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Efficacy of essential oils as antiseptics on the productive characteristics of the mulberry silkworm *Bombyx mori* L.

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ABSTRACT: The study aimed to test the efficacy of three essential oils (basil *Ocimum basilicum* L., lemon *Citrus limon* (L.) Osbeck, and thyme *Thymus vulgaris* L.) as disinfectants, including their positive and negative effects, on the biological and productive parameters of the silkworm *Bombyx mori*. Biological parameters: basil oil treatment at 2000 ppm the highest significant 5^{th} instar larval weight and pupal weight were 2.226 g and 0.787 g. In addition, at the same concentration, recorded the lowest significant mortality percentage and 5^{th} instar larval duration, were 0.787 g and 5.09% respectively. On the other hand, lemon and thyme oils at 4000 ppm come in the second place the same parameters, compared to the control and the chemical disinfectant. While it is equal to the concentration of 8000 ppm for the oils tested in all biological parameters with the control and chemical disinfectant. Economical parameters: basil oil at 2000 ppm and lemon and thyme oils at 4000 ppm had the highest significance for cocoon weights, cocoon shell weight, and silk productivity, which were 1.203 g, 0.220 g, 2.34 cg for basil oil, 1.139 g, 0.210 g, 2.367 cg for lemon oil and 1.265 g, 0.216 g, 2.397 cg for thyme oil, compared with control and disinfectant chemical groups (0.993 g, 0.157 g, 1.49 cg and 0.991 g, 0.160 g, 1.68 cg, respectively). The highest significant difference of cocoon percentages was seen with basil oil at 2000 ppm, compared to the other treatments.

Keywords: Antiseptic; *Bombyx mori*; Nutritional enhancers; Essential oils.

1. INTRODUCTION

Bombyx mori, also known as the silkworm, is one of the most commercially important insects in the world. It feeds on the leaves of the mulberry plant. It is the most unique cultivated silkworm genus and generates the best silk fiber among other genera [1]. The silkworm is subjected to several types of diseases; especially, the larval stage is the most critical, and it is necessary to take appropriate care during this stage. Diseases affecting *B. mori* cause heavy crop losses in sericulture, leading to crop failure and low harvest rates [2]. Different varieties of food plants are found to be effective economically on important characteristics of silk production [3]. Disease-free rearing practices such as bed disinfection and larval body decontamination are known to boost productivity [4].

The use of natural products has increased in the past 20 years. Recent studies have demonstrated the use of plant essential oils as pesticides and good plant protectants [5, 6]. Commercial products derived from essential oils are being developed for a variety of animal and human uses [7]. Aromatherapy, phytotherapy, antibacterial and antifungal applications, and other pharmacological and therapeutic applications are a few uses of essential oils [8].

Some essential oils such as cinnamon, clove, and lavender oils exhibit inhibitory effects against *Bacillus cereus* and *Proteus vulgaris* (flacherie diseases), which are the associated pathogens infecting silkworms. In addition to its beneficial effects on biological and productive qualities, silk production has increased [9, 10].

Essential oils consist of several low-molecular-weight volatile compounds, terpenes, and phenolics that can be extracted from the major plant families Asteraceae, Lauraceae, Myrtaceae, and Lamiaceae. These compounds possess repellent, lethal, and growth-reduction effects on a variety of insects and microorganisms [11]. The chemical compounds found in essential oils have an effect on the growth of the silkworm. A high aldehyde concentration in essential oils has a better effect on silkworm development and cocoon quality, but a low acid concentration in the oils exerts a negative effect [12]. Furthermore, there is extensive research on the use of oils and plant extracts as food additives to improve the biological and productive characteristics of the silkworm [13].

This study was conducted to evaluate the effectiveness of some essential oils as a material disinfectant to protect silkworms from microbial infections, as well as to examine the extent of their safety on larvae and their effect on different productive traits.

2. MATERIALS AND METHODS

2.1. Source and rearing of *B. mori*

The silkworm *B. mori* L. egg box (Egyptian hybrid) was purchased from the Sericulture Research Department., Plant Protection Research Institute, Agriculture Research Center. Dokki, Giza. In the spring, *B. mori* larvae were fed on fresh mulberry leaves (*Morus alba* var. *indica*) in the Plant Protection Department of Fayoum University's laboratory, at 27°C ± 2°C and 75% ± 5% RH [14].

2.2. Source of essential oils

The essential oils of basil (*Ocimum basilicum* L., Lamiaceae), lemon (*Citrus limon* (L.) Osbeck, Rutaceae), and thyme (*Thymus vulgaris* L., Lamiaceae) were purchased from the local market in Fayoum Governorate, Egypt, produced by the organic company for natural oils, cold-pressed and pure natural. Oils were used in the present study for the bioassay experiments on the larvae of *B. mori* as disinfectants and nutritional enhancers.

A chemical bed disinfectant consisting of salicylic acid, paraformaldehyde, benzoic acid, and slaked lime was obtained from the Seric. Res. Dept., Plant Protec. Res. Inst, Agric. Res. Center. Dokki, Giza.

2.3. Bioassay technique

First, the 2nd instar larvae of *B. mori* were divided into three treatment groups (in addition to the control and control sprinkled with the chemical disinfectant). Each treatment was separated into three concentrations, with three replicates for each concentration (each of 50 larvae). Each replicate was reared in a 30 × 15 × 4 cm carton tray.

The larvae in each replicate were fed on treated or untreated mulberry leaves. The treated mulberry leaves were washed with water and immersed in three aqueous serial concentrations of 2000, 4000, and 8000 ppm of each essential oil containing one to three drops of Triton X 100 as the emulsifier. The untreated leaves of the control group were dipped in distilled water containing one to three drops of Triton X-100 for 1 min. In addition, the untreated leaves in the control group with the chemical disinfectant were dipped in distilled water alone.

The leaves were left to dry at room temperature before introducing to the larvae. The larvae are fed four times a day; the leaves treated with essential oils were introduced once a day to the silkworm larvae. On the other hand, in the control disinfectant treatment, the larvae were also sprinkled once a day, this is done daily from the 2nd instar larvae until cocoon formation. Serial concentrations of essential oils were prepared fresh for each treatment. In spring, the entire experiment was conducted twice in a row and the measurements averages of the two experiments were taken.

2.4. Biological parameters

The larval duration was calculated at the end of the 5th instar larvae. The larval mortality percentage was calculated (for the 5th instars) as follows:

Mortality % = Number of dead larvae / total number of larvae × 100

For recording the pupal weights, on the 7th day from cocoon spinning, the cocoons were carefully opened (using a fine cutter) and their pupae and cocoon shells were weighed after cleaning the exuviate to obtain pupal and cocoon shell weights.

2.5. Economical parameters

The economical parameters included cocoon weight, cocoon shell weight, cocooning percentage, cocoon shell ratio, and silk productivity. Cocooning percentages were computed as described previously [15], as follows:

Cocooning percentages = Number of cocoons formed / Total number of larvae kept × 100

The cocoon shell ratio was calculated using the following formula [16]:

Cocoon shell ratio (%) = Fresh cocoon shell weight / Fresh cocoon weight × 100

Silk productivity was determined using the following formula [17]:

Silk productivity per day (cg/day) = Cocoon shell weight / Fifth instar duration (day) × 100

Where, cg = centigram

2.6. Statistical analysis

The results of the bioassays were analyzed using the Duncan test at the 0.05 probability level to determine the least significant differences. The mean ± standard errors (SEs) are represented for each value using the SPSS program software [18].

3. RESULTS

3.1. Biological parameters

Several studies on the influence and efficiency of essential oils on a variety of pests and diseases have been conducted in recent years. Renewable and distinct antibacterial, antifungal, and antiparasitic substances are derived from the biological and structural diversity of their constituents [19-21].

In the present study, we focused on three serial concentrations of the three essential oils of basil (*O. basilium*), lemon (*C. limon*), and thyme (*T. vulgaris*) as preventive agents to protect the silkworm *B. mori* from microbial infections. We also investigated the effect of these concentrations on the biological and productive characteristics of the silkworm.

The biological parameters (5th larval weight, 5th larval duration, 5th larval mortality and pupal weight) of the silkworm were recorded (Table 1 & Fig. 1). The highest significant 5th instar larval weight was 2.226 g with basil oil treatment at 2000 ppm, showing a considerable significant difference from that in the control and control disinfectant groups, where the larval weights were 1.995 and 1.993 g, respectively. The highest significant weights with lemon and thyme oil treatments ranged from 2.000 to 2.096 g at 2000 and 4000 ppm, respectively, whereas the lowest 5th instar larval weight was 1.859 g at 8000 ppm of basil essential oil concentration.

Table 1. Means ± standard error (P≤0.05) of biological parameters for three essential oils efficiency on the 5th larval instar of silkworm *Bombyx mori.*

Materials	Conc. (ppm)	5th larval weight (g)	5th larval duration (day)	5th larval mortality %	Pupal weight (g)
Control		$1.995^{bc} \pm 0.39$	$10.500^{ab} \pm 0.185$	$8.073^{ab} \pm 0.307$	$0.701^{cd} \pm 0.005$
Control + chemical disinfectant		$1.993^{bc} \pm 0.39$	$9.500^{c} \pm 0.185$	$8.063^{ab} \pm 0.307$	$0.703^{cd} \pm 0.005$
Basil *Ocimum basilium*	2000	$2.226^{a} \pm 0.39$	$9.400^{c} \pm 0.185$	$5.090^{e} \pm 0.307$	$0.787^{a} \pm 0.005$
	4000	$1.996^{bc} \pm 0.39$	$10.100^{b} \pm 0.185$	$7.203^{bc} \pm 0.307$	$0.753^{b} \pm 0.005$
	8000	$1.859^{d} \pm 0.39$	$10.800^{a} \pm 0.185$	$8.213^{a} \pm 0.307$	$0.705^{c} \pm 0.005$
Lemon *Citrus limon*	2000	$2.000^{bc} \pm 0.39$	$9.300^{c} \pm 0.185$	$6.300^{cd} \pm 0.307$	$0.700^{cd} \pm 0.005$
	4000	$2.096^{b} \pm 0.39$	$9.200^{c} \pm 0.185$	$5.613^{de} \pm 0.307$	$0.775^{a} \pm 0.005$
	8000	$1.997^{bc} \pm 0.39$	$10.200^{b} \pm 0.185$	$8.260^{a} \pm 0.307$	$0.688^{d} \pm 0.005$
Thyme *Thymus vulgaris*	2000	$2.026^{bc} \pm 0.39$	$10.067^{b} \pm 0.185$	$7.220^{bc} \pm 0.307$	$0.711^{c} \pm 0.005$
	4000	$2.001^{bc} \pm 0.39$	$9.100^{c} \pm 0.185$	$6.093^{d} \pm 0.307$	$0.778^{a} \pm 0.005$
	8000	$1.900^{cd} \pm 0.39$	$10.300^{ab} \pm 0.185$	$8.950^{a} \pm 0.307$	$.698^{cd} \pm 0.005$
Duncan test ″F-Test″		5.87**	9.90**	16.64**	60.97**

(a,b,c,d) letters refer the means significant with the same column. ** refer to significant of ″F-Test″ with the same column.

The lowest 5th instar larval duration of 9.1 and 9.2 days was observed for larvae fed on leaves treated with 4000 ppm concentration of thyme and lemon oils, respectively, followed by larvae fed on leaves treated with 2000 ppm of basil oil (9.4 days), whereas the highest larval duration of 10.8 days was observed at 8000 ppm of basil oil. Prolongation in the duration of the 5th larval stage was observed with an increase in the concentration of all oils, where the concentration of 8000 ppm had the highest duration of the 5th larval stage among all oils. The duration of the 5th larval stage was 9.5 and 10.5 days in the control and control + disinfectant groups, respectively.

The mortality percentage was found to increase at high oil concentrations. Highly significant 5th larval mortality rates were 8.950%, 8.260%, and 8.213% under treatment with thyme, lemon, and basil essential oils at 8000 ppm, respectively. Moreover, the larval mortality rates in the control and control + disinfectant groups were 8.073% and 8.063%, respectively, which were not significant between high concentrations. The lowest mortality rate of 5.090% was recorded in larvae fed on leaves treated with basil oil at a concentration of 2000 ppm.

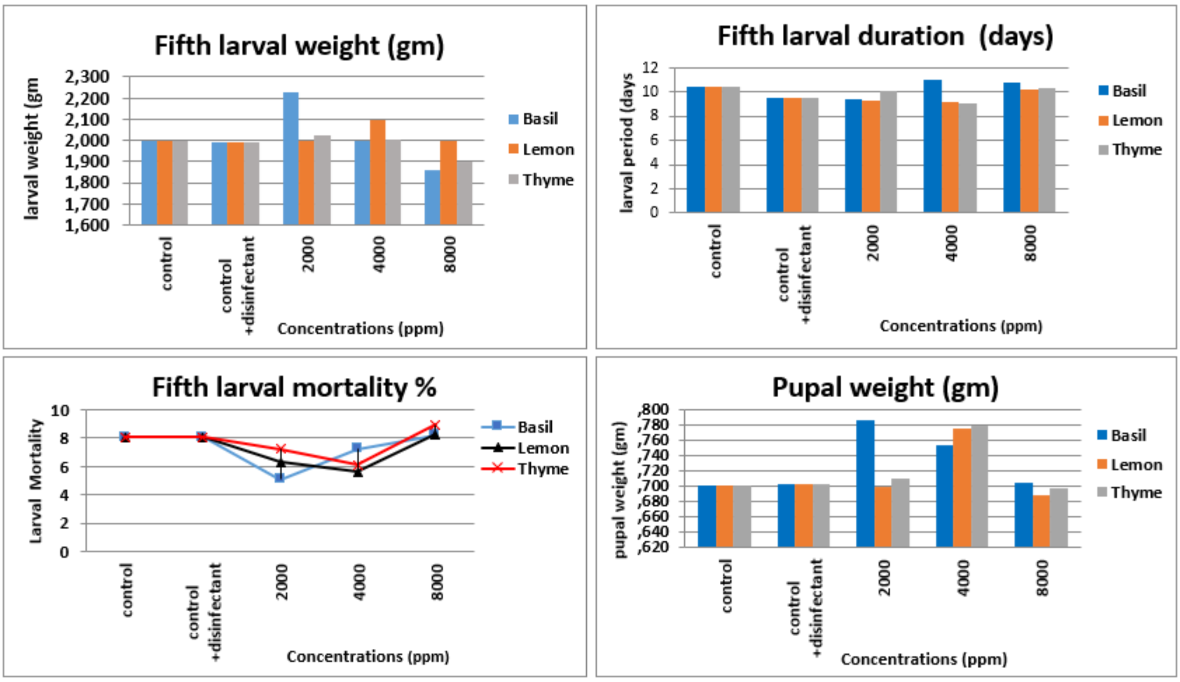

Figure 1. Means of biological parameters for three essential oils efficiency on the 5th larvae instar of silkworm *Bombyx mori*.

There was no significant difference in mortality between larvae fed on leaves treated with high concentrations of all essential oils and larvae fed on untreated leaves. These mortality results indicated the possibility of using low concentrations of essential oils. Basil oil was found to be a safe disinfectant for the silkworm larvae as it resulted in lower mortality than that chemical disinfectants.

Under all treatments of essential plant oils, highly significant pupal weights of 0.787, 0.778, and 0.775 g were observed with basil oil at 2000 and 4000 ppm and thyme and lemon oils, respectively. The lowest pupal weight of 0.698 g was recorded with thyme oil treatment at 8000 ppm. However, the mean weight of the pupa in the control group was 0.701 g.

3.2. Economical parameters

Regarding the economical characteristics of the silkworm *B. mori* (Table 2 & Fig. 2), the highest significant cocoon weights of 1.265 and 1.203 g were observed with thyme and basil oils at 4000 and 2000 ppm, respectively, showing a considerable significant difference from that (0.993 g) in the control group. The lowest cocoon weight of 0.978 g was observed at 8000 ppm of basil oil.

The lowest cocoon percentage of 88.457% was observed with thyme oil treatment at 8000 ppm, and the maximum significant cocoon percentage was 95.503% observed with basil oil treatment at 2000 ppm (Table 2 and Fig. 2). However, the cocoon percentages in the two control groups were 92.073% and 91.160%, respectively.

Cocoon shell weights under treatment with all essential oils appeared to be significantly different, with the highest weight of 0.220 g recorded at 2000 ppm of basil oil, followed by 0.216 and 0.210 g with thyme and lemon oil treatments at 4000 ppm. Compared among all oils, the cocoon shell weight in the two control groups (0.157 and 0.160 g) appeared to be less significant.

Table 2. Means ± standard error (P≤0.05) of economical parameters for three essential oils efficiency on the silkworm *Bombyx mori*.

Materials	Conc. (ppm)	Cocoon weight (g)	Cocoon shell weight (g)	Cocooning percentages %	Cocoon shell ratio %	Silk productivity cg/day
Control		$0.993^{b} \pm 0.057$	$0.157^{c} \pm 0.005$	$92.073^{abcd} \pm 1.229$	$15.807^{a} \pm 0.830$	$1.49^{cd} \pm 0.065$
Control + chemical disinfectant		$0.991^{b} \pm 0.057$	$0.160^{c} \pm 0.005$	$91.160^{bcd} \pm 1.229$	$16.203^{a} \pm 0.830$	$1.68^{c} \pm 0.065$
Basil, *Ocimum basilium*	2000	$1.203^{a} \pm 0.057$	$0.220^{a} \pm 0.005$	$95.503^{a} \pm 1.229$	$17.953^{a} \pm 0.830$	$2.34^{a} \pm 0.065$
	4000	$1.001^{b} \pm 0.057$	$0.163^{c} \pm 0.005$	$90.173^{bcd} \pm 1.229$	$16.397^{a} \pm 0.830$	$1.613^{c} \pm 0.065$
	8000	$0.978^{b} \pm 0.057$	$0.151^{c} \pm 0.005$	$88.050^{d} \pm 1.229$	$15.433^{a} \pm 0.830$	$1.393^{d} \pm 0.065$
Lemon *Citrus limon*	2000	$1.092^{ab} \pm 0.057$	$0.186^{b} \pm 0.005$	$90.110^{bcd} \pm 1.229$	$17.027^{a} \pm 0.830$	$2.00^{b} \pm 0.065$
	4000	$1.139^{ab} \pm 0.057$	$0.210^{a} \pm 0.005$	$93.027^{ab} \pm 1.229$	$18.133^{a} \pm 0.830$	$2.367^{a} \pm 0.065$
	8000	$1.000^{b} \pm 0.057$	$0.167^{c} \pm 0.005$	$89.050^{bcd} \pm 1.229$	$16.650^{a} \pm 0.830$	$1.63^{c} \pm 0.065$
Thyme *Thymus vulgaris*	2000	$1.119^{ab} \pm 0.057$	$0.190^{b} \pm 0.005$	$91.960^{abcd} \pm 1.229$	$17.140^{a} \pm 0.830$	$1.883^{b} \pm 0.065$
	4000	$1.265^{a} \pm 0.057$	$0.216^{a} \pm 0.005$	$92.397^{abc} \pm 1.229$	$16.133^{a} \pm 0.830$	$2.397^{a} \pm 0.065$
	8000	$1.000^{b} \pm 0.057$	$0.162^{c} \pm 0.005$	$88.457^{cd} \pm 1.229$	$16.190^{a} \pm 0.830$	$1.57^{cd} \pm 0.065$
Duncan test ″F-Test″		3.056*	23.90**	3.23**	1.047	32.67**

(a,b,c,d) letters refer the means significant with the same column. ** refer to significant of ″F-Test″ with the same column.

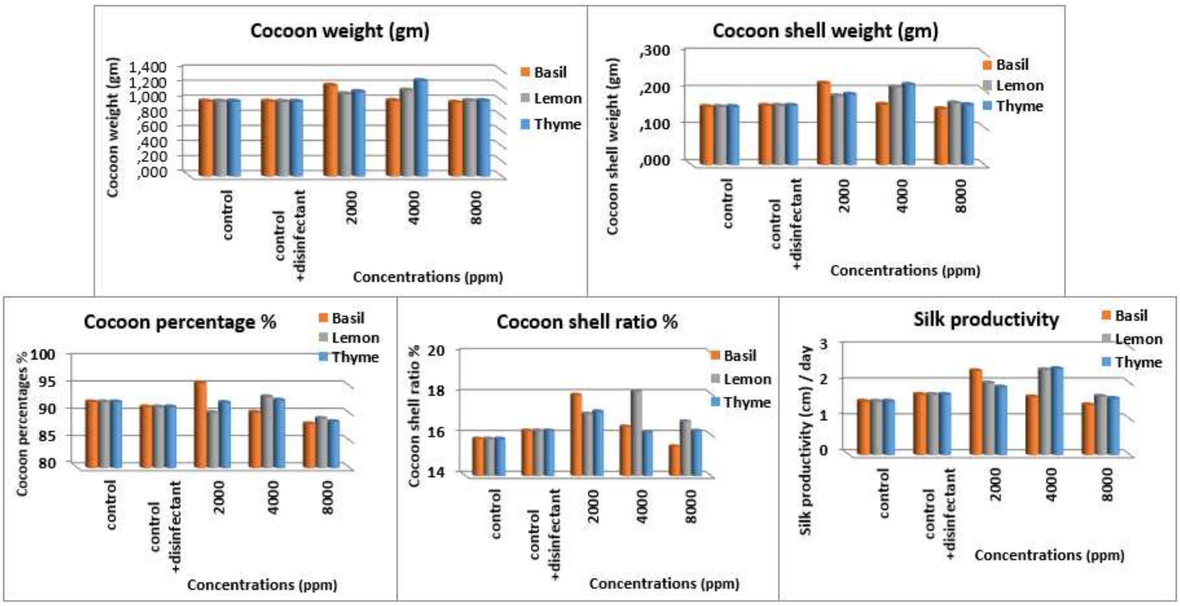

Figure 2. Means of economical parameters for three essential oils efficiency on the silkworm *Bombyx mori*.

Among treatments with the three essential oils, there were no significant differences in the cocoon shell ratio. However, lemon oil treatment resulted in a greater cocoon shell ratio of 18.13% at 8000 ppm compared to 15.8% in the control group.

The feeding efficiency of sericulture is generally determined by the production efficiency of cocoon shells. It was obvious that larvae fed on treated mulberry leaves dipped in the essential oils at various concentrations showed enhanced cocoon shell formation efficiency with all treatments and at higher rates than larvae fed on untreated mulberry leaves (control).

The silk productivity of larvae fed on oil-treated mulberry leaves was higher than that of larvae fed on untreated mulberry leaves, with highly significant differences of 2.397%, 2.367%, and 2.34% under treatment with thyme and lemon oils at 4000 ppm and basil oil at 2000 ppm, respectively, compared to 1.49% and 1.68% in the two control groups.

4. DISCUSSION

To ensure successful rearing and higher cocoons production, silkworm larvae must be protected from infection by their associated microorganisms. Diseases appear clearly on the larvae in the 4th and 5th instars. As a result, we set out to protect the larvae by using oils tested since the beginning of the second instar larvae. The obtained results showed that the oils of basil, lemon, and thyme and reduced significantly the mortality of the 5th instar larvae than the control and chemical disinfectant.

The essential oil of *Ocimum sanctum* is a broad fungitoxicant, with 1.0 µl/ml fumigation reducing the number of *Aspergillus flavus* isolates up to 74.01% [22]. Basil oil contains bioactive chemicals such as linalool (eugenol), methyl cinnamate, and estragole, which have been confirmed as antimicrobial activity [23, 24]. Moreover, was observed high antibacterial activity of essential oils *Citrus limon*, *Ocimum basilicum* and *Thymus vulgaris* [25].

In the present study, the cocoon percentage was increased by 95.5% in the larvae of *B. mori* treated with basil oil at 2000 ppm compared to 92.073% & 91.16% for control and chemical disinfectant respectively, which is consistent with a previous study [26], where the highest cocooning percentages for infected larvae (*Bacillus thuringiensis* and *Beauveria bassiana*) treated with 2% basil leaf extract were 97.3% and 94.0% respectively, compared to 92.0% in the control.

In respect of silk productivity, the oils tested (basil, thyme, and lemon) outperformed the control and chemical disinfection. Another previous study [27] reported that *Ocimum sanctum* is highly efficient against bacterial flacherie and *Staphylococcus* sp., *Bacillus* sp., and *Klebsiella cloacae*, which are associated with silkworm larvae, and can be used to control microbial infections during silkworm rearing and improve silk productivity.

Our results are also consistent with those which subjected *B. mori* larvae to artificial infestation with the conidial solution of *B. bassiana* and *B. thuringiensis* and treated them with basil oil concentrations [28]. Compared with control larvae infected with *B. bassiana* and *B. thuringiensis*, treatment with basil leaf extract increased the larval weight, pupal weight, cocoon weight, and cocoon shell weight and reduced the larval mortality rate. Consequently, the spread of bacterial and fungal infections was slowed. Furthermore, the biological and technological characteristics were enhanced, indicating that basil essential oil can be used in sericulture to improve the quality and quantity of silkworm cocoons.

Our results also correspond with those which found that larval and pupal weights were increased significantly when fed with thyme extract [29]. On the other hand, with an increase in the oil concentrations, we detected decreases in larval and pupal weights, addition increases in mortality and larval duration, which are similar findings to those reported by Dewer and Mona [30].

5. CONCLUSIONS

The essential oils examined in this study may be used as disinfectants for silkworms at low and medium concentrations (2000 ppm & 4000 ppm) to protect them from their associated microbial infections.

Moreover, these oils had no negative impacts on the biological qualities of the larvae. The oil concentrations used in this study resulted in considerable and desirable increases in cocoon weight and silk productivity.

Therefore, these essential oils are important and safe sources of antiseptics for the silkworm *B. mori*, as well as for improving silk productivity, especially basil oil followed by thyme and lemon oils**.**

We recommend using basil essential oil at 2000 ppm concentration or 4000 ppm for lemon and thyme oils as an antiseptic and to increase silk productivity during rearing of the silkworm *B. mori*.

Authors' Contributions: SP: designed the experiment chose the materials used in it, and prepared the tested concentrations. Collect the results in tables, analyzed them statistically, prepared drawings, interpreted data, wrote the manuscript and reviewed it. NS: carried out the experiment design on the larvae and recorded various outcomes and measurements. All authors read and approved the final manuscript.

Conflict of Interest: The authors declare no conflict of interest.

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