Biological and Physiological Effects of Some Honey Bee Products and Its Mixtures as Nutritional Additives on Two Strains of The Mulberry Silkworm *Bombyx mori*

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ABSTRACT

The effect of some honey bee products; royal jelly (RJ 10mg/ml), pollen (P 50mg/100ml), propolis (PR 30mg/100ml), honey (H 1g/100ml) and their mixtures as food additives, on several biological and physiological parameters of the 5th larval instar of (S_1 and S_2) of the silkworm *Bombyx mori* were studied. The above mentioned products, either separately or in combination increased weights of mature larvae, pupae, fresh cocoons and cocoon shells. Larvae fed on mulberry leaves treated with RJ showed a significant increase in weights followed by H and then P in the broad strain. While in the local strain, RJ gave the maximum weights followed by P and then H. The same trend was observed in the total haemolymph protein and the CA surface area in both strains.

Key words: *Bombyx mori*, silkworm, Honey bee products, silk production, haemocytes.

INTRODUCTION

Nutrition plays an important role in improving the growth and development of the silkworm, *Bombyx mori* L. like other organisms. Legay (1958) stated that silk production is dependent on the larval nutrition . The nutritive value of mulberry leaves plays a very effective role in producing good quality cocoons. The nutritional status of mulberry leaves can be improved by enriching them with extra nutrients to increase larval growth and improve cocoon characteristics (Sengupta *et al*. 1992). Mulberry leaves immersed in solutions of 0.5, 1.0 and 1.5% ascorbic acid (vitamin C) solution significantly increased the weights of both larvae and cocoons of silkworm compared with untreated leaves (Miranda *et al*. 1998). Feeding of *B. mori larvae* on fresh mulberry leaves treated with vitamin B complex (0.5%) increased the weights of larvae and cocoon and the shell ratio (Suprakash and Pal, 2002).

Treatments of *B. mori* larvae with propolis extracts (a honeybee product) yielded heavier cocoons and cocoon shells, a higher silk content, and females laid more eggs than controls (Nour *et al*. 1997).

Zannoon (1994) have also reported that, various solutions of bee honey increased the mature weight of the mature larvae, silk gland, fresh cocoons, cocoon cortex and the number of deposited eggs per female. Moreover it gave heavier and longer filaments of reeled cocoons, but did not affect cocoon silk content ratio and filament size. The effects of royal jelly, honey, propolis as supplementary nutrients to the mulberry and caster leaves on the biological parameters, of *B. mori* and *Ph. ricini* were studied by several investigators (El-Karaksy, 1979, El-Sayed, 1999 and Gad, 2006).

Supplementing mulberry leaves with propolis extract showed anabolic effect on silkworm (Cizmark and Metal, 1978, El- Massarawy, 1995 and Gad, 2006). The importance of honey in the nutrition of silkworm was reported by El-Hattab (1985), El- Karaksy et al. (1989), El-Sayed (1999), Gad, (2006) and Mahmoud *et al.* (2012).

The present study aimed to evaluate the effect of the honey bee products (royal jelly, pollen, propolis and honey) and some of their mixtures on the two strains of the silk worm *B. mori* (S_1 and S_2) to improve its productivity. Also, the inter-relationship between the improvements in silk and egg productivity and some physiological parameters were also investigated. These parameters were corpora allata surface area as an indicator of its secretary activity of juvenile hormone (Nour *et al.* 1997) in addition to the total haemolymph proteins.

MATERIALS AND METHODS

The experiment was carried out on two different strains of the mulberry silkworm *Bombyx mori.* The first strain (S_1) Chinese F1 (SN_1 x Iva_1) mulberry silkworm *B. mori* L. was obtained from Coastal Silkworms Coastal Silkworms (Coastalsilkworm.com) and the second strain (S_2) (SA105) was obtained from the Sericulture Research Department (SDA), Giza, Egypt. The insect was reared under the hygrothermic conditions of 25 ± 2°C and 75 ± 5% RH. Both strain larvae were fed on fresh clean mulberry leaves until 4th instar. Only the last larval instar was used in the experiment.

Honey bee products were obtained from the department of Applied Entomology, Alex. University, Egypt. All products were made into final solution of 100ml that contain Royal jelly (RJ) 10mg, Pollen(P) 50mg, Propolis (PR) 30mg and Honey (H) 1g. Mixtures of (RJ) 10mg and (P) 50mg (RJ/P), (RJ) 10mg and (PR) 30mg (RJ/PR), (RJ) 10mg and (H) 1g (RJ/H), (P) 50mg and (H) 1g (P/H) and (P) 50mg and (PR) 30mg (P/PR) were all made in a final volume of 100ml.

The mulberry leaves were dipped in different concentrations of various solutions for five minutes, then left to dry and offered daily to the 5th instar larvae for 48±6hr.Three replicates were made for each concentration using 50 larvae in each. The concentrations were chosen on the light of the previously reviewed studies.

-Biological assessment:

Daily inspection of treated larvae was made until they reached pre-pupal stage . The fresh weights of mature larvae, silk glands, pupae, cocoons and cocoon shells were recorded. The number of deposit eggs/ female moth were considered and silk ratio for untreated larvae were counted in both strains and untreated larvae .Also the percentage of increasing number of deposited eggs/female and cocoon shells weight for some treatments were counted.

-Physiological assessment:

1-Haemolymph estimation.

About 15 larvae of each concentration in both strains were used to collect the haemolymph needed for the haemolymph total protein by using the Biuret method (Jones, 1962).

2- Corpora allata activity.

Corpora allata (CA) volume was used as an indicator of the juvenile hormone (JH) level (Armstrong and Carr, 1964). After dissecting the mature larvae of *B.mori*, permanent mounting of CA were prepared. The surface area of CA was calculated from a planimeter drawing of the gland. Data were statistically analyzed to check the significance of differences between treatments by using F test and L.S.D. (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

- Effect of honey bee products on some biological parameters of two mulberry silkworm *B. mori* strains.

Results shown in Table (1) proved that (RJ) at concentration of 10mg/100ml significantly increased the weights of the treated mature larvae, pupae and cocoon shells by about 82.5, 55.8 and 84.5 %, respectively followed by the treatment of (H) at concentration 1g/100ml, where as the rate of increase amount to 68.4, 55.8 and 61.3%, respectively up to control. Furthermore, the mixture of RJ/H gave the highest weight of larvae, pupae and cocoon shells about 44.3, 42.6 and 66.6 % up to control. On contrast, the mixture (P/PR) gave the lowest economic parameters comparing to other treatments. The included results in Table (1) also indicate that (RJ) and (H) gave the highest rates of deposited eggs with an average of 50.9 and 36.5 %, respectively up to control. Also, the mixture (RJ/H) and (RJ/P) caused an increasing in the rate of deposited eggs about 26.4 and 23.6 %, respectively up to control.

A similar trend was obtained in *B.mori* (S_2) strain, the heaviest weight of mature larvae, pupae and cocoon shells were recorded after treatment with (RJ) about 80.9, 57.6 and 26.7 %, respectively up to control. Moreover, the mixture of (RJ/P) increased the weight of mature larvae, pupae and cocoon shells about 42.1, 35.2 and 21%, respectively up to control. While the mixture (P/PR) gave the lightest weight of mature larvae, pupae and cocoon shells.

-Effect of honey bee products on some physiological parameters:

-The silk gland:

The fresh weight of silk gland of matured (S_1)strain treated larvae exhibited the heaviest mean weight for (RJ) and (H) about 81.5 and 48.6%, respectively up to control. The mixture (RJ/H) increased the silk gland weight about 33.4 % up to control (Fig., 1a).

Figure, (1b) show the effect of honey bee products on (S_2) strain of *B.mori* larvae. Royal jelly treatment gave the heaviest mean weight of silk gland followed by (P) by about 95.6 and 84%, respectively compared to control. The mixture (RJ/H) increased the silk gland weight by about 37.7 % compared to control.

-The CA surface area:

The changes in the surface area of *B.mori* (CA) during the prepupal stage were studied. The present data prove that all the tested products affect the CA surface area and hence the JH level.

As shown in Fig. (2a), RJ treatment significantly increased the CA surface area of (S_1) strain larvae by about 99.4% followed by (H) treatment and (P) 63.3% and 37.3%, respectively) while in the mixture treatments, the most significant increase in the CA surface area was observed when larvae were fed on mulberry leaves treated with RJ/H 33.5 % more than control.

Fig. (2b), show the effect of honey bee products on (S_2) strain of *B. mori* larvae. The maximum increase was observed when larvae fed on mulberry leaves treated with RJ 74.7 % followed by (H) and (P) treatment (36.6% and 36.1 %, respectively more than control). Furthermore, In the mixture treatments, (RJ/P) increased the CA surface area by about 36.8% more than control.

-Total haemolymph protein (THP):

The performed chemical analysis showed a significant effect of all used honeybee products and their mixture on the estimated total haemolymph

protein (THP) of the larvae in both silkworm strains. In the (S_1) strain, the maximum value of THP were recorded in the treatment of (RJ) and (H), respectively followed by 28.6 and 26.2 mg/ml for those treatments of (P) and (PR) 62.9 and 40.9 mg/ml, respectively. Also, the treatment of the mixture (RJ/H) was 28.7 mg/ml while control was 17.5 mg/ml (Fig, 3a). The maximum increase of THP for the (S_2) was 48.7 and 30.5 mg/ml for treatment with RJ and P, respectively followed by 25.9 mg/ml after treatment with (H). The mixture (RJ/P) gave 26.6 mg/ml while control was 15.7 mg/ml (Fig. 3b). The results indicating the effect of the honey bee products that have been used and their mixtures on some biological and physiological parameters are in agreement with the findings of Luscker (1976) and Cheng and Wong (1996).

Singh (1960) reported that injecting the larvae with prepared concentrations of royal jelly increased the weights of larvae, pupae, and cocoon shells. El-Karaksy (1979) concluded that the use of royal jelly with yeast as food additive gave the heaviest weights of larvae, cocoon, silk gland and increased the number of deposited egg / female of *B. mori* and *Ph. ricini*.

El-Karaksy *et al* (1990) found that use royal jelly at concentrations of 2 and 4% enhanced both, the silk production and female fecundity. Also, El-Sayed (1999) reported that the mixture of honey and black cumin seeds increased silk production and the number of deposited eggs/female and gave heaviest weight of larvae, pupae, dry silk gland and increased the total protein of the silk gland .El- Massarawy (1995) and Gad (2006) they found that treatment with propolis extract yielded heavier cocoons and cocoon shells. Also increased the percentage of silk content and increased the number of deposited eggs/ female than those obtained from control.

Table1: Effect of honey bee products on some biological parameters of B. mori (S_1).

Treatment	Larval weight	Weight of pupa (g)	Weight of fresh cocoon(g)	Weight of cocoon shell (g)	Numbr of deposited eggs/female
RJ	3.87±0.01^{a}	0.9848±0.02^{a}	1.256±0.01^{a}	0.2781±0.02^{a}	492.2±3.3^{a}
P	3.36±0.01^{b}	0.9501±0.08^{a}	1.119±0.008^{c}	0.2312±0.01^{b}	429.3±3.6^{a}
PR	2.98±0.02^{c}	0.8911±0.1^{b}	1.122±0.01^{b}	0.2293±0.04^{b}	387.5±2.5^{c}
H	3.57±0.01^{a}	0.9621±0.01^{a}	1.212±0.02^{a}	0.2431±0.03^{ab}	445 ±2.7^{a}
RJ/H	3.06±0.01^{bc}	0.9012±0.02^{b}	1.162±0.03^{d}	0.2510±0.02^{a}	412 ±1.7^{b}
RJ/P	2.91±0.03^{c}	0.8215±0.01^{c}	1.067±0.02^{d}	0.2260±0.04^{b}	403 ±1.2^{b}
RJ/PR	2.86±0.02^{c}	0.7412±0.03^{c}	0.957±0.03^{d}	0.2060±0.01^{d}	399.6±1.8^{bc}
P/H	2.23± 0.03^{d}	0.6760± 0.01^{d}	0.8945±0.03^{d}	0.1619±0.01^{e}	363.7±1.3^{c}
P/PR	2.06±0.01^{d}	0.6512±0.04^{d}	0.8250±0.02^{e}	0.1589±0.03^{e}	344.1±1.7^{c}
Control	2.12±0.04^{d}	0.6320±0.04^{d}	0.792±0.02^{e}	0.1507±0.05^{e}	326±1.3^{d}

RJ: 10mg Royal jelly PR: 30mg Propolis Pl: 50 mg Pollen H: 1 g Honey RJ/P: 10mg Royal jelly+50 mg Pollen RJ/PR: 10mg Royal jelly + 30mg Propolis RJ/H: 10mg Royal jelly +1g Honey P/H :50 mg Pollen+1 g Honey P/PR :50 mg Pollen+ 30mg Propolis.

*Means at each colum followed by the same letter are not significantly different at 0.01

Table 2: Effect of honey bee products on some biological parameters of *B. mori* (S_2).

Treatment	Larval weight (g)	Weight of pupa (g)	Weight of fresh cocoon	Weight of cocoon shell(g)	Numbr of deposited eggs/female
RJ	3.22±0.03^{a}	0.8212±0.02^{a}	1.101±0.01^{a}	0.1800±0.01^{a}	465.2±3.3^{a}
P	2.98 ±0.01^{b}	0.8031±0.03^{a}	0.990±0.02^{a}	0.1770±0.01^{b}	398.3±2.3^{b}
H	2.28±0.01^{c}	0.7701±0.01^{b}	0.956±0.01^{b}	0.1761±0.02^{b}	389 ±2.8^{b}
PR	2.09±0.01^{c}	0.7510±0.01^{b}	0.930±0.008^{b}	0.1692±0.02^{c}	377.6±4.1^{b}
RJ/PR	2.01±0.03^{bc}	0.7034±0.02^{b}	0.8814±0.03^{c}	0.1680±0.007^{c}	349.4±1.9^{c}
RJ/P	2.53 ±0.07^{b}	0.8011±0.009^{a}	0.9892±0.02 ^{a}	0.1781±0.008^{b}	378 ±5.2^{b}
RJ/H	2.41±0.01^{b}	0.7550±0.03^{b}	0.9343±0.03^{b}	0.1693±0.01^{c}	356.2±2.4^{c}
P/H	1.98±0.01^{d}	0.6012±0.06^{c}	0.7131±0.02^{d}	0.1502±0.01^{d}	321±1.1^{d}
P/PR	1.82±0.02^{d}	0.5684±0.01^{c}	0.6881±0.03^{d}	0.1531±0.01^{d}	316.2±1.3^{d}
Control	1.78±0.02^{d}	0.5210±0.04^{c}	0.673±0.02^{d}	0.1420±0.003^{d}	312.2 ± 2.4^{d}

RJ: 10mg Royal jelly PR: 30mg Propolis Pl: 50 mg Pollen H: 1 g Honey RJ/P: 10mg Royal jelly+50 mg Pollen RJ/PR: 10mg Royal jelly + 30mg Propolis RJ/H: 10mg Royal jelly +1g Honey P/H :50 mg Pollen+1 g Honey P/PR :50 mg Pollen+ 30mg Propolis.

*Means at each colum followed by the same letter are not significantly different at 0.01

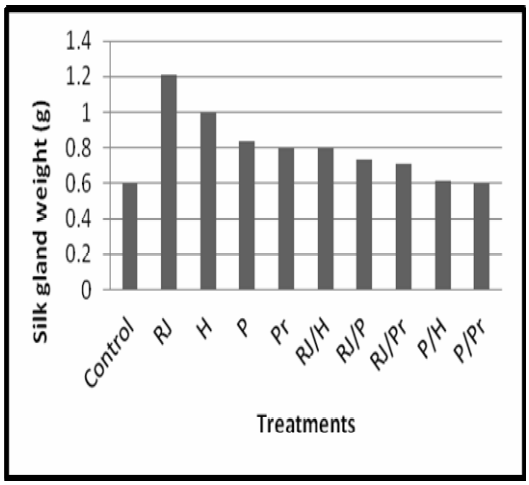

Fig., 1b:Effect of honey bee products on silk glands weight of *B. mori* larvae (S_1).

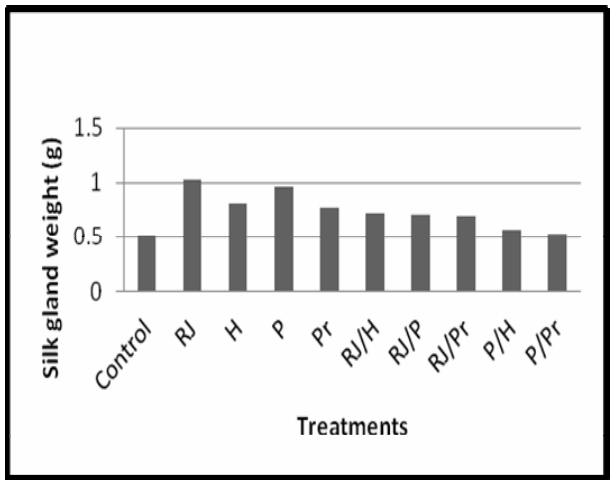

Fig., 1a: Effect of honey bee products on silk glands weight of *B. mori* larvae(S_2).

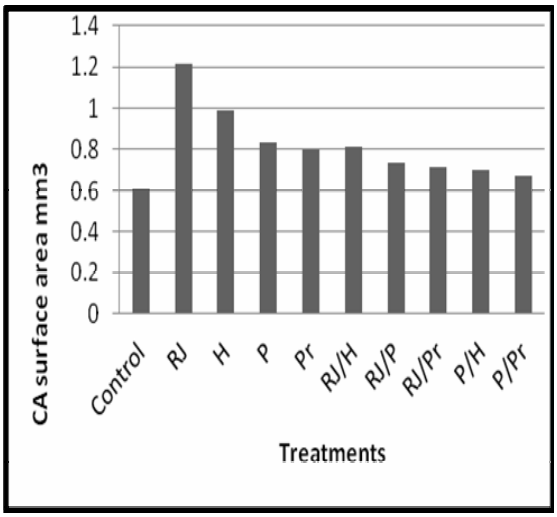

Fig., 2b: Effect of honey bee products on CA surface area of *B. mori* larvae (S_1).

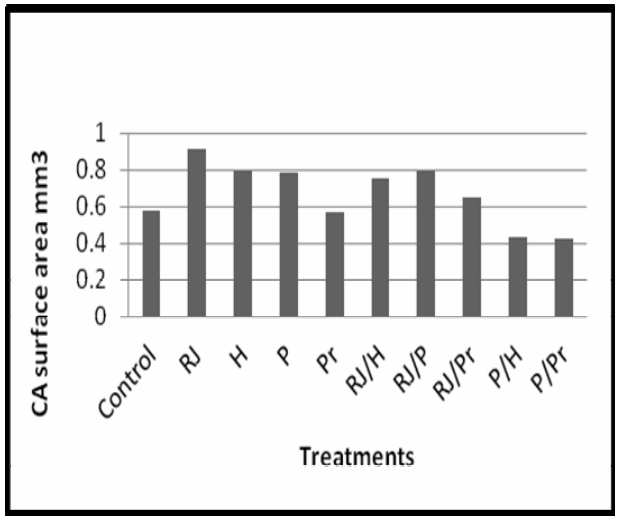

Fig., 2a: Effect of honey bee products on CA surface area of *B. mori* larvae (S_2).

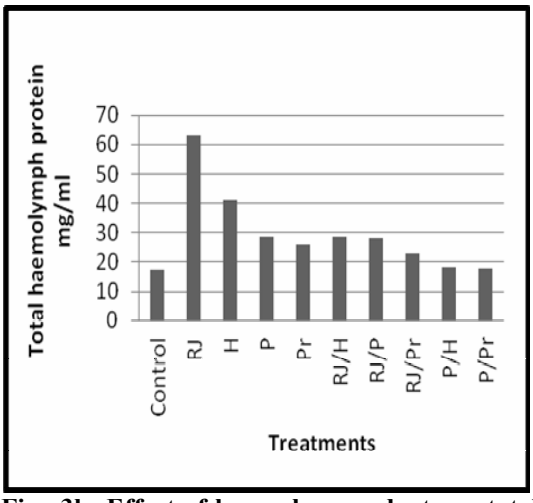

Fig., 3b: Effect of honey bee products on total haemolymph protein of *B. mori* larvae (S_1).

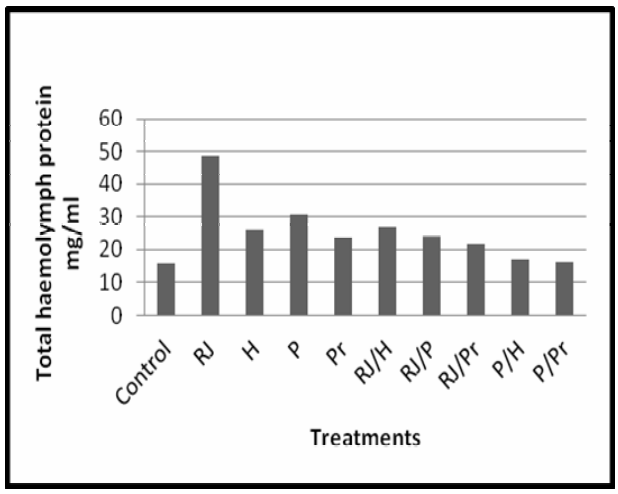

Fig., 3a: Effect of honey bee products on total haemolymph protein of *B. mori* larvae (S_2).

CONCLUSION

The supplementation of honey bee products caused significant effects on all the studied biological and physiological parameters of the mulberry silkworm. In the (S_1) strain, (RJ) increased the cocoon shell weights by about 84.5% and increased the number of deposited eggs / female by about 50.9 % up to control followed by (H) which increased the cocoon shell weights by about 61.3 % and increased the number of deposited eggs / female by about 36.5% more than control. On the other hand, in the local strain (RJ) increased the weight of cocoon shells by about 26.7 % and increased the number of deposited eggs / female by about 48.9 % more than control followed by (P) which increased the cocoon shells weight by about 24.6% and increased the number of deposited eggs / female by about 27.5 % more than control. The mixture of (RJ/ H) increased the weight of cocoon shells by about 19.22 % and increased the number of deposited eggs / female by about 26.4 % more than control while in the (S_2) strain the mixture of (RJ / P) produced increased the weight of cocoon shells by about 25.4 % and increased the number of deposited eggs / female by about 21.07% more than control. It could be concluded that these materials which have been used as feeding stimulants enhanced the feeding behavior of larvae and the total haemolymph content of protein. Also, treatment with (RJ) improved the productivity characters of *B.mori* larvae in both strain especially the broad strain followed by (H), (P) and their mixture with (RJ).

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الملخص العربى

التأثيرات البيولوجية والفسيولوجية لمنتجات نحل العسل وخلائطها كأضافات غذائية علي نوعين من سلالات دودة الحرير التوتية

عبير عبد المجيد جاد

قسم الحشرات الاقتصادية- كلية الزراعة- جامعة الاسكندرية– مصر

تم دراسة تأثير بعض منتجات نحل العسل الغذاء الملكي (10ملجم/١٠٠مل) وحبوب اللقاح(٥٠ ملجم/ ١٠٠مل) والبروبوليس (٣٠ملجم/ ١٠٠مل) والعسل (١جم / ١٠٠مل) وخلائطها) كأضافات غذائية علي العديد من الصفات البيولوجية والفسيولوجية للعمر اليرقي الخامس (س١ – س٢) لدودة الحرير التوتية. وقد اوضحت النتائج ان اضافة منتجات نحل العسل موضع الدراسة سواء منفردة او في مخاليط يزيد من اوزان اليرقات والعذاري والشرانق وقشرة الشرنقة وكذلك عدد البيض المخصب الناتج لكل انثي.

كما اظهرت النتائج ان تغذية يرقات السلالة س١ علي اوراق توت معاملة بالغذاء الملكي اعطي زيادة معنوية في اوزان اليرقات والعذاري والشرانق وقشرة الشرنقة وتبعها المعاملة بالعسل ثم حبوب اللقاح بينما اوضحت الدراسة علي السلالة س٢ ان تغذية اليرقات علي اوراق توت معاملة بالغذاء الملكي اعطت اعلي زيادة في الاوزان يتبعها حبوب اللقاح ثم العسل.

كما تم دراسة تأثير هذه المركبات علي بعض القياسات الفسيولوجية كمستوي بروتينات الدم في دم اليرقات المعاملة وكذلك حجم غدة الجسم الكروي (الكوربورا اللاتا).