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Response of *Moringa oleifera* to Saline Conditions

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ABSTRACT

Moringa oleifera is being cultivated in tropical and sub-tropical areas for nutritional, medicinal and fodder purposes. The present study was conducted to study the seedling growth, physiological and nutritional changes of moringa under different salinity levels (2, 4, 8 & 12 dS m^{-1}). Growth parameters, chlorophyll *a* and *b*, ß- carotene, mineral (sodium, potassium, calcium, magnesium, phosphorous), crude protein, total phenolic contents (TPC) and antioxidants activities of superoxide dismutase (SOD), catalse (CAT) and peroxidase (POD) were investigated. It was found that moringa seedlings survived up to 8 dS m^{-1} with a slight reduction in its biomass, chlorophyll *a*, crude protein and mineral contents and antioxidants' activity increased with increasing salinity. Plant biomass, chlorophyll *a* contents, SOD and POD activity and mineral contents (Ca, K & Mg) were reduced significantly but CAT, total phenolics, β- carotene and chlorophyll *b*, sodium and phosphorous contents increased significantly up to 12 dS m^{-1} as compared to control. A positive correlation was found between salinity levels and root biomass, sodium, phosphorous, chlorophyll *b*, TPC and antioxidants' activity but a negative correlation was recorded between salinity levels and shoot biomass, calcium, magnesium, potassium, crude protein and chlorophyll *a* contents. These findings suggest that moringa can tolerate moderate saline conditions owing to better antioxidant system, activating defensive enzymes and better ionic homeostasis.

Key Words: Antioxidants; Chlorophyll; Pigments; Salinity; Total phenolic contents

INTRODUCTION

Soil salinity is a wide spread problem around the world but it generally affects arid and semi arid regions. It is largely being increased in irrigated lands due to poor drainage, irrigation practices, low rainfall and high transpiration rate. It is reported that about 20% irrigated and 7% of all land is salt affected, which accounts about 1000 million hectare of land (Munns *et al.*, 1999). High salt concentrated soils have damaging effects on the germination and suppress the seedling growth and plant development (Ramoliya & Pandey, 2003). The damage to plant growth is generally being caused by high Na^+ and Cl^- concentration in soil, which impairs the nutrient selectivity of root membranes (Shahbaz & Ashraf, 2007) and ionic imbalance in plants like potassium or calcium deficiency (Chow *et al.,* 1990). Crops differ in response to salinity level and types. In addition to osmotic and ionic imbalance and toxicity salt stress also induces oxidative stress in plants (Rout & Shaw, 2001), which initiates antioxidant system of the plants to cope up with oxidative damage to stressed plants. Many researchers explored the salt resistant plants and their tolerance mechanism. Such research studies help the researchers in screening the salt tolerant plants and exploring their response to salt stress conditions.

Moringa oleifera locally named as sohanjna is a miracle tree having tremendous uses like biopesticides, alley cropping, afforestation, medicines, water purification, biogas, vegetable etc. It is naturally found in diverse habitats with an altitude ranging from 600-1800 m (Jama *et al.*, 1989). It grows best in the areas receiving rainfall less than 400 mm per annum and prefers neutral or slightly acidic soils (Francis & Liogier, 1991). Recently, many uses of moringa have been highlighted and farmers are taking interest to cultivate it as field crop for fodder and vegetable production and as farm forestry.

The decrease in biomass production of many plants under salt stress conditions is mainly attributed to generation of reactive oxygen species (ROS) in chloroplasts (Allen, 1995). ROS diminishes the plant growth in the absence of

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any protective system like antioxidant system. Moringa leaves are rich in minerals having high antioxidant activity rate, which can make them tolerant under salt stress conditions. Salinity is increasing in Pakistan at an alarming rate. Currently, 6.67 m ha of agriculture land is salt affected. According to our best knowledge, no study has been reported to investigate the tolerance of moringa to salinity. Soil salinity may affect moringa plant growth and development. The present study investigated the growth and physiological response of moringa seedlings in saline environment and to determine the antioxidant activity rate in moringa leaves to tolerate salt stress.

MATERIALS AND METHODS

Moringa seeds, collected from the department of Forestry, University of Agriculture Faisalabad, Pakistan were sown in acid washed sand filled pots in completely randomized design (CRD) with five replications. The experiment was conducted in August-October 2009 in greenhouse of department of forestry, university of agriculture Faisalabad-Pakistan. During the experiment, temperature (32□36ºC) and relative humidity (47□56%) was recorded with 14 h average photoperiod. At five leaves stage, four salinity levels i.e., 2 (control), 4, 8 and 12 dS m^{-1} were imposed by using NaCl salt in Hoagland's solution. After 30 days of salt stress, three plants from each replication were randomly selected for observing growth, physiological and mineral analyses. The mean value of each parameter was computed from 15 plants (3 plants from each replication).

Growth parameters: Growth parameters like shoot length, root length, number of leaves and branches per plant, root and shoot fresh and dry weights were recorded by using standard procedures after harvesting. Fresh root and shoot biomass was weighed immediately after harvesting and then was shade dried followed by oven drying at 70±2ºC till constant weight for determining dry matter.

Chlorophyll (*a*, *b*) and β-carotene determination: The protocol devised by Nagata and Yamashta (1992) was followed to determine chlorophyll *a*, *b* and β-carotene contents. One gram moringa leaf sample was ground in 10 mL of 80% acetone and filtered through Whatman No. 1 filter papers. The filtered extract was transferred in cuvette and absorbance was noted at 663, 645, 505 and 453 nm by using UV-spectrophotometer (UV-4000, O.R.I. Germany). Following formulae were used to calculate chlorophyll *a*, chlorophyll *b* and β-carotene contents.

Chlorophyll $a = 0.999\ A_{663} - 0.0989\ A_{645}$

Chlorophyll $b = -0.328\ A_{663} + 1.77\ A_{645}$

β- Carotene $= 0.216\ A_{663} - 1.22\ A_{645} - 0.304\ A_{505} + 0.452\ A_{453}$.

Total phenolic contents (TPC): TPC in moringa leaves were quantified by using the method described by Singleton and Rossi (1965) revised by Waterhouse (2001). Folin Ciocalteu Reagent (2N) and Na_2CO_3 were used as reagents. The absorbance by gallic acid standards (100, 150, 250 & 500 mg L^{-1} gallic acid) and moringa samples was noted at 760 nm by using UV-spectrophotometer (UV-4000, O.R.I. Germany).

Antioxidants assay: Fresh leaves (0.25 g) were homogenized in 2.5 mL of 50 mM cooled phosphate buffer of pH 7.8 and filtered. The filtered mixture was centrifuged at 15000 rpm for 20 minutes at 4ºC, and the supernatant was extracted in other test tube for further analysis. The method described by Giannopolitis and Ries (1977) was used in Superoxide Dismutase (SOD) (EC 1.15.1.1) determination at 560 nm by using UV spectrophotometer (UV-4000, O.R.I. Germany). Catalase (CAT) (EC 1.11.1.6) and peroxidase (POD) (EC 1.11.1.7) activity was determined by Chance and Maehly (1955) method. The enzyme protein was determined by using the method described by Bradford (1976) to express specific activity of SOD, POD and CAT.

Nitrogen (N) and crude protein (CP) analyses: Chapman and Pratt (1961) method was used for nitrogen digestion, distillation and quantification. The sample digestion in sulfuric acid in the presence of a mixture of K_2SO_4: $CuSO_4$: $FeSO_4$ (10: 05: 01) with micro Kjeldhal's apparatus. Crude protein was calculated by multiplying nitrogen contents by 6.25.

Mineral analyses: Moringa leaves and roots were oven dried at 60ºC to a constant weight and ground to pass 2 mm sieve. The samples were digested by using concentrated nitric and perchloric acid (2:1) by following the procedure adapted by (Rashid, 1986). The presence of phosphorus (P) contents was recorded in UV-spectrophotometer at 410 nm. Color was developed with ammonium molybdate ammonium vandate solutions. Flame photometer (Jenway PEP-7) was used to determine potassium (K) and sodium Na^+ in diluted extracts of plant material by using potassium and sodium filter respectively for both minerals (Chapman & Pratt, 1961). Calcium (Ca) and magnesium (Mg) were determined by atomic absorption spectrophotometer (Model: Z-8200).

Statistical analysis: The experiment was conducted twice in completely randomized design (CRD) with five replications. The data were pooled and analyzed using MSTAT-C Program (MSTAT Development Team, 1989). LSD test at 5% level of probability was used to test the differences among mean values (Steel *et al.,* 1997).

RESULTS

Growth parameters: All the parameters were significantly (P<0.05) affected by salt stress (Table I). The present study showed that moringa could tolerate EC up to 8 dS m^{-1}. Moringa seedlings showed stable growth until EC 8 dS m^{-1} while at 12 dS m^{-1} 22.54, 18.6 and 26.4% reduction was recorded in shoot length, fresh and dry weight, respectively. A highly negative correlation (r^2 = 0.91, 0.89 & 0.93) was found between shoot length, fresh and dry weights with increasing salt stress, respectively. A significant increase of 28.84 and 64.94% in root length was observed at 8 and 12

dS m^{-1} but in case of root fresh and dry weights, a significant reduction was recorded as compared to control (1.19 g). At 12 dS m^{-1} moringa root fresh and dry weights were 0.97 and 0.47 g respectively. Positive correlation (r^2 = 0.95) was found between root length and increasing salinity while negative correlation (r^2 = 0.92 & 0.85) was found between salt stress and root fresh and dry weights, respectively (Table I).

Data depicted in (Table I) show significant decrease in number of branches and leaves, while number of roots was significantly increased with increasing salinity. The number of branches of moringa seedlings at 2 dS m^{-1} was counted at 8.67 while 7.73 and 6.35 branches were recorded at 8 and 12 dS m^{-1}, respectively which showed a decrease of 10.84 and 22.75%, respectively in comparison with control. Similarly, moringa leaves were also significantly reduced with increase in salinity in comparison with control. A difference of 34.32% was found between 2 and 12 dS m^{-1} in case of moringa leaves. The number of roots was found significantly increasing with an increase in salinity. At 2 dS m^{-1}, roots were 24.53, which were at par with 4 dS m^{-1} (23.13) while 34.13 and 46.27 root score was found at 8 and 12 dS m^{-1}. Number of branches and leaves were highly negatively correlated (0.95, 0.99, respectively) with increasing salinity while a high positive correlation (0.93) was found between number of roots and higher salinities.

Photosynthetic pigments, total phenolic contents (TPC) and antioxidants (SOD, POD, CAT): Under salt stress the photosynthetic pigments (chl *a*, *b*) of moringa seedlings were affected significantly under salt stress (Table II). A reduction of 42.1% was recorded in chlorophyll *a* as compared to control at 12 dS m^{-1}, which was negatively correlated (r^2 = 0.84) with increasing salinity statistically. Contrariwise, chlorophyll *b*, β-Carotene and total phenolic contents (TPC) were positively correlated (r^2 = 0.95, 0.34, 0.94, respectively) (Table II). An increase of 36.96% was recorded in β-Carotene contents at 12 dS m^{-1} in comparison with control but statistically it was not significant (P>0.05). An increase of 29% in total phenolic contents was also recorded in highly salinized moringa leaves (12 dS m^{-1}) (Table II). SOD, POD and CAT (antioxidants) activities were significantly affected under salt stress (Table II). Maximum SOD activity was recorded at 8 dS m^{-1}. Though, salinity did not affect significantly on SOD activity but a reduction of 17.29% was found in its activity at 12 dS m^{-1}, while POD and CAT activities were significantly affected by increasing salt stress. POD activity increased up to 8 dS m^{-1} but reduced at 12d S m^{-1}, while CAT activity was found increasing at higher salinity level (12 dS m^{-1}).

Mineral contents: Crude protein and mineral contents (Na, N, P, K, Ca, Mg) in moringa leaves and roots were significantly (P<0.01) affected by salt stress. Sodium and phosphorous contents in both leaves and roots increased with increasing salinity, while other minerals (K, Ca & Mg), nitrogen and crude protein exhibited decreasing trend with increasing salinity (Fig. 1). Maximum sodium and phosphorous contents were recorded at 12 dS m^{-1} as compared to control (2 dS m^{-1}) (Fig. 2 & 3). No significant change were observed at 4 dS m^{-1} but a mild change was observed at 8 dS m^{-1} in both crude protein and nitrogen contents while a decrease of 27.60% in nitrogen and crude protein contents was recorded at 12 dS m^{-1} as compared to control. The highly salinized plants had very less potassium contents in their leaves and roots, while potassium contents

Table I: Effect of salinity on the growth characteristics of *M. oleifera*

Salinity Levels (EC: dS m^{-1})	SL (cm)	SFW (g)	SDW (g)	RL (cm)	RFW (g)	RDW (g)	Branches	Leaves	Roots
2 (control)	12.1±0.36 a	0.9±0.01 a	0.53±0.02 a	10.6±0.79 c	1.19±0.08 a	0.57±0.03 a	8.67±0.23 a	73.8±2.53 a	24.5±2.56 c
4	11.8±0.43 a	0.8±0.03 a	0.53±0.02 a	10.6±0.80 c	1.18±0.02 a	0.56±0.03 a	8.53±0.13 ab	69.3±3.70 a	23.1±2.06 c
8	11.1±0.86 a	0.8±0.03 a	0.43±0.03 b	13.7±0.64 b	1.12±0.04 a	0.55±0.01 a	7.73±0.31 b	61.3±3.66 b	34.1±3.11 b
12	9.9±0.39 b	0.7±0.04 b	0.39±0.03 b	17.5±0.81 a	0.97±0.06 a	0.47±0.02 b	6.35±0.51 c	48.5±3.22 c	46.3±3.25 a
P Value	1.039	0.1039	0.4240	0.000	0.3806	0.0498	0.0002	0.0000	0.0000
LSD	0.016	0.015	0.000	1.777	0.2968	0.07344	0.9143	7.723	5.277
R-Square (r^2)	0.91	0.89	0.93	0.95	0.91	0.85	0.95	0.98	0.93

Changes in growth characteristics (SL= shoot length; SFW= shoot fresh weight; SDW= shoot dry weight; RL= root length; RFW= root fresh weight; RDW= root dry weight, number of branches, leaves and roots) of *M. oleifera* under saline conditions (2, 4, 8 and 12 dS m^{-1}). Means showing different letters are statistically different ($p < 0.05$) from each other. Data was computed from five replications consisting of three plants in each replication

Table II: Effect of salinity on the physiological characteristics of *M. oleifera* leaves

Salinity Levels (EC: dS m^{-1})	Chl *a*	Chl *b*	β- carot	TPC	SOD	POD	CAT
2 (control)	2.47±0.16 a	0.69±0.14 c	0.46±0.05 a	68.48±2.86 b	324.09±16.16 c	897.40±71.42 c	80.84±6.69 d
4	2.29±0.01 a	0.55±0.10 c	0.58±0.04 a	68.61±3.53 b	401.1±11.73 ab	1344.6±67.44 ab	108.94±6.71 c
8	1.40±0.12 b	2.21±0.15 b	0.47±0.28 a	83.06±4.90 a	446.10±16.78 a	1558.56±41.16 a	124.41±6.85 b
12	1.43±0.16 b	3.79±0.18 a	0.63±0.02 a	88.44±3.47 a	369.0±13.56 bc	1189.3±85.25 b	142.58±5.48 a
P Value	0.012	0.0000	0.3707	0.0000	0.0009	0.0002	0.0000
R-Square (r^2)	0.84	0.95	0.34	0.94	0.16	0.12	0.92

Physiological changes in (Chl *a*= chlorophyll *a*; Chl *b*= chlorophyll *b*; β- carotene; TPC= total phenolic contents; SOD= superoxide dismutase; POD= peroxidase; CAT= catalase) of *M. oleifera* leaves under saline conditions (2, 4, 8 and 12 dS m^{-1}). Means showing different letters are statistically different ($p < 0.05$) from each other. Data was computed from five replications consisting of three plants in each replication

Fig. 1: Changes in nitrogen and crude protein concentration of *M. oleifera* leaves affected under different saline conditions (2, 4, 8 and 12 dS m^{-1}). ± vertical bars represent standard errors. Data were computed from five replications consisting of three plants in each replication

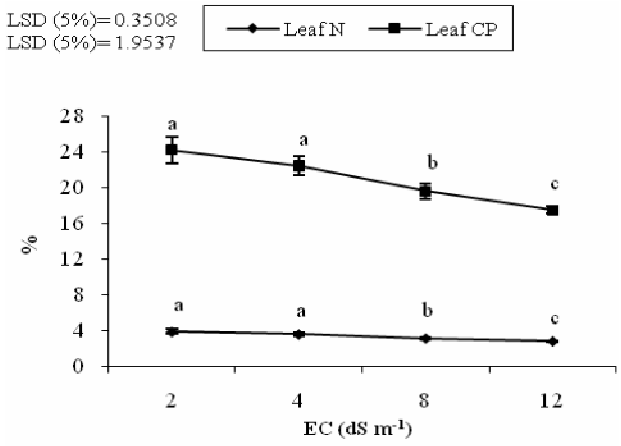
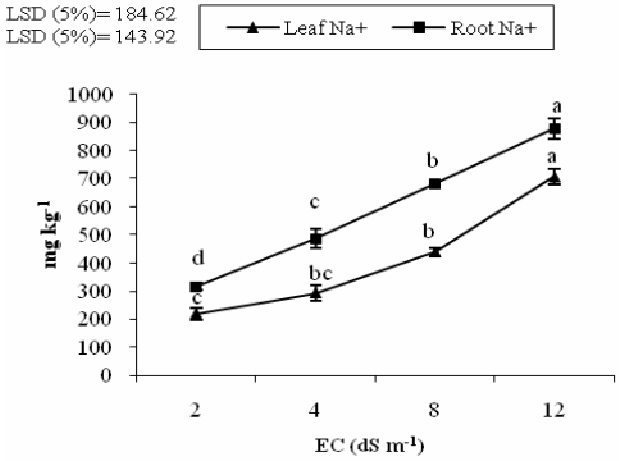


Fig. 2: Changes in sodium concentration of *M. oleifera* leaves and roots affected under different saline conditions (2, 4, 8 and 12 dS m^{-1}). ± vertical bars represent standard errors. Data were computed from five replications consisting of three plants in each replication



in both leaves and roots at 2 dS m^{-1} and 4 dS m^{-1} were statistically at par with each other (Fig. 4). It was recorded that calcium contents in moringa seedlings grown at 2 dS m^{-1} were at 4429.58 and 3021.13 ppm in both leaves and roots, respectively (Fig. 5), which were statistically at par with moringa leaves and roots grown at 4 dS m^{-1} (4415.49 & 2901.41, respectively) but a reduction of 38.16 and 28.67% was recorded in calcium contents of leaves and roots, respectively at 12 dS m^{-1}. Magnesium contents in moringa leaves and roots were found at 11940.88 and 10484 ppm, respectively, which were significantly higher than magnesium contents at 12 dS m^{-1} (6955.83 & 5882.43 ppm)

Fig. 3: Changes in phosphorous concentration of *M. oleifera* leaves and roots affected under different saline conditions (2, 4, 8 and 12 dS m^{-1}). ± vertical bars represent standard errors. Data were computed from five replications consisting of three plants in each replication

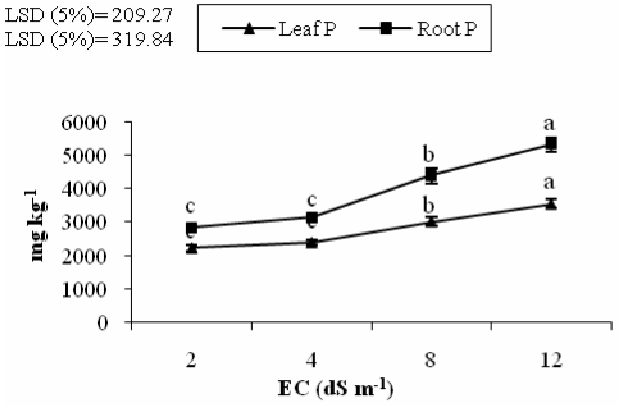
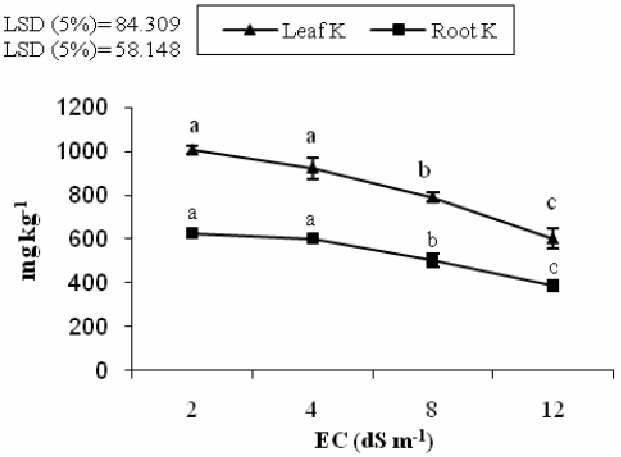


Fig. 4: Changes in potassium concentration of *M. oleifera* leaves and roots affected under different saline conditions (2, 4, 8 and 12 dS m^{-1}). ± vertical bars represent standard errors. Data were computed from five replications consisting of three plants in each replication



magnesium contents, which showed 41.74 and 43.89% reduction, respectively as compared to control (Fig 6). A highly negative correlation (r^2 = 0.99; 0.98, 0.96; 0.97, 0.99; 0.99, respectively) was found between leaf and root mineral contents (K, Ca & Mg) and increasing salinity while Na^+ and P exhibited highly positive correlation (r^2 = 0.97; 0.98, 0.99; 0.99, respectively).

DISCUSSION

Soil salinity affects the plant growth by several physiological and biochemical means like ion toxicity, osmotic stress, nutritional imbalance and biochemical and

Fig. 5: Changes in calcium concentration of *M. oleifera* leaves and roots affected under different saline conditions (2, 4, 8 and 12 dS m^{-1}). ± vertical bars represent standard errors. Data were computed from five replications consisting of three plants in each replication

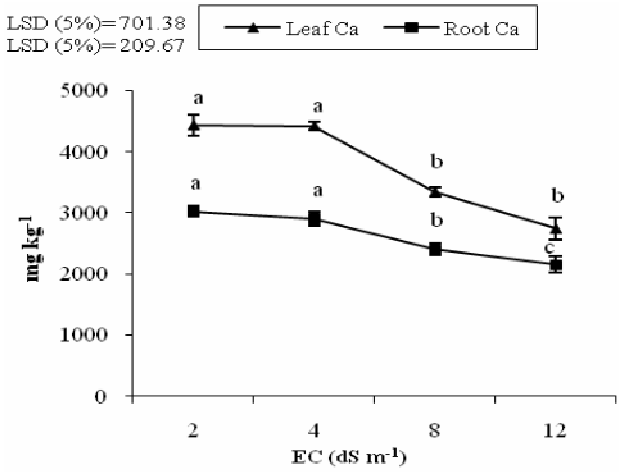
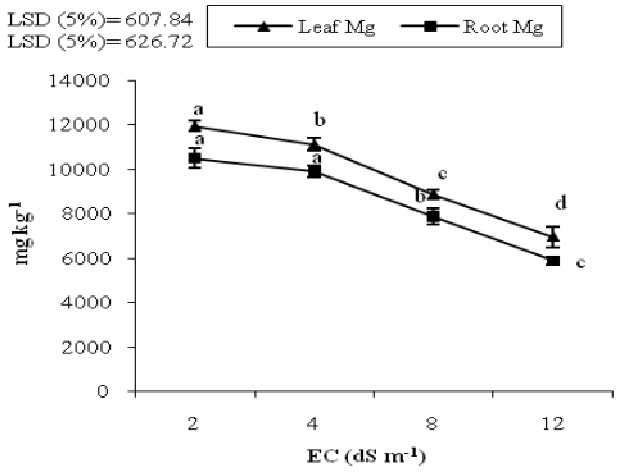


Fig. 6: Changes in magnesium concentration of *M. oleifera* leaves and roots affected under different saline conditions (2, 4, 8 and 12 dS m^{-1}). ± vertical bars represent standard errors. Data were computed from five replications consisting of three plants in each replication



physiological disorders (Kao *et al.*, 2003), which is tolerated by salt tolerant plants through osmoregulation and dehydration avoidance. Different salts play their roles by interacting with each other in accelerating soil salinity (Tester & Davenport, 2003) but the effects of sodium and chloride are very damaging to plant survival especially in woody plants by inhibiting photosynthesis (Flowers, 1988).

The excessive Na^+ inhibits the uptake of other essential minerals like K, Ca and Mg (Al-Karaki, 2000). In the present study, a positive correlation was found between Na^+ and P concentration, while K, Ca and Mg were negatively correlated with Na^+ concentration. This nutritional imbalance resulted in the reduction of number of leaves and branches and stunted shoot growth in melon (Sivritepe *et al.*, 2003), eggplant (Chartzoulakis & Loupassaki, 1997) and pepper (Chartzoulakis & Klapaki, 2000). The increase in root length at higher salinity suggests that moringa roots have tendency to expansion and elongation under stresses conditions. Increased root: shoot ratio is a mechanism of salt tolerance (Flowers & Hajibagheri, 2001; Akhtar *et al.*, 2003). We also found that under saline environment, moringa roots showed more ramification. Pandey *et al.* (1994) in *Prosopis chilensis* and Wahid *et al.* (1999) in sunflower have reported that roots elongation and expansion and increase in root score under saline environment may be attributed to the adaptive behavior of these species.

Kirst (1989) reported that NaCl accumulation in leaf cells cause decrease in photosynthesis. A reduction of 42.1% in chlorophyll *a* contents of moringa leaves was recorded at 12 dS m^{-1}, which caused a big threat to plant survival. The concentration of chlorophyll *b* contents increased with increasing salinity but chlorophyll *a* is mainly responsible for photosynthesis process, which caused the plant cells' damage (Jaleel *et al.*, 2008). It has been reported that salt excess affects the photosynthesis apparatus but the researchers are still unclear about the factors, which are responsible for the inhibition of photosynthetic activity (Steduto *et al.*, 2000) either it is due to stomatal limitations (Brugnoli & Lauteri, 1991) or non-stomatal limitations (Dunn & Neales, 1993).

Abiotic stress induces the production of reactive oxygen species (ROS) (Allen, 1995), which disturbs the cell metabolism (McCord, 2000). Salt tolerant species have a better antioxidant system, which controls the ROS production and enables the plants to survive under abiotic stress (Mittler, 2002). Higher activity rate of antioxidant enzymes (SOD, POD & CAT) under salinity is attributed to the greater ROS scavenging. In the present study, SOD, POD and CAT activities in moringa leaves were significantly higher at higher salt levels as compared to control. No previous study is available to report the salinity effects on antioxidant activity of moringa, although reports are available on plant species like cotton, in which the activity of SOD and POD increased due to built-in salt tolerance mechanism (Rajguru *et al.*, 1999). In this study, maximum SOD activity was recorded when the moringa seedlings were subjected to 8 dS m^{-1} as compared to control while at 12 dS m^{-1}, this activity significantly decreased which might be due to the damage of antioxidant system. Hernandez *et al.* (1993) and Scalet *et al.* (1995) reported increase and decrease of SOD activity of salt tolerant and sensitive pea plants, respectively. In case of CAT, maximum activity was found at 12 dS m^{-1} followed by 8 dS m^{-1}. SOD is responsible for scavenging superoxide, which results in the production of H_2O_2 and O_2. Both these ROS species are scavenged by CAT and POD sometimes with the help of phenolic

compounds (Yang & Poovaiah, 2002). The elevated activity of CAT and POD showed their ability to decompose H_2O_2 generated by the reaction of SOD on superoxide.

In conclusion, moringa can survive better at 8 dS m^{-1} with nominal reduction in its nutritional quality. Moringa antioxidant system is relatively stronger like other salinity tolerant plants, which helped it to survive under abiotic stress conditions. This aspect can be studied in future research studies to evaluate maximum survival capacity of moringa under abiotic and other environmental stress conditions. In this way, moringa cultivation practices can be elaborated more explicitly by keeping in view the soil and water conditions.

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