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THE EFFECT OF SALINITY STRESS ON GERMINATION AND GROWTH CHARACTERISTICS OF *HALOXYLON APHYLLUM* AND *HALOTHAMNUS SUBAPHYLLUS*

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Abstract

Knowing the adaptation of plant species used in rangeland planning of various areas concerning drought and salinity stresses could prove effective in enhancing the success of rangeland restoration plans. The study was conducted to examine the salinity resistance in *Haloxylon aphyllum* and *Halothamnus subaphyllus* at the germination stage and the effect of various levels of soil salinity on the growth characteristics of these two plants in vitro and research greenhouse of the Birjand University of Agriculture as a completely randomized design. Experimental treatments were five salinity levels (0, 3, 10, 20, and 50 Mmhos/cm) in germination and four salinity levels (3, 10, 20, and 50 Mmhos/cm) in a greenhouse experiment with four replications in both. The results showed no significant differences between 3 and 10 Mmhos/cm salinities in terms of the effect on growth characteristics of *Haloxylon aphyllum*, but the Traits examined for 20 Mmhos/cm salinity significantly reduced. There was a difference in the salinity level of *Halothamnus subaphyllus* in terms of the effect on its growth characteristics so that the increase in salinity from 3 to 10 Mmhos/cm significantly reduced all measured traits. However, there were no significant differences between the salinity of 10 and 20 Mmhos/cm. None of the plants could grow at a salinity of 50 Mmhos/cm. The results of the germination test showed that the increase in salinity decreased germination indices in both plant species: *Haloxylon aphyllum* and *Halothamnus subaphyllus*. A significant decrease was seen in seed germination percentage in *Haloxylon aphyllum* with an increase in salinity from 20 to 50 Mmhos/cm and in *Halothamnus subaphyllus* with an increase in salinity to 20 Mmhos/cm. Moreover, the results indicated that salinity has a significant effect on all growth characteristics (fresh and dry weight of radicle and shoot and radicle and shoot length) of *Haloxylon aphyllum* and *Halothamnus subaphyllus*.

Keywords: Rangeland restoration, rangeland, rangeland plants, environmental stress, soil salinity

Introduction

Rangeland is one of the most significant natural resources on earth. Rangelands are very significant in Iran and cover about 90 million hectares (Mesdaghi, 2010). Given its specific geographical location, Iran is considered as a dry and low-rain country, so that more than 64% of it is arid and hyper-arid climatic regions (Ekhtesasi, 2010). Among the characteristics of the area are low or scattered rains and high evaporation rate, leading to the accumulation of minerals in the soil surface layer (Riyahinia, 2011). The studies show that the total area of saline soils in Iran is about 25 million hectares (15% of its land area) (Alizadeh Bonab *et al.*, 2007).

Climate dryness and soil salinity (Sharafi *et al.*, 2019; Tahar *et al.*, 2019; Aslmarz *et al.*, 2019) are of the most significant natural factors preventing the establishment of high-quality rangelands in Iran. Besides the natural factors, human factor contributes to the degradation of rangelands that have arisen in this state, and thus vegetation is in critical condition across a wide range of this country (Kardavani, 2002), which has had adverse effects such as sandstorms, haze, soil erosion, and so on coupled with the loss of livestock (Mesdaghi, 2010).

The salinity and arid and semi-arid climatic stresses in most rangelands show the need for attention to ionic and osmotic stresses. Differences between species in terms of the effect of osmotic stress due to low rainfall along with the stress caused by the presence of ions in the soil will be so important in selecting the appropriate species based on soil and climatic nature (Behtari *et al.*, 2012). Relatively large studies on different plants indicate that with an increase in salinity, radicle length, shoot length as well as seedling dry weight significantly reduce (Okcu, 2005). The decrease in germination and seedling growth under salinity may be due to low osmotic potential of the radicle or soil and inhibition of water uptake, the toxicity of Na^+ or Cl^- ions, or nutrient imbalance (Riyadhnia, 2011). Likewise, salinity affects seed germination and growth by decreasing the water potential and toxicity of specific ions like sodium and chlorine and reduces the nutrient ions like calcium and potassium (Dadkhah, 2006). Ionic toxicity, osmotic stress, lack of some minerals, physiological and biochemical disorders and a combination of these are of the effects of salinity stress on plants (Saleh, 2013). As plant growth starts from germination and seed germination should happen to survive, adapt to environmental conditions and settle in the soil, so success in this period has a key role in later establishment stages of the plant (Mosleh Arani *et al.*, 2011). Given the lack of information and accurate knowledge of rangeland species about their salinity resistance, further studies are needed to better identify native plant species, so that with better identification of them, the native plants can be used to restore and manage rural lands with stress.

The purpose of the study was to examine the effect of various levels of salinity on germination and morphological traits of seed and seedling of *Haloxylon aphyllum* and *Halothamnus subaphyllus* species within in vitro and greenhouse environment.

Materials and Methods

The study was conducted by two separate experiments including a) in vitro cultivation using germinator apparatus and b) cultivation of the plant seed examined in the research greenhouse of Birjand University of Agriculture (pot cultivation) in autumn 2012 on *Haloxylona phylum* and *Halothamnus subaphyllus* seeds. The experiment was performed in a completely randomized design with four replications:

First experiment

The first experiment was seed germination in a germinator. For doing the experiment, first, the seeds were disinfected with 2% sodium hypochlorite solution for two minutes and then were washed three times with water. Petri dishes and the devices were disinfected using alcohol. Then 50 seeds of each plant species were tested in a 10 cm diameter petri dish on Wattman paper bed and 5 ml of saline solution with 0, 3, 10, 20 and 50 Mmhos/cm salinity levels. In order to prevent the evaporation of the solutions, the lids were closed using paraffin. Each petri dish formed an experimental unit. The seeds were planted in germinator at 15 to 25 ° C, 16 h light and 8 h dark. The buds were counted daily. From germination indices, germination speed and percentage were measured and buds were counted daily. Counting continued until no increase was observed in the number of germinated seeds and remained constant for 3 consecutive days. The germination percentage index was calculated according to Equation 1 (Datta and Dayal, 1991).

$$GP = \frac{N}{N_t} \times 100 \qquad ...(1)$$

In this equation, N and N_t, respectively, show the number of germinated seeds and the total number of seeds.

Cumulative germination percentage growth curve versus planting time was plotted to evaluate germination in various treatments, and these curves were used to calculate the time-to-50% germination curves by linear interpolation using Germin program in Excel software environment.

Second experiment

In the second experiment, solutions of 0, 28, 68 and 188 Mmhos/cm sodium chloride were prepared to prepare the soil with desired salinity levels according to soil crop capacity and the soil was made saline to the desired levels using this solution. The result of this was to prepare soil samples with a salinity of 3, 10, 20 and 50 Mmhos/cm. After transferring saline soil to experimental units (pots), the seeds of the studied species were planted. To prepare the seeds, both plants were separated and their seed covers were placed in water for 12 hours before planting. The depth of seed cultivation in pots was three times as large as the diameter of the seeds. The pots were irrigated regularly during the germination and growth stages with 35% moisture discharge. The pots were kept in the greenhouse for three months and finally, after that time passed, the grown seedlings were carefully washed by water and separated from the pots in the soil. Each index - radicle and shoot length - was measured in centimeters using a graded ruler and radicle and shoot fresh weight indices were measured using a digital scale in grams. In centimeters were measured using a graded ruler and radicle fresh weight and shoot fresh weight indices were measured using a digital scale in grams. After determining the fresh weight, the fresh samples were placed in an oven at 75 ° C for 72 hours and the radicle and shoot dry weights were measured using digital scales after drying.

First, using the Kolmogorov-Smirnov nonparametric test, the assumption of the normal distribution of the obtained data for each of the morphological traits of the plants was examined to analyze and compare the data. A variance homogeneity test was performed using Levene's test.

To examine the effect of salinity treatments on the growth characteristics of *Haloxylon aphyllum* and and *Halothamnus subaphyllus* andto compare the salinity levels and significant differences between them by one-way ANOVA and least significant difference test (LSD) were used, respectively. All statistical tests were done in SAS9.1 software.

Results and Discussion

The results of the variance analysis of various salinity treatments on germination percentage and speed of *Haloxylon aphyllum* are shown in Table 1 and for *Halothamnus subaphyllus* in Table 2. The data of these two tables show that the effect of salinity treatments on the percentage and germination speed of both plants was significant ($P \leq 0.05$).

Table1: Analysis of variance of salinity effects on germination indices of *Haloxylon aphyllum* seeds.

		Means of squares	Means of squares
Source of variation	**Degree of freedom**	**Germination percent**	**Germination speed**
Salinity	4	*1385.20	*0.0013
Error	15	62.13	0.00007

* Significant difference at level 0.05

Table 2: Analysis of variance of salinity effects on germination indices of *Halothamnus subaphyllus* seeds.

		Means of squares	Means of squares
Source of variation	**Degree of freedom**	**Germination percent**	**Germination speed**
Salinity	4	*1582.0	*0.0003
Error	15	36.80	0.000056

* Significant difference at level 0.05.

Table 3 shows the comparison of the averages of the effect of salinity on germination indices in *Haloxylon aphyllum* under different salinities. The results showed no significant differences (0.05) between salinity levels 0, 3, 10 and 20 Mmhos/cm in terms of the effect on germination percentage but this index decreased significantly with an increase in salinity from 20 to 50 Mmhos/cm (Table 3).

Table 3: Meancomparison of germination indices in *Haloxylon aphyllum* currant under various salinities.

Saline treatment (Mmohs.cm^{-1})	Germination percent	Germination speed
0	73^{a}	0.058^{a}
3	75^{a}	0.055^{ab}
10	65^{a}	0.043^{bc}
20	70^{a}	0.039^{c}
50	30^{b}	0.012^{d}

In each column, the means have the same common denominator; they have no meaningful differences at the 5% probability level.

The results from the comparison of mean germination percentage of *Halothamnus subaphyllus* under various salinity showed significant differences between 0, 3 and 10 Mmhos/cm salinity levels (P<0.05). However, there were no effects on germination percentage, but increasing salinity from 10 to 20 Mmhos/cm significantly decreased the germination percentage (P≤0.05) (Table 4).

Table 4: Mean comparison of germination indices in *Halothamnus subaphyllus* currant under various salinities.

Saline treatment (Mmohs.cm^{-1})	Germination percent	Germination speed
0	45^{a}	0.024^{a}
3	40^{ab}	0.017^{ab}
10	36^{b}	0.0152^{ab}
20	24^{c}	0.0095^{bc}
50	1^{d}	0.0012^{c}

In each column, the means have the same common denominator; they have no meaningful difference at the 5% probability level.

The results of the analysis of variance of salinity treatments on morphological traits of plants under stress such as radicle length, shoot length, radicle fresh weight, and shoot fresh weight, radicle dry weight and shoot dry weight for *Haloxylon aphyllum* is shown in Table 5 and for in Table 6 *Halothamnus subaphyllus*. Based on these two tables' data, the effect of salinity treatments on all studied traits of these two plants was significant (P≤0.05).

Table 5: Analysis of variance (means square) effect of salinity on the growth characteristics of *Haloxylon aphyllum*.

Source of variation	Degree of freedom	length of radicle (cm)	Length of Plumule (cm)	Fresh weight of radicle (gr)	Fresh weight of Plumule (gr)	Dry Weight of radicle (gr)	Dry weight of Plumule (gr)
Treatment	3	*99.355	*192.815	*0.0013	*0.483	*0.00022	*0.0112
Error	12	1.188	2.415	0.00008	0.0257	0.000004	0.0004

* Significant difference at level 0.05

Table 6: Analysis of variance (mean square) effect of salinity on the growth characteristics of *Halothamnus subaphyllus*.

Source of variation	Degree of freedom	length of radicle (cm)	Length of Plumule (cm)	Fresh weight ofradicle (gr)	Fresh weight of Plumule (gr)	Dry Weight of radic (gr)	Dry weight of Plumule (gr)
Treatment	3	*159.59	*481.90	*0.013	*1.49	*0.0019	*0.054
Error	12	2.5	10.73	0.001	0.075	0.00025	0.0035

* Significant difference at level 0.05)

Comparison of the mean growth characteristics of *Haloxylon aphyllum* showed no significant differences (at level 0.05) between salinity of 3 and 10 Mmhos/cm in terms of effects on measured traits. However, the intended traits decreased significantly under salinity 20 Mmhos/cm and the seeds of the examined plant could not grow at salinity higher than 50 Mmhos/cm (Table 7).

Table 7: Mean comparison of growth characteristics of *Haloxylon aphyllum* in various salinities.

Saline treatment (Mmohs.cm^{-1})	length of radicle (cm)	Length of (cm) Plumule	Fresh weight ofradicle (gr)	Fresh weight of Plumule (gr)	Dry weight of radicle (gr)	Dry weight of Plumule (gr)
3	10.00^{a}	1462^{a}	0.036^{a}	0.75^{a}	0.015^{a}	0.11^{a}
10	10.87^{a}	14.54^{a}	0.040^{a}	0.69^{a}	0.016^{a}	0.105^{a}
20	.37^{b}	11.66^{b}	0.019^{b}	0.36^{b}	0.009^{b}	0.05^{b}
50	0^{c}	^{c}0	0^{c}	0^{c}	0^{c}	0^{c}

In each column, the means have the same common denominator; they have no meaningful difference at the 5% probability.

The data in Table 8 show the comparison of the mean growth characteristics of *Halothamnus subaphyllus* affected by various salinity treatments. The data in this table indicates that the increase in salinity reduced the size of all studied traits in the plant examined. Thus, the increase in salinity from level from 3 to 10 Mmhos/cm 2 significantly reduced all measured traits. However, there were no significant differences between the salinity of 10 and 20 Mmhos/cm. The seeds of this plant could not grow at 50 Mmhos/cm like haloxylonaphyllum.

Table 8: Means comparison of growth characteristics of *Haloxylon aphyllum* in different salinities.

Saline treatment ($Mmohs.cm^{-1}$)	Length of radicle (cm)	Length of (cm) Plumule	Fresh weight ofradicle (gr)	Fresh weight of Plumule (gr)	Dry weight of radicle (gr)	Dry weight of Plumule (gr)
3	14.84^{a}	26.04^{a}	0.13^{a}	1.47^{a}	0.05^{a}	0.27^{a}
10	11^{b}	18.79^{b}	0.06^{b}	0.80^{b}	0.019^{a}	0.12^{a}
20	8.5^{b}	14.70^{b}	0.024^{bc}	0.53^{b}	0.0085^{b}	$0.71b^{c}$
50	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}

In each column, the means have the same common denominator; they have no significant differences at the 5% probability level.

The results showed that salinity had a significant effect on the germination characteristics of both species. No significant decrease was seen in the germination percentage of *Haloxylon aphyllum* seeds with an increase in salinity from 0 to 20 Mmhos/cm, but a significant decrease in seed germination percentage was observed with increase in salinity from 20 to 50 Mmhos/cm. *Halothamnus subaphyllus* showed no significant differences between germination percentage in treatments with 0, 3 and 10 Mmhos/cm salinity but the difference was significant with an increase in salinity to 20 Mmhos/cm. Moreover, the results showed that the lowest germination speed in both plants was in 50 Mmhos/cm treatments and the highest in control treatment. The results of the present study were in line with those of Anvari *et al.* (2009), Rasuli *et al.* (2011), Solimani *et al.* (2011), Bijehkeshavarzi *et al.* (2011), Hosseini Nasr *et al.* (2012), Shahriari (2012), Sadeghipour *et al.* (2013) and Farhangian Kashani (2014). A decrease in plant germination in saline environments can be caused by two reasons: one is the effective absorption reduction because of the osmotic equilibrium leading to aquatic stress for the plant and the other is the ionic toxicity caused by the uptake and accumulation of ions (Anvari *et al.*, 2009). As an environmental factor affecting germination speed, salinity stress besides poisoning the plant reduces water absorption by the seed. On the other hand, sodium and chlorine infiltration into the seed tissue disrupts cell metabolism, especially cell membrane activity leading to increased intracellular material leakage (Vicente *et al.*, 2009). Furthermore, salinity had a significant effect on all growth characteristics (fresh and dry weight of root and shoot, root length and stem length) of *Haloxylon aphyllum* and *Halothamnus subaphyllus*. There were no significant differences between 3 and 10 Mmhos/cm in the case of *Haloxylon aphyllum*. Moreover, the growth characteristics measured in *Halothamnus subaphyllus* increased with an increase in salinity from 3 to 10. There were no significant differences in salinity in this plant with an increase in salinity from 10 to 20 dS/m in both plants, and at 50 dS/m salinity none of the plants could continue growth. This is in line with those of Pourmeidani *et al.* (2011), Rasuoli *et al.* (2011), Solaimani *et al.* (2011), Jafarian Geloudarand Rookhfirouz (2012), Nadali *et al.* (2013), Heidarnejad and Rangbarfarduee (2014) and Jahanbazy *et al.* (2014). Salinity reduces soil water potential and creates physiological drought in the root environment and creates toxicity and disturbance of ions balance (Patel *et al.*, 2009; Sheydaee *et al.*, 2010). Reduced plant growth under salinity may be due to disruption of nutrient uptake, disruption of ionic balance, or reduction of water potential in soil and osmotic stress, or due to alteration of the enzymes effective in plant photosynthetic activity (Heidarnejad *et al.*, 2014). Bojovic *et al.* (2010) argue that salinity inhibits plant growth as a result of osmotic and ionic effects that ultimately leads to plant destruction. Overall, the results showed that germination speed and percentage and growth characteristics of both *Haloxylon aphyllum* and *Halothamnus subaphyllus* decreased under salinity stress. *Haloxylon aphyllum* can adapt up to 10 Mmhos/cm and *Halothamnus subaphyllus* to 3 Mmhos/cm salinity but more salinity reduces the growth characteristics of the two plants. What can be concluded from the results is that *Haloxylon aphyllum* tolerates more soil salinity than *Halothamnus subaphyllus* and growth characteristics of *Halothamnus subaphyllus* decrease more with an increase in salinity compared to *Haloxylon aphyllum*. As both plants are native plants adapted to Iranian arid environments, one can suggest that in similar salinity conditions, *Haloxylon aphyllum* should be used as the main plant and *Halothamnus subaphyllus* as the accompanying one if plant selection is for use in rangeland restoration in saline stress areas.

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