## DIFFERENTIAL ALLELOPATHIC EXPRESSION OF DIFFERENT PLANT PARTS OF ACHILLEA BIEBERSTEINII

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Achillea biebersteinii (Asteraceae) is a perennial medicinal plant and has a wide distributional range in the Mediterranean region. The present study investigated the inhibitory effects of different plant parts of *A. biebersteinii* on germination characteristics and seedling growth of wild barley (*Hordeum spontaneum*). Water extracts were prepared by incubating separately five grams of dried powder of roots, stems, leaves and flowers of *A. biebersteinii* in 100 ml of distilled water for 24 h and distilled water was used as the control. The water extracts from different plant parts of *A. biebersteinii* differed in their effects on the germination and seedling growth of wild barley. Water extracts prepared from leaves and flowers were more suppressive to germination of wild barley than root and stem extracts. The maximum inhibition in radical and plumule growth of germinating caryopses and in root and shoot growth of greenhouse-grown wild barley was recorded for leaf extract followed by flower extract. The lowest Chl a, Chl b and total chlorophyll and protein contents were resulted after exposure to leaf extracts. According to these results, the inhibitory effects of different *A. biebersteinii* plant parts can be arranged in the order: leaf > flower > stem > root.

*Keywords: Achillea biebersteinii* – Allelopathy – chlorophyll pigments – germination – *Hordeum spontaneum* – seedling growth – water extract

### INTRODUCTION

Allelopathy is a common phenomenon in nature and can be defined as the inhibitory or stimulatory effects of one plant species on another one through the release of secondary metabolites (known as allelochemicals) that escape into the environment [35]. These allelochemicals are released into the environment via root exudation, leaching by rains, decomposition of plant materials and volatilization [42]. Allelochemicals are present in almost all plant species and their different parts [43]. Due to their natural origin, allelochemicals are currently applied in organic agricultural practices as a biological tool to control weed instead of using synthetic herbicides [18, 22].

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Several allelochemicals were reported to impose potential phytotoxicity to physiological processes in other plants [8], affecting growth, survival and reproduction [20, 43]. However, allelopathic plants can also be affected negatively by their own allelochemicals, a process known as autointoxication [25]. Allelochemicals produced by plants are reported to interfere with the surrounding environment of other plants which in turn result in alteration in soil biotic factors such as soil microbial activity, or abiotic factors such as soil acidity and fertility, hence playing a significant role in agroecosystems [9, 11, 24].

Besides their use in natural remedies, herbal and medicinal plants are attracting great attention in the community of plant science [2]. Since these plants contain high levels of bioactive phytochemicals, the phenomenon of allelopathy has been reported in several medicinal plants [3, 23, 40].

Achillea biebersteinii (Asteraceae) is one of the most predominant medicinal Achillea species in the Mediterranean region [46]. In folk medicine, A. biebersteinii has been used as a carminatives and in the treatment of intestinal colic and dysentery [5]. Moreover, antimicrobial and anti-inflammatory activities were recorded for this species [6]. For medicinal purposes, essential oils have been purified from A. biebersteinii and were found to be rich in ascaridol, p-cymene, carvenone oxide and camphor [1, 7, 41].

Recently, we have shown that leaf extracts of *A. biebersteinii* impose allelochemical stress on pepper plants [3]. The aim of this study was to further investigate the effects of aqueous extracts from the different parts of *A. biebersteinii* (root, stem, leaf and flower) on seed germination and early growth of wild barley (*Hordeum spontaneum*).

#### MATERIALS AND METHODS

#### Preparation of A. biebersteinii water extracts

Whole mature plants of *A. biebersteinii* were collected at the flowering stage in July, 2012 from natural stands found in Amman, Jordan ( $31^{\circ}57'N$  latitude;  $35^{\circ}65'E$  longitude; 920 m above sea level). Plants roots, stems, leaves and flowers were separated and dried at 45 °C until the weight was constant. Oven-dried tissues were ground to a fine powder by a blender. Five grams of each plant part was separately extracted in 100 ml distilled water (5% extract) for 24 h at room temperature using an orbital shaker set at 120 rpm. The extracts were then filtered through three layer of cheese cloth to remove the tissue debris and centrifuged at  $3500 \times g$  for 30 min. The resulting supernatants were filtered through Whatmann No. 1 filter papers. The pH values of all extracts including distilled water (control) were adjusted to 6.0 with 1N HCl using a pH meter. The osmotic potential (OP) of all extracts was calculated using the following equation [28]:

 $OP = Electrical conductivity \times -0.36$ 

The OP values of all extracts were found to be in the range of -0.087 to -0.15, and under such values, no significant growth inhibition was reported [26]. All extracts were kept refrigerated at 4 °C.

## *Effects of* A. biebersteinii water extracts on germination and early seedling growth of wild barley

Wild-barley (Hordeum spontaneum) caryopses were surface-sterilized in 2% sodium hypochlorite solution for 5 min, then washed four times in distilled water and dried between two filter papers. The sterilized caryopses were soaked in distilled water (control) and respective extract solutions from each plant parts of *A. biebersteinii* for 3 h. After that, 20 uniform seeds of each control and treatment were evenly placed on Petri plates lined with four layers of Whatmann No. 1 filter papers. Initially, filter papers were moistened with 15 ml of respective extract solution in treatment and distilled water in control and Petri plates were incubated in a growth incubator at  $24\pm2$  °C. A 5 ml of extract solution and distilled water were added on alternate days. Germination was recorded daily until eight days. Caryopses were considered germinated after the emergent radical had protruded beyond the seed coat by at least 1 mm. After 8 days, final germination percentage, radical and plumule lengths and their dry weights were recorded. Treatments were arranged in a completely randomized design with eight replications and the experiment was repeated three times.

# Effects of A. biebersteinii water extracts on seedling growth of wild barley

Wild-barley caryopses were sterilized as mentioned above. Four caryopses were planted at equal distance in experimental pots (15 cm in diameter, 25 cm in height) filled with homogenous soil and pots were placed in the greenhouse. Each pot was irrigated every two days with 100 ml of graded concentration of extract solution according to the treatments. The pots irrigated with distilled water were taken as control. After four weeks, data were recorded for root and shoot dry weights, chlorophyll pigments and protein content. Treatments were arranged in a randomized complete block design with six replications and the experiment was repeated three times.

### Determination of chlorophyll content

Chlorophyll pigments from leaf tissues were extracted in 80% acetone for 24 h at 4 °C, and the resulting extracts were centrifuged at  $3500 \times g$  for 5 min. The absorbance of the extracts was measured spectrophotometrically at 645 and 664 nm. Amounts of chlorophyll a (Chl a) and chlorophyll b (Chl b) were quantified according to the following equations [27]:

#### Chl a (mg/g FW) = $11.75 \times A_{663} - 2.35 \times A_{645}$

Chl b (mg/g FW) =  $18.61 \times A_{645} - 3.96 \times A_{663}$ 

After calculation of Chl a and Chl b values, total chlorophyll (Chl a+Chl b) values were determined.

#### Determination of protein content

Leaf tissues of barley seedlings were ground in liquid nitrogen into fine powder. About 300  $\mu$ l of total protein extraction buffer (100 mM NaCl; 50 mM Tris-HCl, pH 7.5; 0.5% (v/v) Triton X-100; 10 mM  $\beta$ -Mercaptoethanol; 2  $\mu$ l·ml<sup>-1</sup> Protease inhibitor mix) was added to the powder in a microfuge tube and the mixture was homogenized. The cell debris was pelleted in a centrifuge at 12,000 rpm and 4 °C for 10 min. Protein concentration in barley seedlings was estimated by a colorimetric assay according to Bradford [12]. To measure the concentration of proteins in leaf extract, 800  $\mu$ l of an appropriate dilution of the protein extracts was mixed with 200  $\mu$ l of Bradford reagent and incubated for 15 min at room temperature. The protein absorbance was recorded at 595 nm, and protein concentration was determined by plotting the OD<sub>595</sub> against BSA standard curve.

#### Statistical analysis

The data were subjected to one-way analysis of variance; differences between treatment means were determined by the least significant difference (LSD) test at the 5% level of probability. Data were analyzed using SAS program [36].

#### RESULTS

The allelopathic potential of water extracts of root, stem, leaf and flower from *A. biebersteinii* was tested on the germination and seedling growth of wild barley.

Final germination percentage of wild barley was significantly reduced when caryopses were subjected to stem, leaf, and flower extracts of *A. biebersteinii*. The germination percentage was reduced by 17.6%, 34.1 and 37.4% with stem, flower and leaf extracts (Table 1). However, applying root extracts showed no effects on germination percentage of wild barley.

The germination rate was significantly influenced by *A. biebersteinii* water extracts. The germination of wild-barley caryopses was delayed as a response of all extracts. The germination rate was 8.69 caryopses /day under the control conditions (distilled water). While a maximum reduction in germination rate (53%) arised from leaf extract followed by flower extract with an average reduction of 48.8%.

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Treatments	Germination (%)	Germination rate	Radical length (cm)	Plumule length (cm)	Radical dry weight (mg)	Plumule dry weight (mg)
Control	96.91 a	8.69 a	10.09 a	7.11 a	7.12 a	8.88 a
Root extract	87.26 ab	5.97 b	8.32 b	5.83 b	6.06 b	7.97 ab
Stem extract	79.81 b	5.61 b	6.79 c	5.47 b	4.04 c	7.30 bc
Leaf extract	60.71 c	4.07 c	4.64 d	3.22 c	2.63 d	5.04 d
Flower extract	63.90 c	4.45 bc	6.12 c	4.68 b	3.15 d	6.78 c

Table 1

Allelopathic effect of *Achillea biebersteinii* water extracts on germination and early seedling growth of wild barley

Means within each column followed by the same letter are not significantly different at  $P \le 0.05$ , according to LSD test.

Radical and plumule growth were significantly and negatively affected by *A. bie-bersteinii* water extracts (Table 1). Leaf extracts resulted in a significantly shorter radicals (4.64 cm) and plumule (3.22 cm) of germinating wild-barley caryopses as compared to other extracts. Stem and flower extracts resulted in almost the same level of inhibition in radical length. While, root, stem and flower extracts resulted in almost the same level of inhibition in radical length. Radical dry weight was reduced by 15, 43, 55.7 and 63% when wild-barley caryopses were treated with root, stem, flower and leaf extracts of *A. biebersteinii*, respectively. Plumule dry weight was found to be less affected by *A. biebersteinii* water extracts. Leaf extracts was the most inhibitory to plumule dry weight with a reduction percentage of 43% (Table 1).

Water extracts of *A. biebersteinii* caused significant alterations in root and shoot dry weights of wild-barley seedlings. As shown in Table 2, root dry weight was decreased to 53.7% of the control when seedlings were treated with leaf extracts of *A. biebersteinii*. Shoot dry weight of wild barley was not significantly affected by treatment with root extracts. While all other extracts were found to decrease shoot dry weight. Leaf extract was shown to reduce shoot dry weight to 24%.

Treatments	Root dry weight (mg)	Shoot dry weight (mg)
Control	32.02 a	260.9 a
Root extract	24.21 b	244.1 ab
Stem extract	21.86 bc	231.5 bc
Leaf extract	14.83 d	198.2 c
Flower extract	18.74 cd	211.1 c

 Table 2

 Allelopathic effect of Achillea biebersteinii water extracts on root and shoot dry weights of wild barley seedlings

Means within each column followed by the same letter are not significantly different at  $P \le 0.05$ , according to LSD test.

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Treatments	Chlorophyll a	Chlorophyll b	Total chlorophyll	Protein content	
Control	9.30 a	6.29 a	15.59 a	16.19 a	
Root extract	7.28 b	6.04 a	13.32 b	15.10 a	
Stem extract	7.04 b	5.89 ab	12.93 b	12.56 b	
Leaf extract	5.83 c	4.66 c	10.49 c	9.44 c	
Flower extract	6.95 b	5.41 b	12.36 b	10.20 c	

 Table 3

 Allelopathic effect of Achillea biebersteinii water extracts on chlorophyll pigments and protein contents of wild barley seedlings

Means within each column followed by the same letter are not significantly different at  $P \le 0.05$ , according to LSD test.

The effect of water extracts of *A. biebersteinii* on chlorophyll pigments was also examined. The amount of *Chl a* was significantly affected by the different extracts applied (Table 3). The maximum reduction (37%) in *Chl a* content was resulted in seedlings treated with leaf extracts. *Chl b* content remained unchanged when treating seedlings with root and stem extracts. Compared to the control (distilled water), the leaf and flower extracts resulted in 26 and 14% reduction in *Chl b*, respectively. The lowest total chlorophyll content was obtained after treating wild-barley seedlings with leaf extract of *A. biebersteinii*. Root, stem and flower extracts resulted in almost the same total chlorophyll content.

Protein content was significantly decreased by treating wild-barley seedlings with stem, flower and leaf extracts. The maximum reduction in protein content (41.7%) was recorded for leaf extract. Protein content in seedlings treated with root extracts was almost the same as in the control.

#### DISCUSSION

The results obtained indicate that water extracts from different parts of *A. biebersteinii* show differential phytotoxic effects on wild barley (*Hordeum spontaneum*), a weed of economic importance in Mediterranean regions. In the current work, leaf extract inhibited more strongly the seed germination and seedling growth of wild barley than any other extracts (Tables 1 and 2). The degree of phytotoxicity of different *A. biebersteinii* plant parts can be arranged in the following order: leaf > flower > stem > root. This findings can be attributed to the higher concentration of water-soluble allelochemicals in *A. biebersteinii* leaves than in roots, stems and flowers, or to the variable composition of bioactive phytochemicals between plant tissues.

Several studies have indicated that different parts of the same allelopathic plants might possess different phytotoxic effects on the growth and development of recipient

plants [14, 37]. In agreement with our results, Hu and Zhang [19] showed that the leaf extract of *Chromolaena odorata* exerted a more pronounced inhibitory effects on seed germination and seedling growth of different herbaceous species that root extract. In a study exploring the allelopathic properties of *Peganum harmala* on wild oat and field bindweed, Sodaeizadeh et al. [40] reported that phytotoxic effects of the leaf extracts were higher when compared to root and stem extracts. This is in contrast to the findings of Han et al. [17], who found that ginger stem extracts exhibited greatest inhibition to seed germination and seedling growth of soybean and chive than leaf and rhizome extracts.

All extracts prepared from different parts of *A. biebersteinii* delayed the germination of wild barley. Allelochemicals were reported to delay the germination process possibly by inhibiting enzymatic activities required for seed germination [31, 35] and alteration in the activity of gibberellic acid [34].

The present study also revealed that growth of radical of germinated wild-barley seeds and root dry weight of seedlings were found to be more sensitive to all types of *A. biebersteinii* water extracts in comparison to plumule growth and shoot dry weight. Abu-Romman [3] reported that root growth of pepper seedlings was more susceptible to leaf extracts of *A. biebersteinii* than shoot growth. Similar results with other allelopathic plants have been reported earlier [4, 7, 30]. It is a well-documented fact that root growth is more susceptible to allelochemicals than shoot growth. This might be resulted from the higher permeability of allelochemicals and higher metabolic rate in root compared to shoot tissues [15, 33]. Ultrastructural changes and tissue browning in root tip were reported after exposure to allelochemicals [8, 13, 16].

Chlorophyll pigments of wild-barley seedlings were negatively affected by *A. bie-bersteinii* water extracts. Reduction in photosynthetic pigments was previously reported as a result of allelochemical stress [21, 38, 39], which could be resulted from chlorophyll degradation or inhibition of chlorophyll biosynthesis [44, 45].

Shoot protein content of wild barley was significantly reduced due to treatment with stem, flower and leaf extracts. Mersie and Singh [29] documented that allelochemicals resulted in enhanced protein degradation in recipient plant. Moreover, some phenolic acids were reported to inhibit the incorporation of certain amino acid into proteins and thus reduce the rate of protein synthesis [10].

### CONCLUSIONS

In conclusion, the present study conferred that water extracts prepared from different organs of *A. biebersteinii* interfere with germination and growth of wild barley. Maximum inhibitory effects on wild-barley germination and growth were exhibited by leaves and least by roots. The present results suggest that *A. biebersteinii* might be used as a natural herbicide for wild barley control and consequently reduce dependence on synthetic herbicide.

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