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Effects of Iron Application on Growth Characters and Flower Yield of *Calendula officinalis* L. Under Water Stress

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Abstract: To evaluate the effects of iron application on growth characters of *Calendula officinalis* L. under end season water deficit condition, an experiment was conducted as split plot at the Research Farm of Faculty of Agriculture, Urmia University (latitude 37.53°N, 45.08°E and 1320m above sea level), Urmia, Iran in 2010. Treatments were irrigation (irrigation disruption at first, second, third harvest and without disruption as control) as main plot and amount of iron fertilizer (0, 0.5, 1 and 1.5 l/ha iron as sub plot were arranged in randomized complete block design with three replications. Data analysis of variance showed the significant effect of irrigation on the stem diameter and significant interaction between irrigation disruption and iron on the numbers of sub stem and leaf, leaf weight, the latest sub stem number, single leaf area, flower diameter and flower yield. The maximum number of sub stem (9.66), single leaf area (1170 mm^2) and leaf weight (8.93 g/plant) were obtained from irrigation disruption after third harvest and 1.5 l/ha iron application. The maximum numbers of leaf (13.82) was observed from irrigation disruption after third harvest and 1 l/ha iron application. The maximum yield of flower (591.7 kg/ha) was observed from control treatment (without irrigation disruption) and 1 l/ha iron application.

Key words: *Calendula officinalis* · Iron · Leaf · Water stress · Yield

INTRODUCTION

Calendula officinalis L is an annual aromatic herb belongs to the Asteraceae family. While the biennial form grows wild in the Southern, Eastern and Central Europe [1], the annual form is more widely cultivated [2]. This medicinal plant contains sesquiterpenes, glycosides, saponins, xanthophylls, triol triterpenes, flavonoids and volatiles [3].

Limited water supply is major environmental constraint in productivity of crop and medicinal plants. Moisture deficiency induces various physiological and metabolic responses like stomatal closure and decline in growth rate and photosynthesis [4]. Drought stress is one of the most important abiotic stress which are generally accompanied by heat stress in dry season [5].

Although, micronutrient elements are needed in relatively very small quantities for adequate plant growth and production, their deficiency may cause great disturbance in the physiological and metabolic processes involved in the plant. Thus, the application of micronutrients fertilizer in the cultivation zone may not be meeting the crop requirement for root growth and nutrient use. The alternative approach is to apply these micronutrients as foliar sprays. Six micronutrients including Mn, Fe, Cu, Zn, B and Mo are known to be required for all higher plants [6]. Iron deficiency (iron chlorosis) is an important nutritional disorder in fruit trees that results from impaired acquisition and use of the metal by plants, rather than from a low level of Fe in soils. The most evident effect of Fe deficiency is a decreased content of photosynthetic pigments, which results in the relative enrichment of carotenoids over chlorophylls and leads to the yellow color that is characteristic of chlorotic leaves [7].

Iron is critical for chlorophyll formation and photosynthesis and is important in the enzyme systems and respiration of plants [8]. Torun *et al*. [9] and Grewal *et al*. [10] reported increased dry matter production for application of micronutrients over control. Macro and micronutrients deficiencies have been reported for different soils and crops [11]. Soyder and Schmidt's [12] research suggests Fe could alter drought resistance through its effects on root growth.

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The importance of Pot marigold as a multi purpose plant (flower, seed, essential oil and seed oil as economic production) and end season water deficit frequency in Iran indicated that investigation of growth under this condition is considered necessary. However, iron as an important nutritional disorder in plants and the scares of studies on iron affection in water deficit condition on *Calendula officinalis* shows the importance of this effect that is the main objective of present study.

MATERIALS AND METHODS

A field experiment was carried out as split plot arrangement based on randomized complete block design with three replications. Experiment was conducted at the Research Farm of Urmia University with latitude of 37.53°N, 45.08°E and 1320 m above sea level in 2010. Experimental units in each replication comprised of 10 line of 2 meters long. Inter and intra row spacing were 0.3 and 0.05 meters, respectively. Treatments were irrigation disruption after first, second and third harvest as well as control in main plots and amount of iron including (0, 0.5, 1 and 1.5 l/ha of Nano Iron Chelate obtained from Khazra Company).

The flowing measurements were recorded at the flowering stage on five repetitive plants in each treatment per replication: stem length (cm), stem diameter (cm), number of sub stem, capitulate diameter (cm), stem weight (g), leaf weight (g), leaf area (mm^2), flower diameter (cm) and flower yield (kg/ha). The dry weight recoded after drying of samples in an oven with 70°C temperature for 72 hours.

The single leaf area was determined by leaf area meter (Area Ueter AM 200). Leaf area index (LAI) measured by LAI meter (model LP-80).

Statistical Analysis: Analysis of variance (ANOVA) on data was performed using the general linear model (GLM) procedure in the SAS software (SAS Institute Inc.). The Student-Neuman Keul's test (SNK) was applied to compare treatment means using the MSTATC software package.

RESULTS

Data analysis of variance (ANOVA) showed the significant effect of irrigation disruption on the Stem diameter ($P \leq 0.05$) and significant interaction effect between irrigation disruption and iron on the numbers of sub stem, numbers of leaf, leaf weight, the latest sub stem number and single leaf area, flower diameter and flower yield ($P \leq 0.01$) (Table1).

Table 1: Effects of iron application on growth characters and flower yield of *Calendula officinalis* L. under water stress

		Means Squares (MS)				
Source of Variation	df	Stem length	Stem diameter	Numbers of sub stem	Numbers of leaves	Leaf weight
Replication	2	6.19	0.00008	1.10	0.79	0.17
irrigation disruption(A)	3	10.10	0.0022*	5.15**	2.68*	9.52**
Error	6	12.88	0.0004	0.93	2.08	0.90
iron(B)	3	31.92	0.00009	3.17*	3.05**	3.90*
Irrigation×iron	9	23.75	0.0007	3.92**	3.14**	7.56**
Error	24	15.67	0.0005	0.88	0.63	1.27
Coefficicient of Variance (%)		11.05	6.15	13.49	6.50	21.43

*and ** Significant at $P \leq 0.05$, $P \leq 0.01$, respectively; df, degree of freedom

Continued Table 1: Effects of iron application on growth characters and flower yield of *Calendula officinalis* L. under water stress

		Means Squares (MS)				
Source of Variation	df	Stem weight	The latest sub stem number	Single leaf area	flower diameter	Flower yield
Replication	2	10.48	0.20	1145.23	0.018	4876.77
irrigation disruption(A)	3	5.71	9.41	79576.57	0.065	43459.60**
Error	6	7.36	0.020	48268.19*	0.047	2005.16
iron (B)	3	4.37	0.08*	35320.30	0.007	9889.87*
Irrigation ×iron	9	10.87	0.08**	156672.01**	0.282**	23084.80**
Error	24	7.28	0.020	23873.26	0.077	2815.05
Coefficicient of Variance (%)		23.03	3.03	25.30	5.52	12.32

* and ** Significant at $P \leq 0.05$, $P \leq 0.01$, respectively; df, degree of freedom

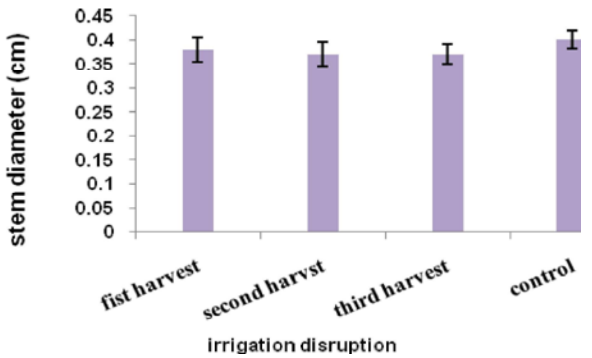

Fig. 1: Effect irrigation disruption on stem diameter of *Calendula officinalis* L. Error bars show the Standard deviation (SD).

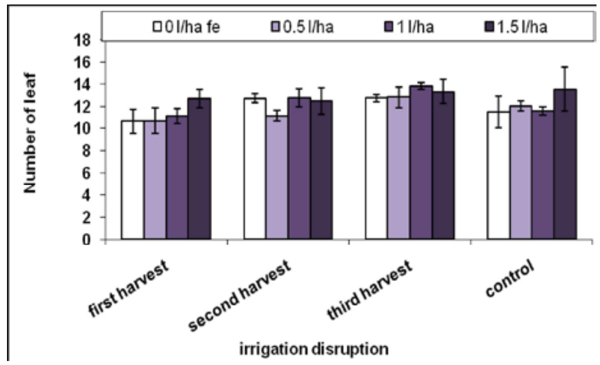

Fig. 3: Effect of iron application on number of leaf of *Calendula officinalis* L. under water stress. Error bars show the Standard deviation (SD).

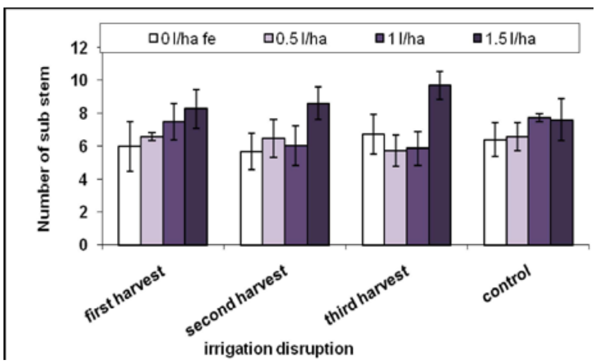

Fig. 2: Effect of iron application on number of sub stem of *Calendula officinalis* L. under water stress. Error bars show the Standard deviation (SD).

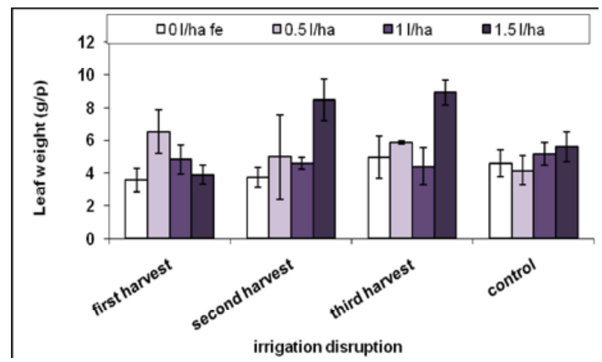

Fig. 4: Effect of iron application on leaf weight of *Calendula officinalis* L. under water stress. Error bars show the Standard deviation (SD).

The maximum stem diameter (0.42 cm) was observed from control treatment (without irrigation disruption). The minimum (0.36 cm) belonged to irrigation disruption after third harvest that was not different with irrigation disruption after second harvest (Figure 1).

The maximum number of sub stem (9.66) was observed from irrigation disruption after third harvest and 1.5 l/ha iron application and the minimum one (5.66) was observed in irrigation disruption after first harvest and control treatment (without iron application) (Figure 2). Despite of greater number of sub stem via higher concentrations of iron in all irrigation levels, this increasing numbers were greater than control treatment of irrigation (Figure 2).

The maximum number of leaf (13.82) was observed from irrigation disruption after third harvest and1 l/ha iron application and the minimum one (10.63) was obtained from irrigation disruption after first harvest and control treatment (without iron application), followed by irrigation disruption after first harvest and 0.5 l/ha iron application (Figure 3).

The maximum single leaf weight (8.93 g/p) was observed at irrigation disruption after third harvest and 1.5 l/ha iron application and the minimum one (3.57 g/p) was obtained from irrigation disruption after first harvest and control treatment (without iron application). The leaf weight increased along with greater amounts of iron, but this augment was bigger in mild water stress than control. Inversely, severe stress, irrigation disruption after first harvest, need to the small amounts of iron to develop their leaves (Figure 4).

The maximum of latest sub stem number (5.1) was observed from irrigation disruption after third harvest and control treatment (without irrigation disruption) and1.5 l/ha iron application and the minimum one (3.0) was obtained from irrigation disruption after first harvest and control treatment (without iron application) (Figure 5).

The maximum single leaf area (1170 mm^2) was observed from irrigation disruption after third harvest and1.5 l/ha iron application and the minimum one (380.5 mm^2) was obtained from irrigation disruption after first harvest and without iron application.

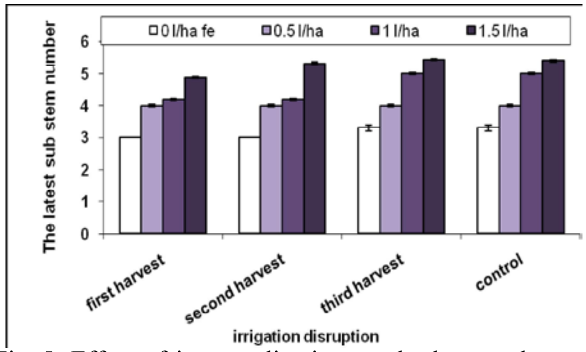

Fig. 5: Effect of iron application on the latest sub stem number of *Calendula officinalis* L. under water stress. Error bars show the Standard deviation (SD).

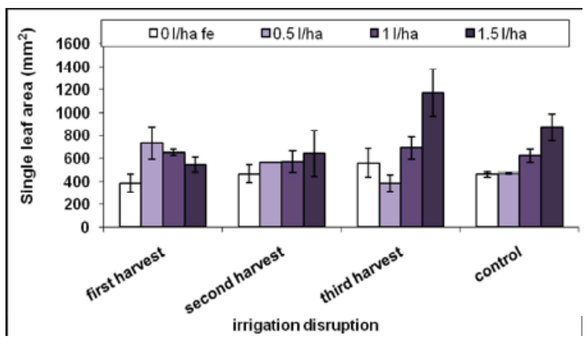

Fig. 6: Effect of iron application on single leaf area of *Calendula officinalis* L. under water stress. Error bars show the Standard deviation (SD).

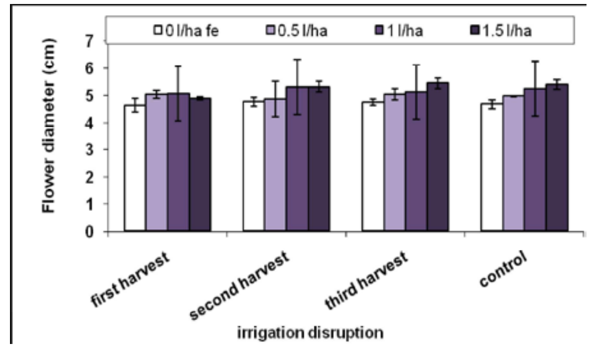

Fig. 7: Effect of iron application on flower diameter of *Calendula officinalis* L. under water stress. Error bars show the Standard deviation (SD).

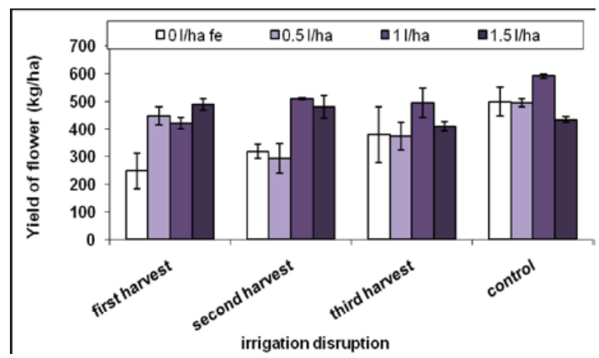

Fig. 8: Effect of iron application on yield of flower of *Calendula officinalis* L. under water stress. Error bars show the Standard deviation (SD).

The single leaf area had the same trends with leaf weight, along with experimental treatments. But, increasing of leaf area was reduced in mild stress earlier than leaf weight (Figure 6).

The greatest capitulate diameter (5.44 cm) belonged to irrigation disruption after third harvest and 1.5 l/ha iron and the smallest diameter (4.64 cm) belonged to irrigation disruption after first harvest and control treatment (without application (Figure 7). Changes in capitulate diameter was more gradual than leaf character like leaf area (Figure 6) and weight (Figure 4).

The highest yield of flower (591.7 kg/ha) was obtained from control treatment (without irrigation disruption) and 1 l/ha iron application. This preferable of iron concentration (1 l/ha) was maintained in irrigation disruption after second and third harvest as the mild water stress. But, the *Calendula* needs to more concentration of iron spray to raise their flower yield. So that the lowest yield of flower (249.11 kg/ha) was obtained from irrigation disruption after first harvest and control treatment (without iron application) (Figure 8).

DISCUSSION

Like our results, Razmjoo *et al*. [13] and Baghalian *et al*. [14] reported that drought stress caused a significant reduction in plant height, shoot weight and flower yield of *Matricaria chamomilla*. Drought stress significantly reduce the plant height, shoot dry weight, flower diameter, flower fresh and dry weight in marigold based on repots of Abdul- Wasea and Khalid, [15].

Although significant reduction in plant height and lateral stem number might be due to decreasing of the evaporation area of leaves and it eventually caused low dry matter at the end of growth period under water deficit stress conditions. Those might be correspond to the fact that under water deficit stress stomata become closed or half-closed and this leads to a decrease in absorbing CO_2 and on the other hand, the plants consume a lot of energy to absorb water, which cause a reduction in producing photosynthetic matters [16]. Many reports showed that drought stress could lead to decrease in plant photosynthesis [17, 18].

The loss of photosynthesis in drought stressed condition result in the loss of dry weight production reported in sunflower, canola, mungbean, common bean, topiary bean and *Sesuvium portulacastrum* plants [19, 20].

Based of present study, the leaf characters (weight, area and numbers) were improved by iron nutrition. However, we found the higher yield of flower in iron treatments. Although changes and increasing in size and yield of marigold (*C. officinalis*) plants were different in each level of water stress. Marschner [21] stated that micronutrient elements have positive effect on photosynthesis in plants. Torun *et al*. [9] and Grewal *et al*. [10] reported increased dry matter production for application of micronutrients over control. Schmidt and Snyder [22] suggested Fe DTPAs influence on shoot growth depended on temperature and growth rate; during warm temperatures and fast growth, succulent leaf tissue absorbs more FeDTPA. Abd El- Wahab [23] reported that micronutrients such as iron, manganese and zinc have important roles in plant growth and yield of aromatic and medicinal plants. Micronutrients, especially Fe acts as metal components of various enzymes and also is associated with saccharide metabolism, photosynthesis and protein synthesis. Iron has important functions in plant metabolism, such as activating catalase enzymes associated with superoxide dismutase, as well as in photorespiration, the glycolate metabolism and chlorophyll content [21, 24]. A balanced fertilization program with macro and micronutrients in plant nutrition is very important in the production of high yield with high quality products [25]. Baghalian *et al*. [14] reported that drought stress caused a significant reduction in plant height, shoot weight and flower yield of *Matricaria chamomilla*. Moreover, Drought stress significantly reduces the plant height, shoot dry weight, flower diameter, flower fresh and dry weight in marigold based on reports of Abdul-Wasea and Khalid [15]. Results of Sadeghipour and Abbasi [26] showed that water stress decreased bacteria activity, number of pods per plant, number of seeds per pod, seed weight and seed yield in soybean.

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