Research Article

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Effect of salt stress on germination and some growth parameters of marigold (*Calendula officinalis* L.)

M. E. Torbaghan*.

Northern Khorasan of Agriculture and Natural Resource Research Center. Khorasan **Iran**.
***Corresponding author**

Marigold (*Calendula officinalis* L.) was cultivated so long as ornamental plant until its medicinal properties was known and it was used as medicinal plant. Marigold type fruit is achene. It has type variation and it sees as three types: carenate, alate and orbicular. Despite of the abundant resources in salinity, little information exists on the effects of salinity on marigold. Thus, the great need exist for detailed understanding reaction of this important economic plant to salt stress conditions. To study tolerance of three type seeds of marigold (*Calendula officinalis* L.) to salinity stress, a laboratory experiment was conducted in Completely Randomize Design Factorial with three replications in North Khorasan Agricultural and Natural Resource Research Center. The main factor was salinity with six levels (0, 1, 3, 5, 7 and 10 dS/m) and sub factor was three types of marigold seed (carenate, alate and orbicular). Results of experiment showed that the germination percent and germination rate decreased with increasing in salinity. Cinarenate had more germination rate than two other types of marigold seeds in all salinity levels. Alate had also more germination rate than orbicular in low salinity levels (0, 1 and 3 dS/m). Salinity decreased radicle and plumule length in all three type seed. Maximum of plumule and radicle length was observed in orbicular and carenate seeds respectively. Plumule wet weight was max in medium salinity levels (3 and 5 dS/m). But, radicle wet weight decreased with salinity increasing. Minimum decreasing in radicle wet weight and maximum salinity tolerant under effect of seed type was observed in carenate seed. Maximum sensitivity to salinity stress was also observed in orbicular marigold seed. Maximum and minimum of radicle to plumule length ratio was observed in 5 and 10 salinity levels respectively and carenate seed of marigold under effect of seed type .

Key words: ***Salinity, seed types (carenate, alate and orbicular), Marigold (Calendula officinalis L.)***

Production of medicinal plant was equivalent to 34084.5 tons in 2001 in Iran. It has been obtained from 81749.1 ha cultivation. Maximum production of medicinal plants relate to khorasan province was 10028.8 tons that it has had 29.4% of total production in the country (Bi-Name, 2001). According to 1999 to 2006 statistics, marigold cultivation was approximately 12.7 ha and the amount of dried flowers production was 10.9 tons that the average yield was 858 Kg/ha (Bi-Name, 2001; Ebrahimi, 2008).

Marigold (*Calendula officinalis* L.) was cultivated as ornamental plant so long until its medicinal properties was recognized and was used as medicinal plant.

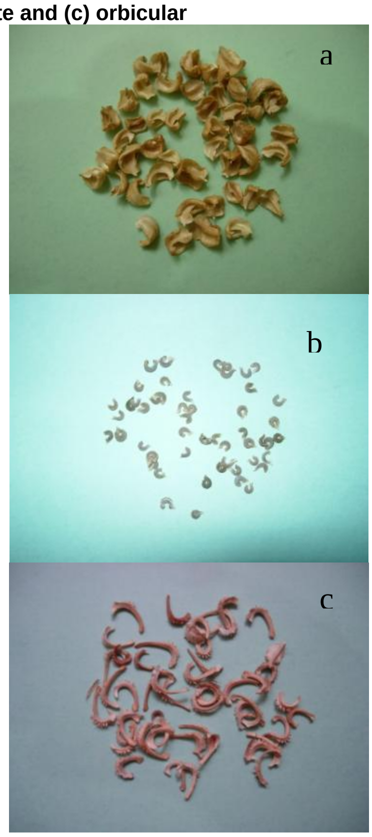
Current affiliation:
Ph.D Student at Ferdowsi University of Mashhad, **Iran**.

Calendula officinalis L. with English name as "pot marigold" is herbaceous plant from *Asteraceae* (*Compositae*) that originates have been reported Mediterranean and West Asia. Origin of this plant has been also mentioned Southern Europe and East Mediterranean (Omid Beygi, 2005; Borm and Van Dijk, 1994; Cromack et al. 1993; Earle et al. 1964; Meizer zu Beerentrup et al. 1987). The name of *Calendula officinalis* L. comes from Latin word (Calend) meaning first day of each month because this plant has a long flowering period. Since the flowers move in the sun radiation, it has been come as sun sing in astronomical texts (Dinda and Craker, 1998). Marigold species had six annual herbaceous plant species in Iran. *C. aurantica* is exclusive to Iran and *C. alata, C. palaestina*, *C.persica*, *C.sancta* and *C. tripterocarpa* species in addition to Iran also grow in Palesrine, Iraq, Saudi

Arabia, Egypt , Libya, Tunisia, Algeria, Morocco, Canary Islands, southern Spain Turkmenistan, Afghanistan, Pakistan and Kashmir, (Mozafariyan, 2003).

Usage of marigold flower is including medicinal (treatment of gastric and intestinal diseases, skin ulcers treatment and anti-inflammatory), cosmetics (supplying various creams) and food (food coloring such as cheese and butter). Also, its seed oil has pharmaceutical and an industrial application (Bernath, 2000; Delia Loggia et al. 1994 and Dinda and Craker, 1998) Marigold has anti fungi and antiviral properties (Mardani Nejad et al. 2003). Recently, evidences of antiviral effects of HIV have been found on the extract flowers of this plant (Kalvatchev et al. 1997). Marigold fruit is seen as achene type with three different type variation alate (Figure. 1), carenate (Figure. 2) and orbicular (Figure. 3) (Cromack. and Smith, 1998).Seed color is grey or light brown. Seeds have suitable vigor for 5 to 6 years (Dinda and Craker, 1998) and in the suitable climatic condition for planting, germinate after 4 to 5 days. Weight of 1000 seed is 8 to 12 gram (Omid Beygi, 2005).

Figure 1. Three types of seed marigold (a) alate, (b) carenate and (c) orbicular



Germination percent in well seeds is 60 to 80% in laboratory conditions and emergence amount is 40 to 60% in filed (Omid Beygi, 2005; Borm andVan Dijk, 1994; Forment et al. 2001).

Medicinal plants including marigold are reach sources of secondary metabolites that means they are basic ingredients of many medicine. Although, these materials are originally made by genetic processes directly. But, making them are clearly under effect of environmental factors, so that environmental factors cause changes in medicinal plants growth and quantity and quality of their ingredients such as alkaloids, glycoside, steroids, volatile oil (essential oil) and etc (Sinebo et al. 2004). Planting economically of a medicinal plant is affordable when its secondary metabolites production is reached to optimal level. With understanding of effective environmental factors in production and selection of appropriate plant varieties (Omid Beygi, 1998; Omid Beygi, 1999) can achieve maximum production (Bi-Name, 2001).

Salinity stress is one of the most extensive damage stresses in arid and semi arid region. But, it exists in semi humid region and costal lands (Iran-Nejad and Shahbazian, 2005).

Salinity problem has been recognized in many years ago and some studies have been also done on it. Scientists often study physiological effects of salinity in plants and of reclamation saline soil (Iran-Nejad and Shahbazian, 2005). The first thing should be examined by each crop plant breeder is natural variety in plant tolerance to salinity and that wild and farm resources are available separately. Many changes can be done in crop tolerance to salinity. Germination seed in saline culture media is the most common method. Salinity effect on germination rate is more than total germination percent. Salinity decrease roots length growth more than shoots. But, this matter is reverse in some plants (Iran-Nejad and Shahbazian, 2005). One of the pre selecting methods in salinity resistance or sensitive to salinity is seed germination in saline solution and observing total seed germination percent and germination rate, and the amount of plumule or radicle growth (Iran-Nejad and Shahbazian, 2005).

Despite of the abundant resources in salinity, little information are available in the salinity effects on marigold. Thus, the great need for detailed understanding of this important economic plant to salinity stress condition exists. Chaparzadeh et al. (2003) studied the effects of anti-oxidative reactions of marigold under salinity conditions. Results of experiment indicated that the most physiological marigold (*Calendula officinalis* L.) parameters were affected by salinity. Total of plant growth, lipid per oxidation and accumulation of hydrogen peroxide were a lot of affected by high salinity conditions and were reduced. Shoots were affected by salinity level more than roots, except of 1000 Mm NaCl salinity that roots growth reduced more than shoots growth.

Abbaspour (2001) studied marigold (*Calendula officinalis* L.) flovonides changes under salinity conditions. In this study, the impact of six NaCl treatments (0, 25, 50, 75, 100 and 150 Mm) was investigated on intensity of photosynthesis, breathing intensity, CO_2 point offset, content chlorophylls, the carotenoids, soluble and insoluble sugars, proteins, proline and robinin and quercetin flavonoids . Results of experiment showed that the intensity of photosynthesis decreased with salinity increasing, but, breathing intensity and CO_2 point offset increased. Also, a and b content chlorophyll decreased in salinity treatments comparing to control. Carotene amount also decreased in all five levels of salinity, but, xanthophyll increased in low salinity and decreased in high salinity levels. Also, in all salinity levels, soluble and insoluble sugars amount of root and leaf increased and decreased, respectively. Quercetin and robinin flavonoids increased both in root, young and old leaves that this increasing was not statistically significant in root but, it was significant in young and old leaves. Also it was determined the amount of quercetin and routine flavonoids was higher in young leaves than the old leaves and root.

The effects of saline irrigation water investigated on yield (fresh and dry weights of flower heads) of *Calendula officinalis* L., essential oil (EO) yield, chemical constituents of the EO and total flavonoids and carotenoids flower heads at three flowering stages, i.e. initial (21 days after bud formation (DABF)), full flowering (81 DABF) and final (111 DABF) (Khalid and Teixeira da Silva, 2010). After plants were treated with different levels of saline irrigation water (0.39, 1.56, 3.13, 4.69, 6.25, 7.81 and 9.38 dS m^{-1}) consisting of NaCl, $CaCl_2$ and $MgCl_2$ salts, the flower head yield and pigment (total flavonoids and carotenoids) content were significantly reduced. Irrigation with saline water increased the EO content and its main components (α-cadinol, γ- and Δ-cadinene). Fresh and dry weights of flower heads and EO increased near 81 DABF while the content of pigments increased by 111 DABF. Saline irrigation water decreased the fresh and dry weights of flower heads, and pigment contents (total flavonoids and total carotenoids) but increased essential oil yield and its main components (α-cadinol, γ- and Δ-cadinene) of *Calendula officinalis* L. Fresh and dry weights of flower heads and essential oil increased towards the full bloom stage of flowering while pigment content, such as total flavonoids and total carotenoids, increased (Khalid and Teixeira da Silva, 2010).

To gain a better insight into long-term salt-induced oxidative stress, some physiological parameters in marigold (*Calendula officinalis* L.) under 0, 50 and 100 mM NaCl were investigated (Chaparzadeh, 2004). Salinity affected most of the considered parameters. High salinity caused reduction in growth parameters, lipid peroxidation and hydrogen peroxide accumulation. Under high salinity stress, a decrease in total glutathione and an increase in total ascorbate (AsA + DHA), accompanied with enhanced glutathione reductase (GR, EC 1.6.4.2) and ascorbate peroxidase (APX, EC 1.11.1.11) activities, were observed in leaves. In addition, salinity induced a decrease in superoxide dismutase (SOD, EC 1.15.1.1) and peroxidase (POX, EC 1.11.1.7) activities. The decrease in dehydroascorbate reductase (DHAR, EC 1.8.5.1) and monodehydroascorbate reductase (MDHAR, EC 1.6.5.4) activities suggests that other mechanisms play a major role in the regeneration of reduced ascorbate. The changes in catalase (CAT, EC 1.11.1.6) activities, both in roots and in leaves, may be important in H_2O_2 homeostasis (Chaparzadeh, 2004).

This research were conducted to study of salt stress on germination and some growth parameters of three types (carenate, alate and orbicular) of marigold (*Calendula officinalis* L.) under laboratory conditions.

MATERIALS AND METHODS

To study of three types of *Calendula officinalis* L. tolerance to salinity stress in laboratory conditions, a factorial experiment was conducted based on completely randomized design with three replications in the laboratory of Agriculture and Natural Resources Research Center, North Khorasan. Experimental main factors, were including salinity as main factor at six levels 0, 1, 3, 5, 7 and 10 dS/m sodium chloride and three types of marigold seed (carenate, alate and orbicular) as sub factor. For this purpose, 54 petri dishes were prepared by disinfecting. Sterling petri dishes were done in the oven at 110 °C temperature and in 24 hours. Marigold seeds were disinfected with 10 percent of sodium hypochlorite and 2 per 1000 Benomile fungicides respectively with 30 seconds for each them. Seeds were washed with sterile distilled water, after each stage. 25 sterile marigold seeds from each three types (carenate, alate and orbicular) were exposure in each petri dish between filter paper and then they were treated with sodium chloride solution (1, 3, 5, 7 and 10 dS/m) and distilled water (control). For preparation of salt solution, laboratory NaCl and distilled water was used. Then the petri dishs were transferred to the incubator with the temperature 25 ± 1 ° C for 20 days for measuring germination. The first stage of germination percent counting began after 48 hours. In order to keep potential solutions constant, the solutions were changed once every three days in each petri dish. Petri were checked every day and the number of germinated seeds was recorded. After 20 days passed from the first day of experiment radicle length mean, plumule length mean, plumule and radicle wet weight,radicle to plumule length ratio and radicle to plumule wet weight was measured and then they were posed in the oven for 48 hours in 60 ºC for dry weight measuring .Marigold seeds germination rate (RG) was calculated by the following formula (MaGuire, 1962).

$$R.G. = \sum_i \frac{M}{D}$$

In the above formula M is the number of germinated seeds in i day and D is the number of days passed from the beginning of the experiment. The data were analyzed with MSTAT-C software and the mean data were compared with Duncan Multiple Range Test at the 0.05 confidence level.

RESULTS

Marigold seeds germination percent decreased under effect of salinity levels rising (Table 1). So that, maximum of germination percent was observed in control (none salinity treatment). Increasing in NaCl salinity levels caused about 83.8, 75.8, 72.5, 35.5 and 22.6 percent decreasing for 10, 7, 5, 3 and 1 dS/m treatments respectively (Table 1).

Study of marigold seeds germination rate was also shown that with salinity increasing in media culture seeds, germination rate decreased (Figure. 2). One dS/m salinity level did not show significant difference with control. But, this level had significant difference with 5, 7 and 10 dS/m salinity levels at 5% confidence level (Figure. 2). Increasing in environmental salinity level showed 83.0, 72.6, 66.1, 33.2 and 12.3 percent decrease in 10, 7, 5, 3 and 1 dS/m treatments compare than to control (Figure. 2).

Maximum of plumule length was affected by environmental salinity. Increasing about 31.5 and 22 percent was showed in 1 and 3 dS/m salinity levels respectively than to control ($P \leq 0.05$) (Table 1). Comparison of other treatments (Table 1) showed that plumule length decreased in all remained treatments than to control. Maximum decrease than to control was observed in 5 and 7 dS/m salinity levels with 39.7 and 35.7 percent than to control respectively that they had statistical significant difference compare to control (Table 1). Maximum of marigold seeds radicle length under effect of environmental salinity was observed in 3 and 1 dS/m salinity levels that they showed increasing about 6.9 and 0.38 percent than to control respectively (Table 1). Radicle length decreased in other levels (treatments) than to none salinity level (Table 1). Maximum decreasing was observed in 7, 10 and 5 dS/m salinity levels with 52.7, 47.9 and 29.1 percent respectively (Table 1). This study showed that 3 and 10 dS/m salinities allocated maximum and minimum wet plumule weight under effect of salinity levels (Figure. 3). Three dS/m salinity level had 39.7 percent increase than to control and 10 dS/m level had 82.8 percent decrease than to none salinity treatment (control) ($P \leq 0.05$) (Figure. 3). Seven dS/m treatment did not show any statistical significant difference with minimum plumule wet weight level (10 dS/m) and it had 79.1 percent decrease than to control (Figure. 3). Wet plumule weight in 5 and 1 dS/m salinities was observed with none statistical significant difference with each other and control (Figure. 3).

The effect of salinity on radicle wet weight had significant difference. Maximum of radicle wet weight was observed in 1 and 3 dS/m treatments under effect of salinity media culture respectively that they had about 71.6 and 12.3 percent increase than to control (none salinity treatment) (Figure. 4). Ten, five and seven dS/m treatments allocated minimum of radicle wet weight respectively. Reduction observed in above levels than to zero treatment (control) was

Table1. The effect of different salinity levels on germination and some growth parameters of *Calendula officinalis* L. seedling

Salinity levels	Germination percent	Plumule length (cm)	Radicle length (cm)	Dry plumule weight (g)	Dry Radicle weight (g)	Radicle to plumule length ratio	Radicle to plumule wet weight ratio	Radicle to plumule dry weirht ratio
S_0	27.56 a	1.553 c	4.183 b	0.004778 b	0.006333 b	2.751 b	0.7414 ab	1.203 b
S_1	21.33 b	2.042 a	4.199 b	0.002444 c	0.007222 a	2.251 d	0.9656 a	2.454 a
S_2	17.78 b	1.896 b	4.473 a	0.008111 a	0.00600 b	2.448 cd	0.5070 ab	0.7791 b
S_3	7.556 c	0.936 d	2.963 c	0.001333 de	0.002222 c	3.136 a	0.3745 b	1.315 b
S_4	6.667 c	0.998 d	1.978 d	0.00100 e	0.002667 c	2.678 bc	0.8880 ab	1.278 b
S_5	4.444 c	1.483 c	2.178 d	0.001667 d	0.001444 d	1.658 e	0.4974 ab	0.9444 b
P value	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0020	0.0000

S_0 = control, S_1 = 1, S_2 = 3, S_3 = 5, S_4 = 7 and S_5 = 10 dS/m NaCl
*Different letters in each column is indicated significant difference at 5% level.

Figure 2. The effect of NaCl salinity levels on germination rate of *Calendula officinalis* L.

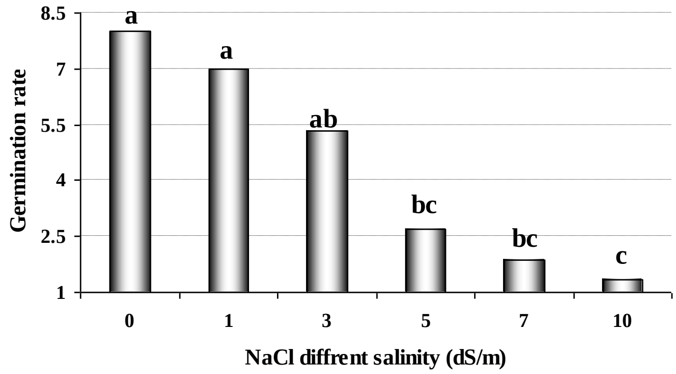
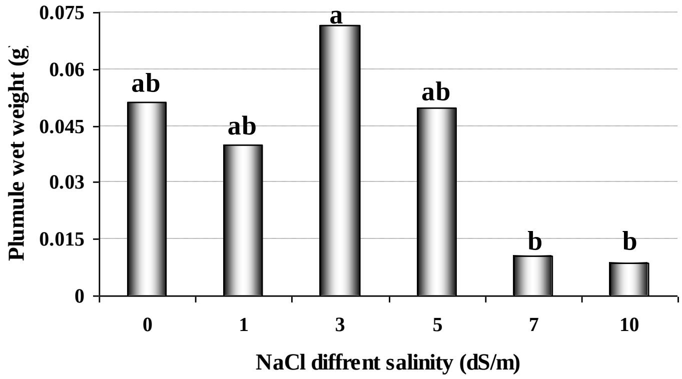
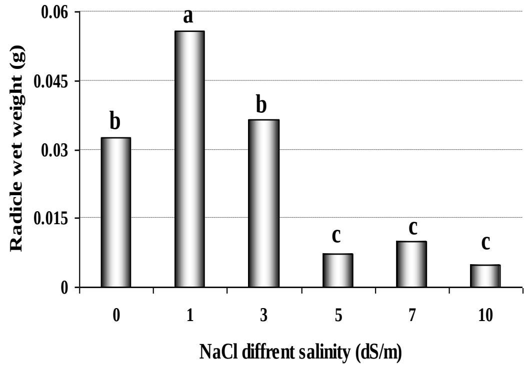


Figure 3. The effect of NaCl salinity levels on plumule wet weight of *Calendula officinalis* L. (g)



about 85.3, 77.4 and 69.2 percent respectively (Figure. 4). Maximum of plumule dry weight under effect of salinity levels was observed in 3 dS/m treatment with 69.7 percent increasing than to control (Table 1). Comparison of other treatments with control was shown about 79.0, 72.1, 65.1 and 48.8 percent decrease in 7, 5 10 and 1 dS/m levels respectively (Table 1). Observed trend for radicle dry weight under effect of environmental salinity in treatments was similar to radicle wet weighy (Table 1). One dS/m treatment had maximum radicle dry weight with statistical significant difference than to control and other treatments. Then, 3 dS/m treatment was the next with none statistical significant difference at 5% confidence level (Table 1). Other treatments decreased than to control (Table 1). Plumule dry weight rising was about 14 percent in 1 dS/m treatment and 10, 5, 7 and 3 dS/m treatments had decreasing than to control was about 77.2, 64.9, 57.8 and 5.2 percent than to control respectively (P≤0.05) (Table 1).

Figure 4: The effect of NaCl salinity levels on radicle wet weight of *Calendula officinalis* L. (g)



Comparison of radicle to plumule length ratio under effect of environmental salinity levels (Table 1) showed that 5 and 10 dS/m levels allocated maximum and minimum length ratio with 13.9 percent increase and 39.7 percent decrease respectively. Comparison of other treatments was also shown that all of the treatments decreased compare to control (none salinity treatment) (Table 1).

Radicle to plumule wet weight was in 1 and 5 dS/m treatments max and min respectively with statistical significant difference with each other (Table 1). Other salinity levels did not show any statistical significant difference with each other and both mentioned treatments (1 and 5 dS/m levels) (P≤0.05). Maximum of radicle to plumule wet weight was observed in maximum treatment (1 dS/m) with 30.2 percent increase and minimum of this ratio was shown in 5 dS/m treatment with about 49.5 percent decrease compare to control (Table 1).

Radicle to plumule dry weight ratio only had significant difference in 1 dS/m salinity with control (103.9 percent increase) and other levels. Other treatments did not show any statistical significant difference with each other (Table 1).

Study of germination percent under effect of marigold seed types showed that germination percent in orbicular type was lower than carenate and alate (Table 2). Maximum of germination rate was observed in carenate, alate and orbicular respectively (Figure. 5). Two types of marigold seeds, carenate and orbicular had statistical significant difference with each other. But, comparison of alate seed did not show any significant difference with two other types seeds (Figure. 5).

Comparison of plumule length in three types of seed showed statistical significant difference (P≤0.05) with each other (Figure. 6). Maximum of plumule length was observed in orbicular, carenate and finally in alate seed respectively (Figure. 6). Comparison of radicle length of marigold seedlings in seed types showed significant difference with each other too (Figure. 7). Maximum radicle length was observed in carenate, alate and then orbicular (Figure. 7).

Figure 5. The effect of seed types on germination rate of *Calendula officinalis* L.

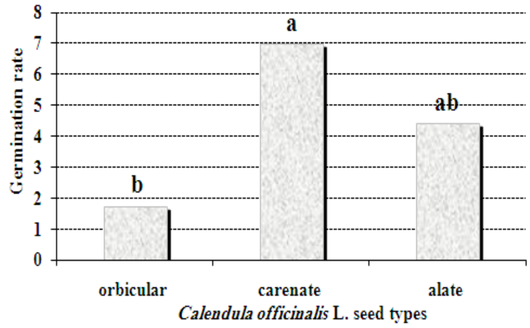


Figure 6. The effect of seed types on plumule length of *Calendula officinalis* L. (cm)

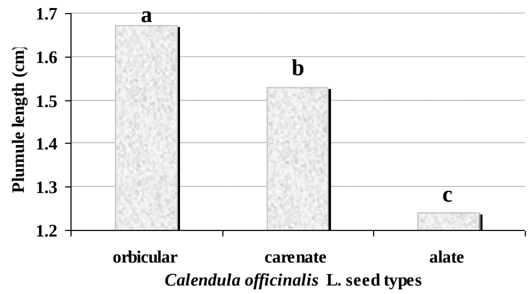
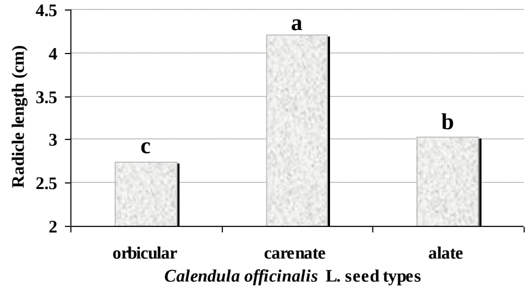


Figure 7. The effect of seed types on radicle length of *Calendula officinalis* L. (cm)



Results of experiment for plumule wet weight did not show any significant difference among marigold seed types (Table 2). But, the comparison of weights with each other showed that plumule wet weight in orbicular seed was lower than two other types' seeds carenate and alate (Table 2) showed statistical significant difference with each other. Although, maximum of radicle wet weight was observed in carenate and then alate and finally orbicular (table 2). Study of plumule and radicle dry weight of marigold seedlings (Figs 8 and 9) showed that carenate, alate and orbicular allocated maximum to

Table 2. The effect of seed types on germination and some growth parameters of *Calendula officinalis* L. seedling

Seed types	Germination percent	Wet plumule weight (g)	Wet Radicle weight (g)	Radicle to plumule length ratio	Radicle to plumule wet weight ratio	Radicle to plumule dry weirht ratio
ST_1	5.556 b	0.007667 a	0.004056 c	2.342 b	0.5938 a	0.8677 b
ST_2	19.33 a	0.05611 a	0.05178 a	2.747 a	0.9053 a	1.923 a
ST_3	17.78 a	0.05200 a	0.01750 b	2.372 b	0.4878 a	1.195 ab
P value	0.0000	0.007	0.0000	0.0000	0.0014	0.0000

ST_1= orbicular, ST_2 = carenate and ST_3= alate
*Different letters in each column is indicated significant difference at 5% level.

Figure 8. The effect of seed types on plumule dry weight of *Calendula officinalis* L. (g)

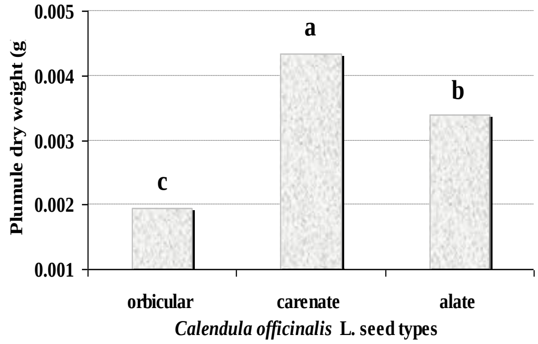


Figure 9. The effect of seed types on radicle dry weight of *Calendula officinalis* L. (g

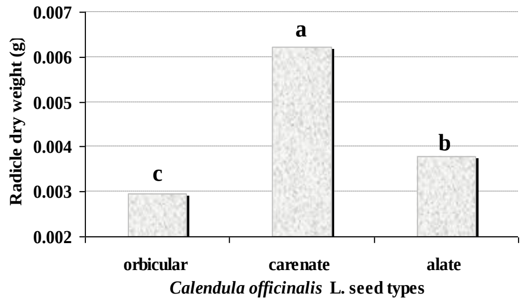
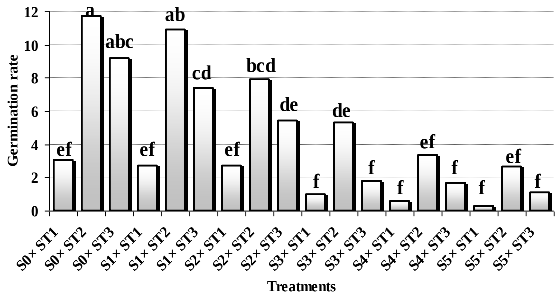


Figure 10. Effect of salinity and seed type on germination rate of *Calendula officinalis* L.



minimum plumule and radicle dry weight with statistical significant difference at 5% level confidence (Figs 8 and 9). Maximum of radicle to plumule length ratio was observed in carenate that it had significant difference with two other types of seed (Table 2). None statistical significant difference (P≤0.05) was observed among marigold seed types in radicle to plumule wet weight ratio (Table 2).

Maximum of radicle to plumule dry weight ratio was observed in carenate, alate and then orbicular (Table 2). Maximum and minimum of radicle to plumule dry weight ratio allocated to carenate and orbicular respectively. Alate did not show any significant difference with two mentioned seeds at 5% confidence level (Table 2).

Marigold seed germination percent under effect of salinity levels and seed type had statistical significant difference with each other in most treatments (Table 3). Maximum of germination percent was observed in alate × none salinity, carenate × 1dS/m salinity and finally in carenate × none salinity treatment respectively that they were in three different separate statistical groups (a, b and c) (Table 3). Minimum of marigold seed germination percent was also observed in orbicular × 7 dS/m salinity, orbicular × 5 and 10 dS/m salinities respectively (Table 3).Totally, germination percent decreased with increasing in salinity. Also, comparison of interaction effect between salinity and seed type (Table 3) showed that in all salinity levels orbicular seed had lower germination percent than two other seed types.

Maximum of germination rate under interaction effect of environmental salinity and seed type was observed in carenate × none salinity treatment and 1 dS/m salinity with none statistical significant difference with each other (P≤0.05) respectively (Figure. 10). None salinity × alate and carenate ×3 dS/m salinity with none statistical significant difference with each other also allocated maximum germination rate next to both maximum treatments (Figure. 10). Results of this study showed that in all salinity levels of 5, 7 and 10 dS/m and orbicular and alate seed type, germination rate was minimum with none statistical significant difference with each

Table 3. Interaction effect of salinity and seed type on germination and some growth parameters of *Calendula officinalis* L.

Treatments	Germination seed	Plumule length (cm)	Radicle length (cm)	Plumule wet weight (g)	Radicle wet weight (g)	Radicle to plumule wet weight ratio	Radicle to plumule dry weight ratio
S_0× ST_1	10.67 ef	1.783e	3.567d	0.009667ef	0.0060fghi	0.6222bcdef	0.6667efg
S_0× ST_2	28.00c	1.480fg	4.893b	0.08700abc	0.04533c	0.5207cdef	1.275cdef
S_0× ST_3	44.00a	1.397gh	4.090c	0.05667bcde	0.04600c	1.081b	1.667bcd
S_1× ST_1	6.667fgh	2.717a	3.180f	0.004333ef	0.002333ghi	0.5333cdef	1.000defg
S_1× ST_2	36.00b	1.970d	5.147b	0.07267abcd	0.1540a	2.126a	4.778a
S_1× ST_3	21.33d	1.440fgh	4.270c	0.04267cdef	0.01067efg	0.2372ef	1.583bcde
S_2× ST_1	9.333ef	2.140c	3.467de	0.02467def	0.01167ef	0.4964cdef	1.206cdefg
S_2× ST_2	21.33d	1.917d	5.727a	0.1073ab	0.05967b	0.5630bcdef	0.5417fg
S_2× ST_3	22.67d	1.550f	4.227c	0.08233abc	0.03800c	0.4617cdef	0.5893fg
S_3× ST_1	2.667hi	0.7333l	2.667g	0.002333f	0.001000i	0.4444def	0.3333g
S_3× ST_2	12.00e	1.210j	4.337c	0.02967def	0.01767de	0.5959bcdef	1.944bc
S_3× ST_3	8.000efg	0.8633k	1.887i	0.1170a	0.003333fghi	0.08315f	1.667bcd
S_4× ST_1	1.333i	0.3333m	1.567j	0.0006667f	0.001333hi	1.000bc	0.6667efg
S_4× ST_2	12.00e	1.243ij	2.000i	0.02500def	0.02433d	0.9815bcd	2.333b
S_4× ST_3	6.667fgh	1.417gh	2.367h	0.006333ef	0.004333fghi	0.6825bcde	0.8333defg
S_5× ST_1	2.667hi	2.333b	2.000i	0.004333ef	0.0020hi	0.4667cdef	1.333cdef
S_5× ST_2	6.667fgh	1.350hi	3.200ef	0.01500ef	0.009667efgh	0.6444bcde	0.6667efg
S_5× ST_3	4.000ghi	0.7667kl	1.333j	0.007000ef	0.002667ghi	0.3810ef	0.8333defg
P_{value}	0.0000	0.0000	0.0000	0.1377	0.0000	0.0001	0.0000

S_0 = control, S_1 = 1, S_2 = 3, S_3 = 5, S_4 = 7 and S_5 = 10 dS/m NaCl
ST_1= orbicular, ST_2 = carenate and ST_3= alate
*Different letters in each column is indicated significant difference at 5% level.

other (Figure. 10). Similar to germination percent, germination rate decreased with salinity increase and carenate seed in all salinity levels than two other seed types and alate seed in low salinity level (0, 1 and 3 dS/m) had better germination rate than to orbicular seed (Figure.10).

Plumule length was maximum in orbicular and 1, 10 and 3 dS/m salinities respectively with statistical significant difference (a, b and c groups) (Table 3). Then, both treatments carenate seed × 1 and 3 ds/m salinities with none statistical significant difference with each other and finally orbicular × none salinity with significant difference with two mentioned treatments allocated maximum plumule length (Table 3).Minimum plumule length under effect of salinity and seed type was observed in orbicular × 7 and 5 dS/m salinities, alate × 10 and 5 dS/m salinities respectively that all of them had significant difference with each other at 5% confidence level (Table 3).

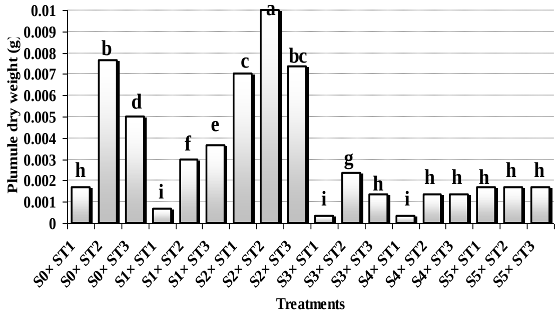
Comparison of radicle length under interaction effect of salinity and seed type showed that (Table 3) maximum of radicle length was observed in carenate × 3 dS/m salinity that it had statistical significant difference with other treatments ($P \leq 0.05$). After that, two treatments (carenate × 1dS/m salinity and none salinity) were in the next statistical group (b) with none significant difference with each other. Alate in 0, 1 and 3 dS/m salinity levels was observed in c statistical group with none significant difference with each other (Table 3). Minimum of radicle length was observed in alate × 10 dS/m salinity, orbicular × 7 dS/m salinity with none significant difference and then in orbicular × 10 dS/m, carenate × 7 dS/m and finally alate × 5 ds/m salinity with none significant difference respectively (Table 3). Results showed that radicle length in *Calendula officinalis* L. seedling affected by increasing salinity more than seed type. As, this decreasing was observed in all of seed types in high salinity levels (Table 3).

Results also indicated that alate × 5 dS/m salinity, carenate × 3 dS/m and none salinity allocated maximum plumule wet weight respectively. But, these treatments did not any significant difference with each other at 5% confidence level (Table 3). Comparison of plumule wet weight was min in all high salinity levels (7 and 10 dS/m) with no attention to *Calendula officinalis* L. seed type (Table 3). In addition to 7 and 10 dS/m salinities plumule wet weight decreased in orbicular and carenate seeds at 5 dS/m salinity and alate × 3 dS/m salinity.

Maximum radicle wet weight was observed in carenate × 1 dS/m salinity and then carenate × 3 dS/m salinity in separate statistical group (a and b) (Table 3). Alate and carenate × none salinity treatments, alate × 3 dS/m was observed with none significant difference with each other after both maximum radicle wet weight treatments. Carenate × 7 dS/m was also observed after mentioned treatments in d statistical group (Table 3). Radicle wet weight decreased with environmental salinity increase. But, minimum decrease and maximum tolerance attention to seed types was observed in carenate and maximum sensitivity to salinity was observed in orbicular seed of marigold too. High sensitivity of orbicular was clear in low salinity levels (0, 1 and 3 dS/m) in radicle wet weight (Table 3).

Study of interaction effect between salinity and seed type on plumule dry weight (Fig 11) showed that all treatments were in separate statistical group in low salinity levels (0, 1,3 and 5 dS/m). Maximum plumule dry weight was observed in carenate × 3 dS/m, carenate × none salinity, alate × 3 dS/m and orbicular × 3 dS/m with statistical significant difference (Figure. 11). Minimum of plumule dry weight was also observed in orbicular and 7, 5 and 1 dS/m treatments (Figure. 11).

Figure 11. Effect of salinity and seed type on plumule dry weight of *Calendula officinalis* L. (g)



Results of interaction effect of salinity and seed type showed that maximum of radicle dry weight was observed in carenate and 1, 0 and 3 dS/m salinities (Fig 12). Maximum of radicle dry weight was observed in carenate × 1 dS/m and none salinity (with none significant difference with each other), alate × none salinity, orbicular × 3 dS/m (Figure. 12). Alate × 1 dS/m, carenate × 3 dS/m treatments were in the same statistical group (d) with no significant difference. With salinity increasing, radicle dry weight decreased similar to radicle wet weight. But, with losing humidity of radicles, special statistical difference was not observed under effect of seed type in radicle dry weight (Figure. 12).

Results of radicle to plumule length ratio under effect of environmental salinity and seed type (Figure. 13) showed that this ratio was higher in medium and high salinity levels (5 and 7 dS/m). Maximum of this ratio was observed in orbicular × 7 dS/m with significant difference with two treatments orbicular and carenate seeds × 5 dS/m (Figure. 13).

Figure.12. Effect of salinity and seed type on radicle dry weight of *Calendula officinalis* L. (g)

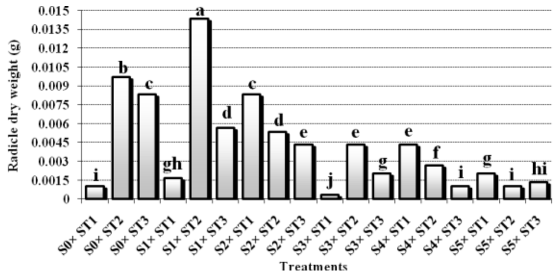
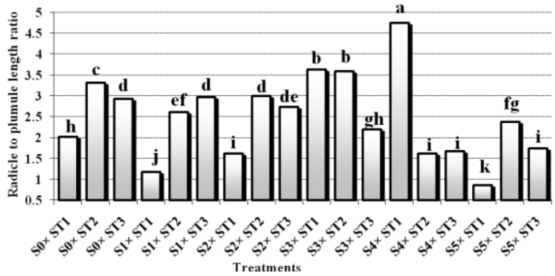


Figure. 13. Effect of salinity and seed type on radicle to plumule length ratio of *Calendula officinalis* L.



Minimum of radicle to plumule length ratio was also observed in orbicular × 10 and 1 dS/m. Study of this ratio showed that maximum and minimum amount of that with attention to salinity level indicated in orbicular seed type (Figure. 13).

Comparison of treatments for radicle to plumule wet weight ratio (Table 3) under interaction effect of salinity and seed type showed that it had not any significant difference amoung treatments. But, only carenate × 1 dS/m, alate × none salinity treatments was observed maximum radicle to plumule wet weight and separate statistical groups (a and b). Minimum of this ratio was observed in alate × 5 dS/m salinity. None special trend among treatments under effect of salinity and seed type was observed for radicle to plumule wet weight ratio (Table 3).This observing was also shown for radicle to plumule dry weight. As, only two treatments, carenate × 1 and 7 dS/m salinities treatments had maximum of this radicle to plumule dry weight with statistical significant difference with each other (Table 3).

DISCUSSION

In addition to results of experiment, germination rate and germination percent of marigold seeds decreased by increasing salinity levels (Table 1). Several studies have been shown that germination percent and germination rate of most plants under effect of salinity decrease, germination delayed at the lower levels of salinity, whereas, final germination percent decreased at higher salinity (Aref and Wander, 1998). Abou El-Fadl et al. (1990) indicated that even though soil salinity more than 2000ppm decreased peppermint (*Mentha arvensis*) plant growth, the EO yield and that of its components increased. By increasing the levels of soil salinity plant growth of *Ocimum basilicum* (basil), damsesea (*Artemisia absinthium*), black cumin (*Nigella sativa*) and sage (*Salvia officinalis*) was significantly decreased but EO and its main components increased (El-Shafy et al. 1991; Abd-El Nabi and Hussein, 1996; Khalid, 2001; Hendawy and Khalid, 2005). Osmotic problem in plants also occurs under drought stress conditions and this term exist about 100 years ago that salinity stress is a type of physiological drought (Kafi and Mahdavi Damghani, 2000). Peppermint essential oil and alkaloid amounts in many plants increase under effect of drought stress because the decomposition of starch and proteins shall simulate the production of such materials (Khaje pour, 1986). In experiment was conducted, maximum of germination percent and germination rate was observed in carenate, alate and orbicular seed respectively (Table 2 and Figure. 5).The seeds have higher germination rate under salinity conditions, in the next of growth stage, seedling establishment and soil solutes avoiding will be more successful (Richards, 1954).Ming et al. (1999) to study the effect of seed type (carenate, alate and orbicular) and different five substrate (vermiculite, sand, soil, commercial mixture and producer' mixture) on *Calendula officinalis* L. germination and seedling development showed that the seed types have no relation with seedling development. But, germination was better in alate and orbicular seeds (Ming et al. 1999). Plumule and radical length had better in 1 and 3 dS/m salinity concentrations rather than to none salinity treatment (control) (Table 1). But, higher salinity levels caused length and weight *Calendula officinalis* L. seedlings decrease. Plumule and radical wet weight was also higher in lower salinity levels (1 and 3 dS/m salinity) (Figs. 3 and 4). Soaking wheat seed in water or NaCl solutions and then drying by air flow, before planting in saline soil with 0.5 percent NaCl , caused germination simulate and growth seedlings of these seeds in soil, also deep root growth of these mentioned seedlings is visible than the seedlings of untreated seeds (Al-Shamma, 1983). Fertilizer in addition to soil fertility is solution salt and when it solves in soil solution osmotic pressure of solution change and this effect is similar to soil salinity (Salardini, 2000). The effect of locations (Gorobilje & Arilje and Kačarevo) and applications of different types of fertilizers (200 kg KAN ha^{-1} ,400 kg NPK (15:15:15) ha^{-1} and control (without fertilising)) on yield and seed quality of the marigold cultivar "domaći oranž"/"domestic orange" was observed and analysed during two growing seasons (2006 and 2007). Results showed that the effect of fertilising on the yield in comparison with control was also significant (Jevđović1 et al. 2008). The highest quality of seed (germination viability, total germination and seed weigt) was recorded in the location of Kačarevo where NPK was applied, while the lowest quality of seed was identified in the location of Gorobilje where KAN was applied. The yield in 2006 was insignificantly higher over all locations and variants, which was caused by a greater amount of precipitation, but seed quality was somewhat better in 2007 as a result of a greater temperature sum (Jevđović1 et al. 2008). Chlorine is the one of essential nutrients for plant and its lack strongly prevented longitudinal growth of root (Khold Barin and Eslam Zade, 2005). Chlorine is also responsible for the emergence of oxygen in the light system II in photosynthesis (Salardini and Mojtahedi, 1988). Comparison between the need of chlorine for growth and the amount of it can be provided by various resources, reveals that under field condition, chlorine deficiency rarely occurs. When that chlorine exist in high amount in root zone have none specially extensive effects on metabolism that can be mentioned such as competition with nitrate uptake, regulate the balance of cations – anions and organic acids metabolisms (Khold Barin and Eslam Zade, 2005). Chlorine is the one of active osmotic materials in cell vacuoles and when external osmotic pressure solutions increases than osmotic pressure of plant cells in addition of disorder of osmotic regulation by plant cells, high levels of sodium and chloride have a direct toxic effects on membrane and enzymatic systems (Kafi and Mahdavi Damghani, 2000) and finally cause decreasing growth and death plant. Carenate seed of *Calendula officinalis* L. in comparison of two other types seed had better condition approximately in all growth parameters. Marigold fruit is achene and it has type variation and is observed as three types carenate, alate and orbicular (Cromack and Smith, 1993). The formation of seeds on receptacle from outside to inside is alate, carenate and orbicular respectively. Considering of time and place of formation, seed vigour is variable. Maximum of seed vigour is observed in alate seed. Carenate seeds have medium seed vigour and orbicular seeds have low seed vigour (Ameri, 2007).

Since the various yield components are affecting on the final yield, the final yield sensitivity to the environmental stresses such as salinity is function of each of the various yield component sensitivity to the stress (Eskandari Torbaghan, 2006). Generally, environmental stresses such as drought and salinity stress cause shoot to root ratio decreasing in different plants. Really, stresses cause root growth increasing. The results of this experiment showed that in low salinity levels, plumule to radicle length ratio had decrease and with environmental salinity increasing (5 and 7 dS/m) this ratio increased. But, in maximum salinity level (10 dS/m) this ratio decreased again (Table 1). Salt stress effects on plants growth are related to cell division and development. Salinity limits growth potential by decreasing it without increasing in cell division period. The number of metaphase stage increase after salinization. This shows that salts have one toxic effect on mitotic division. Mitotic division spindle growth is very sensitive to the medium ionic composition. In halophyte plants, cell division is limited whereby the number of cells decreases. However, longitudinal growth stimulation of cells cause increases of the size of some specific cells and in the other hand in gelycophytes plants with soil salinity increasing, cell division and longitudinal cell growth limit. Salinity induced chloride salts, is strongly limited cell division but increased longitudinal cells are stimulated, while the sulphate salts cause limitation for growth and cell development more than cell division (Iran-Nejad and Shahbazian, 2005). Salinity was more effective on radicle to plumule wet and dry weight ratio than length ratio. As, in lower salinity level (1 dS/m) radicle wet weight was improved than plumule wet weight. The total studied ratios in this research were higher in carenate seed. Study of the effect of different priming types (control, GA_3, Manitol, NaCl and distilled water) on seed germination of two medicinal plants including pot marigold (*Calendula officinalis*) and sweet fennel (*Foeniculum vulgare*) under salinity stress (0, 2.5, 5, 7.5 and 10 ds m^{-1}) showed that with increasing salinity, germination traits such as germination percent, rate and plumule length decreased, but seed priming with GA_3 and NaCl showed lower decrease (Sedghi et al. 2010). In all of the salinity levels, primed seeds (except manitol) possessed more germination rate and plumule length than control. The highest radicle fresh and dry weight in pot marigold was seen at 7.5 ds m^{-1} salinity stress level. It seemed that higher germination rate in pot marigold shows higher tolerance to salinity than sweet fennel. Priming with NaCl and GA_3 caused an increase in germination percent of pot marigold and sweet fennel in various range of salinity, but in lower salinity levels percent of germination was higher than upper ones. The result of this experiment had consistent with the hypothesis that under undesirable conditions such as salinity stress, priming with GA_3 and NaCl can prepare a suitable metabolic reaction in seeds and can improve seed germination performance and seedling establishment(Sedghi et al. 2010).

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