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Phytotoxic effects of volatile oil from *Artemisia scoparia* against weeds and its possible use as a bioherbicide

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ABSTRACT

A study was conducted to assess the bioherbicidal activity of volatile oil hydrodistilled from *Artemisia scoparia* Waldst *et* Kit. (red stem wormwood; Asteraceae) against five weed species, viz. *Achyranthes aspera*, *Cassia occidentalis*, *Parthenium hysterophorus*, *Echinochloa crus-galli*, and *Ageratum conyzoides*. Emergence and seedling growth (in terms of root and shoot length) were significantly reduced in a dose–response bioassay conducted in sand impregnated with *Artemisia* oil (at ≥10, 25, and 50 μg *Artemisia* oil/g sand). In general, the root length was inhibited more as compared to the shoot length and the inhibitory effect was greatest in *P. hysterophorus* followed by *A. conyzoides* and least in *C. occidentalis*. Post-emergence application of *Artemisia* oil (2%, 4%, and 6%, v/v) on 6-week-old weed plants caused visible injury (1- and 7-days after spray) ranging from chlorosis to necrosis to complete wilting of plants. Among the sprayed test weeds, the effect was greatest on *E. crus-galli* and *P. hysterophorus*. *Artemisia* oil treatment resulted in a loss of chlorophyll content and cellular respiration in test weeds thereby implying interference/impairment with photosynthetic and respiratory metabolism. *Artemisia* oil caused a severe electrolyte leakage from *E. crus-galli* (a monocot) and *C. occidentalis* (a dicot) indicating membrane disruption and loss of integrity. The study concludes that *Artemisia* oil has bioherbicidal properties as it causes severe phytotoxicity and interferes with the growth and physiological processes of some weed species.

1. Introduction

Volatile oil released from aromatic plants provides a number of ecological advantages to the plant. For example, they act as pollinator attractants, determinants of vegetation patterning, provide protection against predators and other enemies, and mediate plant–plant interactions including allelopathy (Batish et al., 2008; Singh et al., 2009). Earlier studies have documented that volatile oils and their constituents are potent seed germination inhibitors and retard plant growth (Muller et al., 1964; Barney et al., 2005; Batish et al., 2006; Ens et al., 2009). Muller et al. (1964) demonstrated that volatile oils emanated from aromatic shrubs – *Salvia leucophylla* and *Artemisia scoparia* – reduce the growth of associated plants and cause characteristic vegetation patterning. The growth inhibitory activity of plant essential oils has tremendously increased the interest in exploring volatile oil from aromatic plants for potential weed management (Singh et al., 2003, 2005; Batish et al., 2004; Batish et al., 2008; Dayan et al., 2009). Such studies are important in view of the environmental and human health concerns, and increasing herbicidal resistance linked to synthetic herbicides; thus, there is a need to search for environmentally safer and novel compounds with weed suppressing ability (Singh et al., 2003; Dayan et al., 2009).

The genus *Artemisia* (wormwoods or sagebrushes; Asteraceae) consisting of over 200 species of herbs and shrubs is well known for wide spectrum of biological activities, including medicinal, due to the presence of volatile oils. Of the 37 species found in India, *A. scoparia* (red stem wormwood) is a faintly scented, fast-growing, annual herb that forms monospecific thickets along canals, roadsides, agricultural fields, and wastelands (Singh et al., 2009). Its essential oil has been observed to possess antibacterial (Yeung, 1985) and pesticidal properties (Negahban et al., 2006). Recently, its volatile oil has been reported to inhibit seed germination and seedling growth of three weeds *viz. Avena fatua*, *Cyperus rotundus*, and *Phalaris minor* (Singh et al., 2009). Based on these reports, it is opined that since volatile oil of *A. scoparia* is phytotoxic to weeds, it should be explored for bioherbicidal activity. The present study thus aims to investigate the herbicidal activity of *A. scoparia* volatile oil in terms of effect on plant growth, injury level, and impacts on metabolic processes such as photosynthesis, respiratory activity, and membrane integrity.

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2. Methods and materials

2.1. Extraction of the oil

Volatile oil was hydrodistilled from freshly collected mature leaves of *A. scoparia* Waldst. & Kit. (red stem wormwood; hereafter *Artemisia*) using a Clevenger's apparatus (Singh et al., 2009). Leaves were collected during 3rd week of July 2007 from the plants growing in wastelands around Chandigarh. The oil was light yellow in colour with a yield of 0.21% (v/w, fresh weight basis). It was stored at 4 °C for further use in bioassay and composition determination.

2.2. Chemical characterization of the oil

The chemical composition of the extracted oil was determined by gas chromatography (GC) and gas chromatography–mass spectroscopy (GC–MS) as per Singh et al. (2009). Briefly, GC was performed on a Shimadzu GC-17A Gas Chromatograph fitted with a flame ionization detector (FID), equipped with DB-5 column (60 m × 0.25 mm, i.d.; 0.25 μm film thickness), and using helium (He) as carrier gas (split ratio 1:20; flow rate 1 ml min^{-1}). The oven temperature was programmed from 50 °C (held initially for 2 min) to 260 °C (held for 3 min) at 4 °C min^{-1}. Relative percentages of different constituents were calculated from peak area of total ion chromatograms without any correction factor. The data presented are mean percentages of three analyses.

GC–MS analysis was done on a Shimadzu QP 2010 mass spectrophotometer fitted with SGE-BP20 polar column (30 m × 0.25 mm, i.d.; film thickness 25 μm). Helium (He) was used as a carrier gas at a split ratio of 1:50, and a linear velocity of 38.5 cm s^{-1}. Initially, the oven temperature was 70 °C (held for 4 min) and later increased to 220 °C (held for 5 min) at 4 °C min^{-1}. Mass spectral range was recorded from m/z 40 to 600 amu. Constituents of the oil were identified by (i) the comparison of their retention times with those of pure reference samples procured from the best available sources (Sigma–Aldrich, St. Louis, USA; Fluka, Buchs, Switzerland; Acros, Geel, Belgium; AlfaAeser, Massachusetts, USA; TCI, Tokyo, Japan), (ii) co-elution with available reference compounds, (iii) comparison of their retention indices (RI) relative to homologues *n*-alkanes (C_7–C_{30}; Supelco, Bellefonte, PA, USA), and (iv) computer matching of their mass spectra using library search system HP-5872 (Hewlett-Packard), and consulting data bases of Wiley 275 and NBS 75K Libraries (Singh et al., 2009).

2.3. Dose–response studies

Seeds of *Achyranthes aspera* L., *Cassia occidentalis* L. and *Parthenium hysterophorus* L. were collected locally from wastelands, whereas seeds of *Echinochloa crus-galli* (L.) P. Beauv., and *Ageratum conyzoides* L. were collected from agricultural fields on the outskirts of Chandigarh. These were surface-sterilized with sodium hypochlorite (0.1%, w/v) for 2 min, washed under running tap water (for 5 min) followed by distilled water and stored for further use.

Dose–response studies were conducted under laboratory conditions using sand as growth medium to determine the effect of *Artemisia* oil on growth of test weeds (Singh et al., 2006b). Briefly, 15-cm diameter Petri dishes were lined with a layer of Whatman No. 1 filter paper moistened with 8 ml of distilled water. These were treated with different amounts of *Artemisia* oil to get 5, 10, 25, and 50 μg oil/g sand. After treatment, ~400 g sand was placed on top of the filter circle, sown with test weeds (25 seeds each, except 15 for *C. occidentalis*). One hundred milliliters of distilled water was added to sand, and covered with lids. Petri dishes were then sealed with Parafilm®. A set of Petri dishes without oil for each weed species served as parallel control. Five Petri dishes were maintained as replicates for each treatment and weed species in a randomized block design. Petri dishes were placed in a growth chamber set at 30 ± 2 °C (for all weeds except *A. conyzoides*) or 14 ± 2 °C (for *A. conyzoides*) temperature, 16/8 h L/D photoperiod of 225 μmol m^{-2} s^{-1} and a relative humidity of nearly 80%. After 2 weeks, the number of emerged seedlings, and their root and shoot lengths were measured. Based on growth studies, LC_{50} (least concentration of oil at which 50% inhibition in germination occurs) were determined. The whole experiment was repeated.

2.4. Studies in experimental screenhouse

Another experiment was performed to study the effect of *Artemisia* oil on 6-week-old weed plants raised under controlled conditions in experimental screenhouse. Plants of all the test weeds were raised from the collected seeds in 12 cm diameter earthenware pots. Pots were filled with 750 g garden soil (soil:sand:manure: 3:1:1, w/w) and seven seeds of each weed species were sown per pot. Two-week after emergence, pots were thinned to 5 equal-sized healthy plants per pot. Plants were watered daily and after 3 weeks they were flushed with half-strength Hoagland nutrient solution (Hoagland and Arnon, 1950). When plants were 6-week-old, these were sprayed with 2, 4, and 6% (v/v) solution of *Artemisia* oil or distilled water (as parallel control) using a common garden sprayer at the rate of 100 ml/m^2. For each seed type, a total of 20 pots comprising of 4 treatments and five replicates per treatment were maintained in a completely randomized design. One- and 7-days after spray (DAS), the treated test weed plants were examined for visible injury levels in terms of percent chlorotic and necrotic areas. Further, the leaves were plucked for the determination of chlorophyll content and cellular respiratory activity.

2.5. Estimation of chlorophyll content

Chlorophyll was extracted from leaves (25 mg) in dimethyl sulphoxide (4 ml) as per Hiscox and Israelstam (1979). It was quantified as per Arnon (1949) and expressed on dry weight basis (Rani and Kohli, 1991).

2.6. Determination of cellular respiration

It was determined using 2,3,5-triphenyl tetrazolium chloride (TTC) as per Singh et al. (2002). TTC traps the electrons from respiratory chain and provides an indirect method of measurement of cell respiration (Batish et al., 2007a).

2.7. Relative electrolyte leakage (REL)

REL was determined in sprayed leaves of two weed species (*E. crus-galli* and *C. occidentalis*) to study the effect of *Artemisia* oil on solute leakage and thus membrane integrity as per Singh et al. (2008). Leaf tissue (100 mg) was immersed in distilled water (bathing medium) for 30 min and the conductivity of medium was measured (C1). Thereafter, test tubes containing leaf tissues were boiled for 15 min and the conductivity was again measured (C2). The relative electrolyte leakage (REL) was calculated using following formula and expressed in percent.

$$\%\mathrm{REL} = \left(\frac{C1}{C2}\right) \times 100$$

2.8. Statistical analyses

All the experiments were repeated and the presented data are average of the two experiments. For each analysis, there were five replicated (independent) tissue samples. The data for germination

studies were subjected to dose–response analysis. Dose–response curves were derived by plotting the concentration (on the *x*-axis) and percent response (on the *y*-axis) using GraphPad Prism version 5, and LC_{50} values determined from the dose–response curve. The effect of essential oil on shoot and root length, chlorophyll content, respiratory value, visible injury, and relative electrolyte leakage was analyzed by linear regression models.

3. Results and discussion

3.1. Chemical characterization of the oil

The GC–MS analyses of the oil revealed the presence of 37 components constituting 99.59% (Table 1). Of these, 20 were monoterpenoids (55.18%), 6 were sesquiterpenes (3.56%), and 5 each were other aliphatic (1.66%) and aromatic compounds (39.19%). Among monoterpenoids, hydrocarbons accounted for 50.19%, whereas oxygenated sesquiterpenes accounted for 3.14%. *p*-Cymene (20.5%) was the major monoterpenoid constituent, followed by β-myrcene (13.95%) and (+)-limonene (12.53%), respectively (Table 1). All the oxygenated monoterpenes were ≤1.41%, whereas the amount of all the sesquiterpenes was ≤1.89% (Table 1). The presence of *p*-cymene as the major compound in *A. scoparia* mature leaf oil is in sharp contrast to earlier studies reporting β-myrcene (Singh et al., 2009), methyl eugenol (Basher et al., 1997), or thujone, camphor, and 1,8-cineole (Mirjalili et al., 2007) as the major constituents in the oil.

3.2. Growth studies under laboratory conditions

The emergence of all the test weeds was significantly reduced when sown in *Artemisia* oil impregnated sand (Fig. 1). In general, a dose–response relationship was observed and the emergence declined with the increase in amount of *Artemisia* oil. At 5 μg *Artemisia* oil/g sand, there was no significant effect on emergence of test weeds, except in *A. conyzoides* and *A. aspera*, where ~20% and 27% decrease was observed (Fig. 1). At 25 μg *Artemisia* oil/g sand, the emergence of test weeds was reduced by 31–74%. However, at 50 μg *Artemisia* oil/g sand, only 21%, 17%, and 6% emergence was observed in *P. hysterophorus*, *A. conyzoides* and *A. aspera*, respectively (Fig. 1). From dose–response curves, LC_{50} values were calculated to be 7.39, 8.82, 6.98, 2.12, and 7.04 μg *Artemisia* oil/g sand for *A. aspera, E. crus-galli, C. occidentalis, P. hysterophorus*, and *A. conyzoides*, respectively.

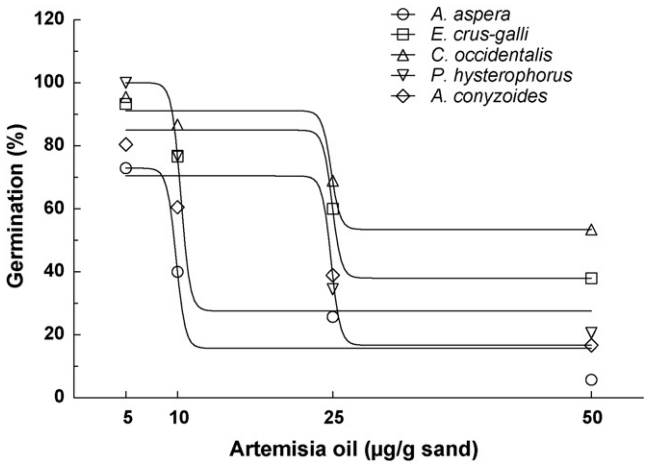

Fig. 1. Dose–response curve showing the effect of *Artemisia* oil on percent germination of test weeds measured 2 weeks after treatment.

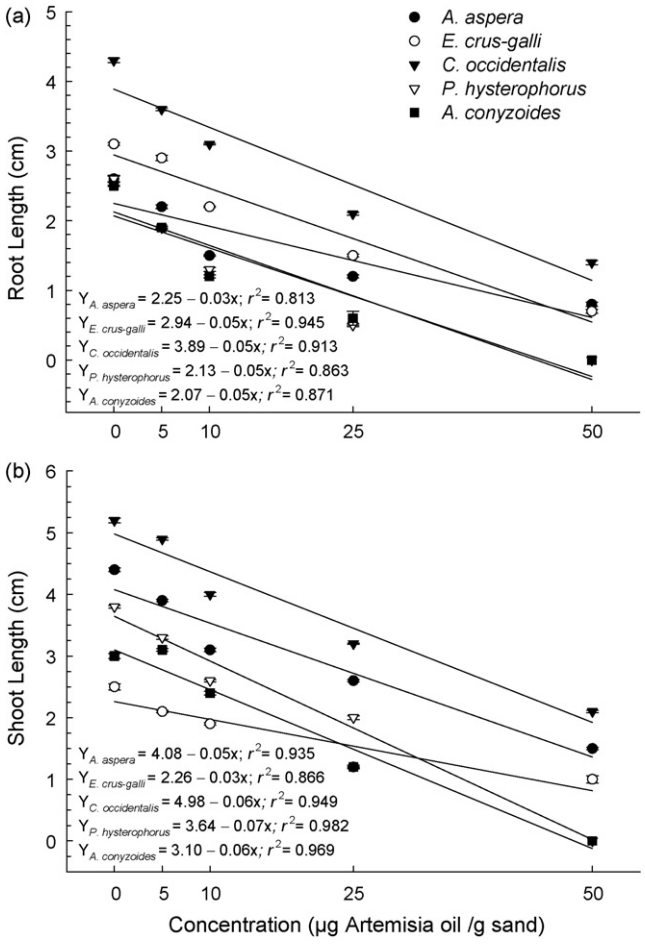

Fig. 2. Effect of *Artemisia* oil on (a) root, and (b) shoot length (cm) of test weeds measured after 2 weeks. Data analyzed by linear regression models and r^2 represents the correlation coefficient. Vertical bars along each data point represent the standard error.

Not only emergence, even the seedling growth measured as root and shoot length was significantly reduced except at 5 μg *Artemisia* oil/g sand (r^2 = 0.81–0.95). At 10 μg *Artemisia* oil/g sand, ~38–52% reduction was observed in root length of test weeds. The reduction was greater with increasing amount of *Artemisia* oil (Fig. 2). At highest concentration (50 μg *Artemisia* oil/g sand), the maximum inhibition in root length was observed in *P. hysterophorus* and *A. conyzoides*, where though seeds germinated but root length was not measurable. In *A. aspera*, *E. crus-galli* and *C. occidentalis* root length declined by 69%, 77%, and 67%, respectively, at 50 μg *Artemisia* oil/g sand (Fig. 2). Likewise, the shoot length of test weeds was significantly (r^2 = 0.87–0.97) reduced in response to *Artemisia* oil. In general, the inhibitory effect was greater on root growth than on shoot growth (Fig. 2). With treatment of 10 and 25 μg *Artemisia* oil/g sand, test weeds exhibited ~23–37% and ~38–68% reduction in root and shoot length, respectively. Further, in response to 50 μg *Artemisia* oil/g sand, shoot length was reduced by ~66% in *A. aspera* and 60% in *E. crus-galli* and *C. occidentalis* (Fig. 2).

The observations made in the present study are parallel to earlier studies documenting the growth inhibitory activity of aromatic plants, including *Artemisia* species and their volatile oils. For example, volatile oil (0.12–0.30 mg ml^{-1}) from *Eucalyptus citriodora* reduced seedling growth and dry weight accumulation in *C. occidentalis*, *Amaranthus viridis*, and *E. crus-galli* by ≥50% (Batish et al., 2004). Angelini et al. (2003) demonstrated that essential oils from three Mediterranean Lamiaceae members *Rosmarinus offici-*

Table 1
Chemical composition of the essential oil extracted from freshly collected mature leaves of *Artemisia scoparia*.

RI[a]	Components[b]	% Age[c]	Identification methods[d]
Monoterpene hydrocarbon			
1161	β-Myrcene	13.95	co-GC, MS, RI
1201	(+)-Limonene	12.53	co-GC, MS, RI
1231	(Z)-β-Ocimene	2.57	co-GC, MS, RI
1244	γ-Terpinene	0.16	co-GC, MS, RI
1250	(E)-β-Ocimene	0.24	co-GC, MS, RI
1269	*p*-Cymene	20.50	co-GC, MS, RI
1455	Isocitronellene	0.24	MS, RI
Oxygenated monoterpenes			
1408	*cis*-Linalool oxide	0.75	MS, RI
1442	*trans*-Limonene oxide	0.35	MS, RI
1463	α-Campholenal	0.18	MS, RI
1547	(−)-Linalool	0.08	co-GC, MS, RI
1587	Dihydrocarvone	0.21	MS, RI
1596	Terpinen-4-ol	0.09	MS, RI
1620	Thujenol	0.21	MS, RI
1723	Neryl acetate	0.26	co-GC, MS, RI
1753	Geranyl acetate	1.41	co-GC, MS, RI
1765	β-Citronellol	0.26	co-GC, MS, RI
2007	Methyl eugenol	0.64	co-GC, MS, RI
2167	Eugenol	0.27	co-GC, MS, RI
2442	Acetylisoeugenol	0.28	MS, RI
Sesquiterpene hydrocarbon			
1523	α-Copaene	0.08	MS, RI
1658	α-Humulene	0.34	MS, RI
Oxygenated sesquiterpene			
1961	*Iso*-caryophyllene oxide	0.10	co-GC, MS, RI
1966	Caryophyllene oxide	0.95	co-GC, MS, RI
2032	Humulene epoxide II	1.89	MS, RI
2081	*trans*-Nerolidol	0.20	co-GC, MS, RI
Other aromatic compounds			
1502	Phenylmethanal	0.80	MS, RI
1940	1,3-Dimethylnaphthalene	0.28	MS, RI
2090	Precocene I	0.19	MS, RI
2096	3,8-Terpineolhydrate	0.67	MS, RI
2229	Acenaphthene	36.86	MS, RI
Other aliphatic compounds			
1361	Hexan-1-ol	0.14	co-GC, MS, RI
1401	*cis*-Hexenol	0.08	MS, RI
1484	(*Z*)-3-Hexenyl isobutyrate	0.08	MS, RI
1986	*Allyl*-butyrate	0.11	MS, RI
2460	Isopropyl tiglate	0.30	MS, RI
2540	Isomenthyl acetate	0.95	MS, RI
Monoterpenoid hydrocarbons			50.19
Oxygenated monoterpenoids			4.99
	Total monoterpenoids		55.18
Sequiterpene hydrocarbons			0.42
Oxygenated sesquiterpenes			3.14
	Total sesquiterpenes		3.56
Other aromatic compounds			39.19
Other aliphatic compounds			1.66
	Total others		40.85
Total identified			99.59

[a] Retention index relative to *n*-alkanes (C_{7-30}) on the SGE-BP20 column.
[b] Compounds presented in order of elution from the SGE-BP20 capillary column.
[c] Percentage based on FID peak area normalization ($n = 3$).
[d] Methods: co-GC, Identification based on retention times of authentic reference compounds on DB-5 column; MS, tentatively identified based on computer matching of mass spectra of peaks with HP-5872, Wiley 275, NBS 75K, and NIST 98 libraries; RI, Tentatively identified based upon matching of retention index with published literature.

nalis, *Thymus vulgaris*, and *Satureja montana* (at 500 ppm) severely reduced germination and seedling growth of weedy species such as *Chenopodium album*, *Portulaca oleracea*, and *E. crus-galli*. Later, Singh et al. (2005) reported that eucalypt oil (at 0.2–5.0 nl ml^{-1}) reduced seed germination and seedling growth of *P. hysterophorus* by 56–100%. Liza López et al. (2008) demonstrated that volatile oil from *Tagetes minuta* (at 100–1000 ppm) inhibited the germination of weed species such as *Taraxacum officinale*, *Mikania cordifolia*, and *Cynodon dactylon*. Recently, Singh et al. (2009) reported that volatile oil from *A. scoparia* (at 0.14–0.35 mg ml^{-1}) inhibited radicle emergence and seedling growth in *Cyperus rotundus*, *A. fatua*, and *Phalaris minor*.

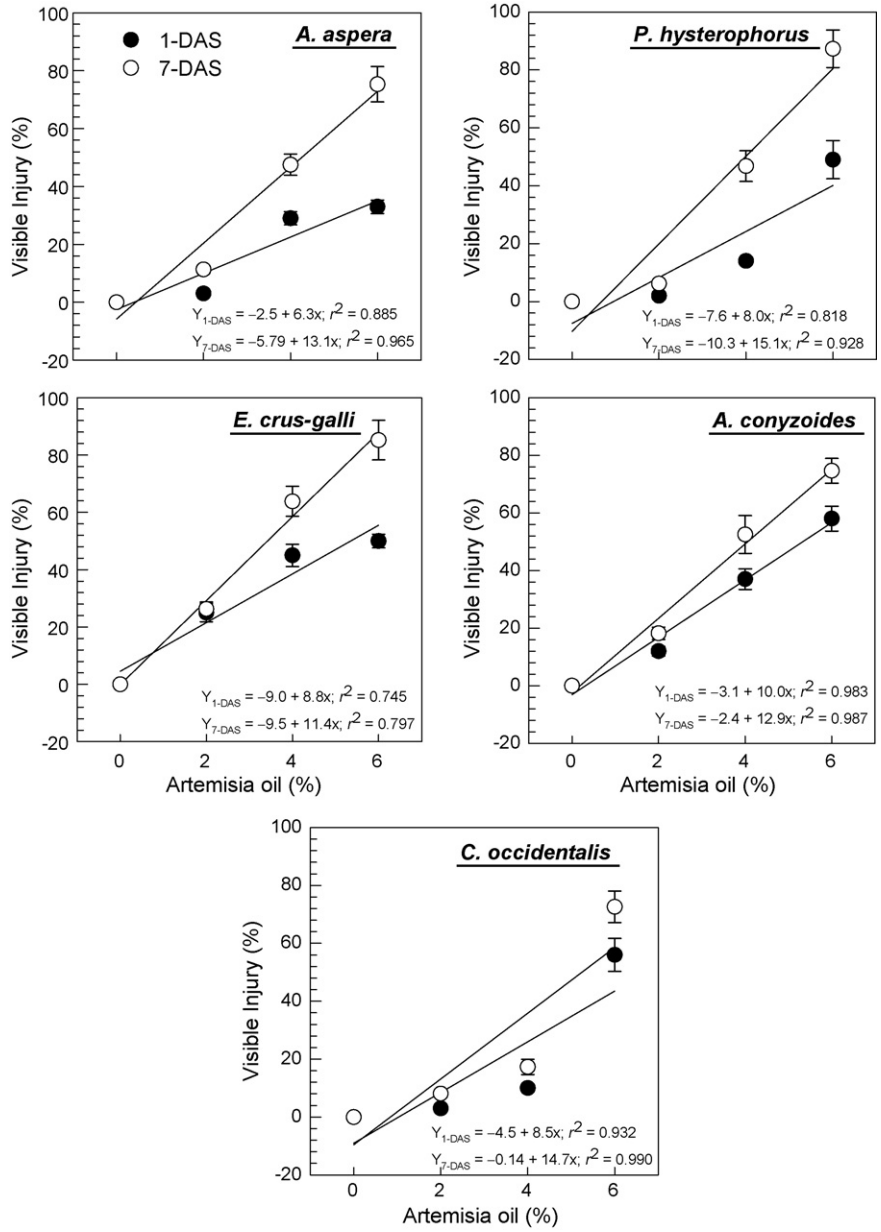

Fig. 3. Effect of *Artemisia* oil on visible injury in 6-week-old plants of test weeds measured 1- and 7-days after spray (DAS). Data analyzed by linear regression models and r^2 represents the correlation coefficient. Vertical bars along each data point represent the standard error.

Not only the essential oils, but their constituents have also been reported to be potent inhibitors of seed germination and root/seedling elongation. For example, monoterpenes such as 1,8-cineole, thymol, geraniol and camphor at 21, 2, 1.9 and 7.4 mg ml^{-1}, respectively, have been reported to inhibit root growth in maize (Zunino and Zygadlo, 2004). Nishida et al. (2005) observed that volatile monoterpenes α- and β-pinene, eucalyptol and camphor (at 200–1250 μM) inhibited root growth of *Brassica campestris*. Likewise, α-pinene (1–10 mM) has been reported to inhibit germination and radicle growth of *C. occidentalis* and *Amaranthus viridis* (Singh et al., 2006a). Singh et al. (2006b) demonstrated that citronellal (5–100 μg g^{-1} sand) inhibited germination and early growth of weedy species such *A. conyzoides*, *C. album*, *P. hysterophorus*, *Malvastrum coromandelianum*, *C. occidentalis*, and *Phalaris minor*. Recently, β-myrcene, a major constituent of *A. scoparia* essential oil, at 0.14–0.35 mg ml^{-1} has also been observed to inhibit emergence and radicle growth in *Cyperus rotundus*, *A. fatua*, and *Phalaris minor* (Singh et al., 2009).

In the present study, the observed growth inhibitory effects on test plants may either be due to synergistic or additive effect of compounds in *A. scoparia* oil. Allelopathy is the result of the simultaneous action of several compounds and often includes compounds whose chemistry is divergent (Einhellig, 2002). This ecological phenomenon is considered to be responsible for dominance and successful colonization of a particular exotic species in invaded plant community (Barney et al., 2005; Ens et al., 2009). It provides an extra competitive advantage to aromatic plants in their natural environment (Batish et al., 2008). Though not much is known about the inhibitory activity of volatile oils and their terpenoid constituents, yet inhibition of cell proliferation in root apical meristems has been postulated as one of the reasons for growth inhibition (Singh et al., 2006a). Koitabashi et al. (1997) demonstrated that essential oils caused accumulation of lipid globules in the cytoplasm, and reduced size of organelles such as mitochondria possibly due to DNA synthesis inhibition or membrane disruption resulting in anatomical and physiological changes. Lorber and Muller (1976)

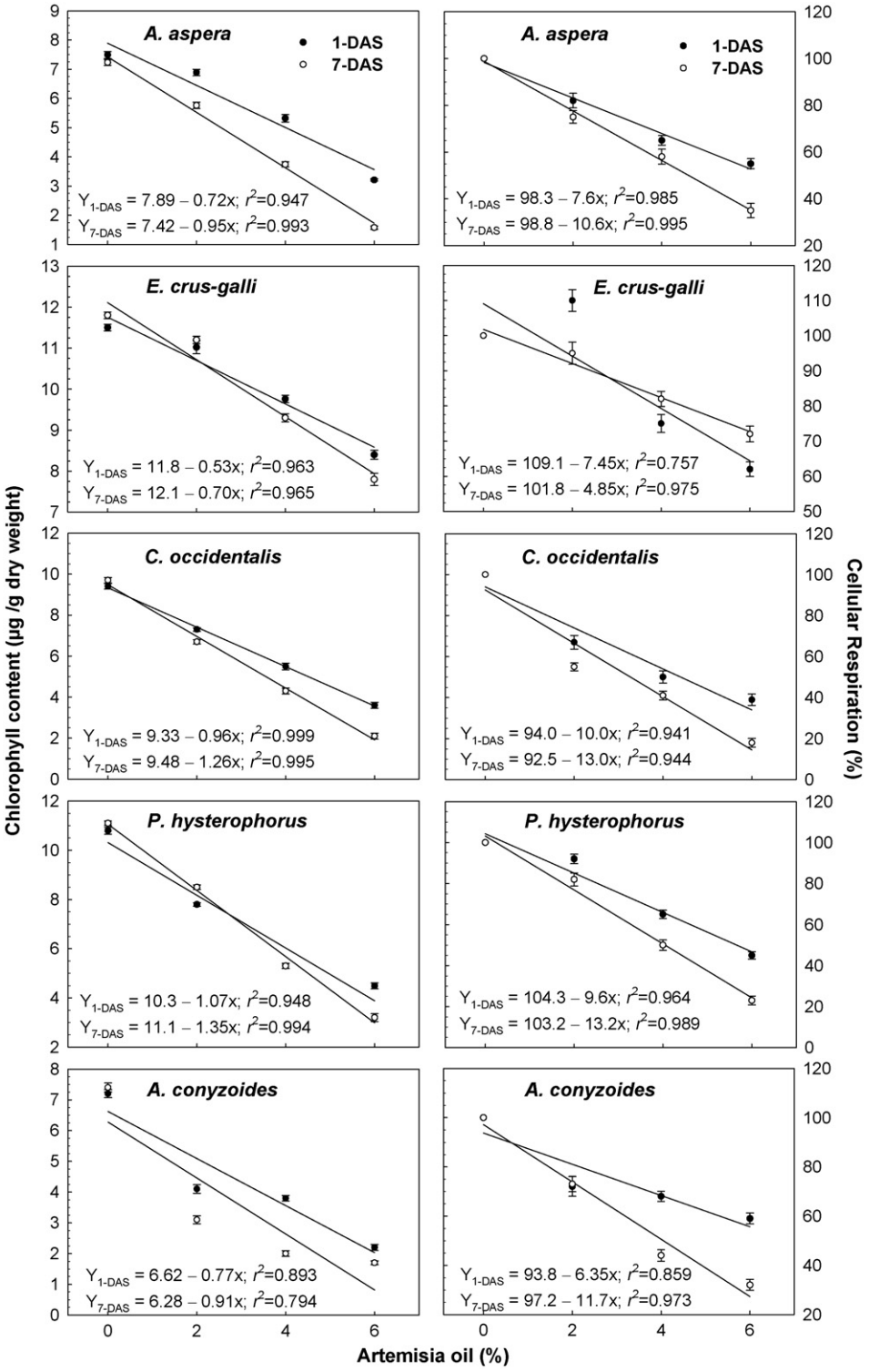

Fig. 4. Variations in total chlorophyll content (µg/mg dry weight; left panel) and cellular respiration (%; right panel) in 6-week-old plants of test weeds measured 1- and 7-days after spray (DAS) with *Artemisia* oil. Data analyzed by linear regression models and r^2 represents correlation coefficient. Vertical bars along each data point represent standard error.

reported structural breakdown and absence of intact organelles in response to volatile oil from *S. leucophylla* resulting in poor root growth. Of late, it has been documented that volatile oils and monoterpenes induce generation of reactive oxygen species resulting in lipid peroxidation and membrane disintegration (Singh et al., 2006a, 2009).

3.3. *Artemisia oil induces visible injury*

In another set of experiment, *Artemisia* oil was applied post-emergent on 6-week-old weed plants to assess the herbicidal activity. The mature plants of test weeds were severely damaged upon spray of *Artemisia* oil and showed visible injury ranging from

chlorosis to necrosis to even complete wilting of plants. In general, the visible injury symptoms observed 1- and 7-DAS treatment increased with increasing concentrations of oil (Fig. 3). The negative effects of *Artemisia* oil further increased with time and weed plants were not able to recover. At 2% *Artemisia* oil, the test weeds except *E. crus-galli* did not show any sign of injury. However, in response to higher concentrations of *Artemisia* oil (≥4%), weed plants 1-DAS showed visible symptoms like chlorosis, necrosis, wilting and drying of leaves, particularly along margins (Fig. 3). On the other hand, 7-DAS with 6% *Artemisia* oil, 60–80% injury was observed in *A. aspera* and *P. hysterophorus*. In *E. crus-galli*, a complete death of plants was observed 7-DAS upon treatment with 6% *Artemisia* oil (Fig. 3). These observations imply that *Artemisia* oil like other herbicides induces severe injuries in plants upon contact. Such observations are parallel to earlier studies demonstrating that volatile oils and even their monoterpenes exhibit herbicidal activity (Tworkoski, 2002; Singh et al., 2005, 2006b; Batish et al., 2007b). Tworkoski (2002) reported that essential oils of *Satureja hortensis*, *T. vulgaris*, *Syzygium aromaticum*, and *Cinnamomum zeylanicum* at 5 and 10% (v/v) sprayed on 12-week-old plants of *C. album*, *Ambrosia artemisiifolia*, and *Sorghum halepense* caused severe visible injury leading to plant death within 24 h. Likewise, Singh et al. (2005) reported death of 4-week-old plants of *P. hysterophorus* sprayed with 75 and 100 μl ml^{-1} *E. citriodora* volatile oil. Batish et al. (2004, 2007b) demonstrated that 5% essential oil from *E. citriodora* caused 50–80% visible injury in *C. occidentalis*, *Amaranthus viridis*, *Phalaris minor* and *E. crus-galli*.

3.4. Artemisia oil affects the chlorophyll content and cellular respiration

A significant reduction was observed in chlorophyll content in leaves of weed plants sprayed with *Artemisia* oil ($r^2 \geq 0.80$). Upon treatment with 2% oil, 1-DAS, the chlorophyll content was reduced by 4–28%. The chlorophyll content declined further in response to treatment with higher concentrations of *Artemisia* oil (Fig. 4). The greatest inhibition in chlorophyll content was observed in *A. conyzoides* 1-DAS with 6% *Artemisia* oil. On the contrary, in other weed plants, the reduction in chlorophyll content was greatest 7-DAS. In response to 6% *Artemisia* oil, 7-DAS, the inhibition in chlorophyll content was greatest in *E. crus-galli* (76%) followed by *A. conyzoides* (73%), *P. hysterophorus* (71%), and *C. occidentalis* (51%) (Fig. 4). The observed loss in chlorophyll content is in agreement to earlier reports that volatile oils and monoterpenes reduce chlorophyll content, and thus interferes with photosynthetic machinery of the plants (Singh et al., 2002; Batish et al., 2004). However, whether the loss of chlorophyll is due to inhibition in chlorophyll synthesis or degradation of existing chlorophyll was not determined. The yellowing of weed leaves upon *Artemisia* oil spray may be the secondary effect due to decrease in chlorophyll content.

Parallel to chlorophyll content, a significant decline in cellular respiration was observed in *Artemisia* oil sprayed plants. In general, the reduction was greatest 7-DAS with $r^2 \geq 0.94$ (Fig. 4). In response to 2% *Artemisia* oil treatment, 1-DAS respiration was reduced by 8–28%. It declined further with increasing concentration of *Artemisia* oil and time. At 6% *Artemisia* oil, cellular respiration decreased by 41–47% in test weeds, except in *P. hysterophorus* where ~55% reduction was observed. The reduction in respiration was the greatest in *E. crus-galli* and *P. hysterophorus*, where ~77% decrease was measured 7-DAS with 6% *Artemisia* oil (Fig. 4). These observations are parallel to earlier reports that volatile oils affect the cellular respiration and thus interfere with energy metabolism of the plants resulting in reduced growth (Singh et al., 2006b, 2009). Earlier, Abrahim et al. (2000) reported that monoterpenes, the constituents of volatile oils, due to high lipophilicity act as uncouplers of oxidative photophosphorylation, suppress respiration and thus imbalance cellular energy. Lorber and Muller (1976) opined that volatile terpenes damage mitochondria and thus negatively impair respiration and energy metabolism.

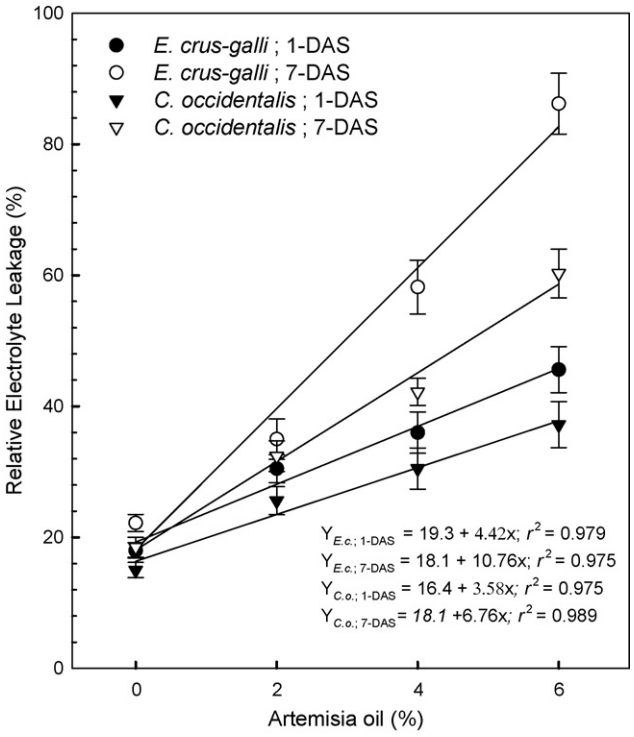

Fig. 5. Effect of *Artemisia* oil on relative electrolyte leakage (%) from leaves of 6-week-old *E. crus-galli* and *C. occidentalis* plants measured 1- and 7-days after spray (DAS). Data analyzed by linear regression models and r^2 represents correlation coefficient. Vertical bars along each data point represent standard error.

3.5. Artemisia oil causes ion leakage

Since spray of *Artemisia* oil caused wilting/desiccation in weed plants, we assessed the electrolyte leakage as the possible reason for observed damage. It was observed that *Artemisia* oil treatment induced 1.7- to 2.5-times REL when measured 1-DAS (Fig. 5). REL increased with passage of time and 7-DAS it was 3.2- to 3.9-times of that in control (Fig. 5). The excessive electrolyte leakage indicates that *Artemisia* oil disrupts the membrane integrity resulting in increased membrane permeability and thus enhanced solute leakage. A changed membrane permeability in turn affects all other physiological and biochemical processes linked to membrane functioning. The observations made in present study are in agreement with earlier reports that essential oils and their constituent terpenes inhibit plant growth through electrolyte leakage (Maffei et al., 2001; Tworkoski, 2002). Singh et al. (2005) observed that volatile oil from *E. citriodora* at concentrations ranging from 5 to 75 μl ml^{-1} caused severe ion leakage from disks of *P. hysterophorus*. Earlier, essential oils from *S. hortensis*, *T. vulgaris*, *S. aromaticum*, and *C. zeylanicum* at 1% (v/v) have been reported to cause rapid electrolyte leakage and cell death in *Taraxacum officinalis* leaf disks (Tworkoski, 2002). Even the monoterpenes such as α-pinene (at 2.5–10 mM) and citronellal (2 mM) have been reported to inhibit root growth of *C. occidentalis* through interference with the membrane integrity due to excessive ion leakage (Singh et al., 2006a,b). However, these studies have been conducted using leaf disks and not with tissue taken from the essential oil sprayed plants.

4. Conclusions

From the present study, it could be concluded that *Artemisia* oil show strong phytotoxicity against weeds (r^2 = 0.75–0.99 for visible injury in sprayed plants) and hence could be useful for developing as a bioherbicide. Among the sprayed test weeds, the phytotoxic effect of *Artemisia* oil was greatest on *E. crus-galli* followed by *P. hysterophorus* with ≥80% injury observed 7-DAS (r^2 = 0.80 and 0.93, respectively). On the contrary, the phytotoxic effect was the minimum against *C. occidentalis*. Nevertheless, the greater injury levels and thus weed suppression by *Artemisia* essential oil could be obtained with the use of adjuvants or development of formulations; however, further work is required in this direction. Furthermore, studies are required to evaluate the herbicidal potential of *Artemisia* essential oil under field conditions and determine the effect on non-target species.

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