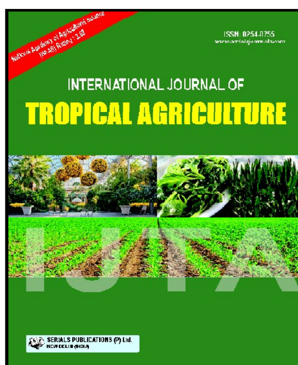

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Phytotoxicity of Volatile Oil of *Artemisia Scoparia* Waldst. and Kit. on Early Growth of two Weeds and Associated Biochemical Changes

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Abstract: We explored the phytoxic effects of volatile oil of *Artemisia scoparia* Waldst. and Kit against two weeds namely *Bidens pilosa* and *Amaranthus viridis*. *Artemisia* oil (1, 2.5, 5 µl) caused a significant reduction in seed germination and early growth (in terms of radicle and plumule length and seedling dry weight) in both the test weeds. In general, a dose dependent effect was observed and growth declined with increase in concentration of oil. We also investigated alterations in contents of proteins and carbohydrates and the specific activity of their hydrolytic enzymes. *Artemisia* oil induced reduction in contents of biomolecules, while the activities of associated hydrolytic enzymes was enhanced. We conclude that volatile oil of *Artemisia scoparia* is phytotoxic towards growth of *B. pilosa* and *A. viridis* and impairs protein and sugar metabolism.

Key words: *Artemisia scoparia*, phytotoxicity, volatile oil, growth inhibition, biochemical changes.

INTRODUCTION

Artemisia scoparia Waldst. and Kit., commonly known as red stem wormwood (Asteraceae) is an annual aromatic plant, well-known for its volatile oils which are extensively used as antimicrobial agent and in pharmaceuticals [1]. Under natural conditions, *A. scoparia* forms thick monospecific strands under diverse habitats such as along the canals, roadsides, uncultivated lands, and even agricultural fields *etc*. In India, the plant is found growing extensively in the states of Punjab, Haryana, Chandigarh, lower parts of Western Himalayas and upper gangetic plains [2].

Few studies show that *A. scoparia* supress the growth of native vegetation due to emanation of volatile oils from its above ground parts thereby, affecting vegetation patterning in an ecosystem. Singh *et al.* [3] reported that volatile oil of *A. scoparia* and its three major constituent monoterpenes *â*-myrcene, *p*-cymene and *dl*-limonene exhibit phytotoxicity and reduced the germination, early growth, chlorophyll content and cellular respiration of *Avena sativa* and *Triticum aestivum* in a dose response manner. A spray treatment of *Artemisia* oil (2, 4, 6%; v/v) has been reported to show strong phytotoxicity against six-week old five weed species *viz. Achyranthes aspera*, *Ageratum conyzoides*, *Echinochloa crus-galli*, *Parthenium hysterophorus* and *Cassia occidentalis* [4]. These studies clearly suggest that volatile oil of *A. scoparia* has a potential to suppress weed growth and may be used as a novel bioherbicide.

A preliminary study revealed that *Artemisia* oil and *â*-myrcene adversely affect the growth of weeds by generation of reactive oxygen species and induction of oxidative stress as indicated by high level of lipid peroxidation, loss of membrane integrity, and high amount of H_2O_2 and conjugated dienes in some weeds namely *Avena fatua* and *Phalaris minor* and *Cyperus rotundus* [5]. However, the exact mechanism of action of *Artemisia* oil is largely unknown. Therefore, keeping in mind that understanding the mechanism of action of oil may help in utilizing the oil in a better and convenient way, a study was performed to investigate the possible mechanism of action of volatile oil of *A. scoparia*. We studied the effect of its oil on growth of two weed species *i.e. Amaranthus viridis* and *Bidens pilosa* along with its effect on biomolecules and activities of associated enzymes.

MATERIALS AND METHODS

Extraction of oil: Volatile oil was extracted from freshly collected leaves of *A. scoparia* growing in the wastelands around Chandigarh, by hydro-distillation using Clevenger's apparatus. Chopped leaves (250 g) were mixed with distilled water in a round bottom flask and boiled for 3 h. The clear yellow oil (0.17% yield; v/w on fresh weight basis) was collected from the nozzle of the condenser, dried under sodium sulfate, and stored at 4°C for identification and bioassay.

Identification of Oil

The oil was analysed by gas chromatography (GC; Shimadzu GC-17A) and gas chromatography–mass spectroscopy (GC-MS) [5]. The gas chromatograph was equipped with a flame ionization detector (FID) and DB-5 column (60 m×0.25 mm, film thickness 0.25 ìm) and helium (He) as carrier gas. The amount of different constituents was determined by peak area and compared with data from GC-MS (Shimadzu QP 2010); equipped with fused silica (SGE BP 20) capillary column (30 m×0.25 mm, i.d., 25 µm film thickness). The identification of different constituents of oil was based on:

1. the comparison of their retention times with those of pure reference samples from Sigma-Aldrich (St. Louis, MO, USA), Fluka (Buchs, Switzerland), Acros (Geel, Belgium), AlfaAeser (Ward Hill, MA, USA), and TCI (Tokyo, Japan);
2. co-elution with available authentic standards;
3. comparison of retention indices (RI) with reference to a homologous series of n-alkanes (C7-C30; Supelco, Bellefonte, PA, USA); and (4) computer matching of mass spectra by using library search system HP-5872 (Hewlett-Packard) and consulting data bases of Wiley 275 and NBS 75K Libraries [5].

Oil Treatment

The seeds of *B. pilosa* and *A. viridis* were collected locally from open wastelands. The seeds were cleaned, surface sterilized (0.1% sodium hypochlorite), washed, dried and stored at room temperature. The

seeds of the test weeds were imbibed overnight and equidistantly placed in Petri dishes on Whatman filter paper moistened with 7 ml distilled water under laboratory conditions. These were exposed to 1, 2.5 and 5 µl of *Artemisia* oil on inner side of Petri dish lids followed by their immediate sealing using parafilm and cello tape so as to avoid vaporization of oil. For each treatment and weed, five replicates were maintained in a randomized block design. The Petri dishes were maintained in a growth chamber set at 16/8 h light/dark period, 25 ± 2°C and 78 ± 2% relative humidity. After a week, the number of seeds germinated was counted and radicle and plumule length of seedlings were measured. These were kept in oven at 60°C for 48 h and their dry weight was determined. The fresh radicles were harvested (~5 cm) and stored at – 20°C until used for further biochemical analysis.

Biochemical Analysis

In brief, the radicles were homogenized in distilled water, centrifuged at 15,000 g for 15 min and the supernatant (radicle extract) collected was stored at 4°C. The radicle extract was used for estimations of total protein content using Folin-Ciocalteu reagent against bovine serum albumin as a standard [6] and total carbohydrates as per Loewus [7] using anthrone reagent (0.2%, w/v, in concentrated sulphuric acid); and expressed as mg g^{-1} fresh weight. The specific activities of hydrolytic enzymes - and *b*-amylases were determined by using starch as a substrate [8] and [9], respectively, and expressed µg min^{-1} mg^{-1} protein. The specific activity of proteases was estimated using casein (1%, *w/v*, in 0.1 M PO_4^{3-} buffer; pH 7.0) as a substrate and tyrosine (50 µg ml^{-1}) as a standard [10] and expressed as µg $h^{-1}mg^{-1}$ protein.

Data Analysis

All the experiments were performed in a randomized block design with at least five replicates. The data are presented as mean ± standard error (S.E.) and analysed by one-way ANOVA followed by the comparison of mean values using *post-hoc* Tukey's test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Oil Composition

GC-MS analysis revealed ~40 components, constituting 99.86% of *Artemisia* oil. Among these, maximum content was composed of twenty monoterpenes (67.2%) of which *b*-myrcene (30%) was the major component followed by *p*-cymene (12.73%). The sesquiterpenes (total 6 in number) constituted 2.61% of total oil content. Other aromatic and aliphatic compounds constituted ~29.01%. Four components accounting ~1.04% remained unidentified (Figure 1). Our studies are in sharp contrast with the earlier studies supporting *b*-myrcene as major compound of the volatile oil of *A. scoparia* [5].

However, few other studies reported thujone, camphor and 1,8-cineole [11] and *p*-cymene [12] as major components. These variations may be attributed to variation in geographical regions, season of collection of leaves, age/growth stage of the plant or other abiotic factors [13].

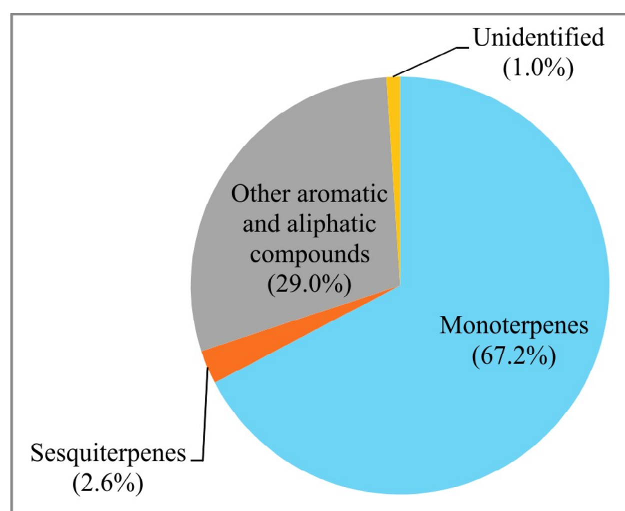

Figure 1: Composition of volatile oil of *Artemisia scoparia*.

Phytotoxic Effect of Volatile Oil on Weeds

The seed germination of both the weeds was adversely affected in response to different treatments of the volatile oil. At 1 µl, though there was insignificant change in seed germination of both the test weeds, however, upon exposure to higher (2.5, 5 µl) treatment, ~21–39% and 29–43% inhibition in the seed germination was observed over control in *B. pilosa* and *A. viridis*, respectively (Figure 2a). Likewise, compared to control, radicle and plumule length of both the weeds were reduced in oil exposed seedlings. With the treatment of 1–5 µl *Artemisia* oil, ~ 4–49% and 18–62% reduction was observed in radicle length of *B. pilosa* and *A. viridis*, respectively (Figure 2b). On the other hand, with exposure to different concentrations of oil, plumule length declined by 4–36% (*B. pilosa*) and 10–46% (*A. viridis*), over control (Figure 2c). Further, oil treatment caused a significant reduction (6–30% in *B. pilosa* and 8–41% in *A. viridis*) in seedling dry weight of both the test weeds (Figure 2d).

In general, more reduction was observed in radicles compared to plumule and early growth of *A. viridis* was more affected compared to *B. pilosa*. Several studies have documented growth inhibitory effects of volatile oils and their constituent monoterpenes of aromatic plants including *Artemisia* sp. [14, 13, 5, 15, 16]. Under natural conditions, oil released from aromatic plant enter the soil and suppress the vegetation in vicinity. Phytotoxic effects of volatile oil have been implicated in vegetation patterning of an ecosystem and successful colonization of a number of invasive weeds [14, 17]. Although few reports indicate that volatile oil and monoterpenes affect cell division in apical meristem and cause oxidative stress [12], however, the exact mechanism of action of *Artemisia* volatile oil remains largely unknown. Therefore, a series of experiments were designed to investigate the mechanism of action of *Artemisia* volatile oil on weeds.

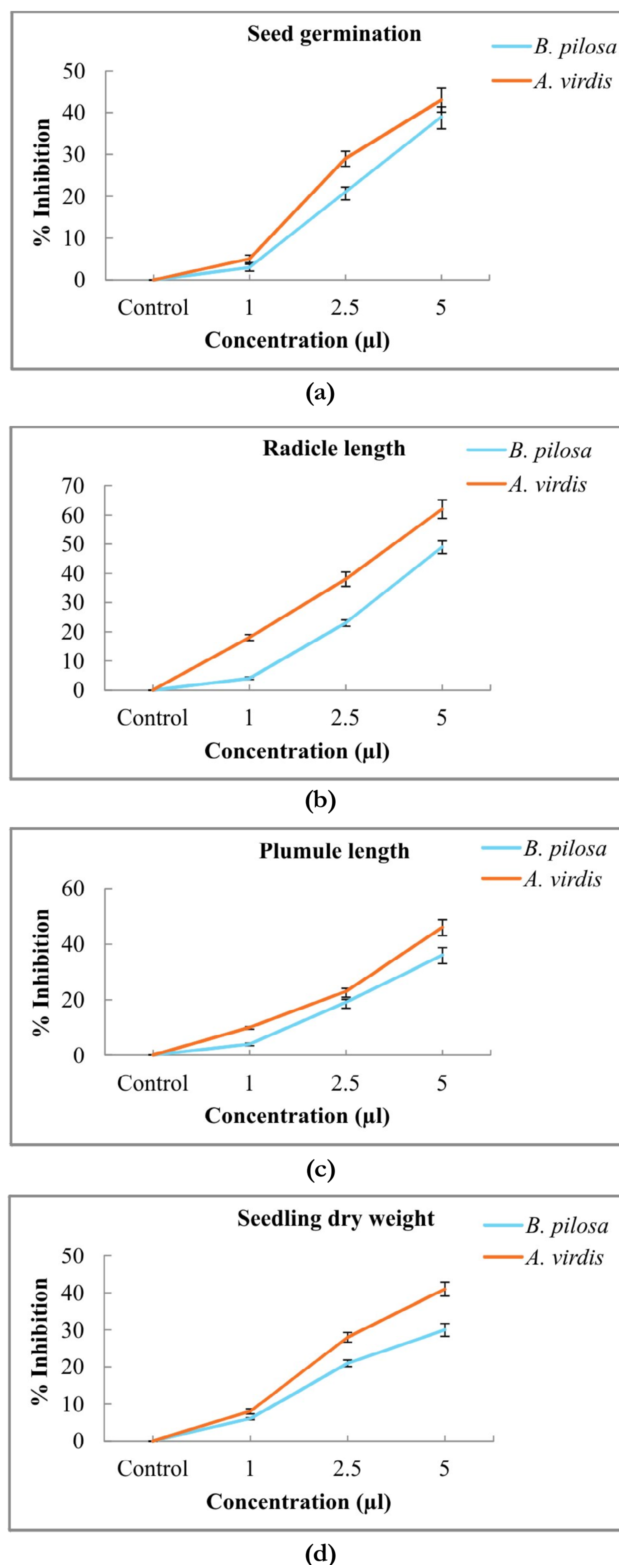

Figure 2: Effect of *Artemisia* oil on (a) seed germination (b) radicle length (c) plumule length (d) seedling dry weight of test weeds.

Bar lines along the lines represent the standard error.

Biochemical Analysis

Table 1
Effect of volatile oil of *A. scoparia* on contents of total proteins and carbohydrates in radicles of test weeds.

	Total proteins ($mg\ g^{-1}$ fresh weight)		*Total carbohydrates ($mg\ g^{-1}$ fresh weight)*	
Conc. (μl)	*B. pilosa*	*A. viridis*	*B. pilosa*	*A. viridis*
Control (0)	23.4±2.8a	11.2±1.4a	15.8±2.2a	9.7±1.6a
1	15.8±2.2b	12.2±1.1a	14.3±1.8a	10.5±1.2a
2.5	12.1±1.9c	8.4±0.9b	10.2±1.3b	7.3±0.8b
5	10.5±1.1c	5.2±0.7c	9.4±1.0b	5.2±0.6c

Values are mean ± SE.

Different alphabets in a column represent significant difference at $P \leq 0.05$ applying Tukey's test.

Total protein content in *B. pilosa* significantly decreased with increase in concentration of oil. With the exposure of 1–5 μl oil, it reduced in the range of 32–55% over control (Table 1). In *A. viridis*, compared to control, slight increase (insignificant at $P \leq 0.05$) was observed at concentration of 1 μl oil. However, at 2.5 and 5 μl concentration, total protein content reduced by 25 and 54%, respectively (Table 1). Likewise, volatile oil treated radicles of test weeds exhibited a significant reduction in the total carbohydrate content over control. An inhibition of ~10 –41% was observed in *B. pilosa* exposed to 1–5 μl oil. On the other hand, in *A. viridis*, a significant reduction (25–46% over control) in the total carbohydrate content was observed at higher concentrations (2.5 and 5 μl) (Table 1).

Table 2 clearly indicates a significant increase in specific activities of hydrolytic enzymes. With the treatment of 1–5 μl oil, activity of *á*-amylases enhanced by 1.3–5.5 and 1.7–4.8 times over control in *B. pilosa* and *A. viridis*, respectively. Likewise, activity of *â*-amylases was more in oil treated radicles compared to the control. In *B. pilosa* and *A. viridis*, its activity increased by ~1.8–6.7 and 1.6–3.8 times with 1, 2.5 and 5 μl *Artemisia* oil, respectively. Further, oil treated test weeds also exhibited a significant increase in proteases activity. It was ~1.5–3.0 and 1.6–2.7 times more in 1–5 μl exposed *B. pilosa* and *A. viridis*, respectively (Table 2).

These observations suggest that *Artemisia* oil reduced the contents of total proteins and carbohydrates while enhanced the activities of hydrolytic enzymes. *á*-amylase is a key enzyme that hydrolyses alpha bonds of large polysaccharides into maltose and glucose [18]. Another key enzyme of carbohydrate metabolism is *â*-amylase which successively removes maltose units from the non-reducing end of *á*-1,4-glucan, thus catalysing the complete conversion of starch to maltose. Proteases are the key enzymes involved in hydrolysis of proteins. Compared to control, activity of these

Table 2
Effect of volatile oil of *A. scoparia* on specific activities of hydrolytic enzymes in radicles of test weeds.

	α-amylases ($mg\ min^{-1}\ mg^{-1}$ protein)		*β-amylases ($mg\ min^{-1}\ mg^{-1}$ protein)*		*Proteases ($\mu g\ h^{-1}\ mg^{-1}$ protein)*	
Conc. (ml)	*B. pilosa*	*A. viridis*	*B. pilosa*	*A. viridis*	*B. pilosa*	*A. viridis*
Control (0)	10.4±0.9a	7.4±0.7a	23.4±2.3a	16.3±0.8a	142.5±3.4a	116.7±2.8a
1	13.4±1.0b	12.5±1.1b	42.2±3.6b	26.1±1.6b	215.6±4.2b	182.8±3.6b
2.5	28.9±2.2c	20.2±1.8c	78.4±4.8c	47.3±2.5c	318.8±4.5c	284.7±4.1c
5	56.8±4.6d	35.4±2.7d	156.2±5.7d	62.5±3.7d	423.2±5.4d	318.1±4.8d

Values are mean ± SE.

Different alphabets in a column represent significant difference at $P \leq 0.05$ applying Tukey's test.

hydrolytic enzymes was more in oil treated radicles. This clearly suggests that volatile oil of *Artemisia* impair the synthesis / accumulation of these macromolecules. This may be attributed to either decline in synthesis of these molecules or enhanced activity of hydrolytic enzymes, more breakdown and hence their lesser accumulation in the oil-treated radicles. These observations are parallel to the earlier studies reporting that allelochemicals adversely affect these molecules and inhibit early growth of plants [19]. Thus, the study concludes that volatile oil of *A. scoparia* is phytotoxic in nature. It adversely affects seed germination and early growth of plants through impairment of protein and sugar metabolism. However, whether this inhibition in response to oil causes alteration of other enzymes involved in defense mechanism in plant roots remains to be explored.

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