Flowering, Physiological and Biochemical Responses of Two *Echinacea* Species to Drought Stress

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Summary

Drought is one of the most important environmental stressors that limit plant's productivity. To evaluate the effects of drought stress on *Echinacea purpurea* and *Echinacea angustifolia* seedlings at the four-leaf stage, an experiment was conducted with four levels of irrigation regimes: 25%, 50%, 75%, and 100% of field capacity (FC) in a CRD based factorial experiment with three replications. Growth indices such as shoot and root dry and fresh weight, were reduced at lower FC in both species, but *E. angustifolia* showed more sensitivity than *E. purpurea*. Drought stress significantly affected flower stem length, flower longevity, flower diameter, and flower anthocyanin in both species. In addition, flowering was not observed in *E. angustifolia* under 50% and 25% FC treatment, but the longest flowering period was recorded in *E. purpurea* under 75% FC treatment (37 days). In this study, leaf carotenoid and anthocyanin contents increased, while the total chlorophyll content decreased under severe drought stress. A reduction of protein content and antioxidant capacity were observed in both species during severe drought stress. The highest amount of electrolyte leakage, malondialdehyde (MDA), total sugars, and proline was observed in *E. angustifolia* under 25% FC treatment. Catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) antioxidant enzymes activity increased at severe drought stress. Overall, the results indicate that *E. purpurea* is more drought tolerant than *E. angustifolia* and *E. purpurea* is a good candidate for arid and semi-arid regions with limited water resources.

Key words

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Introduction

Among environmental stressors, drought has been recognized as the greatest stress that limits plant productivity. Influences of drought stress on the size of plant's root system and shoots, photosynthesis rate, and biochemical responses have been reported (Arabzadeh et al., 2017; Smirnoff, 1993). In addition, drought causes oxidative stress by overproduction of reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$) superoxide radical (O_2-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH). The excessive accumulation of ROS caused by drought stress could lead to cytotoxic oxidative damage to membrane lipids, proteins, photosynthetic pigments, cellular structure, and nucleic acids (Sharma et al., 2012; Smirnoff, 1993).

An efficient antioxidant defensive system is present in plants to counteract oxidative stress including enzymatic and non-enzymatic antioxidant process. The three major anti-oxidative enzymes include catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) that effectively scavenge ROSs. POD and CAT enzymes convert H_2O_2 into H_2O and O_2, while SOD enzyme decomposes the superoxide radical (O_2-) to H_2O_2 (Sharma et al., 2012). Non-enzymatic antioxidants that may protect plants against drought stress includesanthocyanins, carotenoids, proteins, glutathione, ascorbate, alkaloids, phenolic compounds, and flavonoids (Fazeli et al., 2007; Kovinich et al., 2015; Lei et al., 2007).

Compatible solutes appear when plants face drought stress. An accumulation of protective solutes includes sugars (glucose, fructose, sucrose), sugar alcohols (mannitol, glycerol, sorbitol, pinitol, quercitol) proline, glycine betaine, and inorganic ions in plant organs prevent peroxidation of cell organelles (Sharma et al., 2012; Liu et al., 2011; Slabbert and Kruger, 2014).

Water scarcity, especially in arid and semi-arid zones with high rates of population growth, as well as urbanization and industrialization are considered the most critical resources for sustainable development. Water is essential not only for agriculture, industry, and economic growth, but it also is the most important component of the environment, with significant impact on health and nature conservation (Chartzoulakis and Bertaki, 2015). Ornamental perennials are an important part of urban green space and private gardens; however, they are not always properly irrigated and may suffer from drought stress that may affect their flowering (Chylinski et al., 2007; Metwally et al., 2013).

One of the perennial plants belonging to Asteraceae family is the *Echinacea* genus, with nine species native to North America that can be cultivated in different regions of the world for cut flowers, landscape perennial and pharmaceutical aspects (McKeown, 1999). Asadi-Sanam et al. (2015) indicate that the *E. purpurea* species can tolerate freezing conditions by improving protective solutes and antioxidant enzyme activities. Sabra et al. (2012) examined the effects of salinity on three *Echinacea* species by measuring different growth indices and antioxidant enzyme activities. Their results indicate that *E. purpurea* tolerates saline soil more than *E. angustifolia* and *E. pallida*. Liu et al. (2014) report that *E. purpurea* species remediates oil contaminated soil by improving soil enzyme activities. By measuring water relationship parameters and osmotic adjustment between four different ornamental perennials under drought conditions, limited levels of drought tolerance were found in *E. purpurea* species (Chapman and Auge, 1994). Therefore, it can be concluded that the *Echinacea* genus can tolerate various soil conditions and environmental stresses such as cold and saline area. However, only few studies have reported drought tolerance, flowering, physiological and biochemical responses of *Echinacea* species.

Given the importance of *Echinacea* and water scarcity problems, this study aimed to evaluate important physiological and biochemical indices of two *Echinacea* species during several levels of irrigation in order to recognize the tolerance of these plants for landscaping purposes and agriculture production.

Material and Methods

Plant Material and Treatments

This experiment was carried out under greenhouse conditions at the Municipality of Tehran Research, Training, and Consulting Center of Ornamental Plants during the spring and summer in 2016. Tehran, Iran, (35.77N and 51.42E), is at an altitude of 1520 m above sea . level. This experiment used seedlings from two *Echinacea* species (*Echinacea purpurea* and *Echinacea angustifolia*). These seedlings were transplanted to plastic pots at the four-leaf stage (17 cm diameter by 20 cm height) filled with 2 Kg air-dried sand, perlit, loam (2:1:1, by volume) substrate, and a gravel layer at the bottom. The chemical characteristics of the soil mixture were as follows: pH 8.3; electrical conductivity (EC) 2.28 ds/m; organic carbon 0.16 %; N 0.03; P 10.9 ppm; K 155 ppm; Fe 6.7 ppm; Zn 0.9 ppm; Mn 5.9 ppm; Cu 0.3 ppm. Nitrogen determinate by Kjeldahl method (Bremner. 1996), Phosphorus measured spectrophotometrically by Molybdate vanadium method (Chapman, 1961), micro elements (Fe, Zn, Mn, Cu) measured by dry-ashing method, and atomic absorption device (Shimadzu-AA670) (Chapman, 1961), dry-ashing method and flam photometer (Jenway PFP7 Flam Photometer, Staffordshire, UK) used for Potassium determination (Mehlich, 1953).

Pots were under natural sunlight with a light/dark cycle approximately 16/8 hours. The maximum and minimum temperatures during the experiment period were 20±2˚C and 28±2˚C, respectively, and mean relative humidity was approximately 30%. After transplanting the seedlings to pots, the plants were irrigated to the field capacity level for two weeks to prevent drought stress and let the plant establish well. Plants were fertilized by 0.5 g/kg macro complete solid fertilizer (N: P: K=12: 11: 18, Yaramila™) before starting treatments as the base fertilizer.

This experiment was conducted in completely randomized factorial design with three replications, and in each replication were three plants and every plant in one pot (nine experimental units per treatment). Field capacity of the soil was determined to be 20% based on the weight of the soil by pressure plates apparatus (Eijkelkamp soil & water, Giesbeek, Netherland). Drought treatment was applied to plants in four levels of irrigation: 25% (severe stress), 50% (medium stress), 75% (low stress), and 100% (control) of FC. A soil moisture meter (Tlead™ ETP 300, Qingdao, China) measured field capacity depletion, and either all pots were weighed on a scale every day. Water loss was compensated up to field capacity level weight for each treatment. Plants were under these treatments for 90 days (from May to August), after which

physiological and biochemical characteristics that related to drought tolerance were measured.

Growth and Flowering

Measuring of morphological indices including shoot and root weight, shoot and root dry weight and flowering indices such as flower stem length, flower diameter, and flower anthocyanin were measured after 90 days of plants treatment. Flower longevity (number of day from blooming until flower wilting) was measured during the flowering period for each treatment.

Pigments

Total chlorophyll (Chl_{a+b}) and carotenoid were determined spectrophotometrically (Itd T80+ UV/VIS; PG Instruments, Leicestershire, UK) according to the method of Arnon (1967). The absorbance reading was taken at 663 nm for chlorophyll a, 645 nm for chlorophyll b, and 470 nm for carotenoid. Chlorophyll and carotenoid content was expressed as mg/g FW.

Total anthocyanin content was determined according to pH differential method Wrolstad (1976) as modified by Lee et al. (2005) based on the reversible changes of anthocyanins at pH 1.0 and pH 4.5. During extraction, methanol containing 1% (v/v) HCl was used, absorbance was measured at 520 and 700 nm and expressed as cyanidin-3-glocoside (molar extinction coefficient of 26,900 L/mol. cm and molecular weight of 449.2 g/mol) equivalents, mg/L.

Ion Leakage and Lipid Peroxidation (MDA content)

For ion leakage, leaf samples were rinsed with distilled water and immersed in 10 mL of distilled water for 12 hr. The conductivity of the solution (R_1) was determined using a conductivity meter (Jenway 4010 Conductivity meter, Staffordshire, UK). Samples were heated in boiling water for 20 min, then cooled to room temperature. The conductivity of killed tissues (R_2) was again measured. Ion leakage calculated as the percentage of R_1 to R_2 (Lu et al., 2009).

Lipid peroxidation in samples was determined by estimation of malondialdehyde (MDA) content according to the method of Heath and Parker (1968). MDA extract was determined using 20% (w/v) trichloroacetic acid containing 0.5 % (w/v) thiobarbituric acid. MDA content was calculated using the difference between spectrophotometric absorption ratios at 532 and 600 nm wavelengths and 155 mM/cm extinction coefficient.

Total Sugars and Proline

The total sugars were estimated by a modified method of Somogyi (1952) and Phosphomolybdic as reagent. The absorbance was measured by a spectrophotometer at 600 nm. Total sugar content was estimated using a standard curve prepared with glucose and expressed as mg/g FW.

The ninhydrin method determined proline concentration according to Bates et al. (1973) with some modifications. The absorbance was read at 520 nm by the spectrophotometer and toluene as a blank. Proline concentration was calculated using a calibration curve and expressed as μmol proline per g FW.

Protein Content and Antioxidant Capacity

Protein content of the samples was extracted using the method developed by Bradford (1976) and BSA calibration curve was used for the determined amount of protein content and expressed as mg/g FW).

The antioxidant activity was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method (Brand-Williams et al., 1995). The sample's absorbance was measured at 517 nm by spectrophotometer. Percentage of inhibition of DPPH was measured according to the following formula:

$$\text{Inhibition of DPPH (\%)} = [(A_{517Control} - A_{517Sample})/A_{517Control}] \times 100$$

Antioxidant Enzyme Activities

For antioxidant enzyme assays, 0.5 gram of frozen leaf samples were ground to a fine powder in liquid nitrogen and extracted by 1 mL ice-cold 50 mM potassium phosphate buffer (pH=7) containing 0.5 mM EDTA and 2% (w/v) polyvinylpyrrolidone (PVPP). The results were centrifuged (Eppendorf Centrifuged 5417R) at 14,000 rpm for 15 min. at 4°C. The supernatant measured the activities of CAT, POD, and SOD. All steps of the extraction procedure were carried out at 4 ºC.

CAT (EC 1.11.1.6) activity in leaves was determined based on decomposition of H_2O_2 according to the method of Chance and Maehly (1995). The reaction solution (3 mL) contained a 50 mM phosphate buffer (pH=7), 10 mM H_2O_2 and 10 μL of extracted enzyme solution. Decrease in absorbance at 240 nm was read for 1 min. using a spectrophotometer. CAT activity was calculated according to the molar extinction coefficient of H_2O_2 (40 mM/cm) and expressed as μmol/g FW min.

POD (EC 1.11.1.7) activity in leaves was determined based on the oxidation of guaiacol in the presence of H_2O_2 to tetraguaiacol according to the method of Chance and Maehly (1995). The reaction solution (0.5 mL) contained 50 mM phosphate buffer (pH=7), 45 mM guaiacol, 225 mM H_2O_2, and 10 μL of extracted enzyme solution. The increase in absorbance at 470 nm was read for 1 min with a spectrophotometer. POD activity was calculated according to the molar extinction coefficient of tetraguaiacol (26.6 mM/cm) and expressed as μmol/g FW min.

SOD (EC 1.15.1.1) activity in leaves was determined by monitoring the decrease in absorbance of nitro blue tetrazolium (NBT) by enzyme according to the method described by Giannopolitis and Ries (1977). The reaction solution (1 mL) contained a 50 mM phosphate buffer (pH=7), 12 mM riboflavin, 13 mM methionine, 0.1 mM EDTA, 7 mM NBT, and 10 μL of extracted enzyme solution. One unit of SOD was defined as the amount of enzyme that produced 50% inhibition of NBT reduction at 560 nm. SOD activity was expressed as unit per g FW.

Statistical Analysis. The treatment effects were determined by analysis of variance using SAS software (SAS Institute Inc., Cary, NC, USA v.9.4). The values presented in the text indicate mean values of three replicates. Mean comparisons were calculated using Tukey's test at % 0.05 probabilities, and the charts were plotted using Excel software.

Results

Morphological and Flowering Indices

According to the results in Table 1, the effects of different levels of irrigation on shoot weight are significant at $p<0.05$ level. Shoot dry weight as a biomass of plants under different levels of irrigation changed significantly. The highest shoot fresh and dry weight was found in *E. purpurea* with 100% FC treatment, while it decreased significantly at 25% soil FC. More root fresh and dry weight were observed in *E. purpurea* than *E. angustifolia* in various irrigation levels. Root fresh and dry weight considerably reduced at 50% and 25% FC in *E. angustifolia* and 25% FC in *E. purpurea*.

Flower stem length and flower diameter of both species declined by decreasing FC percent, but *E. angustifolia* was severely affected at lower FC percent and inhibition of both traits was seen at 50% and 25% FC. In addition, *E. purpurea* flower longevity decreased only at 25% FC, whereas the longest flowering period of *E. angustifolia* and *E. purpurea* was observed at 100% and 75% FC. Regarding flower anthocyanin, the highest rate of anthocyanin was observed in both species at 100% and 75% FC, but reduced in lower FC. However, the amount of anthocyanin in *E. purpurea* was much higher than in *E. angustifolia* at all FC levels.

Physiological and Biochemical Indices

According to the results in Fig 1a, leaf anthocyanin increased by increasing drought stress severity in both species. The highest content of leaf anthocyanin was observed in *E. angustifolia* in 25% and 50% FC. In the *E. purpurea* species, anthocyanin content did not change among 100%, 75%, and 50% FC irrigation levels. Leaf total chlorophyll (Chl_{a+b}) significantly decreased in *E. angustifolia* species during water shortage. The lowest chlorophyll content was observed in 25% and 50% FC treatments (Fig. 1b). On the other hand, carotenoid as another foliar pigment increased with

intensifying drought stress in *E. purpurea* species. The highest amount of carotenoid observed in *E. purpurea* was under 25% FC treatment while it decreased in *E. angustifolia* at lower FC levels (Fig. 1c).

Electrolyte leakage increased in both species during drought stress, but this trend was more significant in *E. angustifolia* than *E. purpurea*. The highest percentage of electrolyte leakage observed in *E. angustifolia* was during 25% FC (Fig. 1d). Malondialdehyde as a lipid peroxidation biomarker increased in *E. angustifolia* under drought stress, while the lipid peroxidation of *E. purpurea* did not change significantly (Fig. 2a).

The amount of proline increased in both species by decreasing irrigation levels, especially in *E. angustifolia* (Fig. 2b). By increasing drought stress, aggregation of soluble sugars increased in *E. purpurea* and *E. angustifolia* leaves, and the highest value of soluble sugars was seen in *E. purpurea* in 25% and 50% FC treatments (Fig 2c).

The difference between *E. purpurea* and *E. angustifolia* protein content was significant. The lowest level of protein was observed in *E. angustifolia* by 25% FC treatment. No significant difference was observed between 100%, 75%, and 50% treatments in *E. purpurea* or among treatments of the *E. angustifolia* species (Fig 2d). Antioxidant capacity decreased due to severe stress in two species, while *E. purpurea* contained more antioxidants than *E. angustifolia*, although there was no considerable difference within 25% and 50% FC treatments (Fig 3a).

Antioxidant Enzymes Activities

According to the results in Fig. 3b, drought stress significantly increased CAT activity in both species. The highest amount of CAT activity was observed in *E. purpurea* with 25% FC treatment. Severe stress increased POD activity in both species, but this increment was not significant in *E. purpurea*. The highest POD

Table 1. Changes of morphological and flowering indices of *E. purpurea* and *E. angustifolia* under four levels of irrigations, 100% FC (control), 75% FC (low stress), 50% FC (medium stress) and 25% FC (severe stress)

Echinacea angustifolia				*Echinacea purpurea*				Indices
levels of irrigation (% FC)				levels of irrigation (% FC)				
25	50	75	100	25	50	75	100	
0.63e	1.76de	4.43cd	5.59c	10.66b	15.6a	13.06ab	15.33a	Shoot weight (g)
0.28c	0.25c	0.58c	0.59c	2.86b	4.35a	3.68a	4.30a	Shoot dry weight (g)
0.6e	0.95e	3.33de	7.33cd	10.16c	23.61ab	20.63b	28.63a	Root weight (g)
0.22d	0.34d	1.33cd	1.82c	1.81c	4.67b	4.27b	6.29a	Root dry weight (g)
0	0	31e	36de	41.3cd	51.33c	58.30a	51.33b	Flower stem length (cm)
0	0	5.5b	5.8b	6.16b	7.33a	8a	7.5a	Flower diameter (cm)
0	0	16c	16c	22.33b	32.66a	37a	36.33a	Flower longevity (day)
0	0	16.48b	15.64b	16.31b	20.60b	27.23a	22.77ab	Flower anthocyanin (mg/L)

Different letters in rows are for significantly different groups according to Tukey's test ($p<0.05$).

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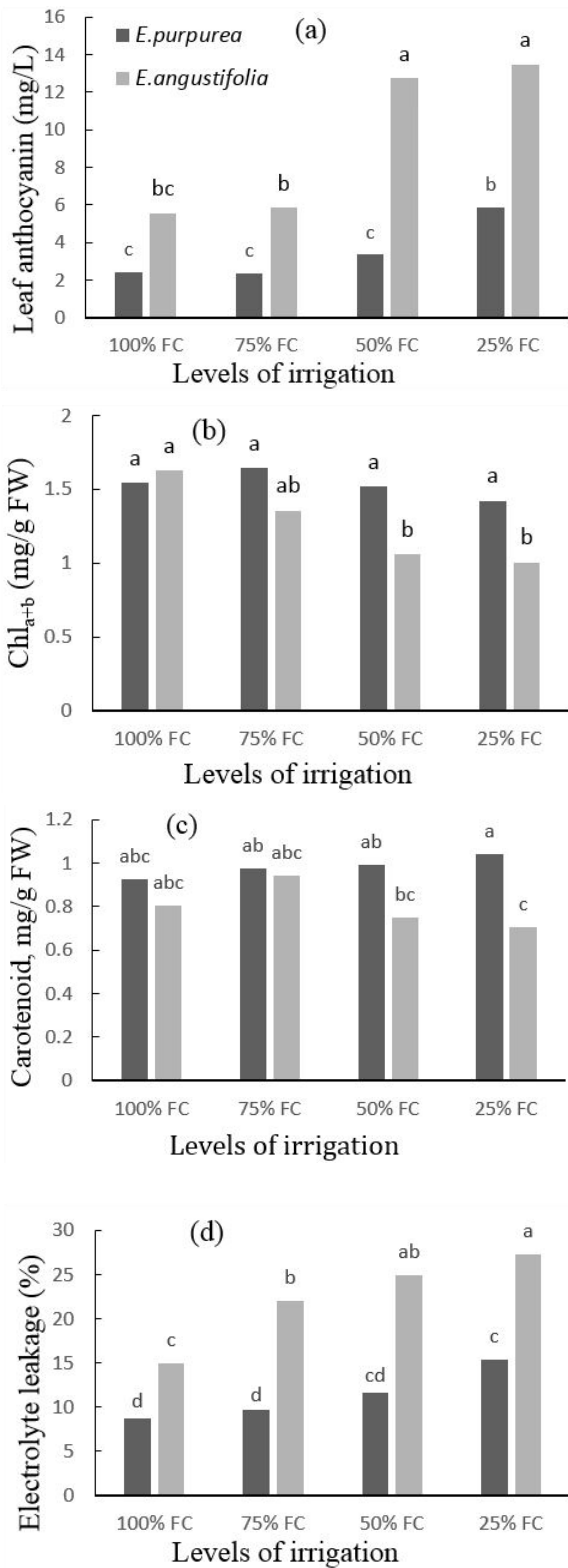
Figure 1. Change of Leaf anthocyanin (a), Total chlorophyll (Chl_{a+b}) (b), Carotenoid (c), and Electrolyte leakage (d) of *E. purpurea* and *E. angustifolia* under four levels of irrigation, 100% FC (control), 75% FC (low stress), 50% FC (medium stress) and 25% FC (severe stress). Different letters are for significantly different groups according to Tukey's test (p<0.05).
▪ *Echinacea Purpurea* ▪ *Echinacea angustofolia*
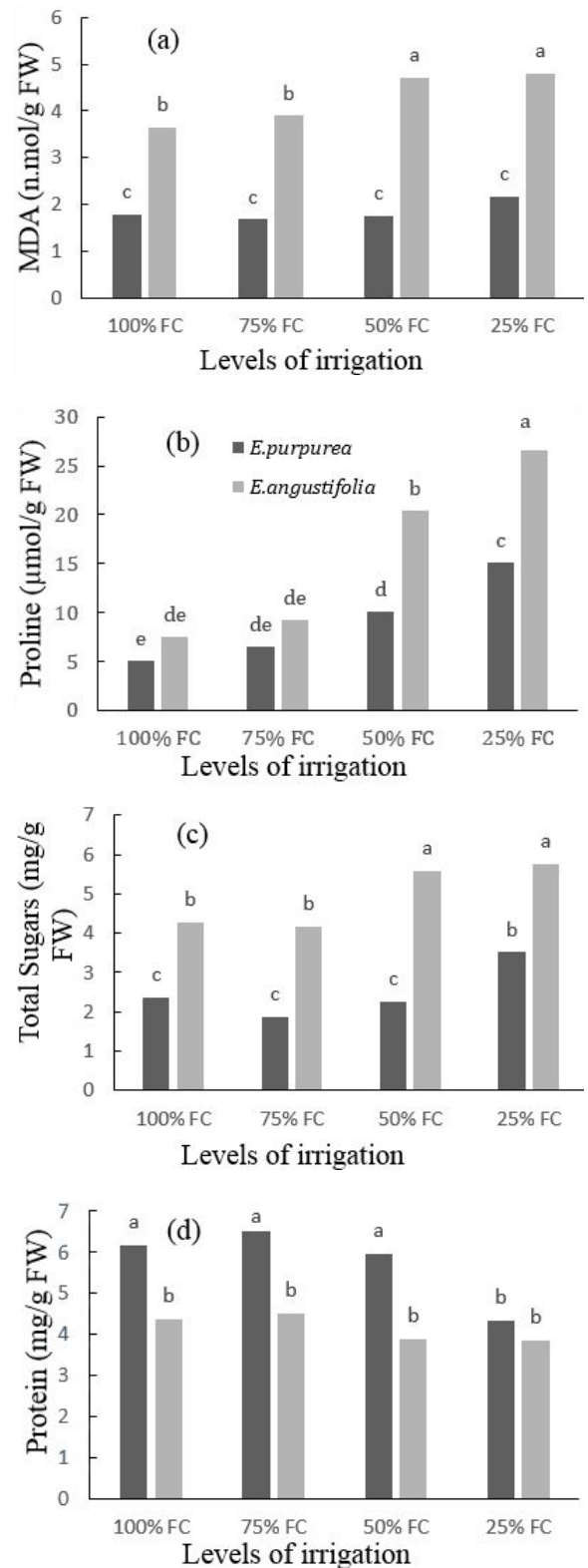
Figure 2. Change of Malondialdehyde (MDA) (a), Proline (b), Total sugars (c), and Protein (d) of *E. purpurea* and *E. angustifolia* under four levels of irrigation, 100% FC (control), 75% FC (low stress), 50% FC (medium stress) and 25% FC (severe stress). Different letters are for significantly different groups according to Tukey's test (p<0.05).
▪ *Echinacea Purpurea* ▪ *Echinacea angustofolia*
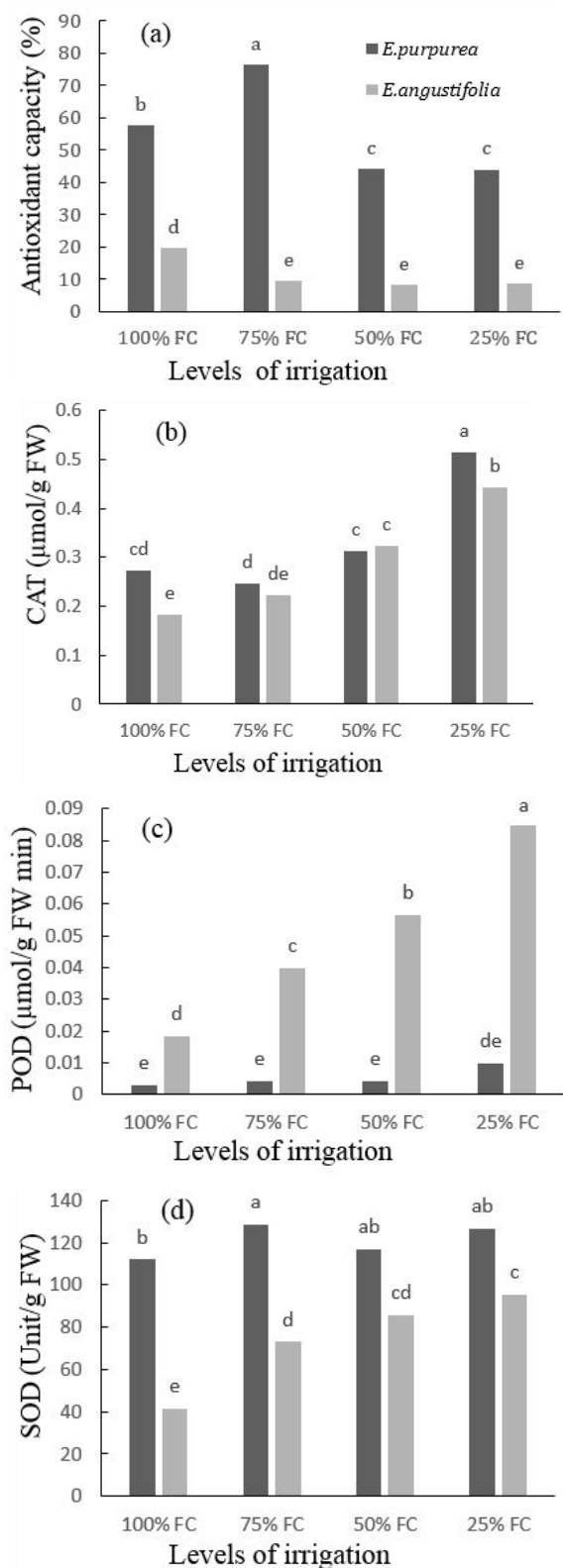
Figure 3. Change of Antioxidant capacity (a), Catalase (b), Peroxidase (c), and Superoxide dismutase (d) of *E. purpurea* and *E. angustifolia* under four levels of irrigation, 100% FC (control), 75% FC (low stress), 50% FC (medium stress) and 25% FC (severe stress). Different letters are for significantly different groups according to Tukey's test (p<0.05).

■ *Echinacea Purpurea* ■ *Echinacea angustofolia*

activity was observed in the *E. angustifolia* species under 25% FC treatment (Fig 3c). Drought stress' effect on SOD activity in both species was additive, and a higher content of SOD activity was seen in *E. purpurea* by 25% FC treatment (Fig 3d).

Discussion

Drought stress affects plant growth in many ways. Reduction in growth ability and biomass are the plants' initial and most important responses to water shortage. Furthermore, decreased plant weight is associated with the loss of water from leaves during drought stress (Fazeli et al., 2007). Measurements of biomass and growth rate in this experiment show that *E. purpurea* responds better than *E. angustifolia* at different irrigation levels. Plants under drought often reduce shoot growth yet elongate roots because elongated roots penetrate deeper in soil to obtain available water (Liu et al., 2011). A single tap root is the main characteristic of all species except *E. purpurea*, which has a fibrous root system (McKeown, 1999). That is why *E. purpurea* survives from drought stress more than *E. angustifolia*. In this study, root weight significantly increased under 50% FC in *E. purpurea*. Other perennials like calendula respond similarly during drought stress by reducing plant growth and flowering (Metwally et al., 2013). Furthermore, reduction in flowering indices under drought stress is observed in impatiens as bedding plants (Chylinski et al., 2007). Flowering reduction under stress cause assimilates saving and in similar manner, not only does flowering quality reduce in both species during decreasing irrigation but also growth and biomass declines in *E. angustifloia* in 50% FC and 25% FC. In addition, no flowering occurred in the *E. angustifolia* species under the lowest irrigation level. By decreasing flower quality under drought stress, anthocyanins also decreased. Decrement of anthocyanin in flower petals relate to flower quality and assimilates saving for drought stress tolerance (Chalker-Scott, 1999).

Plant anthocyanins are induced in response to abiotic stress such as drought, salinity, excess light, and cold (Kovinich, 2015). Anthocyanins could minimize the generation or scavenge reactive oxygen species (ROS); therefore, anthocyanins can protect plants from oxidative damages and cause osmotic adjustment (Close and Beadle, 2003). In this experiment anthocyanin of leaves increased with a water shortage. Therefore, anthocyanin may play a role in scavenging ROS and osmotic adjustment of leaves in both *Echinacea* species. Concentration of Chl_{a+b} declined in both species under severe drought stress. A significant decrease in Chlorophyll content is considered a typical symptom of oxidative stress (Sharma et al., 2012; Liu et al., 2011). Carotenoid content increases in *E. purpurea* due to water shortage, so the accumulation of carotenoid could be accepted as a structural compound of photosynthesis systems and as an antioxidant that scavenges O_2 to protect photosynthetic complex (Liu et al., 2011). Therefore, increases of this pigment under drought conditions in *E. purpurea* rather than *E. angustifolia* relate to its better drought tolerance.

Osmotic adjustment is considered to be an important portion of plants' drought tolerance mechanisms. Evaluations of compatible solutes in different plants under drought stress have shown that proline may participate in osmotic adjustment in some plants (Liu et al., 2011; Slabbert and Kruger, 2014). With increasing drought, plants' proline content increased but it was not related to tolerance of plants (Slabbert and Kruger, 2014). Sun et al. (2013) also

mention that proline accumulation in chrysanthemums under drought results from damage and is not suitable for choosing chrysanthemum drought tolerance. Increment of proline content in *E. purpurea* was observed with lowest irrigation during this experiment and the highest amount of proline was measured in *E. angustifolia* under 25% FC treatment. Soluble sugars play an important role in plants osmotic adjustment (Liu et al., 2011). According to the results by reducing water, leaf soluble sugars considerably increased in both species. The accumulation of soluble sugars is controlled by several mechanisms affecting soluble sugar formation and increasing the leaf soluble sugars concentration contributed with enhancement of relative water content (Slabbert and Kruger, 2014).

Cell membrane of plants affects with different oxidative stressors (Sharma et al., 2012). The degree of cell membrane injury due to water shortage may be easily estimated by electrolyte leakage from the cells (Liu et al., 2011). According to this experiment, *E. angustifolia* species shows more electrolyte leakage than the *E. purpurea* species. Decreasing irrigation may damage cellular membrane and cellular components such as lipids. In addition, MDA content is also used to measure cellular membrane damage due to oxidative stress that leads to lipid peroxidation (Sharma et al., 2012; Smirnoff, 1993). Intense MDA content appears in both species under drought conditions, but higher MDA content is more obvious in *E. angustifolia* species. Boldaji et al. (2012) indicate that by increasing drought stress, ion leakage and MDA content also increase, and this increment in drought sensitive cultivar is more than drought tolerant cultivar.

Drought can induce reactive oxygen species (ROS), leading to oxidative stress. Oxidative stress can damage proteins, organelles, and other cell functions (Sharma et al., 2012; Smirnoff, 1993). The amount of proteins within the treatments is not significantly different in the *E. purpurea* species but reduces with 25% FC irrigation. In *E. angustifolia*, the amount of protein decreases, with the lowest level observed with 25% FC treatment. Fazeli et al. (2007) also report that by increasing drought in sesame cultivars, protein content decreases due to ROS production.

Scavenging the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical has been widely used for determining antioxidant capacity in different plants. Enhancing antioxidant capacity may play an important role in plant stress tolerance (Asadi-Sanam et al., 2015; Khosh-Khui et al., 2012; Lin et al., 2006). By increasing water shortage in *E. purpurea* and *E. angustifolia*, scavenging ability decreases. *E. purpurea* with 75% FC treatment shows the highest antioxidant capacity. Khosh-Khui et al. (2012) state that as drought increases in thyme, antioxidant activity decreases, confirming previous reports on sweet potato under severe drought conditions (Lin et al., 2006). It seems the antioxidant defense system ruined under severe or long-term water deficiency, leads to less antioxidant activity.

Most plant damages caused by various environmental stresses are due to oxidative damage at different cellular levels. Antioxidant enzymes are the most effective factors against oxidative damage in plants under the influence of drought stress (Sharma et al., 2012; Smirnoff, 1993). Antioxidants of CAT, POD, and SOD as key ROS scavengers increase in two species of *Echinacea* during low irrigation and play positive roles in controlling the cellular levels of ROS. CAT is one of the most efficient enzymes, which is activated as hydrogen peroxide (H_2O_2) and increases in cells of plants and animals. CAT and POD enzymes eliminate H_2O_2 into H_2O and oxygen (O_2), and SOD converts superoxide radicals into H_2O_2 and O_2 (Sharma et al., 2012). It seems that in *E. purpurea*, CAT detoxifies H_2O_2 that has not been scavenged completely by POD, confirming Tatari et al. (2018) that antioxidant enzymes (CAT, POD, SOD) scavenge ROS induced under drought stress and lead to plant drought tolerance.

Conclusion

According to biochemical, physiological, and flowering indices of this experiment, *E. purpurea* is more tolerant to drought stress than *E. angustifolia*. Soil moisture reduction up to 50% FC is not harmful to *E. Purpurea* species, while soil moisture level around 50% FC represents a stressful condition for *E. angustifolia* and leads to yield and quality reduction. So the best level of irrigation for *E. Purpurea* species is 50% FC and the best level of irrigation for *E. angustifolia* species is 75% and 100% FC. This estimation proves that *E. purpurea* is suitable for arid and semi-arid regions with limited water resources and also provide information for developing selection and breeding of *Echinacea* species.

Acknowledgement

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