Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

Journal of Entomology and Zoology Studies

ISSN 2320-7078
JEZS 2014; 2 (2): 81-86

Received: 22-02-2014
Accepted: 11-03-2014

Youssef Dewer [1,2]
[1]*Department of Biological Chemistry and Crop Protection, Rothamsted Research, Harpenden, United Kingdom.*

[2]*Bioassay Department, Central Agricultural Pesticide Laboratory, Egypt*

Mona M. Mahmoud
Sericulture Department, Plant Protection Research Institute, Plant Protection Research Station, Agricultural Research Centre, Sabahia Research station, Baccous, P.O. Box 21616, Alexandria, Egypt

Correspondence:
Youssef Dewer
Department of Biological Chemistry and Crop Protection,
Rothamsted Research, Harpenden,
Herts AL5 2JQ, United Kingdom,
Tel: +44 (0) 1582 763133 Ext. 2706,
Email: dewer72@yahoo.com

Effectiveness and safety of some essential oils of aromatic plants on the growth and silk production of the silkworm *Bombyx mori* L.

Youssef Dewer* and Mona M. Mahmoud

ABSTRACT

There are insect pests of mulberry trees (*Morus alba*), that need to be controlled to below economic thresholds. Conventional insecticides control them but can have adverse effects on the development and silk production of the mulberry silkworm *Bombyx mori* L. This experiment evaluated the effect of the essential oils (EOs) of fennel (*Foeniculum vulgare*) and caraway (*Carum carvi*), against larval instars of silkworm by using a leaf-dipping bioassay. The tested EOs had no adverse effects on the growth rate and silk production of the larvae except at the higher concentrations at which these were slightly decreased in the later development stages. This experiment is one of the first attempts to understand the key role of EOs in regulating insect growth and development although further studies at physiological levels are required. In conclusion, these findings could contribute to the IPM of insect pests on mulberry without adversely affecting the silkworm-breeding industry.

Keywords: *Bombyx mori*, *Morus alba*, Essential oil (EOs), bioinsecticides, *Carum carvi, Foeniculum vulgare*.

1. Introduction

Production of mulberry leaves plays a key role in the sustainability of silk industry as the silkworm feeds only on these leaves [1]. The silkworm is an important economic insect which converts mulberry-leaf proteins into the valuable silk proteins. The economy of the sericulture industry depends not only on a high quantity but also on the quality of the mulberry leave [2]. Pests such as insects, together with bacteria and fungi, play an important role in agriculture, causing problems to crops. Mulberry foliage is vulnerable to various insect pests e.g. mealy bugs, armored scale insects, whiteflies and beetles, where the pests not only reduce the yield but also alter the biochemical components in mulberry leaves which become nutritionally inferior. Indeed the insecticides applied to control mulberry pests have a greater impact on the silkworm itself. Generally, insecticide compounds are not advisable for the mulberry ecosystem due to their residual toxicity and deleterious effect on the silkworm rearing [3]. Therefore, we have tested insecticidal compounds of plant origin that may have much less effect on the physiology and growth of silkworm.

Essential oils (EOs) are defined as any volatile oil(s) that have strong aromatic components and that give distinctive odour, flavour or scent to a plant. These are the by-products of plant metabolism and are commonly referred to as volatile plant secondary metabolites. The aromatic characteristics of EOs provide various functions for the plants including attracting or repelling insects, protecting themselves from heat or cold and utilizing chemical constituents in the oil as defence materials [4]. In the search for alternatives to conventional insecticides, EOs extracted from aromatic plants have for decades received much interest as potential bioactive agents (Burt, 2004). Plant EOs show wide and varied bioactivities against both agricultural pests and medically important insect species, ranging from toxicity with ovicidal, larvicidal, pupicidal and adulticidal activities to sub-lethal effects including oviposition deterrence, antifeedant activity and repellent actions; they may also affect biological parameters such as growth rate, life span and reproduction [6,7,8,9,10]. The aim of the present study was to identify the safety and efficacy of EOs from fennel and caraway on the productivity and development of silkworms.

2. Materials and Methods

2.1. Insect culture and rearing conditions

Silkworm eggs of a local hybrid were obtained from the Sericulture Research Department, Plant Protection Research Institute (PPRI), Agricultural Research Center, Giza, Egypt. Larvae were reared in the laboratory at 25 ± 1.5 ºC and 75 ± 5 % relative humidity [11]. The 5th larval instar was employed in our experiments. The newly hatched larvae were fed on fresh clean mulberry leaves (variety Romi) until the 5th instar, which stage was used in our bioassays. For spinning purposes, mature larvae were transferred to mounting frames for cocoon building.

2.2. Preparation of essential oils

Essential oils were derived from two species of Mediterranean plants: fennel (*Foeniculum vulgare*) and caraway (*Carum carvi*). The EOs were extracted from dried ripe seeds (500 g) collected from the local market in Egypt. Seeds were extracted through hydro-distillation using a Clevenger-type apparatus for 4 h under vacuum [12]. The extracted EOs were dried over anhydrous sodium sulphate and stored at 4°C in glass vials. Series of aqueous concentrations of each EO were prepared with Triton X-100 as the surfactant at a rate of 0.1% (v/v).

2.3. Insect Bioassay

A leaf-dipping bioassay method was adapted to evaluate the efficacy of the two plant EOs on the productivity and development of the 5th instar of the silkworm. A series of dilutions of 1, 2, 4, 6 and 8% of the extracted oils were used in the bioassays. The dilutions were prepared from stock solutions of 50% by using distilled water. Fresh dilutions were made on the day of each trial, and each fresh dilution series was used to test the silkworm in a single trial. Mulberry leaves were thoroughly washed with water and then dipped into the test solution for around 1 min. Control leaves were immersed in distilled water with 0.1% Triton X-100. Both treated and control leaves were left to dry at room temperature. Mulberry leaves treated with the EOs were fed to the silkworm larvae (50 larvae/tray x 3 replicates x 10 treatments) up to cocoon formation. Mortality and survival were determined after 24 h of each exposure and the numbers of dead larvae were counted cumulatively. Larvae were considered dead if they did not move when prodded with a fine brush. Observations were also recorded on the cocoon parameters *viz.*, cocoon weight, pupae weight and shell weight.

2.4. Experimental Measurements

The larval duration and mortality were recorded. Also, fresh weights of mature 5th-instar larvae, pupae, fresh cocoons and cocoon shells were recorded. Prior to spinning cocoons, a sample of five mature experimented larvae was dissected and the silk glands were drawn and weighed.

Randomly selected female moths were allowed to mate with male ones; each couple was kept in its perforated paper bag. After oviposition, the number of deposited and fertilized eggs per female moth was counted and recorded. The percentage of fertilized eggs was calculated using the following formula:

Fertilized eggs (%) = (Nº of deposited eggs – Nº non fertilized eggs) X 100 ÷ Nº of deposited eggs

2.5. Statistical analysis

The laboratory bioassays were conducted in a completely randomized design with three replications. The means were separated on the basis of least significant differences (LSD) at the 0.05 probability level. The data as percentages were analysed using methods described by Snedecor [13]. Each value represents the mean ± standard deviation (SD).

3. Results and discussion

Essential-oil extracts of plants are promising alternative natural products for the control of many insect pests [10]. These extracts facilitate the handling and its application, besides they could be cheaper than chemical control. Examining the effect of differing concentrations of fennel and caraway oils on larval mortality and weight of the silkworm (Table 1 and Figures 1; 2A), the larval mortality percentage increased with increasing oil concentrations. The percentage mortality was 15.4% and 69.2% for fennel oil for the concentrations of 1% and 8% respectively. The corresponding mortalities for caraway were 18.5% and 76.9% whereas the control gave 16.9% (Table 1 and Figure 1). These findings are in accord with those of Pitasawat et al (2007), who found that the larvicidal activities of the essential oils (EOs) of fennel and caraway against mosquito larvae *A. aegypti* under laboratory conditions are revealed to the chemical composition of the oils. Whereas carvone was a main constituent representing 32.7% of caraway *oil*, fennel oil contained anethole 85.6% as the major substance.

Furthermore, larvae fed with the treated leaves showed significant differences in their weights during the fifth instar as compared with the control (Table 1 and Figure 2A). The lowest larval weight of 1.919 g was recorded for larvae fed on leaves treated with caraway oil at 8% with significantly different to the untreated, whereas the highest larval weights of 3.064 g, 3.058g and 2.957 g were recorded when larvae fed on leaves treated with 1% of fennel and 1% and 2% of caraway oil, respectively. These results are in agreement with the findings of Shivkumar *et al*. (1995), who observed that 40 mg and 60 mg of prickly chaff flower, *Achyranthes aspera* essential oils sprayed on mulberry leaves and fed to 3, 4, 5 and 6-day old fifth instar larvae reduced larval weight significantly.

Also, in our work the essential oils exerted significant influence on the whole larval duration (Table 1). The lowest duration (31 days) was seen for larvae fed on leaves treated with fennel and caraway oil at 1%, whereas the highest larval duration (34 days) was recorded at 8% concentration for both essential oils in comparison with the control (32 days). The larval period was prolonged for 1-2 days by the different concentrations of oils. A similar trend was observed by Thangavelu and Singh (1994) in Uzi fly, *Bleparipa zebina* Walker.

The mean weight of the silk-glands was increased to 0.592 g and 0.574 g at the end of the 5th larval instar fed on mulberry leaves treated with 1% fennel and caraway oils, respectively (Table 1 and Figure 2B). In contrast, feeding the silkworm larvae on mulberry leaves treated with 8% caraway oil significantly decreased the weight of the silk gland (0.234 g) in comparison with control (0.559 g). Fournier (1979) reported that silk-gland growth reflects the quality and quantity of silk production by silkworms. Our findings are in agreement with those of Hiratsuka (1920) who reported that the final instar in silkworms is characterized by active growth, especially in silk-gland weight. Also, an earlier paper reported that the weight of the silk-gland is positively correlated with the weight of the larval body [19].

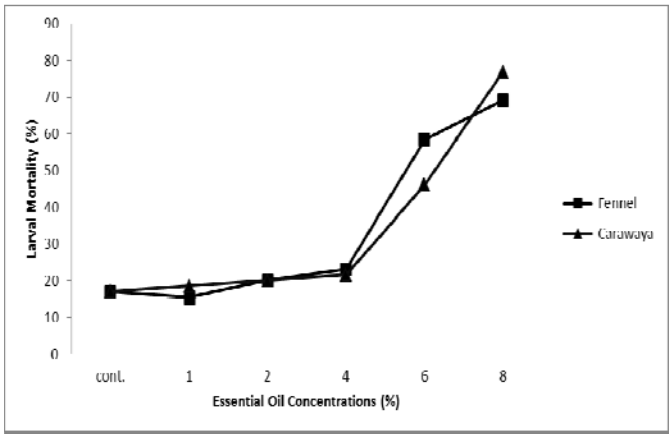

Figure (1): The effect of fennel and caraway essential oils on larval morality percentage of silkworm.

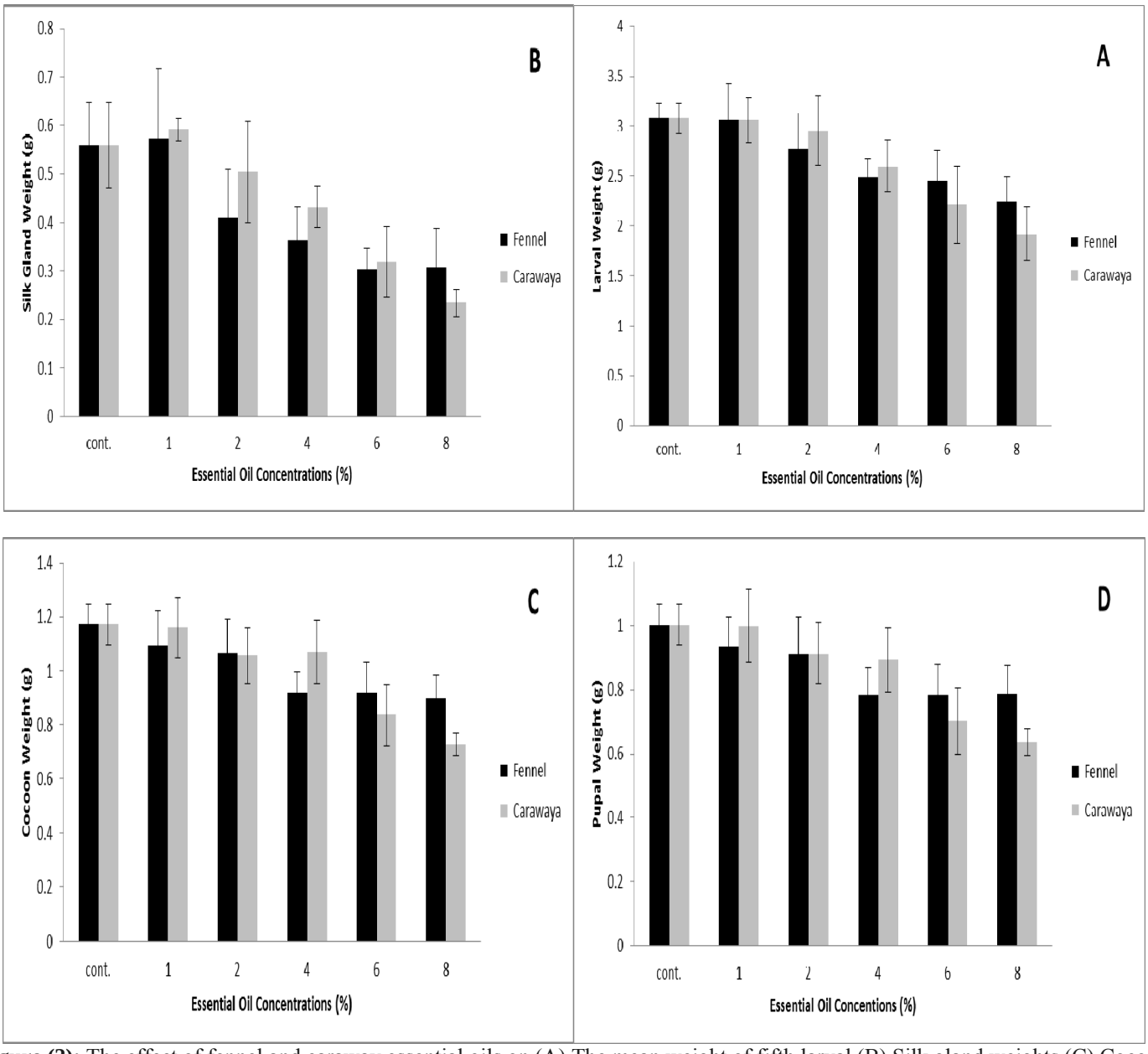

Figure (2): The effect of fennel and caraway essential oils on (A) The mean weight of fifth larval (B) Silk gland weights (C) Cocoon weight and (D) Pupal weight of silkworm. Values are mean ± SD.

Table (1): Efficacy of different concentrations of fennel and caraway oil extracts on some biological parameters of the silkworm.

Treatments	Larval wt (g)	Larval duration (days)	Silk gland wt (g)	Larval Mortality (%)	Pupal wt (g)
Fennel Oil Conc. [%]					
1	$3.064^{a} \pm 0.362$	31	$0.574^{a} \pm 0.142$	15.38	$0.933^{ab} \pm 0.093$
2	$2.776^{bc} \pm 0.352$	32	$0.409^{bc} \pm 0.100$	20	$0.912^{b} \pm 0.114$
4	$2.488^{d} \pm 0.188$	32	$0.362^{cd} \pm 0.070$	23.08	$0.785^{c} \pm 0.085$
6	$2.451^{de} \pm 0.303$	33	$0.304^{de} \pm 0.044$	58.46	$0.785^{c} \pm 0.093$
8	$2.248^{e} \pm 0.243$	34	$0.307^{de} \pm 0.081$	69.23	$0.787^{c} \pm 0.089$
Caraway Oil Conc. [%]					
1	$3.058^{a} \pm 0.224$	31	$0.592^{a} \pm 0.023$	18.46	$0.998^{a} \pm 0.115$
2	$2.957^{ab} \pm 0.344$	32	$0.504^{ab} \pm 0.104$	20	$0.912^{b} \pm 0.095$
4	$2.597^{cd} \pm 0.260$	33	$0.432^{bc} \pm 0.042$	21.54	$0.893^{b} \pm 0.100$
6	$2.215^{e} \pm 0.390$	33	$0.318^{de} \pm 0.073$	46.15	$0.701^{d} \pm 0.103$
8	$1.919^{f} \pm 0.271$	34	$0.234^{e} \pm 0.028$	76.92	$0.635^{d} \pm 0.043$
Control	$3.077^{a} \pm 0.153$	32	$0.559^{a} \pm 0.088$	16.92	$1.001^{a} \pm 0.064$
L.S.D. "F-test"	18.643^{**}	--	10.890^{**}	--	16.395^{**}

Note: the data are expressed as mean ± standard deviation (n =3). Values with same letter differ non-significantly (P≤0.05).

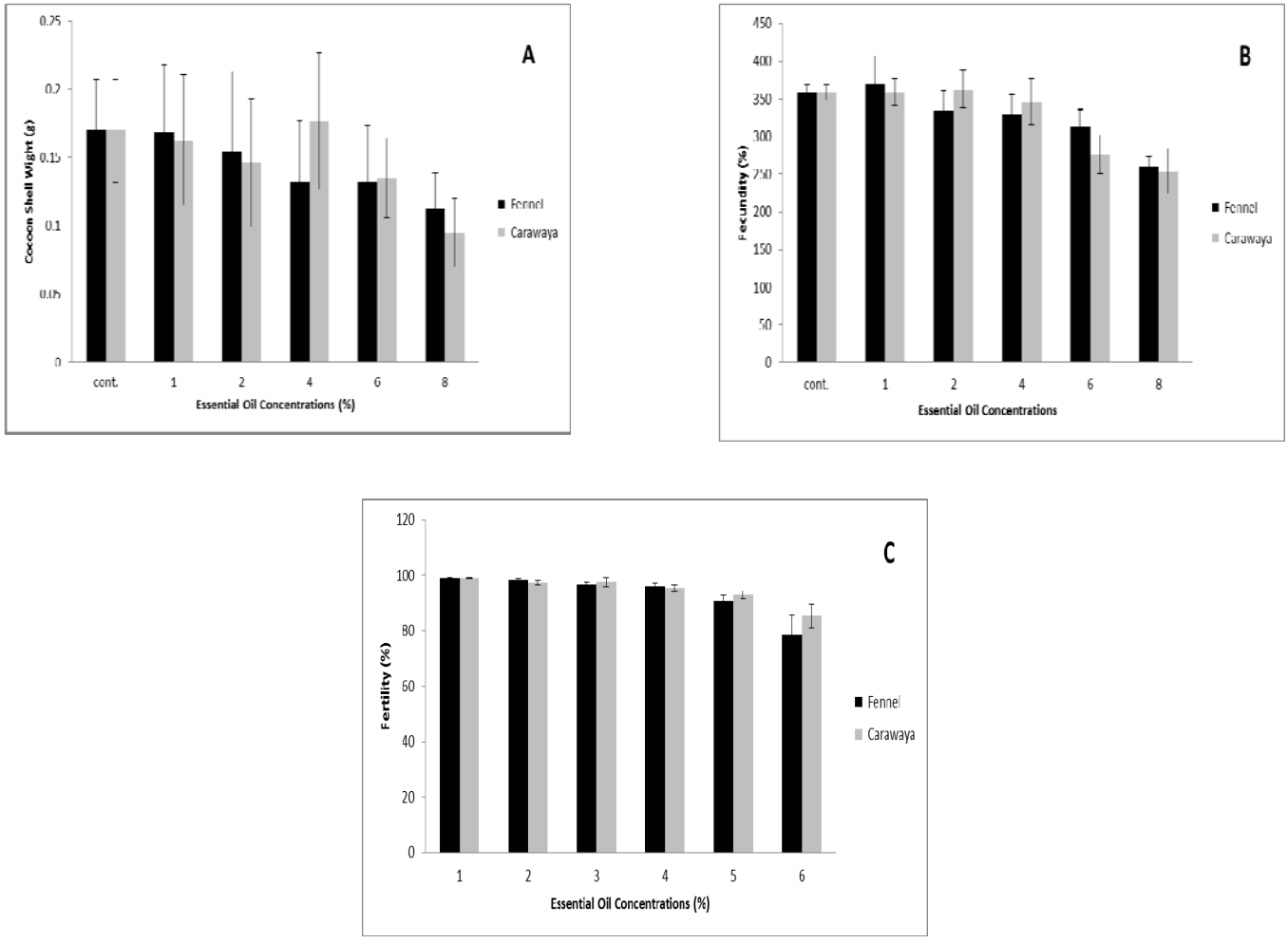

Figure (3): The effect of fennel and caraway essential oils on (A) Cocoon shell weight (B) Fecundity and (C) fertility of silkworm. Values are mean ± SD.

Among the treatments with the two oils, there were not significant differences in the weights of the cocoons formed by the 5th-instar larvae fed on the treated mulberry leaves (Table 2 and Figure 2C). Interestingly, the tested oils had no significant impact on the silk quantity in comparison with control (1.172 g), whereas the highest fresh cocoon weight (1.161 g) was recorded when larvae were fed on leaves treated with 1% caraway oil. Also, this study aimed to explore the possible effects of the selected essential oils on the pupal weight after treating the 5th-instar larvae using the leaf-dipping method. The pupal weight was not changed significantly by the different oil treatments except the caraway oil at 8% which gave a pupal weight of 0.635 g compared to the control of 1.001 g (Table 1 and Figure 2D); the highest pupal weights were observed using 1, 2, 4 and 6% of fennel oil at 0.933 g, 0.912 g, 0.785 g and 0.785 g, respectively, and 1, 2 and 4% of caraway oil which gave pupae weights of 0.998 g, 0.912 g and 0.893 g, respectively. In the light of the above mentioned findings, it seems that the tested concentrations of fennel and caraway essential oils did not have a negative impact on the cocoon and pupal weights. Moreover, the essential oils had positive effects on the weights of the cocoon shells, with caraway oil at 4% increasing the cocoon shell weights of the silkworms by about 0.177 g compared with to the control of 0.170 g (Table 2 and Figure 3A).

Table (2): Efficacy of different concentrations of fennel and caraway oil extracts on silk production of the silkworm.

Treatments	Cocoon wt (g)	Cocoon shell wt (g)	Fecundity value	Fertility (%)
Fennel Oil Conc. [%]				
1	1.093ab ± 0.127	0.169ab ± 0.049	370.4a ± 37.159	98.18^{a} ± 0.564
2	1.067^{b} ± 0.122	0.155^{ab} ± 0.058	335.6^{b} ± 24.835	96.86^{ab} ± 0.714
4	0.918^{c} ± 0.078	0.132^{bc} ± 0.045	330.4^{bc} ± 24.501	96.08^{ab} ± 1.258
6	0.918^{c} ± 0.114	0.132^{bc} ± 0.042	313.2c ± 23.123	90.84^{d} ±1.975
8	0.899^{c} ± 0.088	0.112^{cd} ± 0.027	261^{d} ± 13.076	78.50^{f} ± 6.959
Caraway Oil Conc. [%]				
1	1.161^{a} ± 0.111	0.163^{ab} ± 0.048	359.2^{a} ± 18.198	97.32^{ab} ± 0.735
2	1.058^{b} ± 0.102	0.146^{ab} ± 0.047	362.8^{a} ± 24.933	97.60^{ab} ± 1.606
4	1.070^{b} ± 0.116	0.177^{a} ± 0.050	346.2^{ab} ± 31.276	95.42^{bc} ± 1.254
6	0.837^{c} ± 0.114	0.135^{bc} ± 0.029	276.2^{d} ± 26.347	92.91^{cd} ± 1.360
8	0.730^{d} ± 0.042	0.095^{d} ± 0.025	254^{d} ± 29.656	85.50^{e} ± 4.235
Control	1.172^{a} ± 0.074	0.170^{ab} ± 0.038	359^{a} ± 9.772	98.94^{a} ± 0.236
L.S.D. "F-test"	18.985^{**}	3.542^{**}	14.387^{**}	27.814^{**}

Note: the data are expressed as mean ± standard deviation (n =3). Values with same letter differ non-significantly (P≤0.05).

Fecundity and fertility are the two main factors of cocoon production. Several factors affect the fecundity and fertility of silkworm races including aberrations in sex organs, faulty handling of the moths during mating and egg laying, defective preservation of cocoons and environmental stress during larval rearing and cocoon storage [20]. In this present study, there were no significant differences in the fecundity value and fertility percentage (Table 2 and Figures 3B; 3D) in larvae fed on leaves treated with the lowest concentrations of oils (1, 2 and 4%). The fecundity value (eggs/female) increased significantly at the lowest concentration of 1% and 2% for fennel and caraway oils which gave values of 370.4 and 362.8, respectively, compared to the control 359. Also, the fertility percentage was not affected significantly by using different concentrations of essential oils (Table 2 and Figure 3D). Only the concentration of 8% for fennel and caraway oils showed a significant effect in reducing fertility by 78.5% and 85.5%, respectively, compared to the control of 98.9%.

4. Conclusion

Biopesticides with plant origins have been given new importance in recent years for their use against several insect species. Our findings indicated that both tested essential oils fennel and caraway have no negative effects on the development and silk production of mulberry silkworm except at the highest concentration. Further studies the physiological and biochemistry changes of these oils on silkworm larvae would be useful to gain insight into the efficiency of a safe management process with no effects on beneficial insects.

5. Acknowledgments

Authors are grateful to Dr. Richard Bromilow and Dr. Keith Chamberlain (Department of Biological Chemistry and Crop Protection, Rothamsted Research, Harpenden, UK) for their great effort in revising the manuscript. Special thanks go to Dr. Soad Ramadan (Plant Protection Research Institute, ARC, Egypt) for her comments on an earlier draft. This work was supported by Central Agricultural pesticides Laboratory and Plant Protection Research Institute, Egyptian Agricultural Research Centre.

6. References

1. Tribhuwan S, Mathur SK. The morin factor in mulberry that attracted the *Bombyx mori* L. Indian Silk 1989; 28(5): 39-40.
2. Das PK, Vijaya Raghawan K, Jolly MS. Preliminary observation on the effect of feeding with washed and dusty mulberry leaves on the performance of four races of silkworm, *Bombyx mori* (L.) National seminar on silk research and development (Abstract) 1983; March 10-13, Bangalore. India p-111.
3. Yamamoto M, Toda M, Tanaka K, Sugita T, Sasaki S, Uneyama C, Morikawa K. Study on usage of pesticides

in various countries. Kokuritsu Iyakuhin Shokuhin Eisei Kenkyusho Hokoku 2007; 125, 92-100.
4. Mohan M, Haider SZ, Andola HC, Purohit VK. Essential oils as green pesticides: For sustainable agriculture. Research Journal of Pharmaceutical, Biological and Chemistry Sciences 2011; 2, 10–104.
5. Burt S. Essential oils: their antibacterial properties and potential applications in foods - a review, International Journal of Food Microbiology 2004; 94, 223-253.
6. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils - a review. Food Chemistry and Toxicology 2008; 46, 446–75.
7. Isman MB. Perspective botanical insecticides: For richer, for poorer. Pest Management Science 2008; 64, 8–11.
8. Tripathi AK, Upadhyay S, Bhuiyan M, Bhattachary PR. A review on prospects of essential oils as biopesticide in insect–pest management. Journal of Pharmacognosy and Phytotherapy 2009; 1, 52–63.
9. Ebadollahi A. Iranian Plant Essential oils as sources of natural insecticide agents - a review. International Journal of Biological Chemistry 2011; 5, 266–90.
10. Regnault–Roger C, Vincent C, Arnason JT. Essential oils in insect control: low–risk products in a high–stakes world. Annual Revision of Entomology 2012; 57, 405–24.
11. Krishnaswamy S. Improved methods of rearing young (Chawki) silkworms, Central Silk Board, Bangalore India 1973; p: 24.
12. Aroiee H, Mosapoor S, Karimzadeh H. Control of greenhouse whitefly (*Trialeurodes vaporariorum*) by thyme and peppermint. KMITL Science Journal 2005; 5, 511–514.
13. Snedecor GW. Statistical methods. 5th ed., lowa State University Press, lowa 1956; pp. 534.
14. Pitasawat B, Champakaew D, Choochote W, Jitpakdi A, Chaithong U, Kanjanapothi D, Rattanachanpichai E, Tippawangkosol P, Riyong D, Tuetun B, Chaiyasit D. Aromatic plant-derived essential oil: An alternative larvicide for mosquito control. Fitoterapia 2007; 78, 205–210.
15. Shivkumar GR, Anantha Raman KV, Venkata Rami Reddy KV, Magadum SB, Datta RK, Hussain SS. Effect of Phytoecdysteroids on larval and economic parameters of silk worm, *Bombyx mori* L. Indian Journal of Sericulture 1995; 34, 46-49.
16. Thangavelu K, Singh RN. Integrated pest management in tasar culture. Annals Entomology 1994; 9, 59-65.
17. Fournier A. Quantitative data on the *Bombyx mori* L. silkworm: a review. Biochimie 1979; 61, 283-320.
18. Hiratsuka E. Researches on the nutrition of the silkworm. Bulletin Series Experimental Station in Tokyo 1920; 1, 275 - 315.
19. Ueda S, Kimura R, Suzuki K. Studies on the growth of the silkworm *Bombyx mori*. IV mutual relationship between the growth in the fifth instar larvae and productivity of silk substance and eggs. Bulletin Series Experimental Station Japan 1975; 26, 233-247.
20. Biram SNM, Tribhuwan S, Beera S. Occurrence of unfertilized eggs in the mulberry silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae). International Journal of industrial Entomology 2009; 18, 1 - 7.