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SALINITY TOLERANCE AND RECLAMATION POTENTIAL OF TWO WIDELY DISTRIBUTED SUBTROPICAL TREE SPECIES

ARSLAN ARIF[1*], IRFAN ASHRAF[1], MUHAMMAD SAFDAR HUSSAIN[2] AND RASHID IQBAL[3]

[1]*Department of Forestry, Range and Wildlife Management, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Pakistan*
[2]*Department of Forestry & Range Management, Kohsar University Murree, Pakistan*
[3] *Department of Agronomy, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Pakistan*
**Corresponding author's email: arslanarif.frw.iub@gmail.com, safdarhussain@kum.edu.pk*

Abstract

Soil salinity threatens land productivity and food security, particularly in arid and semi-arid regions worldwide. Phytoremediation is a cost-effective and eco-friendly approach for mitigating salinity impacts in irrigated agricultural landscapes. In this regard, a pot experiment study was conducted to evaluate the salt tolerance and reclamation potential of two commonly planted tree species in subtropical regions globally, *Eucalyptus camaldulensis* and *Syzygium cumini*, under various salinity treatments: Control, 8 dS m^{-1}, and 16 dS m^{-1}. Results revealed that salinity negatively affected the growth and biomass production of both tested species, with more prominent effects on *S. cumini*. *E. camaldulensis* exhibited the lowest reduction in shoot height (22%) and dry biomass (40%), while *S. cumini* showed a 30% and 53% decrease in both traits, respectively, under 16 dS m^{-1} salinity. Correspondingly, increased antioxidant enzyme activities became a defense mechanism in *E. camaldulensis* as peroxidase (2.51–8.41 U mg^{-1} protein), superoxide dismutase (3.12–11.35 U mg^{-1} protein), and catalase (38–83.21 U mg^{-1} protein) at 16 dS m^{-1}. Moreover, *E. camaldulensis* reduced the highest levels of soil SAR and ECe by maintaining the optimum Na, Cl, and K ratios. Thus, the present study suggests that *E. camaldulensis* maintained higher growth and exhibited an effective antioxidant defense mechanism under various salinity levels, suggesting higher potential for the amelioration of saline soils. These results demonstrate a quick and powerful screening mechanism to assess potential reclamation tree species for saline environments.

Key-words: Pakistan, Food insecurity, Tree species, Afforestation, Semi-arid, Woody biomass production, Bioremediation

Introduction

Globally, the demands for food and other agricultural products are expanding with the increase in population, which is expected to reach around 8 billion in 2025 (Jesus *et al*., 2015). To cope with food insecurity under the Sustainable Development Goals (SDG), there is a dire need to increase the area under agricultural productivity by cultivating presently salinity-impacted unproductive lands in arid and semi-arid regions across the (Pandey & Pandey, 2023). Salinity is an ever-increasing phenomenon in irrigated agricultural landscapes and one of the most common forms of soil degradation, which is threatening land productivity, food security, and rural livelihoods, particularly in developing countries (Munns 2002; Nawar *et al*., 2015). Annually, about 1-2% of irrigated arable land is decreasing continuously and causing serious threats to the agroecosystem worldwide (Anon., 2017). About 10–20 million people's livelihoods are destroyed due to salinization and its devastating effects on the natural ecosystem (Vivekananda *et al*., 2014).

Salinity suppresses plant growth and development mechanisms (Roy *et al*., 2014) and plants respond to salt stress depends on through morphological and physiological adjustments (Ma *et al*., 2020). Under severe salt stress conditions, plants face reduced water uptake from soil resulting in osmotic stress (Flexas *et al*., 2004). Salt stress also causes ion toxicity and dehydration in the plant cell (Munns, 2002). Consequently, plant metabolic processes inhibition and growth retardation are often observed following cellular dehydration (Leprince *et al*., 2000). Soil salinity also affects photosynthesis and stomatal closure in plants resulting in the decline of photosynthetic activity (Brugnoli & Lauteri, 1991). Reduction in plant osmotic potential during salt stress may also cause a decline in cell expansion which ultimately leads to a reduction in photosynthesis activity (Lautner, 2013).

Managing salinity is one of the biggest challenges in the Indus Basin areas of Pakistan as salinity hinders crop growth and reduces agricultural productivity of food for human consumptions. Phytoremediation is a cost-effective and eco-friendly way to reclaim salt-affected soils (Zaghloul, 2020). In this scenario, plants have been proved to serve as natural pumps to draw salts and other contaminants from the soil and, in time, restore productivity of impacted lands (Nouri *et al*., 2017). Tree species are preferred over ephemeral plants due to highest biomass production and reclaiming salinity to a significant level along with providing economic returns (Prasad, 2011). Therefore, in the context where reclamation of salt-affected soils becomes crucial, exploring suitable tree species for planting in high salinity landscapes becomes imperative (Kumar *et al*., 2021).

S*yzygium cumini* is widely used medicinal tree which is commonly used for the treatment of various diseases such as diabetes. Being a multipurpose tree species, S. *cumini* has significant importance of antioxidant and antibacterial properties (Banerjee *et al*., 2005; Shafi *et al*., 2002). Extracts of *S. cumini* dry leaves is primary used in biological control of weeds in agricultural lands (Patil *et al*., 1996; Shafique *et al*., 2005). Similarly, *Eucalyptus (Sufaida)* is also important tree species because of high rate of growth, environmental adaptability and superior pulp properties throughout the world (Stape *et al*., 2004). It is an evergreen tree known to extract water from great depths relative to other species and is regarded to be salt-tolerant tree. However, the salinity tolerance potential of both tree species has not been elucidated yet for the reclamation of salinity. The present study aims to test the responses of *E. camaldulensis* and *S. cumini* to different salinity levels on growth, antioxidant enzyme activities and ion accumulation in various plant organs. Comparison of these parameters in the tested tree species may help to develop a better understanding and provide additional information on the salt tolerance mechanisms of *E. camaldulensis* and *S. cumini* for planting in salinity impaired soils.

Material and Methods

The study was carried out from September 2019 to September 2020 as a pot experiment excluding the six-month plant establishment period under greenhouse conditions at the experimental site of the Department of Forestry, The Islamia University Bahawalpur (IUB), Punjab Pakistan (29° 22 N, 71° 45 E; 126 m).

Preparation of soil and planting materials: Fine-grained soil consisting of silt and clay as per the Unified Soil Classification System was collected from the experimental site of the Department of Forestry. Subsequently, before being placed into polythene bags, the soil was sieved using 2 mm sieve and mixed with peat and sand in a 1:1 ratio. Thirty polythene bags were filled with prepared soil for establishing the plants. Similarly, seeds of *S. cumini* and *E. camaldulensis* were obtained from Punjab Forest Research Institute Faisalabad Gatwala. To reduce the risk of fungal and other disease attacks, seeds were sterilized in sodium hypochlorite (20%) for 20 minutes. Finally, three seeds were sown in each plastic bag and irrigated daily with a sprinkler to facilitate germination. Two weeks following seedling emergence, thinning was performed and one healthy plant in each bag was left to grow under ambient conditions in the greenhouse for 6 months.

Experimental procedure: The soil was again collected from the same site and prepared for pot filling. Collected soil was ground sieved through a 2 mm sieve to remove undesirable materials such as weeds and stones if any. Subsequently, three samples were taken from the sieved soil and examined to determine the current soil physio-chemical properties as well as initial level of salinity (Table 1). Following soil analysis, salinity levels were developed with Sodium chloride (NaCl) because this salt is usually present in most of the salt-affected soils. In the control treatment, EC of the soil was maintained at the original level (1.7 dS m^{-1}) while salt was calculated to develop 8 dS m^{-1} and 16 dS m^{-1} EC levels following the range of EC occurring in semiarid agricultural areas of Punjab Pakistan (Hussain *et al*., 2021). To make the solution for treatment applications, the calculated amount of NaCl salt was liquefied (dissolved) in the water, subsequently, the prepared treatment water was sprayed on the soil which was already spread on the plastic sheet. After -3 days, the soil was mixed again and analyzed to verify attainment of desired EC levels. Finally, 7 kg of soil was filled in each pot, and the six-month-old plants were transplanted into the pots. The pot experiment consisted of three treatments with five replications and was arranged using the Complete Randomized Design (CRD). The experiment was left for one-year duration under ambient conditions and irrigated regularly.

Data collection and measurements: After completing the experiment duration, growth parameters, biomass production, antioxidant activities and plant ionic and final soil analysis were performed.

Growth data and Biomass production: Plant height and root length were measured using the measuring tape while stem diameter was noted with the help of digital Vernier Caliper. For biomass measurement, all the individuals were harvested and tagged properly, subsequently, the fresh weight was recorded immediately after harvesting. For measuring the dry weight, the plants were oven-dried at (70°C) for 48 hours to assure attainment of constant weight. Finally, the plant root and shoot dry weight was noted using digital weight balance.

Determination of antioxidant enzyme activities: For measuring the antioxidant enzyme activity, one mature leaf per plant was harvested and immediately transferred to a freezer to prevent enzyme denaturing. The leaves having weight (0.5 g) from each sample were first ground in liquid nitrogen then homogenized in 5 ml of 0.2 mol 1^{-1} phosphate buffer (pH 7.8). This homogenate was centrifuged at 12,000 rpm under chilling condition (4^{0}C) for 15 minutes. Finally, the supernatant was collected in r Eppendorf and photochemical procedure was used to determine the antioxidant enzyme activities. The activity of Superoxide dismutase (SOD) was determined by measuring the ability to decrease the photochemical reduction of nitroblue tetrazolium (NBT) as described by Giannopolitis & Ries (1977), while the method demonstrated by Thomas *et al*., (1982) was used to measure the activity of Peroxidase (POD) and Catalase (CAT).

Ionic analysis: For the ionic analysis, dried leaf powder (0.1 g) was dissolved in 2 mL of 80% perchloric acid and 10 mL of concentrated H_2SO_4 and the mixture was diluted with sterile H_2O to 100 mL. The concentrations of sodium (Na), chlorine (Cl) and potassium (K) in the plant stem, root and leaves were estimated using Flame Photometer using the methods described by Wolf (1982).

Statistical analysis

The data of morphological and physiological traits were analyzed using one-way ANOVA using Statistica (Version 8.1). The significant differences between treatment means were compared using post hoc Tukey's HSD test. All tests and correlations were considered significant at (*$p<0.05$*) and all means were expressed with their standard errors (SE).

Table 1. Chemical properties of the soil used for experiment.

Soil properties	Values
SAR	7.85
ECe (dS m^{-1})	1.6
pH	7.42
Organic matter (%)	1.29
Infiltration rate (mm/hour)	25.7
Saturation (%)	30
Texture	loam

Table 2. Effect of tree species on soil EC (treatment means ±SE).

Treatments	Initial value	*E. camaldulensis*	*S. cumini*
Control	1.8 ± 0.2	^{a}1.28 ± 0.5	^{a}1.70 ± 0.4
8 dS m^{-1}	8 ± 1.1	^{b}6.25 ± 2.4	^{b}7.25 ± 2.1
16 dS m^{-1}	16 ± 2.4	^{c}14.2 ± 3.3	^{c}15.65 ± 3.4

Table 3. Effect of tree species on soil Sodium Adsorption ratio (mmol $L^{-1})^{1/2}$) (treatment means ±SE).

Treatments	Initial value	*E. camaldulensis*	*S. cumini*
Control	7.2 ± 1.4	^{a}6.45 ± 1.1	^{a}6.84 ± 1.1
8 dS m^{-1}	18.5 ± 2.7	^{b}16.6 ± 3.2	^{b}17.45 ± 4.6
16 dS m^{-1}	34.12 ± 1.5	^{c}30.12 ± 5.1	^{c}33.1 ± 6.1

Results

Results from the growth traits revealed that both tree species showed salt sensitivity, but to a different extent, by decreasing root shoot lengths and stem diameter. However, *E. camaldulensis* exhibited lower sensitivity to salt stress by maintaining the highest root shoot lengths and root collar diameter under 16 dS^{-1} EC (Fig. 1). The maximum reduction in shoot height and stem diameter of *E. camaldulensis* was observed as 22.79% and 22.69% at the highest level of salinity. On the other hand, *S. cumini* exhibited more sensitivity to salt stress by showing lower plant height and stem diameter along with severe leaf necrosis or complete mortality of some individuals. The highest reduction in shoot height (30.98%) and stem diameter (41.53%) was noted in *S. cumini* at 16 dS^{-1} EC with respect to control (Fig. 1). Similarly, shoot fresh and dry weight decreased significantly in both species particularly under the highest salt stress treatment. However, *E. camaldulensis* maintained more dry biomass and appeared to be the most tolerant tree species under moderate to high salinity (Fig. 1).

The concentration of Na^{+} and Cl^{-} increased significantly in leaf, stem, and root of both *S. cumini* and *E. camaldulensis* as a function of increasing salinity. The observed response trends of Na^{+} and Cl^{-} concentrations were generally similar for both species. However, *E. camaldulensis* performed better growth than *S. cumini* perhaps by absorbing a low concentration of Na^{+} and Cl^{-} in the various plant organs (Fig. 2). On the other hand, the highest concentration of Na^{+} and Cl^{-} ion was noted in stem and root sections of *S. cumini*, particularly under 16 dS m^{-1} (Fig. 3). Similarly, the concentration of K^{+} ions in *E. camaldulensis* was highest in leaf as compared to stem and root at 16 dS m^{-1} EC while the low leaf concentration of K^{+} ions was observed in *S. cumini* (Fig. 3).

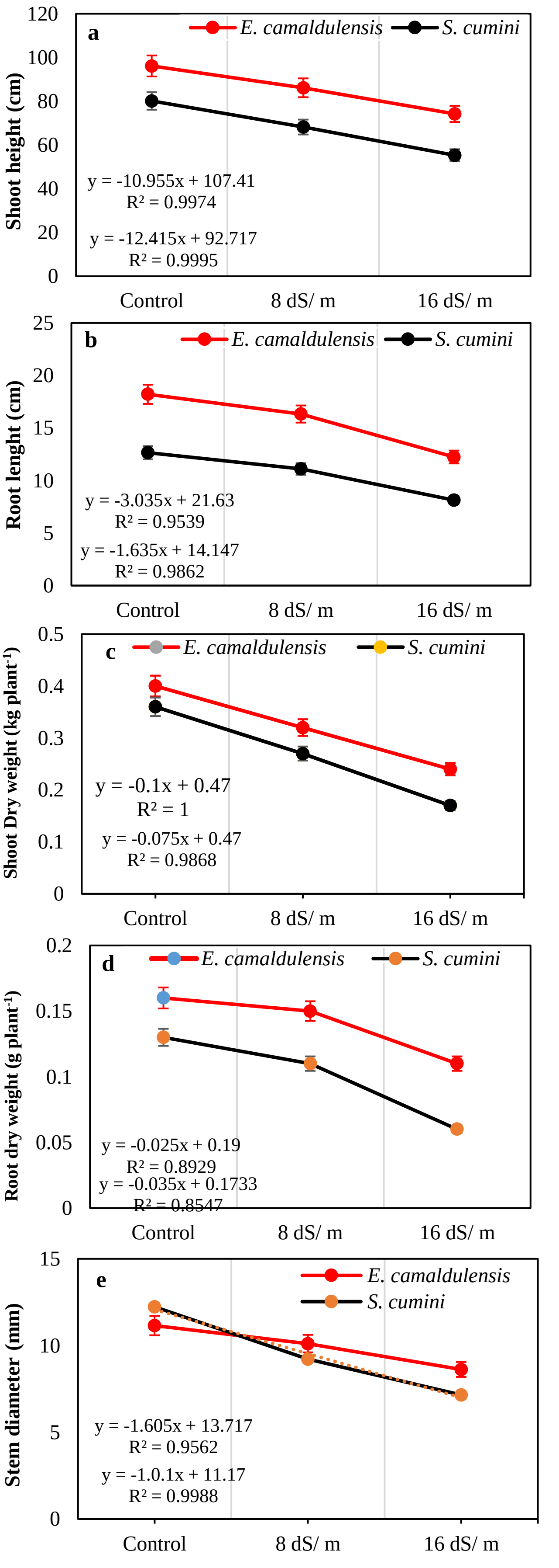

Fig. 1. Effect of salinity on growth traits and biomass production of *E. camaldulensis* and *S. cumini* (Fig; a= Shoot height, b= Root length, c= Shoot dry weight, d= Root dry weight and e= Stem diameter).

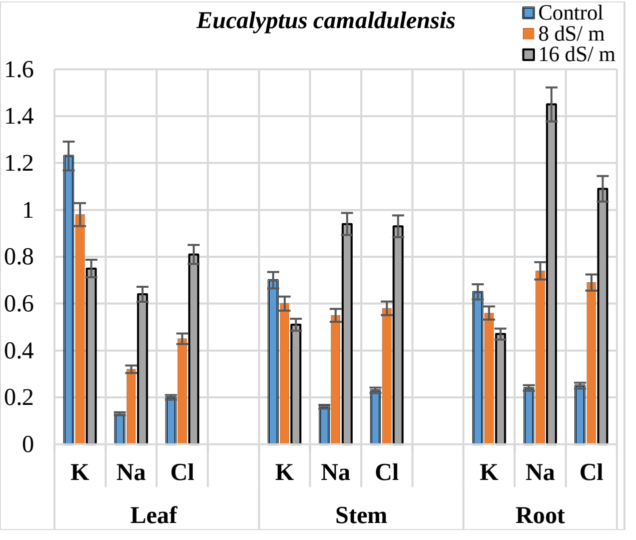

Fig. 2. Effect of salinity on ionic concentrations of leaf, stem and root in *E. camaldulensis.*

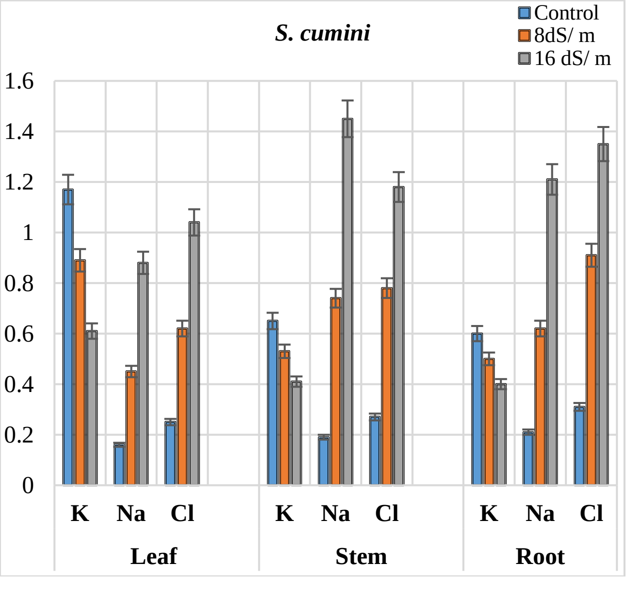

Fig. 3. Effect of salinity on ionic concentrations of leaf, stem and root in *S. cumini.*

Antioxidant enzyme activities increased significantly in both tree species with higher production of POD in *E. camaldulensis*, particularly under the high saline concentration (Fig. 4). In contrast, a low level of POD activity was observed in *S. cumini* at 16 dS m^{-1} EC. Likewise, the activity of SOD also increased simultaneously with an increase in salt stress, where *E. camaldulensis* and *S. cumini* produced 11.35 and 8.56 U mg^{-1} protein SOD activity, respectively. In the same way, a significant increase in CAT activity was noted in both tree species with greater increase in *E. camaldulensis.* and proved to be the first line of defense mechanism against salinity.

E. camaldulensis and *S. cumini* accumulated soil salts and significantly reduced levels of soil EC and SAR from their initial values showing phytoremedial potentials of these plants with *E. camaldulensis* showed the highest reduction in both soil parameters (Tables 2 and 3). It might be due to the strategy of *E. camaldulensis* allocating the extra salts to different plant organs.

Discussion

Plant growth under salt stress: Early plant growth features are known as the important indicators to assess the salt tolerance potential of tree species for planting in saline lands. In the present study, the effects of salinity were found more detrimental to the growth of *S. cumini* that started wilting from the aerial parts apparently due to sudden osmotic stress. This negative effect of salinity on growth and development has also been reported in many other tree species such as olive (Aragues *et al*., 2004) and Australian tree species (Van der Moezel *et al*., 1988). The results of the current study are supported by several previous studies indicating that the reduction in plant growth under salinity stress throughout the life cycle was closely related with decrease water potential and net CO_2 assimilation rate (Okhovatian-Ardakani *et al.*, 2010), specific ion toxicity (Mansour, 2023), nutritional disorder and osmotic stress (Karimi & Tari, 2017). Salt sensitivity of plant species can also be attributed to the production of reactive oxygen species (ROS) under extreme saline conditions that lead to cellular damage and oxidative stress (Vaidyanathan *et al*., 2003). However, salt-tolerant plants also produce hydrogen peroxide (H_2O_2) that plays a significant role in initiating a series of physiological mechanisms that increase plant salt stress tolerance (Hao *et al.*, 2021). This trend was noted in *E. camaldulensis* as this tree species showed a significant salt tolerance mechanism by maintaining optimum growth under extreme salinity. Our results are in line with a recent study ranking *E. camaldulensis* as one of the most salt-tolerant species among five multipurpose tree species (Hussain *et al.*, 2021).

Effect of salinity on ionic balance: Plant ions particularly potassium (K^+) and sodium (Na^+) play a key role in regulating the plant enzyme activities (Blumwald, 2000). Owing to similar physio-chemical properties, both ions compete for the active site of enzymes. In this study, the accumulation of Cl^- and Na^+ ions were high in roots and stem whereas K^+ was accumulated more in leaves of *E. camaldulensis*. The higher rates of root Cl^- and Na^+ accumulation in *S. cumini* and the necrosis of some plants suggest this species has less long-term tolerance to salinity than *E. camaldulensis.* It has been supported from other studies that salt-tolerant tree species exhibit more Na^+ and Cl^- sequestration in roots to control Na^+ entry into leaf tissues (Apse & Blumwald, 2007; Møller *et al*., 2009). Based on these findings, it can be concluded that a parallel mechanism is possibly operating in the roots of *E. camaldulensis* to restrict excessive sequestration of Na^+ in the leaf tissues, which is important to protect the photosynthetic activity of plant species from ion toxicity. This association between Na^+ and Cl^- exclusion (salt tolerance) was also reported by (Van der Moezel *et al*., 1989) in various tree species like Eucalyptus and Casuarina. Similarly, Aswathappa & Bachelard (1986) found that the two salt-tolerant Casuarina species stored low level of Na^+ and Cl^- in shoots as compared to the salt sensitive species indicating a higher level of salt tolerance. This salt exclusion strategy is mainly important in perennial or woody plants in which long term contact to salts can increase the absorption of toxic ions in the leaves (Munns & Tester, 2008). Moreover, Aragues *et al*., (2005) observed that after three years salt tolerance of olive plant suddenly declined due to exposure to salinity and became quite sensitive by increasing the level of toxic ions in the leaves such as Na^+. Andrés *et al*., (2014) indicated that accumulation of K^+ in the plant guard cells could adjust normal stomatal behavior which is helpful to regulate gas exchange activities such as photosynthetic and transpiration rates. Therefore, it is essential to moderate the K^+ homeostasis under salt stress in order to optimize photosynthetic rate, water potential, and cell turgidity (Marriboina *et al*., 2017). In the present study, *S. cumini* showed higher Na^+, Cl^- and less K^+ in aerial plant parts such as shoot and leaves. This suggests that *S. cumini* showed higher affinity towards Sodium and Chloride ion uptake under moderate to highly saline conditions. Therefore, this tree species cannot be grown in the salt-affected soils where the objective is the reclamation of such soils. However, the performance of *E.* under high salinity due to lower accumulation of Na, particularly due to ion compartmentation which controls the mobility of sodium ions throughout the plant.

Effect of salinity on antioxidant enzyme activities: Tree species that flourish in salty environments have a variety of physiological adaptations (Flowers & Colmer, 2008). To combat oxidative stress, these plants produce many types of enzymes (Zulfiqar & Ashraf, 2023). However, the first line of defense against salinity is thought to be the synthesis of antioxidant enzymes in reaction to salts (Numan *et al*., 2018). Different types of antioxidant enzymes are produced by salt-tolerant tree species, but the three most significant ones are catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD). Our results indicate that both tree species displayed enhanced enzymatic activity to varying degrees when exposed to extremely salty circumstances (Fig. 4). But in *E. camaldulensis*, this response was more dramatic, with considerable increases in SOD, CAT, and POD, especially below 16 dS m^{-1}. Our findings concur with those of Hussain *et al*., (2021) who identified *E. camaldulensis* as a salt-tolerant tree species with elevated SOD and POD activity up to 30 dS m^{-1} EC. SOD, one of these antioxidant enzymes, is crucial for the detoxification of reactive oxygen species (ROS), which it does by converting them into H_2O_2 (Apel & Hirt, 2004). Similar to this, CAT is essential to the detoxification of H_2O_2. In plants under stress, a rise in POD activity was observed in stems higher than roots and leaves, according to (Zhang *et al*., 2013). In line with earlier research, *E. camaldulensis* showed a considerable increase in the antioxidant enzyme activities, which may have been caused by the ability to manufacture more antioxidant enzymes in response to the 16 dS m^{-1} salt treatment.

Practical implementation: Even though *E. camaldulensis* gathered the least amount of Na in its leaves, it greatly decreased the amount of Na in the pot soil. This might be because plants use a technique to distribute salts to various organs, including the stem and roots, as was previously mentioned. These results, which are in line with earlier research, show that salt-tolerant tree species have the greatest potential for Na soil extraction (Hussain *et al*., 2021). Harvesting the aboveground biomass enables the efficient removal of Na from the soil since Na accumulates mostly in the above-ground mass of woody plants. Similar findings were made by Tanvir & Siddiqui (2010), who looked into how *Populus. deltoides'* phytoextraction technique ended up being the primary driver of the removal of soil contaminants.

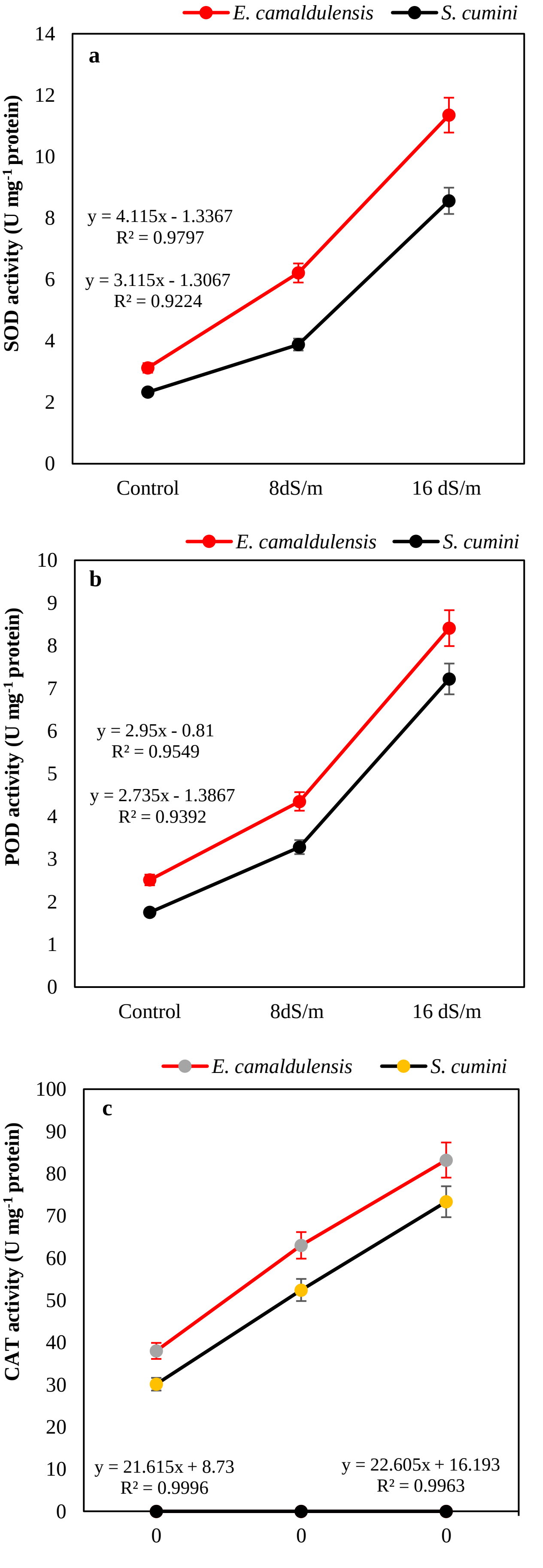

Fig. 4. Effect of salinity on antioxidant enzymes activities (Fig; a= SOD, b= POD and c=CAT).

Conclusions

In the present study, different salt treatments negatively impacted the growth and biomass production of both tree species. However, *E. camaldulensis* showed the highest plant growth indicating the most salt-tolerant tree species under 8 dS^{-1} while *S. cumini* showed significant decrease in growth with severe dieback and appeared to be the less salt-tolerant tree species. In both tree species, antioxidant enzymes (SOD, POD and CAT) activity increased by increasing salinity levels, but the significant increase was noted in *E. camaldulensis* indicating its defense mechanism against higher level of salinity. Furthermore, *E. camaldulensis* exhibited less accumulation of Na and Cl while more retention of K in different plant organs, suggesting more K ratio to Na and Cl was the important salt tolerance mechanism. On the other hand, *S. cumini*, accumulated more Na and Cl contents in leaves and stem with low K, indicating the salt sensitivity of this tree species. This comparative study identifies that plantation of *E. camaldulensis* is better than, *S. cumini* for restoration of salt-affected soils and biomass production. The expansion of agroforestry systems by planting such tolerant species is a sensible strategy would benefit the productivity of saline agriculture landscapes in order to cope with deficiency of staple foods, particularly in the countries facing severe food crises like Pakistan.

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