Research Article

Reverse Osmosis Treatment of Saline Water: An Innovative Approach to Enhance Seedling Production and Biochemical Quality in *Juniperus Phoenicea* L.

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Abstract

To address the water scarcity problem resulting from climate change which has worsened daily, the saltwater reclamation and its reuse for beneficial purposes, innovative techniques are recommended. Cutting edge technologies such as reverse osmosis water purification systems was very pronounced nowadays. This strategy is a common goal of many countries particularly in water stressed countries around the world. The objective of this work is the study of seeds germination, new seedlings production and the extract biochemical quality in *Juniperus phoenicia* L. using different water quality. For these, brown-berried juniper seeds were collected from 30 trees of four Tunisian provenances (Kesra, Elkef, Weslatia, and Mdjez el beb) during May, 2023. Control (Distillated water), reverse osmosis treated water (ROTW), blended water (BW) and well water (WW) were used to initiate germination and to irrigate new seedlings grown in nursery (INRGREF). Compared to the well water characterized by elevated chloride and sodium tenors, electrical conductivity and dry residue; blended water exhibits moderate characteristics after its treatment with reverse osmosis. Data showed that seeds germination and juvenile growth were significantly depending to water quality used. However, seeds germination percentage and germination time were inhibited when seeds imbibition was realized with well water. In another way, the new seedlings growth and their morphological parameters were more pronounced, when irrigated with blended water. In addition, our results showed that the sprouts biochemical quality was improved by irrigation with blended water.

More, Reverse osmosis (RO) is gaining acceptance in water desalination, also, it is adopted to adjust the quality of water resulting in high quality of species considered as sources of biomolecules.

Introduction

Many forestry, ecological and pharmaceutical importance of *Juniperus phoenicea* L. are known. Ability to grow in arid areas with limited level (200 mm) of rainfall, resistance to wind, several soil type [1]. For these reasons, Juniperus could be chosen for stabilizing coastal dunes, windbreak and reforesting dry mountains. Unfortunately, everywhere, juniperus trees have suffered severe degradation due to overexploitation by local populations seeking to meet their needs in areas with limited resources. In addition, red juniper areas are also threatened with extinction due to the lack of natural regeneration. In fact, the germination of juneperus is difficult, [2] because certain physicochemical properties of their seeds (existence of a tough, impermeable and resinous integument,

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embryonic immaturity and dormancy phenomena) (Zine El Abidine et al. 2024). However, this remarkable tree species extinction is more and more accentuated by the climatic changes causing unavailability of water or increase of salt stress factor. Now days, several new innovated techniques were explored to resolve the problems affecting water factor. Reverse osmosis (RO) is one of advanced technologies presenting gain worldwide acceptance in both water treatment and desalination applications [3].

For all these, the present work aims to determine efficiency of salt water (well water) treatment by reverse osmosis technique on germination and production of new sprout of junipers specie needed to rehabilitee degraded areas and to restore forest vegetation. More that, this study highlighted the efficiency of reverse osmosis treated salt water in improvement of biochemical characteristic of junipers as PAM specie excessively demanded in the medicinal field [4].

Materiel and Methods

Sources of Water

Our experiment was conducted using several types of water furnished by JH PURE WATER company (https://purewater.tn/). Reverse osmosis treated water (ROTW, 0g/l NaCl), blended water (BW, 1g/l NaCl) and well water (WW, 6.2g/l NaCl).

Germination and Growth Condition

Seeds of *Juniperus phoenicea* L. were collected from several Tunisian regions characterized by several bioclimate and altitudes: Kesra (SAS/ 7.2m, Elkef (SA/ 440m), Weslatia (48mSAIT, and Mdjez el beb (SAIM/ 94m). Seeds soaked in boiled water for 30min were placed in soil (alveolus) at 2.5cm deep to germinate in green house. When radicle and epicotyl emerged, we calculated germination percent. At this stage, several water type were used for irrigation: Control (Distillated water), reverse osmosis treated water (RO), blended water (BW) and well water (WW).

Three month-aged new sprouts, were used to measure the morphological parameters (shoot and roots lengths) and biochemical quality depending on water quality.

Biochemical measurements

Chlorophyll a, total chlorophyll, carotenoids, total polyphenol compounds, total flavonoids, condensed tannins, proline and soluble sugar contents were determined according to Li et al. (2005), Dehpour et al. (2009), Ba et al. (2010) and Bates et al. [5] Yemm and Willis [6] respectively.

The quantification of malondialdehyde (MDA) amount and hydrogen peroxide (H_2O_2) concentration were conducted using the methods of Heath and Packer [7] and Patterson et al. [8] successively.

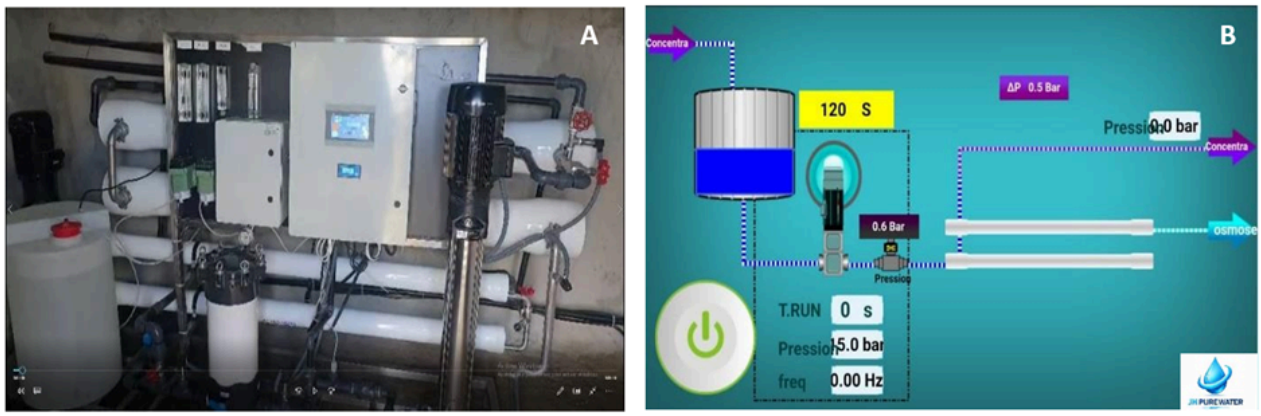


Figure 1: Reverse osmosis station (A) and application of reverse osmosis system automation (B) installed by JH PURE WATER company, Tunisia.

Our experiment was conducted using several types of water furnished by JH PURE WATER company (https://purewater.tn/). Reverse osmosis treated water (ROTW, 0g/l NaCl), blended water (BW, 1g/l NaCl) and well water (WW, 6.2g/l NaCl).

DPPH (2, 2'- diphenyl-1- picrylhydrazyle) and FRAP (ferric reducing antioxidant power) radical-scavenging activities were measured according to Parejo et al. (2002) and Hinneburg (2006) methods.

Statistical Analysis: Data were unanalyzed by ANOVA and Tukey's HSD tests (SPSS Statistics 27) to determine the significant differences between means of treatments at a probability level of 5%.

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Results and Discussion

As shown in Figure 2, the higher germination rate was observed in seeds imbibed by blended water followed by those soaked with reverse osmosis treated water (ROTW) and distillated water (Control). Compared to blended water, use of well water resulted in germination rate inhibition for 89,13%. More that, significant differences in germination rate were observed depending to seeds origin. Results showed that the higher germination rate was observed in seeds collected from kesra. The differences in seed germination between the different provenances, may be correlated with genetic differences between them which could be due to adaptive traits linked to local bioclimatic conditions for the region of collect [9]. Therefore, we selected Kesra provenance to guide this study aiming to verify the effect of water types used on growth and biochemical quality of new seedlings. After three months of growth, different morphologies of Juniperus sprouts were observed (Figure 1).

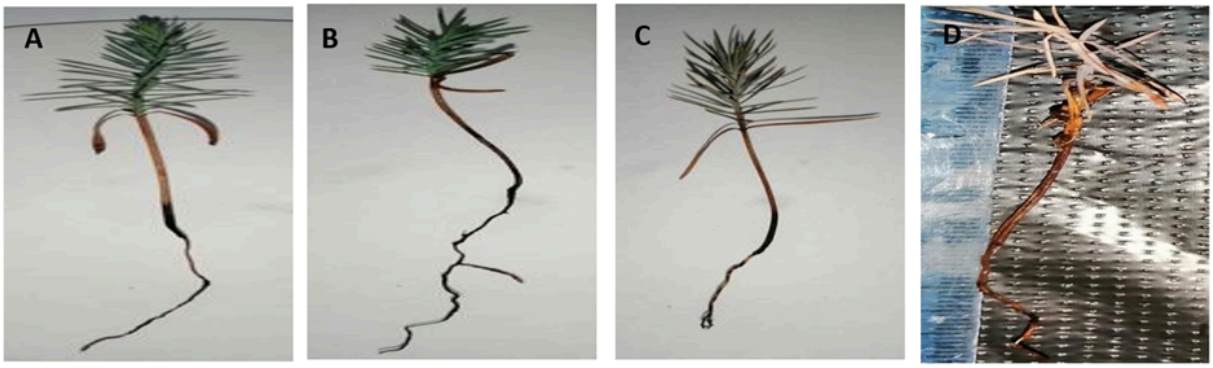
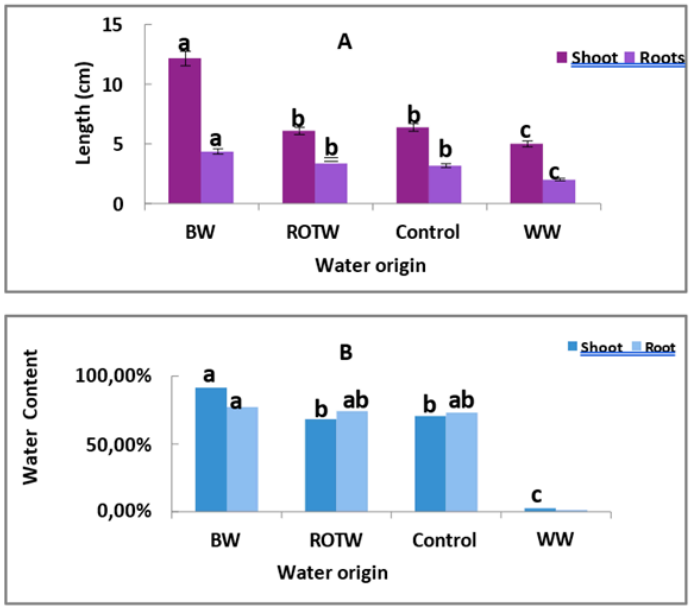


Figure 2: Morphological aspects of Juniperus sprouts irrigated with several water quality: ROTW (A), blended water (B), Control (C) and well water (D).



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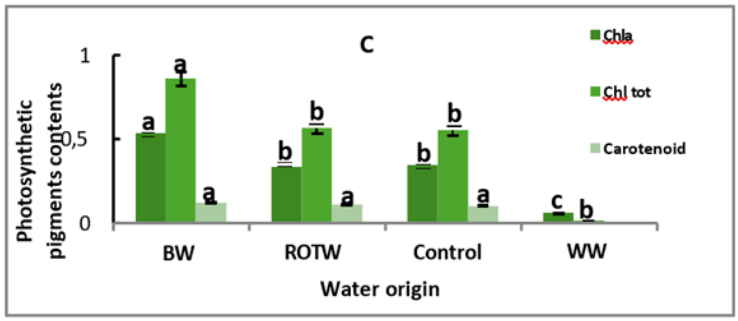


Figure 3: Total length (cm) (A), water content (B) and photosynthetic pigments contents (C) in Juniperus sprouts irrigated with several water quality: ROTW, blended water, distilled water and well water. Data were unanalyzed by ANOVA and Tukey's HSD tests (SPSS Statistics 27).

Length of shoot and roots parts of sprouts were measured. The longer (12,173 cm) shoot part was detected in sprout irrigated by blended water. The shorter (5cm) shoot was observed in juvenile seedlings irrigated with well water. There are no significant differences between levels in sprouts irrigated by ROTW and control one (Figure 3A).

These morphological parameters were correlated to the physiological ones showing that, water content in leaves was more important in seedlings soaked with blended water compared to the other cases (Figure 3 B). Also, tenors of chl a and total chlorophyll were more important in sprouts leaves irrigated with blended water. Whereas, no significant differences in carotenoids tenors were detected. Exceptionally, carotenoids was no present in sprouts soaked with well water (Figure 3C). Total polyphenolic compounds content was higher in sprouts irrigated by blended water. In seedlings soaked by well water, these biomolecules were less accumulated (Table1). The same profile of variation in total flavonoids and condensed tannins contents were noted (Table1).

Table1: Biomolecules contents in *Juniperus phoenicea* L. sprouts irrigated with several water types. Data are means of five replicates. Data were unanalyzed by ANOVA and Tukey's HSD tests (SPSS Statistics 27).

Water Type / Content	Blended Water	Reverse osmosis treated water	Distillated water	Well water
Total polyphenolic compounds	9,018±0236^{a}	7,689±021^{b}	7,021±0.024^{b}	1,022±0.011^{c}
Total flavonoids	0,77033±0.31^{a}	0.526±0.025^{b}	0.534±0.042^{b}	0.109±0.013^{c}
Condensed tanins	0,066±0.005^{a}	0.037±0.001^{b}	0.037±0.002^{b}	0.0101±0.001^{c}
Proline	15,23±0.953^{a}	10,31±0.873^{b}	10,31±0.604^{b}	1,111±0.01^{c}
Soluble sugar	8,253±0.024^{a}	5,102±0.412^{b}	5±0.111^{b}	0,52±0.029^{c}
MDA	0.861±0.302^{b}	0.871±0.017^{b}	0.875±0.023^{b}	8.76±0.412^{a}
H2O2	0.43±0.014^{b}	0.421±0.031^{b}	0.417±0.011^{b}	3.849±0.02^{a}

Proline and total soluble sugar contents were more important in sprouts irrigated with BW than in ROTW and control one. Whereas, the less amounts were detected in seedlings soaked by well water (Table1). This response of Juniperus seedlings produced, was a known plants non-enzymatic possess antioxidant defense systems adopted to resist to salt stress often destroys these protective mechanisms [10-12]. However, when well water was used, the defense mechanism was severely inhibited because the high concentration of salt Yucedag and Gailing [13]. More, as described by wang et al [12], the ultimate reduction in plant performance and survival was the result of increase in ROS levels causing oxidative damage to lipids, proteins, nucleic acids and cellular membranes. Our data showed that Malondialdehyde (MDA) which was produced us result of membrane lipid peroxidation was increased immensely in sprouts irrigated with well water (Table1). Also, our results illustrated the increase of H_2O_2 content in leaves of juvenile seedling soaked by well water [14].

Conclusion

Overall, water quality and seed provenance significantly affected the germination and early growth of *Juniperus phoenicia* L. Germination rate varied significantly according to seed origin and water quality, suggesting that environmental factors, particularly provenance altitude, may influence seed tolerance to different water qualities. Well water negatively affected germination, whereas blended water improved seedling growth and biochemical quality. These findings highlight the potential of reverse osmosis-treated and blended water as sustainable alternatives for *J. phoenicia* nursery production in the context of climate change and its increasing impact on environmental conditions and water availability.

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