

Original Research

# A Cut Flower Cultivation under Saline Conditions: *C. morifolium* Ramat ‘Bacardi’

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Received: 1 July 2021

Accepted: 31 August 2021

## Abstract

Chrysanthemum which belongs to Asteraceae is one of the most important ornamental plants in the world. *Chrysanthemum morifolium* Ramat ‘Bacardi’, a species of this genus, is a very important cut flower used as spray chrysanthemum. Salinity in soil and water is one of the major problems which affect the ornamental aspects and plant growth. It was aimed to determine the effects of salinity on *Chrysanthemum morifolium* Ramat ‘Bacardi’ by analyzing plant growth, flower characters and some physiological traits in this study. Plants were irrigated with seven different levels of salt concentrations (0, 50, 100, 150, 200, 250 and 300 mM NaCl) for 75 days with 3 days intervals in pots under greenhouse conditions. The effects of salinity stress on diameter of flower and disc floret, number of bud and flowers, fresh and dry weights of flower, shoot height, shoot fresh and dry weights, stem diameter, lateral shoot (branch) length and number, inter-node length, relative growth rates, loss of turgidity and ion leakage were investigated in this study. Consequently, flower and disc floret diameters, number of flowers, shoot height, shoot and flower fresh and dry weights, stem diameter, branch length and number, inter-node length were drastically decreased, as the salinity increased, especially after 200 mM. On the other hand, ion leakage significantly increased when salt concentrations increased. According to the results, *C. morifolium* Ramat ‘Bacardi’ is moderately tolerant cultivar and should not be cultivated as a cut flower up to 150 mM NaCl (highly saline) conditions.

**Keywords:** *Chrysanthemum*, cut flowers, ion leakage, plant growth, salinity

## Introduction

Chrysanthemum which belongs to Asteraceae family contains about 200 species and one of the most important ornamental plants [1]. The importance of chrysanthemum stems from their aesthetical and economic values. Chrysanthemum is used as cut,

potted and outdoor plants. The total production area of ornamental plants is 52.477 da and total production number is 1.718.098.240 in Turkey, according to the data of 2019. Some 12.374 da area and 1.093.333.943 production number of it is composed of cut flowers and chrysanthemum has 755 da area, 47.677.050 number in Turkey [2].

Salinity is very serious abiotic stress factor that affects productivity, plant growth, product quality in arid and semi-arid areas [3, 4]. Irrigation water and soil

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salinity is one of the major abiotic challenges worldwide in terms of agriculture [5]. Reducing fresh water has brought to need the reuse of waste waters which generally contain high salt levels for irrigation. Coastal gardens, landscapes and in countries where de-icing salts are applied to roadways during the winter months suffered from salinity problem [6]. Salinity problem affects not only consumed foods but also landscape plants. Salinity can cause osmotic and ionic stress, and decrease plant growth and development, nutrient uptakes [7]. There are several pieces of research to understand the effects of salt stress on plants. However, there are no satisfying studies about ornamental plants which exposed to salinity stress [8]. Although there is limited information about flowering plants which treated salt, it has been determined that increasing salinity can delay flowering, reduce flower number and reduce size of flowers [9]. The aim of the study was to investigate plant growth, flowering parameters, ion leakage and loss of turgidity of *Chrysanthemum morifolium* Ramat 'Bacardi' plants which had economic values and especially used as cut flowers under salt stress.

## Material and Methods

This study was carried out under greenhouse conditions at the Department of Horticulture, Cukurova University in Adana/Turkey, during December 2018 and March 2019. Greenhouse's average temperature was 21.5/15°C. In this study *Chrysanthemum morifolium* Ramat 'Bacardi' 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, 200, 250 and 300 mM NaCl to the irrigation solution (100 mL) for 3 days intervals. Salinity treatments were continued for ten weeks.

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 shoots were washed with distilled water to remove substrate from plants and shoot fresh weight (SFW) and flowers fresh weight (FFW) were weighed using a digital top loading weighing balance. Dry weight of the shoots (SDW) and flowers (FDW) were determined after drying of plant parts at 70°C for 48 h in an thermo ventilated oven. At the beginning of the study, the plant's shoot height, stem diameter and shoot fresh and dry weights were recorded. According to these data relative growth rate (RGR) was determined by following Equation (1):

$$RGR = (D2 - D1)/(t2 - t1) \quad (1)$$

The RGR formula was modified from [5].

Where, D2-D1, is data of shoot length, stem diameter, shoot fresh and dry weight at the end - data the beginning of the experiment; t2-t1 was the time duration for the treatment.

Flower and disc floret diameters, stem diameter, internode length were measured by digital caliper. Shoot and branch lengths were determined using ruler. Flower and branch numbers were determined by counting.

To determine the ion leakage, leaf discs 1 cm diameter were cut from fully expanded and uniform leaves from each of three plants (replicates) per treatment. Discs were softly rinsed in distilled water, blotted with paper, and put 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 (2) [10, 11]:

$$\text{Ion leakage (\%)} = (EC1/EC2) \times 100 \quad (2)$$

Completely expanded leaves were used to detect the loss of turgidity. Initially, fresh weights of leaf discs (1 cm) were recorded, and after discs waited for 4 h on distilled water, turgor weight was determined. Dry weights were determined after waiting 24 h at 70°C. Loss of turgidity was calculated using the following equation (3) [12].

$$\text{Loss of turgidity (\%)} = \left[ \frac{TW - W}{TW} \right] \times 100 \quad (3)$$

W: Sample fresh weight (g), TW: Sample turgid weight (g)

Salt stress experiment was carried out completely randomized experimental design with single factors. Treatments had four 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

Salt stress affected statistically significant all plant growth parameters. All levels of salinity inhibited plant growth parameters and relative growth rates significantly, compared with control plants. In terms of flower parameters, main stem buds (MSB) and buds and flowers in branches (BFB) increased as when salinity level was 150 mM. As salinity increased, relative growth rates of shoot height, stem diameter, shoot fresh and dry weights decreased. Relative

Table 1. Relative growth rates of shoot height (RGR-SH), stem diameter (RGR-SD), shoot fresh and dry weight (RGR-SFW and RGR-SDW) of *C. morifolium* 'Bacardi' plants under different salt concentrations.

	RGR-SH cm day <sup>-1</sup>	RGR-SD mm day <sup>-1</sup>	RGR- SFW g day <sup>-1</sup>	RGR-SDW g day <sup>-1</sup>
0	0.27±0.04a	0.034±0.002a	0.40±0.02a	0.134±0.009a
50	0.18±0.03b	0.026±0.006b	0.28±0.10b	0.099±0.034b
100	0.14±0.07bc	0.023±0.004b	0.23±0.03bc	0.092±0.007bc
150	0.12±0.04bc	0.016±0.001c	0.16±0.01cd	0.069±0.002cd
200	0.09±0.05c	0.015±0.005c	0.15±0.02cd	0.068±0.011cd
250	0.08±0.03c	0.015±0.004c	0.10±0.02de	0.062±0.006d
300	0.07±0.01c	0.010±0.002c	0.05±0.05e	0.042±0.019d
LSD	0.075***	0.007***	0.086***	0.028***

\*\*\*p<0.001, ±shows standart deviation

Table 2. Effects of salt stress on plant growth parameters.

NaCl (mM)	Shoot fresh weight (g)	Shoot dry weight (g)	Shoot diameter (mm)	Shoot Height (cm)	Internode length (cm)	Branch number (Unit)	Branch length (cm)
0	37.69±1.6a	10.13±0.1a	6.1±0.3a	40.0±3.5a	1.5±0.1a	18.3±3.9a	7.0±1.1a
50	29.68±7.4b	7.71±2.4b	5.5±0.6b	33.6±3.2b	1.3±0.2b	15.8±5.3ab	5.2±0.9b
100	25.97±2.5bc	7.16±0.5bc	5.3±0.3b	30.8±6.1bc	1.0±0.1c	12.2±5.7bcd	3.6±0.8c
150	20.98±0.8cd	5.55±0.1cd	4.7±0.2c	29.4±3.9bcd	1.0±0.2cd	10.8±5.5bc	2.7±0.6d
200	19.92±1.5cd	5.51±0.8cd	4.7±0.4c	27.4±3.0cd	0.9±0.2cd	7.2±4.4cde	1.5±0.5e
250	16.75±1.2de	5.04±0.4d	4.7±0.3c	26.5±2.7d	0.816±0.2d	6.5±4.5de	1.1±0.2e
300	13.14±4.1e	3.66±1.3d	4.3±0.3c	26.0±1.5d	0.6±0.1e	4.5±3.4e	0.7±0.3e
LSD	6.112***	1.974***	0.411***	4.290***	0.183***	5.581***	0.830***

\*\*\*p<0.001, ± shows standart deviation

growth of shoot height (0.07 cm day<sup>-1</sup>), stem diameter (0.01 mm day<sup>-1</sup>), shoot fresh weight (0.05 g day<sup>-1</sup>) and shoot dry weights (0.042 g day<sup>-1</sup>) were lowest under 300 mM NaCl concentration (Table 1).

Plant growth parameters were sharply affected when plants were exposed to 250 and 300 mM salinity levels (Table 2). While shoot fresh weight (37.69 g) and shoot dry weight (10.13 g) were highest in control plants, the least values shoot fresh weight (16.75 g, 13.14 g) and shoot dry weight (5.04 g, 3.66 g) were at 250 and 300 mM NaCl concentrations, respectively. Shoot diameter, shoot height, internode length, branch number, branch length were drastically decreased above 100 mM NaCl level.

The highest flower number in the main stem was in 50 mM NaCl (2 unit) concentration and the highest number of main stem buds was in 150 mM (5.5 unit) salinity. Furthermore, the flower and bud numbers in branches were higher in 150 mM NaCl (5.83 unit) level. There were no flowers in 300 mM NaCl, only there were buds (Fig. 1).

Flower diameter was negatively affected by salinity (Table 3). Flower diameter was higher in control plants (5.40 cm). The plants which exposed to 300 mM NaCl had not flowers. Disc floret diameter decreased when salinity reached at 150 mM (13.00 mm). Flower fresh weight decreased as salinity increased. On the other hand, the least value was in 250 mM NaCl in terms of flower dry weight. Effect of salinity on flowers was shown in Fig. 2.

Physiological parameters were also affected from salinity. Ion leakage values increased as salinity increased. The highest ion leakage was at 200, 250 and 300 mM NaCl levels (Fig. 3a). Loss of turgidity was higher at 200 mM NaCl, and lower values were at 0, 50, 250 and 300 mM NaCl (Fig. 3b).

## Discussion

According to the results, *C. morifolium* Ramat 'Bacardi' was negatively affected from high level salt

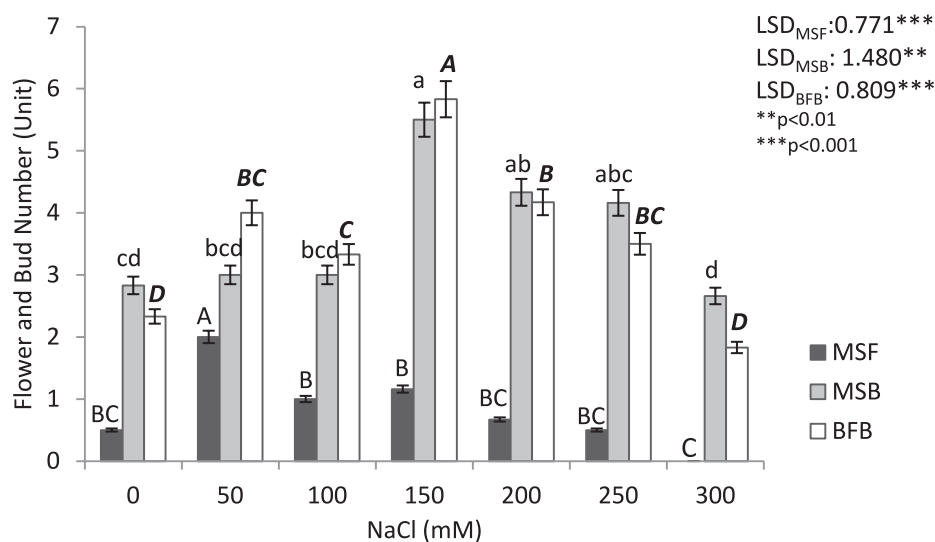


Fig. 1. Numbers of main stem flowers (MSF), main stem buds (MSB) and buds and flowers in branches (BFB) under salinity stress.

concentrations and its flowers lost visual and ornamental quality. The plant height, stem diameter parameters are important for cutting and using as ornaments in a vase. Plant growth is restricted by inhibiting photosynthesis mechanisms, taking excessive salt ions, producing metabolites [5]. Relative growth rates decreased as salinity increased, in this study. Moreover, Cassaniti et al. (2012) [13] exhibited with different levels reducing of relative growth rates on ornamental shrubs. Shoot height, stem diameter, shoot fresh and dry weight, branch number and length, internode length values reduced by salt treatment in this study. Plant height of *Polygonum maritimum* L. reduced with salinity [14]. Plant growth of *Matthiola incana* L. affected by salinity differently depends on the cultivars. Whereas Panamerican cultivars' plant height and shoot fresh weight negatively affected by salinity, Cindirella cultivars' plant height did not affect but shoot fresh weight reduced with salinity [15]. Di Filippo et al. (2020) [16] presented that responses of two ornamental

cultivars of *Glandularia* which exposed to salinity. As a consequence, shoot dry weight of *Dulce Coral* was not affected by salinity, but the dry weight of *Extrema Violeta* decreased as salinity increased. Similarly Akat et al. (2020) [17] showed that salinity reduced to plant growth of *Limonium sinuatum* cultivars. Hooks & Niu (2019) [18] reported that dry weights of four ornamental plants decreased with salt stress. Branch number and length also decreased with salinity, in this study. In parallel Cirillo et al. (2016) [19] found reducing branch number and length in two ornamental shrubs (*Viburnum lucidum* L. and *Callistemon citrinus*). Yasemin (2020) [20] also presented that branch number and length of *Zinnia elegans* and *Zinnia marylandica* decreased as salinity increased. There are several studies that shown reducing plant heights and biomass under saline conditions [16, 14, 17, 21, 22, 6, 23].

There is not too much known the effects of salinity on flowering parameters. Some studies show that salt

Table 3. Effects of salt stress on flowers parameters.

NaCl (mM)	Flower Diameter (cm)	Disc Floret Diameter (mm)	Flower Fresh Weight (g)	Flower Dry Weight (g)
0	5.40±0.5a	15.93±2.5a	1.40±0.4a	0.27±0.09a
50	4.80±0.5b	14.86±1.7ab	1.31±0.2ab	0.26±0.08a
100	4.62±0.5b	15.13±1.4ab	1.03±0.2bc	0.26±0.04a
150	3.93±0.2c	13.00±3.5bc	0.84±0.4cd	0.23±0.10a
200	3.30±0.3d	11.75±1.6c	0.75±0.2cd	0.22±0.06a
250	3.22±0.5d	11.46±3.3c	0.63±0.3d	0.20±0.11b
300	-	-	-	-
LSD	0.454***	2.681***	0.324***	0.095***

\*\*\*p<0.001, ± shows standard deviation

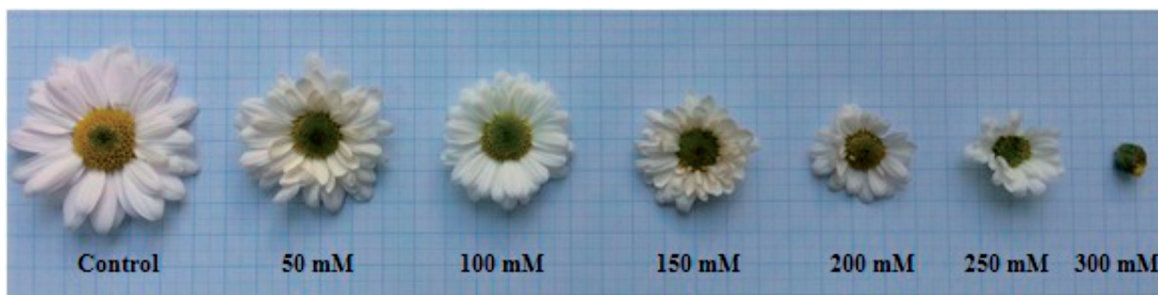


Fig. 2. The aspect of *C. morifolium* flowers under salt (NaCl-mM) stress.

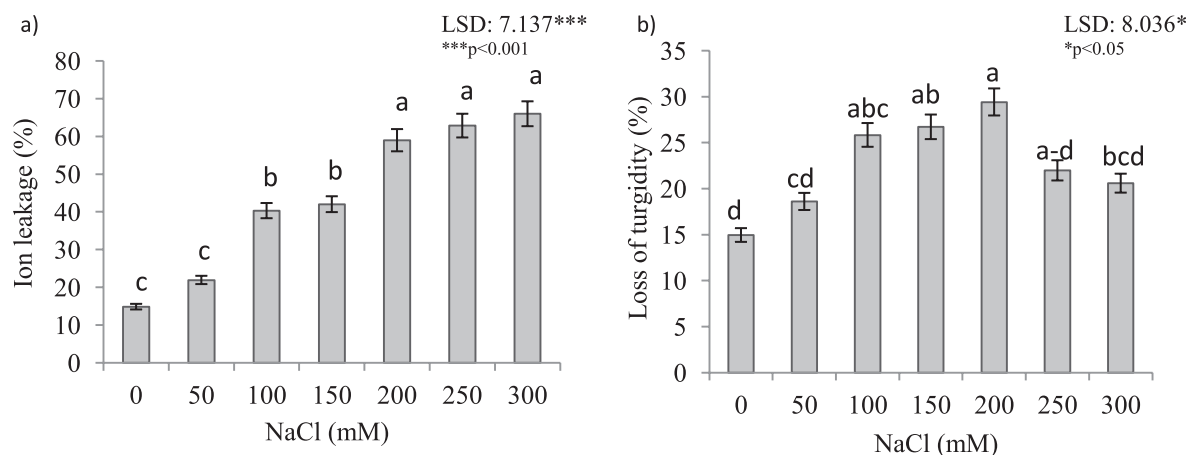


Fig. 3. Ion leakage (%) and loss of turgidity (%) levels in *C. morifolium* 'Bacardi' at different concentrations of NaCl.

stress could delay flowering via some pathways such as insufficient energetic reserves, obstacle of flower structure development, altering hormones balance [9, 24]. In this study, the flowering time was earlier in 50 mM NaCl concentration (data not shown). It can be seen in Fig. 1, the higher number of flowers was obtained from 50 mM NaCl level in the mean stem. On the other hand, Veatch-Blohm & Morningstar, (2011) [25] exhibited that *Zantedeschia aethiopica* cultivars did not affect significantly from salt stress.

Flower quality such as flower yield, flower fresh and dry weights, flower size, flower number and color also negatively affects from salinity [9]. Flower sizes and flower fresh and dry weights also declined by salt stress in *C. morifolium* Ramat 'Bacardi'. However, total number of flowers and buds on main stem and branches increased on 150 mM NaCl levels, the most flower numbers were seen in 50 mM NaCl levels in this study. As salinity increased at 300 mM NaCl concentration, any flowers were recorded and bud numbers declined. In parallel Shillo et al. (2002) [26] found that salinity caused increasing on flower number and reducing stem and inflorescence length. Similar findings were obtained from ornamental sunflower and *Antirrhinum majus*, both of them maintained flower quality under salt stress [27, 28]. On the contrary, flower number reduced under saline conditions in *Matthiola incana* [15]. Sonneveld et al. (1999) [29] reported that flower number of gerbera

and rose decreased with salinity. Flower bud and diameter reduced with salt stress in *Lilium* sp [30], and flower number reduced in *Limonium sinuatum* cultivars [17].

As it is known, excessive salt concentration in areas where plants are grown prevents plants to water uptake [31]. Leaf water potential and osmotic potential of plants decrease depend on the root areas osmotic potential and stress state [32]. Thus, it is possible to demonstrate the stress level of plants by showing the loss of turgor under salt stress conditions. The highest turgidity level was obtained from 200 mM NaCl level, in this study. The turgidity ratio decreased up to 200 mM NaCl concentration. It was found that relative water content of *C. paludosum* [6], Pelargonium [33] and calla lily [34] did not affect from salinity. This ability is associated with the increase in Ca and Mg accumulation in the leaf tissues as well as Na accumulation in plants [33]. On the other hand, Carvalho et al. (2017) [35] reported that relative water content of roses reduced under saline conditions. Relative water content of *Limonium* cultivars also decreased with salt stress [17]. Another indicator of salt stress in plants is the damage to the cellular membrane. There are many studies revealing the increase in ion leakage and membrane damage caused by salt stress. In this study, it was obtained that ion leakage of *C. morifolium* Ramat 'Bacardi' plants increased as salinity increased. Similarly, ion leakage

rates of *Zinnia* cultivars [20], *Hibiscus rose-sinensis* [36] *C. paludosum* [6] increased with salt stress.

### Conclusions

Plant growth parameters, most of flower parameters, ion leakage and water content (maintaining turgor) of *Crysanthemum morifolium* Ramat 'Bacardi' negatively affected from high salinity. Plant height and stem diameter parameters which have importance as cut flowers reduced with salinity. Main stem flower numbers increased at 50 mM NaCl and main stem buds and branch buds increased till 150 mM NaCl level. Up to 150 mM NaCl all of flower parameter values decreased. Ion leakage of plants increased as salinity increased. Loss of turgidity increased to 200 mM NaCl concentration. According to results, *C. morifolium* Ramat 'Bacardi' negatively affected from high level salt concentrations and its flowers lost visual and ornamental quality. As a consequence of this study, *Crysanthemum morifolium* Ramat 'Bacardi' is a moderately tolerant cultivar and but also should not be cultivated as a cut flower under highly (up to 150 mM NaCl) saline conditions.

### Conflict of Interest

The authors declare no conflict of interest.

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