



Efficacy of Biochar and Gypsum in Amelioration of Soil with Saline Water Irrigation in Ornamental Plants

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The present investigation was carried out to evaluate the effects of biochar and gypsum on the physiological and biochemical attributes of ten ornamental plant species grown in saline soil. Six months old ornamental plants were treated against salinity levels of 0, 6 and 9 dS m⁻¹. A set of 300 plants were applied with 100 g of biochar (2% of total pot mass) and another set of 300 plants were applied with 20 g of gypsum according to the treatment schedule. The results showed that, increasing salt concentration in irrigation water decreased plant height, total chlorophyll content, carotenoid content and relative water content and also damaged membrane stability causing electrolyte leakage. Using biochar at 2% of total soil weight, improved the nutrient content of soil, plant growth and plant physiological attributes. Application of gypsum had a major role in mitigating the harmful effects of salt stress on ornamental plants irrigated with saline water.

(Key words: Biochar, Electrolyte leakage, Ornamental plants, Salt tolerance, Soil amendments)

Salt stress is one of the major abiotic stresses, especially in arid and semi-arid regions where the salt content of the soil is naturally higher and the amount of precipitation is insufficient to allow the salts to be washed away. Coastal areas are high in salts, mainly due to the presence of saline groundwater table at shallow depths and frequent brackish water inundation in the low-lying areas. The groundwater influenced by sea and brackish water estuaries reaches the soil surface through capillary rise during the dry season, evaporates from the soil leaving salts behind, finally making the soil saline and unproductive for plants. The salt-laden sands blown by sea winds are also greatly responsible for the formation of coastal salt-affected soils (Ray *et al.*, 2014).

Osmotic stress and ionic stress are the two main challenges that plants cultivated in saline soil must overcome (Silva *et al.*, 2008). Normally, plants ingest water from the soil through their roots via osmosis, but under salt stress, this process becomes more challenging due to an increase in the concentration of solutes in the soil, which reduces growth rate and causes unfavourable

changes in plant metabolism. The osmotic effects, which include decreased cell expansion, cell division, stomatal closure, and inhibition of the formation of new leaves, can be seen right away after salt administration. Long-term exposure to salinity causes ionic stress, which may cause adult leaves to senesce prematurely, decreasing the area used for photosynthetic activity.

Saline soil can be reclaimed and its effects lessened by using specific soil amendments. The most popular soil amendment for sodic soils is gypsum because it supplies superior calcium and sulphur by replacing sodium. It also improves soil penetration, drainage and reduces acidity (Chen and Dick, 2011). A range of organic sources including agricultural waste and woody materials are pyrolyzed at moderate to high temperatures under low oxygen conditions to produce biochar (Ali *et al.*, 2017). It enhances the cation exchange capacity (CEC), soil structure and water holding capacity (WHC) (Ghezzehei *et al.*, 2014). Under salt stress, Ca²⁺, Mn²⁺ and sodium ion (Na⁺) levels are reduced by biochar and K⁺ absorption is improved (Brantley *et al.*, 2016).

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According to projections, the country's current 6.73 million ha of salt-affected soils will nearly triple to 20 million ha by 2050 (CSSRI, 2014). Due to the anticipated growth of the irrigated area and the heavy use of natural resources to meet the demands of an expanding population for food and other means of subsistence, the low-quality waters will also become more of an issue in the near future. Thus, marginal and low-quality waters will become important in these areas and could be used for the irrigation of ornamental plants. Because of this water scarcity, the use of salt-tolerant species in landscaping projects, xeriscape and public areas needs to be considered. Hence, our focus was on finding the physiological pattern which corresponds to salinity stress tolerance in ornamental plants. We also aimed to find out and discuss the role of biochar and gypsum in improving plant tolerance, soil quality and amending saline soil. Hence, the present study was carried out with the ten ornamental plants known to bear aesthetic value to screen out plant species for salt tolerance.

MATERIALS AND METHODS

The experiment was conducted at the College of Horticulture, Anantharajupeta, Dr. Y. S. R. Horticultural University during 2021 and 2022. The experiment site

is located at an altitude of 162 m above mean sea level lying between the 13°59' North latitude and 79°19' East longitudes. The treatments included three factors *viz.*, Factor 1: Ornamental plants -10 nos. (OP₁ to OP₁₀, list given in Table 1), Factor 2: Salt concentrations - 3 levels (C₀, C₁ and C₂) and Factor 3: Soil amendments - 3 nos (A₀, A₁ and A₂). The total number of treatment combinations was 90 (10×3×3) including two replications. Five numbers of plants were grown in each replication. The study period was from January to August 2021 and 2022.

The experiment was conducted in open field conditions. Six months old plants of ten ornamental species were planted in poly bags (9" × 11") each holding 5 kg growing media, containing soil (sandy loam garden soil), sand and FYM (2:1:1). The pH of the growing media was 6.86. There are 90 treatment combinations (10×3×3) with 5 plants in each treatment combination, replicated twice resulting in 900 total number of plants used for the investigation. Among them a set of 300 plants was treated with 100 g of biochar (2% of total soil mass) per each polybag; to another set of 300 plants, gypsum was applied with 20 g of per bag as per the treatment schedule before planting, whereas remaining 300 plants were left untreated (no amendment). Rice husk biochar

Table 1. Details of the statistical design of the experiment conducted in Factorial Completely Randomized Design (FCRD)

S.No.	Factor	Levels in factor (Botanical name)	Common name
1	Factor 1- Ornamental plants	OP ₁ - <i>Ixora coccinea</i> OP ₂ - <i>Tabernaemontana coronaria</i> OP ₃ - <i>Bougainvillea spectabilis</i> OP ₄ - <i>Acalypha wilkesiana</i> OP ₅ - <i>Duranta erecta</i> OP ₆ - <i>Caesalpinia pulcherrima</i> OP ₇ - <i>Rhoeo discolor</i> OP ₈ - <i>Sansevieria trifasciata</i> OP ₉ - <i>Pandanus veitchii</i> OP ₁₀ - <i>Canna indica</i>	Torch tree Crape jasmine Bougainvillea Indian acalypha Golden dew drop Peacock flower Moses in the cradle Snake plant Variegated screw pine Indian shot
2	Factor 2 - Salt concentrations	C ₀ - Control (0.8 dS m ⁻¹) C ₁ - 6 dS m ⁻¹ C ₂ - 9 dS m ⁻¹	
3	Factor 3 - Soil amendments	A ₀ - Control A ₁ - Biochar (2% of total pot mass - 100 g plant ⁻¹) A ₂ - Gypsum (20 g plant ⁻¹)	

with trade name 'Mandy's farm' was purchased from Amazon (an online shopping site) and agricultural grade gypsum with trade name 'SPIC' was purchased from the local market and used in the experiment. All the plants were irrigated regularly with tap water (0.8 dS m^{-1}) up to 15 days of planting. Later the stress treatments were imposed by irrigating plants with the NaCl solution to provide respective concentrations of EC (6 and 9 dS m^{-1}). 3.84 g and 5.76 g NaCl were dissolved in 1000 ml of distilled water to make 6 and 9 dS m^{-1} salt solutions respectively (Rani and Sharma, 2015). The control plants were irrigated with normal tap water (EC = 0.8 dS m^{-1} and 6.8 pH) without any added NaCl. Hand weeding was done regularly as and when needed to keep the poly bags free of weeds and further the top soil around the plant in the polybag was loosened at 15 days interval to provide better aeration for plant growth and development.

Collection of experimental data

Data for various morphological and physiological parameters were recorded starting from 45 days after saline water treatment (DAT), followed by every 45 days intervals for 6 months. A similar procedure was repeated in the second year and the 2 years pooled data (2021 and 2022) is presented here in Tables 2 to 6. The height of the plant was measured from the base of the plant up to the point of emergence of the youngest leaf with a meter scale and expressed in cm.

The total chlorophyll content in the leaves of ornamental plants was analysed by following the procedure standardized by Barnes *et al.* (1992). 500 mg of clean leaf sample was weighed and chopped leaves were immersed in 10 ml of dimethyl sulphoxide (DMSO). Then, the samples were incubated in a hot air oven for four hours at 70°C . Later, it was taken out and cooled at room temperature, 1 ml of the sample solution was diluted to 5 ml with DMSO and the samples were read on a spectrophotometer at 645, 663 nm using pure DMSO as blank. The estimation of total chlorophyll content can be done in a given green plant tissue adopting

Total Chlorophyll (mg/g) =

$$\frac{(20.2 \times A_{645}) + (8.02 \times A_{663}) \times \text{Volume} \times \text{dilution}}{1000 \times \text{weight of the sample (g)}} \dots(1)$$

the following formula proposed by Arnon (1949).

where, A_{645} = Absorption at 645 nm and A_{663} = Absorption at 663 nm. The value of total chlorophyll was expressed as mg g^{-1} fresh weight.

Total carotenoids content in fresh leaves was determined by using dimethyl sulphoxide (DMSO) as the method explained by Wellburn (1994) and samples were read at 480 nm in UV-VIS spectrophotometer.

Total carotenoids (mg g^{-1}) =

$$(1000A_{480} - 1.29C_a - 53.78C_b)/220 \dots(2)$$

where, C_a = Chlorophyll A, C_b = Chlorophyll B

The relative water content of the samples was estimated by using the method proposed by Singh (1977). After measuring 500 mg of the fresh weight of leaves, the leaves were immersed in water overnight, blotted dried and then weighted to get turgid weight. The leaves were dried overnight in an oven at 70°C and reweighed to obtain dry weight. Relative water content was computed by using following equation.

$$\text{RWC (\%)} = 100 \times (\text{FW}-\text{DW})/(\text{TW}-\text{DW}) \dots(3)$$

where, FW = Fresh weight, DW = Dry weight and TW = Turgid weight.

The fully expanded leaves were collected and washed with deionized water, and three circular leaf disks (2 mm in diameter) were cut from the leaves. The prepared disks were placed in glass vials filled with 10 mL of deionized water and placed on the shaker for 5 h. To determine the initial electrical conductivity (EC_1), an electrical conductivity meter was used. The leaf tissues were subsequently placed in an autoclave and heated at 121°C for 15 min to cause full leakage of ions into the bathing solutions. At the end of this period, the EC_2 was recorded and electrolyte leakage (EL, %) was calculated using the formula proposed by Lutts *et al.* (1996).

$$\text{EL (\%)} = (\text{EC}_1/\text{EC}_2) \times 100 \dots(4)$$

RESULTS AND DISCUSSION

Plant height

Morphological assessment on the growth pattern of ten ornamental plants showed that the plant height significantly differed with salinity and amendments

(Table 2). The study on ornamental plants at 45 DAT (Days after Treatment) showed that *Caesalpinia pulcherrima* (OP₆) had the significantly highest plant height (112.00 cm) followed by *Bougainvillea spectabilis* (OP₃) which recorded 76.22 cm, while the lowest plant height of 27.81 cm was noticed in *Rhoeo discolor* (OP₇). This could be due to the inherent genetic makeup of the plant when compared to other species.

Among salt concentrations, increasing salinity to 9 dS m⁻¹ (C₂) significantly reduced the plant height (48.98 cm) and the maximum plant height (61.49 cm) was recorded under control (C₀). This result is probably in response to limited cell expansion resulting from osmotic stress (Munns and Tester, 2008). Reduction in water content and water potential of plants grown in saline soil resulted in internal water deficit, which in turn, reduced the growth of shoot. Similar results of reduced plant height with increasing salinity have been reported by Lakshmaiah *et al.* (2018b) and Patel *et al.* (2010).

Studies on amendments effect revealed that, plants with biochar (A₁) treatment had significantly the maximum plant height (60.39 cm) as compared to gypsum (A₂) (55.04 cm). Biochar improves the ability of the plant to resist environmental stress factors (Thomas *et al.*, 2013). Consistent with the results of this study, the application of biochar increased the growth of plants under salinity stress in previous researches by Akhtar *et al.* (2015) and Mehdizadeh *et al.* (2020). The favourable effects of gypsum on vegetative and flowering parameters may be attributed to readily available Ca and S ions for plant nutrition in addition to improving soil physical and chemical properties by promoting better aggregation, increasing water infiltration and percolation, improving root growth and reclaiming sodic soil by replacing Na with Ca in soil particles (Chen and Dick, 2011). These results of gypsum's effect on increasing plant growth are consistent with the findings of Ashour and Mahmoud (2017).

The plant height was notably influenced by the salt stress, amendments and their interaction. Among interactions, OP₆C₀A₁ has recorded significantly the maximum plant height (126.08 cm), whereas the minimum plant height was recorded with OP₇C₂A₀ (19.43 cm). At 6 and 9 dS m⁻¹ (C₁ and C₂), the significantly highest plant height was encountered in *Caesalpinia pulcherrima* applied with biochar (117.78 and 107.88

cm) followed by gypsum which recorded 111.00 and 103.68 cm respectively. However, the application of biochar and gypsum showed a significant increase in the plant height of all the ornamentals, when compared to salinity-imposed plants with no amendments. The data presented in Table 2 revealed that the plant height at 135 and 180 DAT also showed similar trends as in the above-discussed data.

Total chlorophyll content

The data corresponding to chlorophyll content responded significantly among ornamentals, salt concentrations and amendments. The data at 45 DAT (Table 3) revealed that, among ornamentals total chlorophyll content was found significantly highest in *Sansevieria trifasciata*, OP₈ (5.21 mg g⁻¹), followed by *Bougainvillea spectabilis* (OP₃) with 4.86 mg g⁻¹, while the lowest was recorded in *Duranta erecta* (OP₅) with 2.42 mg g⁻¹ chlorophyll content. The maximum total chlorophyll content in *Sansevieria trifasciata* is due to the plant's ability to have the maximum Na⁺ exclusion ability and favouring maximum K, Mg and N intake, which further leads to high chlorophyll biosynthesis.

The plants in control (C₀) showed significantly the highest chlorophyll content (4.38 mg g⁻¹), while the lowest (3.47 mg g⁻¹) was recorded in C₂. The adverse effect of salt stress on total chlorophyll was due to the accumulation of toxic ions in plant tissue producing reactive oxygen species (ROS) that cause damage to chloroplasts and specific enzymes responsible for the synthesis of photosynthetic pigments. These results are similar to those of Acosta-Motos *et al.* (2015), who reported that salt-tolerant species show increased or unchanged chlorophyll content under salinity conditions whereas chlorophyll levels decrease in salt-sensitive species.

Our study on the amendments effect showed that plants treated with biochar (A₁) recorded significantly the highest chlorophyll content (4.12 mg g⁻¹) compared to gypsum (A₂) (3.91 mg g⁻¹). The present findings are in accordance with Ashour and Mahmoud (2017) and Habba *et al.* (2013) who reported that gypsum treatments increased total chlorophylls, total carbohydrates, N% or K% in salinity-stressed plants. Kanwal *et al.* (2017) also found that biochar usage in wheat increased chlorophyll content probably because of increase in

Table 2. Response of ornamental plants (OP), salt concentrations (C), amendments (A) and their interactions with respect to plant height (cm) (pooled means of two years)

Ornamental Plants (OP)	Salt Concentrations (C)	Amendments (A)																								
		45 DAT						90 DAT						135 DAT						180 DAT						
		A ₀		A ₁		A ₂		Mean		A ₀		A ₁		A ₂		Mean		A ₀		A ₁		A ₂		Mean		
		A ₀	A ₁	A ₂	Mean	A ₀	A ₁	A ₂	Mean	A ₀	A ₁	A ₂	Mean	A ₀	A ₁	A ₂	Mean	A ₀	A ₁	A ₂	Mean	A ₀	A ₁	A ₂	Mean	
OP ₁	C ₀	49.00	60.30	54.85	54.72	56.03	66.35	61.48	63.50	72.53	68.08	68.03	72.30	83.90	78.03	78.08										
	C ₁	44.33	52.78	48.10	48.40	49.83	58.38	53.20	57.83	68.95	62.43	63.07	67.03	78.20	72.45	72.56										
	C ₂	40.30	47.75	43.20	43.75	44.35	52.58	47.40	48.11	51.95	63.08	57.45	59.48	71.43	64.28	65.06										
	Mean	44.54	53.61	48.72	48.96	50.07	59.10	54.03	57.76	68.18	62.61	62.85	66.27	77.84	71.58	71.90										
OP ₂	C ₀	73.30	84.40	80.30	79.33	79.53	91.63	86.13	86.10	95.33	90.73	90.72	94.40	105.85	101.20	100.48										
	C ₁	68.15	77.85	72.85	72.95	74.63	83.33	78.50	78.82	81.75	87.50	87.05	85.72	87.90	98.15	92.08										
	C ₂	61.33	69.20	65.38	65.30	68.35	77.35	73.15	72.95	76.03	85.90	80.88	80.93	79.43	90.35	84.70										
	Mean	67.59	77.15	72.84	72.53	74.17	84.10	79.26	79.18	81.29	89.86	86.22	86.22	98.12	92.53	92.63										
OP ₃	C ₀	78.00	90.20	83.95	84.05	85.58	97.50	90.73	97.50	107.38	103.33	102.73	105.53	118.13	112.13	111.93										
	C ₁	71.05	80.00	75.38	75.48	79.40	88.55	84.48	84.14	89.10	94.28	93.88	92.42	99.70	113.70	105.60										
	C ₂	65.33	73.38	68.73	69.14	72.83	82.58	76.73	77.38	82.95	92.53	87.05	87.51	90.80	103.75	96.53										
	Mean	71.46	81.19	76.02	76.22	79.27	89.54	83.98	84.26	89.85	98.06	94.75	94.22	98.68	111.86	104.75										
OP ₄	C ₀	49.40	61.63	56.50	55.84	60.68	70.28	65.85	65.60	73.90	78.58	78.99	82.05	94.00	87.95	88.00										
	C ₁	45.43	55.55	50.35	50.44	53.63	62.50	57.98	58.03	67.08	71.95	72.03	70.35	75.85	81.50	81.58										
	C ₂	38.90	47.78	42.93	43.20	46.20	55.83	50.70	50.91	60.48	72.45	66.95	66.63	67.93	80.45	74.41										
	Mean	44.58	54.98	49.93	49.83	53.50	62.87	58.18	58.18	67.15	76.30	72.52	71.99	75.28	81.43	81.33										
OP ₅	C ₀	33.48	46.38	40.88	40.24	43.70	58.08	51.60	51.13	50.08	62.98	55.08	56.04	58.03	70.20	63.93										
	C ₁	29.30	41.30	34.33	34.98	39.90	50.45	46.10	45.48	44.13	50.63	49.88	48.21	51.23	63.43	57.23										
	C ₂	26.25	35.20	30.20	30.55	35.58	45.25	40.38	40.40	39.58	50.88	44.73	45.06	44.53	56.45	50.83										
	Mean	29.68	40.96	35.13	35.26	39.73	51.26	46.03	45.67	44.59	54.83	49.89	49.77	51.26	63.36	57.31										
OP ₆	C ₀	120.95	126.08	117.98	121.67	129.83	141.03	135.25	135.37	142.38	152.03	147.73	147.38	158.15	170.10	163.38										
	C ₁	104.25	117.78	111.00	111.01	118.30	128.33	123.53	123.38	133.40	139.30	134.50	135.73	151.25	163.50	157.53										
	C ₂	98.43	107.88	103.68	103.33	109.20	121.50	114.90	115.20	126.93	140.28	134.73	133.98	142.08	156.28	149.43										
	Mean	107.88	117.24	110.88	112.00	119.11	130.28	124.56	124.65	134.23	143.87	138.98	139.03	150.49	163.29	156.95										
OP ₇	C ₀	26.70	37.45	31.55	31.90	31.93	45.78	39.10	38.93	47.78	49.48	41.73	42.77	45.30	51.90	51.48										
	C ₁	21.90	31.55	27.00	26.82	27.50	37.03	33.68	32.73	34.35	39.75	40.78	38.29	38.93	50.90	45.10										
	C ₂	19.43	30.48	24.20	24.70	23.15	34.05	28.88	28.69	28.93	34.03	35.33	33.09	33.45	46.83	39.83										
	Mean	22.68	33.16	27.58	27.81	27.53	38.95	33.88	33.45	33.46	43.42	39.28	38.72	39.23	51.65	45.61										
OP ₈	C ₀	35.43	47.40	42.13	41.65	41.85	54.63	48.70	48.39	48.50	59.48	53.70	53.89	56.15	68.08	63.23										
	C ₁	30.28	42.00	36.55	36.28	36.38	45.93	41.53	41.28	42.13	53.63	47.48	47.74	50.40	63.03	56.65										
	C ₂	22.93	32.85	27.73	27.83	30.58	40.98	35.30	35.62	38.08	46.65	42.10	42.28	45.08	58.10	51.76										
	Mean	29.54	40.75	35.47	35.25	36.27	47.18	41.84	41.76	42.90	53.25	47.76	47.97	50.54	63.07	56.96										
OP ₉	C ₀	37.23	47.40	42.23	42.28	43.50	56.45	49.65	49.87	49.58	60.05	54.50	54.71	57.73	68.75	63.08										
	C ₁	32.25	42.83	36.85	37.31	38.30	48.48	44.08	43.62	44.30	53.70	49.65	49.22	52.08	64.18	57.63										
	C ₂	26.15	38.13	31.78	32.02	32.23	42.98	37.30	37.50	38.93	50.38	44.18	44.49	44.83	57.78	50.95										
	Mean	31.88	42.78	36.95	37.20	38.01	49.30	43.68	43.66	44.27	54.71	49.44	49.47	51.54	63.57	57.22										
OP ₁₀	C ₀	57.45	68.50	63.83	63.26	65.93	75.78	70.30	70.67	73.95	85.15	78.98	79.36	85.23	95.15	90.32										
	C ₁	52.05	63.20	56.98	57.41	58.93	70.05	65.43	64.80	67.53	78.58	73.08	73.06	77.43	91.33	83.33										
	C ₂	45.60	54.40	49.95	52.45	52.45	62.33	57.75	57.51	61.95	74.13	70.48	68.85	67.35	80.23	75.93										
	Mean	51.70	62.03	56.92	56.88	59.10	69.38	64.49	64.33	67.81	79.28	74.18	73.76	76.67	88.90	84.71										
For comparing salt concentrations (C) and amendments (A) levels																										
Ornamental plants (OP)	C ₀	56.09	66.97	61.42	61.49	63.85	75.75	69.88	69.83	72.26	82.89	77.24	77.46	81.49	93.14	87.54										
	C ₁	49.90	60.48	54.94	55.11	57.68	67.30	62.85	62.61	66.16	73.91	71.07	70.38	75.18	87.38	80.90										
	C ₂	44.46	53.70	48.78	48.98	51.49	61.54	60.58	56.25	60.58	71.73	66.37	66.23	67.49	80.16	74.39										
	Mean	50.15	60.39	55.04	55.19	57.67	68.20	62.99	62.95	66.33	76.18	71.56	71.36	74.72	86.89	80.94										
Factor	SEm(±)			CD @ 5%	SEm(±)			CD @ 5%	SEm(±)			CD @ 5%	SEm(±)			CD @ 5%										
Salt concentrations (C)	OPXC	0.081		0.227	0.074			0.209	0.084			0.236	0.081			0.229										
	OPXA	0.044		0.124	0.041			0.115	0.046			0.129	0.045			0.125										
	CXA	0.140		0.394	0.129			0.362	0.146			0.409	0.141			0.396										
	OPXCXA	0.044		0.124	0.041			0.115	0.046			0.129	0.045			0.125										
Amendments (A)	OPXA	0.140		0.394	0.129			0.362	0.146			0.409	0.141			0.396										
	CXA	0.077		0.216	0.071			0.198	0.080			0.224	0.077			0.217										

the photosynthesis rate. Nitrogen and magnesium are constituent elements of the chlorophyll molecule. Findings in a study by Fornes *et al.* (2007) suggested that a decrease in Mg^{2+} uptake was also related to the reduction in the chlorophyll content. Furthermore, in most cases, biochar acts to alleviate the negative effects of salt stress because of its sorptive properties. In saline soils, biochar adsorbs meaningful amounts of added NaCl or may immobilize salt ions as well as produce non-saline microsites to enhance nutrient uptake (Mg^{2+}) for chlorophyll biosynthesis (Thomas *et al.*, 2013).

The interactive response of ornamentals, salinity levels and amendments has shown a significant difference with the highest chlorophyll content (5.73 mg g^{-1}) in $OP_8C_0A_1$. At 6 and 9 $dS \text{ m}^{-1}$ (C_1 and C_2), the significantly maximum total chlorophyll content was noticed in *Sansevieria trifasciata* applied with biochar which recorded 5.43 and 4.97 mg g^{-1} respectively, followed by *Sansevieria trifasciata* with gypsum (5.22 and 4.82 mg g^{-1}) and *Bougainvillea spectabilis* with biochar (5.07 and 4.63 mg g^{-1}). However, the application of biochar and gypsum showed a significant increase in the total chlorophyll content of all the ornamentals, when compared to salinity-imposed plants with no amendments. The data pertaining to total chlorophyll content at 90 DAT showed a significant variation with respect to individual effects of ornamentals, salinity levels and amendments. However, the interaction of ornamentals, salinity levels and amendments are found to be non-significant. A similar trend of significant variation was noticed in the total chlorophyll content of ornamental plants, salinity levels, amendments and their interactions at 135 and 180 DAT (Table 3).

Carotenoid content

The data regarding the carotenoid content during the experimental period was found to be significantly different among the treatments and it is furnished in Table 4. Among the ornamental plants studied *Caesalpinia pulcherrima* (OP_6) had the significantly highest carotenoid content (3.51 mg g^{-1}) followed by *Canna indica* (OP_{10}) with 3.38 mg g^{-1} , while the minimum carotenoid content of 1.59 mg g^{-1} was recorded in *Rhoeo discolor* (OP_7). In different salt concentrations, C_0 resulted in significantly high carotenoid content (2.79 mg g^{-1}) followed by C_1 and C_2 , whereas among amendments, biochar (A_1) recorded the significantly

highest carotenoid content (2.66 mg g^{-1}) compared to gypsum (2.45 mg g^{-1}).

Higher pigment reductions were observed at higher salt concentrations. The reduced rate of photosynthesis associated with salt stress has been attributed to stomatal closure and a decrease in photosynthetic pigment concentrations (Alvarez *et al.*, 2012). Salinity stress while affecting the photosynthetic electron transport chain results in higher production of reactive oxygen species (ROS) in the cells. These events often result in photo-oxidative damage and photoinhibition (Gururani *et al.*, 2015). The findings of Bistgani *et al.* (2019) are in agreement with the present study, where high salinity decreases the carotenoid content in ornamental plant species. Among various interactions studied, the application of biochar followed by gypsum under non-saline treatments, increased all photosynthetic pigments, compared to other interactions. These observations and findings in the present investigation are in conformity with those reported earlier by Ashour and Mahmoud (2017) and Habba *et al.* (2013) who mentioned that adding gypsum to sodic soils improved pigment content. The data of this experiment corresponding to the effect of biochar on salinity are in accordance with the findings of Mehdizadeh *et al.* (2020) who reported that biochar alleviates the negative impact of salt stress on plant pigment content.

Significant differences were observed in the interaction effect of plant species, salt levels and amendments. The maximum carotenoid content was recorded in $OP_6C_0A_1$ (4.13 mg g^{-1}), whereas minimum carotenoid content was recorded in $OP_7C_2A_0$ (1.17 mg g^{-1}). At 6 $dS \text{ m}^{-1}$, significantly maximum carotenoid content was noticed in *Caesalpinia pulcherrima* applied with biochar (3.67 mg g^{-1}) followed by *Caesalpinia pulcherrima* with gypsum (3.49 mg g^{-1}) and *Canna indica* with biochar (3.57 mg g^{-1}) and at 9 $dS \text{ m}^{-1}$, *Caesalpinia pulcherrima* applied with biochar (3.28 mg g^{-1}) had the highest carotenoid content followed by *Canna indica* with biochar (3.23 mg g^{-1}) and *Caesalpinia pulcherrima* with gypsum (3.07 mg g^{-1}). However, the application of biochar and gypsum showed a significant increase in the carotenoid content of all the ornamentals, when compared to salinity-imposed plants with no amendments.

Relative water content

Relative water content is an appropriate method to assess the plant water status, which in turn can be used to screen the plants for stress tolerance. The observations on the effect of different levels of soil salinity on relative water content of ornamentals are presented in Table 5. Significant differences were observed in the relative water content of ornamental plants, salinity levels, amendments and their interactions. At 45 DAT among the ornamental plants studied; *Sansevieria trifasciata* (OP₈) recorded the significantly highest relative water content (94.57%) followed by *Bougainvillea spectabilis* (OP₃) with 92.94%, while *Duranta erecta* (OP₅) had the least RWC of 77.40%. Salt-tolerant plant species are capable of maintaining water potential and cellular integrity, thus there is an increased RWC under high salinity stress.

The study on salt concentrations revealed that C₀ recorded the maximum RWC (93.02%), whereas the minimum RWC (80.53%) was noticed with a high salinity level (C₂). Among amendments, biochar (A₁) recorded the significantly maximum RWC (91.17%) compared to gypsum (88.56%) and control (80.23%). In the present study, it was observed that higher salinity levels resulted in a drastic reduction in the relative water content of ornamental plants. The decrease in RWC may be due to the high salt concentration of the external solution, which causes osmotic stress and dehydration at the cellular level. In addition, lowering the water potential in the protoplast alters the integrity of the photosynthetic apparatus via photo-phosphorolysis and electron transfer. The findings of this experiment were in accordance with the observations reported by Bistgani *et al.* (2019), Butt *et al.* (2021) and Lakshmaiah *et al.* (2018a) who reported that a drastic reduction in RWC may be due to high concentrations of Na⁺ in plants and delay of the osmoregulation of the salinity tolerance threshold.

Regarding the interaction effect of ornamental plants, salinity levels and soil amendments, the highest RWC was recorded in OP₈C₀A₁ (98.40%), whereas the lowest RWC was recorded in OP₅C₂A₀ (57.58 %). At 6 and 9 dS m⁻¹, *Sansevieria trifasciata* applied with biochar (97.38 and 94.45%) have recorded the significantly highest RWC, followed by *Bougainvillea spectabilis* with biochar (95.60 and 94.10%) and

Sansevieria trifasciata with gypsum (95.58 and 91.53%). However, the application of biochar and gypsum showed a significant increase RWC content of all the ornamentals, when compared to salinity-imposed plants with no amendments. Similarly at 90, 135 and 180 DAT, there was a significant decrease in plant RWC with increasing soil salinity. Recorded data at all intervals showed that there was a significant increase in RWC with the use of biochar and gypsum compared to control.

Electrolyte leakage

The data with respect to the electrolyte leakage (EL) was significantly influenced by different salt concentrations, amendments and different plant species were presented in Table 6. Among ornamental plants studied during 45 DAT, the significantly minimum electrolyte leakage was noticed in *Sansevieria trifasciata* (OP₈) with 18.04 %, while *Duranta erecta* (OP₅) was found to have maximum electrolyte leakage (49.67%).

Among the salinity levels studied, significantly least EL was recorded in control (21.46%) and the maximum electrolyte leakage (41.88%) was observed in C₂, whereas among amendments, biochar (A₁) recorded the significantly minimum electrolyte leakage (30.45%), compared to gypsum (33.89%) and control (36.92%). The effect of salt stress and amendments on electrolyte leakage significantly differed among plant species throughout the experimental period. Among the interactions of ornamentals, salt concentrations and amendments, significantly maximum electrolyte leakage (63.25%) was recorded in OP₅C₂A₀, whereas the minimum electrolyte leakage (7.38%) was recorded in OP₈C₀A₁. However, at 6 and 9 dS m⁻¹, *Sansevieria trifasciata* applied with biochar was found with significantly minimum EL of 16.40 and 21.23 % respectively, followed by *Rhoeo discolor* with biochar (17.73 and 22.38%) and *Sansevieria trifasciata* with gypsum (19.45 and 24.78%). However, application of biochar and gypsum showed a significant decrease in EL of all the ornamentals, when compared to salinity-imposed plants with no amendments.

It has been demonstrated that in *Duranta erecta* salinity stress increases cell free radical levels to a degree closely related to membrane damage, thus

Table 6. Response of ornamental plants (OP), salt concentrations (C), amendments (A) and their interactions with respect to electrolyte leakage (%) (pooled means of two years)

Ornamental Plants (OP)	Salt Concentrations (C)	Amendments (A)																							
		Intervals						90 DAT						135 DAT						180 DAT					
		A ₀	A ₁	A ₂	Mean	A ₀	A ₁	A ₂	Mean	A ₀	A ₁	A ₂	Mean	A ₀	A ₁	A ₂	Mean	A ₀	A ₁	A ₂	Mean				
OP ₁	C ₀	34.58	27.18	31.18	30.98	35.03	28.83	32.43	32.09	35.65	29.55	32.85	32.68	36.50	30.55	33.85	33.63								
	C ₁	49.15	45.00	48.45	47.53	51.13	47.08	49.63	49.28	54.20	49.00	51.35	51.52	56.60	50.50	53.65	53.58								
	C ₂	57.80	51.25	56.00	55.02	60.13	52.83	58.08	57.01	62.60	54.55	60.10	59.08	66.05	56.05	63.15	61.75								
	Mean	47.18	41.14	45.21	44.51	48.76	42.91	46.71	46.13	50.82	44.37	48.10	47.76	53.05	45.70	50.22	49.66								
OP ₂	C ₀	25.75	19.18	26.68	23.87	27.23	20.08	23.38	23.56	28.70	21.35	24.20	24.75	30.25	22.50	25.00	25.92								
	C ₁	34.55	30.03	32.43	32.33	37.03	32.08	34.33	34.48	39.20	34.00	36.25	36.48	41.30	35.55	38.40	38.42								
	C ₂	39.75	30.70	36.63	35.69	41.83	33.13	39.53	38.16	44.35	35.30	41.30	40.32	47.20	37.70	44.20	43.05								
	Mean	33.35	26.63	31.91	30.63	35.36	28.43	32.41	32.06	37.42	30.22	33.92	33.85	39.60	31.92	35.87	35.79								
OP ₃	C ₀	17.85	12.30	15.23	15.13	19.38	13.78	16.53	16.56	20.65	14.90	17.25	17.60	22.65	16.30	19.45	19.47								
	C ₁	28.35	24.65	25.90	26.30	30.48	26.28	27.63	28.13	32.35	28.95	29.45	30.25	35.30	31.85	32.50	33.22								
	C ₂	33.60	26.25	29.88	29.91	35.33	28.78	32.73	32.28	38.10	30.80	34.90	34.60	41.65	34.10	37.50	37.75								
	Mean	26.60	21.07	23.67	23.78	28.39	22.94	25.63	25.65	30.37	24.88	27.20	27.48	33.20	27.42	29.82	30.14								
OP ₄	C ₀	37.25	26.20	29.95	31.13	38.13	28.38	30.68	32.39	39.15	29.55	31.85	33.52	20.80	30.55	33.45	28.27								
	C ₁	55.78	52.43	54.45	54.22	57.38	54.48	55.88	55.91	60.35	55.95	59.40	58.57	62.65	57.95	61.35	60.65								
	C ₂	60.63	54.63	57.20	57.48	63.78	57.48	59.98	60.41	66.30	59.20	61.70	62.40	69.80	62.25	64.55	65.53								
	Mean	51.22	44.42	47.20	47.61	53.09	46.78	48.84	49.57	55.27	48.23	50.98	51.49	51.08	50.25	53.12	51.48								
OP ₅	C ₀	39.98	26.28	30.50	32.25	41.38	27.68	32.13	33.73	41.60	29.25	32.85	34.57	42.85	30.75	34.45	36.02								
	C ₁	59.50	53.53	57.68	56.90	61.73	55.48	58.53	58.58	63.60	56.85	60.70	60.38	65.75	59.05	54.95	59.92								
	C ₂	63.25	56.28	60.08	59.87	64.53	59.03	62.33	61.96	67.90	60.95	64.35	64.40	70.70	63.40	66.95	67.02								
	Mean	54.24	45.36	49.42	49.67	55.88	47.39	50.99	51.42	57.70	49.02	52.63	53.12	59.77	51.07	52.12	54.32								
OP ₆	C ₀	24.15	15.88	20.13	20.05	25.48	17.33	21.38	21.39	26.55	18.20	22.55	22.43	27.80	19.60	24.55	23.98								
	C ₁	41.78	39.20	37.55	39.78	43.78	39.33	41.38	41.49	46.35	40.90	43.80	43.68	49.25	44.15	38.25	43.88								
	C ₂	46.30	39.20	43.35	42.95	48.13	41.78	45.98	45.29	51.30	43.85	47.65	47.60	54.95	46.35	50.50	50.60								
	Mean	37.41	30.88	34.42	34.23	39.13	32.81	36.24	36.06	41.40	34.32	38.00	37.91	44.00	36.70	37.77	39.49								
OP ₇	C ₀	14.25	9.93	12.40	12.19	16.58	11.23	13.48	13.76	18.25	12.85	14.80	15.30	19.15	13.60	16.90	16.55								
	C ₁	22.73	17.73	20.45	20.30	25.13	20.23	22.93	22.76	27.95	22.55	25.35	25.28	30.60	25.30	28.45	28.12								
	C ₂	27.03	22.38	24.78	24.73	30.03	24.28	27.48	27.26	33.05	26.10	29.85	29.67	36.30	28.85	38.30	38.15								
	Mean	21.33	16.68	19.21	19.07	23.91	18.58	21.29	21.26	26.42	20.50	23.33	23.42	28.68	22.58	34.55	28.61								
OP ₈	C ₀	10.58	7.38	9.18	9.04	12.33	10.23	10.48	11.01	14.00	11.35	11.35	12.23	15.95	11.20	12.55	13.23								
	C ₁	23.78	16.40	19.45	19.88	25.88	18.63	22.58	22.36	28.40	20.60	24.50	24.50	32.00	23.70	27.25	27.65								
	C ₂	28.53	21.23	25.88	25.21	30.93	24.43	28.53	27.96	33.75	26.30	31.30	30.45	36.80	29.10	34.60	33.50								
	Mean	20.96	15.00	18.17	18.04	23.04	17.76	20.53	20.44	25.38	19.42	22.38	22.39	28.25	21.33	24.80	24.79								
OP ₉	C ₀	29.03	20.48	23.70	24.40	29.93	21.78	24.53	25.41	31.00	22.60	25.75	26.45	32.55	23.55	27.65	27.92								
	C ₁	50.98	45.20	48.10	48.09	52.98	46.93	49.98	49.96	54.70	49.00	51.65	51.78	57.85	51.35	53.40	54.20								
	C ₂	55.18	48.15	52.18	51.83	56.88	51.48	55.23	54.53	58.75	53.30	56.55	56.20	62.45	56.45	58.70	59.20								
	Mean	45.06	37.94	41.33	41.44	46.59	40.06	43.24	43.30	48.15	41.63	44.65	44.81	50.95	43.78	46.58	47.11								
OP ₁₀	C ₀	20.03	11.78	14.80	15.53	21.53	13.03	16.73	17.09	22.10	14.65	17.40	18.05	24.00	15.75	19.30	19.68								
	C ₁	36.15	31.20	34.18	33.84	38.13	33.83	36.43	36.13	40.25	35.80	39.50	38.52	44.00	39.00	41.65	41.55								
	C ₂	39.25	33.18	36.03	36.15	41.68	35.98	39.43	39.03	44.85	37.80	40.65	41.10	48.20	39.90	43.50	43.87								
	Mean	31.81	25.38	28.33	28.51	33.78	27.61	30.86	30.75	35.73	29.42	32.52	32.56	38.73	31.55	34.82	35.03								
For comparing salt concentrations (C) and amendments (A) levels																									
Factor	C ₀	25.34	17.66	21.37	21.46	26.70	19.23	22.17	22.70	27.77	20.43	23.09	23.76	27.25	21.44	24.72	24.47								
	C ₁	40.27	35.37	38.09	37.91	42.36	37.43	39.93	39.91	44.74	39.36	42.20	42.10	47.53	41.84	42.99	44.12								
	C ₂	45.13	38.32	42.20	41.88	47.32	40.92	44.93	44.39	50.10	42.82	46.84	46.58	53.42	45.42	52.20	50.34								
	Mean	36.92	30.45	33.89	33.75	38.79	32.53	35.67	35.66	40.87	34.20	37.37	37.48	42.73	36.23	39.97	39.64								
Ornamental plants (OP)	SEm(±)	0.159	0.087	0.245	0.447	0.142	0.142	0.398	0.167	0.167	0.091	0.257	0.267	0.267	0.146	0.146	0.749								
	Salt concentrations (C)	0.275	0.087	0.245	0.773	0.245	0.245	0.690	0.289	0.289	0.091	0.811	0.462	0.462	0.146	0.146	1.298								
	Amendments (A)	0.087	0.275	0.245	0.447	0.142	0.142	0.398	0.167	0.167	0.091	0.257	0.267	0.267	0.146	0.146	0.749								
	OPXA	0.275	0.087	0.245	0.773	0.245	0.245	0.690	0.289	0.289	0.091	0.811	0.462	0.462	0.146	0.146	1.298								
OPXCXA	CXA	0.151	0.087	0.245	0.447	0.142	0.142	0.398	0.167	0.167	0.091	0.257	0.267	0.267	0.146	0.146	0.749								
	OPXCXA	0.477	0.151	0.424	1.340	0.425	0.425	1.194	0.500	0.500	0.158	1.405	0.444	0.800	0.253	0.253	2.248								

causing high electrolyte leakage, whereas *Sansevieria trifasciata* and *Bougainvillea spectabilis* keep up their cellular membrane integrity, which is considered as the mechanism of salt tolerance (Khan *et al.*, 2010). They may also possess a mechanism of regulation allowing transportation of the excess Na^+ toxic ions from cytosol, and has the capacity to endure the effects of salt excess that was associated with the directed movement of potassium ions (K^+) into cytosol (Alves *et al.*, 2008). In the present investigation, salinity stress gradually decreased the membrane stability, thus leading to higher electrolyte leakage. The Na^+ and Cl^- concentrations in the soil had a significantly positive correlation with cell membrane injury. These results are similar with the observations of Acosta-Motos *et al.* (2015) and Bistgani *et al.* (2019).

By increasing salinity levels, EL was significantly increased but it was decreased with the use of biochar. The application of biochar can improve the water holding capacity of the soil, thus increasing the soil moisture availability which can dilute salts in soil solution thereby maintaining homeostasis and membrane integrity which reduces electrolyte leakage (Akhtar *et al.*, 2015). These results are also in line with Mehdizadeh *et al.* (2020) who reported biochar usage caused a decrease in membrane injury compared to non-biochar usage under salinity stress. Saeed *et al.* (2014) reported that saline soil amended with gypsum is found more effective in reducing salinity hazards to a greater extent and hence improves membrane stability by decreasing EL.

From the present study, it can be concluded that *Sansevieria trifasciata*, *Bougainvillea spectabilis* and *Caesalpinia pulcherrima* showed the least membrane damage and electrolyte leakage in connection with the highest RWC and total chlorophyll content, which made them tolerate high soil salinity and could survive up to 9 dS m^{-1} . Therefore, these species are highly suitable for greening and landscaping in salt-affected coastal ecosystems. However, *Tabernaemontana coronaria*, *Ixora coccinea*, *Canna indica* and *Rhoeo discolor* could tolerate up to 9 dS m^{-1} soil salinity only when biochar and gypsum were used, as these plants had moderate electrolyte leakage. *Pandanus veitchii*, *Acalypha wilkesiana* and *Duranta erecta* could survive moderately up to 6 dS m^{-1} only when biochar and gypsum were used, but could not survive upto 9 dS m^{-1} even when soil

amendments were applied, because these plants had the highest electrolyte leakage, least pigment biosynthesis and RWC, thus leading to salt sensitivity.

Our study revealed that in coastal ecosystems, the application of biochar to salt-affected soils reduces the negative effects of salt stress because of its high Na^+ adsorption capability. Thus plants uptake high N and K^+ , which is responsible for reduced ionic stress, electrolyte leakage, increased chlorophyll biosynthesis, maintaining osmotic balance and improved plant growth. Therefore, biochar application could be an effective method to remediate salt-affected soils and mitigate the harmful effect of salinity stress on ornamental plants as a consequence of its potential to improve soil characteristics. Gypsum performed not much less than biochar in enhancing the physiological and biochemical attributes of the plant and removed the greatest amount of Na^+ from the soil which further supported plant growth.

CONFLICTS OF INTEREST

The authors have no conflicts of interest.

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