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CONTACT TOXICITIES OF OXYGENATED MONOTERPENES TO DIFFERENT POPULATIONS OF COLORADO POTATO BEETLE, *LEPTINOTARSA DECEMLINEATA* SAY (COLEOPTERA: CHRYSOMELIDAE)

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Abstract: In the present study, 12 pure oxygenated monoterpenes at 2 different doses were tested for their toxicity against second and third instar larvae and adults of three different populations of Colorado potato beetle (*Leptinotarsa decemlineata* Say). Some of tested compounds were found to be toxic to larvae and adults, but the degree of toxicity was variable. The mortality range was 20–100%. In general, fenchone, linalool, citronella and menthone showed a strong toxicity against the tested developmental stages; camphor, carvone and linalyl acetate showed moderate toxicity against larvae and adults of Colorado potato beetle and some compounds like fenchol, isomenthol, menthol, nerol and neryl acetate showed the least or no toxicity against the tested developmental stages of *L. decemlineata*. Another important result was that although the tested populations of Colorado potato beetle showed some resistance to Endosulfan (50% WP), there was no resistance to tested oxygenated monoterpenes. The present results indicate that some of these compounds can be used as potential control agents against both larvae and adults of Colorado potato beetle.

Key words: Colorado potato beetle, *Leptinotarsa decemlineta*, Monoterpenes, Pinenes, Endosulfan

INTRODUCTION

Colorado potato beetle, *Leptinotarsa decemlineta* Say (Coleoptera: Chrysomelidae), is one of the beetles best known for its ability to devour vegetables from the nightshade family, such as potato, tomato, eggplants and peppers. The adult beetles as well as their larvae can strip the plants of leaves and ruin an entire crop if left to their own devices (Lawrence and Koundal 2002). This insect has been reported in Iran since 1983, and its distribution in the northwest of Iran is intolerable. Chemical insecticides have been the primary means of controlling these kinds of destructive pests. Nowadays, though, there are many problems associated with the use of synthetic pesticides, like resistance and tolerance to these compounds. The use of chemicals to protect agricultural products is limited and being replaced by environmentally-benign alternatives (Hagstrum and Subramanyam 1996). There is a strong emphasis being placed in recent years, on the production of safe foodstuffs, free from synthetic pesticide residues and chemical additives (Karabelas *et al*. 2009; Kaushik *et al*. 2009). For the possibility of producing quality foodstuffs, it is necessary, among other things, to reduce the risks associated with excessive application of high pesticide doses in primary agricultural production (Kaushik *et al*. 2009). Problems mainly associated with the rise of resistant populations of pathogens and pests, have been the force behind the development of natural and safe bio-pesticides.

One possible way to reduce the high consumption of synthetic insecticides is through the application of Botanical insecticides (BI), generally considered to be environmentally and medically safe (Pavela 2007 a, b; Dayan *et al*. 2009). In addition, many scientists believe that plant extracts or individual compounds of the extracts, can be one of the most efficient alternatives to pest control (Hoffmann and Frodsham 1993; Gonzalez-Coloma *et al*. 1998, 2002, 2004; Zolotar *et al*. 2002; Scott *et al*. 2003, 2004). For instance, it has been demonstrated that monoterpenes, which are important constituents of plant essential oils are easily degradable in soil and water (Misra and Pavlostathis 1997).

Essential oils are natural products that contain natural flavors and fragrances grouped as monoterpenes (hydrocarbons and oxygenated derivatives), sesquiterpenes (hydrocarbons and oxygenated derivatives) and aliphatic compounds (alkanes, alkenes, ketones, aldehydes, acids and alcohols) that provide characteristic odors. Many essential oils isolated from various plant species belonging to different genera, contain relatively high amount of monoterpenes. Insecticidal properties of numerous es-

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sential oils and some monoterpenes against various insect species, have been extensively studied (Don-Pedro 1996; Lee *et al*. 1997, 2003; Prates *et al*. 1998; Isman *et al.* 2001; Kim and Ahn 2001; Park *et al*. 2003; Aslan *et al*. 2004; Papachristos *et al*. 2004; Kordali *et al*. 2006).

The aim of the present study is to assess the toxicity of 12 commercial oxygenated monoterpenes and to compare them with Endosulfan WP 50% (a common used synthetic insecticide for Colorado potato beetle in Iran) against the second and third instar larvae and adults of three different population of *L. decemlineata* . The aim includes finding out what is effective for controlling *L. decemlineata* and what are the probable differences between the sensitivity of different populations to tested oxygenated monoterpenes.

MATERIALS AND METHODS

Chemicals

The pure oxygenated monoterpenes were commercially from Fluka, Sigma, Alfa and Bayer. The compounds tested for toxicity against the Colorado potato beetle were Camphor (Fluka, purity 97%), Carvone (Fluka, purity 99%), Citronellal (sigma, purity 90%), Fenchol (Fluka, purity 98%), Fenchone (Fluka, purity 98%), Isomenthol (Alfa, purity 99%), Linalool (Fluka, purity 97%), Linalyl acetate (Fluka, purity 95%), Menthol (Fluka, purity 99%), Menthone (Fluka, purity 98%), Nerol (Sigma, purity 98%), Neryl acetate (Alfa, purity 98%) and Endosulfan (Trade name: Thiodan) (Bayer, WP 50%).

Insects

The second and third instar larvae and adults of *L. decemlineata* were collected from potato fields in three different cities (Hamedan, Kermanshah & Ardebil). In these cities, this pest cause more than a 30% loss of potato yield and use of synthetic insecticide is the most important method of control. The second and third instar larvae and adults of *L. decemlineata* were reared at 25±3°C, 70±5 relative humidity and 16:8 (L:D) in the Laboratory of the Plant Protection Department at Azad University of Mashhad, Iran. The adults and larvae obtained from laboratory cultures were stored in separate insect cages with appropriate amounts of potato leaves. The number of collected insects was not enough for the experiment. So, the collected insects were kept as stock and their progeny was used for all the experiments. All insects from each farm were fed potato leaves from the same farm, during the time of rearing. All tests were carried out under the same condition and in the same laboratory.

Bioassays using pure compounds

Glass Petri dishes (9 cm wide x 1.5 cm deep) were used as exposure chambers to test the toxicity of pure commercial monoterpenes against the second and third larvae and adults of *L. decemlineata*. Using an automatic pipette, liquid compounds of 7.5 and 15 microliteres were sprayed on Whitman No. 1 paper. The sprayed paper was placed on the inside bottom's of Petri dishes. The solid oxygenated monoterpenes were dissolved in ethanol (500 mg/ml concentration). Using an automatic pipette, 15 and 30 microliters of these solutions, corresponding to 7.5 and 15 mg/Petri dishes, respectively, were sprayed on Whitman No. 1 paper in each Petri dish. Ethanol was vaporized in atmospheric condition for 3 min. Then 15 larvae and adults of *L. decemlineata* were placed on the paper, containing the appropriate amounts of potato leaves. Although there was no direct contact between the oxygenated monoterpenes and the adults and larvae of the Colorado potato beetle, the potato leaves had direct contact with these compounds. Then, the Petri dishes were covered with a lid and transferred into an incubator. The Petri dishes were kept under standard conditions of 25±3 °C, 70±5 relative humidity and a 16:8 (L:D) photoperiod for 4 days. Endosulfan (50 WP) (100 g/l) was used as a positive control in the same conditions above mentioned. Reactive positive control in amounts of 7.5 and 15 µl were applied, corresponding to 2 & 4 mg/Petri dishes, respectively. These doses are among the most recommended doses used to control Colorado potato beetle in Iran. After exposure, the mortality of the adults and larvae was counted at 24, 48, 72 and 96 h. Control treatments without oxygenated monoterpenes were treated in the same way. Each experiment was replicated 4 times at each dose.

Data analysis

The results of mean mortality were subjected to one-way variance analyses (ANOVA), using SPSS 10.0 software package. Differences between means were tested through LSD and values of $p < 0.05$, 0.01 and 0.001.

RESULTS

The toxicity of 12 pure commercial oxygenated monoterpenes was determined against the second and third instar larvae and the adults of three different populations of Colorado potato beetles. Liquid oxygenated monoterpenes at 7.5 and 15 µl/Petri dishes doses, and solid oxygenated monoterpenes at 7.5 and 15 mg/Petri dishes doses, were applied for toxicity tests. Then their toxicities were compared with the toxicity of Endosulfan (50 WP), a commercial insect reactive at 2 and 4 mg/Petri dish doses (Tables 1–9). These tables show that oxygenated monoterpenes exhibited various toxicities against the larvae and the adults depending on exposure time and tested compounds. Although the efficiency of Endosulfan (50% WP) in different populations of Colorado potato beetles was significantly different, the toxicities of different compounds on different populations were similar. There were no significant differences between the mortality percentages because of oxygenated monoterpenes in these three populations (Tables 1–9).

The Ardebil population was the most resistant population to Endosulfan (50% WP) and the Hamadan population was the most sensitive to this compound. But effects of the tested oxygenated monoterpenes on the three populations were similar. Fenchone and linalool were the most effective compounds on the second instar larvae and their effects were analogous with the effects of Endosulfan (50% WP). Although camphor, carvone, citronella and linalyl acetate had some effects on the mortality of the second instars larvae, their effects in tested doses were not substantial. Other compounds like fenchol, isomenthol, menthol, nerol and neryl acetate had the least effect on the second instar larvae (Tables 1–3). Citronella,

Table 1. The toxicity of selected oxygenated monoterpenes against second instar larvae of the Colorado potato beetle (Hamadan population)

Compounds	Dose [mg]	Mean mortality [%][a]			
		24 h	48 h	72 h	96 h
Camphor	7.5	37.4±1.7***	40.8±1.7***	69.7±3.4***	73.1±3.4***
	15	76.5±1.7***	78.2±3.4***	81.6±3.4***	86.7±5.1***
Carvone	7.5	47.6±3.4***	54.4±5.1***	59.5±3.4***	79.9±5.1***
	15	76.5±1.7***	78.2±3.4***	83.3±3.4***	90.1±3.4***
Citronella	7.5	47.6±3.4***	62.9±3.4***	78.2±3.4***	81.6±3.4***
	15	81.6±3.4***	86.7±5.1***	90.1±3.4***	90.1±3.4***
Fenchone	7.5	45.9±6.8***	54.4±5.1***	59.5±3.4***	79.9±5.1***
	15	100.0±0.0***	100.0±0.0***	100.0±0.0***	100.0±0.0***
Fenchol	7.5	8.5±1.7	11.9±3.4	11.9±5.1	17.0±6.8
	15	3.4±1.7	3.4±3.4	5.1±3.4	6.8±5.1
Linalol	7.5	73.1±3.4***	76.5±1.7***	86.7±5.1***	100.0±0.0***
	15	79.9±5.1***	90.1±3.4***	100.0±0.0***	100.0±0.0***
Linalyl acetate	7.5	30.6±5.1**	62.9±3.4***	78.2±3.4***	83.3±3.4***
	15	54.4±5.1***	83.3±3.4***	90.1±3.4***	90.1±3.4***
Isomenthol	7.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	15	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Menthol	7.5	5.1±3.4	23.8±5.1***	28.9±3.4***	37.4±5.1***
	15	23.8±5.1*	25.5±3.4***	34.0±3.4***	42.5±3.4***
Menthone	7.5	32.3±1.7***	32.3±1.7***	34.0±3.4***	37.4±5.1***
	15	52.7±3.4***	54.4±3.4***	57.8±1.7***	57.8±1.7***
Nerol	7.5	15.3±1.7	25.5±1.7***	35.3±3.4***	42.1±3.4***
	15	17.0±1.7	34.0±3.4***	44.2±5.1***	51.0±3.4***
Neryl acetate	7.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	15	3.4±1.7	3.4±1.7	8.5±3.4	10.2±3.4
Endosulfan	2	83.3±6.8***	85.0±6.8***	91.8±8.2***	100.0±0.0***
	4	91.8±8.2***	100.0±0.0***	100.0±0.0***	100.0±0.0***
Control	–	0.0±0.0	1.7±1.7	1.7±1.7	1.7±1.7

*significant at 0.05; **significant at 0.01; *** significant at 0.001 according to the control

[a]mean ±SE of four replicates, each set up with 15 second instar larvae

Table 2. The toxicity of selected oxygenated monoterpenes against second instar larvae of the Colorado potato beetle (Kordestan population)

Compounds	Dose [mg]	Mean mortality [%][a]			
		24 h	48 h	72 h	96 h
Camphor	7.5	35.7±1.7***	42.5±1.7***	68.0±3.4***	73.1±5.1***
	15	78.2±1.7***	78.2±3.4***	81.6±5.1***	86.7±5.1***
Carvone	7.5	49.3±3.4***	56.1±5.1***	57.8±3.4***	78.2±5.1***
	15	76.5±3.4***	76.5±3.4***	83.3±3.4***	88.4±3.4***
Citronella	7.5	45.9±3.4***	64.6±3.4***	76.5±3.4***	83.3±3.4***
	15	81.6±1.7***	88.3±5.1***	90.1±5.1***	90.1±6.8***
Fenchone	7.5	44.2±6.8***	54.4±3.4***	61.2±3.4***	79.9±5.1***
	15	90.1 ± 5.1***	100.0 ± 0.0***	100.0 ± 0.0***	100.0 ± 0.0***
Fenchol	7.5	10.2±1.7	11.9±1.7	13.6±5.1	17.0±6.8
	15	3.4±1.7	3.4±3.4	5.1±3.4	6.8±5.1
Linalol	7.5	71.4±3.4***	78.2±1.7***	86.7±5.1***	90.1±3.4***
	15	78.2±5.1***	90.1±3.4***	100.0±0.0***	100.0±0.0***
Linalyl acetate	7.5	28.9±5.1**	64.6±3.4***	78.2±1.7***	85.0±3.4***
	15	52.7±5.1***	83.3±3.4***	90.1±3.4***	91.8±3.4***
Isomenthol	7.5	0.0±0.0	0.0±0.0	1.7±1.7	3.4±1.7
	15	1.7±0.0	3.4±1.7	3.4±3.4	3.4±3.4
Menthol	7.5	6.8±3.4	22.1±5.1*	30.6±3.4**	35.7±5.1***
	15	25.5±5.1**	27.2±3.4**	34.0±5.1***	42.5±6.8***
Menthone	7.5	32.3±3.4***	34.0±5.1***	37.4±3.4***	40.8±5.1***
	15	52.7±1.7***	56.1±3.4***	57.8±5.1***	59.5±6.8***
Nerol	7.5	13.6±1.7	27.2±1.7**	33.6±3.4***	43.8±3.4***
	15	18.7±1.7	32.3±3.4***	45.9±5.1***	49.3±3.4***
Neryl acetate	7.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	15	5.1±1.7	5.1±3.4	8.5±5.1	10.2±6.8
Endosulfan	2	79.9±3.4***	83.3±6.8***	85.0±6.8***	91.8±8.2***
	4	90.1±3.4***	91.8±8.2***	93.5±5.1***	100.0±0.0***
Control	–	0.0±0.0	1.7±1.7	1.7±1.7	1.7±1.7

*significant at 0.05; **significant at 0.01; ***significant at 0.001 according to the control

[a]mean ±SE of four replicates, each set up with 15 second instar larvae

Table 3. The toxicity of selected oxygenated monoterpenes against second instar larvae of the Colorado potato beetle (Ardabil population)

Compounds	Dose [mg]	Mean mortality [%][a]			
		24 h	48 h	72 h	96 h
Camphor	7.5	35.7±3.4***	40.8±1.7***	69.7±3.4***	71.4±5.1***
	15	76.5±1.7***	78.2±1.7***	81.6±3.4***	85.0±5.1***
Carvone	7.5	51.0±3.4***	54.4±5.1***	59.5±3.4***	79.9±5.1***
	15	74.8±3.4***	76.5±5.1***	83.3±5.1***	86.7±3.4***
Citronella	7.5	47.6± 3.4***	62.9±3.4***	74.8±3.4***	81.6±3.4***
	15	79.9±1.7***	86.7±5.1***	91.8±5.1***	91.8±6.8***
Fenchone	7.5	45.9± 3.4***	57.8±3.4***	62.9±3.4***	79.9±5.1***
	15	91.8±5.1***	100.0±0.0***	100.0±0.0***	100.0±0.0***
Fenchol	7.5	10.2±3.4	11.9±1.7	15.3±5.1	18.7±6.8
	15	3.4±1.7	3.4±3.4	6.8±3.4	8.5±5.1
Linalol	7.5	69.7±3.4***	79.9±1.7***	85.0±5.1***	88.4±3.4***
	15	81.6±5.1***	91.8±3.4***	100.0±0.0***	100.0±0.0***
Linalyl acetate	7.5	30.6±5.1**	66.3±3.4***	78.2±5.1***	85.0±3.4***
	15	51.0±5.1***	85.0±3.4***	88.4±3.4***	90.1±3.4***
Isomenthol	7.5	0.0±0.0	0.0±0.0	3.4±1.7	3.4±3.4
	15	1.7±0.0	5.1±1.7	5.1±3.4	6.8±3.4
Menthol	7.5	6.8±3.4	20.4±5.1*	28.9±3.4**	37.4±5.1***
	15	23.8±5.1*	28.9±3.4**	35.7±5.1***	40.8±6.8***
Menthone	7.5	30.6±3.4**	35.7±5.1***	37.4±3.4***	44.2±5.1***
	15	54.4±1.7***	56.1±3.4***	59.5±5.1***	61.2±6.8***
Nerol	7.5	13.6±1.7	28.9±1.7**	35.3±3.4***	43.8±5.1***
	15	17.0±1.7	34.0±3.4***	45.9±3.4***	51.0±3.4***
Neryl acetate	7.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	15	6.8±1.7	6.8±3.4	8.5±5.1	10.2±6.8
Endosulfan	2	76.5±3.4***	78.2±5.1***	81.6 ±6.8***	85.0±6.8***
	4	86.7±5.1	90.1±6.8***	91.8±3.4***	93.5±5.1***
Control	–	0.0±0.0	1.7±1.7	1.7±1.7	1.7±1.7

*significant at 0.05; **significant at 0.01; ***significant at 0.001 according to the control

[a]mean ±SE of four replicates, each set up with 15 second instar larvae

Table 4. The toxicity of selected oxygenated monoterpenes against third instar larvae of the Colorado potato beetle (Hamadan population)

Compounds	Dose [mg]	Mean mortality [%][a]			
		24 h	48 h	72 h	96 h
Camphor	7.5	0.0±0.0	5.1±1.7	6.8±3.4	6.8±3.4
	15	25.5±3.4**	28.9±3.4**	30.6±1.7**	34.0±5.1***
Carvone	7.5	10.2±1.7	10.2±5.1	13.6±6.8	13.6±6.8
	15	83.3±3.4***	85.0±5.1***	85.0±6.8***	88.4±5.1***
Citronella	7.5	20.4±5.1*	20.4±6.8*	27.2±6.8**	28.9±5.1**
	15	20.4±5.1*	25.5±6.8**	28.9±5.1**	35.7±6.8***
Fenchone	7.5	88.4±5.1***	90.1±6.8***	93.5±6.5***	100.0±0.0***
	15	93.5±6.5***	100.0±0.0***	100.0±0.0***	100.0±0.0***
Fenchol	7.5	23.8±5.1*	27.2±6.8**	28.9±5.1**	35.7±6.8***
	15	10.2±3.4	11.9±5.1	17.0±5.1	20.4±6.8*
Linalol	7.5	13.6±5.1	17.0±5.1	23.7±6.8**	25.5±8.5**
	15	34.0±3.4***	35.7±5.1***	39.1±3.4***	42.5±6.8***
Linalyl acetate	7.5	30.6±1.7***	32.3±3.4***	34.0±3.4***	37.4±5.1***
	15	74.8±3.4***	78.2±5.1***	79.9±5.1***	83.3±6.8***
Isomenthol	7.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	15	0.0±0.0	0.0±0.0	3.4±0.0	5.1±1.7
Menthol	7.5	20.4±6.8*	25.5±6.8**	27.2±6.8**	27.2±6.8**
	15	32.3±3.4***	35.7±5.1***	35.7±6.8***	42.5±6.8***
Menthone	7.5	42.5±6.8***	44.2±6.8***	47.6±5.1***	51.0±6.8***
	15	22.1±3.4*	23.8±5.1**	27.2±5.1**	27.2±6.8**
Nerol	7.5	51.0±6.8***	64.6±5.1***	74.8±3.4***	79.9±5.1***
	15	13.6±5.1	23.8±6.8**	25.4±8.5**	27.2±6.8**
Neryl acetate	7.5	0.0±0.0	0.0±0.0	3.4±1.7	5.1±3.4
	15	23.8±5.1**	25.5±5.1**	27.2±6.8**	30.6±6.8**
Endosulfan	2	74.8±5.1***	78.2±6.8***	79.9±5.1***	85.0±6.8***
	4	86.7±6.8***	90.1±8.2***	91.8±8.2***	96.6±3.4***
Control	-	0.0±0.0	1.7±1.7	1.7±1.7	1.7±1.7

*significant at 0.05; **significant at 0.01; ***significant at 0.001 according to the control

[a]mean ±SE of four replicates, each set up with 15 second instar larvae

Table 5. The toxicity of selected oxygenated monoterpenes against third instar larvae of the Colorado potato beetle (Kordestan population)

Compounds	Dose [mg]	Mean mortality [%][a]			
		24 h	48 h	72 h	96 h
Camphor	7.5	0.0±0.0	3.4±3.4	5.1±3.4	6.8±3.4
	15	27.2±3.4**	28.9±3.4**	32.3±1.7***	34.0±3.4***
Carvone	7.5	10.2±3.4	10.2±5.1	11.9±6.8	13.6±6.8
	15	81.6±3.4***	83.3±5.1***	86.7±6.8***	90.1±5.1***
Citronella	7.5	20.4±3.4*	22.1±6.8*	27.2±6.8**	28.9±6.8**
	15	22.1±5.1*	25.5±5.1**	30.6±5.1**	35.7±6.8***
Fenchone	7.5	86.7±5.1***	90.1±6.8***	95.2±4.8***	100.0±0.0***
	15	93.5±3.4***	100.0±0.0***	100.0±0.0***	100.0±0.0***
Fenchol	7.5	25.5±5.1*	27.2±6.8**	30.6±5.1**	35.7±6.8***
	15	10.2±3.4	13.6±3.4	18.7±5.1	20.4±6.8*
Linalol	7.5	13.6±5.1	17.0±5.1	22.1±6.8**	25.5±8.5**
	15	32.3±3.4***	37.4±5.1***	39.1±5.1***	42.5±6.8***
Linalyl acetate	7.5	30.6±1.7***	34.0±3.4***	35.7±3.4***	39.1±3.4***
	15	73.1±3.4***	78.2±5.1***	81.6±5.1***	83.3±6.8***
Isomenthol	7.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	15	0.0±0.0	0.0±0.0	5.1±0.0	5.1±1.7
Menthol	7.5	22.1±6.8*	23.8±6.8**	27.2±6.8**	28.9±6.8**
	15	30.6±3.4***	34.0±5.1***	35.7±6.8***	44.2±6.8***
Menthone	7.5	42.5±6.8***	45.9±6.8***	47.6±5.1***	51.0±6.8***
	15	22.1±1.7*	23.8±5.1**	27.2±5.1**	28.9±6.8**
Nerol	7.5	52.7±6.8***	64.6±5.1***	73.1±3.4***	79.9±5.1***
	15	13.6±5.1	22.1±6.8**	25.4±8.5**	28.9±6.8**
Neryl acetate	7.5	0.0±0.0	0.0±0.0	3.4±3.4	5.1±3.4
	15	22.1±3.4**	25.5±5.1**	28.9±6.8**	32.3±6.8**
Endosulfan	2	71.4±3.4***	71.4±6.8***	74.8±5.1***	81.6±6.8***
	4	83.3±5.1***	86.7±6.8***	90.1±8.2***	91.8±8.2***
Control	–	0.0±0.0	1.7±1.7	1.7±1.7	1.7±1.7

*significant at 0.05; **significant at 0.01; ***significant at 0.001 according to the control

[a]mean ±SE of four replicates, each set up with 15 second instar larvae

Table 6. The toxicity of selected oxygenated monoterpenes against third instar larvae of the Colorado potato beetle (Ardabil population)

Compounds	Dose [mg]	Mean mortality [%][a]			
		24 h	48 h	72 h	96 h
Camphor	7.5	0.0±0.0	3.4±1.7	5.1±1.7	8.5±3.4
	15	25.5±3.4**	27.2±3.4**	32.3±3.4***	34.0±5.1***
Carvone	7.5	10.2±1.7	10.2±5.1	11.9±5.1	15.3±6.8
	15	79.9±3.4***	83.3±3.4***	88.4±6.8***	90.1±6.8***
Citronella	7.5	18.7± 3.4*	22.1±5.1*	25.5±5.1**	28.9±8.5**
	15	23.8±5.1*	25.5±3.4**	32.3±5.1**	35.7±6.8***
Fenchone	7.5	85.0±5.1***	90.1±8.5***	95.2±4.8***	100.0±0.0***
	15	95.2±4.8***	100.0±0.0***	100.0±0.0***	100.0±0.0***
Fenchol	7.5	23.8±5.1*	27.2±6.8**	32.3±5.1**	35.7±6.8***
	15	10.2±1.7	15.3±3.4	18.7±5.1	20.4±6.8*
Linalol	7.5	13.6±3.4	18.7±5.1	22.1±6.8**	27.2±8.5**
	15	30.6±3.4***	37.4±5.1***	39.1±5.1***	44.2±6.8***
Linalyl acetate	7.5	32.3± 1.7***	34.0±6.8***	37.4±5.1***	40.8±3.4***
	15	71.4±3.4***	79.9±5.1***	81.6±3.4***	83.3±6.8***
Isomenthol	7.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	15	0.0±0.0	0.0±0.0	3.4±1.7	5.1±3.4
Menthol	7.5	20.4±3.4*	25.5±5.1**	28.9±6.8**	30.6±6.8**
	15	30.6±3.4**	35.7±5.1***	35.7±6.8***	44.2±5.1***
Menthone	7.5	42.5±3.4***	45.9±6.8***	49.3±5.1***	51.0±8.5***
	15	22.1±1.7*	25.5±5.1**	27.2±5.1**	30.6± 3.4**
Nerol	7.5	51.0±6.8***	62.9±5.1***	74.8±3.4***	79.9±5.1***
	15	13.6±3.4	23.8±6.8**	27.1±8.5**	28.9±6.8**
Neryl acetate	7.5	0.0±0.0	0.0±0.0	3.4±1.7	5.1±3.4
	15	22.1±5.1**	25.5±5.1**	27.2±6.8**	34.0±6.8**
Endosulfan	2	68.0±1.7***	69.7±3.4***	71.4±3.4***	78.2±5.1***
	4	79.9±3.4***	83.3±3.4***	85.0±6.8***	86.7±6.8***
Control	–	0.0±0.0	1.7±1.7	1.7±1.7	1.7±1.7

*significant at 0.05; **significant at 0.01; ***significant at 0.001 according to the control

[a]mean ±SE of four replicates, each set up with 15 second instar larvae

fenchone, linalool and menthone were the most effective on the adults of Colorado potato beetle, but among these compounds only fenchone was the most effective compound on the third instar larvae (Tables 4–9). Carvone and linalyl acetate in high doses were effective on third instar larvae. Carvone in both tested doses, was effective against the adults of *L. decemlineata*. Other tested oxygenated monoterpens had low effects on these two developmental stages (Tables 4–9).

In general, fenchone in a 15 mg dose, caused 100% mortality in both the second and the third instar larvae after 24 hours in the Hamadan population and after 48 hours in the Kordestan and Ardabil populations (Tables 1–6). Linalool caused 100% mortality in the second instar larvae after 72 hours in three populations. But Isomenthol, in both tested doses, and neryl acetate in only a 7.5 mg dose, had no effects on mortality of the second developmental stages of this pest (Tables 1–3). Isomenthol in a 7.5 mg dose, also had no effect on the mortality of the third instar larvae in three populations (Tables 4–6). Fenchone had 100% mortality against the adult Colorado potato beetles after 24 hours in the Hamadan population, and after 48 hours in Kordestan and Ardabil populations, in both tested doses. In addition, menthone in both doses, killed the adults in three populations after 48 hours (Tables 7–9).

Table 7. The toxicity of selected oxygenated monoterpenes against adult Colorado potato beetles (Hamadan population)

Compounds	Dose [mg]	Mean mortality [%][a]			
		24 h	48 h	72 h	96 h
Camphor	7.5	0.0±0.0	35.7±1.7***	42.5±3.4***	51.0±3.4***
	15	10.2±1.7	39.1±1.7***	73.1±5.1***	86.7±5.1***
Carvone	7.5	25.5±3.4**	66.3±3.4***	83.3±5.1***	93.5±3.4***
	15	34.0±1.7***	86.7±5.1***	100.0±0.0***	100.0±0.0***
Citronella	7.5	88.4±3.4***	100.0±0.0***	100.0±0.0***	100.0±0.0***
	15	66.3±3.4***	73.1±5.1***	83.3±5.1***	88.4±3.4***
Fenchone	7.5	100.0±0.0***	100.0±0.0***	100.0±0.0***	100.0±0.0***
	15	100.0±0.0***	100.0±0.0***	100.0±0.0***	100.0±0.0***
Fenchol	7.5	15.3±1.7	15.3±1.7	32.3±3.4***	39.1±1.7***
	15	0.0±0.0	5.1±1.7	6.8±3.4	20.4±6.8*
Linalol	7.5	73.1±5.1***	78.2±6.8***	83.3±5.1***	88.4±3.4***
	15	74.8±6.8***	88.4±3.4***	100.0±0.0***	100.0±0.0***
Linalyl acetate	7.5	0.0±0.0	15.3±1.7	18.7±3.4	25.5±3.4**
	15	25.5±3.4**	66.3±3.4***	73.1±5.1***	74.8±6.8***
Isomenthol	7.5	15.3±1.7	25.5±3.4**	35.7±1.7***	39.1±1.7***
	15	10.2±1.7	15.3±1.7	23.8±3.4*	27.2±5.1**
Menthol	7.5	3.4±1.7	17.0±3.4	25.5±3.4**	28.9±5.1**
	15	0.0±0.0	15.3±1.7	27.2±5.1**	35.7±1.7***
Menthone	7.5	88.4±3.4***	100.0±0.0***	100.0±0.0***	100.0±0.0***
	15	100.0±0.0***	100.0±0.0***	100.0±0.0***	100.0±0.0***
Nerol	7.5	15.3±1.7	23.8±3.4*	23.8±3.4*	23.8±3.4*
	15	6.8±3.4	10.2±1.7	10.2±1.7	13.6±3.4
Neryl acetate	7.5	23.8±3.4*	25.5±5.1**	25.5±5.1**	27.2±6.8**
	15	10.2±1.7	13.6±3.4	23.8±3.4*	35.7±1.7***
Endosulfan	2	91.8±8.2***	100.0±0.0***	100.0±0.0***	100.0±0.0***
	4	100.0±0.0***	100.0±0.0***	100.0±0.0***	100.0±0.0***
Control	–	0.0±0.0	1.7±1.7	1.7±1.7	1.7±1.7

*significant at 0.05; **significant at 0.01; ***significant at 0.001 according to the control

[a]mean ±SE of four replicates, each set up with 15 second instar larvae

Table 8. The toxicity of selected oxygenated monoterpenes against adult Colorado potato beetles (Kordestan population)

Compounds	Dose [mg]	Mean mortality [%][a]			
		24 h	48 h	72 h	96 h
1	2	3	4	5	6
Camphor	7.5	0.0±0.0	37.4±1.7***	40.8±3.4***	51.0±5.1***
	15	10.2±3.4	40.8±1.7***	71.4±5.1***	86.7±3.4***
Carvone	7.5	23.8±3.4**	66.3±5.1***	81.6±5.1***	93.5±5.1***
	15	35.7±1.7***	85.0±5.1***	100.0±0.0***	100.0±0.0***
Citronella	7.5	88.4±3.4***	100.0±0.0***	100.0±0.0***	100.0±0.0***
	15	64.6±3.4***	74.8±5.1***	83.3±3.4***	90.1±3.4***
Fenchone	7.5	91.8±3.4***	100.0±0.0***	100.0±0.0***	100.0±0.0***
	15	100.0±0.0***	100.0±0.0***	100.0±0.0***	100.0±0.0***
Fenchol	7.5	15.3±1.7	15.3±3.4	34.0±3.4***	37.4±1.7***
	15	0.0±0.0	5.1±3.4	6.8±3.4	20.4±5.1*
Linalol	7.5	71.4±5.1***	79.9±6.8***	83.3±5.1***	88.4±6.8***
	15	74.8±3.4***	90.1±5.1***	100.0±0.0***	100.0±0.0***
Linalyl acetate	7.5	0.0±0.0	13.6±1.7	18.7±5.1	27.2±3.4**
	15	25.5±1.7**	68.0±3.4***	73.1±5.1***	76.5±6.8***
Isomenthol	7.5	15.3±3.4	27.2±3.4**	34.0±1.7***	39.1±0.0***
	15	10.2±1.7	15.3±3.4	23.8±3.4**	28.9±5.1**

1	2	3	4	5	6
Menthol	7.5	3.4±3.4	17.0±3.4	25.5±3.4**	30.6±5.1**
	15	0.0±0.0	15.3±1.7	28.9±5.1**	34.0±1.7***
Menthone	7.5	88.4±1.7***	100.0±0.0***	100.0±0.0***	100.0±0.0***
	15	100.0±0.0***	100.0±0.0***	100.0±0.0***	100.0±0.0***
Nerol	7.5	13.6±1.7	23.8±3.4**	25.5±3.4**	27.2±5.1**
	15	6.8±3.4	10.2±1.7	10.2±3.4	13.6±3.4
Neryl acetate	7.5	23.8±1.7**	25.5±5.1**	25.5±8.5**	27.2±10.2**
	15	11.9±1.7	15.3±3.4	22.1±3.4**	35.7±0.0***
Endosulfan	2	83.3±5.1***	88.4±3.4***	91.8±8.2***	100.0±0.0***
	4	90.1±3.4***	93.5±1.7***	100.0±0.0***	100.0±0.0***
Control	–	0.0±0.0	1.7±1.7	1.7±1.7	1.7±1.7

*significant at 0.05; **significant at 0.01; ***significant at 0.001 according to the control

[a]mean ±SE of four replicates, each set up with 15 second instar larvae

Table 9. The toxicity of selected oxygenated monoterpenes against adult Colorado potato beetles (Ardabil population)

Compounds	Dose [mg]	Mean mortality [%][a]			
		24 h	48 h	72 h	96 h
Camphor	7.5	0.0±0.0	35.7±1.7***	42.5±3.4***	49.3±5.1***
	15	10.2±3.4	39.1±1.7***	69.7±5.1***	85.5±3.4***
Carvone	7.5	23.8±5.1**	64.6±5.1***	81.6±6.8***	91.8±5.1***
	15	34.0±1.7***	83.3±3.4***	100.0±0.0***	100.0±0.0***
Citronella	7.5	90.1±3.4***	100.0±0.0***	100.0±0.0***	100.0±0.0***
	15	62.9±3.4***	76.5±5.1***	83.3±5.1***	90.1±3.4***
Fenchone	7.5	90.1±3.4***	100.0±0.0***	100.0±0.0***	100.0±0.0***
	15	100.0±0.0***	100.0±0.0***	100.0±0.0***	100.0±0.0***
Fenchol	7.5	13.6±1.7	15.3±3.4	35.7±3.4***	37.4±1.7***
	15	0.0±0.0	3.4±3.4	8.5±3.4	20.4±5.1*
Linalol	7.5	69.7±5.1***	81.6±6.8***	83.3±5.1***	88.4±6.8***
	15	73.1±3.4***	90.1±6.8***	100.0±0.0***	100.0±0.0***
Linalyl acetate	7.5	0.0±0.0	13.6±3.4	18.7±5.1	27.2±3.4**
	15	27.2±1.7**	68.0±1.7***	71.4±5.1***	78.2±6.8***
Isomenthol	7.5	15.3±5.1	28.9±3.4**	34.0±1.7***	40.8±1.7***
	15	11.9±1.7	15.3±3.4	22.1±3.4**	28.9±3.4**
Menthol	7.5	3.4±1.7	17.0±5.1	23.8±3.4**	32.3±5.1**
	15	0.0±0.0	15.3±3.4	27.2±5.1**	35.7±1.7***
Menthone	7.5	88.4±3.4***	100.0±0.0***	100.0±0.0***	100.0±0.0***
	15	100.0±0.0***	100.0±0.0***	100.0±0.0***	100.0±0.0***
Nerol	7.5	15.3±1.7	23.8±5.1**	25.5±3.4**	27.2±6.8**
	15	6.8±3.4	10.2±3.4	10.2±3.4	13.6±3.4
Neryl acetate	7.5	22.1±1.7**	25.5±5.1**	25.5±8.5**	27.2±10.2**
	15	11.9±3.4	15.3±3.4	25.5±3.4**	35.7±0.0***
Endosulfan	2	79.9±5.1***	83.3±3.4***	85.0±5.1***	91.8±8.2***
	4	86.7±5.1***	90.1±3.4***	91.8±8.2***	93.5±1.7***
Control	–	0.0±0.0	1.7±1.7	1.7±1.7	1.7±1.7

*significant at 0.05; **significant at 0.01; ***significant at 0.001 according to the control

[a]mean ±SE of four replicates, each set up with 15 second instar larvae

DISCUSSION

The present results show that tested oxygenated monoterpenes have varying degrees of insecticidal activity against both larvae and adults. In general, the mortality increased with increasing doses and exposure times of the monoterpenes. However, with increasing doses, the opposite results were observed for some compounds. Among the tested compounds, fenchone, linalool, citronella and menthone showed strong toxicity against larvae or adults. Some compounds, like comphore, carvone and linalyl acetate had moderate toxicity against at least one developmental stage.

In recent years, several studies were reported on the fumigation toxicity of some pure monoterpenoid constituents against various insect species. In the majority of the studies, it has been cited that different constituents of monoterpenes can be some of the best and safest alternatives to synthetic insecticides, for controlling pests (Don-Pedro 1996; Lee *et al*. 1997, 2003; Prates *et al*. 1998; Kim and Ahn 2001; Park *et al*. 2003; Papachristos 2004). However up till now, no reports have been found in the literature, on the toxic effects of monoterpenes and essential oils against different populations of the Colorado potato beetle. In this respect, this is a first report on the toxicities of some oxygenated monoterpenes on three different populations of *L. decemlineata*. Previously, it had been found that the monoterpenes possess varying insecticidal activities on various insect species and, in general, some oxygenated monoterpenes such as fenchone, linalool, citronella and menthone were found to be more toxic (Don-Pedro 1996; Lee *et al*. 1997, 2003; Prates *et al*. 1998; Kim and Ahn 2001; Park *et al*. 2003; Papachristos 2004). The present results are compatible with previous reports. Lee *et al*. (2003) studied the fumigation toxicity of 22 monoterpenoids to several stored product insects. They indicated that some ketones were more effective than structurally similar alcohols. Similar results are found in the present study when comparing the toxicity of fenchone to fenchol, and menthone to menthol. Likewise, strong toxicities of menthone, linalool and fenchone against *Rhyzopertha dominica* (F.) and *Tribolium costaneum* (Herbest) have been shown by Prates *et al*. (1998). These compounds were also more effective against the Colorado potato beetle in the present study. Similarly, it has been found that fenchone possessed a more toxic effect against different developmental stages of Colorado potato beetles in the present study, and caused over 90% mortality against *Sitophilus granaries* (Kim and Ahn 2001).

It is also important to note, that although the tested endosulfan doses are among the most recommended doses and it is assumed that recommended doses should cause 100% mortality after a 48 hours exposure, the percentage of mortality in insects that were exposed to this compound, decreased in Kermanshah and Ardabil populations. These two populations of Colorado potato beetles may have shown some kind of resistance to the tested synthetic insecticide. The results of present study, however, are not enough to state if there is resistance to this compound in different populations of Colorado potato beetle or not. Further studies need to be conducted for this purpose. According to many scientists, reducing synthetic insecticidal pressure on pest populations is a commonly proposed strategy to delay evaluation of resistance. Selection towards insecticide resistance could be alleviated by replacing at least some of the synthetic insecticide sprays by natural or semi-natural compounds (McGaughey and Reilly 1984; Tabashnik 1994; Caprio 1998; Carriere and Tabashnik 2001).

In conclusion, the development of natural or biological insecticides will help to decrease the negative effects of synthetic chemicals. Negative effects refer to residues in products and insect resistance. In this respect, natural insecticides may also be effective, selective and easily bio-degradable. In the present study, all compounds were found to be toxic against different developmental stages of Colorado potato beetles. In many cases, their toxicities were also identical with the toxicity of commercial Endosulfan (50% WP), widely used as an insect reagent to protect the potato, eggplant and tomato against the Colorado potato beetle. Therefore, in the light of the present results, it is suggested that natural insecticides and/or the plant essential oils containing a high content of these compounds, can be used as new insecticidal reagents against the *L. decemlineata*, Colorado potato beetle. However, further studies need to be conducted to evaluate the cost and safety of these reagents. These compounds should also be tested in greenhouse conditions against this important pest.

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POLISH SUMMARY

KONTAKTOWA TOKSYCZNOŚĆ UTLENIONYCH MONOTERPENÓW DLA RÓŻNYCH POPULACJI STONKI ZIEMNIACZANEJ *LEPTINOTARSA DECEMLINEATA* SAY (COLEOPTERA: CHRYSOMELIDAE)

Przedstawiono ocenę toksyczności 12 czystych, utlenionych monoterpenów zastosowanych w dwóch dawkach, przeciwko drugiemu i trzeciemu pokoleniu larwalnemu oraz dorosłym osobnikom trzech różnych populacji stonki ziemniaczanej (*Leptinotarsa decemlineata* Say). Niektóre z zastosowanych związków wykazywały toksyczność w stosunku do larw i osobników dorosłych, a stopień ich toksyczności był zróżnicowany. Procent śmiertelności szkodnika wahał się w granicach 20–100%. Związki monoterpenowe, takie jak: fenchon, linalol, citronella i menton naogół wykazywały silną toksyczność dla testowanych rozwojowych stadiów szkodnika; kamfora karwon i octan linalolu charakteryzowały się umiarkowaną toksycznością, natomiast takie związki, jak: fenchol, izomentol, mentol, nerol i octan nerylu, wykazywały słabą toksyczność lub jej brak względem testowanych stadiów rozwojowych *L. decemlineata*. Warto podkreślić, że testowane populacje stonki ziemniaczanej charakteryzowały się pewnym stopniem odporności na syntetyczny insektycyd Endosulfan (50% WP). W przypadku testowanych utlenionych monoterpenów nie stwierdzano zjawiska odporności.