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## Original Article

# Allelopathic Effect of *Artemisia herba-alba* Asso. Essential Oil on Seed Germination of *Agropyron desertorum* and *Agropyron cristatum*

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## Abstract

This study was conducted to investigate allelopathic effect of the essential oil of *Artemisia herba-alba* Asso., on seed germination characteristics of *Agropyron desertorum* and *Agropyron cristatum* in a laboratory experiment. Essential oil was extracted from the aerial parts of *Artemisia herba-alba* using a Clevenger-Type apparatus. The volatile chemical compositions of *Artemesia herba-alba* were determined with gas chromatography–mass spectrometry (GC–MS). The crude essential oil was diluted with ethanol to a final concentration of 100 ppm, 500 ppm, 1000 ppm, 1500 ppm and 2000 ppm. Seed germination test was carried out on filter paper moistened with 5 ml of different dilutions of essential oil or distilled water as control. The results showed that increase in essential oil concentration reduced root lengths, seed germination percentage and vigor index of *Agropyron desertorum* and *Agropyron cristatum.*

**Key words:** Allelopathy, Essential oil, *Agropyron desertorum,* Seed germination, Chemical composition, Vigor Index

## Introduction

*Artemisia herba-alba* Asso., commonly known as "desert worm wood", is a perennial dwarf shrub and is a prominent plant of the Irano- Turanian steppe. The genus Artemisia belongs to the tribe Anthemideae which is one of the largest in the Asteraceae family and contains more than 300 species of small herbs and shrubs [1]. *Artemisia herba-alba* Asso., grows in arid and semi-arid climates with 100-230 mm of annual precipitations in Iran. *Artemisia herba-alba* Asso., as a medical and aromatic plant that is known by the colloquial names of " dermaneh". The effects of allelochemicals may play an important role, indirectly, in determining chenopod community structure in the arid and semi-arid zones of Western Australia [2]. Essential oil of *A. herba-alba* significantly reduced the seed germination in the grasses species [3]. Root biomass of Parthenium plants was significantly suppressed by 50 and 100% extracts of both the test allelopathic extracts [4], both concentrations of sorghum extracts significantly reduced shoot biomass, but sunflower extract was effective only at the lower concentration. In vegetative growth stage the oils of *Artemisia herba-alba* were more complicated in comparison with flowering stage's oils [5], 32 compounds in the vegetative stage's oils and 28 compounds in the flowering stage's oils could be identified.

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The objective of this study was to evaluate the effect of the essential oil of *Artemisia herba-alba* Asso., on seed germination characteristics of *Agropyron desertorum* and *Agropyron cristatum* under laboratory conditions.

## Materials and Methods

Plant materials

Aerial parts of *Artemisia herba-alba* Asso., was collected from the National Park of Golestan , Iran, in May 2008. The climate was semi-arid with an average annual temperature of 18 ˚C. National Park of Golestan is located at 36˚ 50′N, 54˚ 26′E. Aerial parts were shade dried for 3 days at room temperature.

Extraction of the essential oil

Dried material was powdered in a Thomas-Wiley laboratory Mill. Essential oil was extracted from the aerial parts of *Artemisia herba-alba* using a Clevenger –Type apparatus. The oils were stored in dark glass bottles in a refrigerator until they were used. The crude essential oil was diluted with ethanol to a final concentration of 100 ppm, 500 ppm, 1000 ppm, 1500 ppm and 2000 ppm. GC and GC/MS analysis : Essential oil components were quantified using a Euro Chrom 2000 from KNAUER chromatograph fitted with a DB-5 capillary column (10 m×0.1 mm, film thickness 0.4 lm) using helium as carrier gas at a flow of 0.9 ml min-1, the injection port and flame ionisation detector at 250 °C and a temperature programmed from 60–285 °C at a rate of 3 °C/min. Quantification was performed using an external standard calibration curve obtained with standard solutions of pure ocimenones. To identify the components of the essential oil, GC–MS studies were performed using a Varian-3400 gas chromatograph coupled to a Saturn II ion trap detector. The column was the same as the GC under the same conditions as stated above. Component identification was based on matching mass spectral fragmentation and retention with commercial MS libraries or with the literature data.
To evaluate the allelopathic activity of different concentrations of *Artemisia herba-alba* essential oil, 50 seeds of *A. desertorum* and *A. cristatum* were placed separately in Petri dishes, which contained two layers of Whatman filter paper moistened with 5ml of mentioned dilutions of essential oil and distilled water as control. The Petri dishes were incubated at 16h light photoperiod and at temperature of 20 ˚C.

Growth measurements

Germination was monitored every day for 13 days, and the seeds of *A. desertorum* and *A. cristatum* were counted when they exhibited a radical extensions of ≥2mm [6]. Root and shoot lengths of the seedlings were measured with a ruler and recorded for the *A. desertorum* and *A. cristatum*. Germination speed was obtained by the following formula [7]: $GS = \sum_{i=1}^{n} \left[ \frac{n}{t} \right]$

Where GS=germination speed, n= number of germinated seeds in the time t, t= number of days from test start time.
Vigor index was measured by the following equation [8]: $VI = (RL+SL) \times GP$
Where VI= vigor index, RL= root length (cm), SL= shoot length (cm), GP=germination percentage.

Statistical analysis

The data were submitted to analysis of variance through the F-test and the mean values were compared by multiple Duncan's test at the probability level of 0.05. Statistical analysis was done with SPSS 11. For windows statistical software package.

## Results

Essential oil of *Artemisia herba-alba* at different concentrations significantly affected the seed germination characteristics of *A. desertorum* and *A. cristatum.* Results showed that the essential oil of *Artemisia herba-alba* significantly decreased seed germination value of *A. desertorum* and *A. cristatum* (Table 1). The lowest germination speed of *A. desertorum* and *A. cristatum* were observed in 2000 ppm treatment (Table 2). The root and shoot lengths of *A. desertorum* and *A. cristatum* decreased significantly with the increase in concentration of the essential oil of *Artemisia herba-alba* (Table 3 and Table 4). The seedling vigor index of the *A. desertorum* significantly decreased as the concentration of essential oil increased (Table 5).

Essential oil composition

A composition of the essential oil isolated from the aerial parts of *A. herba-alba* is shown in Table 4.

**Table 1** Effect of essential oil of *Artemisia herba-alba* at different concentration on the seed germination of *A. desertorum* and *A. cristatum*.

| Essential oil concentrations (ppm) | Germination value (%) | |
|---|---|---|
| | *A. desertorum* | *A. cristatum* |
| 0 (control) | 47 [a] | 47[a] |
| 100 | 25[b] | 22 [b] |
| 500 | 16 [dc] | 15 [dc] |
| 1000 | 10 [d] | 10 [d] |
| 1500 | 5.4 [e] | 5.2 [e] |
| 2000 | 1.4 [f] | 1.2 [f] |
| | *F-value* | |
| Species (A) | 0.01[ns] | |
| Essential oil concentration (B) | 22.9[*] | |
| A×B | 0.51[*] | |

Different letters in table show significant differences based on Duncan's test at P< 0.05.
* Significant at 0.05 levels, [ns] non-significant at 0.05 levels

**Table 2** Effect of essential oil of *Artemisia herba-alba* at different concentration on the germination speed of *A. desertorum* and *A. cristatum*

| Essential oil concentrations(ppm) | Speed germination (day) | |
|---|---|---|
| | *A. desertorum* | *A. cristatum* |
| 0 (control) | 5.4 [a] | 47[a] |
| 100 | 25[b] | 22 [b] |
| 500 | 16 [dc] | 15 [dc] |
| 1000 | 10 [d] | 10 [d] |
| 1500 | 5.4 [e] | 5.2 [e] |
| 2000 | 1.4 [f] | 1.2 [f] |
| | *F-value* | |
| Species (A) | 0.01[ns] | |
| Essential oil concentration (B) | 22.9[*] | |
| A×B | 0.51[*] | |

Different letters in table show significant differences based on Duncan's test at P< 0.05.
* Significant at 0.05 levels, [ns] non-significant at 0.05 levels

**Table 3** Effect of essential oil of *Artemisia herba-alba* at different concentration on the shoot length of *A. desertorum* and *A. cristatum*

| Essential oil concentrations(ppm) | shoot length (cm) | |
|---|---|---|
| | *A. desertorum* | *A. cristatum* |
| 0 (control) | 6.8 [a] | 6.6[a] |
| 100 | 5.2[b] | 5.0 [b] |
| 500 | 3.5[c] | 3.3[dc] |
| 1000 | 2.3[d] | 2.3[d] |
| 1500 | 1.0 [ef] | 1.0[ef] |
| 2000 | 0.1[f] | 0.1[f] |
| | *F-value* | |
| Species (A) | 0.8[ns] | |
| Essential oil concentration (B) | 26.91[*] | |
| A×B | 0.66[*] | |

Different letters in table show significant differences based on Duncan's test at P< 0.05.
* Significant at 0.05 levels, [ns] non-significant at 0.05 levels

GC-MS analysis led to the identifications of 31 compounds. The major compounds were cis-pinocarveol (17.5%), artemisia ketone (13%), trans-sabinene hydrate (8.5%), 1,8-cineole (8%) and aromadendrene (4.7%). Oxygenated monoterpenes constituted the major fraction of the essential oil in *Artemisia herba-alba*.

## Discussion

*Artemisia herba-alba* showed an allelopathic activity on seed germination of *A. desertorum* and *A. cristatum.*. Several enzymes like proteases, lipases and Alfa-amylases play an important role during seed germination. Many enzymatic functions are inhibited by the presence of allelochemicals [3]. The inhibition on seed germination by allelopathic effect could be confounded with osmotic effects on rate of imbibitions, delayed initiation of germination and cell elongation [9]. The seedling vigor index of the *A. desertorum* and *A. cristatum* species tested were significantly decreased ($p< 0.05$) as the concentration of essential oil increased. Essential oil inhibited the growth of seedling roots of *A. desertorum* and *A. cristatum* at concentrations greater than 100 ppm, and increasing the dose increased the inhibition.

**Table 4** Effect of essential oil of *Artemisia herba-alba* at different concentration on the root length of *A. desertorum* and *A. cristatum*

| Essential oil concentrations(ppm) | Root length (cm) | |
|---|---|---|
| | *A. desertorum* | *A. cristatum* |
| 0 (Control) | 0.58 [a] | 0.56[a] |
| 100 | 0.5 [a] | 0.5[a] |
| 500 | 0.38[ab] | 0.37[ab] |
| 1000 | 0.3[b] | 0.28[b] |
| 1500 | 0.15[c] | 0.16[c] |
| 2000 | 0.05[d] | 0.04[d] |
| | *F-value* | |
| Species (A) | 0.002[ns] | |
| Essential oil concentration (B) | 0.3[*] | |
| A×B | 0.01[*] | |

Different letters in table show significant differences based on Duncan's test at $P< 0.05$.
* Significant at 0.05 levels, [ns] non-significant at 0.05 levels

**Table 5** Effect of essential oil of *Artemisia herba-alba* at different concentration on the vigor index of *A. desertorum* and *A. cristatum*

| Essential oil concentrations(ppm) | Vigor index | |
|---|---|---|
| | *A. desertorum* | *A. cristatum* |
| 0 (control) | 348 [a] | 348[a] |
| 100 | 150 [b] | 150[b] |
| 500 | 52[cd] | 54[cd] |
| 1000 | 48[ef] | 47[ef] |
| 1500 | 9.0[g] | 9.0[g] |
| 2000 | 0.0[h] | 0.0[h] |
| | *F-value* | |
| Species (A) | 3.98[ns] | |
| Essential oil concentration (B) | 173.5[*] | |
| A×B | 3.39[*] | |

Different letters in table show significant differences based on Duncan's test at $P< 0.05$.
* Significant at 0.05 levels, [ns] non-significant at 0.05 levels

**Table 6** Chemical composition of the essential oil of *A. herba-alba*

| No. | KI[a] | Compound | % |
|---|---|---|---|
| 1 | 1176 | *Ccis*-pinocarveol | 17.5 |
| 2 | 1063 | Artemisia ketone | 13 |
| 3 | 1259 | *Trans*-sabinene hydrate | 8.5 |
| 4 | 1044 | 1,8-cineole | 8 |
| 5 | 1444 | Aromadendrene | 4.7 |
| 6 | 1657 | α-eudesmol | 4.6 |
| 7 | 989 | Myrcene | 4.5 |
| 8 | 1216 | *Trans*-carveol | 4.1 |
| 9 | 1151 | p-menth-3-en-8-ol | 2.9 |
| 10 | 1741 | (E,Z)-farnesol | 2.9 |
| 11 | 1160 | Isoborneol | 2.8 |
| 12 | 1900 | N-nonadecane | 2.8 |
| 13 | 1419 | E-caryophyllene | 2.5 |
| 14 | 1582 | Caryophyllene oxide | 2.3 |
| 15 | 1227 | Nor-davanone | 2.1 |
| 16 | 1284 | Isobornyl acetate | 2.1 |
| 17 | 1358 | Neoiso-dihydro carveol acetate | 2 |
| 18 | 1506 | Germacrene A | 1.7 |
| 19 | 2100 | N-heneicosane | 1.7 |
| 20 | 1529 | *Trans*-calamenene | 1.5 |
| 21 | 1570 | Caryophyllene alcohol | 1.1 |
| 22 | 1168 | Borneol | 1 |
| 23 | 1768 | Cedryl acetate | 0.9 |
| 24 | 1126 | Chrysanthenone | 0.7 |
| 25 | 1829 | Isopropyl tetradecanoate | 0.7 |
| 26 | 1095 | *Trans*- sabinene hydrate acetate | 0.6 |
| 27 | 1968 | Unknown | 0.6 |
| 28 | 973 | Sabinene | 0.5 |
| 29 | 2076 | N-octadecanol | 0.5 |
| 30 | 1547 | α-calacorene | 0.4 |
| 31 | 1988 | 1-eicosene | 0.4 |
| 32 | 2000 | N-eicosane | 0.4 |

[a] Calculated retention indices.

Present results reveal that the essential oil was most effective phytotoxic on root elongation than shoot length in *A. desertorum* and *A. cristatum*. This may be due to the direct contact between the root and phytotoxic compounds of the essential oil which may inhibit cell division which is highly active in the meristimatic tissue of the growing root [10]. In presence of the allelochemicals, cell elongation is reduced [11]. Some compounds of essential oils can damage the cell wall [12], coagulate the cytoplasm [13], and degrade lipids and proteins [14]. The free lipids within the cytoplasm could be the target of an oxidative action [15], due to increased lipid peroxidation [16] and to lysis [17].The chemical identification and quantification of compounds contained in plant essential oil is an important part of the process of discovering agents of allelopathy. Plants contain thousands of natural products, but not all are allelopathic [18]. In the genus *Artemisia*, over 260 species have been investigated to reveal that contain many classes of secondary metabolites including terpenoids, flavonoids, coumarins, glycosides, steroids and polyacetylenes [19]. Gholami *et al*., [20] showed that Increment of the essential oil concentrations of Artemisia herba-alba, decreased seedling growth parameters and germination percentage in *Medicago sativa* and *Onobrychis sativa*. Several constituents with biological active are produced in *Artemisia* Chanphen *et al*. [21] and Ahmed *et al* [22], showed that five new monoterpenes and seven new sesquiterpene lactones have allelopathic effects; all these were isolated from the aerial parts of *Artemisia suksdorfii.* In our study, the major allelopathic substances present in the essential oil of *A. herba-alba* were cis-pinocarveol, artemisia ketone, trans-sabinene hydrate, 1,8-cineole, aromadendrene and cis- pinocarveol.

## Conclusions

In general, allelochemicals are considered secondary metabolites, usually present in essential oils, and play a major role in influencing germination and growth on other plants. Compounds as phenols, aldehydes, alcohols, among the other, are recognized for their allelopathic property. In this work, 32 chemotypes are detected, in the essential oils of *A. herba-alba*, with the major constituents cis –pinocarveol and artemisia ketone. The essential oil of *A. herba-alba* clearly shows an allelopathic potential. This study demonstrated that, the essential oil of *Artemisia herba-alba* caused decrease in the germination characteristics of *A. desertorum* and *A. cristatum.*

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