

Biochemical Impacts of some Volatile Oils on the Eggs of Greater Wax Moth, *Galleria mellonella* L.

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ABSTRACT

The essential oils of chamomile; *Chamomilla recutita* L., rosemary; *Rosmarinus officinalis* L. and peppermint; *Mentha piperita* L. were tested under laboratory conditions for their ovicidal and biochemical activity against the eggs of greater wax moth, *Galleria mellonella* L. The incubation periods, hatching periods and hatching percentages of the eggs treated by the three oils using spraying technique were significantly retarded at the four concentrations 1%, 2%, 4% and 8%. Generally, the maximum ovicidal activity was recorded at 8% concentrations and the peppermint oil showed marked activity against the eggs of greater wax moth. The longest incubation period (15 days), the longest hatching period (6.67 days) and the highest inhibition of hatchability (5.07%) achieved with peppermint oil treatment, followed by rosemary oil (13.67 days, 5.33 days and 18.25%) then chamomile oil (9 days, 5.67 days and 32.45%), respectively. The chemical constituents of the three essential oils were analysed using gas chromatography (GC). Bisabolol oxide a (43.76 %) and bisabolol oxide b (16.11%) were the major components of chamomile oil whereas, camphor (23.56 %), 1,8-cineole (17.86 %) were for rosemary oil and menthol (47.38 %), menthone (29.99 %) for peppermint oil. Present study demonstrated disturbance in total soluble protein, total lipid contents and inhibition the activity of acid phosphatase and phenoloxidase.

Keywords: Essential oils - *Galleria mellonella* L - Biochemical response

INTRODUCTION

The greater wax moth (GWM), *Galleria mellonella* L., (Lepidoptera: *Pyralidae*), is an economically important pest of honey bee products and beekeeping equipments. Larval stages of the moth feed on the wax, pollen, honey and forming tunnels heavily lined with silken material, making removal by bees difficult. Feeding by larvae causes damage to comb and developing bees (Eischen *et al.*, 1986). Moreover, they have the potential to destroy or damage stored combs. Although wax moth infestations in strong colonies are effectively controlled by worker bees, great losses can occur in queenless colonies and in those exposed to pesticides, disease or the presence of parasitic mites (Romel *et al.*, 1992). Wax moth control strategies are divided into two general categories: apiary management and management of equipments and stored combs away from the colony where, equipments removed from the field may contain eggs or larvae that go undetected prior to storage or honey extraction, i.e technical, biological, physical and chemical methods. (Owayss and Abd-Elgayed, 2007). Despite using of chemicals seemed to be easy and effective, some precautions of safety and contamination of bee products are considered (Fraser, 1997). Moreover, the extensive use of the synthetic insecticides lead to the biological imbalance due to the destruction of beneficial species beside the destruction of pollinating insects such as honey bees. Recently, plant derivatives have been considered as safe source of pesticides with ignorable side effects on the environment (Balandrin *et al.* 1985).

Plants natural products and derivatives are an excellent substitute to synthetic pesticides as they constitute rich sources of bioactive chemicals. Natural products also reduce harmful impacts to human health and the environment; as they are biodegradable to non-toxic products and are found to be highly effective against insecticide resistant insect pests (Arnason *et al.* 1989, Kwon *et al.* 1996 and Koul *et al.* 2008). Most studies that have investigated the efficiency of insect control methods have mostly focused on immature stages or adult and have neglected the egg stage because they are small, or do not feed. Therefore, more studies targeting insect eggs are needed where, the pests would be removed before it has a chance to rise any damage.

The main objective of this work was to estimate the ovicidal activity and biochemical effects of chamomile, rosemary and peppermint essential oils on eggs of greater wax moth as alternative natural products to harmful chemical pesticides.

MATERIALS AND METHODS

Tested oils: Three volatile oils were investigated; chamomile oil (*Chamomilla recutita* L.), rosemary oil (*Rosmarinus officinalis* L.) and peppermint oil (*Mentha piperita* L.). The tested oils were purchased as pure oil from Medicinal and Aromatic Plants Research Department, Horticulture Research Institute, Agriculture Research Center at Dokki, Giza Governorate, Egypt.

Insect rearing technique: Naturally-infested wax combs with greater wax moth were obtained from the apiary of Plant Protection Research Institute, Agriculture Research Center. The infested wax then cut to cubes (feeding medium) and transferred to clean jars. Emerged adults were collected and kept inside egg laying cages provided with paper lids as egg laying substrate. Paper carrying eggs were collected and replaced by new one. Rearing and treatments were conducted under constant conditions $30 \pm 2^{\circ}\text{C}$ and $65 \pm 5\%$ R.H. El-barky *et al.* (2015).

Ovicidal activity of tested oils using spraying technique: Four serial concentrations (1, 2, 4 and 8%) for the tested oils were prepared from the stock solution by dilution with distilled water and tween 80 (as emulsifier). Treatment carried out using spraying technique where, egg-masses of GWM at the developmental age of (1- day- old) were collected from laboratory reared population to study their susceptibility to the tested oils. Eggs treated then transferred to Petri dishes and four replicates were run for each concentration and control (sprayed by distilled water and tween 80). Daily inspection for all treatments was performed. The incubation period, hatching period and hatching percentage were recorded.

Gas chromatography analysis (GC): Gas chromatography analysis of the essential oil samples was accomplished using gas chromatography instrument stands at the Medicinal and Aromatic Plants Research Department, Horticulture Research Institute, Agriculture Research Center at Dokki, Giza Governorate, Egypt with the following specifications. Dschrom 6200 Gas chromatograph equipped with a flame ionization detector Column: BPX-5, 5% phenyl (equiv.),

polysilkphenylenesiloxane 30m × 0.25 mm ID × 0.25µm film., sample size: 1 µl, temperature program ramp increase with a rate of 10 °C/min from 70 to 200 °C, detector temperature (FID): 280 °C, carrier gas: nitrogen, and flow rate: N₂ 30 ml / min; H₂ 30 ml / min; air 300 ml / min. Basic compounds of the essential oils were identified by matching their retention times with those of the authentic samples injected under the same conditions. The relative percentage of each compound was calculated from the area of the peak corresponding to each compound.

Biochemical studies: Egg-masses (0.2 gm weight per treatment) used for biochemical analysis were collected 6 days after sprayed by the tested volatile oils at 8% concentration plus control and kept at -20°C till analysis time. The frozen samples of eggs were homogenized in distilled water using a Teflon homogenizer. Homogenates were centrifuged at 5000 rpm for 10 min at 5°C. The supernatants were used directly for the biochemical analysis of total soluble protein and the activity of acid phosphatase and phenol oxidase whereas, eggs homogenate used for total lipid analysis.

Total soluble protein: Colorimetric determination of total soluble protein in supernatants of eggs homogenate was estimated as mentioned by Gornall *et al.* (1949).

Acid phosphatase (ACP): The activity of acid phosphatase was carried out the same method used by Powell and Smith (1954).

Total lipids: The total lipids in sample of homogenate *Galleria mellonella* eggs were estimated by Schmit (1964) method, using Kits from Diamond Diagnostics.

Phenoloxidase (PO): Phenoloxidase activity was estimated according to method of Ishaaya (1971) using catechol as the substrate.

Statistical analysis: : The statistical analysis was determined by using one way test (ANOVA), between concentrations of the tested oils and the ovicidal activity results were performed according to COHORT SOFTWARE (2005).

RESULTS

Ovicidal activity of the tested volatile oils against the eggs of greater wax moth Incubation period: The incubation period of 1-day-old eggs was significantly elongated as sprayed by the tested oils at different concentrations with the exception of effect of chamomile oil which show slight increase than the control. The longest incubation period (15 days) noticed in peppermint oil treatment at concentration of 8% compared to the untreated one (7.33days) Table (1).

Hatching period: Regarding data in Table (1) the hatching period of the treated eggs were significantly retarded after

exposing to the three oils. The four concentrations show gradual effects on the hatching period where the period increase as oil concentration increase. At 8% concentration peppermint oil, rosemary oil and chamomile oil caused elongation of hatching period from (2.33 days)for control to (6.67, 5.33 and 5.67 days) for the three oils, respectively.

Hatching percentages: The three tested oils induced highly significant decrease in the hatching percentage compared to control (93.94%). Generally, the maximum ovicidal activity observed at 8% concentration and the peppermint oil showed marked activity against the eggs of greater wax moth (5.07%) hatching percent followed by rosemary oil (18.25%) and chamomile oil (32.45) Table (1). Results in the current study showed that, the selected plant essential oils have significant ovicidal activity against *Galleria mellonella* L.

Gas chromatography analysis (GC) of the tested oils: Upon gas chromatography analysis of the tested essential oils, the main components and their percentages are listed in Table (2). Regarding the *Chamomilla recutita* L. oil, bisabolol oxide a (43.76 %), bisabolol oxide b (16.11%), bisabolene oxide a (8.86 %) and α-bisabolol (7.33 %) were the most prominent constituents Fig. (1). Whereas, the major components for *Rosmarinus officinalis* L. were camphor (23.56 %), 1,8-cineole (17.86 %), α-pinene (12.52 %), borneol (10.55 %) and bornyl acetate (8.64%) Fig. (2). The principle constituents of *Mentha piperita* L. were menthol (47.38 %), menthone (29.99 %), limonene (5.50 %), methyl acetate (4.85 %) and β - caryophyllene (3.06 %) Fig. (3).

Table 1. Effect of the tested volatile oils on the incubation period, hatching percentage and hatching period of the greater wax moth eggs using spraying technique.

Tested volatile oils	Concentration	Incubation period (days)	Hatching period (days)	Hatching %
Chamomile oil	1%	8.33 ^{bc}	3.33 ^{ef}	89.35 ^{ab}
	2%	8.67 ^{bc}	4.67 ^{cd}	70.32 ^c
	4%	8.67 ^{bc}	4.67 ^{bcd}	38.52 ^d
	8%	9.00 ^{bc}	5.67 ^{ab}	32.45 ^{de}
Rosemary oil	1%	9.67 ^b	3.67 ^{de}	73.04 ^{bc}
	2%	9.67 ^b	4.33 ^{cde}	67.81 ^c
	4%	10.00 ^b	4.67 ^{bcd}	23.14 ^{de}
	8%	13.67 ^a	5.33 ^{bc}	18.25 ^{ef}
Peppermint oil	1%	9.33 ^b	3.33 ^{ef}	73.76 ^{bc}
	2%	9.67 ^b	3.67 ^{de}	66.79 ^c
	4%	10.33 ^b	5.67 ^{ab}	17.68 ^{ef}
	8%	15.00 ^a	6.67 ^a	5.07 ^f
Control	0.00	7.33 ^c	2.33 ^f	93.94 ^a
LSD _{0.05}		1.86 ^{***}	1.07 ^{***}	16.83 ^{***}

Table 2. Major compounds (%) of chamomile, rosemary and peppermint essentials oils by Gas chromatography analysis.

No.	Chamomile oil		Rosemary oil		Peppermint oil	
	Compound	Area%	Compound	Area%	Compound	Area%
1	Bisabolol oxide a	43.76	Camphor	23.56	Menthol	47.38
2	Bisabolol oxide b	16.11	1,8-cineole	17.86	Menthone	29.99
3	Bisabolene oxide a	8.86	α-pinene	12.52	Limonene	5.50
4	α-Bisabolol	7.33	Borneol	10.55	Methyl acetate	4.85
5	β-Bisabolol	4.89	Bornyl acetate	8.64	β - caryophyllene	3.06
6	β-farnesene	2.53	β - caryophyllene	3.57	Iso-menthol	1.25
7	Trans-dicycloether	2.49	Limonene	3.06	α-pinene	1.14
8	Isosafaurinone	1.89	β - pinene	2.95	1,8-cineole	0.77
9	α-Eudesmol	1.89	α-terpineole	2.04	β -pinene	0.14
10	Caryophyllene oxide	1.88	Eugenol	1.78	Sabinene	0.13

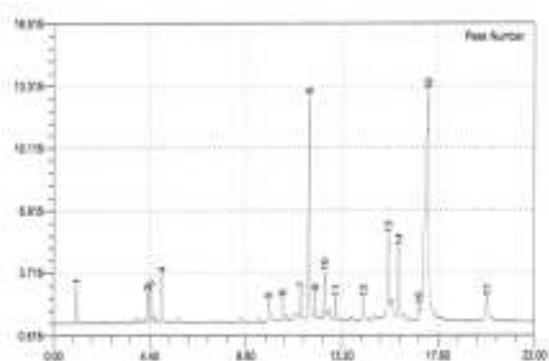


Fig. 1. Chemical composition of chamomile essential oil. 1= α -pinene , 2 = α -farnesene, 3= 1,8-cineole, 4 = β -farnesene, 5 = Isofaurinone, 6 = γ -cadinene, 7 = Caryophyllene oxide, 8 = Bisabololoxide b, 9 = α -Eudesmol, 10 = β -Bisabolol, 11 = Spathylenol, 12 = Unknown, 13 = Bisabolene oxide a, 14 = α -Bisabolol, 15 = Chanazulene, 16 = Bisabolol oxide a, 17 = Trans-dicycloether.

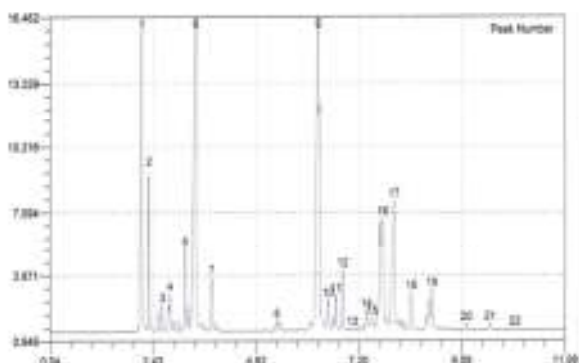


Fig. 2. Chemical composition of rosemary essential oil. 1= α -pinene , 2 = β -pinene, 3= Camphene, 4 = Unknown, 5 = Limonene, 6 = 1,8-cineole, 7 = Unknown, 8 = Unknown, 9 = Camphor, 10 = Unknown, 11 = Unknown, 12 = α -terpineole, 13 = Unknown, 14 = Unknown, 15 = Unknown, 16 = borneol, 17 = bornyl acetate, 18= Eugenol, 19 = β -caryophyllene.

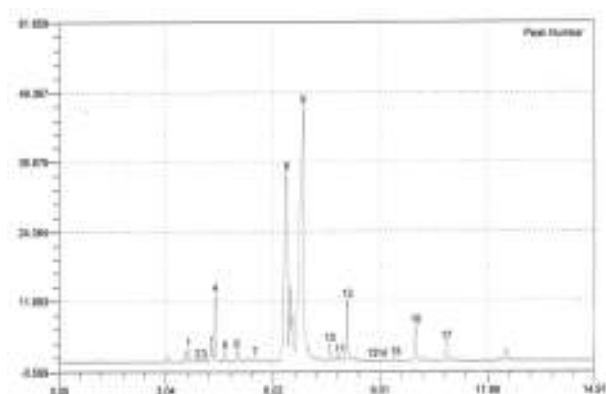


Fig. 3. Chemical composition of peppermint essential oil. 1= α -pinene , 2 = Sabinene, 3= β -pinene, 4 = Limonene, 5 = 1,8-cineole, 6 = Unknown, 7 = Unknown, 8 = Menthone, 9 = Menthol, 10 = Iso-menthol, 11 = Unknown, 12 = Methyl acetate, 13 = Unknown, 14 = Unknown, 15 = Unknown, 16 = β -caryophyllene, 17= Unknown.

Biochemical analysis: The biochemical analysis estimated the change in the level of total lipids and total soluble protein and the activities of detoxification enzymes acid phosphatase (ACP) and phenoloxidase as response of treatment of 1-days old eggs of *Galleria mellonella* L. to the tested volatile oils at 8 % concentration.

Total soluble protein (TSP): The level of total soluble protein in supernatant of the treated eggs decreased as a result of *Chamomilla recutita* L. (42.79 mg/gm. b. wt.) and *Rosmarinus officinalis* L. (50.09 mg/gm. b. wt.) treatments whereas, *Mentha piperita* L. induced an increase in TSP (59.01 mg/gm. b. wt.) as compared to control (55.92 mg/gm. b. wt.) Fig. (4).

Total lipids: The present results in Fig. (5) showed that treatment with plant oils showed an elevation in lipid content with *Chamomilla recutita* (639.32 mg/dl) and *Rosmarinus officinalis* (362.05 mg/dl) compared to control (326.09 mg/dl) whereas, reduction in lipid content recorded with *Mentha piperita* (257.43 mg/dl).

Acid phosphatase (ACP): Data in Fig. (6) revealed that the biochemical parameter (ACP) was inhibited by all treatments, where the highest reduction value (0.792 μ g phenol/min/g.b.wt) observed in *Chamomilla recutita* treatment followed by *Rosmarinus officinalis* (1.43 μ g phenol/min/g.b.wt) and *Mentha piperita* (1.989 μ g phenol/min/g.b.wt) compared to control (2.233 μ g phenol/min/g.b.wt.)

Phenoloxidase (PO): Generally, the three used oils reduced significantly the activity of PO than control Fig. (7), this reduction was cleared with *Mentha piperita* (5 U/g.b.wt) and *Rosmarinus officinalis* (7.14 U/g.b.wt) whereas, *Chamomilla recutita* showed the minimum decrease in PO activity (17.5 U/g.b.wt) than the untreated eggs (26.47 U/g.b.wt.).

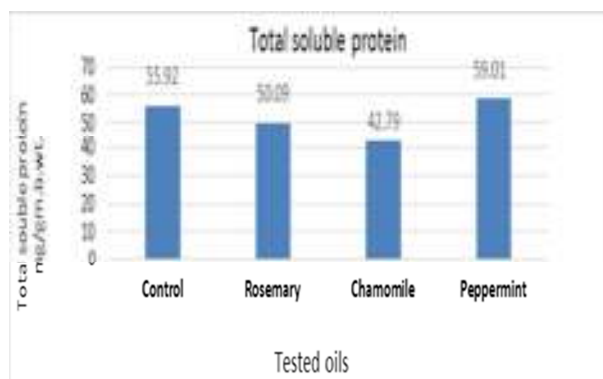


Fig. 4. Biochemical changes in total soluble protein (TSP) of greater wax moth eggs treated by the three volatile oils using spraying technique.

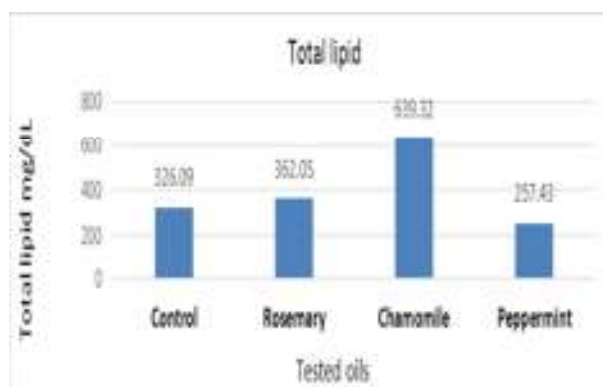


Fig. 5. Biochemical changes in total lipid content of greater wax moth eggs treated by the three volatile oils using spraying technique.

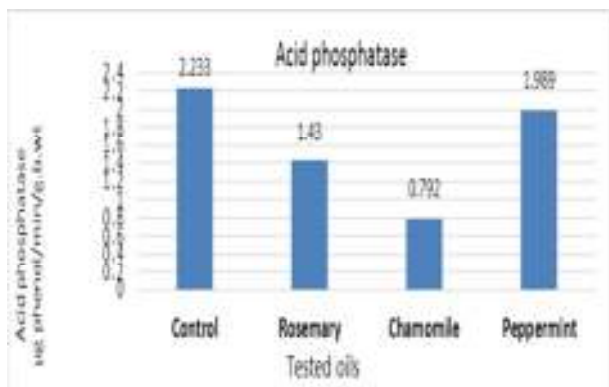


Fig. 6. Biochemical changes in acid phosphatase activity of greater wax moth eggs treated by three volatile oils using spraying technique.

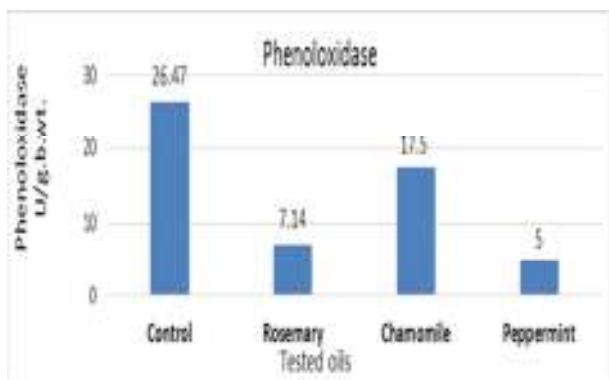


Fig. 7. Biochemical changes in phenoloxidase activity of greater wax moth eggs treated by three volatile oils using spraying technique.

DISCUSSION

Initially the current study was conducted on three selected volatile oils, chamomile oil (*Chamomilla recutita* L.), rosemary oil (*Rosmarinus officinalis* L.) and peppermint oil (*Mentha piperita* L.) for their ovicidal activity against *Galleria mellonella* L. These oils are used as pharmaceuticals and in flavouring, therefore they considered to be less harmful to humans than most traditional insecticides. Moreover, they are easily biodegradable and less deleterious to non-target organisms than pesticides (Baysal, 1997). The GC analysis of the oils cleared the major constituents of the tested three oils. Regarding the *Chamomilla recutita* L. oil, bisabolol oxide a (43.76 %), bisabolol oxide b (16.11%), bisabolene oxide a (8.86 %) and α -bisabolol (7.33%) were the most prominent constituents. whereas, the major components for *Rosmarinus officinalis* L. were camphor (23.56 %), 1,8-cineole (17.86 %), α -pinene (12.52 %), borneol (10.55 %) and bornyl acetate (8.64%). The principle constituents of *Mentha piperita* L. were menthol (47.38 %), menthone (29.99 %), limonene (5.50 %), methyl acetate (4.85 %) and β -caryophyllene (3.06 %). Earlier similar results have been obtained by Presibella *et al.* (2006) who found that bisabolol oxide a, bisabolol oxide b, β -farnesene and trans-dicycloether are the major compounds of *Chamomilla recutita* L. volatile oil. Also, Kadri *et al.* (2011) and Hcini *et al.* (2013) mentioned that camphor, 1,8-cineole, α -pinene, borneol and bornyl acetate were chemical constituents of rosemary essential oil. Mahboubi and Kazempour (2014) found that chemical composition of peppermint essential oil was menthol, menthone, limonene, methyl acetate, β -caryophyllene and α -pinene. The tested four concentrations 1%, 2%, 4% and 8% of the essential

oils, proved to possess highly marked ovicidal activity using spraying technique. These activities expressed by elongation in the incubation period and hatching period beside inhibition in hatchability percentages of the *Galleria mellonella* eggs as compared to the untreated ones. The maximum ovicidal activity was recorded at 8% concentration for the three oils as compared to other concentrations. The high concentration and direct exposure to compounds allowed more entry of the chemical inside the eggshell which affected the embryogenesis Broadbent and Prec (1984). Additionally, exposure of freshly laid eggs of the oriental fruit moth at the developmental age of (1- day- old) to the plant oil formulations found to be effective. Miura *et al.* (1976) revealed that the age of embryos at the time of treatment played an important role with regard to the effectiveness of the chitin synthesis inhibitor, dimilin to *Culex quinquefasciatus*. Additionally, acetylcholine esterase enzyme is not present in early stages of eggs where, this enzyme plays a crucial role on ovicidal action in insect eggs Mehrotra (1960). The treatment of *Galleria mellonella* eggs with various concentrations of the three plant oils formulations significantly retarded the incubation period, hatching period and the hatchability percentages where, peppermint oil showed marked ovicidal activity followed by rosemary oil then chamomile oil compared to the untreated ones. According to Smith and Salkeld (1966), differences in susceptibility to ovicides are due to differential rates of uptake, penetration through the chorion, conversion to active inhibitor; detoxication and failure of the toxic into reach the target. The tested essential oils showed prominent ovicidal response activity, this might be due to the volatile compounds present in the oils. Certain *Mentha piperita* components have been reported to have ovicidal or pesticidal effects. Aggarwal *et al.* (2011) found that methyl acetate was highly ovicidal against the storage *Tribolium castaneum*. Also, Robert (2001) said that Menthone, present in high concentration in peppermint oil, is reported to act as a natural pesticide. Rosemary oil major components have been shown to be an effective insecticide against a range of insects and mite species, with particular efficacy against stored product pests Shaaya *et al.* (1991). In a related study Miresmailli *et al.* (2006) determined 1,8-cineole and α -pinene to be the most toxic constituents of rosemary oil to the two-spotted spider mite [*Tetranychus urticae*. Koch (*Tetranychidae*)] when tested individually. In addition, Murray *et al.* (2008) found that camphor one of major constituents of rosemary oil was the most toxic compound to the armyworm. On other hand El-Khyat *et al.* (2017) observed high mortalities in *Ephestia cautella* populations treated with German chamomile essential oil. Generally, Proteins and lipids are considered the major biochemical nutrients present in the yolk of eggs. Protein is nutrient reserve in insect eggs present as vitellin where, fat body produces vitellogenin which is a source of nutrients for the oocyte and vitellogenins are deposited into the oocyte and stored as vitellin which serves as a food source for the future embryo. Lipid is another nutrient present in insect eggs and about 30-40% of the dry weights of insect oocytes consist of lipids Tufail and Takeda (2009). The present results revealed that treatment with oils at 8% concentration affected biochemical activities which induced disturbance in total soluble protein, total lipid and other enzymes activities. The level of total soluble protein was reduced by both chamomile and rosemary oils. This reduction might reflect the decrease in DNA and RNA synthesis and thus to the reduction in protein content El-barky *et al.* (2008). Whereas, peppermint oil caused an elevation in protein content than control this could be due to detoxification mechanism through increase in protein biosynthesis by its tool (amino acids), as protein helps to synthesize

microsomal detoxifying enzyme, which helps in detoxify the toxicants that entered the insect body Shakoory and Saleem (1991). Lipids consider the most suitable materials for energy reserves, lipids can supply as much as eight times more energy per unit weight, compared to carbohydrates Beenackers *et al.* (1985). In this study, oil treatments caused an increased in lipid content with the exception of peppermint oil was less than untreated one. This reduction in total lipid and increase in total protein with peppermint oil might indicate that lipids converted to proteins in detoxification mechanism against toxicants that enter the insect body as suggested by Shakoory and Saleem (1991). The reason for the lower fat content could be also due to the extended incubation and hatching periods of the treated eggs where they reached 15 and 6.67 days respectively as compared to untreated 7.33 and 2.33 days resp. and the fat reserves might have been utilized for the maintenance during extended periods. Previous studies revealed that the activity of acid phosphatase increased with the progress of embryonic development, while that of alkaline phosphatase remained low in the early stages and increased prior to hatching of larvae. Acid Phosphatase has been shown to be important in the metabolism of the carbohydrates, nucleotides and phospholipids Lambremont (1960). ACP has a role during embryonic development where, yolk reserves undergo breakdown by the yolk cells and vitellogenins converted into simpler nutrients, then transported to the developing embryo for tissue growth and differentiation where, the present study showed that the ACP activity decreased in the eggs as treated by the three essential oils; such decrease reflected block in embryonic development as the activity of this enzyme increase with the progress of embryonic development. Phenoloxidase (PO) is involved in many important physiological processes in insects. During egg development the phenoloxidase system was at its highest activity compared with other stages of development. In fertilized eggs the PO activity increased with age and reached in 3-day-old eggs, about 130 percent that of the newly laid eggs Ishaaya and Navon (1974). Additionally, studies concerning the immune response of insects suggested that (PO) is also critical in the defense reactions of insects against invaders Nigam *et al.* (1997). Our results showed that the highest concentration (8%) of chamomile, rosemary and peppermint oils reduced the PO activity in the supernatant of treated eggs, this reduction might prevent the hardened process of the cuticle that protect the soft component of eggs. Sugumaran (1998) reported that PO has a role in hardened the freshly made cuticle to be quickly converted into hard exoskeleton to protect the soft-bodied insects. This transformation is called sclerotization that protects the eggs, different instar larvae, pupae, nymphs as well as adults. Therefore, it is vital for most if not all insects. Moreover, Hiromori and Nishigaki (2001) added that the decrease of phenoloxidase activity is attributed to weakening of immune system.

CONCLUSION

The selected essential oils may be a potent source of natural ovicidal activity against important bee wax pest spatially, peppermint oil showed marked activity against the eggs of greater wax moth through extend both incubation period, hatching period and inhibition of hatchability. These results are very promising in developing effective and inexpensive approaches to control greater wax moth in early stage before it has a chance to cause any damage.

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التأثيرات البيوكيميائية لبعض الزيوت الطيارة على بيض فراشة الشمع الكبرى *Galleria mellonella* L.

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لقد تم اختبار التأثير السام والنشاط الحيوى الكيمايى لثلاث زيوت عطرية (زيت البابونج *Chamomilla recutita* L. , زيت أكليل الجبل *Rosmarinus officinalis* L.) وأخيراً (زيت النعناع الفلفلى *Mentha piperita* L.) على بيض فراشة الشمع الكبرى *Galleria mellonella* L. تحت الظروف المعملية. حيث تم دراسة تأثير أربع تركيبات (1% , 2% , 4% , 8%) من الزيوت الطيارة بطريقة الرش لتحديد فترة الحضانة وفترة الفقس ونسبة الفقس. وقد سجلت النتائج أن أطول فترة حضانة للبيض (10 يوم) وأطول فترة فقس للبيض (6, 67 يوم) وأقل نسبة فقس للبيض (0, 07%). لزيت النعناع عند تركيز (8%) , يلية زيت أكليل الجبل (8%) (13, 67 يوم , 0, 33 يوم , 18, 25% على الترتيب) ثم زيت البابونج (8%) (9 يوم , 0, 67 يوم , 32, 45% على الترتيب). وقد اظهر التحليل الكيمايى للزيوت العطرية الثلاثة باستخدام جهاز الكروماتوجرافى الغازى ان أكبر مكونات زيت البابونج bisabolol oxide a (43, 76%) و bisabolol oxide b (16, 11%) بينما زيت أكليل الجبل camphor (23, 06%) و 1,8-cineole (17, 86%) وأخيراً زيت النعناع menthol (47, 38%) و menthone (29, 99%). وقد ادت المعاملة بالتركيز 8% للزيوت المختبرة الى حدوث خلل فسيولوجى فى محتوى البروتين الكلى الذائب والمحتوى الكلى للبيبيدات وكذلك نشاط انزيمى الفوسفاتيز الحامضى والفينول اكسيديز لبيض فراشة الشمع الكبرى.