Menoufia J. Plant Prot., Vol. 4 August (2019): 145 - 161

INSECTICIDAL ACTIVITY AGAINST THE GREATER WAX MOTH (*GALLERIA MELLONELLA* L.) AND CHEMICAL COMPOSITION OF FIVE PLANT ESSENTIAL OILS

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Received: May 26, 2019 Accepted: Jun. 1 , 2019

ABSTRACT: *In this study, the toxicity of four concentrations (5-10-15-20%) of five natural essential oils: Lavender (Lavandulaa ngustifolia: Labiatae) – Camphor (Eucalyptus globules: Myrtaceae) – Mint (Mentha spp.: Labiatae – Clove (Syzygium aromaticum: Myrtaceae) - Rosemary (Rosmarinus officinalis: Labiatae) against the third instar larvae of the greater wax worm, Galleria mellonella L. (Lepidoptera : Pyralidae) were evaluated as well as the mortality percentages were computed. Mortality percentages were high at rosemary oil treatments, followed by lavender, then mint, camphor and clove compared to control. In addition, LC_{50} were 7.11-9.45-11.45-13.61-6.45% for lavender essential oil - camphor - mint - clove - rosemary, respectively. The biochemical contents (total protein-alkaline phosphatase-acetylcholinesterase) were measured in the 3rd larvae treated with the tested essential oils. The obtained results indicated an increase in the total protein ratio in all the treatments compared with control. There was a decrease in the activity of alkaline phosphatase and acetylcholinesterase enzymes. The most effective essential oils were rosemary and lavender, compared to the control. The chemical composition of the tested essential oils was measured. The major compounds found in lavender essential oil, were linalool (38.74%) , linalool acetate (29.32%), D limonene (9.04%), triacetien(5.37%), 1.8- cineole (4.71%), camphor (2.82%), exoproneoleacetate (1.8%), a-pinene (1.79%),a- terpineole (1.43%), a-mycene (1.13%). Eucalyptus essential oil contains mainly 1.8-cineole (66.38%), 1-Limonene (16.27%). meanwhile Benzene, methyle (1-methylethyl) (7.44%), 1-phellandrene (4.25%), a-pinene (1.95%) a-terpinene (1.24%), and a-myrcene (1.16%). The major compounds in clove essential oil were trans-caryophyllene in relative concentration of 58.46%, phenol, 2-methoxy-4-(2-propenyl), humulene,1.8-cineole and eugenol were in relative concentration of 9.43%, 7.42%, 1.30%, and 1.87% respectively. the major compounds in mint essential oil were l-menthol in relative concentration (42.14%) ,1-menthone in relative concentration (27.08%) .carvone (8.90%), p-menthone (5.06%), pulegon (2.86%), 1-menthyle acetate (6.01%), and neo-menthol (2.10%). The major components in rosemary essential oil were glycerol triacetien (39.22%) ,1.8-cineole (16.82%), l-menthole (10.64%),a-camphor (8.71%), Pinene (7.16%), d-limonene (7.04%), and as for the minor compounds were p-cymene (1.17%), endobronyole acetate (0.91%), camphene (0.31%), delta 3-carene (0.39%), 1- broneole (0.13%) .*

Key words: *Lavender, mint, clove, rosemary, camphor, essential oils, insecticidal activity, chemical composition*

INTRODUCTION

Wax is one of the most useful products of honey bees. It is used in the pharmaceutical industry, dentistry and cosmetics. Wax contains many nutrients, pollen and honey, and is therefore attacked by various pests (Nurullahoglu and Susurluk, 2001). The wax moth, *Galleria mellonella,* is one of the most devastating and economically important pests of wax in the world (Burges, 1978, Cantwell and Smith, 1970). For economic reasons it is important for beekeepers to control this dangerous pest. Many studies have been conducted to find ways in its control (Burges, 1978). Although the most successful control measure has been the use of insecticides at the larval stage. At the present time, two poisons can be used to protect combs: Paradi chlorobenzene and Phestoxin (Gross, 1994) which can be used to protect all combs in storage except those containing honey intended for human consumption. The odor of Paradichlorobenzene and Phestoxin is readily absorbed by honey, and though the bees do not object to this odor, such honey is unfit for human consumption. The greater wax worm has developed high resistance to insecticides. In addition, the poison residue in the wax is a result of the frequent use of insecticides. In the development of management for *G. mellonella*, control methods need to be explored. An effective biological and environment-friendly control of this pest is the using of natural materials such as plant essential oils or natural materials such as chitosan (Said *et al.,* 2011)

Medicinal and aromatic plants have always important components for human activities , i.e. medicinal drugs, antioxidants, for keeping quality of oils and fats , antimicrobial to some harmful microorganisms (bacteria , Fungi and yeast) as well as being used for food preservation. It also has insecticidal effect . lavender (*Lavendula angustifolia*) belong to family (Labiatae), lavender essential oil continues to be popular today as it has been down the centuries past documented evidence for the use of lavender as a therapeutic agent can be tracted back to the ancient Romans and Greeks, Eucalyptus (*Eucalyptus globulus*) belong to family (Myrtaceae) which contains 133 genera and 3800 species of trees and shrubs this family can be found in temperature, subtropical and tropical regions Eucalyptus, which is a large genus of ever green trees and shrubs containing approximately 700 species the leaves of eucalyptus accumulate a very large number of secondary metabolites and yield hydro-distilled essential oils that possess many biological properties, including antibacterial and antifungal activities , clove (*Syzgium oromaticum*) belong to family (Myrtaceae) this plant has very important oil which has a variety properties such as its antioxidant activity, antiseptic, anti- inflammatory , antimicrobial, insecticidal activity and used in treating the toothache and as spice., mint (*Mentha* spp) belong to family (Labiatae) with vernacular name of (nana felfeli) is traditionally used as antiseptic , stimulant ,carminative agent or it is further used as flavoring agent in cosmetic and pharmaceutical industries throughout the world it their essential oils are used in chewing gums, alcoholic beverages, cosmetics, perfumes, toothpastes and mouth washes. the plant is mainly used as salad, spice and for tea besides mint herbage use for tea besides mint herbage used for wool dyeing , rosemary (*Rosmarinas officinalis*) belong to family (Labiatae) is one of the most effective spices widely used in food processing , and is the only spice commercially available for use as an antioxidant in Europe and united states . These plant are considered to be as medicinal and aromatic plants which are considered grown in the Egyptian newly reclaimed lands. The essential oils from such medicinal and aromatic plants are normally used as therapeutically agent for better tasting foods and also added to different products such as bath product, Hair care, skin care products and soaps.

Undoubtedly, the presence of the medicines and oils prevent the growth of microorganism, consequently, the essential oils of Lavender, Eucalyptus, Clove, Mint and Rosemary were tested in the present investigation to study their insecticidal activity against *G. mollenella* larvae, as well as their chemical composition using Gc-mass spectra.

MATERIALS AND METHODS

1- Plant materials :

The oils of Lavender (*Lavendula angustifolia*), Eucalyptus (*Eucalyptus globulus*), Clove (*Syzygium aromaticum*), Mint (*Mentha* spp) and Rosemary (*Rosmarinus officinalis*) plants were obtained from the local market, Table 1.

2-Insecticdal activity :

2.1-Insect culture:

Galleria mellonella larvae, collected from the domestic hives in Faculty of Agriculture, Menoufia University, and were kept in 2 glass containers (50-75 specimens); these containers consisted of artificial diet as described by Wiesner (1993), these containers with filter paper for pupation. Larvae, pupae, and adults were kept in darkness at 28-31ºC and 60% humidity. Adults were obtained from the first, second, or third generation in the laboratory. Colonies started from wild stock.

2.2-Insect treatments:

Larval mortality and determination of LC_{50} of tested plant essential oils were recorded at four concentrations (5- 10-15 20%), which dissolved on ethanol and dispersed by shaking, then added to the artificial diet as one cm^3 / 5 g diet /containers, while control ones had ethanol only. There were three replicates of each treatment and there were 10 larvae of each replicate. The third instars were used for these treatments and determination of LC_{50}. Percentages of mortality were calculated at 24 and 48 hours after treatment and corrected by the formula given by Abbott' s formula .

$$\text{Abbott's formula} = \frac{\text{Percent test mortality - percent control mortality}}{\text{100-percent control mortality}} \times 100$$

2.3-Biochemical assay:

The third instars larvae were fed on treated artificial diet containing LC_{50} of tested materials for 48 hours and were starved for about 4 hours before being homogenized in distilled water (1/5 w/v) using a Teflon homogenizer surrounded with a jacket of crushed ice for 3 minutes. Homogenates were centrifuged at 3500 r.p.m. for 10 minutes at 5°C and the supernatants were filtered through glass wool to remove fatty materials and used for biochemical studies:

1- Determination acid and alkaline phosphatases were determined according to the method described by Powell and Smith (1954).

Table (1): Latin and family names of the medicinal and aromatic plants under study.

Plant	Scientific name	Family names
Lavender	*Lavandulaa ngustifolia*	*Labiatae*
Eucalyptus	*Eucalyptus globulus*	*Myrtaceae*
Mint	*Mentha Spp.*	*Labiatae*
Clove	*Syzygium Aromaticum*	*Myrtaceae*
Rosemary	*Rosmarinus officinalis*	*Labiatae*

2-Acetylcholinesterase AchE activity was measured according to the method described by Simpson *et al.* (1964) , using acetylcholine bromide (AchBr) as substrate .

3-Total proteins were determined by the method of Bradford (I976).

2.4- Chemical composition of tested plant essential oils:

In order to study the chemical composition of Lavender, Eucalyptus, Clove, Mint and Rosemary essential oils gas chromatography/ mass spectrometry technique was used under the following conditions in Table (2).

3-Statistical analyses:

Collected data were subjected to analysis of variance (ANOVA),. Mean's differentiations were compared using Duncan test at $P < 0.05$.

RESULTS AND DISSECTION

1. Insecticidal activity:

1.1. Toxicity of tested plant essential oils:

Results of the bioassay were recorded after 24 and 48 h from the beginning of treatment. The observed larval mortality increased gradually with the increase of exposed dose of essential oils and time showed high toxicity against the third instars of *G. mellonella* larvae (Table 3 and Fig. 1). The results showed that the tested essential oils had a high toxicity to the larvae, but lavender and rosemary essential oils had a significant toxic effect on the tested larvae and the mortality percentages reached 90 % for lavender essential oil and 100% for eucalyptus essential oil after 48 hours. Table (4) show the value of LC_{50} of tested essential oils. It is clear that lavender and rosemary essential oils are highly toxic more than other tested oils, the value of LC_{50} were 7.11 and 6.45 % for lavender and rosemary essential oils, on the other hand, eucalyptus, clove and mint were less toxic and LC_{50} were 9.45 , 11.45 and 13.61% for eucalyptus, clove and mint essential oils, respectively.

Table (2): The chemical composition assay.

Dx	Condition
Instrument	GC/MS Finnigan Mat SSO 7000
Column	DB-5.50m×0.32mm capillary
Column Packing	(5%phenyl) Methyl poly siloxane
Carrier	Helium
Injection temperature	250 °C
Trans line temperature	250 °C
Oven temperature	50 °C – 250 °C
Programme rate	50 °C rate °C/ min -50 °C – 200 rate 3 °C /min-250 °C rate 5 °C/min-250 °C isothermal for 5 °C/ min

Mass selective detector condition:

Item	Condition
MS ionization voltage	70 ev
Start, stop masses	50-600 amu (Atom mass unit)

Table 3: Mortality percentages of *Galleria mellonella* larvae treated with 4 concentrations of the tested plant essential oils after 24 and 48 hours.

Tested plant oils	Mortality % ± SD after 24 and 48 hours							
	5 % conc.		10% conc.		15% conc.		20% conc.	
	24 h.	48 h.	24 h.	48 h.	24 h.	48 h.	24 h.	48 h.
Lavender	22.76± 12.33	34.63± 17.33	54.33± 18.56	62.33± 18.11	65.33± 33.21	73.33± 18.23	82.62± 35.11	92.25± 38.11
Eucalyptus	10.25± 5.15	33.33± 15.22	54.67± 29.17	62.33± 16.31	65.33± 16.21	68.76± 33.41	73.33± 33.11	75.22± 33.12
Clove	22.32± 19.15	33.36± 14.31	42.25± 28.11	49.33± 27.34	65.33± 16.11	67.35± 33.11	72.25± 23.51	75.33± 32.14
Mint	10.25± 5.17	12.35± 7.56	22.76± 23.45	35.33± 17.61	54.67± 16.11	62.33± 32.41	73.33± 36.11	82.67± 35.13
Rosemary	25.67± 17.33	42.25± 17.33	65.33± 17.21	68.33± 18.22	75.32± 35.27	81.52± 36.77	94.67± 37.23	100± 37.15
Control	0	0	0	0	0	0	0	0

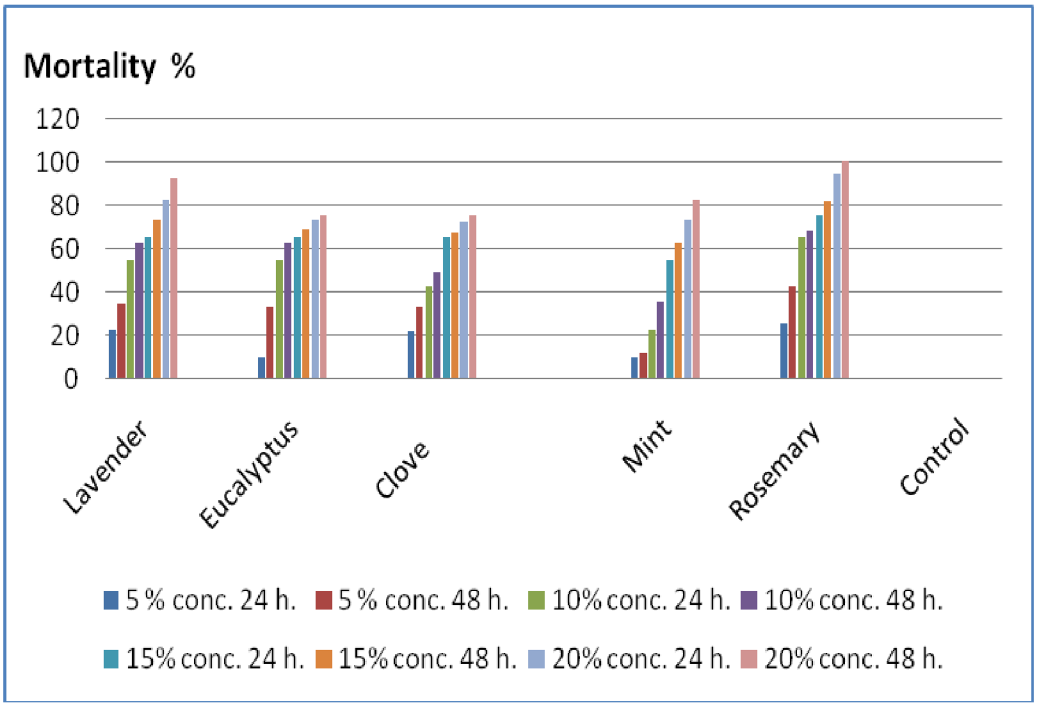

Fig. 1: Mortality percentages of G. melonella larvae treated with tested plant essential oils.

Table 4 : Effect of tested plant essential oils on some biochemical aspects of *Galleria mellonella* larvae treated with LC_{50} of these essential oils.

Tested plant oils	LC_{50} %	Mean ± SD		
		Total protein (mg/g.b.wt)	Alkaline phosphatase (mU/g.b.wt)	AChE (ug AchBr/min/g.b.wt)
Lavender	7.11	49.51±2.29 a	19.11 ± 1.01 cd	329.66 ±17.37 b
Eucalyptus	9.45	44.63±0.72 b	22.76 ±2.08 c	667.21 ±5.57 a
clove	11.45	42.1 ± 1.08 c	20.36 ±2.081 cd	622.66±7.23 a
Mint	13.61	39.73 ±1.1 c	55.66 ±4.04 b	688.33±12.58 a
Rosemary	6.45	36.83 ±1.46 d	16 .21 ± 1.02 d	206.33 ±10.12 c
Control	-	33.4 ± 1.15 e	76 ±4.56 a	659.33 ±8.72 a
L S D	-	2.475	5.032	125.508

Values with different letters are significantly different at P< 0,05 (Duncan test).

These results agreed with Sabrine Attia *et al.* (2015) who investigated the insecticidal activity of *Lavandula angustifolia* Mill, against the pea aphid, *Acyrthosiphon pisum and* found that the LC_{50} of essential oil concentrations were ranged from 5 to 25 µl.l-1 of air. Also, mortality of aphids increased as essential oil concentration increased and LC_{50} values were determined to be 11.2 µl.l-1 of air. *L. angustifolia* essential oil can provide valuable pesticide activity with significantly lower LC_{50} values. Also, Sehari N. H. *et al.* (2018) evaluated the potential insecticide essential oil from rosemary. Insecticides tests were performed in the laboratory by the direct contact method. The results of these tests show that rosemary essential oil has remarkable insecticidal properties. They induced 100% mortality of adult rice weevils to 15µl dose after only 24 hours of treating. The rosemary essential oil in experimental conditions prevents the development of rice weevil larvae Sitophilus oryzae to 15 µl dose. Dhen Najla *et al.* (2013) reported that essential oil of *Euginia carpophyllata* (Myrtaceae) was found to be strong insecticide against *Spodoptera littoralis.* Essential oil of E. carpophyllata was analyzed by GC-MS in order to identify their chemical composition. The main components of essential oil were; apropenylphenolique compound Eugenol (52.82%) and other terpene compounds such as β-Caryophyllene (30.26%), α-Humulene (6.11%) and Eugenyl acetate (2.51%). Different concentrations of the oil were tested and the observed larvae mortality showed high fumigant toxicity against the third instars of S. littoralis larvae. Khani and Asghari (2012) tested essential oils extracted from the foliage of *Mentha longifolia* (L.) (Lamiales: Lamiaceae) in the laboratory for volatile toxicity against two stored product insects, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) and *Callosobruchus maculatus* F. (Coleoptera: Bruchidae). *C. maculatus* was More affected by the tested oils than *T. castaneum. M.*

longifolia showed a strong insecticidal properties against T. *castaneum* (LC50 = 13.05 µl/L air), these results showed that essential oil *from M. longifolia* could be used as potential control agents for stored-product insects. Hai Ping Chen (2014) showed that essential oil of *Cinnamomum camphora* leaves was found to possess strong fumigant and contact toxicity against *Lasioderm aserricorne* adults with LC50/LD50 values of 2.5mg/L and 21.25 µg/adult, respectively.

1.2. Biochemical effects of tested plant oils on *G. mellonella* larvae:

Results in Table (4) show that there were significant differences in the total protein, Alkaline phosphatase and AchE values between treated larvae compared with control . The results show that all tested essential oils increased the total protein content and decreased Alkaline phosphatase of the tested larvae compared with the control. The average content of total protein were : 49.51 ,44.63 ,42.1, 39.73 , 36.83 and 33.4 mg/g.b. wt for Lavender , Eucalyptus, Clove, Mint, Rosemary and control, respectively. The average means of Alkaline phosphatase were: 19.11, 22.76,20.36, 55.66, 16.21and 76 (mU/g.b.wt) for the five oils and control, respectively.

As for AChE, results in Table (4) show that some of the essential oils caused a reduction in the AChE enzyme and the treatment of some other oils caused a decrease in the enzyme ,the averages were: 688.33,667.21, , 622.66, 329.66, 206.33 and 659.33 (ug AchBr/min/g.b.wt) for the tested oils and control , respectively .

Total protein in larvae treated with tested essential oils was found to be higher than in control (Table 4). This finding may be due to the conversion of carbohydrates and lipids to proteins as stated by Kinnear (1968) who suggested that the increased of protein level was due to increased synthesis of new proteins by fat body, haemolymph and other tissues of the larvae. The results are in agreement with Rajendra (1990), and Shakoori and Saleem (1991) who stated that increase in protein content could be due to increase in protein biosynthesis by its tool (amino acids) for the detoxification mechanism, since protein helps to synthesize microsomal detoxifying enzyme, which assists to detoxify the toxicants that entered into the insect body. The obtained results show that alkaline phosphatase activity in treated larvae was less than in control one (Table 4).

In addition, there were some malformations in some treated larvae with tested plant oils (Fig. 2) , this also comes in agreement with Dua *et al.* (2010) and Werdin *et al.* (2010) who stated that the plant essential oils might have insect growth regulator effect. Therefore, the increase in the alkaline phosphatase could be due to juvenile hormone effect of tested oils since juvenile hormone leads to increase in alkaline phosphatase and occurrence of malformations in larvae. Regarding the mode of action of essential oils against insect pests, little information is available but treatment with various essential oils or their constituents cause symptoms that suggest a neurotoxic mode of action (Priestley *et al.*, 2003; Lu and Wu, 2010). In the present study, activity of AChE decreased in all treatments in comparison to control. These findings are coincide with that reported by Chaubey (2011) who found that, fumigation of *S. oryzae* adults with sublethal concentrations of *C. cyminum* and *P. nigrum* essential oils inhibited AChE activity. Previous researchers have reported the competitive inhibition of AChE activity by monoterpenes and monoterpenoids. Most of the essential oil components like cuminaldehyde, limonene, α – pinene and β – phellendrene inhibiting AChE activity (Zapata & Snagghe, 2010, Mona, *et al.*, 2015) .

2. Chemical composition of essential oils:

2.1 Chemical composition of lavender essential oil:

Data presented in Table (5) and illustrated in Fig (2: a) show the chemical composition of lavender essential oil. The major compounds found in lavender essential oil, were linalool (38.74%) and linalool acetate (29.32%), D limonene(9.04%), triacetien (5.37%) , 1.8-cineole (4.71%), camphor (2.82%), exoproneoleacetate (1.8%), a-pinene (1.79%), a- terpineole (1.43%), a-mycene (1.13%)Were presented in amounts less than 10%.The minor compounds in this oil were 3-carene (0.62%) isopulegol (0.36%),Geraniol (0.33%), γterpineole (o,26%), cyclohexanole (0.22%), cyclopentanole (0.14%) isopinocamph-enol (0.18%). The other identified compounds occurred as trace materials such asa-terpinene (0.1%), 1 – phellendrene (0.1%), Glycerol 1.2diacetate (0.10%), verbenol (0.09%), verbenene (0.08%), camphene (0.07%), 2 a-pinene (0.06%), trans-Pinocarveol (0.05%), a -terpinyle acetate (0.04%), isobroneole (0.03%) and other compounds we showed it at the Table (5) the results of the chemical composition of lavender essential oil were in accordance with those reported by Dhen *et al.* (2013) who stated that the chemical composition of lavender (EO) is linalool, linalyleacetate, cymene, terpinene -4-ol and camphor should be between 25-38% , 25-45%, 4-10%, 2-6% and 5% respectively.

2.2. Chemical composition of Eucalyptus essential oil :

The constituents of eucalyptus essential oil are presented in Table (6) and Fig. (2:b). Generally , data revealed that eucalyptus essential oil contains mainly: 1.8-cineole (66.38%), 1-Limonene (16.27%) .meanwhile Benzene , methyle (1-methylethyl) (7.44%), 1-phellandrene (4.25%), a-pinene (1.95%) a-terpinene (1.24%), anda-myrcene (1.16%), were presented in amount less than (10%) ,the other compounds occurred as trace materials such as Terpinene (0.55%), a-pinene (0.32%), 1-4-terpineol (0.07%), 2-caren (0.04%), trans-pinocarveole (0.03%), Camphene (0.02%) and thujene (0.01%). This results were in accordance with those obtained by Dan *et al.*, (2000) in which they mentioned that 1.8-cineole, limonene, αpinene, monoterpenoid P cymene and sesquiterpenoidss pathulenolare the mainly components of eucalyptus leave essential oils .

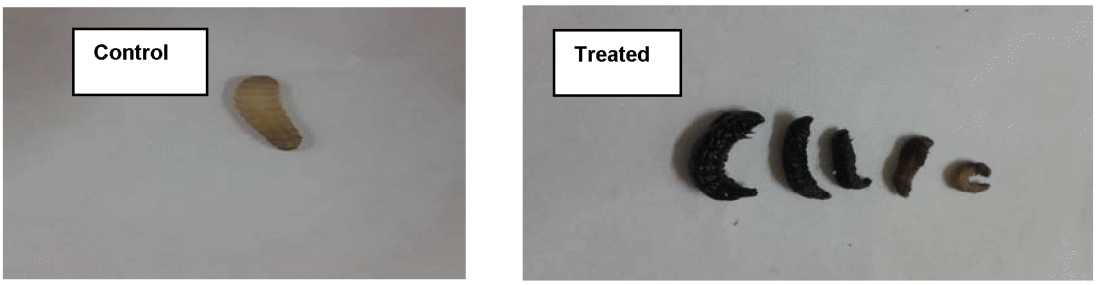

Fig. 2: Malformations in treated larvae with tested plant essential oils.

Table (5): Chemical composition of lavender essential oil.

Compounds	Retention time	Area %
a-pinene	4.68	1.79
Camphene	5.13	0.07
Verbenone	5.23	0.08
2-a pinene	5.86	0.06
a-mycene	6.14	1.13
1-phellendrene	6.71	0.10
a-terpinene	7.02	0.10
D-limonene	7.43	9.04
1.8-cineole	7.56	4.71
3-carene	7.99	0.62
C-terpinene	8.43	0.11
Cyclohexene	9.38	0.22
2.3.3 trimethyle.1.hexene	9.60	0.14
Cyclopentanol	9.75	0.14
Linalool	10.09	38.74
Iso-pinocampheole	10.83	0.18
Isopulegol	10.97	0.03
Plinol A	11.18	0.36
Terpinnene 1-ol	11.49	0.10
Trans-pinocarveol	11.70	0.05
Verbenol	11.90	0.09
Camphor	12.04	2.82
Isoborneole	12.65	0.03
Cyclohexanole	12.90	0.03
Endo-borneole	13.03	0.05
3-cyclohexen-1-ol	13.35	0.05
a-terpineol	14.02	1.43
Gamma-Terpineol	14.17	0.26
Bisyclo3.1.1hept-3-en-2-one,4.6.6trymythyle (CAS)	14.58	0.12
Isopulegol acetate	15.18	0.17
Linalyle acetate	16.05	29.32
Geraniol	16.55	0.06
Dihydrolinalool	16.77	0.33
Exobornenyl-Acetate	17.65	1.80
a-Terpinenyl acetate	20.20	0.04
Triacetin	20.46	5.37
Glycerol 1.2-diacetate	20.75	0.06
Gyranyle acetate	21.64	0.10
Nerryle Acetate	21.86	0.03
Junipene	22.54	0.05

Table (6): Chemical composition of Eucalyptus essential oil.

Compounds	Retention time	Area %
Hexane	3.04	0.00
Hexane	4.04	0.00
2-pinene	4.41	0.00
Thujene	4.49	0.01
a-pinene	4.68	1.95
Camphene	5.13	0.02
Verbenone	5.22	0.02
Sabinene	5.70	0.02
2-a-pinene	5.86	0.32
a-myrcene	6.13	1.16
2.6 octadiene ,2.6dimethyle	6.34	0.07
1-phellendrene	6.70	4.25
a-terpinene	7.02	1.24
Cyclohexen, 1-methyle-4-1methyl	7.22	0.04
Benzene, methyle 1-methylethyl	7.35	7.44
1-limonene	7.45	16.27
1.8-cineole	7.62	66.38
a-ocimene –x	8.00	0.04
Terpinene	8.42	0.55
2-a-pinene	9.00	0.01
2-carene	9.40	0.04
Alloocimene	10.21	0.01
D-fenchylalchol	10.87	0.01
Trans-pinocarreol	11.71	0.03
Trans sabinene hydrate	12.32	0.00
(+) –Broneole	13.04	0.01
1-4-Terpineol	13.38	0.07
a-terpineole	14.12	0.01
3- cyclohexene-1-methanol,aa,4-trimethyle	14.18	0.01
1H-3a,7-methanoazoulene, 3.3.4.7.8.8a hyxahydro-3.6.8.8tetramethyle .	22.84	0.01

2.3. Chemical composition of Clove essential oil:

Data in Table (7) and Fig (2:c) demonstrated the chemical composition of clove essential oil. It is clear from this data that the major compounds in clove essential oil were trans-caryophyllene in relative concentration of 58.46%. Data in Table (7) showed also that phenol,2-methoxy-4- (2-propenyl), humulene, 1.8-cineole and eugenol were in relative concentration of 9.43%,7.42% ,1.30% ,and 1.87% respectively. It is also clear from such data that the following compounds were presented as trace substances : cedrene, isocaryophyllene , Hulemene –

(vl), e.cadinene ,caryophyllene oxide and phenol, 2methoxy -4(2-propenyl) acetate. The obtained results on the composition of clove essential oil were in accordance with those mentioned by (Choi *et al.*, 2014) in which they stated that the highest content of of clove essential oil compounds is eugenol found in flower of 87-96%, β-caryophyllene is highest in leaves 11-19%, the highest eugenole acetate is found in clove leaves 8-21%.

2.4. Chemical composition of Mint essential oil:

Data in Table (8) and Fig. (2:d) demonstrate the chemical composition of mint essential oil. It's clear from these data that the major compounds in mint oil were l-menthol in relative concentration (42.14%), 1-menthone in relative concentration (27.08%) . Data in Table (8) showed that carvone (8.90%), p-menthone (5.06%), pulegon (2.86%), 1-menthyle acetate (6.01%) , neo-menthol (2.10%). It is also clear from such data that the following compounds were presented as trace substances: caryophellene (0.85%), 1.8-cineole (.67%), linalyle propionate (0.32%),linalool (0.25%), neo isomenthol (0.20%),neo menthyle acetate (0.22%), estragole (0.20%), leden (0.16%) the above results for the composition of mint essential oil in were in accordance with those mentioned by Dwivedi *et al* ., (2004) who noted that the peppermint EO different origin contains variable percentages of menthol (20-54%), menthone(5-43%), menthyle acetate (1-29%), and menthofuran (1-8%).

2.5. Chemical composition of rosemary essential oil:

Data in Table (9) and Fig. (2:e) demonstrated the chemical composition of rosemary essential oil. The major components were glycerol triacetien (39.22%), 1.8-cineole (16.82%) ,l-menthole (10.64%), a-camphor (8.71%), Pinene (7.16%), d-limonene (7.04%), and as for the minor compounds werep-cymene (1.17%), endobronyole acetate (0.91%), camphene (0.31%), delta 3-carene (0.39%), 1- broneole (0.13%) .the other identified compounds occurred as trace materials such as: tricyclene,c-terpinene, linalool, terpinene -1-ol, triacetien, caryophyllen oxide, isobroneole, longifoleneisopulegol acetate, exopronyle acetate, acetaldehyde, sabinene, a-myrcene, and the other compounds of rosemary oil at Table (9). The results of the chemical composition of rosemary essential oil were in accordance with those reported by (Mehrosorosh *et al.*, 2014). They mentioned that the major components of rosemary essential oil were α-pinene (23.93%), camphene (8.7%), camphor (10.97%), verbenone (15.44%), p-cymene (7.48%) and 3-octanone (5.63%).

Table (7): Chemical composition of clove essential oil .

Components	Retention time	Area %
1.8-cineole	7.57	1.30
Cedrene	21.14	1.34
Eugenol-acetate	21.75	2.08
Eugenol	22.78	18.96
Caryophellene	22.94	58.46
Isocaryophyllene	23.31	2.31
Hulemene –(vl)	24.22	2.14
Hulemene	24.45	7.42
e.cadinene (CAS)	27.00	0.74
Caryophyllene oxide	29.57	3.37

Table (8): Chemical composition of mint essential oil.

Components	Retention time	Area %
a-pinene (-)	4.69	0.05
Sabinene	5.87	0.06
D-limonene	7.44	0.16
1.8-cineole	7.57	0.76
C-terpinene	8.45	0.07
L-linalool	10.05	0.25
Isoplugeol	12.03	0.05
1-menthone	12.40	27.08
P-menthone	12.73	5.06
Neomenthol	12.90	2.10
L-(-)-menthol	13.32	42.14
(+)-Neo-Isomenthol	13.68	0.20
(+-) Isomenthole	13.82	0.10
Linalyle propionate	14.04	0.32
Estrageole	14.25	0.20
3-Heptanone,2.6dimethyle	14 .81	0.23
Pulegone	15.82	2.86
Carvone	16.17	8.90
2-cyclohexen -1-one ,3-methyle 1-6-(1-metheylethyl) CAS	16.55	0.80
Isomenthyle –Acetate	17.01	0.41
Cyclopentanone ,2-(2-nitro-2heptenyl)	17.59	0.08
1-menthyle acetate	17.78	6.01
Neo menthyle acetate	18.40	0.22
A Bourbonene	21.46	0.24
Trans-caryophyllene	22.95	0.85
Trans-beramotene	23.51	0.12
Lendene	25.86	0.16
Naphthalene	26.85	0.07
e-cadinene (CAS)	27.02	0.06
1s cis-calamenene	27.18	0.11
Isosphathulenol	29.41	0 .9
Verdifloral	30.07	0.08
Guaiol	30.18	0.07
t-cadinol	32.49	0.05
Allithrine	35.32	0.06

Table (9): Chemical composition of Rosemary essential oil.

Compounds	Retention time	Area %
Tricyclene	4.45	0.01
Delta -3-carene	4.68	0.60
Camphene	5.13	0.31
Sabinene	5.70	0.02
2-a-pinene	5.86	7.16
á-myrcene	6.15	0.16
1-phellandrene	6.71	0.19
á-TerpiNene	7.02	0.05
P-cymene	7.34	1.17
D-Limonene	7.43	7.04
1-8-cineol	7.57	16.82
1-Propanol, 2-(2-hydroxypropoxy)	8.07	2.86
1-Propanol, 2-(2-hydroxypropoxy)CAS	8.23	1.91
Delta-3-carene	8.41	0.38
TripRoYlene Glycol 5	8.61	0.02
C-Terpinene	9.37	0.03
2-Propanol,1,1 oxyBis-(CAS)	9.51	0.13
Bis(1-Methyle-2-Hydroxyethyl)Ether	9.67	0.04
Linalool	10.02	0.03
2-Propanol,1,1 oxyBis	10.21	0.01
Acetaldehylde	10.38	0.08
D-Fenchyl alcohol	10.86	0.03
2-Dimethylsiloxytetradecone	11.04	0.01
TerpiNENE-1-Ol	11.48	0.07
Trans-verBenol	11.69	0.02
Camphor	12.05	8.71
IsoBroneol	12.65	0.10
Cyclohexanole, 1-Methyle-4-(1-Methylethenyl)	12.87	0.02
1-Broneol	13.02	0.13
L-(-)-Menthol	13.25	10.64
Linalyl propionate	14.02	1.23
Isopulegol Acetate	15.24	0.01
Exobornyl Acetate	15.97	0.03
Endobornyl Acetate	17.65	0.91
Triacetien	20.59	39.22
Longifolene	22.54	0.02
6- Methyl- 5- hepten-2- one	22.68	0.01
Caryophellene oxide	29.56	0.01
(2,6,6- Trimethylcyclohex-1 enymethanesulfonyl)benzene	39.29	0.01

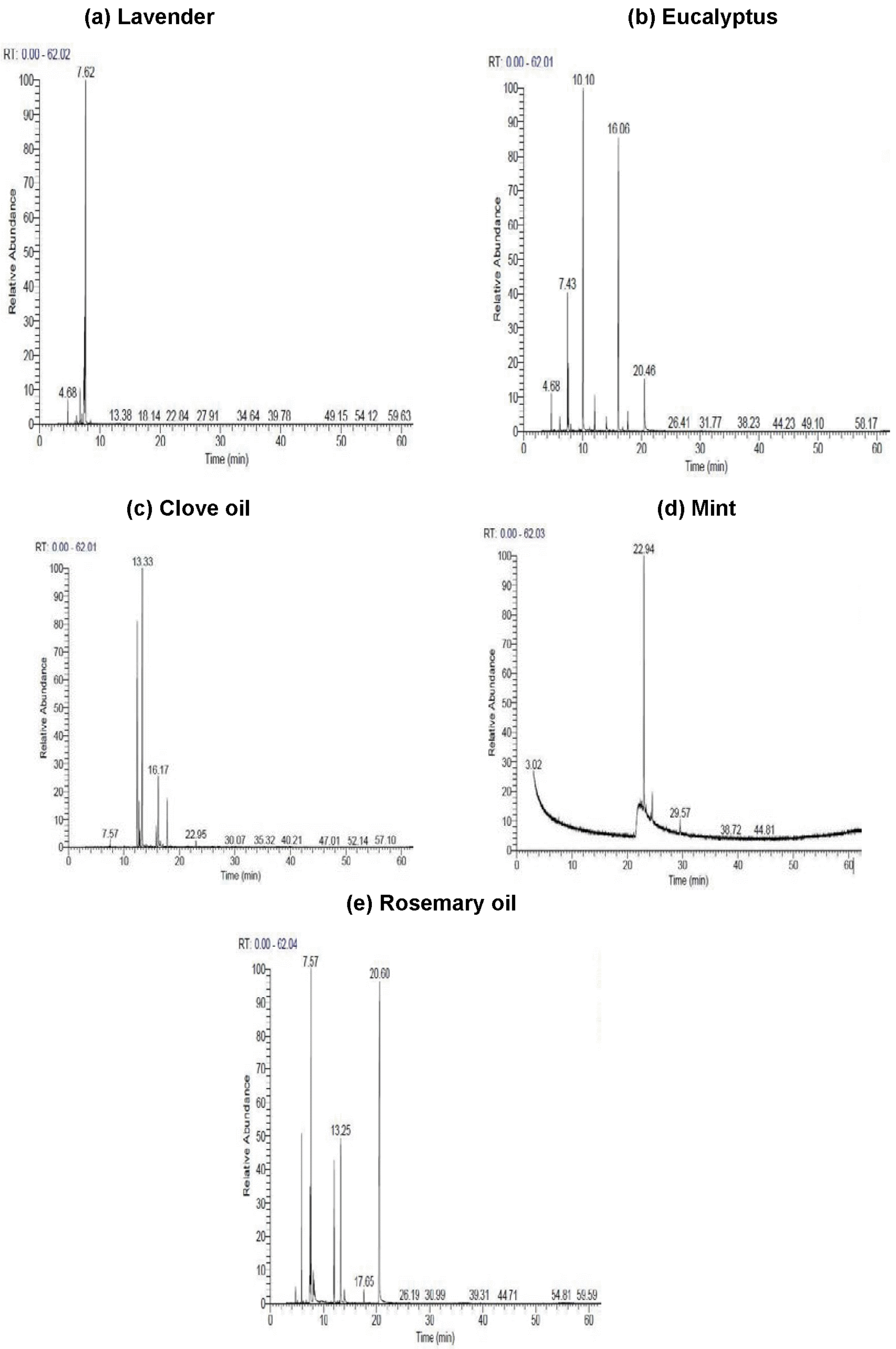

Fig. (2): Chemical composition of tested plant oils

CONCLUSION

From the obtained results of this study, it could be concluded that the tested essential oils had good effect on the *G. mellonella* larvae , where the obtained results showed that rosemary , lavender and eucalyptus successfully controlled the pest, and this effect may be due to the chemical composition of oils. Therefore, the tested essential oils can be successfully used in apiaries to protect stored wax, but this need further studies to determine limits of application.

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التاثير الإبادي للحشرات والتركيب الكيميائي لخمس زيوت اساسية نباتية لمكافحة دودة الشمع الكبيرة

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الملخص العربي

تم دراسة تأثير أربعة تركيزات (٥-١٠-١٥-٢٠ ٪) من خمسة زيوت طبيعية وهى : زيت اللافندر (***Lavandulaa ngustifolia*: Labiatae**) – الكافور (***Eucalyptus globules*** **: Myrtaceae**) – النعناع (***Mentha*** **spp .: Labiatae**) – القرنفل (***Syzygium Aromaticum*****: Myrtaceae**) – روز ماري (***Rosmarinus officinalis*****: Labiatae**) على النسب المئوية للموت في يرقات العمرالثالث لدودة الشمع الكبيرة ***Galleria mellonella*** **L. ((Lepidoptera: Pyralidae**).

اشارت النتائج الي ان اللافندر ، ثم النعناع ، الكافور والقرنفل سببوا اكبر نسبة موت مقارنة بالكنترول ، و تمت دراسة **LC50** للزيوت المختبرة و كانت ٧.١١-٩.٤٥-١١.٤٥-١٣.٦١-٦.٤٥٪ لزيت اللافندر – الكافور – النعناع – القرنفل – روز ماري ، على التوالي و قد تمت دراسة بعض التاثيرات البيوكيميائية للزيوت موضع الدراسة (المحتوي الكلي للبروتين- نشاط انزيم الكالين فوسفاتيز- ونشاط انزيم أستيل كولين استريز) على يرقات العمر الثالث المعامل ، و اوضحت النتائج ان معاملة يرقات دودة الشمع ادي إلى زيادة نسبة البروتين الكلي في جميع المعاملات مقارنة مع الكنترول. وكان هناك انخفاض في نشاط إنزيم الكالين فوسفاتيز وأستيل كولين استريز. وكانت الزيوت الأكثر فعالية روز ماري واللافندر ، على التوالي ، مقارنة مع الكنترول. وتمت دراسة التركيب الكيميائي للزيوت التي تم اختبارها ، حيث كانت المركبات الرئيسية الموجودة في زيت اللافندر ، هي لينالول (٣٨.٧٤ ٪) ، أسيتات لينالول (٢٩.٣٢ ٪) ، دي ليمونين (٩.٠٤ ٪)، ترياسيتين (٥.٣٧ ٪)، ١.٨- سينينول (٤.٧١ ٪)، الكافور (٢.٨٢ ٪)، **Exoproneoleacetate (1.8** ٪)، **a-pinene (1.79** ٪)، **a-terpineole (1.43** ٪)، **a-mycene (1.13** ٪). اما زيت الكافور يحتوي بشكل أساسي على ١.٨ سنتيل (٦٦.٣٨٪) ، ١-ليمونين (١٦.٢٧٪) .بين البنزين ، ميثيل (١-ميثيل إيثيل) (٧.٤٤٪) ، ١-فيلاندادرين (٤.٢٥٪) ، أ-بينين (١.٩٥٪) أ-تربينين (١.٢٤ ٪) ، وأندى ميرسين (١.١٦ ٪) ، و كانت المركبات الرئيسية في زيت القرنفل هي الكاروفيلين عبر التركيز النسبي بنسبة ٥٨.٤٦ ٪ ، الفينول، ٢ ميثوكسي -٤ (بروبنيل) ، هومولين ، كان ١.٨-**cineole** و **eugenol** بتركيز نسبي قدره ٩.٤٣٪ و ٧.٤٢٪ و ١.٣٠٪ و ١.٨٧٪ على التوالي. و كانت المركبات الرئيسية في زيت النعناع هي **l-menthol** بتركيز نسبي (٤٢.١٤٪) ، ١-**menthone** بتركيز نسبي (٢٧.٠٨٪). **carvone (8.90٪)**، **p-menthone (5.06٪)**، **pulegon (2.86٪)**، **1** خلات النمط (٦.٠١ ٪) ، والمنثول الجدد (٢.١٠ ٪). وكانت المكونات الرئيسية في زيت روز ماري هي الجلسرين ترياسيتين (٣٩.٢٢ ٪) ، ١.٨ سينول (١٦.٨٢ ٪) ، المنثول (١٠,٦٤٪) ، الكافور (٨,٧١٪)، بينين (٧,١٦ ٪) ، د-ليمونين (٧.٠٤ ٪)) ، وفيما يتعلق بالمركبات الثانوية ، دبور سيمين (١,١٧ ٪) ، خلات الإندوبرونيل (٠,٩١ ٪) ، الكامفين (٠.٣١ ٪) ، دلتا ٣ كارين (٠.٣٩ ٪) ، ١ - برونونول (٠.١٣ ٪).

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