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# Alleviation of Adverse Effects of Seawater on Growth and Chemical Constituents of *Chrysanthemum morifolium* by Foliar Fe and Mn Applications

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## ABSTRACT

*Chrysanthemum morifolium* plants were grown in a pot experiment to investigate the effects of seawater stress using 0, 2, 4 and 8% seawater (S0, S2, S4 and S8, respectively). The plants grown in sandy soil were foliar-sprayed three times with 5 ppm of Mn and/or Fe in addition to the standard fertilizer applied to the soil. The seawater-stressed treatments had a shorter plant height, lower plant Fresh Mass (FM) and Dry Mass (DM), lower flower head fresh mass and smaller flower diameter than the plants that were not exposed to seawater. These reductions correlated with the seawater concentration and the foliar applications significantly alleviated these adverse effects. The plants watered with S8 and treated with Mn+Fe had a higher FM (446.4) and DM (25.4 g plant$^{-1}$) than the S0-watered plants not treated with the foliar fertilizers (404.9 and 17.4 g plant$^{-1}$), whereas the flower head fresh masses were 96.3 and 108 g plant$^{-1}$, respectively. Increasing the concentration of seawater from S0 to S8 reduced the onset of flowering from 105 to 94 days and the foliar treatment significantly delayed the flowering date, regardless of the type of irrigation water. The S8-watered plants treated with Mn+Fe had significantly higher levels of chlorophylls a and b and carotenoids than the S0-watered plants sprayed with tap water. Furthermore, the Mn and/or Fe-mediated alleviation of seawater stress in the chrysanthemum plants was associated with higher levels of proline as well as reducing and non-reducing sugars. In conclusion, overall, the results support the hypothesis that foliar Fe and Mn treatment can alleviate seawater stress in chrysanthemum plants.

**Key words:** *Chrysanthemum morifolium*, salt stress, micronutrients, pigments, proline, non-reducing sugars

## INTRODUCTION

The cultivated garden chrysanthemum, *Dendranthema×grandiflora* Tzvelv. (*Chrysanthemum ×morifolium* Ramat.), is a member of the Asteraceae family. Chrysanthemum is one of the most important marketable floriculture crops as a cut flower, potted flowering plant and herbaceous perennial (Anderson, 2007).

High salt concentrations in soils inhibiting crop growth and productivity are frequent constraint to agriculture in arid and semi-arid regions. Irrigation with poor quality water is one of the main factors resulting in salt accumulation and decrease of agricultural productivity. As saline soils and saline waters are common around the world, great efforts have been devoted to understand physiological aspects of tolerance to salinity in plants. Salinity imposes both ionic and osmotic stresses on plants (Munns *et al*., 2006). Although, salinity is an important limiting factor for agriculture, plants vary widely in their sensitivity to salt stress. Under saline conditions, soils contain extreme ratios of Na/Ca, Na/K, Ca/Mg and Cl/NO, which can reduce plant growth because of specific ion toxicities and the impact of ionic imbalances on the biophysical and/or metabolic components of plant growth (Grattan and Grieve, 1999).

The exposure to sea aerosol and salt water, which infiltrate the groundwater, may significantly reduce plant growth and production (Cheplick and Demetri, 1999). Unfortunately, Flowers (2004) emphasises that breeding for better $Na^+$ exclusion or osmotic adjustment has not resulted in salt-tolerant cultivars for many cultivated crops. Accordingly, a tolerance to water salinity is important in ornamental plants. Lee and van Iersel (2008) found that chrysanthemums can be grown successfully with 1 g $L^{-1}$ NaCl in the irrigation water without negative impacts on the plant quality, whereas a higher concentration of NaCl severely affected the growth of the plants. However, from a physiological view, a NaCl solution does not mimic seawater that was not purified to remove trace metals (Luther and Tsamakis, 1989). Moreover, the salinity of seawater is a real issue in coastal areas and landscapes where plants are damaged by aerosols that originate from the sea (Ferrante *et al*., 2011).

The nutrients supplied via the roots are restricted under saline conditions because of the effects of drought and salinity. In this case, the efficiency of foliar application is higher than that of soil fertilisation because the required nutrient is supplied directly to the site of demand and the nutrients can be quickly absorbed and independently of root activity and soil water availability (Roemheld and El-Fouly, 1999). Thus, foliar nutrient application under drought and salinity conditions may alleviate a water or nutrient deficit under short-term drought or salt stress conditions. Furthermore, foliar mineral application may be more practical than soil mineral application, in which the minerals can adsorb to the soil particles and are less available to the roots (Sarkar *et al*., 2007; Wissuwa *et al*., 2008).

The aim of the present study was to evaluate the effects of foliar applications of Mn and Fe on the growth and flowering of chrysanthemum plants watered with dilute seawater. To the best of our knowledge, this is the first report regarding the use of diluted seawater for chrysanthemum irrigation.

## MATERIALS AND METHODS

**Plant material and growing conditions:** Two pot experiments were carried out on (*Chrysanthemum morifolium* Ramat cv. Icecap) in a controlled environment of the Plant Production Departments, College of Food and Agricultural Sciences, King Saud University, Riyadh, in two successive winter seasons of 2008/2009 and 2009/2010 seasons. The experiment was carried out in a glass-covered greenhouse with minimum and maximum temperature 15.3 and 31.1°C, respectively; however, the minimum and maximum relative humidity averaged 41 and 70%. Homogeneous seedlings (45 days old) of *C. morifolium* with 2-3 leaf pairs were individually transplanted on the first week of September in 30 cm diameter pots filled with 12 kg sandy soil (12% clay, 1% silt and 87% sand). The soil chemical properties (Jackson, 1973; Black, 1983) are shown in Table 1. Before transplantation, 3 g $pot^{-1}$ of super phosphate (15.5% $P_2O_5$) was mixed with the soil; 2 g $pot^{-1}$ of ammonium sulphate (20.5% N) and 2 g $pot^{-1}$ of potassium sulphate (48% K) were added twice after transplantation (at two weeks intervals, commencing two weeks after transplantation). The daily light integral from 10th, October till 1st November averaged 8.8±2.5 mol $m^{-2}$. The plants were watered with tap water for two weeks before using the diluted Seawater (S), which contains 34000 ppm total salt, as shown in Table 1.

Table 1: Chemical properties of the experimental soli and seawater

| | pH | EC (dS $m^{-1}$) | $CaCO_3$ (%) | $Ca^{++}$ | $Mg^{+2}$ | $Na^+$ | $K^+$ (ppm) | $HCO_3^-$ | $Cl^-$ | $SO_4^{2-}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Experimental soil | 8.40 | 2.10 | 1.00 | 23 | 12 | 14 | 2.79 | 7.10 | 26 | 18.69 |
| Seawater | 8.25 | 53.10 | -- | 4000 | 1272 | 9430 | 380 | 98 | 16312 | 2496 |

**Salinity concentrations:** The concentrations of seawater used for irrigation were 0, 2, 4 and 8% (S0, S2, S4 and S8, respectively), with EC values of 0.5, 2.1, 6.1 and 10.8 dS $m^{-1}$, respectively. Pillsbury and Blaney (1966) concluded that the upper limit for the salinity level of irrigation water is approximately 7.5 dS $m^{-1}$.

**Micronutrient treatments:** All of the plants were watered with 500 mL of the appropriate solution on each treatment and the applied water did not leach from the bottom of the pots. The plants were foliar-sprayed well with tap water, 5 ppm Fe ($FeSO_4$), 5 ppm Mn ($MnCl_2$) or a mixture of Mn and Fe of the same concentrations. Each solution was applied 4 times at 3 weeks intervals, commencing one week after transplantation. Tween-20 (0.1%) was used as a wetting agent. The plants were arranged in a randomised complete block design, with 4 seawater treatments and 4 foliar spray applications. Thus, the experiment included 16 treatments, each with 5 plants and all of the treatments were replicated 4 times. The experiment was repeated in the next season.

**Plant harvesting and chemical treatment:** The plants were harvested after 140 days. Before harvesting, 5 fresh leaf disks (0.6 mm in diameter) from the centre of the 3rd-7th youngest fully expanded leaf were collected to estimate the chlorophyll a and b levels (Lichtenthaler and Wellburn, 1983). The plants were cut above the soil surface and the plant height and dry mass after oven-drying at 80°C were measured. For each treatment, 0.5 g fresh sample of the ray florets were used to measure the carotenoid content, as described previously (Bulda *et al*., 2008). Samples of the flowering heads were collected to assess their mean diameters before drying at 80°C to assess the dry weights of the individual plants. A sample of the dried leaves was used to assess the $Na^+$, $K^+$ and $Cl^-$ levels (Taleisnik and Grunberg, 1994), the reducing and non-reducing sugars according to AOAC (2005) and the proline content following Bates *et al.* (1973).

**Statistical analysis:** The data of the two seasons were summed together and subjected to an ANOVA and the means were evaluated using Tukey's test at the 5% level according to Snedecor and Cochran (1980).

## RESULTS

One of the main effects of seawater stress on chrysanthemum plants was growth reduction, as revealed by significant decreases in plant height, FM, DM and flower head dry mass. The results showed that both the seawater and foliar treatments significantly affected chrysanthemum growth pattern moreover, there was a significant interaction between these two factors. In all cases, an increasing seawater concentration linearly reduced the plant height compared with the tap water-treated plants (69.7 cm). Although, the foliar application of minerals did not affect the height of the S0- or S4-watered plants, treatment with Mn+Fe significantly alleviated the adverse effects on the plant height under the S2 and S4 irrigation levels (Table 2). When the foliar applications of the S0-treated plants increased ($p<0.5$), the FM and DM also increased from 404.9-430.8-446.4 and 17.4-19.5-25.4, respectively, depending on the foliar treatment. The difference in the FM and

Table 2: Effect of Mn and Fe foliar application on the growth of chrysanthemum watered with diluted seawater

| Foliar application | Seawater | Plant height (cm) | FM (g plant$^{-1}$) | DM (g plant$^{-1}$) | Flowering date (days) | Flower-head fresh mass (g plant$^{-1}$) | Flower diameter(cm) |
|---|---|---|---|---|---|---|---|
| Tap water | S0 | $69.7^{b}$ | $404.9^{de}$ | $17.4^{h}$ | $105^{g}$ | $108.0^{c}$ | $3.6^{h}$ |
| | S2 | $64.8^{d}$ | $397.1^{f}$ | $16.5^{i}$ | $104^{gh}$ | $99.0^{h}$ | $3.0^{i}$ |
| | S4 | $62.9^{e}$ | $371.6^{k}$ | $15.4^{j}$ | $100^{ij}$ | $93.0^{m}$ | $2.8^{i}$ |
| | S8 | $59.4^{g}$ | $370.9^{k}$ | $13.5^{l}$ | $94^{k}$ | $89.0^{n}$ | $2.4^{j}$ |
| Mn (5 ppm) | S0 | $72.2^{a}$ | $430.8^{b}$ | $19.5^{de}$ | $112^{de}$ | $112.8^{b}$ | $4.7^{b}$ |
| | S2 | $64.3^{d}$ | $19.1^{cde}$ | $18.2^{g}$ | $110^{f}$ | $103.8^{f}$ | $4.3^{cde}$ |
| | S4 | $62.4^{e}$ | $388.2^{h}$ | $14.3^{k}$ | $105^{g}$ | $97.8^{i}$ | $4.1^{efg}$ |
| | S8 | $58.9^{g}$ | $377.7^{j}$ | $16.3^{i}$ | $99^{j}$ | $93.8^{l}$ | $3.9^{g}$ |
| Fe (5 ppm) | S0 | $72.4^{a}$ | $430.6^{b}$ | $19.6^{d}$ | $114^{c}$ | $112.6^{b}$ | $4.5^{bc}$ |
| | S2 | $64.4^{d}$ | $407.8^{d}$ | $18.5^{f}$ | $111^{ef}$ | $105.0^{e}$ | $4.4^{bcd}$ |
| | S4 | $62.4^{ef}$ | $393.1^{fg}$ | $15.5^{j}$ | $103^{h}$ | $99.0^{h}$ | $4.2^{def}$ |
| | S8 | $59.3^{g}$ | $383.7^{i}$ | $14.3^{k}$ | $100^{ij}$ | $95.0^{k}$ | $4.0^{fg}$ |
| Mn+Fe (5+5 ppm) | S0 | $72.7^{a}$ | $446.4^{a}$ | $25.4^{a}$ | $121^{a}$ | $115.2^{a}$ | $5.3^{a}$ |
| | S2 | $66.8^{c}$ | $418.0^{c}$ | $24.3^{b}$ | $118^{b}$ | $106.3^{d}$ | $4.6^{bc}$ |
| | S4 | $65.0^{d}$ | $401.3^{e}$ | $22.2^{c}$ | $113^{cd}$ | $100.4^{g}$ | $4.4^{cd}$ |
| | S8 | $61.5^{f}$ | $390.8^{gh}$ | $19.3^{e}$ | $101^{i}$ | $96.3^{j}$ | $4.2^{defg}$ |

S0: Tape water, S2: 2% seawater, S4: 4% seawater, S8: 8% Seawater, Fe: $FeSO_4$, Mn: $MnCl_2$, Mn+Fe: A mixture of Mn and Fe, FM: Fresh mass, DM: Dry mass, On the same column means followed by different letters are significantly different at $p<0.05$, Tukey's multiple range test

DM between S0 and S2 was not significant under the same foliar treatment but a significant reduction in the FM and DM was observed for the S8 treatment compared to S2 (Table 2). The plants treated with Mn+Fe had a higher FM and DM (390.8 and 19.3 g plant$^{-1}$) than those watered with S0 and sprayed with tap water (404.9 and 17.4 g plant$^{-1}$). The tap water-irrigated plants treated with Mn+Fe had the highest FM and DM (446.4 and 25.4 g plant$^{-1}$).

The chrysanthemum flower head fresh weights and flower diameters were significantly reduced when the plants were irrigated with diluted seawater, regardless of the foliar application and these reductions were more severe when the concentration of seawater was increased. Unlike the vegetative FM, the flower head fresh mass and flower head diameter were significantly ($p<0.5$) reduced by the diluted seawater, even under the S2 treatment. There was no variation in the flower head fresh mass values (112.8 and 115.2 g plant$^{-1}$, Table 2) among the tap water-irrigated plants and those treated with any of the foliar applications. Nevertheless, all of the plants had higher flower head fresh massed compared with tap water-irrigated plants without foliar applications (108 g plant$^{-1}$). Overall, the Mn and/or Fe treatment alleviated the effects of salt stress under treatment with the same seawater concentration (Table 2).

Increasing the concentration of seawater decreased ($p<0.5$) the time to chrysanthemum flower onset from 105 days (S0-watered plants) to 94 days (S8-watered ones). All of the foliar treatments significantly delayed the flowering date, regardless of the type of irrigation water. Moreover, the application of seawater promoted chrysanthemum flowering, even for the foliar-treated plants. Overall, the order of flowering dates with respect to foliar application was non-treated and Mn-, Fe- and Mn+Fe-treated plants. In all cases, increasing the concentration of the seawater significantly decreased the flower head diameter, which varied between 2.4 cm (non-foliar-treated plants under S8) and 5.3 cm (plants under S0 and treated with Mn+Fe). The foliar application increased ($p<0.5$) the flower head diameter of the S0-watered plants compared with the plants not subjected to a foliar treatment. Moreover, no significant difference was observed for the flower head diameter between S0, non-foliar-treated plants and S2-treated plants with Mn and/or Fe (Table 2).

The leaf content of chlorophylls a and b was affected by the foliar application; moreover, there was a significant interaction ($p<0.5$) between the seawater concentration and foliar application. The chlorophyll a and b contents of the foliar-treated plants increased ($p<0.5$) with an increasing concentration of seawater (Table 3). For the non-foliar-treated plants, the chlorophyll a and b concentration increased under S4 but a further increase in the seawater concentration decreased the chlorophyll a and b levels to 0.3 and 0.10 mg $g^{-1}$ FW, respectively. The foliar application of Fe was more effective than application of Mn ($p<0.5$) on the chlorophyll content because, in most cases under the same irrigation treatment, the Fe-treated plants had higher chlorophyll a and b content than the Mn-treated plants. The highest chlorophyll a and b content (0.45 and 0.16 mg $g^{-1}$ FW, respectively) was observed for the S8 plants treated with Mn+Fe, whereas, the non-foliar-treated plants watered with tap water exhibited the lowest content of these pigments (0.30 and 0.10 mg $g^{-1}$ FW, respectively).

The carotenoid content of the chrysanthemum flowers was significantly variable, between 3.56 and 4.78 mg $g^{-1}$ FW. Increasing the concentration of seawater up to S4 linearly increased ($p<0.5$) the carotenoid content in most cases but a further increase in the seawater concentration reduced the content (Table 3). The Mn-foliar application did not improve the carotenoid content when the plants were watered with diluted seawater, whereas the Fe and Mn+Fe-foliar applications significantly increased the carotenoid content under the same seawater concentration compared with the non-foliar-treated plants. The chrysanthemums watered with S8 and treated with Fe or Mn+Fe had the highest carotenoid content (4.66 and 4.78 mg $g^{-1}$ FW); however, the tap water-irrigated plants that did not receive foliar fertilisation had the lowest carotenoid level (3.56 mg $g^{-1}$ FW).

Table 3: Effect of Mn and Fe foliar application on chlorophylls, carotenoids and proline contents of chrysanthemum watered with diluted seawater

| Foliar application | Seawater | Chl. a mg $g^{-1}$ FM | Chl. b mg $g^{-1}$ FM | Carotenoids mg $g^{-1}$ FM | Proline µg $g^{-1}$ DM |
|---|---|---|---|---|---|
| Tap water | S0 | $0.30^{i}$ | $0.10^{i}$ | $3.56^{o}$ | $7.96^{l}$ |
| | S2 | $0.31^{h}$ | $0.10^{l}$ | $3.71^{m}$ | $9.66^{h}$ |
| | S4 | $0.35^{ef}$ | $0.11^{j}$ | $3.90^{j}$ | $10.50^{d}$ |
| | S8 | $0.40^{c}$ | $0.13^{g}$ | $4.29^{e}$ | $5.58^{n}$ |
| Mn (5 ppm) | S0 | $0.32^{h}$ | $0.11^{k}$ | $3.69^{n}$ | $8.88^{j}$ |
| | S2 | $0.33^{g}$ | $0.12^{i}$ | $3.84^{l}$ | $9.96^{f}$ |
| | S4 | $0.36^{e}$ | $0.13^{g}$ | $4.06^{h}$ | $11.48^{b}$ |
| | S8 | $0.41^{c}$ | $0.14^{d}$ | $4.46^{c}$ | $5.06^{o}$ |
| Fe (5 ppm) | S0 | $0.32^{h}$ | $0.12^{i}$ | $3.87^{k}$ | $9.71^{g}$ |
| | S2 | $0.34^{g}$ | $0.12^{h}$ | $4.02^{i}$ | $9.71^{g}$ |
| | S4 | $0.36^{e}$ | $0.13^{f}$ | $4.24^{f}$ | $11.33^{c}$ |
| | S8 | $0.42^{b}$ | $0.15^{b}$ | $4.66^{b}$ | $5.74^{m}$ |
| Mn+Fe (5+5 ppm) | S0 | $0.34^{fg}$ | $0.12^{h}$ | $4.01^{i}$ | $9.07^{i}$ |
| | S2 | $0.35^{ef}$ | $0.14^{e}$ | $4.16^{g}$ | $10.22^{e}$ |
| | S4 | $0.38^{d}$ | $0.15^{c}$ | $4.37^{d}$ | $11.55^{a}$ |
| | S8 | $0.45^{a}$ | $0.16^{a}$ | $4.78^{a}$ | $4.84^{p}$ |

S0: Tape water, S2: 2% seawater, S4: 4% seawater, S8: 8% seawater, Fe: $FeSO_4$, Mn: $MnCl_2$, Mn+Fe: A mixture of Mn and Fe, FM: Fresh mass, DM: Dry mass, Chl.: Chlorophylls, On the same column means followed by different letters are significantly different at $p<0.05$, Tukey's multiple range test

The chrysanthemum plants accumulated more proline with an increasing concentration of seawater. There was no significant difference in the proline content among the plants watered under the S0 treatment, regardless of the foliar application. However, under the same concentration of seawater, the plants treated with Mn and/or Fe accumulated less proline than the non-treated plants (Table 3). The lowest and highest proline accumulation (4.84 and 11.55 $\mu g\ g^{-1}$) were found for the plants watered with S0 and treated with Mn+Fe and the foliar-treated plants irrigated with S8, respectively. All of the plants accumulated higher reducing and non-reducing sugars with increasing concentrations of seawater. The reducing and non-reducing sugars level of the S4-watered plants treated with Mn+Fe was 2.5 times higher than that of the plants watered with tap water without foliar application (Table 4). However, the plants watered with S8 had the highest values (1.41 and 1.44 as well as 12.01 and 12.26 $\mu g\ g^{-1}$ DM for reducing and non-reducing sugars contents, respectively) when treated with Fe or Mn+Fe, respectively, without a significant difference between them.

An increase in the $K^+$ content of the chrysanthemum leaves was attributed to the concentration of seawater. The plants treated with Mn+Fe had significantly lower concentrations of $K^+$ than the non-treated plants grown under the same seawater concentration. The $K^+$ content increased approximately 4 fold in the S8 watered plants treated with Fe compared with the non-foliar-treated plants watered with S0, which accumulated 17.6 mg $g^{-1}$ DM (Table 4). Almost all of the S0-watered plants had a similar $Na^+$ and $Cl^-$ content, regardless of the foliar treatment. The chrysanthemum plants accumulated more $Na^+$ and $Cl^-$ under the S8 treatment than did plants with the same irrigation treatment under the same foliar treatment. Under the same concentration of seawater, the plants treated with Mn and/or Fe accumulated more $Cl^-$ than the non-treated plants.

Table 4: Effect of Mn and Fe foliar application on $K^+$, $Na^+$, $Cl^-$ content and reducing and non-reducing sugars of chrysanthemum watered with diluted seawater

| | | Conc. (mg $g^{-1}$ DM) | | | | |
|---|---|---|---|---|---|---|
| Foliar application | Seawater | K | $Na^+$ | $Cl^-$ | Reducing sugars | Non-reducing sugars |
| Tap water | S0 | $17.6^{n}$ | $7.5^{p}$ | $10.3^{n}$ | $0.95^{h}$ | $8.92^{k}$ |
| | S2 | $23.5^{m}$ | $22.4^{i}$ | $22.3^{k}$ | $1.26^{e}$ | $9.86^{j}$ |
| | S4 | $48.5^{e}$ | $47.5^{e}$ | $49.4^{g}$ | $1.32^{c}$ | $10.32^{g}$ |
| | S8 | $68.5^{b}$ | $62.4^{a}$ | $71.3^{d}$ | $1.35^{bc}$ | $10.92^{e}$ |
| Mn (5 ppm) | S0 | $24.4^{l}$ | $10.5^{n}$ | $22.4^{k}$ | $1.12^{g}$ | $10.22^{h}$ |
| | S2 | $25.4^{k}$ | $20.3^{k}$ | $45.3^{h}$ | $1.21^{f}$ | $10.98^{e}$ |
| | S4 | $46.5^{f}$ | $37.3^{g}$ | $67.5^{e}$ | $1.31^{cd}$ | $11.34^{d}$ |
| | S8 | $67.5^{c}$ | $57.3^{c}$ | $75.3^{b}$ | $1.37^{b}$ | $11.84^{c}$ |
| Fe (5 ppm) | S0 | $15.4^{p}$ | $11.3^{m}$ | $19.3^{l}$ | $1.14^{g}$ | $10.06^{i}$ |
| | S2 | $36.6^{g}$ | $21.3^{j}$ | $43.3^{i}$ | $1.26^{e}$ | $10.72^{f}$ |
| | S4 | $60.3^{d}$ | $32.3^{h}$ | $62.4^{f}$ | $1.35^{bc}$ | $10.93^{e}$ |
| | S8 | $78.5^{a}$ | $58.3^{b}$ | $80.4^{a}$ | $1.41^{a}$ | $12.00^{b}$ |
| Mn+Fe (5+5 ppm) | S0 | $16.4^{o}$ | $10.3^{o}$ | $18.4^{m}$ | $1.25^{ef}$ | $10.73^{f}$ |
| | S2 | $27.5^{j}$ | $14.4^{l}$ | $34.4^{j}$ | $1.28^{de}$ | $11.39^{d}$ |
| | S4 | $28.4^{i}$ | $44.4^{f}$ | $74.5^{c}$ | $1.36^{b}$ | $11.94^{b}$ |
| | S8 | $30.3^{h}$ | $55.3^{d}$ | $80.3^{a}$ | $1.44^{a}$ | $12.26^{a}$ |

S0: Tape water, S2: 2% seawater, S4: 4% seawater, S8: 8% seawater, Fe: $FeSO_4$, Mn: $MnCl_2$, Mn+Fe: A mixture of Mn and Fe, FM: Fresh mass, DM: Dry mass, On the same column means followed by different letters are significantly different at $p<0.05$, Tukey's multiple range test

## DISCUSSION

The irrigation of chrysanthemum plants with seawater adversely affected their growth pattern including the plant height, Fresh Mass (FM), Dry Mass (DM) and flower head fresh mass and diameter. For most of the investigated parameters the effect of the seawater was positively correlated with the concentration of the seawater. A reduction in growth due to salinity could mainly occur through the inhibition of cell elongation and cell division is the first phase of the growth response to salt stress and could be a result of the osmotic stress of salinity (Munns, 1993). It seems that the effect of seawater on the growth of chrysanthemum might principally be a result of a water deficit rather than salinity, as no obvious toxicity was observed during the plant growth period. There was an approximate 20% reduction in the plant FM, DM and flower head fresh mass in the S8-treated plants. Similar results have been achieved with different crops (Parida and Das, 2005) and salinity has been shown to reduce the flower yield of many ornamental crops (Colom and Vazzana, 2002; Razmjoo *et al.*, 2008).

Cassaniti *et al.* (2012) stated that any negative effects of salts on ornamental plant growth have to be taken into consideration mainly for their influence on the aesthetic value, which is an important aspect of these plants. Seawater stress brought forward chrysanthemum flowering onset, similarly to previous results for some ornamental plants (Shillo *et al.*, 2002). Lee and van Iersel (2008) found that increasing NaCl up to 3 g $L^{-1}$ promoted chrysanthemum flowering, whereas flowering was delayed at 6 and 9 g $L^{-1}$. However, the majority of researchers believe that salinity stress delays flowering. This discrepancy in the flowering time under salinity stress could be a direct effect of toxicity that delays flowering or an indirect effect of osmotic stress that enhances flowering. Early flowering, which means a short growth duration, generally involves a reduction in plant size and leaf area and leads to a complete life cycle before severe drought stress, a known escape strategy (Hirose *et al.*, 2005). The chrysanthemum flower head fresh mass and diameter were linearly reduced by increasing the concentration of seawater. The effects of salinity on flower quality have been observed in many ornamental plants (Cassaniti *et al.*, 2012) and the reduction in the flower head diameter and fresh mass could be due to osmotic stress. Chaparzadeh *et al.* (2004) suggested that a decrease in cell size under unfavourable conditions allows the conservation of energy, thereby launching the appropriate defence response and also reducing the risk of heritable damage.

The chrysanthemum chlorophyll and carotenoid content was increased at the low concentrations of seawater compared with in the tap water-irrigated plants, whereas the higher concentration decreased the pigment levels. These findings are consistent with previous reports on other chrysanthemum species (Chen *et al.*, 2002; Lee and van Iersel, 2008). A low concentration of salts may induce osmotic stress, which reduces plant growth and cell enlargement, resulting in a high content of these pigments; however, a high salt concentration will cause chlorophyll degradation (Tavakkoli *et al.*, 2011; Santos, 2004). Carotenoids in flowers vary considerably and are influenced by many factors, including the environment and salt stress, which decreases the expression of carotenoid biosynthetic genes. Therefore, salt stress plays a significant role in hampering the photosynthesis rate, thereby reducing the yield and the productivity of plants (Babu *et al.*, 2011). Ashraf (1994) suggested that higher levels of chlorophyll were probably maintained by the catabolism of excess proline and the de novo synthesis of glutamic acid, which is a precursor for chlorophyll biosynthesis.

Higher levels of proline as well as reducing and non-reducing sugars accumulated in the plants with an increasing concentration of seawater. Nevertheless, the plants treated with Mn and/or Fe accumulated less proline under seawater stress. Such patterns of reducing and non-reducing sugars and proline accumulation under stress have often been observed in ornamental crops (Chaparzadeh *et al*., 2004). The accumulation of reducing and non-reducing sugars in the leaves might be a response to the excess accumulation of monovalent ions in the vacuole under saline stress. The accumulation of carbohydrates and proline in the cytoplasm could act as osmolytes that, unlike monovalent ions, are not harmful to the enzyme system and membrane stability and help to balance the reduced osmotic potential of the vacuole. Indeed, proline is a compatible organic compound that makes a significant contribution to osmotic adjustment (Singh *et al*., 1996). In addition, a study on the contribution of sugars to osmotic adjustment in the elongating and expanded zones of wheat leaves under drought (Munns and Weir, 1981) showed that sugars were responsible for 55-88% of the osmotic adjustment. In contrast, under saline conditions, sugars accounted for only approximately 13% of the osmotic adjustment in the expanded zone of wheat leaves, whereas cations and anions accounted for approximately 21-30%.

The accumulation of $K^+$, $Na^+$ and $Cl^-$ in the chrysanthemum leaves increased with an increasing concentration of seawater. The higher accumulation of these elements under seawater stress might be due to the physiological mechanisms involved in osmoregulation. According to Munns (1993), plant growth is first inhibited in saline conditions by a water deficit. In general, osmoregulation can use ions from the soil, whereas, in the absence of salinity but in the present of a drought, the necessary solutes, such as carbohydrates, have to be produced within the plant (Hsiao *et al*., 1976). Osmotic adjustment via ion uptake is also more energy efficient than that occurring through the production of organic solutes (Wyn-Jones and Storey, 1981). Thus, plants adapting to salinity should preferentially take up more ions from the soil for osmotic adjustment and this hypothesis is supported by the increased concentrations of most ions in chrysanthemum leaves. Plants that survive under saline conditions accumulate high concentrations of ions, mainly sodium and chloride (Heuer, 2005). Yuncai *et al*. (2008) suggested that the poor growth of plants and the short duration of experiments could be reasons for the high ion concentrations that have been observed under salt stress. Taken together, the results clearly suggest that the actual inhibition of growth under saline conditions is mainly the result of the associated water deficit, a finding that supports the two-phase response of plant growth to salinity proposed by Munns (1993).

The foliar application significantly improved the chrysanthemum plant growth, irrespective of the salinity conditions. In most cases, there were no clear trends for $Na^+$ and $Cl^-$ in response to the foliar treatment, whereas the $K^+$ concentration increased when the plants were treated with Mn+Fe. The results indicated that Mn- and Fe-foliar applications could alleviate the adverse effects of low concentrations of seawater on the growth of chrysanthemum plants. The plants watered with the highest concentration of seawater and treated with Mn+Fe had a significantly higher FM, DM, flower head fresh mass and diameter values than the non-foliar-treated plants watered under the same conditions. Moreover, this foliar treatment brought forward the flowering date by one week but it did not affect the plant height.

The S8-watered plants treated with Mn+Fe had 10% higher FM and DM values than the non-foliar-treated plants irrigated under S0. Nevertheless, there were only 20 and 9% reductions in the flower head fresh mass and diameter, respectively, for the S8-watered plants treated with Mn+Fe compared with the non-foliar-treated plants watered under the S0 treatment. Such an affect might be attributable to the favourable influence of Mn and Fe on metabolism and biological activity and their stimulating effect on photosynthetic pigments and enzyme activity, which, in turn, improve vegetative growth. Iron is involved in many photosynthetic, respiratory and

N-assimilation reactions, as Fe is a cofactor in redox reactions (Mengel and Kirkby, 1982). Manganese is involved in photosynthesis, as it is tightly bound as $Mn^{+2}$ by photosystem 2 and in certain superoxide dismutases; loosely bound $Mn^{+2}$ is the unique activator of some enzymes and, in some cases, is an alternative to $Mg^{+2}$ in the activation of some enzymes (Raven, 1990). Iron and $Mn^{+2}$ may both be growth-limiting nutrients for plants in a number of environments (Marschner, 1986).

## CONCLUSION

Foliar application of 5 ppm Fe and 5 ppm Mn to chrysanthemum plants mitigated the adverse effect of seawater on growth and flowering. The plants watered with 8% seawater and treated with foliar fertilisers were shorter and had a higher mass than the control plants. The treated plants flowered earlier, yet the flower head dry mass and diameter were slightly less than those of the control plants. The foliar application of nutrients improved the tissue content of chlorophylls a and b, carotenoids, proline as well as reducing and non-reducing sugars.

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## REFERENCES

AOAC, 2005. Official Methods of Analysis. 18th Edn., Association of Official Analytical Chemists, Washington, DC., USA.

Anderson, N.O., 2007. Chrysanthemus, *Dendranthema×grandiflora* Tzvelv. In: Flower Breeding and Genetics, Anderson, N.O. (Ed.). Springer, Netherlands, pp: 389-437.

Ashraf, M., 1994. Breeding for salinity tolerance in plants. Crit. Rev. Plant Sci., 13: 17-42.

Babu, M.A., D. Singh and K.M. Gothandam, 2011. Effect of salt stress on expression of carotenoid pathway genes in Tomato. J. Stress Physiol. Biochem., 7: 87-94.

Bates, L.S., R.P. Waldren and I.D. Teare, 1973. Rapid determination of free proline for water-stress studies. Plant Soil, 39: 205-207.

Black, C.A., 1983. Methods of Plant Analysis, Part 1 and 2. American Society of Agronomy Inc., Madisson, WI., USA.

Bulda, O.V., V.V. Rassadina, H.N. Alekseichuk and N.A. Laman, 2008. Spectrophotometric measurement of carotenes, xanthophylls and chlorophylls in extracts from plant seeds. Russian J. Plant Physiol., 55: 544-551.

Cassaniti, C., D.I. Romano and T.J. Flowers, 2012. The Response of Ornamental Plants to Saline Irrigation Water. In: Irrigation-Water Management, Pollution and Alternative Strategies, Show, K.Y. and X. Guo (Ed.). InTech, Croatia.

Chaparzadeh, N., M.L. D'Amico, R.A. Khavari-Nejad, R. Izzo and F. Navari-Izzo, 2004. Antioxidative responses of *Calendula officinalis* under salinity conditions. Plant Physiol. Biochem., 42: 695-701.

Chen, F., S. Chen and W. Guo, 2002. Salt tolerance identification of three species of chrysanthemums. Acta Horticultarea, 618: 299-305.

Cheplick, G.P. and H. Demetri, 1999. Impact of saltwater spray and sand deposition on the coastal annual *Triplasis purpurea* (Poaceae). Am. J. Bot., 86: 703-710.

Colom, M.R. and C. Vazzana, 2002. Water stress effects on three cultivars of *Eragrostis curvula*. Italy J. Agron., 6: 127-132.

Ferrante, A., A. Trivellini, F. Malorgio, G. Carmassi, P. Vernieri and G. Serra, 2011. Effect of seawater aerosol on leaves of six plant species potentially useful for ornamental purposes in coastal areas. Sci. Hortic., 128: 332-341.

Flowers, T.J., 2004. Improving crop salt tolerance. J. Exp. Bot., 55: 307-319.

Grattan, S.R. and C.M. Grieve, 1999. Mineral Nutrient Acquisition and Response by Plants Grown in Saline Environments. In: Handbook of Plant and Crop Stress, Pessarakli, M. (Ed.). Marcel Dekker, New York, ISBN-10: 0072402318, pp: 203-229.

Heuer, B., 2005. Photosynthetic Carbon Metabolism of Crops Under Salt Stress. In: Handbook of Photosynthesis, Pessarakli, M. (Ed.)., 2nd Edn., Taylor and Francis Group, UK.

Hirose, T., T. Kinugasa and Y. Shitaka, 2005. Time of Flowering, Costs of Reproduction and Reproductive Output in Annuals. In: Reproductive Allocation in Plants, Reekie, E.G. and F.A. Bazzaz (Eds.). Academic Press, Burlington, MA., pp: 159-188.

Hsiao, T.C., E. Acevedo, E. Fereres and D.W. Henderson, 1976. Water stress, growth and osmotic adjustment. Phil. Trans. R. Soc. Lond. B., 273: 479-500.

Jackson, M.L., 1973. Soil Chemical Analysis. 1st Edn., Prentice Hall of India Pvt. Ltd., New Delhi, India.

Lee, M.K. and M.W. van Iersel, 2008. Sodium chloride effects on growth, morphology and physiology of chrysanthemum (*Chrysanthemum morifolium*). Hortscience, 43: 1888-1891.

Lichtenthaler, H.K. and A.R. Wellburn, 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem. Soc. Transactions, 11: 591-592.

Luther, G.W. and E. Tsamakis, 1989. Concentration and form of dissolved sulfide in the toxic water column of the ocean. Marine Chem., 27: 165-177.

Marschner, H., 1986. Mineral Nutrition of Higher Plants. Academic Press, London.

Mengel, K. and E.A. Kirkby, 1982. Principles of Plant Nutrition. 3rd Edn., International Potash Institute, Bern, Switzerland.

Munns, R. and R. Weir, 1981. Contribution of sugars to osmotic adjustment in elongating and expanded zones of wheat leaves during moderate water deficits at two light levels. Aust. J. Plant Physiol., 8: 93-105.

Munns, R., 1993. Physiological processes limiting plant growth in saline soils: Some dogmas and hypotheses. Plant Cell Environ., 16: 15-24.

Munns, R., R.A. James and A. Lauchli, 2006. Approaches to increasing the salt tolerance of wheat and other cereals. J. Exp. Bot., 57: 1025-1043.

Parida, A.K. and A.B. Das, 2005. Salt tolerance and salinity effects on plants: A review. Ecotoxicol. Environ. Saf., 60: 324-349.

Pillsbury, A.F. and H.F. Blaney, 1966. Salinity problems and management in river systems. Proc. ASCE (IR), 92: 77-90.

Raven, J.A., 1990. Predictions of Mn and Fe use efficiencies of phototrophic growth as a function of light availability for growth and of C assimilation pathway. New Phytolgis., 116: 1-18.

Razmjoo, K., P. Heydarizadeh and M.R. Sabzalian, 2008. Effect of salinity and drought stresses on growth parameters and essential oil content of *Matricaria chamomila*. Int. J. Agric. Biol., 10: 451-454.

Roemheld, V. and M.M. El-Fouly, 1999. Foliar nutrient application: Challenge and limits in crop production. Proceedings of the 2nd International Workshop on Foliar Fertilization, April 4-10, Bangkok, Thailand, pp: 1-34.

Santos, C.V., 2004. Regulation of chlorophyll biosynthesis and degradation by salt stress in sunflower leaves. Sci. Hortic., 103: 93-99.

Sarkar, D., B. Mandal and M.C. Kundu, 2007. Increasing use efficiency of boron fertilizers by rescheduling the time and methods of application for crops in India. Plant Soil, 301: 77-85.

Shillo, R., M. Ding, D. Pasternak M. Zaccai, 2002. Cultivation of cut flower and bulb species with saline water. Sci. Hort., 92: 41-54.

Singh, A.K., D. Chakravarthy, T.P.K. Singh and H.N. Singh, 1996. Evidence for a role for L-proline as a salinity protectant in the cyanobacterium (*Nostoc muscorum*). Plant Cell Environ., 19: 490-494.

Snedecor, G.W. and W.G. Cochran, 1980. Statistical Methods, 8th Edn., Iowa State University Press, USA.

Taleisnik, E. and K. Grunberg, 1994. Ion balance in tomato cultivars differing in salt tolerance. I. Sodium and potassium accumulation and fluxes under moderate salinity. Physiol. Plant., 92: 528-534.

Tavakkoli, E., F. Fatehi, S. Coventry, P. Rengasamy and G. McDonald, 2011. Additive effects of $Na^+$ and $Cl^-$ ions on barley growth under salinity stress. J. Exp. Bot., 62: 2189-2203.

Wissuwa, M., A.A. Ismail and R.D. Graham, 2008. Rice grain zinc concentrations as affected by genotype native soil-zinc availability and zinc fertilization. Plant Soil, 306: 37-48.

Wyn-Jones, R.G. and R. Storey, 1981. Betaines. In: Physiology and Biochemistry of Drought Resistance in Plants, Paleg, L.G. and D. Aspinal (Eds.). Academic Press, Sydney, pp: 172-204.

Yuncai, H.U., B. Zoltan and U. Schmidhalter, 2008. Effect of foliar fertilization application on the growth and mineral nutrient content of maize seedlings under drought and salinity. Soil Sci. Plant Nutr., 54: 133-141.