

# Investigation of Physiological and Biochemical Response of *Echinacea purpurea* under Salinity Stress <sup>†</sup>

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**Abstract:** *Echinacea purpurea* is an Important medicinal plant that contains valuable medicinal compounds that have a tremendous effect on stimulating the body's immune system to fight off viral and bacterial agents. To evaluate salinity stress tolerance in *Echinacea purpurea*, an experiment was conducted using a diverse population. The seeds used in this experiment were the result of selecting superior genotypes in terms of chicoric acid content and drought tolerance. Considering the medicinal value of *Echinacea purpurea* and the high area of saline soils in Iran, the purpose of this study is to investigate the possibility of cultivating this plant in saline soils. In this experiment, salinity stress at two levels of 0 and 60 mM of NaCl started when the plant was in the 6-leaf stage and continued for 14 days. The results showed a significant decrease in the amounts of photosynthetic pigments, potassium element under salinity stress. Also, under salinity condition, the amount of sodium ions in the shoots, ion leakage, and total phenol increased, but there was no significant change in the amount of proline and antioxidant capacity and chlorophyll fluorescence parameters. It seems that among the genotypes under salinity stress, based on the results obtained from results of genotypes under stress, genotypes 34, 46, 90, 89, 79, and 165 have high levels of proline, phenolic compounds, and antioxidant properties. These genotypes were in a better position in terms of these parameters and were placed in a separate cluster in cluster analysis, so these can be selected as tolerant genotypes.

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**Keywords:** Salinity stress; proline; Polyphenol compounds; antioxidant capacity; Elements

## 1. Introduction

Medicinal and aromatic plants are valuable products; The natural products of these plants are small volume but very valuable and have many applications in various industries such as food, beverages, food supplements, perfumery, cosmetics and medicine [1].

Purple coneflower (*Echinacea purpurea* L.) is a perennial and herbaceous medicinal plant belonging to the family Asteraceae. All parts of *Echinacea purpurea* including, leaves, flowers, and roots are widely used in the preparation of pharmaceutical products. These products are used to stimulate the immune system and for treating respiratory disorders and viral infections [2]. In this sense, this medicinal plant is famous for its effects on the immune system (2). The use of *Echinacea* products has dramatically increased: sales in 2013 increased by 94.7% over those in 2012, making it the 8th most commonly sold herb in the United States [3]. By 2014, sales of *Echinacea* had increased by 79% from 2013 and it was the third most commonly sold herb in the United States with the sales surpassing \$50 million [4].

Salinity stress results in an excessive generation of ROS [5]. Elevated CO<sub>2</sub> mitigates the oxidative stress caused by salinity, involving lower ROS generation and better maintenance of redox homeostasis as a consequence of higher assimilation rates and lower

photorespiration [6]. The leaf area in *Echinacea purpurea* decreases significantly due to high NaCl concentrations. Increasing concentrations of NaCl leads to an increase in the levels of shoot phenol, shoot flavenoids and proline content [7]. Increases in Na<sup>+</sup> and Cl<sup>-</sup> during salt stress have resulted in decreased levels of N, P, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> in fennel, *Trachyspermum ammi*, peppermint, lemon verbena, *Matricaria recutita*, *Achillea fragratissima* [1,8,9]. Both chlorophyll a and b along with the total chlorophyll content were decreased in centaury, *Teucrium polium*, *Thymus vulgaris*, *Zataria multiflora*, *Ziziphora clinopodioides* and *Satureja hortensis* [10]. A decrease in protein content during salt stress was reported in *Catharanthus roseus* [11].

Considering the medicinal value of *Echinacea purpurea* and the high area of saline soils in Iran, the purpose of this study is to investigate the possibility of cultivating this plant in saline soils.

## 2. Materials and Methods

The seeds used in this experiment are the result of selecting superior genotypes in terms of chicoric acid content and drought tolerance [12]. The uniformly sized seedlings reaching the height of 10–12 cm (60 days after germination) were transplanted into 10 L pots. Also, to warrant optimal nutritional support, plants were regularly fed with a diluted nutrition solution (fertigation) during the experiment [13].

Analysis of variance of morphological and phytochemical data was performed based on the relevant experiment. At 6-leaf stage, salinity stress started at two levels of control (no salinity), and 60 mM of NaCl continued for 14 days. Data analysis of physiological and biochemical properties of different genotypes of *Echinacea purpurea* were performed using Minitab V.14 software.

### 2.1. Chlorophyll Assays

The content of chlorophyll and carotenoids were measured by Arnon [14] method and the adsorption rate was read by spectrophotometer at 663, 645, and 470 wavelengths.

### 2.2. Total Polyphenol Content

TPC was measured according to the method of Stankovic [15].

### 2.3. DPPH-Radical Scavenging Assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was performed according to the method of Stankovic [15].

### 2.4. Relative Water Content

The method of Cameron et al. [16] was used to measure the relative content of leaf water.

### 2.5. Chlorophyll Fluorescence

All chlorophyll fluorescence parameters ( $F_0$ ,  $F_m$ ,  $F_v/F_m$ ) were measured by a portable chlorophyll fluorescence meter (handyPEA, Hansatech Instruments, King's Lynn, UK).

### 2.6. Ion Leakage

Ion leakage was measured based on Sullivan & Ross [17].

### 2.7. Proline

Free proline was measured according to the method of Bates et al. [9], and the absorbance was read at 520 nm spectrophotometrically.

### 2.8. Elements Measurement

Ca<sup>2+</sup>, Na<sup>-</sup>, K<sup>+</sup> and Cl<sup>-</sup> Ions was measured based on Tahmasebi [18].

### 3. Results and Discussion

The analysis of variance results showed that there was a significant difference between salinity and control in terms of chlorophyll a and b, total chlorophyll, carotenoids, total polyphenol, ion leakage, sodium and potassium Ions (Table 1).

**Table 1.** Mean of Echinacea genotypes in two levels of control and salinity in terms of Physiological and biochemical traits.

Traits	t-Value	p-Value	Salinity Level	Control Level	Difference
Chlorophyll a (mg/g fw)	12.33	0.00	0.40	1.28	0.87
Chlorophyll b (mg/g fw)	12.48	0.00	0.32	1.05	0.73
Total chlorophyll (mg/g fw)	13.39	0.00	0.72	2.34	1.61
Carotenoid (mg/g fw)	2.32	0.024	0.081	0.14	0.056
TPC (mg/g fw)	2.85	0.006	329	235	93.70
Dpph (%)	1.91	0.06	11.98	14.01	202
RWC (%)	0.33	0.744	66.70	67.60	0.93
Fv/Fm	0.35	0.729	0.70	0.68	0.02
Ion leakage (%)	6.53	0.00	88.30	56	32.30
Proline (mg/g fw)	1.87	0.07	52.40	41.10	11.36
Ca <sup>2+</sup> (%)	0.12	0.91	4.66	4.72	0.07
Na <sup>-</sup> (%)	4.54	0.00	0.21	0.10	0.11
K <sup>+</sup> (%)	2.20	0.034	0.79	0.51	0.27
Cl <sup>-</sup> (%)	1.75	0.89	3.84	3.17	0.67

#### 3.1. Chlorophyll Assays

Based on results of genotypes under salinity stress, the highest amounts of chlorophyll a, chlorophyll b, carotenoids and total chlorophyll were observed in genotypes (34), (27, 34 and 83), (95 and 89) and (34), respectively and the lowest amounts of chlorophyll a, chlorophyll b, carotenoids and total chlorophyll were observed in genotypes (25, 161, 163, 32, 164, 36, 37, 141, 142 and 147), (49), (25, 161, 163, 32, 36, 37, 155 and 159) and (161, 163, 37, 38, 169, 142, 36 and 40), respectively. The results show that the amount of chlorophyll a, chlorophyll b, carotenoids and total chlorophyll in salinity stress decreased by 68.75, 69.52, 42.14 and 69.23%, respectively, compared to the control treatment. Sabra [19] showed that the application of 50 mM NaCl on Echinacea purpurea decreases chlorophyll a, chlorophyll b and carotenoids by 16.67, 23.01 and 14.77%, respectively.

#### 3.2. Total Polyphenol Content

Based on results of genotypes under salinity stress, the highest amounts of TPC was observed in genotypes (46 and 165) and the lowest amounts of TPC observed in genotypes (161, 42, 155 and 159), respectively. The results show that the TPC in salinity stress increased by 40.00%, respectively, compared to the control treatment. Cappellari [20] showed that the application of 75 mM NaCl on Mentha piperita increases TPC by 17.39%.

#### 3.3. DPPH-Radical Scavenging

Based on results of genotypes under salinity stress, the highest amounts of antioxidant activity was observed in genotype (137), and the lowest amounts of chlorophyll a, chlorophyll b, carotenoids and total chlorophyll were observed in genotypes (34, 41, 43, 155, 150 and 156), respectively. The results show that the amount of antioxidant activity in salinity stress decreased by 14.49%, respectively, compared to the control treatment. Khorasaninejad [7] showed that the application of 75 mM NaCl on Echinacea purpurea increases antioxidant activity by 56.2%.

### 3.4. Relative Water Content

Based on results of genotypes under salinity stress, the highest amounts of relative water content was observed in genotype (156), and the lowest amounts of chlorophyll a, chlorophyll b, carotenoids and total chlorophyll were observed in genotypes (81, 165, 41, 47, 40, 161, 140 and 141), respectively. The results show that the amount of RWC in salinity stress decreased by 1.33%, respectively, compared to the control treatment. Zrig [21] showed that the application of 100 mM NaCl on *Thymus vulgaris* decreases RWC by 31.00% after two weeks treatment.

### 3.5. Chlorophyll Fluorescence

Based on results of genotypes under salinity stress, the highest amounts of Quantum Efficiency of Photosystem II was observed in genotypes (50 and 166), respectively and the lowest amounts of Quantum Efficiency of Photosystem II was observed in genotype (80). The results show that the amount of Fv/Fm in salinity stress increased by 2.94%, respectively, compared to the control treatment.

### 3.6. Ion Leakage Percentage

Based on results of genotypes under salinity stress, the highest amounts of Ion leakage percentage was observed in genotypes (25, 162, 34, 164, 35, 36, 50, 159 and 169), respectively and the lowest amounts of Ion leakage percentage was observed in genotypes (140 and 154), respectively. The results show that the amount of Ion leakage percentage in salinity stress increased by 32.3%, respectively, compared to the control treatment. Sabra [19] showed that the application of 50 mM NaCl on *Echinacea purpurea* increases Ion leakage percentage by 8.00%.

### 3.7. Proline Content

Based on results of genotypes under salinity stress, the highest amounts of proline content was observed in genotypes (34, 79, 89 and 90), respectively and the lowest amounts of proline content was observed in genotypes (82, 137 and 33), respectively. The results show that the amount of proline content in salinity stress increased by 27.49%, respectively, compared to the control treatment. Zrig [21] showed that the application of 100 mM NaCl on *Thymus vulgaris* increases proline content by 188.46% after four weeks treatment.

### 3.8. Elements Content

Based on results of genotypes under salinity stress, the highest amounts of Calcium, Sodium, Potassium and Chlorine percentage were observed in genotypes (79), (49), (48) and (84), respectively and the lowest amounts of Calcium, Sodium, Potassium and Chlorine percentage were observed in genotypes (39, 160, 42, 47, 142, 144, 159, 150, 154 and 147), (27, 161, 43, 135 and 169), (43 and 135) and (37 and 93), respectively. Sabra [19] showed that the application of 50 mM NaCl on *Echinacea purpurea* increases Sodium, Potassium and Chlorine percentage by 7.72, 1.60 and 30.20%, respectively.

## 4. Conclusions

Applying abiotic stresses is one of the ways to change the amount and components of the active ingredient of medicinal plants, in which a lot of research has been done. In general, based on physiological and biochemical results, it can be said that *Echinacea* has the ability to adapt to salinity stress conditions, and by selecting genotypes tolerant to salinity stress in breeding programs, the conditions for cultivating this plant in areas with salines water and soil can be provided. It seems that among the genotypes under salinity stress, based on the results obtained from histogram and cluster analysis of genotypes under stress, genotypes 34, 46, 90, 89, 79, and 165 have high levels of proline, phenolic compounds, and antioxidant properties. These genotypes were in a better position in

terms of these parameters and were placed in a separate cluster in cluster analysis, so these can be selected as tolerant genotypes.

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