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Full Length Article: Effects of Salinity Stress on Seed Germination Characteristics of Two Medicinal Species *Thymus kotschyanus* and *T. daenensis*

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Manuscript Received: 22/09/2011 Manuscript Accepted: 22/05/2012

Abstract. The genus *Thymus* is perennial medicinal plants belong to the Lamiaceae family that is used for different propose in pharmaceutical industry. Environmental stresses, particularly salinity, are considered as the major factor reducing plant growth. The purpose of this study was to investigate the effects of salt stress on germination characteristics and seedling morphology in two medicinal species of T. kotschyanus and T. daenensis. A factorial experiment was conducted using completely randomized design with four replications and seven salinity levels. The salinity factor were included of 0, 50, 100 150, 200, 250 and 300 mm NaCl. Data were collected for germination percentage, germination rate, time means of germination, radicle length, plumule length, seed vigor, and allometry ratios. The overall means of germination percentage, germination rate, radicle length, and seed vigor were lower for T. daenensis as 41.44, 0.28, 1.15 and 1.87 than those for T. kotschyanus as 54.32, 0.37, 1.81 and 2.8, respectively, indicating more tolerant to salinity for T. kotschyanus than that for T. daenensis. Results showed that by increasing salinity, the germination percentage, rate of germination, plumule length, radicle length, and seed vigor were reduced in both species. The slop of reduction was higher for T. daenensis than T. kotschyanus. In other words, T. kotschyanus was more tolerate to salinity than T. daenensis. It was concluded that this genus could be cultivated in environments, which plants are not in expose to salinity.

Key words: Salinity stress, Germination characteristics, Medicinal plants, *Thymus kotschyanus, Thymus daenensis.*

Introduction

Medicinal plants play an important role in the provision of health care in many developing countries. They also provide a stable economic return to local communities especially through the sale of wild-harvested material. Most of the genus Thymus L. (Labiatae) are medicinal plants that consist of about 215 species of herbaceous perennials and sub shrubs. They originated from Mediterranean region (Jamzad, 2010). This genus is presented in Iranica flora by 14 species, four of which (Thymus carmanicus, T. daenensis subsp. and Т. daenensis daenensis subsp. lancifolius, T. persicus and T. trautvetteri) are endemic (Jamzad, 2010). The Persian name of the genus is "Azorbeh" and/or "Avishan" (Safari et al., 2010). Thymus species are commonly used as herbal tea, flavoring agents (condiment and spice) and medicinal plants (Burnett, et al., 2005). Among the species grown in Iran, T. daenensis Celak and T. kotschyanus Boiss. are more widely used for these purposes. Infusion and decoction of aerial parts of Thymus species are used as tonic, carminative, digestive, antispasmodic, antiinflammatory, expectorant and for the treatment of colds in Iranian traditional medicine (Moghimi, 2005). Recent studies have showed that Thymus species have strong antibacterial, antifungal, antiviral, ant parasitic, and antioxidant activities (Omidbayg, 2007). The aromatic and medicinal properties of the genus Thymus have made it one of the most popular plants throughout the world. It is believed that a part of these activities is due to the volatile constituents. Therefore. there is а considerable research interest towards the compositional analysis of Thymus essential oils (Sandra and Bicchi, 1987).

Salinity is one of the environmental factors having a critical influence on seed germination, seed physiology and plant establishment. Salinity affects imbibitions, germination and radicle elongation. It reduces substrate water potential, thereby restricting water and nutrient uptake by plants (Safarnezhad and Hamidi, 2008). Salinity may also cause ionic imbalance and toxicity. Because substrate salinity fluctuates through the growing season, a plant may be exposed to different salinity levels, at various stages of development, with potentially significant consequences on population dynamics (Hosseini and Rezvani Moghadam, 2006). Regarding medicinal importance of Thymus genus, specially T. kotschyanus and T. daenensis, and high Extension of this genus in saline sodic soil in Iran, the aim of this study was to investigate the effect of salt stress on seed germination characteristics of T. kotschyanus and T. daenensis.

Materials and Method

Thymus (T. kotschyanus and T. daenensis) seeds were collected from Ghazvin and Isfahan province, Iran in 2009. A Factorial experiment was conducted for species in two levels and salinity in seven levels based on completely randomized design with four replications. The salinity levels were 0, 50, 100 150, 200, 250 and 300 mm NaCl. Twenty five seeds for each treatment were placed in three petri dishes (25×3) . The seeds were sterilized using Carboxin Tiram, and then the seeds rinsed with distilled water and transferred to germinator 25±1°C.

The germination percentage was recorded every two days. Rate of germination was estimated using modified Timpson's index of germination velocity (Khan and Ungar, 1984). Mean Germination Time (MGT) was calculated in order to assess the rate of germination (Ellis and Roberts, 1981).

$$MGT = \frac{\sum D.N}{n}$$

Where:

N= the number of seeds which in D day grow,

n = the total number of seeds grown and

D= is the number of days from the date of germination.

The germination rate index was obtained by reversing MGT at the end of this period. The normality of data was checked and non-normal data transformed by ArcSin to verification of this hypothesis. ArcSin transformation was used for germination percentage before analysis (Khan *et al.*, 2006). Experimental data was analyzed by MSTATC software. The comparisons among treats were made using Duncan's Multiple Range Tests (DMRT) at %5 level of probability.

Results

The results of analysis of variance showed significant effects of salinity for all of traits (P \leq 0.01). The species effect was also significant for all of traits except plumule length and the time of germination (P \leq 0.01). The species x salinity interaction effect was significant for only radicle length, germination percent, and seed vigor (Table 1).

The overall means of germination percentage, germination rate, radicle length, and seed vigor were lower for T. daenensis as 41.44, 0.28, 1.15 and 1.87 than those for T. kotschyanus as 54.32, 0.37, 1.81 and 2.8, respectively, indicating more tolerant to salinity for *T. kotschvanus* than that for T. daenensis. The results showed that by increasing salinity, the germination percentage, rate of germination, plumule length, radicle length, and seed vigor were reduced in both species (Tables 3 and 4). The slop of reduction was higher for T. daenensis than that of T. kotschyanus. In other words, T. kotschyanus was more tolerate to salinity than T. daenensis.

For speed of germination the average values of *T. kotschyanus* was significantly higher than *T. daenensis*, while for algometric index the higher

values were obtained for *T. daenensis* (Figs. 1 and 2 and Table 1). For plumule length, there was no difference between two species (Table 1). By increasing the salinity levels, the means time of germination and Allometry ratios were increased in both of species (Tables 3 and 4).

The results showed that critical point of salinity was 150 mil/molar/lit. Therefore, by increasing to this point, the plumule length and radicle length were less than 1 mm. However, for germination percentage, the seeds of both species were germinated up to 250 mil/molar/lit and for 300 mil/molar/lit concentration, no seeds was germinated.

Discussion

In both species of *Thymus*, by increasing of salinity, germination percentage, rate of germination, plumule length and radicle length had decreased. Increasing salinity caused the reduction in water, nutrient uptake and osmotic imbalance in the environment of plumule radicles, ion toxicity and ultimately reduction in germination percentage and rate of germination, and finally led to decrease plant establishment. Our results were in agreement with some publications (Stephanie et al., 2005; Burnett et al., 2005; Boromanerezazade and Kocheki, 2005 and Fallahi et al., 2008). High concentration of salinity caused the impairment in seedling growth and its physiological processes. Hosseini et al. (2006) in the study of the effects of drought and salinity stress on seeds characteristics Isabgol (Plantago of ovata), and Safarnezhad et al., (2007) in study of salinity stress of Nigella sativa found that by increasing in salinity stress, germination percentage and speed of germination were decreased and the salinity stress led to reduction of plant growth. Our study

indicates that when salinity levels increased, the plumule length and radicle length of both species decreased. This increasing morphology in and physiological processes of plant may be due to decreasing plants access to water and nutrient due to the osmotic imbalance and impairment of biological activity and metabolic of plant. Tarzi (1995)investigated the effect of salinity on builder compound Cuminum cyminum in cultivation tissue and mature plant and showed that by increasing salinity effect, the plumule length had decreased. This was due to due impairment of ion toxicity and biological metabolic processes. Our results are in agreement with Fallahi et al., (2008) in which they showed that with increasing in salinity levels, the seedling length had decreased and minimum and maximum length of seedling were observed for control and 300 Mm NaCl treatments, respectively. The results of the present study were in agreement with those of Salami et al., (2006) in study of the effect of salinity stress on Cuminum cyminum and

Valeriana officinalis and Safarnejad and (2008)study of Hamidi in the morphological characters of Foeniculum vulgare under salt stress in which they showed that with increasing in salinity levels, seed vigor, the ability of plant for survival and normal living were decreased. consequently, based on the results, the published studies and regarding high medicinal values of this genus and their sensitivity to salinity stress, we recommend that the genus is cultivated in environments that plants are not in expose to salinity.

Acknowledgment

The researchers would like to appreciate the research vice-presidency in Young Researchers Club, Ardestan Branch, Islamic Azad University, Ardetan, Iran, for their support and coordination to fulfill the research plan of "The effect of salinity and drought stresses on seed germination and early growth of two species of medicine from Labiteae Family" and the present study.

Table 1. The Results of analysis of variance for seed characteristics of *T. kotschyanus* and *T. daenensis*

| S.O.V | | DF | F Mean of squares | | | | | | |
|----------|---|----|-------------------|-------------|-------------|-------------|-------------|--------------|--------------|
| | | | Radicle | Plumule | Germination | Time Means | Germination | Seed | Allometry |
| | | | Length | Length | Rate | Germination | Percentage | Vigor | Ratios |
| Species | | 1 | 1.48** | 0.01 | 0.14^{**} | 0.200 | 3020** | $2.9.0^{**}$ | 0.042^{**} |
| Salinity | | 6 | 8.03** | 3.59^{**} | 0.21^{**} | 1.38^{**} | 6383** | 20.6^{**} | 0.36** |
| Salinity | х | 6 | 0.39^{**} | 0.200 | 0.700 | 0.35 | 328.1* | 0.56^{**} | 0.006 |
| Species | | | | | | | | | |
| Error | | 28 | 0.30 | 0.02 | 0.004 | 0.25 | 128.7 | 0.06 | 0.004 |
| CV% | | | 20.3 | 21.9 | 24.8 | 25.8 | 29.8 | 17.9 | 5.2 |
| 4 | • | | 10/11 | 1 . | 1 | | | | |

*, ** Significant at %5 and %1 level respectively.

| Table 2. Mean of interaction effects of species and salinity treatments for the seed |
|--|
| characteristic |

| Species | Germination Percentage | Time Means Germination | Germination Rate | Plumule Length | Radicle Length | Seed Vigor | Allometry Ratios |
|----------------|---------------------------|---------------------------|---------------------|-------------------|-------------------|---------------|---------------------|
| T. daenensis | 41.44 b | 4.49 a | 0.28 b | 1.08 a | 1.15 b | 1.87b | 1.19 a |
| T. kotschyanus | 54.32 a | 3.60 b | 0.37 a | 1.14 a | 1.81 a | 2.8 a | 0.87 b |

Means with different superscript letters in a row are significantly (P<0.05) different

| Treat | Germination F | ercentage | Time Means C | Bermination | Germination Rate | | |
|-------|---------------|----------------|--------------|----------------|------------------|----------------|--|
| | T. daenensis | T. kotschyanus | T. daenensis | T. kotschyanus | T. daenensis | T. kotschyanus | |
| 0 | 83.9 a | 83.9 a | 2.34 d | 1.91 b | 0.43 a | 0.55 a | |
| 50 | 53.8 b | 79.4 a | 3.09 d | 1.79 b | 0.34 ab | 0.56 a | |
| 100 | 42.0 b | 69.1 a | 3.58 cd | 2.25 b | 0.29 b | 0.45 ab | |
| 150 | 22.7 с | 61.2 a | 4.62 c | 3.21 b | 0.22 bc | 0.31 b | |
| 200 | 5.7 cd | 23.6 b | 8.25 b | 5.63 a | 0.12 c | 0.18 c | |
| 250 | 2.9 d | 8.7 b | 10.00 a | 6.80 a | 0.10 c | 0.16 c | |
| 300 | 0.0 e | 0.0 c | - | - | 0.0 d | 0.0 d | |
| Means | 41.44 | 54.32 | 4.49 | 3.60 | 0.28 | 0.37 | |

Table 3. Mean of interaction effects of species and salinity treatments for germination percentage, time means of germination and germination rate

Means with different superscript letters in a row are significantly (P<0.05) different

Table 4. Mean of interaction effects of species and salinity treatments for plumule length, radicle length, seed vigor and allometry ratios

| Treat | Plumule Length | | Radicle Length | | Seed vigor | | Allometry Ratios | |
|-------|----------------|-------------|----------------|-------------|------------|-------------|------------------|-------------|
| | Т. | Т. | Т. | Т. | Т. | Т. | Т. | Т. |
| | daenensis | kotschyanus | daenensis | kotschyanus | daenensis | kotschyanus | daenensis | kotschyanus |
| 0 | 1.88 a | 1.94 a | 2.28 a | 3.60 a | 4.08 a | 5.44 a | 0.83 b | 0.54 b |
| 50 | 1.36 b | 1.46 b | 1.50 b | 2.23 b | 2.27 b | 3.51 b | 0.91 b | 0.66 b |
| 100 | 0.78 c | 0.85 c | 0.67 c | 1.20 c | 0.97 c | 1.85 c | 1.20 ab | 0.72 b |
| 150 | 0.27 d | 0.29 d | 0.14 d | 0.19 d | 0.17 d | 0.40 d | 1.80 a | 1.57 a |
| 200 | 0.0 e | 0.00 e | 0.00 e | 0.00 e | 0.00 e | 0.00 e | - | - |
| Means | 1.08 | 1.14 | 1.15 | 1.81 | 1.87 | 2.81 | 1.19 | 0.87 |

Means with different superscript letters in a row are significantly (P<0.05) different



Fig. 1. Mean effects of species on the rate of germination

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Fig. 2. Mean effects of species on the allometry ratios

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