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Effects of Water Stress on Seed Germination of *Thymus koteschanus* Boiss. and Hohen and *Thymus daenensis* Celak

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Abstract: Study on growth status of medicinal plants in different water stress conditions can be considered as the cultivation guide for resistant plant species. The current study was carried out to investigate the effects of water stress on seed germination of *Thymus koteschanus* and *Thymus daenensis*. To achieve this goal, polyethylene glycol (PEG) was used to provide appropriate water potentials (0, -0.3, -0.6, -0.9, -0.12, -0.15 and -0.18 MPa). A completely randomized design was applied as the experimental design with three replications and all data were analyzed using SAS software.Mean comparisons was done through LSD test. The results showed significant differences between two studied thymes and maximum germination velocity and vigor index were recorded for *Th. koteschanus*. There were also significant differences among all studied traits in different treatments as they were decreased with increase of water stress and no germination was recorded under sever water stress. According to the results, it could be concluded that the effcts of water stress on germination of Thyme was negative and caused a reduction in germination parameters.

Key words: Water stress · Thyme · *Thymus koteschanus* · *Thymus daenensis* · Germination

INTRODUCTION

Water stress is one of the main constraints for plant growth and most common environmental stresses worldwide. The effect of water stress on plant growth and yield is clearly dependent on genotype [1]. Water is one of the scarce resources in Iran affected by precipitation. Water stress in plants occurs when the amount of water uptake is less than water losses and this may be due to excessive water loss, reduced uptake or both cases [2]. Water stress affects different aspects of plant growth and causes reduction and delay in seed germination.

Reduction of osmotic potential and total water potential with swelling loss, stomata closing and growth reduction are of water stress characteristics. Sever water stress will result in reduced photosynthesis and disruption of physiological processes, growth stop and eventually plant death [3]. Water potential is the most effective parameter in water absorption and seed swelling as water stress reduces water absorption. Seed germination of all plant species need a minimum of water to be absorbed and swelled and that is why osmotic potential should not be less than a certain amount. With decreasing osmotic potential, water absorption by seeds and consequently germination are reduced [4].

Seed germination velocity is utmost important to fast establishment and reduced loss of the seedlings. In arid regions, deficiency of soil moisture affects seed germination and seedling establishment [5,6].

Thymus genus of the Lamiaceae family contains almost 215 species of prenial forbs and small shrubs native to the Mediterranean region and also grows in parts of southern Europe, Africa and some parts of Asia [7]. In Iran 14 species of this genus naturally grow in several parts of the country [8]. *Thymus* species are widely used in different parts of the world as drinks, food flavors (spices and condiments) and medicinal plants [7]. *Thymus* is a robust and dense shrub distributed in a wide range of montainous rangelands as dominant and companion species. Having aboundant stems relatively short and woody which gives a pulvinate crown to this species along with robust and dense roots play a key role in soil stabilization and also prevent water erosion in mountainous and sharp slope regions [9].

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Although metabolic and physiologic responses of agricultural plants have been studied, less studies have been done on medicinal and range species. Results of the investigation on the effects of water and salinity stresses on seed germination of *Thymus vulgaris* showed that water stress significantly decreased seed germination percentage [10]. In an experiment, the effects of water stress were studied on two Lemongrasses namely *Cymbopogon nardus* and *Cymbopogon pendulus* and a significant reduction was observed in height, leaf length, leaf area and weight under moderate water stress treatments [11]. Baher Nik *et al*. [12] studied the metabolic changes resulting from water stress in *Satureja hortensis*. The results showed that water potential of leaf samples was reduced by increasing water stress from -0.5 to -1.5 MPa and relative water content(RWC) of leavs was also decreased [12]. In another study, Babaei *et al*. [13] investigated the effects of water stress on morphological traits of *Thymus vulgaris*. Their results showed that water stress had significant effect on growth parameters and vegetative organ yield [13].

Plant height, number of side shoots, shoot dry and fresh weight, root volume, root dry and fresh weight and root length were reduced with increasing water stress. Soil water reservior is one of the most important environmental factors that controls germination and seedling establishment [14]. In researches performed by Baalbaki *et al*, 1999, Hucl, 1993, Karan *et al*, 1985, Manga, 1998 and Swarn *et al*, 1999 respectively on seeds of indian millet, soybean, pea, bean and wheat, they reported that germination percentage and germination rate decreased with increase of water stress. They also expressed that germination rate had more sensitivity to the changes of water stress in comparison with germination percentage [15-19].

Considering the importance of medicinal plants propagation and the role of seeds in growth and production of these valuable plants, in the current study, the effects of different levels of water stress on seed germination of thyme were evaluated so that tolerance level of the species in germination stage to water stress can be determined.

MATERIALS AND METHODS

In order to study the effects of water stress on seed germination in *Thymus kotschyanus* and *Thymus daenensis*, seeds of *Th. koteschanus* and *Th. daenensis* were respectively collected from Babyn village, Qazvin province in 2003 and Fereydounshahr, Isfahan province in 2002. To evaluate the effect of different water potentials on seed germination of the mentioned species, the experiment was performed in the incubator with a temperature of 19 to 23 ° C and 12-hour intermittent light including three replications of 20 seeds (each petri dish as one replication).

In each sterile Petri, 20 seeds were placed equally with a suitable distance from each other on Whatman filter paper grade 40.

Water potential gradient was induced using PEG (Polyethylene Glycol) 6000 in levels of -0.3, -0.6, -0.9, -1.2, -1.5 and -1.8 MPa [20]. Zero concentration was also considered as the control treatment. Seed germination data were recorded daily until constant percentage was calculated in each Petri for seven consecutive days.

In the seed germination experiment final germination percentage (GF), germination rate (GR) and mean germination time (MGT) were measured and studied. MGT was calculated to assess the germination rate [21]. MGT and GR were calculated as follows:

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

$$GR = \frac{1}{MGT}$$

Where, N is the number of seeds which grow in D day, n is the total number of seeds grown and D is the number of days from the date of germination.

At the end of this period, the length of the plumule and radicle after the emergence of the first two new leaflets were recorded. The important index of allometry was determined by calculating the length ratio of the plumule to the radicle.

Seed vigor index was calculated with the following formula [22]:

Vigor index (VI) = [seedling length (mm) × germination percentage]/100

The arcsine square root transformation of GF was used to ensure normal distribution and homogeneity of variance [23, 24].

Drought resistance experiment was conducted in a completely randomized factorial design. To study the differences among plants and treatments for germination traits, an analysis was carried out by means of two-way ANOVA (SAS version 9.1 programs) and the LSD test ($P<0.05$) was used to test differences between treatment means.

RESULTS

Results of ANOVA analysis indicated that species effects in terms of germination rate and seed vigor index were significant ($P<0.05$) and also treatment effects were significant in all factors examined ($P<0.01$), while species x treatments interaction effects showed no statistical significant differences in studied factors (Table 1).

Table 1: ANOVA of water-stress resistance

		MS						
SOV	d.f	Germination %	Mean germination time	Germination rate	Plumule length (cm)	Radicle length (cm)	Vigor index	Allometry ratios
Species	1	38.1	0.06	0.02*	0.006	0.13	0.12*	0.002
Treatment	6	10868.6**	1.41**	2.09**	0.57**	1.34**	1.7**	0.047**
Treatment x Species	6	28.37	0.03	0.07	0.01	0.04	0.04	0.0006
Error	28	54.16	0.12	0.01	0.01	0.04	0.02	0.001
Coefficient Variation(%)		23.24	23.90	17.75	22.98	19.51	14.78	4.66

*P<0.05, **P<0.01

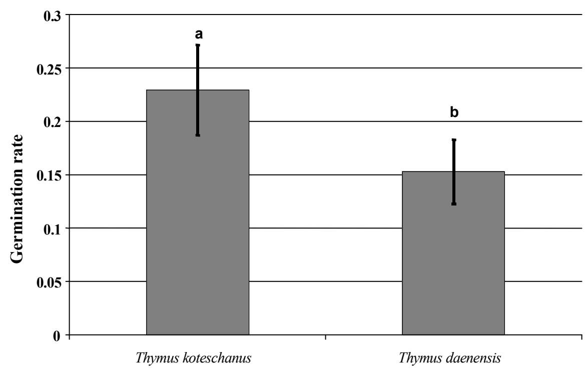

Fig. 1: Mean effects of species on germination rate

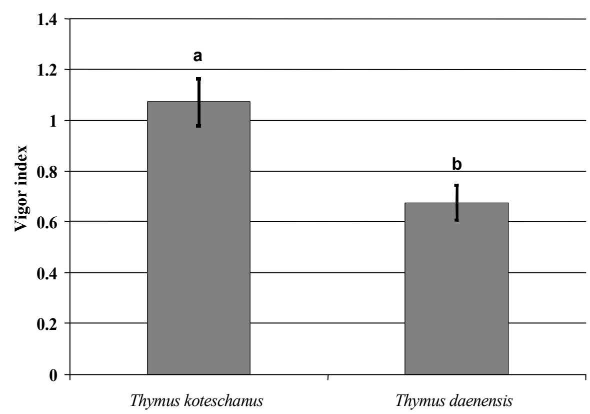

Fig. 2: Mean effects of species on Vigor index

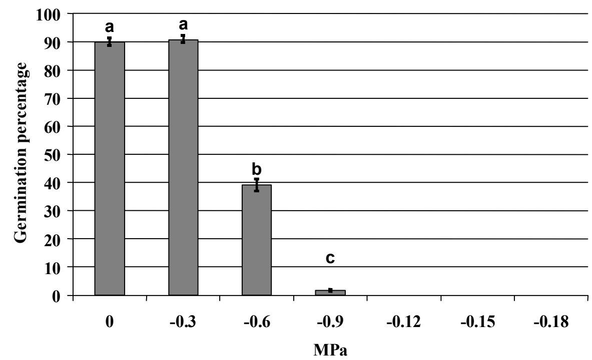

Fig. 3: Mean effects of water-stress treatments on germination percentage

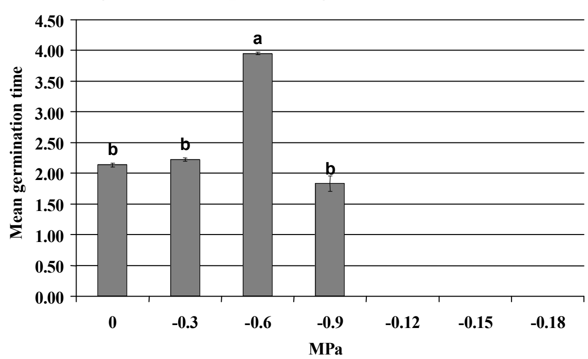

Fig. 4: Mean effects of water-stress treatments on mean germination time

As shown in Table 1, significant species effects were just observed in germination rate and vigor index (Fig.1 and 2). Maximum germination rate and vigor index were recorded for *Th. Koteschanus*.

Statistical analysis of treatment effects in different water stress levels shows no significant differences in germination between -0.3 MPa and control treatments, while it was significant for other treatments. Germination percentage was also obviously decreased when water stress increased more than -0.3 MPa as no germination was recorded at -0.9 MPa and more water stress (Fig. 3).

Mean germination time was also significant in different water stress levels and maximum was recorded at -0.6 MPa. Water stress more than the mentioned value caused a decrease in studied factor as average germination percentage decreased to zero at water stress more than -0.9 MPa (Fig. 4).

Significant differences were seen among different treatments in germination rate at 5% level of confidence (Fig. 5). Although no significant differences were identified between -0.3 MPa and control traetments. Germination rate was decreased with increase of water stress more than -0.3 MPa as this value reached zero in the last three treatments.

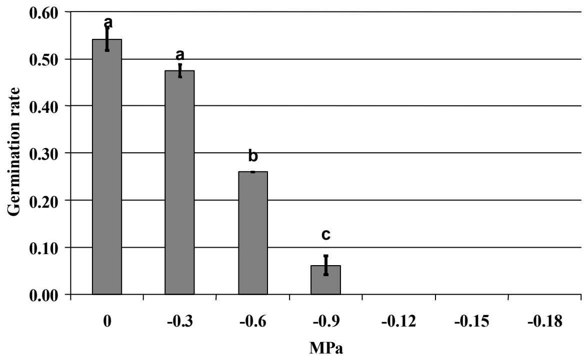

Fig. 5: Mean effects of water-stress treatments on germination rate

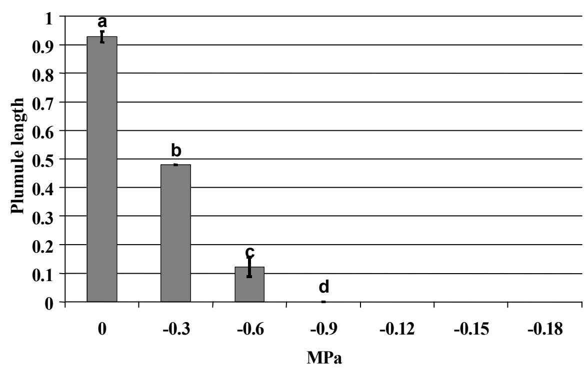

Fig. 6: Mean effects of water-stress treatments on Plumule length

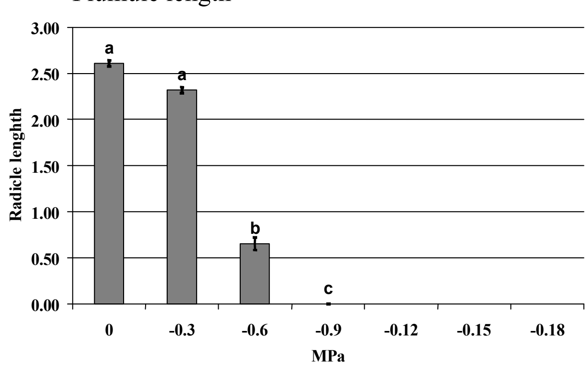

Fig. 7: Mean effects of water-stress treatments on radicle length

Statistical analysis of treatment effects on plumule length of both studied thymus species showed that this trait was also significantly decreased with increasing water stress and this value was zero in levels more than -0.9 MPa (Fig. 6)

No significant differences were identified between control and -0.3 MPa treatments in radicle lenghth in both thymus species but in other treatments it was significantly decreased with increase of water stress (Fig. 7).

The same trend was also seen in vigor index of both species as this trait was decresed with increase of water stress. Although no significant differences were

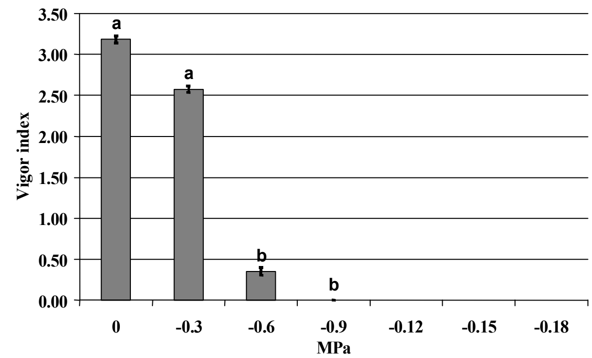

Fig. 8: Mean effects of water-stress treatments on vigor index

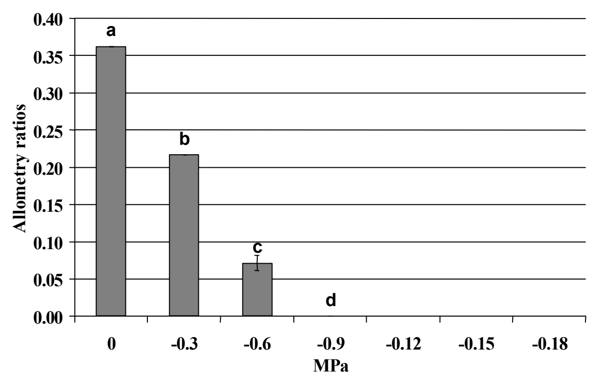

Fig. 9: Mean effects of water-stress treatments on Allometry ratios

seen between control and -0.3 MPa treatments, increase of water stress more than -0.3 MPa caused a considerable reduction in vigor index as it reached zero in levels more than -0.9 MPa (Fig. 8).

As well as other traits, significant differences were identified among different treatments in allometric ratios as it was decreased with increase of water stress and reached zero in levels more than -0.9 MPa (Fig. 9).

CONCLUSION

Results of the current study showed that species effects for germination percentage and vigor index were significant at level of 5% while treatment effects were significant for all studied tratis at level of 1%. Species × treatment interaction effects for all traits were not significant statistically.

Our results indicated that *Th. koteschanus* was more resistant to water stress than *Th. daenensis* in view of germination rate and vigor index. In other words, it can be stated that *Th. koteschanus* is more drought tolerant than *Th. daenensi*s under similar water stress treatment.

Reduction in germination due to the water stress can be related to low water absorption by seeds. If the water absorption by seeds is adversly affected or slow absorption of water occurs, metabolic activity within the seed germination will take place slowly. As a result, radicle emergence from the seed will take longer hence germination rate decreases [25]. It is noteworthy that the effects of water stress treatments on germination percentage, germination rate, radicle and plumule length and also vigor index were significant as the maximum value of the studied traits was recorded at 0 MPa (control treatment). Increase of water stress caused a reduction in all traits as in severe water stress i.e. more than -0.9 MPa no growth was seen. This indicates that water stress has negative effects on germination behaviour of this species. Hassani [26] studied the effect of water stress on *Ocimum basilicum* and showed that radicle growth is less affected by water stress in comparison with plumule and no significant differences were observed for germination percentage upto -0.41 MPa while there was no germination at -1.35 MPa [26].

Ghani *et al*. [27] studied reactions of different Achillea species to drought stress and showed that different water stress levels and type of the species had significant effects on germination percentage, germination rate and radicle length. Interaction effect of water stress and species on the mentioned traits was also significant. According to the results, radicle length of Achillea species was less affected by water stress and germination percentage was better than other factors to evaluate water stress resistant in *Achillea* [27]. Our results are in good agreement with the research conducted by Boromand and Koochaki [28] as they also indicated that in medicine plants of *Trachyspermum ammi*, *Foeniculum vulgare* and *Aniethum graveolens* with increase of water stress, germination rate, germination percentage, radicle and plumule length were decreased and water stress showed more intensive negative effects on germination rate and germination percentage than that of salinity stress as no germination was seen at -1.5 MPa water stress [28]. Abroud *et al.* [29] showed a significant difference between water stress levels in view of germination rate and germination percentage of *Melilotus officinalis* at level of 1% confidence. These traits were decreased with increasing water stress and maximum germination rate and germination percentage were recorded at the potential of zero bar [29]. The similar results were observed in our study as the maximum value of all studied traits was recorded at the potential of zero.

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