

## Effects of NaCl and Na<sub>2</sub>SO<sub>4</sub> on germination and initial growth phase of *Halostachys caspica*

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### Abstract

Current research on effect of increasing concentrations (0 (control), 100, 200, 300, 400 and 500 mM) of different salts including NaCl and Na<sub>2</sub>SO<sub>4</sub> on germination and initial growth phase of *Halostachys caspica* were studied. The experimental design was completely randomized design with three replications. Characters of percentage, speed and index of germination, seed healthy index, radicle, plumule and plant length were measured. For analyzing of results were used of ANOVA, Duncan test and parried T- test sample in SPSS software. It was compared with germination under control condition, the most of characters of NaCl salt were not affected by 100 mM NaCl but were affected significantly different by 100 mM Na<sub>2</sub>SO<sub>4</sub> salt. The results showed that effect of same concentration NaCl and Na<sub>2</sub>SO<sub>4</sub> on radicle, plumule and plant length are significantly different but on percentage, speed and index of germination and seed healthy index the different aren't significantly. The results showed that germination percentage were severely inhibited by 500 mM NaCl but no affected severely by 500 mM Na<sub>2</sub>SO<sub>4</sub>. However, the results showed that *Halostachys caspica* in growth characters such as, radicle, plumule and plant length is more sensitive to Na<sub>2</sub>SO<sub>4</sub> than NaCl salt but in germination characters such as percentage, peed and index of germination is more sensitive to NaCl than Na<sub>2</sub>SO<sub>4</sub> salt. At least we can put *Halostachys caspica* chloridephyte and sulfatephyte group of halophytes.

**Keywords:** *Halostachys caspica*; NaCl; Na<sub>2</sub>SO<sub>4</sub>; Germination; Chloridephyte; Sulfatephyte

### 1. Introduction

World population is increased continuously and over 800 million hectares of land throughout the world are salt effected (FAO, 2005) then the halophytes plants are more worthy than before for us. Optimum and sustainable utilization of halophytes would play an important role to cope with the reduction of yield due to salinity by providing different raw materials for food, chemical industry and medicinal purposes in addition to elimination of soil and wind erosion (Flowers, 1999 & Larcher, 1995). Salt stress is probably the first

environmental factors that organisms face to it in evolution stages. Halophyte plants are founded of salty and arid regions of world. Biosalinity researches lead our studies to resistant plants with salinity and are impacted to use of salt waters such as sea, salinity lakes and drainage waters. Seed of halophytes under natural conditions are usually subjected to salt stress dominated by NaCl, however, other chloride, sulfate and carbonate salts, singly as well as can also affect seed germination and other factors significantly (Duan, 2004), (Khan, 2002) and (Beweley, 1994). Saline soils of Iran are formed from the accumulation of various chloride and sulfate salt dominated by NaCl and Na<sub>2</sub>SO<sub>4</sub> salts (Jafari, 1993 & Szabolic, 1992). Important difficulties of salinity for plants are due to increasing of NaCl (Glenn, 1997) and Na<sub>2</sub>SO<sub>4</sub> (Martin, 1993) salts. *Halostachys caspica* is halophyte (*Chenopodiaceae*) that in

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order to resistant with high salt measure is absorbed and stored a lot of water and has succulent stems. Large canopy cover is effected to conservation and fixed of soil and salt special adage of lakes and wet land. The plant could be used for improvement and sustainable development in salty regions due to excellent germination and easily generation with correct management (Moghimi, 2004). *Halostachys caspica* is not used in initial growth and green stage as forage but could be used as forage for livestock after seeding special in autumn and winter season with preference value respectfully camel, goat and sheep (Zhao, 2001). Researchers reported that *Halostachys caspica* is one of the dominant species of salty community and region such as Urmia and Qom lakes. (Asri, 1997) and (Akhani & Ghorbanli 1993). (Strogonov, 1964) divided halophyte plants in two groups as chloridephyte and sulfatephyte and put *Atriplex verruciferum*, *Atriplex canescens* in chloridephyte group and *Suaeda glaea*, *Haloxylon sp* in sulfatephyte group. Of course, seed germination and seedling emergence are critical to the survival of plants and salt- affected area (Khan, 2002). Then Current research was studied on the resistant of salts stress (NaCl and Na<sub>2</sub>SO<sub>4</sub>) on germination of *Halostachys caspica*. (De-yu, 2007) showed that germination of *Suaeda salsa* inhibition was in the following order Na<sub>2</sub>SO<sub>4</sub> > NaCl but (Dashtkeyan, 2000) on *Rubia tinctorum*, showed that germination was in the following order NaCl>Na<sub>2</sub>SO<sub>4</sub>+NaCl>Na<sub>2</sub>SO<sub>4</sub>. (Shalka, 2006) on *Urochondra setulosa* and Indulkar on *Sorghom* showed that germination was in the following order NaCl>Na<sub>2</sub>SO<sub>4</sub>. Seed of halophytes respond to salinity stress the initial germination process is delayed under salt stress (Ungar & Keiffer, 1997). Reddy and Vora (1983) showed that seed germination of Bara delayed and radicle and plumule and decreased significantly with increased salinity of Na<sub>2</sub>SO<sub>4</sub>, NaCl and KCl. Jie (2005) reported that germination percentage of *Halostachys caspica* was not affected by 100 mM NaCl, while severely inhibited by 500 mM NaCl. (Assareh, 2003) on three species *Eculyptus camaldulensis*, *Eculyptus salubris* and *Eculyptus tetragona* and (Tajbakhsh, 2001) on *Hordeum* showed that percentage, speed, index of germination, seed healthy index, radicle, plumule and plant length decreased significantly with increased salinity of NaCl. Results of such as same researches could be used for improvement and sustainable development in salty regions in addition to elimination of soil and wind erosion and forage for livestock in arid and saline regions.

## 2. Materials and Methods

Seeds of *Halostachys caspica* were collected from Qom Lake. The seeds were surface sterilized with %70 alcohol for 15 second and washed with distilled water three times with germination experiment being started immediately. Then the seeds were surface sterilized with 1000 ppm for 15 minutes and washed with distilled water three times. Germination were carried out in Petri dishes (9 cm in diameter) on two layers of filter paper moistened with 5 ml distilled water (0 (control), 100, 200, 300, 400 and 500 mM NaCl and Na<sub>2</sub>SO<sub>4</sub>) solution and covered Petri dishes with Paraffin. Three replicates of 30 seeds were used for each treatment. Germination experiment was carried out 20°C temperature with an 8-h dark, 16-h light photoperiod. Germination was recorded every three day along 30 days. Characters of percentage speed and index of germination, seed healthy index, radicle, plumule and plant length were measured. Before statistical analysis in order to ensure homogeneity of variance was used Kolmogrov-smirnov test. Statistical analysis was carried out using SPSS 10. One way ANOVA was carried out to determine differences among treatment groups of characters. Treatment means were compared by Duncan test to determine whether differences among means were significant between treatments within each salinity concentration of NaCl and Na<sub>2</sub>SO<sub>4</sub>. Parried T-Test sample was carried out to determine differences among same concentration treatment of NaCl and Na<sub>2</sub>SO<sub>4</sub>. Characters were calculated with following formulas

Germination index (GI=  $(\sum T_i N_i)/S$  where  $T_i$  is I days after started experiment,  $N_i$  is number of seed germinated along I day, S is total of seeds (30).

Germination speed (GS=  $\sum n_i/D_i$  where  $n_i$  is the number of germination in special day and  $D_i$  is number days after started experiment).

Seed healthy index (SHI= (plant length (mM) \*germination percentage) /100

## 3. Results

Kolmogrov-Smirnov test showed that all groups variance are homogene. Results of one way ANOVA and Duncan test of NaCl salt are shown in Tables 1 and 2 and Na<sub>2</sub>SO<sub>4</sub> salt in Tables 3 and 4. Parried T-test sample of same concentration treatment of NaCl and Na<sub>2</sub>SO<sub>4</sub> carried out in Table 5.

Table 1. One way ANOVA of characteristic of *Halostachys caspica* due to NaCl salt concentrations and their interaction

Characters	Source of variables	Sum of squares	df	F
Radicle length	Between groups	126.81	5	10.63**
	Within groups	28.63	12	
	Total		17	
Plumule length	Between groups	35.82	5	12.1**
	Within groups	7.1	12	
	Total		17	
Plant length	Between groups	223.32	5	10.37**
	Within groups	51.71	12	
	Total		17	
Germination percentage	Between groups	21344.41	5	24.53**
	Within groups	2088.8	12	
	Total		17	
Seed healthy index	Between groups	349.89	5	20.76**
	Within groups	40.45	12	
	Total		17	
Germination speed	Between groups	213.51	5	24.54**
	Within groups	20.88	12	
	Total		17	
Germination index	Between groups	19.21	5	24.52**
	Within groups	1.88	12	
	Total		17	

\*\* Indicated significant difference at  $p=0.01$

Table 2. Results of Duncan test of characteristic of *Halostachys caspica* due to NaCl salt concentrations (Different letters in the same column indicate significant difference at  $p=0.01$ )

	Radicle length	Plumule length	Plant length	Germination percentage	Seed healthy	Germination speed	Germination index
0(control)	7.22a	4.66a	11.89a	95.56a	11.47a	9.56a	2.87a
100 mM	7.44a	4.89a	12.33a	100a	10.34a	10a	3a
200 mM	8.44a	3.43bc	11.89a	83.33ab	9.74a	8.33ab	2.5ab
300 mM	5.11bc	2.77c	7.89bc	65.56b	5.13b	6.56b	1.98b
400 mM	2.44cd	2.66c	5.11cd	23.33c	1.18c	2.33c	0.71c
500 mM	0.67d	0.67d	2.44d	11.11c	0.44c	1.11c	0.23c

One way ANOVA indicated significant ( $p=0.01$ ) effect of various concentration (0,100, 200, 300, 400 and 500 mM NaCl) on all characters measured of *Halostachys caspica* (Table 1). Duncan test indicated except of plumule, compared with the control, all characters were not affected by 200 mM NaCl and the plant tolerate easily salt stress by 200 mM NaCl (Table 2). While Duncan test indicated except of plumule, compared with the control, all characters were severely inhibited by 400 and 500 mM NaCl (Table 2). Maximum seed and speed germination was obtained in 100 mM NaCl (Table 2). Rate of radicle, plumule and plant length were not decreased with increase of concentration in 300 by 400 mM NaCl but percentage, speed and index of germination, seed healthy index were decreased with increase of concentration in 300 by 400 mM NaCl (Table 2). Rate of percentage, speed and index of germination and plumule were not decreased with increase of concentration in 200 by 300 mM NaCl but, radicle, plant length and seed healthy index were decreased with increase of concentration in 200 by 300 mM NaCl (Table2). However results showed that *Halostachys caspica* in characters of percentage, speed and index of germination,

seed healthy index and plant length tolerate easily salt stress by 200 mM NaCl and increased rate of their by 100 mM NaCl (Table 2). Results indicated that radicle, plumule and plant length (growth characters) are more sensitive than germination percentage, speed and index of germination and seed healthy index (generation characters) to increase of NaCl salt.

One way ANOVA indicated significant ( $p=0.01$ ) effect of various concentration (0,100, 200, 300, 400 and 500 mM  $\text{Na}_2\text{SO}_4$ ) on all characters measured of *Halostachys caspica* (Table 3). Duncan test indicated compared with the control, characters of radicle, plumule, plant length and seed healthy index were severely inhibited by 100 mM  $\text{Na}_2\text{SO}_4$  salt and showed that the plant is very sensitive to  $\text{Na}_2\text{SO}_4$  salt but germination index and speed were not decreased with increased by 200 mM  $\text{Na}_2\text{SO}_4$  salt and showed the plant rather tolerate by 200 mM  $\text{Na}_2\text{SO}_4$  salt (Table 4). Results indicated that radicle, plumule and plant length (growth characters) and seed healthy index are more sensitive than germination percentage, speed and index (generation characters) with increased of  $\text{Na}_2\text{SO}_4$  salt (Table 4).

Results showed that effect of each same concentration of NaCl and  $\text{Na}_2\text{SO}_4$  salt (100,

200, 300 and 400 mM) on percentage, speed and index of germination and seed healthy index characters have similar responses and were not significantly different but these characters in concentration 500 mM of NaCl was significantly lower in comparison with 500 mM of Na<sub>2</sub>SO<sub>4</sub>. Rate of radicle and plant length in all each same concentrations of NaCl and

Na<sub>2</sub>SO<sub>4</sub> salt (100, 200, 300, 500 mM) except 400 mM and Plumule in all each same concentrations (100, 200, 300 mM) except 400 and 500 mM in Na<sub>2</sub>SO<sub>4</sub> salt was significantly lower in comparison NaCl salt. However the plant in Plumule, radicle and plant length is more sensitive to Na<sub>2</sub>SO<sub>4</sub> than NaCl salt.

Table 3. One way ANOVA of characteristic of *Halostachys caspica* due to Na<sub>2</sub>SO<sub>4</sub> salt concentrations and their interaction

Characters	Source of variable	Sum of squares	df	F
Radicle length	Between groups	59.04	5	12.83**
	Within groups	11.05	12	
	Total		17	
Plumule length	Between groups	20.44	5	12.51**
	Within groups	3.92	12	
	Total		17	
Plant length	Between groups	137.31	5	12.24**
	Within groups	26.93	12	
	Total		17	
Germination percentage	Between groups	5316.87	5	7.58**
	Within groups	1688.53	12	
	Total		17	
Seed healthy index	Between groups	164.56	5	11.22**
	Within groups	35.02	12	
	Total		17	
Germination speed	Between groups	53.24	5	7.58**
	Within groups	16.85	12	
	Total		17	
Germination index	Between groups	4.78	5	7.55**
	Within groups	1.52	12	
	Total		17	

\*\* . Indicated significant difference at p=0.01

Table 4. Results of Duncan test of characteristic of *Halostachys caspica* due to Na<sub>2</sub>SO<sub>4</sub> salt concentrations (Different letters in the same column indicate significant difference at p=0.01)

	Radicle length	Plumule length	Plant length	Germination percentage	Seed healthy index	Germination speed	Germination index
0(control)	7.22a	4.66a	11.89a	95.57a	11.47a	9.56a	2.87ab
100 mM	2.55b	1.67b	4.22b	98.89a	4.18b	9.89a	2.97a
200 mM	2.55b	1.89b	4.44b	98.89a	4.36b	9.89a	2.97a
300 mM	2.66b	1.89b	4.55b	75.55bc	3.47b	7.55bc	2.27bc
400 mM	2.11b	1.9b	4.77b	62.22c	2.99b	6.22c	1.87c
500 mM	2.11b	1.7b	4.44b	57.22c	2.59b	5.78c	1.73c

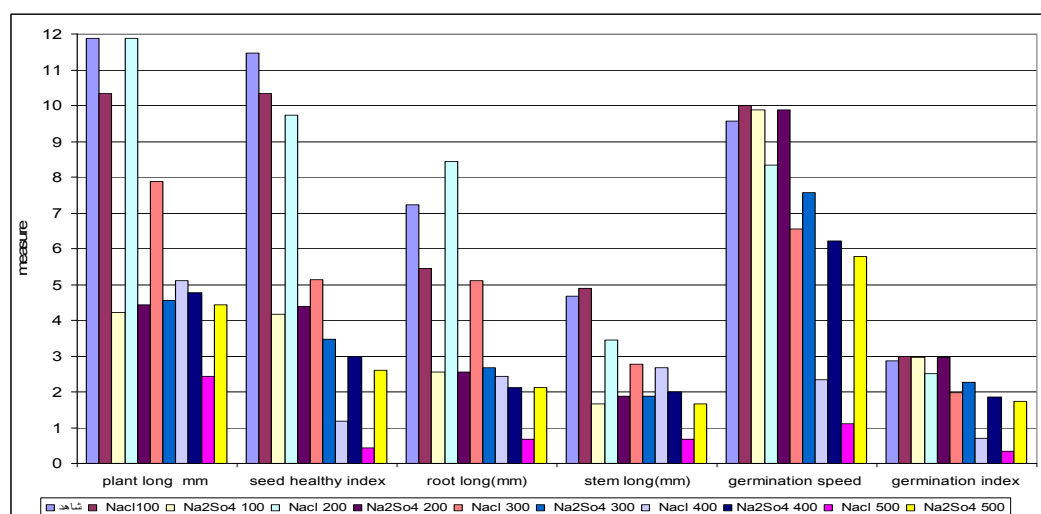


Fig.1. Mean final all characters measured (except germination percentage) of *Halostachys caspica* in different concentrations of NaCl and Na<sub>2</sub>SO<sub>4</sub> salts

Table 5. Paired T-test sample compared of same concentration treatment of NaCl and Na<sub>2</sub>SO<sub>4</sub>

Sources	Compared of treatments	Different of variance treatment	t	df	significant
Radicle length	NaCl100- Na <sub>2</sub> SO <sub>4</sub> 100	2.72	3.194	8	0.013*
	NaCl200- Na <sub>2</sub> SO <sub>4</sub> 200	2.57	6.871	8	0.001**
	NaCl300- Na <sub>2</sub> SO <sub>4</sub> 300	1.94	3.773	8	0.005**
	NaCl400- Na <sub>2</sub> SO <sub>4</sub> 400	0.71	1.414	8	0.195ns
	NaCl500- Na <sub>2</sub> SO <sub>4</sub> 500	1.13	3.833	8	0.005**
Plumule length	NaCl100- Na <sub>2</sub> SO <sub>4</sub> 100	1.48	6.526	8	0.001**
	NaCl200- Na <sub>2</sub> SO <sub>4</sub> 200	1.67	2.8	8	0.023*
	NaCl300- Na <sub>2</sub> SO <sub>4</sub> 300	0.61	4.438	8	0.002**
	NaCl400- Na <sub>2</sub> SO <sub>4</sub> 400	1	2	8	0.081ns
	NaCl500- Na <sub>2</sub> SO <sub>4</sub> 500	1.32	2.268	8	0.053ns
Plant length	NaCl100- Na <sub>2</sub> SO <sub>4</sub> 100	3.55	5.163	8	0.001**
	NaCl200- Na <sub>2</sub> SO <sub>4</sub> 200	2.56	8.741	8	0.001**
	NaCl300- Na <sub>2</sub> SO <sub>4</sub> 300	2.45	4.082	8	0.004**
	NaCl400- Na <sub>2</sub> SO <sub>4</sub> 400	2.78	3.59	8	0.729ns
	NaCl500- Na <sub>2</sub> SO <sub>4</sub> 500	2.61	2.309	8	0.05*
Germination percentage	NaCl100- Na <sub>2</sub> SO <sub>4</sub> 100	1.92	1	2	0.423ns
	NaCl200- Na <sub>2</sub> SO <sub>4</sub> 200	11.71	2.3	2	0.148ns
	NaCl300- Na <sub>2</sub> SO <sub>4</sub> 300	23.34	0.742	2	0.535ns
	NaCl400- Na <sub>2</sub> SO <sub>4</sub> 400	21.43	3.143	2	0.088ns
	NaCl500- Na <sub>2</sub> SO <sub>4</sub> 500	8.81	9.168	2	0.012*
Seed healthy index	NaCl100- Na <sub>2</sub> SO <sub>4</sub> 100	2.48	4.292	2	0.05*
	NaCl200- Na <sub>2</sub> SO <sub>4</sub> 200	1.01	9.123	2	0.012*
	NaCl300- Na <sub>2</sub> SO <sub>4</sub> 300	1.98	1.451	2	0.284ns
	NaCl400- Na <sub>2</sub> SO <sub>4</sub> 400	0.96	3.271	2	0.082ns
	NaCl500- Na <sub>2</sub> SO <sub>4</sub> 500	0.50	7.406	2	0.018*
Germination speed	NaCl100- Na <sub>2</sub> SO <sub>4</sub> 100	0.19	1	2	0.423ns
	NaCl200- Na <sub>2</sub> SO <sub>4</sub> 200	1.17	2.296	2	0.149ns
	NaCl300- Na <sub>2</sub> SO <sub>4</sub> 300	2.34	0.739	2	0.537ns
	NaCl400- Na <sub>2</sub> SO <sub>4</sub> 400	2.14	3.142	2	0.088ns
	NaCl500- Na <sub>2</sub> SO <sub>4</sub> 500	0.88	9.198	2	0.012*
Germination index	NaCl100- Na <sub>2</sub> SO <sub>4</sub> 100	5.77	1	2	0.423ns
	NaCl200- Na <sub>2</sub> SO <sub>4</sub> 200	0.35	2.302	2	0.148ns
	NaCl300- Na <sub>2</sub> SO <sub>4</sub> 300	0.7	0.742	2	0.525ns
	NaCl400- Na <sub>2</sub> SO <sub>4</sub> 400	0.65	3.143	2	0.088ns
	NaCl500- Na <sub>2</sub> SO <sub>4</sub> 500	0.26	9.165	2	0.012*

\*\* Indicated significant difference at p=0.01 \* Indicated significant difference at p=0.05

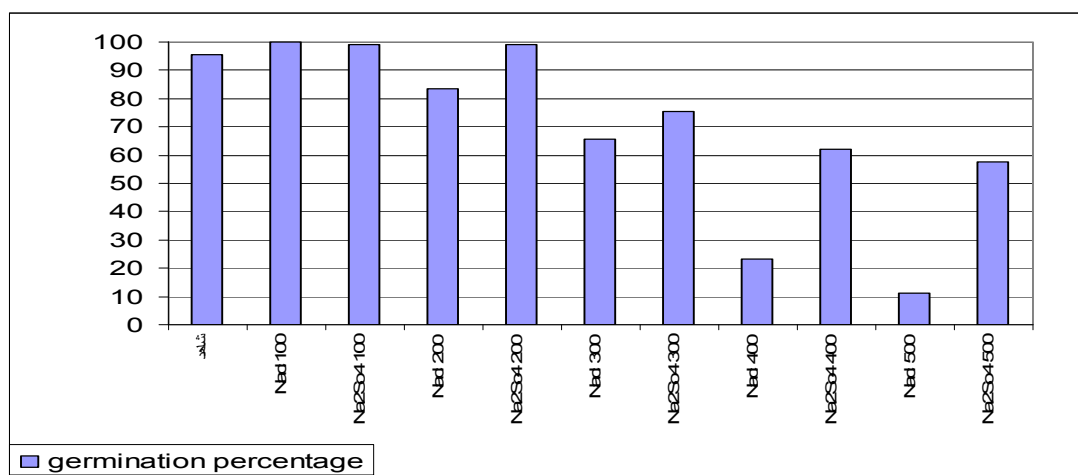


Fig.2. Mean final germination percentage of *Halostachys caspica* in different concentrations of NaCl and Na<sub>2</sub>SO<sub>4</sub> salt

Rate of germination percentage, index and speed in all same of concentrations Na<sub>2</sub>SO<sub>4</sub> salt was more than with comparison NaCl salt and radicle, plant length and seed healthy index in all same of concentrations (except 500 mM) Na<sub>2</sub>SO<sub>4</sub> salt was lower with comparison NaCl salt (Fig1,2). Rate of germination percentage in concentrations 100 and 200 mM of NaCl and

Na<sub>2</sub>SO<sub>4</sub> salts was more than with comparison controlled (Fig. 2). However rate of germination percentage in concentrations 400 and 500 mM of NaCl was severely inhibited but in concentrations 400 and 500 mM Na<sub>2</sub>SO<sub>4</sub> salt was not severely affected and is almost 60% (Fig. 2).

#### 4. Discussion and Conclusion

Results showed that Compared with control condition percentage, speed and index of germination were increased by 200 mM NaCl and Na<sub>2</sub>SO<sub>4</sub> salts. Jie. Song (2005) on *Halostachys caspica* and Shariat (2000) on *Poterium sanguisorba* showed that were increased germination percentage, speed and index by 100 to 150 mM of NaCl salt too. But Assareh (2003) on three species *Eculyptus camaldulensis*, *Eculyptus salubris* and *Eculyptus tetragona* and Tajbakhsh (2001) on *Hordeum*, Poresmail(2000) on *Nitraria schoberi*, *Suaeda fruticosa*, Bagheri (1998) on *Kochia prostrata*, *Eurotia ceratoides*, *Elymus junceus*, Afzali (2000) on *Melilotus officinalis*, *Trifolium fragiferum*, Farkhah(2000) on *Salsola dendroides pall*, *Alhaji persarum*, *Aleluropus lagopoides* and Assadian (1987) on *Medicago* showed that compared with control condition germination percentage, speed, and index, seed healthy index, radicle, plumule and plant length decreased significantly with increased salinity of NaCl salt. The results showed that germination percentage and speed were severely inhibited by above 400 mM NaCl such as Jie. Song, (2005) on *Halostachys caspica*, E.B.kurkova, (2002) on *Seidlitzia rosmarinus*, Shalka, (2006) on *Urochondra setulosa* and Poresmail (2000) on *Nitraria schoberi*, *Suaeda fruticosa* showed that germination percentage and speed were severely inhibited by above 400 mM NaCl salt. The results showed that germination percentage and speed were not severely inhibited by above 400 mM Na<sub>2</sub>SO<sub>4</sub> salt and is almost 60 % but Shalka, (2006) on *Urochondra setulosa* showed that germination percentage and speed were severely inhibited by above 400 mM Na<sub>2</sub>SO<sub>4</sub> salt. However, the results showed that *Halostachys caspica* in germination characters such as germination percentage, speed and index is more sensitive to NaCl than Na<sub>2</sub>SO<sub>4</sub> salt such as Dashtkeyan (2000) on *Rubia tinctorum*, Shalka, (2006) on *Urochondra setulosa*, Strogonov (1964) on

*Haloxylon sp* and Indulkar on *Sorghom* showed that germination of was in the following order NaCl>Na<sub>2</sub>SO<sub>4</sub>+NaCl> Na<sub>2</sub>SO<sub>4</sub> but De-yu, (2007) showed that germination of *Suada salsa* inhibition was in the following order Na<sub>2</sub>SO<sub>4</sub>>NaCl. *Halostachys caspica* were not decreased significantly with increased salinity of NaCl in growth characters such as radicle, plumule and plant length by 300 mM but more concentration of 300 mM NaCl occurred a shock for plant and compared with control condition were decreased significantly by 500 mM while in the growth characters salt the shock occurred by and more concentration of mM 100 of Na<sub>2</sub>SO<sub>4</sub> compared with control condition were decreased significantly. Rate of radicle was affected lower than plumule in each of NaCl and Na<sub>2</sub>SO<sub>4</sub> salts but Jafari (1993) showed that plumule was affected lower than radicle. Ungar & Keiffer, (1997) showed that Seed of halophytes in the initial germination process is delayed and decreased under salt stress but this research showed that *Halostachys caspica* germination percentage and speed increased by 200 mM of NaCl and Na<sub>2</sub>SO<sub>4</sub> salts. *Halostachys caspica* with comparison control was increased the growth characters in NaCl salt and increased the germination characters in Na<sub>2</sub>SO<sub>4</sub>. Then, we can put this plant in obligate halophyte groups such as Moghim (2004), Meiri (1984) and Zhao, (2005) reported that *Halostachys caspica* is obligate halophyte and has been adapted to saline and wet land regions. Results showed that *Halostachys caspica* has different interaction to NaCl and Na<sub>2</sub>SO<sub>4</sub> salts; however *Halostachys caspica* in the germination characters has been adapted to Na<sub>2</sub>SO<sub>4</sub> and in the growth characters adapted to NaCl salt. Then we can put *Halostachys caspica* chloridephyte and sulfatephyte group of halophytes. *Halostachys caspica* plant has excellent power germination about 100% (Moghimi, 2004) and could be used for improvement and sustainable development, elimination of soil and wind erosion and forage for livestock in arid and saline regions.

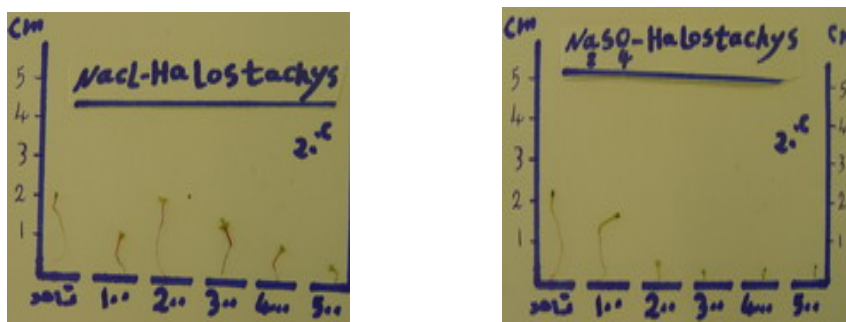


Fig.3. Picture of plant length in different concentrations of NaCl and Na<sub>2</sub>SO<sub>4</sub>

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