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Germination and seedling growth of barley as affected by *Artemisia annua* water extract

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Abstract

Laboratory and greenhouse pot experiments were conducted to assess the allelopathic effects of *Artemisia annua* water extract on germination and growth of barley. Lower concentrations of *A. annua* water extract (0.5 and 1.0 %) did not affect the germination of barley seeds. However, higher concentrations (1.5-3.5 %) resulted in significant reductions in the germination percentage. Seedling growth of barley was also affected by *A. annua* water extract. Both shoot and root lengths were negatively affected by *A. annua* water extract and the degree of inhibition was concentration dependent. When barley seedlings were subjected to 0.5% extract concentration, shoot length was inhibited by 6% while root length showed 18.5% inhibition over control seedlings. The lowest shoot and root lengths were recorded at 3.0 and 3.5 % water extract. At 0.5 % extract concentration, shoot and root fresh and dry weights were significantly unaffected compared with the control. Shoot fresh weight was significantly unaltered when seedlings were treated with 1.0 % extract. However, at the same concentration root fresh weight, shoot dry weight, and root dry weight were inhibited by 44.7, 33.3, and 40 %, respectively. The present results confirmed that root growth (length and weight) was more sensitive to *A. annua* water extract than shoot growth. The present results also indicated the presence of water soluble allelochemicals in *A. annua* that are able to inhibit growth of barley.

Keywords: *Artemisia annua*, barley, germination, growth inhibition, water extract.

Introduction

Allelopathy, as an ecological phenomenon, refers to the positive or negative impacts of one plant species (as well as microorganisms) on the germination, growth, and physiological functions of other plant species through the release of phytochemical compounds (allelochemicals) into the environment (Rice, 1984; Weir et al., 2004; Inderjit et al., 2006). Allelochemicals are produced by any organ of the plant and are mainly secondary metabolites, such as flavonoids, alkaloids, tannins, phenolic acids, lignins, coumarins, and terpenoids (Inderjit and Duke, 2003; Li et al., 2010).

These chemicals vary among plant species and organs and are released into the environment by different processes; root exudation, volatilization, leaching, and tissue decomposition in soil (Parvez et al., 2004; Gniazdowska and Bogatek, 2005). Allelopathic activity has been reported in several herbal and medicinal plants, due to the array of active phytochemicals produced (Khanh et al., 2005; Sodaeizadeh et al., 2009; Abu-Romman et al., 2015).

Artemisia annua L (Asteraceae) is an annual medicinal plant widely distributed around the world and used in folk medicine to treat different ailments. *A. annua* is rich in terpenoids, phenols, flavonoids, and other shikimate pathway metabolites (Brown, 2010; Carbonara et al., 2012). This plant is a major source of the sesquiterpene lactone artimisinin, which is one of the most efficient drugs used in the treatment of the infectious disease malaria (van der Kooy and Sullivan, 2013). *In vitro* studies performed with *A. annua* also showed that this medicinal plant exhibits other therapeutic properties. It has been reported that *A. annua* possesses anticancer activity (Lai et al., 2013). Moreover, some studies indicated that *A. annua* tea infusions were highly effective against HIV (Wu et al., 2001; Lubbe et al., 2012).

Some reports have demonstrated the allelopathic nature of *A. annua* and its secondary metabolite artemisinin. In a field study, Lydon et al. (1997) reported growth inhibition of soybean and maize by incorporating *A. annua* leaves into soil. Similar results were shown by Delabays et al. (2008). The phytotoxic nature of artimisinin was first reported by Duke et al. (1987). Germination of lettuce and mung bean were shown to be inhibited in response to artimisinin (Chen and Leather, 1990; Chen et al., 1991; Panamanik et al., 2008; Jessing et al., 2014). The present study intended to further investigate the impacts of *A. annua* water extract on germination and seedling growth of barley.

Results and Discussion

The allelopathic potential of *A. annua* water extract on the germination of barley three days after treatment is shown in Fig 1 and 2. At low concentrations (0.5 and 1.0 %), the germination of barley seeds was not significantly affected compared to control. However, at 1.5 and 2.0 % of water extract, the germination was significantly reduced and scored 65.00 and 46.67 %, respectively. The lowest germination percentages (< 10%) were recorded when barley seeds were treated with 2.5-3.5 % of *A. annua* water extract (Fig 1). Allelopathic action of leaf tissues and extract fractions were previously reported in *A. annua* and other *Artemisia* species

Table 1. The effect of different concentrations of water extract of *Artemisia annua* on shoot and root lengths (cm) of barley seedlings. Data are presented as the mean ± SD. Values with the same letter are not significantly different as determined by Tukeys HSD test at P< 0.05.

Extract concentration (%)	Shoot length (cm)	Root length (cm)
0	14.09 ± 1.74[a]	9.37 ± 1.33[a]
0.5	13.25 ± 2.61[ab]	7.63 ± 1.02[b]
1.0	11.58 ± 2.73[b]	6.47 ± 0.99[c]
1.5	9.24 ± 3.32[c]	5.01 ± 0.89[d]
2.0	7.09 ± 2.44[d]	3.08 ± 1.15[e]
2.5	5.87 ± 2.22[de]	2.51 ± 1.15[e]
3.0	3.97 ± 1.62[ef]	1.40 ± 1.04[f]
3.5	2.73 ± 1.37[f]	1.33 ± 0.60[f]

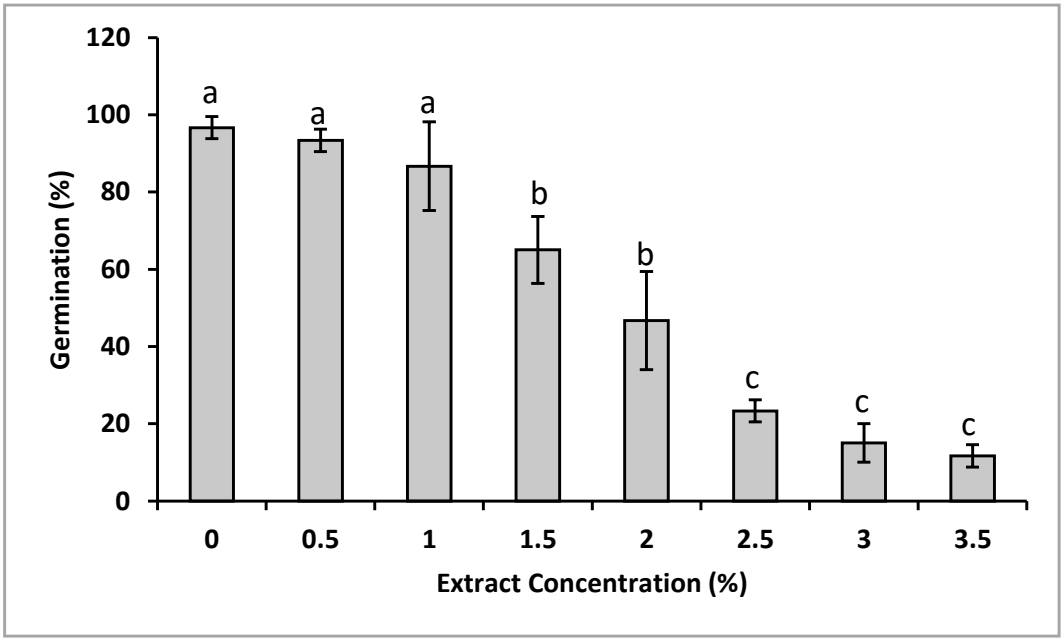

Fig 1. The effect of different concentrations of water extract of *Artemisia annua* on germination percentage of barley. Data are presented as the mean ± SD. Values with the same letter are not significantly different as determined by LSD test.

(Passim and Rodrigues, 1999; Delabays et al., 2008; Panamanik et al., 2008; Kegode et al., 2012; Jessing et al., 2014).

The observed reduction in barley germination under treatment of *A. annua* water extract could be attributed to the presence of water-soluble inhibitory allelochemicals. Several phytotoxins have been identified in *A. annua* (Jessing et al., 2014). Artemisinin, a highly oxygenated sesquiterpene of *A. annua*, was found to inhibit germination of different plant species (Duke et al., 1987; Chen et al., 1991; Lydon et al., 1997). Allelochemicals are reported to inhibit and delay the germination process by altering membrane permeability, mitochondrial respiration, enzymatic activities, and protein synthesis (Ferreira and Aquila, 2000; Kato-Noguchi et al., 2010; Ertani et al., 2016; Ozaki and Kato-Noguchi, 2016).

A pot experiment was conducted under greenhouse conditions to explore the allelopathic influence of *A. annua* water extract on the seedling growth of barley after one week of treatment.

Both shoot and root lengths were negatively affected by *A. annua* water extract and the degree of inhibition due to the extract increased with increasing extract concentration (Table 1). Similar effects were reported by Mallik et al. (2015) of *Artemisia dubia* on wheat and field mustard.

When barley seedlings were subjected to 0.5% extract concentration, shoot length was inhibited by 6% while root length showed 18.5% inhibition compared to control seedlings. The lowest shoot and root lengths were recorded at 3.0 and 3.5 % water extract. The data in Table 1 illustrated clearly that root length was more affected by *A. annua* water extract compared to shoot length.

Root and shoot elongation of lettuce were found to be inhibited by artemisinin in a dose-dependent manner (Yan et al., 2015). The inhibitory action of artemisinin on target species growth could be attributed to the overproduction of reactive oxygen species and subsequence lipid peroxidation and arrested cell division (Dayan et al., 1999; Yan et al., 2015). Moreover, artemisinin was reported to possess strong anti-algal activity against *Microcystis aeruginosa* (Ni et al., 2012).

At 0.5 % extract concentration, shoot and root fresh and dry weights were significantly unaffected compared with the control plants (Table 2). Shoot fresh weight was significantly unaltered when seedlings were treated with 1.0 % extract. However, at the same concentration root fresh weight, shoot dry weight, and root dry weight were inhibited by 44.7 %, 33.3 %, and 40 %, respectively. Growth parameters measured in Table 2 generally showed reduction with elevated extract concentrations. Similarly, Moussavi-Nik et al. (2011) reported reduction in fresh and dry weights of *Plantago ovate* in response to treatment with *A. annua* water extract.

The results in Figure 1 and Table 1 confirmed that root growth (length and weight) was more sensitive to putative allelochemicals present in *A. annua* water extract than shoot

Table 2. The effect of different concentrations of water extract of *Artemisia annua* on fresh and dry weights (g) and chlorophyll content of barley seedlings. Data are presented as the mean ± SD. Values with the same letter are not significantly different as determined by Tukeys HSD test at P< 0.05.

Extract concentration (%)	Fresh weight (g)		Dry weight (g)		Chlorophyll content
	Shoot	Root	Shoot	Root	
0	0.64 ± 0.09^{a}	0.47 ± 0.11^{a}	0.06 ± 0.009^{a}	0.037 ± 0.003^{a}	30.13 ± 2.8^{a}
0.5	0.61 ± 0.11^{a}	0.42 ± 0.10^{a}	0.05 ± 0.009^{ab}	0.035 ± 0.004^{a}	29.90 ± 2.1^{a}
1.0	0.54 ± 0.04^{a}	0.26 ± 0.07^{b}	0.04 ± 0.008^{bc}	0.022 ± 0.005^{b}	27.23 ± 1.4^{a}
1.5	0.52 ± 0.05^{ab}	0.20 ± 0.06^{bc}	0.04 ± 0.007^{bcd}	0.018 ± 0.006^{b}	28.29 ± 1.7^{a}
2.0	0.36 ± 0.05^{bc}	0.11 ± 0.06^{cd}	0.03 ± 0.004^{cd}	0.015 ± 0.003^{b}	29.54 ± 2.0^{a}
2.5	0.29 ± 0.10^{c}	0.08 ± 0.04^{cd}	0.02 ± 0.008^{de}	0.007 ± 0.004^{c}	29.49 ± 2.3^{a}
3.0	0.22 ± 0.13^{cd}	0.03 ± 0.03^{d}	0.01 ± 0.007^{ef}	0.002 ± 0.002^{c}	29.76 ± 1.6^{a}
3.5	0.08 ± 0.04^{d}	0.02 ± 0.01^{d}	0.01 ± 0.004^{f}	0.002 ± 0.003^{c}	27.49 ± 1.3^{a}

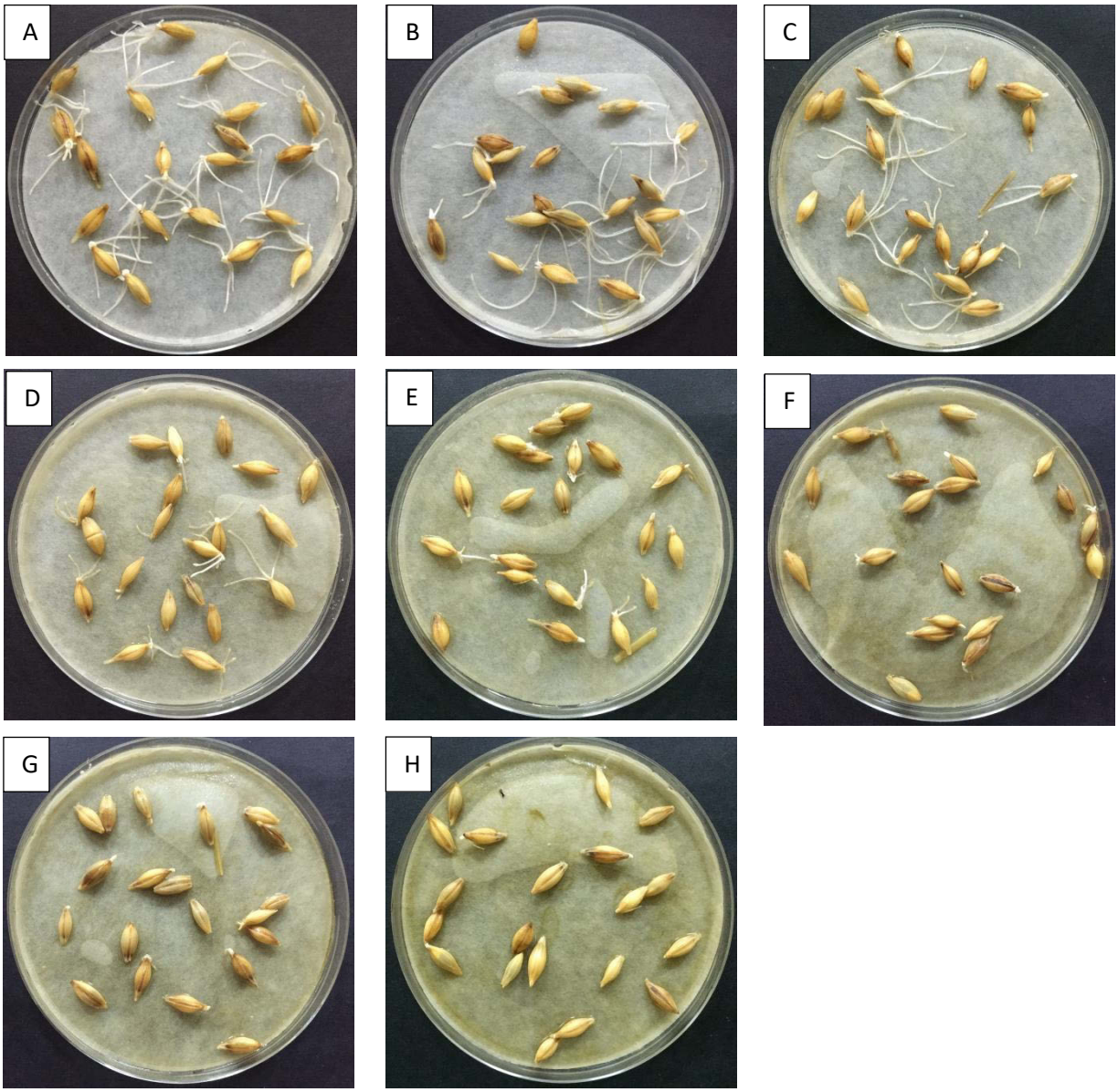

Fig 2. The effect of different concentrations of water extract of *Artemisia annua* on germination of barley seeds were A: control, B: 0.5%, C: 1.0%, D: 1.5%, E 2.0%, F: 2.5%, G: 3.0% and H: 3.5%.

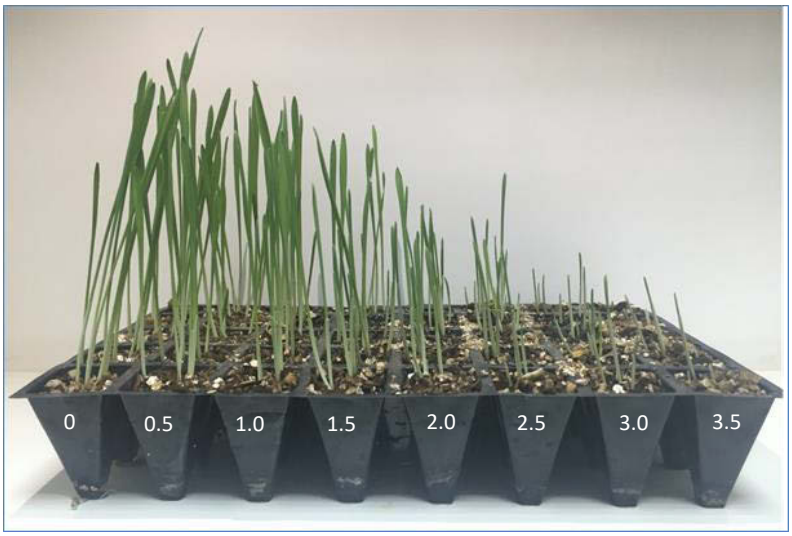

Fig 3. The effect of different concentrations (0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5%) of water extract of *Artemisia annua* on growth of barley seedlings.

growth. Similar observations were previously reported (Khan and Kato-Noguchi, 2016). A study conducted by Abu-Romman (2011) showed that water extract of *Achillea biebersteinii* (Asteraceae) greatly inhibited root growth compared to shoot growth of pepper. The highest root sensitivity to allelochemicals could be attributed to the direct contact of the root system to these toxic phytochemicals and the high permeability of root tissues (Nishida et al., 2005; Abu-Romman and Ammari, 2015; Abd El-Gawwad, 2016)
Allelochemical stress was reported to reduce chlorophyll content in different affected species (Gulzar and Siddiqui, 2014; Sarkar and Chakraborty, 2015; Abu-Romman, 2016). However, in the present study total chlorophyll content of barley seedlings was not significantly affected in response to *A. annua* water extract (Table 2). This result is in line with the findings of Benyas et al. (2010), who showed that water extract of the weed *Xanthium strumarium* did not affect total chlorophyll content of lentil seedlings.

Materials and Methods

Plant material and extract preparation

Leaves of *Artemisia annua* were collected from Tulkarm in the West Bank (32°18.6222′ N, 35°1.7178′ E, 117 m above sea level). The collected leaves were dried at 65°C for 48 h until constant weight and ground to a fine powder. A 10% (w/v) extract was prepared by soaking 10 g of the powder in 100 mL distilled water for 24 h on a horizontal shaker at 110 rpm. The extract was filtered through a double layer of cheesecloth and then filtered through Whatman no 1 filter paper. The extract was stored at 4°C until use. Different concentrations (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5%) of the extract were used in laboratory and green house experiments.

Laboratory experiment

Twenty seeds of barley (*Hordeum vulgare* L.) were spread on a filter paper in 9 cm Petri dishes. For each petri plate 3 mL of each concentration was added. The plates were sealed with parafilm and incubated at 25°C in the dark. After 3 days, final germination percentages were recorded. Seeds were considered germinated if the root length exceeds half the length of the seed. Treatments were arranged in a completely randomized design with three replications and the experiment was repeated three times.

Pot experiment

Seedling cell trays (5 cm in diameter eye) were filled with peat moss vermiculite mixture (2:1 v/v). Five barley seeds were sown in each cell before irrigation with *A. annua* aqueous extracts. Each cell was irrigated with 10 mL of each concentration. Controls were irrigated with tap water. One week after incubation at 25°C, number of germinated seeds was recorded. Shoot and root lengths were measured. After recording the fresh weight of the germinated seedlings, the seedlings were placed in a drying oven at 65°C for dry weight measurement. In addition to that, chlorophyll content was measured using Chlorophyll Meter SPAD-502Plus. Treatments were arranged in a randomized complete block design with three replications and the experiment was repeated three times.

Statistical analysis

All experiments were done in triplicates and repeated three times. Statistical analysis was done using XlStat (Adinosoft). Significant differences were computed using ANOVA after Tukeys HSD test at $P< 0.05$

Conclusion

Allelopathic effect *A. annua* water extract toward barley was observed in the current study. Water extract of this medicinal plant inhibited germination of barley seeds. Moreover, seedling growth was also negatively affected by treatment with *A. annua* water extract in a concentration-dependent manner. These results indicated the presence of water soluble allelochemicals in *A. annua*. The medicinal plant *A. annua* must be examined for its allelopathic action on other economic crops and noxious weeds, particularly under field conditions. Moreover, further study is needed to characterize potential allelochemicals present in different plant parts of *A. annua*.

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