GERMINATION RESPONSE OF POTENTIAL HALOPHYTE *HALOXYLON STOCKSII* IN DIFFERENT SALTS AND PHOTOPERIODS

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Abstract

The effect of different salts (NaCl, Na_2SO_4, $MgCl_2$, $CaCl_2$, KCl) on the seed germination of a potential halophytic species *Haloxylon stocksii* were studied. Germination was carried out in different salinity levels (0, 100, 200, and 300, 400 and 500 mM) at day: night temp/ 30-20^o C in a 12h light/dark photoperiod. Highest percent seeds germinated in distilled water control. Increase in salt concentration decreased the seed germination in all salts treatments. Germination percentage was higher in light treatment as compared to dark. After 20 days an ungerminated seed of saline condition transferred in to non saline condition (water) and the maximum percent recovery was obtained at highest concentrations in all salts treatment.

Introduction

Saline soil is characterized with high concentration of soluble salts, especially those of sulphates and chlorides of Na, Ca, Mg and small quantities of carbonates and bicarbonates (Tobe *et al*., 2002, 2004). Most of the plants have been examined under the influence of only Na^+ salts or ea water and very small information available for the comparative effect of Na, Mg and other salts on plants germination or growth (Atia *et al*., 2011, Panuccio *et al*., 2014). Because the saline soil contains multiple salts that alter the physiological and biochemical functions of plants, therefore, it is necessary to investigate the tolerance of different plants under various salts concentrations.

The plants growing in saline environment, germination is very sensitive stage in their life cycle. This stage is considered as an identifier stage for the plants for their survival at the soil condition in which later plant will exist (Ungar, 1982). Salinity is known to affect many aspects of plants and induces numerous changes. One of the major effects of salinity is the inhibition of seed germination (Saeed and Ahmad, 2013).

Increase salinity stress causes reduction in total number of seeds germinating and delaying the initiation of the germination process. Due to low water potential of the soil halophyte seeds become dormant. (Zehra and Khan, 2007, Saeed *et al*., 2014). Rate of germination increases with an increase in temperature and best germination of the halophytes found in our region was obtained at a temperature regime of (20-35^{o}C).Temperature and salinity interaction select the rate (germination velocity) and total germination percentage of halophyte seeds (Gul and Weber, 1999). Germination of various halophyte seeds occur at times when there is an optimal combination of day length, temperature and salinity (Gul and Weber, 1999).The interactive effect of light, temperature and salinity have been reported in a number of species (Zaheer and Khan, 2010, Shaikh *et al*., 2013, Zehra *et al*., 2013)

Halophytes have ability to sustain their viablility even in prolonged hypersaline conditions. It has been observed in our climatic region, seed of halophytes germinate quickly after monsoon rain as the substantial reduction in soil salinity of the rhizosphere due to leaching of salts.

Haloxylon stocksii is one of the succulent perennial halophyte of the family Chenopodiaceae. It is distributed from southern Sindh and Baluchistan up to northern Himalayans mountain valley (Stewart, 1972). In high saline patches it is found with *Sueada fruticosa, Salsola baryosma* and *Sporobolus arabicus*. It is known as potential halophyte species used in medicines (Ilahi, 2008). The salt tolerance of *Haloxylon stocksii* is investigated by Khan and Ungar (1996) with NaCl salt only. The objective of the proposed research is to evaluate the germination response of *Haloxylon stocksii* under different salts treatments. Due to medicinal importance of the species, it would be beneficial for agriculture and pharmaceutics.

Materials and Methods

Seeds of *Haloxylon stocksii* were collected from the saline community of Karachi university campus. The seeds were separated from inflorescence and surface sterilized with 1% Clorox solution and with distilled water..

Germination Experiment: Seed Germination was carried out in 50 x 9 mm diameter air tight plastic Petri dishes. Twenty five seeds were placed on each Petri dish with 5 ml solution of (0, 10, 20, 30, 40 and 500mM) of

NaCl, Na_2SO_4, $CaCl_2$, $MgCl_2$ & KCl. The petri dishes were arranged in randomized complete block design (RCBD) with four replicates. Seeds with salinity treatments were incubated in germinators maintained at temperature regimes 20-30º C with 12h: 12h light :dark photoperiod. Germination was recorded for 20days at every alternate day. Seed germination experiment was also conducted in complete darkness by using photographic envelops at above mentioned temperature regimes and germination was recorded just at 20th days. Emergence of radical showed the seed germination. The rate of germination was estimated by using modified Timson Index of germination velocity, ΣG/t, where *G* is the percentage of seed germinated at 2-day intervals and *t* is the total germination period (Timson, 1965).

Recovery Germination Experiment: To study seed recovery germination, the ungerminated seeds of saline solution were shifted into distilled water after 20 days. These seeds were also placed under similar conditions used for germination of seeds in salinity. The recovery percent was determined by the index *(a-b) / (c-b)* X100, where *a* is the total number of seeds germinated after being transferred to distilled water, *b* is the total number of seeds germinated in saline solution, and *c* is the total number of seeds.

Statistical analysis: Data was statistically analyzed by SPSS version 9.0 (SPSS Inc., 1999). The significance level was analyzed by Bonferroni test ($P < 0.05$).

Results and Discussion

Generally optimal seed germination found in non-saline condition, whereas the raising salinity levels reduce the seed germination of halophytes (Khan *et al*. 2002). Similar findings were also observed in the present study of *Haloxylon stocksii* in which highest seed germination was in control condition while the salinity treatment significantly inhibited the germination (Fig. 1). Increased level of salinity negatively influences germination and imbibitions, physiological processes, including photosynthesis, respiration, transpiration, membrane properties, nutrient imbalance, enzymatic and metabolic activities, which leads to plant death (Mahjan and Tutija, 2005, Hassanuzzaman *et al*., 2012, Zehra *et al*., 2013, Saeed *et al*., 2014). The data presented in (Table. 1), showed the inhibition in germination rate of *Haloxylon stocksii.* Germination rate inhibition was also reported in *Eragrostis ciliaris, Dicanthium annulatum* (Shaikh *et al*., 2013) and *Phragmites karka* (Zehra *et al*., 2013). At the time of germination, water uptake and seed imbibitions occur which activates metabolic processes (Bewely and Black, 1994) but due to high salt concentration of the medium the water uptake is low which cause the delay in germination.

Seed germination is not only depends on dissolved salts concentration dependent but it also depends the nature of the salt (Ryan *et al*., 1975, Atia *et al*., 2011). Limited information is available about the germination of halophytes under the influence of single salt (Houle *et al*. 2001, Atia *et al*., 2006, Liu *et a*., 2006). Saline soil recognized as a mixture of various salts, which influence the physiological and biochemical response of the plants. (Tobe *et al*., 2002, 2004). Data presented in (Fig. 1) showed the percent germination of *Haloxylon stocksii* in the order of $CaCl_2 > Na_2SO_4 > NaCl > MgCl_2 > KCl$. Lesser germination inhibition of *Haloxyalon stocksii* was in $CaCl_2$ as compared to other sas one lts. Calcium lessens the salt stress and promotes germination and plant growth (Ebert *et al*., 2002; Munns, 2002; Shaikh *et al*., 2007, Zehra *et al*., 2012). Na_2SO_4 was also proved by present study that it is less effective for seed germination of *Haloxylon stocksii* under higher salinities. Sulphate is one of the component of sulfur conataining aminoacids (cysteine and methionine) and many other compounds (ferredoxin and glutathione) which plays key physiological functions (Llanes *et al*., 2005). The inhibition of germination in *Haloxylon stocksii* is mainly attributed due to KCl (Fig. 1), which cause significant inhibition. Although, potassium is involved in certain metabolic processes including protein and starch synthesis and enzyme activation (Alkaraki, 2001), but its toxicity is also reported in other halophytes (Egan & Ungar, 1998; Zehra *et al*., 2013). Potassium may cause ion toxicity, because it cannot be able to transport across membranes, and cause disorder of various metabolic functions (Egan *et al*., 2001). The salts effect on halophytes has been investigated, but it is still controversial, weather the effect is osmotic (Gul and Weber, 1998), ionic (Tobe *et al*., 2002) or combined (Sosa *et al*., 2005, Atia *et al*., 2011).

Seed germination of *Haloxylon stocksii* is light dependent while the germination is significantly inhibited in dark (Fig. 2). Similar results were found in *Allenrolfia occidentalis* (Gul and Weber, 1999), *Arthrocnemum indicum* (Saeed *et al*., 2011), *Suaeda heterophylla* (Hameed *et al*., 2013), *Leptochloa chinensis* (Benvenuti *et al*., 2004). Baskin and Baskin(1995) has been reported photoperiod as most effective environmental factor for germination. Generally seed acquire the dormancy during the unfavorable condition, which later breaks in the presence of light and it is necessary for the initiation of germination.

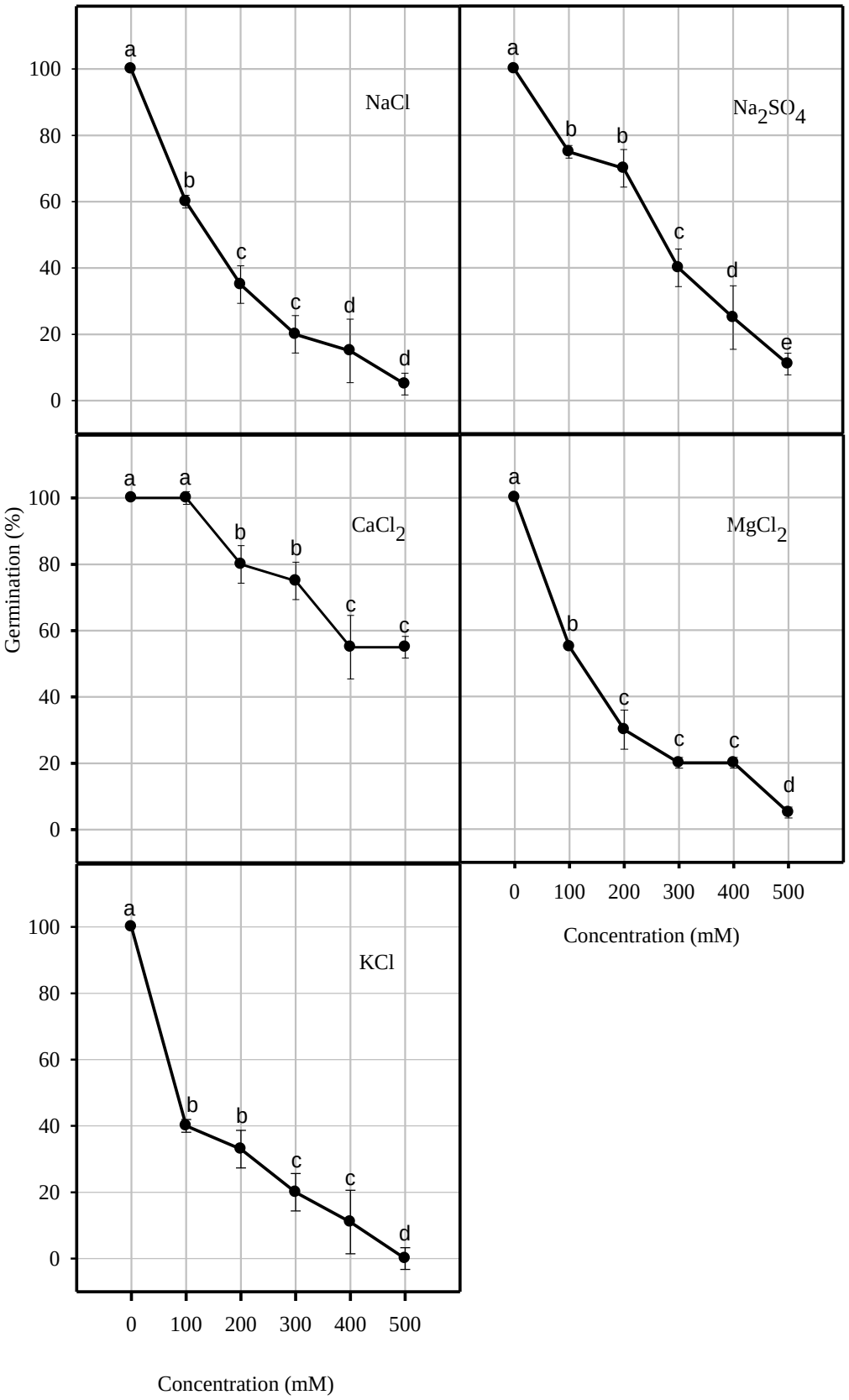

Fig. 1. Percent seed germination of *Haloxylon stocksii* in different salts at 20-30°C in light/dark (12h/12h) photoperiod.

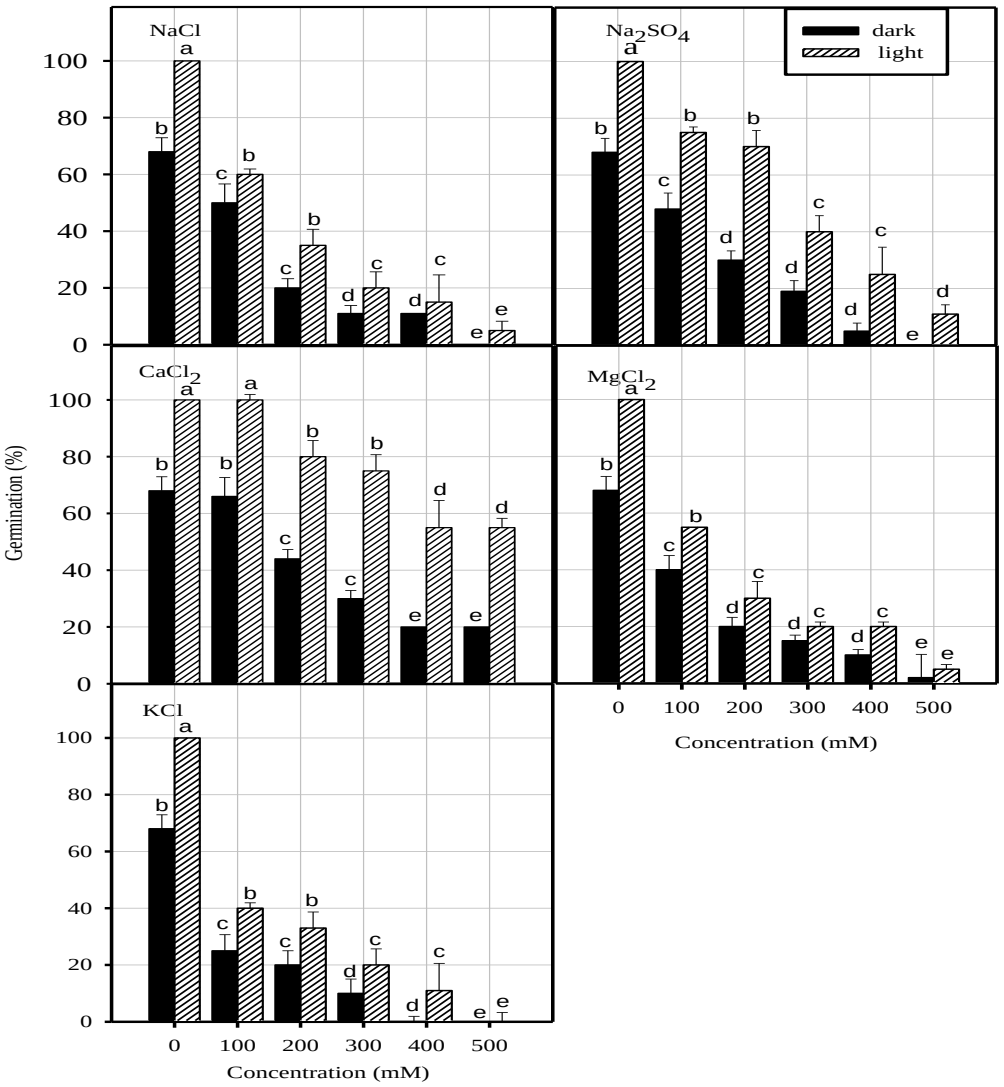

Fig. 2. Percent seed germination of *Haloxylon stocksii* in 12h light :12h dark and 24 h complete darkness.

Halophyte seeds remained viable under higher saline condition and initiate germination under favorable condition (Keiffer and Ungar, 1995). Highest percent recovery of *H. stocksii* seeds was observed at higher salinities when transferred to distilled water (Fig. 3). Same results were described by Zehra *et al*., 2013 in *Phragmites karka*. Recovery rate in *H. stocksii* seeds is also increased with increase in salinity in all salt treatments (Table. 2). This data indicates that halophyte seeds have the ability to remain in the soil in extreme environmental conditions and germinate as the salinity level decreases after rainfall (Ungar, 1962; Okusyana, 1977; Zid and Boukhris, 1977). It also shows these plant species adapted the significant startegy during their course of evolution, by which they become able to best fit even in the extreme environmental conditions (Ungar, 1982).

The present study clearly demonstrated that the germination of *Haloxylon stocksii* is greatly affected in the presence of different salts at optimal temperature. The germination is totally light dependent, and change in photoperiod and the combinations of the environmental conditions cause differential germination response.

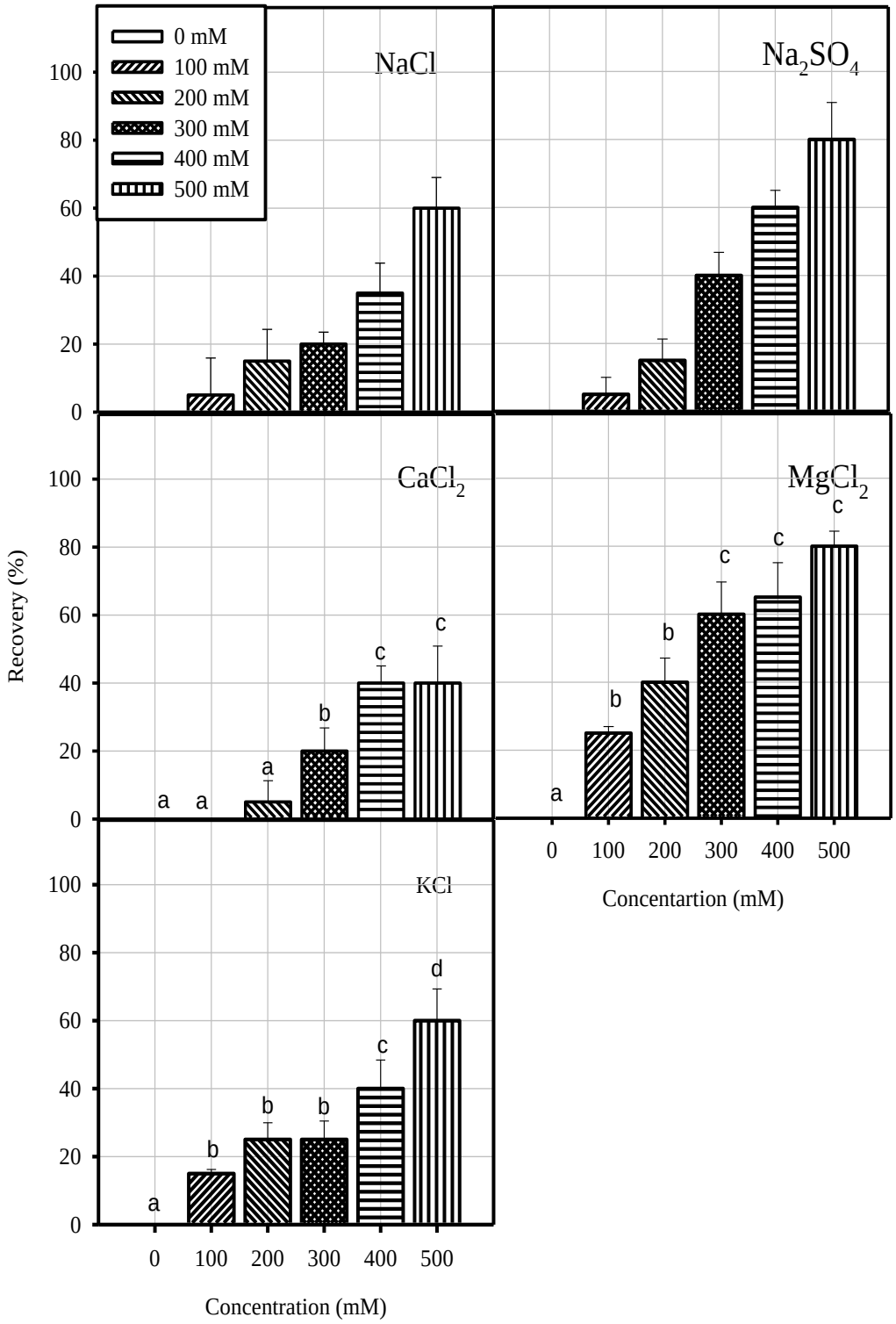

Fig.3. Mean final percent recovery of *Haloxylon stocksii* in various salinity treatments of different salts. Bars represent mean + SE

Table 1. Effect of different salts on germination rate (mean ± S.E.) of *Haloxylon stocksii*. Significant differences between treatments was calculated by Bonferroni test at $p < 0.05$.

Salinity	Rate of germination in different salts									
	NaCl		Na_2SO_4		$CaCl_2$		$MgCl_2$		KCl	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark
0 mM	45.4±2.1[a]	68.5±0.5[a]	45.4±2.1[a]	68.5±0.5[a]	45.4±2.1[a]	68.5±0.5[a]	45.4±2.1[a]	68.5±0.5[a]	45.4±2.1[a]	68.5±0.5[a]
100 mM	42.1±0.7[b]	55.1±0.8[b]	44.0±1.2[a]	55.7±0.2[b]	45.4±2.1[a]	65.1±0.1[a]	41.7±1.2[a]	40.2±0.0[b]	17.6±1.3[b]	0.9±0.21[b]
200 mM	20.9±2.0[b]	10.5±0.1[c]	41.1±0.9[a]	20.9±0.2[c]	44.2±2.0[a]	50.2±0.1[b]	15.9±1.1[b]	10.5±0.2[c]	9.9±3.8[c]	0.4±0.1[b]
300 mM	10.4±0.0[c]	10.0±0.0[c]	20.0±0.2[b]	12.2±0.0[c]	44.0±1.7[a]	24.7±0.0[c]	8.7±0.0[c]	2.2±0.0[c]	0.0±0.0[d]	0.0±0.0[c]
400 mM	1.3±0.0[c]	1.0±0.0[d]	8.1±0.0[c]	2.7±0.0[d]	25.3±1.5[b]	15.1±0.0[d]	5.0±0.0[c]	1.4±0.0[c]	0.0±0.0[d]	0.0±.0.0[c]
500 mM	1.0±0.0[c]	0.0±0.0[d]	5.0±0.0[c]	0.0±0.0[d]	15.7±1.1[c]	9.2±0.0[d]	1.2±0.0[c]	0.2±0.0[d]	0.0±0.0[d]	0.0±0.0[c]

Table 2. Effect of different salts on rate of recovery (mean ± S.E.) of *Haloxylon stocksii*. Significant differences between treatments was calculated by Bonferroni test at $p < 0.05$.

Salinity	Rate of recovery germination in different salts				
	NaCl	Na_2SO_4	$CaCl_2$	$MgCl_2$	KCl
0 mM	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
100 mM	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.39±0.22[a]	0.65±0.38[a]
200 mM	0.55±0.26[a]	0.72±0.18[a]	0.00±0.00[a]	0.62±0.23[a]	1.28±0.41[b]
300 mM	1.15±0.05[a]	1.00±0.14[b]	0.96±0.05[b]	0.80±0.14[a]	1.25±0.10[b]
400 mM	1.850.05[a]	1.85±0.10[b]	1.30±0.10[c]	1.15±0.10[b]	2.30±0.06[c]
500 mM	2.20±0.08[b]	2.00±0.00[c]	1.85±0.15[c]	1.90±0.13[b]	2.85±0.15[c]

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