

Phytotoxicity of Major Constituents of the Volatile Oil from Leaves of *Artemisia scoparia* Waldst. & Kit.

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The phytotoxicity of the three major monoterpene constituents of the essential oil from leaves of *Artemisia scoparia* Waldst. & Kit. (redstem wormwood) was investigated. GC/GC-MS analysis revealed that the essential oil (yield 0.84%) is a complex mixture containing 19 monoterpenes, 7 sesquiterpenes and 15 other compounds – aliphatic alcohols, ketones, aromatic hydrocarbons and esters. The three major monoterpenes were β -myrcene (30.2%), *p*-cymene (12.8%) and *dl*-limonene (12.4%). The essential oil and the three monoterpenes exhibited phytotoxicity and reduced germination, seedling growth, chlorophyll content and percent respiration of *Avena sativa* and *Triticum aestivum* in a dose-response manner. The inhibitory effect of monoterpenes was comparatively smaller than of the crude essential oil and β -myrcene was most toxic followed by *p*-cymene, whereas limonene was least toxic. The study suggests that *A. scoparia* oil and β -myrcene can be explored for phytotoxicity against weeds.

Key words: *Artemisia scoparia*, β -Myrcene, Phytotoxicity

Introduction

Artemisia (commonly wormwoods; family Asteraceae) is a diverse genus with over 200 species of perennial herbs and shrubs distributed in various parts of the world (Anonymous, 1993). It is known for the presence of volatile oils that are extensively used in medicines and as antimicrobial agent (Anonymous, 1993). Additionally, a few of the *Artemisia* species inhibit the germination and growth of other plants through their volatile oils/terpenes and are responsible for the characteristic patterning of vegetation. These include *A. californica* (Muller *et al.*, 1964), *A. tridentata* (Weaver and Klarich, 1977), *A. princeps* var. *orientalis* (Yun *et al.*, 1993), and *A. vulgaris* (Barney *et al.*, 2005). However, not all species of *Artemisia* have been explored for their allelopathic potential, though they contain essential oil and possess a characteristic aroma. *Artemisia scoparia* (redstem wormwood) is one such species that grows extensively in India, particularly in the lower parts of western Himalayas and Gangetic plains, and forms monospecific strands. It has a dark reddish stem with numerous small leaves and grows up to ~1.5 m in

height. All the above ground parts of *A. scoparia* are aromatic and contain volatile essential oil that possesses medicinal, antifungal and antimicrobial properties (Anonymous, 1993). However, very little is known about the role of the essential oil in imparting allelopathic nature to the plant and thus regulating the vegetation in its vicinity. In the present study, the allelopathic/phytotoxic properties of the volatile essential oil from above ground parts of *A. scoparia* was investigated to identify the bioactive constituents of its oil. To explore the phytotoxicity of *A. scoparia* essential oil we selected 2 plants – *Avena sativa* (oat) and *Triticum aestivum* (wheat).

Materials and Methods

Collection of plant material and extraction of the essential oil

Green leaves were collected from nearly 5-week-old plants of redstem wormwood (*Artemisia scoparia* Waldst. & Kit.) growing in wild strands around the campus of Panjab University, Chandigarh, India in May 2007. They were used for oil extraction through steam-distillation in a Clev-

ger's apparatus. Green leaves (~200 g) were mixed with 750 mL of distilled water in a 2 L round bottom flask fitted with a condenser and heated for 3 h. The oil was collected from the nozzle of the apparatus and dried over sodium sulfate and stored at 4 °C till further use.

GC/GC-MS analysis of the essential oil

GC was done on a Shimadzu gas chromatograph (model GC-14B) fitted with a FID (flame ionization detector), Supelco wax column (60 m × 0.25 mm i. d., film thickness 0.25 µm) and using N₂ as carrier gas. The oven temperature was initially held isothermally at 40 °C for 4 min, then increased to 220 °C at the rate of 4 °C/min and finally held for 5 min. Relative amounts of different chemical components were determined by computer-based peak area normalization without any correction factor.

GC-MS was performed on a Perkin-Elmer mass spectrophotometer (Model Q Mass 910) equipped with a fused silica (BP 21) capillary column (30 m × 0.25 mm i. d., film thickness 0.25 µm) and using He as carrier gas. The oven temperature was initially held isothermally at 40 °C for 7 min, then increased to 190 °C at the rate of 5 °C/min and finally held for 20 min. Injector and detector temperature were 230 °C and 250 °C, respectively.

The constituents were identified based on computer matching of mass spectra using the library search system HP-5872 (Hewlett-Packard), consulting data bases (Wiley 275 and NBS 75K), libraries, and NIST 98 (McLafferty, 1988; Stein, 1990), and compilation by Adams (1995).

Phytotoxicity of the essential oil and its major constituents

To assess the role of the essential oil in regulating the vegetation in the vicinity of *A. scoparia*, the phytotoxicity of the essential oil and its major monoterpenes (β -myrcene, *dl*-limonene and *p*-cymene) was studied against *Avena sativa* (oat) and *Triticum aestivum* (wheat). 20 Seeds (pre-imbibed for 16 h in distilled water) of each test plant were placed in 15 cm diameter Petri dishes each lined with a single layer Whatman no. 1 filter circle moistened with 7 ml of *Artemisia* essential oil or either of the three monoterpenes or distilled water (as control). Three concentrations – 0.14, 0.35 and 0.70 mg/mL – of the essential oil and the major monoterpenes were used. In total, there were 13

treatments including control. There were five Petri dish replicates for each treatment including control. We used the oil and monoterpenes as mg/mL rather than mM, since the molecular weight of the essential oil was unknown. All the Petri dishes were maintained in a growth chamber set at (23 ± 2)/(18 ± 2) °C day/night temperature, a 14 h photoperiod (06.00 AM to 08.00 PM) of ~260 µmol m⁻² s⁻¹ photon flux density and a relative humidity of ~75%. After 7 d, percent germination and seedling length were measured. The leaves were removed and analyzed for chlorophyll content and respiratory activity. Chlorophyll was extracted with dimethyl sulfoxide and analyzed spectrophotometrically (Hiscox and Israelstam, 1979) and calculated using the equation of Arnon (1949). The respiratory activity was determined using 2,3,5-triphenyl tetrazolium chloride according to Steponkus and Lanphear (1967).

The observed data were analyzed by one-way ANOVA and their significance in response to the essential oil or the major monoterpenes was determined over control by *post hoc* Dunnett's test.

Results and Discussion

GC and GC-MS analyses revealed that the essential oil (yield 0.84%) from *Artemisia scoparia* leaves is a complex mixture of 41 constituents (19 monoterpenes, 7 sesquiterpenes and 15 other compounds – aliphatic alcohols, ketones, aromatic hydrocarbons and esters) that are isolated between 4 to 38.4 minutes (data not presented). Among them, the major monoterpenes were β -myrcene (30.2%), *p*-cymene (12.8%) and *dl*-limonene (12.4%) (Table I). This is in sharp contrast to earlier reports showing that *A. scoparia* oil is rich in camphor, 1,8-cineole and β -caryophyllene (Cha *et al.*, 2005), in α - and β -thujone (Farzaneh *et al.*, 2006), and in capillene (Demirci *et al.*, 2005). Studies in the past have demonstrated the antimicrobial and fungicidal activity of *A. scoparia* essential oil (Yun *et al.*, 1993; Cha *et al.*, 2005; Farzaneh *et*

Table I. The three major monoterpene components of the essential oil from leaves of *Artemisia scoparia* as revealed by GC-MS analysis.

Component	Retention time [min]	Relative area (%)	Kovats index
β -Myrcene	4.16	30.2	1161
<i>dl</i> -Limonene	4.82	12.4	1201
<i>p</i> -Cymene	6.44	12.8	1269

al., 2006); however, nothing has been done to explore the phytotoxicity of the oil. It is imperative in view of the increasing reports that volatile oils have a good potential for the use as weed management agent under organic farming (Singh *et al.*, 2003; Batish *et al.*, 2004, 2007). We therefore analyzed the phytoinhibitory activity of *A. scoparia* essential oil and its three major monoterpenes (β -myrcene, *p*-cymene and *dl*-limonene) against *T. aestivum* and *A. sativa*.

The essential oil from leaves of *A. scoparia* significantly (at $p < 0.01$) inhibited the germination, early growth, chlorophyll content and respiration of both the test plants (Table II). A parallel reduction in germination was observed in response to β -myrcene and *p*-cymene (except on wheat), whereas there was no effect for *dl*-limonene. Likewise, there was a significant (at $p < 0.01$) reduction in seedling length, chlorophyll content and percent respiration in both the test plants in response to all the three monoterpenes, though the effect was variable (Table II). In general, the crude essential oil was more phytotoxic than its major components β -myrcene, *p*-cymene and *dl*-limonene, and the inhibitory effect was more on *A. sativa* than on *T. aestivum*. Among the monoterpenes, β -myrcene was most potent followed by *p*-cymene and *dl*-limonene (Table II). Earlier studies have shown that volatile essential oil from aromatic plants (herbs, shrubs and trees) reduces the germination and growth of other plants (Muller *et al.*, 1964; Batish *et al.*, 2006). The growth retardatory effect of essential oil of *A. scoparia* and its major monoterpene components parallels the observed scattered vegetation around *A. scoparia*. Further, the study bears significance in view that essential oils are being explored for their weed/pest suppressing properties (Singh *et al.*, 2003). The reduction in chlorophyll content in response to *A. scoparia* essential oil and its major monoterpenes is in agreement with earlier reports indicating a negative effect of essential oils and monoterpenes (Abraham *et al.*, 2000; Singh *et al.*, 2002a, b, 2005; Batish *et al.*, 2004). Earlier, Weaver and Klarich (1977) have documented that volatile monoterpenes from *A. tridentata* reduce the respiration in young and juvenile wheat plants.

The present study concludes that *A. scoparia* essential oil and its three major monoterpenes (β -myrcene, *p*-cymene and *dl*-limonene) reduce germination, growth, chlorophyll content and respiration of target species – *T. aestivum* and *A. sativa*.

Table II. Effect of *Artemisia scoparia* essential oil and its major components β -myrcene, *dl*-limonene and *p*-cymene on germination, seedling length, total chlorophyll content and percent respiration of *Avena sativa* and *Triticum aestivum*. Data are presented as mean \pm SE.

Conc. [mg/mL]	Germination (%)		Seedling length [cm]		Chlorophyll content [μ g/mg tissue]		Percent respiration	
	<i>A. sativa</i>	<i>T. aestivum</i>	<i>A. sativa</i>	<i>T. aestivum</i>	<i>A. sativa</i>	<i>T. aestivum</i>	<i>A. sativa</i>	<i>T. aestivum</i>
0 (Control)	100 \pm 0	100 \pm 0	18.6 \pm 0.25	18.3 \pm 0.60	13.2 \pm 0.46	12.5 \pm 0.17	100 \pm 0	100 \pm 0
<i>A. scoparia</i> oil								
0.14	60.0 \pm 1.30 b	84.7 \pm 1.73 b	10.0 \pm 0.38 b	14.0 \pm 0.29 b	6.6 \pm 0.34 b	6.1 \pm 0.37 b	62.6 \pm 1.02 b	77.6 \pm 1.12 b
0.35	41.7 \pm 2.10 b	63.2 \pm 2.12 b	7.6 \pm 0.21 b	12.8 \pm 0.19 b	3.7 \pm 0.12 b	3.5 \pm 0.29 b	51.9 \pm 1.84 b	63.7 \pm 1.34 b
0.70	25.0 \pm 2.30 b	41.7 \pm 2.90 b	2.6 \pm 0.10 b	7.6 \pm 0.16 b	0.8 \pm 0.07 b	2.0 \pm 0.14 b	21.6 \pm 1.38 b	61.4 \pm 0.45 b
β -Myrcene								
0.14	82.7 \pm 1.73 b	94.7 \pm 2.13 a	11.8 \pm 0.24 b	15.1 \pm 0.24 a	7.1 \pm 0.29 b	6.5 \pm 0.26 b	62.2 \pm 2.1 b	78.4 \pm 1.46 b
0.35	61.7 \pm 2.12 b	86.1 \pm 1.85 b	8.2 \pm 0.19 b	13.9 \pm 0.37 b	3.9 \pm 0.17 b	4.1 \pm 0.17 b	46.1 \pm 1.02 b	75.4 \pm 1.05 b
0.70	43.7 \pm 2.90 b	61.7 \pm 2.30 b	4.7 \pm 0.31 b	11.0 \pm 0.38 b	0.6 \pm 0.06 b	2.2 \pm 0.09 b	17.7 \pm 1.87 b	66.9 \pm 1.20 b
<i>dl</i> -Limonene								
0.14	100 \pm 0	100 \pm 0	15.6 \pm 0.78 a	16.9 \pm 0.70	10.8 \pm 0.47 b	10.1 \pm 0.32 b	89.2 \pm 2.4 a	93.4 \pm 2.4 a
0.35	100 \pm 0	100 \pm 0	12.4 \pm 0.25 b	14.7 \pm 0.29 b	8.7 \pm 0.37 b	7.9 \pm 0.19 b	73.2 \pm 2.1 b	81.2 \pm 3.1 b
0.70	100 \pm 0	100 \pm 0	8.7 \pm 0.37 b	12.9 \pm 0.41 b	3.4 \pm 0.22 b	3.5 \pm 0.24 b	46.8 \pm 1.9 b	67.9 \pm 2.48 b
<i>p</i> -Cymene								
0.14	90.4 \pm 2.06 a	100 \pm 0	12.1 \pm 0.67 b	10.4 \pm 0.19 b	11.0 \pm 0.38 b	8.1 \pm 0.16 b	78.5 \pm 3.17 b	80.2 \pm 4.36 b
0.35	81.3 \pm 1.82 b	100 \pm 0	8.9 \pm 0.75 b	6.3 \pm 0.23 b	3.9 \pm 0.11 b	6.8 \pm 0.21 b	47.4 \pm 2.45 b	62.8 \pm 3.78 b
0.70	72.8 \pm 1.39 b	100 \pm 0	5.0 \pm 0.84 b	2.5 \pm 0.17 b	0.8 \pm 0.08 b	2.4 \pm 0.16 b	30.9 \pm 3.02 b	49.4 \pm 5.29 b

Within a column for a particular treatment, a and b represent significant difference from respective control at $p < 0.05$ and 0.01 applying *post hoc* Dunnett's test.

However, the inhibitory effect of the monoterpenes was comparatively lesser than that of the crude essential oil, and among them β -myrcene was most toxic followed by *p*-cymene, whereas limonene was least toxic. The phytotoxicity of the oil and β -myrcene qualify them to be explored for possible phytotoxicity against a wide range of weeds.

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