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## Evaluation of Herbicidal Potential of Essential Oils and their Components under *In vitro* and Greenhouse Experiments

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**ABSTRACT.** The present study aimed to evaluate the phytotoxic potential of essential oils. For this purpose, 18 essential oil samples extracted from Korean plants and 64 commercial essential oils were screened for their phytotoxic potential against the seedling growth of *Brassica napus* L. (rapeseed). Among the 82 samples, 11 commercial oils (cinnamon, citronella, clove, cumin seed, geranium, jasmine, lemongrass, palmarosa, pimento, rose otto and spearmint) strongly inhibited the seedling growth with GR<sub>50</sub> value <150 µg mL<sup>-1</sup>. Major components from these effective essential oils were identified by solid phase microextraction/gas chromatography-mass spectrometry (SPME/GC-MS). GC-MS analyses revealed that the effective samples mainly consist of benzyl benzoate, carvone, citral, citronellol, eugenol, geraniol, D-limonene and terpinene. Subsequently, bioactivity of these individual components was evaluated against the seedling growth of *B. napus*, *Echinochloa crus-galli* and *Aeschynomene indica*. The components from different chemical groups exhibited different potency in inhibiting the seedling growth with varied GR<sub>50</sub> values ranged from 29 µg mL<sup>-1</sup> to >1,000 µg mL<sup>-1</sup>. In the greenhouse experiment, citral and geraniol completely suppressed the growth of all the tested 10 plants at 100 kg ha<sup>-1</sup>. In conclusion, the individual essential oil components geraniol and citral could be used as natural herbicides for weed management.

**Key words:** Citral, Essential oil, Geraniol, Herbicide

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### Introduction

Weeds are one of the most important pests causing economic losses in the world agriculture. In commercial crop cultivation, the competitions caused by the growth of weeds are influencing the reduction of crop yield and quality of their products. The weed control can be achieved by manual, herbicidal or biological control methods (Hulme, 2012). Manual control method using hand weeding is a good weed control strategy, but requires more number of workers and also consuming more time. The use of synthetic herbicides to control weeds is common and the most effective method. Although synthetic herbicides have showed promising results, the continuous use of synthetic herbicides produce negative impacts on human health and environment, and linked to increasing herbicidal resistance in weed species (Vyvyan,

2002; Batish et al., 2007). Thus, there is an important to search for environmentally safer and novel compounds with more effective, more specific targets for the management of weeds. In this regard, allelopathy is one of the alternative methods to control weed species biologically through the production and release of phytotoxic chemicals from different parts of living or decomposing plant materials (Weston, 1996).

Phytotoxic compounds may help to reduce the use of synthetic herbicides and environmentally friendly method to attain high quality agricultural products (Singh et al., 2003; Khanh et al., 2006). Recently, many studies have investigated the phytotoxic potentials of plant extracts and individual compounds and their ability to control weeds in crop production. Among the various natural plant products, essential oils constitute an important group of that provide a versatile source of bioactive components. Essential oils are

natural, volatile and complex mixtures of terpenes in addition to some other non-terpene components as phenylpropanoids (Buchbauer, 2010). A number of studies have reported that the essential oils and their components are potent inhibitors of seed germination and retard plant growth (Batish et al., 2007; Kaur et al., 2010; Yun et al., 2013). In phytotoxic activity, plant cuticle is the first barrier for diffusing the active component into the leaf tissue. Essential oils are known to promote the penetration of the active component through solubilizing or disrupting the nature of the cuticular waxes (Izadi-Darbandi et al., 2013).

The present study was undertaken to evaluate the phytotoxic potential of essential oils and their components. For this purpose, essential oils extracted from different plants in Korea and commercially available essential oils were screened through a seed bioassay of *Brassica napus* (rapeseed). Further, major components of effective essential oil samples were identified by SPME/GC-MS analysis. In addition, a greenhouse experiment was carried out using effective individual essential oil components against different plant species.

## Materials and Methods

### Essential oils and individual chemicals

A total of 64 commercially available essential oils were

purchased from Aroma House, Seoul, Republic of Korea (Table 2). Benzyl benzoate, carvone, citral, citronellol, eugenol, geraniol, D-limonene, and terpinene were purchased from Sigma-Aldrich (St. Louis, MO, USA).

### Extraction of essential oils

Fresh plant parts of 18 plants (Table 1) were collected in June 2012 from different places in Republic of Korea. The essential oil was extracted from the samples by steam distillation for 60 min (1 kg sample) using a Clevenger-type apparatus. The collected essential oils were dried with anhydrous sodium sulfate and stored under refrigeration (4°C).

### Seed materials

The seeds of rapeseed (*Brassica napus* L.), Indian jointvetch (*Aeschynomene indica* L.), velvet leaf (*Abutilon theophrasti* Medik.), cotton (*Gossypium hirsutum* L.), soybean (*Glycine max* L.), roundleaf morning-glory (*Ipomoea angulata* Lam.) barnyardgrass (*Echinochloa crus-galli* var. *echinata* (Willd.) Honda), Southern crabgrass (*Digitaria ciliaris* (Retz.) Koeler), green foxtail (*Setaria viridis* L.), annual bluegrass (*Poa annua* L.) and maize (*Zea mays* L.) were purchased from local market, Chuncheon, Republic of Korea. Undersized or damaged seeds were discarded.

**Table 1.** Inhibitory activity of essential oils extracted from Korean plants against the seedling growth of *Brassica napus*.

S. No.	Plant names	Family name	Plant parts	GR <sub>50</sub> (µg mL <sup>-1</sup> ) <sup>z</sup>
1	<i>Abies holophylla</i> Maxim	Pinaceae	Needles	556
2	<i>Abies koreana</i> E.H. Wilson	Pinaceae	Cones	1,552
3	<i>Abies nephrolepis</i> (Trautv.) Maxim.	Pinaceae	Needles	3,042
4	<i>Picea koraiensis</i> Nakai	Pinaceae	Needles	3,632
5	<i>Pinus bungeana</i> Zucc. ex Endl.	Pinaceae	Needles	>5,000
6	<i>Pinus densiflora</i> Siebold & Zucc.	Pinaceae	Needles	>5,000
7	<i>Pinus koraiensis</i> Siebold & Zucc.	Pinaceae	Needles	1,582
8	<i>Pinus parviflora</i> Siebold & Zucc.	Pinaceae	Needles	898
9	<i>Cosmos bipinnatus</i> Cav.	Compositae	Flowers	2,718
10	<i>Dendranthema indicum</i> (L.) DesMoul.	Compositae	Flowers	318
11	<i>Ligularia fischeri</i>	Compositae	Leaves	1,657
12	<i>Ligularia stenocephala</i> (Maxim.) Matsum. & Koidz.	Compositae	Leaves	2,272
13	<i>Juniperus chinensis</i> L.	Cupressaceae	Leaves	>5,000
14	<i>Chamaecyparis obtuse</i> Siebold & Zucc.	Cupressaceae	Leaves	942
15	<i>Thuja orientalis</i> L.	Cupressaceae	Leaves	>5,000
16	<i>Aralia cordata</i> var. <i>continentalis</i> (Kitag.) Y. C. Chu	Araliaceae	Roots	1,134
17	<i>Glechoma grandis</i> (A. Gray) Kuprian	Labiatae	Whole plant	567
18	<i>Metasequoia glyptostroboides</i> Hu & W. C. Cheng	Taxodiaceae	Cones	1,225

<sup>z</sup>GR<sub>50</sub> values were calculated from four replicates of each sample.

**Table 2.** Inhibitory activity of commercial essential oils against the seedling growth of *Brassica napus*.

S. No.	Essential oil	GR <sub>50</sub> (µg mL <sup>-1</sup> ) <sup>z</sup>	S. No.	Essential oil	GR <sub>50</sub> (µg mL <sup>-1</sup> )
1	Angelica	2,832	33	Lemongrass	64
2	Basil	484	34	Lime	1,577
3	Bergamot	1,308	35	Magnolia	275
4	Black pepper	1,156	36	Majoram	399
5	Cajaput	241	37	Mandarin	3,762
6	Camphor	763	38	Myrtle	906
7	Caraway	223	39	Neroli	337
8	Cardamom	1,748	40	Niaouli	911
9	Carrot seed	2,374	41	Nutmeg	1,401
10	Cedar wood	1,382	42	Orange	2,007
11	Chamomile German	>5,000	43	Patchouli	1,206
12	Chamomile Roman	1,561	44	Palmarosa	26
13	Cinnamon	79	45	Peppermint	209
14	Citronella	79	46	Petitgrain	457
15	Clary sage	684	47	Pimento	52
16	Clove	48	48	Pine	1,338
17	Coriander	315	49	Rose absolute	599
18	Cumin seed	149	50	Rosemary	873
19	Cypress	>5,000	51	Rose otto	70
20	Eucalyptus	>5,000	52	Rose wood	1,349
21	Fennel	667	53	Sage	537
22	Fir	1,019	54	Sandalwood	>5,000
23	Frankincense	1,370	55	Savory	208
24	Galbanum	1,458	56	Spearmint	149
25	Geranium	81	57	Tagetes	758
26	Ginger	2,443	58	Tangerin	575
27	Grapefruit	1,351	59	Teatree	425
28	Hyssop	180	60	Thyme	218
29	Jasmine	107	61	Vanilla	>5,000
30	Juniper	>5,000	62	Vetiver	855
31	Lavender	492	63	Yarrow	503
32	Lemon	>5,000	64	Ylang ylang	705

<sup>z</sup>GR<sub>50</sub> values were calculated from four replicates of each sample.

### Seed bioassay

The seed bioassay of essential oils was evaluated on seeds of *Brassica napus* (rapeseed). To accomplish this experiment, 1% agar in distilled water was used as growth medium. The rapeseeds were surface sterilized with 0.5% sodium hypochlorite for 3 min then washed with sterile distilled water. Essential oils were prepared with series of concentrations from 0-5,000 µg mL<sup>-1</sup> (diluted using 0.01% Tween 20 v/v). The seeds were placed in a 24-well cell culture plate contains 1% agar medium (5 seeds per well). Then, one mL of each test

solution was added to respective wells with four replicates per treatment. The plates were covered with plastic bags to maintain humidity and allowed to germinate in the growth chamber at 25°C/ 23°C (day/night), 60% relative humidity, and 250 µmol m<sup>-2</sup> s<sup>-1</sup> light intensity for 5 days.

After the incubation period, the effect of essential oils on seedling growth was determined by measuring the weight of the seedlings. Inhibitory activity of essential oils against rapeseed growth was calculated based on growth rate<sub>50</sub> (GR<sub>50</sub>)

values (effective concentrations capable of inhibiting 50% of plant growth). Further studies were carried out with most effective essential oil samples.

### SPME conditions

One mL of essential oil was introduced into SPME vial. The SPME device coated (fused-silica fiber) with a 100  $\mu\text{m}$  layer of polydimethylsiloxane (Supelco, Bellefonte, PA, USA) was used for extraction of the plant volatiles and the vial was sealed with a silicone septum. They were exposed in the SPME vial at 60°C for 30 min and immediately introduced in the gas chromatography injector.

### Gas chromatography/mass spectrometry (GC/MS) analysis

GC-MS analysis was performed with a Varian CP 3800 gas chromatography equipped with a VF-5 MS polydimethylsiloxane capillary column (30  $\times$  0.25 mm  $\times$  0.25  $\mu\text{m}$ ) and a Varian 1200 L mass detector (Varian, CA, USA). Helium was used as a carrier gas at the rate of 1 mL min<sup>-1</sup>. Oven temperature was kept at 50°C for 5 min initially, and then raised with rate of 5°C min<sup>-1</sup> to 250°C min<sup>-1</sup>. The injector temperature was set at 250°C. The mass spectra were recorded in the electrospray ionization mode at 70 eV in a scan range of 50-600 m z<sup>-1</sup>. The major components of essential oils were identified by comparing the retention indices of the GC peaks obtained using homologous series of n-alkanes (C<sub>8</sub>-C<sub>20</sub>) with those reported in literature (Adams, 2007). The mass spectra of the peaks were also matched with standards reported in literature and National Institute of Standards and Technology (NIST, 3.0) library.

### Effect of major components on seedling growth

The individual major components, namely benzyl benzoate, carvone, citral, citronellol, eugenol, geraniol, D-limonene and terpinene were used to evaluate their inhibitory activity against the seedling growth of *Brassica napus*, *Echinochloa crus-galli* and *Aeschynomene indica*. The effect of individual components on seedling growth was carried out by following the procedure as mentioned earlier in the seed bioassay section.

### Greenhouse experiment

For greenhouse experiment, eight pure compounds, namely benzyl benzoate, carvone, citral, citronellol, eugenol, geraniol, D-limonene and terpinene were used to evaluate their herbicidal potential. In this experiment, five dicot plants (*Aeschynomene indica*, *Abutilon theophrasti*, *Gossypium hirsutum*, *Glycine max* and *Ipomoea angulata*) and five monocot plants (*Echinochloa crus-galli*, *Digitaria ciliaris*, *Setaria viridis*, *Poa annua* and *Zea mays*) were used. The nursery trays were filled with sandy soil.

Ten seeds of each species were sown separately in each tray

(350 cm<sup>2</sup>). Five days after seed sowing, different concentrations (25, 50 and 100 kg ha<sup>-1</sup>) of eight pure individual components were prepared separately (Tween-20 0.01% v/v) and sprayed (1000 L ha<sup>-1</sup>) using CO<sub>2</sub> pressure belt-driven track sprayer (R & D sprayer, 8002 EVB nozzle, 40 psi, 40 cm height). The observation was made post spray treatment of the test materials and the data were recorded at 3, 7 and 14 days after treatment by visually counting the plants in each treatment.

### Statistical analysis

The seed bioassay was conducted with four replications and the statistical analysis was carried out by analysis of variance (ANOVA) followed by Duncan's test, and values of P < 0.05 were considered significantly different. The data were evaluated with SPSS 18.0 software package (SPSS Inc., Chicago, IL, USA).

## Results and Discussion

### Phytotoxic effect of essential oils on seedling growth of rapeseed

It is well known that phytotoxic compounds from plants are considered to be safe and beneficial to the environment and human beings (Khanh et al., 2006). A variety of plant species have phytotoxic effects on weed species. The growth inhibitory activity of essential oils has remarkably increased the interest in exploring essential oil from plants for potential weed management. Germination and seedling growth bioassays are important preliminary screening methods to determine phytotoxic potential of plant extracts and compounds. In the present study, herbicidal activity of 18 essential oil samples extracted from Korean plants and 64 commercial essential oil samples was evaluated by seed bioassay using rapeseed. The results are expressed as GR<sub>50</sub> that is an effective concentration capable of inhibiting the seedling growth of rapeseed by 50% (Table 1 and 2). Among the 18 essential oil samples extracted from Korean plants, *D. indicum* showed higher inhibitory activity (GR<sub>50</sub> of 318  $\mu\text{g mL}^{-1}$ ) followed by *A. holophylla* (GR<sub>50</sub> of 556  $\mu\text{g mL}^{-1}$ ) and *G. grandis* (GR<sub>50</sub> of 567  $\mu\text{g mL}^{-1}$ ).

In the case of commercial oil samples, GR<sub>50</sub> values against rapeseed seedling growth were ranged between 26 and >5,000  $\mu\text{g mL}^{-1}$ . Out of 64 commercial essential oil samples, 11 oils (cinnamon, citronella, clove, cumin seed, geranium, jasmine, lemongrass, palmarosa, pimento, rose otto and spearmint) showed remarkable inhibitory activity against rapeseed seedling growth with GR<sub>50</sub> values of below 150  $\mu\text{g mL}^{-1}$ . Among them, palmarosa oil showed the highest inhibitory activity on seedling growth (GR<sub>50</sub> of 26  $\mu\text{g mL}^{-1}$ ) followed by clove (GR<sub>50</sub> of 48  $\mu\text{g mL}^{-1}$ ) and pimento (GR<sub>50</sub> of 52  $\mu\text{g mL}^{-1}$ ) oils. Previously, many authors have investigated the inhibitory effect of essential oil from various aromatic plants. Essential oil from *Artemisia scoparia* Waldst. & Kit.

reduced the emergence and seedling growth of weed species such as *Achyranthes aspera* L., *Cassia occidentalis* L., *Parthenium hysterophorus* L., *Echinochloa crus-galli* (L.) P. Beauv. and *Ageratum conyzoides* L. (Kaur et al., 2010). The essential oils from the aerial parts of catmint (*Nepeta meyeri* Benth.) effectively inhibited the seedling growth of weed species such as *Amaranthus retroflexus* L., *Bromus danthoniae* Trin., *Bromus intermedius* Guss., *Chenopodium album* L., *Cynodon dactylon* L., *Lactuca serriola* L., and *Portulaca oleracea* L. by inducing oxidative stress (Mutlu et al., 2011). Poonpaiboonpipat et al. (2013) reported that the essential oil from *Cymbopogon citratus* Stapf remarkably inhibited germination and seedling growth of *E. crus-galli* and affecting  $\alpha$ -amylase activity of seeds.

### Identification of major components using SPME/GC-MS

Further studies in relation to identification of chemical components were carried out with these 11 effective essential oil samples. In order to identify the major components from the effective 11 essential oil samples, SPME-GC/MS analyses were performed. Area percentage of major components identified from the essential oil samples is presented in Table 3. Eugenol was detected as a major component in clove (92.27%), cinnamon (91.89%) and pimento (72.9%) oils. In citronella oil, the major components were citronellal (50.56%) and geraniol (24.52%). The main components of the essential oil from cumin seed oil were 2-methyl-3-phenyl-propanal (42.48%), safranal (15.88%), terpinene (12.48%) and cymene (10.30%). Citronellol was found to be a major component in geranium (38.41%) and rose otto (58.64%) oils. In jasmine oil, benzyl benzoate (35.88%) and benzyl acetate (30.80%) were found to be main components. Citral (52.59%) and  $\beta$ -citral (33.66%) were major ones in lemongrass oil. Geraniol (86.56%) and carvone (76.65%) are found to be main components in palmarosa and spearmint oils, respectively. The results revealed that the analyzed essential oil samples mainly composed of oxygenated monoterpenes.

### Phytotoxic effect of individual essential oil components

Based on the results of chemical composition, individual components namely, benzyl benzoate, carvone, citral, citronellol, eugenol, geraniol, D-limonene and terpinene were used to evaluate their inhibitory activity against the seedling growth of three different species. All the tested 8 components effectively inhibited the seedling growth of *B. napus*, *E. crus-galli* and *A. indica* (Table 4). Among the three plants, *B. napus* and *E. crus-galli* are more susceptible than *A. indica*. Citral showed significantly higher inhibitory activity ( $P < 0.05$ ) against *B. napus* with  $GR_{50}$  of  $34 \mu\text{g mL}^{-1}$ . In the case of *E. crus-galli*, geraniol exhibited significantly higher inhibitory activity

**Table 3.** Chemical composition of 11 effective essential oil samples.

Sample	Compound name	Area %	Component group
Cinnamon	Eugenol	91.89	Alcohol
Citronella	Citronellal	50.56	Aldehyde
	Geraniol	24.52	Alcohol
Clove	Eugenol	92.27	Alcohol
Cumin seed	Cymene	10.30	Hydrocarbon
	Terpinene	12.48	Hydrocarbon
	2-Methyl-3-phenyl-propanal	42.48	Aldehyde
	Safranal	15.88	Aldehyde
Geranium	Citronellol	38.41	Alcohol
	Geraniol	31.73	Alcohol
Jasmine	Benzyl acetate	30.80	Acetate
	Benzyl benzoate	35.88	Acetate
Lemongrass	Citral	52.89	Aldehyde
	$\beta$ -Citral	33.66	Aldehyde
Palmarosa	Geraniol	86.56	Alcohol
	Geranyl acetate	11.47	Acetate
Pimento	Eugenol	72.9	Alcohol
	Caryophyllene	6.3	Hydrocarbon
Rose otto	Citronellol	58.64	Alcohol
	Geraniol	15.48	Alcohol
Spearmint	Limonene	16.70	Hydrocarbon
	Carvone	76.65	Ketone

( $P < 0.05$ ) of seedling growth than other components with  $GR_{50}$  of  $29 \mu\text{g mL}^{-1}$ . Out of eight components, terpinene and D-limonene showed lower inhibitory activity. The herbicidal potential of tested compounds varied enormously against the three plant species. Four components of 8 (geraniol, citronellol, citral and carvone) are coming under oxygenated monoterpene group, two components (terpinene and limonene) are monoterpene and eugenol is a phenylpropanoid and benzyl benzoate is esters of benzyl alcohol and benzoic acid.

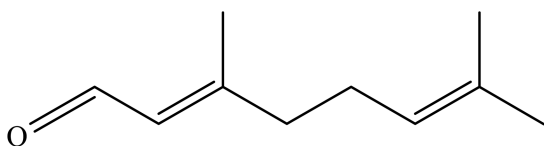
Previous studies have shown that essential oils and their individual components isolated from various plant species, exhibited potent herbicidal effects on weed germination and primary root growth of several other species. Martino et al. (2010) studied the anti-germinative potential of twenty seven monoterpenes, including monoterpene hydrocarbons and oxygenated ones, against seed germination and subsequent primary radicle growth of *Raphanus sativus* L. and *Lepidium sativum* L. Among the 27 components tested, geraniol, borneol,  $\beta$ -citronellol and  $\alpha$ -terpineol are the most active components. Further, the authors reported that the radicle

**Table 4.** Effect of individual essential oil components on seedling growth of *Brassica napus*, *Echinochloa crus-galli* and *Aeschynomene indica*.

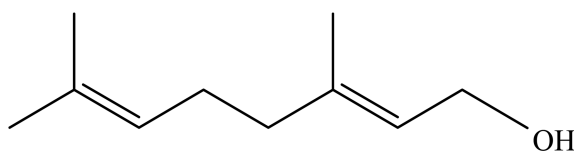
S. No.	Compound Name	GR <sub>50</sub> (µg mL <sup>-1</sup> ) <sup>z</sup>		
		<i>Brassica napus</i>	<i>Echinochloa crus-galli</i>	<i>Aeschynomene indica</i>
1	Benzyl benzoate	205 <sup>c</sup>	>1,000 <sup>e</sup>	547 <sup>f</sup>
2	Carvone	88 <sup>d</sup>	50 <sup>b</sup>	259 <sup>e</sup>
3	Citral	34 <sup>a</sup>	283 <sup>d</sup>	163 <sup>d</sup>
4	Citronellol	66 <sup>c</sup>	67 <sup>c</sup>	135 <sup>a</sup>
5	Eugenol	43 <sup>b</sup>	72 <sup>c</sup>	155 <sup>c</sup>
6	Geraniol	82 <sup>d</sup>	29 <sup>a</sup>	144 <sup>b</sup>
7	D-Limonene	574 <sup>f</sup>	>1,000 <sup>e</sup>	936 <sup>h</sup>
8	Terpinene	>1,000 <sup>g</sup>	280 <sup>d</sup>	891 <sup>g</sup>

<sup>z</sup>GR<sub>50</sub> values were calculated from four replicates of each sample. Mean values followed by different superscripts in a column are significantly different ( $P < 0.05$ ).

#### Citral (C<sub>10</sub>H<sub>16</sub>O)



#### Geraniol (C<sub>10</sub>H<sub>18</sub>O)



**Fig. 1.** Structure of the compounds citral and geraniol.

elongation of two test species was inhibited mainly by alcohols and ketones. Similar to our data, various authors have reported that the essential oil components (1,8-cineole, camphor citronellal, citronellol, linalool,  $\alpha$ -pinene and limonene) effectively inhibited seed germination and seedling growth (Abraham et al., 2000; Kordali et al., 2007; Singh et al., 2002, 2006). In the present study, the results showed that the oxygenated monoterpenes (carvone, citronellol, citral, eugenol, and geraniol) had higher inhibitory activity on seedling growth than monoterpene hydrocarbons. Vokou et al. (2003) studied the effects of 47 individual monoterpenoids from different chemical group, acting alone or in pairs, on seed germination and subsequent seedling growth of *Lactuca sativa*, and they concluded that the most active components were terpinen-4-ol, dihydrocarvone, and two carvone stereoisomers.

### Phytotoxic effect of individual components under greenhouse experiment

Seed bioassay was an important preliminary screening method to determine the phytotoxic potential of plant extracts or compounds. However, greenhouse and field experiments are important criteria in order to understand the efficacy of herbicidal compounds under field conditions for further utilization of products commercially. Based on the results from seed bioassay, a greenhouse experiment was conducted using eight individual components such as benzyl acetate, carvone, citral, citronellol, eugenol, geraniol, D-limonene and terpinene against 5 monocot plants (*D. ciliaris*, *S. viridis*, *P. annua*, *E. crus-galli* and *Z. mays*) and 5 dicot plants (*A. indica*, *A. theophrasti*, *G. hirsutum*, *G. max* and *I. angulata*). The phytotoxic activity of 8 components showed considerable variation among the plant species tested. Except D-limonene and terpinene, all other components showed appreciable phytotoxic activity against the tested plants at the highest concentration (100 kg ha<sup>-1</sup>). The most phytotoxic components among them were geraniol and citral. After 14 days of spray treatment, citral and geraniol showed potent phytotoxic activity by totally killed all the tested 10 plants at the concentration of 100 kg ha<sup>-1</sup>. Moreover, citral and geraniol also killed all the tested plants at the concentration of 50 kg ha<sup>-1</sup> with the exception of *P. annua* (Table 5). The compound citral completely suppressed the growth of *A. indica*, *A. theophrasti*, *G. hirsutum*, *I. angulata* and *Z. mays* even at the lowest concentration (25 kg ha<sup>-1</sup>). Whereas geraniol suppressed the growth of *A. indica* and *A. theophrasti* at the lowest concentration tested. However, the concentrations of citral and geraniol used in this study were higher than that of commercial herbicides (4 kg ha<sup>-1</sup>). Therefore, further large scale field studies are required to understand the phytotoxic effect of these compounds.

The most effective components, citral and geraniol are coming under the group of oxygenated monoterpene. The primary oxidation products of geraniol/nerol are geranial (citral A) and neral (citral B) known together as citral (Dapurkar et al., 2011). Citral and geraniol exhibit various biological properties and found abundantly in large number of aromatic plants. These are the most important avoring compounds used widely in beverages, foods, and fragrances for their characteristic avor prole. Previous studies have stated that the phytotoxic effects of these compounds might be due to anatomical and physiological changes in seedlings by reducing some organelles like mitochondria, accumulation of lipid globules in the cytoplasm, inhibiting the synthesis of DNA or disruption of membranes and suppression of metabolic enzymes activity that involved in glycolysis and in oxidative pentose phosphate pathway (Podesta and Plaxton, 1994; Muscolo et al., 2001; Nishida et al., 2005). The essential

**Table 5.** Herbicidal effect of individual essential oil components with early post-emergence treatment on ten plants in a greenhouse.

Component Name	Dose (kg ha <sup>-1</sup> )	<i>Abutilon theophrasti</i>	<i>Aeschynomene indica</i>	<i>Gossypium hirsutum</i>	<i>Glycine max</i>	<i>Ipomoea angulata</i>	<i>Echinochloa crus-galli</i>	<i>Digitaria ciliaris</i>	<i>Setaria viridis</i>	<i>Poa annua</i>	<i>Zea mays</i>
Benzyl benzoate	25 <sup>z</sup>	4 <sup>y</sup>	2	0	3	2	2	2	0	0	5
	50	7	10	0	4	3	3	7	2	2	10
	100	10	10	10	10	9	7	10	10	6	10
Carvone	25	1	4	0	2	1	1	0	1	1	6
	50	10	9	10	5	4	3	4	7	2	9
	100	10	10	10	10	10	6	10	10	10	10
Citral	25	10	10	10	6	10	3	2	8	2	10
	50	10	10	10	10	10	10	10	10	4	10
	100	10	10	10	10	10	10	10	10	10	10
Citronellol	25	10	2	10	4	4	3	7	7	3	9
	50	10	10	10	10	6	4	8	10	4	10
	100	10	10	10	10	10	7	10	10	10	10
Eugenol	25	6	9	4	4	1	1	1	2	1	2
	50	10	10	10	9	10	2	3	3	5	8
	100	10	10	10	9	10	10	10	10	10	9
Geraniol	25	10	10	0	7	3	4	4	6	2	9
	50	10	10	10	10	10	10	10	10	6	10
	100	10	10	10	10	10	10	10	10	10	10
D-Limonene	25	0	0	0	0	0	0	0	0	0	9
	50	0	0	0	1	0	0	2	1	2	10
	100	1	10	0	2	0	3	3	10	4	10
Terpinene	25	0	0	0	0	0	0	0	0	0	1
	50	0	8	2	1	1	1	0	0	0	7
	100	2	8	2	3	2	2	7	1	2	10

<sup>z</sup>Each treatment has 10 plants with four replicates. Herbicidal activity was determined 14 days after treatment by visual injury.

<sup>y</sup>Results were expressed as 0-no effect; 10-totally killed.

oil of *Artemisia scoparia* inhibited germination and plant root growth by generating ROS-induced oxidative stress (Singh et al., 2009). The mechanism behind its phytotoxic effect might be affecting chlorophyll content, cellular respiration and electrolyte leakage of weed plants (Kaur et al., 2010). Sanchez-Moreiras et al. (2008) suggested that the inhibitory effects of essential oils have been associated with their influence on the regulation of shoot elongation and cell division of the target plants. In addition, plant essential oils have been shown some other mechanisms including delay crystallization, reduce the volatilization and photo-degradation of the herbicides on the leaf surface (Bunting et al., 2004; Si et al., 2004; Ramsey et al., 2006). Phytotoxic compounds released from plants that aid

them in both interspecific and intraspecific competitions (Meyer et al., 2007). Overall results showed that the citral and geraniol have strong herbicidal potential than other compounds. The findings of present investigation indicated that the individual components provided a good platform to develop novel and effective herbicides.

## Conclusions

The present study reveals that the different essential oil samples showed a considerable variation in the phytotoxic effect on rapeseed seedling growth. The effective essential oil samples mainly composed of oxygenated monoterpenes. The

results confirmed that citral and geraniol provided excellent phytotoxic activity under *in vitro* seed bioassay as well as in greenhouse experiment than other essential oil components. It could be concluded that citral and geraniol may be favorably used for incorporating in agricultural practices as natural herbicides for the management of weeds. Further studies in relation to mechanism of action and field experiment are under progress.

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