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The Toxicity of Selected Monoterpene Hydrocarbons as Single Compounds and Mixtures against Different Developmental Stages of Colorado Potato Beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae)

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ABSTRACT

In the present study, 6 pure monoterpene hydrocarbons were tested as single compounds and mixtures for their toxicity against the second and third instars larvae and adults of Colorado potato beetle (*Leptinotarsa decemlineata* Say). In order to measure the toxicity of compounds tested insects were placed in glass Petri dishes, in which monoterpene hydrocarbons had been sprayed. Tested compounds used in 2 different doses, 5 and 10 mg/Petri dish. The majority of tested compounds were found to be toxic to the larvae and adults; however, the percentage of mortality varied from 15 to 100%. In general, Limonene and Myrcene showed strong toxicity against the second instars larvae and adults. α-Pinene had relatively high toxicity against the adults. However, γ-Terpinene was more toxic against the second instars larvae. The toxicity of Camphene was low against all developmental stages. In mixtures, γ-Terpinene displayed antagonistic effects but other compounds showed effects varying from no observable to additive. According to the outcome of this study these 6 compounds can be used as potential control agents against both larvae and adults of Colorado potato beetle either as single compounds or in mixtures.

Key words: Botanical insecticides, potato key pest, *Leptinotarsa decemlineta*, monoterpene hydrocarbons, pinenes

INTRODUCTION

The Colorado potato beetle, *Leptinotarsa decemlineata* Say, is one of the most destructive and devastating insect pests in many countries all over the world and uncontrolled CBP populations can completely defoliate potato plants and cause a complete loss of tuber production (Hare, 1990). The Colorado potato beetle is a polyphagous pest and can feed on different species of vegetables of the nightshade family such as potatoes, tomatoes, 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; Kordali *et al.*, 2006). This insect has been reported in Iran since 1983 and its distribution in the northwest of Iran is intolerable (Khorram *et al.*, 2010).

Chemical insecticides have been the primary means of controlling these kinds of pests but because of many problems associated with the use of synthetic pesticides like resistance and tolerance to these compounds, use of chemicals to protect agricultural products is limited and being replaced by environmentally-benign alternatives (Subramanyam and Hagstrum, 1996). Hence, there is a need to develop natural and safe bio-pesticides such as the methods used in Integrated Pest Management (Park *et al.*, 2003; Aslan *et al.*, 2004; Dadji *et al.*, 2011; Mulungu *et al.*, 2011). Thus, there is an increasing interest in research concerning with the development of new alternative pesticides, such as insect growth regulators, fungal pathogens, toxic natural products including plant essential oils, extracts and secondary metabolites.

Many scientists believe that plant extracts or individual compounds of them can be one of the most efficient alternatives to pest controls (Hoffmann and Frodsham, 1993; Gonzalez-Coloma *et al.*, 1998, 2002, 2004; Zolotar *et al.*, 2002; Scott *et al.*, 2003, 2004). Natural products are relatively safe to environment and mammalian health as compared with synthetic chemicals as a result of high compatibility of organisms with natural compounds during thousands of years (Baier and Webster, 1992; Oparaeke and Kuhiep, 2006). For instance, it has been demonstrated that monoterpenes which are important constituents of plant essential oils, are easily degradable in soil and water (Keita *et al.*, 2000; Tapondjou *et al.*, 2002).

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, acides and alcohols) that provide characteristic odors. Many essential oils isolated from various plant species belonging to different genera contain relatively high amounts of monoterpenes. Insecticidal properties of numerous essential oils and some monoterpenes have been extensively studied against various insect species (El Nahal *et al.*, 1989; Xie *et al.*, 1995; Haque *et al.*, 2000; Isman *et al.*, 2001; Kim and Ahn, 2001; Tunc *et al.*, 2000).

The aim of the present study is assess the toxicity of six monoterpene hydrocarbons as single compounds and mixtures against the second and third instars larvae and adults of *L.decemlineata* to find the efficiency of these compounds to control of *L. decemlineata*.

MATERIALS AND METHODS

Chemicals: The pure monoterpene hydrocarbons were commercially from Fluka, Sigma, Aldrich and Bayer. The compounds tested for toxicity against Colorado potato beetle were Camphene (Fluka, purity 90-95%), Limonene (Fluka, purity 98%), Myrcene (Aldrich, purity 98%), α-pinene (Fluka, purity 95-97%), β-pinene (Fluka, purity 95%), γ-terpinene (Sigma, purity 95%) and Thiodan ® (Bayer, WP 50%).

Insects: The second and third instars larvae and adults of *L.decemlineata* (Say) were collected in April 2008 and 2009 from potato fields of Bojnord (situated in the northeast of Iran and 250 km far away from Mashhad, the second biggest city in Iran), where this pest caused more than 30% loss of potato yield and the use of synthetic insecticide was the most important mean of control. Then they were reared in the laboratory at 25±3°C, 70±5 relative humidity and 16:8 (L:D) in the laboratory of Plant Protection Department at Azad University of Mashhad from April to August of each years. The adults and larvae obtained from laboratory cultures stored in separate insect cages including appropriate potato leaves. The number of collected insects was not enough for the experiment. So, they were kept as stock and their progeny was used for all experiments. All insects from each farm were fed by potato leaves from the same farm during the time of rearing. Tests are also carried out under the same condition and in the same laboratory.

Bioassays using pure compounds: Glass Petri dishes (12 cm wide×2 cm deep) were used as exposure chambers to test the toxicity of pure commercial compounds against the second and third larvae and adults of *L.decemlineata*. The 5 and 10 μL of liquid compounds were impregnated to Whitman No.1 Paper which was placed on the bottom of Petri dishes, by an automatic pipette. The solid compounds were solved primarily in ethanol (500 mg mL^{-1} concentration) and then 10 and 20 μL of these solutions, corresponding to 5 and 10 mg/Petri dishes, respectively were impregnated to Whitman No.1 paper in each Petri dish by using an automatic pipette. Ethanol was vaporized in atmospheric condition for 3 min. Then 15 larvae and adult of *L.decemlineata* were placed on the filter paper, containing the appropriate amounts of potato leaves. Thus, although there was no direct contact between the monoterpene hydrocarbons and insects, the potato leaves had direct contact with these compounds. After that Petri dishes were covered with a lid and transferred into incubator and then kept under standard conditions of 25±3°C, 70±5 relative humidity and 16:8 (L:D) photoperiod for 4 days. After exposure, the mortality of the adults and larvae was counted at 48 and 96 h. Control treatments without monoterpene hydrocarbons were treated in the same way. Each experiment was replicated for 3 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

Toxicity of single compounds: The toxicity of six pure commercial monoterpene hydrocarbons individually and combined with each other was determined against the second and third instars larvae and the adults of Colorado potato beetles. Liquid monoterpenes at 5 and 10 μL/Petri dishes doses and solid monoterpenes at 5 and 10 mg/Petri dishes doses were applied for toxicity tests and their toxicities were compared to control. The data showed that monoterpene hydrocarbons exhibited various toxicities against the larvae and the adults depending on exposure times and tested compounds.

Although the mortality generally increased with increasing doses of the compounds and exposure times, there were some exceptions in the toxicity of Limonene on second instars larvae and the toxicity of Myrcene, α-pinene and β-pinene on adult stage, where the toxicity of the compounds in higher concentration was lower or equal with their toxicities in lower concentration (Table 1). Limonene was more effective against the second instars larvae and adults; however it was found to be less toxic against third instars larvae. Although γ-terpinene was the most effective compound against the second instars larvae, it was one of the least effective compounds against the third instars larvae and adults. Myrcene and α-pinene were among the most effective compounds only against adults but their toxicities on the third instars larvae were the lowest. β-pinene was the only compound which showed high toxicity against all tested stages of Colorado potato beetle and its toxicity was the highest against adults and lowest against the third instars larvae (Table 1). The

Table 1: The toxicity of monoterpene hydrocarbons individually against second and third instars larvae and adults of Colorado potato beetle

		2nd larvae		3rd larvae		Adults	
		Mean	Mortality (%)[a]	Mean	Mortality (%)[a]	Mean	Mortality (%)[a]
Compound	Dose (mg)	48 h	96 h	8 h	96 h	48 h	96 h
Camphene	5	1.7±1.7	3.4±1.7	3.4±1.7	6.8±3.4	6.8±5.1	10.2±1.7
	10	18.7±1.7	22.1±3.4*	17.0±3.4	25.5±5.1**	10.2±3.4	17.0±3.4
Limonene	5	54.4±3.4***	64.7±5.1***	27.2±1.7**	32.3±3.4***	62.9±3.4***	66.3±5.1***
	10	51.0±1.7***	59.6±6.8***	30.6±3.4***	35.7±5.1***	66.3±5.1***	68.0±1.7***
Myrcene	5	49.3±5.1***	54.4±3.4***	27.2±3.4**	34.0±3.4***	69.7±3.4***	69.7±1.7***
	10	52.7±6.8***	59.5±5.1***	32.3±5.1***	42.5±5.1***	64.6±5.1***	66.3±3.4***
α-pinene	5	37.4±3.4***	40.8±1.7***	3.4±1.7	5.1±3.4	68.0±3.4***	69.7±1.7***
	10	39.1±5.1***	44.2±3.4***	8.5±1.7	18.7±1.7	68.0±5.1***	68.0±1.7***
β-pinene	5	52.7±3.4***	56.1±5.1***	42.5±3.4***	47.6±3.4***	66.3±1.7***	66.3±5.1***
	10	66.3±5.1***	64.7±3.4***	59.5±5.1***	62.9±5.1***	62.9±3.4***	64.6±1.7***
γ-terpinene	5	64.7±3.4***	66.4±5.1***	10.2±1.7	20.4±5.1*	27.2±3.4**	32.3±3.4***
	10	68.0±5.1***	69.7±3.4***	22.1±3.4*	25.5±3.4**	30.6±5.1***	35.7±5.1***
Control	-	1.7±1.7	1.7±1.7	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

* Significant at 0.05; ** Significant at 0.01; *** Significant at 0.001 according to Control. [a] Mean±SE of three replicates, each set up with 15 insects

toxicity of camphene was very low against all developmental stages and in most of the cases its toxicity was not significant. α-pinene and γ-terpinene showed insignificant toxicity against the third instars; however, their toxicity against other developmental stages were moderate or high (Table 1).

Toxicity of two-compound mixtures: When monoterpene hydrocarbons were compared as mixtures possessing two compounds, their toxicity in some cases was different from their toxicity as single compounds. Camphene was only compound which did not show any effect on other compounds. In other words, toxicity of Limonene, Myrcene, α-pinene, β-pinene and γ-terpinene were similar when they were tested individually and mixed with Comphene. The same situation was observed in Limonene+Myrcene, Myrcene+γ-terpinene and α-pinene+β-pinene mixtures. The effects of the Limonene+β-pinene mixture were additive but the mixtures of Limonene+α-pinene and Limonene+γ-terpinene showed antagonistic effect (Table 2). Although the mixtures of Myrcene+α-pinene and Myrcene+β-pinene had additive effects, the mixture of Myrcene+γ-terpinene showed no observable effect. α-pinene+β-pinene mixture showed no observable effect; however, the mixture of γ-terpinene with each of these compounds showed an antagonistic effect. When mixture toxicity is less than the toxicity of the individual compounds used in the mixture, it means that these compounds have antagonistic effects. In other words, they decrease the toxicity of each other. For additive effects, mixture toxicity is higher than the toxicity of each compound but it is important to consider that this value varies from case to case (Table 2-7). In general, camphene didn't have any effects on other compounds and γ-terpinene showed an antagonistic effect when it was mixed with other compounds. The effects of Limonene and Myrcene varied from no observable to additive (Table 2).

Table 2: The toxicity of Paired monoterpene hydrocarbons against the second larvae of Colorado potato beetle

		2nd larvae		
		Mean	Mortality (%)[a]	
Compound	Dose (mg)	48 h	96 h	Effects between compounds
Camphene+Limonene	5	56.1±1.7***	63.0±1.7***	No observable effects
	10	51.0±3.4***	61.3±3.4***	No observable effects
Camphene+Myrcene	5	49.3±3.4***	56.1±3.4***	No observable effects
	10	54.4±6.8***	59.5±6.8***	No observable effects
Camphene+α-pinene	5	39.1±3.4***	42.5±3.4***	No observable effects
	10	39.1±5.1***	42.5±6.8***	No observable effects
Camphene+β-pinene	5	51.0±3.4***	57.8±5.1***	No observable effects
	10	66.3±1.7***	68.1±6.8***	No observable effects
Camphene+γ-terpinene	5	63.0±5.1***	64.7±1.7***	No observable effects
	10	66.3±1.7***	69.7±8.5***	No observable effects
Limonene+Myrcene	5	54.5±3.4***	59.5±1.7***	No observable effects
	10	59.5±3.4***	63.0±3.4***	No observable effects
Limonene+α-pinene	5	42.5±5.1***	44.2±3.4***	Antagonistic effect
	10	44.2±3.4***	47.6±5.1***	Antagonistic effect
Limonene+β-pinene	5	57.8±6.8***	66.4±5.1***	Additive effects
	10	73.1±1.7***	69.7±6.8***	Additive effects
Limonene+γ-terpinene	5	61.3±1.7***	63.0±3.4***	Antagonistic effect
	10	64.7±6.8***	66.4±8.5***	Antagonistic effect
Myrcene+α-pinene	5	56.1±1.7***	66.3±5.1***	Additive effects
	10	59.5±3.4***	69.7±3.4***	Additive effects
Myrcene+β-pinene	5	71.4±5.1***	73.1±1.7***	Additive effects
	10	86.7±3.4***	88.4±3.4***	Additive effects
Myrcene+γ-terpinene	5	66.4±6.8***	66.4±6.8***	No observable effects
	10	68.0±3.4***	68.0±1.7***	No observable effects
α-pinene+β-pinene	5	52.7±1.7***	56.1±5.1***	No observable effects
	10	64.6±3.4***	66.3±5.1***	No observable effects
α-pinene+γ-terpinene	5	34.0±5.1***	40.8±3.4***	Antagonistic effect
	10	37.4±3.4***	42.5±3.4***	Antagonistic effect
β-pinene+γ-terpinene	5	51.0±1.7***	52.7±3.4***	Antagonistic effect
	10	64.6±1.7***	61.3±5.1***	Antagonistic effect
Control	-	0.0±0.0	1.7±1.7	-

* Significant at 0.05; ** Significant at 0.01; *** Significant at 0.001 according to Control. [a]Mean±SE of three replicates, each set up with 15 second instars larvae

The mixture of Myrcene+β-pinene had the highest toxicity against the second and third instars larvae, followed by the Limonene+β-pinene mixture that showed relatively high toxicity against these developmental stages (Table 2, 3). The least toxic mixtures to the second and third instars larvae were Camphene+α-pinene and α-pinene+γ-terpinene. It is important to notice that in general, toxicity of mixtures against the second instars larvae was more than the toxicity of the same mixtures against the third instars larvae (Table 2, 3).

Most of the mixtures had high toxicity on the adults and only mixtures of Camphene+γ-terpinene and Limonene+γ-terpinene showed relatively low toxicity. The highest

Table 3: The toxicity of Paired monoterpene hydrocarbons against the third larvae of Colorado potato beetle

		3rd larvae		
		Mean	Mortality (%)[a]	
Compound	Dose (mg)	48 h	96 h	Effects between compounds
Camphene+Limonene	5	25.5±1.7**	32.3±5.1***	no observable effects
	10	30.6±1.7***	37.4±3.4***	no observable effects
Camphene+Myrcene	5	27.2±5.1**	34.0±1.7***	no observable effects
	10	30.6±5.1***	42.5±5.1***	no observable effects
Camphene+α-pinene	5	1.7±1.7	6.8±3.4	no observable effects
	10	10.2±3.4	18.7±5.1	No observable effects
Camphene+β-pinene	5	44.2±5.1***	44.2±3.4***	No observable effects
	10	57.8±3.4***	61.2±3.4***	No observable effects
Camphene+γ-terpinene	5	11.9±1.7	22.1±1.7*	No observable effects
	10	20.4±5.1*	25.5±6.8**	No observable effects
Limonene+Myrcene	5	32.3±5.1***	37.4±1.7***	No observable effects
	10	37.4±3.4***	44.2±5.1***	No observable effects
Limonene+α-pinene	5	6.8±3.4	8.5±1.7	Antagonistic effect
	10	11.9±1.7	20.4±3.4*	Antagonistic effect
Limonene+β-pinene	5	47.6±5.1***	51.0±5.1***	Additive effects
	10	64.6±6.8***	66.3±3.4***	Additive effects
Limonene+γ-terpinene	5	6.8±3.4	17.0±1.7	Antagonistic effect
	10	17.0±5.1	18.7±1.7	Antagonistic effect
Myrcene+α-pinene	5	32.3±1.7***	42.5±3.4***	Additive effects
	10	40.8±3.4***	47.6±1.7***	Additive effects
Myrcene+β-pinene	5	69.7±5.1***	71.4±6.8***	Additive effects
	10	78.2±3.4***	86.7±8.5***	Additive effects
Myrcene+γ-terpinene	5	25.5±3.4**	35.7±1.7***	No observable effects
	10	32.3±1.7***	44.2±6.8***	No observable effects
α-pinene+β-pinene	5	44.2±1.7***	45.9±3.4***	No observable effects
	10	57.8±3.4***	62.9±1.7***	No observable effects
α-pinene+γ-terpinene	5	3.4±3.4	5.1±1.7	Antagonistic effect
	10	8.5±1.7	17.0±3.4	Antagonistic effect
β-pinene+γ-terpinene	5	37.4±1.7***	42.5±3.4***	Antagonistic effect
	10	52.7±5.1***	57.8±3.4***	Antagonistic effect
Control	-	1.7±1.7	1.7±1.7	-

* Significant at 0.05; ** Significant at 0.01; *** Significant at 0.001 according to Control. [a] Mean±SE of three replicates, each set up with 15 third instars larvae

toxicity belonged to Myrcene+α-pinene, followed by the Myrcene+β-pinene mixture. The toxicity of other mixtures was similar and in general, it can be said that these compounds were more effective against the adults of Colorado potato beetle individually and in mixtures in comparison to their toxicity against other developmental stages (Table 4).

Toxicity of three-compound mixtures: When three monoterpene compounds were mixed, results showed that effects between three compounds in mixtures possessing γ-terpinene were antagonistic. In other words, antagonistic effect of this compound on other compounds was predominant effect (Table 5-7).

Table 4: The toxicity of Paired monoterpene hydrocarbons against the adult of Colorado potato beetle

Compound	Dose (mg)	Adults Mean Mortality (%)[a] 48 h	96 h	Effects between compounds
Camphene+Limonene	5	61.2±3.4***	66.3±5.1***	No observable effects
	10	68.0±3.4***	62.9±3.4***	No observable effects
Camphene+Myrcene	5	69.7±6.8***	69.7±6.8***	No observable effects
	10	66.3±1.7***	64.6±1.7***	No observable effects
Camphene+α-pinene	5	64.6±5.1***	66.3±3.4***	No observable effects
	10	68.0±8.5***	69.7±1.7***	No observable effects
Camphene+β-pinene	5	66.3±1.7***	68.0±3.4***	No observable effects
	10	64.6±5.1***	66.3±1.7***	No observable effects
Camphene+γ-terpinene	5	25.5±3.4**	34.0±3.4***	No observable effects
	10	30.6±1.7***	35.7±1.7***	No observable effects
Limonene+Myrcene	5	61.2±3.4***	68.0±5.1***	No observable effects
	10	68.0±3.4***	66.3±3.4***	No observable effects
Limonene+α-pinene	5	59.5±5.1***	62.9±3.4***	Antagonistic effect
	10	61.2±6.8***	62.9±5.1***	Antagonistic effect
Limonene+β-pinene	5	71.4±5.1***	73.1±3.4***	Additive effects
	10	66.3±3.4***	69.7±5.1***	Additive effects
Limonene+γ-terpinene	5	25.5±3.4**	27.2±1.7**	Antagonistic effect
	10	27.2±1.7**	28.9±3.4**	Antagonistic effect
Myrcene+α-pinene	5	91.8±5.1***	93.5±3.4***	Additive effects
	10	95.2±4.8***	96.9±3.1***	Additive effects
Myrcene+β-pinene	5	88.4±3.4***	91.8±5.1***	Additive effects
	10	86.7±5.1***	90.1±6.8***	Additive effects
Myrcene+γ-terpinene	5	69.7±3.4***	66.3±1.7***	No observable effects
	10	62.9±5.1***	68.0±5.1***	No observable effects
α-pinene+β-pinene	5	66.3±3.4***	68.0±3.4***	No observable effects
	10	68.0±3.4***	68.0±1.7***	No observable effects
α-pinene+γ-terpinene	5	40.8±5.1***	45.9±1.7***	Antagonistic effect
	10	51.0±3.4***	54.4±3.4***	Antagonistic effect
β-pinene+γ-terpinene	5	52.7±3.4***	54.4±5.1***	Antagonistic effect
	10	56.1±5.1***	59.5±3.4***	Antagonistic effect
Control	-	1.7±1.7	1.7±1.7	-

* Significant at 0.05; ** Significant at 0.01; *** Significant at 0.001 according to Control. [a] Mean±SE of three replicates, each set up with 15 adult insects

In general, the toxicity of three-compound mixtures on the second instars larvae was higher than on the third instars larvae. The mixtures of Limonene+Myrcene+β-pinene and Camphene+Limonene+β-pinene showed the highest toxicity against the second and third instars larvae, followed by the mixture of Myrcene+α-pinene+β-pinene. The lowest toxicity against these developmental stages was associated with Myrcene+α-pinene+γ-terpinene mixture (Table 5, 6).

The three-compound mixture toxicity on the adults of Colorado potato beetle displayed the highest values among the three developmental stages tested. The mixtures of

Table 5: The toxicity of mixture of three monoterpene hydrocarbons against the second larvae of Colorado potato beetle

Compound	Dose (mg)	2nd larvae Mean Mortality (%)[a] 48 h	96 h	Effects between compounds
Camphene+Limonene+Myrcene	5	59.5±1.7***	69.7±3.4***	Additive effects
	10	61.3±3.4***	66.4±5.1***	Additive effects
Camphene+Limonene+α-pinene	5	47.6±5.1***	44.2±3.4***	Antagonistic effect
	10	45.9±1.7***	52.7±1.7***	Antagonistic effect
Camphene+Limonene+β-pinene	5	61.3±1.7***	68.0±1.7***	Additive effects
	10	78.2±3.4***	76.5±3.4***	Additive effects
Camphene+Limonene+γ-terpinene	5	56.1±5.1***	57.8±3.4***	Antagonistic effect
	10	59.5±3.4***	59.5±5.1***	Antagonistic effect
Limonene+Myrcene+α-pinene	5	49.3±3.4***	59.5±3.4***	No observable effects
	10	52.7±1.7***	62.9±1.7***	No observable effects
Limonene+Myrcene+β-pinene	5	63.0±1.7***	68.0±1.7***	Additive effects
	10	78.2±3.4***	81.6±3.4***	Additive effects
Limonene+Myrcene+γ-terpinene	5	49.3±5.1***	51.0±1.7***	Antagonistic effect
	10	51.0±3.4***	62.9±3.4***	Antagonistic effect
Myrcene+α-pinene+β-pinene	5	56.1±3.4***	64.6±3.4***	Additive effects
	10	66.3±5.1***	73.1±1.7***	Additive effects
Myrcene+α-pinene+γ-terpinene	5	30.6±1.7***	34.0±3.4***	Antagonistic effect
	10	34.0±3.4***	37.4±1.7***	Antagonistic effect
α-pinene+β-pinene+γ-terpinene	5	49.3±5.1***	51.0±3.4***	Antagonistic effect
	10	59.5±3.4***	61.3±5.1***	Antagonistic effect
Control	-	0.0±0.0	1.7±1.7	-

* Significant at 0.05; ** Significant at 0.01; *** Significant at 0.001 according to Control. [a] Mean±SE of three replicates, each set up with 15 second instars larvae

Table 6: The toxicity of mixture of three monoterpene hydrocarbons against the third larvae of Colorado potato beetle

Compound	Dose (mg)	3rd larvae Mean Mortality (%)[a] 48 h	96 h	Effects between compounds
Camphene+Limonene+Myrcene	5	37.4±3.4***	42.5±5.1***	Additive effects
	10	42.5±5.1***	47.6±3.4***	Additive effects
Camphene+Limonene+α-pinene	5	11.9±1.7	17.0±1.7	Antagonistic effect
	10	17.0±3.4	28.9±1.7**	Antagonistic effect
Camphene+Limonene+β-pinene	5	52.7±1.7***	56.1±5.1***	Additive effects
	10	68.0±3.4***	71.4±5.1***	Additive effects
Camphene+Limonene+γ-terpinene	5	3.4±3.4	15.3±1.7	Antagonistic effect
	10	11.9±1.7	15.3±3.4	Antagonistic effect
Limonene+Myrcene+α-pinene	5	27.2±3.4**	34.0±3.4***	No observable effects
	10	32.3±1.7***	40.8±5.1***	No observable effects
Limonene+Myrcene+β-pinene	5	64.6±3.4***	66.3±5.1***	Additive effects
	10	73.1±1.7***	79.9±5.1***	Additive effects
Limonene+Myrcene+γ-terpinene	5	6.8±3.4	17.0±3.4	Antagonistic effect
	10	17.0±1.7	20.4±1.7*	Antagonistic effect
Myrcene+α-pinene+β-pinene	5	51.0±3.4***	56.1±3.4***	Additive effects

Table 6: Continued

Compound	Dose (mg)	3rd larvae Mean Mortality (%)[a] 48 h	96 h	Effects between
	10	68.0±1.7***	73.1±1.7***	Additive effects
Myrcene+α-pinene+γ-terpinene	5	1.7±1.7	1.7±1.7	antagonistic effect
	10	5.1±1.7	11.9±3.4	Antagonistic effect
α-pinene+β-pinene+γ-terpinene	5	6.8±3.4	10.2±5.1	Antagonistic effect
	10	11.9±5.1	17.0±1.7	Antagonistic effect
Control	-	1.7±1.7	1.7±1.7	-

* Significant at 0.05; ** Significant at 0.01; *** Significant at 0.001 according to Control. [a] Mean±SE of three replicates, each set up with 15 third instars larvae

Table 7: The toxicity of mixture of three monoterpene hydrocarbons against the adult of Colorado potato beetle

Compound	Dose (mg)	Adults Mean Mortality (%)[a] 48 h	96 h	Effects between compounds
Camphene+Limonene+Myrcene	5	76.5±1.7***	79.9±3.4***	Additive effects
	10	73.1±3.4***	73.1±5.1***	Additive effects
Camphene+Limonene+α-pinene	5	56.1±3.4***	59.5±3.4***	Antagonistic effect
	10	61.2±5.1***	62.9±3.4***	Antagonistic effect
Camphene+Limonene+β-pinene	5	76.5±6.8***	76.5±5.1***	Additive effects
	10	69.7±3.4***	73.1±3.4***	Additive effects
Camphene+Limonene+γ-terpinene	5	17.0±3.4	20.4±5.1*	Antagonistic effect
	10	20.4±5.1*	25.5±6.8**	Antagonistic effect
Limonene+Myrcene+α-pinene	5	68.0±6.8***	69.7±5.1***	No observable effects
	10	66.3±3.4***	68.0±3.4***	No observable effects
Limonene+Myrcene+β-pinene	5	81.6±3.4***	86.7±5.1***	Additive effects
	10	79.9±1.7***	83.3±3.4***	Additive effects
Limonene+Myrcene+γ-terpinene	5	18.7±3.4	27.2±3.4**	Antagonistic effect
	10	25.5±1.7**	28.9±1.7**	Antagonistic effect
Myrcene+α-pinene+β-pinene	5	81.6±3.4***	86.7±5.1***	Additive effects
	10	85.0±1.7***	91.8±3.4***	Additive effects
Myrcene+α-pinene+γ-terpinene	5	51.0±3.4***	57.8±1.7***	Antagonistic effect
	10	59.5±5.1***	59.5±5.1***	Antagonistic effect
α-pinene+β-pinene+γ-terpinene	5	56.1±1.7***	59.5±3.4***	Antagonistic effect
	10	57.8±1.7***	61.2±1.7***	Antagonistic effect
Control	-	1.7±1.7	1.7±1.7	-

* Significant at 0.05; ** Significant at 0.01; *** Significant at 0.001 according to Control. [a] Mean±SE of three replicates, each set up with 15 adult insects

Limonene+Myrcene+β-pinene and Myrcene+α-pinene+β-pinene showed the highest toxicity on the adults, followed by Camphene+Limonene+Myrcene and Camphene+Limonene+β-pinene mixtures that had relatively high toxicity against this growth stage. The lowest toxicity against adults was related to the mixtures of Camphene+Limonene+γ-terpinene and Limonene+Myrcene+γ-terpinene, where the toxicity of mixtures was less than 30% (Table 7).

DISCUSSION

The results of this study showed that monoterpene hydrocarbons tested as single compounds and mixtures exhibit insecticidal activity against three tested developmental stages of Colorado potato beetle; however, the toxicity of the different compounds on the three growth stages varied. Among the tested single compounds, β-pinene showed relatively strong toxicity against both larvae and adults and Limonene and Myrcene had relatively high toxicity against the second larval stage and adults. γ-terpinene was the most toxic compounds among tested compounds against the second larvae and α-pinene was the most toxic compound against only adults.

In recent years, several studies were reported on the fumigation toxicity of some pure monoterpenoid constituents on various insect species and in their majority it has been stated that different constituents of monoterpenes can be one of the best and safest alternatives for synthetic insecticides (Prates *et al*., 1998; Papachristos *et al*., 2004; Kordali *et al*., 2007). There is only one study done on the effects of monoterpenes and essential oils on the Colorado potato beetle (Kordali *et al*., 2007) but no report was found in related literature on the effects of mixtures of pure monoterpene hydrocarbons. In this respect, this is the first report on the toxicity of monoterpene hydrocarbons as single compounds and in mixtures that provides some information on the possible interactions between the different compounds of the monoterpene hydrocarbon group.

It has been stated previously that monoterpenes possess varying insecticidal activities on the various insect species and that, in general, some monoterpene hydrocarbons such as Limonene, Myrcene and α-pinene and β-pinene were found to be more toxic (Don-Pedro, 1996; Lee *et al*., 1997, 2003, 2004; Kordali *et al*., 2007). The present results are in agreement with previous reports, especially the results presented by Kordali *et al*. (2007). Lee *et al*. (2003) studied the fumigation toxicity of 22 monoterpenoids to several stored product insects and indicated that some compounds like Camphene and γ-terpinene are more effective on larval stages than adults. Kordali *et al*. (2007) showed that the toxicity of Comphene against different developmental stages of Colorado potato beetle was low to moderate and γ-terpinene showed high toxicity against the second instars larvae and adults. Those results are supported the results of the present study, where the toxicity of Comphene was relatively low against all developmental stage of this key pest and γ-terpinene had high toxicity only on the second developmental stages of tested insect. Likewise, strong toxicity of Menthone, 1,8-cineole and Limonene against *Rhyzopertha dominica* (F.) and *Tribolium costaneum* (Herbest) have been shown by Prates *et al*. (1998). The result of this study also showed that Limonene was one of the most effective compounds against the Colorado potato beetle. In addition, similar results have been presented by Kordali *et al*. (2007) on α-pinene, β-pinene and Limonene where they exhibited high toxicity against this key pest. Similarly, it has been shown that α-pinene and γ-terpinene, that exhibit more toxic effects on adults and the second instars larvae of Colorado potato beetles in the present study, also caused over 90% mortality against *Sitophilus granaries* (Kim and Ahn, 2001).

When Comphene was mixed with only one other compound, had no effect or at least no measurable effect in comparison with other five compounds. This suggests that Comphene did not interact or had a very low antagonistic effect on other compounds. Limonene and Myrcene showed additive effects in mixtures possessing β-pinene but effects of these compounds on 4 other compounds varied from antagonistic to additive effect and thus there is no definite answer as to what the exact effects of these compounds are. γ-terpinene showed a low or moderate antagonistic effect when it was mixed by other compounds; however, its antagonistic effect was more observable when it was mixed with two other compounds. Although α-pinene and β-pinene showed additive effects in mixtures consisting of two or three compounds, antagonistic effects of γ-terpinene was much stronger than the additive effects of these compounds. γ-terpinene influenced the effects of other compounds in mixtures possessing two or three single compounds and decreased the toxicity of other compounds but the magnitude of this decrease differed between cases.

It is important to consider that these data are not enough to derive an exact conclusion on the effects of single compounds on each other in mixtures. In order to understand the interaction between different compounds it is probably better to analyze the compounds and find some information about the chemistry of single compounds and possible chemical reactions between compounds when they are mixed. It is possible that the chemical structure of compounds changes during interaction with other compounds and produce metabolites which are more or less toxic than original compounds (Stankovic *et al.*, 2004; Denloye *et al.*, 2006; Alyokhin *et al.*, 2007; Baker *et al.*, 2007). In addition, evaluation of the effects of mixtures possessing four or more single compounds will probably produce more precise data on the possible interactions between these toxic compounds.

In conclusion, the development of natural or biological insecticides will help decrease the negative effects of synthetic chemicals such as residues in products, insect resistance and environmental pollution (Nassar and Abdulah, 2005). In this respect, natural insecticides may also be effective, selective, easily bio-degradable and relatively low pollution for environment (Ramasubramanian and Regupathy, 2004). In the present study, the majority of the compounds examined as single compounds or mixtures were found to be toxic against different developmental stages of Colorado potato beetles but in general, the toxicity of these compounds on the adults were higher than the toxicity of same compounds against larval stages. This may be related to the physiology of insects in different developmental stages. Therefore, in the light of the present results, it can be suggested that these compounds and/or the plant essential oils which contain a high enough content of these compounds can be used as new insecticidal reagents against *L.decemlineata*, Colorado potato beetle. However, further studies need to be conducted to evaluate the cost and safety of these reagents.

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