

## POTENTIAL OF PRIMING IN IMPROVING GERMINATION, SEEDLING GROWTH AND NUTRIENT STATUS OF *CALOTROPIS PROCERA* UNDER SALINITY

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### Abstract

In the present study, various priming methods were applied to assess the germination response, growth and nutritional value of *C. procera* under different salinity levels. In screening phase, seeds were primed with distilled water, ascorbic acid (50 mg L<sup>-1</sup>), NaCl (0.3%) and thiourea (0.3%), grown under salinity levels: 40, 80, 120, 160, 300, 340, 380 and 420 mM NaCl. No germination was observed above 160 mM of NaCl and salinity up to this level was selected for further experiment. In the second phase, primed seeds were germinated in pots under controlled conditions to study the growth and nutritional value of *C. procera*. The results indicated that ascorbic acid (50 mgL<sup>-1</sup>) at 120 mM salinity level was the most effective treatment for growth attributes: shoot and root length (15.10 cm, 16.30cm), shoot fresh and dry weight (8.05 g, 1.61 g), root fresh and dry weight (6.06 g, 1.73 g), respectively. Chlorophyll content was higher at 120 mM salinity level with 0.3% of thiourea. Nutrients like potassium (K<sup>+</sup>), cadmium (Cd<sup>2+</sup>) and zinc (Zn<sup>2+</sup>) were greater at 120 mM salinity level with ascorbic acid while Na<sup>+</sup> concentration was highest at 160 mM salinity level in both shoot and root (9118 ppm, 2657 ppm) with 0.3% of NaCl priming as compared to other nutrients. It was concluded that under high salt stress, priming with ascorbic acid could enhance seed germination, growth behavior, and nutritional value compared to other treatments by triggering antioxidant defense mechanisms in the seed of *C. procera*.

**Key words:** *Calotropis procera*, Defense mechanism, Germination, Nutritional value, Seed priming.

### Introduction

Germination is considered as a crucial factor in controlling population dynamics. Seed germination is of prime importance for the persistence of species including weeds and crop plants (Yang *et al.*, 2008). According to United Nations Environment Program, desertification has become a major environmental issue and has increased upto many folds in recent 20 years around the globe (Zare *et al.*, 2011; Farajollahi *et al.*, 2014). Vegetation protects the soil, especially against wind erosion in the desert ecosystem as desert plants, have the multifaceted tactics that help them to grow in xerophytic conditions (Yates *et al.*, 2000; Yasin *et al.*, 2016).

Salinity has adverse effects on vegetation production especially in desertic climate around the globe (Bijeh, 2012; Khan *et al.*, 2013). Ion toxicity because of accumulation of various salts along with the imbalanced amount of Na<sup>+</sup> and Cl<sup>-</sup> results in oxidative stress which ultimately reduces seed germination, plant growth and its vigor (Amor *et al.*, 2005; Bijeh, 2012). Moreover, seeds exaggerated by salinity, drought, and toxicity of different ions results in a series of metabolic changes and cause complete failure of germination (Ashraf & Foolad, 2005; Ghoulam *et al.*, 2002). Nowadays, seed priming has become common practice to increase the rate and uniformity of seed germination and emergence. It is an easy and low-cost pre-sowing germination treatment which reduces the poisonous impacts of toxic salts, decrease the problem of salinity in plants and improve seed germination and growth under salinity stress (Pastor *et al.*, 2013; Farooq *et al.*, 2019).

*Calotropis procera* also known as giant milkweed because of the production of latex is a member of the

Apocynaceae family. It is a perennial shrub which attains a height of 2m (Rathore & Meena, 2010) and considered as a weed in most countries of the world (Farajollahi *et al.*, 2014; Sayed & Mohamed, 2000). *C. procera* is most commonly found in tropics, subtropical Africa and the Middle East along with some Asian countries like Pakistan, India, Afghanistan, and Iran (Batoool *et al.*, 2020, Azhar *et al.*, 2014; Sharma *et al.*, 2012; Boutraa, 2010).

*C. procera* has xerophytic characteristics with high nutrients and excellent medicinal properties and can tolerate harsh conditions of salinity and drought. Most of the time it is used as allopathic medicine for the treatment of various diseases due to its active pharmacological actions (Akindele *et al.*, 2017). The bark, root, and leaves of this plant are consumed as medication for curing ulcer, liver, spleen, malaria, piles, bronchitis asthma, tumor and cancer (Chan *et al.*, 2017). Moreover, the species can be optionally used as livestock feed during a drought in arid areas around the globe (Boutraa, 2010).

Previously, few studies about the effect of salinity on seed germination and seedling establishment of *C. procera* were carried out (Chan *et al.*, 2017; Farajollahi *et al.*, 2014; Taghvaei *et al.*, 2012; Boutraa, 2010) but seed priming is the most promising method to pre-condition stress tolerance in vegetation before germination. The present research work was designed to evaluate germination response, growth behavior and nutritive value of *C. procera* under the combined effect of seed priming and different salt concentrations and to evaluate the priming technique which reduces the adverse effects of salinity on seed germination, growth and nutritive quality of *C. procera*.

## Materials and Methods

**Plant material:** *Calotropis procera* seeds were collected from Lal Sohanra National Park, Bahawalpur, Pakistan. The research was conducted in two phases i.e. in lab and greenhouse of the respective department in 2017 to evaluate the response of *Calotropis* to salinity.

**Culture conditions:** Different salinity levels i.e. 40, 80, 120, 160, 300, 340, 380 and 420 mM NaCl were developed by adding the respective quantity of salt. Before priming, seeds were surface-sterilized for 5 minutes with sodium hypochlorite solution, rinsed with distilled water and then air-dried for 28 hours. Treatments included control without any treatment, seeds primed with distilled water (hydropriming), ascorbic acid 50 mgL<sup>-1</sup> (osmopriming), NaCl (halopriming), and thiourea 0.3% (osmopriming) each for 24 h. Petri dishes covered with a double layer of moistened filter papers were used for seed germination. A climate chamber with 22°C temperature was used for the germination test. Nine seeds were placed in each Petri plate and moistened with 1 ml saline solution at regular intervals based on its dry condition. Germination was observed on the first day and continued till 15 days. No germination was noticed at >160 mM salinity level. Therefore, up to 160 mM salinity levels were further selected for the greenhouse study.

**Evaluation of plant growth:** The combined effect of different priming techniques and salinity levels on growth and nutritional quality of *C. procera* was observed in the glasshouse experiment. After filling the pots a total of 9 seeds treated with priming material were sown in each pot. Irrigation was applied according to the requirements of plants. After three weeks of emergence, 3 healthy plants were taken for further study and the declared levels of salt stress were prompted.

**Morphological characters:** Plants were harvested after 3 months of stress for measuring morphological characters such as fresh and dry weights of root and shoot, root and shoot length, leaves per plant and roots. Spectrophotometer was used to determine the chlorophyll content (Sumanta *et al.*, 2014).

**Mineral analysis:** Nutrients like Na and K were investigated by adopting the dry ash procedure explained by Chen *et al.*, (2005) while Cu, Zn, Cd, and Mn were estimated by following the standard protocol described by Fontes *et al.*, (2014).

## Statistical analysis

Data regarding growth and nutritional parameters were analyzed using statistics 8.1 statistical software. Means of all parameters were compared through Tukey's least significant difference test at 5% probability level.

## Results

**Germination (%) of *C. procera*:** The results showed that germination and growth of *C. procera* were initially

increased upto 120 mM salinity level and then started decreasing. The germination percentage was significantly ( $p < 0.05$ ) influenced by all priming treatments. Osmopriming with ascorbic acid (50 mgL<sup>-1</sup>) and thiourea (0.3%) were found to be the most effective treatments under salinity compared to control, hydro- and halopriming. Moreover, osmopriming with ascorbic acid performed best at all salinity levels and demonstrated maximum germination: 80%, 89%, 96%, and 68% respectively, against all salinity levels when compared with other treatments (Fig. 1).

**Growth behavior and Chlorophyll content:** The growth parameters were also significantly ( $p < 0.05$ ) affected by all priming treatments. Highest shoot fresh and dry weights, shoot length, leaves per plant and roots were found at 120 mM stress for osmopriming with ascorbic acid. However, maximum root length was computed at 120 mM salt stress for 0.3% of thiourea with an increase of 57.79%, 38.48%, and 77.17% compared with 40, 80 and 160 mM salt stress (Table 1). Likely, the highest chlorophyll content was measured at 120 mM salinity level with osmopriming of thiourea and it was higher by 9.13% and 19.30% when compared with all other treatments. The chlorophyll content was 9.62% and 12.55% less under 80 and 40 mM stress levels respectively with the osmopriming of thiourea compared to 120 mM salinity. A further reduction of 28.45% was noticed for osmopriming with thiourea and 29.57% with ascorbic acid at 160 mM NaCl (Fig. 2).

**Mineral contents of *C. procera*:** The mineral contents in shoot portion of *C. procera* at different salinity levels were in the order 160 mM > 40 mM > 80 mM > 120 mM for osmopriming with ascorbic acid and thiourea except sodium (Na<sup>+</sup>) which was maximum at 160 mM salt stress with halo priming. Highest potassium (K<sup>+</sup>), cadmium (Cd<sup>2+</sup>) and zinc (Zn<sup>2+</sup>) contents in shoot were observed for osmopriming with ascorbic acid while copper (Cu<sup>2+</sup>) and manganese (Mn<sup>2+</sup>) with thiourea priming at all salinity levels that were significantly different ( $p < 0.05$ ) when compared with other priming treatments including control. The highest concentration of K<sup>+</sup> (5865.3 ppm), Cd<sup>2+</sup> (0.65 ppm) and Zn<sup>2+</sup> (42.1 ppm) in shoot portion was found for ascorbic acid priming while Cu<sup>2+</sup> and Mn<sup>2+</sup> showed highest concentration (40.2 ppm and 22.3 ppm) with thiourea at 120 mM salt stress. However, maximum Na<sup>+</sup> concentration was measured at 160 mM salt stress and was increased by 12.43%, 8.43% and 3% respectively, when compared with 40 mM, 80 mM and 120 mM salt stress with halopriming (Table 2). Similarly, the concentration of all nutrients in roots increased with increasing salt stress and was significantly influenced by all priming treatments ( $p < 0.05$ ). The maximum K<sup>+</sup> (1825 ppm), Cd<sup>2+</sup> (0.84 ppm), Cu<sup>2+</sup> (14.06 ppm) and Zn<sup>2+</sup> (5.92 ppm) were found with ascorbic acid priming at 120 mM salt stress in roots whereas Na<sup>+</sup> with 0.3% halopriming at 160 mM salt stress. Manganese (Mn<sup>2+</sup>) was highest for osmopriming with thiourea compared to other treatments with maximum concentration at 120 mM and minimum at 40 mM NaCl stress (Table 3).

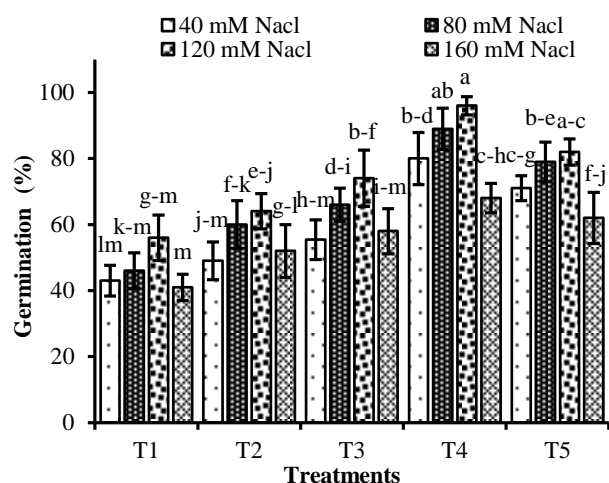


Fig. 1. Germination percentage response of *C. procera* against different priming techniques and salinity levels. T<sub>1</sub>: Control; T<sub>2</sub>: Hydropriming; T<sub>3</sub>: Halopriming (0.3% NaCl); T<sub>4</sub>: Osmopriming (50 mgL<sup>-1</sup> ascorbic acid); T<sub>5</sub>: Osmopriming (0.3% thiourea).

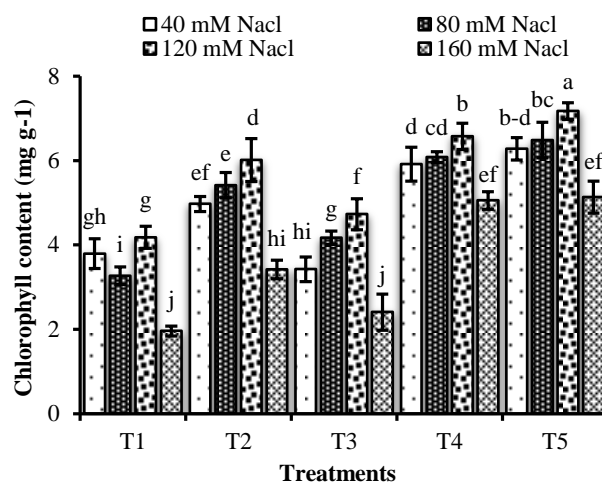


Fig. 2. Chlorophyll Content of *C. procera* against different priming techniques and salinity levels. T<sub>1</sub>: Control; T<sub>2</sub>: Hydropriming; T<sub>3</sub>: Halopriming (0.3% NaCl); T<sub>4</sub>: Osmopriming (50 mgL<sup>-1</sup> ascorbic acid); T<sub>5</sub>: Osmopriming (0.3% thiourea).

Table 1. Effect of different priming treatments on growth behavior of *C. procera* under various salinity levels.

Salinity levels	Treatments	SFW (g)	RFW (g)	SDW (g)	RDW (g)	SL (cm)	RL (cm)	Number of leaves	Number of roots
40 (mM NaCl)	T <sub>1</sub>	1.30 <sup>i</sup>	1.23 <sup>ef</sup>	0.54 <sup>gh</sup>	0.24 <sup>fg</sup>	5.23 <sup>g-j</sup>	1.90 <sup>g</sup>	4 <sup>ij</sup>	3 <sup>e-h</sup>
	T <sub>2</sub>	1.42 <sup>h-i</sup>	1.31 <sup>ef</sup>	0.63 <sup>e-h</sup>	0.59 <sup>c-f</sup>	2.81 <sup>j</sup>	2.80 <sup>g</sup>	6 <sup>h-j</sup>	6 <sup>a-c</sup>
	T <sub>3</sub>	2.25 <sup>f-i</sup>	2.29 <sup>de</sup>	0.60 <sup>f-h</sup>	0.38 <sup>e-g</sup>	6.10 <sup>e-i</sup>	5.60 <sup>ef</sup>	11 <sup>e-f</sup>	4.67 <sup>a-f</sup>
	T <sub>4</sub>	5.23 <sup>bc</sup>	2.38 <sup>de</sup>	1.30 <sup>a-c</sup>	0.93 <sup>cd</sup>	7 <sup>d-i</sup>	8.67 <sup>cd</sup>	18.45 <sup>ab</sup>	6.33 <sup>ab</sup>
	T <sub>5</sub>	3.60 <sup>d-f</sup>	2.22 <sup>de</sup>	1.14 <sup>b-d</sup>	0.41 <sup>e-g</sup>	12 <sup>b</sup>	10.33 <sup>bc</sup>	15.29 <sup>bd</sup>	3.33 <sup>d-h</sup>
80 (mM NaCl)	T <sub>1</sub>	2.73 <sup>d-h</sup>	2.73 <sup>cd</sup>	0.60 <sup>f-h</sup>	0.53 <sup>d-g</sup>	7.07 <sup>d-i</sup>	3.60 <sup>fg</sup>	6.16 <sup>gh</sup>	3.67 <sup>c-h</sup>
	T <sub>2</sub>	3.77 <sup>de</sup>	2.40 <sup>de</sup>	0.73 <sup>d-g</sup>	0.23 <sup>fg</sup>	5 <sup>i-j</sup>	4.13 <sup>fg</sup>	7 <sup>g-i</sup>	4.67 <sup>a-f</sup>
	T <sub>3</sub>	3.90 <sup>cd</sup>	2.23 <sup>de</sup>	1.07 <sup>b-e</sup>	0.70 <sup>c-e</sup>	8.33 <sup>c-e</sup>	7.67 <sup>de</sup>	13.67 <sup>c-e</sup>	1.33 <sup>h</sup>
	T <sub>4</sub>	5.93 <sup>b</sup>	5.20 <sup>ab</sup>	1.21 <sup>bc</sup>	1.40 <sup>ab</sup>	10.78 <sup>bc</sup>	8.99 <sup>cd</sup>	18.51 <sup>ab</sup>	5.60 <sup>a-d</sup>
	T <sub>5</sub>	3.27 <sup>d-g</sup>	3.60 <sup>bc</sup>	1.14 <sup>b-d</sup>	0.30 <sup>e-g</sup>	8.33 <sup>c-e</sup>	11.77 <sup>b</sup>	17 <sup>a-c</sup>	5.33 <sup>a-e</sup>
120 (mM NaCl)	T <sub>1</sub>	2.50 <sup>e-i</sup>	2.23 <sup>d-e</sup>	0.32 <sup>gh</sup>	0.30 <sup>e-g</sup>	6.03 <sup>e-i</sup>	7.20 <sup>de</sup>	10 <sup>fg</sup>	4.33 <sup>b-g</sup>
	T <sub>2</sub>	1.30 <sup>i</sup>	3.63 <sup>bc</sup>	0.60 <sup>f-h</sup>	0.50 <sup>d-g</sup>	7.60 <sup>d-h</sup>	7.23 <sup>de</sup>	14.33 <sup>b-e</sup>	5.67 <sup>a-d</sup>
	T <sub>3</sub>	2.87 <sup>d-g</sup>	1.90 <sup>d-f</sup>	1.32 <sup>a-c</sup>	0.13 <sup>g</sup>	7.67 <sup>d-g</sup>	12.00 <sup>b</sup>	14 <sup>c-e</sup>	3.33 <sup>d-h</sup>
	T <sub>4</sub>	8.05 <sup>a</sup>	6.06 <sup>a</sup>	1.61 <sup>a</sup>	1.73 <sup>a</sup>	15.10 <sup>a</sup>	12.47 <sup>b</sup>	19.33 <sup>a</sup>	7.33 <sup>a</sup>
	T <sub>5</sub>	3.23 <sup>d-g</sup>	4.03 <sup>b</sup>	1.50 <sup>ab</sup>	1 <sup>bc</sup>	9.20 <sup>cd</sup>	16.30 <sup>a</sup>	17.67 <sup>b</sup>	5 <sup>a-f</sup>
160 (mM NaCl)	T <sub>1</sub>	1.89 <sup>g-i</sup>	0.92 <sup>f</sup>	0.26 <sup>h</sup>	0.26 <sup>e-g</sup>	5.20 <sup>h-j</sup>	3.77 <sup>fg</sup>	2.67 <sup>j</sup>	2.23 <sup>gh</sup>
	T <sub>2</sub>	2.99 <sup>d-g</sup>	0.97 <sup>f</sup>	0.39 <sup>gh</sup>	0.23 <sup>fg</sup>	5.27 <sup>f-i</sup>	3.20 <sup>fg</sup>	4.67 <sup>ij</sup>	5 <sup>a-f</sup>
	T <sub>3</sub>	3.20 <sup>d-g</sup>	1.97 <sup>d-f</sup>	0.70 <sup>d-h</sup>	0.43 <sup>e-g</sup>	7.70 <sup>d-f</sup>	8.27 <sup>cd</sup>	7.33 <sup>g-i</sup>	4.67 <sup>a-f</sup>
	T <sub>4</sub>	3.97 <sup>cd</sup>	2.97 <sup>b-d</sup>	1.42 <sup>a-c</sup>	0.50 <sup>d-g</sup>	7.97 <sup>de</sup>	8.91 <sup>cd</sup>	12.15 <sup>d-f</sup>	5.09 <sup>a-f</sup>
	T <sub>5</sub>	3.23 <sup>d-g</sup>	2.23 <sup>de</sup>	1 <sup>c-f</sup>	0.27 <sup>e-g</sup>	7.90 <sup>de</sup>	9.20 <sup>cd</sup>	9 <sup>f-h</sup>	2.67 <sup>f-h</sup>

T<sub>1</sub>: Control; T<sub>2</sub>: Hydropriming; T<sub>3</sub>: Halopriming (0.3% NaCl); T<sub>4</sub>: Osmopriming (50 mgL<sup>-1</sup> ascorbic acid); T<sub>5</sub>: Osmopriming (0.3% thiourea); SWF: Shoot fresh weight; RFW: Root fresh weight; SDW: Shoot dry weight; RDW: Root dry weight; SL: Shoot length; RL: Root Length.

**Discussion**

The present study investigated whether seed priming improved plant resistance to mitigate salt stress in *C. procera* under various salinity levels. Higher salt stress significantly reduced the germination percentage (Fig. 1), seedling growth (Table 1), chlorophyll contents (Fig. 2) including mineral contents in the shoot (Table 2) and root of *C. procera* (Table 3). Various physiological disturbances caused by salinity include low water availability is the main reason for reduced plant growth and nutrients availability (Azooz *et al.*, 2013). Seed priming is one of the most efficient and economical techniques to mitigate the adverse effects of salinity (Matias *et al.*, 2018; Farooq *et al.*, 2019).

The germination percentage increased initially up to 120 mM salinity level and then decreased with further increase in salinity under all priming methods. This is due to the reduction in osmosis potential of solution and higher toxic ions reducing imbibition, thereby reducing germination (Sun *et al.*, 2000). Similarly, the growth attributes of *Calotropis* plants were decreased at higher salinity levels but improved with hydropriming and osmopriming (Taghvaei *et al.*, 2012). According to Ibrahim (2013), the biomass and nutrients in *Calotropis procera* decreased at higher salinity stress. This is due to the accumulation of a higher amount of salts in leaf, induce toxicity and ultimately result in leaf shedding and growth inhibition (Munns, 2002).

**Table 2. Effect of different priming treatments on mineral contents of shoot of *C. procera* under various salinity levels.**

Salinity levels	Treatments	Na <sup>+</sup> (mg kg <sup>-1</sup> )	K <sup>+</sup> (mg kg <sup>-1</sup> )	Cd <sup>2+</sup> (mg kg <sup>-1</sup> )	Cu <sup>2+</sup> (mg kg <sup>-1</sup> )	Mn <sup>2+</sup> (mg kg <sup>-1</sup> )	Zn <sup>2+</sup> (mg kg <sup>-1</sup> )
<b>40</b> (mMNaCl)	T <sub>1</sub>	2240.3 <sup>n</sup>	2163 <sup>no</sup>	0.28 <sup>cd</sup>	13.3 <sup>lm</sup>	16 <sup>cd</sup>	4.5 <sup>k</sup>
	T <sub>2</sub>	2662 <sup>k-m</sup>	2617 <sup>kl</sup>	0.39 <sup>b</sup>	19.5 <sup>f-i</sup>	8.6 <sup>k-n</sup>	11 <sup>ij</sup>
	T <sub>3</sub>	8117.3 <sup>d</sup>	3375.4 <sup>gh</sup>	0.16 <sup>fg</sup>	15.4 <sup>j-k</sup>	12.4 <sup>g-i</sup>	15.6 <sup>gh</sup>
	T <sub>4</sub>	<sup>g</sup> 7173.6	5433 <sup>b</sup>	0.59 <sup>a</sup>	23.2 <sup>de</sup>	11.5 <sup>h-j</sup>	37.1 <sup>b</sup>
	T <sub>5</sub>	4913 <sup>j</sup>	4659 <sup>de</sup>	0.42 <sup>b</sup>	31.5 <sup>b</sup>	7.5 <sup>mn</sup>	27 <sup>d</sup>
<b>80</b> (mMNaCl)	T <sub>1</sub>	2414.7 <sup>mn</sup>	2251.6 <sup>mn</sup>	0.29 <sup>c</sup>	14.7 <sup>kl</sup>	18.1 <sup>c</sup>	4.8 <sup>k</sup>
	T <sub>2</sub>	2779 <sup>kl</sup>	2829.6 <sup>jk</sup>	0.41 <sup>b</sup>	20.9 <sup>e-h</sup>	9.4 <sup>j-m</sup>	12.6 <sup>hi</sup>
	T <sub>3</sub>	8409.6 <sup>c</sup>	3416.3 <sup>gh</sup>	0.17 <sup>e-g</sup>	17.5 <sup>i-k</sup>	13 <sup>f-h</sup>	17.1 <sup>fg</sup>
	T <sub>4</sub>	<sup>f</sup> 7452.5	5607 <sup>ab</sup>	0.61 <sup>a</sup>	25.4 <sup>cd</sup>	13.8 <sup>e-g</sup>	40.2 <sup>ab</sup>
	T <sub>5</sub>	5143.2 <sup>ij</sup>	4707 <sup>d</sup>	0.42 <sup>b</sup>	33.1 <sup>b</sup>	9.9 <sup>l</sup>	28.9 <sup>cd</sup>
<b>120</b> (mMNaCl)	T <sub>1</sub>	2625 <sup>lm</sup>	2484 <sup>lm</sup>	0.30 <sup>c</sup>	18.7 <sup>g-i</sup>	22.3 <sup>a</sup>	5.2 <sup>k</sup>
	T <sub>2</sub>	2866.3 <sup>kl</sup>	3209.7 <sup>hi</sup>	0.46 <sup>b</sup>	22.4 <sup>d-f</sup>	10.2 <sup>jk</sup>	13.9 <sup>hi</sup>
	T <sub>3</sub>	8853.3 <sup>b</sup>	3611 <sup>g</sup>	0.21 <sup>d-g</sup>	21.5 <sup>e-g</sup>	20.3 <sup>b</sup>	19 <sup>f</sup>
	T <sub>4</sub>	<sup>e</sup> 7820.6	5865.3 <sup>a</sup>	0.65 <sup>a</sup>	27.5 <sup>c</sup>	15.1 <sup>de</sup>	42.1 <sup>a</sup>
	T <sub>5</sub>	5347.5 <sup>i</sup>	5056.6 <sup>c</sup>	0.44 <sup>b</sup>	40.2 <sup>a</sup>	10.8 <sup>h-j</sup>	31.3 <sup>c</sup>
<b>160</b> (mMNaCl)	T <sub>1</sub>	2776.7 <sup>kl</sup>	1956.7 <sup>o</sup>	0.22 <sup>c-g</sup>	10.4 <sup>m</sup>	14.9 <sup>d-f</sup>	4.3 <sup>k</sup>
	T <sub>2</sub>	2892 <sup>k</sup>	2481 <sup>lm</sup>	0.23 <sup>c-f</sup>	12.3 <sup>lm</sup>	6.9 <sup>n</sup>	9 <sup>j</sup>
	T <sub>3</sub>	9118 <sup>a</sup>	3056.4 <sup>ij</sup>	0.15 <sup>g</sup>	13.7 <sup>l</sup>	10.4 <sup>i-k</sup>	13.2 <sup>hi</sup>
	T <sub>4</sub>	<sup>de</sup> 7896.2	4414.2 <sup>ef</sup>	0.29 <sup>c</sup>	18.3 <sup>h-j</sup>	8 <sup>l-n</sup>	30.5 <sup>c</sup>
	T <sub>5</sub>	5591 <sup>h</sup>	4236.5 <sup>f</sup>	0.25 <sup>c-e</sup>	22.9 <sup>de</sup>	7.1 <sup>n</sup>	23.7 <sup>e</sup>

T<sub>1</sub>: Control; T<sub>2</sub>: Hydropriming; T<sub>3</sub>: Halopriming (0.3% NaCl); T<sub>4</sub>: Osmopriming (50 mgL<sup>-1</sup> ascorbic acid); T<sub>5</sub>: Osmopriming (0.3% thiourea)

**Table 3. Effect of different priming treatments on mineral contents of root of *C. procera* under various salinity levels.**

Salinity levels	Treatments	Na <sup>+</sup> (mg kg <sup>-1</sup> )	K <sup>+</sup> (mg kg <sup>-1</sup> )	Cd <sup>2+</sup> (mg kg <sup>-1</sup> )	Cu <sup>2+</sup> (mg kg <sup>-1</sup> )	Mn <sup>2+</sup> (mg kg <sup>-1</sup> )	Zn <sup>2+</sup> (mg kg <sup>-1</sup> )
<b>40</b> (mMNaCl)	T <sub>1</sub>	648.3 <sup>o</sup>	445.2 <sup>i</sup>	0. <sup>m</sup>	1.93 <sup>l</sup>	1.32 <sup>k</sup>	0.46 <sup>j</sup>
	T <sub>2</sub>	817 <sup>l-n</sup>	582.6 <sup>e-i</sup>	0.15 <sup>lm</sup>	3.14 <sup>k</sup>	2.68 <sup>h</sup>	0.89 <sup>ef</sup>
	T <sub>3</sub>	2078 <sup>c</sup>	1042.7 <sup>d</sup>	0.18 <sup>i-l</sup>	4.55 <sup>ij</sup>	4.17 <sup>fg</sup>	0.82 <sup>f-h</sup>
	T <sub>4</sub>	1092.6 <sup>jk</sup>	1361.3 <sup>bc</sup>	0.71 <sup>c</sup>	7.45 <sup>g</sup>	4.57 <sup>d-f</sup>	1.15 <sup>cd</sup>
	T <sub>5</sub>	1488 <sup>fg</sup>	653.6 <sup>e-h</sup>	0.31 <sup>ef</sup>	5.33 <sup>hi</sup>	5.29 <sup>c</sup>	0.71 <sup>g-i</sup>
<b>80</b> (mMNaCl)	T <sub>1</sub>	713 <sup>no</sup>	496 <sup>hi</sup>	0.14 <sup>lm</sup>	3.5 <sup>k</sup>	1.51 <sup>jk</sup>	0.51 <sup>j</sup>
	T <sub>2</sub>	883.7 <sup>lm</sup>	652 <sup>e-i</sup>	0.17 <sup>j-m</sup>	6.14 <sup>h</sup>	2.86 <sup>h</sup>	0.94 <sup>ef</sup>
	T <sub>3</sub>	2213.6 <sup>c</sup>	1152.3 <sup>d</sup>	0.21 <sup>h-k</sup>	8.21 <sup>g</sup>	4.33 <sup>ef</sup>	0.84 <sup>e-h</sup>
	T <sub>4</sub>	1205 <sup>ij</sup>	1487.6 <sup>b</sup>	0.76 <sup>bc</sup>	11.85 <sup>b</sup>	4.79 <sup>c-e</sup>	1.24 <sup>c</sup>
	T <sub>5</sub>	1596 <sup>ef</sup>	663 <sup>e-h</sup>	0.35 <sup>e</sup>	9.48 <sup>ef</sup>	6.1 <sup>b</sup>	0.78 <sup>f-h</sup>
<b>120</b> (mMNaCl)	T <sub>1</sub>	887.6 <sup>lm</sup>	549 <sup>f-i</sup>	0.22 <sup>h-j</sup>	5.85 <sup>h</sup>	2.07 <sup>i</sup>	0.69 <sup>hi</sup>
	T <sub>2</sub>	977 <sup>kl</sup>	714 <sup>ef</sup>	0.24 <sup>g-i</sup>	9.28 <sup>ef</sup>	3.74 <sup>g</sup>	0.99 <sup>de</sup>
	T <sub>3</sub>	2414.3 <sup>b</sup>	1205.6 <sup>cd</sup>	0.28 <sup>fg</sup>	10.8 <sup>c-d</sup>	5.08 <sup>cd</sup>	0.87 <sup>e-g</sup>
	T <sub>4</sub>	1395 <sup>gh</sup>	1825 <sup>a</sup>	0.84 <sup>a</sup>	14.06 <sup>a</sup>	5.92 <sup>b</sup>	2.16 <sup>a</sup>
	T <sub>5</sub>	1787 <sup>d</sup>	736 <sup>c</sup>	0.47 <sup>d</sup>	11.63 <sup>bc</sup>	6.59 <sup>a</sup>	1.28 <sup>c</sup>
<b>160</b> (mMNaCl)	T <sub>1</sub>	790.3 <sup>m-o</sup>	502 <sup>g-i</sup>	0.16 <sup>k-m</sup>	4.14 <sup>jk</sup>	1.93 <sup>ij</sup>	0.61 <sup>ij</sup>
	T <sub>2</sub>	890.7 <sup>lm</sup>	667.7 <sup>e-g</sup>	0.21 <sup>h-k</sup>	8.49 <sup>fg</sup>	3.1 <sup>h</sup>	1.18 <sup>c</sup>
	T <sub>3</sub>	2657 <sup>a</sup>	1096.5 <sup>d</sup>	0.25 <sup>f-h</sup>	9.72 <sup>c</sup>	4.46 <sup>ef</sup>	0.93 <sup>ef</sup>
	T <sub>4</sub>	1303 <sup>hi</sup>	1794 <sup>a</sup>	0.81 <sup>ab</sup>	12.56 <sup>b</sup>	4.85 <sup>c-e</sup>	1.67 <sup>b</sup>
	T <sub>5</sub>	1713 <sup>de</sup>	692.3 <sup>ef</sup>	0.42 <sup>d</sup>	10.1 <sup>de</sup>	5.30 <sup>c</sup>	1.21 <sup>c</sup>

T<sub>1</sub>: Control; T<sub>2</sub>: Hydropriming; T<sub>3</sub>: Halopriming (0.3% NaCl); T<sub>4</sub>: Osmopriming (50 mgL<sup>-1</sup> ascorbic acid); T<sub>5</sub>: Osmopriming (0.3% thiourea)

Results indicated that seed priming improved the germination, seedling growth and reduced  $\text{Na}^+$  accumulation and improved uptake of beneficial nutrients. Among priming hydro-, halo- and osmopriming mitigates the adverse effects of salinity compared to control by increasing germination rate and initial plant growth (Oliveira *et al.*, 2019). For example, seedling growth and seed germination were increased due to seed priming with glycine betaine under salinity (Zhang & Rue, 2012) but the most effective treatment to improve seed performance was NaCl in hot pepper (*Capsicum annuum* L.) under normal and saline conditions (Khan *et al.*, 2009). To exterminate the antagonistic effect of salinity at the germination stage, different salts and chemicals were used as priming agents in maize (Molazem & Azimi, 2015), and fine aromatic rice also exhibited similar results (Afzal *et al.*, 2012). Moreover, germination rate increases due to the swelling of the embryo inside the primed seed that facilitates water absorption (Elouaer & Hannachi, 2012), while radical protrusion starts after the stimulation of pre-germination metabolic processes due to seed priming (Farooq *et al.*, 2007).

The reduction in the amount of chlorophyll under higher salinity levels generally occurs due to the deterioration of the membrane (Al Sohbi *et al.*, 2005). The chlorophyll content of *C. procera* increased initially and then decreased at higher salinity levels. More or less similar findings have been explained by Khan *et al.*, (2013). In the present study, the concentration of all nutrients increased up to 120 mM stress level with ascorbic acid priming and then decreased, however, higher  $\text{Na}^+$  concentration was noticed both in root and shoot with increasing salinity as compared to other nutrients. These variations might be due to greater uptake of  $\text{Na}^+$  under higher salt stress which restrained the uptake of several nutrients like Ca, Mg, K, Cd, Zn (Nouman *et al.*, 2012). Our findings showed a similar trend of nutrients availability as reported by (Nouman *et al.*, 2012) in the root and shoot of *M. oleifera* with higher  $\text{Na}^+$  and Phosphorous (P) contents with increasing salt stress. Moreover, various medicinal plants including *C. procera* have been documented as a rich source of  $\text{Na}^+$  and  $\text{K}^+$  while containing the least contents of Mn and Cd (Jabeen *et al.*, 2012). These variations might be due to the difference in the germination time of seeds under priming or due to the effect of different environmental aspects (Yacoubi *et al.*, 2011).

## Conclusion

Seed priming is helpful in reducing the risk of poor stand establishment under a wide range of environmental conditions. In the present study *C. procera* exhibited maximum germination, growth attributes, and nutritional content at 120 mM salt stress under osmopriming with ascorbic acid and thiourea. As, *C. procera* is a good source of active ingredients, the findings will be helpful to plant this medicinal shrub and a good source of active ingredients and might be used in various remedies. The results obtained will be useful in planting of this potential shrub on large scale, especially for medicinal purposes as *C. procera* is a good source of active ingredients and can

be used in many remedies. Moreover, application of priming can enhance seedling establishment and field performance of this important species on saline soils due to its ability to tolerate salts and can be helpful for the reclamation of deserts. This information could ultimately help in the sustainable development of the arid zones.

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