Advances in Environmental Biology, 8(21) October 2014, Pages: 510-515

AENSI Journals

Advances in Environmental Biology

ISSN-1995-0756 EISSN-1998-1066

Journal home page: http://www.aensiweb.com/AEB/

The effect of Salinity and Drought Stress on Seed Germination, Seedling Growth and Biochemical Changes in Marigold

Mahrokh Rashidi and Mehrab Yadegari

Department of Agronomy and Medicinal Plants, Faculty of Agriculture, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.

ARTICLE INFO

Article history:
Received 4 September 2014
Received in revised form 24 November 2014
Accepted 1 December 2014
Available online 25 December 2014

Keywords:
Calendula officinalis
environmental stress
growth

ABSTRACT

One of the effects of the decrease of water and salinity in soil is the reduction of growth and development and change in field development under such changes. In order to examine the effect of drought and salinity stress on germination indexes and proline change of marigold under the drought stress [0, -0.2, -0.4, -0.6, -0.8 and -1 MPa] and salinity stress [0, -0.2, -0.4, -0.6 and -0.8 MPa], two experiments were conducted in a randomized design with three replication. Results showed that drought and salinity stresses were significantly effective on germination percentage, germination rate, mean time to germination, normal seedling percentage, root and shoot length, Proline content, seed vigor, seedling dry weight, duration of reaching to %5 [D05], %10 [D10] and %20 percent of germination [D20]. Drought and salinity stresses caused reduction in germination percentage, germination rate, normal seedling percentage, root and shoot length, seed vigor, seedling dry weight and other hand increased the Proline content duration of reaching to %5 [D05], %10, [D10], %20 [D20] germination, proline and mean time to germination. The highest germination percent with the average of 84 percent was related to control condition, but with the increase in the salinity levels to -0.8 MPa and drought stress to -1 MPa germination percentage was 24 and 23 percent respectively. Generally the highest amount of other measured indexes were related to control condition [non-stress] in both salinity and drought stress condition.

To Cite This Article: Mahrokh Rashidi and Mehrab Yadegari., The effect of Salinity and Drought Stress on Seed Germination, Seedling Growth and Biochemical Changes in Marigold ***Adv. Environ. Biol., 8(21),*** *510-515, 2014*

INTRODUCTION

Environmental stresses such as drought and salinity stresses are of abiotic stresses that affect the germination, growth and crop yield [1,35,36,37]. The presences of great amount of salinity in the soil, which is resulted from the water of irrigation, face the plant with salinity stress. Low osmotic potential and salinity intensive density which are the two main characteristic of salty environments are potentially toxic for the plants. Also the lack of water resources and the rainfall shortage in recent year has resulted in drought stress in the plants [20, 32].

Drought stress which causes one of the most important prevailed environmental stresses that will face the agricultural products with limitation and reduce the efficiency of using the semiarid districts [2; 28]. Germination is the first phase in the plant development which is one of the important and sensitive phases in the plant life cycle and is a key process in plant germinating [9]. Seed germination is a complex physiological process that is actually a reaction to environmental signals such as temperature, water potential, light, nitrate and other factors. Environmental stress such as drought and salinity stress result in reduction in germination indexes and the reduction of germination under the effect of drought stress and salinity can be attributed to the reduction of water sucking by seeds. If water sucking by the seed disordered, metabolic activity of germination decreased, consequently the period in which radicle released from the seed will be prolonged and subsequently germination rate will be reduced [2, 3]. Seeds for germination must suck enough water; solution substantial in the medium such as PEG cause the reduction of water sucking by the seed and subsequently postpone or stop the germination [17, 32]. Drought stress and water sucking limitation by the seed with effects on the transforming of seed storage and protein synthesis in embryo can be possibly the cause of the reduction of germination [2]. Salinity will affect the germination of seeds and their growth by reducing the water potential and special ions toxicity such as Na and Cl and also the reduction of nutritional ions which needed by the plant such as calcium

Corresponding Author: Mehrab Yadegari, Department of Agronomy and Medicinal Plants, Faculty of Agriculture, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.
Tel: +98 9133814318; Fax: +98 3813361093; E-mail: mehrabyadegari@gmail.com

and potassium [11]. Several studies showed that germination rate and percentage reduced by the increase of salinity [10,14, 21,22,24, 25, 26, 29].

The plants in environmental stresses like drought, salinity, and heat store adjusting osmotic substantial in contrast with the stresses. Pressures adjusting osmotic substantial mostly consisting of amino acids, sugar and some of mineral ions and hormones. Proline is one of the active amino acids in osmotic adjustment phenomenon that plays a key role in the creation and preserving the osmotic pressure [13, 27, 30]. Proline is a soluble amino acid in water that its amount will be increased in free stress conditions. Increase of this amino acid is the indicator of plant's resistance against stress condition. Proline protect proteins and cell membrane from the damage of ions intensive density. The medical plants are one of the natural recourses of Iran that with scientific recognition, sowing, development and correct beneficiary can play an important role in society health, employment and non-oil exports. *Calendula officinalis* L., also known marigold is annual herb [8]. It grows wild in the southern, eastern and central Europe [12, 16, 19]. It is usually multi stemmed with a strong tap root. The vegetative parts of the plant are mid green while the stems are angular and covered in fine hairs. The composite flowers could be yellow or orange, which blossom in the spring-summer seasons. Marigold is cultivated for its flowers with receptacle or flowers without receptacle [31] which are used as the medical raw material. The flower contains essential oils which are used for high blood-fat and treatment of inflammation intestine organs. Flavonoids, carotenoids and polyphenols are the active ingredient contents reported previously for *C.officinalis* flowers [18, 23]. The most essence in this plant is formed at full blooming [0.97%] and the least of essence [0.13%] produced before flowering [7, 33].

MATERIAL AND METHODS

In order to examine the effect of drought and salinity stress on germination indexes and changes of proline amount in marigold, two experiments conducted in complete randomized design with three replication in the Islamic Azad University Branch of Ahwaz Laboratory. The drought stress experiment was conducted in 6 levels with osmotic pressure 0, -0.2, -0.4, -0.6, -0.8 and -1 MPa[Michael Coffman method] and with using PEG [Poly Ethylene Glycol 6000]. The salinity stress experiment was conducted in 5 levels with osmotic pressure 0, -0.2, -0.4, -0.6 and -0.8 MPa by using NaCl on seeds of marigold. Seeds of *C.officinalis* var Qazvin, obtained from the PakanBazr Company, Isfahan, Iran. The seeds at first are sterilized with hypo chloride sodium %3 in 2 minutes and then washed by 3 times superficially with distilled water and 50 seeds transferred to glass Petri dish with 10 cm diameter and for the duration of the experiment 5ml solution with different levels was added to each Petri dish and for 14 days were transferred to a 20±1 ºC temperature with darkness condition and the number of geminated seeds in each day was counted and recorded.

After the termination of germination term, below indexes were measured in both drought and salinity conditions: germination rate, germination percentage, mean time to germination, normal seedling percentage, duration to 5 [D05], 10 [D10], 20 [D20] percent germination rootled and shoot length, the seed vigor and the dry weight of seedling was measured, and in a larger amount were sowed in tray proline index was conducted based on Bates et al methods [5].

All data were subjected to ANOVA using the statistical computer package SAS and treatment means separated using Duncan‘s multiple range test at P<0.05 level.

Results:

The effect of salinity stress on germination indexes of marigold seed:

According to the results of variance analysis it was observed that the effect of different levels of salinity stress on all traits of marigold seed germination were significant in a probable 1% level [table 1]. This matter shows the difference between different levels of salinity stress in different germination indexes such as germination percentage, germination rate, mean time to germination, normal seedling percentage, rootled and shoot length, seed vigor and the dry weight of seedling, duration to 5 [D05], 10 [D10], 20 [D20] percent germination and proline. In different plants also it is reported that salinity stress has a significant effect on germination indexes [4,22, 28, 29].

Table 1: Analysis of Variance of the effect of different levels of salinity stress on seeds of marigold germination indexes. Gp: germination percentage. GR: germination rate. MTG: mean time of germination. NSP: normal seedling percentage. Sl: shoot length. Rl: rootled length. SVI: seed vigor. SDW: dry weight of seedling. T05: duration to 5 percent germination. T10: duration to the start of germination. T20: duration to 20 percent germination.

S.O.V	df	GP	GR	MTG	NSP	SL	RL	SVI	SDW	T05	T10	T20	Prolin
Salinity	4	1930.26**	59.84**	1.29**	2849.73**	10.83**	35.81**	810879.22**	0.002**	4118.54**	6873.76**	24259.73**	0.04**
Error	10	10.66	0.32	0.19	9.6	0.13	0.18	1394.18	0.00001	81.25	296.83	364	0.004
C.V %	-	5.81	7.39	6.01	6.41	12.58	7.37	6.74	8.4	24.55	28.23	27364	15.09

**significant at 1% probability level.

The results of mean comparison of the effect of different salinity levels on marigold seed germination indexes showed that with the increase in different levels of salinity stress, germination indexes such as germination percentage, germination rate, normal seedling percentage, rootled length, shoot length, seed vigor and seedling dry weight were significantly reduced, but mean time to germination, duration to 5 percent germination [D05], duration to the start of germination [D10] and the duration to 20 percent germination [D20] and proline were significantly increased [table 2].

The highest germination percentage with the means of 84.67 %, germination rate with the means of 13.2 seed in day, normal seedling percentage with the means of 82%, shoot length with the means of 5.7cm, rootled length with the means of 9.67cm and seedling dry weight with the means of 0.075 gr were related to control condition.

The lowest mean time to germination, the period of reaching to 5, 10 and 20 percent of germination and proline were respectively with the means of 7.9, 7.85, 15.71, 53.33 hr., and 0.32 were observed in free stress condition [table 2]. Salinity resulted from the reduction of water potential and the toxicity of special ions such as sodium and Cl and also reduced of nutritional ions which is needed by the plant such as Cl and potassium affected the germination and germination rate of seeds [11, 30]. Maybe the reduction in the germination rate in the levels above the salinity stress is the reduction in water potential and consequently reduction in the rate of sucking water and the toxic effect of sodium ion. So that the seeds will suck water with a lower rate and the metabolic activities of seed will become slower and consequently cell development and root release will become slower and germination rate will be reduced. The reduction in normal seedling percentage with the increase of salinity stress levels can be because of the toxic effect of ions that will influence the normal seedling growth and in this way results in the reduction in the normal seedling percentage. Since the increase in stress level, the metabolic activities for increasing the division activity and cell development reduced, it is possible that the reason for the reduction in the rootled and shoot growth is the reduction in cell division in stress condition. Seed vigor is an index of germination that is the product normal seedling percentage and seedling length or seedling dry weight. In this experiment the seed vigor index was produced from normal seedling percentage in seed vigor [4]. Hence, the seed vigor with the reduction of normal seedling percentage and seedling length with the increase in salinity stress reduced. The reason for the reduction of seedling dry weight with the increase in salinity stress is the reduction in seedling growth. Generally with the increase in salinity stress the power for sucking water with seeds reduced and the needed time for sucking water increased and consequently the start of germination processes reduced and also there was a disorder it and the time to the start of germination increased. Also with the increase in stress the duration of the start of germination reduced [29]. Other reports also mentioned that salinity stress will cause a reduction in germination indexes in many of the plants [6; 32, 34].

Table 2: Mean Comparisons of the effects of different levels of salinity stress on marigold seed germination indexes. Gp: germination percentage. GR: germination rate. MTG: mean time of germination NSP: normal seedling percentage. SL: shoot length. Rl: rootled length. SVI: seed vigor. SDW: dry weight of seedling. T05: duration to 5 percent germination. T10: duration to the start of germination. T20: duration to 20 percent germination

Salinity Mpa	GP	GR	MTG	NSP	SL	RL	SVI	SDW	T05	T10	T20	Prolin
0	84.67^{a}	13.2^{a}	7.09^{d}	82^{a}	5.7^{a}	9.67^{a}	1260.07^{a}	0.075^{a}	7.85^{e}	15.71^{e}	53.33^{e}	0.32^{e}
-0.2	76^{b}	11.07^{b}	7.19^{cd}	72^{b}	3.97^{b}	8.6^{b}	904.47^{b}	0.063^{b}	13.04^{d}	36^{d}	88.67^{d}	0.36^{de}
-0.4	58^{c}	7.27^{c}	8.06^{b}	51.33^{c}	2.37^{c}	5.87^{c}	422.87^{c}	0.044^{c}	20^{c}	42.67^{c}	156^{c}	0.38^{c}
-0.6	38^{d}	2.93^{d}	8.33^{ab}	29.33^{d}	1.93^{d}	3.7^{d}	165.2^{d}	0.033^{d}	44.67^{b}	72^{b}	162.67^{b}	0.48^{b}
-0.8	24^{e}	1.7^{e}	8.65^{a}	6.67^{e}	0.83^{e}	1.27^{e}	14.13^{e}	0.008^{e}	98^{a}	138.67^{a}	288^{a}	0.62^{a}

The effects of drought stress on germination index in seeds:

According to the results, we observed that the effects of different levels of drought stress on all of the traits of the germination of the seeds were significantly [in a probable %1 level- table 3]. It was also reported in different plants that drought stress was significantly effective on germination indexes [2, 10,14].

Table 3: variance analysis of the effect of different levels of drought stress on marigold seed germination indexes. Gp: germination percentage. GR: germination rate. MTG: mean time of germination. NSP: normal seedling percentage. SL: shoot length. Rl: rootled length. SVI: seed vigor. SDW: dry weight of seedling. T05: duration to 5 percent germination. T10: duration to the start of germination. T20: duration to 20 percent germination.

S.O.V	df	GP	GR	MTG	NSP	SL	RL	SVI	SDW	T05	T10	T20	Prolin
Drought	5	1993.06**	71.36**	0.73**	2490.48**	8.9**	33.27**	758829.56**	0.002**	2268.01**	8147.27**	29455.82**	0.35**
Error	12	6.88	0.27	0.21	10.44	0.2	0.09	2374.9	0.000006	25.67	208.03	293.33	0.006
C.V %	-	4.63	6.03	6.58	7.19	11.75	4.06	7.71	4.62	19.21	25.82	13.35	11.07

**significant at 1% probability level.

The results of mean comparison of the effect of different levels of drought stress on seeds of marigold germination indexes showed that with the increase in different levels of drought stress of germination indexes such as germination percentage, germination rate, normal seedling percentage, rootled length, shoot length, the seed vigor and seedling dry weight were significantly reduced, but mean time to germination, duration to 5 percent germination [T05], duration to the start of germination [D10] and duration to 20 percent germination [D20] and proline were significantly increased [table 4]. The highest germination percentage with the means of 84.67 % in the control condition and the lowest germination percentage with the mean of 23.33 in the drought stress condition -1 MPa was observed [table 4]. The highest germination rate with the mean of 13.2 in day, normal seedling percentage 82 %, shoot length with the mean of 5.7 cm, rootled length with the mean of 9.67 cm, seed vigor with the mean of 1260.7 and seedling dry weight with the mean of 0.075 were respectively related to control condition [table 4]. The lowest mean time to germination, period of reaching to 5, 10 and 20 percent of germination and proline were respectively with the means of 67, 27 hr., and 0.77 were observed in control condition [table 2]. With the increase in different levels of drought stress, seed accessing to water was reduced and the activity of the enzymes which are responsible for changing in germination also was reduced and in this way it is possible that the germination percentage will be reduced [2 , 32]. With the increase in different levels of drought stress, seed accessing to water was reduced and the activity of the enzymes which are responsible for changing in germination also was reduced and in this way it is possible that the germination percentage will be reduced.

The reduction in germination rate can be related of the reduction in water sucking and the increase in the duration of root release. Mayer and Poljakoff [16] reported that the cause of germination rate and the creation of abnormal seedling is the lack of enough energy for starting the related process of germination. If the water sucking by seed disordered or if sucking decreased, metabolic activities of germination inside the seed will be done slowly and consequently the duration of radicle release from the seed will be increased and so germination rate will be reduced [9]. Maybe the reduction in the normal seedling percentage is because of water shortage in an intensive stress condition that in which the seedling has not the ability to growth normally. Reduction in the normal seedling percentage under the stress condition was reported by Ansari et al. [3]. With the increase of negative potential of water the rootled length significantly reduced. In the drought stress

s condition the reduction of water sucking by the seed caused in the reduction in metabolic activities of seed, the reduction of hormone release and enzymes activities and consequently will cause disorder in seedling growth. The more seed vigor is greater it means that the seed has more power in growth under the stress condition. It is showed that seed vigor in different plants will be reduced with the increase in different levels of drought stress and the reason for this reduction in normal seedling percentage and seedling length is specified [2, 3]. The reason for the reduction in seedling dry weight of wheat is the reduction of the ability for transforming nutritional substantial their transfer from cotyledon to embryonic axis that these results are similar by our observations in this research [2, 29].

Generally in stress conditions the plant will prepare its resistance to oppose this condition by increasing proline amount. The plants in different environmental conditions will synthesis solutes with low molecules weight that is generally called compatible solutes. These compatible solutes consist of acid amine [proline, glycine], sugar, sugar alcohol, ions, organic acids, amides, amines, betaine groups that are synthesis in reaction to stress and do not interfere with normal biochemical reactions of cell.

Slama et al. [27] mentioned that the amount of the proline of grass was increased three times than the control one under the effect of drought stress. It is suggested that the reason for this matter is the provocation of biosynthesis activity of proline and the control of catabolic enzyme.

Table 4: Mean Comparisons of the effects of different levels of drought stress on marigold seed germination indexes. Gp: germination percentage. GR: germination rate. MTG: mean time of germination. NSP: normal seedling percentage. SL: shoot length. Rl: rootled length. SVI: seed vigor. SDW: dry weight of seedling. T05: duration to 5 percent germination. T10: duration to the start of germination. T20: duration to 20 percent germination.

Drought Mpa	GP	GR	MTG	NSP	SL	RL	SVI	SDW	T05	T10	T20	Prolin
0	84.67^{a}	13.23^{b}	7.09^{de}	82^{a}	5.7^{a}	9.67^{d}	1260.06^{a}	0.075^{ab}	7.85^{e}	15.71^{e}	53.33^{e}	0.41^{f}
-0.2	86^{a}	14.61^{a}	6.42^{f}	70.66^{b}	5.5^{a}	10.77^{a}	1149.67^{b}	0.08^{a}	6.67^{f}	13.33^{f}	44^{f}	0.47^{e}
-0.4	62^{b}	8.98^{c}	6.99^{e}	49.33^{c}	4.3^{b}	10.07^{b}	709.47^{c}	0.071^{b}	11.33^{d}	22.67^{d}	80^{d}	0.51^{d}
-0.6	49.33^{c}	5.84^{d}	7.37^{cd}	39.33^{d}	3.4^{c}	8.13^{d}	453.33^{d}	0.052^{c}	18.33^{c}	44^{c}	97.33^{c}	0.67^{c}
-0.8	34.67^{d}	4.04^{e}	7.61^{bc}	29.33^{e}	2.8^{d}	6.03^{e}	201.53^{e}	0.037^{d}	36^{b}	100^{b}	198.67^{b}	0.83^{b}
-1	23.33^{e}	1.72^{f}	8.19^{a}	5.66^{f}	1.17^{e}	1.9^{f}	16.33^{f}	0.01^{e}	78^{a}	139.33^{a}	296^{a}	1.33^{a}

Conclusion:

The results showed that the drought and salinity stress caused the reduction in germination percentage, germination rate, normal seedling percentage, rootled and shoot length, seed vigor and seedling dry weight, and increase in the reaching to 5 percent germination [D05], the duration to 10% of germination [D10], the duration to 20 percent germination [D20], proline and the mean time of germination. The reduction in germination indexes can be attributed to the increase of proline amount in the condition of high levels of drought and salinity stress. The seed vigor index is related to the normal seedling percentage and seedling length and the reason of the reduction in seed vigor is because of the reduction in rootled and shoot length and also the reduction in normal seedling percentage under high stress level condition. The reduction in seedling growth also will cause in the reduction in seedling dry weight. The produced seedling under the drought and salinity stress condition increased their proline amount in order to prevent from the intensive damage which can be a defensive mechanism against stress condition.

REFERENCES

[1] Alam, M.Z.,T. Southbury, R.E.L. Naylor and M.A. Rashid, 2003.Water uptake and germination pattern of rice seeds under iso-osmotic solutions of Nacl and PEG and different concentration of CaCl and combination of Nacl and CaCl .Pakistan Journal of Biological Science, 6(12): 1059-1066.

[2] Ansari, O., H.R. Choghazardi, F. Sharif Zadeh and H. Nazarli, 2012. Seed reserve utilization and seedling growth of treated seeds of mountain rye [*Secalemontanum*] as affected by drought stress. CercetăriAgronomiceîn Moldova, 2(150): 43-48.
[3] Ansari, A., R. TavakolAfshari, F. Sharifzadeh and A. Shaianfar, 2013.Consumption of storage materials and germination of mountain rye [*Secalemontanum*] under salinity stress and priming. Journal of Plant Science, 44(2): 181-189. [In Persian].
[4] Ansari, O., M.S. Azadi, F. Sharifzadeh and E. Younesi. 2013. Effect of hormone priming on germination characteristics and enzyme activity of mountain rye [*Secalemontanum*] seeds under drought stress conditions. Journal of Stress Physiology & Biochemistry, 9(3): 61-71.
[5] Bates, L.S., R.P. Waldern and I.D. Teave, 1973. Rapid determination of free proline for water stress studies. Plantand Soil, 39: 205-207.
[6] Bybordi, A. and J. Tabatabaei, 2009. Effect of salinity stress on germination and seedling properties in canola cultivars [*Brassica napus*L.]. Not Bot Hort Agro bot Cluj, 37(2): 71-76.
[7] Carreon, S.P., G.C. Iimenez and J.L. Vega, 2002.Genotoxic and antigenotoxic properties of *Calendula officinalis*extracts in rat liver cell cultures treated with diethylnitrosamine. Toxicology inVitro, 16: 235-258.
[8] Danielski, L., L.M.A.S. Campos., L.F.V. Bresciani, 2007. Marigold [*Calendula officinalis*L.] oleoresin: Solubility in SC-CO_2 and composition profile.Chemistry Engineering Procedures, 46: 99-106.
[9] De, F. and R.K. Kar, 1994. Seed germination and seedling growth of Mungbean [*Vignaradiate* L.] under water stress induced by PEG-6000. Seed Science and Technology, 23: 301-304.
[10] Kaya, M.D., G. Okcu, M. Atak, Y. Cıkılı and O. Kolsarıcı,2006. Seed treatments to overcome salt and drought stress during germination in sunflower [*Helianthus annuus*L.]. Eur. J. Agron, 24: 291-295.
[11] Khan, M.A. and S. Gulzar. 2003. Germination responses of Sporobolusioclados: A saline desert grass. Journal of Arid Environments, 55: 453–464.
[12] Lucia, H.D., B.L. Karina and D.A. Janete, 2009. Topical *Calendula officinalis* L. successfully treated exfoliatedchelates: a case report Cases Journal, 2: 9077.
[13] Lutts, S., V. Majerus and J.M. Kinet. 1999. NaCl effect on proline metabolism in rice seedlings. PhysiologiaPlantarum, 105: 450-458.
[14] Macar, T.K., O. Turan and Y. Ekmekci. 2009.Effects of water deficit induced by PEG and NaCl on chickpea [*Cicerarietinum*L.] cultivars and lines at early seedling stages. G.U. Journal of Science, 22: 5-14.
[15] Martin, R.J. and B. Deo. 2000. Effect of plant population on *Calendula officinalis* flower production. New Zealand J. of Crop and Horticulture Science, 28(1): 37-44.
[16] Mayer, A.M. and A. Poljakoff-Mayber, 1989. The germination of seeds. 4ed. Oxford: Pergamum Press.
[17] Mohammed, El, M., M. Benbella and A. Talouizete. 2002. Effect of sodium chloride on sunflower [*Helianthus annuus*L.] seed germination. Helia, 25(3)]: 51-58.
[18] Mrda, J., B. Marinković and G. Jaćimović. 2007. Effect of row spacing on calendula [*Calendula officinalis* L.] flowers production. International Symposium “Trends in European Agriculture Development”, Scientific Papers Faculty of Agriculture, 39(1): 77-82.
[19] Noor, E., F.M. Azhar and A.L. Khan, 2001. Differences in responses of *Gossypiumhirsutum* L. varieties to NaCl salinity at seedling stage. International Journal of Agricultural Biology, 3[4]: 345-347.
[20] Okoh, O.O., A.P. Sadimenko, O.T. Asekun and A.J. Afolayan, 2008.The effects of drying on the chemical components of essential oils of *Calendula officinalis*L. African Journal of Biotechnology, 7(10): 1500-1502.
[21] Parida, A.K., V.S. Dagaonkar, M.S. Phalak and L.P. Aurangabadkar, 2008. Differential response of the enzymes involved in proline biosynthesis and degradation in drought tolerant and sensitive cotton genotypes during drought stress and recovery. ActaPhysiologiaePlantarum, 30: 619-627.
[22] Patade, V.Y., K. Maya and A. Zakwan. 2011. Seed priming mediated germination improvement and tolerance to subsequent exposure to cold and salt stress in capsicum. Research Journal Seed Science, 4[3]: 125-136.
[23] Re, T.A., D. Mooney, E. Antignac, E. Dufour and G. Nohynekn, 2009. Application of the threshold of toxicological concern approach for the safety evaluation of calendula flower [*Calendula officinalis*L.] petals and extracts used in cosmetic and personal care products. Food Chemistry Toxicology, 47: 1246-1254.
[24] Reddy, B.V.S., P. Sanjana Reddy, F. Bidinger and M. Blummel. 2003. Crop management factors influencing yield and quality of residues. Field Crops Research, 84: 57-77.
[25] Saki Nejad, T., 2011. Effect of drought stress on stomata resistance changes in corn. Journal of American Science, 7(9): 27-31.
[26] Shahid, M., M.A. Pervez and M.Y. Ashraf, 2011. Characterization of salt tolerant and salt sensitive pea [*Pisumsativum*L.] genotypes under saline regime. Pak Journal life Science, 9-2.
[27] Slama, I., D. Messedi.,T. Ghanaya., A. Savoure and C. Abdelly. 2005.Effects of water deficit on growth and proline metabolism in *Sesuviumportulacastrum*. Environmental and Experimental Botany, 56: 231-238.

[28] Soltani, A., M. Gholipoor and E. Zeinali, 2006 - Seed reserve utilization and seedling growth of wheat as affected by drought and salinity. Environmental and Experimental Botany, 55: 195-200.
[29] Soltani, A., S. Galeshi, E. Zenali and N. Latifi. 2001. Germination seed reserve utilization and growth of chickpea as affected by salinity and seed size. Seed Science and Technology, 30: 51-60.
[30] Sudhakar, P.R., M.P. Reddy and K.Veeranjaneyulu, 1993. Effect of salt stress on the enzymes of proline synthesis and oxidation in green seedling. Journal of Plant Physiology, 141: 621-623.
[31] Varban, D., M. Marcel., R.Varban and S. Muntean, 2008. The influence of the nutrition space upon the production at *Calendula officinalis* L. specie, in the condition of Cluj-Napoca. Scientific Papers Faculty of Agriculture, 40(1): 235-238.
[32] Yadegari, M., 2014. Study of ratio and speed germination of twelve medicinal plants under several treatments of salinity. Advances in Environmental Biology, 8(2): 425-430.
[33] Yadegari, M., 2013. Foliar Application of Fe, Cu, Mn and B on growth, yield, and essential oil yield of marigold [*Calendula officinalis*]. Journal of Applied Science and Agriculture, 8[5]: 559-567.
[34] Yildirim, E. and I. Guvenc, 2006. Salt tolerance of pepper cultivars during germination and seedling growth. Turkish Journal of Agriculture and Forestry, 30: 347-353.
[35] Al-Tawaha, A., G. Al-Karaki, A. Massadeh, 2013. Comparative response of essential oil composition, antioxidant activity and phenolic contents spearmint [Mentha spicata L.] under protected soilless vs. open field conditions, Advances in Environmental Biology, 7(5): 902-910.
[36] Al-Tawaha, A., G. Al-Karaki, A. Massadeh, 2013.. Antioxidant activity, total phenols and variation of chemical composition from essential oil in sage [Salvia officinalis L.] grown under protected soilless condition and open field conditions. Advances in Environmental Biology, 7(5): 894-901
[37] Al-Tawaha, A., G. Al-Karaki, A. Massadeh, 2014. Variation of Chemical composition, antioxidant and total phenols of essential from thyme [Origanum syriacum L.] grown under open field conditions and protected soilless condition. American-Eurasian Journal of Sustainable Agriculture, 8(12): 20-26.