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Growth of Various Calendula Seeds under Saline Irrigation

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Abstract

A pot experiment was conducted to observe the growth responses from various Calendula (*Calendula officinalis*) seeds grown by using saline irrigation during the year 2015.Calendula seeds of three different shapes are commonly sold together in packets. Thirty five seeds of each shape viz.Carenate, alate and orbicular were separated and grown in the pots. The irrigation was maintained with electrical conductivity (EC) at 0, 1, 3, 5 and 7 dSm^{-1}. The experiment was run in Completely Randomized Design with five replications. The best results for mean seed germination (49.14%), germination time (2.10 days), seedling vigor index (1023.8), shoot length (2.18 cm), number of leaves per seedling (13.40), electrolytes leakage (42.77%)and leaf chlorophyll content (41.54) were observed from plants where distilled water was used. However, fresh weight of the shoot (5.43 g), root (0.45 g), and days to initiate flower bud (89.30) were recorded the bestwhere irrigation was maintained at EC level of 1 dSm^{-1}. Out of three shapes of calendula seeds, carenate seeds got minimum days to germination (3.48), the highest germination (76.11%), seedling vigor index (1044.10), number of leaves per seedling (10.68) and minimum days to flower bud (93.72). The plants from orbicular seeds got the maximum root length (13.19 cm), fresh weight of shoot (4.23 g) and root (0.42 g). It is concluded that the most of the germination and growth related attributes of the seedlings are sensitive with the increased salinity levels of irrigation water.

Key words: Calendula seeds, salinity, germination, seedling, growth.

Introduction

Calendula (*Calendula officinalis*), also known as the pot marigold, is one of the most attractive flowers grown widely in garden as well as for landscape purpose. It is a short lived aromatic herbaceous plant of the Asteraceae family. It is mainly grown as a bedding and border plant or as a cut flower. The plant height goes usually ranges from 50-80 cm. Various calendula species produce different flowers with diverse colours including pale yellow, orange-red, and with 'double' flower heads with ray florets replacing some or all of the disc florets. The flowers may appear all year long where conditions are suitable [1, 2].

Calendula is commercially planted by seeds. Generally calendulas seeds are sold in the market in packets contain seeds of different shapes and growers usually plant these different types of seeds by broadcast method of plantation to raise the nursery of the calendula. They don't know which type/shape of seeds actually germinate and grow. Few seed selling companies may be sold these seeds on the basis of different shapes of the seeds. The present study is mainly focusing on the specific seed type plantation on the basis of seed shape to evaluate germination and growth responses of the seedlings grown by using saline irrigation water. As soil salinity is a major constraint to the successful germination and stand establishment of the seedlings [3, 4). It is well documented that more than 800 million ha of soil suffers from salinity worldwide, and most of the land is affected by sodium chloride (NaCl) [5]. Several lines of evidence indicated that low or higher level of salinity revealed adverse effects on germination of seed and growth and yield of most of the crop species (2). Lower levels of salinity generally slow down germination process, whereas higher levels not only inhibit seed germination but also adversely affect the germination percentage [6].

Good quality water plays a significant role in successful flower production. However, scarcity of good quality water occurs globally which is biggest threat to agriculture and floriculture as well. Under these conditions the application of saline irrigation water to flowers and other cultivated crops may be inevitable. Most of the flower species are not tolerant to salinity, especially caused by sodium chloride. The accumulation of salts in the root zone of flowers has injurious effects on the growth of flowers. Symptoms caused by high levels of salt include leaf tip burn, reduced height and yield [7].

The growing and development of salt tolerant flower species is promising and sustainable approach for successful and profitable cultivation of flowers. Most flowers species including calendula are glycophytes and comprises of diverse species that can tolerate low to moderate [8]. Most of growers related to floriculture believe that flowers are highly sensitive to salinity. However, several lines of evidences have suggested that moderately saline waters can be successfully applied to certain flower species without compromising acceptable growth and productivity [9, 10, 11]. In the past, most of the studies related to salinity tolerance have been conducted on trees, shrubs and perennial herbaceous plants [12]. However, few studies were also performed on cut flowers which showed some level of tolerance of salinity [13].

Available reports demonstrate that wide genetic variation occurs for salinity tolerance in various genotypes among ornamental plants and also genotypes of the same ornamental species. The Among numerous plant species being studied in landscapes there is a great variation of salt tolerance in species and even cultivars within a species [14]. Therefore, the present experiment was conducted to compare the germination and growth responses of *calendula officinalis* seeds of different shapes under various salinity levels.

Material and methods

The experiment was conducted at the horticulture garden, Department of Horticulture Sindh Agriculture University, Tandojam, Sindh, Pakistan. Three types of Calendula seeds viz. carenate, alate and orbicular were used in the present study to observe germination and growth responses of the seedlings by using various levels of irrigation water. The electrical conductivity (EC) of irrigation water was maintained at 0, 1, 3, 5 and 7 dSm^{-1}. Thirty five seeds of each type were planted in earthen pots contained potting mixture. The potting mixture was prepared by mixing equal ratio of sand, farm yard manure and garden soil. This growing mixture was analyzed for soil texture by Bouyoucos Hydrometer method [15] pH of 1:5 soil-water extract by pH meter, EC of 1:5 soil-water extract by EC meter and organic matter byWalkley-Black method [16]. The soil was sandy clay loam, non-saline in nature with EC 0.71 dSm^{-1} and slightly alkaline in reaction with pH 7.4. The soil was also low in organic matter (0.72%). The mixture of growing medium was filled in earthen pots leaving approximately one inch space at the top. The experiment was conducted in Completely randomized design (CRD) with five replications. The data was recorded for seed germination (%), germination time (days), seedling vigor index (SVI), shoot length (cm), root length (cm), fresh weight of the shoot (g), fresh weight of the root (g), electrolyte leakage of leaf (%), leaf chlorophyll content and days taken to initiate flower bud.

Seed germination percentage -Germination was checked on every alternative day for upto 7^{th} day of plantation and the germination percentage was calculated by using following equation as described by Larsen and Andreasen [17].

$$GP = \Sigma n / N \times 100$$

where n is number of germinated seeds at each counting and N is total seeds in each treatment.

Germination Time (GT) was calculated by using following equation of Ellis and Roberts [18]

$$MGT = \frac{\sum Dn}{\sum n}$$

Where n is the number of seeds germinated on day D and D is the number of days as counted from the beginning of germination.

Seedling Vigor Index (SVI) was calculated by using following formula, Abdul-Baki and Anderson [19].

Vigor index (VI) = [seedling length (cm)× germination percentage]

Two months old seedlings were kept under observation for length and weight of shoot and root, electrolyte leakage and chlorophyll content of leaf.

Electrolyte leakage of leaf was measured by taking leaf discs of size 1 cm^2 and weight 0.5 g from random samples of leaf. The leaf discs were rinsed well with deionized water prior to incubation in 25 ml of deionized water for 3 h at room temperature. After incubation, the conductivity (value A) of the bathing solution was measured with the conductivity meter. The petal discs were boiled with bathing solution for 15 min to lyse all cells. After cooling at room temperature, the conductivity (value B) of the bathing solution was again measured. The electrolyte leakage was expressed as percent value according to the formula given below.

Electrolyte leakage of leaf % = (Value A/Value B) 100

The data was statistically analyzed by using software Statistixs 8.1 and interpreted on the basis of Duncan's Multiple Range Test (DMRT).

Results

The experiment was conducted to observe the seed germination and growth responses from different types of *Calendula* seeds grown by using saline irrigation water. The significant results observed in relation to seed germination and the data is presented in the Table 1.Carenate seeds had the highest mean germination of 76.11% followed by Orbicular seeds (30.51%). However no response was observed from the Alate seeds. The response of these three types of seeds was also observed significant with various salinity levels of irrigation water. Out of these three types of seeds, Carenate seeds were observed tolerant to saline irrigation water producing germination of 81.71 and 73.13% in saline irrigation water that had EC of 3 and 5.00 dSm^{-1} respectively. However on the basis of mean data of salinity levels of irrigation water, germination was observed decreased with the increasing levels of salinity in irrigation water. The seed germination was adversely affected by salinity levels and plants irrigated by saline water of EC 7.00 dSm^{-1} resulted in lowest mean seed germination of 15.04%. However, the highest mean germination of 49.14% was observed from control irrigation.The seed germination was inversely proportional to increasing salinity levels and there was a simultaneous decrease in seed germination of calendula with increasing water EC levels used for their irrigation. However, regardless the salinity levels, Carenate showed most encouraging results for germination which indicates an ability in Carenate seeds to tolerate salinity and grow well under saline water irrigation.

Carenate seeds of calendula germinated earlier in mean 3.48 days as compared to Orbicular that took 4.32 days (Table 1). This indicates more viability of Carenate seeds than the Orbicular. In case of salinity effect, the increasing salinity level markedly delayed the germination of calendula seeds and plants irrigated by saline water of EC 7.00 dSm^{-1} took maximum days (6.30) for germination.While plants irrigated by water of reduced EC levels of 5.00 $dSm^{-1,}$ 3.00 dSm^{-1} and1.00 dSm^{-1} took 4.50, 3.80 and 2.80 days for seed germination, respectively. However, the calendula seeds took only 2.10 days for germination time under control treatment. The germination time was simultaneously increased with increasing salinity levels. However, irrespective the salinity levels, Carenate seeds displayed earlier seed germination than the Orbicular.

The higher seedling vigour index (SVI) was mainly associated with higher seed germination and other seeding growth attributes. The highest seedling vigor index (1463.3) was observed from theCarenate plants irrigated with the water that had EC 1 dSm^{-1} (Table. 2). However statistically similar results (1263.9) were also observed from the plants irrigated by the water with EC of 3 $dSm^{-1}$and control treatment (1248). The seedling vigour index was inversely proportional to salinity and as the EC level of irrigation water increased from 3 to 7 dSm^{-1}, there was a concurrent decrease in the seedling vigour index. On the basis of calendula seed types carenate had better mean seedling vigor index (1044) as compared to orbicular (470.3). The mean 1023.8 SVI was observed from control treatment followed by the results 1011.3 from plants irrigated with water had EC of 1 dSm^{-1}.

The shoot length was observed the highest(2.18 cm) from control treatment followed by the results of 1.86 cm obtained from the plants which were irrigated with the water had EC of 1.00 dSm^{-1} (Table 3.).The salinity effect indicated that increasing salinity levels decline the shoot length and plants irrigated by saline water of highest EC level of 7.00 dSm^{-1} resulted in minimum shoot length of 1.04 cm. In contrast to shoot length, the highest root length (14.30 cm) was observed in the plants irrigated with saline water with EC of 3 dSm^{-1}. These results are non-significant with the results (13.62 cm) obtained from the plants irrigated by saline water with EC of 1 dSm^{-1}. However, the increasing EC levels of irrigation water above 3 dSm^{-1} reduced root length of the plants. On the basis of seed type comparison, significantly longer roots (13.19 cm) were noted from the plants grown from orbicular seeds as compared to Carenate seeds (11.89 cm).

Fresh weight of the shoot and root were observed decreased when irrigation water had EC above than 1 dSm^{-1} (Table 4). The plants from Orbicular seed types had more fresh weight of shoot (4.23 g) and root (0.42 g) in comparison to

Carenate. On the basis of salinity levels of irrigation water, maximum fresh weight of shoot (5.42 g) and root (0.45 g) was recorded from the plants irrigated with the water had EC levels of 1.00 dSm^{-1} as compared to control. The effect of salinity showed that mean fresh weight of shoot (2.28 g) and root (0.24 g) were the lowest fromthe plants irrigated by saline water of higher EC level 7.00 dSm^{-1}. However, the plants received irrigation had EC levels of 5.00 and 7.00 dSm^{-1} were statistically non-significant with each other for fresh weight of shoot and root ($P>0.05$).

The number of leaves of calendula was significantly higher (10.68) in Carenate than the plants of Orbicular (8.640) seeds (Table 2). The lowest number of leaves per plant (8.60) were observed from the plants irrigated with the water had EC of 7.00 dSm^{-1}. However, the plants irrigated with the distilled water produced 13.40 number of leaves per plant, while number of leaves per plant was almost similar under all salinity levels. The results clearly indicated that under saline irrigation water, severe adverse effect on the number of leaves $plant^{-1}$ of calendula is experienced in this study as compared to control treatment.

Chlorophyll content and electrolyte leakage of leaves was assessed in *Calendula* leaves and the data is presented in Table 5. The analysis of variance indicated that different salinity levels had significant ($P<0.05$) effect on chlorophyll content and electrolyte leakage of leaves; while effect of seed types and interactive effect of seed types and salinity levels on these parameter were non-significant. The mean chlorophyll content ranges from 26.63 to 41.54 producing the highest chlorophyll content from control treatment. These results of mean chlorophyll content were observed decreased with the increasing levels of salinity in irrigation water. The lowest chlorophyll content (26.30) and the highest leakage of electrolytes (70.81%) were observed from the plants irrigated with the water had EC level of 7.00 dSm^{-1}.These results are statistically similar with the results obtained from the plants irrigated with the water had EC level of 5.00 dSm^{-1}. No significant differences were observed from the plants grown from carenate and orbicular seeds.The plants of the control treatment and the plants received irrigation with the EC level of 1.00 dSm^{-1} exhibited statistically similar results for chlorophyll content and leakage of electrolytes.

Discussion

Cut-flower production has gained significance importance in the worldwide and rapid changes have noticed in production, storage, classification and marketing of cut-flowers [20]. Floriculture remained quite a neglected segment of agriculture in Pakistan [21]. and organizations unluckily could not develop awareness on account cut-flower production among the farming communities. The present experiment was conducted to compare the germination and growth responses of different types of seeds of *Calendula* under various salinity levels of irrigation water. Very little work reported on different types of calendula seeds.

Seed germination is one of the most fundamental and vital phases in the growth cycle of plants that determine plant establishment and the yield of the crops. It is well established that salt stress has negative correlation with seed germination and vigor [22]. Higher level of salt stress inhibits the germination of seeds while lower level of salinity induces a state of dormancy [23]. Seed germination was observed decreased with the increased levels of salinity. These reports are in accordance with the results of Gharineh [24] who treated the calendula seeds with 1, 3, 5, 7 and 10 dSm^{-1} of sodium chloride solution and found significant decrease in germination percentage with each increased level of germination. They reported 27.38 germination percent from the seeds of *Calendula* grown with distilled water with EC 0 dSm^{-1}. However the water with EC 1, 3, 5, 7 and 10 dSm^{-1} significantly decreased germination percent from 27.38 to 3.97. Almost similar results were reported by Torbaghan [25] under varied levels of salinity. However he obtained the highest germination percentage (19.33) from alate seeds of calendula followed by orbicular and carenate. Ming [26]. reported better germination from alate and orbicular seeds of calendula. In contrast in the present study no germination was observed from alate seeds and the highest germination percentage was observed from carenate seeds. This may be due to plantation of seeds in different growing conditions as Torbaghan grew seeds in incubator at constant temperatures by using filter paper as compared to open soil conditions of pot. Decreasing in germination percentage with increasing salinity can reduce water absorption and the increasing of ions in around and creation water stress influence was attributed to osmotic imbalance. Ions increasing influence high solute ionic toxicity caused by reduced germination of the species. Thereby decreasing water potential, plants cannot absorb water and is faced with water shortages. Germination time delayed in salinity. Sedghi [27] reported that mean germination time increased by raising salinity levels, such that the lowest MGT was at control level and the highest was at 10 ds m^{-1} salinity level.

The other growth parameters other than germination related attributes decreased with the higher levels of salinity. Seedling vigor index is an important factor for rapid stand establishment and early growth of the plants as reported by Tabrizian and Osareh [28]. It is mainly based on the seedling length and germination percentage. The seedling length includes shoot and root lengths which decreased when salinity was above 1dS m^{-1}.

Fresh weight of the shoot and root were also decreased when irrigation water had EC above than 1 dS.m$^{-1.}$ Jamil [29] reported that fresh root weight was affected more than fresh shoot weight. These results are similar with the results of Jamil [30]. Vaidez-Aguilar [31] examined the effect of EC levels of 2, 4, 6, 8, and 10 dSm^{-1} on growth of marigold and reported a 20% to 25% decrease in plant height, leaf dry weight (DW), and shoot DW when irrigated with 4 dS.m^{-1} water. Carter and Grieve [32] determined the effects of higher soil EC levels on the growth of marigold water with salinity levels with electrical conductivities of 2.5 (control), 4.0, 6.0, 8.0, and 10.0 dS.m^{-1} and there were adverse effects on plant shoot fresh weight, number of leaves, number of shoots. Niu [33] irrigated zinnia plants with saline solutions at electrical conductivity (EC) of 1.3, 3.0, 4.2, 6.0, or 8.2 dS m^{-1} and reported that salt damage symptoms were more severe at higher EC levels. Sayyed [34] reported that after two weeks treatment of salt stress in *Tageteserecta* fresh biomass of plant increased significantly ($P<0.001$) in 50 and 100 mM but decreased in 150 mM and 200 mMNaCl.

Chlorophyll is the main pigment responsible for photosynthesis. Under adverse circumstances, the chlorophyll level is a good indicator of the photosynthesis function. It has been found that the chlorophyll level decreases with aggravated salt stress [35] due to enzymatic chlorophyll degradation [36, 37]. Munns [38] stated that reduction in fresh and dry biomass caused by suppression of plant growth under saline conditions may either be due to decreased availability of water or to the toxicity of sodium chloride. The decrease in Chlorophyll content under salt stress is a commonly reported phenomenon and in various studies and the chlorophyll concentration were used as a sensitive indicator of the cellular metabolic state [39]. Bayat [40] reported that salinity decreased the growth, Chlorophyll reading values, flower number per plant, shoot and total dryweight, plant height and leaf area of calendula plants under salt stress. Bizhani [41]. reported that plants became shorter, their leaf area, chlorophyll content and overall growth was stunted as EC of irrigation water increased. Shoot dry weight of zinnias reduced by 93% at EC 12.5 dSm^{-1} as compared with that of the control. Sayyed

[34] conducted an experiment on salt stress in *Tageteserecta*. The salinity concentration of NaCl solution (50 mM, 100 mM, 150 mM and 200 mM) were used. Results indicated that *Tageteserecta* is moderately tolerant to salt stress. Plant height, root length, no of leaves, fresh and dry biomass, Chlorophyll a, b and caroteneoids exhibited reduction under higher concentration of NaCl.

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Table-1 **Seed germination (%) and days taken to germination observed from different types of *Calendula* seeds grown by using various levels of saline irrigation water.**

Salinity levels of irrigation water	**Seed germination (%)**			**Mean**	**Days to germination**		**Mean**
	Carenate	Alate	Orbicular		Carenate	Orbicular	
Distilled water (control)	97.71 a	0.00 f	49.71 c	**49.14 A**	2.00 e	2.20 e	**2.10 E**
1.00 dSm^{-1}	96.00 a	0.00 f	35.42 d	**43.80 B**	2.80 de	2.80 de	**2.80 D**
3.00 dSm^{-1}	81.71 b	0.00 f	32.56 d	**38.09 C**	3.20 d	4.40 c	**3.80 C**
5.00 dSm^{-1}	73.13 b	0.00 f	21.71 e	**31.61 D**	3.40 d	5.60 b	**4.50 B**
7.00 dSm^{-1}	32.00 d	0.00 f	13.14 e	**15.04 E**	6.00 ab	6.60 a	**6.30 A**
Mean	**76.11 A**	**0.00 C**	**30.51 B**	**-**	**3.48 B**	**4.32 A**	**-**

Table 2. **Seedling vigor index and number of leaves per seedling as observed from different types of *Calendula* seeds grown by using various levels of saline irrigation water**

Salinity levels of irrigation water	**Seedling vigor index (SVI)**		**Mean**	**Number of leaves per seedling**		**Mean**
	Carenate	Orbicular		Carenate	Orbicular	
Distilled water (control)	1248.0 a	799.6 b	**1023.8 A**	12.00 b	14.80 a	**13.40 A**
1.00 dSm^{-1}	1463.3 a	559.3 c	**1011.3 A**	11.00 bc	6.80 e	**8.90 B**
3.00 dSm^{-1}	1263.9 a	532.1 c	**898.00 B**	10.80 bc	7.20 e	**9.00 B**
5.00 dSm^{-1}	854.50 b	285.90 d	**570.20 C**	10.00 bc	6.80 e	**8.40 B**
7.00 dSm^{-1}	390.90 cd	174.80 d	**282.85 D**	9.60 cd	7.60 de	**8.60 B**
Mean	**1044.10 A**	**470.30 B**	**-**	**10.68 A**	**8.64 B**	**-**

Table-3 **Shoot and root length (cm) observed from different types of *Calendula* seeds grown by using various levels of saline irrigation water**

Salinity levels of irrigation water	**Shoot Length (cm)**		**Mean**	**Root length (cm)**		**Mean**
	Carenate	Orbicular		Carenate	Orbicular	
Distilled water (control)	2.16	2.20	2.18 A	10.60	13.80	12.20 BC
1.00 dSm^{-1}	1.76	1.96	1.86 B	13.48	13.76	13.62 AB
3.00 dSm^{-1}	1.54	1.70	1.62 B	14.00	14.60	14.30 A
5.00 dSm^{-1}	1.32	1.28	1.30 C	10.36	11.86	11.11 C
7.00 dSm^{-1}	0.96	1.12	1.04 C	11.00	11.914	11.457 C
Mean	**1.548**	**1.652**	**-**	**11.89 B**	**13.19 A**	

Table-4 **Fresh weight of Shoot and root (g) observed from different types of *Calendula* seeds grown by using various levels of saline irrigation water**

Salinity levels of irrigation water	**Fresh weight of shoot (g)**		**Mean**	**Fresh weight of root (g)**		**Mean**
	Carenate	Orbicular		Carenate	Orbicular	
Distilled water (control)	2.76 c	5.53 a	4.14 C	0.254 cde	0.525 a	0.389 B
1.00 dSm^{-1}	5.36 a	5.49 a	5.42 A	0.389 b	0.509 a	0.449 A
3.00 dSm^{-1}	4.24 b	5.36 a	4.80 B	0.236 def	0.530 a	0.383 B
5.00 dSm^{-1}	2.938 c	2.46 c	2.70 D	0.328 bc	0.207 ef	0.267 C
7.00 dSm^{-1}	2.25 c	2.30 c	2.28 D	0.162 f	0.316 bcd	0.239 C
Mean	**3.51 B**	**4.23 A**	-	**0.274 B**	**0.417 A**	-

Table 5. **Chlorophyll content and electrolyte leakage of leaves (%) observed from different types of *Calendula* seeds grown by using various levels of saline irrigation water**

Salinity levels of irrigation water	**Chlorophyll content of leaves**		**Mean**	**Electrolyte leakage of leaves (%)**		**Mean**
	Carenate	Orbicular		Carenate	Orbicular	
Distilled water (control)	42.46	40.54	**41.54 A**	37.50	48.05	**42.77 C**
1.00 dSm^{-1}	40.67	40.61	**40.67 AB**	54.26	50.55	**52.40 BC**
3.00 dSm^{-1}	39.57	35.74	**37.65 B**	57.90	64.72	**61.31 AB**
5.00 dSm^{-1}	28.87	30.21	**29.54 C**	66.29	64.93	**65.61 AB**
7.00 dSm^{-1}	25.00	28.26	**26.63 C**	73.29	68.33	**70.81 A**
Mean	**35.33**	**35.08**	**-**	**57.85**	**59.325**	