

Research Article

## Insecticidal activity of five medicinal plant essential oils against the cabbage aphid, *Brevicoryne brassicae*

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**Abstract:** Essential oils are volatile mixtures of hydrocarbons with diverse functional groups. In the current study the effect of essential oils from five medicinal plants including *Zataria multiflora* and *Nepeta cataria* (Lamiaceae), *Tagetes minuta* and *Artemisia sieberi* (Asteraceae), and *Trachyspermum ammi* (Apiaceae) were analyzed using GC-mass spectrometry. The toxicity of these plant essential oils against the adult stage of the cabbage aphid, *Brevicoryne brassicae* L. (Hemiptera: Aphididae) was studied using a fumigant assay. GC/Mass analysis revealed that the main essential oils varied between species. The most abundant components in *N. cataria*, *Z. multiflora*, *T. ammi*, *T. minuta* and *A. sieberi* were 4a- $\alpha$ , 7- $\beta$ , 7a- $\alpha$ -nepetalactone (76.8%), carvacrol (62.1%),  $\gamma$ -terpinene (27.1%), limonene (13.0%) and artemisia ketone (48.0%) respectively. The fumigant assays showed that all of these essential oils were toxic to *B. brassicae* in a dose-dependent manner. The essential oils of *N. cataria* at 126, 63, 31, 16, 8, and 3  $\mu\text{l L}^{-1}$  air caused 94, 76, 52, 46, 36, and 24% mortality within 24 hours, respectively. The same trend was seen when essential oils of the other plants were tested against *B. brassicae*. These plant essential oils have great potential to be used in integrated pest management especially in greenhouses or other closed systems.

**Keywords:** Essential oils, GC/Mass analysis, Fumigant toxicity, *Brevicoryne brassicae*

### Introduction

The Cabbage aphid (*Brevicoryne brassicae* L.) is widely distributed and it is a severe pest of oil-seed and horticultural brassicaceous crops. This aphid was identified in Europe for the first time and later on it has been reported worldwide in most countries with a temperate climate (Ellis and Singh, 1993; Singh and Ellis, 1993). The aphid feeds exclusively on cultivated and wild cruciferous plants. The

aphid's major plant hosts are Broccoli, Brussels sprouts, cauliflower, and head cabbage. It also feeds on other species of the family Cruciferae, however, its damage is usually less severe than on cabbage hosts. Cabbage aphid populations, if not controlled, often build high densities. Thus, heavily infested plants acquire a grayish appearance due to the mass of aphid bodies on the foliage (Amin and ElDefray, 1981; Trumble, 1982; Pickel *et al.*, 1983).

In addition to the direct damage, damage is caused by aphid contamination of foliage. The cabbage aphid can also be a vector of plant viruses which over 30 viruses are known to be transmitted by *B. brassicae*. Amongst them *cauliflower mosaic* and *cabbage ring spot virus* are two destructive viruses that are transmitted

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by this aphid. More importantly cabbage aphid transmits cauliflower mosaic more effectively because this virus concentrates in the young tissues of the plant, which is the preferred feeding site of the aphid (Broadbent, 1956; Miles, 1989; Khattab, 2007).

The main method of *B. brassicae* control is pesticide use. However, development of resistance to pesticides is an important issue (Yan-ping *et al.*, 2006). Increasing number of resistant insect and mite species to synthetic pesticides is associated with the use of chemicals that indiscriminately affect both natural enemies and pest itself. In addition, environmental pollution and food contamination by synthetic pesticides are other scenarios that should be considered seriously. Thus, it is necessary to develop new alternatives and explore safe control methods with no residual activity or adverse effects on the non-target animals. Many plants produce secondary metabolites with diverse structures (Barenbaum 1995). These chemicals especially in the form of essential oils act as a plant protection agent against micro-organisms and insect herbivores (Choi *et al.*, 2004; Kazana *et al.*, 2007). The essential oils are the complex mixture of 20-60 organic compounds that give characteristic odor and flavor to leaves, flowers, fruits, seeds, barks and rhizomes. Several essential oils have been known to show bactericidal, fungicidal, virucidal and insecticidal properties (Bakkali *et al.*, 2008). Essential oils generally are safer to mammals, birds and fish than commercial pesticides (Isman, 2006). These compounds have been known to have different mode of action including repellency and antifeedant activities, disruption of molting and cuticle, retardation of growth and fecundity, inhibition of oviposition and disruption of embryonic development (Cosimi *et al.*, 2009; Sertkaya *et al.*, 2010). Because of rapid action of these natural chemicals against insects and mites, it has been concluded that these compounds possess neurotoxic effects especially affecting octopamine pathways and GABA-Gated chloride ion channels (Isman, 2006). Different

methods including fumigant toxicity, repellency activity and antifeedant activity have been used to test the effects of plant essential oils on different insects and mites (Trigg and Hill, 1996; Chou *et al.*, 1997; Lee *et al.*, 2004; Ceferino *et al.*, 2006; Cosimi *et al.*, 2009; Nerio *et al.*, 2009).

Although some studies have been done regarding the effect of essential oils against *B. brassicae* (Moharramipour and Sahaf, 2006; Pavela, 2006; Isik and Gorur, 2009), the current study was undertaken to evaluate the effect of essential oils from five different plant species including *Zataria multiflora* and *Nepeta cataria* from Lamiaceae, *Tagetes minuta* and *Artemisia sieberi* from Asteraceae and *Trachyspermum ammi* from Apiaceae against *B. brassicae*. Also, the constituents of these plants essential oils were analyzed using gas chromatography/mass spectrometry.

## Materials and Methods

### Insect's cultures

The insect, *B. brassicae* was obtained from department of Plant Protection, Faculty of Agriculture, University of Shiraz. *B. brassicae* were reared on *Brassica napus* at  $25 \pm 2$  °C and  $55 \pm 5\%$  Relative Humidity, and a photoperiod of 16: 8 L: D in greenhouse.

### Plant species

Five medicinal plant species including *Z. multiflora*, *N. cataria*, *T. minuta*, *A. sieberi*, and *T. ammi* were collected in 2010 from Fars Province, Iran, mainly at flowering stage.

### Essential oil extraction

Plant leaves and stems were air dried in shade, at room temperature and chopped into small pieces. Then, essential oils were extracted based on Motazedian *et al.*, (2012). Plant materials were submitted for 3h to water-distillation using a Clevenger- type apparatus (Papachristos and Stamopoulos, 2004). Anhydrous sodium sulphate was used for water removal, and oils were stored in sealed vials at 4 °C before using in assays.

### Essential oil analysis

The constituents of essential oils was determined using GC/MS based on Ayvaz *et al.* (2010) method. The oils were analyzed using a Shimadzu QP 5050 system fitted with a Free Fatty Acid Phase (50 m × 0.32 mm i.d.) (0.25 µm df) capillary column. Then, the oven temperature was programmed at 6 °C/min from 120 °C to 230 °C and held at 230 °C for 30 min. Other operating conditions were: carrier gas, He with a flow rate of 1.1 ml/min; injector and detector temperatures were 250 and 280 °C, respectively; split ratio, 1: 10. The carrier gas was helium GC/MS analysis was performed on a GC mentioned above coupled with a Thermoquest-Finnigan Trace GC-MS Mass system. The other operating conditions were the same conditions as described above, mass spectra were taken at 70 eV. Mass range was from m/z 35–465 amu and an ionization current of 150 mA. The compounds were identified by comparison of their mass spectra with those of Wiley and Nilst Tutore Libraries (Adams, 2001).

### Insecticidal activity

Toxicity of essential oils on the *B. brassicae* was essentially done based on Pascual-Villalobos and Robledo (1998). Briefly, glass Petri plates (90 × 10 mm) were used as a chamber for the determination of test materials on the insect. To test essential oil toxicity, ten adults of the same age (1-48 hour of age) from the stock colonies were transferred onto excised *B. napus* leaves (2 cm diam.) placed with its dorsal side on four layers of wet (saturated with distilled water) filter paper in a Petri dish using a soft paint brush. The insects were allowed to settle for half an hour before being exposed to the essential oil. The application of the essential oil was based on Soylu *et al.*, (2006). To prevent a direct contact between the insects and oils, the desired oil quantities were applied on filter paper (5 × 2 cm) fixed on the inner surface of the Petri dish. Preliminary tests were done to choose the right doses. Each filter paper received 0.25, 0.5, 1, 2, and 4 µl of essential oils using micropipette (Eppendorf®), 0.1-2.5 µl) which corresponds to 3.9, 7.9, 15.7, 31.5, 62.9,

and 125.8 µl L<sup>-1</sup> air. Plates were then sealed with parafilm to prevent any loss of essential oils. Each concentration (treatment) was replicated five times with each replicate consisting of 10 adult insect. The control consisted of a similar setup but without essential oils. Mortality was recorded after 24 hours of exposure. Aphid incapable of moving after a slight touch with a fine brush was considered as dead.

### Data analysis

Data were corrected using Abbot's formula (Abbot 1925). The lethal concentration was obtained using probit analysis (Robertson *et al.*, 2007) and Polo Pc software (LeOra software, 1987). Percentage of mortality values for different doses were subjected to analysis of variance (by one way of ANOVA) followed by LSD test when significant differences were shown at  $\alpha = 0.05$  (SAS institute 1997).

### Results

#### Essential oils constituents

The GC-Mass analysis of essential oils of *N. cataria*, *Z. multiflora*, *T. ammi*, *T. minuta*, and *A. sieberi* are presented in table 1. The main constituents of *N. Cataria* main essential oils were 4a- $\alpha$ ,7- $\beta$ ,7a- $\alpha$ -nepetalactone (76.8%), 4a- $\alpha$ ,7- $\beta$ ,7a- $\beta$ -nepetalactone (18.6%), and Thymole (1.1%). The main constituents of *Z. multiflora* essential oils were Carvacrol (62.1%), Thymol (7.4%),  $\alpha$ -Pinene (2.8%) and Myrcene (2.0%). Composition of *T. ammi* essential oils were thymol (45%), Y-Terpinene (27.1%), P-Cymene (15.1%), and myrcene (13%).

*T. minuta* main essential oils compositions were Limonene (13.0%), Piperitenone (12.2%),  $\alpha$ -Terpineol (11%), z-ocimenone (5.1%), Docosane (5.0%), Z-Tagetone (5.7%), Tricosane (4.2%), Verbenone (3.7%), and Caryophyllene oxide (3%). *A. sieberi* main essential oils constituents were Artemisia ketone (48.0%), 1,8 Cineol (20%), Selin-11en-4-a-ol (4.6%), yomogi alcohol (3.8%), and lavandulol (2.8%).

**Table 1** Chemical composition of the essential oils obtained from five medicinal plants, *N. cataria*, *Z. multiflora*, *T. ammi*, *T. minuta*, and *A. Sieberi* using GC/Mass.

| Compounds   | <i>N. cataria</i> | <i>Z. multiflora</i> | <i>T. ammi</i> | <i>T. minuta</i> | <i>A. Sieberi</i> |
|---|-------------------|----------------------|----------------|------------------|-------------------|
| 4a- $\alpha$ ,7- $\beta$ ,7a- $\beta$ -nepetalactone  | 76.8              | -                    | -              | -                | -                 |
| 4a- $\alpha$ ,7- $\beta$ ,7a- $\alpha$ -nepetalactone | 18.6              | -                    | -              | -                | -                 |
| carvacrol   | -                 | 62.1                 | -              | -                | -                 |
| thymol  | 1.1               | 7.4                  | 45.0           | -                | -                 |
| $\gamma$ -terpinene                                   | -                 | -                    | 27.1           | -                | -                 |
| p- cymene   | -                 | 0.1                  | 15.3           | -                | 0.9               |
| Myrcene   | -                 | 2.0                  | 1.3            | -                | -                 |
| 11- dodecenol   | 0.7               | -                    | -              | -                | -                 |
| Trans caryophyllene                                   | 0.7               | -                    | -              | -                | -                 |
| $\alpha$ - Pinene                                     | -                 | 2.8                  | 0.2            | 0.5              | 0.2               |
| Cis-linalool oxide                                    | -                 | 1.2                  | -              | -                | -                 |
| Trans-linalool oxide                                  | -                 | 1.2                  | -              | -                | -                 |
| 3-octyl acetate                                       | -                 | 1.2                  | -              | -                | -                 |
| $\alpha$ - humulene                                   | 0.5               | 1.1                  | -              | -                | -                 |
| Carvacrol acetate                                     | -                 | 0.7                  | -              | -                | -                 |
| E-isocitral   | -                 | 0.6                  | -              | -                | -                 |
| $\beta$ -pinene                                       | 0.4               | 0.4                  | 0.1            | -                | 0.3               |
| 1,8-cineole   | -                 | 0.1                  | -              | -                | -                 |
| Triplaol  | 0.3               | -                    | -              | -                | -                 |
| Terpinen-4-ol   | -                 | -                    | 0.2            | -                | 1.5               |
| linalol   | -                 | -                    | 0.2            | -                | -                 |
| Chavicol  | -                 | -                    | -              | -                | -                 |
| $\alpha$ -terpinene                                   | -                 | -                    | 0.2            | -                | -                 |
| $\alpha$ -thujene                                     | -                 | 0.2                  | 0.2            | -                | -                 |
| 1-cyclohexan-1-yl-methyl ketone                       | 0.2               | -                    | -              | -                | -                 |
| camphene  | -                 | 0.1                  | -              | -                | -                 |
| 1-octene-3-one  | -                 | 0.1                  | -              | -                | -                 |
| Sabinene  | -                 | 0.1                  | -              | -                | -                 |
| $\alpha$ -phellandrene                                | -                 | 0.3                  | -              | -                | -                 |
| Delta-3-carene  | -                 | 0.1                  | -              | -                | -                 |
| Octyl acetate   | -                 | 0.3                  | -              | -                | -                 |
| Geranyl acetate                                       | -                 | 0.2                  | -              | -                | -                 |
| Alleo-aromadendrene                                   | -                 | 0.2                  | -              | -                | -                 |
| bicyclogermacrene                                     | -                 | 0.1                  | -              | -                | -                 |
| Limonene  | -                 | -                    | -              | 13.0             | -                 |
| Piperitenone  | -                 | -                    | -              | 12.2             | -                 |

**Table 1 Continued**

| Compounds                                  | <i>N. cataria</i> | <i>Z. multiflora</i> | <i>T. ammi</i> | <i>T. minuta</i> | <i>A. Sieberi</i> |
|--|-------------------|----------------------|----------------|------------------|-------------------|
| $\alpha$ -terpineol                        | -                 | 0.2                  | -              | 11.0             | 0.90              |
| Z-ocimene                                  | -                 | -                    | -              | 5.1              | -                 |
| Docosane                                   | -                 | -                    | -              | 5.0              | -                 |
| Z-tagetone                                 | -                 | -                    | -              | 5.7              | -                 |
| Tricosane                                  | -                 | -                    | -              | 4.2              | -                 |
| Verbenone                                  | -                 | -                    | -              | 3.7              | -                 |
| Caryophyllene oxide                        | 0.50              | 0.2                  | -              | 3.0              | 0.09              |
| dihydrotagetone                            | -                 | -                    | -              | 1.2              | -                 |
| 1-methylel-4-isopropenylbenzene            | -                 | -                    | -              | 1.0              | -                 |
| Cis- epoxy ocimene                         | -                 | -                    | -              | 2.6              | -                 |
| Trans-epoxy ocimene                        | -                 | -                    | -              | 1.2              | -                 |
| $\beta$ -Bisabolone                        | -                 | -                    | -              | 2.0              | -                 |
| E- tagetone                                | -                 | -                    | -              | 2.0              | -                 |
| Linalyl propionate                         | -                 | -                    | -              | 0.9              | -                 |
| Piperitone                                 | -                 | -                    | -              | 6.0              | -                 |
| Piperitone oxide                           | -                 | -                    | -              | 1.2              | -                 |
| $\beta$ - Caryophyllene                    | 0.06              | -                    | -              | 2.5              | -                 |
| Spathulenol                                | -                 | -                    | -              | 0.7              | -                 |
| Heptadecane                                | -                 | -                    | -              | 0.7              | -                 |
| Neophytadiene                              | -                 | -                    | -              | 0.6              | -                 |
| Octadecane                                 | -                 | -                    | -              | 0.9              | -                 |
| Nonadecane                                 | -                 | -                    | -              | 1.2              | -                 |
| Heneicosane                                | -                 | -                    | -              | 2.5              | -                 |
| Artemisia ketone                           | -                 | -                    | -              | -                | 48                |
| 1,8-cineol                                 | -                 | -                    | -              | -                | 20                |
| Selin-11-en-4-a-ol                         | -                 | -                    | -              | -                | 4.6               |
| Yomogi alcohol                             | -                 | -                    | -              | -                | 3.8               |
| lavandulol                                 | -                 | -                    | -              | -                | 2.8               |
| Cadium-4-en-7-ol                           | -                 | -                    | -              | -                | 2.5               |
| Artemisia alcohol                          | -                 | -                    | -              | -                | 1.2               |
| Trans verbenol                             | -                 | -                    | -              | -                | 0.8               |
| Santoline teriene                          | -                 | -                    | -              | -                | 0.3               |
| Lavandulol acetate                         | -                 | -                    | -              | -                | 1.3               |
| Geranyl acetate                            | -                 | -                    | -              | -                | 0.8               |
| Lavandulol isovalerate                     | -                 | -                    | -              | -                | 1.5               |
| Lavandulol 2- methyl butyvate              | -                 | -                    | -              | -                | 1.5               |
| Caryophyllene 4 (14), 8 (15)- diexe-5-a-ol | -                 | -                    | -              | -                | 1.2               |
| B- eudesmol                                | -                 | -                    | -              | -                | 1.4               |
| Total                                      | 99.86             | 83                   | 89.8           | 90.6             | 94.19             |

### Toxicity assay

As shown in Table 2, the vapors of the essential oils from five medicinal plant species were toxic to *B. brassicae*. In all plant species when high dose ( $125.8 \mu\text{L}^{-1}$  air) was used the insect mortality was more than 90% (Table 2). Also, it was found that the effect of all plant species essential oils on the aphid was in a dose dependant manner. For example essential oils of *N. cataria* at 125.8, 62.9, 31.5, 15.7, 7.9, and  $3.0 \mu\text{L}^{-1}$  air produced 94, 76, 52, 46, 36, and 24 % mortality within 24 hours, respectively (Table 2). The same trend was seen when essential oils from the other plant species including *Z. multiflora*, *T. minuta*, *A. sieberi*, and *T. ammi* were tested against the aphid (Table 2).  $\text{LC}_{10}$ ,  $\text{LC}_{50}$  and  $\text{LC}_{90}$  of *N. cataria* against the aphid was 2(0.00- 3), 21(14-34), and 297(150-710)  $\mu\text{L}^{-1}$  air, respectively (Table 3). Toxicity of the other plant species is given in Tabel 3. Six different doses were employed for each essential oil and

differences between doses were found to be significant ( $P < 0.001$ ) in all five essential oils used in the assays (Table 4). Figure 1 compares percentage mortality of *B. brassicae* caused by six different concentrations of each plant species. As seen in Figure 1 when low dose ( $3.0 \mu\text{L}^{-1}$  air) of essential oil was used *Z. multiflora* caused low mortality (less than 20%) whereas *T. minuta* caused high (more than 30%) mortality. Figure 2 shows regression lines of the five plant essential oils against the aphid. As seen in the Figure 2 line slopes of different essential oils are quite different showing different toxicity rate against the aphid. For example the lowest and the highest slopes belongs to *T. minuta* and *T. ammi*, respectively (Fig. 2). The results showing the effectiveness of *T. ammi* essential oil against *B. Brassicae* was high. *T. minuta* has the lowest slope showing that its effectiveness on the aphid is not as pronounced as *T. ammi* (Fig. 2).

**Table 2** The mortality percentage of the essential oils from five medicinal plants, *Z. multiflora*, *N. cataria*, *T. minuta*, *A. sieberi*, *T. ammi*, against *B. brassicae*.

| Concentration<br>( $\mu\text{L}^{-1}$ air) | Mean mortality (%) <sup>1</sup> |                   |                   |                   |                      |
|--|---------------------------------|-------------------|-------------------|-------------------|----------------------|
|  | <i>N. cataria</i>               | <i>T. minuta</i>  | <i>A. sieberi</i> | <i>T. ammi</i>    | <i>Z. multiflora</i> |
| 125.8                                      | 94.0a                           | 98.0a             | 96.0a             | 96.0a             | 94.0a                |
| 62.9                                       | 76.0b                           | 84.0ab            | 72.0b             | 78.0b             | 70.0b                |
| 31.5                                       | 52.0c                           | 74.0b             | 50.0bc            | 48.0c             | 54.0bc               |
| 15.7                                       | 46.0c                           | 56.0c             | 40.0dc            | 40.0cd            | 46.0cd               |
| 7.9  | 36.0cd                          | 40.0cd            | 32.0dc            | 34.0ed            | 34.0de               |
| 3.0  | 24.0d                           | 34.0d             | 26.0d             | 22.0e             | 18.0e                |
| 0.0 (control)                              | 4.0e                            | 4.0e              | 2.0e              | 2.0f              | 0.0 f                |
| $\text{LC}_{10}$ <sup>2</sup>              | 2 (0.00- 3)                     | 1 (0.00- 2)       | 1 (0.00- 3)       | 2 (0.00-5)        | 2 (0.00- 3)          |
| $\text{LC}_{50}$                           | 21 (14-34)                      | 12 (8-16)         | 25 (16- 42)       | 23 (11- 32)       | 21 (15-32)           |
| $\text{LC}_{90}$                           | 297 (150-710)                   | 117 (66-343)      | 472 (172- 796)    | 286 (86- 639)     | 289 (131- 696)       |
| Slope $\pm$ S.E                            | 1.124 $\pm$ 0.219               | 1.277 $\pm$ 0.219 | 1.000 $\pm$ 0.208 | 1.168 $\pm$ 0.214 | 1.130 $\pm$ 0.201    |
| df   | 4                               | 4                 | 4                 | 4                 | 4                    |
| $\chi^2$                                   | 1.7052                          | 0.8723            | 1.7601            | 3.8192            | 0.871                |

1. Mean values (n = 5 replicates with ten adults aphid per replicate) followed by different letters in the same column differ significantly at  $\alpha = 0.05$  according to LSD test. The estimated lethal concentration values ( $\mu\text{L}^{-1}$  air) for each essential oil was given using probit analysis.

2. Values in parentheses indicate 95% confidence limits.

**Table 3** LC<sub>10</sub>, LC<sub>50</sub>, and LC<sub>90</sub> of five essential oils *Z. multiflora*, *N. cataria*, *T. minuta*, *A. sieberi*, *T. ammi* against *Brevicoryne brassicae*.

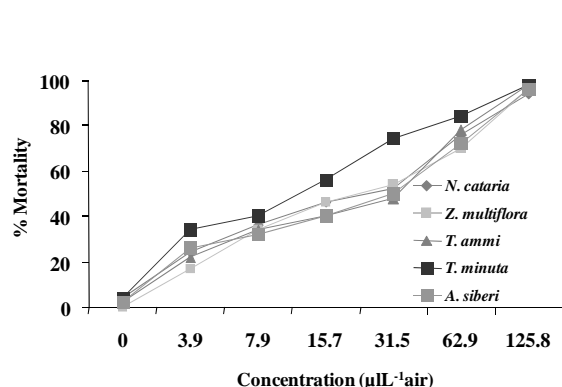
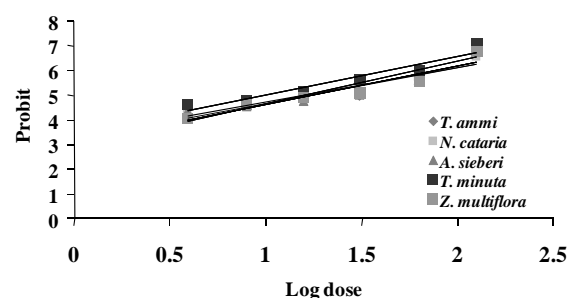
| Essential oils       | N   | LC <sub>10</sub> <sup>1</sup> | LC <sub>50</sub> <sup>1</sup> | LC <sub>90</sub> <sup>1</sup> | Slope ± S. E  | χ <sup>2</sup> |
|----------------------|-----|-------------------------------|-------------------------------|-------------------------------|---------------|----------------|
| <i>T. ammi</i>       | 350 | 2 (0.00-5)                    | 23 (11-32)                    | 286 (86- 639)                 | 1.168 ± 0.214 | 3.8192         |
| <i>Z. multiflora</i> | 350 | 2 (0.00- 3)                   | 21 (15-32)                    | 289 (131- 696)                | 1.130 ± 0.201 | 0.871          |
| <i>T. minuta</i>     | 350 | 1 (0.00- 2)                   | 12 (8-16)                     | 117 (66- 343)                 | 1.277 ± 0.219 | 0.8723         |
| <i>A. sieberi</i>    | 350 | 1 (0.00- 3)                   | 25 (16- 42)                   | 472 (172- 796)                | 1.000 ± 0.208 | 1.7601         |
| <i>N. cataria</i>    | 350 | 2 (0.00- 3)                   | 21 (14-34)                    | 297 (150-710)                 | 1.124 ± 0.219 | 1.7052         |

1. Values of LC<sub>10</sub>, LC<sub>50</sub> and LC<sub>90</sub> μL<sup>-1</sup>air estimated 24 h post application using probit analysis.

**Table 4** Effects of essential oils of *Z. multiflora*, *N. cataria*, *T. minuta*, *A. sieberi*, *T. ammi* against *Brevicoryne brassicae*.

| Source of variation         | <i>N. cataria</i> |        | <i>T. minuta</i> |        | <i>A. sieberi</i> |       | <i>T. ammi</i> |       | <i>Z. multiflora</i> |       |         |
|-----------------------------|-------------------|--------|------------------|--------|-------------------|-------|----------------|-------|----------------------|-------|---------|
|                             | df                | MS     | F                | MS     | F                 | MS    | F              | MS    | F                    | MS    | F       |
| Dose (μL <sup>-1</sup> air) | 6                 | 46.447 | 24.08**          | 51.247 | 26.97**           | 47.98 | 16.63**        | 53.53 | 55.11**              | 51.51 | 31.63** |
| Error                       | 28                | 1.92   |                  | 1.90   |                   | 2.88  |                | 0.97  |                      | 1.62  |         |
| Total                       | 34                |        |                  |        |                   |       |                |       |                      |       |         |

For each plant essential oil, six doses and one control were used. M. S. = Mean Square,  $P < 0.001$ .

**Figure 1** Comparison of mortality percentage of five essential oils *Z. multiflora*, *N. cataria*, *T. minuta*, *A. sieberi*, *T. ammi* against *B. brassicae*.**Figure 2** Regression lines of the effect of five essential oils including *Z. multiflora*, *N. cataria*, *T. minuta*, *A. sieberi*, *T. ammi* against *B. brassicae*.

## Discussion

In the current study essential oils of five plant species from three different plant families were evaluated using GC/Mass spectroscopy and bioassays. GC/Mass analysis showed that each plant species contains a wide range of essential oil components. For example total number of essential oil compound in *N. cataria*, *Z. multiflora*, *T. ammi*, *T. minuta* and *A. sieberi* were 11, 25, 9, 26, and 21 compounds, respectively. However, only a limited number of compounds constitute the major components. The major compounds which constitute more than 10% of essential oil of *C. cataria* were two compounds of 4a- $\alpha$ ,7- $\beta$ ,7a- $\alpha$ -nepetalactone (18.6%) and 4a- $\alpha$ ,7- $\beta$ ,7a- $\beta$ -nepetalactone (76.8%). In *Z. multiflora* only one compound exists in its essential oil with a quantity of higher than 10% which is Crvacrol (62.1%). In *T. minuta* only two compounds including Limonene (13%) and Piperitenone (12.2 %) were major constituents of essential oils while in *A. sieberi* had only one compound including 1,8-Cineol (20%) which was the major constituent of essential oil. The number of compounds, their amounts and the yield of essential oils amongst different and even the same species vary considerably in different studies. The variation between studies may be due to the method of isolation, plant location, plant varieties and time of harvest (Bakkali et al., 2008).

Studies showed that plant essential oil components including  $\gamma$ -terpinene (fumigant toxicity against adults of *Tribolium castaneum*, Moravej et al., 2009), pulegone (against *Sitophilus oryzae*, *Tribolium castaneum*, *Musca domestica* and *Blattella germanica*, Lee et al., 2002a), (E)-anethole (Adulticidal against *Plodia interpunctella*, Ebadollahi and Ashori, 2011), carvacrol, thymol (Toxicity against the workers of the *Odontotermes obesus* termite, Gupta et al., 2011 and Contact and fumigant toxicity against adult male and female of *Blattella germanica*, Yeom et al., 2012) and  $\alpha$ -terpinene (against adults of *Sitophilus zeamais* and *Acanthoscelides obtectus*, Bittner et al., 2008) linalool (Fumigant toxicity on adult of *Sitophilus granarius* and

*Tribolium castaneum*. Ebadollahi & Mahboubi, 2011), eugenol (against *Sitophilus granarius*, *S. zeamais*, *Tribolium castaneum* and *Prostephanus truncates* were investigated in the laboratory by Obeng-ofori and Reichmuth, 1997; Isman, 2006).

Current study showed that tested plant species essential oils had fumigant toxicity toward the cabbage aphid but with varying degrees. However, since there are overlaps between obtained upper and lower limits it seems that there was not significant differences in the obtained values.

Toxicity of Citronella (*Cymbopogon winterianus*) and Alfazema (*Hyptis suaveolens* (L.)) oils was studied against *Hyadaphis foeniculi* Passerini (Hemiptera; aphidae) and its predator *Cylonedra sangninea*. The experiment showed the high toxicity of alfazama against aphid and attractive effect to the predator (Abramason 2006). Also, studies on the effect of seven essential oils on poultry red mite *Dermanyssus gallinae* and non target invertebrates *Tenebrio molitor* revealed that while the oils were toxic to *D. gallinae*, they had less toxicity to *T. molitor* (George et al., 2009).

In conclusion, as seen in the current study and in the other studies, plant essential oils in addition to being toxic against postembryonic stages of insect and mite herbivores, they have oviposition- and feeding deterring activities (Topuz and Erler 2007; Motazedian et al., 2012). Thus it could be mentioned, based on the current study, that plant essential oils possess a great potential compounds that could be used in the integrated pest management program in order to control insect pests both in field and especially in the greenhouses or other closed systems.

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## References

- Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18: 265-267.

- Abramson, C., Wanderley, I., Paulo, A., Wanderley, M. J. A. and Alexandre, M. J. S. 2006. Effect of essential oil from citronella and alfazema on fennel aphids *Hyadaphis foeniculi* Passerini (Hemiptera: Aphididae) and its predator *Cycloneda sanguinea* L. (Coleoptera: Coccinellidae). *American Journal of Environmental Sciences*, 3: 9-10.
- Adams, R. P. 2001. Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy, 3rd edn. Allured Publishing.
- Amin, A. H. and ElDefray, G. M. 1981. The biology of the cabbage aphid, *Brevicoryne brassicae* (L.), in Egypt. *Bulletin of the Entomological Society of Egypt, Economic Series*, 63: 111-118.
- Ayvaz, A., Sagdic, O., Karaborklu, S., Ozturk I 2010. Insecticidal activity of the essential oils from different plants against three stored-product insects. *Journal of Insect Science*, 10: 1-13.
- Bakkali, F., Averbeck, S., Averbeck, D. and Idaomar, M. 2008. Biological effects of essential oils a review. *Food and Chemical Toxicology*, 46: 446-475.
- Berenbaum, M. R. 1995. Chemical defense: theory and practice. *Proceedings of the National Academy of Sciences of the United States of America*, 92: 2-8.
- Broadbent, L., Burt, P. E. and Heathcote, G. D. 1956. The control of potato virus diseases by insecticides. *Annals of Applied Biology*, 44 (2): 256-273.
- Ceferino, T. A., Julio, Z., Mougabure, C. G., Fernando, B., Eduardo, Z. and Maria, I. P. 2006. Fumigant and repellent properties of essential oils and component compounds against Permethrin-resistant. *Pediculus humanus capitis* (Anoplura: Pediculidae) from Argentina. *Journal of Medical Entomology*, 43: 889-895.
- Chou, J. T., Rossignol, P. A. and Ayres, J. W. 1997. Evaluation of commercial insect repellents on human skin against *Aedes aegypti* (Diptera: Culicidae). *Journal of Medical Entomology*, 34: 624-630.
- Choi, W. I., Lee, S. G., Park, H. M. and Ahn, Y. J. 2004. Toxicity of plant essential oils to *Tetranychus urticae* (Acari: Tetranychidae). *Journal of Economic Entomology*, 97: 553-558.
- Cosimi, S., Rossi, E., Cioni, P. L. and Canale, A. 2009. Bioactivity and qualitative analysis of some essential oils from Mediterranean plants against stored-product pests: Evaluation of repellency against *Sitophilus zeamais* Motschulsky, *Cryptolestes ferrugineus* (Stephens) and *Tenebrio molitor* (L.). *Journal of Stored Products Research*, 45: 125-132.
- Ellis, P. R. and Singh, R. 1993. A review of the host plants of the cabbage aphid, *Brevicoryne brassicae* (Homoptera: Aphididae). *International Organization for Biological and Integrated Control of Noxious Animals and Plants/ West Palaearctic Regional Section Bulletin*, Darmstadt, 16: 192-201.
- Enan, E. E. 2005. Molecular and pharmacological analysis of an octapamin receptor from American cockroach and fruit fly in response to plant essential oils. *Archives of Insect Biochemistry and Physiology*, 59: 161-171.
- George, D. R., Sparagano, O. E. A., Port, G., Okello, E., Sheil, R. S. and Guy, J. H. 2009. Repellence of plant essential oils to *Dermanyssus gallinae* and toxicity to the non target invertebrates *Tenebrio molitor*. *Veterinary Parasitology*, 162: 129-134.
- Hopkins, W. G. and Hüner, N. P. A. 2004. *Introduction to Plant Physiology*. John Wiley & Sons. Inc. USA.
- Isman, M. B. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annual Review of Entomology*, 51: 45-66.
- Kazana, E., Pope, T. W., Tibbles, L., Bridges, M., Pickett, J. A., Bones, A. M., Powell, G. and Rossiter, J. T. 2007. The cabbage aphid: a walking mustard oil bomb. *Proceedings of the Royal Society B: Biological Sciences*, 274: 2271-2277.
- Khattab, H. 2007. The defense mechanism of cabbage plant against phloem-sucking aphid (*Brevicoryne brassicae* L.). *Australian Journal of Basic and Applied Sciences*, 1 (1): 56-62.
- Klingauf, F., Bestman, H. J., Vostrowsky, O. and Michaelis, K. 1983. The effect of essential oils on insect pests. *Mitteilungen der Deutschen Gesellschaft für Allgemeine und Angewandte Entomologi*, 4: 123-126.



- Lamiri, A., Lhaloui, S., Benjilali, B. and Berrada, M. 2001. Insecticidal effects of essential oils against hessian fly, *Mayetiola destructor* (Say). *Field Crops Research*, 71: 9-15.
- Lee, S., Tsao, R., Peterson, C. J. and Coats, J. R. 1997. Insecticidal activity of monoterpenoids to western corn rootworm (Coleoptera: Chrysomelidae), two spotted spider mite (Acari: Tetranychidae), and house fly (Diptera: Muscidae). *Journal of Economic Entomology*, 90: 883-892.
- LeOra software 1987. POLO PC: A user guide to Probit or Logit 786 analysis. LeOra software, Berkeley, California.
- Miles, P. W. 1989. The responses of plant to the feeding of Aphidoidea: principles. in: Minks, A. K., Harrewijn, P. (Eds.), *World Crop Pests*. Elsevier-New York, (2c), pp. 1-63.
- Motazedian, N., Ravan, S. and Bandani, A. R. 2012. Toxicity and repellency effects of three essential oils against *Tetranychus urticae* Koch (Acari: Tetranychidae). *Journal of Agricultural Science and Technology*, 14: 275-284.
- Nerio, L. S., Olivero-Verbel, J. and Stashenko, E. E. 2009. Repellent activity of essential oils from seven aromatic plants grown in colombia against *Sitophilus zeamais* Motschulsky (Coleoptera). *Journal of Stored Products Research*, 45: 212-214.
- Obeng-Ofori, D. and Reichmuth, C. 1997. Bioactivity of eugenol, a major component of *Ocimum suave* (Wild.) against four species of stored product Coleoptera. *International Journal of Pest Management*, 43: 89-94.
- Robertson, J. L., Preisler, H. K. and Russell, R. M. 2007. PoloPlus: Probit and Logit Analysis User's Guide. LeOra Software, Petaluna, CA, USA.
- Papachristos, D. P. and Stamopoulos, D. C. 2004. Fumigant toxicity of three essential oils on the eggs of *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). *Journal of Stored Products Research*, 40: 517-525.
- Pascual-Villalobos, M. J. and Ballesta-Acosta, M. C. 2003. Chemical variation in an *Ocimum basilicum* germplasm collection and activity of the essential oils on *Callosobruchus maculatus*. *Biochemical Systematics and Ecology*, 31: 673-679.
- Pascual-Villalobos, M. J. and Robledo, A. 1998. Anti-insect activity of plant extracts from the wild flora in Southern Spain. *Biochemical Systematics and Ecology*, 27: 1-10.
- Quarles, W. 1999. Grow pesto for your pests! *Common Sense Pest Control* 15 (4): 13-19.
- Pickel, C., Mount, R. C., Zalom, F. G. and Wilson, L. T. 1983. Monitoring aphid on Brussels sprout. *California Agriculture*, 37 (5-6): 24-25.
- SAS Institute. 1997. SAS/ ST User's Guide for Personal 826 Computers. SAS Institute, Cary, NC.
- Sertkaya, E., Kaya, K. and Soylu, S. 2010. Acaricidal activities of the essential oils from several medicinal plants against the carmine spider mite (*Tetranychus cinnabarinus* Boisd.) (Acarina: Tetranychidae). *Industrial Crops and Products*, 31 (1): 107-112.
- Singh, R. and Ellis, P. R. 1993. Sources, mechanisms and bases of resistance in cruciferae to the cabbage aphid, *Brevicoryne brassicae*. *International Organization of Biological and Integrated Control of Noxious Animals and Plants/ West Palaearctic Regional Section Bulletin*, Darmstadt, 16 (5): 21-35.
- Soylu, E. M., Soylu, S. and Kurt, S. 2006. Antimicrobial activities of the essential oils of various plants against tomato late blight disease agent *Phytophthora infestans*. *Mycopathologia*, 161 (2): 119-28.
- Tapondjou, A. L., Ader, C., Bouda, H. and Fontem, D. A. 2002. Efficacy of powder and essential oil from *Chenopodium ambrosoides* leaves as post-harvest grain protectants against six-stored beetles. *Journal of Stored Products Research*, 38: 395-402.
- Trigg, J. K. and Hill, N. 1996. Laboratory evaluation of eucalyptusbased repellent against four biting arthropods. *Phytotherapy Research*, 10: 43-6.
- Trumble, J. T. 1982. Aphid (Homoptera: Aphididae) population dynamics on Broccoli in an interior valley of California. *Journal of Economic Entomology*, 75 (5): 841-847.
- Toba, H. H. 1962. Studies on the Host Range of Watermelon Mosaic Virus in Hawaii. *Plant Disease*, 46: 409-410.
- Tunc, I. and Sahinkaya, S. 1998. Sensitivity of two greenhouse pests to vapours of essential oils. *Entomologia Experimentalis et Applicata*, 86: 183-187.

## فعالیت حشره‌کشی اسانس پنج‌گونه گیاه دارویی روی شته کلم، *Brevicoryne brassicae*

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**چکیده:** اسانس‌ها مخلوطی از ترکیبات هیدروکربن فرار با گروه‌های عاملی متفاوت هستند. در مطالعه حاضر اثر اسانس‌های پنج گیاه دارویی شامل، *Zataria multiflora* and *Nepeta cataria* (Lamiaceae)، *Tagetes minuta* and *Artemisia sieberi* (Asteraceae)، and *Trachyspermum ammi* (Apiaceae) با استفاده از GC-Mass آنالیز شدند. سمیت این اسانس‌ها علیه حشرات کامل شته کلم *Brevicoryne brassicae* L. (Hemiptera: Aphididae) با استفاده از زیست‌سنجی تدخینی بررسی شدند. آنالیز GC-Mass نشان داد که ترکیبات اصلی اسانس در بین گونه‌های مختلف متفاوت می‌باشد. فراوان‌ترین ترکیب در *Z. multiflora*، *N. cataria*، *T. ammi*، *T. minuta* و *A. sieberi* به ترتیب 7- $\beta$ ، 4a- $\alpha$ ، nepetalactone (۷۶٫۸ درصد)، Carvacrol (۶۲٫۱ درصد)،  $\gamma$ -terpinene (۲۷٫۱ درصد)، limonene (۱۳ درصد) و Artemisia ketone (۴۸ درصد) بودند. سمیت تدخینی نشان داد که همه این اسانس‌ها برای شته مومی کلم به صورت وابسته به غلظت سمی بودند. اسانس گیاه *N. cataria* در غلظت‌های ۳، ۸، ۱۶، ۳۱، ۶۳ و ۱۲۶ میکرولیتر در لیتر هوا به ترتیب باعث ۲۴، ۳۶، ۴۶، ۵۲، ۷۶، و ۹۴ درصد مرگ و میر در مدت ۲۴ ساعت شدند. روند یکسانی در مورد سایر اسانس‌های گیاهی بر علیه شته کلم دیده شد. این اسانس‌های گیاهی دارای پتانسیل قوی جهت کاربرد در مدیریت تلفیقی آفات مخصوصاً در گلخانه‌ها و محیط‌های بسته دارند.

**واژگان کلیدی:** اسانس، آنالیز Gc-Mass، سمیت تدخینی، *Brevicoryne brassicae*