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# Evaluation of Certain Types of Honey, Essential Botanical Oils and Their Mixtures on the Productivity of the Mulberry Silkworm *Bombyx Mori* L.


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## ABSTRACT

**The present work was initiated to study the effect of supplementing mulberry leaves with different types of honey, essential botanical oils and their tested mixtures at different concentrations on the 5th larval instar of the mulberry silkworm *Bombyx mori* L., for improving certain biological and reproductive parameters, in particular, silk and egg production. The results indicated that treatment of mulberry leaves with Fenchone oil at both tested concentrations of 5 and 10mg/100ml water significantly increased the weight of silk gland and cocoon shell in comparison to the other performed treatments.**

**Compared to the untreated control, the evaluated types of honey to more or less extent increased the egg productivity. In this concern, Seder honey followed by Carob honey type gave highest increase over control (43.460 and 42.137%, in respect). Also, admixing Seder honey with Fenchone oil significantly increased the assessed biological and reproductive parameters when compared to the other evaluated honey types/botanical oils mixtures and untreated control.**


## INTRODUCTION

Sericulture or silk farming is the rearing of silkworms for the production of raw silk. As a fact, the silkworm *Bombyx mori* L. feeds mainly on mulberry leaves. Kumaraj *et al*. (1972) reported that the important factor influencing the growth and production of the silkworm is the nutritional factor.

The silkworm *B. mori* L. require certain essential carbohydrates, proteins, amino acids, fatty acids, sterols and minerals for the growth of silk gland and for higher production of egg and silk (Ito, 1978).

For improving the nutritional value of mulberry leaves, various food additives were used as supplementary nutrients (Moustafa and El-Karaksy, 1988; El-Sayed and Mesbah, 1992 a &b and El-Sayed *et al*., 1998).

The importance of honey in the nutrition of silkworm was reported by El-Hattab (1985), El-Karaksy *et al*. (1989) and El-Sayed (1999).

The physico–chemical properties of honey in each region are related to the type of growing plants and flowers visited by honey bee workers to get their food from. Therefore, recognition of botanical components is very important. Mulberry leaves contain adequate amounts of fatty acids to maintain good growth of the silkworm *B. mori* (Horie and Watanabe, 1980). Furthermore, the addition of certain fatty acids and vegetable oils as supplemented nutrients to leaves for providing sterol requirement has been confirmed by Ito and Nakasone (1967).

The present study aimed to evaluate the effect of different types of honey (Carob [*Ceratonia siliqua*], Seder *[Ziziphus* sp.] and Black Cumin honeys [*Nigella sativa*]), different botanical essential oils (Dill [*Anethum graveolens*], Fenchone [*Foeniculum vulgare*] and Rosemary oil [*Rosmarinus oficinalis*]) and their mixtures on the egg and silk productivity of *Bombyx mori* L.

## MATERIALS AND METHODS

The experiment was carried out on the Chinese F1 hybrid 9F7X mulberry silkworm *Bombyx mori* L. The insect was reared under the hygrothermic conditions of 25± 2ºC and 75± 5 % R.H. The larvae were fed on fresh clean mulberry leaves until 4th instar. Only the last larval instar was used in the experiment and the larvae were grouped in separated trays.

### Substances used

Certain types of honey, oils and their mixtures were chosen as supplementary nutrients for the silkworm *B. mori;* these substances were as follows:


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**Types of honey:**

1. **Carob honey (*Ceratonia siliqua* )**

Carob honey is somewhat rare, dark and crystallizes easily and acquires a soft coarse texture. It has a medium of aromatic intensity. It tastes very similar to chocolate.

2. **Seder Honey ( *Ziziphus* sp. )**

This honey strengthens the nervous system, increase physical and mental activity and fights fatigue. It also strengthens the immune system, works on stimulating glands and useful for infertility.

3. **Black Cumin honey ( *Nigella sativa* )**

It contains all the good qualities and benefits of black cumin seeds. It has been recommended for the chest and asthma problems, strengthens heart and purifies the blood and urinary tract. Black Cumin honey is very useful for cases of inflamed urinary or/and gall bladder.

All types of honey are used at a concentration of 1g/100ml distilled water. Carob and Seder honey were brought from Libya while Black Cumin honey was available in Egypt.

**Types of oils:**

1. **Dill oil (*Anethum Graveolens*)**

It has a grass like smell and pale yellow colour with a watery viscosity. Dill oil is considered as non–toxic, non-irritant and non-sensitizing but, it is too powerful.

2. **Fenchone oil (*Foeniculum vulgare*)**

It is a natural organic compound classified as a monoterpene and a ketone. It is a colorless oily liquid. It has a structure and an odor similar to camphor. It has been found to be the main constituent essential oil of *Lavandula stoechas* supsp. *stoechas* leaves and inflorescences (39.9 and 21.0%, respectively) (Tzakou *et al*., 2009)

3. **Rosemary oil (*Ros marinus oficinalis*)**

This oil is high in iron, calcium and vitamin $B_6$. Rosemary essential oil is potentially toxic if ingested. Large quantities of rosemary can cause adverse reactions (that can be fatal). Consuming large quantities of rosemary should be avoided.

All types of oils preparations were used at concentrations of 50 and 100 ppm (5 and 10 mg/100 ml distilled water) and were available in Egypt.

**Mixtures**

The mixtures that have been evaluated in the present investigation are shown in Table 1.

Therefore, the experiments included fifteen treatments and control. Every treatment was replicated three times. Each replicate contained 50 larvae of the fifth instar. Mulberry leaves were dipped in the prepared concentrations of the tested materials.

After the leaves being dried, they were introduced to the larvae. For control, larvae were supplied with leaves treated with distilled water.

**Table 1. The evaluated mixtures of different types of honey and botanical oils and their rate of application**

| Food Additives mixture | Rate of Application |
|---|---|
| Car/Dil | The mixtures of all types of honey and oils were used at 1g honey + 5 mg oil/100 ml distilled water |
| Car/Fen | |
| Car/Ros | |
| Sed/ Dil | |
| Sed/ Fen | |
| Sed/ Ros | |
| BC/ Dil | |
| BC/ Dil | |
| BC/ Dil | |

Car= Carob, Sed = Seder and BC = Black cumin Honey types
Dil= Dill, Fen= Fenchone and Ros= Rosemary botanical oils

**Estimation of the studied parameters**

Fresh weights of mature 5$^{th}$ instar larvae, pupae, fresh cocoons and cocoon shells were weighted. Durations of larvae and pupae were recorded. Prior to spinning cocoons, a sample of five mature larvae was dissected and the silk glands were drawn and weigh. Randomly elected ten raised female moths were allowed to mate with male moths; each couple was kept in its numerical paper bag. After oviposition, the number of deposited eggs/ female moth was counted and recorded.

Simple correlation between quantitative characters was done, considering the relations between cocoon, cocoon shell and weight of silk gland.

Data were statistically analyzed on the basis of "F" test and the least significant difference test (L.S.D.) was carried out at level 0.05 (Sendecor, 1956).

## RESULTS AND DISCUSSION

**Effect of the tested materials on biological parameters of the silkworm *Bombyx mori* L.**

The results presented in Table 2 show that the mean weights of the full grown larvae were significantly different. It is found that the use of Dill, Rosemary and Fenchone oils at the concentration of 5mg/100 ml gave the heaviest larval weights of 3.693, 3.566 and 3.459 g, respectively, whereas the rate of increase amounted to 36.598%, 31.988% and 27.966% over the control larvae(2.703 g), in respect. On the other hand the mixtures of Black Cumin (BC) or Seder honey (Sed) (1g) with Rosemary oil at the concentration rate of 5mg/100 ml gave the lightest larval weight of 2.557 and 2.524 g, respectively and indicated a decrease of larval

weight comprised 5.439 and 6.625% less than that of the control larvae, respectively.

The shortest duration of the fifth larval instar was 10 days and that was recorded for those larvae fed on mulberry leaves treated with each of Carob (Car), Seder (Sed) and Black Cumin (BC) honey. Vice versa, the longest 5$^{th}$ larval duration (13 days) was recorded for the larvae fed on the mixture of Seder/Rosemary; but still less than that of the control larvae (14 days).

The fresh weights of silk glands of matured treated larvae exhibited the heaviest mean weight for the mixture of Black Cumin / Fenchone (0.559 g) with an increase of 29.987% over control. That weight was 0.532, 0.516, 0.503 and 0.498 g for the treatments of Seder / Fenchone, Fenchone (10mg), Seder/Rosemary and Fenchone (5mg), respectively (Table 2).

**Table 2. Effect of the tested materials on the inspected biological parameters of *Bombyx mori* L.**

| Treatments | Weight of larvae (g) | 5$^{th}$ instar duration (days) | Larval duration (days) | Weight of silk gland (g) | Weight of pupa (g) | Pupal duration (days) | % increase over control | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Weight of larva | Weight of silk gland | Weight of pupa |
| **Car** | 2.907 d ±0.115 | 10 | 38 | 0.438 cd ±0.024 | 1.153 bc ±0.148 | 14 | 7.527 | 26.394 | 3.497 |
| **Sed** | 2.899 d ±0.211 | 10 | 38 | 0.470 bcd ±0.018 | 1.23 ab ±0.2 | 14 | 7.236 | 34.786 | 11.153 |
| **BC** | 2.720 e ±0.145 | 10 | 38 | 0.439 cd ±0.032 | 1.161 bc ±0.136 | 14 | 0.614 | 27.304 | 3.799 |
| **Dil$_1$** | 3.693 a ±0.141 | 16* | --- | --- | --- | --- | 36.598 | --- | --- |
| **Dil$_2$** | 3.206 c ±0.262 | 16* | --- | --- | --- | --- | 18.608 | --- | --- |
| **Fen$_1$** | 3.459 b ±0.167 | 13 | 42 | 0.498 abc ±0.028 | 1.33 a ±0.187 | 16 | 27.966 | 45.798 | 17.863 |
| **Fen$_2$** | 3.297 c ±0.248 | 13 | 42 | 0.516 ab ±0.029 | 1.247 ab ±0.219 | 17 | 21.954 | 36.726 | 22.022 |
| **Ros$_1$** | 3.566 ab ±0.232 | 16* | --- | --- | --- | --- | 31.988 | --- | --- |
| **Ros$_2$** | 3.195 c ±0.208 | 16* | --- | --- | --- | --- | 18.192 | --- | --- |
| **Car/Dil$_1$** | 2.712 e ±0.173 | 15* | --- | --- | --- | --- | 0.325 | --- | --- |
| **Car/Ros** | 2.608 ef ±0.142 | 15* | --- | --- | --- | --- | - 3.528 | --- | --- |
| **Sed/Fen$_1$** | 2.897 d ±0.232 | 12 | 42 | 0.532 ab ±0.07 | 1.138 bc ±0.173 | 15 | 7.155 | 24.761 | 25.756 |
| **Sed/Ros$_1$** | 2.524 f ±0.154 | 13 | 43 | 0.503 abc ±0.058 | 1.024 cd ±0.162 | 16 | -6.625 | 12.194 | 18.998 |
| **BC/Fen$_1$** | 2.931 d ±0.167 | 12 | 42 | 0.559 a ±0.050 | 1.186 b ±0.092 | 16 | 8.413 | 29.987 | 32.136 |
| **BC/Ros$_1$** | 2.557 f ±0.26 | 12 | 42 | 0.420 d ±0.036 | 0.897 d ±0.129 | 16 | -5.439 | -1.698 | - 0.708 |
| **Control** | 2.703 e ±0.175 | 14 | 43 | 0.423 d ±0.137 | 0.912 d ±0.086 | 17 | | | |
| **L.S.D.** | 0.139 | | | 0.074 | 0.141 | | | | |

**Honeys : Car** = Carob honey, **Sed**=Seder honey and **BC** =Black Cumin honey (1g)
**Oils : Dil** =Dill oil, **Fen** =Fenchone oil and **Ros**=Rosemary oil
$_1$=5mg/100 ml water and $_2$=10 mg/100 ml water
*Didn't complete

The lightest weight (0.420 g) was recorded for the mixture Black Cumin/ Rosemary and that weight was somewhat less than that of the control by 1.698%.

A similar trend was obtained for the pupal weight, so the heaviest weight of pupa was obtained from Fen. (5mg) that comprised 1.33 g with 17.863% increase over control. Lightest pupal weight (0.897 g) was gained from the treatment of BC/Ros with a decrease of 1.698 than that of the control.

Car, Sed and BC honey gave shortest pupal duration similar to that of larval one.

These results are in accordance with those obtained by Hashida (1961) who stated that honey is a valuable agent that can be added to mulberry leaves and also those reported by Govidan *et al.*, (1988) El-Sayed (1989 and 1994), El-Karaksy and Idriss (1990), Muniandy (1995), Sarker *et al.* (1995) and Manoharan (1997) who found that the detected parameters of fitness component of *Philosamia ricini* Boisd. or *Bombyx mori* L. larvae were significantly affected by the evaluated food additives.

El- Sayed *et al.* (1996) found that the tested Black Cumin (BC) dilutions at 10 and 20% (w/v) gave the heaviest weights of larvae, silk gland and male and female pupae of the mulberry silkworm *B. mori* L. Also, El-Sayed (1999) reported that the mixture of honey and Black Cumin seeds gave the heaviest weights of larvae, pupae and dry silk gland. Abd El-Aziz (2002) evaluated some vegetable oils as food additives for the silkworm *Bombyx mori* and she found that all tested vegetable oils recorded higher mean of $5^{th}$ larval instar weight at the lower concentration than that recorded at the higher concentrations.

The results also declared that the use of Dill and Rosemary oils alone or/and the mixtures of Carob honey with either Fenchone or Rosemary oils must be not recommended as food additives for the silkworm because the larvae fed on the leaves treated with these food additives were found to overgrow, became weaken and exceeded the time of spinning; in addition to the infection of some diseases that appeared on them. This may be due to the powerful and fatal effects of suggested oils which led to a prolongation of larval duration and the appearance of diseases.

**1- Effect of the tested materials on silk and egg production of *Bombyx mori* L.**

Results in Table 3 elucidate the effect of the tested materials on the silk and egg production. The Fenchone oil at 5mg/100ml ($Fen_1$) gave the heaviest fresh cocoon weight of 1.749 g with an increase of 53.383% over control. The lightest weight (1.24 g) was recorded for the tested mixture of BC/Ros with an increase amounted to 8.704% over control.

With the same trend, both the concentrations of Fenchone oil ($Fen_1$ and $Fen_2$) gave the heaviest weights of cocoon shell (0.419 and 0.432 g, in respect). Lightest weights of obtained cocoon shells were revealed for all tested types of honey (Car, Sed and BC), but still have an increase over the control by 38.667, 45.857 and 32.485%, respectively.

The included results in Table, 3 also indicate that Car and Sed gave the highest rates of deposited eggs with an average of 558.6 and 563.8 eggs/female followed by the oil $Fen_1$ and the mixture of Sed /Fen with 27.531 and 28.396% increase over control.

The lowest calculated average (393 eggs/ female) was recorded for the larvae of the untreated control which gave minor results in most tests.

These results are in agreement with the findings of Babock and Rutscky (1961) who reviewed lipids in insect eggs, Gupta and Kamboj (1962) who suggested that lipids provide the energy of secretary metabolism in *B. mori* L. Also, El-Hattab (1985) found the fed larvae on semi – artificial diet containing honey gave higher number of deposited eggs/ female; El-Karaksy *et al.* (1990) stated that the use of honey at different concentrations of 2 and 4% enhanced both the silk production and female fecundity.

El-Sayed (1999) reported that the mixture of honey and Black Cumin (BC) seeds increased silk production and number of deposited eggs/female. Abd El - Aziz (2002) found that the treatment of larvae with tested vegetable oils indicated a high efficiency on the mean weight of cocoon shell and number of deposited eggs/female at the low concentration of 0.05%. Moreover, Zah *et al.* (2011) using different sources of fat on four different silkworm hybrid strands, observed an increase in the individual mass and silk quantity compared to the control.

**2- Correlation between some quantitative characters of silkworm *Bombyx mori* L. during its rearing periods**

Due to the presence of counteracting relationship between certain quantitative characters of the mulberry silkworm themselves, it was necessary to study these relationships in order to provide practical information for the establishment of adequate and favourable principles for selection determining the resulting effects and the limits of selection. Though, these correlated quantitative characters are exhibited in Table 4.

Simple correlation coefficients were calculated between certain studied biological and physiological parameters that included cocoon weight, cocoon shell weight and silk gland weight.

**Table 3. Effect of the tested materials on silk and egg production of *Bombyx mori* L**

| Treatments | Weight of cocoon (g) | Weight of cocoon shell (g) | No. of deposited eggs/female | % increase over control | | |
|---|---|---|---|---|---|---|
| | | | | Weight of cocoon | Weight of cocoon shell | No. of deposited eggs |
| **Car** | 1.469 cd ±0.157 | 0.316 ef ±0.023 | 558.6 a ±48.153 | 28.848 | 38.667 | 42.137 |
| **Sed** | 1.562 bc ±0.224 | 0.332 def ±0.028 | 563.8 a ±28.908 | 37.000 | 45.857 | 43.460 |
| **BC** | 1.464 cd ±0.150 | 0.302 f ±0.060 | 494.6 bc ±17.096 | 28.339 | 32.485 | 25.852 |
| **Fen$_1$** | 1.749 a ±0.190 | 0.419 ab ±0.034 | 501.2 b ±13.217 | 53.383 | 83.735 | 27.531 |
| **Fen$_2$** | 1.680 ab ±0.260 | 0.432 a ±0.059 | 485.2 bc ±11.189 | 47.335 | 89.785 | 23.460 |
| **Sed/Fen** | 1.525 bcd ±0.193 | 0.386 bc ±0.053 | 504.6 b ±10.526 | 33.698 | 69.456 | 28.396 |
| **Sed/Ros** | 1.386 de ±0.165 | 0.362 cd ±0.03 | 432.8 e ±35.940 | 21.537 | 58.921 | 10.127 |
| **BC/Sed** | 1.555 bc ±0.102 | 0.368 cd ±0.027 | 459 cd ±23.065 | 36.316 | 61.639 | 16.793 |
| **BC/Ros** | 1.240 ef ±0.162 | 0.342 de ±0.040 | 447.2 de ±16.361 | 8.704 | 50.328 | 13.791 |
| **Control** | 1.140 f ±0.105 | 0.228 g ±0.028 | 393 f ±10.368 | | | |
| **L.S.D.** | 0.157 | 0.036 | 36.663 | | | |

**Car** = Carob honey **Sed**=Seder honey **BC** =Black Cumin honey
**Dil** =Dill oil **Fen** =Fenchone oil **Ros**=Rosemary oil
$_1$=5mg/100 ml water and $_2$=5mg/100 ml water
*Didn't complete

**Table 4. Correlation matrix between certain quantitative characters of the silk worm *B. mori***

| | Wt. of cocoon | Wt. of cocoon shell | Wt. of silk gland |
|---|---|---|---|
| **Wt. of cocoon** | 1 | | |
| **Wt. of cocoon shell** | 0.801 | 1 | |
| **Wt. of silk gland** | 0.614 | 0.717 | 1 |

From the represented data in Table, 4 the positive strong relationship were detected between weight of cocoon and weight of cocoon shell (r = 0.801). The same positive highly correlation was found between weight of cocoon shell and weight of silk gland (r = 0.717). Moreover, there was a positive correlation between weight of cocoon and weight of silk gland (r = 0.614) but somewhat less than the other parameters..

These results are in agreement with the findings of Mahmoud(2005) who obtained high correlations between cocoon shell weight and silk gland weight of the parents of *Bombyx mori* L. and their F1, F2 and F3 progenies.

In conclusion, the obtained results of the present study proved the significant efficiency of the used honey types, botanical oils and their mixtures for improving the biometrics of *Bombyx mori* L. that lead to increase the silk and egg production

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## الملخص العربي

# تأثير بعض الأعسال والزيوت النباتية وخلائطها على إنتاجية دودة الحرير التوتية

منى ماهر محمود، حسن على مصباح، نجدة أحمد السيد

يهدف هذا البحث دراسة تأثيرإضافة بعض أنواع الأعسال المختلفة والزيوت النباتية وخلائطها إلى أوراق التوت بتركيزات مختلفة لتقييم بعض القياسات البيولوجية والإنتاجية ليرقات العمرالخامس لدودة الحرير التوتية المتغذية على تلك الأوراق المعاملة بهدف زيادة إنتاجية كل من الحرير والبيض.

هذا وقد أدت أضافة ومعاملة أوراق التوت بالتركيزات المختبرة من زيت الشمر(5، 10مجم/100مل ماء) إلى زيادة وزن غدة الحرير وقشرة الشرنقة معنوياً بالمقارنة مع المعاملات الأخري المختبرة.

وأوضحت النتائج أيضاً أن تغذية اليرقات على أوراق توت معاملة بأنواع مختلفة من العسل أدت إلى زيادة فى عدد البيض الموضوع حيث أدت المعاملة بعسل السدر إلي زيادته تبعه في ذلك المعاملة بعسل الخروب(43,160، 42,137% زيادة عن الكنترول علي التوالي). أيضاً أدت معاملة أوراق التوت بخليط عسل السدر مع زيت الشمر إلى زيادة معنوية فى القياسات البيولوجية وإنتاجية الحرير بالمقارنة مع خلائط الأعسال والزيوت الأخري المختبرة والكنترول.