# Antifeedant Activity and Toxicity of Some Plant Essential Oils to Colorado Potato Beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae)

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**Abstract**

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Essential oils of *Satureja khuzistanica* Jamzad, *Ocimum basilicum* L., *Myrtus communis* L., *Thymus daenensis* Celak, *Mentha spicata* L., and *Eugenia caryophyllus* (Sprengel) were evaluated for nutritional indices and mortality of the 4$^{th}$ instar larvae and adults of *Leptinotarsa decemlineata* (Say). Relative growth rate, relative consumption rate, efficiency of conversion of ingested food and feeding deterrent index were measured. Results showed that the most efficient essential oil on the 4$^{th}$ instar larvae and adults was *S. khuzistanica* ($LC_{50}$ = 23.36 and 167.96 ppm, respectively). Even if all essential oils were effective on feeding deterrence of both stages of *L. decemlineata*, the essential oil of *S. khuzistanica* was the most effective. So, these essential oils can be used as potential control agents against both stages of *L. decemlineata*.

**Keywords**: *Satureja khuzistanica*; *Ocimum basilicum*; *Myrtus communis*; *Thymus daenensis*; *Mentha spicata*; *Eugenia caryophyllus*; mortality; nutritional indices

Over the last 50 years, the control of Colorado potato beetle, *Leptinotarsa decemlineata* (Say), has been mostly based on the use of synthetic insecticides. In this way, resistance of this pest to insecticides remains a serious threat to potato production (Dickens 2002; Gouamene-Lamine *et al*. 2003). This pest is a destructive pest of potato throughout the world and in Iran especially in Ardabil (northwest of Iran) (Nouri-Ganbalani 1989). Due to the use of insecticides it is needed to develop selective and environmentally friendly materials that will result in better management of Colorado potato beetle (CPB) (Cutler *et al*. 2007). Antifeedant, insecticidal, growth-inhibiting, and antiovipositional effects (Pavela *et al.* 2008; Rani &Murty 2009) of botanical insecticides on pests are well documented (Isman 2006). Botanical insecticides have found increasing popularity both in integrated and in ecological pest management. This is due to their special nature. Firstly, these products are considered as safe for the health and for the environment; secondly, they usually contain a mixture of several dozens of active substances, and thus do not cause pest resistance (Pavela 2011). Among botanical insecticides, essential oil is one of the best suggestions that are natural products and can negatively affect the food consumption of insects; they are known as feeding deterrents or antifeedants (Wawrzyniak 1996).

Therefore one of the promising sources for controlling this pest is using the essential oils of some of these plants. Several studies have assessed the ability of plant extracts and essential oils and their

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constituents as antifeedants and insecticides against CPB (Kordali *et al.* 2007; Pavela 2010). *Piper nigrum* L. and *Piper tuberculatum* Jacq. extracts have a strong insecticidal effect on larvae and adults of CPB (Scott *et al.* 2003), *Plantago major* L. and *Tilia cordata* Mill. extracts have an antifeedant activity against CPB adults (Kutas *et al*. 2003). Secondary metabolites extracted from *Geraniaceae* plants were effective on feeding and development of CPB (Lamparski & Wawrzyniak 2005), extract from *Humulus lupulus* L. and *Xanthium strumarium* L. had the highest toxic effect on all stages of CPB (Gokce *et al*. 2006; Erdogan & Toros 2007), extract from *Satureja hortensis* L. and *Thymus vulgaris* L. had strong deterrent effects on the 4$^{th}$ instar larvae (Pavela *et al*. 2009). Essential oil of *Flourensia oolepis* Blake is a feeding inhibitor of the CPB adults (Garcia *et al*. 2007). Methanol extracts obtained from *Angelica archangelica* L., *Grindelia camporum* E., and *Inula auriculata* Boiss. & Balansa have antifeedant activities on the fourth instar larvae of CPB (Pavela 2010).

Because of the long history of using plants in the treatment of different human diseases, controlling pests, most of the botanical insecticides are believed to be safer and more effective than synthetic insecticides, so the plants chosen in this study are based on their safety to humans and environment and medicinal properties. However, there are no reports concerning the toxicity and nutritional indices activity of essential oils from these plants that were mentioned above against CPB. We aimed here to asses and compare nutritional indices and insecticidal activity of essential oils from six medicinal plants against the fourth instar larvae and adults of CPB.

## MATERIAL AND METHODS

***Plant material and essential oil extraction and analysis of essential oil***. The fresh leaves and flowers of *Satureja khuzistanica* Jamzad and *Thymus daenensis* Celak were collected from the 2200 m highland of Zagros Mountains in Lorestan province on May 13–15, 2013. *Ocimum basilicum* L. and *Myrtus communis* L. were collected on May 20, 2013 from a greenhouse of the Medicinal Plant Institute of Shahid Beheshti University (Tehran, Iran), *Mentha spicata* L. and *Eugenia caryophyllus* (Sprengel) were collected from a plant garden of Shiraz (south of Iran) on May 25, 2013. Then they were dried naturally on laboratory benches at room temperature (23–27°C) until they were crisp. Dried leaves and flowers were subjected to hydrodistillation using a modified Clevenger-type apparatus in order to obtain essential oil. Conditions of extraction were as follows: 50 g of leaves and flowers, 500 ml distilled water and 2 h distillation for each plant. Then the essential oils were stored in a refrigerator (Electrosteel, Tehran, Iran) at 4°C. Anhydrous sodium sulphate was used to remove water from oils after they were obtained.

GC analysis was carried out on Varian CP 3800 equipped with DB-5 capillary column (25 m × 0.25 mm; 0.25 µm film thickness). The oven temperature was maintained at 60°C for 1 min, then programmed at 4°C/min to 250°C and for 10 min stopped at this temperature. Other operating conditions were as follows: carrier gas N, at a flow rate of 1.1 ml/min; injector temperature 250°C; detector temperature 280°C. Mass system, the operating conditions were the same as described above, but the carrier gas was helium. Mass spectra were taken at 70 eV. Detection of compounds using different parameters and retention index, study of the range and comparison of their mass spectra with those published in the literature (Adams 1995) and presented in the MS computer library and standard compound.

***Insect***. The first generation adults of *L. decemlineata* were collected from potato fields of Ardabil (northeast of Iran) and newly ecdysed fourth instar larvae were obtained from a colony reared on potato, *Solanum tuberosum*, cv. Agria, in a plastic container (10 × 20 × 30 cm) in a growth chamber Binder K.B.W.F 240 L set at 24–26°C, 60–70% RH and 16 : 8 h (L : D) photoperiod.

***Bioassay of essential oils***. Glass Petri dishes (9 cm in diameter) were used as exposure chambers to test the toxicity of essential oil against the fourth instar larvae and adults of CPB (Kordali *et al.* 2007). Preliminary experiments were done, then the concentrations were determined with logarithmic distance. Potato leaves were impregnated at different concentrations (diluted with acetone), put in glass Petri dishes and then dried for 30 min at room temperature. Ten individuals were transferred to each Petri dish for each developmental stage and experimental treatment. The Petri dishes were covered with a lid and transferred into an incubator, under standard conditions as described above. Control treatments were with acetone in the same way. Each experiment was replicated six times at each dose. The number of live and dead insects was counted after 24 h exposure. The insects were considered to be dead when they showed no leg or antennal movements. The mortality data were corrected by using Abbott's formula

(Abbott 1925) and they were subjected to probit analysis to estimate $LC_{50}$ values (Finney 1971) using the SAS statistical program.

***Nutritional indices assay***. For evaluation of nutritional indices we used the methods described by Huang *et al.* (2000) with some modifications by Pavela (2010). The experiment was done in Petri dishes (9 cm in diameter). The 4th instar larvae of CPB were kept without food for 3 h before the experiment. Damp filter paper was laid on the bottom of the dishes, and 6 disks (2 cm in diameter inside each Petri dish from potato leaves) were placed on the filter paper. The weight of the leaf was determined at the beginning of the experiment. The solutions were prepared from the formulations by further dilution in acetone to produce four different concentrations (10, 12, 14, 16 ppm); then the solvent was allowed to evaporate for 10 min at room temperature. Disks to which only the solvent had been applied were used as the control. The two starved 4th instar larvae of CPB were placed into the centre of each Petri dish. The weight of the larvae before and after each experiment was determined, and then Petri dishes containing the insects were transferred to the growth chamber set at the above stated conditions. Leaves were changed every day during the bioassay. The amount of leaves consumed by the larvae was estimated by reweighing the left leaves of potato and compared to the control. The experiment was counted for 5 days and observations were recorded every 24 hours. This experiment was also done for adults like above just to estimate the FDI. The nutritional indices were calculated for larvae according to Huang *et al.* (2000) formula:

Relative Growth Rate (RGR):

$$RGR = (A - B)/(B \times day)$$

where: A – weight of live insect after experiment (mg to each insect); B – weight of insect before experiment (mg to each insect)

Relative Consumption Rate (RCR):

$$RCR = D/(B \times day)$$

where: D – dried weight of food consumed by insect (mg)

Efficacy of Conversion of Ingested Food (ECI):

$$ECI = RGR/RCR \times 100\%$$

Feeding Deterrence Index (FDI):

$$FDI = [(C - T)/C] \times 100\%$$

where: C – food consumed in control (mg); T – food consumed in treatment (mg)

This study was conducted in a completely randomised and factorial experiment with 5 replications. Factors included four concentrations and control of each plant. ANOVA and Tukey's multiple range tests at a 5% level were used for means and comparison of means.

## RESULT

***Chemical constituents of essential oil***. The chemical constituents of the essential oil of six medicinal plants, the retention indices, and the percentage of the individual components and the yield of each essential oil are summarised in Table 1. In *S. khuzistanica* the major compound was carvacrol (81.1%), and the analysis of *T. daenensis* essential oil revealed that thymol (72.3%) was the main product. The chemical constituents of *O. basilicum* and *M. communis* have shown that linalool (54.34%) and α-pinene (23.0%) accounted for the highest percentage, respectively. In the essential oil of *E. caryophyllus* and *M. spicata*, eugenol (68.4%) and carvone (18.7%) were the components with the highest percentage, respectively.

***Bioassay of essential oils***. The $LC_{50}$ values in this study showed that the CPB was affected significantly by different essential oils. Both stages were more susceptible to the essential oil of *S. khuzistanica* than to the other treatments, the $LC_{50}$ value of this oil for the 4th instar larvae and adults was 23.36 and 167.96 ppm, respectively (Table 2). The 4th instar larvae were more susceptible stages to the essential oils compared to adults. $LC_{50}$ values of *S. khuzistanica*, *T. daenensis*, *O. basilicum*, *M. spicata*, *M. communis*, and *E. caryophyllus* were 23.36, 43.71, 103.58, 75.31, 58.77, and 69.06 ppm, respectively, for the 4th instar larvae (Table 2).

***Nutritional indices assay***. The result showed that the effect of six essential oils on RGR of the 4th instar larvae of CPB at different concentrations was also significantly different; RGR index was significantly reduced with increased concentrations of all essential oils (Table 4). This showed that 16 ppm is the best concentration for forcing CPB to use less food and to have less growth. It was found that the essential oil of *S. khuzistanica* had the highest effect and that of *T. daenensis* had the lowest effect on RCR compared with the essential oils of the other medicinal plants in the fourth instar larvae (Table 4). Also, ECI is significantly reduced with increasing concentrations in all essential oils in the fourth instar larvae. At the lowest concentration (10 ppm) of the essential oil of *S. khuzistanica*, *O. basilicum*, *M. communis*, *T. daenensis*, *M. spicata*, and *E. caryophyllus* the ECI was

Table 1. Chemical constituents of the essential oil from six medicinal plants

| Compound | Retention index | Composition (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | *S. khuzistanica* | *T. daenensis* | *O. basilicum* | *M. communis* | *M. spicata* | *E. caryophyllus* |
| α-Thujene | 931 | 0.3 | 2.3 | – | 0.6 | – | – |
| α-Pinene | 934 | 0.04 | 0.7 | 0.30 | 23.0 | 0.8 | – |
| Camphene | 957 | 0.11 | 0.2 | – | – | 0.3 | – |
| Sabinene | 953 | – | – | 0.29 | – | 0.4 | – |
| β-Pinene | 968 | 0.1 | 0.1 | 0.65 | 0.4 | 1.0 | – |
| Myrcene | 958 | 1.1 | 1.6 | 0.79 | 0.2 | – | – |
| 1,8-Cineole | 1040 | – | – | 7.70 | 20.3 | 2.9 | 0.3 |
| z-β-ocimene | 1021 | – | – | 0.33 | – | – | – |
| *cis*-Sabinene hydrate | 1038 | 0.3 | – | 0.26 | – | – | – |
| *trans*-Sabinene hydrate | 1071 | – | 0.5 | – | – | – | – |
| α-Terpinolene | 1106 | 0.2 | – | – | – | – | – |
| Terpinolene | 1046 | – | – | 0.11 | 0.5 | 0.1 | – |
| Linalool | 1109 | 1.2 | – | 54.34 | 12.3 | 1.0 | 0.6 |
| *cis*-Epoxy ocimene | 1080 | – | – | 0.17 | – | – | – |
| Camphor | 1106 | – | – | 0.20 | – | – | – |
| γ-Terpinene | 1076 | 2.3 | 4.8 | | – | 0.8 | 0.1 |
| α-Terpinene | 1022 | – | 1.8 | – | 0.1 | 0.1 | – |
| α-Terpineol | 1109 | 0.26 | – | 0.55 | 3.3 | | – |
| Borneol | 1181 | 0.52 | – | 1.12 | – | – | – |
| 4-Terpineol | 1184 | – | 0.2 | – | 0.2 | – | – |
| *p*-Menth-1-one-8-ol | 1193 | – | – | 0.16 | – | – | – |
| Bornyl acetate | 1203 | – | – | 1.19 | – | – | – |
| Thymol | 1297 | 0.44 | 72.3 | – | – | 2.1 | 0.3 |
| Carvacrol | 1304 | 81.1 | 7.1 | 0.35 | – | 6.8 | 0.3 |
| Eugenol | 1256 | – | – | 7.52 | – | – | 68.4 |
| α-Copaene | 1297 | – | – | 0.24 | – | – | 0.05 |
| β-Caryophyllene | 1463 | 0.38 | 2.5 | 4.52 | – | 3.4 | 1.7 |
| Caryophyllene oxide | 1600 | 0.73 | 0.1 | – | 0.1 | 0.1 | 0.8 |
| Aromadendrene | 1535 | – | – | 0.33 | – | – | – |
| α-Humulene | 1622 | – | – | 0.56 | 1.5 | – | 0.4 |
| γ-Muurolene | 1634 | – | – | 0.57 | – | – | – |
| Germacrene D | 1500 | – | – | 0.57 | – | – | – |
| β-Guaiene | 1522 | – | – | 4.25 | – | – | – |
| Δ-Cadinene | 1530 | – | – | 0.98 | – | – | – |
| *cis*-Calamenene | 1537 | – | – | 1.51 | – | – | – |
| α-Muurolene | 1545 | – | – | 2.84 | – | – | – |
| *trans*-Nerolidole | 1565 | – | – | 0.23 | – | – | – |
| 10-Epi-eudesmol | 1587 | – | – | 0.53 | – | – | – |
| 1-Epi-cubenol | 1632 | – | – | 0.78 | – | – | – |
| τ-Cadinol | 1659 | – | – | 5.36 | – | – | – |
| α-Cadinol | 1671 | – | – | 0.46 | – | – | – |
| α-Bisabolol | 1694 | – | – | 0.12 | – | – | – |
| 2-Hexyl-(e)-cinnamaldehyde | 1754 | – | – | 0.11 | – | – | – |
| β-Phellandrene | 1035 | – | 0.1 | – | – | – | – |
| *o*-Cymene | 1026 | – | – | – | – | – | 0.1 |
| *p*-Cymene | 1029 | 3.3 | 5.4 | – | 0.3 | 0.7 | – |
| Limonene | 1033 | 1.02 | 0.1 | – | 17.8 | | 0.1 |
| α-Phellandrene | 1010 | 0.1 | 0.1 | – | 0.4 | – | – |
| Δ-3-Carene | 1016 | – | – | – | 0.4 | – | – |
| Terpinen-4-ol | 1193 | 1.14 | – | – | – | 0.3 | – |
| *p*-Menth-1-en-8-ol | 1203 | 0.23 | – | – | – | – | – |
| Carvacrol methyl ether | 1256 | 0.29 | – | – | – | – | – |
| (e,e)-α-Farnesene | 1535 | 0.19 | – | – | – | – | – |
| β-Bisabolene | 1544 | 2.72 | – | – | – | – | – |
| *cis*-α-Bisabolene | 1574 | 0.59 | – | – | – | – | – |

Table 1 to be continued

| Compound | Retention index | Composition (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | *S. khuzistanica* | *T. daenensis* | *O. basilicum* | *M. communis* | *M. spicata* | *E. caryophyllus* |
| β-Eudesmole | 1634 | 0.37 | – | – | – | – | – |
| Eugenol acetate | 1245 | – | – | – | – | – | 22.9 |
| Methyl eugenol | 1402 | – | – | – | 1.6 | – | 0.1 |
| Isoeugenol | 1449 | – | – | – | | – | 0.1 |
| *trans*-β-Ocimene | 1407 | – | – | – | 0.2 | – | – |
| Methyl acetophenone | 1063 | – | – | – | 0.8 | – | – |
| Humulene oxide | 1635 | – | – | – | 0.1 | – | 0.1 |
| *trans*-Caryophyllene | 1442 | – | – | – | 1.7 | – | – |
| (e)-2-Hexenal | 848 | – | – | – | 0.5 | – | – |
| Isobutyl isobutyrate | 910 | – | – | – | 1.1 | – | – |
| Flavesone | 1553 | – | – | – | 0.3 | – | – |
| Neryl acetate | 1362 | – | – | – | 0.2 | – | – |
| Geranyl acetate | 1381 | – | – | – | 3.1 | – | – |
| α-Terpinyl acetate | 1356 | – | – | – | 1.8 | – | 0.2 |
| *p*-Arill arisol | 1202 | – | – | – | 1.2 | – | – |
| Linalyl acetate | 1255 | – | – | – | 4.6 | – | – |
| Methyl citronellate | 1258 | – | – | – | 0.3 | – | – |
| Carvone | | – | – | – | – | 18.7 | |
| Methyl chavicol | 1198 | – | – | – | – | | 1.8 |
| Chavicol | 1253 | – | – | – | – | | 0.4 |
| Spathulenol | 1593 | – | – | – | – | 0.5 | 0.2 |
| Sylvestrene | 1032 | – | – | – | – | 5.1 | – |
| *cis*-Dihydrocarvone | 1205 | – | – | – | – | 18.6 | – |
| *trans*-Dihydrocarvone | 1212 | – | – | – | – | 2.7 | – |
| *trans*-Piperitone epoxide | 1265 | – | – | – | – | 9.8 | – |
| Geranial | 1271 | – | – | – | – | 2.8 | – |
| Geranyl acetate | 1379 | – | – | – | – | 5.1 | – |
| Yield (%) | | 1.5 | 1.6 | 2 | 1.3 | 1.5 | 1.2 |
| Total (%) | | 99.05 | 100 | 99.99 | 98.64 | 99.8 | 100 |

8.357, 5.589, 6.361, 5.309, 6.994, and 5.968%, respectively, and at the highest concentration (16 ppm) the ECI was 3.118, 3.228, 3.153, 2.279, 4.099, and 2.526%, respectively, and the essential oils that were studied in this research show a significant difference at all concentrations from the control (Table 4).

With increasing concentrations of essential oils of *S. khuzistanica*, *O. basilicum*, *M. communis*, *T. dae-*

Table 2. $LC_{50}$ values of six medicinal plant essential oils in adults and 4th instar larvae of *Leptinotarsa decemlineata*

| Plants | Adults | | | | | 4th instar larvae | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $LC_{50}$ (ppm) (95% CL) | slop ± SE | *df* | $\chi^2$ | *P*-value | $LC_{50}$ (ppm) (95% CL) | slop ± SE | *df* | $\chi^2$ | *P*-value |
| *Satureja khuzistanica* | 167.96 (156.84–181.00) | 4.64 ± 0.59 | 34 | 13.58 | 0.99 | 23.36 (20.65–26.59) | 2.60 ± 0.26 | 34 | 12.64 | 0.99 |
| *Thymus daenensis* | 226.99 (203.62–256.23) | 2.93 ± 0.36 | 34 | 10.29 | 1.00 | 43.71 (38.46–50.04) | 2.50 ± 0.30 | 34 | 13.13 | 0.99 |
| *Ocimum basilicum* | 196.35 (180.10–214.98) | 3.75 ± 0.43 | 34 | 13.50 | 0.99 | 103.58 (92.44–115.00) | 3.02 ± 0.40 | 34 | 24.49 | 0.88 |
| *Mentha spicata* | 259.73 (246.16–275.92) | 5.96 ± 0.72 | 34 | 10.26 | 1.00 | 75.31 (68.43–82.61) | 3.53 ± 0.39 | 34 | 15.70 | 0.99 |
| *Myrtus communis* | 314.18 (296.83–336.29) | 5.73 ± 0.70 | 34 | 11.22 | 0.99 | 58.77 (51.16–68.60) | 2.30 ± 0.28 | 34 | 15.32 | 0.99 |
| *Eugenia caryophyllus* | 243.85 (230.23–262.22) | 5.79 ± 0.74 | 34 | 8.71 | 1.00 | 69.06 (57.56–86.37) | 1.74 ± 0.24 | 34 | 23.03 | 0.92 |

Table 3. The effects of essential oils of six medicinal plants on Feeding Deterrence Index (FDI %) (mean ± SE) of the adults of *Leptinotarsa decemlineata*

| Concentration (ppm) | *S. khuzistanica* | *O. basilicum* | *M. communis* | *T. daenensis* | *M. spicata* | *E. caryophyllus* |
|---|---|---|---|---|---|---|
| 10 | 15.57 ± 0.73$^{d}$ | 11.95 ± 0.52$^{d}$ | 7.73 ± 0.78$^{d}$ | 16.19 ± 1.10$^{d}$ | 11.90 ± 0.65$^{d}$ | 11.05 ± 0.80$^{d}$ |
| 12 | 29.64 ± 0.91$^{c}$ | 21.18 ± 0.99$^{c}$ | 16.44 ± 0.98$^{c}$ | 24.09 ± 1.21$^{c}$ | 20.26 ± 1.01$^{c}$ | 20.98 ± 0.96$^{c}$ |
| 14 | 44.90 ± 1.01$^{b}$ | 27.49 ± 1.18$^{b}$ | 22.17 ± 1.26$^{b}$ | 31.49 ± 1.61$^{b}$ | 33.83 ± 1.10$^{b}$ | 27.42 ± 1.13$^{b}$ |
| 16 | 57.66 ± 1.14$^{a}$ | 35.07 ± 1.14$^{a}$ | 28.09 ± 1.35$^{a}$ | 33.91 ± 1.11$^{a}$ | 39.26 ± 0.82$^{a}$ | 31.88 ± 1.12$^{a}$ |
| *P* | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| *F* | 267.2 | 95.76 | 52.16 | 38.93 | 81.76 | 186.54 |
| *df* | 3 | 3 | 3 | 3 | 3 | 3 |

Different letters in the same column indicate significant differences ($P \leq 0.05$) between treatments according to ANOVA and Tukey's Multiple Range Test

*nensis*, *M. spicata*, and *E. caryophyllus* from 10 ppm to 16 ppm FDI in larvae increased 64.53, 26.35, 23.17, 26.8, 31.76, and 15.83%, respectively (Table 4). This result continued in adults, with increasing concentrations of essential oils of *S. khuzistanica*, *O. basilicum*, *M. communis*, *T. daenensis, M. spicata*, and *E. caryophyllus* from 10 ppm to 16 ppm FDI increased 42.09, 23.12, 20.36, 17.72, 27.36, and 20.83%, respectively (Table 3).

## DISCUSSION

Natural products or botanical pesticides are an excellent alternative to synthetic pesticides as a means to reduce negative impacts on the human health and the environment (Mohan *et al.* 2011). One possible way to reduce the high consumption of synthetic insecticides is the application of botanical insecticides, generally considered to be environmentally and medically safe (Pavela 2007; Dayan *et al.* 2009). Among them essential oils are the best-known substances and act as fumigants, insecticides, repellents, and antifeedants (Shaaya *et al.* 1997). Chemical constituents of essential oils grouped as monoterpenes (hydrocarbons and oxygenated derivatives), sesquiterpenes (hydrocarbons and oxygenated derivatives), and aliphatic compounds (alkanes, alkenes, ketones, aldehydes, acids, and alcohols) provide characteristic odours and belong to different genera (Isman *et al.* 2001; Kim & Ahn 2001; Kordali *et al.* 2007). The chemical composition of essential oil depends on chemotype, leaf and flower colours, aroma and origin of plants (Sajjadi 2006). In the chemical composition of essential oils we found that carvacrol (81.1%) and thymol (72.3%) were the main products of *S. khuzistanica* and *T. daenensis*, respectively; Sajjadi and Khatamsaz (2003) also found thymol (73.9%) in *T. daenensis* and Moazeni *et al.* (2012) found that carvacrol (94.9%) was the major compound of *S. khuzistanica.* According to Zini *et al.* (2011) carvone (29%) was the major constituent of *M. spicata* and Merchan Arenas *et al.* (2011) reported that eugenol (60.5%) was the most important constituent, which is the same as our finding with different percentage. Sambul *et al.* (2011) showed that linalool and Ozek *et al.* (1995) showed that alpha-pinene were the major constituents of *O. basilicum* and *M. communis*, respectively; according to our findings the main composition of essential oils was the same but the percentage was different because of that described above.

According to the results the concentration that was used for an antifeedant experiment was below the mortality concentration (Table 2), so the dose that was used was an efficient dose without killing the insect but had an antifeedant activity. The results of this study showed that the essential oil of *S. khuzistanica* has strong toxicity and nutritional indices activity against the 4$^{th}$ instar larvae and adults of CPB. This plant is used in folk medicine in the southern part of Iran for relief of toothache, strengthening the gum, healing the wound, as well as antimicrobial (Amanlu *et al.* 2004), anti-inflammatory activities (Amanlu *et al.* 2005), and antioxidant properties of this plant (Abdollahi *et al.* 2003) were reported, so the use of essential oil of this plant can be safe for humans and environment. Also, may be because its main constituent (carvacrol) was so effective. This component has a broad insecticidal activity and acts against agricultural and stored-product insects (Ahn *et al.* 1998; Isman 2000). Kordali *et al.* (2007) reported that 1,8-cineole, fenchone, β-pinene, and γ-terpinene can be used as potential control agents against both the larvae and adults of CPB; Safaei

Table 4. The effects of essential oils of *S. khuzistanica* and *T. daenensis* on nutritional indices (mean± SE) of the fourth instar larvae of *Leptinotarsa decemlineata* (the essential oil was used in potato leaves)

| Concentration (ppm) | *S. khuzistanica* | | | | *T. daenensis* | | | | *M. spicata* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | RGR | RCR | ECI | FDI | RGR | RCR | ECI | FDI | RGR | RCR | ECI | FDI |
| | (mg/mg/h) | | (%) | | (mg/mg/h) | | (%) | | (mg/mg/h) | | (%) | |
| 0 | 0.033 ± 0.001$^{a}$ | 0.334 ± 0.004$^{a}$ | 9.949 ± 0.371$^{a}$ | | 0.024 ± 0.000$^{a}$ | 0.312 ± 0.001$^{a}$ | 7.935 ± 0.293$^{a}$ | – | 0.027 ± 0.001$^{a}$ | 0.323 ± 0.003$^{a}$ | 8.395 ± 0.282$^{a}$ | – |
| 10 | 0.021 ± 0.000$^{b}$ | 0.257 ± 0.002$^{b}$ | 8.357 ± 0.066$^{b}$ | 21.645 ± 0.676$^{d}$ | 0.013 ± 0.000$^{b}$ | 0.246 ± 0.002$^{b}$ | 5.309 ± 0.250$^{b}$ | 20.721 ± 0.611$^{d}$ | 0.018 ± 0.000$^{b}$ | 0.268 ± 0.007$^{b}$ | 6.994 ± 0.424$^{b}$ | 18.191 ± 1.151$^{d}$ |
| 12 | 0.012 ± 0.000$^{c}$ | 0.171 ± 0.003$^{c}$ | 7.128 ± 0.163$^{c}$ | 47.662 ± 1.002$^{c}$ | 0.007 ± 0.000$^{c}$ | 0.202 ± 0.005c | 3.709 ± 0.102c | 35.210 ± 1.484$^{c}$ | 0.014 ± 0.000$^{c}$ | 0.229 ± 0.004$^{c}$ | 6.243 ± 0.285$^{bc}$ | 31.001 ± 0.768$^{c}$ |
| 14 | 0.006 ± 0.000$^{d}$ | 0.109 ± 0.003$^{d}$ | 6.019 ± 0.181$^{c}$ | 65.800 ± 0.927$^{b}$ | 0.005 ± 0.000$^{c}$ | 0.168 ± 0.003d | 3.283 ± 0.316c | 46.9109 ± 0.853$^{b}$ | 0.010 ± 0.0000$^{d}$ | 0.191 ± 0.004$^{d}$ | 5.192 ± 0.372$^{cd}$ | 39.989 ± 0.761$^{b}$ |
| 16 | 0.001 ± 0.000$^{e}$ | 0.054 ± 0.001$^{e}$ | 3.118 ± 0.458d | 82.738 ± 0.232$^{a}$ | 0.002 ± 0.000$^{d}$ | 0.106 ± 0.005e | 2.279 ± 0.676d | 67.085 ± 1.547$^{a}$ | 0.005 ± 0.000$^{e}$ | 0.151 ± 0.003$^{e}$ | 4.099 ± 0.165$^{d}$ | 55.436 ± 1.101$^{a}$ |
| *P* | 0.000 | 0.000 | 0.263 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| *F* | 301.33 | 139.49 | 80.42 | 114.82 | 169.64 | 364.43 | 9.90 | 269.22 | 110.13 | 184.14 | 26.79 | 264.45 |
| *df* | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 3 |
| | *O. basilicum* | | | | *M. communis* | | | | *E. caryophyllus* | | | |
| | RGR | RCR | ECI | FDI | RGR | RCR | ECI | FDI | RGR | RCR | ECI | FDI |
| | (mg/mg/h) | | (%) | | (mg/mg/h) | | (%) | | (mg/mg/h) | | (%) | |
| 0 | 0.025 ± 0.001$^{a}$ | 0.324 ± 0.003$^{a}$ | 7.819 ± 0.550$^{a}$ | – | 0.026 ± 0.000$^{a}$ | 0.329 ± 0.005$^{a}$ | 7.945 ± 0.326$^{a}$ | – | 0.026 ± 0.001$^{a}$ | 0.336 ± 0.006$^{a}$ | 7.751 ± 0. 231$^{a}$ | – |
| 10 | 0.015 ± 0.000$^{b}$ | 0.269 ± 0.003$^{b}$ | 5.589 ± 0.229$^{b}$ | 16.930 ± 0.486$^{d}$ | 0.016 ± 0.000$^{b}$ | 0.264 ± 0.002$^{b}$ | 6.361 ± 0.169$^{b}$ | 17.944 ± 1.064$^{d}$ | 0.016 ± 0.001$^{b}$ | 0.278 ± 0.003$^{b}$ | 5.968 ± 0.387$^{b}$ | 16.236 ± 0.839$^{d}$ |
| 12 | 0.009 ± 0.000$^{c}$ | 0.217 ± 0.008$^{c}$ | 4.201 ± 0.215$^{c}$ | 32.120 ± 2.635$^{c}$ | 0.014 ± 0.000$^{c}$ | 0.244 ± 0.002$^{c}$ | 5.829 ± 0.136$^{b}$ | 25.339 ± 0.520$^{c}$ | 0.010 ± 0.0000$^{c}$ | 0.239 ± 0.008$^{c}$ | 4.314 ± 0.226$^{c}$ | 27.938 ± 1.107$^{c}$ |
| 14 | 0.006 ± 0.000$^{cd}$ | 0.187 ± 0.006$^{d}$ | 3.390 ± 0.276c | 41.033 ± 1.069$^{b}$ | 0.009 ± 0.000$^{d}$ | 0.200 ± 0.005$^{d}$ | 4.718 ± 0.273$^{c}$ | 38.934 ± 1.473$^{b}$ | 0.006 ± 0.000$^{cd}$ | 0.215 ± 0.004$^{d}$ | 3.196 ± 0.184$^{cd}$ | 35.740 ± 0.783$^{b}$ |
| 16 | 0.004 ± 0.000$^{d}$ | 0.132 ± 0.004$^{e}$ | 3.228 ± 0.242$^{c}$ | 60.284 ± 1.722$^{a}$ | 0.005 ± 0.000$^{e}$ | 0.173 ± 0.004$^{e}$ | 3.153 ± 0.229$^{d}$ | 46.220 ± 1.581$^{a}$ | 0.004 ± 0.000$^{d}$ | 0.187 ± 0.002$^{e}$ | 2.526 ± 0.323$^{d}$ | 43.911 ± 0.653$^{a}$ |
| *P* | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| *F* | 91.46 | 182.52 | 33.83 | 116.14 | 168.60 | 207.66 | 57.25 | 107.99 | 95.74 | 114.98 | 57.36 | 186.86 |
| *df* | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 3 |

RGR – relative growth rate; RCR – relative consumption rate; ECI – efficiency of conversion of ingested food; FDI – Feeding Deterrence Index; different letters in the same column indicate significant differences ($P \leq 0.05$) between treatments according to ANOVA and Tukey's Multiple Range Test

Khoram *et al.* (2011) reported that limonene and myrcene have a strong toxicity to the 2$^{nd}$ instar larvae and adults and α-pinene has a relatively high toxicity to adults. In this study we found that carvacrol can exert a high effect on nutritional indices of this pest.

The 4$^{th}$ instar larvae and the adults of the CPB cause the greatest damage to the potatoes, eggplants, and tomatoes, which results in a significant loss of the yield of these crops (Nouri-Ganbalani 1989), therefore in this study the effect of the essential oils was investigated on nutritional indices and mortalities of the fourth instar larvae and the adults of CPB. The adults were less sensitive to plant essential oils than the larvae. Adult tolerance to plant extracts and insecticides was previously reported by Scott *et al.* (2003), who found that adults were 10-fold less

susceptible to *P. tuberculatum* extract than early instar larvae, Gokce *et al.* (2006) also reported that *Humulus lupulus* L. extract had a higher effect on the 4th instar larvae than on the adults of CPB.

Faster growth occurs when the amount of food consumed by insect increases and the efficiency of food that is consumed is different according to the quality of food. So, if the insect consumes a good diet, its feeding rate increases. In this experiment with increases of concentrations of essential oils of all six plants, RGR, RCR, and ECI of the CPB decreased. In fact, with the tendency of insect to consume food, growth rate and food consumption decreased. Also, with increased concentrations of all essential oils FDI increased.

Effects of essential oils on nutritional indices have been studied by different researchers (Haung *et al*. 2000; Taghizadeh Saroukolai & Behzadi 2010). Erdogan and Toros (2007) reported that the maximum consumed leaf area was in the control extraction of *X. strumarium* amounting to 5.70 ± 0.123 $cm^2$ and the least consumed leaf area by adults of CPB was at the 20% concentration and it was 0.08 ± 0.0.053 $cm^2$. According to Gokce *et al.* (2012) the effect of different concentrations of *H. lupulus* extract on *L. decemlineata* adult FDI was decreased after 24 h, so at the lowest concentration (0.4 mg/ml) FDI was 3.5% and at the highest concentration (40 mg/ml) no feeding occurred and it was 100%. Liu and Ho (1999), Zapata *et al.* (2009), and Pavela *et al.* (2009) also reported a reduction in feeding indices after treatment of their respective insects with plant extracts or essential oils. Lower RGR, RCR, and ECI probably lead to larval growth retardation and formation of smaller pupa, which results in reduced fecundity and longevity of the adult insect and makes them susceptible to diseases and natural enemies (Khosravi *et al*. 2010). Also, reductions of nutritional indices of the 5th instar larvae of *Spodoptera littoralis* (Boisduval) induced by *Reynoutria* sp. extract were reported by Pavela *et al.* (2008).

Pest management in agriculture, forestry and managed landscapes has often relied on toxic, broad-spectrum insecticides with negative impacts on natural enemies, pollinators, and other non-target organisms (Isman 2002). So, purification and synthesis of active compound may allow these natural plant essential oils to compete successfully with conventional insecticide. Development of natural insecticides will help to decrease the negative effects of synthetic chemicals such as residues in products, insect resistance, and environmental pollution. On the basis of these results we would suggest that the essential oil from *S. khuzistanica* plays a role in pest control due to its antifeedant and toxicity effects. In this way there is an increasing interest not only in the synthesis of chemical analogues of natural compounds more available for practical use, but also in finding the structural elements that evoke insect antifeedant activity, but given the costs and practical aspects, e.g. their low content in plants, natural antifeedants are difficult to apply on a large scale (Gabrys *et al*. 2006). If cost-effective commercial problems are solved, essential oils obtained from plants can be used as part of integrated pest management strategies. Therefore, large quantities of plant material must be processed to obtain sufficient quantities of essential oils for commercial-scale tests, situation which also requires breeding these plants in great quantities.

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