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Research Article

INFLUENCE OF PLANT DENSITY AND IRRIGATION METHOD ON THE GROWTH, FLOWERING AND QUANTITY OF ESSENTIAL OIL OF *CALENDULA OFFICINALIS* L.

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ABSTRACT
Calendula officinalis L. is an important medicinal and ornamental plant. This study evaluated the effect of plant density (20, 40, 60 and 80 plants/m^2) and irrigation methods (flooding, furrow and ridge and sprinkler) on quantity and quality features of calendula. Experiment was designed in factorial on the basis of completely randomized block design with 3 replicates in 36 plots. Studies were carried out on experimental field in Islamic Azad University, Rasht, Iran. Investigated characteristics were plant dry weight, flower number/m^2, stem number/plant, flower dry weight, flower diameter, and quantity of essential oil. Based on analysis of variance, the effect of plant density and irrigation method on all measured characters was significant at 0.05 and 0.01 level of probability. Maximum of flower dry weight/surface unit (149.252 g), flower number/m^2 (857.267), and amount of essential oil/100 g dried flower (0.232 mL) were obtained at the density 60 plants/m^2. Maximum of plant dry weight (76.1867 g), and stem number (10.744) were obtained at the 20 plants/m^2. Maximum flower diameter (52.111 mm) was calculated in plants grown under the 20 plants/m^2. The best method of irrigation was furrow and ridge.

Key words: *Dry weight, flower production, Iran, pot marigold, flooding irrigation, furrow and ridge irrigation, sprinkler irrigation*

INTRODUCTION
English marigold (*Calendula officinalis* L.) belongs to the Asteraceae family; it is an annual, aromatic, medicinal and ornamental herb (Bcerentrup and Robbelen, 1987; Cromack and Smith, 1988). The leaves and flowers of marigold are applied in horticulture, medicine, cosmetics, perfume, pharmaceutical preparation, food and other industries (Marczal, 1987; Muusa et al., 1992; Van Wyk and Wink, 2004; Gazim et al., 2008). One of the main components of active ingredient in *Calendula officinalis* L. are essential oils which the most of them synthesize in its orange petals (Omidbaighi, 2005). Flower essence is using for food and medicine (Hamburger et al., 2003; Janke, 2004; Jimenez-Medina et al., 2006).
Plant density, irrigation regime, sowing date and plant age are effective factors on quantity and quality of plants products (Tiwari et al., 2003; Saif et al., 2003; Okoh et al., 2007; Morteza et al., 2009). Plant density and irrigation method are the two important factors that affect directly on the yield and flower number, amount of essential oils and yield components (Marisol Berte et al., 2003; Saif et al., 2003; Tiwari et al., 2003). There are several reports about the effect of genetically and environmental factors on essential oils of *Calendula officinalis* L. (Miguel et al., 2004; Danielski et al., 2006; Okoh et al., 2008).
The objective of this study was to investigate the effects of three irrigation methods (flooding, furrow and ridge and sprinkler) in relation to four planting densities (20, 40, 60 and 80 plants/m^2) on the growth, flowering and quantity of essential oil of *Calendula officinalis* L.

MATERIALS AND METHODS

Field experiments were conducted in Ghazian area located in northern part of Iran on a clay loam soil, since … until …. Seeds of marigold (*Calendula officinalis* L.) were prepared from Pakan Bazr Co., Isfahan, Iran. Properties of the soil in studied region are observed in Table 1. Plot area was 6.3 m^2 (3.5 m × 1.8 m), totally; 36 plots and total surface was 376.2 m. Initially, chemical fertilizer including; urea-superphosphate triple-potassium chloride (222.5:133.5:120 kg/ha) and manure (40 ton/ha) was applied to the main plots at planting time. Distance between plots and blocks were 0.5 m and 1 m, respectively. Used plant spacing was 12.5, 6.25, 4.2 and 3.1 cm for plant density (D) of 20, 40, 60 and 80 plants/m^2, respectively; row distance was 40 cm. Thinning was performed 2 weeks' after cultivation. Irrigation was carried out in 8 days intervals, when there was no rainfall. Hoeing was conducted in three stages: 15, 30 and 45 days after planting. The chemical analysis of the growing medium is presented in Table 1. Experiment was designed in factorial test on the basis of completely randomized block design with 3 replications. Interaction of two factors; irrigation method (flooding, furrow and ridge and sprinkler) and plant density (20, 40, 60 and 80 plants/m^2) (totally, 36 plots) was used in this experiment. Plant dry weight, flower dry weight, flower number/m^2, stem number/plant, flower diameter, and quantity of essential oil were evaluated. For determination of plant dry weight, five plants was selected from center of each plot, cut from crown and then dried in Oven at 72°C, following obtaining of fresh weight. For determination of flower dry weight, five plants was selected from center of each plot, dried for 6 h at 35-45°C and then weighted. Totally, flowers were harvested at 3 stages, and total dry weight calculated as yield. Flower number and stem number were obtained by account of five plants from center of each plot. The mean of flower diameter in each plot was determined by measurement of the completely open flower

Table 1: The main chemical properties of the growing medium.

Soil texture	Nitrogen (%)	Phosphorus (ppm)	Potassium (ppm)	Organic matter (%)	EC (mmhos/cm)	pH	Soil depth (cm)
Clay	0.3	7	84	0.7	0.59	6.33	0-30

Table 2: Analysis of variance (ANOVA) for the effect of plant density and irrigation on the quantity and quality traits of Calendula officinalis L.

Source of variations	df	MS					
		Essential oil	Number of stem	Number of flower per m^2	Flower diameter	Dry weight of bush	Flower dry weight
Irrigation (I)	2	0.005**	2.034*	29904.664*	18.599^{ns}	411.646**	577.452*
Density (D)	3	0.010**	25.192**	431786.48**	141.188**	930.269**	5515.741**
I × D	6	0.000^{ns}	0.092^{ns}	13905.697^{ns}	20.169^{ns}	12.603^{ns}	82.031^{ns}
Error	22	0.001	0.484	6728.934	27.681	32.216	106.468
Total	36	-	-	-	-	-	-
CV (%)	-	15.70	7.82	11.53	10.90	8.32	8.57

**: Significant at α = 1%, *: Significant at α = 5%, ns= Not significant

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Table 3:Mean comparison of the effect of plant density and irrigation on some quality and quantity traits of *Calendula officinalis* L.

Treatments	Essential oil (ml)	Number of stem	Number of flower per m^2	Flower diameter (mm)	Dry weight of bush (g)	Flower dry weight (g)
I_1	0.1783b	8.433b	691.9759b	49.418a	61.6558b	113.877b
I_2	0.2167a	9.250a	768.542a	48.408a	72.9500a	127.680a
I_3	0.2092a	8.750ab	674.767b	46.942a	69.9942a	119.567ab
D_1	0.1633b	10.744a	436.167c	49.778a	76.1867a	93.566d
D_2	0.2244a	9.500a	638.144b	52.111a	73.3367a	131.253b
D_3	0.2322a	8.11c	857.267a	48.334ab	69.8178a	149.252a
D_4	0.1856b	6.889d	915.267a	42.790b	53.4589b	107.429c
I_1D_1	0.1500a	10.233a	447.333^{ns}	52.233^{ns}	71.640a	85.587
I_1D_2	0.1100a	9.333a	627.333	55.967	66.210a	130.82
I_1D_3	0.2000a	7.833a	864.400	46.000	62.340a	141.8233
I_1D_4	0.1733a	6.333b	828.833	43.470	46.433a	97.2767
I_2D_1	0.1767a	11.333a	450.467	48.900	80.840a	101.4167
I_2D_2	0.2400a	9.833a	657.000	52.232	79.273a	140.8867
I_2D_3	0.2500a	8.500a	983.600	48.900	75.763a	154.52
I_2D_4	0.2000a	7.332a	983.100	43.500	55.923a	113.8967
I_3D_1	0.1633a	10.667c	410.700	48.200	76.080a	93.6933
I_3D_2	0.2433a	9.333a	630.700	48.033	74.527a	112.0533
I_3D_3	0.2467a	8.000a	723.800	50.133	71.350a	151.4133

*In each column, means with the similar letters are not significantly different at 5% level of probability using LSD test

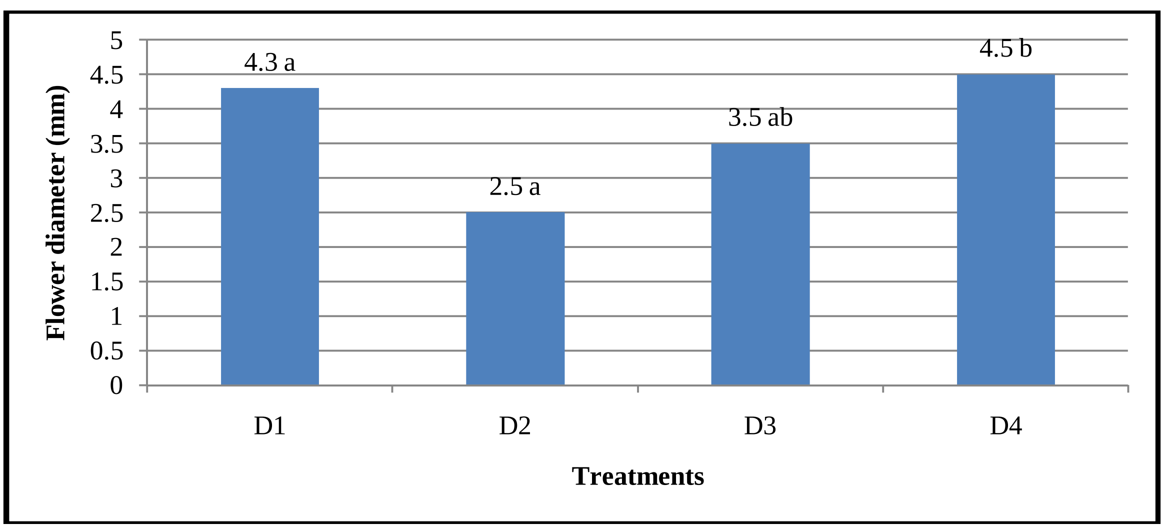

Fig 1:Effect of different irrigation methods and plant density on flower diameter

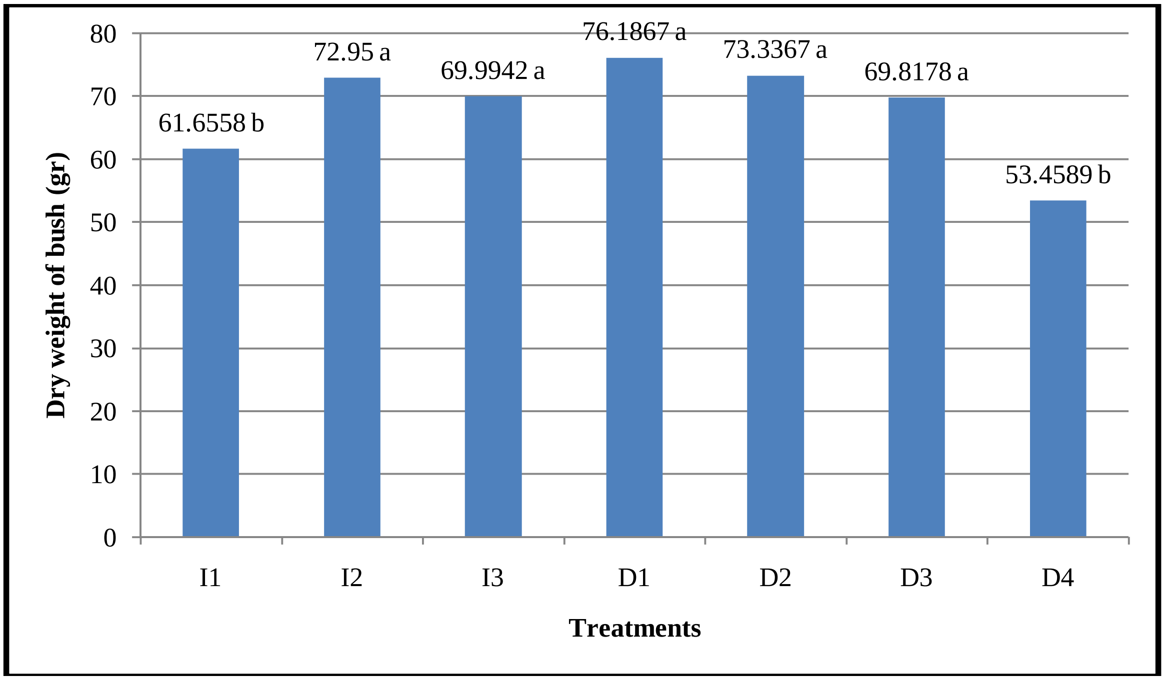

Fig 2: Effect of different irrigation methods and plant density on bush dry weight.

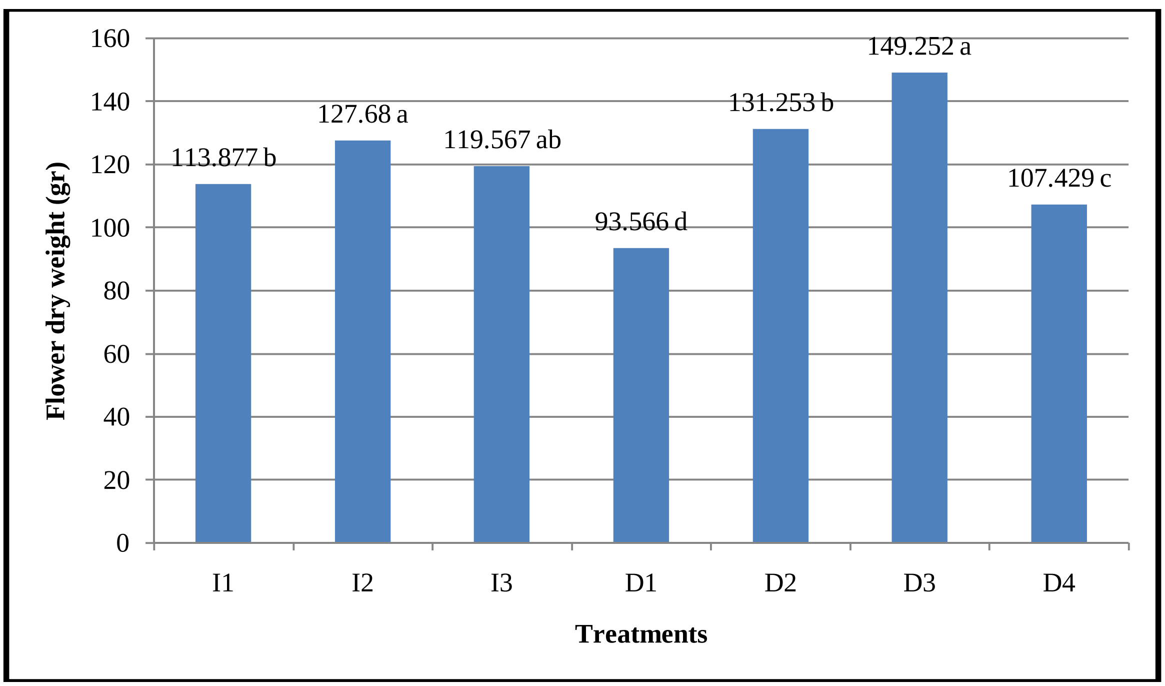

Figure 3: Effect of different irrigation methods and plant density on flower dry weight.

and dividing of obtained number on flowers number. For determination of the essential oil, the plant materials (flowers) were dried in 45°C. The essential oil was obtained in a Clevenger apparatus by steam distillation. Thus, the 50 g of dried plant materials was extracted with 1000 ml of water. The water collected was re-extracted with 0.5 ml hexane. The essence and hexane was separated from water physically and weighted until the plant essence obtained. Data processing of the results was carried out by an EXCEL. Analysis of variance (ANOVA) was done using SPSS statistical software and means were compared using Duncan's test.

DISCUSSION

Plant density is dependent on the plant characters, growth period, time and method of cultivation, soil conditions, plant length, sun light, and weeds. There is a need to use optimum plant density for maximum growth and flowering of pot marigold. Plant density has been recognized as a major factor determining the degree of competition between plants (Heitholt and Sassenrath-Cole, 2010). It is expected to decrease growth and flowering of pot marigold as the density increases. Most of quantity and quality traits of pot marigold yield are high at low planting density (Berimavandi et al., 2011). The use of high plant density increases competition for light, water and nutrients. Thus, finding the optimum plant density that produces the maximum yield is important.

Irrigation is an important and effective factor on growth and flowering traits, because it is associated with many environmental factors, which influence plant growth and development. Availability of sufficient amount of moisture optimizes the metabolic process in plant cells and increases the effectiveness of the mineral nutrients (Saif et al., 2003). Water availability is one of the most limiting factors for plant production and drought stress had a strong effect on plants. There exists a need to improve management techniques for irrigation methods in order to conserve water, and reduce groundwater contamination. Various types of irrigation such as furrow, alternate furrow, drip, flooding, sprinkler and surface irrigations are used in all over the world. Different irrigation methods can alter growth and total productivity of biomass. Among these methods, furrow irrigation in which soil surface is used to channel and infiltrate water is used widely throughout the world because of its simplicity and low capital costs (Mostafazadeh-Fard et al., 2009).

RESULTS

Number of stem

The analysis of variance presented in Table 2 showed that the effect of irrigation method (I) and plant density (D) on the number of stem per plant was significant at 0.05 and 0.01 level of probability, respectively. Interaction effect of I and D on the number of stem per plant was no significant (Table 2). Mean comparison obtained from the data showed that the largest and smallest number of stem per plant was obtained from furrow and ridge irrigation method in plant density of 20 (I_2D_1) (11.333) and flooding irrigation method in plant density of 80 (I_1D_4) (6.333), respectively (Table 3). Among different irrigation methods, the furrow and ridge method (I_2) had larger number of stem per plant (9.250) than flooding (I_1) (8.433) and sprinkler (I_3) (8.750) methods (Table 3). Also, the number of stem per plant in planting density of 20 (D_1) (10.744) was higher than that of other densities. In plant density of 80 (D_4), minimum number of stem (6.889) was obtained (Table 3).

Number of flower

The analysis of variance presented in Table 2 showed that the effect of irrigation method (I) and plant density (D) on the number of flower per square meter was significant at 0.05 and 0.01 level of probability, respectively. Interaction effect of I and D on the number of flower per square meter was no significant (Table 2). Mean comparison obtained from the data showed that the maximum and minimum number of flower per square meter was obtained from furrow and ridge irrigation method in plant density of 60 (I_2D_3) (983.600) and sprinkler irrigation method in plant density of 20 (I_3D_1) (410.700), respectively (Table 3). The number of flower per square meter in plants irrigated with furrow and ridge method and cultivated in plant density of 80 (I_2D_4) (983.100) was suitable and almost near to I_2D_3 (Table 3). Among different irrigation methods, the furrow and ridge method (I_2) had maximum number of flower per square meter (768.542) than flooding (I_1) (691.976) and sprinkler (I_3) (674.767) methods (Table 3). Also, the number of flower per square meter in planting density of 80 (D_4) (915.26) was higher than that of other densities. In plant density of 20 (D_1), minimum number of flower per square meter (436.167) was obtained (Table 3).

Flower diameter

Based on analysis of variance (Table 2), the effect of plant density on the flower diameter was significant at 0.01 level of probability. There is no significant difference in the flower diameter due to the effect of irrigation method and interaction effect of plant density and irrigation method (Table 2). The highest flower diameter was obtained from density of the 40 plants and flooding irrigation (I_1D_2) (55.967 mm) (Table 3). The lowest flower diameter was obtained from density of the 80 plants (D_4) (42.790 mm) (Table 3). Among different irrigation methods, the flooding method (I_1) caused maximum flower diameter (49.418 mm). Minimum flower diameter (46.942 mm) was calculated in plants treated with sprinkler (I_3) method (Table 3). Among different plant density, 40 plants per square meter (D_2) caused maximum flower diameter (52.111 mm). Minimum flower diameter (42.790 mm) was calculated in plants cultivated in 80 plants per square meter (D_4) (Table 3).

Plant dry weight

The data presented in Table 2 indicated that, the effect of plant density and irrigation method on the plant dry weight was significant at 0.01 level of probability. No interaction effect of plant density and irrigation method on the plant dry weight was significant. The most plant dry weight (80.840 and 79.273 g) was obtained in plants irrigated with furrow and ridge method and cultivated in plant density of 20 (I_2D_1) as well in plants irrigated with furrow and ridge method and cultivated in plant density of 40 (I_2D_2), respectively (Table 3). The least plant dry weight (46.433 g) was obtained in plants irrigated with flooding method and cultivated in plant density of 80 (I_1D_4). Among different irrigation methods, the furrow and ridge method (I_2) and flooding method (I_2) caused highest (72.9500 g) and lowest (61.6558 g) plant dry weight, respectively (Table 3). Among different plant density, 20 and 80 plants per square meter (D_1 and D_4) caused highest (76.187 g) and lowest (53.460 g) plant dry weight, respectively (Table 3).

Flower dry weight

The analysis of variance presented in Table 2 showed that the effect of irrigation method and plant density on the flower dry weight was significant at 0.05 and 0.01 level of probability, respectively. Interaction effect of irrigation method and plant density on the flower dry weight was no significant (Table 2). Mean comparison obtained from the data showed that the highest and lowest flower dry weight was obtained from furrow and ridge irrigation method in plant density of 60 (I_2D_3) (154.52 g) and flooding irrigation method in plant density of 20 (I_1D_1) (85.587), respectively (Table 3). Among different irrigation methods, the furrow and ridge method (I_2) had higher flower dry weight (127.680 g) than flooding (I_1) (113.877 g) and sprinkler (I_3) (119.567 g) methods (Table 3). Also, flower dry weight in planting density of 60 (D_3) (149.252 g) was higher than that of other densities. In plant density of 20 (D_1), lowest flower dry weight (93.566 g) was obtained (Table 3).

Essential oils

The effect of irrigation method and plant density was significant on the content of essential oil. The interaction effect of these two factors was no significant on the content of essential oil (Table 2). Maximum essential oil (0.2500 mL) was obtained when pot marigold was planted in 60 plants density and irrigated with furrow and ridge method (I_2D_3). Minimum essential oil (0.1100 mL) was obtained when pot marigold was planted in 40 plants density and irrigated with flooding method (I_1D_2) (Table 3). At irrigation methods, maximum and minimum essential oil (0.2167 and 0.1783 mL) was achieved in furrow and ridge (I_2) and flooding (I_1) methods, respectively. At planting densities, maximum and minimum essential oil (0.2322 and 0.1633 mL) was achieved in plant densities of 60 (D_3) and 20 (D_1) plants, respectively (Table 3).

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