, *Bombyx mori*, stevia leaf extract, physiological characteristics, larvae, doses

1. Introduction

Sericulture has both an agricultural and an industrial parts, the agricultural part comprises raising silkworms and growing mulberries, while industrial part is based on the process production of silk thread. In China, Uzbekistan, India, Vietnam, Sri Lanka, and Thailand, more than 30 million families rely primarily on the mulberry silkworm as a source of income (Li *et al.,* 2006). For several socioeconomic factors, natural silk manufacturing has received increased attention in developing nations, particularly Egypt. The mulberry silkworm, *Bombyx mori* L., provides over ninety percent of the silk produced worldwide. For the monophagous silkworm, *B. mori* mulberry leaves are its only food supply, moreover, the production of mulberries alone accounts for more than 60 percent of the entire cost of cocoon production. Silk production depends on critical variables, including, silkworm strain, mulberry leaves which provided as food, breeding season, genotypes and other significant elements (Bongale *et al.,* 1997). For mulberry silkworm breeders, these factors are constant, thus the variable factor is fortification agents when added to the normal diet boost its nutritional value and significantly increase silk yield. Therefore, numerous researchers have introduced particular substances and nutrients as food additives such as juvenile hormone (JH) analogues, minerals, vitamins, proteins, various plant extracts, amino acids, royal jelly carbohydrates and probiotic to get a higher yield of the silkworms and fertility (Gangwar 2009; Shahin *et al.,* 2013a; Nijagal *et al.,* 2017).

Juvenile hormone has a distinct terpenoid structure (methyl ester of epoxy farnesoic acid), which most insects produced in six different forms. Numerous developmental and physiological processes, including behaviour, metamorphosis, reproduction, immunity, ageing and caste differentiation, have been shown to be regulated by JH, which is predominantly synthesised and secreted from the corpus allatum (Goodman and Cusson 2012). A number of researchers have discovered a correlation between the lengthening of the last larval instar and the increase in silk protein biosynthesis caused by JH and its derivatives, which are used as growth regulators (Akai *et al.,* 1971; Nihmura, *et al.,* 1974). Also, JH accelerates vitellogenesis by prompts the vitellogenin generation and uptake in insect ovary (Parthasarathy *et al.,* 2010). More focus has been placed on stevia (*Stevia rebauidana*), a sweet herb

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that is a healthier alternative to white sugar (Soejarto 2002; Yadav *et al.,* 2011). Stevia is currently permitted for use as a sweetener, food additive, and ingredient in a variety of food categories, including beverages, desserts, and sweets (Yadav *et al.,* 2011). It has been found eight glycosidic diterpenes in stevia leaves that have sweetening actions, and this compound composition is similar to that of JH terpenoid structure (Tateo *et al.,* 1999). For this reason, a foliar application of stevia leaf extract on mulberry leaves has been done in order to assess the effects of biological and economic characteristics on the final larval instar of the mulberry silkworm.

2. Materials and Methods

2.1. Specimens

A Chinses hybrid silkworm eggs came from the Sericulture Research Department of the Plant Protection Research Institute of the Agricultural Research Center in Giza, Egypt. Larvae were raised in a lab setting at 25±2 °C and 75±5 % relative humidity. It was regular to clean the bed and get rid of any sick larvae. The larvae received free access to fresh mulberry leaves four times every day.

2.2. Stevia leaves extract

The air-dried leaves of *Stevia rebaudiana* (25g) were pulverised and extracted with 400 ml of distilled water using the merceration method. Then, the crude extract was filtered and kept at 4°C until needed.

2.3. Experimental design

To get the experimental concentrations (0.005, 0.01, 0.05, and 0.1%), distilled water was added to the aqueous leaf extract of *S. rebaudiana*. These concentrations were selected in accordance with earlier tests that were done. The fifth instar larvae were fed leaves that had been treated with stevia leaf extract four times each day. The control larvae were given mulberry leaves, washed in distilled water. Each treatment was repeated three times, with 50 larvae in each replicate.

2.4. Biological parameters

At the wandering stage, weights of larvae and silk glands were noted. In addition, measurements of pupae, fresh cocoons, cocoon shells, and cocoon shell ratios were recorded. The quantity of eggs laid by each female was counted and noted.

2.5. Physiological parameters

2.5.1. Preparation of midgut extracts

The midgut of the dissected larvae was collected and homogenised in a buffer containing 0.8% NaCl. Centrifuging the homogenates at 7,000 x g for 10 min. The supernatant was taken and stored at –20 °C until use.

2.5.2. Protease activity assay

According to the manufacturer's instructions, the Protease quantitative kinetic assay Kit (Cat. No. K781-100, BioVision Inc., Milpitas, CA, USA), was used to evaluate the activity of the protease. Spectrophotometer measurements were made of the samples at 490 nm using (UNICO, SP2100 UV, China).

2.5.3. Protein estimation

Hemolymph was collected into sterile tubes with a few crystals of phenylthiourea at the wandering stage. Hemocytes and cell debris were then removed by centrifuging the solution at 10,000 g for 10 min. Ten larvae's cell-free hemolymph samples were used right away or kept frozen at 20 °C until needed. Using bovine serum albumin as a reference, the total amount of hemolymph protein was calculated spectrophotometrically in accordance with Bradford's method (1976).

2.6. Statistical analysis

The Duncan's multiple range function in SAS was used to analyse the variance of the data.

3. Results

3.1. Effect of supplementing with stevia leaf extract on the biological and economic characteristics.

The average weights of fifth instar *B. mori* larvae fed on mulberry leaves treated with stevia leaf extract at various doses (0.005, 0.01, 0.05, and 0.1%) are shown in Table1. Maximum larval weights were reached during the spinning stage with significant changes in the mean values of larval weights in all concentrations except for 0.005%, resulting in a noteworthy rate of increase at 0.05% treatment to record 77.7% over the control of mature larvae. The silk gland weights significantly increase at the spinning stage and shown superiority to all concentrations compared to the control (Table 1). Their weights were (579, 639, 893, and 782 mg), indicating respective differences of 0.005, 0.01, 0.05 and 0.1%, comparing with the control (498 mg). At the end of the fifth larval instar, 0.05% treatment raised the silk gland weight to a maximum of 79.3% over the control. At the 0.005, 0.01, 0.05 and 0.1% treatments, a significant rise in pupal mean weights was noticed. For 0.05 and 0.1% their corresponding weight increases were 44.9 and 39.8%, respectively. Fresh cocoon weights increased dramatically by roughly 51.5 and 44.3% for 0.05 and 0.1%, respectively. Weights of cocoon shells significantly increased with treatments of 0.005, 0.01, 0.05, and 0.1%. It appears without a doubt that the mulberry silkworm's cocoon shell weights were raised by stevia leaf extract, particularly a striking increase of about 90.5 and 70.4% for the 0.05 and 0.1% treatments, respectively. The current study's findings showed a substantial change in the cocoon shell ratio between the 0.05 and 0.1% treatments. In comparison to (312.1 eggs/moth for the control), the means of the deposited eggs were (487.8 and 442.3 eggs/moth) for the 0.05 and 0.1% concentrations, respectively. Application of stevia leaf addition led to an extension of *B. mori*'s last instar larval duration. The larval period is extended over the control by two days when feed mulberry leaves at a concentration of 0.05% treatment, and by two days and half at a concentration of 0.1% treatment, respectively.

Table 1: Effects of stevia leaf extract of the economic and biological characteristics of the mulberry silkworm, *Bombyx mori* L.

Stevia leaf conc.	Weight of Larvae (g)	Weight of silk gland (g)	Weight of pupae (g)	Weight of fresh cocoons (g)	Weight of cocoons shell (g)	cocoon shell ration %	No. of deposited eggs
Control	2.04^{d} ± 0.115	0.498^{d} ± 0.042	1.815^{e} ± 0.034	2.126^{e} ± 0.046	0.311^{e} ± 0.016	14.61^{d} 0.494	312.1^{e} ± 6.056
0.005%	2.15^{d} ± 0.111	0.579^{dc} ± 0.027	1.907^{d} ± 0.041	2.239^{d} ± 0.046	0.332^{d} ± 0.005	14.83^{d} 0.124	339.8^{d} ± 4.051
0.01%	2.64^{c} ± 0.145	0.639^{c} ±0.037	2.069^{c} ± 0.054	2.455^{c} ± 0.051	0.385^{c} ± 0.008	15.69^{c} 0.511	395.7^{c} ± 5.329
0.05%	3.62^{a} ± 0.046	0.893^{a} ± 0.065	2.630^{a} ± 0.054	3.222^{a} ± 0.058	0.592^{a} ± 0.007	18.37^{a} 0.259	487.8^{a} ± 2.802
0.1%	3.26^{b} ± 0.1253	0.782^{b} ± 0.070	2.537^{b} ± 0.035	3.067^{b} ± 0.040	0.530^{b} ± 0.006	17.29^{b} 0.11136	442.3^{b} ± 8.130
L.S.D	**0.207**	**0.093**	**0.081**	**0.088**	**0.017**	**0.630**	**10.144**

- Values represent the average of three replicates, each with 10 larvae.
- Means with the same letter do not differ at $P \leq 0.05$.

3.2. Effect of supplementing with stevia leaf extract on the physiological characteristics.

Protease activity.

Of all the samples treated with stevia leaf extract, it was discovered that the 0.05% and 0.1% had the maximum enzyme activity. The increases in enzyme activity at the above-mentioned concentrations were 70.60 and 48.10%, respectively. The values of protease activity were (23.47, 29.47, 37.38 and 32.45 µg tyrosine/ml/g tissue), compared with control (21.91 µg tyrosine/ml/g tissue) as shown is figure 1)

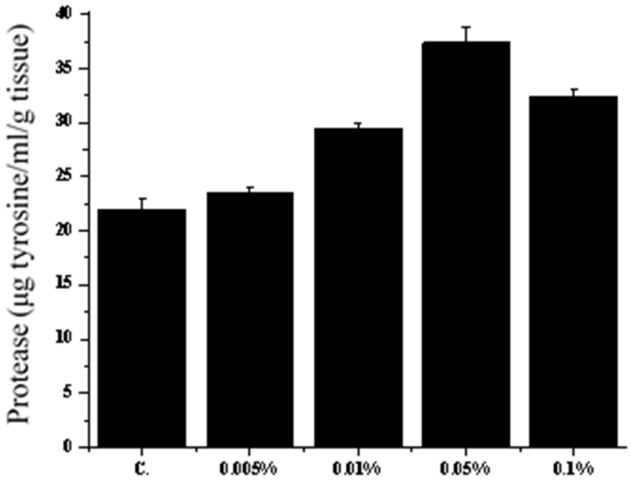

Fig. 1: The influence of stevia leaf extract supplementation on the protease activity of *B. mori* fifth instar larvae. The bars represent mean values with standard errors of the mean.

3.3. Effect of administering stevia leaf extract on levels of total hemolymph protein

At the end of the fifth larval instar, the hemolymph of treatments with stevia leaf extract generally exhibited an upward trend in the level of total protein (Figure 2). The recorded averages of total hemolymph protein concentrations during the mature larval stage (spinning stage) were 104.6, 111, 127.67, and 120.28 mg/ml, respectively, with significant differences at in all concentrations 0.005, 0.01, 0.05, and 0.1%, comparing with control 100.3 mg/ml.

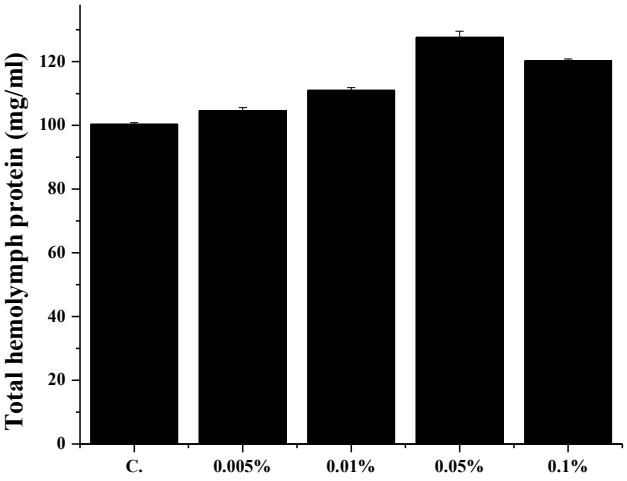

Fig. 2: The impact of stevia leaf extract supplementation on the total hemolymph protein of *B. mori* fifth instar larvae. The bars show mean values along with standard error.

4. Discussion

4.1. Effect of supplementing with stevia leaf extract on the biological and economic characteristics

Since mulberry is the only food source for the silkworm *Bombyx mori*, nutrition is crucial to Bombyx's growth and development. In this investigation, all stevia leaf extract concentrations with the exception of 0.005% showed significant variations in the mean values of larval weights. The difference in size between hatching and larval maturity is 10,000 times greater in *B. mori* larvae, which reach their maximum weights a day before maturation (Grekov *et al.,* 2005; Radjabi 2010). According to Slansky and Scriber (1985), the type and amount of food consumed had an impact on final body weight, growth rate, time needed for development, survival, and reproductive capacity. As a result of the current findings, both larval and silk gland growth rates were dramatically increased in the 0.05 and 0.1% stevia

leaf extract treatments. The results obtained are consistent with (Hiratsuka 1920) who observed that *B. mori* final instar is characterised by active growth particularly, in silk gland weight. Larval diet effects growth, development, and silk gland function in *B. mori* (Akai *et al.,* 1988). Previous research indicated a favourable correlation between the weight of the silk gland and the weight of the larval body (Fournier 1979; Ueda *et al.,* 1975). The quality and amount of silk produced by silkworms are reflected in the expansion of the silk glands. The efficient utilization of the increased amount of protein/amino acids and other nutrients in the mulberry leaves may be the cause of the increase in the silk gland weight during the fifth larval instar (Chandraskar *et al.,* 2007). Seventeen amino acids were existing in the stevia leaf extract, which may have increased the food utilization and growth rate of larvae and silk glands (Abou-Arab and Abu-Salem 2010). The findings of the current study also showed that there is a significant difference between control and all concentrations of stevia leaf extract in terms of pupal weight and silk productivity. *B. mori* needs a set of vital sugars, carbohydrates, amino acids, proteins, and vitamins for optimal growth, survival, and the development of its silk productivity (Sengupta *et al.,* 1972, Etebari and matindoost 2004). It has been shown that the stevia leaves contain minerals, including calcium, potassium, magnesium, zinc, sodium, and iron, which may have boosted the production of silk (Kim *et al.,* 2011). The data in this study demonstrate that feeding on mulberry leaves treated with all different concentrations of stevia leaf extract had a significant positive impact on fecundity. These results are corroborated by Ito (1978), who found that β-carotene and vitamin E both have a discernible influence on growth and the quantity of eggs laid by moths. Ascorbic acid and lettuce seed oil, have been linked to an increase in the quantity of silkworm eggs in earlier studies (Chauhan and Singh 1992; Shahin *et al.,* 2013a). Stevia leaves distinguished by a high concentration of vitamins like folic acid and vitamin C have been identified, which may have enhanced and increased fertility (Kobus-Moryson and Gramza-Michatowska 2015). Based on the aforementioned results, it appears that supplementing with stevia leaf extract affected the silkworm's growth, which in turn affected the quantity of silk produced and fecundity. The amount of stevia leaves present has an immediate relationship with the lengthening of the larvae period; treatments at 0.05 and 0.1% have an impact on growth and silk production. Juvenile hormone (JH) and its derivatives have been utilized by a number of researchers to control the growth of silkworms and boost silk production (Akai 1971; Chang *et al.,* 1972; Murakoshi *et al.,* 1972). Chang (1972) noted that when the larvae were treated with methoprene derivatives, there was an increase in pupal weight with huge cocoon production. When the larval growth was delayed by two to three days, silk protein biosynthesis increased along with an increase in the weights of cocoon and shell (Akai 1971; Shimada *et al.,* 1979). Stevia leaves contain glycosidic terpenes with a structure resembling that of JH terpenes, which may have improved and boosted the lengthening of the larvae and silk yield (Tateo *et al.,* 1999; Abou-Arab and Abu-Salem 2010).

4.2. Effect of supplementing with stevia leaf extract on the physiological characteristics

Protease activity

The silkworm enzyme system is crucial in determining how effectively the larvae convert the organic mulberry leaves into usable macromolecules, protease basically catalyzes the breakdown of protein into amino acids. The last instar consumed more than 80% of mulberry leaves during its life, food is efficiently used at this stage to support the silkworm metabolism and to produce proteins for the silk formation (Lokesh *et al.,* 2006). The midgut protease, which is generated at a faster rate during the silkworm larval stage, is an essential enzyme that aids in converting mulberry protein to silk protein (Kumari *et al.,* 1997). The results of this study showed that stevia leaf extract treatments exhibit increased protease activity. Similar results for the JHA treatments were previously reported, and they amply proved the greater protease activity (Magadum *et al.,* 1989).

4.3. Effect of administering stevia leaf extract on levels of total hemolymph protein

During the final larval instar of the current study, total hemolymph protein content increased significantly in all of the treatments with stevia leaf extract. Nagajyothi *et al.* (2010) published similar findings, noting that during the growth of the fifth instar larvae, the level of total proteins showed a rising trend in the silk gland. The silkworm hemolymph protein contents increased at the last stage due to more silk was generated along with reproductive organs (Kawaguchi *et al.*, 1993; Mahmoud 1988; Shahin *et al.,* 2013b). According to Sasaki and Noda (1973), silkworms create a significant amount of silk proteins for the creation of the cocoons. This study demonstrates how the amino acids, minerals,

vitamins, and nutrients found in stevia leaves affect hemolymph protein content during the fifth instar larvae.

5. Conclusion

It has been notable that using stevia leaf extract in sericulture techniques increases silk yield, particularly a startling increase of nearly 90.5 and 70.4% of the 0.05 and 0.1% treatments, respectively. The current study clearly showed that foliar stevia leaf treatment boosted hemolymph protein concentration as well as midgut protease activity. It follows that the addition of stevia leaf extract at a dosage of 0.05% has a positive impact on raising the quantity of silk production and moth fertility by improving the digestion of mulberry leaves and resulting increase in the protein content of hemolymph.

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