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Effect of Salinity on Growth and Gas Exchanges in Seedlings of Pinus nigra Subsp. Pallasiana

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ABSTRACT

Aims Salinity, due to its remarkable effects on physiology and performance of plant is considered as a world major problem in arid zone ecosystems. Pinus *nigra subspecies* pallasiana is known as a nurse and pioneer species. The aims of this study were to determine growth responses and gas exchanges of the seedlings of *Pinus nigra* subspecies under different salinity stress in the greenhouse environment.

Materials & Methods In this experimental Study, Seedlings of *Pinus nigra* was investigated under salinity stress in 6 NaCl levels including 0, 50, 100, 150, 200 and 250mM as completely randomized design in greenhouse conditions. Height and diameter growth and gas exchanges parameters were determined in day 90 (August) and biomass allocations in day 150 (November). For data analysis One-Way ANOVA, Duncan's test and SPSS 19 software were used.

Findings The highest survival appeared in zero and 50mM with 100 and 93.33%, respectively. Salinity stress decreased survival, height and diameter growth and also biomass of root, shoot, root:shoot and total of seedling. With increasing salinity, photosynthesis, stomatal conductance, transpiration reduced. Higher survival, better growth and gas exchanges were detected below 50mM NaCl salinity.

Conclusion Salinity has adverse effects on growth and gas exchanges of *P. nigra* seedlings during the studied period. Survival in 50mM NaCl has a high percentage, but in higher salt concentrations (200 and 250mM NaCl) it drastically reduce. Similarly, decrease in seedling performance was found in severe salinity levels. This species has a relatively good resistance to 50mM NaCl.

Keywords Biomass; Growth; Photosynthesis; Turkish Black Pine; Stomatal Conductance

CITATION LINKS

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Introduction

About 6% of the world area (800 million hectares) is exposed to salinity [1]. In Iran there are about 25 million hectares salt lands, and half irrigated lands is saline or exposed to saline [2]. Soil salinity is becoming an increasingly serious constraint to plant growth in many parts of the world; this is particularly common in semi-arid and arid zones [3]. Salinity caused by sodium chloride than other salts with more intensity impacts on young tissue growth; these salts negatively effect on plant tissues, photosynthesis, cell division and stop or restrict their growth [4].

Soil salinity with creating high osmotic pressure at rhizosphere causes disrupting the balance of nutrients and toxicity by some ions such as chloride, sodium, and boron and reduces the availability of water and nutrients and performance in plants [5]. By increasing the concentration of the solutes by more than a tolerance threshold of the plant both growth rate and plant size decreases. Tolerance threshold and growth speed depend on the type of plant species.

Salinity also affects plant physiology activities, so that photosynthesis as major factor of energy absorption is affected by salinity severity [6]. Because of stomatal closure induced by abscisic acid and adverse effect of salts on the chloroplasts (especially electron transport and secondary processes) photosynthesis is disrupted [7]. On the other hand, reduction of leaf water potential has resulted in stomatal closure, disruptions in the supply of carbon dioxide required to plant and decreased photosynthesis [8]. Reduction of photosynthetic activity under salinity conditions usually decreases stomatal conductance and transpiration [9]. Salinity also leads to reduction of water absorption and an increase in sodium concentration, nutrient imbalances, and disruption in plant biochemical reaction [10].

Several studies have been done about the effect of salinity on Pine genus. Croser *et al.* [11] by investigation on *Picea mariana*, *P. glauca* and *Pinus banksiana* for 6 weeks found that *P. glauca* was the most sensitive species so that increased salt caused a decrease in height, total weight, root length and lateral roots of plants. Findings of Kayama *et al.* [12] on spruces (*Picea abies* and *P. glehnii*) of planted along roadsides in northern Japan indicated the salinity effect on reduction of height growth, diameter growth, photosynthesis, transpiration and water potential in these areas. Sadeghi *et al.* [13] showed that increased NaCl concentration greater than 50mM decreased growth of *Pinus eldarica* seedlings. Jimenez-Casas and Zwiazek [14] with investigation on response of *Pinus leiophylla* seedlings to salinity (0, 100, 150 and 200mM NaCl for 64 days) found that by increasing salinity, decreased the diameter growth biomass, photosynthesis, stomatal conductance and chlorophyll. In general it can be cited that a large number of plants in their natural sites are destroyed annually, due to the increasing salinity [15]. Cristiano et al. [16] investigated the growth and ecophysiology of evergreen sclerophyll species of Two Mastic Tree (*Pistacia lentiscus* L.). Accessions largely distributed in dry areas of the Mediterranean basin. The seedlings were subjected to three different levels of salinization by adding 0, 400, and 800g of salts 100L^{-1} of substrate, respectively.

Salinity reduced the values of all the examined parameters, both morphological and physiological. Results suggested that *P. lentiscus* can tolerate and accumulate salt at high concentrations in nursery conditions. Afforestation and the restoration of degraded areas, including appropriate actions to cope with salinization the water and soil resources. On the other hand, identity of resistant species to environmental stresses provides success of the restoration program of natural sites and increase the richness and biodiversity.

Turkish black pine (*Pinus nigra* J.F.Arnold subsp. Pallasiana (Lamb.) Holmboe.) from the family of Pinacea, has the greatest extent of occurrence of all the recognized subspecies, It has been recorded from Cyprus; from the Krym (Crimea) along the Black Sea coast to Krasnodar; and in Turkey (western Anatolia) and Greece. In Cyprus; Russian Federation (European Russia, Krasnodar); Turkey; Ukraine (Krym) it is finds as native in elevations between 100 and 1900m a.s.l. In Turkey, although it is scattered, but still abundant, especially in the coastal mountains. It occurs in larger stands in both the Pontic and Taurus Mountains, and is widespread, but very scattered in the interior of Anatolia. Here it occupies cooler north-facing slopes and ravines, often in rocky terrain. In the Taurus Mountains it can form more or less pure stands, or is mixed with *Cedrus libani* [17]. This subspecies has of importance as a timber tree with moderate growth and its height reaches to over 20m. It is resistant to drought and easily establishes and grows on poor soils and sandy-limestone. No range wide threats have been identified with this subspecies. Increasing frequencies of wildfires could pose a localized threat. Reforestation with this species to protect soil from erosion and create windbreak is very common and successful in Mediterranean and semi-Mediterranean areas [18]. It can be used as a nurse and pioneer tree in reforestation projects of sites with shallow and poor soils. In Iran, afforestation with this species in Kheyroud-Kenar Experimental Forest showed that plants after 6 years had a relatively good success and with 70.6% survival rate. In west areas of Iran it has been planted in some provinces so that in reforestation Hassan-Abad Forest Park of Sanandaj its survival in comparison with other species was favorable and over 80% [19].

So far no research has been reported in relation with influence of salinity stress on seedling production and seedling establishment of this species at saline environments. Due to the need of seedling production and reforestation with Turkish black pine in arid regions of Iran and extensive use of it in reforestation projects, as well as green space of urban and suburban regions, exact understanding of vegetative responses of this plant to salinity stress are of great importance. In the present study hypothesized that Turkish black pine was able to tolerate NaCl salinity more than 50mM.

The aims of the present study were to determine growth responses and gas exchanges of the seedlings of *Pinus nigra* subspecies under different salinity stress in the greenhouse environment.

Materials and Methods

The present study is experimental.

Plant species and experimental design: The 2-year-old *P. nigra* var. pallasiana seedlings that contained clay-loam-sand soil from Lajim nursery of Savadkoh city were planted in polyethylene pots (15×15×25cm) and provided and transferred to the greenhouse.

Out of them 90 seedlings, with an averaged collar diameter and shot height 5.40±0.90mm and 29.20±2.50cm, respectively, were selected and under exposed to salinity treatment. Experiment was performed in a completely randomized design, with salinity treatment in 6 levels of 0, 50, 100, 150, 200 and 250mM NaCl (approximately equal to 5, 10, 15, 20 and 25ds/m, respectively) and 3 replicates and 5 seedlings per replicate.

To provide the proper drainage, three holes with the diameter of 5mm was created at bottom of the pots and until the end of the period, control treatment with fresh water and salinity treatments with concentrations determined as above were irrigated with regard to field capacity (the amount by which the soil gets wet).

To avoid soil leaching was attempted to do irrigation as gradual so that no water exits out of the pot' holes, as far as possible. An experiment was conducted in greenhouse conditions for 150 days. Measurements were done on two occasions, one after 90 days (in August) for determining parameters of physiological attributes and morphological characteristics (height growth and diameter growth) and also survival rate. The measurement of August for height growth, diameter growth and survival rate was for this reason that the seedlings exposed to high salinity levels were being weakened and it was anticipated that some seedlings in higher salinity levels might have not able to survive at the end of the growing season. The second measurement was done after 150 days (in November) for assessing biomass allocations.

Physiological parameters: For measuring the gas exchange parameters such as photosynthesis, transpiration, stomatal conductance using a portable infrared gas analyzer Model LCpro+(ADC BioScientific Ltd.; Hertfordshire; UK) were applied. For this purpose, only from mature leaves of the lower part of the seedlings (those leaves in final stages of growth) were measured [20]. Instantaneous Water Use Efficiency (WUE; WUEi=A/E) was calculated by dividing photosynthetic rate by transpiration. Measurements of physiological parameters were performed at a sunny day and from hours 9-11:30' and in constant conditions of CO_2 (350ppm), relative humidity (60 to 80%) and leaf temperature (25 to 30˚C) [21].

Survival and morphological parameters: At the end of the period, seedlings were counted and survival percent of them in each salinity level recorded. Shoot height as well as collar diameter was measured at the end of the period and with subtracting from initiation size their growth were determined.

At the end of the experiment, one seedling was taken from each replicate and after washing soil particles around root, each seedling was divided into two parts including root and shoot and thus dried at 70°C for 48h [20] and weighted using digital balance with 0.0001g precision. So, root, shoot, total biomass and root: shoot biomass were determined.

Statistical Analysis: Before data analysis, data normality with Kolmogorov-Smirnov's test and homogeneity of variance with Levene's test was checked. In case of normality and homogeneity of variance, One-Way ANOVA and for comparison of means Duncan's test was conducted. Statistical analysis of data with SPSS 19 software and drawing graphs with excel 2013 software was conducted. Because, at the end of experiment (November) the seedlings exposed to 200 and 250mM NaCl salinity were dead, data for biomass allocation at these salinity levels was zero.

Findings

Salinity had significant effect on all parameters measured in *P. nigra* seedlings (Table 1).

Table 1) One-way ANOVA in measured traits of *P. nigra* in different salinity levels

Variables	F-value	P-value
Survival	28.20	0.0001*
Height growth	52.47	0.0001*
Diameter growth	42.71	0.0001*
Root dry biomass	83.89	0.0201*
Shoot dry biomass	367.77	0.0201*
Root biomass/shoot biomass ratio	24.01	0.0001*
Total dry biomass	312.64	0.0001*
Photosynthesis	125.67	0.0001*
Stomatal conductance	3133	0.0001*
Transpiration	38.87	0.0001*
Water use efficiency	34.73	0.0001*

*Significantly at p<0.01

Under salinity of zero and 50mM, survival was the highest (100 and 93.33%, respectively). Survival decreased to 46-48% in 100 and 150mM and to 26-28 in 200 and 250mM NaCl (Diagram1-A). Salinity decreased the diameter growth and height growth, so that with increasing salinity the amount of these variables were decreased (Diagram 1-B and C).

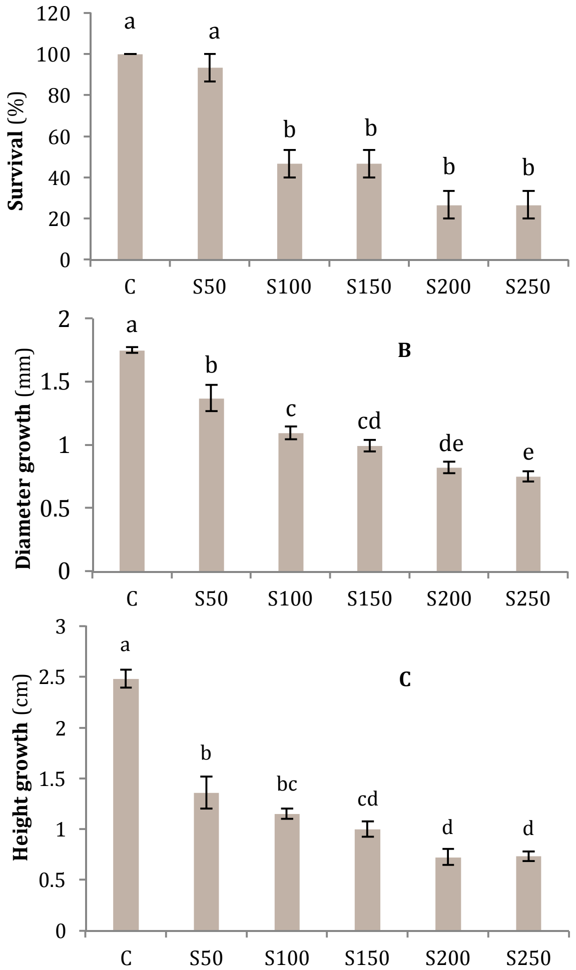

Diagram 1) Plant survival (A), diameter growth (B) and height growth (C) of *Pinus nigra* seedlings under different salinity conditions (C: Control, S0: Control, S50: Salinity of 50mM NaCl, S100: Salinity of 100mM NaCl, S150: Salinity of 150mM NaCl, S200: Salinity of 200mM NaCl and S250: Salinity of 250mM NaCl. Salinity lasted 90 days. Different letters indicates significant differences between treatments at P<0.05 based on Duncan's test)

There was a significant difference in the effect of salinity on photosynthesis of seedlings (Table 1). The highest photosynthesis allocated to the control and the lowest to 250mM NaCl, which was resulted in decrease of 78.8% compared to control (Diagram 2-A).

Effect of salinity on stomatal conductance was the same with that of photosynthesis. Stomatal conductance was significantly highest in control treatment and lowest in 250mM NaCl (Diagram 2-B). Salinity affected transpiration rate whereas the highest NaCl concentration caused a decrease 55% compared to the control (Diagram 2-C).

With increasing salinity to 150mM NaCl, WUE increased but it strictly decreased in higher salinities. In other words, the lowest was found in treatments of 250mM NaCl and highest in treatments of 150mM NaCl (Diagram 2-D).

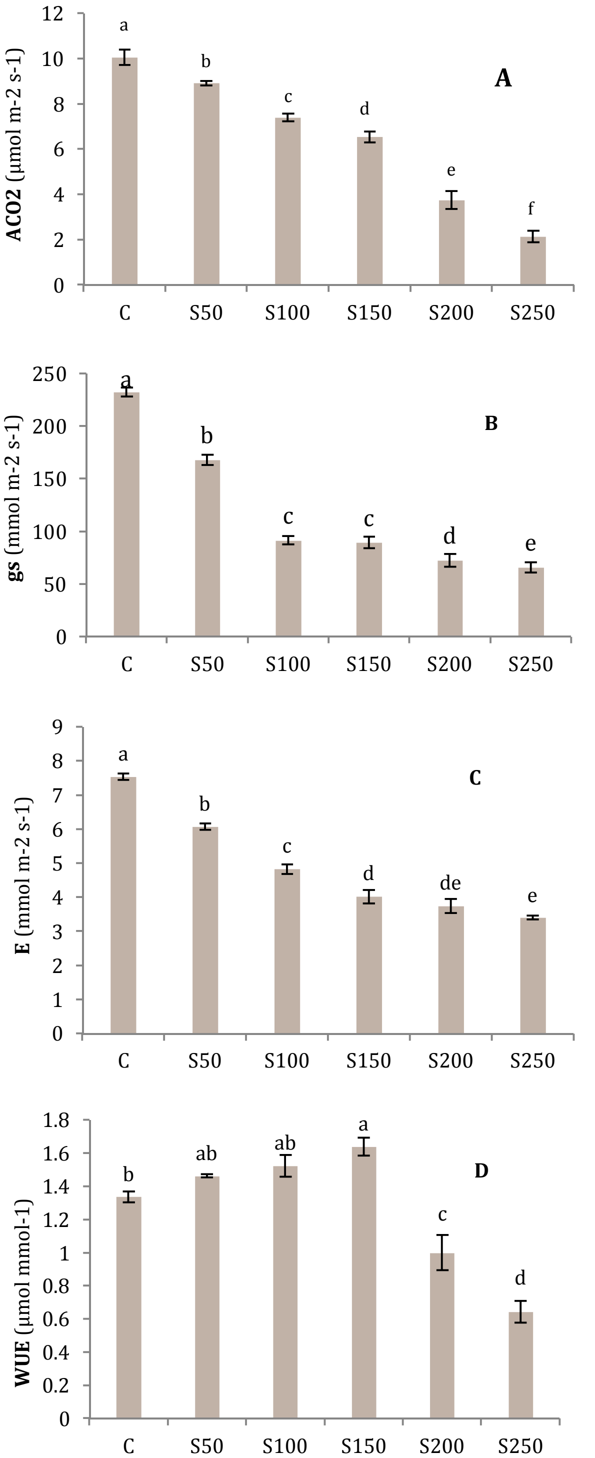

Diagram 2) Photosynthesis (A_{CO2}; A), stomatal conductance (g_s; B), transpiration (E; C), water use efficiency (WUE; D) of *Pinus nigra* seedlings under different salinity conditions (C: Control, S0: Control, S50: Salinity of 50mM NaCl, S100: Salinity of 100mM NaCl, S150: Salinity of 150mM NaCl, S200: Salinity of 200mM NaCl and S250: Salinity of 250mM NaCl. Salinity lasted 90 days. Different letters indicates significant differences between treatments at P<0.05 based on Duncan tests)

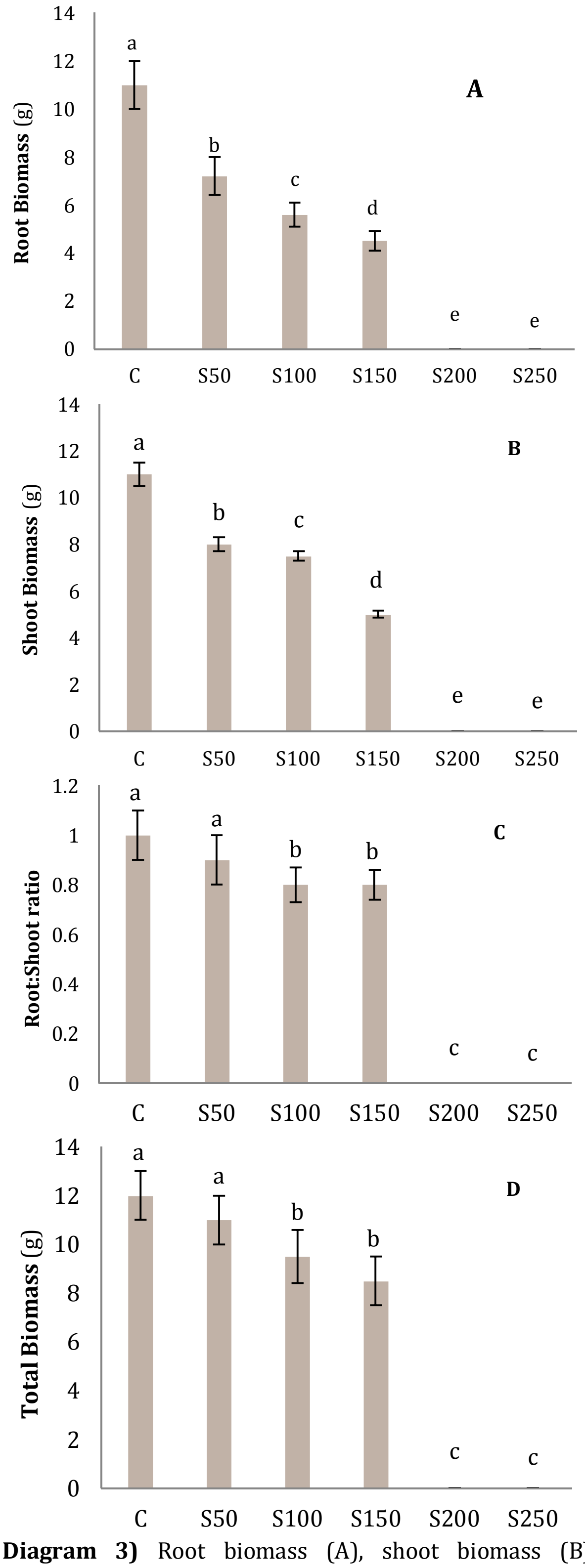

Diagram 3) Root biomass (A), shoot biomass (B), root:shoot ratio (C) and total biomass (D) of Pinus nigra seedlings under different salinity conditions (C: Control, S0: Control, S50: Salinity of 50 mM NaCl, S100: Salinity of 100 mM NaCl, S150: Salinity of 150 mM NaCl, S200: Salinity of 200 mM NaCl and S250: Salinity of 250 mM NaCl. Salinity lasted 90 days. Different letters indicates significant differences between treatments at P < 0.05 based on Duncan tests. In the end of experiment, the seedlings as affected by 200 and 250 mM NaCl salinity died and therefore their means were zero)

Salinity stress reduced the root and shoot biomass of seedlings (Diagram 3-A and 3-B). The ratio of root biomass to shoot biomass was decreased by salinity, but there was no significant difference between control and treatment of 50mM NaCl (Diagram 3-C). The total biomass decreased under salinity stress after reduction in other organs, so that it was more in control than in other treatments, although there was no significant difference between control and salinity of 50mM NaCl, as well as 100 and 150mM NaCl (Diagram 3-D).

Discussion

The aims of the present research were to determine growth responses and gas exchanges of its seedlings under different salinity stress in the greenhouse environment.

The results of the present study showed that *P. nigra* seedlings at the end of 90 days in levels of control and 50mM NaCl had the highest survival percentage (100% and 93.33%), In severe salinity levels, the growth of seedlings was decreased and the mortality increased, probably due to the toxicity of Na^+ and Cl^- ions and imbalance in nutrient caused by this ions [22].

There was no sign of stress, such as aging, necrosis and leaf drop in control and 50mM NaCl treatment. In consistent with this findings, Croser *et al*. [11] found that survival rate in seedlings of *Pinus banksiana* like *Picea mariana* was the highest at 50mM NaCl (71.5% and 67.5%, respectively), it decreased 28 to 12.5% in 100mM NaCl and to zero in 250mM NaCl.

In line with the results of Abdullahi *et al*. [23] on *Acer negundo* and *Thuja orientalis*, Sadeghi *et al*. [13] on *Pinus eldarica* and Sokhna Sarr *et al*. [24] on *Senegalia Senegal*, in the present study salinity stress decreased diameter growth and shoot growth of seedling. The reason for this could be due to osmotic stress happened in the soil, reducing the relative water content and soil water potential in plant and the ionic balance [25]. Adverse effect of salinity on the growth can also be explained by toxicity of sodium ions, chlorine and osmotic stress [26]. Salinity also leads to reduces in the absorption of soil minerals so that an intensive reduction in absorption often occurs on the size of plant diameter. Decreasing height growth in salinity conditions causes by reduction in absorb of material and transport of substance from root to leaf [27].

Corresponding to the reports of Sadeghi *et al*. [13] and Hafsi *et al*. [28] in the present study, salinity had significant effect on biomass allocations. Salinity stress changes influence the growth rate of the plant. Due to salinity, the amount and activity of growth hormones such as auxins, gibberellins, cytokinins and other growth stimulator agents such as putrescin are reduced while growth reducer materials such as abscess acid is increased. Generally, these changes can reduce growth in plants [29].

The same as results of Kayama *et al*. [12] on *Picea abies* and *P. glehnii* in the present study with increasing salinity level the photosynthesis was decreased. Like many physiological studies, inhibition of plant growth affected by salinity is together with decreased in photosynthesis [30]. In the present investigation, decreasing photosynthesis due to salinity stress depends on affects toxicity caused by accumulation of sodium and chlorine, reduction carbon dioxide (due to stomatal closure), accelerating the aging (due to salt accumulation and subsequently leaf fall), change in enzyme activity (due to structural changes in the cytoplasm) and lack of consumption of smylatha (due to reduce growth) [31].

In accordance with Qui *et al*. [32], Cristiano *et al*. [16] and Sokhna Sarr *et al*. [24] in the present results, salinity stress significantly reduced stomatal conductance which can be related to reduction in number and density of leaf stomata. So that mesophyll cell in plants under salinity stress compared to the control is smaller and denser and the access of carbon dioxide is decreased. This can be also stated that besides reduction of stomatal conductance, some non-stomatal limitations including possible injuries applied to photon systems are involved in reduction of photosynthesis under salinity conditions [33].

In this study, the rate of transpiration of *P. nigra* seedlings decreased with increasing salinity level. In general, under salinity stress osmotic potential of soil, water uptake and subsequently transpiration and plant growth is reduced and with the continued salinity stress, destruction of plant occurred (due to the accumulation of Na^+ and Cl^- in the leaf) [34]. Results of Jimenez-Casas and Zwiazek [14] on *Pinus leiophylla* confirms decrease of transpiration and growth characteristics of plant under salinity stress.

In this study, water use efficiency was increased in salinity up to 150mM NaCl, but it was reduced at higher salinities (200 and 250mM NaCl), which greatly is consistent with results of Kaya *et al.* [35]. Hester *et al.* [36] reported that in lower levels of salinity, osmotic effects caused by salinity on its ionic toxicity was dominant, damage to plant photosynthesis was lower compared to transpiration, and therefore with increased salinity water use efficiency increased. But in higher salinities, with increasing stress caused by the toxicity of saline soluble ions increased the respiration and decreased the photosynthesis. This is resulted in reduces the amount of organic matter produced by plants and thus decrease water use efficiency in high salinities. Reina-Sanchez *et al.* [37] reported that reduced water uptake was led to decrease in absorption of toxic ions in saline environment.

The research for more complete adaptability of this plant to saline environments, various salts (with different concentrations) at longer periods can be followed by other researchers.

Considering to the saline resistance of this species, it may be suggested that *Pinus nigra* subspecies pallasiana to be used for seedling production in nurseries as well as the development of green spaces and restoration of sites where the NaCl concentration is lower than 50mM.

The limitations of this research include due to lack of adequate funding, measurement some of biochemical parameters was not possible in order to reinforcement and more accurately recommend the applied aspect of the present study and the suggestions for the future researches includes use of bio-fertilizers such as *Arbuscular mycorrhizal* fungi to resistant the black pine seedlings to salt stress, Study of the effect of salinity stress on morphological and physiological performance of black pine trees at the time that occur these stress in nature.

Conclusion

Salinity has adverse effects on growth and gas exchanges of *P. nigra* seedlings during the studied period. Survival in 50mM NaCl has a high percentage, but in higher salt concentrations (200 and 250mM NaCl) it drastically reduced. Similarly, decrease in seedling performance was found in severe salinity levels. This species has a relatively good resistance to 50mM NaCl and it can be used for seedling production in nurseries, development of green spaces and restoration of arid areas of country where the salinity is lower than 50mM.

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