

## Response of *Eucalyptus camaldulensis*, *Eucalyptus microtheca* and *Eucalyptus intertexta* Seedlings to Irrigation with Saline Water

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**Abstract:** A pot experiment was carried out in a green house at the Research and Experiments Station of the Faculty of Agriculture and Food Sciences, King Saud University, Riyadh, Saudi Arabia. Seedlings of *Eucalyptus camaldulensis* Dehn, *Eucalyptus intertexta* R. T. Baker and *Eucalyptus microtheca* F. Muell were irrigated with saline water by adding sodium chloride to tap water. Four treatments represented four gradual levels of salinity were applied as a control and low, medium and high saline water represented by sodium chloride solutions with EC =1, 4, 8 and 16 dsmG<sup>l</sup>, respectively. Increasing Na<sup>+</sup> concentrations in irrigation water decreased the diameter, height, root length, number of leaves, average leaf size and total leaf area of the seedlings. These resulted in decreases in Leaf, stem, roots and consequently total plant dry weight indicating a reverse trend between these traits and salinity levels. Increasing salinity decreased LWR and increased SWR, while RWR did not change. RGR, LWR, SLA and LAR of *Eucalyptus* species all decreased with increasing the concentration of sodium chloride in irrigation water, while NAR was not affected. Relative water content of the leaves decreased with increasing salinity. Concentrations of Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup> and Na<sup>+</sup>: k<sup>+</sup> ratio increased with increasing salinity.

**Key words:** *Eucalyptus camaldulensis* % *Eucalyptus intertexta* % *Eucalyptus microtheca* % Salinity % Growth

### INTRODUCTION

Saline or salt-affected soils are common in arid and semiarid regions [1]. Salinity in arid and semiarid areas could be caused by poor irrigation water which contains considerable amounts of salts. Also, low rainfall, high evaporation rate and poor water management could cause salinity related problems in these areas [2]. In warm and dry areas, salt concentrations increase in the upper soil layer due to evapotranspiration exceeding precipitation [3]. Saline conditions adversely affect the growth and survival of glycophytes [4]. Plant growth is generally inhibited by salt stress [5]. Many investigators have reported retardation of germination and growth of seedlings at high salinity [6]. Munns [7] explains that plant growth is affected because a high build-up of salt kills the photosynthetically active leaves, which in turn affects the supply of carbohydrates or hormones to the actively growing parts. Amacher *et al.* [8] describe general

plant responses to different soil salinity ranges. They consider that soil salinity of 0-2 dS mG<sup>l</sup> is mostly negligible, while at 2-4 dS mG<sup>l</sup> growth of sensitive plants may be restricted, at 4-8 growth of many plants is restricted, at 8-16 only tolerant plants grow satisfactorily and above 16 only a few very tolerant plants grow satisfactorily.

In Saudi Arabia, saline water is one of the most frequent environmental stresses that face growing plants [9]. This because groundwater represents more than 90% of water used in agricultural irrigation and it classified as very saline water [10]. Al-Matroud [11] reported that salinity of groundwater from Riyadh Region (Central part of Saudi Arabia) has electrical conductivities (EC) ranged between 1.34 and 7.84 dS mG<sup>l</sup> and dominated by sodium chloride cations. Therefore, afforestation efforts in arid area usually fail due to non-availability of fresh waters and hesitancy for utilizing saline ground water resources [12].

The growth of some *Eucalyptus* species is negatively affected by salt stress [13]. However, the response differs at different salinity levels and between species [14]. *Eucalyptus camaldulensis* and *E. microtheca* grow in different areas in Saudi Arabia. *E. camaldulensis* was the first species introduced since over forty years and planted in many afforestation projects over the country. Nevertheless, the last very hot summers that prevailed in the central part of Saudi Arabia, particularly in years 1998 and 2002 showed that *E. microtheca* surpassed *E. camaldulensis* in tolerating these harsh conditions. On the other hand, the existence of *E. intertexta* is limited to few sites within the country.

The present study aims at comparing the morphological and physiological response of *E. camaldulensis*, *E. microtheca* and *E. intertexta* to irrigation with saline water at seedlings stage.

## MATERIALS AND METHODS

**Plant Material and Cultural Technique:** One month-old seedlings of *Eucalyptus camaldulensis* Dehn, *Eucalyptus intertexta* R.T. Baker and *Eucalyptus microtheca* F. Muell produced from seeds were collected from healthy trees grown in the Research Station of Prince Sultan Research Centre for Environment, Water and Desert near Riyadh, Saudi Arabia.

Seeds were cleaned and tested for their germination percentage, then sown in 40 × 40 cm trays containing a mixture of sand and peat moss (1:1 v/v). The sown seeds were left to germinate without any pretreatment under green house conditions included 32 and 17°C at day and night, respectively with 12 h day light and were well-watered every other day. After occurrence of the first seven true leaves on the seedlings, they were transferred to 30 cm rim diameter and 40 cm deep plastic pots containing washed sand (Table 1). Before and during imposing the treatments, the seedlings were watered with Hoagland's nutrient solution once a week in addition to a regular watering regime every other day.

**Treatments:** Because of it is easier in application and control [15], sodium chloride was used to prepare solutions varied in their salinity level. Four treatments represented four gradual levels of salinity were applied to the seedlings through adding sodium chloride to tap water in order to have a solution with a specific electrical conductivity (EC) [16]. The treatments were irrigation with tap water as a control and low, medium and high saline water represented by sodium chloride solutions with EC = 1, 4, 8 and 16 dsmG<sup>l</sup>, respectively.

To insure a good distribution of salts and to avoid their accumulation in one side of the pot, watering was adding to the pots using a rose. The pots were placed on sand to allow drainage of the surplus water. The amount of water added to each pot every week was determined according to the average amount of water lost by evapotranspiration of the weight of the pot [16]. This amount was 2 L for each pot every week during the period from December to March, 2.5 L during the period from May to September and 2.25 during the period from October to November.

**Measurements:** Before applying the treatments, height, diameter and total leaf area of five seedlings from each species were measured to be used in calculating other growth variables. After applying the treatments, a fourth of the number of the seedlings of each species were harvested monthly. Heights, diameters, total leaf areas, number of leaves, root lengths of the harvested seedlings were measured. Total leaf area was scaled using Automatic area meter (Model AAC-400, Hayshai Denkoh Co., LTD. Tokyo, Japan). Leaf, stem and root fresh weights of each seedling were determined. These parts were oven dried in an oven at 60°C for 48 h and their dry weights were determined then their total plant dry weights were calculated.

Allocation of dry weight to leaves, stem and roots of each seedling was calculated as a proportion of total plant dry weight. Relative growth rate (RGR), leaf area ratio (LAR), specific leaf area (SLA) and net assimilation rate

Table 1: Chemical analysis of the saturated best of sand used for growing seedlings

	Ca <sup>++</sup>	Mg <sup>+</sup>	Na <sup>+</sup>	K <sup>+</sup>	Cl <sup>-</sup>	CO <sub>3</sub> <sup>-2</sup>	SO <sub>4</sub> <sup>-2</sup>	HCO <sup>-3</sup>	SAR	
pH	EC(dsmG <sup>l</sup> )	meq LG <sup>l</sup>								
8.2	2.3	29.5	3.15	2.93	0.22	3.25	0.25	27.9	1.0	0.66

(NAR) all were calculated according to Evans [17]. This was carried out using the following equations:

$$\begin{aligned} \text{RGR} &= (\text{Log}_e W_2 - \text{Log}_e W_1) / t_2 - t_1 \\ \text{LAR} &= \text{TLA} / \text{TWt} \\ \text{SLA} &= \text{TLA} / \text{LWt} \\ \text{NAR} &= (W_2 - W_1 / L_2 - L_1) \times (\log_e L_2 - \log_e L_1 / t_2 - t_1) \end{aligned}$$

Where:  $\text{Log}_e$  = natural logarithm,  $W_2$  and  $W_1$  = total plant dry weight at the beginning and the end of each harvest (g),  $t_2 - t_1$  = the length of the period before each harvest (month),  $\text{TLA}$  = total leaf area ( $\text{cm}^2$ ),  $\text{TWt}$  = total plant dry weight,  $\text{LWt}$  = leaf dry weight and  $L_1$  and  $L_2$  are the total leaf areas.

**Quantifying Relative Water Content (RWC):** Fresh weights of a leaf samples from each harvest were determined immediately after harvesting and submerged in distilled water for 24 h, then, the saturated weight of the leaves was determined and placed into an oven at  $70^\circ\text{C}$  for 48 h until weight constancy. Relative water content (RWC) was calculated [18] using the following equation:

$$\text{RWC} = (\text{FW} - \text{DW}) / (\text{SW} - \text{DW}) \times 100$$

Where:  $\text{FW}$ ,  $\text{SW}$  and  $\text{DW}$  are fresh, saturated and dry weight of the sample (g), respectively.

**Determination of Chlorine:** Chlorine concentration in the leaves of the seedlings was determined only at the last harvest using dry burning [19]. Chlorine was extracted from one gram of leaf sample using 100 ml of 1N nitric acid ( $\text{HNO}_3$ ) for two hours then the mixture was filtered and its pH was adjusted using 0.005 Silver nitrate solution [20]. Chlorine concentration was calculated as the following:

$$\text{Cl}(\text{ppm}) = \frac{(\text{volume} \times \text{normality} \times \text{extract volume} \times 35.5 \times 10^3)}{\text{volume of the solution used in determination}}$$

**Determination of Sodium and Potassium:** Sodium and potassium concentration in the leaves of the seedlings was determined only at the last harvest using Flame photometer. Leaves were oven-dried to constant weight at  $70^\circ\text{C}$ , ground and digested in a solution of 98% sulphuric acid ( $\text{H}_2\text{SO}_4$ ) supplemented with Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) (30%, v/v). Concentrations of sodium and potassium in plant leaves were calculated as the following:  $\text{Na}(\text{ppm}) = (\text{R} \times \text{Sw}) \times 10^4$ , where  $\text{R}$  = apparatus reading,  $\text{Sw}$  = sample weight.

**Statistical Design and Analysis:** The Statistical design used in carrying out the present experiment was a complete randomized design (CRD) in a factorial arrangement [21] included salinity factor with four levels, three eucalyptus species and four monthly sequential harvests. There were seven replications of these 28 treatment combinations.

Data was analyzed through analysis of variance procedure (ANOVA) using SAS computer program [22]. The differences between factors means were distinguished using L. S. D. test.

## RESULTS

**Growth of the Stem and Roots:** Analysis of variance showed significant effects of increasing the concentration of sodium chloride on the stem height of the seedlings ( $P < 0.0001$ ). The seedlings grown under irrigation with high saline solution ( $16 \text{ dsmG}^1$ ) had average stem height were 52.3 cm followed by that of those grown under  $8 \text{ dsmG}^1$  (62.3 cm) then that of those grown under  $4 \text{ dsmG}^1$  (69.9 cm) and finally that of the seedlings irrigated with tap water ( $1 \text{ dS mG}^1$ ) (78.1 cm) (Table 2). This result indicates the existence of a reverse relationship between salinity level and the height of plant.

Stem height differed significantly between species across treatments and harvests ( $P = 0.0003$ ), where the mean height of *E. microtheca* seedlings was greater than those of the other two species; which were not significantly different (Table 2). Stem heights of the seedlings increased significantly from harvest to the next ( $P < 0.0001$ ). The average stem heights across treatments and species were 54.3, 62.9, 70.8 and 79.5 in H1, H2, H3 and H4, respectively (Table 2).

There were interactions between species and harvests ( $P = 0.007$ ) (indicated increasing the magnitude of species effects from harvest to harvest).

There was also treatments  $\times$  harvests interaction ( $P > 0.0001$ ) indicated changing the effects of treatments from harvest to the next (Table 2).

Increasing salinity in irrigation water significantly decreased the diameter of the seedlings ( $P > 0.0001$ ) indicating a reverse trend between diameter growth and salinity levels. Average stem diameter increased progressively from harvest to the next ( $P < 0.0001$ ) across treatments and species, with treatments  $\times$  harvest interaction ( $P = 0.0034$ ) indicated increasing the magnitude of treatment effects from harvest to the next. Increasing salinity in irrigation water significantly decreased the average root length of the seedlings ( $P < 0.0001$ ) (Table 2).

Table 2: Growth of the stem and roots of *E. microtheca*, *E. camaldulensis* and *E. intertexta* seedlings under different levels of salinity in irrigation water

Trait	EC (dS mG <sup>l</sup> )	Species			Treatment mean
		<i>E. microtheca</i>	<i>E. camaldulensis</i>	<i>E. intertexta</i>	
Stem height (cm seedlingG <sup>l</sup> )	1	80.28	78.25	75.83	78.12 <sup>a</sup>
	2	70.66	69.22	69.80	69.89 <sup>b</sup>
	3	65.25	58.60	62.90	62.25 <sup>c</sup>
	4	61.56	54.37	55.91	57.28 <sup>d</sup>
Species mean		*69.44 <sup>a</sup>	65.11 <sup>b</sup>	66.11 <sup>b</sup>	
Stem diameter (cm seedlingG <sup>l</sup> )	1	0.48	0.49	0.48	0.48 <sup>a</sup>
	2	0.42	0.42	0.43	0.42 <sup>b</sup>
	3	0.40	0.38	0.40	0.39 <sup>c</sup>
	4	0.38	0.36	0.37	0.37 <sup>c</sup>
Species mean		0.42 <sup>a</sup>	0.41 <sup>a</sup>	0.42 <sup>a</sup>	
Root length (cm seedlingG <sup>l</sup> )	1	26.50	26.35	25.54	26.13 <sup>a</sup>
	2	24.54	23.31	23.82	23.89 <sup>b</sup>
	3	22.71	22.56	23.58	22.95 <sup>b</sup>
	4	21.32	21.41	21.14	21.29 <sup>c</sup>
Species mean		23.77 <sup>a</sup>	23.41 <sup>a</sup>	23.52 <sup>a</sup>	

\*Means followed by different superscript letters are significantly different according to L.S.D. test at  $P=0.05$

Table 3: Growth of the leaves of *E. microtheca*, *E. camaldulensis* and *E. intertexta* seedlings under different levels of salinity in irrigation water

Trait	EC(dS mG <sup>l</sup> )	Species			Treatment mean
		<i>E. microtheca</i>	<i>E. camaldulensis</i>	<i>E. intertexta</i>	
No. of leaves (leaf seedlingG <sup>l</sup> )	1	49.32	46.39	46.21	47.31 <sup>a</sup>
	2	42.33	41.32	39.11	40.92 <sup>b</sup>
	3	39.40	36.64	32.60	36.21 <sup>c</sup>
	4	36.35	30.46	29.50	32.10 <sup>d</sup>
Species mean		*41.85 <sup>a</sup>	38.70 <sup>b</sup>	36.86 <sup>b</sup>	
Total leaf area (cm <sup>2</sup> seedlingG <sup>l</sup> )	1	662.73	811.01	794.19	755.98 <sup>a</sup>
	2	466.25	581.89	583.78	543.97 <sup>b</sup>
	3	351.57	377.99	439.08	389.55 <sup>c</sup>
	4	248.52	296.72	327.88	291.04 <sup>d</sup>
Species mean		432.27 <sup>b</sup>	516.90 <sup>a</sup>	536.23 <sup>a</sup>	
Average leaf size (cm <sup>2</sup> leafG <sup>l</sup> )	1	12.74	16.49	16.31	15.18 <sup>a</sup>
	2	10.54	13.72	13.81	12.69 <sup>b</sup>
	3	8.64	11.62	11.16	10.47 <sup>c</sup>
	4	6.59	10.50	9.89	8.99 <sup>d</sup>
Species mean		9.63 <sup>b</sup>	13.08 <sup>a</sup>	12.79 <sup>a</sup>	

\*Means followed by different superscript letters are significantly different according to L.S.D. test at  $P=0.05$

**Growth of the Leaves:** Increasing the concentration of NaCl in the irrigation water decreased the number of leaves ( $P>0.0001$ ), average leaf size ( $P>0.0001$ ) and consequently total leaf area ( $P>0.0001$ ) of the seedlings (Table 3). These traits were reduced steadily with increasing the salinity level of the irrigation water, while they increased from harvest to the next ( $P>0.0001$ ) across both species and treatments. *E. microtheca* had mean number of leaves across treatments accounted for 41.85 leaf seedlingG<sup>l</sup> which was significantly greater than those of the other two species (Table 3).

However, the average leaf size of *E. microtheca* was significantly lower than those of the other two species.

**Dry Weight Production:** Analysis of variance procedure revealed significant effects of treatments ( $P<0.0001$ ) on leaf, stem roots and consequently total plant dry weight. Under high saline irrigation water the seedlings produced

less dry weight and *vice versa* (Table 4). *E. microtheca* had significantly lower leaf, stem and total dry weight comparing with either *E. camaldulensis* or *E. intertexta* which had similar values ( $P<0.0001$ ). There were no significant differences between species in root dry weight. Dry weight of the seedlings increased from harvest to the next ( $P<0.0001$ ). There were treatment  $\times$  harvest interactions indicating that the magnitude of treatments effects on dry weight changed from harvest to harvest ( $P<0.0001$ ); except for root dry weight ( $P<0.005$ ). There were treatment  $\times$  species interactions for leaf ( $P=0.0009$ ), stem ( $P=0.01$ ) and total dry weight ( $P=0.005$ ) indicating changing the magnitude of treatment effects between species (Table 4).

**Dry Weight Partitioning:** Increasing sodium chloride concentration in irrigation water decreased the proportion of dry weight partitioned to leaves (leaf weight ratio,

Table 4: Dry weight production of *E. microtheca*, *E. camaldulensis* and *E. intertexta* seedlings under different levels of salinity in irrigation water

Trait	EC(dS mG <sup>l</sup> )	Species			Treatment mean
		<i>E. microtheca</i>	<i>E. camaldulensis</i>	<i>E. intertexta</i>	
Leaf dry weigh (g seedlingG <sup>l</sup> )	1	4.17	5.69	5.34	5.07 <sup>a</sup>
	2	3.08	4.29	4.36	3.91 <sup>b</sup>
	3	2.55	2.95	3.30	2.93 <sup>c</sup>
	4	2.03	2.56	2.52	2.37 <sup>d</sup>
Species mean		*2.96 <sup>b</sup>	3.87 <sup>a</sup>	3.88 <sup>a</sup>	
Stem dry weight (g seedlingG <sup>l</sup> )	1	1.65	2.17	1.81	1.88 <sup>a</sup>
	2	1.52	1.96	2.06	1.85 <sup>a</sup>
	3	1.43	1.52	1.74	1.56 <sup>b</sup>
	4	1.29	1.44	1.48	1.40 <sup>c</sup>
Species mean		1.47 <sup>b</sup>	1.77 <sup>a</sup>	1.77 <sup>a</sup>	
Root dry weight (g seedlingG <sup>l</sup> )	1	1.02	1.17	1.30	1.16 <sup>a</sup>
	2	0.87	1.00	0.98	0.95 <sup>b</sup>
	3	0.77	0.72	0.75	0.75 <sup>c</sup>
	4	0.61	0.64	0.58	0.61 <sup>d</sup>
Species mean		0.82 <sup>a</sup>	0.88 <sup>a</sup>	0.90 <sup>a</sup>	
Total dry weight (g seedlingG <sup>l</sup> )	1	6.84	8.91	8.45	8.07 <sup>a</sup>
	2	5.47	7.25	7.40	6.71 <sup>b</sup>
	3	4.76	5.19	5.79	5.25 <sup>c</sup>
	4	3.93	4.64	4.57	4.38 <sup>d</sup>
Species mean		5.25 <sup>b</sup>	6.50 <sup>a</sup>	6.55 <sup>a</sup>	

\*Means followed by different superscript letters are significantly different according to L.S.D. test at P=0.05

Table 5: Partitioning of dry weight of *E. microtheca*, *E. camaldulensis* and *E. intertexta* seedlings into leaves, stem and roots under different levels of salinity in irrigation water

Trait	EC(dS mG <sup>l</sup> )	Species			Treatment mean
		<i>E. microtheca</i>	<i>E. camaldulensis</i>	<i>E. intertexta</i>	
Leaf weigh ratio	1	62	64	64	63.3 <sup>a</sup>
	2	57	60	60	59 <sup>b</sup>
	3	55	58	58	57 <sup>c</sup>
	4	53	57	56	55 <sup>c</sup>
Species mean		*56.8 <sup>b</sup>	59.8 <sup>a</sup>	59.5 <sup>a</sup>	
Stem weigh ratio	1	23	22	21	22 <sup>d</sup>
	2	27	26	27	26.7 <sup>c</sup>
	3	29	28	29	28.7 <sup>b</sup>
	4	32	30	31	31 <sup>a</sup>
Species mean		27.8 <sup>a</sup>	26.5 <sup>a</sup>	27 <sup>a</sup>	
Root weigh ratio	1	14.5	13.4	14.6	14.2 <sup>a</sup>
	2	15.1	13.6	12.7	13.8 <sup>a</sup>
	3	15.7	13.3	12.7	13.9 <sup>a</sup>
	4	14.9	13.1	12.1	13.4 <sup>a</sup>
Species mean		15.1 <sup>a</sup>	13.4 <sup>b</sup>	13.0 <sup>b</sup>	

\*Means followed by different superscript letters are significantly different according to L.S.D. test at P=0.05

LWR) (P<0.0001) and increased that partitioned to stem (stem weight ratio, SWR) (P<0.0001), while RWR did not change (Table 5). *E. microtheca* had significantly lower LWR (P<0.0001) and greater root weight ratio (RWR)

(P= 0.0049) comparing with the other two species which had closed values (Table 5). From harvest to the next both SWR and RWR increased steadily (P<0.0001) while LWR decreased onwards (P<0.0001).

Table 6: Effects of irrigation with saline water on specific leaf area, leaf area ratio and relative growth rate of *E. microtheca*, *E. camaldulensis* and *E. intertexta* seedlings

Trait	EC (dS mG <sup>l</sup> )	Species			Treatment mean
		<i>E. microtheca</i>	<i>E. camaldulensis</i>	<i>E. intertexta</i>	
Specific leaf area (SLA) (cm <sup>2</sup> gG <sup>l</sup> leaf dry weight)	1	152.57	139.26	145.54	145.79 <sup>a</sup>
	2	146.63	130.54	132.00	136.39 <sup>b</sup>
	3	132.41	126.05	130.78	129.75 <sup>c</sup>
	4	117.42	115.13	127.11	119.89 <sup>d</sup>
Species mean		*137.26 <sup>a</sup>	127.75 <sup>b</sup>	133.86 <sup>a</sup>	
Leaf area ratio (LAR) (cm <sup>2</sup> gG <sup>l</sup> total plant dry weight)	1	93.68	87.53	92.61	91.27 <sup>a</sup>
	2	82.39	77.91	78.83	79.71 <sup>b</sup>
	3	71.59	71.82	74.85	72.75 <sup>c</sup>
	4	61.46	64.56	70.53	65.52 <sup>d</sup>
Species mean		77.28 <sup>ab</sup>	75.46 <sup>b</sup>	79.21 <sup>a</sup>	
Net assimilation rate (NAR)	1	0.0499	0.0479	0.0501	0.0493 <sup>a</sup>
	2	0.0495	0.0381	0.0573	0.0483 <sup>a</sup>
	3	0.0474	0.007	0.0558	0.0367 <sup>a</sup>
	4	0.0411	0.0002	0.0038	0.0264 <sup>a</sup>
Species mean		0.0470 <sup>a</sup>	0.0233 <sup>a</sup>	0.0503 <sup>a</sup>	
Relative growth rate (g gG <sup>l</sup> mG <sup>l</sup> )	1	0.51	0.42	0.40	0.445 <sup>a</sup>
	2	0.38	0.37	0.33	0.363 <sup>ab</sup>
	3	0.30	0.25	0.32	0.291 <sup>b</sup>
	4	0.28	0.08	0.19	0.184 <sup>c</sup>
Species mean		0.371 <sup>a</sup>	0.282 <sup>b</sup>	0.312 <sup>ab</sup>	

Table 7: Effects of irrigation with saline water on Relative leaf water content (RWC) of *E. microtheca*, *E. camaldulensis* and *E. intertexta* seedlings

Trait	EC(dS mG <sup>l</sup> )	Species			Treatment mean
		<i>E. microtheca</i>	<i>E. camaldulensis</i>	<i>E. intertexta</i>	
Relative leaf water content (RWC)	1	81.84	83.82	80.17	81.94 <sup>a</sup>
	2	71.13	74.05	71.10	72.09 <sup>b</sup>
	3	64.79	65.18	65.11	65.03 <sup>c</sup>
	4	62.32	60.69	58.85	60.62 <sup>d</sup>
Species mean		*70.02 <sup>ab</sup>	70.94 <sup>a</sup>	68.81 <sup>b</sup>	

Table 8: Effects of irrigation with saline water on the concentration (ppm) of sodium (Na<sup>+</sup>), potassium (K<sup>+</sup>), chlorine (Cl<sup>-</sup>) and Na<sup>+</sup>: K<sup>+</sup> ratio in the leaves of *E. microtheca*, *E. camaldulensis* and *E. intertexta* seedlings under irrigation with saline water

Trait	EC (dS mG <sup>l</sup> )	Species			Treatment mean
		<i>E. microtheca</i>	<i>E. camaldulensis</i>	<i>E. intertexta</i>	
Na <sup>+</sup> concentration (ppm)	1	1162.53	1276.42	1233.07	1224.00 <sup>d</sup>
	2	2664.69	2107.37	2592.27	2454.78 <sup>c</sup>
	3	3712.31	3423.66	3385.03	3507.00 <sup>b</sup>
	4	3899.25	4605.85	4730.13	4411.74 <sup>a</sup>
Species mean		*2859.70 <sup>a</sup>	2853.33 <sup>a</sup>	2985.13 <sup>a</sup>	
K <sup>+</sup> concentration (ppm)	1	2047.93	2334.09	2342.12	2241.38 <sup>c</sup>
	2	4337.28	3393.55	3721.78	3817.54 <sup>b</sup>
	3	5387.25	5609.95	4513.85	5170.35 <sup>a</sup>
	4	4576.53	5331.89	4730.35	4879.59 <sup>a</sup>
Species mean		4087.25 <sup>a</sup>	4167.37 <sup>a</sup>	3827.03 <sup>a</sup>	
Na <sup>+</sup> : k <sup>+</sup> ratio	1	0.566	0.546	0.527	0.55 <sup>c</sup>
	2	0.616	0.622	0.698	0.65 <sup>b</sup>
	3	0.689	0.611	0.753	0.68 <sup>b</sup>
	4	0.852	0.869	0.992	0.90 <sup>a</sup>
Species mean		0.68 <sup>b</sup>	0.66 <sup>b</sup>	0.74 <sup>a</sup>	
Cl <sup>-</sup> concentration (ppm)	1	3106.26	2995.99	3478.13	3193.46 <sup>d</sup>
	2	5753.91	7116.69	6778.59	6549.73 <sup>c</sup>
	3	7014.50	8706.76	8446.68	8055.98 <sup>b</sup>
	4	9387.58	9487.42	11784.79	10219.93 <sup>a</sup>
Species mean		6315.56 <sup>c</sup>	7076.72 <sup>b</sup>	7622.05 <sup>a</sup>	

\*Means followed by different superscript letters are significantly different according to L.S.D. test at P=0.05

**Growth Analysis:** Relative growth rate (RGR), leaf weight ratio (LWR), specific leaf area (SLA) and leaf area ratio (LAR) of *Eucalyptus* species all decreased with increasing the concentration of sodium chloride in irrigation water ( $P < 0.0001$ ,  $P < 0.0001$  and  $P < 0.0001$ , respectively) (Table 6). Both *E. microtheca* and *E. intertexta* seedlings had significantly greater RGR (0.37 and 0.31 g g month<sup>-1</sup>, respectively) than that of *E. camaldulensis* (0.28 g g month<sup>-1</sup>) as an average of all salinity treatments. This trend was also more or less true for SLA and leaf area ratio. Net assimilation rate (NAR) was not affected by salinity but, increased from harvest to the next ( $P < 0.05$ ) (Table 6).

**Relative Water Content:** Relative leaf water content (RWC) decreased with increasing salinity ( $P < 0.0001$ ). *E. camaldulensis* had a RWC was greater than that of *E. intertexta* but did not vary from that of *E. microtheca* ( $P < 0.05$ ) (Table 7). There were species x harvests interactions indicated changing the magnitude of RWC for species from harvest to harvest ( $P < 0.0001$ ). RWC of *E. microtheca* was the greatest in both the first and last harvest, while *E. camaldulensis* had the least RWC in the third and fourth harvest. RWC of *E. intertexta* decreased by the second harvest then increased (Table 7).

**Concentration of Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup> and Na<sup>+</sup>: K<sup>+</sup> Ratio:** With increasing salinity the concentrations of Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup> and Na<sup>+</sup>: K<sup>+</sup> ratio increased in the leaves of the seedlings ( $P < 0.0001$ ) (Table 8). There were no differences between eucalyptus species in the concentration of Na<sup>+</sup> and K<sup>+</sup> in their leaves. However, *E. intertexta* had Na<sup>+</sup>: K<sup>+</sup> ratio was greater than either that of the other two species ( $P = 0.0251$ ). Cl<sup>-</sup> concentration in the leaves ranged from 6315.56 to 7076.72 to 7622.05 ppm for *E. intertexta*, *E. camaldulensis* and *E. microtheca*, respectively ( $P < 0.0001$ ) (Table 8).

## DISCUSSION

Growth of *Eucalyptus* species investigated in this study decreased significantly due to increasing sodium chloride concentration in irrigation water.

In most of the variables measured there was an increase from harvest to the next which is logic, as the plants were in an early stage of growth. This is considered the most vital developmental stage for seedlings until they establish as fully grown individuals [23]. The detrimental effects of salinity on plants may also vary with developmental stage [24]. Often, growth and survival following emergence are not affected by low and moderate

salinity levels [25]. However, high substrate salinity may be more detrimental to plants in their early growth stages than to seeds [26].

All the growth variables measured in the present study decreased with increasing the level of salinity except stem dry weight which increased steadily. Decreasing the growth of stem height of woody species due to increasing the level of salinity was extensively reported [2, 4, 27-32]

Decreasing stem diameter of the *Eucalyptus* seedlings with increasing salinity level concurs with other findings [30, 32]. Contradictory, Catchpoole *et al.* [33] reported no significant effect of salt treatment on the mean growth in tree height or diameter of *Eucalyptus globulus* spp. *globulus*. El-Juhany and Aref [31] also, found unaffected stem diameter of *Conocarpus erectus* L. seedlings in high salt treatment. Often, growth and survival following emergence are not affected by low and moderate salinity levels [25]. However, high substrate salinity may be more detrimental to plants in their early growth stages [26].

Increasing the concentration of NaCl in irrigation water reduced root length of *Eucalyptus* seedlings. This result is in conformity with the finding of Ramoliya and Pandey [4, 29] for *Salvadora oleoides* and *Cordia rothii*, respectively. Also, reducing root length due to salinity was reported by Gama *et al.* [2]. As an average of the four salinity treatments, *E. microtheca* had stem height was greater than that of *E. camaldulensis* or *E. intertexta*. But, the responses of stem diameter and root length to the increase in the concentration of NaCl were not significantly different between the three *Eucalyptus* species.

Reduction in both the number of leaves and average leaf size in this study resulted in reducing total leaf area. Reducing total leaf area due to salinity was obtained by Gebauer *et al.* [34] Ramoliya and Pandey [4, 29] Chen *et al.* [35]. El-Juhany and Aref [31] reported significant decreases in the number of leaves and average leaf size resulted in reducing total leaf area of *C. erectus* seedlings in high salt treatment. Although *E. microtheca* seedlings had greater number of leaves however, their leaves were smaller in size so that they had lower leaf area comparing with *E. camaldulensis* or *E. intertexta*, which did not vary significantly in these traits.

The reductions in leaf, stem, roots and total dry weight of *Eucalyptus* seedlings with increasing salinity level are in consistence with many other previous results on the effect of salinity in dry matter production [29, 31, 34]. However, the species in this study had a trend for leaf, stem and total dry weight

production was similar to that of total leaf area mentioned above. Root dry weight did not differ between species. This concurs with the finding of the research work carried out by Mehari *et al.* [32] who found *Acacia nilotica* and *A. tortilis* responding similarly to salinity.

Increasing sodium chloride concentration in irrigation water increased dry weight partitioned to stem (SWR) at the expense of that proportioned to leaves (LWR), while RWR did not change. However, Houle *et al.* [36] found stem mass ratio decreased in salinity treatment. *E. microtheca* had significantly lower LWR and greater root weight ratio (RWR) comparing with the other two species which had closed values. The above ground part of the plant is more affected than that of root at high salinity [37]. El-Juhany and Aref [31] found both SWR and RWR of *C. erectus* seedlings increased at high salinity at the expense of LWR. Also, the proportion of dry weight allocated to roots of *Prosopis alba* increased with increasing NaCl levels [38]. More recently, Perica and Goreta [39] found a linear increase of root to plant ratio and consequently decrease of shoot to plant ratio as salinity increased for Olive Cultivars.

Decreasing relative growth rate (RGR) of *Eucalyptus* species with increasing the concentration of sodium chloride in irrigation water in the present study concurs with other results [40-43]. Decreased SLA may be contributed to this decrease in RGR. Ramoliya and Pandey [4] found SLA of *Cordia rothii* decreased in high salinity treatment. In contrast, Houle *et al.* [36] reported that salinity treatment had no effect on SLA. While decreasing RGR of plants due to salinity is well documented, attributing this decrease to decreasing NAR (including SLA and LWR) or LAR varies according to plant species or age of plant or growing conditions and may be to other factors. In the present study decreasing RGR was a result of decreasing LAR including decreases in both LWR and SLA as NAR was unaffected. Rodríguez *et al.* [44] found the relative growth rate (RGR) and net assimilation rate (NAR) decreased in salinity treatments, while leaf area ratio (LAR) remained unchanged. However, Dodd and Donovan [45] stated that variation in RGR was correlated with NAR and not LAR.

Decreased Relative water content (RWC) with increasing the concentration of sodium chloride in irrigation water in the present study concurs with the result reported by Meloni *et al.* [38] and Rodríguez *et al.* [44]. In contrast, Gebauer *et al.* [34] asserted that water content of leaf tissue of *Tamarindus indica* increased as salinity concentration increased.

By the last harvest, the seedlings that were irrigated with saline water had a significant increase of Na<sup>+</sup> and Cl<sup>-</sup> ions levels in their leaves, while K<sup>+</sup> ions decreased. Similar results were obtained by Gebauer *et al.* [34] Rodríguez *et al.* [44] and Perica and Goreta [39].

Na<sup>+</sup>: K<sup>+</sup> ratio increased with increasing the concentration of sodium chloride in the irrigation water. This result is in consistence with the findings of previous studies [34]. Both *E. microtheca* and *E. camaldulensis* had lower Na<sup>+</sup>: K<sup>+</sup> ratio than that of *E. intertexta*. Maintaining relatively low K<sup>+</sup>: Na<sup>+</sup> ratios in the leaves of these two species is likely to be one of the key determinants of plant salt tolerance.

Our study showed that *E. microtheca* and *E. camaldulensis* tolerated the levels of salinity (EC) applied up to 4 dSmG<sup>l</sup>. Webb *et al.* [46] classified both *Eucalyptus camaldulensis* and *E. microtheca* within the species tolerant to saline soils. Moreover, the Department of Natural Resources of New South Wales government (NSW) [47] placed both *Eucalyptus camaldulensis* and *Eucalyptus microtheca* within class 3 in which the plants tolerate very saline conditions (8<sup>+</sup> dS/m). However, Sun and Dickinson [48] found that *E. camaldulensis* was salt-tolerant and it is preferable for reclamation of salt-affected land, particularly when the salinity is moderate or low. In the Ecocorp Data Sheets [48], FAO reported that *Eucalyptus intertexta* tolerates low (<4 dS/m) to medium (4-10 dS/m).

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