Drought stress is one of the most important problems in agricultural production that limits the growth and development of crops, especially in arid and semi-arid regions of the world [Waraich et al. 2011, Bayat et al. 2016]. Drought stress has a negative effect on plant growth and yield by altering physiological, biochemical and molecular processes [Zhang and Huang 2013, Khan et al. 2017]. Drought stress results in growth retardation, stomatal closure, a disorder in photosynthesis, a reduction in nutrient uptake, and causes a significant increase in the level of reactive oxygen species (ROS) [Farooq et al. 2009, Bayat et al. 2016].

There are several methods to mitigate the negative effects of environmental stresses on plant growth and yield [Shanker et al. 2014]. It seems that seed priming is the most economical, simple and low-risk approach to improve germination, emergence, seedling establishment and greater yield in many crops especially under adverse abiotic stress such as drought [Bajebhaj 2010]. Seed priming is a pre-sowing treatment that activate the metabolic processes of germination,

but the emergence of the radicle does not occur [Chen and Sung 2001]. There are various seed priming techniques including thermopriming, hydropriming, nutrient priming, hormonal-priming, osmopriming, and chemical priming [Ibrahim 2016].

Selenium (Se) is a necessary trace element for humans and plants [Tapiero et al. 2003]. Several studies demonstrate that Se at low concentrations improves physiological and biochemical processes in plants [Saffaryazdi et al. 2012, Nawaz et al. 2016]. Moreover, Se treatment has been reported to improve resistance to certain abiotic stresses, such as drought [Nawaz et al. 2015, Jiang et al. 2017], salinity [Astaneh et al. 2018], UV-irradiation [Pennanen et al. 2002], and heavy metal [Kumar et al. 2012]. Priming with selenium is an applicative technique that combines the positive effects of seed priming with an improved of Se supply, which may enhance plant tolerance against environmental adversities.

Pot marigold (*Calendula officinalis* L.) is an annual herbaceous plant of the family Asteraceae with bright or yellow-orange flowers that used for medicinal and ornamental purposes [Dole and Wilkins 2005]. Pot marigold is well known for its pharmacological effects such as anti-tumor, anti-mutagenic, anti-HIV, anti-inflammatory, antiviral and cytotoxic properties [Amirghofran et al. 2000, Re et al. 2009]. Despite ameliorative effects of selenium on crop plants has been given considerable attention under drought stress, there is no information about the response of pot marigold plant to seed priming with Se under drought stress. Therefore, this study was aimed to evaluate the effect of seed priming with Se on growth and some physiological and biochemical parameters of pot marigold plants grown under both control and drought stress conditions.

**MATERIALS AND METHODS**

**Plant materials, growth conditions, and experimental design.** This study was done in the greenhouse of the Department of Horticultural Science at the University of Birjand, Birjand, Iran in April 2018. The seeds of pot marigold (*Calendula officinalis* L. cv. ‘Orange Star’) were supplied from Pakan seed Company, Iran. The seeds were disinfected with sodium hypochlorite solution (5%) for 5 min and washed 3 times with distilled water. The seeds were primed at 25°C for 24 h in solution of Se (Na₂O₄Se, Sigma-Aldrich, Germany) at concentrations of 0, 0.5, 1, 1.5, 2 and 4 mg L⁻¹. After seed priming, the solutions decanted off and the seeds washed with distilled water and air-dried. Then three seeds were sown into 2 L plastic pots filled with sandy loam soil containing 22.8% clay, 32% silt, and 45.2% sand. Some soil properties were pH = 7.4, EC = 1.16 dS m⁻¹ and FC = 22%. After emergence, one plant was kept in each pot. The plants were irrigated three times per week to keep soil moisture at field capacity for two months until the plants reached full flowering stage. Then, the plants were exposed to two irrigation treatments: 1) control (well-watered): plants kept at field capacity; and 2) drought stress: irrigation was completely suspended for 7 days. The experiment was conducted under light intensity of 600 μmol m⁻² s⁻¹, mean day/night temperatures of 25/15°C, the relative humidity of 50–60% and a 14 h photoperiod. The experiment was based on the completely randomized design with twelve treatments and three replications per treatment. The number of measurements for each parameter was 9 times.

**Measurements.** Seventy days after sowing the seeds, the measurements were recorded. Maximum root length and number of leaves per plant were measured. The total leaf area was estimated by a Laser Leaf Area Meter (LI-3100, LI-COR Company, USA). The pot marigold plants were then harvested and washed using tap water. Shoots were excised from the roots, and then oven dried at 78°C to a constant weight. Dry weights of shoot and root were measured [Khan et al. 2016].

Relative water content (RWC) was calculated using the following equation:

\[
\text{RWC} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100,
\]

where FW is leaf fresh weight, TW is leaf turgid weight and DW is leaf dry weight [Gonzalez and Gonzalez-Vilar 2003].

To determine electrolyte leakage (EL), fresh leaves (0.5 g) were dispersed with distilled water (10 mL) in test tubes. The test tubes were then shaken at 24°C for 24 hours. At this step, the primary electrical conductivity (EC1) was measured by the electrical conductivity meter (Model Jenway, Germany). The test tubes were then transferred to an autoclave (121°C)
for 15 min and secondary electrical conductivity (EC2) was determined [Lutts et al. 1996]. Finally, the EL values were calculated using the following equation:

$$EL = \frac{(EC1/EC2) \times 100}{1}$$

The photosynthetic pigments were determined spectrophotometrically (Model Unico 2100, China) at 663 and 645 nm [Arnon 1949]. Leaf total carbohydrate content was determined according to Irgoyen et al. [1992] method using an anthrone reagent.

Total phenolic and flavonoid content, and free radical scavenging activity (FRSC) were determined according to the methods of Singleton and Rossi [1965], Yoo et al. [2008] and Koleva et al. [2002], respectively. To prepare leaf extracts, 1 g of the sample was homogenized with 10 mL of methanol at 24°C for 24 h. The homogenate was filtered and then centrifuged at 6000 rpm for 15 min. The FRSC was calculated using the following equation:

The FRSC (% inhibition) =

$$= 1 - \frac{A_{\text{Sample}(515 \text{ nm})}}{A_{\text{Control}(515 \text{ nm})}} \times 100.$$  

**Statistical analysis.** Data were subjected to analysis of variance (ANOVA) using the JMP 8 Software (SAS Campus, Cary, NC, USA). The difference between the mean values was evaluated by using LSD at the 5% level of probability.

**RESULTS**

**Plant growth.** The results showed that the effects of drought stress, Se and drought × Se interaction on the total leaf area and number of leaves per plant were significant. Drought stress decreased total leaf area and number of leaves per plant as compared to the control plants. Soil drying decreased the number of leaves per plant and total leaf area by 47% and 77%, but the reductions were 22% and 59% when 2 mg L⁻¹ Se treatment was applied to the drought-stressed plants, as compared to the control, respectively. Under the control condition, the highest leaf number and total leaf area were obtained from 2 mg L⁻¹ Se treatment (Tab. 1). Drought stress reduced root length; however, Se supplementation improved this parameter. The highest root length was observed from 1.5 mg L⁻¹ Se under drought stress. Under well-watered condition, all the levels of Se increased root length compared to the control plants (Tab. 1). Dry weight of root, shoot and total biomass of pot marigold plants were significantly affected by drought, Se and drought × Se interaction. Root, shoot and total dry weight significantly decreased under drought stress. Reduction of the mean root, shoot and total dry weight by drought were 59%, 56% and 57% compared to the control plants, respectively (Tab. 2). However, supplemental Se improved the accumulation of dry matter in all parts of pot marigold plants under water stress. The highest root dry weight

**Table 1.** Effect of seed priming with Se on number of leaves per plant, leaf area and root length of pot marigold (*Calendula officinalis* L. cv. ‘Orange Star’) plants under well-watered and drought stress conditions

<table>
<thead>
<tr>
<th>Se (mg L⁻¹)</th>
<th>Number of leaves per plant</th>
<th>Leaf area (cm²)</th>
<th>Root length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>drought</td>
<td>control</td>
</tr>
<tr>
<td>0</td>
<td>13.33 ±0.33 b</td>
<td>7.00 ±0.59 d</td>
<td>397.02 ±14.23 b</td>
</tr>
<tr>
<td>0.5</td>
<td>13.66 ±0.35 ab</td>
<td>9.00 ±0.58 cd</td>
<td>388.60 ±16.06 b</td>
</tr>
<tr>
<td>1</td>
<td>15.66 ±1.85 a</td>
<td>8.66 ±0.66 cd</td>
<td>435.76 ±12.15 b</td>
</tr>
<tr>
<td>1.5</td>
<td>15.68 ±0.88 a</td>
<td>9.02 ±0.56 cd</td>
<td>503.96 ±27.05 a</td>
</tr>
<tr>
<td>2</td>
<td>15.92 ±0.57 ab</td>
<td>9.33 ±0.90 c</td>
<td>424.02 ±23.24 b</td>
</tr>
<tr>
<td>4</td>
<td>14.33 ±0.88 ab</td>
<td>8.66 ±0.34 cd</td>
<td>428.01 ±5.69 b</td>
</tr>
</tbody>
</table>

Values are mean ±SE. Different letters indicate significant differences according to LSD test at P < 0.05
Table 2. Effect of seed priming with Se on root, shoot and total dry weight of pot marigold (*Calendula officinalis* L. cv. ‘Orange Star’) plants under well-watered and drought stress conditions

<table>
<thead>
<tr>
<th>Se (mg L⁻¹)</th>
<th>Control root dry weight (g)</th>
<th>Drought root dry weight (g)</th>
<th>Control shoot dry weight (g)</th>
<th>Drought shoot dry weight (g)</th>
<th>Control total dry weight (g)</th>
<th>Drought total dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.306 ±0.006 c</td>
<td>0.123 ±0.003 f</td>
<td>0.803 ±0.072 c</td>
<td>0.350 ±0.011 e</td>
<td>1.11 ±0.066 c</td>
<td>0.473 ±0.012 f</td>
</tr>
<tr>
<td>0.5</td>
<td>0.383 ±0.031 b</td>
<td>0.206 ±0.007 de</td>
<td>1.15 ±0.047 a</td>
<td>0.44 ±0.058 de</td>
<td>1.53 ±0.074 a</td>
<td>0.653 ±0.062 de</td>
</tr>
<tr>
<td>1</td>
<td>0.346 ±0.014 bc</td>
<td>0.210 ±0.010 de</td>
<td>0.933 ±0.060 bc</td>
<td>0.406 ±0.026 de</td>
<td>1.28 ±0.052 bc</td>
<td>0.616 ±0.028 def</td>
</tr>
<tr>
<td>1.5</td>
<td>0.496 ±0.026 a</td>
<td>0.246 ±0.004 d</td>
<td>0.903 ±0.093 c</td>
<td>0.430 ±0.036 de</td>
<td>1.40 ±0.112 b</td>
<td>0.676 ±0.040 de</td>
</tr>
<tr>
<td>2</td>
<td>0.460 ±0.012 a</td>
<td>0.220 ±0.011 de</td>
<td>1.08 ±0.078 ab</td>
<td>0.550 ±0.030 d</td>
<td>1.54 ±0.084 a</td>
<td>0.770 ±0.032 d</td>
</tr>
<tr>
<td>4</td>
<td>0.383 ±0.023 b</td>
<td>0.180 ±0.010 c</td>
<td>0.810 ±0.025 c</td>
<td>0.373 ±0.031 e</td>
<td>1.19 ±0.039 c</td>
<td>0.553 ±0.030 ef</td>
</tr>
</tbody>
</table>

Values are mean ±SE. Different letters indicate significant differences according to LSD test at P < 0.05

Table 3. Effect of seed priming with Se on chlorophyll content of pot marigold (*Calendula officinalis* L. cv. ‘Orange Star’) plants under well-watered and drought stress conditions

<table>
<thead>
<tr>
<th>Se (mg L⁻¹)</th>
<th>Chlorophyll a (mg g⁻¹ FW⁻¹)</th>
<th>Chlorophyll b (mg g⁻¹ FW⁻¹)</th>
<th>Total chlorophyll (mg g⁻¹ FW⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control drought</td>
<td>Control drought</td>
<td>Control drought</td>
</tr>
<tr>
<td>0</td>
<td>1.27 ±0.040 ef</td>
<td>0.79 ±0.047 g</td>
<td>0.57 ±0.063 cde</td>
</tr>
<tr>
<td>0.5</td>
<td>1.58 ±0.046 cd</td>
<td>1.27 ±0.066 ef</td>
<td>0.55 ±0.013 de</td>
</tr>
<tr>
<td>1</td>
<td>1.80 ±0.101 ab</td>
<td>1.37 ±0.035 ef</td>
<td>0.71 ±0.050 bc</td>
</tr>
<tr>
<td>1.5</td>
<td>1.90 ±0.153 a</td>
<td>1.40 ±0.078 de</td>
<td>0.85 ±0.022 a</td>
</tr>
<tr>
<td>2</td>
<td>1.68 ±0.080 bc</td>
<td>1.37 ±0.026 ef</td>
<td>0.78 ±0.026 ab</td>
</tr>
<tr>
<td>4</td>
<td>1.58 ±0.006 cd</td>
<td>1.19 ±0.046 f</td>
<td>0.71 ±0.051 bc</td>
</tr>
</tbody>
</table>

Values are mean ±SE. Different letters indicate significant differences according to LSD test at P < 0.05

was recorded from 1.5 mg L⁻¹ Se, whereas the highest total dry weight was obtained from 2 mg L⁻¹ Se, suggesting that seed priming with Se mitigated the growth inhibition induced by drought. Under well-watered condition, supplemental Se also increased shoot, root and total dry weight (Tab. 2).

The **RWC and EL.** The leaf RWC was significantly affected by drought, Se and drought × Se interaction. The RWC decreased under drought stress condition. However, Se treatments were effective to significantly increase the RWC under drought stress. There were no significant differences among Se treated plants under both well-watered and drought-stressed conditions. Application of 1.5 mg L⁻¹ Se increased the RWC of drought-stressed plants by 15% compared to the control plants (Fig. 1). Effect of drought stress, Se and drought × Se interaction on the leaf EL was significant. The leaf EL was intensively increased by drought treatment; however, Se treatments significantly decreased this parameter. Under drought stress condition, the lowest EL was obtained from the plants treated with 1.5 mg L⁻¹ Se, which indicates a 19% decrease compared to the control. Under well-watered, seed priming with Se decreased the EL compared to the control plants (Fig. 1).

**Chlorophyll content.** Chlorophyll content was significantly affected by drought, Se and drought × Se interaction. Drought stress decreased chlorophyll a, b
Figure 1. Effect of seed priming with Se on relative water content (RWC) and electrolyte leakage (EL) of pot marigold (*Calendula officinalis* L. cv. ‘Orange Star’) plants under well-watered and drought stress conditions. Values followed by the same letter are not significantly different according to LSD test at \( P < 0.05 \). Vertical bars indicate ±SE.
Figure 2. Effect of seed priming with Se on root and leaf total soluble sugar of pot marigold (*Calendula officinalis* L. cv. ‘Orange Star’) plants under well-watered and drought stress conditions. Values followed by the same letter are not significantly different according to LSD test at $P < 0.05$. Vertical bars indicate ±SE.

and total chlorophyll by 37%, 33%, and 36% when compared to the control plants. However, all the levels of Se increased chlorophyll a, b and total chlorophyll under drought stress and the highest values of those were obtained from 1.5 mg L\(^{-1}\) Se (Tab. 3).

**Total soluble sugar.** Total soluble sugar in the root and leaf tissues were significantly affected by drought, Se and drought × Se interaction. Drought stress increased total soluble sugar in the root and leaf tissues. The water-stressed plants primed with 2 and 4 mg L\(^{-1}\) Se had the highest total soluble sugar accumulations in the leaves and the roots, respectively. Mean total soluble sugar accumulation in the roots was higher than the leaves under both drought and control conditions (Fig. 2).

**Total flavonoid and phenolic content and FRSC.** The results showed that the effects of drought stress, Se and drought × Se interaction were significant on the total flavonoid and phenolic content, and FRSC. Drought stress increased total flavonoid and phenolic content and FRSC by 16%, 18% and 7% in comparison to the control plants. Moreover, Se supplementation increased total flavonoid and phenolic content and FRSC under well-watered and drought-stressed conditions and the highest values of those were obtained from 1.5 mg L\(^{-1}\) Se (Tab. 4).

**DISCUSSION**

Drought stress restricted growth indices of calendula plant, while seed priming with Se was helpful in alleviating the adverse effect of drought stress. Moreover, under unstressed condition, seed priming with lower concentrations of Se improved growth parameters as compared to non-primed ones. Decreasing effects of drought on plant growth have been reported in *Salvia nemorosa* [Bayat and Naseri Moghadam 2019], in cucumber [Mardani et al. 2012] and in wheat [Nawaz et al. 2013]. The first sign of water scarcity is the reduction of turgor pressure, which reduces the growth and development of the plant by reducing the division and elongation of the cells [Farooq et al. 2009]. The growth promoting ability with the application of Se were reported by Nawaz et al. [2015] in wheat and Habibi [2013] in barely. Nawaz et al. [2013] reported that seed priming with Se significantly increased root length and total biomass of drought-stressed wheat seedlings. Valabadi et al. [2010] reported that supplemental Se improved growth parameters such as dry weight of root and shoot, total biomass, root length and leaf area of *Brassica napus* under drought stress. The Se induced higher growth rate may result from the benefits of well-preserved membrane systems, which are essential for photosynthesis and respiration [Astaneh et al. 2018]. Overall, the improvement of the growth in Se treated plants could be related to its function in regulating water relation, photosynthesis, antioxidant defense systems, uptake of mineral nutrients, and activation of plant growth stimulating hormones [Saffaryazdi et al. 2012, Nawaz et al. 2016]. In this study, our results also confirmed that Se supplementa-

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**Table 4.** Effect of seed priming with Se on total flavonoid and phenolic content and free radical scavenging activity (FRSC) of pot marigold (*Calendula officinalis* L. cv. ‘Orange Star’) plants under well-watered and drought stress conditions

<table>
<thead>
<tr>
<th>Se (mg L(^{-1}))</th>
<th>Total flavonoid content (mg g(^{-1}) FW(^{-1}))</th>
<th>Total phenolic content (mg g(^{-1}) FW(^{-1}))</th>
<th>FRSC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>drought</td>
<td>control</td>
</tr>
<tr>
<td>0</td>
<td>0.40 ±0.014 g</td>
<td>0.42 ±0.033 fg</td>
<td>1.97 ±0.040 e</td>
</tr>
<tr>
<td>0.5</td>
<td>0.42 ±0.043 fg</td>
<td>0.50 ±0.026 cde</td>
<td>3.40 ±0.107 c</td>
</tr>
<tr>
<td>1</td>
<td>0.46 ±0.034 efg</td>
<td>0.55 ±0.013 c</td>
<td>3.49 ±0.158 bc</td>
</tr>
<tr>
<td>1.5</td>
<td>0.78 ±0.017 b</td>
<td>0.92 ±0.010 a</td>
<td>3.59 ±0.142 bc</td>
</tr>
<tr>
<td>2</td>
<td>0.47 ±0.040 def</td>
<td>0.51 ±0.030 cde</td>
<td>3.21 ±0.144 cd</td>
</tr>
<tr>
<td>4</td>
<td>0.46 ±0.011 efg</td>
<td>0.53 ±0.020 cd</td>
<td>2.86 ±0.149 d</td>
</tr>
</tbody>
</table>

Values are mean ±SE. Different letters indicate significant differences according to LSD test at P < 0.05.
tion improved growth parameters through the increase in chlorophyll content, RWC and antioxidant activities under drought stress.

According to the results, the leaf RWC decreased under drought stress. However, treatment with Se at lower concentrations significantly enhanced this parameter. Similar results were reported in *Trifolium repens* [Wang 2011], in olive [Proietti et al. 2013] and in maize [Nawaz et al. 2016]. The RWC of the leaf can show the tolerance of the plant to drought stress, and its reduction is one of the most important effects of drought [Karimi et al. 2015]. Yao et al. [2009] reported that Se increased RWC by improving the growth and development of the root system and reducing transpiration rate under water stress. In this study, a positive relationship was observed between RWC and root length, total biomass, chlorophyll content, total soluble sugar, and antioxidant activity.

In this study, drought stress significantly increased the EL of leaf, while seed priming with Se decreased this parameter. Similar results were reported in wheat [Nawaz et al. 2015] and in spring barley [Habibi 2013]. The EL is widely used as a criterion for evaluating drought stress tolerance in plants. The present results indicated that drought stress significantly increased the leaf EL of pot marigold plants, which suggest an injury of cell membranes. Under drought stress, peroxidation of membrane lipids can initiate by ROS, which results in loss of membrane semi-permeability [Anjum et al. 2011]. It is demonstrated that the application of Se (low level) can reduce peroxidation of membrane lipids by increasing the antioxidant capacity of plants exposed to water stress [Yao et al. 2009].

In this study, the chlorophyll content decreased under drought stress. However, treatment with Se significantly increased this parameter. These results are similar to the findings on wheat and *Lycium chinense* [Yao et al. 2009, Dong et al. 2013]. The ROS are produced in the cell during the drought stress, which causes the destruction of the photosynthetic system and ultimately the decomposition of chlorophyll [Farooq et al. 2009]. The increase in chlorophyll content of Se-primed pot marigold exposed to water stress may be related to the protective role of Se from chloroplast enzymes and thus increasing the biosynthesis of photosynthetic pigments [Pennanen et al. 2002].

In our study, the accumulation of total soluble sugar in the leaf and root tissues increased significantly under water stress conditions in both Se primed and non-primed plants. Similar results were reported by Nawaz et al. [2013] in wheat and Hajiboland et al. [2015] in alfalfa. Organic osmolytes, such as soluble sugar, accumulate in plants in response to abiotic stress [Hsu and Kao 2003]. Emam et al. [2014] stated that Se treatment increased the concentration of organic osmoprotectants in plant tissue of rice. The accumulation of total soluble sugar by Se helped plants to maintain water relations under drought stress conditions. Selenium may contribute to the activity of amylase enzyme under drought stress, which results in starch degradation [Nawaz et al. 2015].

In the present study, the total flavonoids, phenols, and FRSC of pot marigold leaves increased significantly by drought stress. The production of the ROS such as superoxide, hydrogen peroxide and hydroxyl radicals increase under drought stress that damage lipids, nucleic acids, proteins, and carbohydrates [Anjum et al. 2011]. In order to decrease the levels of ROS, plants develop antioxidant enzymatic and non-enzymatic defense systems [Farooq et al. 2009]. The increase in phenols and flavonoids improves FRSC of plant cells to reduce the damage caused by oxidative stress. In this study, supplemental Se increased total flavonoids, phenols, and FRSC which is in agreement with the findings of Nawaz et al. [2015] in wheat and Habibi [2013] in barley. Kaur et al. [2014] reported that Se supplementation could scavenge the ROS through regulation of the level of antioxidants. Astaneh et al. [2018] reported that Se application increased the phenolic content of garlic leaves by increasing the activity of phenylalanine ammonia-lyase enzyme. The appropriate concentration of Se decreases the $H_2O_2$ levels in plants exposed to drought stress through the reactivation of FRSC especially $H_2O_2$-quenchers like glutathione peroxidase [Kumar et al. 2012, Andrade et al. 2018].

**CONCLUSION**

The results of this study showed that Se supplementation at lower concentrations had beneficial effects on pot marigold plants both under control and water stress conditions. Treatment with Se alleviated...
negative effects of drought stress through improvement of water relations, the integrity of cell membranes, accumulation of soluble sugars and increased antioxidant activity. According to the results of this study, seed priming with Se (especially 1.5 mg L⁻¹) can be recommended in the areas affected by drought.

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REFERENCES


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