

Allelopathic effect of *Arundo donax*, a mediterranean invasive grass

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Abstract.

Arundo donax often dominates plant communities by forming large stands throughout Mediterranean climate zones. The objective of this study was the investigation of allelopathic property of *A. donax* leaves on germination and seedling growth of lentil (*Lens culinaris*) under laboratory and greenhouse conditions. Four concentrations of aqueous extract (2, 4, 6, and 10%) were compared with the control (distilled water). The germination percentage of lentil seeds was significantly reduced at extract concentrations $\geq 6\%$. Aqueous extracts of *A. donax* at concentrations of 6 and 10% significantly reduced the germination percentage of lentil with reduction percentages of 21.3% and 47.4%, respectively. Aqueous extract of *A. donax* significantly delays the germination process of lentil and reduces the seedling vigor index. Root and shoot growth of lentil seedlings were significantly inhibited by the extract. Root growth was more suppressed than shoot growth in response to *A. donax* aqueous extract. The results suggested that *A. donax* aqueous extracts possesses allelopathic potential which could explain in part the dominance competitiveness for this invasive species. Further, the *A. donax* extract or its active substances could be used for the development of bio-herbicides as alternatives of commercial herbicides.

Keywords: *Arundo donax*, Allelopathy, Bio-herbicide, Invasive plant, Lentil.

Abbreviations: GR_ growth rate; LSD_ least significant difference; SVI_ seedling vigor index.

Introduction

Arundo donax (giant reed) is a perennial herbaceous grass (Poaceae) originated from eastern Asia and widespread throughout the Mediterranean climate zones (Mariani et al., 2010). This species has been listed on the Global Invasive Species Database as one of the world's worst invasive alien species (GISD, 2013). In Jordan, the invasive *A. donax* plants are abundant in the Jordan Valley especially on road sides near water streams, agricultural lands and along King Abdullah canal. *A. donax* spreads vegetatively and is characterized by vigorous growth rate, high yield capacity, broad environmental tolerance, and ability to displace native vegetation forming monotypic stands, and therefore, destroying wildlife habitats (Dudley, 2000; Herrera and Dudley, 2003; Angelini et al., 2005). In spite of being an invasive species, *A. donax* has been cultivated in several parts of the world for several purposes, such as producing high quality fiber for papermaking and being suitable for soil phytoremediation and wastewater treatment (Manios et al., 2002; Coelho et al., 2007; Papazoglou et al., 2007). This plant has been considered as a promising energy crop utilized for biofuel production (Scordia et al., 2012; Pilu et al., 2013). Most recently, leaves of *A. donax* were reported to be potentially used as a raw material of low cost eco-biosorbent for cadmium (Ammari, 2014). Allelopathic potential is one of

the strategies used by invasive plants to become successful and replace other native ones (Callaway and Ridenour, 2004; Greer et al., 2014). Allelochemicals are mainly plant secondary metabolites (Putnam, 1988) and vary in concentration according to the species and plant organ (Friedman and Waller, 1995; Gatti et al., 2010). Most allelochemicals are liberated into the environment by different processes, namely exudation, leaching, volatilization and decomposition (Weir et al., 2004). The negative impacts of allelochemicals on target plants are complex and diverse and are considered as biotic stress called allelochemical stress (Cruz-Ortega et al., 2002; Singh et al., 2009). Allelochemicals are reported to modulate growth and development of target plants through affecting vital processes (Rice, 1984), such as membrane permeability (Galindo et al. 1999), water and nutrient uptakes (Barkosky and Einhellig, 2003; Fageria and Stone, 2006), cell division (Soltys et al., 2011), photosynthesis (Yu et al., 2003) and respiration (Chai et al., 2013). Moreover, allelochemicals are reported to exert their effects in target plants by inducing oxidative damages through the production of reactive oxygen species (Bais et al., 2003; Bogatek and Gniazdowska, 2007). Therefore, the following study was conducted to evaluate the allelopathic potential of *A. donax* on germination and seedling growth of lentil.

Results and Discussion

Allelopathy is usually referred to the impact of phytochemicals on growth and development of receptor plants. Allelopathy attributes to the dominance success and the higher competitive ability of invasive plants (Ridenour and Callaway, 2001; Stinson et al., 2006; Jarchow and Cook, 2009). Moreover, enhanced allelochemical content of invasive plant species could be a defensive strategy against herbivore and pathogen attacks (Zhang et al., 2009; Watling et al., 2011). No previous information are found in literature documenting the allelopathic activity of *A. donax* on receptor plants. In the present study, laboratory and greenhouse experiments were conducted to evaluate the allelopathic potentiality of the aqueous extract at different concentrations of *A. donax*. The effects of different concentrations of *A. donax* aqueous extract were tested on the germination process of lentil (Table 1). Germination percentage of lentil seeds was not affected in response to lower concentrations of the extracts (2 and 4%). Aqueous extracts of *A. donax* at concentrations of 6 and 10% significantly reduced the germination percentage of lentil with reduction percentages of 21.3% and 47.4%, respectively (Table 1). The germination rate (GR) was 7.13 seeds/day under control. Except for the 2% extract concentration, all *A. donax* extracts resulted in a significant reduction in GR (Table 1). The maximum reduction (47.1%) in GR resulted from the 10% extract concentration followed by the 6% extract concentration with an average reduction of 31%. Seedling vigor index (SVI) was significantly reduced by the presence of *A. donax* extracts. The highest extract concentration was the most inhibitory with reduction percentage of 68% (Table 1). The results shown in Table 1 clearly indicated that aqueous extract of *A. donax* delayed the germination process of lentil and reduced the SVI in a dose-dependent manner. Allelochemicals are known to reduce seed germinability possibly by altering the enzymatic activities needed for the immobilization and conversion of reserve carbohydrates during germination (Lara-Núñez et al., 2009). Bousquet-Mélou et al. (2005) have demonstrated that aqueous extracts of *Medicago arborea*; a Mediterranean invasive shrub, strongly lowered the germination of yellow flax seeds. Other reports have also documented the strong allelopathic pressures of invasive plant species on germination process of different receptor plants (Dorning and Cipollini, 2006; Qin et al., 2006; Yuan et al., 2012; Ghebrehiwot et al., 2013; Uddin et al., 2014). However, some invasive plants exhibited no allelopathic interference to outperform native plant species (Ridenour and Callaway, 2001). The effects of aqueous extract of *A. donax* on seedling growth of lentil are provided in figures 1 and 2. Root length was significantly and negatively affected by *A. donax* aqueous extract (Fig. 1). All concentrations of *A. donax* aqueous extract caused significant reductions in root length, root fresh weight and root dry weight. The 2 and 4% extract concentrations resulted in almost the same level of inhibition in root growth. The greatest inhibition in root growth was recorded for the 6 and 10% extract concentrations (Fig. 1). Shoot growth was significantly altered by *A. donax* aqueous extract (Fig. 2). The lower concentrations of the extract (2 and 4%) did not significantly affect the shoot growth of lentil. However, shoot growth was inhibited only by the treatment with the 6 and 10% extract concentrations. The 10% extract concentration was the most inhibitory to shoot length and shoot fresh and dry weights with reduction percentages of 40%, 50%

and 54%, respectively. The observed growth inhibition in lentil seedlings in response to *A. donax* aqueous extract could result from allelochemicals-mediated oxidative stress and retardation of cell proliferation (Bais et al., 2003; Nishida et al., 2005). The results of the current study indicated that root growth of lentil seedlings was more susceptible to *A. donax* extract compared to shoot growth. This observation might result from the more intensive and direct contact between roots and allelochemicals and the higher permeability between root tissues and these chemicals (Nishida et al., 2005; Wang et al., 2009; Abu-Romman, 2011). Several potential allelochemicals with antifungal activities have been characterized in *A. donax*., including indoles, ketones, esters and alcohols (Hong and Hu, 2007; Hong et al., 2010). Among them, gramine (3-(dimethylaminomethyl) indole, an alkaloid) was very inhibitory to the freshwater algae *Microcystis aeruginosa* (Hong et al., 2009). The presence of these allelopathic compounds could explain the observed inhibitory effects of *A. donax* on germination and growth of lentil.

Materials and Methods

Preparation of aqueous extract

Leaf samples were collected from *Arundo donax* stands from the Jordan valley (32°1'N; 35°48'E; 230 m below sea level). The collected leaf samples were rinsed repeatedly under running tap water to remove any debris. Leaf tissues were dried in a drying oven at a temperature of 65 °C for 48 h and ground to a fine powder. The aqueous extract was prepared by soaking 2, 4, 6, and 10 g of dry ground leaves of *A. donax* in 100 ml of distilled water and agitated at room temperature for 24 h on a horizontal shaker set at 100 rpm. The extract was filtered through Whatman no. 1 filter paper and stored at 4 °C until use. The distilled water was used as a control (0% extract).

Laboratory experiment

Seeds of lentil (*Lens culinaris* Medik.) were surface sterilized by soaking them in 3% sodium hypochlorite solution for 5 min, thereafter, washing them four times in sterile distilled water. Twenty five seeds were evenly placed on three layers of Whatman no. 1 filter papers in sterilized 9 cm Petri plates. Initially, 15 ml of extract solution from each concentration was added to each Petri plate and controls were obtained using distilled water. All Petri plates were placed in growth chamber at 24±2 °C. Five ml of each aqueous extract and distilled water were added on alternate days. Germination was recorded daily until seven days. Seeds were considered to have germinated when root protrusion beyond the seed coat reached at least 1 mm. After seven days, final germination percentages were recorded. The germination rate (GR) was calculated by dividing the number of germinating seeds each day by the number of days and summing the values. Seedling vigor index (SVI) was recorded and calculated as germination % × radical length (cm) (Abdul-baki and Anderson, 1973). Treatments were arranged in a completely randomized design with ten repetitions and the experiment was repeated three times.

Table 1. The effects of different concentrations of aqueous leaf extract of *Arundo donax* on germination of lentil. Data are presented as the mean \pm SD. Values in a column followed by the same letter are not significantly different as determined by LSD_{0.05} test.

Extract concentration (%)	Germination (%)	Germination rate (GR)	Seedling vigor index (SVI)
0	100 \pm 0.00a	7.13 \pm 0.31a	34.44 \pm 2.73a
2	100 \pm 0.00a	6.56 \pm 0.15ab	28.99 \pm 1.85ab
4	100 \pm 0.00a	6.04 \pm 0.18b	26.09 \pm 1.44b
6	78.7 \pm 3.42b	4.91 \pm 0.19c	18.75 \pm 2.03c
10	52.6 \pm 5.88c	3.77 \pm 0.24d	10.86 \pm 1.22d

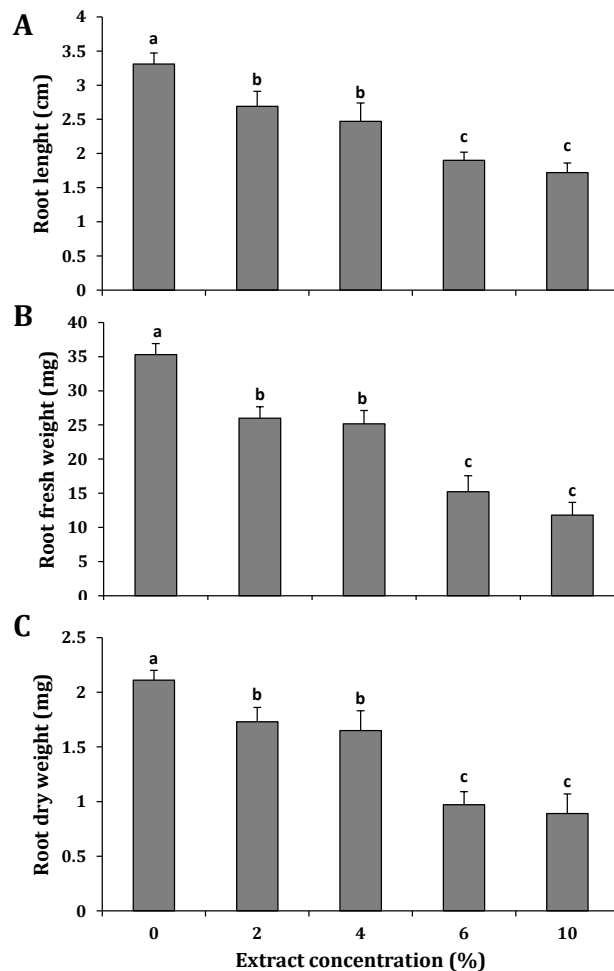


Fig 1. The effects of different concentrations of aqueous leaf extract of *Arundo donax* on root growth of lentil: (A) root length; (B) root fresh weight; (C) root dry weight. Data are presented as the mean \pm SD. Values with the same letter are not significantly different as determined by LSD_{0.05} test.

Greenhouse experiment

Seeds of lentil were sterilized as described above. Four seeds were planted at equal distance in 1 L plastic pots filled with peatmoss and pots were placed in a natural lit greenhouse. Each pot was irrigated every day with 50 ml of graded concentration of extract solution according to the treatments. The pots irrigated with distilled water were considered as the control. After three weeks, data were recorded for root and shoot lengths, fresh weights, and dry weights.

Treatments were arranged in a randomized complete block design with eight repetitions and the experiment was repeated three times.

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 and statistical significance

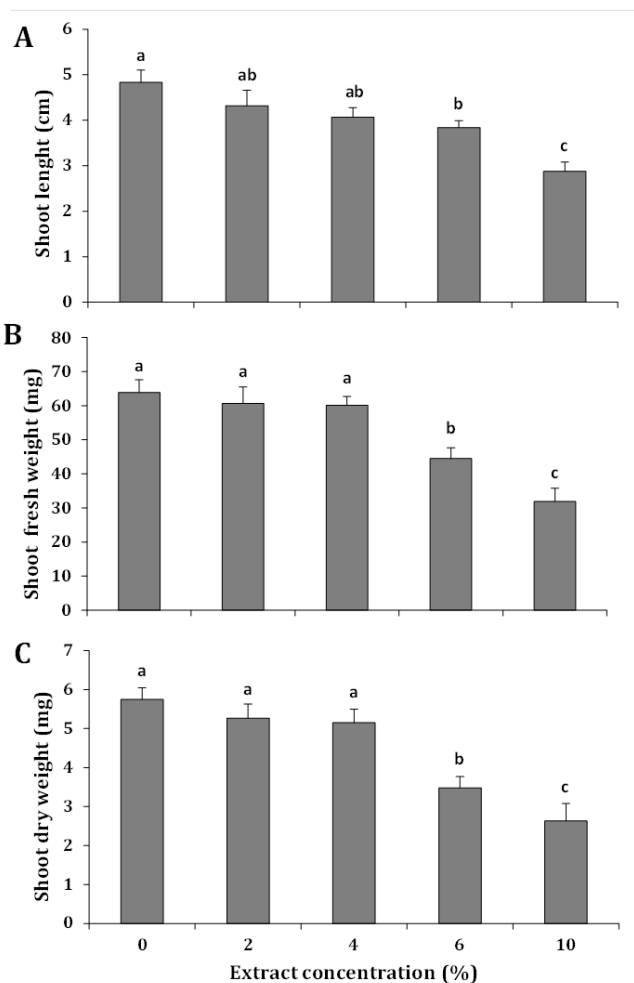


Fig 2. The effects of different concentrations of aqueous leaf extract of *Arundo donax* on shoot growth of lentil: (A) shoot length; (B) shoot fresh weight; (C) shoot dry weight. Data are presented as the mean \pm SD. Values with the same letter are not significantly different as determined by LSD_{0.05} test.

was evaluated at $P \leq 0.05$. Data were analyzed using SAS program (SAS Institute, 1988).

Conclusion

In conclusion, the results of the present study have demonstrated that *A. donax* aqueous extracts possess allelopathic effects on germination and seedling growth of lentil. The allelopathic potential of *A. donax* could explain its successful monotypic stands and dominance in Mediterranean regions. We suggest that *A. donax* aqueous extract should be considered as a potential bioherbicide.

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