Inhibitory Effects of Monoterpenes on Seed Germination and Seedling Growth

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Monoterpenes, the chemical constituents of essential oils found in plants, are known biologically active compounds. The present study was conducted to investigate the inhibitory effects of 30 monoterpenes including monoterpene hydrocarbons and oxygenated monoterpenes on seed germination and seedling growth of *Amaranthus retroflexus, Chenopodium album* and *Rumex crispus* under laboratory conditions. The monoterpenes were applied at contents of 10 and 20 μl for liquid compounds and 10 and 20 μg for solid compounds. The results show that most of the monoterpenes significantly inhibited seed germination and seedling growth of the tested plants. Oxygenated monoterpenes including β-citronellol, nerol and terpinen-4-ol completely inhibited seed germination and seedling growth of all tested plants. Their inhibitory effects were also stronger than that of the herbicide 2,4-D. In general, monoterpenes were less effective against seed germination and seedling growth of *C. album* as compared with *R. crispus* and *A. retroflexus*. Phytotoxic effects of monoterpene hydrocarbons were found to be lower than those of oxygenated monoterpenes. The alcohol derivatives of oxygenated monoterpenes were also found to be more phytotoxic as compared with their acetate derivatives. Based on the present results, it can be concluded that the oxygenated monoterpenes can be used as potential bio-herbicides.

Key words: Allelopathy, Herbicidal Effect, Monoterpenes

Introduction

Scientists have focused on the increase of food production needed for the fast expansion of the world's population. Weeds are the major problem in world agriculture because they cause losses in crop yields. The discovery of synthetic herbicides such as 2,4-D and 2,4,5-T within the last 50 years successfully reduced labour costs spent on weed management and enhanced crop productivity. Therefore, farmers have increased herbicide use. However, the intensive use of synthetic herbicides resulted in soil and groundwater contamination, and development of weed resistance (Abrahim *et al.*, 2000). Herbicides at high concentrations increase the risk of toxic residues in agricultural products. The use of synthetic herbicides has also some unfavourable effects for the environment and mammalian health due to their slow biodegradation in the environment. Therefore, there is a need for new herbicides less harmful for mammalian health and the environment. There is a considerable interest in the development of natural products and bio-herbicides (Dudai *et al.*, 1993, 1999; Duke *et al.*, 2000; Tworkoski, 2002). Thus, researchers have focused on new potential herbicides including various plant extracts, plant secondary metabolites, plant essential oils and monoterpenes (Putnam and Duke, 1978; Rice, 1984; Duke *et al.*, 1988; Dudai *et al.*, 1993, 1999; Tworkoski, 2002; Angelini *et al.*, 2003; Scrivanti *et al.*, 2003). Among these, potent plant inhibitory effects of some monoterpenes have been shown (Robinson, 1983; Duke, 1991; Koitabashi *et al.*, 1997; Abrahim *et al.*, 2000; Singh *et al.*, 2002, 2004; Scrivanti *et al.*, 2003; Topal *et al.*, 2006; Vokou *et al.*, 2003; Zunino and Zygadlo, 2004; Nishida *et al.*, 2005). Bio-herbicides have been also shown different and selective herbicidal mechanisms in comparison to their synthetic derivatives (Rizvi *et al.*, 1980; Duke *et al.*, 2000).

The allelopathic properties of plants and their metabolites may be effectively used for biological weed management in crop production (Rice, 1984). Essential oils contain natural flavours and fragrances grouped as monoterpenes (hydrocarbons and oxygenated derivatives), sesquiterpenes

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(hydrocarbons and oxygenated derivatives) and aliphatic compounds (alkanes, alkenes, ketones, aldehydes, acids and alcohols) that provide characteristic odours. Many essential oils isolated from various plant species belonging to different genera contain relatively high amounts of monoterpenes. Allelopathic activities of numerous essential oils and their monoterpenes have been extensively studied on seeds of several species (Jimenez-Osornio *et al.*, 1996; Dudai *et al.*, 1999; Abrahim *et al.*, 2000; Singh *et al.*, 2002, 2004; Tworkoski, 2002; Angelini *et al.*, 2003; Scrivanti *et al.*, 2003; Vokou *et al.*, 2003; Mao *et al.*, 2004; Zunino and Zygadlo, 2004).

Amaranthus retroflexus, Chenopodium album as well as *Rumex crispus* are important weeds in cultivated areas of Turkey; therefore they cause loss in crop yields. The aim of the present study was to investigate the potential inhibitory effects of some commercial monoterpenes on seed germination and seedling growth of *A. retroflexus, C. album* and *R. crispus*.

Experimental

Individual monoterpenoids

The compounds tested were *allo*-ocimene (Fluka), borneol (Fluka), bornyl acetate (Sigma), camphene (Fluka), camphor (Fluka), 3-carene (Aldrich), carvone (Fluka), 1,8-cineole (Sigma), citronellal (Sigma), β-citronellene (Fluka), β-citronellol (Fluka), dihydrocarvone (Alfa), fenchol (Fluka), fenchone (Fluka), geranyl acetate (Alfa), isomenthol (Alfa), limonene (Fluka), limonene oxide (Aldrich), linalool (Fluka), linalyl acetate (Fluka), menthol (Fluka), menthone (Fluka), myrcene (Aldrich), nerol (Sigma), neryl acetate (Alfa), α-pinene (Fluka), β-pinene (Fluka), γ-terpinene (Sigma), terpinen-4-ol (Aldrich), α-terpineol (Merck) and 2,4-D (Takimsan, Istanbul, Turkey).

Seed germination and seedling growth experiments

The seeds of *A. retroflexus, C. album* and *R. crispus* were collected in Erzurum region (Turkey) in October 2004. Empty and undeveloped seeds were discarded by floating in tap water. To avoid possible inhibition caused by toxins from fungi or bacteria, the seeds were surface-sterilized with 15% sodium hypochlorite for 20 min (Tinnin and Kirkpatrick, 1985) and then rinsed with abundant distilled water. Two layers of filter paper were placed on the bottom of each Petri dish (9 cm diameter) and then 50 seeds of *A. retroflexus*, *C. album* and *R. crispus* were placed on the filter paper (Tefera, 2002). Then, 10 ml of distilled water was added to each Petri dish. Lids of Petri dishes were closed with Whatman no. 1 filter paper wrapping up tightly with a transparent ribbon.

Treatment and experimental design

The treatments were applied at 10 and 20 μl/Petri dish contents of liquid compounds and 10 and 20 μg/Petri dish contents of solid compounds. Seeds and filter papers were moistened with 10 ml of distilled water. 10 and 20 μl of the liquid compounds were dripped on the paper placed on the lid using a micropipette. Solid compounds were dissolved in ethanol (1:1, w/v). Whatman no. 1 filter papers were impregnated with the appropriate amounts of the solutions (10 and 20 μg/Petri dish), placed on the lid of Petri dishes and then kept to evaporate the ethanol. Petri dishes were closed with an adhesive tape to prevent escaping of volatile compounds and kept at (23 ± 2) °C on a laboratory bench supply with 12 h of fluorescent light and humidity of 80% (Dudai *et al.*, 1993). After 10 d, the number of germinated seeds and seedling lengths were estimated. Germination was measured as the percentage of seeds from which a radicle emerges. The treatments were arranged in a completely randomized design with three replications including controls.

Statistical analysis

Data of seed germination and seedling growth 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 were considered significantly different.

Results and Discussion

Monoterpenes are chemical constituents of plant essential oils. In the present study, inhibitory effects of 30 commercial monoterpenes including monoterpene hydrocarbons and oxygenated monoterpenes were tested on seed germination and seedling growth of *A. retroflexus, C. album* and *R. crispus*. The present results showed that the tested compounds have inhibitory effects on seed germination and seedling growth of the tested weeds (Tables I, II). Different degrees of inhibition were

Table I. Inhibitory effects of monoterpene hydrocarbons on seed germination and seedling growth of *A. retroflexus, C. album* and *R. crispus.*

Compound	Dose	*A. retroflexus*			*C. album*			*R. crispus*		
		Germination[a] (%)	Seedling growth [mm][a] Root	Aerial part	Germination[a] (%)	Seedling growth [mm][a] Root	Aerial part	Germination[a] (%)	Seedling growth [mm][a] Root	Aerial part
Control	–	76.5 ± 1.0	28.9 ± 1.0	16.9 ± 0.05	61.3 ± 0.7	41.0 ± 1.3	9.7 ± 0.3	75.3 ± 4.7	24.9 ± 1.3	11.4 ± 0.3
Camphene	10 μg	80.0 ± 3.1	21.5 ± 1.3***	12.2 ± 0.7***	52.7 ± 1.2	51.3 ± 2.2**	4.8 ± 1.3**	83.3 ± 7.5	29.6 ± 1.4	9.4 ± 0.1
	20 μg	45.3 ± 8.4***	26.3 ± 1.6	15.4 ± 7.0	53.3 ± 5.4	54.5 ± 2.3**	9.8 ± 3.0	88.0 ± 1.2*	31.0 ± 1.3*	9.6 ± 0.1
3-Carene	10 μl	16.7 ± 6.4***	11.0 ± 1.1***	10.6 ± 1.0***	52.3 ± 1.5	15.0 ± 0.7***	8.1 ± 0.3	69.0 ± 2.9	21.4 ± 1.8	7.9 ± 0.4
	20 μl	4.0 ± 2.3***	5.0 ± 1.4***	2.9 ± 1.2***	46.7 ± 2.7**	13.1 ± 0.7***	8.1 ± 0.2	58.0 ± 3.5**	15.0 ± 1.3***	9.8 ± 0.0
β-Citronellene	10 μl	61.3 ± 6.7**	25.7 ± 1.2*	16.8 ± 0.7	52.3 ± 1.3	41.9 ± 1.6	8.6 ± 0.3	56.7 ± 3.0**	32.7 ± 1.9**	9.9 ± 0.2
	20 μl	60.7 ± 5.9**	27.9 ± 1.2	18.2 ± 0.6	54.3 ± 4.3	36.2 ± 1.3	7.9 ± 0.3	75.3 ± 8.0	37.1 ± 1.3**	10.0 ± 0.2
Limonene	10 μl	10.0 ± 3.5***	18.7 ± 4.2***	9.5 ± 1.6***	57.0 ± 2.1	35.1 ± 1.2***	13.9 ± 2.0**	60.7 ± 6.7*	31.3 ± 1.7*	10.4 ± 0.2
	20 μl	9.3 ± 4.4***	18.0 ± 4.3***	11.1 ± 2.2***	53.3 ± 1.5	31.2 ± 1.0***	7.9 ± 0.6	72.0 ± 8.0	41.3 ± 1.7***	8.7 ± 0.2*
Myrcene	10 μl	9.3 ± 3.5***	10.9 ± 2.3***	7.9 ± 1.6***	55.7 ± 2.8	23.2 ± 1.1***	16.6 ± 1.8	74.0 ± 2.0	34.3 ± 1.5**	10.3 ± 0.2
	20 μl	35.3 ± 12.7***	16.8 ± 1.2***	9.6 ± 0.5***	56.3 ± 1.4	5.3 ± 0.7***	7.1 ± 2.5	80.0 ± 6.4	32.4 ± 1.3**	10.1 ± 0.2
allo-Ocimene	10 μl	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	46.7 ± 6.1**	3.9 ± 0.1***	1.6 ± 0.3***	56.0 ± 5.8**	2.1 ± 0.0***	4.1 ± 0.0***
	20 μl	0.7 ± 0.7***	0.3 ± 0.3***	0.3 ± 0.3***	50.3 ± 2.2**	3.6 ± 0.1***	0.9 ± 0.2***	65.3 ± 7.7	2.7 ± 0.0***	5.8 ± 0.0***
α-Pinene	10 μl	32.7 ± 2.4***	24.7 ± 2.2*	14.3 ± 1.2*	48.0 ± 3.2*	47.5 ± 2.0**	9.5 ± 0.4	68.0 ± 7.0	27.0 ± 2.0	11.1 ± 0.2
	20 μl	34.0 ± 4.0***	24.2 ± 2.2*	13.6 ± 1.2**	53.3 ± 1.8	47.3 ± 1.9**	9.4 ± 0.3	75.0 ± 4.7	29.8 ± 1.5*	10.4 ± 0.2
β-Pinene	10 μl	5.3 ± 1.8***	16.9 ± 4.3**	10.1 ± 2.9**	54.0 ± 2.1	23.5 ± 1.4***	9.9 ± 0.3	57.3 ± 1.8**	36.8 ± 1.9**	8.7 ± 0.2*
	20 μl	14.0 ± 3.1***	13.9 ± 2.1***	8.5 ± 1.3***	53.3 ± 3.8	28.5 ± 1.7***	9.5 ± 0.3	62.3 ± 0.9*	22.8 ± 2.2	7.8 ± 0.2*
γ-Terpinene	10 μl	32.0 ± 9.0***	22.0 ± 1.4***	14.3 ± 0.9*	55.3 ± 3.0	31.3 ± 1.1***	14.8 ± 1.8**	88.7 ± 3.7*	33.9 ± 1.0**	8.9 ± 0.0*
	20 μl	21.3 ± 5.2***	14.5 ± 1.9***	11.0 ± 1.3***	45.3 ± 0.7***	34.0 ± 1.3***	14.3 ± 2.1**	82.0 ± 1.1	33.5 ± 1.5**	9.7 ± 0.2
2,4-D	10 μl	6.0 ± 2.3***	1.8 ± 0.1***	2.0 ± 0.3***	55.7 ± 1.4	5.4 ± 0.2***	10.1 ± 0.3	66.7 ± 5.8	12.8 ± 1.0***	18.4 ± 0.7***
(Positive control)	20 μl	2.0 ± 0.0***	1.3 ± 0.3***	2.8 ± 1.8***	57.0 ± 6.2	5.0 ± 0.2***	9.3 ± 0.3	70.7 ± 2.5	16.3 ± 1.5***	16.7 ± 0.5***

[a] Mean ± S.E. of three replicates, each set up with 50 weeds.
* Statistically significant difference at $p < 0.05$.
** Statistically significant difference at $p < 0.01$.
*** Statistically significant difference at $p < 0.001$ according to control.

Table II. Inhibitory effects of oxygenated monoterpenes on seed germination and seedling growth of *A. retroflexus, C. album* and *R. crispus.*

Compound	Dose	*A. retroflexus*			*C. album*			*R. crispus*		
		Germination[a] (%)	Seedling growth [mm][a] Root	Aerial part	Germination[a] (%)	Seedling growth [mm][a] Root	Aerial part	Germination[a] (%)	Seedling growth [mm][a] Root	Aerial part
Control	–	76.5 ± 1.0	28.9 ± 1.0	16.9 ± 0.05	61.3 ± 0.7	41.0 ± 1.3	9.7 ± 0.3	75.3 ± 4.7	24.9 ± 1.3	11.4 ± 0.3
Alcohols										
Borneol	10 μg	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	9.0 ± 0.6***	1.7 ± 0.2***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
	20 μg	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	9.7 ± 0.3***	1.4 ± 0.1***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
β-Citronellol	10 μl	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
	20 μl	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
Fenchol	10 μg	3.3 ± 1.8***	3.7 ± 1.2***	2.8 ± 1.2***	2.3 ± 1.4***	1.6 ± 0.4***	0.8 ± 0.8***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
	20 μg	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	4.7 ± 3.2***	1.7 ± 0.2***	2.8 ± 1.0**	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
Isomenthol	10 μg	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	24.0 ± 1.7***	3.5 ± 0.2***	0.2 ± 0.1***	87.3 ± 0.7	3.7 ± 0.1***	4.7 ± 0.1***
	20 μg	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	21.0 ± 0.6***	3.2 ± 0.2***	0.0 ± 0.0***	77.3 ± 3.8	3.0 ± 0.1***	4.2 ± 0.1***
Linalool	10 μl	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	1.0 ± 0.6***	0.8 ± 0.3***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
	20 μl	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	1.0 ± 0.6***	0.8 ± 0.3***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
Menthol	10 μg	2.0 ± 2.0***	5.2 ± 3.1***	4.4 ± 2.3***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
	20 μg	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	10.1 ± 0.3***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
Nerol	10 μl	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0****	0.0 ± 0.0***	0.0 ± 0.0***
	20 μl	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
Terpinen-4-ol	10 μl	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
	20 μl	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
α-Terpineol	10 μg	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	14.3 ± 2.7***	2.7 ± 0.8***	4.4 ± 2.0**	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
	20 μg	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	2.3 ± 0.9***	2.0 ± 0.5***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
Epoxides										
1,8-Cineole	10 μl	12.0 ± 2.0***	14.2 ± 2.1***	11.4 ± 2.0***	43.7 ± 4.9**	18.7 ± 1.4***	12.0 ± 0.4	36.0 ± 15.3***	4.9 ± 0.5***	4.6 ± 0.3***
	20 μl	16.0 ± 3.1***	16.8 ± 2.2***	14.6 ± 1.6	49.0 ± 5.5*	36.4 ± 1.7	11.0 ± 0.4	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
Limonene oxide	10 μl	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	47.3 ± 6.9**	2.3 ± 0.1***	2.9 ± 0.3**	10.0 ± 3.1***	2.1 ± 0.2***	0.7 ± 0.5***
	20 μl	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	45.0 ± 4.7***	1.7 ± 0.1***	0.5 ± 0.2***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
2,4-D (Positive control)	10 μl	6.0 ± 2.3***	1.8 ± 0.1***	2.0 ± 0.3***	55.7 ± 1.4	5.4 ± 0.2***	10.1 ± 0.3	66.7 ± 5.8	12.8 ± 1.0***	18.4 ± 0.7***
	20 μl	2.0 ± 0.0***	1.3 ± 0.3***	2.8 ± 1.8***	57.0 ± 6.2	5.0 ± 0.2***	9.3 ± 0.3	70.7 ± 2.5	16.3 ± 1.5***	16.7 ± 0.5***

Table II (continued).

Compound	Dose	*A. retroflexus*			*C. album*			*R. crispus*		
		Germination[a] (%)	Seedling growth [mm][a] Root	Aerial part	Germination[a] (%)	Seedling growth [mm][a] Root	Aerial part	Germination[a] (%)	Seedling growth [mm][a] Root	Aerial part
Control	–	76.5 ± 1.0	28.9 ± 1.0	16.9 ± 0.05	61.3 ± 0.7	41.0 ± 1.3	9.7 ± 0.3	75.3 ± 4.7	24.9 ± 1.3	11.4 ± 0.3
Ketones and aldehydes										
Camphor	10 μg	12.0 ± 5.0***	12.1 ± 2.2***	9.2 ± 1.8***	27.0 ± 1.5***	2.6 ± 0.2***	8.7 ± 0.3	82.0 ± 1.1	3.7 ± 0.1***	4.1 ± 0.3***
	20 μg	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	22.3 ± 7.3***	9.0 ± 2.6***	9.0 ± 0.3	4.0 ± 2.0***	1.7 ± 0.3***	0.0 ± 0.0***
Carvone	10 μl	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
	20 μl	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.7 ± 0.7***	1.0 ± 0.1***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
Citronellal	10 μl	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	3.3 ± 0.7***	1.3 ± 0.1***	0.0 ± 0.0***	75.3 ± 4.4	31.1 ± 1.9*	10.0 ± 0.3**
	20 μl	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	1.3 ± 0.3***	1.5 ± 0.3***	0.0 ± 0.0***	68.7 ± 1.3	31.8 ± 2.1*	10.4 ± 0.2**
Dihydrocarvone	10 μl	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	2.0 ± 2.0***	1.8 ± 0.3***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
	20 μl	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	3.7 ± 1.2***	5.4 ± 0.2***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
Fenchone	10 μl	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	22.3 ± 0.9***	2.1 ± 0.1***	0.5 ± 0.1***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
	20 μl	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	26.7 ± 5.8***	2.7 ± 0.1***	4.3 ± 0.6**	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
Menthone	10 μl	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	42.7 ± 0.7***	4.8 ± 1.3***	6.0 ± 1.8*	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
	20 μl	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	25.7 ± 1.5***	3.0 ± 0.2***	2.5 ± 0.3**	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
Esters										
Bornyl acetate	10 μl	6.7 ± 1.8***	1.8 ± 0.2***	1.0 ± 0.0***	36.7 ± 5.0***	4.1 ± 0.0	0.5 ± 0.1***	58.7 ± 4.0**	8.9 ± 0.9***	5.8 ± 0.2***
	20 μl	4.7 ± 0.7***	1.3 ± 0.2***	1.0 ± 0.0***	34.3 ± 4.9***	24.0 ± 1.1	26.8 ± 2.7***	55.3 ± 13.1**	13.4 ± 1.3**	6.9 ± 0.2***
Geranyl acetate	10 μl	2.7 ± 0.7***	2.5 ± 0.3***	1.0 ± 0.0***	37.7 ± 4.6***	8.4 ± 0.7***	4.7 ± 0.4**	56.3 ± 9.9**	15.5 ± 1.3*	8.9 ± 0.5**
	20 μl	0.7 ± 0.7***	1.0 ± 0.1***	0.3 ± 0.3***	36.3 ± 3.4***	5.2 ± 0.3***	3.1 ± 0.3**	57.3 ± 1.3**	12.7 ± 0.8**	10.4 ± 0.3**
Linalyl acetate	10 μl	2.0 ± 1.2***	2.0 ± 0.7***	1.0 ± 0.3***	42.0 ± 5.6***	19.2 ± 1.4***	6.7 ± 0.3*	66.7 ± 6.4	32.6 ± 1.7*	7.3 ± 0.1***
	20 μl	6.0 ± 3.1***	2.8 ± 0.4***	0.9 ± 0.1***	42.0 ± 4.6***	10.8 ± 0.8***	9.8 ± 1.5	68.0 ± 3.0	14.7 ± 1.4**	6.7 ± 0.2***
Neryl acetate	10 μl	5.3 ± 2.9***	5.4 ± 0.9***	0.9 ± 0.1***	38.0 ± 2.6***	6.5 ± 0.0***	4.3 ± 0.5**	63.3 ± 4.7	11.0 ± 0.8**	8.6 ± 0.2**
	20 μl	14.0 ± 0.0***	5.0 ± 0.5***	1.0 ± 0.0***	47.3 ± 0.7**	6.6 ± 0.1***	4.3 ± 0.3**	66.7 ± 5.5	16.5 ± 0.6**	9.2 ± 0.2**
2,4-D	10 μl	6.0 ± 2.3***	1.8 ± 0.1***	2.0 ± 0.3***	55.7 ± 1.4	5.4 ± 0.2***	10.1 ± 0.3	66.7 ± 5.8	12.8 ± 1.0***	18.4 ± 0.7***
(Positive control)	20 μl	2.0 ± 0.0***	1.3 ± 0.3***	2.8 ± 1.8***	57.0 ± 6.2	5.0 ± 0.2***	9.3 ± 0.3	70.7 ± 2.5	16.3 ± 1.5***	16.7 ± 0.5***

[a] Mean ± S.E. of three replicates, each set up with 50 weeds.
* Statistically significant difference at $p < 0.05$.
** Statistically significant difference at $p < 0.01$.
*** Statistically significant difference at $p < 0.001$ according to control.

observed when compared with control groups, whereas oxygenated monoterpenes have more potent herbicidal effects on seed germination as compared with monoterpene hydrocarbons. Some oxygenated monoterpenes were strong inhibitors and among them, β-citronellol, nerol and terpinen-4-ol totally suppressed the germination of the assayed species (Table II). Their phytotoxic effects were also higher than that of the commercial herbicide 2,4-D. Furthermore, as can be seen from Tables I and II, *A. retroflexus* was more sensitive than *C. album* and *R. crispus* against some monoterpene hydrocarbons. As shown in Table I, monoterpene hydrocarbons have less or no phytotoxic effects on the seed germination of *C. album*. In addition to these results, alcohol derivatives of oxygenated monoterpenes were more phytotoxic than their acetate derivatives.

In general, the tested compounds suppressed the seedling growth of the assayed species (Tables I, II) as compared with the control. In some cases, monoterpenes did not affect seedling growth of the assayed species, whereas seed germination was inhibited. For instance, while two doses of β-citronellene reduced the seed germination of *A. retroflexus*, they did not affect the seedling growth (Table I). The opposite results were also obtained for some monoterpene hydrocarbons. For instance, some monoterpenes did not affect the seed germination of *C. album*, whereas seedling growth of this species was significantly increased and/or reduced by some monoterpene hydrocarbons. As shown in Table I, limonene, myrcene and β-pinene, significantly reduced the seedling root growth of *C. album,* whereas they inhibited the seed germination (Table I). It is interesting to find that camphene and α-pinene increased the root growth of *C. album*. Our results also indicate that there are no correlations between the treatment dose and herbicidal effect. In addition to the findings above mentioned, in the light of the present results given in Tables I and II, the herbicidal effects of monoterpenoids seem to be not selective.

Previous research showed that monoterpenes and essential oils isolated from various plant species possess potent herbicidal effects on weed germination and seedling growth of various plant species (Koitabashi *et al.,* 1997; Abrahim *et al.*, 2000; Singh *et al.*, 2002, 2004; Tworkoski, 2002; Angelini *et al.*, 2003; Scrivanti *et al.*, 2003; Zunino and Zygadlo, 2004). In these researches, monoterpene hydrocarbons showed to have lower inhibitory effects than oxygenated monoterpenes (Singh *et al.*, 2002, 2004; Vokou *et al.*, 2003). The allelopathic effects of 47 monoterpenes on the seed germination of *Lactuca sativa* seedlings have been previously reported by Singh *et al.* (2004). These researchers found that the monoterpenes showed various allelopathic effects on seed germination and the hydrocarbons were the least inhibitors in comparison to oxygenated monoterpenes (Vokou *et al.*, 2003). The effect of four oxygenated monoterpenes (citronellal, citronellol, cineole and linalool) on the germination and growth of *Cassia occidentalis* was also investigated (Singh *et al.*, 2002). All tested compounds reduced seed germination, whereas citronellal and linalool completely inhibited seed germination of *C. occidentalis* (Singh *et al.*, 2002). Similar results were found in the present study. As shown in Tables I and II, the inhibitory effects of monoterpene hydrocarbons on seed germination and seedling growth of three plant species tested were lower than those of oxygenated monoterpenes. Relatively high herbicidal effects of oxygenated monoterpenes in comparison to monoterpene hydrocarbons can be attributed to the higher solubility of oxygenated monoterpenes in the test medium. Previously, it has been shown that oxygenated monoterpenes possess relatively high solubility in water as compared with the solubility of monoterpene hydrocarbons in water (Weidenhamer *et al.*, 1993; Fischer *et al.*, 1994). Our results also showed that alcohol derivatives of oxygenated monoterpenes were more phytotoxic than their acetate derivatives (Table II). Similarly, this can be attributed to the high solubility in water of alcohols that are polar compounds as compared with acetates.

In the present study, some oxygenated monoterpenes exhibited potent weed suppressing effects on weed germination and seedling growth of the tested weeds, despite there is no evidence of the herbicidal mechanism of these compounds. It is well known that monoterpenes in essential oils, have phytotoxic effects against various weed species, cause anatomical and physiological changes in plant seedlings probably due to the accumulation of lipid globules in cytoplasm, reduction in organelles including mitochondria and nuclei, inhibition of DNA synthesis, and disruption of membranes surrounding mitochondria and nuclei (Koitabashi *et al.*, 1997; Zunino and Zyagadlo, 2004; Nishida *et al.*, 2005). In a recent study, it has been shown that some monoterpenes inhibit both cell-

nuclear and organelle DNA synthesis in root apical meristem of *Brassica campestris* (Koitabashi *et al.*, 1997; Nishida *et al.*, 2005). Furthermore, in a different study, the content of chlorophyll was found to be less in plants treated with monoterpenes as compared to control (Singh *et al.*, 2002, 2004). In the view of these results mentioned above, in the present study, the seedling growth inhibition of plant species subjected to monoterpenes may be attributed to a reduction of cell division (Koitabashi *et al.*, 1997; Singh *et al.*, 2004; Zunino and Zygadlo, 2004; Nishida *et al.*, 2005).

In conclusion, *Amaranthus retroflexus, Chenopodium album* and *Rumex crispus* are important weeds in cultivated areas of cultured plants. The present results show that many oxygenated monoterpenes completely inhibited seed germination and seedling growth of these weeds. Their phytotoxic effects were even higher than that of 2,4-D, despite there is no evidence of the herbicidal mechanism of the monoterpenes. According to present results, it can be concluded that pure monoterpenes possess herbicidal effects and these compounds may be used as potential bio-herbicides. In this respect, bio-herbicides may be also effective, selective, biodegradable and relatively less toxic to the environment. However, further studies also need to be conducted to evaluate the costs, applicability, safety and herbicidal mechanism as well as phytotoxicity against the cultured plants of monoterpenes as potential herbicides.

Abrahim D., Braguini W. L., Kelmer-Bracht A. M., and Ishii-Iwamoto E. L. (2000), Effects of four monoterpenes on germination, primary root growth, and mitochondrial respiration of maize. J. Chem. Ecol. **26**, 611–624.

Angelini L. G., Carpanese G., Cioni P. L., Morelli I., Macchia M., and Flamini G. (2003), Essential oils from Mediterranean Lamiaceae as weed germination inhibitors. J. Agric. Food Chem. **51**, 6158–6164.

Dudai N., Poljakoff-Mayber A., Mayer A. M., Putievsky E., Ravid U., and Katzir E. (1993), Inhibition of germination and growth by volatiles of *Micromeria fruticosa.* Acta Horticult. (ISHS) **344**, 123–131.

Dudai N., Poljakoff-Mayber A., Mayer A. M., Putievsky E., and Lerner H. R. (1999), Essential oils as allelochemicals and their potential use as bioherbicides. J. Chem. Ecol. **25**, 1079–1089.

Duke S. O. (1991), Plant terpenoids as pesticides. In: Handbook of Natural Toxins. Toxicology of Plant and Fungal Compounds (Keeler R. F. and Tu A. T., eds.). Marcel Dekker, New York, Vol. 6, pp. 269–296.

Duke S. O., Paul R. N., and Lee S. M. (1988), Terpenoids from the genus *Artemisia* as potential pesticides. ACS Symposium Series **380**, 375–382.

Duke S. O., Dayan F. E., Romangni J. G., and Rimando A. M. (2000), Natural products as sources of herbicides: current status and future trends. Weed Res. **40**, 99–111.

Fischer N. H., Williamson G. B., Weidenhamer J. D., and Richardson D. R. (1994), In search of allelopathy in the Florida schrub – the role of terpenoids. J. Chem. Ecol. **20**, 1355–1380.

Jimenez-Osornio F. M. V. Z. J., Kumamoto J., and Wasser C. (1996), Allelopathic activity of *Chenopodium ambrosioides* L. Biochem. Syst. Ecol. **24**, 195–205.

Koitabashi R., Suzuki T., Kawazu T., Sakai A., Kuroiwa H., and Kuroiwa T. (1997), 1,8-Cineole inhibits root growth and DNA synthesis in the root apical meristem of *Brassica campestris* L. J. Plant Res. **110**, 1–6.

Mao L. X., Henderson G., and Laine R. A. (2004), Germination of various weed species in response to vetiver oil and nookatone. Weed Technol. **18**, 263–267.

Nishida N., Tamotsu S., Nagata N., Saito C., and Sakai A. (2005), Allelopathic effects of volatile monoterpenoids produced by *Salvia leucophylla*: Inhibition of cell proliferation and DNA synthesis in root apical meristem of *Brassica campestris* seedlings. J. Chem. Ecol. **31**, 1187–1203.

Putnam A. R. and Duke W. B. (1978), Allelopathy in agroecosystems. Annu. Rev. Phytopathol. **16**, 431–451.

Rice E. L. (1984), Allelopathy, 2nd ed. Academic Press, London.

Rizvi S. J. H., Mukerji D., and Mathur S. N. (1980), A new report on possible source of natural herbicide. Ind. J. Exp. Biol. **18**, 777–778.

Robinson J. B. (1983), The Organic Constituents of Higher Plants. Cordus Press, North Amherst, Massachusetts, USA.

Scrivanti L. R., Zunino M. P., and Zygadlo J. A. (2003), *Tagetes minuta* and *Schinus areira* essential oils as allelopathic agents. Biochem. Syst. Ecol. **31**, 563–572.

Singh H. P., Batish D. R., Kaur S., Ramezani H., and Kohli R. K. (2002), Comparative phytotoxicity of four monoterpenes against *Cassia occidentalis.* Ann. Appl. Biol. **141**, 111–116.

Singh H. P., Batish D. R., Kaur S., Vaid S., and Kohli R. (2004), Weed suppressing ability of some monoterpenes. Z. Pflanzenkr. Pflanzenschutz – J. Plant Dis. Plant Protect. **111**, 821–828.

Tefera T. (2002), Allelopathic effects of *Partenium hysterophorus* extracts on seed germination and seedling of *Eragrostis tef*. J. Argon. Crop Sci. **188**, 306–310.

Tinnin R. O. and Kirkpatrick L. A. (1985), The allelopathic influence of broadleaf tree and shrubs on seedling of Douglas fir. Forest Sci. **31**, 945–952.

Topal S., Kocacaliskan I., and Arslan D. (2006), Herbicidal potential of catechol as an allelochemical. Z. Naturforsch. **61c**, 69–73.

Tworkoski T. (2002), Herbicide effects of essential oils. Weed Sci. **50**, 425–431.

Vokou D., Douvli P., Blionis G. J., and Halley J. M. (2003), Effects of monoterpenoids, acting alone or in pairs, on seed germination and subsequent seedling growth. J. Chem. Ecol. **29**, 2281–2301.

Weidenhamer J. D., Macias F. A., Fischer N. H., and Williamson G. B. (1993), Just how insoluble are monoterpenes. J. Chem. Ecol. **19**, 1799–1807.

Zunino M. P. and Zygadlo J. A. (2004), Effect of monoterpenes on lipid oxidation in maize. Planta **219**, 303–309.