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Comparison of solute-specific effects on seed germination characteristics of SM seed (*Silybum marianum*) at the same osmotic potential under salinity and drought stress conditions

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ABSTRACT

Salinity and drought tension, are the most important environmental factors that reduce growth and production plants in Iran, germination reduction and seedling establishment are the most important problems of these soils; knowledge of resistance to drought and salinity and also adaptation ratio in hard situations, is a prerequisite for implementing strategies of dry and saline soils resuscitation that can be irrigated with saline water. This test was examined in order to separate the effects of drought and salinity and different levels of concentration on SM (Silybum Marianum) germination characteristics in above tension conditions in Hamadan Agriculture and Natural Resources Research Center. The test was implemented in a completely randomized design with 8 treatments and 4 design replications and or reviewing of drought treatment from Glycol polyethylene 6000 in 4 levels with osmotic potentials (0 and -3.5 and -7.2 and -10.8 bar). Also for surveying of salinity treatment effect, sodium chloride salt was used in 4 concentration levels with osmotic potentials (0 and -3.5 and -7.2 and -10.8 bar). SM seeds were put separately after disinfection within sterilized Petri-dishes and were added 10 cc of sodium chloride salt and polyethylene glycol separately with different concentrations to each of them. Within experiment days, the rate of seed germination and also shoot and root length was measured and recorded; at the end of the experiment, fresh and dry weight of roots and shoots were measured. Results showed that concentration increase in salt and dry environments, caused germination reduction and other factors were measured, so that high concentrations of drought and salinity treatments have reduced the germination rate among 40 to 50 percent, respectively. Therefore, it is concluded that SM (Silybum Marianum) seeds resistance in drought conditions tolerance is better than saline, which its reason is concerned to specific effects of salts in saline conditions that reduces enzyme activity and less cell membrane permeability. Higher treatment levels of salt concentration cause root and shoot length reduction respectively 11 and 2 percent in relation with drought treatment conditions and then salts specific effects is significance at .01 and caused increase of the stem and roots length under drought tension conditions in related of the salinity. Higher treatment levels of salt concentration also reduces root and shoot dry weight, respectively 7.34 and 31.3 percent relative to drought treatment conditions, so the solute-specific effects are significant at level of 1 percent and increase root and shoot dry weight under drought tension rather than salinity. In fact, the specific effects of salts decrease enzyme activity and record of cell division in stem and root that leads to a drop in the growth of SM seedlings under salinity tension compared with drought tension.

Keywords: SM (*Silybum Marianum*), Salt- specific effect, Seed germination, Drought stress, Salinity stress, Hamedan

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INTRODUCTION

Herbal plants are rich repositories of many drugs essential ingredients. Although the effective ingredients are basically made by genetic process guidance but making them is influenced by environmental factors markedly, so that environmental factors cause changes in medicinal plants growth, the amount and quality of effective ingredient materials (3).

SM (Silybum marianum) is a Mediterranean medicinal plant that needs hot weather and abundant sunshine during the growing. **SM** seeds can survive up to 9 years under soil. Optimum temperature for germination is happened when the seed passes intermittently sixteen-hour duration at 2 to 15 ° C and then an 8-hour period at temperatures of 10 to 30 ° C. The beginning of this plant germination is simultaneous with the start of rainfall in November and in late May, it gets to flower and after producing seeds it will dry.

SM is capable to finish its life cycle by precipitation (about 240 mm) and therefore this plant can be planted for dry areas like Khuzestan and Fars (1).

Water is one of the main factors on germination activator and capability of water availability reduces by decrease in osmotic potential of soil; osmotic potential has a direct impact on water absorption rate and thus plant germination (9, 15 and 28).

Sharafi (14), in an experiment with the effect survey of different levels of salinity (0, -0.3, -0.6, -0.9 and -1.2 Mega Pascal=MPa) and drought (0, -0.3, -0.6, -0.9, -1.2 MPa) on SM germination showed that all surveyed traits like seedling growth and uniformity of germination in both experiments were affected by drought and salinity stress.

Rezaei in survey of cumin seed germination (Cuminum cyminum) affected by different levels of salinity stress (0, 100, 105, 200 mg in sodium chloride liter) showed the highest percentage of germination and dry root weight was related to 150 mg treatment of sodium chloride and also the minimum length of peduncle was concerned to 200 mg treatment of sodium chloride concentration (10).

Judy and colleagues (6) in survey of anise seed germination (Pimpinella anisum) affected by dehydration stress, showed that seed germination percentage, peduncle and root length and seed vigor decreased by stress effect.

Barzegar and Rahmani (5) reported that there was a significant difference between values of percentage averages and germination speed of hyssop seed (Hyssopus officinalis) under affection of drought stress levels (0, -1, -3, -6, -9 bar), and with increasing stress intensity, the values of these traits got lower.

In another experiment with drought treatment deed with concentration (-3, -6, -12 bar) on Hyssop plant, it was observed that on the third day after germination in concentration of -3 bar, the number of germinated seeds reached their maximum numbers and in concentration of -6 bar, the number of buds and the germination rate was reduced so that in -9 and -12 bar, germination was stopped completely (16).

In an experiment with survey of different drought levels effect 0, 50, 100, 150 and 200 mmol NaCl on herbs Sage(*Salvia officinalis*), Indian Senate(*Cassia aculata)*, SM(*Silybum marianum)*, Cannabis(*Cannabis sativa*), Bitter Daphnia(*Sisymbrium sophia*), Roman Chamomile(*Anthemis nobillis*) and German chamomile(*Matricaria chamomilla*) was observed that with concentration salinity increase, germination percentage, germination rate, root length, shoot length, dry weight, seed vigor and length ratio of shoot to root decreased.

The results showed that there was a significant difference between herbal plants in all surveyed traits. Also dry weight and seed vigor had the highest coefficient of variation and the length ratio of shoot to root and germination percentage had the lowest coefficient of variation.

The highest value of germination percentage, shoot length, seed vigor and the length ratio of shoot to root was related to cannabis while Daphnia had the highest rate of germination, salvia had the largest root length and SM had the highest dry weight (17).

In another experiment with survey of the salinity stress levels (0, -2, -4, -6 and -8 bar) on components of seedling growth of 10 medicinal plant SM(*Silybum marianum*), Psyllium(*Plantago psylium*), Ns(*Nigella sativa*), Citrullus Colocynthis, Flax(*Linum usitatissimum*), Artichoke(*Cynarpa scolymus*), Basil(*Ocimum basilicum*), Fennel(*Foeniculum vulgare*), Safflower(*Carthamus tinctorius*) and Hyssop, it was observed that with salinity increasing in all plants, root and peduncle length and root and peduncle dry weight decreased; seedling length in Citrullus Colocynthis, fennel, hyssop and flax was decreased with salinity levels increasing from control value linearly, while in plants SM, artichoke, flax, black beans and basil with salinity increasing of -2 bar, seedling length began to fall slowly and in safflower with salinity increase of -4 bar, seedling length got decreased.

In plants choosing for Seedling dry weight of Black seed and cotton began to fall in salinity -2 and -4 bar respectively and in SM and artichoke in salinity -6 bar it began to fall. The results showed that among this herbal plants, Safflower, SM and artichoke have high resistance to salinity in component of the seedling growth and plants, Citrullus Colocynthis and Nigella were members of salt-sensitive plants in the seedling stage (2).

Choosing plants for cultivation, resistance to salinity and low water always should be considered, especially during germination and emergence; thence in one hand, usual evaluation under field conditions is time-consuming and on the other hand, is influence by many uncontrollable factors such as soil factors, climate and farming operations, therefore it is necessary using a laboratory method under controlled conditions, of rapid and relatively accurate assessment of plants response can be possibly provided to drought and salinity stress (20).

SM in terms of massed active ingredients is important in treating disorders liver, bile, and many other diseases and also favorable compatibility with the climatic conditions in Iran (3). Accordingly, the study of this plant resistance to drought and salinity stress is important to promote the cultivation of this plant.

MATERIALS AND METHODS

To investigate the effect of different levels of drought and salinity stress on germination and characteristics of, herbal seedling plant SM, two separate experiments was conducted in a completely randomized design with four replications under laboratory conditions; the first experiment was consisted of four levels of drought potential (0, -3.6, -7.2, -10.8 bar) which according Mitchell and Kaufman instruction (29) was prepared using the chemical polyethylene glycol 6000 (PEG-6000).

The second experiment also included four potential levels of salinity (0, -3.6, -7.2, -10.8 bar) and by electrical conductivity was respectively (0, 8.5, 17.1 and 25.5 mmho/Cm) which NaCl was used to establish the levels of salinity (30); also, distilled water was used for stress surface of zero establish in both experiments.

Before starting the experiment, Petri dish sets and bed seed (Wattman paper) were sterilized in an autoclave at 120 ° C for 2 hours, then 25 seeds were disinfected with 10 percent sodium hypo chloride dilution for 30 seconds and after washing with distilled water were transferred on filter paper in Petri-dishes, for drought and salinity treatments, 5 ml glycol poly ethylene solution and 5 ml sodium chloride solution was added to each Petri-dish respectively. Petri-dishes were weighed and the first weight of each was recorded and in recommended temperature 25±1° C was placed in the germinator.

All Petri-dishes were weighed daily and distilled water was added to them to different size of their initial weight with their first weight at the beginning of experiment. This action was performed to prevent potential change of any solution by water evaporation, afterwards germinated seeds (according to exit the root shell to the size of 2 mm from seed testa) were measured and recorded daily in each Petri-dish (19, 23 and 24).

After 10 days of the experiment, seed germination percentage was calculated, then root length and shoot length of seeds were measured with a ruler, to determine the dry weight of root and shoot, samples were first washed with distilled water and after removing the root and shoot, they were put in oven with temperature 70 ° C for 18 hours and finally, root and shoot dry weight was measured (18).

Statistical analysis of data from two experiments was performed to compare the concentrations independently and to compare stress type in equal concentrations and in the same situations by SAS statistical software. Comparing all averages by Duncan's multiple test range was conducted in level 5%. All graphs were plotted with Excel software.

RESULTS AND DISCUSSION

Effects of salinity stress

Germination percentage

Based on variance analysis results, levels effect drought stress on seed germination percentage was significant (Table1). Germination percentage in control treatment was in its highest range compared with other treatments. It seems high germination percentage is related to high SM cell membranes and absence of the solute effect. Also in regards of salinity stress levels effect, it determined that to stress level at -10.8 bar, there was a significant difference between seed germination percentage with difference control, so germination percentage was reduced by 50 percent (Table1). Test results showed SM high resistance to salinity stress and also the results of EhteshamNia (2) indicate that SM seeds have high resistance to salinity on germination stage. Also Seyed Sharifi (13) in an experiment with study on four salinity levels (0, 25, 100 mmol) NaCl on SM germination showed that salinity stress has significant effect on germination percentage and germination uniformity and germination percentage average in salinity potential 25 and 50 mmol NaCl decreased compared to control (distilled water) respectively 17.2 and 43 percent. Furthermore Sharafi (14) in addition of survey on different salinity levels effect on SM seed germination observed that time to reach that stage 10 percent of germination and germination rate, has been influenced by of salinity. The above findings regarding the effects of different salinity levels on SM, shows high resistance in this plant at germination stages which the results of this experiment was consistent with their.

Some researchers believe that salinity stress has got effects by osmotic pressure increase and water absorption reduction by seeds and in addition, by sodium and chlorine ions toxic effects, may affect seed germination (12, 32).

Root and shoot length

Root and shoot length decreased with increasing salinity potential (Fig. 1). Maximum length of root and shoot in salt less potential (control) and minimum was in potential -10.8 bar which was significant rather than control and other treatments ($p<0.50$); root and shoot length in comparison to control treatment at osmotic potential -10.8 bar decreased respectively 57 percent and 83 percent respectively. It can be concluded from above findings that salinity stress increase caused growth reduction and peduncle and root length got dipped.

EhteshamNia (2) in an experiment with studying the salinity potential levels (0, -2, -4, -6, -8 bar) on seedling growth characteristics of **SM** concluded that with increasing salinity levels, from potential of -2 bar, Seedling length began to fall slowly which is consistent with the results of this study.

Also Mehdikhani (17) by studying salinity effect on SM showed with increasing salinity, root length and shoot length was reduced which this is also consistent with the results.

Some studies show that the germinated seeds at saline environments have shorter stem and root and NaCl has severe inhibitor effect on emergence of fetal tissues than other causing salinity materials (25, 26).

Root and shoot dry weight

Effect of salinity stress levels was significant on SM root and peduncle dry weight (Table 1). Changes in root and shoot dry weight showed decrease trend up to osmotic potential -10.8 bar, so that reduction in potential above was 72 percent and 83 percent of control treatment for root and shoot dry weight respectively (Fig. 2).

Mehdikhani (17) with study the effect of salinity on SM showed that root and shoot dry weight decreased by reducing salinity potential. EhteshamNia (2) in addition of salinity effect on SM reported that seedling dry weight began to fall with increasing salinity potential of -6 bar. He concluded SM plant is resistant to salinity stress. It seems osmotic potential reduction and ion toxicity effects disordered root and shoot growth process by increasing salinity levels which causes reducing seedling dry weight. Some studies results on canola are also confirmed this issue (33).

Decrease in germination characteristics studied in this experiment can be related to reducing the rate and pace of water absorption (21, 22) and also negative effect of negative osmotic potentials of salt and ion toxicity on enzymatic hydrolysis process of seeds storage materials and then to build new tissues by hydrolyzed materials (32).

Effects of drought stress

Germination Percentage

Effect of different levels of drought was significant on seeds germination percentage (Table 2). Germination percentage in control treatment had significant statistical differences with other drought potential. In addition, drought potentials of -7.2 and -10.8 bar had the lowest percentage of germination (Table 2).

It can be concluded that increasing drought stress has been caused reducing germination percentage. Also, germination in drought potential of -10.8 bar reduced 38 percent compared to control treatment. According to Yazdani reports (2009), SM seeds were capable of germination up to -20 bar by reducing water potential and in this potential it was done up to 16 percent of germination.

Sharafi (14) with survey of drought different levels effect on SM germination showed that there is a reverse and negative relationship between some characteristics like seedling growth and germination uniformity under drought stress and its reason is hard seed coat in water absorption. On the other hand drought stress had no significant effect on arrival time to the stage of 10 percent germination and germination rate. The above findings indicate a high SM resistance to drought stress.

It is expressed (28) that if water absorption is impaired by seeds, germination metabolic activity will be taken slowly within the seed. So root outgoing period will be increasing from seed and then germination will be decreases.

Root and shoot length

Drought potential levels caused significant difference in root and shoot length (Table 2). With increasing drought stress severity in relation to control, root length was significantly decreased (Fig. 3). With increasing drought stress, only to -3.6 bar, shoot length was decreased 53 percent and root length was decreased 13 percent. Comparing the results of two experiments drought stress and salinity stress it was determined that shoot length got more damage than root length (Tables 1, 2). Root length decrease reported with increasing water by Tackle (31) and one of the causes of shoot length reduction in drought stress conditions has been found reduction or non-transfer of nutrients from seed storage tissues to the fetus; generally germinated seeds in environments under stress conditions have shorter shoot and root (25).

Dry root and shoot weight

Dry root weight was gradually decreased with increasing drought stress**.** The highest dry root weight was related to level of stress less (control)**.** Dry shoot and root weight got significantly less with increasing drought stress from control treatment to lower osmotic potential, so that in level -10.8 bar, the amount of weight loss was respectively 51 and 64 percent for shoot and root in comparison with control treatment (Figure 4).

Dry root and shoot weight reduction due to increased drought stress is natural and Sharafi results (14) indicate that with increasing levels of drought stress, SM dry root and shoot weight decreased. There are also some reports that drought stress in some herbs like basil and fenugreek causes weight loss in root and shoot (7, 11).

Table 1: Comparison of mean traits of the different salinity levels in the SM

Potential drought (bar)	**Germination %**	**Root length**	**Shoot length**	**Shoot to root ratio**	**Root dry weight**	**Shoot dry weight**
Control	92.976^{a}	68.585^{a}	65.750^{a}	0.9587^{a}	0.0155^{a}	0.17425^{a}
-3.6	79.762^{b}	51.425^{b}	27.00^{b}	0.5251^{b}	0.00950^{b}	0.07350^{b}
-7.2	59.643^{c}	40.230^{c}	17.300^{c}	0.4300^{c}	0.00650^{c}	0.04725^{c}
-10.8	50.238^{d}	29.225^{d}	11.150^{d}	0.3815^{d}	0.00425^{d}	0.03025^{c}

Numbers that have common letters in each column, they have not significantly different at 5% level (p<0.05).

Table 2: Comparison of mean traits of the different drought levels in the SM

Potential drought(bar)	**Germination%**	**Root length**	**Shoot length**	**Shoot to root ratio**	**Root dry weight**	**Shoot dry weight**
Control	93.452a	67.778a	64.550a	0.9523 a	0.01525 a	0.1710a
-3.6	86.548^{b}	58.983^{b}	30.00^{b}	0.5086^{b}	0.011250^{b}	0.11975^{b}
-7.2	69.762^{c}	47.998^{c}	18.800^{c}	0.3917^{c}	0.00775^{c}	0.09725^{c}
-10.8	55.00^{d}	36.268^{d}	12.300^{d}	0.3391^{c}	0.00550^{d}	0.083250^{d}

Numbers that have common letters in each column, they have not significantly different at 5% level (p<0.05).

Table 3. Results of treatment groups based on Duncan test.

Compared and grouped treatments								
Seedling treatments	**PEG-1**	**Nacl-1**	**PEG-2**	**Nacl-2**	**PEG-3**	**Nacl-3**	**PEG-4**	**Nacl-4**
Germination	**93.452^{a}**	**92.976^{a}**	**86.548^{a}**	**69.762^{b}**	**69.762^{b}**	**59.643^{c}**	**55.03^{cd}**	**50.238^{d}**
Root length	**68.585^{a}**	**67.778^{a}**	**58.983^{b}**	**51.425^{c}**	**47.998^{c}**	**40.230^{d}**	**36.268^{e}**	**29.225f**
Shoot length	**65.750^{a}**	**64.550^{a}**	**30.00^{b}**	**27.00^{c}**	**18.800^{d}**	**17.300^{d}**	**12.300^{e}**	**11.150^{e}**
Root dry weight	**0.0155^{a}**	**0.0151^{a}**	**0.0110^{b}**	**.0096^{c}**	**.0079^{d}**	**0.0065^{e}**	**.0053^{f}**	**.0043^{f}**
Shoot dry weight	**0.174^{a}**	**0.1709^{a}**	**0.1196^{b}**	**0.0973^{c}**	**0.083^{cd}**	**0.0734^{d}**	**0.0472^{e}**	**0.0304^{f}**

Salinities treatments with osmotic potentials [Ψs= (0,-3.6,-7.2,-10.8) bar] are treatments codes of apposite:
PEG; 1,2,3,4

Droughts treatments with osmotic potentials [Ψs= (0,-3.6,-7.2,-10.8) bar] are treatments codes of apposite:
Nacl; 1,2,3,4

Numbers that have at least one common letter in each column, they have not significantly different with Duncan test at 5% level

Table 4. Comparison of treatments percentage decrease in terms of salinity stress than drought stress.

Treatments	Control treatment	-3.6bar treatment	-7.2bar treatment	-10.8bar treatment
Germination	0	-6.31	-9.64	-4.29
Root length	0	-12.04	-12.16	-10.09
Shoot length	0	-5.68	-2.82	-2.09
Root dry weight	0	-10.81	-8.99	-7.43
Shoot dry weight	0	-27.85	-29.71	-31.32

Fig 1: Mean of shoot and root drought in drought stress

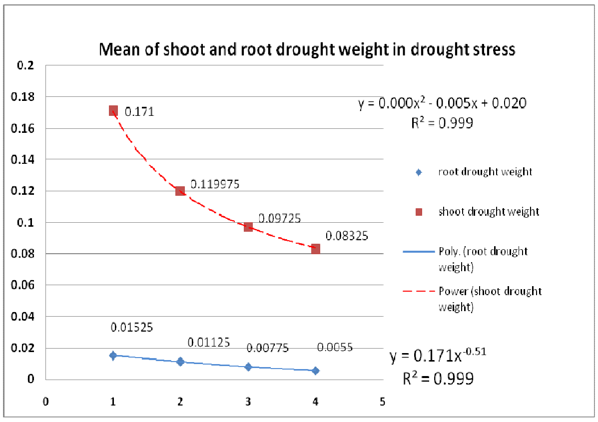


Fig 2: Mean of shoot and root drought weight in drought stress

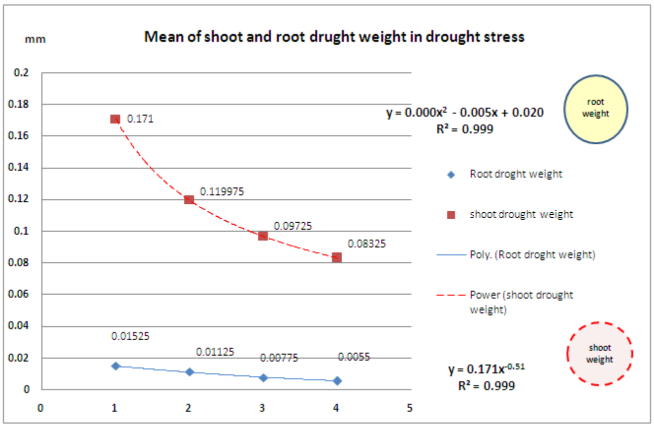


Fig: 3 Mean of shoot and root length in drought stress

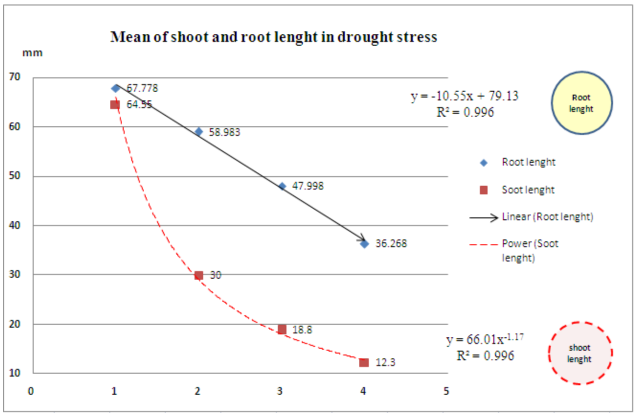
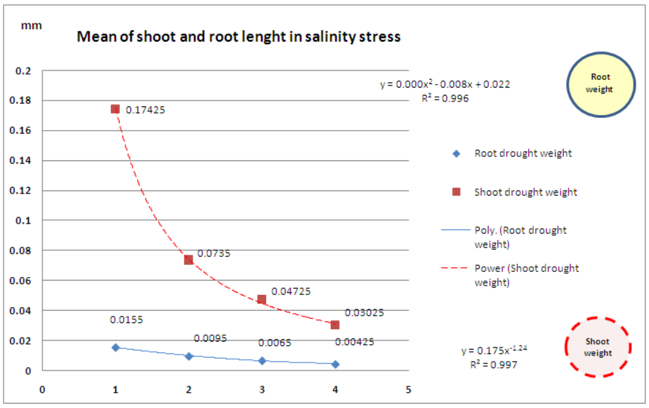


Fig: 4 Mean of shoot and root length in salinity stress



CONCLUSION

Comparison of drought and salinity at equal concentrations
After checking out the effects average of drought and salinity stress and their concentrations on SM germination it can be presumed that seeds under drought and salinity stress have less germination percentage than control and treatments concentration go higher and osmotic potential and water absorption go less**,** percentage germination of the seed has fallen to that rate**.**

These results is consistent with Marchner and colleagues researches (1995) to review the status of nutrients in drought and salinity stress conditions and concluded that germination process reduction due to drought stress is related to water absorption reduction by seeds and thereupon metabolic activity within the seed takes place slowly and sometimes stops**.**

Effect of salinity and drought type had has a very significant effect on germination characteristics**,** root and shoot length and also dry root and shoot weight and concentration levels difference of these two treatments on characteristics above was significant at level 1% and in both cases, drought treatment is better than salinity treatment**.**

Comparing the results of two experiments has a conclusion that SM as a kind of herbal plant has high resistance in suffering drought and salinity on germination characteristics because seed germination percentage has been 60 and 50 percent in drought and salinity stress respectively to stress level -10.8 bar.

This salinity rate in salt concentration is equivalent electrical conductivity of 25 Mm per cm that puts this plant in the salt-resistant plants category because more than 50 percent of seeds have the ability to deploy from establishment and survival point.

In Yazdani's research (2009), salinity stress tolerance and SM seeds survival have been reported up to -20 bar**.** Anyway, it is necessary to perform some experiments for achieving more accurate results to determine biochemical changes in seeds and also damage to cell membranes in determining electrical conductivity**.**

But based on Table 3 information, the amount of injury caused by salinity stress in all studied traits has been more than drought stress which the reason is concerned to specific effects of salts and toxic conditions of some elements such as sodium and chloride in saline environments**.**
This conclusion is consistent with the results of Chadhou and Rajender research (1995) that suggest negative impact of osmotic potential caused by the presence of salt and their toxicity on enzymatic hydrolysis processes of seed storage substances and they believe that salts toxicity cause impaired Starch hydrolysis processes, thus it puts a negative effect on germination and growth of plant cells.
Root and shoot in salinity stress are shorter than relation to drought stress (Fig. 1 and 2) that this result confirmed Kartji and colleagues research (1994) who surveyed the effects of salinity stress and drought stress began in the early stages of vegetative sunflower and they concluded that germinated seeds in salinity stress conditions have been shorter stem and root and sodium chloride in comparison with other causing salt materials has stronger inhibitory effect on fetal tissues**.**

Based on the results of Tables 3 and 4, the amount of root injury in salinity stress conditions is more than drought stress so in grouping above, root length in treatment levels of -3.6 and -7.2 and -10.8 bar stand in different classes (Fig. 1 and 2) which indicates statistical significant difference between salinity and drought treatments at level 5 percent while it is not observed such this situation in stem length except treatment of -3.6 bar and from this point, treatments are lied in a group**.**

Dry shoot weight under salinity stress conditions is less than being in drought stress (Fig. 4) and as noted in above chart, dry shoot weight at treatment levels of -3.6 and -7.2 and -10.8 bar are lied in different classes in relation to each other and there is a significant difference in the level of 5 percent statistically whereas about dry root weight, it is observed the same situation only up to treatment level of -7.2 bar but with less intensity (Fig. 3).

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