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Effect of Saline Water on Some Morpho-Physiological Traits of Neem (*Azadirachtaindica* L.) Tree Seedlings

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Abstract:

A field experiment was conducted to find out the effect of saline water with different EC levels (control/canal water, 04, 06, 08 and 12 dS m^{-1}) on some morpho-physiological traits of neem tree seedlings. About 30-day-old local neem tree seedlings were planted in a pit ($1\frac{1}{2}$ ft. wide, × $1\frac{1}{2}$ ft. deep and x $1\frac{1}{2}$ ft. length) filled with 20 kg river-bed sand. Plants were continuously irrigated for four months as per planned treatments and replications (three). After four months of planting, the results related to height, number of branches, stem girth, leaf area and chlorophyll (a, b and total) contents were recorded from plants. In addition to that youngest fully expanded leaves were harvested from each plant for extraction of sap and, analysis of Na^+, K^+ and K^+/Na^+ ratio. The results indicated that compared to control (freshwater), the seedlings irrigated with 4, 6, 8 and 12 EC (dS m^{-1}) water showed 12, 29, 33 and 41 % reduction in height; 16, 42, 47 and 58% reduction in the number of branches per plant; 5, 14, 16 and 26% reduction in stem girth; 0.034, 0.17, 0.31 and 0.56% reduction in leaf area; 23, 26, 24 and 20% reduction in chlorophyll b and 28 23, 21, and 21% reduction in total chlorophyll contents, respectively. The negative effect of water *salinity on various traits were found to be associated with higher Na^+ and lower K^+ concentrations and narrow K^+/Na^+ ratio determined in the leaf sap. As seedlings irrigated with 02, 04, 08 and 12 ECiw water accumulated 16, 52, 60 and 69% more Na^+ and 25, 42, 61 and 73% less K^+ in leaf sap, respectively over control. Hence they displayed 76, 87 and 93% lower K^+/Na^+. It can be concluded from the study that at seedling stage desi local neemtreeis tolerant to saline irrigation, thus it can successfully be grown with saline water having ECiw up to 08 (dS m^{-1}). However, it can survive with 12 ECiw water, it shows larger reductions in some important traits.*

Key words: Saline, Traits, Chlorophyll, Freshwater, Ratio and Neem tree.

INTRODUCTION

Desertification is a land degradation process mainly occurs in arid and semi-arid regions, resulting from climatic change and human activities (Le Houerou, 1975; Warren, 1996; UNEP, 1992). The land area prone to desertification has been estimated at 3.5-4.0 billion ha or 57-65% of the total land area of dry land ecosystems (UNEP, 1991). The land area affected by soil degradation alone (excluding vegetation degradation) ranges from 1.02 (UNEP, 1991) to 1.14 billion ha (Oldman and Van Lynden, 1998). The estimates of current rate of desertification also vary widely the annual rate of desertification is about 5.8 million hectares, with 55% occurring in range land and 45% in rain-fed crop land. Affordable afforestation programs in arid areas suffer mainly from low rainfall and high evapotranspiration (Whitehead and Beadle, 2004; Gazal*et al.*, 2006), while the quantity of water required for the photosynthesis process amounts to only about 0.01% of the total quantity of water used by the plants (Mengel and Kirkby, 1987).The climate of irrigated areas in Pakistan is mostly hot and summer temperature reaches a peak of 52°C in the plains. Average annual rainfall ranges between 250–300 mm and is distributed mostly in the Monsoon July/August receive 80% of total rainfall (Qadir*et al.,* 2003). Evaporation losses around the year are estimated at 75% of the precipitation, while the amount of rainfall used as transpiration is estimated to be as lows as 5% (Nimbkar*et al*., 1986; Johnson *et al*., 2005).

Salinity is one of the major environmental stresses affecting the performance of many plant species in arid and semi-arid regions. It is estimated that over 800 million hectares of land in the world are affected by both salinity and sodicity. Salinity has various effects on plant physiological processes such as increased respiration rate and ion toxicity, decreased leaf net CO_2 assimilation rate and membrane disruption (Gupta *et al*., 2002; Munns 2005). Salinity often leads to decrease in chlorophyll contents and photosynthetic rates (Lee *et al*., 2004; Kao *et al*., 2006). It can seriously change the photosynthetic carbon metabolism, leaf chlorophyll content, and photosynthetic efficiency (Sharkey *et al*., 1985). The major factors responsible for salinity are seepage of water from canal system and its evaporation from the surface of adjoining soils, rising of water table due to excessive percolation from the canal system, and over-irrigation practices, inadequate availability of water, irrigate on with brackish tube well water (Panhwar*et al*., 2003). The problem of irrigation system in Pakistan is unscientific water management practice, improper natural drainage and inappropriate reclamation procedures. Pakistan is facing shortage of good quality water for agriculture, particularity because of increased cropping intensity and competition from non-agricultural sectors for freshwater resources (Murtaza*et al*., 2009; Ghafoor*et al*., 2010). Most of the ground water is poor quality and unfit for crop use. This fact requires technology development and adoption. Use of brackish water for irrigation can increase the resource base for irrigated agriculture in

Pakistan, which will help to meet the increased food requirements of the country (Mohtadullah*et al*., 1993; Ghafoor*et al*., 2002) for irrigation of approximately 17 mha of land (Anonymous, 1998). Water resources at present as well as for future are insufficient to meet the needs of cultivated area under this irrigation system. However, current water supplies are about 30% short on an annual basis. For supplementing canal supplies, about 47.5 MAF of water is being pumped to grow crops (Mohtadullah*et al*., 1993). According to many reports (Malik *et al*., 1984; Ahmad, 1993; Ghafoor*et al*., 1999), this pumped groundwater in Pakistan is hazardous for irrigation and requires special management strategies. Soil salination/sodication is a natural consequence of using such saline and sodic waters to irrigate the crops (Minhas, 1986: Van Schilfgaarde, 1994).

Afforestation programmes for the area with poor quality ground water require the proper selection of tree species and planting techniques. As the main problems in these areas is high salinity water, hence the tree species should be those which are tolerate to salt-stress environments (Tomar*et al.,* 1998). Because salt-stress directly affects the growth and establishment of plants. Whereas the tolerant plants tolerate such an adverse environment through various mechanisms. Reports on various defense mechanism of plant species in response to stress caused by salinity include the enhanced production of intracellular solutes, osmic protectants and oxidative enzymes. *Azadirachtaindica* A. Juss, is one of the important multipurpose agro-forestry tree species, has not been studied in detail for its reaction to salinity stress. Although, the plant species is grown very extensively for its timber and medicinal purpose and is ideal for reforestation and rehabilitation of degraded, semi-arid and arid lands (Kumar *et al*., 2002).The neem thrives well in dry areas throughout the tropics and subtropics with rainfall as low as 150-250 mm per annum. The neem tree is gaining importance throughout the world, because it not only provides solutions to rehabilitation of degraded lands (Palsson and Jaenson, 1999; Deans *et al*., 2003; Gajalakshmi and Abbasi, 2004; Xuan *et al*., 2004; Arya, 2006; Jabbar*et al*., 2006; Rugutt*et al*., 2006), but it is used as source of organic pesticides (Prakash and Srivastava, 2006; Bakshi and Wadhwa, 2007; Senthil*et al*., 2007; Peer *et al*., 2008), herbal medicines and affordable timber as well. This study was planned to investigate the influence of saline water on some morpho-physiological attributes and accumulation of Na^+ and K^+ in leaves of neem tree seedlings.

MATERIALS AND METHODS

The experiment was conducted in a natural environment at the Centre for Bio saline Agriculture, Department of Soil Science, Sindh Agriculture University Tandojam. About 30-day-old seedlings of local desi neem tree seedlings were purchased from a commercial nursery of Sindh Agriculture University, TandoJam. The seedlings were planted by openings a pit of about $1\frac{1}{2}$ ft (wide) × $1\frac{1}{2}$ ft (deep) and × $1\frac{1}{2}$ ft (length) pits. Each pit was filled with 20 kg pure river-bed sand. The neem tree seedlings were planted at the center of each sand filled pit. There was single plant in each pit. The pits were set as the Randomized Complete Block Design with three replications and five saline water ECiw levels (dS m^{-1}) treatments viz: T_1:Canal water (0.34), T_2: 4.0 (dS m^{-1}), T_3:6.0 (dS m^{-1}), T_4:8.0 (dS m^{-1}) and T_5: 12.0 (dS m^{-1}) were used in the experiment. The seedlings in all treatments were irrigated with fresh/canal waterup to one week of planting, whereas after one week of planting the plants in saline water treatments were stressed continuously with salty waters as per plan with artificially prepared NaCl solution having different EC levels. In the control treatment pits, plants were only irrigated with-fresh/canal water, continuously up to four months.

***Soil analyses before planting*:**

The soil used in the pits was analyzed for some physico-chemical properties (EC, pH, $CaCO_3$, etc) before planting of seedlings. Following methods were adopted for soil analyses: pH and EC (at 1:2 ratio) were recorded by digital meters (Schott Lab 960, and Sartorius PB-11, respectively) lime content ($CaCO_3$) and organic matter were determined through acid neutralization method and Walkley Black method, respectively, (Ryan *et al*. 2001).

***Observations recorded*:**

Some morpho-physiological traits of neem tree seedlings were recorded after four months of stress Plant height (cm), Number of branches per plant, Stem girth (cm) and Leaf area (cm^2). The chemical analyses was done after four months of planting, fully mature leaves were sampled from each plant for extraction of sap and analysis of Na^+ and K^+ using flame photometer (USDA, 1956). The K^+/Na^+ ratio was also calculated using the values of Na^+ and K^+. The Chlorophyll *a* and *b* contents were determined through spectrophotometer using the Acton method of Bowel *et al*. (1986).

***Statistical analysis*:**

All the data were analyzed through Student Edition of Statistix(SXW), version 8.1 (copyright 2005 Analytical Software, USA).

RESULTS

This open field pit experiment was conducted to find out the effect of saline water with different ECiw levels on neem tree seedlings(*Azadirachtaindica*L.). The data generated from the study of some basic characteristics determined before planting are given in the Table-1.The pure river sand which was alkaline in reaction, low in organic matter and had no salinity (ECiw 2.2 dSm^{-1}) or sodicity problem was selected for pits.

Table-1.Some characteristics of the sand used in the pits

Properties	**Values**
Texture	100% river sand
Organic matter	0.21
EC(dSm^{-1})	2.2
pH(H_2O)	8.3

The results related to the effect of saline water on some morpho-physiological traits of neem tree seedlings, Plant height (cm), Number of branches per plant, Stem girth (cm) and Leaf area (cm^2) in (Fig:-1 to Fig:-4).It is evident from the results that increasing EC_{iw} of irrigation water significantly ($P<0.05$) decreased the Plant height (cm), Number of branches per plant, Stem girth (cm) and Leaf area (cm^2) of neem tree seedlings. Compared to control without saline water (Control/Canal water 0.34), the plants irrigated with 04, 06, 08 and 12 EC_{iw} ($dS\ m^{-1}$) water showed12, 29, 33 and 41 %, reduction in height, 16, 42, 47 and 58% reduction in the number of branches per plant, 5, 14, 16 and 26 % reduction in stem girth and 0.034, 0.17, 0.31 and 0.56% reduction in leaf area, respectively. Moreover, the results on the effect of saline water on chlorophyll contents *a, b* and total chlorophyll contents are presented in (Fig:-5 to Fig:-7). Compared to control without saline water (Control/Canal water 0.34), the plants irrigated with 04, 06, 08 and 12 EC_{iw} ($dS\ m^{-1}$) water showed23, 26, 24 and 20% reduction in chlorophyll *b* and 28 23, 21, and 21% reduction in total chlorophyll contents, respectively. Whereas, the chlorophyll *a* content of leaves compared to control/fresh water irrigated plants, the seedlings receiving saline water for irrigation did not show much variation for chlorophyll content.

The data on the concentration of $Na^+$and K^+determined in the leaf sap and K^+/Na^+ ratio are plotted against ECiw values of saline water in the (Fig:-8 to Fig:-10).Compared to control without saline water (Control/Canal water 0.34), the plants irrigated with 04, 06, 08 and 12 EC_{iw} (dS m^{-1}) water showed 16, 52, 60 and 69% more Na^+ and 25, 42, 61 and 73% less K^+, hence displayed 76, 87, and 93%, lover K^+/Na^+ respectively.

Fig

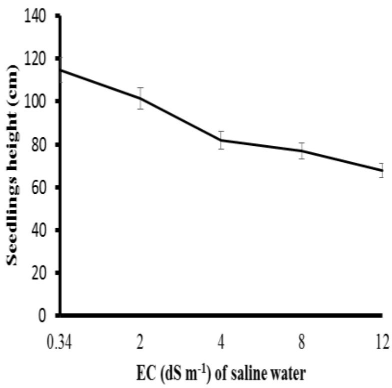

Fig:-1. Effect of saline water on height (cm) of neem tree seedlings

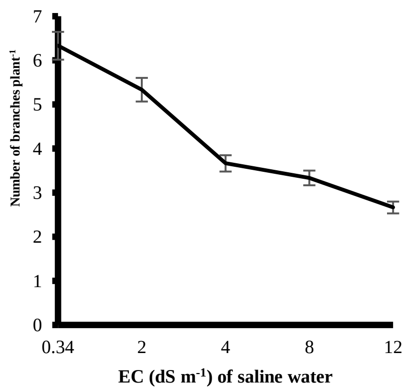

Fig:-2. Effect of saline water on number of branches emerged on neem tree seedlings

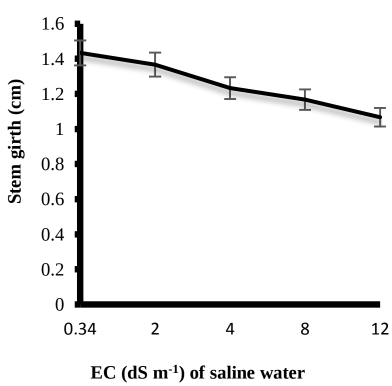

Fig:-3. Effect of saline water on stem girth of neem tree seedlings

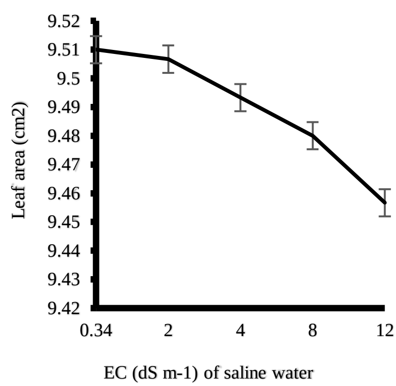

Fig:-4. Effect of saline water on the area of fully mature leaf sampled from neem tree seedlings

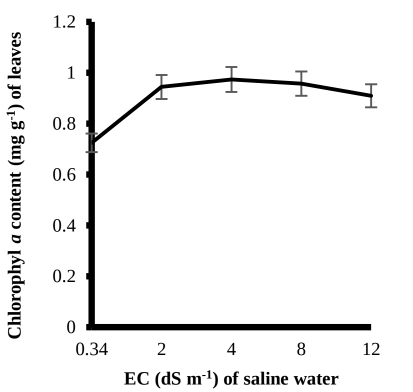

Fig:-5. Effect of saline water on the content of chlorophyll *a* (mg g^{-1}) of the leaves of neem tree seedlings

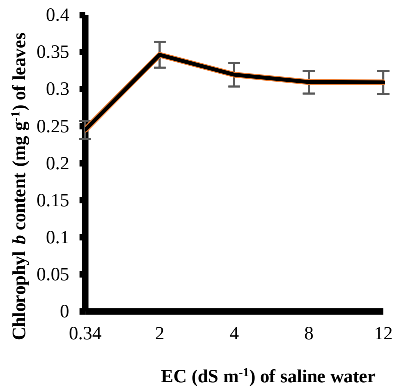

Fig:-6. Effect of saline water on the of chlorophyll *b* (mg g^{-1}) content of the leaves of neem tree seedlings

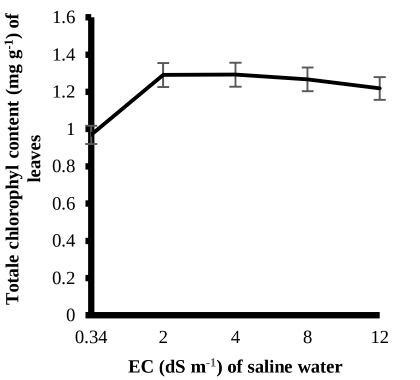

Fig:- 7. Effect of saline water on the total chlorophyll content (mg g^{-1}) of leaves of neem tree seedlings

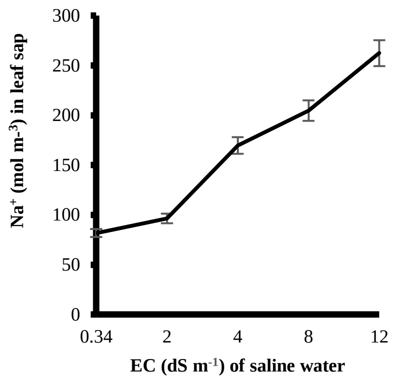

Fig:-9. Effect of saline water on the concentration of Na^+ (molm^{-3}) determined in the leaves of neem tree seedlings

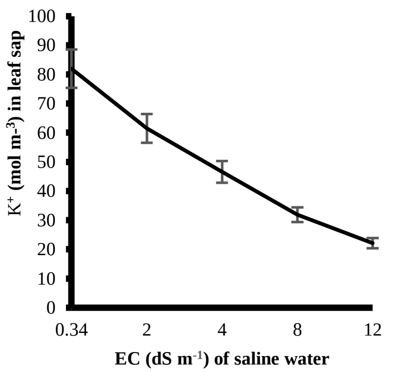

Fig:- 8. Effect of saline water on the concentration of K^+ (mol m^{-3}) determined in the leaves of neem tree seedlings

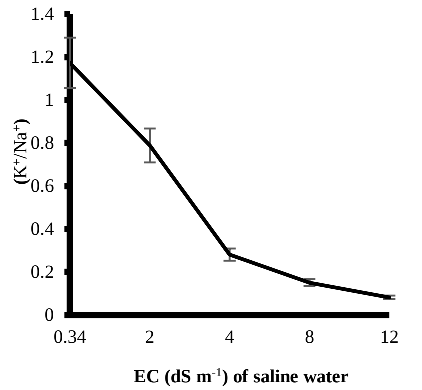

Fig:-10. Effect of saline water on the K^+/Na^+ ratio determined in the leaves of neem tree seedlings

DISCUSSION

Desertification is a land degradation process mainly occurs in arid and semi-arid regions (Warren, 1996). The salinity is one of the major environmental stresses adversely affecting on many plant species (Gupta *et al*., 2002). The major factors responsible for salinity developments are seepage of water from canals, over-irrigation practices, inadequate availability of water and use of brackish ground water (Panhwar*et al*., 2003). The problem of irrigation systems is unscientific water management practices, disturbances with natural drainage inappropriate reclamation procedures, shortage of good quality water for agriculture, increased cropping intensity and competition of non-agricultural sectors for freshwater resources (Murtaza*et al*., 2009; Ghafoor*et al*., 2010). Most of the ground or drainage water is poor in quality, this fact requires technology development and adoption for using that lower quality water for cultivation of plants (Ghafoor*et al*., 2002). Use of poor quality brackish water in appropriate, way for irrigation or forestry can increase the resource base for irrigated agroforestry (Mohtadullah*et al*., 1993).Afforestation programs for the areas where groundwater is of a poor quality require the proper selection and screening of tree species and planting techniques. As the main problem of these areas is water shoratage(Tomar*et al.,* 1998).The neem (*Azadirachtaindica*A. Juss) tree has been reported as is moderately tolerant to salinity stressand is well grown in arid areas of tropics and subtropics region(Dagar, 1998; Palsson and Jaenson, 1999).

The present study was carried out to evaluate performance of local neem(*Azadirachtaindica*L.) tree seedlings grown with saline waters of 0, 02, 04, 08 and 12 ECiw (dS m^{-1}). The results obtained from the study discussed here indicated that compared to canal water, irrigating neem tree seedlings with saline water of 0, 02, 04, 08 and 12 ECiw (dS m^{-1}) showed 12, 29, 33 and 41%reduction inplant height (Fig. 1), number of branches per plant (Fig. 2), stem girth (Fig. 3), leaf area (Fig. 4), chlorophyll *a,* (Fig. 6), chlorophyll *b*(Fig. 7) and total chlorophyllcontents (Fig. 8). These findings are comparable with of those Greenway and Munns(1980), who also reported that increasing salinity levels decreased stem girth and plant height, leaf area and chlorophyll content. Chlorophyll *a* and*b*contents of the Thai neem tree seedlings decreased strongly with increasing NaClconcentration (Cha-uma*et al*., 2004). Several other workers reported that the salts are harmful to most plant organelles, cells, tissues and organs, because they induce negative water potential in the soil, disturb cell membrane permeability, inhibit nutrient uptake and induce anoxia or oxidative stress.The biochemical and physiological responses in terms of the photosynthetic capability of the plant under salt-stress environment get disturbed (Panhwar, 2002; Do and Rocheteau, 2002;Cha-uma, 2004; Sixto*et al*., 2005; Pesarkali and Touchane, 2006; Khan *et al*., 2009).

The present results indicated the Na^+ and K^+ contents determined in the sap obtained from youngest fully expanded leaves of neem tree seedlings were significantly affected by saline water. Compared to other treatments, the seedlings irrigated with 12 ECiw (dS m^{-1}) water had much more Na^+ and less K^+. The K^+/Na^+ ratio was also affected by increasing Na^+ in the leaf sap of neem seedlings (Figures 9, 10 and 11). These results are comparable to Marcar*et al.*, (1995), Rawant and Banerjee(1998)., and Saqib*et al.*(2000) they also reported that higher Na^+ and lower K^+ due to imbalance in nutrient uptake may result in excessive accumulation of Na^+ and Cl^- in leaves of plant tissue. However increase Na^+ concentrationthe $K^+$was decreased. Several previous researchers have used K^+/Na^+ratio as an indicator of salinity tolerance (Saqib*et al.*, 2004; Saqib*et al.*, 2005; Munns, 2005) as a high K^+/Na^+ratio in the cytosol is essential for normal cellular functions of plants (Chinnusamy, *et al.*, 2005). In leaves of poor plants, K^+/Na^+ratio was quite lower than found in good plants. Low K^+/Na^+ratio in the plant leaves tissue may be the result of loss in capacity to maintain K^+ selectivity above Na^+ at higher external salinity (Huang and Van Steveinck, 1989).

CONCLUSION

It can be concluded from the study that at seedling stage desi local neem tree seems to be tolerant to saline irrigation, this tree can be grown with saline water having EC up to 08 (dS m^{-1}). Although, it can survive with 12 ECiw water, it shows larger reductions in some important traits, at this time high salinity level.

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