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Effects of different NaCl Concentrations on germination and seedling growth of *Amaranthus hybridus* and *Celosia argentea*

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Salinity refers to the salt content of any given system. By nature, arid soils are naturally saline. Soils could also acquire salinity due to some agricultural practices like irrigation. There is need therefore for search on halophytes that could adapt to such soils and be used to reclaim soils contaminated with salts. Thus, this present study focused on investigating *Amaranthus hybridus* and *Celosia argentea* with respect to their potentials and suitability for use in saline environments. This study was conducted in two phases; germination (laboratory-based) and seedling tests (field-based). Shoot length, root length, root/shoot ratio, total length, fresh weight, dry weight, dry matter content, leaf area index, leaf number as well as relative water content were parameters used for assessing results for the field-based test. Germination results showed that only the control (0 mM) showed 100 % germination in both species. Germination percentages decreased steadily with increasing NaCl concentrations in both species. Growth was steadily stimulated in both species at lower NaCl levels with best growth stimulation at 50 mM NaCl but was adversely affected by higher NaCl concentration levels (75, 100 and 150 mM). Both species showed almost the same phenomenon for fresh weight, dry weight, dry matter, leaf area index, number of leaves as well as relative water concentration. These show that both species would best be cultivated under moderate than low or high saline concentration. The findings in this study showed that it is best for seedlings of both species of plants to be raised in nurseries free of NaCl to attain 100% germination and after which, the seedlings could then be transplanted to moderately saline soils for maximum growth and development. This has strong implications for maximizing food productivity and ensuring food security.

Key words: Germination, early seedling growth, NaCl, salinity, *Amaranthus hybridus, Celosia argentea*.

INTRODUCTION

Salinity refers to the salt content of any given system. According to Herr (2005), salinity results from the build-up of the following minerals which are deposited by evaporating water: Na+, K+, Ca^{2}+, Mg^{2}+, Cl^{-}, HCO^{3-}, and

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SO_2^{4-}, respectively. Addition of salts to water lowers its osmotic potential, resulting in decreased availability of water to the root cells (Sairam et al., 2002). High salt concentrations could affect various physiological processes in plants. Ratnakar and Rai (2013) stated that high salt concentration hampers vital processes such as seed germination, seedling growth and vigour, vegetative growth, flowering as well as fruit set. Thus, high salt concentration ultimately reduces crop yield and the quality of the produce (Sairam and Tyagi, 2004).

According to Galston et al. (1980), soil salinity is becoming a serious worldwide problem as more land is irrigated thoroughly and heavily fertilized. Salinity is one of the major abiotic stresses in arid and semi-arid regions that substantially reduce the yield of major crops by more than 50% (Bray, 2000). Salinity affects agricultural production in a large proportion worldwide (Byordi et al., 2010). Salinity affects about 7% of the world's land area, amounting to about 930 million ha (Munns, 2002), thus seriously limiting crop production, especially the sensitive ones (Zadeh and Naeini, 2007). The problems of salinization are increasing, either due to bad irrigation, drainage or agricultural practices (Al-Seedi and Gatteh, 2010).

Cuartero et al. (2006) opined that although salt stress affects all growth stages of a plant, but that seed germination and seedling growth stages are known to be more sensitive for most plant species. In addition, Ibid (2006) further stressed that germination and seedling stages are predictive of plant growth responses to salinity.

Amaranthus hybridus is a robust annual herb (Olorode, 1984), that is cultivated for its nutritional value (Oguntona, 1998) and used as food by man and other animals. *C. argentea*, on the other hand, is a short-lived annual herb, slow growing and more drought-resistant than *A. hybridus* (Ogunwenmo et al., 2010). Both *A. hybridus* and *C. argentea* belong to the family, Amaranthaceae (Dutta and Dutta, 2008).

Byordi (2010) studied the influence of salt stress on seed germination, growth and yield of Canola cultivars. It was found that significant differences existed between influence of salt stress on the cultivars during germination and vegetative growth respectively. Al-Seedi and Gatteh (2010) while studying the effects of salinity on seed germination, growth and organic compounds of Mung bean plant [*Vignia radiate (L.) Wilczek*] found that an increase in the salinity caused a corresponding decrease in germination rate, growth parameters and the carbohydrate content of the Mung bean plant. Ratnakar and Rai (2013) observed while studying effects of NaCl salinity on seed germination and early seedling growth of *Trigonella foenum-Graecum L. Var Peb* that increasing NaCl concentrations caused a gradual decrease in root length, shoot length, fresh weight and dry weight of the growing seedling. In addition, Katambe et al. (1998) stated while studying the effects of germination and seedling growth of two *Atriplex* species *(Chenopodiaceae*) that NaCl caused a greater increase in nuclear volume than iso-osmotic PEG solutions. In addition, Ogunwenmo et al. (2010) studied effects of brewery, textile and paint effluent on seed germination of *A. hybridus* and *C. argentea* and stated that industrial effluents affected seed germination at different rates.

However, information pertaining to the effects of salinity due to varying concentrations of NaCl on *A. hybridus* and *C. argentea* are hard to come by. It is important to study the effects of different NaCl concentrations on germination and seedling growth of both crop species as that would help in establishing the tendencies of both crop species to be used in saline environments, most especially as there appears to be some level of universality in the occurrence of NaCl in most saline environments.

MATERIALS AND METHODS

Salt preparation

Salt solution of 1 M NaCl was prepared by dissolving 58.8 g of NaCl crystals in a universal bottle upon which distilled water was added onto to make up 1 L. Corresponding rations of 25/1000, 50/1000, 75/1000, 100/1000 and 150/1000 gave 25, 50, 75,100 and 150 mM respectively. Pure distilled water was considered to be 0 mM, thus represented the control. The experiment was conducted in two phases; germination and growth tests, respectively.

Germination test

This was conducted in the Botany Laboratory of Ambrose Alli University, Ekpoma, Edo State, Nigeria. It is located on latitude 06° 42 ´´N and longitude 06° 08 ´´E. It lasted for ten (10) days, from 20th June, 2001 to 30th June, 2001. Seeds of both *A. hybridus* and *C. argentea* used for the study were obtained from the Federal Department of Agriculture, Ubiaja, Edo State, Nigeria. Approximate seed sizes of both species were randomly selected and soaked in distilled water for 2 h before being transferred into glass Petri dishes. Ten (10) seeds of each crop species were sown into each glass Petri and these were replicated ten (10) times each for 0, 25, 50, 75, 100 and 150 mM respectively. Thus, total number of seeds per concentration (from 0 to 150 mM) amounted to 100 seeds each. The seeds were placed between folds of moistened filter paper in the glass Petri dishes at room temperature of 27.5°C. The seeds of both species in the glass Petri dishes were moistened every 12 h with varying concentrations (0, 25, 50, 75, 100 and 150 mM) of NaCl salt and observations were recorded every 24 h for radical emergence as indicative of germination. Seeds were considered to have germinated when up to 1 mm radicle emergence from the seed was noticed.

Seedling growth test

Black polythene bags measuring 25 x 25 cm were all filled with sandy-loamy soil from the Experimental Garden of Botany department, Ambrose Alli University, Ekpoma, Edo State, Nigeria. Fifteen seeds were randomly chosen and sown into each potted bag at a depth of 1 cm and after germination; only ten (10) seedlings considered 'healthier' were allowed to grow. Masking

Table 1. Effect of varying concentrations of NaCl on seed germination of *Amaranthus hybridus* in percentages.

NaCl (mM)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
0	81	87	94	100	100	100	100	100	100	100
25	54	68	76	79	80	80	80	81	81	82
50	49	63	70	71	72	72	73	74	74	75
75	31	48	51	55	58	61	62	62	62	63
100	27	36	50	53	54	54	54	55	55	55
150	21	39	42	44	45	46	46	47	47	47

tapes were used to label the bags appropriately. Salt solutions of NaCl corresponding to 25, 50, 75, 100 and 150 mM were used for watering the plants in the potted bags on twelve (12) hourly bases, throughout the period of experimentation. Distilled water was labeled 0mM and considered as the control. The experimental period lasted for eight (8) weeks. At the end of the eighth (8th) week, results were collected for parameters like shoot length, root length, root/shoot ratio, total length, fresh weight, dry weight, dry matter content, leaf area index, leaf number as well as relative water content, respectively.

Shoot length, root length and total length were measured with a ruler. Shoot to root ratio was determined mathematically through simple ratio. Numbers of seeds were determined manually. Before shoot and root lengths were determined, seedlings were uprooted carefully to avoid breakage from the medium. Particles stuck to the roots and shoots were carefully removed before measurements were taken. Each seedling was then measured on a weighing balance for fresh weight while for dry weight, the same seedlings were dried at 80 °C till constant weights were obtained and records taken. Leaf area index was determined following the methods of Gunkel and Mulligan (1953) in which leaf discs were punched from five (5) leaves using a cork-borer of diameter 0.82 cm. The discs of known area of the punched leaves were dried to constant weight at 80°C for 24 h hand the total leaf area per plant was then calculated using the relationship:

$$\text{Leaf Area Index} = \frac{\text{Leaf Dry Weight} \times \text{Disc Area}}{\text{Disc Dry Weight}}$$

Relative water content was determined following the method of Handley and Jennings (1977). In which case, four (4) plants from each treatment in both plant species were separated into leaf, stem and root tissues. The materials were dried at 80°C for 24 h and their corresponding dry weights measured. It is then calculated using this relationship:

$$0 \text{ Relative Water Content} = \frac{\text{Fresh weight} \times \text{Dry weight}}{\text{Dry weight}} \times 100$$

RESULTS AND DISCUSSION

From Table 1, it was observed that the control experiment attained 100% germination on the fourth day after sowing. Also, only the control was found to attain 100% germination at the end of the period of experimentation unlike other salinity levels that could not attain 100% germination. Generally, it was observed that germination percentage decreased steadily with increasing NaCl concentrations. This is in agreement with the works of several authors; Nasir (2002), Al-Seedi (2004), Herr (2005), Al-Seedi and Gatteh (2010) and Ratnakar and Rai (2013) to mention but a few. This could be attributed to specific ion effect (Hassen, 1999) or osmotic stress (Zekri, 1993) which later led to reduced water intake into seeds for enhancement of germination.

In addition, Begum et al. (2010) stated that germination of seeds depends on the utilization of reserved food materials of the seed and, Ratnakar and Rai (2013) opined that salinity interferes with the process of water absorption by the seeds which subsequently inhibits the hydrolysis of food reserves which ultimately delays and decreases seed germination.

Table 2 shows that germination percentages were highest in the first three days after planting than the other days. The control attained 100% germination within the first three days whereas the other experimental sets could not attain up to 90% even on the tenth day after sowing. Also, apart from 25 mM NaCl level which attained 50% germination on the third day, no other experimental set (50, 75, 100 and 150 mM NaCl) attained up to 50% germination rate.

According to Ratnakar and Rai (2013), this may be due to the fact that the increased amount of NaCl disturbed ionic balance of plant cells and also caused imbalances in plant nutrients which must have affected germination percentages. In addition, it could be deduced that *A. hybridus* tolerated varying concentrations of NaCl better than *C. argentea* during seed germination. Hence, *A.hybridus* is a better halophyte than *C. argentea*.

Length measurements

According to Ratnakar and Rai (2013), root and shoot lengths are the most important parameters for studying salt stress. This is obvious as roots are in direct contact with soil salinity and the effects are then translocated and manifested along the shoot, thus the need for evaluation of salt stress effects in terms of root/shoot ratio as well as total lengths. Shoot length, root length and total length behaved in a similar pattern in both *A. hybridus* and *C. argentea* (Table 3). Growth was steadily stimulated in

Table 2. Effect of varying concentrations of NaCl on seed germination of *Celosia argentea* in percentages.

NaCl (mM)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
0	86	93	100	100	100	100	100	100	100	100
25	41	47	50	51	52	52	53	54	55	55
50	18	29	35	40	40	41	41	42	43	43
75	14	26	29	31	32	33	33	33	33	33
100	11	16	18	20	22	22	22	22	22	22
150	6	11	12	14	15	15	15	15	15	15

Table 3. Effect of varying concentrations of NaCl on growth parameters of *Amaranthus hybridus*.

NaCl (mM)	Shoot length	Root length	Root /shoot ratio	Total length	Fresh weight	Dry weight	Dry matter	Leaf area index	Rel. water index	Leaf No.
0	19.21±0.24	5.06±0.12	0.26	24.29±0.53	7.24±0.32	0.68±0.01	9.39	2.03±0.11	757.11	10
25	12.43±0.21	5.87±0.18	0.47	18.37±0.41	4.22±0.37	1.46±0.03	34.61	3.08±0.09	2284.21	13
50	13.46±0.19	6.74±0.24	0.50	20.25±0.43	7.03±0.43	0.63±0.02	8.96	2.15±0.07	2166.67	14
75	10.81±0.18	4.79±0.15	0.44	15.66±0.31	6.84±0.39	0.56±0.02	8.19	1.62±0.06	1125.81	11
100	9.43±0.17	4.18±0.13	0.44	13.69±0.29	3.01±0.14	0.51±0.01	16.94	1.28±0.05	1105.71	10
150	7.97±0.15	3.34±0.09	0.44	11.42±0.26	2.43±0.09	0.49±0.02	20.17	1.04±0.03	244.83	8

both species at lower NaCl salinity levels with best growth stimulation at 50 mM NaCl where they achieved highest values respectively. However, both species were adversely affected by higher NaCl salinity levels (75 mM, 100 and 150 mM) where increased NaCl salinities caused a corresponding decrease in root length, shoot length as well as total lengths owing to adverse salinity effects on cell differentiation and elongation. Hence, at lower NaCl salinity levels, both studied parameters are good halophytes.

Osmotic differences could explain this phenomenon where by lower NaCl salinity levels (25 and 50 mM) positively influences solutes to readily cross the cell membranes into the cytoplasm of the cells but at higher salinity levels, active metabolic pumps prevents accumulation of these ions (Katembe et al., 1998). Built up toxic ions emanating from continuous exposure to higher NaCl levels could lead to decreased availability of some essential nutrients (Werner and Finkelstein, 1995). In addition, Heidari et al. (2001) while studying the effects of NaCl concentrations on *Helianthus annus* suggested that reduction in plant growth is due to decreasing turgur pressure in the soils under saline environment. Furthermore, evidence from root/shoot ratio showed that shoot length was more stimulated at 25 and 50 mM that root length where as the effects remained the same at higher salinity levels (75, 100 and 150 mM) in *A. hybridus* (Table 3) as compared to *C. argentea* where root length was more negatively affected than shoot length with increasing NaCl levels (Table 4).

Weight measurements

This includes fresh weight, dry weight and dry matter contents. Fresh weight was most stimulated at 0 mM NaCl level in *A. hybridus* whereas 50 mM NaCl concentration influenced fresh weight more than the other NaCl levels in *C. argentea*. Both species showed decreasing fresh weights with increasing NaCl levels, from 50 mM to 150 mM NaCl concentrations. This implies that 50 mM NaCl concentration most positively stimulated water uptake into cytoplasm of cells in both species, apart from 0 mM NaCl in *A. hybridus*. Reduced fresh weights could be attributed to water deficits as established by Cha-Um and Kirdmanee (2009) while studying maize seedlings under NaCl influences and Ratnakar and Rai (2013) who observed a similar trend while investigating *Trigonalla foenum-graecium L. Var. Peb* under NaCl exposure.

Dry weight

Dry weight was highest at 25 mM NaCl concentration in *A. hybridus* as compared to *C. argentea* where highest value occurred at 50 mM NaCl level. Also, both species showed decreasing dry weight values with increasing salinities from 25 mM NaCl in *A. hybridus* and 50 mM NaCl concentrations in *C. argentea*. This is in line with the study of Turan et al. (2009) while investigating NaCl salinity effects in maize plants. Dadkhan and Grifiths

Table 4. Effect of varying concentrations of NaCl on growth parameters of *Celosia argentea.*

NaCl (mM)	Shoot length	Root length	Shoot /root ratio	Total length	Fresh weight	Dry weight	Dry matter	Leaf area index	Rel. H_2O index	Leaf no.
0	15.33±0.32	5.21±0.19	0.34	20.54±0.27	3.61±0.31	0.38±0.01	10.53	1.36±0.12	545.16	10
25	16.97±0.35	5.84±0.21	0.34	22.85±0.29	3.04±0.27	0.41±0.01	13.49	2.88±0.15	1172.73	15
50	18.28±0.28	5.97±0.18	0.33	24.26±0.24	8.64±0.38	0.76±0.02	8.80	2.47±0.14	1142.86	16
75	13.46±0.22	4.07±0.12	0.30	17.54±0.21	5.58±0.27	0.33±0.02	5.91	2.24±0.12	1066.67	11
100	12.22±0.18	3.23±0.11	0.26	15.45±0.17	5.41±0.22	0.31±0.01	5.73	2.19±0.10	1004.35	10
150	12.16±0.13	3.04±0.09	0.25	15.21±0.13	3.24±0.18	0.21±0.01	6.48	2.11±0.08	900.08	9

(2006) attributed such a decrease in dry weight to greater reduction in uptake and reduction in utilization of mineral nutrients by plants under salt stress while Jafari et al*.* (2009) opined that it could be due to reduced rate of photosynthesis.

Dry matter was highest at 25 mM NaCl level in both species studied. There was a gradual decrease to 75 mM NaCl concentration until an increase started occurring from 100 up to 150 mM NaCl concentration in both species. This could be attributed to accumulation of salt particles within the tissues of both species.

Leaf area index

Mean leaf area index was found to be highest at 25 mM NaCl level in both species studied. After which, there was a corresponding decrease in average leaf area index in both species with increasing NaCl salinities. Reduction in leaf area index could have serious implications for chlorophyll content, thus a reduction in photosynthetic ability of plants. This explains the direct relationship existing in this study between leaf area index and parameters as root length, shoot length as well as total lengths of the species under study. Ungar (1991) opined that higher salinities cause decreases in assimilation of CO_2 through the effect of stomatal opening and sufficiency of photosynthesis process. High salinity could affect stroma volumes of chloroplasts (Price and Hendry, 1991) and the protein bonds of green pigments, thus causing a decrease in chlorophyll content (Rivera and Heras, 1973) in plants.

Relative water concentration (RWC)

RWC decreased with increasing salinities in both *A. hybridus* and *C. argentea* from 25 mM through 150 mM NaCl concentrations. This phenomenon could best be explained by salt induced stress since increasing NaCl levels have tendencies of decreasing water absorption into the cells of plants thereby causing physiological desiccations. This is in agreement with the findings of Abbas and Latif (2005) who established a decrease in the growth of jute seedlings under NaCl stress, which they attributed to low water absorption into cells as the salinity increased.

Leaf number

Both *A. hybridus* and *C. argentea* are cultivated chiefly for their vegetative parts, thus, it becomes pertinent to evaluate their leaf number. Highest number of leaves in both species was noticed among seedlings growing in 50 mM NaCl concentration. In addition, seedlings with higher total shoot lengths seemed to have higher number of leaves. The numbers of leaves first increased from 0 to 50 mM NaCl concentrations, before a gradual decrease from 75 mM through 150 mM NaCl levels were observed. This shows that moderate NaCl levels (50 mM) positively influenced leaf formation and development as compared to lower (0 and 25 mM) and higher (75, 100 and 150 mM) NaCl concentrations in both *A. hybridus* and *C. argentea*. This shows that both species would be most productive and best cultivated under moderate than low or high NaCl concentrations.

Conclusion

Findings in this study have shown that both *A. hybridus* and *C. argentea* are good halophytes that could sustainably be cultivated in farmlands contaminated with NaCl-induced salinities either due to natural aridity or man-made soil salinization through buildup of salts as a result of extensive use of fertilizers and/or irrigation practices. Although, both species responded to varying NaCl concentrations in different ways, however, both tended to show almost a similar trend.

On the one hand, germination of seeds of both species was adversely affected with increasing NaCl concentrations. Conversely, seedling growth was positively stimulated at moderate levels and adversely affected at varying rates at low (25 mM) and higher (100 and 150 mM NaCl levels in *A. hybridus* and 75, 100 and 150 mM

NaCl levels in *C. argentea*), respectively. Thus, it is being recommended that seedlings of both species of *A. hybridus* and *C. argentea* should be raised in nurseries free of NaCl influences (0 mM), that is, salt-free soils and as soon as maximum germination has been attained, the seedlings could then be transferred to moderately NaCl saline soils for maximum growth and development. There is therefore need to enlighten farmers on these findings to enhance maximum productivity and help ensure food security.

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