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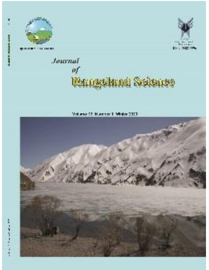

Research and Short Length Article:

Effect of Salinity and Drought Stresses on Seed Germination of *Thymus satureioides*

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Abstract. Salinity stress and drought stress are the major environmental constraint for promoting medicinal plants in the arid lands of Morocco. To extend the cultivation of *Thymus satureioides* in Tafilalet zone, Morocco, we investigate (in 2020) the effects of salinity and drought stresses on seed germination traits of *T. satureioides*. Salinity treatments included six concentrations of NaCl (0, 1, 2.5, 5, 7 and 10 g/l) and drought stress; five osmotic potentials of PolyEtylenGlycol 4000 (0, -0.1, -0.3, -0.6 and -0.9 MPa). Results showed that *T. Satureioides* tolerated salinity till the concentration of 2.5g/l of NaCl, which was statistically not different from control. Then, a highly significant reduction in germination percentage was recorded with the increasing salt concentration, only 38% seed germination was recorded for 5 g/l and a complete inhibition was started from 7 g/l and 10 g/l of NaCl. Decreasing osmotic potentials inhibited progressively and significantly seed germination, the germination percentage for -0.1 MPa, -0.3 MPa, and -0.6 MPa were 85.3%, 65.3%, 6.6% respectively. The two stresses (Drought and salinity) had negative impacts on seed germination, but the drought effect was accentuated than salinity in reducing germination.

Key words: *Thymus satureioides*, Germination, Salinity, Drought

Introduction

The *Thymus L* genus belongs to the *Lamiaceae* family, including more than 215 species, suited to the Mediterranean region's hot and dry climate. In Morocco, known locally as Azukni or Zaitra, 21 species are described, 13 of which are endemic (Benabid, 2000).

Thymus satureioides Cosson is an aromatic plant endemic to Morocco and Algeria, typical of arid habitats. In this area, *T. satureioides* is the most used species in herbal medicine (Bellakhdar *et al.,* 1991). However, growing this wild species necessitates a detailed understanding of its germination and growth responses to environmental stresses such as salinity and drought. These two factors are serious obstacles for field crops, especially in the arid regions of Morocco. Salinity is defined as the mean abiotic factor that limits crop production (Koyro, 2006). High soil salinity or saline water used for irrigation may exert an adverse effect upon seed germination in the field, exposing the seeds to stress (Khan and Ungar, 1997).

Moisture conditions play a crucial role in regulating germination in dry environments (Flores and Briones, 2001). Seed germination is modulated by soil water potential, and different species have different minimum water potentials at which germination can occur (Daws *et al.,* 2008). Many authors have reported on the negative impact of water stress and salinity on the germination of some Thymus species (Belaqziz *et al.,* 2009; Khoshsokhan *et al.,* 2012; Abbad *et al.,* 2011).

Nevertheless, no studies have investigated the effect of abiotic stresses on the germination of *T. satureioides*.

The objective of this study was to evaluate the effect of water stress and salt stress on the germination of seeds of *T. satureioides*.

The findings of this study offer new information about germinability requirements of *T. satureioides* in saline and arid habitats to enhance the chance of successful plant propagation.

Material and Methods

Site information and Sampling Method

Mature seeds were collected in July 2020 from Ait Hani, which is located in the province of Tinghir in the southeast of Morocco whose coordinates are (Latitude:31.75° N, Longitude -5.48° W, and altitude of 1992m).

Before starting the study, the seeds were stored dry in paper bags at an ambient temperature of about between 12-22°C. In the laboratory, the inflorescence was manually shaken to isolate the seeds, and then a 45x stereomicroscope was used to select good seeds. Afterward, seeds were soaked for 10 minutes in a solution of 5% sodium hypochlorite (NaOCl) before being used them in the experiments.

Research Method

For each test, 150 seeds of *T. satureioides* are divided into three lots of 50 seeds. The seeds were germinated in sterilized Petri dishes, containing two layers of filter paper (Whatman filter paper No. 1).

Sodium chloride (NaCl) was used as a salt stress simulator with six levels (0, 1, 2.5, 5, 7 and 10 g/l) of NaCl and five levels PolyEtylenGlycol 4000 were used (0, -0.1, -0.3, -0.6 and -0.9 MPa) to simulate drought stress.10 ml of the designated treatment solution was added to each Petri dish and 10 ml of distilled water was used as a control. The osmotic potential has been calculated from the polynomial regression linking the osmotic potential to the PEG concentration (Money, 1989).

The experiment was conducted in a programmed incubator with 14 hours of light with higher temperature, and 10 hours of darkness at 15/05 °C day/night temperatures.

Seed Germination Traits

Final germination percentage (FGP), Mean Time Germination (MTG), and Time to 50% germination (T50) were determined for each seed location to estimate the germination capacity and rate using the following formulas:

$$FPG\ (\%) = \frac{Ng}{Nt} \times 100$$

Where:
FGP= Final germination percentage
Nt =the total number of seeds
Ng = the number of germinated seeds ISTA (2015).

$$MTG = \sum_{i=1}^{k} \frac{NiTi}{\sum_{i=1}^{k} N_i}.$$

Where,
MTG= Mean Time Germination
N_i = the number of germinated seeds in the i^{th} time interval,
T_i is the time from the start of the experiment to the i^{th} interval,
k is the total number of time intervals (Ranal and Santana 2006).

$$T50 = \frac{\left(\frac{N}{2} - ni\right)(tj - ti)}{nj - ni}.$$

Where:
T50= Time to 50% germination
N =the final number of germination
Nj=ni cumulative number of seeds germinated by adjacent counts at times tj
ti =when ni<N/2< nj (Farooq *et al*., 2005)

SPSS 18 was used to analyze the data (IBM Corp, 2010). One-way ANOVA tested for the significance of the main effects of water stress and salt stress. The Newman and Keuls test (SNK) was used to compare means, and significance was determined at 95 % confidence limits.

Results and discussion

Effect of salinity on seed germination

The Table 1 shows that the germination kinetics depend on the NaCl concentration. Indeed, the germination percentage was statistically not different between the control and the treatments of 1 g/l and 2.5 g/l of NaCl. Nevertheless, the germination percentage was significantly reduced for the other concentration. Then the Final germination percentage (FGP) was statistically decreased gradually for the 5g/l of NaCl concentration. Complete inhibition of germination started for seed imbibed with of 7 g/l and 10 g/l of NaCl. The results show a significant effect of NaCl concentration on Mean Time Germination (MTG) and time to 50% germination (T50), it can thus be concluded that the salinity acts negatively on the germination of *T. satureioides* seeds by increasing MTG and T50. Many authors have reported a negative effect of salt stress on germination in several plant species (Liopa-Tsakalidi et *al.*, 2011; Keshavarzi, 2012; Sharma *et al.*, 2014). Our results are fortified by the results of Belaqziz *et al*. (2009), who found that salt stress has a negative response on the germination of *Ocimum basilicum* and *Thymus maroccanus*. However, the germination percentage, of *T. daenensis* and *T. kotschyanus* were relatively resistant to salinity (Khoshsokhan *et al.*, 2012). Other studies (Al-Karaki, 2001) show that NaCl impacts germination in two ways: by completely inhibiting it at dosages exceeding tolerance limits, or by delaying it by stressing the seed. Seed emergence may be inhibited by salinity due to specific salt toxicity, water inhibition failure, or a combination of these factors (Manzoor et al., 2017). Osmotic potential (ψ0) is the major factor affecting seed emergence, where high saline levels decrease H_2O availability to seeds, and consequently limiting their hydration and causing germination failure (Zhou and Xiao, 2010; Nedjimi, 2013). Furthermore, the physiological state affects seed absorption of water, causing a reduction in plant hormones and enzyme production, which inhibits seedling growth (Bor *et al.*, 2003; Younis *et al.*, 2008). Groome *et al.* (1991) showed that a reduction in germination percentage was caused by an increase in external osmotic pressure, which influences seed water absorption, and/or an accumulation of Na^+ and Cl^- ions. Increasing NaCl levels has resulted in lower germination percentages, which can be explained by a reduction in water absorption caused by salinity and/or toxic effects of Na^+ and Cl^- ions on the germination process (Singah et al, 1988).

Table 1. Means of seed germination traits of *T. satureioides* at different concentrations of NaCl
Values are represented as means ± S.D

Treatments concentration of NaCl	Maximal germination%	Time to 50% Germination (days)	Germination time (days)
Control (0g/L)	93±1.73^c	11.98±0.72^b	11.12±0.53^b
1.0 g/L	94±3.05^c	11.93±0.39^b	11.93±0.22^b
2.5 g/L	86±3.05^c	15.04±0.63^c	15.15±0.87^c
5.0 g/L	38±3.46^b	17.68±0.24^c	17.16±0.32^d
7.0g/L	0.0 ±0.0^a	0.0 ±0.0^a	0.0 ±0.0^a
10.0 g/L	0.0 ±0.0^a	0.0 ±0.0^a	0.0 ±0.0^a

Means of the column with different letters are significantly different ($p<0.05$)

Effect of drought stress on seed germination

Drought stress has a similar impact to salinity stress in terms of reducing seed germination with increasing osmotic potential values.

The percentages of germination of *T. satureioides* seeds reaching their maximum of 93% and 85.33%, respectively at treatment 0 MPa and -0.1 MPa, the germination was strongly decreased at -0.6 MPa, to 6.66%, while the pressure -0.9 MPa inhibited completely the germination (Table 2). The evolution of T50 and mean times germination as a function of water potential show that increasing water stress leads to a reduction not only in germination percentage but also in the mean time germination and T50 of *Thymus satureioides* seeds. The mean time germination increases significantly ($p<0.05$) with increasing water stress, the lowest times were recorded in the control 11.98 days which remains insignificant with -0.1MPa water stress. The same T50 results show that they are significantly affected ($p < 0.05$) by water stress. These results may be due to the poor adaptation to drought. In addition, *T. satureioides* is mainly located in the mountains and distributed between arid and subhumid bioclimates (Fennane *et al*., 2007). Many authors have reported on the negative impact of hydric stress on germination in a variety of species (Bakhshandeh, *et al.*, 2011; Rios-Rojas *et al.*, 2014; Zhou *et al.*, 2015; Torabi, *et al.*, 2016). Similarly, Abbad *et al.* (2011) showed that the resistance limits of the two species *Thymus maroccanus* and *Thymus broussonetii* to water stress are between -0.32 and -0.53 MPa. The decrease in osmotic potential progressively inhibited the germination of seeds of *T. daenensis* and *T. kotschyanus* (Khoshsokhan, *et al.*, 2012). Taiz and Zeiger (2002) and Schulze *et al.* (2005) show that low imbibitions rates cause a halt in cell division and elongation at the level of growing roots. In the Mediterranean climate, rain falls unpredictably, probably not enough to maintain the water content of the soil is a critical value that favors germination.

Table 2. Means of seed germination traits of *T. satureioides* at different water potentials
Values are represented as means ± S.D

Treatments water potential	Maximal germination%	Time to 50% Germination (days)	Germination time (days)
Control (-0.0 MPa)	93±1.73[c]	11.12±0.53[b]	11.98±0.72[b]
-0.1 MPa	85.33±2.66[c]	13.14±0.4[c]	12.74±0.41[b]
-0.3 MPa	56.66±3.52[b]	17.06±0.72[d]	17.24±0.42[c]
-0.6 MPa	6.66±0.66[a]	21.58±0.08[e]	22.11±0.11[d]
-0.9 MPa	0.0 ±0.0[a]	0.0 ±0.0[a]	0.0 ±0.0[a]

Means of the column with different letters are significantly different ($p<0.05$)

Conclusion

Water had a decisive effect on the germination process. There was a strong negative of NaCl concentration and osmotic potential on seed germination. The concentration of 2.5g/l NaCl did not affect the germination percentage. At -0.6 MPa the germination was strongly decreased. With concentrations 7 and 10 g/l of NaCl and osmotic pressure -0.9 MPa, the germination percentage is canceled. These results could be used to develop protocols for propagation and ex-situ conservation of this medicinal plant.

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