

Growth and Carbon Partitioning of *Juniperus procera* Seedlings under Salinity

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Abstract: The adverse effects of salinity on the growth of plants are well documented. These range between apparent symptoms and reduction of plant growth to death of the plant. *Juniperus procera* is the most widespread tree species that dominates the natural forests in southwestern Saudi Arabia and suffers from low capacity of the natural regeneration. Thus, there is a crucial need to grow the seedlings of this species in the nursery from local seeds and then planting them in these forests. Groundwater represents the main source of water used in agricultural irrigation and it classified as very saline water. A pot experiment was carried out using a randomized complete design with five replications and two treatments, mainly irrigation with high saline water (3500 ppm NaCl) and low saline water (500 ppm NaCl) to examine the growth of the *J. procera* seedlings under saline irrigation. The results revealed decreasing stem height, number of branches, total leaf area and consequently leaf, stem, root and total dry weight. Net assimilation rate (NAR) decreased due to high saline irrigation. There were also decreases in specific leaf area (SLA), leaf area ratio (LAR) and relative growth rate (RGR) but not significant. Irrigation with high saline water decreased the amount of both structural (SCC) and non-structural carbon compounds (NSCC). On the other hand, the percentage of non-structural carbon compounds (NSCC%) in the leaves, stem and roots of *J. procera* seedlings decreased in the high saline irrigation treatment at the expense of structural carbon compounds (SCC%). The ratios of non-structural/structural carbon compounds (NSCC/SCC%) in the leaves, stem and the whole-plant of *J. procera* seedlings decreased significantly due to increasing the concentration of NaCl in the irrigation water from 500 ppm to 3500 ppm. The results suggest that *J. procera* seedlings tolerated the conditions of irrigation with saline water (*i.e.* 3500 ppm) and survived without showing any apparent injury.

Key words: *Juniperus procera* • Salinity • Growth analysis • Structural and non-structural carbon compounds

INTRODUCTION

Salinity is one of the most limiting environmental stress factors for plant growth. Ozturk *et al.* [1] mentioned that salinity is one of the most limiting environmental stress factors for plant growth. Salts come with the irrigation water and are accumulated and concentrated in the soil as water evaporates and is taken up by plants. Sodium chloride is the most important salt affecting plant growth in its different stages. Ionic toxicity and the decline in osmotic pressure which interact under saline conditions represent the harmful effect of salinity [2, 3]. In Saudi Arabia, Aref *et al.* [4] asserted that saline water is one of the most frequent environmental stresses that

face growing plants. This because of depending on the groundwater which represents more than 90% of the water used in agricultural irrigation and it classified as very saline water [5]. Afforestation efforts in the arid areas usually fail due to the non-availability of freshwaters and hesitancy for utilizing saline groundwater resources [6].

Producing tree seedlings need proper silvicultural practices in the nurse include irrigation with water of good quality and quantity. However, since using groundwater in nurseries is inevitable it is necessary to examine the tree species to grow them under such conditions in terms of tolerating salinity. *Juniperus procera* is the most tree species that dominates the natural forests in

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southwestern Saudi Arabia. Unfortunately, this species suffers from a low capacity for natural regeneration [7]. Thus, there is a crucial need to solve this problem by growing seedlings in the nursery from local seeds and then planting them in these forests.

Although the effects of salinity on the growth of tree seedlings have been extensively studied however, the response of *Juniperus procera* seedlings to salinity is rather few, if any. The present study aims to examine the response of *J. procera* seedlings to irrigation with saline water under greenhouse conditions.

MATERIALS AND METHODS

Plant Materials: One-year-old seedlings of African pencil-cedar (*Juniperus procera* Hochst. ex Endl) were obtained from the nursery of Raidah Reserve, affiliated to the Saudi Wildlife Authority in Abha City, Asir region. The seedlings were transferred to the experiment site at the College of Food and Agricultural Sciences at King Saud University, Riyadh with taking due precautions to preserve them during transportation. The seedlings were placed on a table in a greenhouse with a fan cooling system and has heat and lighting controls, with the temperature was 24/18 Celsius day/night.

The seedlings were transferred to pots of 25 cm size, using soil consisting of sand and peat moss mixture in a ratio of 1: 2 v/v. Watering of the pots continued for 50 days until the plant became firm before imposing the treatments.

Preliminary Measurements: Preliminary measurements were carried out prior to the experiment on 7 seedlings. They were plant height and diameter, total leaf area and leaf and total plant fresh weights. These parts were then dried by placing them in an oven with 72°C for 72 hours and then the dry weight of each was estimated.

Experimental Design and Treatments: A randomized complete design with five replications was used to examine the growth of the *J. procera* seedlings under saline irrigation. 30 seedlings were distributed into two equal groups represented low salt and high salt treatments. Salt concentrations of 500 ppm and 3500 ppm were prepared by dissolving the required quantities of NaCl pure salt in distilled water (*i.e.* 500 g and 3500 g in each litre of distilled water, respectively). The *J. procera* seedlings were watered every 3 days with either concentration with washing the pots with tap water every 45 days to prevent the accumulation of salt in the soil.

The concentration of the salt solutions was measured before applying to the seedling using a salinity measuring device(TDS & EC (Hold) –Runilex). The seedlings grown in low salt treatment were receiving irrigation water containing 500 ppm sodium chloride (NaCl) while the high salt ones were receiving irrigation water containing 3500 ppm. The rate of water added to each pot in each watering time (*i.e.* every 3 days) was 350 ml.

Harvesting and Measurements: The seedlings were harvested after 180 days since the starting of the treatment. The stem height from the soil surface to the top of the seedling was measured using a metric ruler, while stem diameter at a 3-cm height on the stem of the seedling was measured by a caliper. Total leaf areas of all seedlings were scaled immediately after harvesting using CI-202 Laser Leaf Area Meter - CID Bio-Science. The number of branches for each seedling was counted and their root length was measured using a ruler after extracting from the soil.

Determining of Dry Weight and Plant Growth Analysis: The leaves, stem and roots of each seedling were dried in an oven with 72°C for 72 hours and then the dry weight of each was determined. Relative growth rate (RGR), leaf area ratio (LAR), specific leaf area (SLA) and net assimilation rate (NAR) all were calculated according to Evans [8]. This was carried out using the following equations:

$$RGR = (\text{Log } W_2 - \text{Log } W_1) / t_2 - t_1$$

$$LAR = TLA / TW$$

$$SLA = TLA / LWt$$

$$NAR = (W_2 - W_1 / L_2 - L_1) \times (\text{Log } L_2 - \text{Log } L_1 / t_2 - t_1)$$

where: Log = natural logarithm, W_2 and W_1 = total plant dry weight at the beginning of the experiment and at the time of the harvest, $t_2 - t_1$ = the length of the period between the two measurements (week), TLA = total leaf area (cm^2), TW = total plant dry weight, LWt. = leaf dry weight and L_1 and L_2 are the total leaf areas at the beginning of the experiment and at the time of harvest.

Quantification of Structural and Non-Structural Carbon Compounds: Structural and non- structural carbon compounds in the leaves, stems and roots was quantified using an extraction procedure previously described by Browning [9] and modified by Ibrahim [10]. Non-structural carbon compounds included both starch and non-structural carbohydrates.

Preparation of Samples: Oven-dried samples of the leaves, stem and roots of the seedlings grown in both the irrigation treatments were ground in an electric grinder. The ground material was then sieved through a set of laboratory sieves. The particles that passed the 40-mesh sieve and remained on the 60-mesh sieve were dried in an oven at 60-70°C until weight constancy was achieved. Three grams of the dried ground material was transferred into a white winceyette sack that was 2 × 5 cm in size. Empty sacks were tested for leach ability by being subjected to the extraction procedure.

Extraction Procedure: A sample of 2-5 g with 40-60-mesh particle size was weighed in a glass thimble and placed into a 100 cm Soxhlet extractor. Then, 125 ml of 95% ethanol was placed in a 250 mL round bottom distillation flask. Extraction was carried out for a period of 4-8 h. After extraction, the thimble was removed from the extractor and placed upright on absorbent tissue for approximately three days to air dry the sample at room temperature. After the drying period, the thimble and its contents were weighed and the ethanol-soluble extractives were calculated.

The ethanol-extracted contents of the thimble were transferred to a 250 ml Pyrex glass beaker. After the addition of 100 ml of distilled water, the beaker was placed in a boiling water bath for 3 h. After the hot water extraction, the contents of the beaker were filtered through a medium-fast filter paper (Whatman No. 1) and washed with small amounts of hot water. The filter paper and the precipitate were then placed in an aluminum can and dried at 105±1°C to constant weight.

Calculations: The weight of structural carbon compounds (SCC) was obtained by subtracting the can weight from the oven-dry weight of the can and its contents. The weight of the non-structural carbon compounds (NSCC) is equal to the difference between the dry weight and SCC weight. SCC can also be obtained from the dry weight minus the sum of both ethanol and water extractives, which represent the NSCC weight [10].

Statistical Analysis: Data were analyzed with analysis of variance (ANOVA) using SAS software [11]. The differences between the means were determined using Fisher's least significant difference (LSD) test at $P<0.05$. Data were log or arcsine transformed when necessary.

RESULTS

Plant Growth: The Reduction of plant growth due to irrigation with saline water has been extensively reported. Analysis of variance of the results showed that increasing the concentration of sodium chloride (NaCl) in the irrigation water from 500 ppm to 3500 ppm led to significant decreases ($P<0.0001$) in the growth of stem height, total leaf area and the number of branches of *J. procera* seedlings, the decreases accounted for 23, 32 and 17%, respectively (Table 1). The decreases in stem diameter and root length due to the high saline water irrigation were not significant.

Dry Matter Production: The analysis of variance showed that the means of leaf, stem and root dry weights and consequently the mean total plant dry weight of

Table 1: Mean seedling height (cm plant⁻¹), stem diameter (cm plant⁻¹), total leaf area (cm² plant⁻¹), number of branches (branch plant⁻¹) and root length (cm plant⁻¹) of *Juniperus procera* seedlings grown under irrigation with saline water containing 500 ppm or 3500 ppm NaCl after 180 days in the greenhouse

Trait	Treatments (NaCl concentration)	
	500 ppm	3500 ppm
Seedling height (cm plant ⁻¹)	25.40 ^a	19.56 ^b
Seedling diameter (cm plant ⁻¹)	00.71 ^a	00.63 ^a
Total leaf area (cm ² plant ⁻¹)	170.4 ^a	115.86 ^b
Number of branches (branch plant ⁻¹)	14.80 ^a	11.00 ^b
Root length (cm plant ⁻¹)	55.53 ^a	46.00 ^a

*The same letters in each of two consecutive horizontal boxes mean that they do not significantly different at the $P<0.05$ level

Table 2: Means of specific leaf area (SLA), leaf area ratio (LAR), net assimilation rate (NAR) and relative growth rate (RGR) of *Juniperus procera* seedlings grown under irrigation with saline water containing 500 ppm or 3500 ppm NaCl for 180 days in the greenhouse

Trait	Treatments (NaCl concentration)	
	500 ppm	3500 ppm
Specific leaf area (cm ² g ⁻¹ leaf dry weight)	26.66 ^a	23.06 ^a
Leaf area ratio (cm ² g ⁻¹ total dry weight)	10.02 ^a	09.77 ^a
Net assimilation rate (cm ² g ⁻¹ week ⁻¹)	00.11 ^a	00.06 ^b
Relative growth rate (g g ⁻¹ week ⁻¹)	00.11 ^a	00.09 ^a

*The same letters in each of two consecutive horizontal boxes mean that they do not significantly different at the $P<0.05$ level

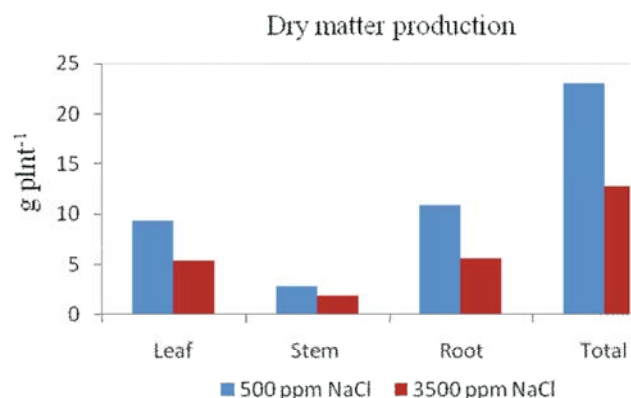


Fig. 1: Mean leaf, stem, root and total dry weight (g plant⁻¹) of *Juniperus procera* seedlings grown under irrigation with saline water containing 500 ppm and 3500 ppm of NaCl after 180 days in the greenhouse

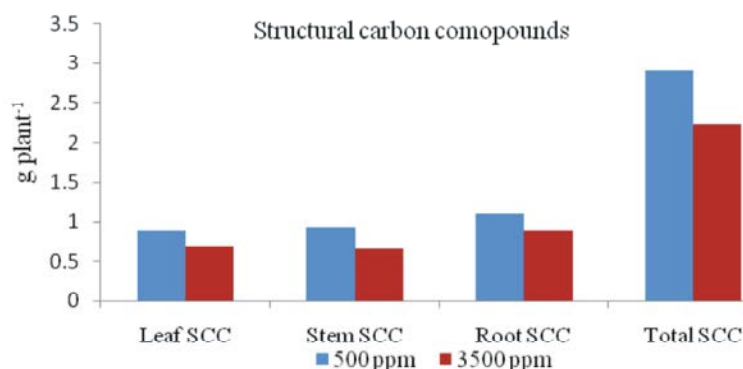


Fig. 2: Mean leaf, stem, root and total structural and non-structural carbon compounds (SCC) (g plant⁻¹) of *J. procera* seedlings grown under irrigation with saline water containing 500 ppm and 3500 ppm of NaCl after 180 days in the greenhouse

J. procera seedlings decreased significantly ($P<0.05$), ($P<0.05$), ($P=0.0029$) and ($P=0.005$) and by 42, 34, 49 and 45%, respectively as a result of increasing the concentration of NaCl in the irrigation water from 500 ppm to 3500 ppm (Figure 1).

Growth Analysis: The Analysis of variance revealed significant decreases in the net assimilation rate (NAR) of *J. procera* seedlings grown under irrigation with saline water containing 3500 ppm of NaCl after 180 days in the greenhouse. On the other hand, the means of specific leaf area (SLA), leaf area ratio (LAR) and consequently relative growth rate (RGR) were not affected (Table 2).

Structural and Non-Structural Carbon Compounds: The analysis of variance procedure revealed that the mean of the amount of structural carbon compounds in the

leaves, stem, roots and whole-plant of *J. procera* seedlings grown in the irrigation water that containing 3500 ppm NaCl all decreased significantly ($P<0.0001$) and by 22, 29, 19 and 23% (Fig. 2). The amount of non-structural carbon compounds in the same plant parts also decreased ($P<0.005$) and by 39, 38, 16 and 30% (Fig. 3).

The percentage of non-structural carbon compounds (NSCC%) in the leaves, stem and roots of *J. procera* seedlings decreased in the high saline irrigation treatment at the expense of structural carbon compounds (SCC%) (Fig. 4).

On the other hand, the ratios of non-structural/structural carbon compounds in the leaves, stem and the whole-plant of *J. procera* seedlings were decreased significantly ($P<0.001$) due to increasing the concentration of NaCl in the irrigation water from 500 ppm to 3500 ppm (Table 3).

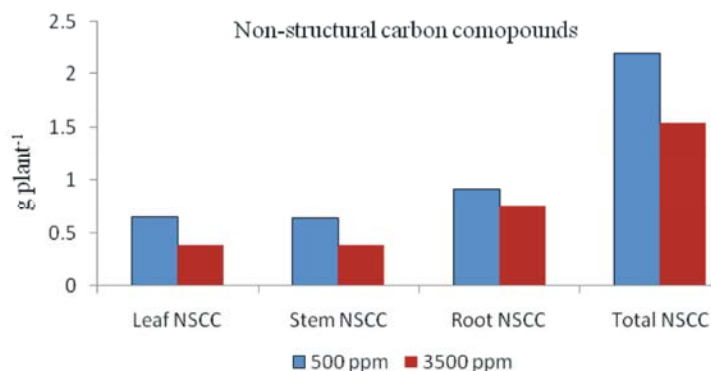


Fig. 3: Mean leaf, stem, root and total structural and non-structural carbon compounds (NSCC) (g plant⁻¹) of *Juniperus procera* seedlings grown under irrigation with saline water containing 500 ppm and 3500 ppm of NaCl after 180 days in the greenhouse

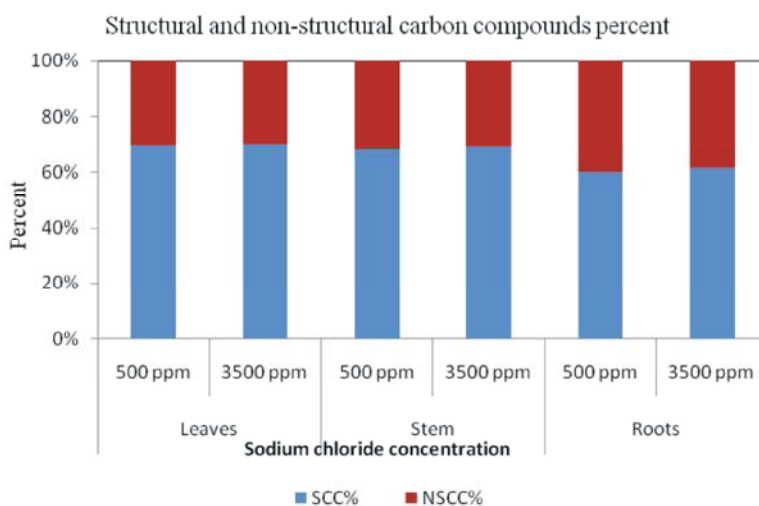


Fig. 4: The mean percentages of the structural carbon compounds (SCC%) and non-structural carbon compounds (NSCC%) in the leaves, stem and roots of *J. procera* seedlings grown under irrigation with saline water containing 500 ppm and 3500 ppm of NaCl after 180 days in the greenhouse

Table 3: The means of the non-structural/structural carbon compounds ratio (NSCC/SCC) in the leaves, stem, roots and whole plant of *J. procera* seedlings grown under irrigation with saline water containing 500 ppm and 3500 ppm of NaCl after 180 days in the greenhouse

Trait	Treatments (NaCl concentration)	
	500 ppm	3500 ppm
NSCC/SCC ratio in the leaves	72.94 ^a	56.94 ^b
NSCC/SCC ratio in the stem	68.52 ^a	58.42 ^b
NSCC/SCC ratio in the roots	84.51 ^a	86.18 ^a
NSCC/SCC ratio in the whole-plant	75.65 ^a	68.96 ^b

*The same letters in each of two consecutive horizontal boxes mean that they do not significantly different at the $P < 0.05$ level

DISCUSSION

Growth of the Seedlings: Many researchers reported decreasing the growth of plants due to increasing sodium chloride in the irrigation water. For instance, Munns [12] mentioned that plant growth is affected because a high

build-up of salt kills the photosynthetically active leaves, which in turn affects the supply of carbohydrates or hormones to the actively growing parts.

The growth suppression in plants under salinity is thought to be related to the increased energy expenditure by plants to combat osmotic and ionic stresses due to salt

[13]. High salt concentration treatment in the present study caused a reduction in the height growth of *J. procera* seedlings by 23%. This result concurs with previous reports (e.g. [1, 14-17]).

The number of branches of the *J. procera* seedlings decreased in the high saline irrigation treatment and by 17% of that in low salt treatment. Similar results were obtained by Ozturk *et al.* [1] and El-Juhany and Aref [15] for instance. Decreasing total leaf area in the present study due to high saline irrigation concurs with several published results (e.g. [15-20]).

Many authors stated that increasing salinity reduces the total leaf area as an adaptation mechanism to the stress experienced by the plant. This reduction may be achieved through inhibiting leaf initiation [10, 21] or decreasing leaf size [22, 23] or accelerating leaf senescence and consequently leaf shedding [24]. In the present study, the reduction in the total leaf area seems to have been achieved by reducing the number of branches and restricting the expansion of the new leaves, however. Munns and Tester [25] mentioned that the growth response to salinity stress represents as the rate at which growing leaves expand is reduced, new leaves emerge more slowly and lateral buds develop more slowly or remain quiescent, so fewer branches or lateral shoots form.

Dry Matter Production: The decreasing total dry weight of *J. procera* seedlings and its components (*i. e.* leaves, stem and root) due to irrigation with high saline water irrigation concurs with previous similar results for woody species (e.g. [15-17, 26] for leaf dry weight, [15, 16] for stem dry weight and [16] for root dry weight). The reduction of the total dry weight of *J. procera* seedlings in high saline irrigation treatment thus was expected due to the decreases of its components. This result concurs with other findings for tree species such as those of Rawat and Banerjee [27]; El-Juhany and Aref [15]; El-Juhany *et al.* [16].

Growth Analysis: We applied growth analysis as it considered being a standard approach to the study plant growth and productivity (e.g. Wilson [28]). Net assimilation rate (NAR) of *J. procera* seedlings is the only growth analysis parameter among those measured in the present study (*i.e.* RGR, NAR, SLA, LAR) that was reduced in the treatment of high saline irrigation. Decreasing NAR due to salinity has been reported previously [15, 29-32]. Other studies showed unaffected NAR in saline conditions (e.g. [16, 33]).

In the present study, RGR was not affected due to increasing NaCl concentration in irrigation water which is expected as LAR and SLA did not change also. Similar results were obtained (e.g. [15, 34, 35]). However, many authors reported decreasing RGR due to salinity (e.g. [16, 36]). El-Juhany *et al.* [16] asserted that while decreasing RGR of plants due to salinity is well documented, attributing this decrease to decreasing NAR (including SLA and LWR) or LAR varies according to plant species or age of the plant or growing conditions and maybe to other factors.

Structural and Non-Structural Carbon Compounds:

Partitioning of dry matter to structural and non-structural carbon compounds is likely to be an appropriate approach to achieve a better understanding of assimilates distribution in the plant, coupling morphological features and physiological processes [10]. Poljakoff-Mayber and Lerner [37] asserted that the different growth responses to salinity can result from changes in the allocation and partitioning of photoassimilates. To increase their salt tolerance, plants accumulate compatible solutes in their cells to maintain turgor as a physiological mechanism. In some plants, osmotic adjustment results from the synthesis of compatible organic solutes in the cytoplasm, including proline, glycine, betaine and other amino acids, as well as sugars [38]. In the present study, the amount of structural carbon compounds in the leaves, stem, roots and whole-plant of *J. procera* seedlings grown in the irrigation water containing 3500 ppm NaCl all decreased significantly. Also, the amount of non-structural carbon compounds in the same plant parts decreased significantly.

The decrease in the amount of structural and non-structural compounds in the leaves, stems, roots and whole plant in *J. procera* seedlings growing in high salinity treatment is logical as the sum of both represents the total dry weight of the plant.

The percentage of non-structural carbon compounds (NSCC%) in the leaves, stem and roots of *J. procera* seedlings decreased in the high saline irrigation treatment at the expense of structural carbon compounds (SCC%). Similar results were obtained previously [39, 40]. Contradictory, El-Juhany *et al.* [41] reported a significant increase of the NSCC in leaves and stems of *Eucalyptus* sp. seedlings with increasing salinity level in the irrigation water. On the other hand, Salinity did not alter leaf or root contents of soluble carbohydrates of coconut palm seedlings [42].

It seems that the degree at which the plant uses the mechanism of altering carbon partitioning depends on several factors include the magnitude of salinity, the age of the plant, the ability of the plant to tolerate the imposed level of salinity and the duration of exposure to salinity condition.

The ratio of non-structural to structural carbon compounds (NSCC/ SCC) in an organ reflects the function of this organ and its physiological performance [10]. The ratio of NSCC/SCC in the leaves, stem and whole-plant of *J. procera* seedlings grown in the high saline water irrigation treatment showed significant decreases comparing with those of the same parts of the seedlings grown in the low saline water irrigation treatment. Similar results were obtained by Ghosh *et al.* [43] who found both the water-soluble carbohydrates and the total nonstructural carbohydrates contents decreased by salt stress at the later stages of the plant's life.

On the contrary, the ratio of NSCC/SCC increased in leaves and stems of *Eucalyptus* seedlings with increasing salinity concentration in irrigation water [41].

CONCLUSION

Although the salinity level imposed in this study caused decreases in most growth traits measured, however, these decreases were not acute. This suggests that *Juniperus procera* seedlings tolerated the conditions of irrigation with saline water (*i.e.* 3500 ppm) without losing much growth, survived, looked shiny and did not show any apparent damage. As the research work on the effects of salinity on *Juniperus procera* is few, if any, we recommend carrying out experiments on the effect of salinity on seed germination and on the growth of trees of this species.

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