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Research article

EFFECT OF INCREASING SEVERITY OF DROUGHT STRESS ON MORPHOLOGY AND ESSENTIAL OIL OF POT MARIGOLD

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ABSTRACT: Flower heads of *Calendula officinalis* L. are used for medicinal or culinary purposes. To evaluate effect of water deficit stress on morphology and yield of *Calendula officinalis*, a split plot experiment was carried out based on randomized complete block design with three replications in 2011. Treatments were irrigation (irrigation after 30, 60, 90 and 120 mm evaporation from class A pan) as main plots and gradual rise intensification of water deficit (increasing the irrigation intervals after first irrigation cycle amounted 0, 5, 10 and 15 mm evaporation) as sub plots. Results of ANOVA showed significant interaction between irrigation and increasing stress strength on capitulate diameter, percentage of essential oil, yields of dried flower and essential oil. The highest yields of flower (934.7 kg/ha), essential oil (4.92 kg/ha) and single dried flower weight were obtained from irrigation after 30 mm evaporation, and control treatment of water deficit strength (0 mm evaporation). The highest essential oil percent (0.77%) belonged to irrigation after 120 mm and increasing of 15 mm evaporation. In conclusion, increasing water deficit caused to reduction all traits, except the essential oil content that it raised along with sever stress.
Key Words: *Calendula officinalis,* capitulate, essential oil, irrigation, yield.

INTRODUCTION

Calendula officinalis L. (English marigold, pot marigold) belongs to the Asteraceae (Composite) family and is native to Mediterranean region, it is an annual plant with pinnate divided leaves and bright or yellow orange daisy-like flowers which are used as a decorative plant in horticultural industry and for medicinal uses and food industry to confer both color and flavor to foods [10,14]. Pot marigold is well known for its pharmacological effects such as anti-inflammatory, antiviral, anti-HIV, anti-tumor, anti-mutagenic and cytotoxic properties and it is used mainly for treating cutaneous and internal disease [4,25]. The main chemical constituents of *C. officinalis* include terpenoids, phenolic acids, flavonoids, isorhamnetin, carotenoids, glycosides, vitamin C and sterols [25; 5], which have antioxidant activities and play important role in human health [20].

Drought stress is one of the most important environmental stresses affecting agricultural productivity around the world and may result in considerable yield reductions [8,19]. Water deficit resistance refers to plant ability to grow and reproduce satisfactorily under drought conditions, and drought acclimation is its ability to slowly modify its structure and function so that it can tolerate drought better [30]**.** Drought affects nearly all the plant growth processes; however, the stress response depends upon the intensity, rate, and duration of exposure and the stage of crop growth [21]. It has been established that drought stress is a very important limiting factor at the initial phase of plant growth and establishment. It affects both elongation and expansion growth [6,7,18,27]. Deficit irrigation is a water management method in which water will be saved with accepting little yield reduction without any severe damage to the plant. In deficit irrigation the water amount reduces compared to normal irrigation at each irrigation level [13]. Accordingly in an experiment conducted by Jorat et al [16] on two forage sorghum cultivars, under irrigation treatments (irrigation after 70, 100 and 130 mm accumulative evaporation from evaporation class A pan), the highest forage yield was produced by Speedfeed cultivar at all irrigation levels.

In another study by Emam et al [12] on *Phaseolus vulgaris* L., the deficit irrigation was induced by reducing water volume of consecutive irrigation as well 100, 75, 50 and 25% of field capacity by weight. The results showed that in 50 and 25% water stress levels, all plant pods of both cultivars were aborted. Drought stress increased secondary metabolites in medicinal plants such as *Hypericum brasiliense* [1] and *Ocimum americanum* L. [17], and did not showed any change of essential oil in *Lippia berlandieri* Schauer [11]. But the purposes of this study were: to delineate the effect of increasing water deficit stress on yield of flower and essential oil, essential oil percentage and growth of flower capitulate and determine the best irrigation regime in calendula.

MATERIALS AND METHODS

Experimental Site

To investigate the effect of irrigation intervals and increasing water deficit stress on morphology and yields of dried flower and essential oil of *Calendula officinalis*, a field experiment was carried out as split plot based on complete blocks design with three replications. The experiment was conducted at Research Farm of Urmia University (latitude of 37.53°N, 45.08°E and 1320 m above sea level) in 2011. Experimental units in each replication composed of 8 line of 2 m long. Inter-row and inter-plant spacing was 0.3 and 0.05 m, respectively. Water stress applied on the 4-5 leaf stage of plant growth. The field was kept weed free by hand weeding. Treatments were irrigation regimes (irrigation after 30, 60, 90 and 120 mm evaporation from class A pan) as main factor allocated to main plots and (0, 5, 10 and 15 mm evaporation from class A pan) increase to main factors as sub factor, allocated to subplots.

Measurements

Capitulate diameter (cm) was measured at the flowering stage on five plants in each plot. Fresh flower heads were collected from each treatment from the flowering stages, air dried and weighed to extract the essential oil. Dry flower heads (10 g) from each of these treatments were hydro distilled for 3 h using a Clevenger-type apparatus [9]. The essential oil content was measured as a percentage of weight. Also yield of dried flower (kg/ha) was calculated according to the below formula.

Yield of essential oil= flower yield × essential oil percentage

Statistical Analysis

Analysis of variance (ANOVA) of data was performed using the general linear model (GLM) procedure in the SAS software [26]. The student-Neuman Keul's test (SNK) was applied to compare treatments means using the MSTATC software package.

RESULTS

Results of analysis of variance (ANOVA) showed the significant interaction between irrigation and increasing stress strength on capitulate diameter, essential oil percentage, and flower and essential oil yields ($P \leq 0.01$) (Table1).

Table 1. The effect of increasing severity of drought stress on some characteristics of flower in *Calendula officinalis*

Source of variation	df	Mean square (Ms)				
		Capitulate diameter	essential oil Percentage	Yield of flower	Yield of essential oil	single dried flower weight
Replication	2	0.0045	0.000165	851.5	147.73	0.000165
Irrigation(A)	3	8.1164**	0.09383**	901266.0**	190751.46**	0.938278**
Error	6	0.0048	0.00008	121.3	85.48	0.000084
Stress strength(B)	3	0.3833**	0.00423**	23653. 7**	4319.16**	0.004228**
A × B	9	0.0681**	0.00051**	1384.0**	407.24**	0.000515**
Error	24	0.0025	0.00004	42.1	32.42	0.000043
Coefficient of variance (%)		1.11	1.06	1.21	1.83	1.06

** Significant at 1% probability level; df, degree of freedom.

Means comparison revealed that the maximum capitulate diameter (6.11cm) was obtained from irrigation after 30 mm and control treatment of water deficit strength (0 mm evaporation), and minimum of it (3.58 cm) was observed at irrigation after 120 mm and 15 mm additive evaporation per each irrigation cycle (Figure 1). Capitulate diameter was decreased along with increasing irrigation interval from 30 to 120 mm evaporation and stress severity from 0 to 15 mm evaporation at each irrigation cycle.

The highest percentage of essential oil (0.77 %) was obtained from irrigation after 120 mm and increased stress by 15 mm evaporation. And minimum essential oil content (0.52 %) belonged to irrigation after 30 mm and strength of 5 mm evaporation (Figure 2).
Maximum yield of flowers (934.7 kg/ha) was obtained from irrigation after 30 mm and control treatment of water deficit strength (0 mm evaporation), and the least (185.54 kg/ha) was observed from irrigation after 120 mm and increasing strength of 15 mm evaporation (Figure 3).
The highest yield of essential oil (4.92 kg/ha) was obtained from irrigation after 30 mm and control treatment of water deficit strength (0 mm evaporation), and the lowest (1.43 kg/ha) was observed in irrigation after 120 mm and increasing strength of 15 mm evaporation per each irrigation cycle (Figure 4). With increasing intervals of irrigation, essential oil yield was decreased. The greatest weight of single dried flower (0.12 g) was obtained from irrigation after 30 mm and control treatment of water deficit strength (0 mm evaporation), and the least amount (0.08 g) was obtained from irrigation after 120 mm and increasing strength of 15 mm evaporation per each irrigation cycle (Figure 5).

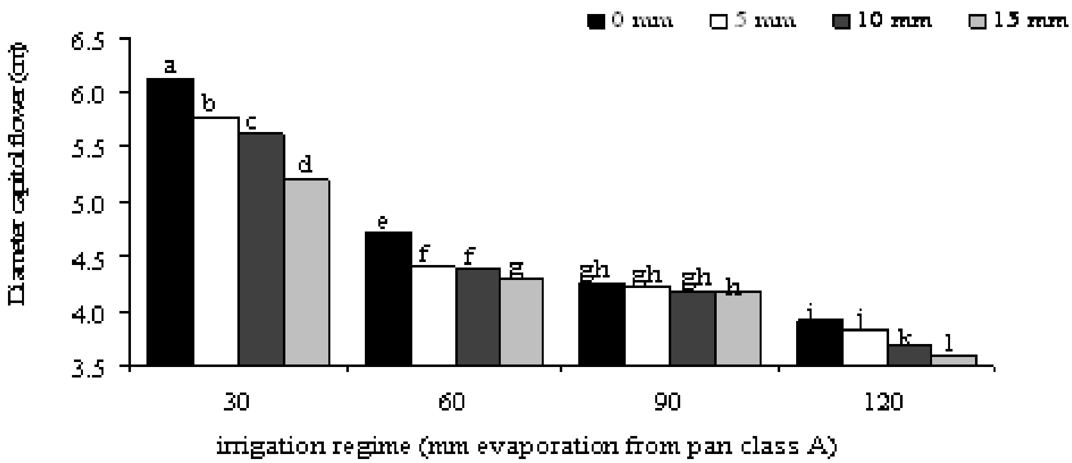

Figure 1. Means comparison of capitulate diameter in *Calendula officinalis* L. under different irrigation regime in one harvest. The same letters show non significant differences

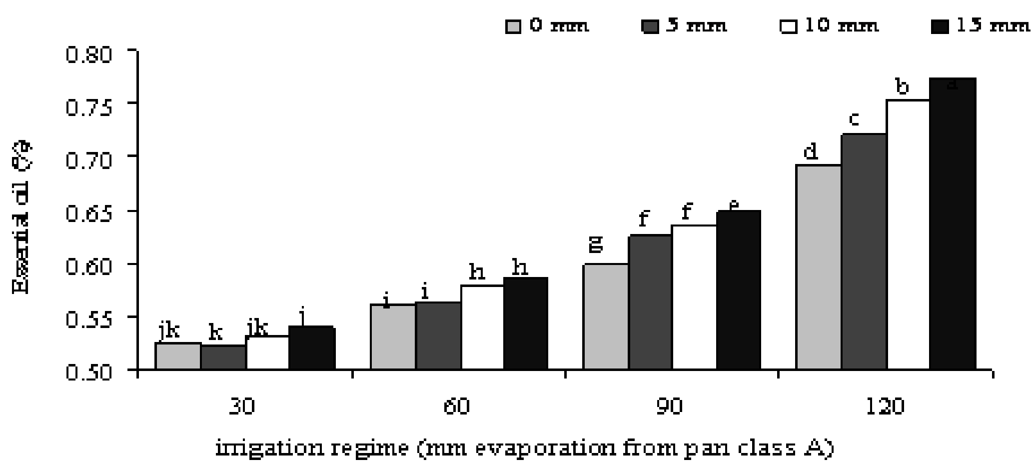

Figure 2. Means comparison of essential oil percentage in *Calendula officinalis* L. under different irrigation regime in one harvest. The same letters show non significant differences

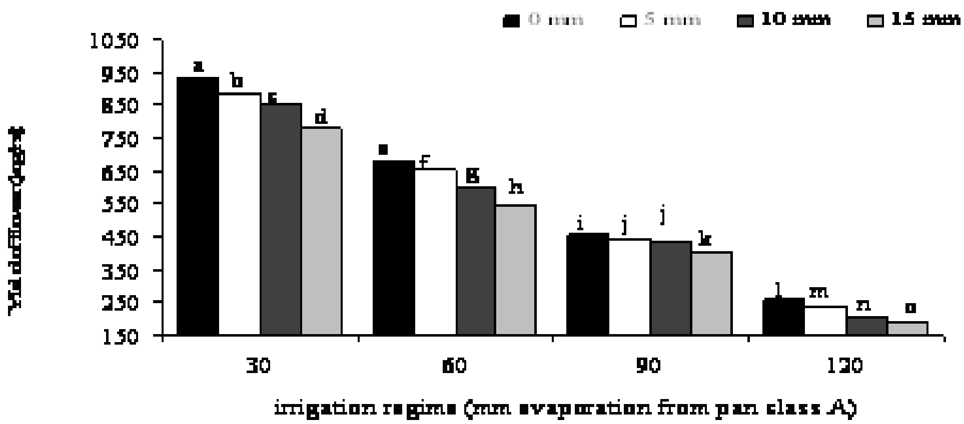

Figure 3. Means comparison of yield of flower in *Calendula officinalis* L. under different irrigation regime in one harvest. The same letters show non significant differences.

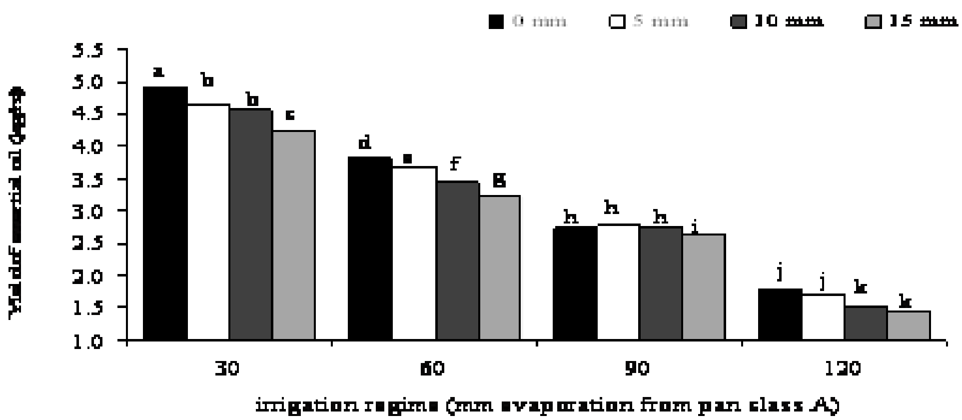

Figure 4. Means comparison of yield of essential oil in *Calendula officinalis* L. under different irrigation regime in one harvest. The same letters show non significant differences

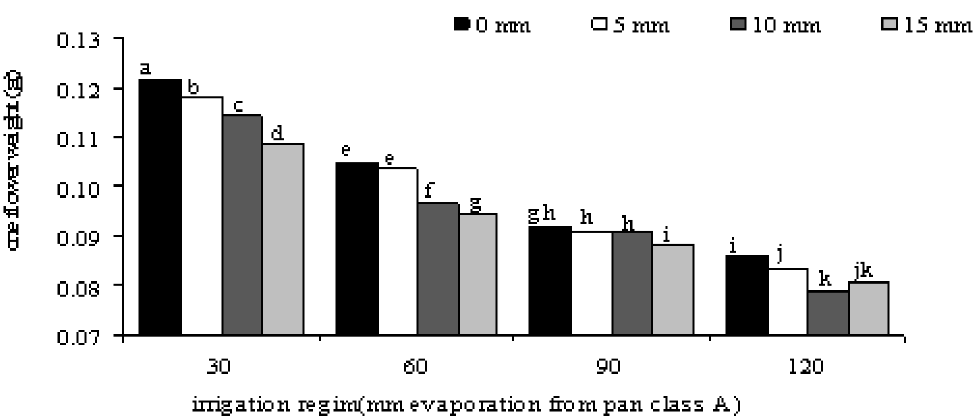

Figure 5. Means comparison of single dried flower weight in *Calendula officinalis* L. under different irrigation regime in one harvest. The same letters show the non significant differences

Correlation analysis (Table 2) of capitulate diameter with yield of flower, yield of essential oil and single dried flower weight were positive and significant ($P \leq 0.01$). However, there were positive and significant correlation between yield of flower with yield of essential oil and flower weight. Correlation between essential oil yield and single dried flower weight was significantly positive, but it was negative between capitulate diameter and essential oil percent. There is a negative significant correlation between essential oil percentage and yield of flower and essential oil.

Table 2. Correlation coefficients between measured traits in *Calendula officinalis* L

Variables	Capitulate diameter	Percentage of essential oil	Yield of flower	Yield of essential oil
Capitulate diameter				
Percentage of essential oil	-0.85**			
Yield of flower	0.95**	-0.95**		
Yield of essential oil	0.94**	-0.96**	0.99**	
Single dried flower weight	0.97**	-0.90**	0.98**	0.97**

**: Significant at 1% probability level.

DISCUSSION

Our results indicated that the severe drought stress decreased yield of dried flower, essential oil and capitulate diameter, but the essential oil percentage was greater than before along with increasing irrigation interval (from irrigation after 30 to 120 mm evaporation). Well irrigated plants led to the highest yield of flower and essential oil. In a research conducted by Pirzad & Shokrani [24] the maximum capitulate diameter in *Calendula officinalis* L. was observed in control treatment (without irrigation disruption). Drought stress increases the essential oil percentage of more medicinal and aromatic plants, because in case of stress, more metabolites are produced in the plants and substances prevent from oxidization in the cells. But essential oil content was reduced under drought stress, because the interaction between the amount of the essential oil percentage and flower yield is consider important as two components of the essential oil content. In that, exerting stress condition increases the essential oil percentage, but flower yield decreases by the drought stress [3]. Pirzad et al [23] reported that the lowest yield of essential oil in *Matricaria chamomilla* L. was obtained at 55 % field capacity is due to water deficit. Damage resulting from water deficit stress is related to the detrimental effects of dessication on protoplasm and to an increase in solute concentration as the protoplasm volume shrinks, which may itself have serious structural and metabolic consequences. The integrity of membranes and proteins is also affected by dessication, which in turn leads to metabolic dysfunctions. In addition to membrane damage, numerous studies have shown that cytosolic and organellar proteins may undergo substantial loss of activity or complete denaturation when dehydrated. Photosynthesis can be affected by water stress in two ways, closure of the stomata and effects on the structural integrity of the photosynthetic machinery [15]. Omidbaigi et al [22] measured essential oil content in *Ocimum basilicum* under different irrigation regimes. Percentage of essential oil were 1.12, 1.04, 1.26 and 0.99 in plants irrigated with 100, 85, 70 and 55 % field capacity, respectively. Shokrani et al [28] reported that results showed an increase in essential oil content and yield with increasing water stress severity and higher yield of essential oil obtained from mild water stress. Singh & Ramesh [29] reported that water deficit stress reduced the oil yield of rosemary on a hectare basis. Khalid [17] evaluated the influence of water stress on the yield of essential oil of two species of *Ocimum basilicum* L. (sweet basil) and *Ocimum americanum* L. (American basil). For both species under water stress, essential oil percentage and the main constituents of essential oil increased. The yield of flower and essential oil in coriander were achieved under non stress conditions, but the highest percentage of oil was achieved under water stress conditions [2].

CONCLUSION

It can be concluded that increasing water deficit stress decreased some morphological traits of Calendula and these increased percentage of essential oil. But this increase of essential oil percent can not lead to raise the yield of essential oil, because it reduce along with irrigation intervals and water deficit stress strength (gradual increase of irrigation intervals) as well as the flower yield.

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