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# COMPARATIVE ADVERSE EFFECTS OF PEG- OR NaCl-INDUCED OSMOTIC STRESS ON GERMINATION AND EARLY SEEDLING GROWTH OF A POTENTIAL MEDICINAL PLANT *MATRICARIA CHAMOMILLA*

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**Abstract**

The adverse effects of salinity and PEG-induced water stress on growth of *Matricaria chamomilla* were assessed at the germination stage using osmotic solution of NaCl (0, -0.175, -0.358, -0.541, -0.716 MPa), and polyethylene glycol (PEG 6000) (0, -0.05, -0.1, -0.2, -0.3 MPa), respectively. Effect of NaCl salinity stress (0, 40, 80, 120 and 190 m*M* NaCl) at early growth stage of chamomile was also studied. The levels of NaCl and PEG-induced water stress were determined in first experiment. Both seed germination rate and seedling growth reduced with increasing osmotic potential of growth medium either due to NaCl or due to PEG. However, PEG-induced osmotic stress caused more growth inhibition compared with NaCl-induced osmotic stress. Seeds were not germinate at -0.8 MPa of NaCl and -0.4 MPa of PEG. In conclusion, adverse effects of salt stress on germination and early growth of chamomile is due to $Na^+$ accumulation in addition to its osmotic stress.

**Keywords**: seedling growth, seed germination, osmotic stress, abiotic stresses, water stress

## Introduction

Abiotic stresses including drought and salinity are currently the major factors which reduce crop productivity world-wide. Excessive amounts of salts in soil severely reduced the seed germination and further seedling growth and this has been well documented in the literature (Greenway & Munns, 1980; Bewley & Black, 1982; Ashraf, 1994; 2004; Munns, 1993; 2002; 2005). This has been ascribed due to salt-induced osmotic stress or due to its toxic effects or combination of both of these (Greenway & Munns, 1980; Ashraf, 1994; 2004; Munns, 1993; 2002; 2005). However, Munns is of view that major contribution in salt-induced growth reduction at initial phase of salinity is due to the osmotic stress (Munns, 1993; 2002; 2005). Furthermore, Neumann (1997) concluded in his extensive review responses of cultivars of a same species vary to salt-induced osmotic stress or PEG-induced water stress.

In Iran (mostly arid and semiarid with saline-alkaline soil), there are more than 7500 plant species which most of them have valuable active substances. One of the most important specie of them is Chamomile, an annual plant belongs to Asteraceae (Compositae) family, grows widely in various ecological zones of Iran. Its flowers have an active substance that is called essential oil in which the most important constituent is chamazulene that is used widely in pharmaceutical, food, perfumery and flavouring industry (Kacuric 1979; Galambosi & Holm 1991). The annual world consumption of

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chamomile flowers is more than 4000 ton (Franz *et al.*, 1986). Recently its cultivation in Tehran, Lorestan, Khoozestan, Fars and Isfahan provinces has started and several drugs have produced from its essential oil. This is very important to reduce chemical drugs and increase individual health .

In view of importance of Chamomile as potential medicinal herb, which is being used for treatment of a number diseases (Simpson, 2001), an experiment was conducted to assess whether salt induced reduction in growth at early growth stages due to either its osmotic effect or due to its toxic effect.

## Materials and Methods

This study was carried out at the Department of Soil Science, College of Agriculture, Isfahan University of Technology, Iran. Germination and early seedling growth (21 days) of chamomile were studied using distilled water (control) and under osmotic potentials of 0, - 0.175, -0.358, -0.541 and -0.716 MPa, for NaCl (Coons *et al.*, 1990) and 0, -0.05, -0.1, -0.2, and -0.3 MPa polyethylene glycol (PEG 6000) (Michel & Kaufmann, 1973). Osmotic potentials selected according to pretreatments. Also it showed that seed germination was totally inhibited at -0.8 MPa of NaCl and -0.4 MPa of PEG .

Seeds were sterilized with 0.5% sodium hypochlorite solution for 1 min. Thereafter, they were washed twice with distilled water. Four replicats of 50 seeds for each treatment, or for the control, non-treated seeds, were placed on one layer of filter paper in 90 mm Petri dishes. Water or fresh salt solutions were added periodically maintaining the filter paper wet during the course of the experiment. The experiment was arranged in a completely randomized design. Germination was carried out in a germination chamber with a regime of 12 h light at 20 $^{o}$C. The number of germinated seeds was counted every day during 21 days from the start of the test. After 21 days the seedlings were harvested and the shoot and stem length were measured. Data for germination were subjected to arcsine transformation before analysis of variance. Data ware subjected to statistical analysis using ANOVA, a statistical package available from SAS.

Early growth of chamomile was investigated at four salinity levels using irrigation with saline water of 0, 40, 80, 120 and 190 m*M* NaCl, respectively. Salt solutions were prepared in half strength Johnson nutrient solution (Johnson *et al.*, 1957). The final concentrations of NaCl in the nutrient solutions were achieved after two weeks and continued during 70 days. The pH of nutrient solution was adjusted to 5.5 by adding KOH or $HNO_3$ as needed. The nutrient solutions were renewed every 15 days during the growing period.

Plants were harvested at flowering stage, separated into roots and shoots, and their fresh weights were determined. The shoots were washed twice with distilled water and dried at 85 °C for 48 h to determine their dry weights. To determine shoot and root concentrations of Na, the dried samples were ground, ashed at 550 $^{0}$C for 8 h, and the ashed material was then dissolved in HCl (Chapman & Pratt 1961). Concentrations of Na and K in the digest solutions were measured on a flame photometer (Cottenie *et al.*, 1982).

## Results

Final germinations were significantly reduced in NaCl and PEG (Fig. 1, 2 and Table 1). Also it showed that seed germination was totally inhibited at -0.8 MPa of NaCl and -0.4 MPa of PEG. (Levels of osmotic stress used in experiment were below the level of osmotic stress [PEG-induced or NaCl induced] where no seed germination was observed).

There was a little reduction in germination in solutions of PEG. In contrast, a more significant reduction in germination was observed in solutions of NaCl. This plant differed significantly in its response to salt and osmotic stress (PEG). With reducing osmotic potentials, both NaCl and PEG delayed the onset of germination, reduced final percent germination, decreased germination rate and shoot length.

Plant growth significantly ($P$<0.05) increased with increasing NaCl concentration up to 40 mM while decreased at higher salinity levels proportional to the salt rate (Table 2). Infact, the highest and lowest shoot fresh and dry weight was found in 40 and 190 m*M*

**Table 1. Final germination percentage and shoot length of *Matricaria chamomilla* as influenced by NaCl and PEG.**

| Treatments | Mean Squares |
|---|---|
| *Final Germination (%) – NaCl* | 0.01 ** |
| *Final Germination (%) – PEG* | 0.15 * |
| *Shoot length – NaCl* | 0.05 ** |
| *Shoot length – PEG* | 0.05 * |
| P < 0.05 *; P < 0.01 ** | |

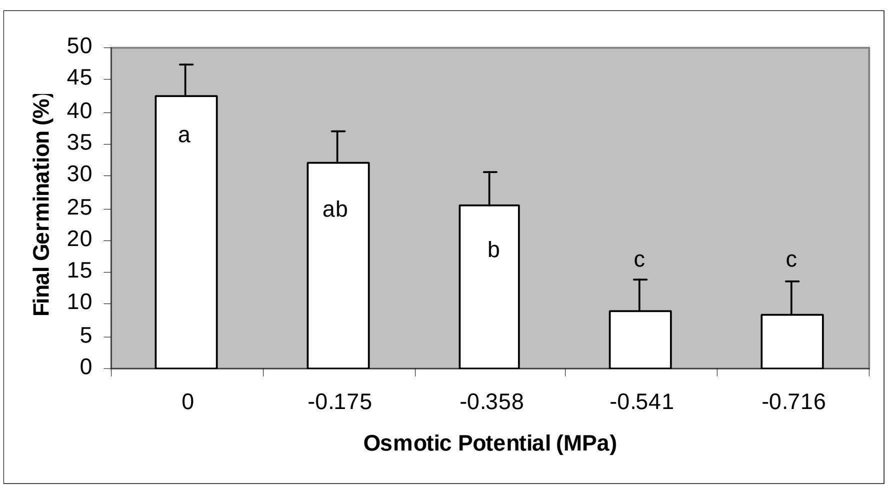


Fig. 1. Germination percentage of *Matricaria chamomilla* seeds as influenced by salinity. Values with the same superscript letters are not significantly different at P< 0.05.

**Table 2. Averages of $Na^+$ (%) in shoots and roots. Averages of shoots fresh and dry weights (g/plant) of *M. chamomilla* as influenced by NaCl concentrations.**

| NaCl rate | Na | | Shoot | |
|---|---|---|---|---|
| (m*M*) | Roots | Shoots | Fresh Weight | dry weight |
| 0 | $0.08^{c}$ | $0.11^{c}$ | $30.95^{b}$ | $6.19^{b}$ |
| 40 | $4.78^{b}$ | $2.78^{b}$ | $26.5^{a}$ | $7.95^{a}$ |
| 80 | $5.92^{ab}$ | $4.87^{a}$ | $26.24^{bc}$ | $5.65^{bc}$ |
| 120 | $6.12^{ab}$ | $5.75^{a}$ | $14.54^{bc}$ | $5.08^{bc}$ |
| 190 | $6.49^{a}$ | $6.30^{a}$ | $20.9^{c}$ | $4.18^{c}$ |

Values with the same superscript letters are not significantly different at P < 0.05.

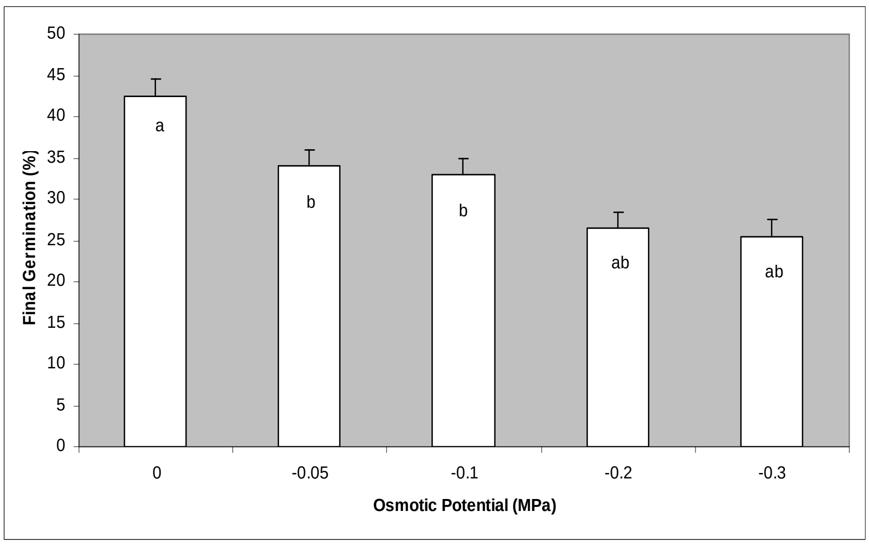


Fig. 2. Germination percentage of *Matricaria chamomilla* seeds as influenced by PEG. Values with the same superscript letters are not significantly different at P > 0.05.

NaCl treatments, respectively. There was no significant difference in the shoot fresh and dry weight between control and 80 m*M* NaCl treatments. Increasing salinity level resulted in significant ($P<0.05$) increases in shoot and root Na concentration, although more accumulation of Na was found in the root than in the shoot (Table 2).

## Discussion

Chamomile differed significantly in its response to salt and osmotic stress (PEG). Such responses have been reported by many workers over a wide range of halophytic and glycophytic plant species (Hampson & Simpson, 1990a, b; Falleri, 1994; Huang & Redmann, 1995; Katembe *et al.,* 1998; Raza *et al.,* 2006; 2007). The general view is that a decrease in water potential gradient between seeds and their surrounding media adversely affects seed germination and subsequent growth processes. The physical process of water uptake leads to the activation of metabolic processes as the dormancy of the seed is broken following hydration (Katembe *et al.,* 1998). Elevated NaCl and PEG concentrations slowed down water uptake by seeds, thereby inhibiting the germination process. In this research, it was found that PEG to be more inhibitory to germination. These results agree with those of Hampson & Simpson (1990a, b) for wheat, as well as Huang & Redmann (1995) for barley and Brassica, Gulzar & Khan (2001) for *Aeluropus lagopoides*. However, such findings are not universal. For example, Roundy *et al.,* (1985) and Katembe *et al.,* (1998) observed the opposite in wild rye, wheat grass and Atriplex species. However, tolerance to high salt stress may also be due to the tolerance of these plant species (halophytic) to high salt-induced osmotic stress. Unlike PEG, NaCl may readily cross the cell membrane into the cytoplasm of the cell unless an active metabolic pump prevents accumulation of the ions (Katembe *et al.*, 1998). In the present study, sodium concentration significantly increased in the shoot and root of *M. chamomilla* with increasing the NaCl concentration (Table 2). However, germination was more sensitive to PEG-induced stress. It has been reported that PEG-induced osmotic stress can cause hydrolysis of storage compounds that further lower the internal osmotic potentials of the seed (Hampson & Simpson, 1990a). In conclusion, chamomile is more sensitive to osmotic stress at germination stage. However, at early growth stage both salt-induced osmotic stress and Na toxicity reduced growth.

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