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Effects of salicylic acid foliar application on morpho-physiological traits of purslane (*Portulaca olaracea* L.) under salinity stress conditions

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Abstract
Purslane, *Portulaca olaracea*, is an annual plant used as potherb and traditional medicine. A factorial experiment was carried out to investigate the effects of different levels of salicylic acid (0.0, 0.5 and 1.0 mM) on growth characteristics and photosynthetic pigments of purslane under salinity stress (0.0, 40, and 80 mM). The treatment combinations were arranged in a completely randomized design with three replications. The measured traits were as follows: root and shoot dry weights, root and shoot lengths, leaf length and width, relative water content, number of leaves, total chlorophyll, chlorophyll a, b and carotenoids. Results showed that salinity had negative and decreasing effects on the majority of morphological traits and relative water content, whereas SA increased the growth and pigment contents of the purslane plant. For example, application of salicylic acid (1 mM) increased the content of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids from 0.43, 0.23, 0.65 and 4.49 mg/g under normal condition to 0.65, 0.46, 1.11 and 8.45 mg/g under saline condition, respectively. In conclusion, application of SA as a foliar agent improved the detrimental effects of salinity stress.

Keywords: Medicinal plants; Photosynthetic pigments; *Portulaca olaracea* L.; Salicylic acid; Salinity stress; Tolerance**.**

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Introduction

Purslane, *Portulaca olaracea*, belongs to the Portulacaceae family. This herb is a rich source of vitamins, flavonoids, alkaloids, polysaccharides, omega-3 fatty acids (especially alpha-linolenic and gamma-linolenic acids), terpenoids, sterols, proteins and minerals and has also been reported as the super food for the future (Chugh *et al.* 2019). It has been used as a folk medicine in many countries, acting as a diuretic, febrifuge, antiseptic and antispasmodic (Lee *et al.* 2012).

Salinity stress is one of the important environmental stresses worldwide that affects plant growth and yield. In contrast to some other stresses faced by the plant in part of the growth period, salinity stress influences the whole growth period through osmotic and ionic stresses (Agarwal *et al.* 2015). Salinity not only reduces the production of agricultural crops, but also affects the physical and chemical properties of soils. As a result, the fertility of cultivated lands is lost partially or completely. Salinity affects ionic balance, osmosis and metabolism of the plant by changing salts concentrations in the plant growth medium. In addition to the above consequences, salinity stress also leads to oxidative effects similar to other abiotic stresses (Chookhampaeng 2011).

Salicylic acid (SA), which is produced by the root cells, is related to a group of phenolic compounds. SA regulates various physiological processes such as germination, ion absorption, photosynthesis and plant growth (El-Tayeb 2005).

It is also an important intermediate molecule in the plant reaction against environmental stresses (Senaratna *et al*. 2002). SA is one of the elicitors producing secondary metabolites through physiological pathways and thus increase the amount of antioxidants (Ghasemzadeh *et al.* 2012). SA has been shown to significantly reduce ionic leakage and accumulation of toxic ions in plants (Zhou *et al.* 2009), and to mitigate the impacts of environmental stresses through the increase of growth regulating hormones, such as auxins and cytokinins (Shakirova *et al*. 2003).

Considering that salinity stress is one of the major limiting factors of agricultural production in most regions of the world, including Iran, and the use of plant growth regulators could be beneficial in improving and eliminating the effects of salinity stress, this research investigated the effect of foliar application of SA under salinity on morpho-physiological characteristics of the purslane plant.

Materials and Methods

This study was conducted in October 2017 under greenhouse conditions (12 hours of light, temperature of 18-25 ^{o}C, relative humidity of 60-80%) at Shahid Bakeri Higher Education Center (Miandoab, Iran). The effect of SA at three concentrations (0.0, 0.5 and 1.0 mM) and salinity at three levels (0.0, 40 and 80 mM; EC= 0, 3.6 and 7.2 ds/m) on growth characteristics and photosynthetic pigments (chlorophyll a, chlorophyll b, total carotenoids) was examined using completely randomized design with three replications.

The purslane seeds were collected from the local population of Miandoab city, Iran. Before starting the experiment, seeds were disinfected with 5% sodium hypochlorite for one minute, and then washed with distilled water three times to remove the remaining disinfectant from the seed surface. Then, the disinfected seeds were planted in plastic pots (depth 24 cm, diameter 20 cm). The soil consisted of 1/3 clay, 1/3 manure, and 1/3 sand, which were disinfected under sunshine before mixing. The initial EC of the soil was 5.1 ds/m. The results of the soil analysis are shown in Table 1.

Twenty seeds were planted in each pot, and salinity (NaCl) was applied at three levels (0.0, 40 and 80 mM). SA (0.0, 0.5 and 1.0 mM) was sprayed twice (at the fourth leaf stage and three week later).

Measurement of morpho-physiological traits

The measured growth characteristics were as follows: root dry weight, shoot dry weight, root length, shoot length, leaf length, leaf width, leaf relative water content (RWC) and leaf number. Chlorophyll and total carotenoids were assessed based on Arnon *et al.* (1959), through extraction with 80% acetone and reading the solution absorbance by a spectrophotometer at 645, 663 and 470 nm wavelengths using the following formulae:

Table 1. Chemical properties of the soil used in this study.

pH	EC	CL	Na	HCO3
7.67	5.08 (ds/m)	21.65 (me/l)	21.11 (me/l)	4.01 (me/l)

$$\text{Chlorophyll a} = [12.7\ (A663) - 2.69\ (A645)] \times V/W1000$$
$$\text{Chlorophyll b} = [22.9\ (A645) - 4.68\ (A663)] \times V/W1000$$
$$\text{Total chlorophyll} = [20.2\ (A645) + 8.02\ (A663)] \times V/W1000$$
$$\text{Total carotenoids} = [1000\ A470 - 1.9\ Ca - 63.14\ Cb]/214$$

where, V = Volume of filtered solution (supernatant from centrifugation), A = Optical absorbance at wavelengths of 663, 645 and 470 nm and W = Fresh weight (g) of the sample.

Statistical analysis

After analysis of variance, means were compared by Duncan's multiple range test at 5% probability level. Data were analyzed by the SPSS software.

Results and Discussion

Although we used three salinity levels (0, 40 and 80 mM) in this investigation, but purslane did not tolerate the 80 mM NaCl plus the initial EC of 5.1 ds/m in the soil, and all plants died under this condition. Therefore, the data were analyzed at two salinity levels of 0 and 40 mM. Results of the analysis of variance showed that salinity and SA significantly affected all traits under study, except root length and leaf width. For leaf width, only the effect of SA was significant. The interaction of salinity × SA was also significant for all traits, except leaf length and leaf width (Table 2).

Our results show that salinity had a negative effect on all morphological traits (except root length and leaf width) and RWC of purslane (Table 3, Figures 1-5). SA is an important intermediate molecule in reaction of plants against environmental stresses. Treatment of purslane by SA played a positive role in relation to all traits under study (Table 3, Figures 1-5). This result is probably due to the effect of SA on elongation and cell division within the plant meristem resulting in growth improvement (Shakirova *et al.* 2003).

Root length of purslane increased under 40 mM salinity with rising SA concentrations, so that 1 mM SA spray led to a significant increase in root length as compared to the control (Figure 1A). Considering the initial EC of 5.1 ds/m in the soil, purslane plant had the salinity tolerance threshold of 40 mM in this soil and the higher concentration of 80 mM destroyed the plant.

Shoot length of purslane dropped with increasing the salinity level. However, SA at the concentration of 0.5 mM elevated the shoot length as compared to the control (Table 3). Although the mechanism by which SA increases root and shoot growth in plants is not well understood, it is assumed to regulate the elongation and cell division together with other substances as auxins (Shakirova *et al.* 2003). Root dry weight decreased with rising the salinity level. SA at 40 mM salinity raised root dry weight significantly as compared to the control (Figure 2A). Salinity stress reduced the shoot dry weight of purslane. Also, spraying of 0.5 mM SA elevated the shoot dry weight of purslane plants as compared with the control plants (Figure 2B). Salinity stress prevents water absorption and reduces the water potential for cell turgor, thereby, lowers the plant weight. According to Khorsandi *et al.* (2010), increasing salinity level reduced the plant height, shoot number and length, stem

Table 2. Analysis of variance of effect of salinity (S) and salicylic acid (SA) on morpho-physiological traits of purslane (*Portulaca olaracea* L.).

SOV	df	Mean squares											
		Root length	Shoot length	Root dry weight	Shoot dry weight	Relative water content	Leaf length	Leaf width ×100	Leaf number	Chl a ×100	Chl b ×100	Caroten-oids	Total Chl ×100
S	1	6.93	211**	3.38**	18.8**	216**	1.09**	0.4	648**	1.2**	1.3**	1.99**	4.6**
SA	2	2.77	9.7**	0.23**	3.28**	30.9**	0.342*	6.8*	1042**	4.9**	4.7**	15.7**	17**
S × SA	2	6.63*	3.9**	0.05*	0.11*	9.5**	0.105	2.2	58.5**	1.5**	0. 9**	3.54**	4.3**
Error	12	1.62	1.07	0.01	0.019	0.51	0.051	1.3	5.9	0.03	0.01	0.026	0.05

SOV: sources of variation; * and **, significant at 5% and 1% levels of probability, respectively; Chl a: Chlorophyll a; Chl b: Chlorophyll b; Total Chl: Total chlorophyll.

Table 3. Shoot length, leaf length and leaf width of purslane (*Portulaca olaracea* L.) as affected by NaCl salinity and salicylic acid.

Factor	Level	Shoot length (cm)	Leaf length (cm)	Leaf width (cm)
Salinity	0 mM	28.62 a	3.81 b	1.72 a
	40 mM	21.78 b	4.31 a	1.69 a
Salicylic acid	0 mM	24.51 b	3.79 b	1.59 b
	0.5 mM	26.67 a	4.20 a	1.80 a
	1 mM	24.43 b	4.20 a	1.74 a

The same letters in each column for each factor indicate non-significant difference at p≤ 0.05 (Duncan multiple range test).

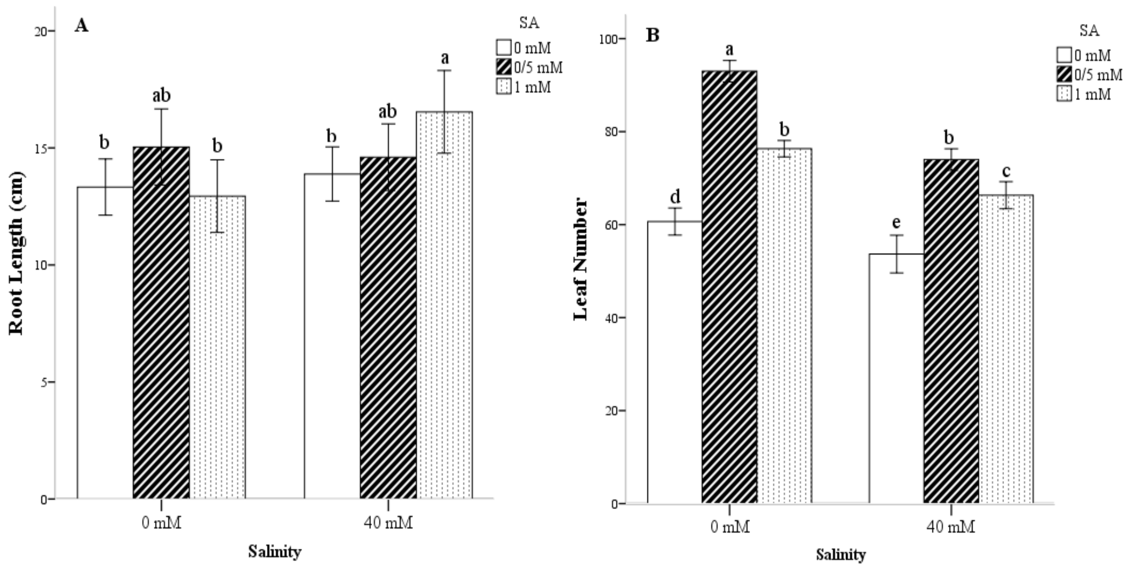

Figure 1. Effects of different concentration of salicylic acid on the root length (A) and leaf number (B) of *Portulaca olaracea* under salt stress; Means for each trait that followed by the same letter(s) are not significantly different (p≤ 0.05) by Duncan's multiple range test.

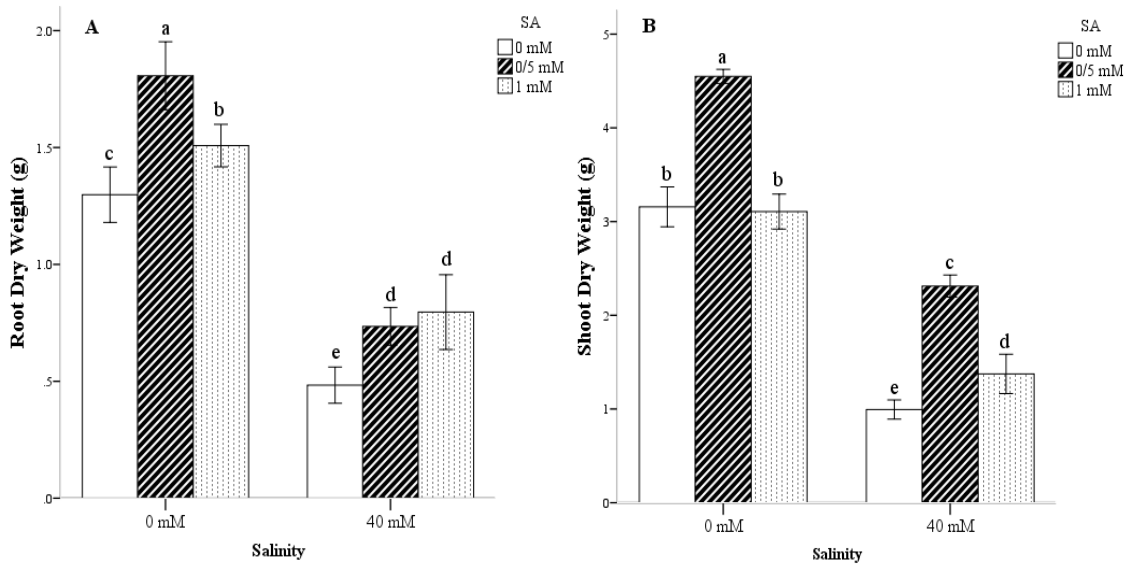

Figure 2. Effects of different concentration of salicylic acid on the root dry weight (A) and shoot dry weight (B) of *Portulaca olaracea* under salt stress; Means for each trait that followed by the same letter are not significantly different (p≤ 0.05) by Duncan's multiple range test.

diameter, internode interval and the fresh and dry vegetative yield in Agastache (*Agastache foeniculum* Kuntz.). Significant decrease in root dry weight of bean plants was reported by Ashraf and Bashir (2003) under the salinity stress. Dry weight loss can result from reduced leaf area and decreased photosynthesis rate due to biochemical constraints caused by water shortages, including reduction of photosynthetic pigments, especially chlorophyll (Lawlor 2002). On the other hand, SA increased the dry weight of corn seedlings under salinity stress conditions (Khodary 2004). Lawlor (2002) stated that the use of SA at non-salinity condition elevated dry weight of aerial parts.

Leaf length rose with salinity as opposed to the control (Table 3); however, this increase is not justified and can be attributed to measurement and/or sampling error. Contrarily, salinity reduced leaf number in this experiment (Figure 1B). Khorsandi *et al*. (2010) also reported a decrease in leaf number and leaf area by increasing the salinity level in the Agastache plant. SA increased purslane leaf length and leaf width as compared to the control plants (Table 3). Furthermore, application of 0.5 mM SA increased leaf number significantly under both normal and saline conditions (Figure 1B).

Salinity reduced leaf RWC in the purslane plant; however, SA increased RWC at both normal and saline conditions (Figure 3). The highest effect of SA on RWC under saline condition was observed at 0.5 mM concentration, indicating its positive impact on improving resistance to salinity stress. The decreased RWC content due to salt stress results from reduced water absorption by roots on one hand, and increased water transpiration through the leaves on the other hand, ultimately leading to the leaf stomatal closure. The elevation in RWC by the SA treatment can be attributed to the role of this substance in improving

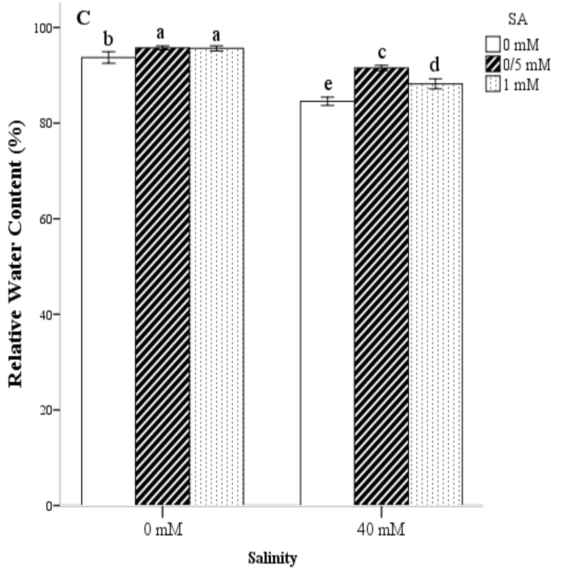

Figure 3. Effects of different concentration of salicylic acid on the relative water content of *Portulaca olaracea* under salt stress; Means that followed by the same letter are not significantly different (p≤ 0.05) by Duncan's multiple range test.

antioxidant defense system, reducing the oxidative stress, increasing the membrane stability, and synthesis of some protective substances against stresses (Janda *et al.* 1999; Shakirova *et al.* 2003).

The greatest effect of SA on chlorophyll a, chlorophyll b, total chlorophyll and carotenoids was obtained under 1.0 mM concentration (Figures 4 and 5). This great effect has caused the increased mean of these pigments at 40 mM NaCl on the average of three SA concentrations as compared to the normal condition. Previous studies reported the reduced photosynthesis of plants under salinity stress conditions, which was attributed to the reduction of chlorophyll content, increase in chlorophyll fluorescence, stomatal closure (Ashraf 2004), reduction of carboxylase activity, and higher chlorophyllase activity (Abd El-Aziz *et al*. 2006). Salinity stress in the fleawort plant was shown to reduce chlorophyll content (Heidari *et al*. 2011). On the other hand, application of SA has increased the chlorophyll content in canola (*Brassica napus*) (Baghai *et al*. 2002) and the carotenoids content in saffron (Tajik *et al.* 2015).

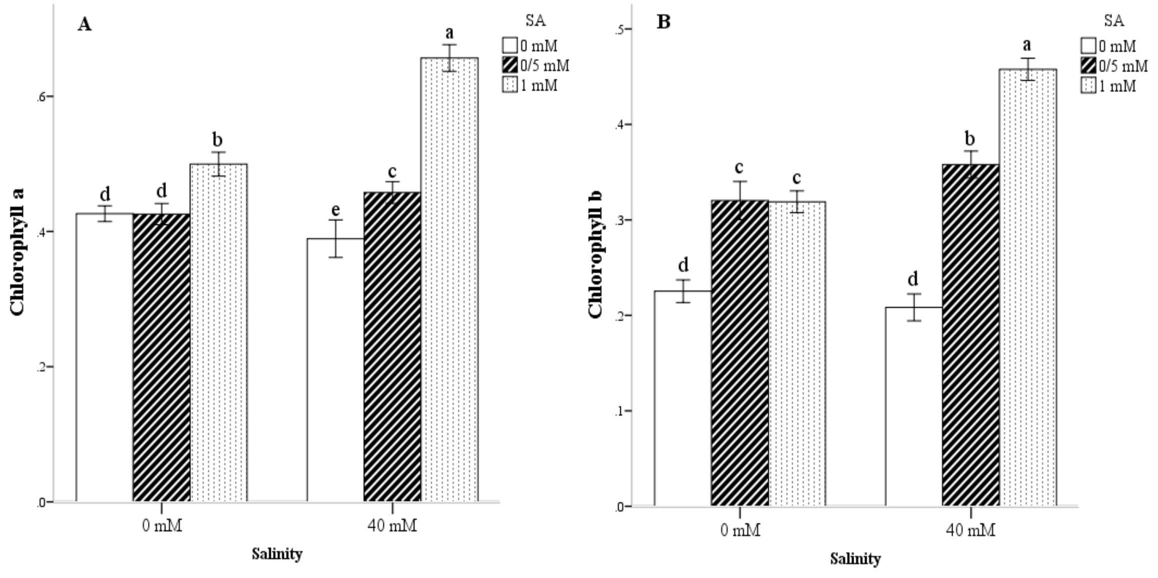

Figure 4. Effects of different concentration of salicylic acid on the chlorophyll a (A) and chlorophyll b (B) contents of *Portulaca olaracea* under salt stress; Means for each trait that followed by the same letter are not significantly different (p≤ 0.05) by Duncan's multiple range test.

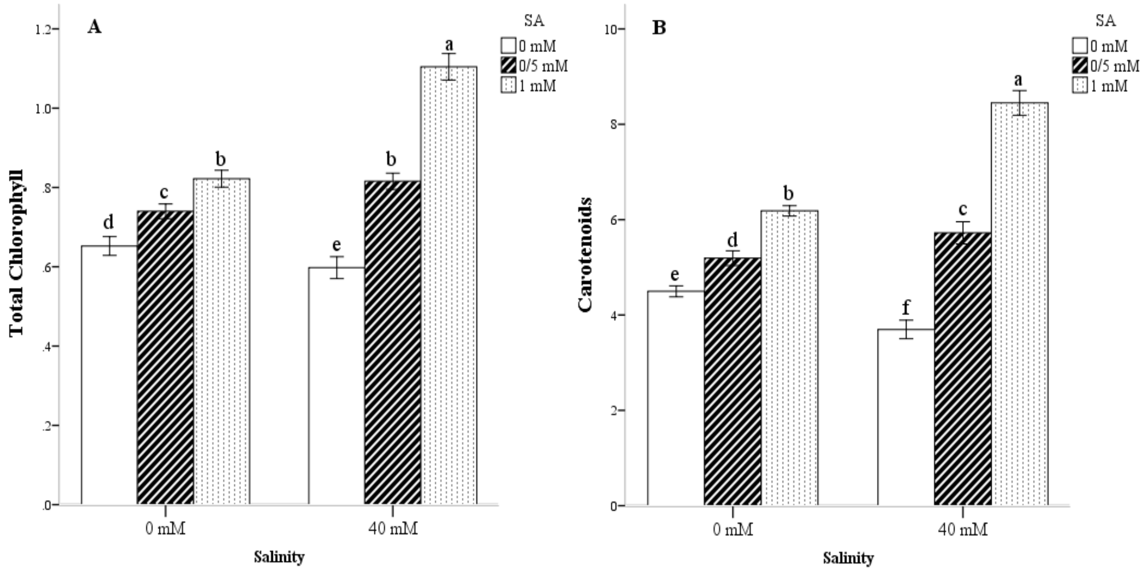

Figure 5. Effects of different concentration of salicylic acid on the total chlorophyll (A) and carotenoids (B) contents of *Portulaca olaracea* under salt stress; Means for each trait that followed by the same letter are not significantly different (p≤ 0.05) by Duncan's multiple range test.

Conclusions

SA improved shoot length, leaf length and leaf width on the average of normal and saline conditions, root length at 40 mM NaCl, and leaf number, root and shoot dry weight and RWC under both conditions. Also the concentration of 1 mM SA showed the greatest effect on the improvement of resistance to salinity through elevations in carotenoids, chlorophylls a and b and total chlorophyll. Hence, SA is an appropriate agent in acceleration of purslane growth in the saline environment. The commercial cultivation of purslane can help to fulfill the increased demands of fresh vegetables and improve sustainability of agriculture, especially in saline areas.

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اثر محلول‌پاشی اسید سالیسیلیک بر صفات مورفوفیزیولوژیکی خرفه (*Portulaca olaracea* L.) تحت تنش شوری

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چکیده

خرفه با نام علمی *Portulaca olaracea* متعلق به تیره Portulacaceae گیاهی یک ساله است که به عنوان خوراکی و نیز داروی سنتی استفاده می‌شود. در این راستا، آزمایشی به صورت فاکتوریل در قالب طرح بلوک های کامل تصادفی در سه تکرار با هدف بررسی اثر سطوح مختلف اسید سالیسیلیک (۰، ۰/۵ و ۱ میلی مولار) بر صفات رشدی و رنگیزه‌های فتوسنتزی گیاه دارویی خرفه تحت تنش شوری (۰، ۴۰ و ۸۰ میلی مولار) طراحی و اجرا شد. ترکیبات تیماری در قالب طرح بلوک‌های کامل تصادفی با سه تکرار ارزیابی شدند. صفات مورد اندازه‌گیری به این شرح بودند: وزن خشک ریشه و اندام هوایی، طول ریشه و اندام هوایی، طول و عرض برگ، محتوای آب نسبی، تعدادبرگ، کلروفیل a، b و کل و کارتنوئیدها. نتایج نشان داد که شوری اثر منفی و کاهنده بر اکثر صفات مورفولوژیکی و نیز محتوای آب نسبی داشت، در حالی که اسید سالیسیلیک باعث افزایش رشد و مقدار رنگیزه‌های گیاهی در خرفه شد. به عنوان مثال، کاربرد اسید سالیسیلیک (۱ mM) موجب افزایش محتوای کلروفیل a، کلروفیل b، کلروفیل کل و کاروتنوئیدها در شرایط شوری (به ترتیب ۰/۶۵، ۰/۴۶، ۱/۱۱ و ۸/۴۵ میلی گرم برگرم) نسبت به گیاهان شاهد (به ترتیب ۰/۴۳، ۰/۲۳ ، ۰/۶۵ ۴/۴۹ میلی گرم برگرم) شد. به طور خلاصه، محلول‌پاشی اسید سالیسیلیک موجب کاهش اثرات زیانبار شوری بر رشد گیاه خرفه شد.

واژه‌های کلیدی: اسید سالیسیلیک؛ تحمل؛ تنش شوری؛ رنگیزه‌های فتوسنتزی؛ گیاهان دارویی؛ *Portulaca olaracea* L.