ADVANCES in NATURAL and APPLIED SCIENCES

ISSN: 1995-0772
EISSN: 1998-1090
2016 April 10(4): pages 372-378

Published BY **AENSI Publication**
http://www.aensiweb.com/ANAS
Open Access Journal

Testing the effect of temperature and salinity on germination behavior of *Thymus Fontanesii* Boiss. & Reut.

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Received 25 January 2016; Accepted 28 March 2016; Available 10 April 2016

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ABSTRACT
To study the germination behavior and response of *Thymus fontanesii* (*Lamiaceae*), the laboratory experiments were carried out to assess the effects of temperature and salinity on seed germination. For this, seeds were submitted at different constant temperatures of 5, 10, 15, 20, 25, 30, 35 and 40 °C and various sodium chloride (NaCl) concentrations of 0, 1, 2, 4, 6, 8, 10, 12 and 14 g L^{-1}. Temperatures between 5 and 30 °C seem to be favorable for germination of this species. Germination regresses by either an increasing or decreasing temperature from the optimum (20 °C). The highest Final germination percentage (88%) has been obtained at 1 g L^{-1} NaCl. However, the increase of solution osmolalities (NaCl) declines progressively seed germination. The mean germination time (**MTG**) increased with increasing salinity. Temperature of 35 °C and salt concentration of 12 g L^{-1} are the tolerance threshold of *T. fontanesii*. By these results it should be noted that this species has ability to adapt to climate change and soil salinization in the germination stage.

KEYWORDS: Germination; Salinity; Temperature; Tolerance*; Thymus fontanesii*.

INTRODUCTION

Successful establishment of plants largely depends on successful germination. Germination is a crucial stage in the life cycle of plants and tends to be highly unpredictable over space and time. Several environmental factors such as temperature, salinity, light and soil moisture simultaneously influence germination [1, 2]. Seed germination behavior in relation to thermal and salt stress is very important to determine the colonization capacity of a species [3].

Temperature is a determining factor for seed germination [4]. The establishment of plants in arid and semi arid regions is often limited by the temperature even though the conditions of humidity are favorable [5]. Knowledge of temperature effects on germination may be useful to evaluate the germination characteristics or the establishment potential among range species [6]. Temperature changes may affect a number of processes controlling seed germinability, including membrane permeability and the activity of membrane-bound and cytosolic enzymes [7].

High salinity is the most widespread soil problem limiting plant distribution and productivity [8]. Tolerance to salinity during germination is critical for the establishment of plants growing in saline soil of arid regions [9]. An increase in salinity induces a reduction in the percentage of germinating seeds [10]. Germination failures in saline soils are often a result of high salt concentrations in the seed-planting zone because of the upward movement of soil solution and subsequent evaporation in the soil surface; this has been attributed to both

To Cite This Article: Mohammed Dadach and Zoheir Mehdadi., Testing the effect of temperature and salinity on germination behavior of *Thymus Fontanesii* Boiss. & Reut. **Advances in Natural and Applied Sciences**. 10(4); Pages: 372-378

osmotic and toxic effects [11]. Seed germination under saline conditions occurs after high precipitation where soil salinity is usually reduced due to leaching [12].

A considerable number of medicinal and grass species are locally adapted and considered as native to arid zones of the world [13, 14]. The potential of water and salinity stress tolerance and the economical value of medicinal and aromatic plants make them suitable alternative crops in dry land agro-ecosystems [15].

Thymus is considered a genus characteristic of the Mediterranean, as representatives of all its sections except *Thymus* sect. *Serpyllum* (Mill.) Benth. and sect. *Hyphodromi* (A. Kern.) Halácsy (subsect. *Serpyllastru*m Villar) found in the other Regions [16]. *T. fontanesii* is a spontaneous and an aromatic plant, belonging to the *Lamiaceae* family and native to Algeria and Tunisia, used by local people for its medicinal properties. The whole plant is used in traditional as an antispasmodic, carminative, anti-cough, antiseptics [17].

At present, growing medicinal plants is an important sector in agriculture and is the main source of extracting and producing raw materials for manufacturing current drugs. That is why in most developed countries research centers have been established specifically for medicinal plants. These centers identify and introduce numerous active ingredients in plants, together with their desirable effects [18].

However, no information is available concerning seed germination responses to temperature and salinity of *T. fontanesii*. For that, Experiments were conducted to investigate the effects of temperature regimes and different levels of NaCl concentrations on germination under laboratory controlled conditions. Information from this study provides basic knowledge about germination requirements of *T. fontanesii*.

MATERIEL AND METHODS

Plant materiel:

Mature seeds were collected in late summer 2014 from *Tessala mount* in Algeria (35°16’19.19” N; 00°46’02.14” W at 802m). This mount is semi-arid with a typical Mediterranean climate with average annual rainfall is between 290 mm and 420 mm,

Germination experiments:

Seeds were surface sterilized in 1% sodium hypochlorite solution for 5 min, to avoid fungus attack, then thoroughly rinsed with deionised water [19] and air-dried before being used in the germination experiments. 90 mm Petri dishes containing two disks of Whatman No. 1 filter papers with 5 ml of test solution were prepared. Each treatment consisted of 4 replicates with 25 seeds. Seeds were considered germinated when radical appeared [20].

Effect of temperature and salinity:

The preliminary experiment was carried out in order to determine the optimal temperature. Germination experiments were conducted in darkness and in incubators set at 5, 10, 15, 20, 25, 30, 35 and 40 °C. Distilled water equal to the mean water loss from dishes was added to maintain humidity.

The secondly experiment was conducted to evaluate the effect of exposure seeds to different salinity treatments (NaCl) of 1, 2, 4, 6, 8, 10, 12 and 14 g L^{-1} , distilled water was used as control (0 g L^{-1}). Germination tests were performed at the optimal temperature (20 °C) and in darkness. Irrigation with different NaCl concentrations equal to the mean loss from dishes was added every two days.

Data analysis:

Final germination percentage (FGP), initial germination day (IGD) and mean time of germination (MTG) are parameters to determine for studying germination behavior.

MTG was calculated as follows: $MTG = \Sigma_i (n_i x\, d_i)/N$

Where n_i is the number of seeds germinated at day i, d_i is the incubation period in days, and N is the total number of germinated seeds [21].

Statistical analysis:

Germination variables percentages were transformed before statistical analysis to ensure homogeneity of variance. Data were analyzed using SPSS for windows, version 20. A one-way analysis of variance (ANOVA) was performed on all results. Duncan’s multiple range tests was used to estimate least significant range between means.

RESULTS AND DISCUSSION

Temperature effects:

Germination is significantly affected by temperature. The seeds of *T. fontanesii* are able to germinate at the temperature range between 5 and 35 °C, this germination has been totally inhibited at 40 °C (Table 1). The maximum FGP was obtained at temperature of 20 °C (Fig. 1), than the germination decreased above and below this optimum. The MTG also varied significantly. The lowest MTG was noticed at temperature of 15 °C and it increased with increasing or decreasing temperature. Concerning IGD, the germination at 20 °C started at 1.7 days and the longest IGD was remarked at temperature of 35 °C with a delay of 10 days (Table 1).

Table 1: Germination characteristic variables of *T. fontanesii* seeds at various temperatures for two weeks (mean ± S.E., n = 4).

Temperature (°C)	Parameters		
	FGP (%)	IGD (days)	MTG(days)
5	40 ± 10^{a}	5.7^{b}	13.1 ± 1.2^{a}
10	57 ± 2^{a}	3.3^{ab}	10.2 ± 0.6^{c}
15	85 ± 4^{b}	2.0^{a}	4.62 ± 0.5^{b}
20	88 ± 5^{b}	2.0^{a}	5.03 ± 0.6^{b}
25	67 ± 6^{a}	2.3^{a}	9.46 ± 1.5^{c}
30	43 ± 6^{a}	4^{ab}	10.8 ± 1.6^{ac}
35	8 ± 2^{c}	10^{c}	10.9 ± 0.8^{ac}
40	0^{d}	-	-
F- value	80.78*	1.63**	20.29*

Different lowercase letters (column) show significant differences between the averages, F-probabilities are indicated by symbols: * significant differences at $p < 0.05$, **significant differences at $p < 0.01$, according to Duncan multiple comparisons test

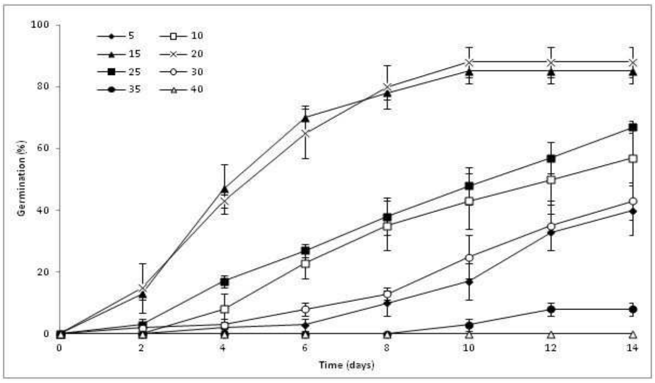

Fig. 1: Cumulative germination percentage. Seeds incubated for four weeks at different incubation temperatures (°C). Bars represent ± S.E. (n = 4). The confidence intervals were calculated at the threshold of 5%.

Salinity effects:

Salinity has significantly affected germination of *T. fontanesii* seeds (Table 2 and Fig. 2). The highest FGP was noted at treatment of 1 g L^{-1} NaCl (88%) followed by control (87%). Germination started to decrease significantly above the concentration of 2 g L^{-1} NaCl. However, higher NaCl concentrations (10 and 12 g L^{-1}) exhibit a substantial reduction in seed germination and which it was inhibited at 14 g L^{-1} NaCl. At salinity treatment less than 10 g L^{-1} NaCl germination began on second day, whereas, for concentration of 12 g L^{-1} NaCl germination started at 3.7 days. MTG is also significantly affected by salt stress. It has been found that MTG progressed with increasing salinity.

Table 2: Germination characteristic variables of *T. fontanesii* seeds at various salinity levels for two weeks (mean± S.E., n = 4).

NaCl (g L^{-1})	Parameters		
	FGP (%)	IGD (days)	MTG(days)
0	87 ± 2^{a}	2^{a}	4.5 ± 0.4^{bc}
1	88 ± 6^{a}	2^{a}	3.9 ± 0.5^{ab}
2	70 ± 6^{b}	2^{a}	3.6 ± 0.3^{a}
4	62 ± 2^{b}	2^{a}	4.0 ± 0.3^{ab}
6	52 ± 6^{c}	2^{a}	4.4 ± 0.1^{abc}
8	47 ± 2^{c}	2^{a}	4.9 ± 0.4^{cd}
10	35 ± 4^{e}	2^{a}	5.6 ± 0.8^{de}
12	20 ± 4^{f}	3.4^{b}	6.0 ± 0.2^{e}
14	0^{g}	-	-
F- value	80.74*	0.75**	11.66*

Different lowercase letters (column) show significant differences between the averages F-probabilities are indicated by symbols: * significant differences at $p < 0.05$, **significant differences at $p < 0.01$, according to Duncan multiple comparisons test.

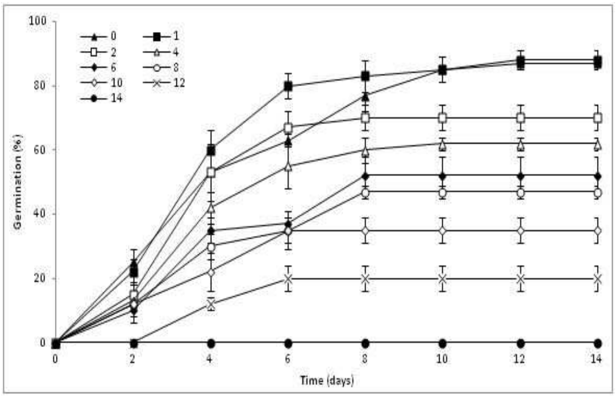

Fig. 2: Cumulative germination percentage. Seeds incubated for two weeks at different salinity levels (g L^{-1}). Bars represent ± S.E. (n = 4). The confidence intervals were calculated at the threshold of 5%.

Polynomial analysis:

Polynomial regression analysis was used to determine the relationships between FGP with temperature and salinity. It was found that there is a strong relationship between FGP / temperature with a regression coefficient of 0.94 (Fig. 3) and between FGP / salinity with a regression coefficient of 0.96 (Fig. 4).

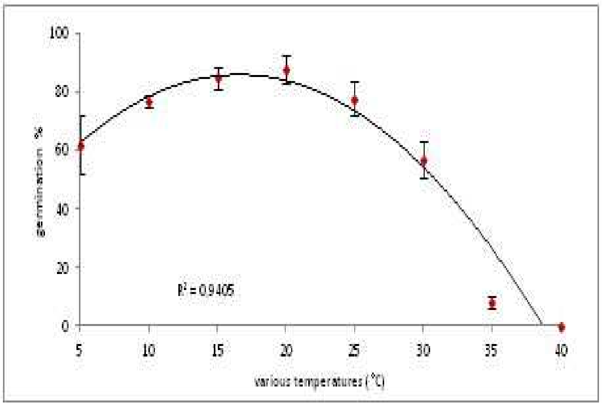

Fig. 3: Polynomial plot for final germination percentage (FGP) at various temperatures regimes. Bars represent ± S.E. (n = 4). The confidence intervals were calculated at the threshold of 5%.

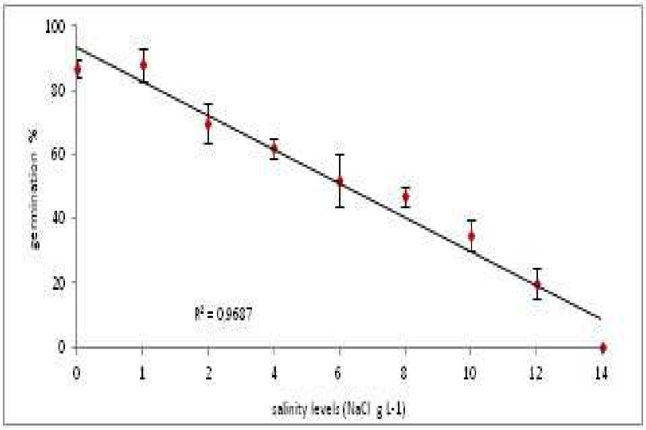

Fig. 4: Regression plot for final germination percentage (FGP) at different NaCl concentration. Bars represent ± S.E. (n = 4). The confidence intervals were calculated at the threshold of 5%.

Relationship between FGP and MTG at various salinity levels:

In added, it was noted that there is a clear distinction between the effect of salt on the FGP and MTG. This found is evident when examining (Fig. 5). Actually, salt stress affects significantly germination rate (estimated by the increase of MTG).

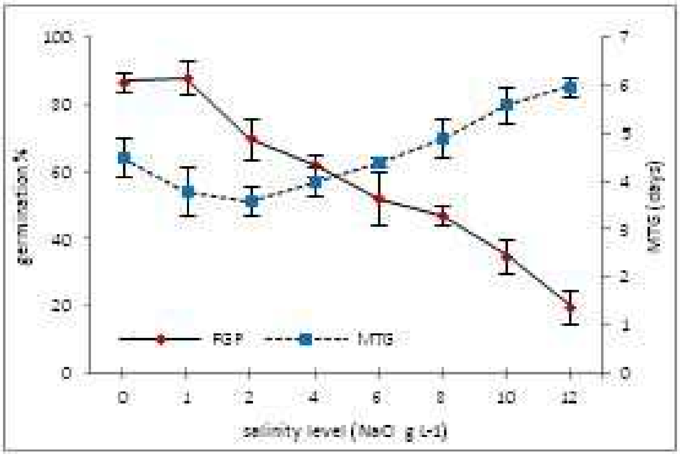

Fig. 5: Effect of various NaCl concentrations on final germination percentage (FGP) and mean time of germination (MTG) at 20 °C. Bars represent ± S.E. (n = 4). The confidence intervals were calculated at the threshold of 5%.

The results showed that the *T. fontanesii* seeds are viable insofar as their germination is possible in a wide temperature range extending from 5 °C to 35 °C. The less FGP was obtained at 35 °C (8 %); at 40 °C the germination was completely inhibited. However, the maximum FGP were noted at temperature range between 10 °C to 25 °C with a peak registered at 20 °C. At this thermal range it was noted the shortest MGT and IGD. This thermal optimum also characterizes other Mediterranean species in arid and semi-arid regions belonging to the *Lamiaceae* family such as *Lavandula dentata*, *Teucrium gnaphalodes*, *Thymbra capitata* and *Thymus hyemalis* [22,23].

Germination behavior of *T. fontanesii* is similar to those of some medicinal plants that in fact retain a high germination percentage in a wide range of temperatures but this germination become low or it totally decline at 35 °C [13]. Our results also join those of Corme [24] who reported that seeds of *Salvia officinalis* and *Salvia sclarea* (*Lamiaceae*) germinate satisfactorily in a wide range of temperature extending respectively between 10 - 25 °C and 10 - 30 °C. Mijani *et al.* [25] have noted that the highest germination percentage of *Hyssopus officinalis*, *Ocimum basilicum* and *Origanum vulgare* occurred between 20 - 30 °C. Furthermore, it was reported in the results of Nadjafi *et al.* [19] and Atashi *et al.* [26] that the seeds germination of *Thymus daenensis* and *Melissa officinalis* are completely inhibited at 40 °C, this is what has been found for the seeds of our species. This can be explained by the fact that in such temperature induces a secondary dormancy in seeds that were previously able to germinate [27]. The delay of germination establishment at temperature of 35 °C can be due to the adaptation of *T. fontanesii* to cooler temperatures and need a higher thermal time to germinate; this has been explained by Trudgill *et al.* [28] when they have studied some British herbaceous plants.

Several studies have shown that the application of salt stress in the step of germination is a reliable test to evaluate the resistance of many plants [29,30]. Seeds of *T. fontanesii* have recorded a maximum germination at the salt concentration of 1 g L^{-1} NaCl and at control. Beyond these concentrations, we have remarked a reduction of FGP and elongation of MTG. Germination is canceled at 14 g L^{-1} NaCl and the tolerance threshold is equivalent to 12 g L^{-1} NaCl. Khoshsokhan *et al.* [31] showed that *Thymus kotschyanus* and *Thymus daenensis* seeds germinate better in absence of salt, that what has been reported also by Niazi *et al.* [32] on *Hordeum vulgare* and Khan and Gulzar [9] on *Sporobolus ioclados.* Furthermore, Belaqziz *et al.* [33] studied the salt stress effects on germination of *Thymus maroccanus* noted that these species exhibit a negative response to salinity resulting in the decrease of seed germination. Other studies of various medicinal herbs such as artichoke [34] and Hyssop [35] have also shown that with increasing salinity, germination characteristic values decrease.

It was also found that the average IGD are equivalent to 2 days, repeated for all the saline concentrations tested, except for the concentration of 12 g L^{-1}. In this context, Ghavami and Raminwe [36] have noticed that the salt tolerance term during germination is used only to involve situations where the seed has the ability to germinate quickly in salt stress conditions. The negative response of *T. fontanesii* seed germination in saline conditions may be due to a potential reduction in cellular water or a decrease in hydration of proteins and the enzymatic activity involved in the germination process [37,38].

Conclusion:

For this study, we can conclude that seeds of *T. fontanesii* are able to germinate in a wide range of temperatures and capable of developing a strategy for salt tolerance. These data certainly serve as part of the cultivation of this endangered taxon. The salt effect at the germination stage requires other investigations to better understand the physiological mechanisms adopted by this species to survive in constraining natural environmental conditions characterizing the semi-arid and arid regions.

ACKNOWLEDGEMENTS

We would like to thank members of Plant Biodiversity Laboratory for their help, especially Pr. Ali Latreche and Pr. Belhassaini Hachemi. We thank also Mr Sofiane Bouazza for his help in statistical analysis.

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