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Seedling Tolerance and Phasiological Response to Short-Term Soil of Three Eucalypts Species

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Short Research Article

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ABSTRACT

The present paper involves a detailed comparison between the salt tolerance and physiological responseof three eucalypt species occurring within the Swan Coastal Plain, Western Australia. *Eucalyptus gomphocephala* DC (Myrtaceae) (common name 'Tuart') is restricted to the calcareous (limestone), brown or yellow sand of the coastal Spearwood dunes. *Eucalyptus marginata* Sm. (common name 'Jarrah') is a small tree on the porous, well-drained sandy soils of the Bassendean dunes Plain, and a much larger tree on the Darling Range. *Corymbia calophylla* (Lindl.) K.D. Hill & J. A. S. Johnson (common name 'Marri'), and has a similar distribution to that of Jarrah, but is more common on wetter, well drained soils. This investigate implemented to find out the seedling tolerance of these three species to soil-induced stressor, namely salinity via addition of sodium chloride solution. Tolerance assessment measured changes in seedling growth, leaf allocation and leaf physiology after 70-80 days. Neither *E. marginata* and *C. calophylla* could tolerate the highest salinity (0.25 M NaCl solutions) with 9-13% survival, although *E. marginata* was clearly the least tolerant with 52% reduction in relative growth rate and a 88% in transpiration rates. *E. gomphocephala* was the most tolerant to salt stress in terms of survival and growth parameters.

Keywords: Eucalypt salt tolerance; eucalypt physiological response; eucalypt growth.

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1. INTRODUCTION

Soil salinity may cause physiological response in plants, and hence influencing plant growth, due to changes in cell osmotic potentials, tissue ion toxicity, or by causing preventing inorganic soil nutrient causing tissue deficiencies [1]. Under saline conditions the concentration of soluble salts increases in the rhizosphere, which influences root pressure and hence soil water availability, decreasing the uptake of water and certain soil nutrients [2]. Increasing salinity in the root medium interferes with uptake and translocation of Ca^{2+} and K^+ and sometimes excludes K^+ [3]. Soil salt stress is a dominant factor influencing plant survival; growth and productivity within the genus *Eucalyptus* [4] as well as other genera that have the potential to be influenced by soil salinity. Different species, and even provenances within species, differ in their ability to tolerate soil salinity [5]. Salinity is one the major environmental issues affecting the Australian vegetation [6,7] with an estimated 5.6 million ha of salt affected land [8]. Salinization in Western Australia was in first observation reviewed [9] who observed that the decline in native plant species' abundance was caused by increases in soil and stream salinity. About 77 percent of Western Australia land has affected by salinity as well as risk of increasing secondary dry land salinity [10]. Much of Perth's Swan Coastal Plain has groundwater salinity that rarely exceeds 1,000 mg/L total dissolved solids, with the greatest salinities occurring where soils impede rainfall infiltration and have high evaporation rates, or at salt water interfaces of rivers and deep aquifers [11]. This low level of groundwater salinity is a type of 'primary salinity', resulting from the presence of naturally high dissolved salt concentrations in the soil solution, or marine incursions [12]. Agricultural lands experience 'secondary salinisation', caused by the removal of deep-rooted, perennial native vegetation in favour of growing shallow-rooted, usually annual crops or pasture land, or other land uses that causes underlying water tables to rise [13]. *Eucalyptus camaldulensis* (River Red Gum) is the most widely distributed eucalypt along Australian river systems, well known for its tolerant to salinity and waterlogging, showing an >85% survival rate under a variety of saline soil condition [14]. Although *E. camaldulensis* trees was second most tolerant to salt at 0.4% NaCl compared with 5 tree species growth for one year in pot experiment [15]. High narrow-sense heritability found out for shoot dry weight within *E. grandis* and *E. globules* attributed to salt treatment [16]. The *Eucalyptus occidentalis* is known as extremely salt tolerant [10], however a limited information available on how that salt tolerance be inherited. In a natural system, the potential to be impacted by increased salinity due to anthropogenic interference, an understanding of how key components of the native flora might respond to salt stress is important. There is possible for substantial benefits in resolving salinity problems during growing plants with deeper roots such as Eucalyptus. This paper aims to investigate the salt tolerance and physiological behaviour of seedlings of three common Eucalypt species by growing in soils watered with five salinity solutions 0.00, 0.05, 0.10, 0.15, 0.25 Molarity NaCl.

2. METHODOLOGY

2.1 Experimental Design

Seeds of the three eucalypt species were purchased from Nindethana Seed Service (Albany, Western Australia), and germinated in shallow trays filled with white sand in a naturally lit glasshouse at Curtin University (Western Australia). Trays were initially partly submerged in a larger tray of water containing pervicur fungicide (2 ml L^{-1}) to minimize seedling death resulting from fungal infection. Every 3-4 days the trays were rewatered.

Seedlings remained in these trays until they had obtained a height of approximately 3 cm. A total of 120 seedlings of each species were than transplanted into individual pots (7cm wide and 7cm long by 8cm deep) filled with soil at a ratio of four parts white sand to two parts peat. Transplanted seedlings were carefully watered twice weekly until the seedlings had 4-6 leaves or were approximately 6 cm tall. The day before applying the salinity treatments, five seedlings of each species were randomly selected for harvesting, with each seedling divided into stem, root and leaf components. Biomass of stem, root and leaves were calculated after drying the samples in a drying oven at 80ºC for 48 hours ($g\ g^{-1}$). The remaining seedlings were randomly divided into five salinity treatments (0.00, 0.05, 0.10, 0.15 and 0.25 M NaCl) for each species. Each treatment was watered with the appropriate salt solution, twice a week for a total of 81 days. Solutions were poured onto the soil surface using a thin nozzle watering can to avoid wetting the leaves. The total number of leaves and height was recorded for each seedling prior to adding the salt solutions, and remeasured before the final harvest.

2.2 Physiology and Growth Measurements

In the days leading up to the final harvest, about ten seedlings per treatment and species were randomly chosen for chlorophyll and physiological measurements. Chlorophyll content (SPAD-502 meter, Konica Minolta, Japan), and stomata conductance (steady state porometer, LI-1600, Li-Cor, Nebraska, USA) were measured on the youngest fully expanded leaf. All measurements were recorded during the mid-morning in full sunlight. Stomata conductance was measured a second time 14 days afterwards to assess for physiological recovery after watering with tap water (Stomatal conductance units recorded ≈mol $m^{-2}\ s^{-1}$). Plant height was measured from soil level to the top of the apical meristem, thus providing a consistent measurement between species. Percentage relative water content was measured on a different subset of seedlings as [(saturation weight – dry weight)/ (fresh weight – dry weight)] x 100 of the youngest fully expanded leaves. Saturation weight was obtained by floating leaf discs in deionised water overnight in a darkened container. At the end of the experiment, seedling were harvested into stem, leaf and root components. For each seedling all leaves were digitally scanned fresh and total leaf area measured using the image J software (http://rsb.info.hih.gov/ij). All plant material was oven dried at 80ºC for 48 hours, and the dry weights of each component recorded. Various growth such as biomass (g) and leaf area ratio ($mm^2\ g^{-1}$) allocation parameters were then calculated for each treatment and species. These included relative growth rate (RGR ≈ mg g^{-1} day^{-1}), leaf area ratio (LAR ≈ $mm^2\ g^{-1}$), leaf weight ratio (LWR ≈ $g\ g^{-1}$) and shoot to root ratio as defined by McGraw and Garbutt system.

2.3 Statistical Analysis

Interaction between species and treatment was analysed by factorial ANOVA and statistical difference between treatments within species was analysed by one-way ANOVA using SPSS version 16.0 (SPSS inc. Chicago, USA). Homogeneity of variances was assessed using Levene's test and log transformed as required, with data presented as untransformed means. Scheffe's test was used for multiple comparisons when a significant relationship between treatments occur ($P \leq 0.05$). Values followed by the same letter were found to not be significantly different.

3. RESULTS AND DISCUSSION

3.1 Survival

Both *C. calophylla* and *E. marginata* exhibited similar survival patterns with 34-42 of the original 120 seedlings surviving after 11 weeks subjected to weekly watering of 0.15 M NaCl solution, and 11-15 seedlings surviving at 0.25 M solution (Table 1a). For the same solutions, *E. gomphocephala* had 70 - 82 seedlings survive compared to 99% survival for the control treatment. Comparisons between the salt tolerance of our studied eucalypt species demonstrated that *C. calophylla* was the least tolerant to soil salinity. Declining seedling growth began when 0.10 M NaCl solutions, or greater were provided. The next tolerant was *E. marginata* and most tolerant *E. gomphocephala*. This was supported by a 68% (*C. calophylla*), 56% (*E. marginata*) and 13% (*E. gomphocephala*) reductions in relative growth rate between the highest NaCl concentration (0.25 M) and the control (0.0 M). Although *E. gomphocephala* survival rate declined with an increased salinity, it was always greater than the other eucalypts. The survival data suggests that *E. gomphocephala* seedlings have shown ability to cope with a weekly dosage of NaCl solution much greater than 0.25 M, and at least survived for more than 11 weeks under moderately saline conditions.

Table 1. (a) Percentage of surviving seedlings after 11 weeks growing in soils watered with NaCl solutions0.00, 0.05, 0.10, 0.15 and 0.25 M and (b). The mean of (±SE) leaves number of three eucalypt species after 9 weeks growing in soils watered with NaCl solutions0.00, 0.05, 0.10, 0.15 and 0.25 M

a

Concentration / Species	0.00 M	0.05 M	0.10 M	0.15 M	0.25 M
C. calophylla	100	85	73.3	35	12.5
E. gomphocephala	99.1	90.8	84.1	68.3	58.3
E. marginata	99.1	80.3	68.3	28.3	9.1

b

Treatments	*C. calophylla*	*E. gomphocephala*	*E. marginata*	*C. calophylla*
0.00 M	15.66±1.45^{a}	13.33±2.40	10.33±0.33^{a}	0.00 M
0.05 M	11.66±1.45^{a}	14.66±0.66	9.33±0.66^{a}	0.05 M
0.10 M	10.66±1.33^{a}	12.00±1.15	8.33±0.88^{a}	0.10 M
0.15 M	9.66±1.45^{a}	12.66±0.33	7.33±1.33.^{a}	0.15 M
0.25 M	8.00±1.15^{b}	12.00±1.15	5.33±0.66^{b}	0.25 M
P- value	*	NS	*	P- value

** = 0.05, ** = 0.005, *** = 0.0005, NS= not significant*

Salinity treatment had effect in leaf number, which increased at lower salt concentration in all species, but decreased at higher salt concentration as compared with the control (Table 1b) where symptoms of leaf salt damage such as burned leaves of the plant was noticed at concentrations >0.1 M for both *C. calophylla* and *E. marginata*, but not as obvios in *E. gomphocephala*. As has been documented for other eucalypts, and indeed other plant species, growing plants in every increasing saline soil will ultimately have a negative effect on seedling growth, leaf biomass and leaf area allocation, with the amount of salinity tolerated depending on species, provenance or genotype [17]. In the current experiment, it could be concluded that *E. gomphocephala* and *E. marginata* are more likely to invest less leaf area (on both a total leaf dry mass and total dry plant mass basis) at the same time as salinity levels increase, the long-term (i.e. greater than 11 weeks). Although, implications of growing these eucalypts under these stressful conditions is unknown, particularly as a continue watering of NaCl solutions may eventually cause soil salt levels to rise to toxic levels.

3.2 Plant Growth

All three species produced significantly shorter seedlings at NaCl concentrations >0.15 M (Fig. 1). Statically, there was no significant difference in height between seedlings growing in the control treatment (0.00 M) comparing to the 0.05 and 0.10 M treatments. A significant relationship between treatment concentration and growth calculation was established ($P \leq 0.05$) based on Scheffe post-hoc testing (Table 2a). The values followed by the same letter (^{a}&^{b}) point to no important difference. All species show declining relative growth rate (RGR) compared with the 0.0 M treatment. The *E. gomphocephala* demonstrated the highest RGR for every treatment with range between 41- 47.3 mg g^{-1} day^{-1} (Table 2b). Both *C. calophylla* and *E. marginata* had a 32 and 44% respective in RGR at 0.25 M compared to the control, compared with 87% for *E. gomphocephala* (Table 3b). In general, there was a decrease in RGR with increasing salinity concentration, with the greatest decrease occurring between the 0.15 and 0.25 M treatments. None of the three eucalypts displayed any significant difference in shoot-to-root (StR) biomass ratios between salinity treatments (Table 2c). Growth data appeared to have no significant influence on seedling height in the 0.05 and 0.10 M treatments compared to control treatment (0.00 M). This suggests continue cells elongation in plants under low salinity concentrations and is similar to results obtained on other eucalypts [18,19]. However, a decrease growth in high level of salinity is mostly a result of decreasing cell turgor potential which has implications for cell elongation and cell division [20,18]. In study, Salt tolerance of *E. gomphocephala* may be attributed to one, or more, of the following: (1) salt exclusion; (2) osmotic effects [6]. (3) storage of accumulated salts in mature leaves in spices. Salt exclusion from the shoot has been described as a vital mechanism of salt tolerance [21,22,6]. Due to the energy demanding production of osmoregulators, an overall reduction in growth is expected [21]. It has been described that salt excluding species will generally have a lower overall growth rate than salt stressed non-excluding species [21,6]. The three species may have the capacity for osmotic adjustment in saline environments. This involves the production of various organic solutes in the cytoplasm, which help mitigate high salt concentrations accumulating in the vacuole, yet have no adverse effect on enzyme and cell membrane function [22].

Generally however, salinity reduces vegetative growth of all non-halophyte species [22] including those involved in the trial, irrespective of potential mechanisms of avoiding or tolerating saline conditions.

3.3 Leaf Investment

Increased soil salinity had no significant effect on the relative biomass allocation in leaf mass compared with the total plant mass (Leaf Weight Ratio) for any of the three eucalypts species (Table 3a), but did influence the Specific Leaf Area (measured as total dry mass of leaves divided by total projected leaf area) and Leaf Area Ratio (measured as total projected leaf area divided by the total plant dry mass), at least for *E. gomphocephala* and *E. marginata* (Tables 3b and 3c).

Table 2. (a) Difference in height of three eucalypts species to increasing salinity treatments after 81 day trial period, (b). Relative growth rate of three eucalypts species to increasing salinity treatments after 81 day trial period and (c). The mean (± SE) of biomass partitioning (shoot-to-root ratio) of three eucalypt species to increasing salinity treatments after 81 day trial period

		a	
Treatments	***C. calophylla***	***E. gomphocephala***	***E. marginata***
0.00 M	28.70±1.53[a]	15.00±0.88[a]	10.50±1.10[a]
0.05 M	16.70±1.00[a]	10.40±0.88[a]	10.30±0.76[a]
0.10 M	16.0±0.71[a]	16.00±1.14[a]	10.60±0.90[a]
0.15 M	11.2±0.95[b]	10.80±0.72[b]	4.30±0.42[b]
0.25 M	9.70±0.47[b]	5.80±0.59[b]	3.30±0.36[b]
P- value	**	***	***
		b	
0.00 M	30.9	47.3	39
0. 05 M	28.4	46.0	36.9
0.10 M	20.9	42.5	35.2
0.15 M	18.5	43	20.11
0.25 M	9.96	41.3	17.3
		c	
0.0 M	5.84±0.64	7.70±1.46	13.74±1.73
0.05 M	3.53±0.07	4.41±0.52	4.96±1.38
0.10 M	3.20±0.39	5.75±0.94	11.50±6.13
0.15 M	8.41±2.90	7.36±1.74	6.30±1.95
0.25 M	7.15±2.93	6.33±1.36	3.02±0.63
P -Value	NS	NS	NS

** = 0.05, ** = 0.005, *** = 0.0005, NS= not significant, NS= not significant*

3.4 Relative Chlorophyll and Water Content

Increasing salt concentration had significant difference of relative chlorophyll content in all *Eucalypts* species (*E. calophylla, gomphocephala* and *marginata*) with (P= 0.0001, 0.001 and 0.0001), although increasing salinity treatment reduced relative chlorophyll content. *E. gomphocephala* had the greater chlorophyll content in all treatment compared with other species, and there is slightly different of chlorophyll content between control and other treatment in this specie. *E. calophylla* chlorophyll content with 0.25 M having approximately 40% (17.14 SPAD units) as the control (44.28 SPAD units).*E. marginata* there is no great different of chlorophyll content between treatments up to 0.15 M, but with 0.25 M the chlorophyll content having roughly 60% (28.83 SPAD units) as the control (46.47 SPAD units) (Table 4a). Relative chlorophyll content significantly decreased in the three eucalypt investigated. *C. calophylla* had the lowest relative chlorophyll content compared with the control treatment, while *E. gomphocephala* had least reduction of chlorophyll content. Studies have shown increasing salinity levels results in lower chlorophyll concentration [23] which has been discussed as being attributed to the disintegration of chloroplasts due to increased chlorophylls activities [22,24]. A reduction in chlorophyll content reduces an individual's ability to photosynthesize and effects overall plant growth [25]. No data was obtained for *C. calophylla* and *E. marginata* with 0.15 and 0.25 M NaCl as the plants died before measurements could be taken. Leaf relative water content decreased with increasing salt concentration for all species, except *C. calophylla* (Table 4b).

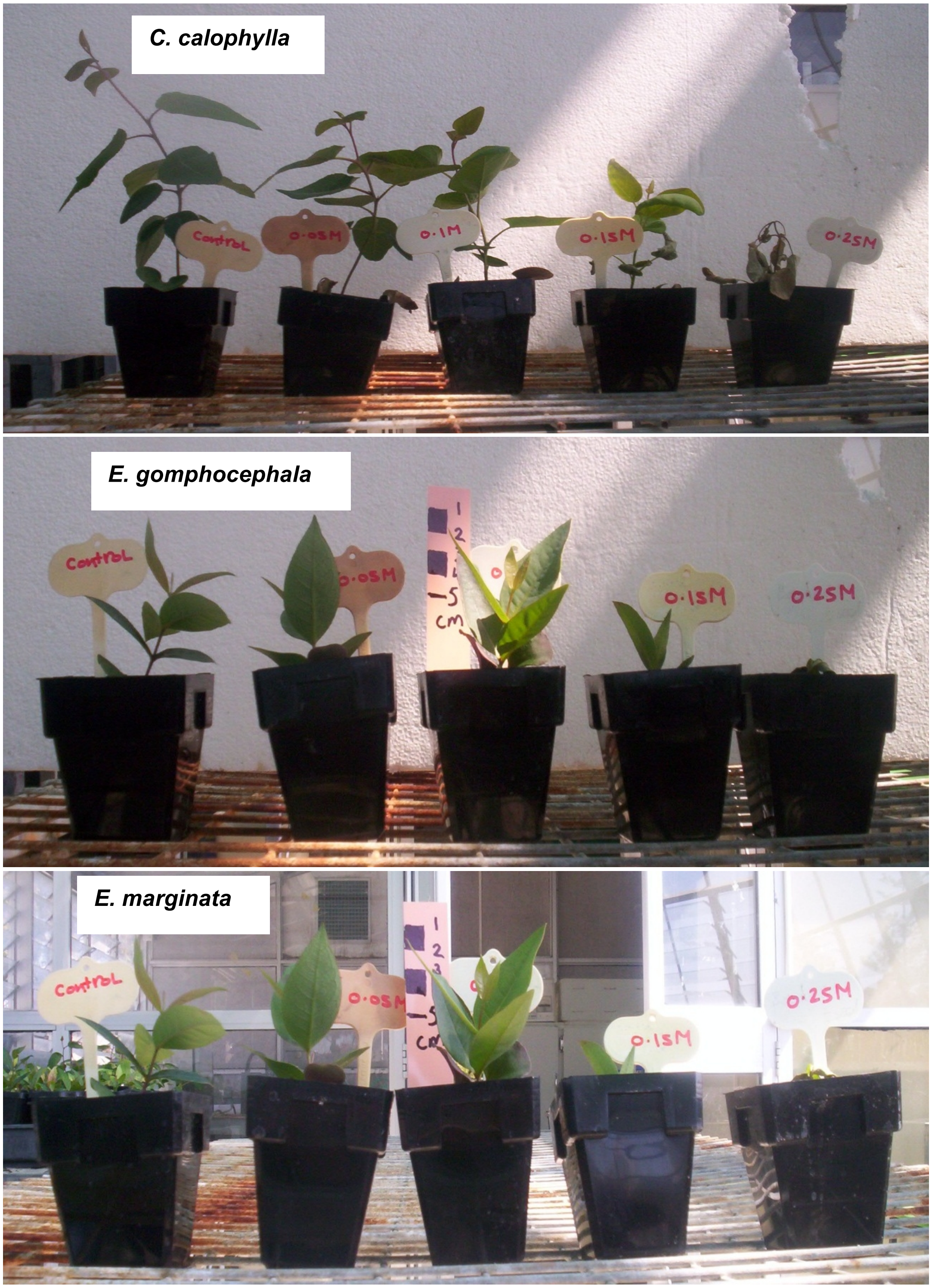

Fig. 1. Photographs showing relative heights of the three eucalypts species between NaCl solution treatments

Table 3. (a) Mean (± SE) leaf weight ratio (g g^{-1}) of three eucalypt species to increasing salinity treatments after 81 day trial period, (b). Specific leaf area (m^2 g^{-1}) of three eucalypt species to increasing salinity treatments after 81 day trial period and (c). Mean (± SE) leaf area ratio (m^2 g^{-1}) of three eucalypts to increasing salinity treatments after 81 day trial period

	a		
Treatments	***C. calophylla***	***E. gomphocephala***	***E. marginata***
0.00 M	0.61±0.01^{a}	0.66±0.02^{a}	0.73±0.01^{a}
0.05 M	0.51±0.07^{a}	0.67±0.04^{a}	0.66±0.06^{a}
0.10 M	0.61±0.02^{a}	0.65±0.04^{a}	0.70±0.02^{a}
0.15 M	0.64±0.03^{a}	0.76±0.04^{a}	0.71±0.03^{a}
0.25 M	0.49±0.16^{a}	0.63±0.18^{a}	0.69±0.04^{a}
P- Value	NS	NS	NS
	b		
0.00 M	193.0±17.1^{a}	185.9±9.0^{a}	158.1±6.9^{a}
0.05 M	120.7±6.8^{a}	88.9±8.6^{ab}	98.2±8.7^{b}
0.10 M	127.8±4.0^{a}	98.2±30.8^{b}	93.8±1.8^{b}
0.15 M	123.3±6.8^{a}	111.5±9.9^{b}	104.8±4.4^{b}
0.25 M	159.2±15.1^{a}	117.2±2.5^{b}	103.6±3.9^{b}
P- Value	NS	**	**
	c		
0.00 M	119.1±13.1^{a}	122.9±6.5^{a}	115.7±6.1^{a}
0.05 M	62.8±10.3^{a}	60.1±7.0^{b}	63.4±2.8^{b}
0.10 M	78.8±1.0^{a}	63.3±19.6^{b}	66.2±2.7^{b}
0.15 M	79.2±4.8^{a}	84.7±6.1^{a}	74.2±1.8^{b}
0.25 M	109.0±25.2^{a}	75.1±12.1^{b}	72.1±2.9^{b}
P- Value	NS	**	**

**=0.05, ** = 0.005, ***0.0005, NS= not significant*

E. gomphocephala and *E. marginata* having a significant difference in relative water content. Relative water content also decreased with increasing salt concentration especially in*E. marginata* and *E. gomphocephala*, but not *C. calophylla,* despite *C. calophylla* being overall the least tolerant. The results suggest that *C. calophylla* had the ability to avoid the water stress induced by salinity than *E. marginata* and *E. gomphocephala*, With increase NaCl concentration, the water uptake by plants is mostly slowed down thereby the RWC decreased. It led to reduction of the fresh weight and observed in all studied species, clearly in salt-sensitive *C.calophylla*. This is consistent with fact that the Inhibition of growth and decrease in water content induced by water stress has been universally observed even in tolerant plants. Although growth level is a visible mark of plant performance can be expressed under stress, it is to result from the sum of the adaptive mechanism that is adapted by a given species.

3.5 Stomatal Conductance and Transpiration

No data is presented for *C. calophylla* and *E. marginata* for 0.15 and 0.25 M treatments as plants died before measurements were taken. Increasing salinity concentration caused substantial decline of stomatal conductance in the three species (Table 5a). This decline was great from 0.05 M and above; however the difference between treatments were significantly for *E. gomphocephala* and *E. marginata*; for *C. calophylla* the difference between treatments was not significant. The transpiration rates of *E. gomphocephala* and *E. marginata* were found to have been significantly affected with increasing salinity treatment levels with (P<0.04), with the maximum reduce occurring for *E. gomphocephala* at 0.25 M by approximately (95% decrease) compared with control (0.0 M), followed by *E .marginata* (85% decrease) and *C. calophylla* with about (65% decrease) (Table 5b). Stomatal conductance and transpiration in this study was found to be significant, except *C. calophylla*. Stomata cconductance and transpiration rate declined with increasing salt concentration, which was the *E. gomphocephala* had the greater decreased comparing with the other two studied species. This may be due to the information that lowered water potentials in the root be able to activate a signal from root to shoot , for example abscisic acid, which has been recommended to be the operating mechanism [26]. Though an another reason for decreased Stomata cconductance and transpiration might be that the inhibition of photosynthesis caused by salt increase in the mesophyll make an raise in Intercellular CO_2 concentration, which decrease the stomatal aperture [27].

Table 4. (a) Relative chlorophyll content (SPAD units) of three eucalypts species subjected to varying salinity regimes and (b). Water content of three eucalypts species subjected to varying salinity regimes

		a	
Treatments	***C. calophylla***	***E. gomphocephala***	***E. marginata***
0.00 M	44.3±1.8^{a}	52.9±1.6^{a}	46.5±1.6^{a}
0.05 M	41.8±2.1^{a}	56.8±2.0^{a}	47.2±3.1^{a}
0.10 M	38.1±1.2^{a}	50.6±1.9^{a}	48.1±2.7^{a}
0.15 M	34.5±2.6^{a}	52.4±1.2^{a}	47.4±2.7^{a}
0.25 M	17.1±1.8^{b}	46.3±1.4^{a}	28.8±2.5^{b}
P Value	***	**	**
		b	
0.00 M	88.18±3.88^{a}	81.60±6.48^{a}	82.54±1.67^{a}
0.05 M	73.35±15.15^{a}	82.27± 7.36^{a}	82.33±8.87^{a}
0.10 M	61.86±17.2^{a}	78.40±18.82^{b}	74.43±6.73^{ab}
0.15 M	NA	76.20±6.40^{a}	NA
0.25 M	NA	74.76±16.44^{a}	NA
P- Value	NS	*	*

**=0.05, ** = 0.005, ***0.0005, NS= not significant, NA= not available*

Table 5. (a) Stomatal conductance (mol m^{-2} s^{-1}) and (b). Transpiration (mmol m^{-2} s^{-1}) of three eucalypt species to increasing salinity treatments after 81 day trial period

		a	
Treatments	***C. calophylla***	***E. gomphocephala***	***E .marginata***
0.00 M	0.051±0.01^{a}	0.23±0.08^{a}	0.33±0.11^{a}
0.05 M	0.023±0.01^{a}	0.05±0.02^{a}	0.04±0.00^{a}
0.10 M	0.020±0.00^{a}	0.07±0.00^{a}	0.05±0.02^{ab}
0.15 M	NA	0.02±0.01^{ab}	NA
0.25 M	NA	0.02±0.02^{ab}	NA
P- Value	NS	*	*
		b	
0.00 M	1.57±0.35^{a}	7.68±2.68^{a}	9.95±3.19^{a}
0.05 M	0.59±0.32^{a}	1.56±0.80^{a}	1.24±0.19^{a}
0.10 M	0.83±0.18^{a}	1.47±0.46^{a}	1.14±0.53^{a}
0.15 M	NA	0.67±0.43^{a}	NA
0.25 M	NA	0.34±0.60^{b}	NA
P- Value	NS	*	*

*NA= not available, *= 0.05, ** = 0.005, *** = 0.0005, NS= not significant*

4. CONCLUSION AND RECOMMENDATIONS FOR FUTURE WORK

The study has been demonstrated that the increasing salt concentration in soil will ultimately have a negative effect on physiological functions of seedling, however this effect was different between the species and term, due to the fact that the research proved the highest tolerance level was of *E. gomphocephala* compared to the tolerance level of *C. calophylla* but not as low as *E. marginata*. Novel results are offered within this paper and much models can be suggested for simulation via this methodology. The findings of paper confirm complexity of the this type of simulated models on open lands, however gives a new approaches for such applications. An additional focus on other environments circumstances and plants could identify the solutions for other environmental problems.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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