

BIOLOGICAL AND PRODUCTIVITY CHARACTERS OF MULBERRY SILKWORM, *BOMBYX MORI* L. FED ON LEAVES TREATED WITH LEMON JUICE AND ANTIBIOTIC E-MOX

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ABSTRACT: *The effect of three concentrations of lemon juice and E-mox (antibiotic) (1, 2 and 3%) on growth and silk production of mulberry silkworm Bombyx mori L. was investigated. It was found that the higher concentration (3%) of E-mox as oral administration increased the grown larval, silk gland, pupal weights and fecundity as well as, cocoon weight, cocoon shell weight, silk content ratio, reeled filament weight, length and size. The obtained results suggest the use of lemon juice and antibiotic E-mox as a profitable supplementary diet for silkworm to improve its growth and productivity as well as health maintenance and growth promotion.*

Key words: *Antibiotic, Silkworm, Bombyx mori , Lemon juice, Health maintenance, Nutritional additives.*

INTRODUCTION

Silk production, depends on the quality and nutritional status of the mulberry leaf. Recently, the enrichment of mulberry leaf with a multitude of exogenous factors, such as vitamins, minerals, antibiotics, hormones and study their impact on, has become the order of traditional research in sericulture .

There are many factors that influence the success of production of silk. In recent years, some antibiotic agents have been used for larval growth enhancement and improvement of silkworm production (Thilagavathi, *et al.* 2013). Silkworm is affected by several diseases which causes heavy crop losses. Various (antibiotics) are extensively employed to prevent the attack of diseases to the silkworm thereby, help in, increasing the productivity of silk. The beneficial action of the antibiotics has been attributed to the oral feeding of them along with mulberry leaves, which reduced significantly the incidence of flacherie and grasserie (Anonymous, 1980; El-Karaksy and Idriss (1990) and Mahmoud, 1988). Several antibiotics were found to be effective in reducing the mortality of

silkworms without affecting the cocoon parameters and increase the important larval and cocoon parameters and then produce bad quantity and quality of natural silk (Radha *et al.*, 1980 and Rahmathulla *et al.*, 2006).

Review of literature reveals that the nutritional-enrichment studies were based largely on the application of expensive commercial sugars, proteins, vitamins and minerals. The combined effects of most of these exogenous nutrients could be achieved through a single natural nutrient, called lemon juice (LJ), which is a rich source of multiple vitamins and minerals (Markus and Sass, 2003). Further, lemons are perennial and inexpensive fruits, readily available in all seasons. Despite its high nutritional value, the impact of lemon juice has not been examined with reference to silkworm growth and economic parameters of sericulture.

Citrus flavonoids have a large spectrum of biological activity including antibacterial, antifungal, antidiabetic, anticancer and antiviral activities (Ortuno *et al.*, 2006).

One important nutrient that attracted the attention of researchers in this field is vitamin C or L- ascorbic acid. This vitamin is present in the mulberry leaf and plays many vital roles in *B. mori* (Lombardi, 1964). It has been identified as a powerful anti- oxidant, potential phago-stimulant, efficient growth promoter and booster of silk production in the silkworm (Ito, 1961; and Hussain and Javed, 2002). The present study aimed to evaluate the effect of the lemon juice and antibiotic E-mox as nutritional additives on silkworm *B. mori* to improve its growth and productivity as health maintenance or growth promotion.

MATERIALS AND METHODS

The present experiments were carried out during spring season of 2014 in laboratories of Sericulture Research Department, Plant Protection Research of Sharkia Branch, Institute, Agriculture Research Center, Egypt to study the biological and technological effects of lemon juice and E-mox (antibiotic) as a nutritional additive on silkworm *Bombyx mori* larvae growth.

The local hybrid of the silkworm *Bombyx mori* was used.

1. E-mox (antibiotic):

E-mox (antibiotic 1 g product by Egyptian International Pharmaceutical Industry Co. (EIPICO). Each vial contains: Amoxicillin sodium equivalent 1 g Amoxicillin activity. Standard stock solution : 1g of amoxicillin was dissolved in 100 ml of distilled water (1000 mg × 1000 µg/100 ml) which is equivalent to 10000 µg/ml, from this solution 1ml was taken and added to 99 ml of distilled water (10000 µg /100ml) which is equivalent to 100 µg/ml, known as standard stock solution. For the preparation of 3 µg/ml concentration, 3ml of standard stock solution was added to 97ml of distilled water. Likewise for 2 µg/ml concentration, 2ml of

standard stock solution was added to 98ml of distilled water.

2. Lemon juice:

Fresh fruits of *Citrus lemon* L. were washed in running tap water, surface sterilized with 70% alcohol, rinsed with distilled water and cut with a sterile knife and the juice pressed out into a sterile universal container separately then filtered into another sterile container to remove the seeds and other tissues. Bottles were aseptically opened and 3 milliliter (3 ml) of each sample from crude juice was added to 97 ml of distilled water to prepare 3% concentration. Likewise for 2% concentration, 2ml of lemon juice was added to 98ml of distilled water then 1% concentration, 1ml of lemon juice was added to 99ml of distilled water.

Rearing of silkworm was carried out under laboratory conditions ($28 \pm 2^{\circ}\text{c}$ and $70 \pm 5\%$ R.H.) according to Krishnaswami (1978). Larval bed was cleaned daily. Cleaning net was used for removing the remained dried food and feces. Chicken egg cartons plates were used as montages for cocoon spinning Zannoon and Omera (1994).

At the beginning of the 4th instar, larvae were divided into 7 groups with three replicates each of 50 larvae. Each group was fed on mulberry leaves treated with one concentration of the tested material for one meal only then fed on untreated leaves the rest of the day, while the 7th group was fed on mulberry leaves dipped on distilled water. Mulberry leaves were dipped in the concentrations of treated antibiotic E-mox and Lemon juice for 5 minute and left to dry then offered to larvae. The control group of leaves was treated only with distilled water.

The following parameters were studied:
Biological aspects:

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Larval weight (gm); Silk gland weight (gm); Pupal weight (g) and Fecundity.

Cocoon indices: weight of fresh cocoon (gm); Weight of cocoon shell (gm); Silk content ratio (%): Silk content ratio was calculated according to Tanaka (1964) formula:

$$\text{Silk content ratio (\%)} = \frac{\text{Cocoon shell weight}}{\text{Cocoon fresh weight}} \times 100$$

Filament characters:

Length of reelable filament (m); Weight of reelable filament (gm); Size of reelable filament (dn), Tanaka (1964): Size of silk filament (dn.) = (Weight of filament (g)/ Length of filament (m) × 9000.

Statistical analysis:

Data obtained were statistically analyzed according to Snedecor and Cochran (1967) methods using computer software Costat program.

RESULTS AND DISCUSSION

Biological aspects:

1- Larval weight (g):

Data in Table (1) revealed that larvae fed on mulberry leaves supplemented with lemon juice and E-mox (3%) showed a significant increases about (4.21 and 4.04g) in larval weights, respectively. While a significant decrease about 3.15 and 3.26g was caused when larvae fed on leaves treated with lemon juice and E-mox concentration (1%), in comparison with 3.48g for control. Results showed that there are high significant differences between concentrations.

2- Silk gland weight (g):

Obtained data in Table (1) cleared that larvae fed on mulberry treated with lemon juice and E-mox (3%) presented a significant high values of silk gland weight

(1.12g), while low concentration (1%) cause decreased value (0.97 and 0.95g) for tested treatment. Control group was recorded 1.04g for silk gland weight. Statically analysis show that there are high significant between concentration.

3- Pupal weight (g):

Table 1 cleared that the 3% concentration of lemon juice and E-mox gave the highest result (1.57 and 1.52g) of pupal weight, respectively than control was (1.09g). Whereas, the lowest results (0.99g) was recorded when larvae fed on mulberry leaves treated with concentration of E-mox 1%. Results showed that there are high significant differences between concentrations.

4- Fecundity:

The highest mean number of eggs laid by female resulted from larvae treated by concentrations (3%) recording (766.6 and 706.60 egg/female) than control for lemon juice and e-mox, respectively. While the numbers of eggs laid by female was decreased to (475.8 egg/female) for 1% concentration of E-mox; in comparison with (588.2 egg/female) recorded for control larvae, (Table 1). There are high significance among used concentrations.

According to the obtained results, feeding silkworm larvae on mulberry leaves treated with high concentrations of lemon juice and E-mox enhanced the larval weight, silk gland weight, pupal weight and fecundity of silkworm. These results are in close correlation with Ito, (1961) who recorded relationship of ascorbic acid supplementation and growth of silkworm. Supplementation of vitamin C to mulberry leaves improved economic traits in the silkworm as suggested by Chauhan & Singh (1992) and Sengupta *et al.* (1992).

Table (1): Effect of Lemon juice and E-mox on biological characters of *B. mori*.

Treatment	Conc.	Larval weight (gr.)	Silk gland weight (gr.)	Pupal weight (gr.)	Fecundity (No. of Total egg/female)
Lemon juice	1%	3.15 ^a ±0.025	0.97 ^c ±0.008	1.09 ^d ±0.027	594.0 ^{cd} ±15.19
	2%	3.59 ^c ±0.042	1.02 ^b ±0.009	1.21 ^c ±0.020	610.0 ^{cd} ±13.352
	3%	4.21 ^a ±0.061	1.12 ^a ±0.007	1.57 ^a ±0.035	766.6 ^a ±17.394
E-MOX (Antibiotic)	1%	3.26 ^e ±0.052	0.95 ^c ±0.018	0.99 ^d ±0.045	475.8 ^e ±9.145
	2%	3.64 ^c ±0.049	1.04 ^b ±0.016	1.32 ^b ±0.037	626.4 ^c ±10.782
	3%	4.09 ^b ±0.027	1.12 ^a ±0.012	1.52 ^a ±0.027	706.6 ^b ±7.619
Control		3.48 ^d ±0.028	1.04 ^b ±0.024	1.09 ^d ±0.046	588.2 ^d ±12.893

Means in each column followed by different letter(s) are significantly different at 5% level.

Also, Hussain and Javed (2002) and Etebari and Mantindoost (2005), identified ascorbic acid as a powerful anti-oxidant, potential phago-stimulant, efficient growth promoter and booster of silk production in the silkworm. Moreover, Aabids and Kanika (2011) and Singh and Ahmed (2012) found that supplementation of vitamin C in the last larval stages of silkworm exerted significant improvement in larval weight.

Ganesh *et al.* (2013) indicated that the ascorbic acid exhibits the presence of certain growth stimulant activity. Thulasi (2013 and 2014) Minimum effective concentration of lemon juice (LJ) has a positive impact on *Bombyx mori*, with reference to larval growth, protein profiles and economic parameters of sericulture. Lemon juice is suggested as a profitable supplementary diet for silkworm.

Antibiotics play a vital role in improvement of disease resistance and growth enhancement in the animals and insects. Furthermore, antibiotics approved for four purposes: disease treatment, disease prevention, disease control and for health maintenance or growth promotion and to certain extent help in the

improvement of silk production (Radha *et al.*, 1980), Shoukry *et al.* (1998), El-Sayed and Mosbah (1992), Rahmathulla *et al.* (2003 and 2006), Venkatesh and Srivastava (2010). Also, Saad *et al.* (2012), Anadakumar *et al.* (2012), Thilagavathi *et al.* (2013) who observed that antibiotics administration with different concentrations significantly improved the rearing performance like larval duration, larval weight, growth index, and the better performances were recorded with the increase of antibiotics concentration. These findings are in accordance with the present study as the high concentration of E-mox resulted the higher values of the studied parameters.

5. Cocoon indices:

5.1. Cocoon weight (g):

The highest recorded weight of cocoons was 3.36 and 3.28g when larvae fed on mulberry leaves treated with 3% concentration of lemon juice and E-mox, respectively in comparison to (2.34g) recorded for control (Table 2). The lowest weight was 2.02 and 2.05g recorded for 1% concentration of lemon juice and E-mox, respectively. Results showed that there are

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high significant differences among concentrations.

5.2. Cocoon shell weight:

Data obtained in Table (2) showed that, the larvae fed on mulberry leaves treated with lemon juice (3%) caused increases in produced cocoon shells weight recording 0.46g than same concentration of E-mox and control (0.40 and 0.38g), respectively. While, the lowest cocoon shell weight was recorded 0.33g for low concentration (1%) of lemon juice and E-mox. Results showed that there are high significant differences among concentrations.

5.3. Silk Ratio:

The obtained results in (Table 2) showed positive relationship between the used concentrations of lemon juice and silk ratio ranged from 20.42% to 24.30%, while it was ranged from 19.65 to 22.82% in E-mox concentrations. The lowest percent of silk ratio was recorded (19.65%) for lowest concentration of E-mox, in comparison with (20.25%) for control. Results showed that there are high significant differences between the concentrations.

6. Technological aspects :

6.1. Filament length (m.):

Data in Table (3) cleared that the filament length was the longest value (1.159.40 and

1138.80 m) for high concentration of lemon juice and E-mox, respectively, compare to control (1093.6m). The short filament was resulted for larvae fed on mulberry leaves treated with low concentration of E-mox (958.60m). Results showed that there are high significant differences among concentrations.

6.2. Filament weight:

The obtained results indicated also that the weight of the silk filament increased (0.32 and 0.28g) when larvae fed on mulberry leaves treated with lemon juice and E-mox at concentration of 3%, respectively. All concentrations caused silk filament increase than control (0.20g). Statistical analysis revealed that there are high significant differences among the concentrations.

6.3. Silk filament size:

Silk filament size presented an increase (2.98 and 2.94 dn.) compare to control which recorded (2.44g), when the larvae fed on mulberry leaves treated with lemon juice and E-mox at 3% concentration, respectively. All used concentrations enhanced the silk filament size (Table, 3). Statistical analysis cleared high significant differences among the concentrations.

Table (2): Effect of Lemon juice and E-mox on cocoon indices of *B. mori* .

Treatment	Conc.	Cocoon weight (gr.)	Shell cocoon weight (gr.)	Silk ratio %
Lemon juice	1%	2.02 ^c ± 0.102	0.33 ^d ± 0.011	20.42 ^c ± 0.034
	2%	2.37 ^b ± 0.024	0.41 ^b ± 0.012	23.37 ^b ± 0.060
	3%	3.36 ^a ± 0.055	0.46 ^a ± 0.013	24.30 ^a ± 0.112
E-MOX (Antibiotic)	1%	2.05 ^c ± 0.045	0.24 ^a ± 0.003	19.65 ^d ± 0.233
	2%	2.41 ^b ± 0.19	0.33 ^d ± 0.001	20.14 ^{cd} ± 0.117
	3%	3.28 ^a ± 0.067	0.40 ^{bc} ± 0.004	22.82 ^b ± 0.528
Control		2.34 ^b ± 0.044	0.38 ^c ± 0.008	20.25 ^c ± 0.012

Table (3): Effect of Lemon juice and E-mox on silk filament characters of *B. mori*.

Treatment	Conc.	Silk filament L. (m.)	Silk filament W. (gr.)	Silk filament S. (dn.)
Lemon juice	1%	1098.4 ^{bc} ±9.882	0.25 ^c ±0.007	2.21 ^c ±0.012
	2%	1127.0 ^{abc} ±2.549	0.29 ^b ±0.005	2.51 ^b ±0.029
	3%	1159.4 ^{ab} ±3.059	0.32 ^a ±0.010	2.98 ^a ±0.067
E-MOX (Antibiotic)	1%	958.6 ^d ±2.731	0.23 ^d ±0.005	2.47 ^b ±0.018
	2%	1084.2 ^c ±19.024	0.25 ^{cd} ±0.003	2.60 ^b ±0.140
	3%	1138.8 ^a ±1.685	0.28 ^b ±0.004	2.94 ^a ±0.072
Control		1093.6 ^c ±23.716	0.20 ^e ±0.004	2.44 ^b ±0.044

Means in each column followed by different letter(s) are significantly different at 5% level.

The obtained results cleared that, feeding silkworm larvae on mulberry leaves treated with high concentrations of lemon juice and E-mox enhanced the cocoon indices and silk filament parameters. These results are in agree with Ito, (1961) who found that, supplementation of vitamin C to mulberry leaves improved economic traits in the silkworm as suggested by studied of Chauhan & Singh (1992) and Sengupta *et al* (1992). Also, Hussain and Javed, 2002 and Etebari and Mantindoost, 2005, who identified ascorbic acid as a powerful antioxidant, potential phago-stimulant, efficient of silk production in the silkworm. Moreover, Markus and Sass, (2003) clear that, If, the mulberry diet is reinforced with ascorbic acid, it is possible to show improvement in the sericulture industry. Aabid and Kanika (2011) and Singh and Ahmed (2012) found that supplementation of vitamin C in the last larval stages of silkworm exerted significant improvement in cocoon weight and shell weight. Ganesh *et al* (2013) was indicated that the ascorbic acid exhibits the presence of certain growth stimulant activity, cocoon (length, width and weight), yield of cocoon (%), cocoon shell weight (g), cocoon shell ratio (%), silk thread length (mts.) and silk

thread denier (%). Thulasi (2013 and 2014) found that the minimum effective concentration of lemon juice (LJ) has a positive impact on *Bombyx mori*, with reference to economic parameters of sericulture.

Antibiotics in silkworm are approved for four purposes: disease treatment, disease prevention, disease control and for health maintenance or growth promotion and to certain extent help in the improvement of silk production (Radha *et al.*, 1980 and Venkatesh, and Srivastava, 2010). Antibiotics are also known to improve the growth of larvae Shoukry *et al.* (1998), El-Sayed and Mosbah (1992), Rahmathulla *et al.* (2003 and 2006), Saad *et al.* (2012), Saad *et al.* (2014), Anadakumar *et al.* (2012) and Thilagavathi *et al.* (2013) observed that antibiotics administration with different concentrations significantly improved the rearing performance like single cocoon weight, single shell weight and shell ratio, average filament length, non breakable filament length, raw silk recovery percentage, denier, reelability and neatness and the better performances were recorded with the increase of antibiotics concentration.

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The present study indicated that enriching mulberry leaves with the lemon juice and E-mox can be used to increase the silk yield in commercial silkworm rearing.

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الصفات البيولوجية والإنتاجية لدودة الحرير التوتية بومبيكس موري المغذاة على اوراق
معاملة بعصير الليمون والمضاد الحيوي إي - موكس

محمود سعد ابراهيم سعد

باحث بمعهد بحوث وقاية النباتات - مركز البحوث الزراعية

الملخص العربي

تم دراسة تأثير ثلاثة تركيبات (١ ، ٢ ، ٣%) من كل من عصير الليمون والمضاد الحيوي ايه - موكس على بيولوجى وانتاج الحرير لدودة الحرير التوتية بومبيكس موري. وقد وجد أن التركيز الاعلى (٣%) من المواد المستخدمة بطريقة الغمر قد أدى الى زيادة كلا من وزن اليرقات، غدة الحرير، العذراء ، عدد البيض الموضوع لكل فراشة ، وكذلك وزن الشرنقة الطازجة ، قشرة الشرنقة ، ونسبة الحريرفى قشرة الشرنقة، وزن الخيط الحريرى، وطول الخيط الحريرى ، وحجم الخيط الحريرى.

اوضحت الدراسة اهمية استخدام عصير الليمون والمضاد الحيوي ايه- موكس كاضافات غذائية تكميلية لدودة الحرير التوتية لتحسين نموها وانتاج الحرير.