ECOPERSIA

DOI:10.18869/modares.Ecopersia.4.3.1493 **2016, 4 (3): 1493-1503**

Effect of Extract of Fast Growing Species *Trifolium alexandrium* L. on Germination, Photosynthetic Pigments and Nutrient Uptake of *Prosopis cineraria* (L.) Druce

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Received: 28 June 2016 / Accepted: 13 September 2016 / Published Online: 30 September 2016

ABSTRACT In a completely randomized design, the effect of the extract from *Trifolium alexandrium* (0, 0.2 and 0.4%) on the germination, some morphological characteristics, photosynthetic pigments, and nutrients uptake of *Prosopis cineraria* was evaluated. The highest germination rate and percentage (9.16 and 21%, respectively) were recorded in the 0.2% treatment. The maximum and minimum lengths of radicle (6.58 and 3.16 cm) and pedicel (6.56 and 14.23 cm), and dry weight were recorded in the control and 0.4% treatments, respectively. The highest level of chlorophyll a (16.80 mg g^{-1} fresh weight) was found in the control. The highest and lowest chlorophyll b (9.65 and 7.96 mg g^{-1} fresh weight) were measured in the 0.2%and control, respectively. The extract adversely affected the nutrient uptake by *P. cineraria*, the minimum and maximum of which were recorded at the 0.4%and control treatments, respectively. In general, although the extract of *T. alexandrium* increased the *P. cineraria* germination in the 0.2% treatment, the increased concentrations led to its reduced growth and nutrient uptake. Therefore, the simultaneous cultivation of these two species in the dry lands is not recommended.

Key words: *Allelopathy, Plant growth, Plant extract, Photosynthetic pigments*

1 INTRODUCTION

Allelopathyis one of the important issues in rehabilitation of rangelands, which has received less attention (Gholami *et al*., 2011). Allelopathic effects, which can be seen in different plants species (Ricki Maryshany *et al*., 2015), is a form of plant interference by means of interaction of plants with their released chemicals (Trezzi *et al*., 2016), which play an important role in biodiversity and ecosystem functions (Iman and Zakaria, 2006). Various compounds in plants can affect various aspects of plant communities (Narwal, 2004; Iman and Zakaria, 2006). Such compounds are classified as secondary plant materials or sub-materials of metabolic pathways of plants (Narwal, 2004), which are derived from the branches and leaves or secreted by the roots to the environment (Naseem *et al*., 2009).Chemicals released by plants can act as natural herbicides or pesticides by controlling weeds (Narwal, 2004). Allelopathic studies on plants can, therefore, lead to discovery of new natural herbicides and growth inhibitors.

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There are more than 8000 plant species in Iran (Mozaffarian, 1996) with limited studies on their allelopathic properties. Leguminosae include important flowering plants with a wide distribution (Rechinger, 1984). *Prosopis cineraria* and *Trifolium alexandrinum* are two legume species found in Iran. *P.cineraria* is a native of the arid environments of several Western Asiacountries (Bohra and Ghosh, 1980) and also found in extreme conditions with an annual rainfall as low as 15 cm and high alkaline and saline soils (Rechinger, 1984). The seed of *P. cineraria* has a hard germination (Manga and David, 1995; Sacheti and Al-Areimi, 2000). *T. alexandrinum* is a fast growing annual legume and one of the most important forage crops in the Mediterranean and the Middle–Eastregions (Hackney *et al*., 2007).

The most important strategy for reclamation and conservation of rangeland ecosystems is selecting suitable plant species. However, even with most adaptable species to be used for the rangeland, the improvement projects are likely to fail, if *allelopathy* properties are neglected (Bagheri and Mohammadi, 2011). Studies have shown that the germination and growth of *Chenopodium album* decreased under the allelopathic effect of *Atriplex nummulariat* stem extracts (Bouchikh-Boucif *et al*., 2014). Sadaqa *et al*. (2010) also reported that the extract of *Amaranthus graesizans* had reduced the shoot and root dry weight of *Allium cepa* by 94% and 96%, respectively. The negative impacts of *Artemisia sieberi* on seed germination, stem and root of three important rangeland species has also been reported (Bagheri and Mohammadi, 2011). Similarly, Kazerooni Monfared *et al*. (2013) reported the negative effects of the aqueous extract of *T. alexandrium* shoot on seed germination of some weed species, and Hejazi *et al*. (2004) reported *T. alexandrium* extract decreased the growth of *Heliantusannus*. In the present study, the effect of *T. alexandrium* extract on the germination, morphological characteristics and nutrient uptake of *P. Cineraria* was studied, since it is one of the most important species in southern Iran and it has hard germination. Our hypothesis was that *T. alexandrium* extract acted as an enhancing growth on the germination, growth and nutrient uptake of *P. cineraria*.

2 MATERIALS AND METHODS

2.1 Plant extraction

The collected *T. alexandrium* samples were dried in the shade and ground to powder. Then, 190 g of the powder was put in a plastic bottle, then filled with 1 L ethanol and placed on a shaker for 24 hours. The resulting solution was filtered out and the extract was obtained using maceration method.

2.2 Preparation of pots

Soil was collected from the depths of 0–30 cm with a 5.5 cm diameter corer, then mixed, sieved to 4 mm, and its moisture content adjusted to 70% water–holding capacity (WHC).Soil characteristics were determined (Table 1); for texture, laser diffractometry was used(Wang *et al*., 2012), pH was measured in a 1:5 soil to distilled water slurry after 1 hour of agitation(Thomas, 1996),using a digital pH–meter (Model 691, Metrohm AG Herisau, Switzerland); for electrical conductivity (ECe), an EC–meter (DDS-307, Shanghai, China)was used (Rhoades, 1996);for the available phosphorus (AP) and potassium (AK), the methods of Bray and Kurtz (1954) andthe flame photometry (Knudsen *et al*., 1982) were applied, respectively; the total N was analyzed calorimetrically using of Kjeldahl (Bremner, 1996).

[DOR: 20.1001.1.23222700.2016.4.3.4.9]

Table 1 General characteristics of the soil used in the experiment

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

2.3 Germination medium

Plastic pots (diameter10× diameter 15× height 45 cm) were filled with the sieved and dried soil. After disinfecting the seeds of *P. cineraria* with fungicide solution, 30seedswere buried evenly throughout each pot, at least 3 cm from the edge. The pots were treated every two days with the extract of *T. alexandrium* (ml/L of distilled water) at concentrations of 0.2%, 0.4%, and 0% (only distilled water as control).The work was conducted in four replications under the greenhouse conditions (temperature 23±5°C, humidity 60%). The pots were irrigated with tap water as needed and the experiment was terminated 14 days after cultivation. The parameters measured included germination percentage and rate, radicle and pedicel length, seedling dry weight, photosynthetic pigments, and nutrient uptake by *P. cineraria*.

2.4 Calculation of plant properties

Germinated seeds were counted daily until the germination had been completed (Farajollahi *et al*., 2012; Ebrahimi and Miri, 2016). On the last counting day (the 14^{th} day), the radicle and pedicle lengths of 10 randomly selected seedlings from each pot were measured with a caliper, then washed with distilled water and placed in an oven (Dena-Iran) at 70 ºC for 48 hr for drying, the weight of which was also measured. The germination rate and germination percentage measured according to Eqs. 1 and 2.

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

Where GR, Ni and Di were germination rate, number of germinated seeds in each day and: counted day, respectively (Maguire, 1962).

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

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

To measure chlorophyll a and bcontent, totalchlorophyll, and carotenoids, 100 mg of fresh tissue was pulverized inside a porcelain mortar with 5 ml of 80% acetone, then centrifuged. The solution was transferred to centrifuge tubes, and the remnant in the mortar was washed twice with 5 ml of 80% acetone, the solution of which was added to the tubes. Then, the tubes were centrifuged for 10 min at 6000 rpm, the solution of which was transferred to a 250 mm flask, and its volume was adjusted to 25 ml with 80% acetone. Chlorophyll contents was read at wavelengths of 470, 663, 645 nm, using spectrophotometer (WPA–S2000) (Arnon, 1967).The contents of chlorophyll a, b and carotenoids wereestimated according to theEqs.3, 4, 5. Total chlorophyll was calculated by sum of chlorophyll a and b in terms of milligrams per gram of sample weight (Arnon, 1967).

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

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

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

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

[DOR: 20.1001.1.23222700.2016.4.3.4.9]

2.4 Calculation of plant nutrient uptake

The wet oxidation method was used for the samples digestion to measure the absorbed elements. For this purpose, 0.3 g of the plant was transferred to digestion pipes, then 2.5 ml of a mixture of sulfuric acid, salicylic acid, selenium and hydrogen peroxide was added. The sample was shaken well and left for2hrs, then heated for 2 hr at 100 °C, and cooled. Then, one ml hydrogen peroxide was added and heated to330 °C until the digestion was over when the extract turned colorless or pale yellow, which lasted for almost 2 hrs. After drying, 48.3 ml of distilled water were added to the pipes and, stirred. The next day, the stirring operation was repeated, and was put to itself to be deposited. Then, the elements of N, P, K, Zn, Mn were measured (Einhelling and Leather, 1988). The amount of Mn and Zn were measured using atomic absorption spectrophotometery (GBC Avanta, Australia). Nitrogen was measured usingKjeldahlmethod (Gerhardt 9801/Ac), phosphorus through the colorimetric method by a spectrophotometer (JENWAY 640), and K was measured using a flame photometer (Ryan *et al*., 2001).

2.5 Statistical analysis

The data were statistically analyzed using the SAS 8.1. The statistical processing was mainly conducted by analysis of variance (ANOVA), and the normality of data was tested using Kolmogorov–Smirnov. When needed, log transformation of the data was conducted to achieve normality. Equality of variance among treatments was tested using Levene's test for homogeneity of variance. Duncan test post hoc analysis was performed to define which specific mean pairs were significantly different.

3 RESULTS

3.1 Effect on germination and morphological properties

The extract of *T. alexandrium* significantly affected the germination rate and percentage of *P. cineraria*, the highest of which were recorded for the 0.2% treatment, while the lowest were found in the control treatment (Table 2).

The extract of *T. alexandrium* also significantly affected the radicle and pedicle lengths of *P cineraria*. Increased concentration of the extract was associated with the decreased lengths of the radicle and pedicle (Table 2). The highest radicle and pediclelengths were recorded in the control treatment, while the minimum lengths were found in the 0.4% extract treatment. The dry weight of *P. cineraria* was also significantly reduced with the increased concentration of the extract. The maximum dry weight was measured in the control treatment, while the lowest dry weightwas found inthe 0.4% treatment (Table 2).

Table 2 Germination and morphological properties of *P. cineraria*treated with *T. alexandrium* extract

Extract (%)	Germination rate	Germination percentage	Radicle length (cm)	Pedicle length (cm)	Total dry weight (g pot^{-1})
Control	7.00 ±0.51c	33.3±2.50c	6.58±0.40a	14.23±1.20a	0.86±0.01a
0.2	21.00±2.22a	91.6±4.3a	5.23±0.40b	12.27±1.75a	0.76±0.01b
0.4	12.00 ±1.13b	58.3 ±2.30b	3.16±0.30c	6.56±1.09b	0.54±0.02c

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

[DOR: 20.1001.1.23222700.2016.4.3.4.9]

3.2 Effect on photosynthetic pigment

The photosynthetic pigments of *P. Cineraria* were affected under *T. alexandrinum* extract treatment, however inconspicuously. The chlorophyll was inversely affected with the increased extract concentrations, so that the lowest level of chlorophyll a was recorded in the 0.4% extract treatment (Table 3), which was significantly different from the control treatment. The highest level in chlorophyll b was found in the 0.2% treatment, followed by the 0.4%. Overall, the highest level in the total chlorophyll was recorded in the 0.2% treatment. The highest and lowest levels of carotenoids were related to 0.4%and the control treatments, respectively (Table 3).

Table 3 Photosynthetic pigments of *P. cineraria* treated with *T. alexandrium* extract

Extract (%)	Chlorophyll a (mg g^{-1} fresh weight)	Chlorophyll b (mg g^{-1} fresh weight)	Total Chlorophyll (mg g^{-1} fresh weight)	Carotenoid (mg g^{-1} fresh weight)
Control	16.80±2.40a	7.96±1.10c	24.577±1.40b	1.18±0.09b
0.2	15.56±2.31a	16.79±1.43a	32.34±2.40a	1.27±0.09b
0.4	12.48 ±2.01b	9.65 ±1.40b	22.13±2.30b	3.51±0.50a

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

3.3 Effect on nutrient uptake

The extract of *T. alexandrium* affected the nutrient (N, P, K, Zn and Mn) uptake by *P. cineraria*, although inconspicuously (Table 4). Differences in N, Mn and Zn contents were non–significant between the control and 0.4% treatments. Potassium content showed non–significant difference between the 0.2 and 0.4% treatments. In general, the maximum and minimum nutrient uptakes were recorded in the control and 0.4% extract treatments, respectively.

Table 4 Nutrient uptake by *P. cineraria* treated with *T. Alexandrium* extract

Extract (%)	N (mg g^{-1})	P (mg g^{-1})	K (mg g^{-1})	Zn (mg g^{-1})	Mn (mg g^{-1})
Control	2.75±0.03b	0.43±0.03a	0.43±0.01a	0.13±0.01b	0.17±0.01b
0.2	3.53±0.03a	0.30±0.03b	0.23±0.00b	0.16±0.01a	0.18±0.01a
0.4	2.86 ±0.03b	0.23 ±0.00c	0.21±0.00b	0.12±0.01b	0.15±0.01b

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

4 DISCUSIONS

Decomposition of plant in the soil have been shown to have allelopathic effects by releasing compounds such as phenolic acids that can have negative effects on the germination or growth performance in some plants (Purvis *et al*., 1985; Hoffman *et al*, 1996; Naseem *et al*., 2009). These effects are selective and depend on the concentration and type of residue and, may lead to stimulating the growth inhibitory effects in plants. Low concentration of *T. alexandrium* extract had a positive effect on the germination of *P. cineraria*. Because the allelopathic phenomenon much depends on the concentration of the allelochemicals, and any change in the amount of these materials leads to

[DOR: 20.1001.1.23222700.2016.4.3.4.9]

different inhibition and stimulation effect (Chon and Kim, 2002; Koloren, 2007). It is interesting to note that allelopathic substances in low concentrations may have positive or negative effects on the target species, but high concentrations are always associated with ambiguous changes (Ismail and Chong, 2002). The reason for reduced germination at 0.4% extract can be related to the enzyme activities such as amylase that plays an important role in seed germination (Soltanipor *et al*., 2007; Bagheri and Mohammadi, 2011). The results indicate that allelopathic compounds may but not sure reduce plant germination with effect on hormones, such as gibberellin, which is important in plant germination, as well as the effect on the activity of special enzymes, such as amylase and proteinase, which are essential in the process of germination. In addition, reduction in the germination stage may be attributed to change in the activity of enzymes that affect the transfer of storage compositions during germination. Inhibiting substances secreted from different plant organs, may result in the accumulation of phenolic compounds and reduced germination percentage (Ghorbanli *et al*., 2008).

Accumulation of phenolic compounds is considered as a defense response against biotic and abiotic stresses that reduces the plant growth and germination (Hirt and Shinozaki, 2004). For example, germination percentage of some weeds species (Kazerooni Monfared *et al*., 2013) and sunflower (Hejazi *et al*., 2004) were significantly affected hen treated with the extract of *T. alexandrium*. In addition, Ghorbanli *et al*. (2008) reported that germination percentage of *Avenalodoviciana* was significantly affected by the extract of *Artemisia* extract.

SafwanIshak *et al*. (2016) found decreased germination, root growth and fresh weight of some plants with increased concentrations of extract from *L. leucocephala*.

The results of the present study showed that increase in the concentration of *T. alexandrium* extract was associated with decrease in lengths of the radicle and pedicle as well as reduced dry weight of *P. cineraria*. The reduced radicle and pedicle lengths may be due to hormonal balance and, reduced shoot growth. Some mechanisms of allelopathic activities are similar to plant hormones. *Decreased* seedlings' length of plant which are exposed to allelopathic compounds may be due to the negative effect of the extract on cell division or cell elongation, which in addition to longitudinal growth of the plant, inhibiting substances in extract can have a negative impact on weight plant (Qasem, 2001). Root is the first part that absorbs allelopathic materials directly from the environment, and so compared to other traits may be more affected (Kazerooni Monfared *et al*., 2013). This is due to the fact thatthe impact on root growth reduces water absorption and, thereby, reduces the seedlings' length (Chon *et al*., 2002). Reductions in the root growth under allelopathic stress in some plants have reportedly been higher than the shoot growth (El-Khatib*et al*., 2016). Increased antagonistic effects as the result of increase in the extract concentration have also been reported (Saraei*et al*., 2011; Samedani and Baghestani, 2005; Gong *et al*., 2016).

One reason for the reduced growth rate during allelopathic stress has been attributed to changes in the mitochondrial respiration rate that, in turn, decreases the ATP production, which can cause changes in other cellular processes, such as ion adsorption and growth. Decreased plant growth in the presence of allelopathic compounds is associated with the reduced mitosis of root and shoot's meristem cells and therefore the length of root and shoot will be reduced (Bertin *et al*., 2003).

The effects of allelopathic materials on the photosynthetic pigments have already been demonstrated (Hejazi, 2001; Ricki Maryshany

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et al., 2015).In the present study, *T. alexandrium* extract showed negative effect on the contents of photosynthetic pigments in *P. cineraria*. The reason for the reduced chlorophyll contents at high concentrations may be decomposition of chlorophyll and carotenoid or their reduced synthesis (Chaniago *et al*., 2006; Ricki Maryshany *et al*., 2015). The decreased chlorophyll contents as the result of allelochemical may be a secondary effect (Babu and Kandasamy, 1997).

Nutrient uptake is an important factor for plant growth and development, the rate of which can be affected by the accumulation of allelopathic factors (Mallik, 2005; Mohammaddoust Chamanabad *et al*., 2014). Both increases and decreases in nutrient uptake have been reported for plants that are subjected to the allelopathic conditions. Unstable situation of minerals in receiver plants is created by leaching of plant debris, root exudates and allelopathic debris, the effects of which may directly be related to plants competition, and indirectly through microorganisms that stabilize the nutrients (Alam *et al*., 2001). Special allelochemical (flavonoids and phenolic acids) prevent the minerals uptake through disrupting the normal actions of membrane in the root cells. Allelochemical can reduce cellular ATP content through inhibition of electron transport and oxidative phosphorylation, which are two mitochondrial membrane actions, as well as change the membrane permeability to inorganic ions uptake (Bhowmik and Doll, 1984). Yu and Matsui (1997) found that the root exudates of cucumbers and their analogues prevented the uptake of $H_2PO^{-4}$by the seedlings. Mohammaddoust Chamanabad *et al*. (2014) reported that fresh and dry extract of *Sinapisarvensis* and *Cirsiumarvense* increased K concentrate in the tested plant.

5 CONCLUSION

In the present study, extract of *T. alexandrium* influenced all traits of *P. cineraria*. However, the lower concentration had positive effect on the germination percentage. Given the positive impact of *T. alexandrium* on increasing germination of *P. cineraria*, the former plant can be used to increase the germination of *P. cineraria* that has germination problem. But given the negative impact of *T. alexandrium* on the growth and nutrient uptake in of *P. cineraria*, cultivation of two plants is not recommended in arid rangelands.

6 ACKNOWLEDGEMENTS

The assistance of Department of Range and Watershed Management and the laboratory of Soil and Water Engineering Faculty (University of Zabol) is gratefully acknowledged.

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[DOR: 20.1001.1.23222700.2016.4.3.4.9]

تاثیر عصاره گونه سریع‌الرشد *Trifolium alexandrium* L. در جوانه‌زنی، رنگیزه‌های فتوسنتزی و جذب عناصر

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تاریخ دریافت: ۸ تیر ۱۳۹۵ / تاریخ پذیرش: ۲۳ شهریور ۱۳۹۵ / تاریخ چاپ: ۹ مهر ۱۳۹۵

چکیده: در یک طرح کاملا تصادفی با چهار تکرار، تاثیرعصاره *Trifolium alexandrium* در مقادیرصفر (کنترل)، ۰/۲، ۰/۴ درصد بر جوانه‌زنی، برخی خصوصیات موفولوژیک، رنگیزه‌های فتوسنتزی و جذب عناصر غذایی توسط *Prosopis cineraria* بررسی شد. بیش‌ترین درصد و سرعت جوانه‌زنی (به‌ترتیب ۹/۱۶ و ۲۱ درصد) در غلظت ۰/۲درصد محاسبه شد. بیش‌ترین و کم‌ترین طول ریشه‌چه (۶/۵۸ و ۳/۱۶ سانتی‌متر)، ساقه‌چه (۱۴/۲۳ و ۶/۵۶ سانتی‌متر) و وزن خشک گیاه به‌ترتیب در تیمار شاهد و غلظت ۰/۴درصد اندازه‌گیری شد. بیش‌ترین مقدار کلروفیل a (۱۶/۸۰ میلی‌گرم بر گرم وزن تر) مربوط به شاهد بود. بیش‌ترین و کم‌ترین کلروفیل b (۹/۶۵ و ۷/۹۶ میلی‌گرم بر گرم وزن تر) در تیمار ۰/۲ درصد و تیمار شاهد اندازه‌گیری شد. عصاره در مقایسه با تیمار شاهد باعث کاهش جذب نیتروژن، پتاسیم، فسفر، روی و منگنز توسط بافت‌های *P. cineraria* گردید. کم‌ترین و بیش‌ترین مقدار جذب عناصر غذایی به‌ترتیب در غلظت ۰/۴ عصاره و تیمار شاهد به‌دست آمد. به‌طور کلی هرچند عصاره*T. Alexandrium* در غلظت ۰/۲ درصد باعث افزایش جوانه‌زنی *P. cineraria* شد، اما افزایش غلظت، منجربه به کاهش رشد و جذب عناصر غذایی گردید و کشت این دو گونه گیاهی باهم در مراتع خشک توصیه نمی‌شود.

کلمات کلیدی: دگرآسیبی، رشد گیاهان، عصاره گیاهی، رنگدانه‌های فتوسنتزی

[DOR: 20.1001.1.23222700.2016.4.3.4.9]