Effects of *Artemisia sieberi* extract on growth and nutrients uptake of *Peganum harmala*

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Abstract

This study was conducted to determine the effects of *Artemisia sieberi* extract (0.2%, 0.4%, and control) on germination, some morphological characteristics, photosynthesis pigments, and nutrients uptake of *Peganum harmala*. Results showed that the extract of *A. sieberi* had a significant effect on seed germination and morphological characteristics of *P. harmala*. The highest seed germination was calculated in 0.2% treatment. The lowest seed germination was observed in the control treatment. The highest and lowest radicle lengths were calculated in the control and 0.4% treatments, respectively. With increasing concentration of the extract, dry weight and pedicel length of *P. harmala* decreased. *A. sieberi* extract had phytotoxic effect on chlorophyll contents of *P. harmala*. The highest photosynthesis pigments were related to the control treatment, and by increasing the concentration of extract, photosynthesis pigments decreased. The highest contents of nitrogen and zinc were found in 0.2% treatment. The lowest amounts of nitrogen and zinc were related to 0.4% treatment. The highest amount of phosphorus was related to the control treatment and with increasing concentration of extract, phosphorous content decreased. 0.2% treatment exhibited the highest potassium content while the lowest amount of potassium was related to the 0.4% treatment. In general, results showed that *A. sieberi* besser, increased nutrients uptake of *P. harmala*, in 0.2% treatment, but increased concentrations of the extract prevented germination and growth of *P. harmala*. It is recommended not to cultivate the two plants together if we consider *P. harmala* as a medicinal plant.

Keywords: seed germination; plant extract; chlorophyll contents; Allelochemicals; *Peganum harmala*

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Introduction

Environmental factors play a crucial role in the growth of plants in natural ecosystems (Mahdavi et al., 2013). Some factors such as light (Alonso-Amelot, 2007), drought (Delshadi, 2015), temperature (Ranjbar, 2014), mineral nutrients (Ardakani and Mafakheri, 2011), and biochemicals (Al-Watban and Salama, 2012; Mohammaddoust Chamanabad et al., 2014) are considered as the most important environmental factors affecting plant growth.

Allelopathy is one of the most important interference methods of the plants that can help

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Table 1
Some physical and chemical characteristics of the soil used in the experiment

Texture	N (%)	P (mg kg^{-1})	K (mg kg^{-1})	EC (dS m^{-1})	pH
Loamy sand	0.09	0.23	90	1.06	8.46

to know plant interaction (Mohammaddoust Chamanabad et al., 2014). Allelopathy is a biological phenomenon by which an organism produces one or more allelochemicals that influence the germination, growth, survival, and reproduction of other organisms (Stamp, 2003).

Allelopathic compounds play an important role in biodiversity of ecosystems (Iman and Zakaria, 2006). In recent years, allelopathy has received much attention to understand the interrelationships of plants in natural ecosystems. Studies suggest that allelochemicals contain secondary compounds with allelochemical or phytotoxic activity which cause growth inhibition (Iman and Zakaria, 2006; Al-Watban and Salama, 2012). However, allelopathy may alter the available resources in the environment (Wardle et al., 1996; Al-Watban and Salama, 2012) and researchers believe they are a joint action of some secondary metabolites such as phenolic compounds, flavonoids, etc. (Berhow and Voughn, 1999; Narwal, 2004).

Peganum harmala is a perennial plant from *Zygophyllaceae* family which grows in arid and semi-arid areas (steppe areas and sandy soils) (El Gendy and El-Kadi, 2009). Many parts of the plant including seeds, fruits, and roots have been used as traditional medicine in Iran and other countries. Many studies have reported different effects of *P. harmala* and/or its active alkaloids (particularly harmaline) (Tahraoui et al., 2007; Moloudizargari et al., 2013). It has also been shown in various pharmacological studies that *P. harmala* extract and its main active alkaloids, harmine and harmaline, have different cardiovascular effects such as bradycardia, decreasing systemic arterial blood pressure, and total peripheral vascular resistance, increasing pulse pressure, peak aortic flow, cardiac contractile force (Aarons et al., 1977), and Vasorelaxant (Berrougui et al., 2006).

Artemisia is one of the widely distributed genera of the *Asteraceae* family which is widely distributed in the arid areas of Iran (Mozaffarian, 1996). This plant has forage value for livestock and also medicinal properties for humans. Some pharmacological effects of *A. Siberia* such as spasmolytic, germicidal (Zargari, 1996), insecticidal (Negahban et al., 2007), and anticandidal (Mahboubi et al., 2008) were reported.

Natural resources are the basis of biodiversity in ecological systems and identification of effective factors in the system can provide a suitable context for sustainable exploitation of them (Al-Rowaily et al., 2015; Chillo et al., 2015). For effective management of rangelands, knowing the proper procedures is necessary. Among these methods, we can consider to restore rangelands with suitable plants. But, we must keep in mind that even if most adaptable species are used for rangeland, regardless of allelopathy properties, the project is likely to fail (Bagheri and Mohammadi, 2011).

The objectives of this study were: (1) to investigate the effects of extract of *A. sieberi* which is a fast growing plant species on morphological characters (germination, biomass, radical, and pedicel length) and nutrients uptake of *P. harmala* under greenhouse conditions and (2) to identify the effects of *A. sieberi* extract on photosynthesis pigments of *P. harmala*.

Materials and Methods

Pot preparation and plant extract

The present study was carried out in a greenhouse condition, with the environmental conditions, temperature 23±5 °C, humidity 60%, and moisture content 70% water-holding capacity. The experimental design was completely randomized with four replications. Soil was selected from Deging village, located in Khash city (Sistan and Baloochestan province, Iran). Soil sampling was obtained from the depth of 0-30 cm with a 5.5 cm diameter hand driven corer and mixed. All soil samples were sieved to 4 mm and moisture contents were adjusted to 70% water–holding capacity (WHC). Characteristics of the soil

are listed in Table 1. The soil texture (loamy sand) was determined using laser diffractometry (Wang et al., 2012). The soil pH was determined in a 1:5 soil to distilled water slurry after 1 hour of agitation using a digital pH–meter (Thomas, 1996) and the electrical conductivity (ECe) was measured using an EC-meter (Rhoades, 1996). Available phosphorus (AP) was measured by the method of Bray and Kurtz (1954). Total soil N was determined calorimetrically with a continuous flow ion analyzer following wet digestion in sulfuric acid using a Kjeldhal (Bremner, 1996); Available potassium (AK) was measured by flame photometry method (Knudsen et al., 1982).

In order to prepare the plant extracts, tissues of *A. sieberi* were collected from Tighab rangeland in Iranshahr city. Plant samples were dried in the shade before they were ground. Then, 190 g plant powder was placed in a plastic bottle. Ethanol (1 l) was poured on powder samples which were then placed on a shaker for 24 hours. The resulting solution was filtered out and the extract was obtained. After soil sieving, 3 kg of the dried soil was stored in plastic pots. Moisture contents were adjusted to 70% water-holding capacity (WHC). Seeds were disinfected with fungicide solution. In all treatments, 30 seeds of the plants were buried evenly in each pot at least 3 cm from the edge. Treatments comprised the extract of *A. sieberi* (0.2% (2 ml per 1000 ml distilled water), 0.4% extract (4 ml per 1000 ml distilled water), distilled water (control). Pots were irrigated with tap water every other day as needed and the experiment was terminating 14 days after cultivation. The parameters measured included germination percentage and rate, radicle and pedicel length, seedling dry weight, photosynthesis pigment, carotenoids, and nutrients uptake from the soil by *P. harmala*.

Seed germination was counted and recorded daily (Ebrahimi and Miri, 2016). This was done every 24 hours until the germination had been completed. On the last counting day (the 14^{th} day), the radicle and pedicle lengths and the dry and fresh weights of seedlings were measured. After 14 days when the number of the germinated seeds were fixed and the growth period terminated, the germination properties including the germination rate and percentage were measured according to Equations 1 and 2:

$$GR = \sum Ni / Di \quad (1)$$

GR: germination rate; Ni: number of germinated seeds in each day; Di: counted day (Mereddy et al., 2000).

$$GP = (n/N)\ 100 \quad (2)$$

GP: germination percentage; n: total number of the germinated seeds during counting; N: total number of the germinated seeds in each petri dish (Behbodian et al., 2005).

In each pot 10 seedlings were randomly chosen and the radicle and pedicle lengths were calculated by a caliper. Then, in order to determine the dry weight, samples were washed with distilled water. The plants were then placed in an oven (Dena-Iran) at 70 °C for 48 h and the dry weight were measured. Chlorophyll a, b, and carotenoids were measured by method of Arnon (1967).

Total chlorophyll was calculated by sum of chlorophyll a and b.

$$\text{Chlorophyll a} = (19.3 \times A663) - (0.86 \times A645)\ (v/100w) \quad (3)$$

$$\text{Chlorophyll b} = (19.3 \times A645 - 3.6 \times A663)\ V/ \quad (4)$$

$$\text{Carotenoids} = 100\ (A470) - 3.27\ (\text{mg chl.a}) - 104\ (\text{mg chl.b})/227 \quad (5)$$

V: volume of filtrated solution (upper solution of centrifuges); A: absorption of light at wave lengths of 663, 645 and 470 nm; W: wet weight of the sample (g).

Plant nutrients uptake

The wet oxidation method was used in order to measure the elements absorbed by the plant and plant samples digestion. Contents of N, P, K, Zn, and Mn were measured in the treated *P. harmala* plants (Einhelling and Leather, 1988). Mn and Zn contents in the extract were obtained from wet digestion of plant samples using atomic absorption spectrophotometer (GBC Avanta,

Australia). Nitrogen was measured by titration, by Kjeldhal method (model Gerhardt 9801/Ac), phosphorus content was obtained through the colorimetric method and a flame photometer using a spectrophotometer (model (JENWAY 640) (Rayan et al., 2001).

Statistical Analysis

Statistical analyses of the experimental data were performed using SPSS. 18. All reported results were the means of four replicates and deviations were calculated as the standard error of the mean (SEM). The statistical processing was mainly conducted by analysis of variance (ANOVA). Duncan test post hoc analysis was performed to define which specific mean pairs were significantly different. A probability of 0.05 or lower was considered as significant.

Results

Germination and morphological properties

Results of the effect of *A. sieberi* extract on germination of *P. harmala* are shown in Fig (I). As Fig. (I) suggests, *A. sieberi* extract significantly affected germination of *P. harmala* (P<0.01). The highest germination percentage (64.5%) and germination rate (23.66%) were calculated in 0.2% treatment. The lowest germination rate and percentage were related to the control treatment. Results showed significant effects of *A. sieberi* extracts (P<0.05) on the radical and pedicel length of *P. harmala* (Fig. II). The highest radicle length (1.06 cm) was calculated in the control treatment. The lowest radicle length (1.03 cm) was related to the 0.4% treatment. With increasing concentration of the extract, the pedicel length of *P. harmala* decreased. The highest pedicel length of the plant was measured in the control treatment and the minimum pedicel length was related to 0.4 % treatment (Fig. II). The extract of *A. sieberi* significantly reduced the dry weight of *P. harmala* and by increasing the concentration of *A. sieberi* extract, dry weight of *P. harmala* decreased. Maximum plant dry weight was measured in the control treatment and the lowest value was related to 0.4 % treatment (Fig. II).

Photosynthetic pigment

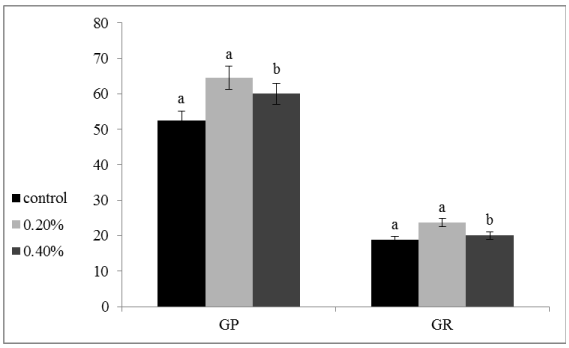

Fig. I. Effects of *A. sieberi* extract on germination rate and germination percentage of *P. harmala* seeds; vertical bars show ±SE (Standard Error)

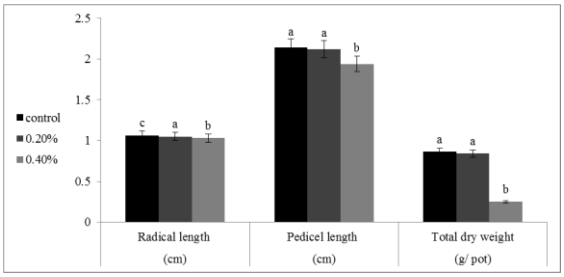

Fig. II. Effects of *A. sieberi* extract on morphological properties of *P. harmala*; vertical bars show ± SE (Standard Error)

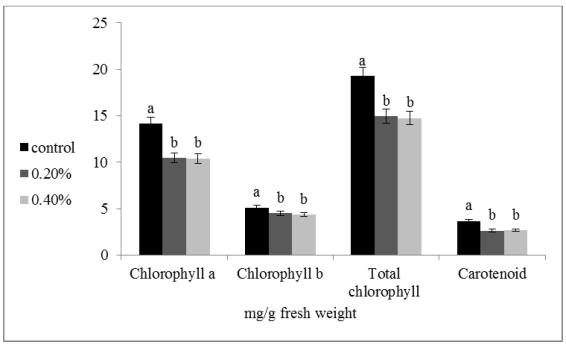

Fig. III. Effects of *A. sieberi* extract on photosynthesis pigments of *P. harmala*; vertical bars show ± SE (Standard Error)

Figure (III) shows the phytotoxic effect of *A. sieberi* extract on the photosynthetic pigments of *P. harmala* (P<0.05). *A. sieberi* extract reduced the chlorophyll contents of *P. harmala*. The highest chlorophyll a was related to the control and with increasing the concentration of *A. sieberi* extract, chlorophyll a decreased. A similar trend was observed for chlorophyll b. Maximum total chlorophyll was related to the control plants and minimum value was related to the 0.4 % treatment.

Table 2
Nutrients uptake of *P. harmala* under different *A. sieberi* extract

Extract concentration	N (%)	P (%)	K (%)	Zn (%)	Mn (%)
Control	3.29±0.2^{b}	0.31±0.02^{a}	0.42±0.03^{b}	0.19±0.01^{b}	0.18±0.01^{a}
0.2%	3.48±0.2^{a}	0.26±0.01^{b}	0.48±0.03^{a}	0.28±0.01^{a}	0.20±0.01^{a}
0.4%	3.28±0.2^{b}	0.23 ±0.01^{b}	0.21 ±0.01^{c}	0.18±0.01^{b}	0.19±0.01^{a}

*Values within a column followed by the different letters are significantly different ($p<0.05$, means ± SE).

Nutrients uptake

Table 2 shows significant effect of *A. sieberi* extract on uptake of N, P, K, and Zn by tissues of *P. harmala* ($P<0.05$). Differences in the Mn contents were not significant (Table 2). The N and Zn contents were found to be greater in 0.2% treatment compared with the control and 0.4 % treatments. However, differences in N and Zn contents were not significant between the control and 0.4 % treatments. The lowest amount of N and Zn were related to the 0.4 % treatment. The highest amount of phosphorus was related to the control treatment and with increasing concentration of *A. sieberi* extract, P content decreased. The 0.2% treatment resulted in a higher K content compared to the other treatment. The lowest amount of K content was related to the 0.4 % treatment.

Discussion

Plants grow in different ecosystems and under the influence of different factors, including the biochemicals as the vital determinants of the quantity of the plants (Ricki Maryshany, 2015; Ebrahimi et al., 2017). Allelopathy effects are most often described based on visual changes in plant (Skrzypek et al., 2015). There is a delay or inhibition of seed germination (Rassaeifar et al., 2013), growth inhibition or stimulation of plants (Uddin et al., 2014). These actions depend on the concentration of the active substances containing allelochemicals (Skrzypek et al., 2015).

Plant are more sensitive during germination than the other growth stages. In the present study, low concentration of the extract had a positive effect on germination. Because the allelopathic phenomenon much depends on the concentration of the allelochemicals, changes in the amount of these materials may lead to different inhibition and stimulation effect (Chon and Kim, 2002; Koloren, 2007; Ebrahimi et al., 2017). Allelopathy effects are selective and depend on the concentration and type of the residue and, may lead to stimulating the growth inhibitory effects in plants (Naseem et al., 2009; Ebrahimi et al., 2016).

Ismail and Chong (2002) reported that allelopathic substances at low concentrations may have positive or negative effects on the target species, but high concentrations are always a deterrent. The reason for reduction of plant germination can be related to the activity of enzymes such as amylase that plays an important role in seed germination (Bagheri and Mohammadi, 2011). Results indicated that allelopathic compounds reduce plant germination affecting hormones such as gibberellin, which is important in plant germination, as well as the activity of special enzymes such as amylase and proteinase, which are essential in the process of germination (Ghorbanli et al., 2008). Kazerooni Monfared et al. (2013), studied the germination of some weed species under the effect of *T. alexandrium* L., and reported that with increasing extract concentration, germination percentage decreased. Ghorbanli et al. (2008) reported that seed germination significantly was affected by different concentrations of extracts of *Artemisia* spp.

Results showed that with increasing the concentration of the extract, the pedicel and radicle lengths and dry weight of *P. harmala* decreased. Allelopathic compounds by having an impact on the root growth and by reducing the formation of capillary roots, reduce water absorption in plants and seedling length (Chon et al., 2002). Kazerooni Monfared et al. (2013) showed that root is the first plant part that absorbs allelopathic materials directly from the

environment, and so compared to other traits may be more affected by allelopathic materials. Reducing the radicle and pedicel lengths may be due to hormonal balance. Some mechanisms of allelopathic activities are similar to plant hormones. Allelopathic compounds through having an impact on root growth can reduce water absorption in plants and thereby reduce the length of seedling (Chon et al., 2002). Decreased seedling radicle length in the plants exposed to allelochemicals may be due to the negative effects of the extract on cell division or cell elongation. In addition to longitudinal growth of the plant, inhibiting substances in extract can have a negative impact on plants' weight (Qasem, 2001). Decreased plant growth in the presence of allelopathic compounds is associated with high mitosis stop of root and shoot meristem (Bertin et al., 2003). Samedani and Baghestani (2005) reported that the effect of different species of *Artemisia* spp extract on the root and shoot of *Avena ludoviciana* was significantly different. One reason for reduction of the plant growth during allelopathic stress is changes in mitochondrial respiration rate, which decreases the production of ATP.

Results showed that *A. sieberi* extracts had inhibition effects on photosynthesis pigments of *P. harmala*, reflecting the allelopathic potential of the plant. High concentrations of the extracts had a higher degree of inhibition. Reducing photosynthesis pigments affects seedling photosynthesis, root and shoot length, and plant vigor (Ricki Maryshany, 2015). Research has shown that decreased plant growth in the presence of allelochemicals is associated with decreased chlorophyll which in turn may be a secondary effect caused by the performance of special allelochemicals (Babu and Kandasamy, 1997; Skrzypek et al., 2015). Chlorophyll content is closely related to plant dry matter production (Skrzypek et al., 2015); therefore, reduction in chlorophyll content results in decreased photosynthesis and hence plant growth. Rice (1995) showed that water-soluble materials prevent the roots of some plants. The remains of some plants have allelopathic properties in the soil and release compounds such as phenolic acids after harvesting which has negative effects on the germination of some plants or their performance (Hoffman, 1996). Naseem et al. (2009) demonstrated that the production and releasing allelochemicals in the soil by plants can affect germination and plant growth. These effects are selective and depend on the concentration and type of residue and may lead to stimulating the growth inhibitory effects in plants. Allelopathic materials can disrupt their neighboring plants and affect the amount of photosynthesis pigments in them. The reason for reducing chlorophyll at high concentrations may be decomposition of chlorophyll and carotenoid pigments or reduction of their synthesis (Tripathi and Kori, 1999). Reactions and processes like cell division, hormone production, cell membrane stability and permeability, photosynthesis, and respiration can be raised as the purpose and effect point for allelopathic material (Hejazi, 2001).

Results showed that low concentration of *A. sieberi* extract had positive effects on uptake of N, K, Zn, and Mn. Nutrient uptake is an important factor for plant growth and development. Some studies have shown low concentrations of extract had positive effects on the nutrients uptake. The allelopathy depends on the concentration of the allelochemicals (Koloren, 2007; Ricki Maryshany, 2015). Ricki Maryshany (2015) showed that high concentrations of allelochemicals are deterrent but at low concentrations they may have positive effects on the nutrient uptake of the plants. Both increases and decreases in nutrient uptake have been reported for plants that are subject to the allelopathic conditions change. Unstable situation of minerals in receiver plants is created by leaching of plant debris, root exudates, and allelopathic debris (Bhowmik and Doll, 1984; Alam et al., 2001). It is interesting to note that these effects may be directly related to plants competition and may be indirectly done through microorganisms related to stabilization of nutrients such as nitrogen (Alam et al., 2001). Some allelochemicals prevent the minerals uptake by plant roots and the mechanism of action of these compounds is through disrupting the membrane normal actions in plant cells. Allelochemical can reduce cellular ATP content through inhibition of electron transport and oxidative phosphorylation applied, as well as changes in the membrane permeability property compared to inorganic ions uptake (Bhowmik and

Doll, 1984; Alam et al., 2001). Yu and Matsui (1997) studied the effects of root exudates of cucumbers, aromatic carboxylic acids in root exudates, and their analogues on the uptake of NO_3, H_2PO^{-4}, SO_4^{-2}, K^+, Ca^{+2}, Mg^{+2} and Fe^{+2} in cucumber seedlings. Root exudates prevent the uptake of all ions into the ion H_2PO^{-4}. Bhowmik and Doll (1984) observed that the remains of *Ambrosia artemisifolia* and *Setaria glauca* increased K uptake by 21-48% in *Zea mays*.

In the present study, germination, growth, and nutrients uptake of *P. harmala* were affected by *A. sieberi* extract. By increasing the concentration of *A. sieberi* extract, germination and growth of *A. sieberi* decreased. This could be due to an increased amount of allelopathic materials. However, at lower concentrations of the extract, a positive effect was observed on the uptake of nutrients. In the present study, the extract was not analyzed but results of different studies showed that some compounds could be introduced as inhibitory factors of the studied characteristics of *P. harmala*. Given the negative impact of *A. sieberi* on growth of *P. harmala*, it is recommended not to cultivate the two plants together if we consider *P. harmala* as a medicinal plant.

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