Weed Science 2016 64:191–200

PRE and POST Herbicidal Activity of Monoterpenes against Barnyard Grass (*Echinochloa crus-galli*)

Neama A. A. Gouda, Mona M. G. Saad, and Samir A. M. Abdelgaleil*

Monoterpenes, the major constituents of essential oils, are known for their diverse biological activities. This study was conducted to assess the herbicidal effect of six monoterpenes viz. (*R*)-carvone, 1,8-cineole, cuminaldehyde, (*S*)-fenchone, geraniol, (*S*)-limonene, and (*R*)-linalool on barnyardgrass under laboratory and glasshouse conditions with a view to explore the possibility of their utilization for future weed management. The effect of monoterpenes on chlorophyll contents and total phenolic compounds was also evaluated. The inhibitory effects of monoterpenes on seed germination and seedling growth were tested at concentrations of 1, 2, 4, 6, and 8 mM. The results showed that geraniol and (*R*)-carvone caused greatest reduction of seed germination with complete inhibition at the concentrations > 2 mM. Similarly, these two compounds were the most potent inhibiters for root and shoot growth. In general, monoterpenes were less effective against seed germination than seedling growth. Furthermore, the inhibition of root growth by all compounds was greater than that of shoot growth. In foliar application treatments under glasshouse conditions, the monoterpenes reduced the fresh and dry weights, and shoot length of two-leaf stage barnyardgrass at concentrations of 1 and 2%. In addition, the tested monoterpenes caused phytotoxicity symptoms, mainly chlorosis and necrosis, followed by weed death. Complete weed control was observed in the treatments with 1 and 2% of geraniol, and 2% of cuminaldehyde. Further, a reduction of chlorophyll contents and total phenolic compounds of barnyardgrass leaves was noticed, indicating that the monoterpenes cause adverse effect on photosynthesis and weed metabolism. Based on the results of this study, it can be concluded that the monoterpenes, particularly geraniol, (*R*)-carvone, and cuminaldehyde, can be used as potential natural herbicides.
Nomenclature: Barnyardgrass, *Echinochloa crus-galli* (L.) Beauv., (*R*)-carvone, 1-8-cineole, cuminaldehyde, (*S*)-fenchone, geraniol, (*S*)-limonene, (*R*)-linalool.
Key words: Chlorophyll contents, herbicidal activity, monoterpenes, natural herbicides, phenolic compounds.

During the last two decades, the use of plant secondary metabolites as weed control agents has attracted considerable attention. This is mainly due to public awareness about serious environmental and human health problems associated with intensive use of synthetic herbicides (Narwal 1999). The development of weed resistance to many currently used herbicides is another challenge facing food production all over the world (Duke et al. 2002). Plant natural products may have potential as weed management agents and can serve as templates for the development of new biodegradable herbicides. Among the plant natural products, essential oils and their major constituents, monoterpenes, are being explored for the discovery of new herbicides (Isman 2000).

Monoterpenes, the main constituents of essential oils, give plants their unique odoriferous properties because of their low boiling points. Several hundred monoterpenes have been isolated from plants and their structures identified. They are biosynthesized from geranyl pyrophosphate, the ubiquitous acyclic C_{10} intermediate of the isoprenoid pathway (Windholz et al. 1983). Monoterpenes can be classified into two major groups: monoterpene hydrocarbons that include acyclic, monocyclic, and dicyclic aliphatic and aromatic monoterpenes, and oxygenated monoterpenes that include acyclic, monocyclic, and dicyclic monoterpenoids. The latter group includes many alcohols, aldehydes, ketones, ethers, esters, and acids (Templeton 1969). Monoterpenes possess a wide spectrum of biological activities that are important in food chemistry, chemical ecology, and the pharmaceutical industry (Schewe et al. 2011). It has been also confirmed that monoterpenes are involved in numerous ecological functions in plants,

10.1614/WS-D-15-00045.1

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such as protection against herbivores and microbial diseases, attraction of pollinators, and in allelopathy (Langenheim 1994). The natural toxic effects of monoterpenes make them useful as potential alternative pest control agents as well as good lead compounds for the development of safe, effective, and fully biodegradable pesticides (Isman 2000; Kohli et al. 1998; Romagni et al. 2000).

Barnyard grass, an annual grass, is widely spread throughout the world. It has been reported to cause problems in at least 61 countries and in at least 36 different crops (Holm et al. 1991). It is a major weed in paddy fields as it competes with rice (*Oryza sativa* L.) and causes reduction in rice yield. It reduces crop yields by removing up to 80% of the soil nitrogen. Competition from 25 barnyardgrass plants m^{-2} can cause 50% reduction in rice yield (Chin 2001).

The inhibitory effect of monoterpenes on seed germination and seedling growth of plant and weed species under laboratory conditions and as PRE growth inhibitors has been reported. For example, Reynolds (1987) and Vokou et al. (2003) described inhibitory effects of monoterpenes belonging to several chemical classes on the seed germination and seedling growth of garden lettuce (*Lactuca sativa* L.). Singh et al. (2002) demonstrated inhibitory effect of four monoterpenes, citronellol, citronellal, cineole, and linalool, on the germination, seedling length, and biomass of coffee senna [*Senna occidentalis* (L.) Link]. Moreover, Vaughn and Spencer (1993) explained the inhibitory effect of 18 monoterpenes on germination and growth of nine different plant species. De Martino et al. (2010) studied the potential biological activity of 27 monoterpenes, including monoterpene hydrocarbons and oxygenated ones, against seed germination and subsequent primary radicle growth of radish (*Raphanus sativus* L.) and garden cress (*Lepidium sativum* L.). In addition, the possible modes of phytotoxic action of monoterpenes were described by several studies. For instance, monoterpenes may cause their phytotoxic effects through inhibiting cellular and mitochondrial respiration (Macias et al. 2007), inhibiting DNA synthesis (Nishida et al., 2005), disrupting mitotic activity in the growing cells (Dayan et al. 2000), causing oxidative damage through enhanced generation of reactive oxygen species (ROS) (Singh et al. 2006), inhibiting of electron transfer and mitochondrial ATP production (Abrahim et al. 2003), inhibiting of cell proliferation and disrupting of the activity of metabolic enzymes involved in glycolysis (Amri et al. 2012; Kaur et al. 2010).

However, the POST herbicidal activity of monoterpenes under glasshouse conditions applied as foliar application has not yet been reported. Nevertheless, there have been few studies on the POST herbicidal activity of essential oils (Batish et al. 2004, 2007; Kaur et al. 2010; Poonpaiboonpipat et al. 2013; Singh et al. 2005; Tworkoski 2002). Therefore, the first objective of the present study was to evaluate the PRE and POST herbicidal activity of seven monoterpenes, namely (*R*)-carvone, 1,8-cineole, cuminaldehyde, (*S*)-fenchone, geraniol, (*S*)-limonene, and (*R*)-linalool on barnyardgrass under laboratory and glasshouse conditions. The second objective was to measure effects of monoterpenes on chlorophyll contents and total phenolic compounds after foliar application to determine the possible mechanisms of action.

Materials and Methods

Monoterpenes. Seven monoterpenes were purchased from Sigma-Aldrich Chemical Co. (Steinheim, Germany). The tested compounds were ketones ((*R*)-carvone [98%] and (*S*)-fenchone [98%]), an ether (1-8-cineole [99%]), an aldehyde (cuminaldehyde [98%]), alcohols (geraniol [98%] and (*R*)-linalool [95%]), and a hydrocarbon ((*S*)-limonene [96%]).

Test Weed. Seeds of a field biotype barnyard grass were obtained from Faculty of Agriculture Farm, Alexandria, Egypt. Uniform seeds were selected for the test while undersized and damaged seeds were discarded. Germination of the seeds was tested before use and was 70% 9 d after sowing.

Laboratory Phytotoxic Bioassay. A bioassay based on germination and subsequent seedling growth was carried out to study the phytotoxic effects of the seven monoterpenes on seeds of barnyard grass. The solutions of tested monoterpenes were initially prepared in dimethyl sulfoxide (DMSO) and then diluted with distilled water containing 0.02% of an emulsifying agent (Triton-X 100) to give the concentrations of 1, 2, 3, 4, 6, and 8 mM. The treatments with distilled water containing DMSO (0.5% v/v) and Triton-X 100 (0.02%) were taken as the controls. The use of DMSO and Triton-X 100 at these concentrations did not reduce germination or plant growth compared to a water only control. Three replicates, each of 20 seeds, were prepared for each treatment using glass Petri dishes (9 cm) lined with Whatman No. 2 filter paper. Six milliliters of each concentration was added to individual Petri dishes.

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Afterward, Petri dishes were placed in the bottom of 0.1 mm thick polyethylene bags (15 by 30 cm) that were expanded to contain air and then closed at the top with rubber bands to prevent the loss of moisture. The Petri dishes were placed in a growth chamber at 26 ± 2 C with a 12-h photoperiod. Nine days after sowing, the germination was determined by counting the number of germinated seeds and the lengths of root and shoot were measured. The growth inhibition percentages of root and shoot lengths were calculated from the following equation: I (%) = [1 − T/C] × 100; where T is the root or shoot length of treatment (cm) and C is the root or shoot length of control (cm).

POST Herbicidal Activity under Glasshouse Conditions. Twenty seeds of barnyardgrass were planted in each plastic pot (15 cm diameter by 20 cm height) filled with clay soil (clay [55.61%], silt [26.48%], sand [17.91%], pH [7.1], electrical conductivity [1.6 d Sm^{-1}], total nitrogen [0.4%] and organic matter [1.1%]). Seedlings were grown in the greenhouse under natural sunlight at 30 ± 2 C. Fifteen days after sowing, monoterpene solutions were sprayed on the foliage of weed seedlings at concentrations of 1 and 2% (5 ml for each replicate). The solutions of monoterpenes were prepared in distilled water containing 0.02% (v/v) Triton X-100 as a surfactant. Treatments were replicated three times. The experiment was performed in a completely randomized design within the glasshouse and was repeated twice. Treated plants were kept under observation for 5 d after treatment. Effects of monoterpenes on visual injury of plants (burning, necrosis, chlorosis, leaf distortion, and stunting) were recorded. After 5 d, the heights and fresh weights of plant were measured. The shoots were oven-dried at 80 C for 48 h and the dry weights were recorded.

Estimation of Chlorophyll a and Chlorophyll b Contents. Fresh leaves of barnyardgrass (100 mg) were homogenized in 80% aqueous acetone (5 ml). The homogenate was filtered through Whatman filter paper no. 1. The final volume was adjusted to 5 ml by acetone (80%). Chlorophyll a and chlorophyll b contents were determined spectrophotometrically using Unico 1200-Spectrophotometer at 663 nm for chlorophyll a and 647 nm for chlorophyll b. Concentrations were calculated using Lichtenthaler's equation (Lichtenthaler 1987) and expressed as μg g^{-1} weight.

Measurement of Total Phenolic Compounds. Total phenolic compounds were measured according to the procedures described by Poonpaiboonpipat et al. (2013). Briefly, 2.5 ml ethanol was added to 0.5 g of barnyardgrass fresh leaves and kept in the freezer for 48 h. The frozen samples were homogenized and centrifuged at 10,000 rpm for 10 min. To 1 ml of supernatant, 1 ml ethanol, 5 ml distilled water, and 0.5 ml 50% Folin-Ciocalteu reagent were added. The mixture was left in the dark at room temperature for 5 min. Then, 1 ml sodium bicarbonate solution (5%) was added to the mixture. The reaction mixture was kept in the dark at room temperature for 1 h. The absorbance was measured at 765 nm by using Unico 1200-Spectrophotometer. The total phenolic content was expressed as mg gallic acid equivalent g fresh $weight^{-1}$ (mg GAE g fw^{-1}). The inhibition percentages of total phenolic content were calculated from this equation: I (%) = [1 − T/C] × 100; where T is the concentration of total phenolic (mg g fw^{-1}) in treatment and C is the concentration of total phenolic in control (mg g fw^{-1}).

Statistical Analysis. Germination percentages, root and shoot lengths, shoot growth, and chlorophyll a and b contents were subjected to one-way analysis of variance followed by Student–Newman–Keuls test (Cohort Software Inc. 1985) to determine significant differences among mean values at the probability level of 0.05.

Results and Discussion

Inhibition of Seed Germination under Laboratory Conditions. The inhibitory effect of the seven monoterpenes on the seed germination of barnyardgrass 9 d after sowing is shown in Table 1. The results demonstrate that the tested monoterpenes caused significant inhibition of seed germination in a concentration dependent manner. Geraniol (EC_{50} = 0.98 mM) was the most potent inhibitor for seed germination, followed by (*R*)-carvone (EC_{50} = 1.29 mM). These two compounds caused complete inhibition (100%) of seed germination at the concentrations > 2 mM. At concentrations of 1 mM, geraniol and (*R*)-carvone caused 52.6 and 36.8% germination inhibition, respectively. In addition, geraniol and (*R*)-carvone strongly inhibited germination at 2 mM with germination inhibition rates of 89.4 and 73.7%, respectively. (*S*)-Limonene and (*R*)-linalool inhibited germination from 27.3 to 72.7% and from 15.8 to 80.0%, respectively, whereas cuminaldehyde and (*S*)-fenchone inhibited germination from 26.2 to 63.2% and from 10.4 to 57.8%, respectively. The results also showed that

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Table 1. Effect of monoterpenes on barnyardgrass seed germination 9 d after sowing[a]

	Germination[b]			
Conc	(*R*)-Carvone	1,8-Cineole	Cuminaldehyde	(*S*)-Fenchone
mM	% ± SE (I %)			
0	63.3 ± 3.34 a[c] (0.0)	73.3 ± 3.34 a (0.0)	63.3 ± 3.34 a (0.0)	63.3 ± 3.34 a (0.0)
1	40.0 ± 5.80 b (36.8)	63.3 ± 3.34 ab (13.6)	46.7 ± 3.34 b (26.2)	56.7 ± 3.34 a (10.4)
2	16.7 ± 3.34 c (73.7)	63.3 ± 3.34 ab (13.6)	43.3 ± 3.34 b (31.6)	40.0 ± 5.80 b (36.8)
4	0.0 ± 0.0 c (100.0)	56.7 ± 3.34 b (22.6)	33.3 ± 3.34 c (47.4)	36.7 ± 3.34 b (42.2)
6	0.0 ± 0.0 c (100.0)	40.0 ± 5.80 c (45.4)	30.0 ± 5.80 c (52.6)	33.3 ± 3.34 b (47.4)
8	0.0 ± 0.0 c (100.0)	36.7 ± 3.34 c (50.1)	23.3 ± 3.34 c (63.2)	26.7 ± 3.34 b (57.8)
EC_{50}	1.26	7.47	4.61	5.67

	Germination		
Conc	Geraniol	(*S*)-Limonene	(*R*)-Linalool
mM	% ± SE (I %)		
0	63.3 ± 3.34 a (0.0)	73.3 ± 3.34 a (0.0)	63.3 ± 3.34 a (0.0)
1	30.0 ± 5.80 b (52.6)	73.3 ± 3.34 a (0.0)	53.3 ± 3.34 a (15.8)
2	6.7 ± 6.70 c (89.4)	53.3 ± 3.34 b (27.3)	36.7 ± 3.34 b (42.0)
4	0.0 ± 0.0 c (100.0)	33.3 ± 3.34 c (54.6)	23.3 ± 3.34 bc (63.2)
6	0.0 ± 0.0 c (100.0)	20.0 ± 3.34 c (72.7)	23.3 ± 3.34 bc (63.2)
8	0.0 ± 0.0 c (100.0)	20.0 ± 5.80 c (72.7)	13.3 ± 3.34 c (80.0)
EC_{50}	0.98	3.51	3.02

[a] Data are expressed as means ± SE from experiments with three replicates of 20 seeds each.
[b] Abbreviations: EC_{50}, concentration of compound inhibiting germination 50%; I, inhibition.
[c] Mean values within a column sharing the same letter are not significantly different at the 0.05 probability level.

1,8-cineole (EC_{50} = 7.47 mM) did not reduce germination at concentrations < 2 mM.

The inhibitory effect of tested monoterpenes in germination of barnyardgrass was not reported except 1,8-cineole and (+) carvone (He et al. 2009; Romagni et al. 2000). However, some of the tested monoterpenes were reported to possess herbicidal effects on weed germination of other plant and weed species. For example, 1,8-cineole inhibited seed germination of redroot pigweed (*Amaranthus retroflexus* L.), common lambsquarters (*Chenopodium album* L.), curly dock (*Rumex crispus* L.), and maize (*Zea mays* L.) (Abrahim et al. 2000; Kordali et al. 2007). Similarly, limonene and linalool caused seed germination inhibition of maize and coffee senna (Abrahim et al. 2000; Singh et al. 2002). In addition, Vokou et al. (2003) stated that geraniol, linalool, carvone, and fenchone reduced seed germination of lettuce.

Inhibition of Seedling Growth under Laboratory Conditions. The effect of the tested monoterpenes on root growth of barnyardgrass 9 d after treatment is presented in Table 2. The seven monoterpenes strongly inhibited the root growth and geraniol and (*R*)-carvone were the most potent compounds at the tested concentrations. The EC_{50} values for geraniol and (*R*)-carvone were 0.15 and 0.38 mM, respectively. (*S*)-Fenchone and (*R*)-linalool revealed remarkable root growth inhibition as EC_{50} values were lower than 1 mM. Moreover, 1,8-cineole, (*S*)-limonene, and cuminaldehyde were the less effective among the tested compounds.

On the other hand, the tested monoterpenes caused variable inhibitory effect on shoot growth of barnyardgrass with geraniol (EC_{50} = 0.78 mM) and (*R*)-carvone (EC_{50} = 0.96 mM) being the most potent inhibitors for shoot growth (Table 3). Furthermore, (*R*)-linalool (EC_{50} = 1.13 mM) showed strong shoot growth inhibition, while 1,8-cineole and (*S*)-limonene had the least shoot growth inhibition at all the tested concentrations. These results are consistent with those previously reported on the root and shoot growth inhibition of tested monoterpenes. Compounds such as 1,8-cineole, limonene, geraniol, (+)-carvone, and linalool have been described as potent root and shoot growth inhibitors against other plant and weed species (Barton et al. 2014; He et al. 2009; Nishida et al. 2005; Singh et al. 2002; Vokou et al. 2003; Zunino and Zygadlo 2004).

The results indicated that the tested monoterpenes exhibited greater inhibitory effects on seedling growth than on seed germination. A similar finding was described by Leather and Einhellig (1985) who demonstrated that bioassays determining seedling growth of many allelochemicals are usually more

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Table 2. Effect of monoterpenes on barnyardgrass root growth 9 d after sowing[a]

	(*R*)-Carvone		1,8-Cineole		Cuminaldehyde		(*S*)-Fenchone	
Conc	Root length	I[b]	Root length	I	Root length	I	Root length	I
mM	cm	%	cm	%	cm	%	cm	%
0	4.8 ± 0.15 a[c]	0.0	5.9 ± 0.09 a	0.0	3.4 ± 0.15 a	0.0	3.4 ± 0.15 a	0.0
1	0.7 ± 0.13 b	85.4	5.9 ± 0.09 a	0.0	3.1 ± 0.09 a	8.8	1.4 ± 0.06 b	58.8
2	0.3 ± 0.10 c	93.8	3.9 ± 0.07 b	33.9	2.3 ± 0.15b	32.4	0.7 ± 0.06 c	79.4
4	0 ± 0.0 c	100.0	3.7 ± 0.07 c	37.3	1.4 ± 0.07 c	58.8	0.4 ± 0.03 d	88.2
6	0 ± 0.0 c	100.0	3.0 ± 0.12 d	49.2	1.0 ± 0.09 d	70.6	0.2 ± 0.01 e	94.1
8	0 ± 0.0 c	100.0	1.2 ± 0.09 e	79.7	0.6 ± 0.09 e	82.4	0.2 ± 0.01 e	94.1
EC_{50}	0.38		4.43		3.22		0.71	

	Geraniol		(*S*)-Limonene		(*R*)-Linalool	
Conc	Root length	I	Root length	I	Root length	I
mM	cm	%	cm	%	cm	%
0	3.4 ± 0.15 a	0.0	5.9 ± 0.09 a	0.0	3.4 ± 0.15 a	0.0
1	0.2 ± 0.03 b	94.1	5.9 ± 0.09 a	0.0	1.4 ± 0.12 b	58.8
2	0.1 ± 0.07 b	97.1	3.9 ± 0.06 b	33.8	0.4 ± 0.03 c	88.2
4	0 ± 0.0 b	100.0	3.6 ± 0.06 c	39.0	0.1 ± 0.03 d	97.1
6	0 ± 0.0 b	100.0	1.1 ± 0.09 d	81.4	0 ± 0.0 d	100.0
8	0 ± 0.0 b	100.0	0.6 ± 0.06 d	89.8	0 ± 0.0 d	100.0
EC_{50}	0.15		3.39		0.84	

[a] Data are expressed as means ± SE from experiments with three replicates of 20 seeds each.
[b] Abbreviations: EC_{50}, concentration of compound inhibiting root growth 50%; I, inhibition.
[c] Mean values within a column sharing the same letter are not significantly different at the 0.05 probability level.

sensitive than those measuring germination. In addition, all of the tested monoterpenes had greater inhibitory effects on root growth than on shoot growth except for cuminaldehyde, which inhibitory effects on shoot growth were similar for root growth. These results are supported by earlier studies of inhibitory effects of monoterpenes on seedling growth (Chowhan et al. 2011; Singh et al. 2006; Zhao et al.

Table 3. Effect of monoterpenes on barnyardgrass shoot growth 9 d after sowing[a]

	(*R*)-Carvone		1,8-Cineole		Cuminaldehyde		(*S*)-Fenchone	
Conc	Shoot length	I[b]	Shoot length	I	Shoot length	I	Shoot length	I
mM	cm	%	cm	%	cm	%	cm	%
0	3.7 ± 0. 5 a[c]	0.0	3.7 ± 0.03 a	0.0	5.5 ± 0.1 a	0.0	5.5 ± 0.10 a	0.0
1	1.7 ± 0.15 b	54.1	3.9 ± 0.03 a	−5.4	3.5 ± 0.02 b	36.4	4.4 ± 0.07 b	20.0
2	0.5 ± 0.03 c	86.5	3.9 ± 0.03 a	−5.4	2.9 ± 0.09 c	47.3	3.0 ± 0.09 c	45.5
4	0 ± 0.0 c	100.0	3.7 ± 0.03 a	0.0	2.3 ± 0.12 d	58.2	2.7 ± 0.09 c	50.9
6	0 ± 0.0 c	100.0	3.1 ± 0.18 b	16.2	1.7 ± 0.12 e	69.1	2.1 ± 0.2 1 d	61.8
8	0 ± 0.0 c	100.0	1.7 ± 0.09 c	54.1	1.1 ± 0.09 f	80.0	1.4 ± 0.15 e	74.5
EC_{50}	0.96		7.76		2.17		3.25	

	Geraniol		(*S*)-Limonene		(*R*)-Linalool	
Conc	Shoot length	I	Shoot length	I	Shoot length	I
mM	cm	%	cm	%	cm	%
0	5.5 ± 0.1 a	0.0	3.7 ± 0.03 a	0.0	5.5 ± 0.1 a	0.0
1	1.9 ± 0.13 b	65.5	3.7 ± 0.03 a	0.0	3.1 ± 0.18 b	43.6
2	0.6 ± 0.58 c	89.1	3.6 ± 0.3 a	2.7	1.4 ± 0.06 c	74.5
4	0 ± 0.0 c	100.0	3.4 ± 0.06 b	8.1	1.0 ± 0.07 d	81.8
6	0 ± 0.0 c	100.0	1.4 ± 0.06 c	62.2	0.4 ± 0.09 e	92.7
8	0 ± 0.0 c	100.0	1.0 ± 0.09 d	73.0	0.2 ± 0.03 e	96.4
EC_{50}	0.78		5.91		1.13	

[a] Data are expressed as means ± SE from experiments with three replicates of 20 seeds each.
[b] Abbreviations: EC_{50}, concentration of compound inhibiting shoot growth 50%; I, inhibition.
[c] Mean values within a column sharing the same letter are not significantly different at the 0.05 probability level.

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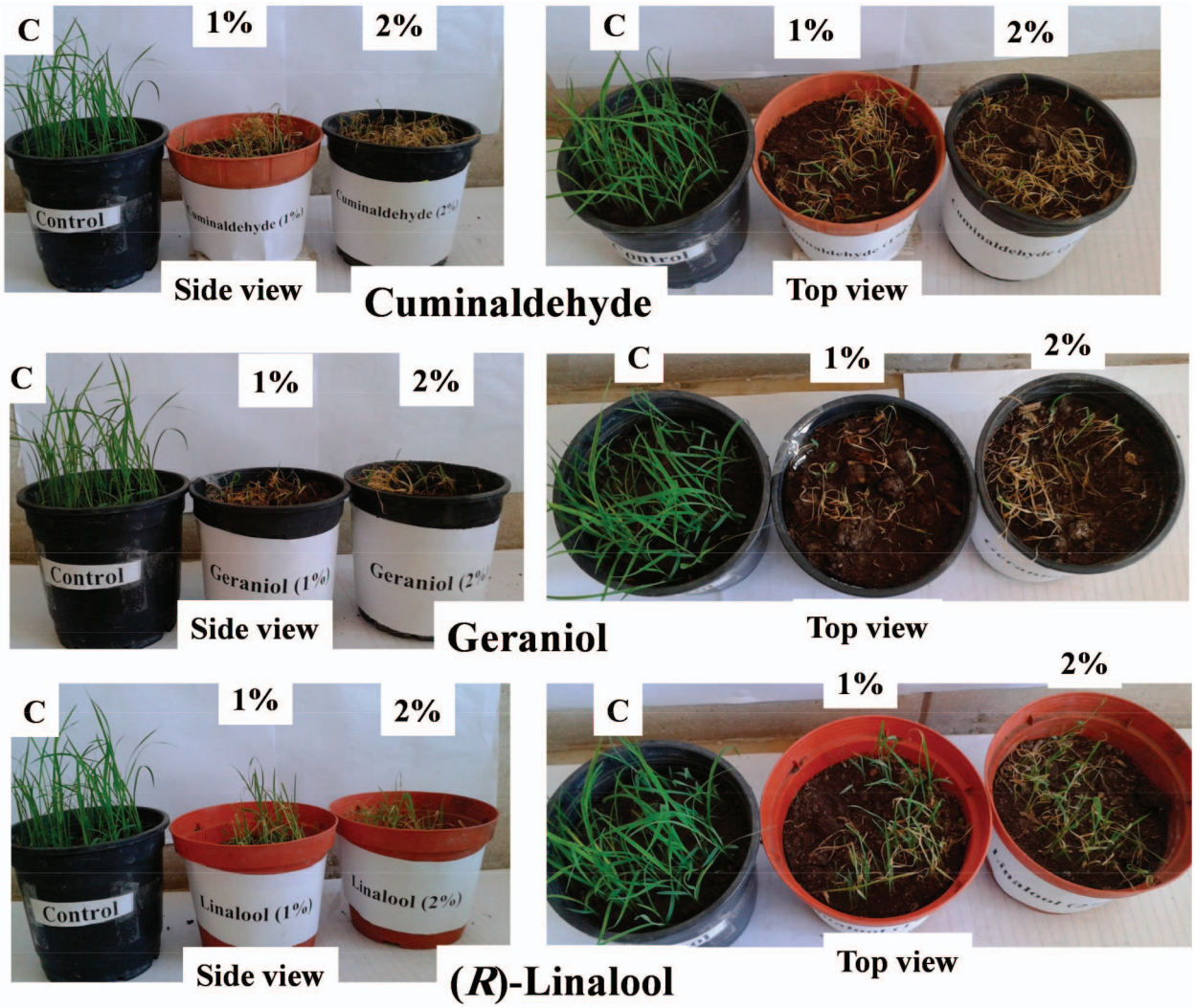

Figure 1. Herbicidal effects of cuminaldehyde, geraniol, and (*R*)-linalool on two-leaf stage barnyardgrass 5 d after foliar application. (C): control treatments sprayed with water containing 0.02% (v/v) Triton X-100. (1%) treatments strayed with 1% of monoterpene solutions containing 0.02% Triton X-100. (2%) treatments strayed with 2% of monoterpene solutions containing 0.02% Triton X-100. (Color for this figure is available in the online version of this article.)

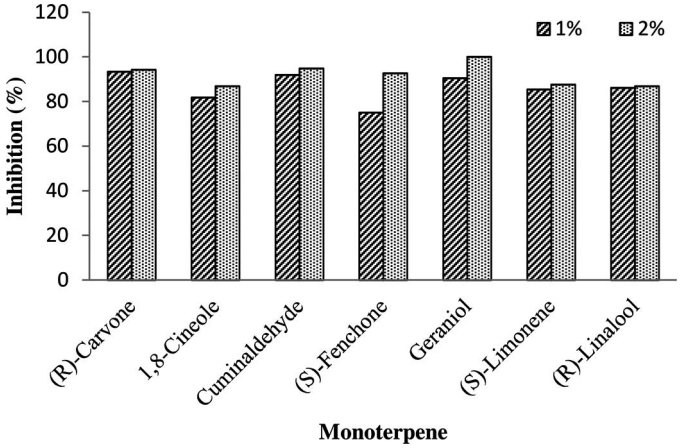

Figure 2. Effect of monoterpenes on fresh weight of two-leaf stage barnyardgrass 5 d after foliar application with concentrations 1 and 2%.

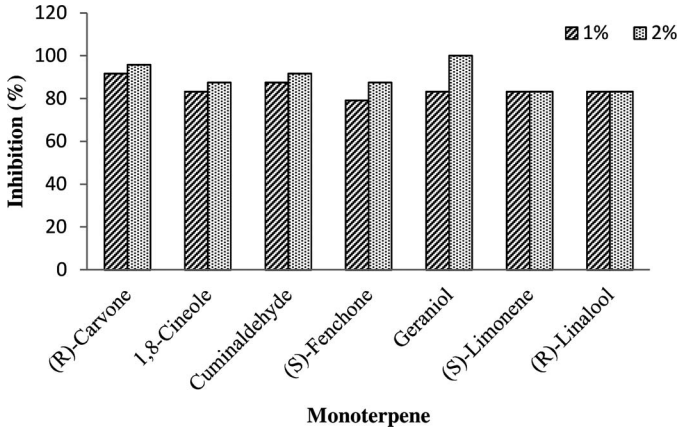

Figure 3. Effect of monoterpenes on dry weights of two-leaf stage barnyardgrass after 5 d after foliar application with concentrations 1 and 2%.

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Table 4. Effect of monoterpenes on shoot growth of two-leaf stage barnyardgrass 5 d after foliar application[a]

Conc	(*R*)-Carvone		1,8-Cineole		Cuminaldehyde		(*S*)-Fenchone	
	Shoot length	I [b]	Shoot length	I	Shoot length	I	Shoot length	I
%	cm	%	cm	%	cm	%	cm	%
0	18.4 ± 0.12 a[c]	0.0	18.4 ± 0.12 a	0.0	18.4 ± 0.12 a	0.0	18.4 ± 0.12 a	0.0
1	3.5 ± 0.76 b	81.0	8.6 ± 0.49 b	53.3	5.0 ± 0.29 b	72.8	7.3 ± 0.44 b	60.3
2	3.0 ± 0.57 b	83.7	6.5 ± 0.76 c	64.7	3.9 ± 0.43 c	78.8	4.8 ± 0.66 c	73.9

Conc	Geraniol		(*S*)-Limonene		(*R*)-Linalool	
	Shoot length	I	Shoot length	I	Shoot length	I
%	cm	%	cm	%	cm	%
0	18.4 ± 0.12 a	0.0	18.4 ± 0.12 a	0.0	18.4 ± 0.12 a	0.0
1	6.5 ± 0.29 b	64.7	6.2 ± 0.16 b	66.3	5.5 ± 0.29 b	70.1
2	5.5 ± 0.29 c	70.1	5.0 ± 0.44 c	72.8	5.2 ± 0.16 b	71.7

[a] Data are expressed as means ± SE from experiments with three replicates.
[b] Abbreviation: I, inhibition.
[c] Mean values within a column sharing the same letter are not significantly different at the 0.05 probability level.

2011). This finding might be predictable, because it is likely that roots are the first to absorb the allelochemicals compounds from the media (Turk and Tawaha 2002).

Herbicidal Activity of Monoterpenes under Glasshouse Conditions. The effects of tested monoterpenes 5 d after treatment with a foliar application of 1 and 2% solution on fresh weight, dry weight, and shoot growth of two-leaf stage barnyardgrass are summarized in Figures 1, 2, and 3, and Table 4. At the two concentrations (1 and 2%), the tested monoterpenes drastically reduced the fresh and dry weights of the weed compared to control. The inhibition rates of fresh weight ranged from 75.1 to 93.4%, and from 86.9 to 100.0% at the concentrations of 1 and 2%, respectively. In the case of the dry weight, the inhibition rates ranged from 79.2 to 91.4%, and from 83.3 to 100.0% at concentrations of 1 and 2%, respectively.

The shoot growth of two-leaf stage barnyardgrass treated with 1 and 2% of monoterpenes was reduced compared with control. At concentrations of 1 and 2%, (*R*)-carvone caused the greatest reduction in shoot growth with 81.0 and 83.3% growth inhibition, respectively, while 1,8-cineole caused the least reduction in shoot growth with 53.3 and 64.7% growth inhibition, respectively. Foliar application of tested monoterpenes on two-leaf stage barnyardgrass caused some phytotoxicity symptoms. The main symptoms were chlorosis and necrosis, followed by the plant death (Figure 1). Severe phytotoxicity symptoms were observed on plants treated with geraniol, cuminaldehyde, and (*R*)-linalool. Complete control of plants was achieved by the foliar application of 1 and 2% of geraniol, and 2% of cuminaldehyde.

In the literature, there are no reported studies on the post-emergent herbicidal activity of monoterpenes. However, some essential oils were described to possess herbicidal activities causing severe injuries in growing weeds after foliar application (Batish et al. 2004, 2007; Kaur et al. 2010; Poonpaiboonpipat et al. 2013; Singh et al. 2005; Tworkoski 2002). For example, the essential oils of true cinnamon (*Cinnamomum zeylanicum* L.), summer savory (*Satureja hortensis* L.), clove [*Syzygium aromaticum* (L.) Merr. & L.M. Perry], and thyme (*Thymus vulgaris* L.) caused severe visible injury and plant death of common ragweed (*Ambrosia artemisiifolia* L.), common lambsquarters, and johnsongrass [*Sorghum halepense* (L.) Pers.] when applied at 5 and 10% (v/v) on 12-wk-old plants (Tworkoski 2002). Similarly, the oil of lemonscented gum [*Corymbia citriodora* (Hook.) K.D. Hill & L.A.S. Johnson] caused death of 4-wk-old plants of Santa Maria feverfew (*Parthenium hysterophorus* L.) sprayed with 75 and 100 µl ml^{-1} (Singh et al. 2005). Likewise, the oil of lemonscented gum produced 50 to 80% visible injury in slender amaranth (*Amaranthus viridis* L.), coffee senna, barnayrdgrass, and littleseed canarygrass (*Phalaris minor* Retz.) (Batish et al. 2004, 2007). In addition, the essential oil lemon grass [*Cymbopogon citratus* (DC ex Nees) Stopf] applied on barnyardgrass at concentrations of 1.25, 2.5, 5, and 10% (v/v) 28 d after sowing in greenhouse caused leaf wilting (Poonpaiboonpipat et al. 2013). POST application of redstem wormword (*Artemisia scoparia* Waldst. & Kit.) (2, 4, and 6%, v/v) on

https://doi.org/10.1614/WS-D-15-00045.1 Published online by Cambridge University Press

Table 5. Effect of monoterpenes on chlorophyll a and b contents (μg g^{-1} FW) of two-leaf stage barnyardgrass 5 d after foliar application[a]

	(*R*)-Carvone		1,8-Cineole		Cuminaldehyde	
Conc	Chl a	Chl b	Chl a	Chl b	Chl a	Chl b
%	μg g^{-1} (I %)[b]	μg g^{-1} (I %)	μg g^{-1} (I %)	μg g^{-1} (I %)	μg g^{-1} (I %)	μg g^{-1} (I %)
0	21.48 a[c] (0.0)	11.06 a (0.0)	21.48 a (0.0)	11.06 a (0.0)	21.48 a (0.0)	11.06 a (0.0)
1	9.66 b (55.0)	5.78 b (47.7)	23.68 a (-10.2)	11.0 a (5.0)	2.84 b (86.8)	1.76 b (84.1)
2	4.10 c (80.9)	2.48 c (77.6)	12.22 b (43.1)	6.72 b (39.2)	2.34 b (89.1)	1.64 c (85.2)

	(*S*)-Fenchone		Geraniol		(*S*)-Limonene	
Conc	Chl a	Chl b	Chl a	Chl b	Chl a	Chl b
%	μg g^{-1} (I %)[b]	μg g^{-1} (I %)	μg g^{-1} (I %)	μg g^{-1} (I %)	μg g^{-1} (I %)	μg g^{-1} (I %)
0	21.48 a (0.0)	11.06 a (0.0)	21.48 a (0.0)	11.06 a (0.0)	21.48 a (0.0)	11.06 a (0.0)
1	20.18 b (6.0)	11.0 a (5.0)	3.18 b (85.6)	2.22 b (79.9)	17.76 b (17.3)	0.96 b (91.3)
2	12.22 c (43.1)	7.12 b (35.8)	1.40 c (93.5)	0.96 c (91.3)	12.48 c (41.9)	0.98 b (91.1)

	(*R*)-Linalool	
Conc	Chl a	Chl b
%	μg g^{-1} (I %)	μg g^{-1} (I %)
0	21.48 a (0.0)	11.06 a (0.0)
1	11.42 b (46.8)	6.70 b (39.4)
2	6.96 bc (67.6)	3.92 c (64.6)

[a] Data are expressed as means ± SE from experiments with three replicates.
[b] Abbreviation: I, inhibition.
[c] Mean values within a column sharing the same letter are not significantly different at the 0.05 probability level.

6-wk-old plants of devil's horsewhip (*Achyranthes aspera* L.), coffee senna Santa Maria feverfew, barnyardgrass, and tropical whiteweed (*Ageratum conyzoides* L.) caused visible injury ranging from chlorosis to necrosis to complete wilting of plants (Kaur et al. 2010).

Effect of Monoterpenes on Chlorophyll Contents. Monoterpene treatments at concentrations of 1 and 2% caused significant reduction in leaf content of chlorophyll a and chlorophyll b as shown in Table 5. Geraniol and cuminaldehyde caused the greatest inhibition in chlorophyll a, while 1,8-cineole and (*S*)-limonene caused the least inhibition. Geraniol inhibited chlorophyll a by 85.5 and 93.5% at concentrations of 1 and 2%, respectively. Similarly, cuminaldehyde reduced chlorophyll b by 86.8 and 89.1% at concentrations of 1 and 2%, respectively. (*S*)-Limonene, cuminaldehyde, and geraniol exhibited the greatest reduction in chlorophyll b. The reduction in chlorophyll content observed in this study is in agreement with earlier reports indicating that the monoterpenes had a potential to reduce chlorophyll content. For example, monoterpenes such as 1,8-cineole, citronellol, citronellal, linalool, and β-pinene were reported to reduce chlorophyll content (Chowhan et al. 2011; Kaur et al. 2010; Romagni et al. 2000; Singh et al. 2002). The mechanism of chlorophyll reduction by monoterpenes is not fully understood. However, it has been suggested that the reduction of chlorophyll content may be due to inhibition of biosynthesis of chlorophyll and/or degradation of chlorophyll.

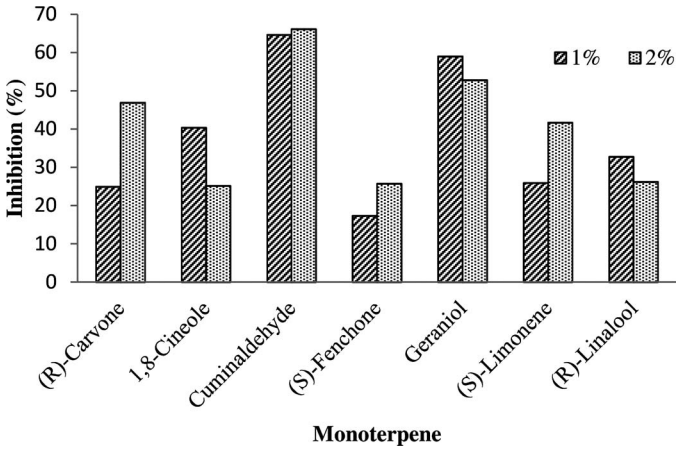

Figure 4. Effect of monoterpenes on total phenolic contents of two-leaf stage barnyardgrass 5 d after foliar application with concentrations 1 and 2%.

https://doi.org/10.1614/WS-D-15-00045.1 Published online by Cambridge University Press

Effect of Monoterpenes on Phenolic Compounds. The inhibitory effect of tested monoterpenes at concentrations 1 and 2% on total phenolic content of leaves is presented in Figure 4. The monoterpenes decreased the concentration of phenolic compounds at the two tested concentrations. The inhibition of phenolic compounds caused by (*R*)-carvone, cuminaldehyde, (*S*)-fenchone, and (*S*)-limonene increased with increasing the concentration. However, in the case of 1,8-cineole, geraniol, and (*R*)-linalool, the inhibition of phenolic compounds was decreased with increasing of concentration. Cuminaldehyde and geraniol caused the greatest reduction in phenolic compounds at the two tested concentrations. Similar findings were previously reported on the effect of α-pinene and β-pinene in phenolic compounds of maize (Areco et al. 2014). In contrast, some allelochemicals were shown to increase the phenolic content (Djanaguiraman et al. 2005).

Regarding the relationship between the chemical structure and herbicidal activity of tested monoterpenes, the results of this study showed that geraniol, an alcohol, had the greatest herbicidal activity in PRE and POST experiments. Moreover, (*R*)-carvone, a ketone, showed strong herbicidal activity in PRE experiments and cuminaldehyde, an aldehyde, revealed strong herbicidal activity in POST experiments. In contrast, 1,8-cineole, an ether, and a monoterpene hydrocarbon, limonene, exhibited the weakest herbicidal activities. These findings are in agreement with those previously reported on the phytotoxic activities of monoterpenes (De Martino et al. 2010; Vokou et al. 2003). Our results are also supported by general conclusions indicating that oxygenated monoterpenes are more phytotoxic than the hydrocarbon monoterpenes (Kordali et al. 2007; Vaughn and Spencer 1993).

Concerning the mechanism of action of tested monoterpenes, the results showed that the monoterpenes caused inhibition in chlorophyll contents and phenolic compounds indicating that these compounds may affect the plant photosynthesis and biosynthesis of secondary metabolites such as phenolic compounds. The phenolic compounds have an important role in plant defense mechanism against pests and pathogens (Khatun et al. 2008). These findings are in agreement with those reported by Batish et al. (2004) who indicated that the monoterpenes may cause their phytotoxic effect by affecting the photosynthetic machinery and energy metabolism of plant.

Introducing new natural herbicides may help to overcome weed resistance and environmental pollution caused by extensive use of synthetic herbicides. Based on the results of this study, oxygenated monoterpenes such as geraniol, (*R*)-carvone, and cuminaldehyde exhibit strong PRE and POST herbicidal activity aginst barnyardgrass in both laboratory and glasshouse experiments. Therefore, these monoterpenes could be useful as potential bio-herbicides and as lead structures for the development new herbicides. However, further studies are needed to determine the suitable formulation, cost, selectivity, safety and phytotoxicity against crops before commercialization.

Acknowledgments

This work was supported by the Alexandria University Research Fund (ALEX-REP, 2010–2013).

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Received March 20, 2015, and approved September 30, 2015.

Associate Editor for this paper: Steven Seefeldt, University of Alaska at Fairbanks.