

**PHYTOCHEMICAL AND TOXICOLOGICAL STUDIES ON  
SOME ESSENTIAL OILS OF AROMATIC PLANTS AGAINST  
*TETRANYCHUS URTICAE* KOCH.**

**A.A. BARAKAT<sup>1</sup>, H.M.A. BADAWEY<sup>1</sup>, A.M.I. FARRAG<sup>2</sup> AND E.  
M. BAKR<sup>2</sup>**

<sup>1</sup>*Dept. of Econ. Entomology and Pesticides, Fac. Agric., Cairo Univ.*

<sup>2</sup>*Dept. of Veg. Acar., Plant Prot. Res. Inst., Agric. Res. Center.*

(Received 7-5-2006)

### INTRODUCTION

The search for natural compounds having pest suppressing potential continues in an attempt to find alternatives which pose less risk to the humans and the environment than the conventional pesticides. Among natural products there are certain essential oils currently used in the food, perfume, cosmetic and pharmaceutical industries, which is promising in pest control particularly in controlled environment such as greenhouse or granaries. The potential of essential oils to act as fumigants has been demonstrated for stored product pests (Klingauf *et al.*, 1983; El-Nahal *et al.*, 1989; Shaaya *et al.*, 1991; Rice & Coats 1994 and Sarac & Tunc 1995) as well as for parasitic mites of honey bee (Delaplane, 1992 and Calderone & Spival, 1995). These results indicate that essential oils may be efficacious and safe replacements for conventional synthetic fumigants. There is few research on the effects of essential oils of spice plants and their components on mites, from which was a work dealt with the influence of phenolic and monoterpene compounds on *Tetranychus urticae* Koch (Larson and Berry 1984). The present study aimed to identify the crude oil components of parsley seed (*Petroselinum sativum* L.), coriander herb (*Coriandrum sativum* L.) and sweet basil herb (*Ocimum basilicum* Linn.) by GC/MS analysis and to assess the toxicity of their main components; eugenol, limonene, decanal, undecanal, linalool and eucalyptol against the different stages of *T. urticae*. It also aimed to study the acaricidal activity of parsley and coriander fractions and identify their promising fractions.

## MATERIAL AND METHODS

### 1- Identification of essential oil components of parsley seeds and coriander herb oils by GC/MS.

The essential oils of parsley seed, coriander herb, sweet basil herb and their promising fractions were identified by GC/MS analysis. Hewlett Packard 6890 gas chromatography apparatus linked to HP 5973 mass-selective detector (MSD) was used. HP-SMS cross linked 5% HP-ME Siloxane column (30m length X 0.25 mm X 0.25  $\mu$ m film thickness). The injector temperature was 200 °C, oven temperature was programmed from 70 to 250 °C at a rate of 4 °C / minutes with an initial holding time of 8 minute, oven temperature was then increased to 250 °C with a final holding time of 5 minute, carrier gas helium. MSD conditions were as follows: Capillary direct interface temperature, 250 °C; ion source temperature 250 °C; ionization voltage 70 eV; mass range 33-300 mu; electron multiplier voltage 2200 V.

### 2- Treatment of eggs, protonymphs and adults

The tested *T. urticae* were reared under laboratory conditions of  $25 \pm 0.5$  °C and  $70\% \pm 5$  R.H. according to Darwish (1991). The method of Barakat *et al.*, (1985) was followed to study the efficacy of the standard chemical compounds; eugenol, limonene, decanal, undecanal, linalool and eucalyptol against the eggs of *T. urticae*. Series of aqueous concentrations were prepared with Triton X-100 as surfactant at the rate of 0.1 %. The one day old eggs were sprayed by a glass atomizer with a serial of concentrations for each oil. Eggs were incubated at  $25 \pm 0.5$  °C and  $70\% \pm 5$  R.H. for six days till hatching and the percentage of hatchability was determined and corrected by Abbott's formula (1925). The LC<sub>50</sub>, LC<sub>90</sub> and slope values were calculated according to Finney (1971).

Newly emerged protonymphs were used to evaluate the acaricidal activity of the tested materials by using the direct spray technique of Abo-Shabana (1980). Twenty protonymphs were transferred to the lower surface of lima bean disc (1 inch in diameter) using fine brush. Four discs were used as four replicates for each treatment. Discs were placed upside-down on moist cotton wool in petri dishes. The disc surfaces carrying the protonymphs were directly sprayed with the aqueous solution of the tested material using a glass atomizer. Control treatment was sprayed with aqueous solution of Triton X-100 at a rate of 0.1 %. The mortality was calculated after 24 h of spraying and corrected by Abbott's formula (1925). The LC<sub>50</sub>, LC<sub>90</sub> and slope values were calculated according to Finney (1971).

Direct spray technique of Abo-Shabana (1980) was also used to determine the acaricidal activity of the tested essential oil components and the fractions of parsley and coriander oils against adult females.  $LC_{50}$  values of the tested crude oils were used to evaluate the acaricidal activity of each fraction. This was made by applying the fractions at the same concentration as those of the  $LC_{50}$  of the crude oil.

### 3- Separation of essential oil fractions of parsley and coriander

Parsley seed and coriander herb oils were fractionated by TLC using silica gel GF plates with benzene as mobile phase according to the method of Su and Horvat (1981). The TLC spots were detected under UV lamp at 254 nm. A sample of candidate essential oils (0.4 g/ml acetone) was applied and after developing of the plates, the silica gel layer at each fraction was abraded and washed on filter paper. The fraction was eluted with acetone, which was evaporated using rotary evaporator. The remaining residue was weighed and kept under freezing till used.

## RESULTS AND DISCUSSION

### 1- Identification of crude oil components of parsley seed

Parsley seed, sweet basil herb and coriander herb oils were chosen to be identified by GC/MS analysis because they gave high efficacy against the different stages of *T. urticae* according to the previous data of our working group (Badawy 2005). Table (1) presents the compound name, retention time ( $R_t$ ), percent area, molecular weight (M.W.) and chemical formula of the detected compounds of parsley seed crude essential oil. Forty-six compounds were identified in this crude oil by their scan and after being compared with library data of the instrument. Data indicated that the principal chemical group in the parsley seed crude essential oil was phenylpropanoid compounds with an amount of 84.42 %, as percent area. These compounds were apiol isomers (42) and (43) amounting to 28.69% and 12.80 % with total percentage of 41.49 and  $R_t$  29.27 & 30.66 min.; followed by myristicine isomers (28, 29, 30, 31 and 38) amounting to 5.57, 6.62, 9.46, 4.55 and 13.40 % with total percentage of 39.60 % and  $R_t$  21.22, 21.72, 22.25, 22.53 and 26.37 min, respectively.

In addition to apiol and myristicine, another four phenylpropanoid components were detected; elemicin (33) with 2.94 %, eusarone (34) with 0.14 %, trans-asarone (37) with 0.22 % and delta-guaiene (27) with 0.03 %. Sixteen monoterpene compounds appeared with total percentage area of 7.42. These compounds were alpha pipene (2) with 2.48 % area, beta-pinene (4) with 2.23 % area, limonene (8) with 2.03 % area, gamma-terpinene (10) with 0.16 % area, beta-myrcene (5) with 0.12 % area, alpha-

terpinolene (12) with 0.12 % area, alpha-santalol (20) with 0.08 % area, camphene (3) with 0.05 % area, alpha-thujene (1) with 0.05 % area, trans-beta-ocimene (9) with 0.04 % area, pinocarvone (17) with 0.02 % area, trans sabinene hydrate (11) with 0.01 % area, 1(7) ,5,8-o-menthatriene (13) with 0.01 % area, alpha-terpipene (7) with 0.01 % area and alpha phellandrene (6) with 0.01 % area.

In the crude oil of parsley seeds, sesquiterpenes were present in fourteen compounds with an amount of 3.25 % of crude oil. The other detected compounds in Table (1) were in small amounts and identified as phthalide, aldehyde, hydrocarbon, acetamide and phenolic compounds.

### **2- Identification of crude oil components of coriander herb**

Data in Table (2) showed that the dominant chemical group in coriander herb oil was aldehydes with total amount of 73.74 %. The dominant aldehyde was 2-decenal (17), amounting to 37.23 % as percent area with  $R_t$  17.68 min., followed by decanal (15), 2-dodecenal (22), undecenal (19), koiganal (26), undecanal (18) and dodecanal (21) amounting to 17.90, 7.68, 4.45, 2.88, 2.29 and 1.31 %, respectively (as percent area) with  $R_t$  15.31, 23.96, 20.66, 29.83, 18.72 and 21.96 min., respectively. Monoterpenes were 17.89 as percent area. Linalool (12) was the dominant monoterpene, amounting to 14.16 % with  $R_t$  11.42, followed by gamma terpinene (10), alpha pinene (3), camphor (13), limonene(9), terpinene-4-ol(14), camphene (4) and beta pinene (5) amounting to 1.40, 0.96, 0.78, 0.24, 0.18, 0.10 and 0.07 % with  $R_t$  9.47, 5.27, 12.70, 8.35, 13.97, 5.71 and 6.57 min., respectively. In addition to aldehydes and monoterpenes some compounds which belong to hydrocarbons (1.73%), quinoline (1.34%) and carbazole (1.14%) were detected.

### **3- Identification of crude oil components of sweet basil herb**

Forty-three compounds were identified in this crude oil by their scan, after comparing them with library data of the instrument. Data in Table (3) showed that linalool (15) was the dominant compound, with amount of 41.62 % as percent area and  $R_t$  11.96 min.

Linalool was followed by eugenol (33) amounting to 20.51 % with  $R_t$  20.90 min, eucalyptol (10) amounting to 9.19 % with  $R_t$  8.61 min, alpha-bergamotene (38) with an amount of 7.42% with  $R_t$  22.91 min. and germacrene-d isomers (40, 42) amounting to 5.08 % with  $R_t$  23.68 and 24.33 min. In addition to the first three dominant monoterpenes i.e., linalool, eugenol and eucalyptol with an amount of 71.32%, twenty three monoterpene compounds were found in crude

**TABLE (I)**  
Chemical constituents in the crude essential oil of parsley seeds.

No.	Compound	R <sub>i</sub> (min)	Percent Area	M.W.	Chemical formula
1	alpha.-thujene	4.19	0.05	136.24	C <sub>10</sub> H <sub>16</sub>
2	alpha pipene	4.52	2.48	136.24	C <sub>10</sub> H <sub>16</sub>
3	Camphene	4.90	0.05	136	C <sub>10</sub> H <sub>16</sub>
4	Beta-pinene	6.02	2.23	136.24	C <sub>10</sub> H <sub>16</sub>
5	Beta-myrcene	6.39	0.12	136	C <sub>10</sub> H <sub>16</sub>
6	Alpha-phellandrene	6.90	0.01	136.24	C <sub>10</sub> H <sub>16</sub>
7	Alpha-terpipene	7.37	0.01	136	C <sub>10</sub> H <sub>16</sub>
8	Limonene	8.01	2.03	136.24	C <sub>10</sub> H <sub>16</sub>
9	trans-beta-ocimene	8.23	0.04	136	C <sub>10</sub> H <sub>16</sub>
10	Gamma-terpinene	9.06	0.16	136	C <sub>10</sub> H <sub>16</sub>
11	trans sabinene hydrate	9.54	0.01	154.14	C <sub>10</sub> H <sub>18</sub> O
12	Alpha-terpinolene	10.21	0.12	136.13	C <sub>10</sub> H <sub>16</sub>
13	1(7),5,8-o-menthatriene	11.20	0.01	134.11	C <sub>10</sub> H <sub>14</sub>
14	n-isobutylphenoxy acetamide	11.44	0.01	207.13	C <sub>12</sub> H <sub>17</sub> NO <sub>2</sub>
15	trans-alpha-bisabolene	12.10	0.01	204.19	C <sub>15</sub> H <sub>24</sub>
16	Benzenepropanal	12.36	0.01	134.07	C <sub>9</sub> H <sub>10</sub> O
17	Pinocarvone	13.27	0.02	150	C <sub>10</sub> H <sub>14</sub> O
18	trans-beta-farnesene	14.13	0.19	204.19	C <sub>15</sub> H <sub>24</sub>
19	Myrtenal	14.58	0.22	150	C <sub>10</sub> H <sub>14</sub> O
20	Alpha-santalol	15.48	0.08	152.12	C <sub>10</sub> H <sub>16</sub> O
21	Gamma-cadinene	15.99	0.03	204.19	C <sub>15</sub> H <sub>24</sub>
22	Beta-selinene	16.98	0.88	204.19	C <sub>15</sub> H <sub>24</sub>
23	Beta-selinene	17.65	0.48	204.19	C <sub>15</sub> H <sub>24</sub>
24	Alpha-selinene	18.38	0.12	204.19	C <sub>15</sub> H <sub>24</sub>
25	beta bisabolene	19.23	0.10	204.19	C <sub>15</sub> H <sub>24</sub>
26	trans-caryophyllene	19.45	0.21	204.19	C <sub>15</sub> H <sub>24</sub>
27	Delta-guaiene	19.61	0.03	204.19	C <sub>15</sub> H <sub>24</sub>
28	Myristicine	21.22	5.57	192.21	C <sub>11</sub> H <sub>12</sub> O <sub>3</sub>
29	Myristicine	21.72	6.62	192.21	C <sub>11</sub> H <sub>12</sub> O <sub>3</sub>
30	Myristicine	22.25	9.46	192.21	C <sub>11</sub> H <sub>12</sub> O <sub>3</sub>
31	Myristicine	22.53	4.55	192.21	C <sub>11</sub> H <sub>12</sub> O <sub>3</sub>
32	Zingiberene	22.74	0.08	204.36	C <sub>15</sub> H <sub>24</sub>
33	Elemicin	23.66	2.94	208.26	C <sub>12</sub> H <sub>16</sub> O <sub>3</sub>
34	Euasarone	24.14	0.14	208.26	C <sub>12</sub> H <sub>16</sub> O <sub>3</sub>
35	Beta-selinene	24.51	0.61	204.19	C <sub>15</sub> H <sub>24</sub>
36	Alpha-selinene	24.74	0.29	204.19	C <sub>15</sub> H <sub>24</sub>
37	Trans-asarone	25.09	0.22	208.26	C <sub>12</sub> H <sub>16</sub> O <sub>3</sub>
38	Myristicine	26.37	13.40	192.21	C <sub>11</sub> H <sub>12</sub> O <sub>3</sub>
39	Elemicin	27.12	1.94	208.26	C <sub>12</sub> H <sub>16</sub> O <sub>3</sub>
40	Euasarone	27.49	0.08	208.26	C <sub>12</sub> H <sub>16</sub> O <sub>3</sub>
41	Carotol	28.19	0.25	222.2	C <sub>13</sub> H <sub>26</sub> O
42	Apiol	29.27	28.69	222.24	C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>
43	Apiol	30.66	12.80	222.24	C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>
44	Isobutylidenphthalide	31.47	0.96	188.08	C <sub>12</sub> H <sub>12</sub> O <sub>2</sub>
45	Isobutylidenphthalide	32.02	1.53	188.08	C <sub>12</sub> H <sub>12</sub> O <sub>2</sub>
46	Hexadecane	32.31	0.16	214.17	C <sub>16</sub> H <sub>22</sub>

TABLE (II)

Chemical constituents in crude essential oil of coriander herb.

No.	Compound	R <sub>f</sub> (min)	Percent Area	M.W.	Chemical formula
1	Octane	2.70	0.03	112	C <sub>8</sub> H <sub>18</sub>
2	Nonane	4.29	0.66	128	C <sub>9</sub> H <sub>20</sub>
3	Alpha-pinene	5.27	0.96	136.24	C <sub>10</sub> H <sub>16</sub>
4	Camphene	5.71	0.10	136	C <sub>10</sub> H <sub>16</sub>
5	beta-pinene	6.57	0.07	136.24	C <sub>10</sub> H <sub>16</sub>
6	Decane	7.27	0.14	142	C <sub>10</sub> H <sub>22</sub>
7	Octanal	7.46	0.56	130	C <sub>8</sub> H <sub>16</sub> O
8	para cymene	8.22	0.44	134.22	C <sub>10</sub> H <sub>14</sub>
9	Limonene	8.35	0.24	136.24	C <sub>10</sub> H <sub>16</sub>
10	gamma-terpinene	9.47	1.40	136	C <sub>10</sub> H <sub>16</sub>
11	Cyclooctane	10.04	0.32	113	C <sub>8</sub> H <sub>17</sub>
12	Linalool	11.42	14.16	154.25	C <sub>10</sub> H <sub>18</sub> O
13	Camphor	12.70	0.78	152.24	C <sub>10</sub> H <sub>16</sub> O
14	terpinene-4-ol	13.97	0.18	154.25	C <sub>10</sub> H <sub>18</sub> O
15	Decanal	15.31	17.90	156.2	C <sub>10</sub> H <sub>20</sub> O
16	1-methyl-2-cyclopenten-1-ol	16.50	0.27	98.07	C <sub>6</sub> H <sub>10</sub> O
17	2-decenal	17.68	37.23	154.25	C <sub>10</sub> H <sub>18</sub> O
18	Undecanal	18.72	2.29	170.17	C <sub>11</sub> H <sub>22</sub> O
19	Undecenal	20.66	4.45	168.17	C <sub>11</sub> H <sub>20</sub> O
20	geranyl acetate	21.12	0.41	196.15	C <sub>12</sub> H <sub>20</sub> O <sub>2</sub>
21	Dodecanal	21.96	1.31	184.18	C <sub>12</sub> H <sub>24</sub> O
22	2-dodecenal	23.96	7.68	182.18	C <sub>12</sub> H <sub>22</sub> O
23	Tridecanal	25.05	0.26	198.2	C <sub>13</sub> H <sub>26</sub> O
24	Trans-2-tridecenal	26.83	0.31	196.2	C <sub>13</sub> H <sub>24</sub> O
25	Tetradecanal	28.04	0.35	212.21	C <sub>14</sub> H <sub>28</sub> O
26	Koiganal	29.83	2.88	266.26	C <sub>18</sub> H <sub>34</sub> O
27	(trans)-2-nonadecene	38.52	0.14	266.3	C <sub>19</sub> H <sub>38</sub>
28	3,4-diphenylpyrazole-1-dl	41.61	0.26	221.11	C <sub>15</sub> H <sub>11</sub> DN <sub>2</sub>
29	1,4-dihydroxy-2-(3-hydroxybutyl)-9,10 anthraquinone	41.75	0.24	312.1	C <sub>18</sub> H <sub>16</sub> O <sub>5</sub>
30	Cyclohexanone, 4-methyl	42.04	0.14	112	C <sub>7</sub> H <sub>12</sub> O
31	2-phenyl-indolizine	42.99	0.33	193.09	C <sub>14</sub> H <sub>11</sub> N
32	Benzene, 1-fluoro-4-isothiocyanat	44.28	0.24	153.01	C <sub>7</sub> H <sub>4</sub> FNS
33	2-Dibenzofuranamine	44.85	0.28	185	C <sub>12</sub> H <sub>11</sub> NO
34	2-phenylethynylquinoline	46.32	1.34	232	C <sub>17</sub> H <sub>12</sub> O
35	1,2,3,4-Tetrahydro-11H-benzo[a]carbazole	46.71	0.92	221.12	C <sub>16</sub> H <sub>15</sub> N
36	1,2,3,4-Tetrahydro-11H-benzo[a]carbazole	48.66	0.22	221.12	C <sub>16</sub> H <sub>15</sub> N
37	Nordextromethorphan	50.20	0.24	257.18	C <sub>17</sub> H <sub>23</sub> NO
38	7-ethoxy-5-methoxy-2,2-dimethylchromanone	50.58	0.27	250.12	C <sub>14</sub> H <sub>18</sub> O <sub>4</sub>

TABLE (III)

Chemical constituents in crude essential oil of sweet basil.

No.	Compound	R <sub>t</sub> (min)	Percent Area	M.W.	Chemical formula
1	alpha-pinene	5.24	0.53	136.24	C <sub>10</sub> H <sub>16</sub>
2	Camphene	5.69	0.14	136.24	C <sub>10</sub> H <sub>16</sub>
3	Sabinene	6.46	0.44	136.24	C <sub>10</sub> H <sub>16</sub>
4	beta-pinene	6.59	0.98	136.24	C <sub>10</sub> H <sub>16</sub>
5	Myrcene	7.02	0.78	136.24	C <sub>10</sub> H <sub>16</sub>
6	3-octanol	7.31	0.02	126.1	C <sub>8</sub> H <sub>14</sub> O
7	1-phellandrene	7.48	0.08	136.24	C <sub>10</sub> H <sub>16</sub>
8	delta-3-carene	7.67	0.02	136.24	C <sub>10</sub> H <sub>16</sub>
9	alpha-terpinene	7.92	0.09	136	C <sub>10</sub> H <sub>16</sub>
10	1,8-cineole ( eucalyptol )	8.61	9.19	154.25	C <sub>10</sub> H <sub>18</sub> O
11	Trans-beta ocimene	9.11	1.46	136	C <sub>10</sub> H <sub>16</sub>
12	gamma-terpinene	9.45	0.07	136	C <sub>10</sub> H <sub>16</sub>
13	Trans-sabinene hydrate	9.85	0.03	154.136	C <sub>10</sub> H <sub>18</sub> O
14	alpha-terpinolene	10.53	0.23	136	C <sub>10</sub> H <sub>16</sub>
15	Linalool	11.96	41.62	154.25	C <sub>10</sub> H <sub>18</sub> O
16	Camphor	12.98	0.77	152.24	C <sub>10</sub> H <sub>16</sub> O
17	Citronellal	13.18	0.17	154.25	C <sub>10</sub> H <sub>18</sub> O
18	Endo-borneol	13.70	0.50	154.25	C <sub>10</sub> H <sub>18</sub> O
19	Terpinene-4-ol	14.07	0.36	154.25	C <sub>10</sub> H <sub>18</sub> O
20	alpha-terpineol	14.66	0.96	154	C <sub>10</sub> H <sub>18</sub> O
21	Estragole	14.84	1.53	148.2	C <sub>10</sub> H <sub>12</sub> O
22	n-octyl acetate	15.21	0.28	160	C <sub>9</sub> H <sub>20</sub> O <sub>2</sub>
23	Geraniol	15.97	0.10	154.25	C <sub>10</sub> H <sub>18</sub> O
24	Carvone	16.42	0.08	150.1	C <sub>10</sub> H <sub>14</sub> O
25	Linalyl acetate	16.73	0.27	180.15	C <sub>12</sub> H <sub>20</sub> O
26	Trans-geraniol	16.97	0.13	154.25	C <sub>10</sub> H <sub>18</sub> O
27	1-bromo-1-cyclopropylpentane	17.44	0.06	190.04	C <sub>8</sub> H <sub>15</sub> Br
28	Pinocarveylacetate	17.80	1.21	193	C <sub>12</sub> H <sub>18</sub> O <sub>2</sub>
29	3,8,8-trimethyltetrahydronaphthalene	18.46	0.01	174.14	C <sub>13</sub> H <sub>18</sub>
30	Methyl cinnamate	18.61	0.03	162.07	C <sub>10</sub> H <sub>10</sub> O <sub>2</sub>
31	Exo-2-hydroxycineole	19.67	0.06	170.13	C <sub>10</sub> H <sub>18</sub> O <sub>2</sub>
32	alpha-cubebene	19.91	0.08	204.19	C <sub>15</sub> H <sub>24</sub>
33	Eugenol	20.90	20.51	164	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>
34	beta-elemene	21.44	1.52	204.19	C <sub>15</sub> H <sub>24</sub>
35	Methyl eugenol	21.96	0.22	178	C <sub>11</sub> H <sub>14</sub> O <sub>2</sub>
36	Zingiberene	22.09	0.20	204.36	C <sub>15</sub> H <sub>24</sub>
37	Trans-caryophyllene	22.27	0.41	204.36	C <sub>15</sub> H <sub>24</sub>
38	alpha-bergamotene	22.91	7.42	204.19	C <sub>15</sub> H <sub>24</sub>
39	alpha-humulene	23.41	0.87	204.36	C <sub>15</sub> H <sub>24</sub>
40	Germacrene-d	23.68	0.80	204.19	C <sub>15</sub> H <sub>24</sub>
41	Alloaromadendrene	24.01	0.22	204.19	C <sub>15</sub> H <sub>24</sub>
42	Germacrene-d	24.33	4.28	204.19	C <sub>15</sub> H <sub>24</sub>
43	Bicyclogermacrene	24.72	1.27	204.19	C <sub>15</sub> H <sub>24</sub>

essential oil of sweet basil with small areas. These compounds were trans-beta ocimene (11), beta-pinene (4), alpha-terpineol (20), myrcene (5), camphor (16), alpha-pinene (1), endo-borneol (18), sabinene (3), terpinene-4-ol (19), linalyl acetate (25), alpha-terpinolene (14), methyl eugenol (35), citronellal (17), camphene (2), trans-geraniol (26), geraniol (23), alpha-terpinene (9), 1-phellandrene (7), carvone (24), gamma-terpinene (12), exo-2-hydroxycineole (31), trans-sabinene hydrate (13) and delta 3-carene (8). It can be concluded that all monoterpene compounds cover 79.79 % of crude essential oil components of sweet basil. Eight compounds were detected and identified as sesquiterpene. These compounds were alpha-bergamotene (38), germacrene-d (42), beta-elemene (34), bicyclogermacrene (43), alpha-humulene (39), germacrene-d (40), trans-caryophyllene (37) and alpha-cubebene (32) with total amount of 16.65%.

#### 4- Acaricidal activity of parsley and coriander oil fractions

Parsley seed and coriander herb oils, which proved high potency against *T. urticae*, as previously found by our working group, were selected to be fractionated by TLC.  $R_f$  values of parsley oil fractions were tabulated in Table (4). Six fractions were obtained in parsley seed oil after developing by benzene with  $R_f$  values of 0.98, 0.91, 0.77, 0.50, 0.33 and 0.02. These fractions were tested to evaluate their effect against adult females. This was achieved by applying the fractions at the same concentration as those of the  $LC_{50}$  of the parsley crude oil. As it appears in Table (4), fraction 3 provides the highest potency against adult females of mites and gave 65.95% mortality. Fraction 5 gave poor potency with 9.31 % mortality. Non of the other fractions appear to have toxic effect against mites adult females.

**TABLE (IV)**

Efficiency of the TLC fractions of parsley seeds and coriander herb oils against the adult females of *T. urticae*.

No.	Parsley seeds oil fractions		Coriander herb oil fractions	
	$R_f$	Mortality (%)	$R_f$	Mortality (%)
1	0.98	0	0.95	57.4
2	0.91	0	0.83	94.2
3	0.77	65.95	0.72	62.9
4	0.50	0	0.61	14.6
5	0.33	9.31	0.46	9.0
6	0.02	0	0.26	6.3
7	---	---	0.03	12.5



Developing coriander herb oil by benzene revealed seven spots with  $R_f$  values of 0.95, 0.83, 0.72, 0.61, 0.46, 0.26 and 0.03. These fractions were adjusted to obtain 0.77% concentration as  $LC_{50}$  of crude coriander oil against adult females. Fraction 2 provided the highest potency against adult females of mites and gave 94.2% mortality, followed by fractions 3 and 1 with 62.9 and 57.4%, respectively. Fractions 4, 7, 5 and 6 have poor potency as follows; 14.6, 12.5, 9.0 and 6.3% mortality, respectively.

#### 5- Identification of most potent fractions.

The most potent fractions of parsley seed (no. 3 with  $R_f$  0.77) and coriander herb (no. 2 with  $R_f$  0.83) oils against mite adult females were identified by GC/MS analysis (Table 5). Four isomers of apiol were detected and identified in the fraction no. 3 of parsley seed amounting to 2.63, 5.46, 0.05, and 91.86 as percent area with  $R_t$  22.46, 22.56, 23.02, and 23.33 min, respectively.

TABLE (V)

Chemical constituents of fraction no. 3 of parsley seed oil and fraction no. 2 of coriander herb oil.

Oil	No.	Compound	Percent area	$R_t$	M.W.	Chemical formula
Parsley	1	Apiol	2.63	22.46	222.24	$C_{12}H_{14}O_4$
	2	Apiol	5.46	22.56	222.24	$C_{12}H_{14}O_4$
	3	Apiol	0.05	23.02	222.24	$C_{12}H_{14}O_4$
	4	Apiol	91.86	23.33	222.24	$C_{12}H_{14}O_4$
Coriander	1	Decanal	70.7	8.86	156.2	$C_{10}H_{20}O$
	2	Undecanal	16.5	10.27	170.167	$C_{11}H_{22}O$
	3	Tetradecanal	12.8	11.61	212.21	$C_{14}H_{28}O$

In fraction no. 2 of coriander herb oil, three aldehyde compounds were identified by comparing them with instrument data base library. Decanal was the dominant compound in this fraction amounting to 70.7 % with  $R_t$  8.86 min. followed by undecanal and tetradecanal amounting to 16.5 and 12.8 as percent area with  $R_t$  10.27 and 11.61 min., respectively. These results confirmed that the main toxic component in parsley crude oil is apiol, while the aldehyde compounds decanal, undecanal and tetradecanal are the principal and toxic components in the crude oil of coriander herb.

#### 6- Toxicity of the main components of the tested crude essential oils to *T. urticae* stages

The available main components (eugenol, limonene, decanal, undecanal, linalool and eucalyptol) in the candidate crude essential oils were tested against the

different stages of *T. urticae*. All tested materials gave good control to the different stages of *T. urticae* except eucalyptol which had no effect against mite eggs (Table 6). Eugenol was the most potent material against mite eggs followed by limonene, decanal, undecanal and linalool. Limonene was the most effective substance against mite protonymphs, while eucalyptol was the least potent one. The tested substances could be classified into three categories. The first category includes three compounds which had LC<sub>50</sub> values less than one percent (limonene, eugenol and linalool). The second category includes decanal and undecanal with LC<sub>50</sub> values; 1.00 and 1.45 %. The third category includes only one compound (eucalyptol). The descending order of the tested materials according to their activity against adult females of *T. urticae* at LC<sub>50</sub> level was limonene, eugenol, decanal, linalool, undecanal and eucalyptol.

**TABLE (VI)**

Comparative toxicity of some essential oil components to the different stages of *T. urticae*.

Substance	Eggs			Protonymphs			Adults		
	LC <sub>50</sub> (%)	LC <sub>90</sub> (%)	Slope	LC <sub>50</sub> (%)	LC <sub>90</sub> (%)	Slope	LC <sub>50</sub> (%)	LC <sub>90</sub> (%)	Slope
Eugenol	0.99	3.04	2.63	0.53	1.29	3.29	0.71	1.51	3.89
Limonene	1.49	6.74	1.95	0.28	0.81	2.75	0.59	2.24	2.21
Decanal	1.95	5.14	3.04	1.00	2.19	3.77	1.03	2.68	3.08
Undecanal	1.96	4.36	3.69	1.45	3.85	3.03	1.65	3.43	4.04
Linalool	3.14	7.31	3.50	0.97	1.90	4.41	1.34	2.48	4.79
eucalyptol	>100	>100	---	2.41	6.18	3.14	3.06	7.62	3.24

In general, the results confirmed that the main components in crude oils of parsley seeds (apiol), coriander herb (decanal, undecanal and linalool) and sweet basil herb (linalool, eugenol and eucalyptol) had good acaricidal activity. It seems that there is connection between effectiveness and the main components of the crude essential oils. On the contrary, limonene compound was found only in crude oil of parsley seeds with a small amount (2.03%), which was not detected in its fractions but it was the most effective standard materials against protonymphs and adults of *T. urticae*.

The main constituents of essential oils tested were phenylpropanoid (isomers of apiol and myristicine) with total percentage of 81.09 for parsley seed, aldehydes (decanal and undecanal) with total amount of 73.74% for coriander and monoterpenes (linalool, eugenol and eucalyptol) with amount of 71.32% for sweet basil. Forty-six compounds were identified in the tested crude oil of parsley seed. These results were confirmed by MacLeod *et al.*, (1985) who identified 45 constituents in parsley leaves oil including the following:  $\alpha$ -pinene;  $\beta$ -pinene, myrcene, limonene;  $\alpha$ -phellandrene, p-cymene,  $\alpha$ -terpinolene, 1,3,8-p-menthatriene, 4-iso-propenyl-1-methylbenzene  $\alpha$ -

terpineol, p-methylacetophenone,  $\alpha$ -elemene, apiol and myristicin. Simon and Quinn (1988) reported that the parsley fresh leaves oil consisted of  $\alpha$ -pinene,  $\beta$ -pinene, myrcene,  $\alpha$ -phellandrene,  $\beta$ -phellandrene, terpinene, terpinolene and 1-methyl-4-isopropenylbenzene, 1,3,8-p-menthatriene, thymol, myristicin and apiol. Padma *et al.*, (1999) found that eugenol, methylchavicol, linalool, isoeugenol and methyl isoeugenol are the main active constituents in *Ocimum* spp. (*O. basilicum*, *O. sanctum*) and were responsible for the pesticidal properties.

The present results indicated that decanal, undecanal and linalool have powerful acaricidal activities against the different stages of *T. urticae*. Limonene had superiority against both protonymph and adult stages followed by eugenol, while in case of the egg stage eugenol appeared to have the superiority followed by limonene. Eucalyptol had the lowest potency against the protonymph and adult mite stages and had no effect against mite eggs. The present study confirmed that the toxicity of the crude essential oils of parsley, coriander and sweet basil to the different stages of *T. urticae* was due to their main components; Apiol, decanal & undecanal and linalool & eugenol & eucalyptol, respectively. These results agree with those of Watanabe *et al.*, (1989) who reported that linalool was highly toxic to the house dust mites *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and *Tyrophagus putrescentiae*. Perrucci *et al.*, (1994) reported that the essential oil of *Lavandula angustifolia* (lavender) and linalool had very powerful acaricidal activities against *Psoroptes cuniculi*.

Most of the main components of the tested essential oils proved to be toxic against many pests according to different investigations. Thorsell *et al.*, (1989) analyzed the toluene extract of *Mentha spicata* by GC/MS and found several components which were tested for their ability to repel the fly *Protophormia terraenovae*. The tested substances; eucalyptol, limonene, p-cymene,  $\gamma$ -terpinene, dihydrocarvyl-acetate, beta -pinene, beta-myrcene, eugenol and alpha -humulene seemed to have a deterrent effect mainly by contact of the fly with the treated bait. Sangwan *et al.*, (1990) found that greatest nematocidal activity was shown by eugenol, linalool and geraniol, which were toxic against all nematodes tested. Shaaya *et al.*, (1991) reported that terpinen-4-ol and eucalyptol were active against *Rhizopertha dominica*. Linalool and alpha-terpineol were active against *Oryzaephilus surinamensis*. Bhatnagar *et al.*, (1993) found that the essential oil of *Ocimum basilicum* and its major constituent, methyl chavicol and eugenol were effective against *Anopheles stephensi* Liston, *Aedes aegypti* Linnaeus and *Culex quinquefasciatus*. Obeng-Ofori *et al.*, (1996) suggested that eugenol and eucalyptol

were highly toxic to the grain borer, *Prostephanus truncates*. Ho *et al.*, (2001) reported that eucalyptol, which is one of the basil constituents showed an activity as a fumigant towards the rice weevil, *Sitophilus oryzae*. Also, Eun *et al.*, (2001) found that linalool and pinene have toxic effect against *S. oryzae*. Thus, most of above mentioned studies indicate that toxicity of the crude essential oils of parsley, coriander and sweet basil to the different stages of *T. urticae* was due to its major components.

## SUMMARY

The main chemical constituents of the crude essential oils tested were apiol and myristicine for parsley seed, decanal and undecanal for coriander herb and linalool & eugenol & eucalyptol for sweet basil herb according to GC/MS analysis. The most potent fractions of parsley seed and coriander herb oils against mite adult females were identified by GC/MS analysis. Four isomers of apiol were identified in third fraction of parsley oil and amounting to 2.63, 5.46, 0.05, and 91.86 as percent area, respectively. While three aldehyde compounds were identified in the second fraction of coriander herb oil. Decanal was the dominant compound amounting to 70.7 % followed by undecanal and tetradecanal amounting to 16.5 and 12.8 as percent area. Decanal, undecanal and linalool appear to be highly potent against the different mite stages. Limonene had superiority against both protonymphs and adults mite stages followed by eugenol, while in case of egg stage eugenol appear to had the superiority followed by limonene. Eucalyptol showed the least potency against protonymph and adult mite stages and had no effect against mite eggs. The main components of the tested essential oils had good acaricidal activity. It seems that there is connection between effectiveness and the principal components of the crude essential oils.

## REFERENCES

- ABBOTT, W.S. (1925):** A method of computing the effectiveness of an insecticide. (*J. Econ. Entomol.*, 18 : 265-267).
- ABO-SHABANA, M.A. (1980):** Acaricidal Action of some New Pesticides with Special Reference to Insect Growth Regulator. (*Unpublished Ph.D.Thesis, fac. of Agric., Zagazig Univ.*, pp 243).
- BADAWY, H.M.A.; A. A BARAKAT; A.M.I. FARRAG and E.M. BAKR (2005):** Biological activity of several essential oils against *Tetranychus urticae* Koch. (*Bull. Ent. Soc. Egypt, Econ. Ser.*, 32: 69-78).

- BARAKAT, A. A.; G. M. SHEREEF; S.A. ABDALLAH and S.A.A AMER (1985):** Effect of pesticides and plant extracts on some biological aspects of *Tetranychus urticae* Koch. (*Bull. Ent. Soc. Egypt, Econ. Ser., 14 : 225-232*).
- BHATNAGAR, M.; K.K. KAPUR; S. JALEES; and S.K. SHARMA (1993):** Laboratory evaluation of insecticidal properties of *Ocimum basilicum* Linnaeus and *O. sanctum* Linnaeus plant's essential oils and their major constituents against vector mosquito species. (*J. Entomol. Res., 17 (1): 21-26*).
- CALDERONE, N. W. and SPIVAL, M. (1995):** Plant extracts for control of parasitic mite *Varroa jacobsoni* (Acari: Varroidae) in colonies of the Western honey bee (Hymenoptera: Apidae). (*J. Econ. Entomol., 88: 1211-1215*).
- DARWISH, M.A. (1991):** Studies on Mites of Medicinal and Ornamental Plants in Field and Storage with Biological Studies on some Predaceous Species. (*Unpublished Ph.D. Thesis, Fac. of agric., Cairo Univ., pp 172*).
- DELAPLANE, K. S. (1992):** Controlling tracheal mites (Acari: Tarsonemidae) in colonies of honey bees (Hymenoptera: Apidae) with vegetable oil and menthol. (*J. Econ. Entomol, 85: 2118-2124*).
- EL-NAHAL, A.K.M.; SCHIMIDT, G.H. and RISHA, E.M. (1989):** Vapours of *Acarus calamus* oil – a space treatment for stored product insects. (*J. Stored Prod. Res., 25: 211-216*).
- EUN, L.S.; L.B. HO; C.W. SIK; P.B. SOO; K.J. GYU and B. C. CAMPBELL (2001):** Fumigant toxicity of volatile natural products from Korean spices and medicinal plants towards the rice weevil, *Sitophilus oryzae* (L.). (*Pest Management Science, 57 (6) :548-553*).
- FINNEY, D.J. (1971):** Probit Analysis. (*Cambridge Univ. Press. pp 333*).
- HO, L.B.; C.W. SIK; L.S. EUN and P.B. SOO (2001):** Fumigant toxicity of essential oils and their constituent compounds towards the rice weevil, *Sitophilus oryzae* (L.). (*Crop Protection, 20 (4) : 317-320*).
- KLINGAUF, F., BESTMANN, H.J., VOSTROWSKY, O. and MICHAELIS, K. (1983):** Wirkung von aetherischen Oelen auf Schadinsekten. (*Mitteilungen der Deutschen Gesellschaft für Allgemeine und Angewandte Entomologie, 4: 123-126*).

- LARSON, C.K. and BERRY, R. E. (1984):** Influence of peppermint phenolics and monoterpenes on two-spotted spider mite (Acari: Tetranychidae). (*Environ. Entomol.*, 13: 282-283).
- MacLeod, A.; C.H. Snyder and G. Subramanian (1985):** Volatile aroma constituents of parsley leaves. (*Phytochemistry*, 24 (11): 2623-2627).
- OBENG-OFORI, D.; C. H. REICHMUTH; J. BEKELE and A. HASSANALI (1996):** Efficacy of products derived from indigenous plants for the control of the larger grain borer (*Prostephanus truncatus*). (*International Conference, Brighton, UK, 18-21 November: 379-384*).
- PADMA, V; K. SUMAN; S. SATYAWATI; P. VASUDEVAN; S. KASHYAP and S. SHARMA (1999):** Bioactive botanicals from basil (*Ocimum* sp.). (*J. Sci. Indust. Res.*, 58 (5): 332-338).
- PERRUCCI, S; P.L. CIONI; G. FLAMINI; I. MORELLI and G. MACCHIONI (1994):** Acaricidal agents of natural origin against *Psoroptes cuniculi*. (*Parassitologia Roma.*, 36 (30): 269-271).
- RICE, P. J. and COATS, J. R. (1994):** Insecticidal properties of several monoterpenoids to the housefly (Diptera: Muscidae), red flourbeetle (Coleoptera: Tenebrionidae) and southern corn rootworm (Coleoptera: Chrysomelidae). (*J. Econ. Entomol.*, 87: 1172-1179).
- SANGWAN, N.K.; B.S. VERMA; K.K. VERMA and K.S. DHINDSA (1990):** Nematicidal activity of some essential plant oils. (*Pesticide Science*, 28 (3): 331-335).
- SARAC, A. and TUNC, I. (1995):** Toxicity of essential vapours to stored-product insects. (*Z. Pflanzenkrankheiten und Pflanzenschutz*, 102: 69-74).
- SHAAYA, E.; RAVID, U.; PASTER, N.; JUVEN, B.; ZISMAN, U. and PISSAREV, V. (1991):** Fumigant toxicity of essential oils against four major stored-product insects. (*J. Chem. Ecol.*, 17: 499-504).
- SIMON, J.E. and J. QUINN (1988):** Characterization of essential oil of parsley. (*J. Agric. Food Chem.*, 36 (3): 467-472).
- SU, H.C.F. and R. HORVAT (1981):** Isolation, identification and insecticidal properties of *Piper nigrum* amides. (*J. Agric. Food Chem.*; 29: 115-118).

- THORSELL, W.; A. MIKIVER; E. MALM and M. MIKIVER (1989):** Fly repellents from extracts of *Mentha spicata* x *crispata* - studies of *Protophormia terraenovae*. (*Entmol. Tidskr.*, 110 (3) : 109-112).
- WATANABE, F.; S. TADAKI; M. TAKAOKA; M. ISHINO and I. MORIMOTO (1989):** Killing activities of the volatiles emitted from essential oils for *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and *Tyrophagus putrescentiae*. (*Shoyakugaku Zasshi. Japanese Journal of Pharmacognosy*, 43(2): 163-168).