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Effects of different levels of NaCl and $CaCl_2$ on seed germination characteristics of *Melissa officinalis* L. and *Ocimum basilicum* L.

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Summary: More than 15 million hectares of Iran's land areas are suffering from salinity. We investigated the effects of two types of salt (NaCl, $CaCl_2$) in different levels on the germination and initial growth of two medicinal and aromatic plants, *Melissa officinalis* L. and *Ocimum basilicum* L. The experiment was carried out in Qazvin Agriculture and Natural Resource Research Center based on a randomized complete block design, for 14 days in a germinator. Sweet basil germinated above 50% in each saline treatment (EC=10 dSm^{-1}), in alkali treatment ($CaCl_2$=18800 mg/L) and in sodic-saline treatment (Ec=10 dSm^{-1}; SAR=5.6), however at the highest concentrations germination was slowed down. Lemon balm showed a higher sensitivity: except the lowest NaCl treatment, each other ones inhibited germination and its rate to some extent. The combined treatments of both salts increased the stress reactions. However, seedling growth was less effected. The results showed a stimulating effect of NaCl treatment in 2500 mg/L (EC<5 dSm^{-1}) for both species concerning germination and seedling growth.

Key words: Lemon balm, sweet basil, salinity, sodicity, stress tolerance

Introduction

Recently, in Iran there is increasing interest in cultivation of medicinal and aromatic plants due to their economic advantages compared with the other horticultural crops (*Ibrahimi,* 2002). Within these medicinal species, lemon balm and sweet basil are two of the more common and easy to grow ones in Iran's medicinal plants section.

Melissa officinalis L. (lemon balm) from the family *Lamiaceae* is an aromatic perennial subshrub native to the eastern Mediterranean region and Western Asia. The leaves are used as a herbal tea for their aromatic, digestive, and antispasmodic properties in nervous disturbance of sleep and for gastrointestinal disorders (*Mencherini* et al., 2007).

Ocimum basilicum L. (sweet basil) is an aromatic annual plant belonging also to the family *Lamiaceae*. It is native in tropical Asia, perhaps North-East Africa, and cultivated mainly in North-Africa, Europe, and South-West Asia. The species is an important source of essential oils used extensively in perfumery, food and cosmetic industries (*Hiltunen & Holm,* 1999).

Low degree of viability and unsuccessful germination in different regions of Iran, especially in the irrigated areas, which are covered by saline and alkali soils have always been a problem for producers. In irrigated areas of arid and semiarid regions salinity causes osmotic stress and reduction in plant germination and subsequently in plant growth and yield productivity (*Çiçek & Çakirlar,* 2002). Water deficiency is occurring in arid and semiarid parts of the world with an estimate of 6150 million hectares (*Koochaki,* 2000). On the other side, most saline water and soil resources of the world are located in these areas. From the total areas of saline soils of the world in arid and semiarid regions, which is about 955 million hectares, approximately 18 million hectares belong to Iran (*Koochaki & Mahallati,* 1994).

During plant life, seed germination is one of the most sensitive stages of plant growth which is controlled by environmental factors as well as by physiological processes. Germination includes those events that commence with imbibitions of water by the dormant, usually dry seed and terminate with the elongation of the embryonic axis (*Lambers* et al., 2008).

Water availability is basically important for seed germination. Since salinity is one of the most important environmental factors influencing water availability via osmotic stress, testing its effect on germination is considered a crucial step in research works.

In discussing the effects of salts in the soil, it is distinguished between high concentrations of Na^+, referred to as sodicity and high concentration of total salts, referred to as salinity. The two factors are often related, but in some areas Ca^{2+}, Mg^{2+} and $So^{2}_{4}{}^{-}$, as well as NaCl can contribute substantially to salinity. The high Na^+ concentration of a sodic soil can not only injure plants directly but also degrade the soil structure, decreasing porosity and water permeability (*Taiz & Zeiger,* 2002).

The intensity of salinity response is also mediated by environmental reactions and depending upon the composition of the saline solution. Ion toxicities or nutritional deficiencies may arise because of a predominance of a specific ion or competition effects among cations or anions (*Bernstein* et al., 1974). Sometimes nutritional deficiency symptoms are generally similar to those that occur in the presence of salinity. Calcium deficiency symptoms are common when Na/Ca ratio is high in soil water (*Shannon & Grieve,* 1999).

Usually, salinity is measured in unit of electrical conductivity (EC) and sodicity is measured in unit of sodium absorption ratio (SAR), (*Van de Graaff & Patterso,* 2001). SAR is determined by the concentrations of solids dissolved in water extracted from the soil, where sodium, calcium, and magnesium are in milli-equivalents/liter:

$$SAR = \frac{[Na^{+}]}{\sqrt{\frac{1}{2}([Ca^{2+}]+[Mg^{2+}])}}$$

51 randomly taken soil samples have been measured in Iran land farms and the variation of SAR were found between 1.5 to 11.8 milli-equivalents per litre (*Seilsepour* et al., 2009).

In order to find the optimum utilization of saline and alkali soils, scientific experiments are needed to check the tolerance of different crops. In contrary of numerous data, information on the adverse effects of salt-alkaline stress on seed germination is hardly available. Therefore the present study was initiated to investigate the influence of different concentrations and combinations of NaCl and $CaCl_2$ on the germination and early seedling's growth parameters (germination rate, germination percentage, radicle length, plumule length, seedling fresh weight and seedling dry weight) of sweet basil and lemon balm.

Material and Methods

The experiment was carried out in the laboratory of Qazvin Agricultural and Natural Resource Research Center in 2008 as a Completely Randomized Design with three replications. Two different levels of $CaCl_2$ (6400 and 18800 mg/L), three different levels of NaCl (2500, 5000, 8600 mg/L), 6 different levels of matrix-combination based on SAR (sodium absorption ratio) between $CaCl_2$ and NaCl and one control, all together 12 treatments *(Table 1)* were applied. The ratio and concentration of the salts were calculated according to Electrical Conductivity (EC) and Sodium Absorption Ratio (SAR).

Table 1. Different concentrations and combinations of NaCl and $CaCl_2$

T	Treatments mg/L	T	Treatments mg/L
1	Distilled water	7	NaCl 2500 + $CaCl_2$ 6400 EC=5 SAR 3.3
2	NaCl 2500 EC<5	8	NaCl 5000 + $CaCl_2$ 6400 EC=10 SAR 5.6
3	NaCl 5000 EC<10	9	NaCl 8600 + $CaCl_2$ 6400 EC=15 SAR 11.25
4	NaCl 8600 EC<15	10	NaCl 2500 + $CaCl_2$ 18800 EC=5 SAR 5.6
5	$CaCl_2$ 6400	11	NaCl 5000 + $CaCl_2$ 18800 EC=10 SAR 11.25
6	$CaCl_2$ 18800	12	NaCl 8600 + $CaCl_2$ 18800 EC=15 SAR 22

The seeds of lemon balm and sweet basil were commercial material without variety name, assured by the Medicinal Plants Section of Iran's Forests and Rangeland Research Institute.

The 20 selected seeds for each treatment were surface sterilized in 0.25% sodium hypochlorite (NaClO) solution for 5 minutes (Jafarzadeh and Aliasgharzad, 2001). After washing the seeds with distilled water, the seeds were placed on filter paper (Whatman No. 1, sterilized on 180 °C for 8 hours) in 8 cm in diameter sterile plastic Petri dishes. About 10 milliliters of prepared solutions were added to each Petri dish, so that about half the volume of each seed was immersed. The Petri dishes were covered by lids and wrapping with parafilm, they were kept in a Germinator (SET 550 G) for 14 days with diurnal regime of day/night temperatures at 25°/20 °C and 70 percent of humidity. Artificial light was provided for a 16/8 hours photoperiod. This light and temperature regime was chosen to represent the mid-spring temperatures, when these species germinate in open field cultivation.

Germinating seeds were monitored every day starting from the second day after the beginning of the test. Seeds were considered as having germinated with the emergence of 2 mm of radicule (*Abbassi & Koochaki,* 2008). The daily growth of each seedling was measured under the graded stereoscope respectively and the length of radicles and plumules was recorded. After the lapse of experimental period, germination capacity (in%) and the rate of germination were evaluated. Germination rate (GR) was determined using a Maguire index (*Maguire,* 1962) described as follows:

$$GR = \frac{\text{Number of germinated seeds}}{\text{Days of first count}} + \cdots + \frac{\text{Number of germinated seeds}}{\text{Day of final count}}$$

After recording the fresh mass of seedlings the plants were kept in an oven for drying at 60 °C for 2 days to determine the dry mass. The root and shoot lengths are the most important parameters for salt stress because roots are in direct contact with soil and absorb water from soil and shoots are the assimilating organs and places of substance transport and supply it to the rest of the plant. For this reason, radicle and plumule lengths were measured as an important clue to the response of plants to salt stress (*Jamil & Rha,* 2004).

For statistical analysis the software SAS, and for sketching diagrams Ms Excel were employed. Means were compared using Duncan's Multiple Range Test at 95% significance. In the cases where the variance homogeneity could not be proved even for the transformed data, or because of not normally distributed data, the non parametric Kruskal-Wallis Test was used instead of the ANOVA for analysing the effect of our treatments on fresh and dry mass of the plants.

Results

It was found that seed germination was significantly affected by salinity and alkalinity. The highest germination capacity was determined in 2500 Mg/L NaCl with EC<5 (T-2), *(Figure 1)*. The same result was found in the case of germination rate *(Figure 2)*.

We established, that at lower concentrations of NaCl and $CaCl_2$ (treatment 2), positive effects were observed both on germination capacity and germination rate. Increased NaCl concentration caused a decrease both in sweet basil and lemon balm germination capacity. However, for increasing concentrations of $CaCl_2$, sweet basil was less sensitive and showed no reduction of germination capacity only a decrease of germination rate at the higher dosages. Lemon balm proved to be more sensitive to $CaCl_2$ than to NaCl concentrations. It influenced first of all the germination rate.

It seems to be obvious, that the effect of NaCl is highly depending on the concentration of $CaCl_2$. The higher concentrations of $CaCl_2$ were tolerable only in presence of no more than 2500 mg/L NaCl. It is interesting, that as for the germination capacity, lemon balm, and as for the germination rate basil showed higher tolerance *(Figure 1 and 2)*.

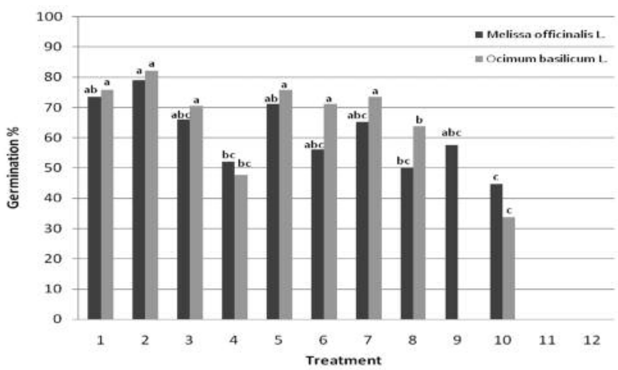

Figure 1. The effect of salinity and alkanity on germination capacity of lemon balm (*Melissa officinalis* L.) and sweet basil (*Ocimum basilicum* L). (Letters show the significant differences in 95% significance level)

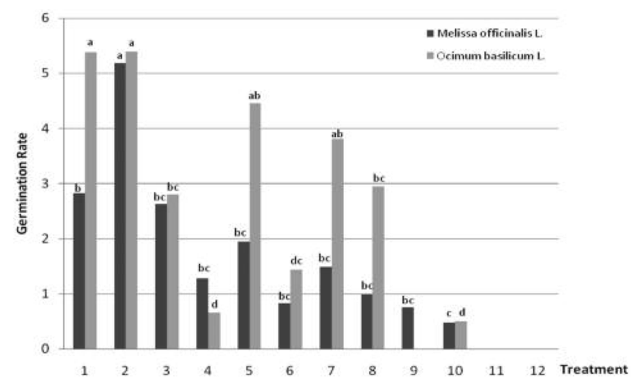

Figure 2. The effect of salinity and alkanity on germination rate of lemon balm (*Melissa officinalis* L.) and sweet basil (*Ocimum basilicum* L). Germination rate was calculated according to the formula of Maguire (1962). Letters show the significant differences in 95% significance level.

At highest concentrations in combination of NaCl and $CaCl_2$ (T-11 and T-12), neither of the species was able to germinate.

Results indicated that sweet basil could germinate more than 70% in saline treatment which is representing the EC value of saline soils (Ec=10 dSm^{-1}) and alkali treatment ($CaCl_2$=18800 Mg/L) and more than 60% in sodic-saline treatment (Ec=10dSm^{-1}& SAR=5.6).

Lemon balm could germinate in more than 50% in saline treatment (Ec=15 dSm^{-1}) and alkali treatment ($CaCl_2$=18800 Mg/L) and 50% in sodic-saline treatment (Ec=15dSm^{-1}& SAR=11.25).

Figure 3 shows that the highest and lowest dry mass of lemon balm was recorded in T-2 (EC<5) and T-3 (EC<10), respectively. The highest and lowest fresh mass of sweet basil was established in T-1 (distilled water) and T-3 (EC<10), respectively. The dry mass of lemon balm significantly declined especially at high concentrations of NaCl and combined treatment *(Figure 3)*. 2500 mg/L of NaCl had a significant effect in increasing the dry mass.

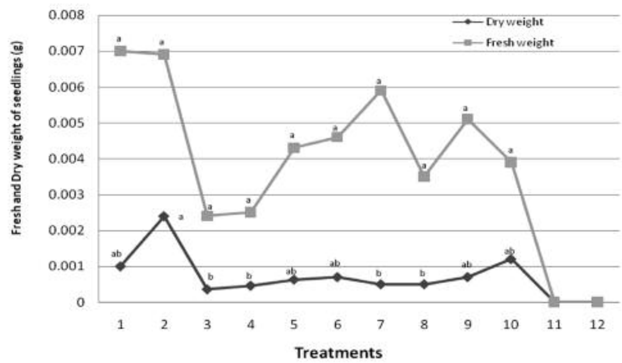

Figure 3. The effect of various levels of NaCl and $CaCl_2$ on the dry and fresh mass of *Melissa officinalis* L. seedling (Letters show the significant differences in 95% significance level).

The longest plumules and radicles of lemon balm were found out in control treatment (T-1), *(Figure 4)*. On the other side, the shortest ones were measured T-9 (EC=15 and SAR=11.25) and T-10 (EC=5 and SAR=5.6), respectively. 2500 mg/L of NaCl had no negative effect on lemon balm radicle length but the plumule length was significantly decreased in all treatments compared with distilled water.

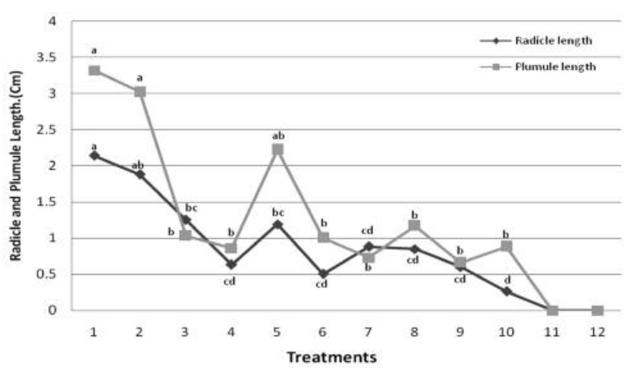

Figure 4. The effect of various levels of NaCl and $CaCl_2$ on the radicle length and plumule length of *Melissa officinalis* L. (Letters show the significant differences in 95% significance level).

The highest and lowest dry mass of sweet basil was recorded in T-4 (NaCl 8600 EC<15) and T-10 (EC=5 & SAR=5.6), respectively *(Figure 5)*. The highest and lowest fresh mass of sweet basil was determined in T-5 ($CaCl_2$ 6400 Mg/L) and T-10 (EC=5 & SAR=5.6), respectively. Sweet basil is more sensitive to increasing concentrations of $CaCl_2$. Low concentration of $CaCl_2$ caused the highest fresh mass but at concentrations of 18800 mg/L of it, the lowest dry and fresh masses were measured. Increased levels of NaCl up to 5000Mg/L (EC=10) had no significant effect on sweet basil dry mass.

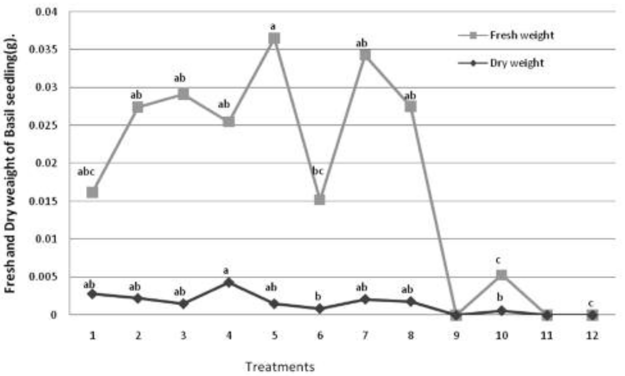

Figure 5. The effect of various levels of NaCl and $CaCl_2$ on the dry and fresh mass of seedlings of *Ocimum basilicum* L. (Letters show the significant differences in 95% significance level).

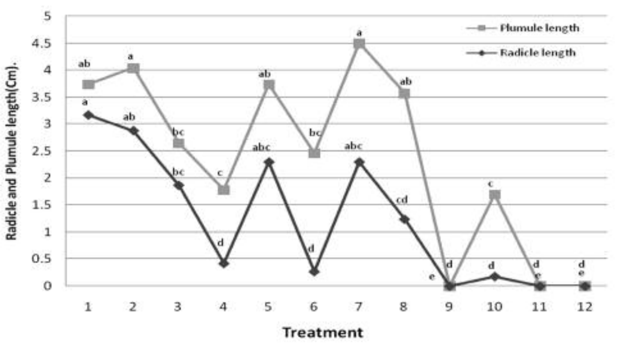

Figure 6. The effect of various levels of NaCl and $CaCl_2$ on the radicle length and plumule length of *Osimum basilicum* L. (Letters show the significant differences in 95% significance level).

It was also measured that the longest plumules of sweet basil was found in T-7 (EC=5 & SAR=3.3) while longest radicles length of sweet basil were measured in the control. Increased levels of NaCl and $CaCl_2$ either separately, or in combination with each other had negative effect on sweet basil's radicle length. It means, that growth of sweet basil seedlings is more sensitive to NaCl compared with lemon balm.

Discussion

Considering all the results obtained from this study, we concluded that both species are moderately tolerant to salinity. Concentrations above 5000 mg/L of NaCl decreased the germination percentage until about 50% but only the highest and combined stress treatments fully inhibited it.

The reaction of both species is not the same concerning germination and seedling growth afterwards. While lemon balm reacted more sensitive during germination, the germinated seedlings showed a hiher tolerance compared to basil.

It's assumed that in addition to toxic effects of certain ions, higher concentration of salts diminished the water potential in the medium which hinders water absorption by germinating seeds and thus reduces germination (*Maas & Nieman,* 1978). There are numerous references in literature which indicate negative effects of salinity and water deficiency on seed germination. Nevertheless, in this experiment we found a stimulatory effect on germination at 2500 mg/L (EC<5) concentration of NaCl. The effect is even more pronounced considering the rate of germination and seedling growth which may be of high practical importance under natural cultivation conditions. Similar results have been found by *El-Darier & Youssef,* (2000) in case of garden cress (*Lepidium sativum L.).*

It was concluded that salinity and alkalinity reduced both germination capacity and rate. The combined effects may show independent reactions from those reached in the presence of individual compounds.

For the future, it seems to be important to find the optimum tolerance levels of these species as well as to investigate their production during the whole life cycle under the saline conditions.

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