

## Acaricidal Activity of Some Lamiaceae Plant Essential Oils against *Tetranychus urticae* Koch

**Sanaa, A. A. Amer<sup>\*</sup>; Fatma, S. A. Mohamed<sup>\*\*</sup>; A. M. Kamel<sup>\*\*\*</sup>;  
Zakeya, E. A. Darwish<sup>\*\*</sup>; Huda, E. Hussein<sup>\*</sup> and Marwa, E. El-Desouky<sup>\*</sup>**

<sup>\*</sup>Plant Protection Dept., National Research Center (N R C), Dokki, Giza, Egypt

<sup>\*\*</sup>Invertebrates, Zoology Dept., Faculty of Sci., AL-A Zhar Univ., for Girls, Cairo, Egypt

<sup>\*\*\*</sup>Medicinal and Aromatic Plants Dept., N R C, Dokki, Giza, Egypt

### ABSTRACT

Three plant essential oils, namely, *Mentha longifolia* L., *Salvia officinalis* L. and *Dracocephalum moldavicae* L. were analyzed by GC/MS and tested for their toxicity and repellency against the tetranychid mite *Tetranychus urticae* Koch. *D. moldavicae* proved to be the most effective against *T. urticae* followed by *S. officinalis* and *M. longifolia*. The LC<sub>50</sub> values were 1.57 & 0.58; 2.24 & 3.33 and 2.85 & 3.52 for adults and eggs, respectively. However, all essential oils tested showed high repellency, shortened female longevity and oviposition period as well as reduction in the total number of deposited eggs. This result may be correlated to the differences in the chemical structure of these oils based on GC/MS analysis.

**Key Words:** Acaricidal, Lamiaceae, *Tetranychus urticae*, *Mentha longifolia*, *Salvia officinalis*, *Dracocephalum moldavicae*, Essential oils.

### INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch is considered to be one of the most economically important pests. It is responsible for significant yield losses in many horticultural, ornamental and agricultural systems mainly annual crops and vegetables worldwide, and affects plants by direct feeding, thereby reducing the area of photosynthesis activity causing leaf abscission (Helle & Sabelis, 1985).

In recent years, the use of synthetic organic pesticides in crop pest control programs around the world has resulted in damage to the environment, pest resurgence, pest resistance to pesticides and lethal effects on non-target organisms (Gorman *et al.*, 2002; Cohen, 2006; Hardman *et al.*, 2006; Kim *et al.*, 2007 and van Leeuwen, *et al.*, 2010).

So, increasing concern about potential problems associated with continued long-term use of toxic pesticides requires development of biodegradable and environmentally safe alternatives.

Among alternative strategies, the use of aromatic plants and their essential oils represented a rich source of bioactive chemicals (Regnault-Roger, 1997).

Aromatic plants have minimal toxicity on mammals, minimal development of resistance and act as oviposition-deterrent, antifeedant and growth inhibitors (Ibrahim *et al.*, 2001 and Bakkali, *et al.*, 2008).

More than 2,000 species of plants are known to possess acaricidal properties (Klocke, 1989) and family Lamiaceae is one of them. This family has received the most attention in the search for biologically active natural products against many pests (e. g.: Mansour *et al.*, 1986; Tunc & Sahinkaya, 1998 and Calmasur *et al.*, 2006). So, much attention has been focused on these plants and their constituents as potential sources of commercial acaricides.

The objectives of the present work were:-

- 1- To identify the chemical constituents of wild mint: *Mentha longifolia* L., sage; *Salvia officinalis* L. and dragon head, *Dracocephalum moldavicae* L. oils.
- 2- To determine the efficiency of these oils and their acaricidal activities against *T. urticae*.
- 3- Deterrent effects of these oils and effects on some biological aspects of *T. urticae* were also studied.

### MATERIALS AND METHODS

#### Mite rearing:

*T. urticae* was collected from infested kidney bean, *Phaseolus vulgaris* L., and reared in the laboratory at N R C, under controlled conditions of 26±2°C and 70±5 % R.H.

#### Plant materials:

Plants that proven to contain essential oils were chosen from family Lamiaceae and were identified by Prof. Dr. Loutfy Boulos, Department of Plant Taxonomy, National Research Center *Mentha longifolia* was obtained from Mehalit Zaiad village -

Gharbia while *S. officinalis* was obtained from the Faculty of Agriculture, Cairo University farm, and *D. moldavicae* from N R C, experimental farm. All plants were collected at flowering stage.

#### Separation of essential oils:

A dried aerial part of each plant was crushed, then essential oils were extracted by hydro-distillation apparatus (Clevenger-type) for 3-hrs. The obtained oil from each plant was dried over anhydrous sodium sulfate, and after filtration, kept in the freezer until used.

Different concentrations of the oils were prepared using two drops of Triton X-100 to serve as emulsifier.

#### GC/MS analysis of essential oil:

The analysis of essential oil was performed using THERMO trace 2000 gas chromatography linked to FINNIGAN SSQ7000 mass spectrometer. Ionization mode: Eleven 70. Column: capillary column of fused silica, DB-5 (5% phenyl methyl siloxane), 30 m length and 0.25  $\mu\text{m}$  thickness. The carrier gas was helium at 1ml/min. The volume of the injected sample was 1 $\mu\text{l}$  in split less mode. Initial temp. 60°C for 15 min, and increasing from 60°C to 220°C, with a rate of 5°C/min., the final temp. was 250°C for 15 min., ionization voltage was 70 eV. The major components of essential oils were identified on the basis of comparison of their retention indexes and mass spectra with those of published data (Adams, 2001).

#### Treatments:

##### Toxicity effects of *Mentha longifolia*, *Salvia officinalis* and *Dracocephalum moldavicae* on *T. urticae* eggs and adult females:

Eggs (0-24 hr age) or adult females of the same age were transferred to the lower surface of raspberry leaf discs (3cm. diameter) and sprayed with different concentrations from each essential oil using glass atomizer. Each test contained 5 concentrations and each concentration had 5 replicates (20 females/replicate and 40 eggs/replicate). In each test, a control was included using distilled water and two drops of Triton X-100. The mortality of the females was recorded after 48 hr and after 6 days for eggs.

##### Repellency test procedure for *T. urticae* females:

Raspberry leaf discs (5 cm diam.) were prepared with lower surfaces upside-down in Petri- dish, lined with moist cotton wool. One half of each disc was painted with series of aqueous concentrations beginning with LC<sub>50</sub> values of each essential oil and each LC<sub>50</sub> value was diluted to each half and so on, till the fourth concentration, while the other half left

untreated served as control. Ten females were put on the mid-rib of the leaf. Orientation of the females on treated or control half was recorded after 2, 4, 6, 24 and 48-hr. The number of eggs laid on each half were recorded after 48 hr. Tests were replicated 10 times for each concentration.

##### Effect of LC<sub>50</sub> of essential oils on some biological aspects of *T. urticae* females:

Newly emerged mated females were sprayed with LC<sub>50</sub> of each essential oil using glass atomizer, then twenty females of the surviving individuals were singly transferred to treated raspberry leaf discs with LC<sub>50</sub> of each essential oil in Petri-dishes. Twenty replicates were used in the experiment and a control was included also. The biological parameters tested were pre-oviposition and oviposition periods, female longevity, total number of eggs deposited by females and also egg hatchability.

All experiments were carried out in the laboratory at 26 $\pm$ 2 °C and 70 $\pm$ 5 % R.H.

##### Statistical analysis:

Corrected mortality was counted according to Abbott's formula. Corrected mortality (%) was submitted to probit analysis according to the formula described by Finney (1971).

The repellency was calculated according to Lwande *et al.*, (1985):

$$D = (1 - T/C) \times 100$$

Where:

T = mean number of eggs/female in treatment.

C = mean number of eggs/female in control.

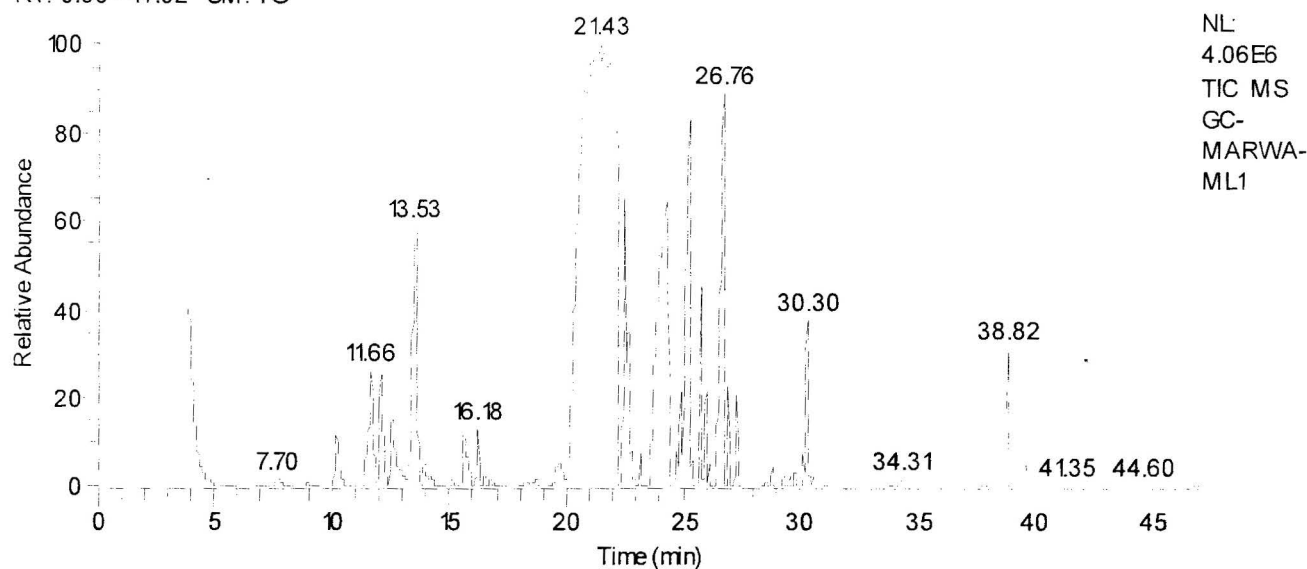
Biological data were statistically analyzed using the SPSS program, version 11; a significance of results was obtained by one-way ANOVA. Duncan's Multiple Range Test was used to compare means according to Duncan.

## RESULTS AND DISCUSSION

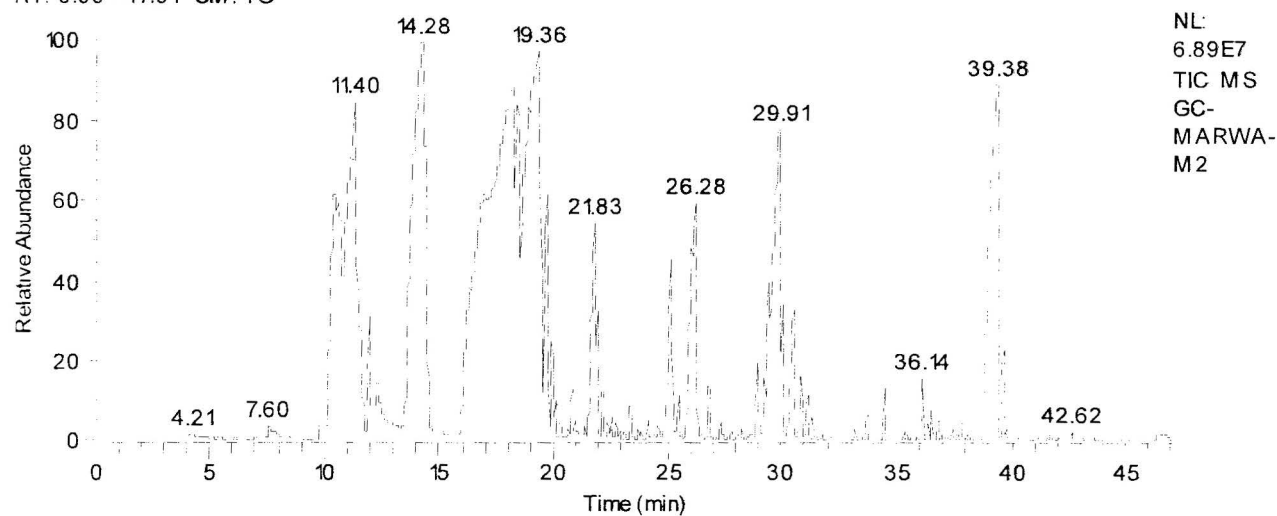
#### GC/MS analysis of essential oils

Table & Fig. (1) show the quantitative analysis of *M. longifolia* oil. The obtained data showed that oil was mainly characterized by high concentration of total terpene compounds (84.19%) and low concentration of hydrocarbons (2.55%). Cis-Piperitone oxide and trans-piperitone oxide were the major compounds (34.42% and 19.31%), respectively. Similarly, *S. officinalis* oil (Table & Fig. 2) and *D. moldavicae* oil (Table & Fig. 3), were characterized by higher concentration of total terpenes (88.35 & 93.32%), respectively. The major compound of *S. officinalis* oil was found to be 1, 8- cineole (18.64%) and to be geranyl acetate (29.99%) in *D. moldavicae* oil.

RT: 0.00 - 47.02 SM: 7G

Fig. (1): Gas liquid chromatogram of wild mint oil, *Mentha longifolia*.

RT: 0.00 - 47.01 SM: 7G

Fig. (2): Gas liquid chromatogram of sage oil, *Salvia officinalis*.

RT: 0.00 - 47.01 SM: 7G

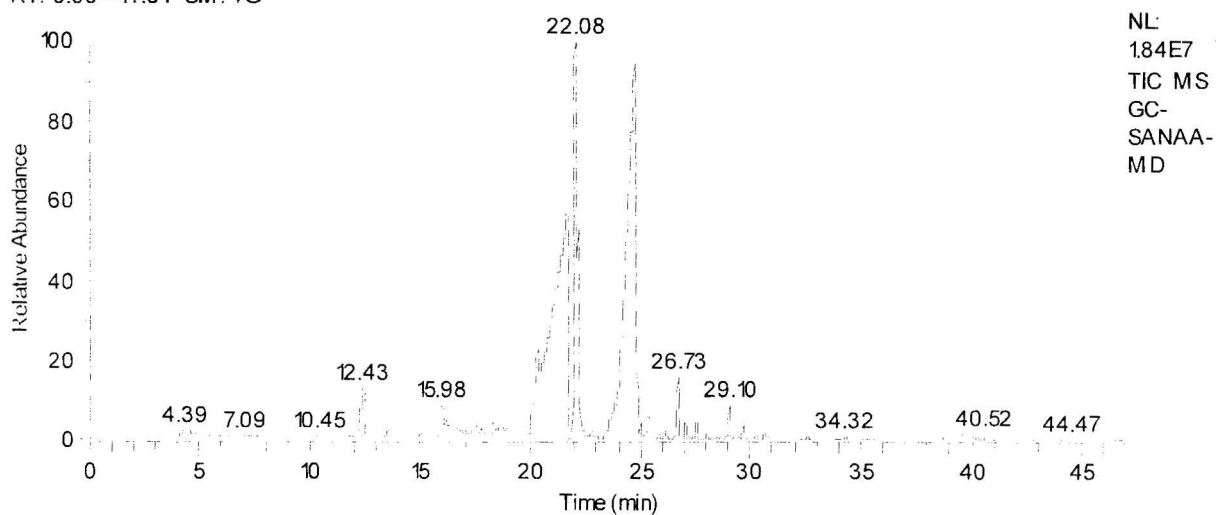
Fig. (3): Gas liquid chromatogram of dragon head oil, *Dracocephalum moldavicae*.

Table (1): GC /MS analysis of the essential oil of wild mint, *Mentha longifolia*

I. C No.	Compound	R <sub>t</sub> (min.)	Area (%)
* 1	$\alpha$ -Pinene	10.18	0.86
* 2	Sabinene	11.66	1.98
* 3	Myrcene	12.09	1.38
* 4	1,8-Cineole	13.53	4.28
* 5	Ocimene	16.51	0.08
* 6	Thujyl alcohol	18.71	0.08
* 7	Menthene isomer	19.63	0.43
* 8	Cis-Piperitone oxide	21.43	34.42
* 9	Trans-Piperitone oxide	21.58	19.31
* 10	Elemene	22.49	3.60
* 11	trans-Caryophyllene	25.27	6.61
* 12	Farnesene	25.78	1.38
* 13	Humulene	25.97	0.71
* 14	Cubebene	26.74	6.24
* 15	bicyclogermacrene	26.93	0.40
* 16	Cadinene	27.28	0.62
* 17	Caryophyllene oxide	28.82	0.10
* 18	Cadinol	30.29	1.71
	Total area of terpens		84.19
* 19	2-Hexenal	7.71	0.14
* 20	3-Octanol	12.56	1.04
* 21	Isopentyl pentanoate	15.59	0.93
* 22	3-octanyl acetate	16.18	0.44
	Total area of hydrocarbons		2.55

Table (2): GC/MS analysis of the essential oil of sage, *Salvia officinalis*

I. C No.	Compound	R <sub>t</sub> (min.)	Area (%)
* 1	$\alpha$ - Pinene	10.44	5.11
* 2	Camphene	11.38	8.04
* 3	$\beta$ -pinene	11.98	1.08
* 4	1,8-Cineole	14.25	18.64
* 5	trans-Caryophyllene	16.31	1.95
* 6	Thujone	16.62	0.99
* 7	Camphor	19.37	13.26
* 8	Borneol	19.73	2.98
* 9	Endobornyl acetate	21.82	4.31
* 10	Trans-Caryophyllene	25.17	2.71
* 11	Aromadendrene	25.51	0.32
* 12	$\alpha$ - Humulene	26.26	5.51
* 13	Ledene	26.81	0.38
* 14	(-)-Caryophyllene oxide	28.99	0.64
* 15	Veridiflorol	29.89	8.59
* 16	Aristolene epoxide	30.86	2.51
* 17	13-Epimanool	39.37	11.33
	Total area of terpens (%)		88.35

Table (3): GC/MS analysis of the essential oil of dragon head, *Dracocephalum moldavicae*

I.C No.	Compound	R <sub>t</sub> (min.)	Area (%)
* 1	limonene	13.56	0.23
* 2	Delta-3-carene	14.16	0.15
* 3	Trans- Linalool oxide	14.99	0.13
* 4	D- Linalool	15.97	1.46
* 5	Trans-2-Pinanol	16.16	1.02
* 6	L- linalool	16.50	0.52
* 7	Nerol oxide	17.48	0.25
* 8	Limonene oxide	17.78	0.24
* 9	Verbenol	18.32	0.54
* 10	citrial	20.32	2.41
* 11	trans-Geraniol	21.63	24.07
* 12	Nerol	22.07	28.16
* 13	Neryl acetate	23.86	0.21
* 14	$\alpha$ - humulene	26.11	0.24
* 15	Geranyl acetate	4.79	29.99
* 16	Cubebene	26.72	1.61
* 17	Farnesene	27.06	0.38
* 18	Cadinene	27.57	0.33
* 19	Caryophyllene oxide	29.09	1.18
* 20	$\alpha$ - Cadinol	30.32	0.11
* 21	Lavandulyl acetate	32.59	0.09
	Total area of terpens		93.32
* 22	6-Methyl-5-hepten-2-one	12.41	2.45
* 23	2,6-octadienoic acid ,3,7-dimethyl-,methyl ester	22.81	0.26
* 24	9,12,15-octadecatrienoicacid ,2,3-bis (acetyloxy) propyl ester	24.99	0.11
* 25	Cis - caryophyllene	25.35	0.89
* 26	5,9,-undecadien-2-one,6,10-dimethyl-,(E)-(GAS)	25.86	0.12
* 27	3- Butyl- 4-methyl-2-methylthiophene	28.05	0.14
* 28	2- pentadecanone,6,10,14-trimethyl-	34.32	0.11
* 29	Neopentyl difluoro(3-phenyl-phenyl) methane sulfonate	39.59	0.25
* 30	7-hydroxy- 6-keto-drimanol	40.12	0.12
* 31	2,6- octadiene,3,7- dimethyl-1-(2-propenyloxy)-	40.52	0.13
	Total area of hydrocarbons		4.58

R<sub>t</sub>: retention time

\*: Confirmation of compounds by published data.

I. c: Identification of compound

Table (4): Toxicity of *Mentha longifolia*, *Salvia officinalis* and *Dracocephalum moldavicae* plant essential oils against adult females and eggs of *Tetranychus urticae*.

Toxicity parameter	<i>D. moldavicae</i> oil		<i>S. officinalis</i> oil		<i>M. longifolia</i> oil	
	adults	eggs	adults	eggs	adults	eggs
I.C <sub>50</sub> %	1.57	0.58	2.24	3.33	2.85	3.52
Lower Limit	1.44	0.17	1.90	2.81	2.22	2.79
Upper Limit	1.73	0.82	2.79	4.32	4.85	5.01
Folds	1.81	6.07	1.27	1.06	1	1
Toxicity index	100	100	70	17	55	16
Slope	3.93	2.29	1.79	3.01	2.27	2.07
I.C <sub>90</sub> %	3.32	2.11	11.60	8.88	10.43	14.62

### Toxicity effects of *Mentha longifolia*, *Salvia officinalis* and *Dracocephalum moldavicae* on *T. urticae* eggs and adult females

Table (4) shows that all essential oils tested had toxic effects against females and eggs of *T. urticae*. *D. moldavicae* oil was the most potent oil tested against females ( $LC_{50}= 1.57$  &  $LC_{90}=3.32\%$ ) and eggs ( $LC_{50}= 0.58$  &  $LC_{90}= 2.11$ ) of *T. urticae*, while *M. longifolia* oil was the least toxic oil tested on females ( $LC_{50}= 2.85$  &  $LC_{90}=10.43\%$ ) and eggs ( $LC_{50}= 3.52$  &  $LC_{90}= 14.62$ ). Eggs of *T. urticae* treated with *D. moldavicae* oil were more susceptible than females. Results also showed that *S. officinalis* and *M. longifolia* oils were more effective to females than eggs. When *S. officinalis* oil was used as a fumigant for *T. urticae*, it had more toxic effect on females than on eggs (Choi *et al.*, 2004).

Several authors demonstrated the efficiency of various essential oils derived from plants belonging to the family Lamiaceae, (e. g.: *Satureja hortensis* L., *Thymus vulgaris* L., *Ocimum basilicum*, *Micromeria fruticosa* L., *Nepeta racemosa* L., *Origanum vulgare* L. and *Marjorana hortensis* L.) against *T. urticae* (Aslan *et al.*, 2004; Calmasur *et al.*, 2006 and Afify *et al.*, 2009). It is worth to mention that Baker (2003) proved that females of *T. urticae* were more sensitive to *Petroselinum sativum* L., *Coriandrum sativum* oils (family: Apiaceae) than eggs.

The essential oils composition including, limonene, 1,8- cineole, linalool, anethole, camphor, geraniol  $\alpha$ - terpinene, found in the this study, were reported to be responsible for the toxic effect on eggs and females of *T. urticae* and

*T. cinnabarinus* Boisd by Erler & Tunc. (2005). El-Zemity *et al.*, (2009) and Badawy *et al.*, (2010).

### Repellency test for *T. urticae* females:

Table (5) shows that the tested plant essential oils strongly deterred *T. urticae* females (99.85, 99.51 and 97.19%, respectively) at  $LC_{50}$  of each essential oil. The deterrent effect increased at all concentrations in dragon head oil, while the percentage of repellency decreased at lower concentrations of sage oil.

Essential oils of *Mentha viridis* L., *Mentha piperita* L., *Rosmarinus officinalis* L., *M. hortensis*, *O. basilicum*, *Lavandula officinalis* L. and *Mintha spicata* L. deterred females of *T. urticae* from settlement and feeding on treated parts. Amer *et al.*, (2001); Momen *et al.*, (2001); Refaat *et al.*, (2002) and Omar *et al.*, (2009)

### Effect of $LC_{50}$ of essential oils on some biological aspects of *T. urticae* females:

Data in Table (6) indicated that all treatments at level of  $LC_{50}$  were highly significantly shortened the oviposition period, female longevity and reduced the number of eggs laid per female; while prolonged pre-oviposition period of eggs compared with control.

*Dracocephalum moldavicae* oil had a highly significant remarkable shortness on oviposition period and female longevity, (ANOVA;  $F= 269.46$  &  $190.54$ ,  $df_{1,23,76}$ ,  $P= 0.000$ ), respectively.

Pre-oviposition period was significantly prolonged by *M. longifolia* oil, (ANOVA;  $F= 55.94$ ,

Table (5): Relative distribution and repellency of *Tetranychus urticae* on treated leaf discs with *Mentha longifolia*, *Salvia officinalis* and *Dracocephalum moldavicae* essential oils

Concentration (%)	%Distribution of mites on treated leaf discs after					Avg. no. of eggs/female after 48h		Repellency %
	2h	4h	6h	24h	48h	T	C	
<i>M. longifolia</i> oil								
2.85	2	2	1	1	1	0.05	10.20	99.51
1.42	2	2	2	2	1	0.06	11.61	99.48
0.71	2	3	3	2	2	0.10	12.72	99.21
0.35	15	11	6	13	22	1.32	12.10	89.67
<i>S. officinalis</i> oil								
2.24	1	2	3	2	7	0.3	10.68	97.19
1.12	2	2	2	5	19	0.61	10.76	94.33
0.56	2	4	3	14	37	3.33	7.71	55.81
0.28	11	12	13	28	51	5.25	6.03	12.94
<i>D. moldavicae</i>								
oil 1.57	1	1	0	0	0	0.01	6.56	99.85
0.78	1	0	1	0	1	0.03	6.74	99.55
0.39	4	0	1	0	6	0.11	7.44	98.52
0.19	5	0	1	6	9	0.14	8.53	98.36

T= treated.

C= control.

Table (6): Effect of *Mentha longifolia*, *Salvia officinalis* and *Dracocephalum moldavicae* essential oils on some biological aspects of *Tetranychus urticae*

Oils	Biological aspects (Mean ± S.E.)						% Egg hatchability	% Reduction in eggs no.
	Pre-oviposition period	Oviposition period	Female longevity	Total no. of eggs / female	No. of eggs/ female/day	Incubation period		
<i>D. moldavicae</i>	1.40 ±0.15 <sup>b</sup>	1.40 ±0.23 <sup>c</sup>	3.25 ±0.28 <sup>c</sup>	3.15 ± 0.50 <sup>c</sup>	2.1 ±0.23 <sup>c</sup>	3.25 ±0.33 <sup>b</sup>	87.31	95.2
<i>M. longifolia</i>	3.01 ±0.20 <sup>a</sup>	2.48 ±0.28 <sup>c</sup>	8.20 ±0.65 <sup>b</sup>	5.80 ±0.72 <sup>c</sup>	2.22 ±0.20 <sup>c</sup>	3.63 ±0.28 <sup>ab</sup>	80.31	91.34
<i>S. officinalis</i>	1.43 ±0.14 <sup>b</sup>	5.18±0.28 <sup>b</sup>	6.90 ±0.27 <sup>b</sup>	26.65 ±1.94 <sup>b</sup>	5.11 ±0.19 <sup>b</sup>	4.27 ±0.11 <sup>a</sup>	61.21	65.57
Control	0.33 ±0.02 <sup>c</sup>	16.71 ±0.71 <sup>a</sup>	23.35 ±1.02 <sup>a</sup>	128.15 ±7.28 <sup>a</sup>	7.64 ±0.25 <sup>a</sup>	3.67 ±0.02 <sup>ab</sup>	98.6	-
F	55.94**	269.46**	190.54**	242.59**	140.97**	3.45*		
df <sub>1,2</sub>	3,76	3,76	3,76	3,76	3,76	3,76		
P	0.000	0.000	0.000	0.000	0.000	0.021		

Different letters in a column denote a significant difference.

\* =  $P \leq 0.05$

\*\* =  $P \leq 0.01$

df<sub>1,2</sub> 3, 76,  $P = 0.000$ ). The incubation period of eggs resulted from females treated with *S. officinalis* oil significantly prolonged, (ANOVA;  $F = 3.45$ , df<sub>1,2</sub> 3, 76,  $P = 0.021$ ).

A highly significant reduction in the total number of eggs laid per female for all essential oils was recorded. Interestingly, oils of *D. moldavicae* and *M. longifolia* showed the highest reduction in the fecundity (95.2 & 91.34%) and the highest percentage of egg hatchability (87.31 & 80.31%), respectively. The lowest reduction in the fecundity (65.57%), and the lowest eggs hatchability (61.21%) was reported in *S. officinalis* oil.

These results are in agreement with that of Ibrahim and Amer (1992) who demonstrated that essential oil from *Callistemon lanceolatus* Dc. (family: Myrtaceae) had a strong effect on some biological aspects of *T. urticae* females since the female longevity and oviposition period were shortened while, the pre-oviposition and incubation periods of deposited eggs were prolonged. Similarly, oils from *T. vulgaris*, *M. viridis*, *M. piperita*, *R. officinalis*, *M. hortensis*, *L. officinalis*, and *M. spicata* caused a reduction in the total number of eggs laid by females *T. urticae*. (EL-Gengaihi *et al.*, 1996; Amer *et al.*, 2001; Momen *et al.*, 2001; Refaat *et al.*, 2002; and Omar *et al.*, 2009).

The present results clearly confirm that essential oils from aromatic plants belonging to the family Lamiaceae revealed acaricidal activity against eggs and females of *T. urticae*. Essential oils from dragon head plant showed the highest acaricidal activity against *T. urticae* and this result could be due to the higher concentrations of terpenes compound, transgeraniol (24.07%), nerol (28.16%) and geranyl acetate (29.99%). Each compound has a chemical structure allows the compound to penetrate and go directly to the active site to make its action. So, the

differences of the biological activity of oils depend on the differences of the chemical structure of the compounds (Carveior *et al.*, 1982).

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