

Interactive Effects of NaCl Saline Stress and KCl Irrigation on Growth and Physiological Properties of Common Matricariae

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ABSTRACT

Utilization of various water sources is necessary due to increasing population and the consequent need of agricultural development. One of the main concerns in the coming years is soil salinity and its related stress. Therefore of the important medicinal plants which is extensively cultivated throughout the world (*Matricaria chamomilla* L. from the Asteraceae family) was treated in a factorial experiment based on a completely randomized design with NaCl at 4 levels (0, 40, 70, 100 mM) and KCl at 2 levels (0 and 0.7 mM) and 3 replicates in order to evaluate the alleviating effects of K⁺ on NaCl stress. Physiological and morphological traits such as leaf area, fresh and dry weights, relative water content, etc. were measured and evaluated under stress condition. Results showed a significant effects on most measured traits at statistical levels ($P \leq 0.05$ and $P \leq 0.01$). It also revealed that Na⁺ is more effective. The highest and lowest values were observed in different treatments of NaCl (0-100 mM). Morphological traits were more affected by NaCl (salinity) stress, so that shoot fresh weight, shoot dry weight and root fresh weight decreased by 59, 36 and 81% respectively from control to highest Na⁺ level, likewise electrolyte leakage, relative water content, proline and chlorophyll content decreased from 73.11, 51.45, 0.122 and 76.28 to 62.19, 70.32, 0.094 and 20.08 respectively. Na⁺ and K⁺ contents were not significant at any stress level. Generally, it seems that using KCl on plants irrigated instead of NaCl, alleviated the harmful effects of Na⁺ ion and improved the growth properties of *Matricaria chamomilla* under stress.

Keywords: Ionic stress, irrigation water, *Matricariae chomamilla*, medicinal plant, salinity.

INTRODUCTION

Matricaria chamomilla L. (syn: *M. recutita* L.; German chamomile) exist in the Asteraceae (Compositae) family and is one of the most widely used medicinal plants throughout the world (Salamon, 1992).

Increasing population and the consequent need of agricultural products is expanding, which requires the utilization of various sources of water. The main problem to be considered in using different sources of water is the salinity stress. Many crop species suffer a decline in growth while exposed to salinity stress. Many studies denote the interaction between NaCl and the elevated Ca²⁺ and K⁺ concentrations on plants (Reid and Smith, 2000; Naz *et al.*, 2010a; 2010b). Salt stress inhibits the uptake and transport of K⁺ and Ca²⁺, nutrients that influence plant growth (Morishita *et al.*, 1986; Glores *et al.*, 2001).

Potassium is an essential activator for some enzymes in cytosol, and Na⁺ can rarely substitute

these biochemical functions. K⁺ has often been also considered to play a role in osmotic stress and salt toxicity remediation (Shirazi *et al.*, 2005; Abdul Majid *et al.*, 2007). It has been shown that application of K⁺ improved growth and yield under water stress possibly by regulating photosynthesis (Gupta *et al.*, 1989).

Salinity affects Na⁺/K⁺ ratio in plants, and the cultivars that have the ability to minimize this ratio may be more salt tolerant than those with higher Na/K ratio (Benzyl and Reuveni, 1994; Lingle *et al.*, 2000). High ionic concentration competes with the uptake of other nutrients, especially K⁺, leading to K⁺ deficiency. High concentration of NaCl increases Na⁺ and Cl⁻ but decreases Ca²⁺, Mg²⁺ and K⁺ levels in a number of plants (Khan *et al.*, 2003). There is a negative correlation between Na⁺ and K⁺ concentration in roots and leaves.

It has been shown that Proline accumulates quickly, and is considered to function in salt stress

adaptation (Berteli et al., 1995), by protecting plant tissue against osmotic stress and/or acting as enzyme protector (Solomon et al., 1994; Liu and Zhu, 1997). Accumulation of proline in plants under stress may offer multiple benefits to the cell. For example, it was concluded that free proline and glycinebetaine accumulation in the shoot were possible indicators for salt tolerance in the maize genotypes studied (Mansour et al., 2005).

Therefore the main aim of this study was to evaluate the improving effects of KCl irrigation on NaCl salinity stress. No significant studies with similar treatments have been conducted on other plants.

MATERIAL AND METHODS

Treatment and Experimental Conditions:

This experiment was conducted in greenhouse conditions on *Matricariae chomomilae* L. Fully grown young seedlings were placed in 30 cm diameter plastic pots filled with a mixture of sand and practical soil (as media culture) for 2 weeks in order to be established before the start of treatments. Mean day and night temperatures were recorded as 24/16 °C with a light intensity of 16 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and 45% RH. Stress treatments were applied using a factorial experiment based on a completely randomized design with NaCl at (0, 40, 70, and 100 mM) and KCl (0 and 0.7 mM) applied twice a week via soil drenching. Consequently various morphological, physiological and biochemical characteristics were studied as mentioned below:

Leaf Area, Root Volume and Dry Weights:

Leaf surface was determined with a Li-3100 area meter (LI, Lincoln, Nebraska, USA). Roots, leaves, and stems were then dried (70 °C for 48 h) and the dry weights were recorded.

Chlorophyll Content

Chlorophyll content was determined using Dere et al. (1998) method. 200 mg of fresh leaves was homogenized and extracted with 10 ml methanol 96% (v/v %). After this process, absorbance was read at 666 and 653 nm chlorophyll a and b wavelength. Calculation equations used are as below:

$$\text{CHL a} = 15.65 A_{666} - 7.340 A_{653}$$

$$\text{CHL b} = 27.05 A_{653} - 11.21 A_{666}$$

$$\text{CHL t} = \text{CHL a} + \text{CHL b}$$

Relative Water Content:

To measure Relative Water Content (RWC), two excised leaves per plant were weighed (fresh weight, FW) and placed in plastic bags in the dark with their petioles plunged in distilled water overnight to allow them to reach full turgor and, hence, to determine their turgid weight (TW). These leaves were then dried at 70 °C for 24 h and their dry weight (DW) was recorded. Then RWC

was calculated using the bellow equation (Abbaszadeh et al., 2008):

$$\% \text{ RWC} = (\text{FW} - \text{DW}) \times 100 / (\text{TW} - \text{DW})$$

Electrolyte Leakage:

Electrolyte leakage was calculated by following the standard method of Pinhero and Fletcher (1994).

Proline Content:

The proline content was estimated by Bates et al. (1973) method. The plant material was homogenized in 3.3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 10,000 rpm. The supernatant was used for the estimation of the proline content. The reaction mixture consisted of 2 ml of acid ninhydrin and 2 ml of glacial acetic acid, which was boiled at 100 °C for 1 h. After the termination of reaction in ice bath, the reaction mixture was extracted with 4 ml of toluene, and absorbance was read at 520 nm.

Antioxidant Activity by DPPH:

Antioxidant activity was estimated by the Abe et al. (1998) method. 100 mg of fresh plant material (leaf) was extracted by methanol 99% (v/v %). The extracts were then centrifuged at 3500 rpm for 5 minutes and mixed with DPPH solution. After an incubation period of 30 min. at 25 °C, the absorbance at 517 nm was recorded. The free radical-scavenging activity of each solution was then calculated as percent inhibition according to the following equation:

$$\% \text{ inhibition} = (A_{\text{blank}} - A_{\text{sample}}) \times 100 / A_{\text{blank}}$$

Statistical Analysis:

Data were analyzed in completely randomized design ANOVAs using JMP8. Data means were separated by the LSD test at ($P \leq 0.05$ and 0.01).

RESULTS

Analysis of variance of measured traits is shown in tables 1 and 2. Results showed significant effects on most traits measured at statistical levels ($P \leq 0.05$ and $P \leq 0.01$) and NaCl stress was observed to be more effective (Table 1).

Leaf Area and Root Volume:

Salinity stress showed a significant effect in both leaf area and root volume ($P \leq 0.05$ and $P \leq 0.01$). But leaf area was mainly affected by K^+ treatments and root volume is mainly affected by Na^+ levels (Table 1). The highest and lowest leaf area values (1357.42 and 792.32 cm^2) were observed in control treatment and 100 mM salinity treatment, respectively. Root volume decreased from 81.66 to 21.67 as did the leaf area.

Shoot, Root and Leaf Fresh and Dry Weight:

According to table 1, salinity stress significantly affected the shoots fresh and dry weights. K^+ treatment was observed as the major cause of shoot fresh weight gain, but Na^+ was the major cause considered for shoot dry weight gain (Table

1). On the other hand all simple and interaction treatments decreased root fresh weight in examined plants significantly ($P \leq 0.01$ and 0.05), whereas root dry weights were only affected by

Na^+ (Table 1). Shoot fresh weight, shoot dry weight and root fresh weight decreased by 59, 36 and 81 % respectively from control to the highest Na^+ level.

Table 1. Analysis of variance in different measured traits of *Matricariae Chomamoliae* in response to NaCl stress.

| SOV | Electrolyte leakage (%) | Leaf area (cm ²) | Shoot fresh weight (g) | Shoot dry weight (g) | Root volume |
|-------|-------------------------|------------------------------|------------------------|----------------------|-------------|
| K | 132.58* | 1154917* | 4197.35** | 0.57 ns | 150 ns |
| Na | 151.57* | 385905 ns | 818.6 ns | 99.11** | 3650 ** |
| K×Na | 156.47* | 169140 ns | 592.51 ns | 11.33 ns | 50 ns |
| Error | 46.46 | 338453 | 342.6 | 7.48 | 112.5 |

Continue of Table 1. Analysis of variance in different measured traits of *Matricariae Chomamoliae* in response to NaCl stress.

| SOV | Root fresh weight (g) | Root dry weight (g) | Relative water content (%) | Proline content (μmol/gdw) | Chlorophyll content (mg/gfw) | Antioxidant activity (%) |
|-------|-----------------------|---------------------|----------------------------|----------------------------|------------------------------|--------------------------|
| K | 1370.78** | 31.88 ns | 396.99** | 0.015 ** | 80.26 ns | 1.28 ns |
| Na | 6367.76** | 449.87** | 325.69** | 0.006 ** | 4106.58 ** | 0.416 ns |
| K×Na | 232.15* | 10.27 ns | 78.45* | 0.0001 ns | 551.72 ns | 0.414 ns |
| Error | 132.16 | 10.89 | 17.74 | 0.00082 | 539.96 | 1.5 |

Table 2. Mean comparison of Morphological characteristics of *Matricariae Chomamoliae* in response to NaCl stress.

| Treatment | Leaf area (cm ²) | Shoot fresh weight (g) | Shoot dry weight (g) | Root volume | Root fresh weight (g) | Root dry weight (g) |
|-----------|------------------------------|------------------------|----------------------|-------------|-----------------------|---------------------|
| NaCl (mM) | | | | | | |
| 0 | 1357.42a [†] | 70.32a | 12.75a | 81.66a | 87.47a | 22.57a |
| 40 | 1197.58a | 54.82ab | 9.87a | 56.66b | 54.05b | 9.35b |
| 70 | 992.24b | 52.41ab | 4.5b | 50b | 22.74c | 5.82bc |
| 100 | 792.32c | 42.04b | 4.64b | 21.67c | 16.65c | 3c |
| KCl (mM) | | | | | | |
| 0 | 1344.19a | 68.12a | 7.79a | 50.02a | 37.67b | 9.03a |
| 0.7 | 805.6b | 41.67b | 8.01a | 55.04a | 52.78a | 11.34a |

[†]Means with different letters in each column are significantly different at $P \leq 5\%$.

Table 3. Biochemical characteristics of *Matricariae Chamamoliae* in response to NaCl irrigation.

| Treatment | Electrolyte Leakage (%) | Relative Water Content (%) | Proline Content (μmol/g dw) | Chlorophyll Content (mg/g fw) |
|-----------|-------------------------|----------------------------|-----------------------------|-------------------------------|
| NaCl (mM) | | | | |
| 0 | 73.11a | 49.21b | 0.122a | 76.27a |
| 40 | 72.34a | 48.44b | 0.054c | 47.55b |
| 70 | 70.67a | 64.21a | 0.062bc | 18.36b |
| 100 | 62.19b | 51.45b | 0.094ab | 20.08b |
| KCl (mM) | | | | |
| 0 | 71.93a | 49.26b | 0.058b | 39.22a |
| 0.7 | 67.23a | 57.39a | 0.108a | 44.04a |

[†]Means with different letters in each column are significantly different at $P \leq 5\%$.

Chlorophyll Content:

According to the results of this experiment, salinity stress significantly affected the total chlorophyll content within Na^+ treatments ($P \leq 0.01$) (Table 1). Increasing levels had greater effects. The highest chlorophyll content (76.27 mg/g FW) was observed in control treatment whereas the lowest (20.08 mg/g FW) in recorded severe stress (100 mM) condition.

Relative Water Content:

K^+ , Na^+ and the interaction show significant effects on RWC at $P < 0.01$ and 0.05 respectively (Table 1). RWC was significantly affected by salinity stress, with the highest value in control and the lowest at NaCl (40 mM) (Table 2). Although all treatments resulted in RWC decrease, K^+ application improved RWC in all NaCl levels (Fig. 1).

Electrolyte Leakage:

K^+ , Na^+ and interaction levels of these treatments were considered significant within electrolyte leakage (Table 1). Electrolyte leakage was noticeably affected by salinity stress (Table 2). Interaction effect is also significant and is shown in Fig. 2. Control and (100 mM) treatments show the highest (73.11) and lowest (62.19) amounts respectively.

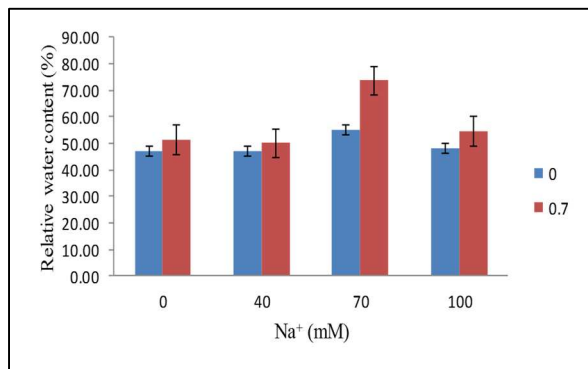


Fig. 1. Interaction of NaCl and KCl and their effect on relative water content.

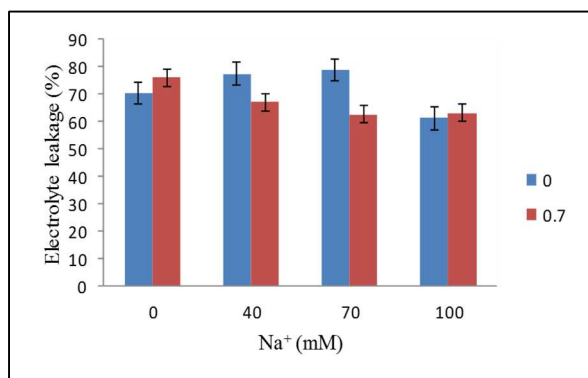


Fig. 2. Interaction of NaCl and KCl and their effect on electrolyte leakage.

Proline Content:

Results of variance analysis shows remarkable variations among K^+ and Na^+ treatments in proline content, whereas interaction treatments were not significant (Table 1). The results show a significant effect of salinity stress on proline content ($P \leq 0.01$). The highest (0.122 $\mu\text{mol/g DW}$) and lowest (0.094 mg/g DW) proline content was determined for control and mild stresses (40 mM), respectively (Table 2).

Results on Antioxidant Activity was not significant at any statistical level. **PROVIDE MORE INFORMATION on Antioxidant Activity.**

DISCUSSION

Results of a physiological study revealed that root and shoot dry weights of *H. maritimum* decreased significantly with increasing salinity (Hafsi et al, 2007). This effect was due to the

combination of decreased leaf formation, shoot and root length, and leaf surface area. Reduction in dry weight reflects the increased metabolic energy cost and reduced carbon gain. It also reflects salt impacts on tissues (Karimi et al., 2005) reduction in photosynthetic rates (Ziska et al., 1990; Ashraf, 2004) and attainment of maximum salt concentration tolerated by the fully expanded leaves (Hu et al., 2000). Decrease in root length and weight in response leads to a reduction in root volume generally. Leaf area substantially decreased when NaCl was the root medium (Shariat Jafari et al., 2009). Reduction in dry weight reflects the increased metabolic energy cost and reduced carbon gain due to salinity (Ashraf, 2004). Interactive effects of Ca^{2+} and K^+ caused lower vegetative and reproductive dry weight, denoting higher investment in adjustment of increasing cations and anions for better performance under saline conditions (Ahmad et al., 2006; Ashraf et al., 2008; Ahmad et al., 2010).

Plant salinity tolerance is a multifaceted physiological trait. The ability of plants to maintain a high cytosolic K^+/Na^+ ratio is likely to be one of the determining keys of plant salt tolerance (Maathuis and Amtmann, 1999; Bizidet et al., 1988; Haddad and Coudret, 1991). The selective uptake of K^+ as opposed to Na^+ is considered to be one of the important physiological mechanisms contributing to salt tolerance in many plant species (Ashraf and Khanum, 1997). After supplemental Ca^{2+} and K^+ were added to the slightly salinized (less than 30 mM) growth medium of lentil, the productivity of this plant was improved in the presence of salinity. Similar result was observed in our experiment.

The main cause of leaf number reduction in the last three weeks of lentil might be due to accumulation of more cations, mainly Na^+ , or anions, particularly Cl, in the leaves that need energy in order for the plant to adjust them (Kafi et al., 2012).

Salinity forces qualitative and quantitative variations on pigment composition of leaves which is dependent on plant species and salinity level. In more researches, decreased level of chlorophyll pigments in plants grown under NaCl salinity stress has been reported (Bethke and Drew, 1992; Heuer and Nadler, 1998). Reduction in leaf area as the primary effect of salinity results in a decline of the photosynthesis rate. Probably, the main reason is the reduced chlorophyll content of plants which is related to the increase in toxic ions and osmotic stress by salinity in leaf, this bring about chlorophyll degradation. Reduction in chlorophyll levels in plants under stress can be related to the increase in the activity of chlorophyll degrading enzymes (Chlorophyllase) (Bertrand and Schoefs, 1999).

Leaf relative water content (RWC) is an index representing the amount of water in the plant organs and shows the ability of a plant in maintaining water under stress conditions (Abbaszadeh et al., 2008). So in a controlled environment for an experiment, the measured LRWC shows the response of a plant; the higher the measured amount, the greater the ability of a treatment for keeping water (Abbaszadeh et al., 2008).

It is obvious that proline plays an adaptive role in the tolerance of plant cells to salinity by increasing the concentration of osmotic active components in order to equalize the osmotic potential of the cytoplasm (Wataad et al., 1983). It has been shown that Proline accumulates quickly and is considered to function in salt stress adaptation (Berteli et al., 1995), by protecting plant tissue against osmotic stress and/or acting as enzyme protector (Solomon et al., 1994; Liu and Zhu, 1997). Accumulation of proline in plants under stress may offer multiple benefits to the cell.

In plants, K^+ plays an essential role as an osmoticum and charge carrier (Ashraf et al., 2008). Cherel (2004) reported that potassium plays an important role in balancing membrane potential and turgor, activating enzymes, regulating osmotic pressure, stoma movement and tropisms. Salinity stress disturbs the uptake and accumulation of essential nutrients (Shannon and Grieve, 1999). Generally, Ca^{2+} and K^+ are decreased in plants under saline conditions. These decreases could be due to the antagonism of Na^+ and K^+ at uptake sites in the roots, the effect of Na^+ on K^+ transport into the xylem or the inhibition of uptake processes (Al-Harbi, 1995). The positive effects of supplemental Ca^{2+} and K^+ on grain yield of lentil was due to lowering exchangeable sodium percentage (ESP) of the growth medium, because as much as the rate of other cations in the growth medium increases the rate of sodium decrease. This increase of K^+ content had improved the Na-K balance in plant tissue which in turn facilitated the plant growth. The occurrence of high K^+ in plant had a share in reducing the damage caused by high Na^+ concentration (Nessim et al., 2008).

In the present experiment antioxidant activity of leaf extract depended on the salt concentration in the medium. It has been found that the radical scavenging activity of the extract enhanced the whole range of salt concentration but not linearly, which agrees with Rezazadeh et al. (2012).

Supplemental Ca^{2+} and K^+ is known to be useful for overcoming the negative impact of high salinity where the growth medium may become saline at some time during the crop growth cycle (Irfan and Murat, 2004). When K^+ was added showed the root and shoot characters of cucumber embryos improved (Irfan and Murat, 2004). Most authors agree that K^+/Na^+ homeostasis is a key feature of plant salinity tolerance (Gorham et al., 1990; Rubio et al., 1995; Dubcovsky et al., 1996; Maathuis and Amtmann, 1999; Cuin and Shabala, 2006; Volkov and Amtmann, 2006). Reduction in dry weight reflects the increased metabolic energy use and reduced carbon gain. It also reflects salt impacts on tissues (Karimi et al., 2005) reduction in photosynthetic rates (Ziska et al., 1990; Ashraf, 2004) and attainment of maximum salt concentration tolerated by the fully expanded leaves (Hu et al., 2000).

K^+ application not only stimulated the negative effects of salinity on growth, but also reduced dry matter accumulation particularly at low and medium stress. In plants, K^+ plays an essential role as an osmoticum and charge carrier (Ashraf et al., 2008). The capacity of plants to maintain a high cytosolic K^+/Na^+ ratio is likely to be one of the key determinants of salt tolerance. Generally, Ca^{2+} and K^+ decrease in plants under saline conditions. These decreases could be due to the antagonism of Na^+ and K^+ at uptake sites in the roots, the effect of Na^+ on K^+ transport into the xylem or the inhibition of uptake processes (Al-Harbi, 1995).

CONCLUSION

Plant salinity tolerance is a multifaceted physiological trait. Results showed significant effects on most traits measured at statistical levels ($P \leq 0.05$ and $P \leq 0.01$) and Na^+ was observed to be more effective. Matricariae was mainly considered as a sensitive crop but KCl irrigation imposed a rather good alleviating effect on NaCl stress.

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