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Original Research Article

Growth and Physiological Responses of '*Chrysanthemum paludosum*' under Salinity Stress

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ABSTRACT

***Chrysanthemum paludosum*, belongs to Asteraceae family, is a perennial medicinal herb and ornamental plant. It has aesthetical values together with several medical effects such as antibacterial, antifungal, insecticidal, antioxidant, nervous, cytotoxic etc. Excess of salinity in soils is one of the major problems which reduce plant growth. The purpose of this study was to determine the effects of salinity on *C. paludosum* by assaying plant growth and some physiological traits. *C. paludosum* plants were irrigated with five different levels of NaCl concentrations (0, 50, 100, 150, 200 mM) for 30 days with 3 days intervals in pots under greenhouse conditions. The effects of salinity stress on diameter of flower and disc floret, number of flowers, shoot height, root length, thickness of root collar and stem, fresh weights of root and shoot, dry weights of root and shoot, leaf chlorophyll concentration (SPAD readings), moisture content on wet basis, relative water content and ion leakage were investigated. According to the results, flower diameters, disc florets, number of flowers, shoot height, root collar thickness, root and shoot fresh weights were negatively affected in 150 and 200 mM NaCl treatments. In parallel, ion leakage also highly increased in 150 and 200 mM NaCl treatments. Leaf chlorophyll concentrations decreased by increasing salinity levels.**

Keywords: Abiotic stress, *Chrysanthemum*, Membrane injury, NaCl, Plant growth, SPAD

INTRODUCTION

Chrysanthemum belongs to the Asteraceae family and comprises of about 200 species are commonly available in Asia, northeastern Europe and most species originate from East Asia (Chae 2016). Stems, leaves and flowers of Chrysanthemum have wide medicinal values such as efficient drugs for various diseases in traditional medicinal field, herbal tea (Gupta et al. 2013, Chae 2016). Chrysanthemum has also much attention in the field of biomolecules research thanks to antifungal, antibacterial and anti-inflammation properties (Ma et al. 2016). *Chrysanthemum paludosum* [*Leucanthemum paludosum*] commonly called Mini marguerite, Baby Marguerite, White Buttons, Snow Daisy is native to Western Europe. *C. paludosum* could use as an alternative for pest control. The flower methanolic extracts of *C. paludosum* has significant insecticidal activity and owing to its petroleum ether and methanol extracts, it has activity against bacterial and yeasts strains (Haouas et al. 2008, Sassi et al. 2008). It is a vigorously growing herbaceous perennial for ornamental purposes (Wang et al. 2014, Kapoor et al. 2015). Recently *C. paludosum*, is planted both solo and groups with other plants, in flower beds and home gardens, besides it has a wide range of use as pot plant.

Salinity is one of the most important abiotic stress factors that effects product quality and productivity in arid and semi-arid regions. Salt stress negatively affects plant growth and development by causing osmotic and ionic stress. These negative effects alter depending on the type of applied salt, the intensity and duration of the stress, the genotype and the development stage of plant exposure to the stress. Salt stress causes many problems at morphological, cellular, physiological and molecular levels and in various development processes in plants. Salinity may reduce the chance of survival due to various metabolic events and photosynthetic activities negatively in plants. While some of plants show susceptibility to these conditions, some of them survive with tolerance mechanisms that are induced with various morphological, physiological, biochemical and molecular responses (Parida and Das 2005, Yılmaz et al. 2011, Köksal et al. 2016). There are physiological and biochemical responses which provide tolerance mechanisms against the salinity such as ion regulations, synthesis of osmotic regulators and antioxidant systems in plants (Grattan and Grieve 1999, Parida and Das 2005, Aşık et al. 2009). However, few studies have dealt specifically with ornamental plants used in landscapes

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(Marosz 2004, Cassaniti et al. 2009, Cassaniti et al. 2012). The importance of the salinity problem is increasing for landscape plants as well as vegetable and fruit crops. Because the scarcity of water has led to the reuse of waste waters which generally include high salinity levels for irrigation of urban green areas (Niu and Rodriguez 2006, Navarro et al. 2008, McCammon et al. 2009). Salinity is also a big problem in coastal gardens and landscapes (Ferrante et al. 2011) and in countries where de-icing salts are applied to roadways during the winter months (Parida and Das 2005).

As the value of ornamental plants has gradually increased, interest to unconventional plant species have increased (Köksal et al. 2013). In this regard, medicinal and aromatic plant species owing to their aesthetic and functional features is considering as popular alternatives. These plants like Chrysanthemum, planted either solitary or in groups, have a wide range of use in flower beds, home gardens and plant pots (Arslan and Yanmaz 2010; Korac and Khambholja 2011).

The response of many plants which exposed to salinity has been reported in several studies (Navarro et al. 2008, Rodriguez et al. 2005). The salt stress tolerance of many ornamental plants that are used outdoor and indoor is not well known. In areas which suffer from salinity problem, there is a limited information for landscape designers and growers of ornamental plants to recommend the suitable plant species. In this study, we focused on understanding growth and physiological response of *C. paludosum* under different salinity levels.

MATERIALS AND METHODS

Plant material and growth conditions

This study was carried out in greenhouse at the Department of Horticulture, Cukurova University in Adana/Turkey. In this study *C. paludosum* plants were used as plant material. Two-months-old plants were supplied from a commercial nursery, and they were transferred into plastic pots (12.3 x 8.3 x 11.6) containing peat. The plants were irrigated with different NaCl concentrations by the addition of 0, 50, 100, 150 and 200 mM NaCl to the irrigation solution for 3 days intervals. Salinity treatments were continued for four weeks.

Determination of plant growth parameters

At the end of the experiment, plants were harvested in order to determine plant growth parameters. The peat substrate was softly removed from the plants, the roots were washed with distilled water, and the plants were divided into shoot and root. Root fresh weight (RFW) and shoot fresh weight (SFW) were weighed using a digital top loading weighing balance. Following that, the dry weight of the roots (RDW) and shoots (SDW) were determined after drying of plant parts at 70 °C for 48 h in an thermo ventilated oven. Moreover, flower diameter, disc floret, root collar and stem diameters were measured by digital caliper. Schema related to measurement of flower and disc floret diameter was presented in Figure 1. Root and shoot lengths were also determined by using a ruler. Flower numbers on each plant were determined by counting.

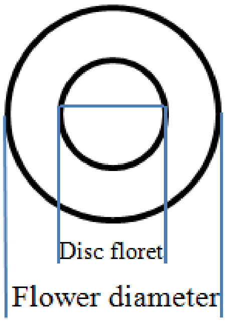

Figure 1. Schema of flowering parameters measurements.

Physiological analysis

In order to determine the ion leakage, 1 cm diameter leaf discs were cut from fully expanded and uniform leaves from each of three plants (replicates) per treatment. Leaf discs were softly rinsed in distilled water, blotted with paper, and placed in test tubes (three discs per tube) containing 20 ml of distilled water. Test tubes were shaken on a shaker for 4 h and electrical conductivity of each sample was measured using a conductivity meter (EC600 model, Extech Instruments A Flir Company) for first measurement (EC1). Then, leaf discs were killed in the same solution by autoclaving, and final conductivity was measured at room temperature (EC2). Ion leakage was calculated using the following equation (Arora et al. 1998, Gülen et al. 2007):

$$Ion\ leakage\ (\%) = \frac{\mathrm{EC1}}{\mathrm{EC2}}\ x\ 100$$

Moisture content of wet basis was calculated from difference of fresh weight and dry weight of shoot samples by following formula (Koksal et al. 2016).

$$MCwb = \left[\frac{\mathrm{W - DW}}{\mathrm{W}}\right]\ x\ 100$$

MC_{wb} : Moisture content wet basis (%), W: Shoot fresh weight (g), DW: Shoot dry weight (g)

For determination of the leaf relative water content (RWC), completely expanded leaves were used. Fresh weights of leaf discs (1 cm) were firstly recorded, and turgor weight was detected after discs waited for 4 h on distilled water. Dry weights were determined after 24 h at 70°C (Van Laere et al. 2011).

$$RWC\ (\%) = \left[\frac{\mathrm{W - DW}}{\mathrm{TW - DW}}\right]\ x\ 100$$

RWC: Relative water content (%) W: Sample fresh weight (g), DW: Sample dry weight (g), TW: Sample turgid weight (g)

Leaf chlorophyll concentration was evaluated using a portable SPAD-502 meter (Minolta, Japan). Readings were taken from the leaves of three replicates at the end of the experiment (Cimen et al. 2014).

Statistical analysis

Salinity treatment experiment was carried out completely randomized experimental design with single factors. Treatments had five replications with five plants each. Data were subjected to ANOVA and the means were separated using the LSD multiple range test at $P \leq 0.05$. All the statistical analyses were performed using the JMP8 Software package.

RESULTS

Plant growth parameters

Different salinity levels affected statistically significant plant growth. As seen in Table 1, except the root length and stem diameter, all plant growth parameters were statistically significant. Figure 2 also represented the general effects of salinity on the *C. paludosum* plants. Whereas root length did not differ statistically, shoot height was found significant. When plants were exposed to 150 and 200 mM salinity levels, the lowest shoot heights were obtained at the value of 14.87 and 13.77 cm, respectively. While in root collar thickness was seen difference, stem thickness was not found significant, statistically. The highest values of root collar thickness were detected in the 0, 50 and 100 mM NaCl treatments. When salinity levels were 150 and 200 mM, it was found the lowest values related to root collar thickness.

Figure 2. *C. paludosum* plants grown in pots with different salinity levels.

Table 1. Effect of different salinity levels on plant growth parameters of *C. paludosum.*

Parameters	***NaCl concentration (mM)***					***LSD***
	0	**50**	**100**	**150**	**200**	
Root length	23.33±2.08	23.33±1.16	27.33±1.53	21.67±4.51	20.00±4.58	---^{NS}
Shoot height	20.50±2.29^{a}	19.33±2.19^{a}	17.10±0.95^{ab}	14.87±1.63^{b}	13.77±2.60^{b}	*3,672***
RC-thickness	6.98±1.04^{a}	5.70±0.64^{ab}	6.02±0.99^{a}	4.48±0.09^{bc}	4.23±0.23^{c}	*1,294***
S-thickness	6.48±0.64	6.06±0.59	5.40±0.74	5.46±0.55	4.94±0.29	---^{NS}
Root FW	7.15±0.69^{ab}	8.83±1.95^{a}	9.19±2.27^{a}	5.42±1.76^{bc}	3.27±1.70^{c}	*3,197**
Shoot FW	54.78±5.53^{a}	52.73±6.27^{a}	54.23±1.61^{a}	37.39±8.10^{b}	25.43±1.48^{c}	*9,364****
Root DW	0.55±0.07^{b}	0.83±0.16^{a}	0.84±0.15^{a}	0.48±0.15^{b}	0,33±0.14^{b}	*0,252***
Shoot DW	3.77±0.50^{a}	3.75±0.14^{a}	3.52±0.26^{ab}	3.06±0.20^{b}	3.15±0.23^{b}	*0,529**
F-diameter	40.34±2.28^{a}	37.05±3.42^{ab}	32.82±1.31^{bc}	29.02±4.76^{c}	26.87±4.30^{c}	*6,287**
DF-diameter	12.88±1.36^{a}	11.17±1.26^{ab}	10.40±0.39^{b}	10.00±1.07^{bc}	8.13±1.19^{c}	*2,014***
Flower number	12.89±1.32^{b}	15.69±1.30^{a}	16.03±1.68^{a}	15.33±1.13^{a}	11.54±0.00^{b}	*1,937***

The values (mean and standard deviation) followed by different letters are significantly different at least significant difference test (LSD). Significance, ^{ns}: not significant, *: 5%, **: 1%, ***: 0.1%.

RC-thickness: Root color thickness, S-thickness: Stem thickness, F-diameter: Flower diameter, DF-diameter: Disc floret diameter, FW: Fresh weight, DW: Dry weight

Root and shoot fresh weights were higher in 0, 50, 100 mM salinity level (Table 1, Figure 3). In terms of root FW, the lowest values were found in 150 and 200 mM level of NaCl at the values of 5.42 and 3.27 g, respectively. On the other hand, shoot FW values dramatically decreased in 150 and 200 mM salinity levels. When the 150 and 200 mM NaCl treatments compare with 0 mM NaCl, the values decreased at the respective rates of 31.7 and 53.6 %. While the highest root DW values were found in 50 and 100 mM NaCl, the lowest values were in 0, 150 and 200 mM treatments. In parallel with shoot FW, similar results were determined for shoot DW. The lowest values of shoot DW were found in 150 and 200 mM salinity levels.

Figure 3. Morphological overview of *C. paludosum* plants grown in pots with different salinity levels.

Diameter of flower and disc floret were negatively affected by the increasing salinity levels (Table 1 and Figure 4). The highest flower diameter and disc floret were shown in 0 and 50 mM NaCl concentration. In the 50, 100, 150 and 200 mM NaCl concentrations, flower diameter decreased at the respective rates of 8.2, 18.6, 28.1 and 33.4 %. In parallel flower diameter, disc floret diameter in the 50, 100, 150 and 200 mM NaCl concentrations also decreased at the rates of 13.3, 19.3, 22.4 and 36.9 %, respectively. The highest flower number was found in 50, 100 and 150 mM salinity level. On the other hand, the least flower number was detected in 0 and 200 mM NaCl.

Figure 4. Flowers of *C. paludosum* which affected from salinity stress.

Physiological Analysis

In the study, ion leakage, leaf chlorophyll concentrations (SPAD readings), and moisture content of wet basis of *C. paludosum* plants grown with different salinity levels were significantly affected (Figure 5). The ion leakage remarkably increased in leaf tissues as the salinity levels increased (Figure 5a). Ion leakage was the highest in 200 mM NaCl treatment (79%). In 0, 50, 100, 150 mM NaCl concentrations, ion leakage increased at the respective rates of 15, 37, 62 and 69 %.

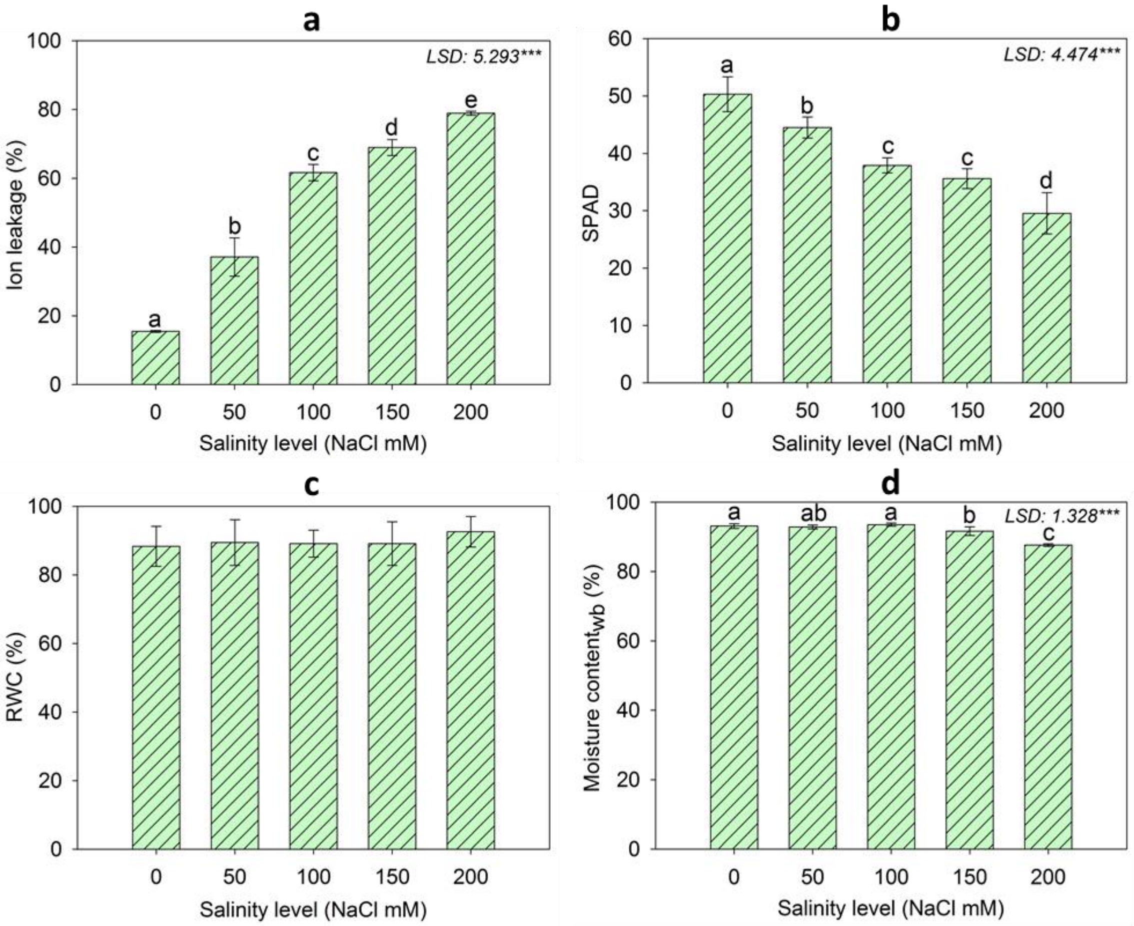

Figure 5. Physiological responses of *C. paludosum*: a. Ion leakage, b. Leaf chlorophyll concentration (SPAD), c. Relative water content (RWC), d. Moisture content.

Leaf chlorophyll concentrations (SPAD readings) reduced by the increasing salinity levels (Figure 5b). The highest leaf chlorophyll concentration (50.30) was in the 0 mM level of salinity (control group). When the NaCl concentration was 200mM, SPAD reading was found the lowest value. (29.55). In the 50, 100, 150 and 200 mM NaCl levels, leaf chlorophyll concentrations decreased at the respective rates of 11.5, 24.7, 29.2 and 41.3 % comparing with 0 mM NaCl treatment.

The relative water content (RWC) did not differ significantly. *C. paludosum* plants saved a large amount of water in the leaf discs (Figure 5c). On the other hand, the wet-basis moisture content (MCwb) of shoots decreased in the higher saline conditions. The wet-basis moisture contents of shoots in the 0, 50, 100, 150, and 200 mM NaCl applications were 93.11, 92.84, 93.52, 91.64, and 87.63 %, respectively. There were no significant differences in the wet-basis moisture content of the shoots in the 0, 50 and 100 mM NaCl treatments. MCwb decreased as the salinity stress increased above 150 mM. In the 150 and 200 mM NaCl treatments, the wet-basis moisture contents of the shoots decreased at the respective rates of 1.47 and 5.48 % compared to the control (Figure 5d).

DISCUSSION

Salinity lead to several alterations at morphological, physiological and biochemical processes in plants (Sevengor et al. 2011). Data presented in Table 1 indicated that plant growth negatively influenced by increasing salinity levels. In our study *C. paludosum* plants maintained shoot height, root collar thickness, fresh and dry biomass (root and shoot) up to 100 mM NaCl. However flower and disc floret diameter dramatically decreased by increasing salinity stress. Plant growth retardation and biomass loss of most of species under salinity stress were revealed in many previous studies (Senaratna et al. 2000, Alpaslan and Gunes 2001, Kaya et al. 2003, Navarro et al. 2008, Villarino and Mattson 2011, Lolaei 2012, Köksal et al. 2014, Köksal et al. 2016). Zidan et al. (1990) reported that salt stress has inhibited effects on cell division and/or cell elongation in growing roots, stems and leaves tissues. Razmjoo et al. (2008) emphasized that increased salinity (0, 84, 168, 252 and 336 mmol l^{-1} NaCl) caused reduction in the number of branches per plant, flowers per plant, peduncle length, head diameter, fresh and dry flower weight on *Matricaria chamomilla* L. Similarly, Heidari and Sarani (2012) stated that, shoot fresh weight of Chamomile (*Matricaria chamomilla* L.) decreased by increasing salinity from 0 to 150 mM. Baghalian et al. (2008) indicated that fresh flower weight of chamomile was significantly affected from salinity level. In parallel, Bayat et al. (2013) also reported that shoot, root and total dry weight, plant height, leaf area of calendula plants were lower at salt stress treatment as compared to non-saline conditions. Moreover, they also stated negative effects of saline conditions on ornamental quality of calendula. Salinity treatment significantly decreased both number of flower per plant and flower diameter of calendula plants. These results support our finding related to plant growth parameters on Chrysanthemum (*C. paludosum*).

As seen in Figure 5, salt stress generally reduced physiological activities of *C. paludosum*. It is well known that electrolyte leakage is an indicator for the extent of membrane damage of salt-stressed plants. Salt stress impairs membrane permeability and causes increasing of ion leakage. Ashraf and Harris (2004) reviewed that salt-sensitive species showed an increase in electrolyte leakage in the leaves under salinity, too. In our study, ion leakage increased by salt treatment on *C. paludosum*. Similarly, low values of electrolyte leakage were recorded in the controls (0 mM) while the presence of NaCl in the rooting medium induced a significant increase in electrolyte leakage in the leaves of *H. rosa-sinensis* (Trivellini et al. 2014). Leaf chlorophyll concentration (SPAD) of *C. paludosum* significantly decreased with the increasing salt stress in our study. In accordance with, Bayat et al. (2013) showed that salt stress led to decrease in SPAD reading as compared to the non-saline conditions. Many researchers also indicated that chlorophyll concentration dramatically decreased in the leaves of various plants with increasing NaCl concentration. (Downton et al. 1985, Stepien and Klobus 2006 and Yildirim et al. 2008).

Determination of water content indicated on a tissue fresh or dry mass basis have been generally replaced by measurements based on the maximum amount of water a tissue can hold (Yamasaki and Dillenburg 1999). Loss of water content under salinity stress were previously revealed in tomato by Rodriguez et al. (1997)

and Tuna et al. (2007), in marigold by Köksal et al. (2016). As a result of present study, relative water content (RWC) did not alter while moisture content of wet basis (MC_{wb}) of shoots decreased in the higher saline conditions (150 and 200 mM). On the other hand, as considering all NaCl treatments, it was determined that moisture content values were between about 88% and 94%. These results were indicated that water content and cell turgor may relatively preserve under saline conditions on *C. paludosum*. Similarly Matsumara et al. (1998) identified as a moderate tolerant plant to *C. paludosum*. They emphasized that an increase in ion concentrations (Na^+ and Cl^-) induced from salinity largely contributed to a decrease in leaf osmotic potential and this adaptation allows plants to keep cell turgor by continuing water uptake.

CONCLUSIONS

As a summary, most of morphological and physiological parameters of *C.ryshanthemum paludosum* negatively were affected by increasing salinity levels. *C. paludosum* plants maintained shoot height, root collar thickness, fresh and dry biomass (root and shoot) up to 100 mM NaCl. However flower and disc floret diameter dramatically decreased by increasing salinity. Whereas ion leakage increased with salt treatment, leaf chlorophyll concentration significantly decreased. For this reason ion leakage and SPAD reading were considered as useful tools for determining effects of salinity on *C. paludosum*. Furthermore, stability on relative water content and partial reduction on moisture content indicated that water content and cell turgor may relatively preserve under saline conditions on *C. paludosum*. As a consequence of the study, *C. paludosum* was considered as a moderate salt-tolerant plant.

Thus, *C. paludosum* can be grown successfully on most agricultural soils and can be irrigated with alternative water sources which include NaCl until critical values (100mM in this study).

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