



Using some different essential oils for improving silk production of mulberry silkworm, *bombyx mori* L.

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Abstract: The effect of some essential oils (basil, mint, olive, thyme and citronella) as food additives to mulberry leaves was evaluated during the last larval instar on silkworm biological parameters. Treatment of mulberry leaves with different concentrations (0.125, 0.25, 0.5 and 1%) of both commercial and crude basil oil extractions hadn't showed positive effect on the most biological parameters except number of deposited eggs which was positively increased by 33.2, 33.4 and 35.1% at concentrations of 0.125, 0.5 and 1% respectively. Commercial peppermint oil revealed insignificant increase in all treatments. While crude peppermint oil at 1% concentration conducted significant increase only in the mean weight of silk gland and number of deposited eggs. Olive oil recorded insignificance among all treatments in most of the biological parameters under study. While the number of deposited eggs increased significantly at 0.125 and 0.25% treatments. Insignificant result among all treatments of thyme oil comparing to control, except for silk gland and number of deposited eggs. Mature larval weight revealed insignificance in all treatments with citronella oil comparing to control with slight increase at 0.25 and 1% concentrations. While fresh cocoon, pupae and cocoon shell weights showed significant decrease in all treatments except on 0.5%. Silk gland recorded insignificant decrease at concentration of 0.25%. Numbers of deposited eggs were higher than control in all treatments with significant increase (46.4%) at 0.5%.

keywords: *Bombyx mori*, oils, sericulture.

1. Introduction

Various extract of medicinal plants has been tested by supplementation to *Bombyx mori* L. food and were seen to influence the body weight, silk gland weight and the silk thread length [1]. Basil oil (*Ocimum basilicum*) has a great importance because of their wide spectrum of biological action. The extract of *Ocimum basilicum* showed a great effect on growth and maturation of silkworm, decreased the maturation time and the treated larvae start producing silk earlier than the control [2]. So, there is a great possibility of using such leaf extracts as prophylactic measure for rearing silkworm [3]. Treatment of silkworm larvae with *Ocimum sanctum* showed improvement in larval performance and commercial parameters. A significant increase in the larval weight, cocoon yield, average cocoon weight, pupal weight, shell weight and silk ratio % at 3%

concentration [4]. Basil leaves (*Ocimum basilicum*) enhanced some biological aspects of silkworm. Treating mulberry leaves with its leaves extract at 3% enhanced female fecundity and give the highest cocooning percentage [5]. The effect of peppermint oil with different concentrations as disinfectants on infected *Bombyx mori* enhancing biochemical parameters and the activity of the enzyme responsible for production of silk were explained by [6]. Treating mulberry leaves with olive oil at 0.25 and 0.5% by two ways, spraying the leaves and filter paper soaked in olive oil placed between leaves on four silkworm races showed negative effect on its weight and length [7]. Adding different concentrations of marjoram (*Majorana hortensis*) and thyme (*Thymus vulgaris*) oils (1%, 0.5%, and 0.25%) and their botanical

extracts (2%, 1%, and 0.5%) as a nutritional additives offered to silkworm larvae during the 4th and 5th larval instars. recorded a remarkable increase in larval growth owing to increase in food utilization and also, improved larval physiological characteristics when treated with Thus, treatment with this oil proved to be more efficient in rearing mulberry silkworm as its improved food acquisition, high ingestion, high efficiency of conversion of food ingested to body substance [8]. The current study is an attempt to evaluate the effect of essential oils (basil, mint, olive, thyme and citronella) as food additives on mulberry silkworm biological parameters.

2. Materials and methods

1. Insects:

Imported Bulgarian hybrid mulberry silkworm, *Bombyx mori* L. was used in the current study. Rearing technique was done as followed, in Sericulture Research Department, Plant Protection Research Institute, Ministry of Agriculture and Land Reclamation. Each treatment and control have three replicates, each replicate contained 30 larvae of 5th instar that reared on cardboard boxes (30 *15*10). The daily weights of mature larvae, silk glands, fresh cocoon, pupa and cocoon shells were recorded. Mulberry leaves sprayed with oils and introduce to silkworm larvae at the first day of 5th instar for three times.

2. Chemicals:

Commercial basil oil (*Ocimum basilicum*),peppermint (*Mentha piperita*), and thyme (*Thymus vulgaris*) essential oils were purchased from EL Captain Company (Cap Farm) for extracting nutriments oil and natural herbs and cosmetics, Al Obour City, Cairo , Egypt. Crude oils basil (*Ocimum basilicum*), peppermint (*Mentha piperita*), olive (*Olea europaea*) and citronella (*Cymbopogon citratus*), were obtained from Sakha Horticulture Research Institute, Kafr-

The present data express the mean \pm SD of 20 observations for (larvae, fresh cocoon, pupae and cocoon shell) and 3 replicate for silk gland and no. of eggs.

Similar letters (a, b, c) means non-significant differences at the same columns at ($P \geq 0.05$).

Elsheikh, Egypt. Commercial oils were dissolved in ethyl alcohol 70% then diluted with distilled water for different concentrations (0.125, 0.25, 0.5 and 1%) according to [9] and the control was sprayed with distilled water and alcohol. While crude oils were dissolved in tween 20, then diluted with distilled water for obtaining concentrations (0.125, 0.25, 0.5 and 1%) Control was sprayed with distilled water and tween 20.

3. Statistical Analysis

Statistical analysis was performed using analysis of variance (ANOVA); means were compared using LSD and Duncan's test according to Snedecor and Cochran (1982) using Statistical Software V.9.0.

3. Results and Discussion

Evaluation of basil oil extraction:

Data in table (1) illustrate the effect of both commercial and crude basil (*Ocimum basilicum*) oils on mean weights of mature larvae, fresh cocoons, pupae, cocoon shell, silk gland and number of deposited eggs per female of *Bombyx mori* larvae fed with mulberry leaves treated with oils with different concentrations of (0.125, 0.25, 0.5 and 1%) at first day of last larval instar. Larvae fed with commercial basil oil at all concentrations presented a lower and significant decrease in the mean weights of mature larvae, fresh cocoons, pupae, cocoon shell, and silk gland and an insignificant increases in number of deposited eggs by 41% and 37.7% at concentrations of 0.25 and 0.5%, respectively in comparison to untreated one. Treatment with extracted basil oil at all concentrations showed the same trend in all parameters, number of deposited eggs, gives the highest percentage by 33.2, 33.4 and 35.1% ,respectively at concentrations of 0.125, 0.5 and 1% in comparison to untreated larvae.

silkworm, *Bombyx mori*, fifth larval instar.

*Commercial basil oil dissolved on 70% alcohol and then diluted with distilled water.

** Crude basil oil dissolved with tween20 and diluted with distilled water.

Table (1): Mean values of studied parameters under the effect of basil commercial and crude oils on mulberry

Oil	Concentration%	Larval weight(g)	Fresh Cocoon Weight(g)	Pupal Weight (g)	Cocoon shell Weight(g)	Silk gland weight (g)	No. of eggs
Commercial Basil *	Control	3.09±0.38 a	1.31±0.21 a	1.05±0.17 a	0.24±0.04 a	0.71±0.07 a	321±103 a
	0.125%	2.66±0.27 b	1.15±0.18 b	0.95±0.17 b	0.19±0.04 b	0.61 ±0.07 ab	329±36 a
	0.25%	2.57±0.28 b	1.12±0.14 b	0.91±0.13 b	0.18±0.03 bc	0.63±0.10 ab	451±42 a
	0.5%	2.69±0.23 b	1.12±0.17 b	0.93±0.15 b	0.17±0.03 c	0.52±0.13 b	442±6 a
	1%	2.66±0.34 b	1.12±0.17 b	0.92±0.15 b	0.18 ±0.04 bc	0.60±0.02 ab	327±106 a
Crude Basil **	Control	2.5±0.39 ab	1.22± 0.26 a	0.96±0.21 a	0.22± 0.05 a	0.67±0.04 ab	356±48 b
	0.125%	2.51±0.25 b	1.06± 0.13 a	0.83±0.12 a	0.19±0.03 b	0.55±0.02 c	474±37 a
	0.25%	2.52± 0.23 b	1.13±0.14 a	0.91±0.12 a	0.20±0.03 ab	0.71±0.03 a	411±46 ab
	0.5%	2.73± 0.37 a	1.15± 0.14 a	0.92±0.13 a	0.19±0.02 ab	0.62±0.03 b	475±72 a
	1%	2.46± 0.35 b	1.12± 0.23 a	0.89± 0.19 a	0.24±0.04 a	0.67±0.05 ab	481±27 a

2. Evaluation of peppermint oil extraction:

Data revealed that the mean weights of mature larvae, fresh cocoons, pupae, cocoon shell, silk gland and number of eggs of *B. mori* last larval instar fed with both mint oil at concentrations of 0.125, 0.25, 0.5 and 1%. The results in table (2), revealed that larvae fed with commercial mint oil at all concentrations presented insignificant changes in the mean

weights of mature larvae, fresh cocoons, pupae, cocoon shell, silk gland and the no. of deposited eggs in comparison to untreated one, larvae fed on crude mint oil followed the same trend at all concentrations for all parameters except silk gland mean weight and number of deposited eggs, concentrations of 1% gave the highest and significant increase over control by 13.4 and 21.1%, respectively.

Table (2): Mean values of studied parameters under the effect of peppermint commercial and crude oils on mulberry silkworm, *Bombyx mori*, fifth larval instar

Oil	Concentrations %	Larval weight(g)	Fresh Cocoon Weight(g)	Pupal Weight (g)	Cocoonshell Weight(g)	Silk gland weight (g)	No. of eggs
	Control	3.09±0.38 ab	1.31±0.21 a	1.05±0.17 a	0.24±0.04a	0.71±0.07 ab	321±103 ab
	0.125%	3.16±0.36 a	1.38±0.23 a	1.12±0.18a	0.22±0.06 a	0.77±0.09 a	450±53 ab
	0.25%	3.03±0.33 ab	1.38±0.21 a	1.11±0.19 a	0.25±0.04 a	0.74±0.05 ab	438±23 ab
	0.5%	3.18±0.23a	1.31±0.17 a	1.06±0.15 a	0.22±0.41 a	0.71±0.92 ab	521±20 a
	1%	2.91±0.39 b	1.36±0.22 a	1.09±0.19 a	0.23±0.05 a	0.60±0.08 b	408±13 ab
Crude peppermint **	Control	2.59±0.39 ab	1.22± 0.26 a	0.96±0.21 a	0.22± 0.05 a	0.67±0.04 b	356±48 b
	0.125%	2.44±0.32 c	1.01±0.07 b	0.79±0.08 a	0.21±0.04 a	0.58±0.02 c	347±25 b
	0.25%	2.84±0.34 a	1.21±0.27 ab	0.96±0.23 a	0.22±0.053 a	0.75±0.07 ab	391±15 ab
	0.5%	2.64±0.03abc	1.05±0.13 ab	0.85±0.10 a	0.19±0.06 a	0.72±0.06 ab	419±38 ab
	1%	2.75±0.42 ab	1.05±0.29 ab	0.84±0.25 a	0.19±0.05 a	0.76±0.03 a	431±63 a

The present data express the mean ±SD of 20 observations for (larvae, fresh cocoon, pupae and cocoon shell) and 3 replicates for silk gland and no. of eggs.

Similar letters (a, b, c) means non-significant differences at the same columns at (P≥0.05).

*Commercial peppermint dissolved on 70% alcohol and then diluted with distilled water.

** Crude peppermint oil dissolved with tween20 and diluted with distilled water.

3 Evaluation of olive, thyme and citronella oil extractions:

Larvae fed on mulberry leaves sprayed with olive oil at concentrations of 0.125 and 0.25% showed the highest increase in number of deposited eggs 504±58 and 483±9 compared

with that of untreated one 356±48 with significant increase ratio by 41.6 and 35.7%, respectively and didn't affect the biological parameters of treated silkworm enhance only the moth fecundity ratio (Table: 2).

Thyme (*Thymus vulgaris*) oil showed insignificant changes in all biological parameters except the concentration of 0.25% showed a significant increase in both silk gland mean weight and number of deposited eggs by 17.4% and 37.4%, respectively comparing to control (Table 3). Larvae fed with thyme oil at 1% presented the highest increase in all biological parameters. The results showed a significant increase in mature larvae by 15.7% higher, fresh cocoons by 7.1%, pupal weights by 9.4%, cocoon shell by 4%, silk gland by

13% and finally 51.4% increase in number of deposited eggs when compared to control larvae.

Table (3) showed insignificant changes in the mean weight of mature larvae fed with citronella oil (*Cymbopogon citratus*) extract at all concentrations. Fresh cocoons, pupae and cocoon shell presented significant decrease when larvae fed with citronella oil extract at

concentration of 0.125, 0.25 and 1% and insignificant changes at 0.5%. Silk gland also showed only significant decrease for larvae fed with citronella oil at 0.25% and insignificant changes at other concentrations in comparison to control. While, the number of deposited eggs showed an increase at all concentration, with significance only (46.4%) at 0.5% comparing to control

Table (3): Mean values of studied parameters under the effect of olive, thyme and citronella oils on mulberry silkworm, *Bombyx mori*, fifth larval instar.

Oil	Concentrations%	Larval weight(g)	Fresh Cocoon Weight(g)	Pupal Weight (g)	Cocoonshell Weight(g)	Silk gland weight (g)	No. of eggs
Olive oil *	Control	2.59±0.39 ab	1.22± 0.26 a	0.96±0.21 a	0.22± 0.05 a	0.67±0.04 ab	356±48 b
	0.125%	2.54±0.31 a	1.15±0.19 a	0.96±0.15 a	0.18±0.05 b	0.60±0.02 b	504±58 a
	0.25%	2.59±0.39 a	1.22±0.25 a	0.99±0.20 a	0.22±0.06 ab	0.66±0.03 ab	483±9 a
	0.5%	2.64±0.41 a	1.27±0.18 a	1.01±0.16 a	0.24±0.04 a	0.71±0.07 a	435±60 ab
	1%	2.61±0.33 a	1.07±0.18 a	0.85±0.16 a	0.20±0.05 ab	0.68±0.06 ab	457±100 ab
Thyme oil **	0.125%	2.66±0.35 b	1.17±0.22 a	0.93±0.19 a	0.19±0.05 a	0.64±0.03 c	407±49 bc
	0.25%	2.61±0.28 b	1.07±0.19 a	0.94±0.17 a	0.21±0.04 a	0.79±0.09 a	489±33 ab
	0.5%	2.67± 0.37 b	1.15±0.20 a	0.93±0.18 a	0.19±0.04 a	0.69±0.05abc	428±107 abc
	1%	2.99±0.39 a	1.31±0.22 a	1.05±0.19 a	0.23±0.05 a	0.76±0.05 ab	539±52 a
Citronella oil ***	0.125%	2.54±0.28 a	1.00±0.14 b	0.81±0.09 b	0.16±0.05 b	0.69±0.06 a	414±97 ab
	0.25%	2.60±0.26 a	1.01±0.19 b	0.81±0.16 b	0.17±0.04 b	0.56±0.03 b	387±24 b
	0.5%	2.59±0.28 a	1.07±0.18 ab	0.85±0.15 ab	0.19±0.04 ab	0.67±0.07 a	521.3±48 a
	1%	2.61±0.33 a	1.02±0.14 b	0.80±0.13 b	0.18±0.04 b	0.60±0.07 ab	399±82 b

The present data express the mean ±SD of

20 observations for each (larvae, fresh cocoon, pupae and cocoon shell) and 3 for silk gland and no. of eggs.

Similar letters (a, b, c) means non-significant differences at the same columns at ($P \geq 0.05$).

* Olive oil dissolved with tween20 and diluted with distilled water.

**Thyme oil dissolved on 70% alcohol and then diluted with distilled water.

***Citronella oil dissolved with tween20 and diluted with distilled water.

Discussion

1. Evaluation of basil oil extraction:

Basil plants (*Ocimum basilicum*) known as "Queen of herb" is one of the medicinal plants that having a biological active chemical compound. Basil oil extract has not only an antimicrobial property [10], but also playing an important role in improving the economical traits as larval weight, pupal weight, cocoon weight and silk ratio [11] and found that supplementation of (*Ocimum basilicum*) to silkworm improved larvae and cocoon parameters, with the best results at 2%

concentration. Also, [3,4] reported that enriching mulberry leaves with *Ocimum basilicum* leaf extracts and feeding it to *B. mori* enhanced the growth rate of the larvae stimulate it to start producing silk earlier than the control. Basil also, has a role in improvement of some important biological and physiological parameters in mulberry silkworm [5], basil leaves (*Ocimum basilicum*) extracts at concentrations of 1, 2 and 3% led to an improvement in some important biological and physiological parameters, such as the cocooning, pupation percentage and emergency percentage as well as the number of eggs laid by the female. In the current study commercial basil oil at concentration 0.125, 0.25, 0.5 and 1% revealed that the mean weights of mature larvae, fresh cocoons, pupae, cocoon shell, silk gland and number of eggs were not positively affected. However, number of deposited eggs (moth fecundity ratio) presented a positive and significant increase (33.2, 33.4 and 35.1%) especially when larvae fed with crude basil oil at concentrations of 0.125, 0.5 and 1%, respectively. Current results are in agreement with [12] who found increasing number of

fertilized eggs of *B. mori* larvae treated with *Aloe-vera*, and were agreed with the results of [13,5] in the fecundity of moth only, while differ with the results of other biological traits due to the variance of concentration used in each study.

2. Evaluation of peppermint oil extraction:

Peppermint *Mentha piperita* is a medicinal plant have been used for centuries in traditional medicine because of their therapeutic value [14]. Mint essential oil was also, used as antibacterial against *Salmonella* micro-organism [15]. The effects of dexamethasone antibiotic and peppermint oil with different concentrations was used as a disinfectant on infected silkworm *B. mori* larvae with bacteria [6]. In current study, commercial and crude peppermint oil extractions were used as a stimulant at low concentrations of 0.125, 0.25, 0.5 and 1% in last larval instar of mulberry silkworm. The results revealed that crude mint oil enhanced significantly only the silk gland mean weight and the number of deposited eggs (moth fecundity) by 13.4 and 21.1%, respectively higher than control at concentration of 1%. This finding is in agreement with that obtained by [16], who proved that fecundity increased when the silkworm, *B. mori* larvae treated with the volatile oils and [12] who found increasing number of fertilized eggs of *B. mori* larvae treated with *Aloe-vera*. It is also in agreement with [5], who noticed a highest fertility percentage of egg in silkworm larvae treated with concentration of 3% of basil oil.

3. Evaluation of olive, thyme and citronella oil extraction:

Virgin olive oil (*Olea europaea* L.) is known as one of the good vegetable oils given its nutritional benefits in the human diet [17]. It also acts as antibacterial activity against bacteria strain isolated from gut tissue of silkworm *B. mori* [18]. Silkworm nutrition research has been considered in the recent years [7], who reported a strongly positive enhancement for the silkworm hybrid when using leaves fed with olive oil: the silk percentage remained constant, but the total mass of the raw cocoon and the shell weight were both higher by 15% compared to control group. In the current study olive oil treatment

has no effect on the biological parameters of treated silkworm but enhance only the moth fecundity ratio. Larvae fed on mulberry leaves sprayed with olive oil at concentrations of 0.125 and 0.25% showed the highest increase in the number of deposited eggs, 504 ± 58 and 483 ± 9 compared with that of untreated 356 ± 48 with a significant increase ratio about 41.6 and 35.7% respectively. This finding are in agreement with [12] who found increasing number of fertilized eggs of *B. mori* larvae treated with *Aloe-vera* and agreed with those investigated by [16,5], who proved that fecundity increased when the silkworm, *B. mori* larvae treated with the volatile oils.

Thyme, (*Thymus vulgaris*) is an aromatic plant of the Lamiaceae family and has received major attention as both a pharmaceutical and therapeutic agent across the globe. It contains thymol, carvacrol and other chemical component such as flavonoids which have antimicrobial activity [19, 20]. The primary pharmacological effects of thyme arise from thymol and carvacrol, which are the most important bioactive compounds that this plant contains [21]. In the current investigation, the larvae fed with thyme oil at 1% presented the highest and significant increase in all biological parameters. The results showed a significant increase in mature larvae by 15.7%, fresh cocoons by 7.1%, pupal weights by 9.4%, cocoon shell by 4%, silk gland by 13% and finally 51.4% increase in number of deposited eggs when compared to control larvae. These finding is agreement with the previous results of [8], who revealed a remarkable increase in larval growth owing to increase in food utilization and also improved larval physiological characteristics, and agreed with the finding of [16], who proved that fecundity increased when the silkworm, *B. mori* larvae treated with the volatile oils. Previous results [22,23,24] demonstrated that supplementation of *Clerodendrum multiflorum* and *Xanthium indicum* L. plant extracts to mulberry leaves enhanced the economic characteristics of *B. mori*. This is due to the physiological stimulation by plant extracts on silkworm larvae leading to remarkable larval growth leading to increased food consumption and cocoon weight.

Citronella, an aromatic grass belonging to the Poaceae family which gives essential oils upon steam distillation. It is an antifungal agent, anti-parasitic agent, a potent mosquito repellent and antibacterial agent [25]. In the current study silkworm larvae fed with mulberry leaves sprayed with citronella (*Cymbopogon citratus*) oil at 1, 0.5, 0.25 and 0.125% showed no significant changes between control and treated larvae in all biological traits, but only, the number of deposited eggs showed an increase for larvae fed with citronella oil at concentration of 0.5% with significant increase by 46.4% in comparison to control. This finding is in agreement with that finding by [16], who proved that fecundity increased when *B. mori* larvae treated with oils. Also, [26,27] recorded gradual increase in fecundity, hatchability [28] and [12] found increasing number of fertilized eggs of *Bombyx mori* larvae treated with *Aloe-vera*.

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