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Salinity Effects due to CaCl on Germination and Seedling Growth of *Amaranthus hybridus* and *Celosia argentea*

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ABSTRACT
Salinity is one of the environmental factors that affect seed germination and consequent establishment in saline environments. Heavy fertilization and irrigation seem to complicate soil-$CaCl_2$ salinity problems. $CaCl_2$ solution was prepared following standard methods and procedures. Corresponding serial dilutions gave 25mM, 50mM, 75mM, 100mM and 150mM while distilled (0mM) water constituted the control. Germination and field experiments were conducted. Results for growth experiment 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. The control (0mM) attained 100% germination within the first three days whereas the other experimental sets (50mM, 75mM, 100mM and 150mM $CaCl_2$ salinity levels) couldn't attain up to 50% even on the tenth day after sowing in both species. Growth parameters were generally stimulated at lower salinities and adversely affected at higher salinities in both species. It is hereby recommended that seedlings of both species should be raised in nurseries that should be free of $CaCl_2$-salinity and after maximum germination is attained, the seedlings could then be transferred to low $CaCl_2$-saline soils for best results. These findings have strong positive implications for attaining food security.
Keywords: Germination; Seedling Growth; Soil Salinity; CaCl; *Amaranthus hybridus*; *Celosia argentea*.

INTRODUCTION
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 SO_2^{4-}(Herr, 2005). Salinity is one of the environmental factors that have critical influence on the germination of halophyte seeds and plant establishment (Katembe et al, 1998). 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. Hence, according to Sairam and Tyagi (2004), high salt concentration ultimately reduces crop yield and the quality of the produce.

Seeds of many halophyte species show optimal germination in fresh water environments (Khan and Ungar, 1984), necessitating a need for reduction in soil salinity that occurs under field conditions following spring rains (Breen *et al.*,1977). According to Katembe et al (1998), salinity affects imbibitions, germination and root elongation.

Soil salinity is becoming a serious worldwide problem as more land is irrigated thoroughly and heavily fertilized (Galston *et al*, 1980). 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). Munns (2002) reported that salinity affects about 7% of the world's land area, amounting to about 930 million ha, thus seriously limiting crop production, especially the sensitive ones (Zadeh and Naeini, 2007). And, the trend could continue as according to Al-Seedi and Rai (2010), the problems of salinization are increasing, either due to bad irrigation, drainage or agricultural practices.

According to Ashraf (1993), salt tolerance in plants is a complex phenomenon, which depends on a number of inter-related factors based on morphological, biochemical and physiological processes. Germination and seedling growths are predictive of plant growth responses to salinity (Cuertero et al, 2006). Both Amaranthus hybridus and Celosia argentea belong to the family, Amaranthaceae (Dutta and Dutta, 2008). Amaranthus hybridus is a robust annual herb (Olorode, 1984), cultivated for its nutritional value (Oguntona, 1998) and used as food by man and many other animals. Celosia argentea, on the other hand, is a short-lived annual herb, slow growing and more drought-resistant than Amaranthus hybridus (Ogunwenmo *et al*., 2010). Both Amaranthus hybridus and Celosia argentea are chiefly grown for their vegetative parts, hence has chances of manifesting salinity effects faster.

The most common adverse effect of salinity on the crop of Brassica is the reduction in plant height, size and yield as well as deterioration of the quality of the product (Kumar, 1995). Also, Byordi (2010) studied the influence of salt stress on seed germination, growth and yield of Canola cultivars and found that significant differences existed between influence of salt stress on the cultivars during germination and vegetative growth respectively. Ratnakar and Rai (2013) studied effects of NaCl salinity on seed germination and early seedling growth of Trigonella foenum-Graecum L. Var Peb and were able to establish that increasing NaCl concentrations caused a gradual decrease in root length, shoot length, fresh weight and dry weight of the growing seedling. Al-Seedi and Gatteh (2010) studied the effects of salinity on seed germination, growth and organic compounds of Mung bean plant (Vignia radiate (L.) Wilczek) and found out that an increase in the salinity caused a corresponding decrease in germination rate, growth parameters and the carbohydrate content of the Mung bean plant. Furthermore, Katambe *et al*. (1998) while studying the effects of germination and seedling growth of two *Atriplex species* (Chenopodiaceae), found out that NaCl caused a greater increase in nuclear volume than iso-osmotic PEG solutions.

This work was informed by the decision to help fill existing vacuum since studies on the effects of salinity as a result of varying concentrations of $CaCl_2$ on *Amaranthus hybridus* and *Celosia argentea* are hardly available. Thus, it becomes very important to study the relationship between the effects of varying concentrations of $CaCl_2$ salinity on germination and seedling growth of both crop species since such information would help establish the chances for the suitability 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 $CaCl_2$ in most saline environments. This is the basis of this research work.

MATERIALS AND METHODS

A. Salt Preparation

Salt solutions of 1M $CaCl_2$ solution was prepared by dissolving 110.99g of $CaCl_2$ crystals in a universal bottle upon which distilled water was added onto to make up 1 liter. Corresponding rations of 25/1000, 50/1000, 75/1000, 100/1000 and 150/1000 gave 25mM, 50mM, 75mM,100mM and 150mM respectively. Pure distilled water was considered to be 0mM, thus represented the control.

B. Germination Test

This was conducted within 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 *Amaranthus hybridus* and *Celosia 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 two (2) hours 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 0mM, 25mM, 50mM, 75mM, 100mM and 150mM respectively. Thus, total number of seeds per concentration (from 0mM to 150mM) 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 in the Botany Department of Ambrose Alli University, Ekpoma, Edo State, Nigeria. The seeds of both species in the glass Petri dishes were moistened every twelve (12) hours with varying concentrations (0mM, 25mM, 50mM, 75mM, 100mM and 150mM) of $CaCl_2$ salt and observations were recorded every twenty four (24) hours for radical emergence as indicative of germination. Seeds were considered to have germinated when up to 1mm radicle emergence from the seed is noticed.

C. Seedling Growth Test

Black polythene bags measuring 25cmx25cm 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 1cm and after germination; only ten (10) seedlings considered 'healthier' were allowed to grow. Masking tapes were used to label the bags appropriately. Salt solutions of $CaCl_2$ corresponding to 25mM, 50mM, 75mM, 100mM and 150mM 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 0.82cm. The discs of known area of the punched leaves were dried to constant weight at 80°C for twenty four (24) hours and 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 twenty four (24) hours and their corresponding dry weights measured. It is then calculated using this relationship;

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

RESULTS AND DISCUSSIONS

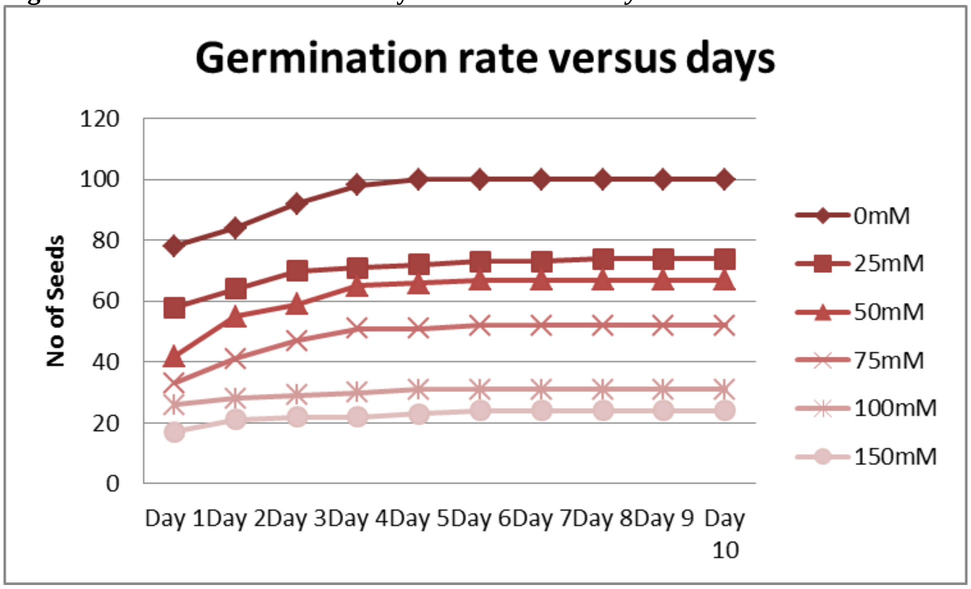
Table 1 Effect of varying Concentrations of $CaCl_2$ on Seed Germination of *Amaranthus hybridus*

S/No	CaCl	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
1	0mM	78	84	92	98	100	100	100	100	100	100
2	25mM	58	64	70	71	72	73	73	74	74	74
3	50mM	42	55	59	65	66	67	67	67	67	67
4	75mM	33	41	47	51	51	52	52	52	52	52
5	100mM	26	28	29	30	31	31	31	31	31	31
6	150mM	17	21	22	22	23	24	24	24	24	24

Table 1 above showed that the control experiment attained 100% germination on the fifth day after sowing whereas corresponding salinities of $CaCl_2$ for 25mM, 50mM, 75mM, 100mM and 150mM attained 74%, 67%, 52%, 31% and 24% respectively at the end of the experiment. 25mM, 50mM and 75mM attained more than 50% germination whereas 100mM and 150mM showed less than 50% germination at the end of the germination experiment. The trend noticed in this study is in consonance with findings by authorities like Nasir (2002), Al-Seedi (2004), Herr (2005), Al-Seedi and Gatteh (2010) and Ratnakar and Rai (2013) etc. Hassen (1999) attributed this trend to specific ion effect, while Zekri (1993) opined it could be due to osmotic stress.

Begum et al (2010) stated that germination of seeds depends on the utilization of reserved food materials of the seed. In addition, Ratnakar and Rai (2013) established that salinity interferes with the process of water absorption by the seeds which subsequently inhibits the hydrolysis of food reserves, this could lead to a resultant delay and decrease in seed germination.

Figure 1 Germination rate over days for *Amaranthus hybridus*



As shown in table 2 above, germination percentages were highest in the first three days after planting than the other days in all salinity levels studied in this experiment. The control attained 100% germination within the first three days whereas the other experimental sets (50mM, 75mM, 100mM and 150mM $CaCl_2$ salinity levels) couldn't attain up to 50% even on the tenth day after sowing, apart from 25mM which showed 53% on the third day and later 58% germination percentages on the tenth day. Generally, it was also shown that germination percentages decreased with increasing $CaCl_2$ salinities for *Celosia argentea*.

Table 4. Effect of varying concentrations of $CaCl_2$ on Seed Germination of *Celosia argentea*.

S/No	CaCl	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
1	0mM	85	94	100	100	100	100	100	100	100	100
2	25mM	43	48	53	55	55	56	57	58	58	58
3	50mM	22	27	30	30	31	31	31	32	32	32
4	75mM	14	19	23	25	26	27	27	27	27	27
5	100mM	09	11	14	16	18	18	18	18	18	18
6	150mM	07	08	10	11	11	11	11	11	11	11

In this study, $CaCl_2$ seemed to exhibit the same behaviour as NaCl (Ratnakar and Rai, 2013). They observed gradual decrease in germination of *Trigonella foenum-Graecum* L. Var with increasing NaCl salinities. Thus, they attributed this phenomenon to 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. Furthermore, it could be safe to state that *Amaranthus hybridus* seeds tolerated $CaCl_2$ salinity during germination better than *Celosia argentea*, thus a better halophyte than *Celosia argentea* for soils contaminated with $CaCl_2$.

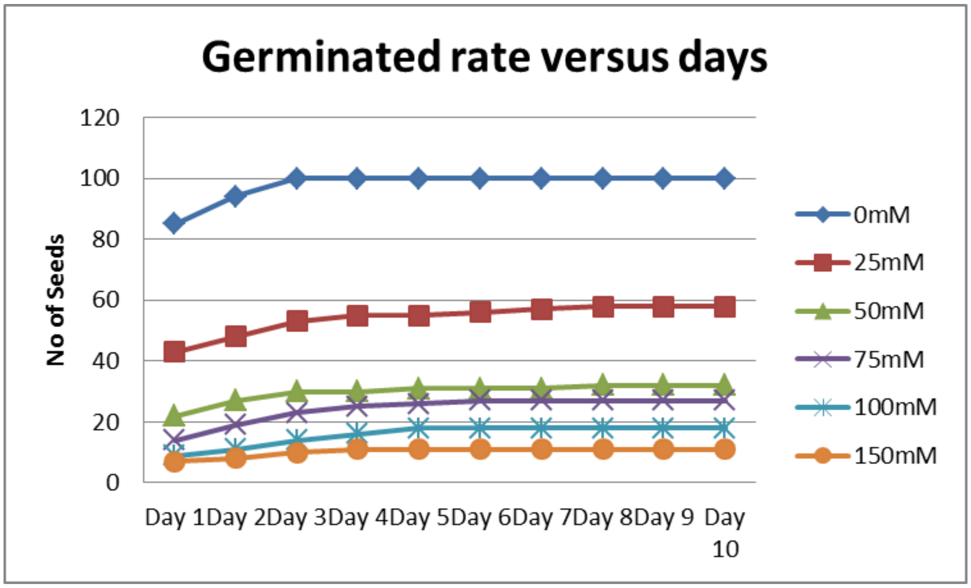


Figure 1 Germination rate over days for *Celosia argentea*.

Effect of varying Concentrations of $CaCl_2$ on Growth parameters of *Amaranthus hybridus* and *Celosia argentea*

1.3.1 **Length Measurements:** This includes root length, shoot length, total length as well as root/shoot ratio. Roots are in direct contact with saline soils while the effects manifest in both roots and shoots of plants, thus the need for evaluating the parameters. Shoot length and total lengths were highest in the control (0mM $CaCl_2$ salinity) while root length was highest at 25mM $CaCl_2$ in *Amaranthus hybridus* whereas in *Celosia argentea*, the highest values were observed at 25mM $CaCl_2$ salinity level. This is an indication of growth stimulation at those salinity levels. However, both species were adversely affected by higher $CaCl_2$ salinity levels (50mM, 75mM, 100mM and 150mM) where increased $CaCl_2$ salinities caused a corresponding decrease in root length, shoot length and that of total lengths and this could be due to adverse salinity effects on cell differentiation and elongation.

$CaCl_2$ salinity followed the same trend as NaCl (Katembe et al, 1998 and Heidari et al, 2001). The positive influences could be attributed to osmotic differences since solutes readily crosses the cell membranes into the cytoplasm of the cells at lower salinities whereas higher salinity levels activates metabolic pumps to prevent accumulation of ions (Katembe et al, 1998). While studying the effects of salinity on *Helianthus annus*, Heidari et al (2001) suggested that reduction in plant growth could be due to decreasing turgur pressure in the soils under saline environment. Also, Werner and Finkelstein (1995) opined that build up of toxic ions owing to continuous exposure to higher salinity levels could lead to decreased availability of some essential nutrients. This explains the increase in length at lower $CaCl_2$ salinities and decrease in length at higher $CaCl_2$ salinities in length measurements observed in both species. Furthermore, evidence from root/shoot ratio showed that shoot length was more adversely affected than root length with increasing $CaCl_2$ salinities except at 50mM $CaCl_2$ in Celosia argentea (see tables 6 & 7 below).

Table 6 shows Effect of varying Concentrations of $CaCl_2$ on Growth parameters of *Amaranthus hybridus*

S/No	$CaCl_2$	Shoot Length	Root Length	Root /Shoot Ratio	Total Length	Fresh Weight	Dry Weight	Dry Matter	Leaf Area Index	Rel. H_2O Index	Leaf No.
1	0mM	19.21±0.24	5.08±0.11	0.27	24.27±0.55	7.27±0.26	0.68±0.01	9.35	2.03±0.13	757.14	14
2	25mM	11.15±0.22	5.16±0.13	0.46	16.31±0.38	6.66±0.38	0.56±0.02	8.41	3.51±0.17	1033.61	11
3	50mM	9.62±0.18	4.14±0.11	0.43	13.77±0.29	4.97±0.25	0.55±0.01	11.07	3.85±0.16	605.88	10
4	75mM	9.21±0.16	3.89±0.08	0.42	13.11±0.25	3.84±0.23	0.34±0.01	8.85	2.75±0.14	105.71	9
5	100mM	8.27±0.12	3.23±0.06	0.39	11.50±0.18	3.15±0.18	0.27±0.01	8.57	2.26±0.11	98.53	8
6	150mM	7.83±0.07	3.07±0.04	0.39	10.91±0.11	2.88±0.11	0.13±0.01	4.51	2.05±0.08	91.82	7

Table 7 Effect of varying Concentrations of $CaCl_2$ on Growth parameters of *Celosia argentea.*

S/No	$CaCl_2$	Shoot Length	Root Length	Root /Shoot Ratio	Total Length	Fresh Weight	Dry Weight	Dry Matter	Leaf Area Index	Rel. H_2O Index	Leaf No.
1	0mM	15.35±0.31	5.22±0.20	0.34	20.59±0.28	3.60±0.31	0.38±0.01	10.56	1.37±0.12	545.17	13
2	25mM	16.84±0.44	5.77±0.18	0.34	22.61±0.22	4.06±0.36	0.52±0.02	12.81	1.47±0.09	1057.87	14
3	50mM	14.11±0.31	5.01±0.22	0.36	19.12±0.18	3.24±0.27	0.33±0.01	10.19	1.46±0.09	900.02	12
4	75mM	13.72±0.24	4.17±0.18	0.30	17.89±0.14	1.82±0.21	0.26±0.01	14.29	2.01±0.21	733.33	10
5	100mM	12.22±0.19	3.26±0.13	0.27	15.49±0.13	1.34±0.01	0.21±0.01	15.67	2.03±0.18	650.14	9
6	150mM	10.51±0.17	2.77±0.07	0.26	13.33±0.07	1.05±0.01	0.18±0.01	17.14	1.96±0.12	561.41	8

1.3.2 Weight Measurements: Fresh weight, dry weight and dry matter contents all witnessed weight measurements.

Fresh weight was most stimulated at 0mM $CaCl_2$ salinity in *Amaranthus hybridus* (see table 6 above) where as 25mM $CaCl_2$ salinity level influenced fresh weight more than the other salinity levels (see table 7 above) in *Celosia argentea*. Both species showed decreasing fresh weights with increasing $CaCl_2$ salinity levels. This implies that 25mM $CaCl_2$ salinity most positively stimulated water uptake into cytoplasm of cells in both species, apart from 0mM $CaCl_2$ in *Amaranthus hybridus*. Reduced fresh weights could be attributed to water deficits (Cha-Um and Kirdmanee, 2009 and Ratnakar and Rai, 2013) . Dry weight followed the same trend as fresh weight in *Amaranthus hybridus* and *Celosia argentea*. This agrees with the works of Dadkhan and Grifiths (2006) who 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 50mM $CaCl_2$ salinity level in *Amaranthus hybridus* and 150mM in *Celosia argentea*. While there was gradual decrease with increasing $CaCl_2$ salinity level in *Amaranthus hybridus* from 50mM $CaCl_2$ salinity level, dry matter increase steadily with increasing $CaCl_2$ salinity levels from 50mM $CaCl_2$ salinity level. It seemed *Amaranthus hybridus* has a greater propensity to breakdown bioaccumulated salt particles than *Celosia argentea* where it is suspected that $CaCl_2$ particles bioaccumulated within the cytoplasm of cells.

1.3.3 **Leaf Area Index**: Mean leaf area index was found to be highest at 50mM $CaCl_2$ salinity level in *Amaranthus hybridus* and 100mM $CaCl_2$ in *Celosia argentea.* Reduction in leaf area index could have serious implications for chlorophyll content, thus a reduction in photosynthetic ability of plants. Leaf area index decrease from 50mM $CaCl_2$ with increasing $CaCl_2$ salinity levels in *Amaranthus hybridus* whereas *Celosia argentea* increased steadily with increasing $CaCl_2$ salinity, except at 150mM $CaCl_2$ salinity level. Differences in genetic differences must have influenced this trend. Salinity levels could affect stroma volumes of chloroplasts (Price and Hendry, 1991).

1.3.4 **Relative Water Concentration**: Relative Water Content decreased with increasing salinity in both *Amaranthus hybridus* and *Celosia argentea* from 25mM through 150mM $CaCl_2$ salinity levels. Salt stress which could result in low water absorption (Abbas and Latif, 2005) could be responsible for this behaviour.

1.3.5 **Leaf Number:** Highest number of leaves in both *Amaranthus hybridus* and *Celosia argentea* showed decreasing numbers of leaves with increasing $CaCl_2$ salinity levels from 25mM $CaCl_2$ salinity levels. Leaf number showed a direct proportional relationship with length (root length, shoot length and total length) and weight (fresh and dry weights) parameters.

CONCLUSION

It is evident from this study that both *Amaranthus hybridus* and *Celosia argentea* good halophytes for $CaCl_2$ contaminated soils. Thus, whether it is a naturally occurring arid saline soil or farmlands that acquired salinity owing to anthropogenic activities like irrigation or use of fertilizers that must have induced $CaCl_2$ salinity into such soils, both *Amaranthus hybridus* and *Celosia argentea* could be cultivated in such farmlands. Though, both species responded to $CaCl_2$ salinities in different ways.

Firstly, germination of seeds of both species was adversely affected with increasing $CaCl_2$ salinities. Thus increasing $CaCl_2$ salinity level caused a corresponding decrease in the percentage rate of germination in both species under study. Secondly, *Celosia argentea* seemed to withstand $CaCl_2$ salinities more than *Amaranthus hybridus* seedling, owing to it being more positively stimulated at lower $CaCl_2$ salinities and more adversely affected at higher $CaCl_2$ concentrations in both species studied. It is thereby recommended that seedlings of both species should be raised in nurseries that should be free of (0mM) $CaCl_2$ salinity and after maximum germination is attained, the seedlings should then be transferred to low

$CaCl_2$ saline soils for best results. Finally, there is need for farmers to be well enlightened on these findings so that the best use is made of $CaCl_2$ contaminated soils to help guarantee food security.

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