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Improvement of the Yield and Essential Oils Quantitative in Calendula (*Calendula officinalis* L.) by Using Different Planting Arrangement and Potassium Fertilizer

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Abstract

This study was done to evaluate the effect of different planting arrangements (square, lozenge and rectangular) and various concentrations of potassium sulfate (K_2SO_4) fertilizer (0, 100, 200 and 300 kg/ha) on yield and essential oils quantitative of *Calendula officinalis* L., a medicinal and ornamental plant. The experiment was done as a factorial in randomized completely blocks design (R.C.B.D.) with 3 replications. The results showed that the plant height, the number of flowers, flowers dry weight, potassium content of aerial part, flower essential oil and carotenoids content in petals were significantly increased. The highest plant height (54.18 cm) was calculated from plants grown under 200 kg/ha K_2SO_4 and rectangular arrangement. Maximum number of flowers per square meter (727.93), flower dry weight per square meter (140.58 g), potassium content of aerial part (0.9 mg/L), essential oils content per square meter (0.27 g) and carotenoids content in petals (8.24 mg/L) were obtained from plants grown under soil conditions containing 300 kg/ha K_2SO_4 and with square arrangement. The results of current study recommend using of 300 kg/ha of K_2SO_4 and square planting arrangement for increasing the yield and essential oils content of calendula.

Keywords: Essential oils, Flower heads, *Calendula officinalis* L., Carotenoids.

INTRODUCTION

Marigold (*Calendula officinalis* L.) is the species that belongs to the family of Asteraceae. Large numbers of the species from this family are using in medicine, horticulture, industries, and some of them have important role in everyday life as food (Meda *et al.*, 2005; Khalid and Da Silva, 2010). Marigold flowers are used as the medical row material. The pharmacological activity of marigold is related to the content of several classes of secondary metabolites such as essential oils and carotenoids (Vidal-Ollivier *et al.,* 1989). Cultivation of medical plants has advantages in relation to assembling the medical plants from the nature. It can be accomplished by choosing the right plant species, soil, the optimal sowing date, the right plant nutrition, the best planting arrangement, the harvest, and etc (Međa *et al*., 2005). There are more advantages, like the high yield, obtaining the quality row material without additive and impurity (Međa *et al.,* 2005). *Calendula officinalis* L. was originated from the Mediterranean region, but it can be cultivated broad wise Europe, often as the decorative plant (Cromack and Smith, 1998).

Potassium (K) is a key essential plant nutrient, although it is not a constituent of any plant part. It acts as catalyst for many of the enzymatic processes which are necessary for plant growth. It also regulates the opening and closing of stomata which affect CO_2 uptake for photosynthesis (Somida, 2002). Potassium acts as an important nutrient in plant metabolism, enhancing carbohydrates synthesis, positively affecting water transport in the xylem and cell elongation. It is most abundant cation in cell cytoplasm and vacuole and plays an important role in cell turgid (Marchner, 1995). Most important role of potassium is functional and enhances plant production and resistance of plants against biological and environmental stresses (Malakouti *et al.*, 2009). Most plants are needed to high amount of potassium and uptake it between 50 and 300 kg/ha from the soil (Laegreid *et al.*, 1999).

Planting arrangement is the geometric conditions of plants in different rows, which can be altered by changing the rows and plant distances, also to set the plants on cultivation rows (Moadab Shabestari and Mojtahedi, 1993). Plant arrangement has important effects on vegetative and reproductive growth of plants (Murphy *et al.,* 1996). The most important effects of plant arrangement on the plant yield are distribution of solar radiation and photosynthesis rate (Steringfield and Taeher, 1987). The rate of light absorption is influenced by plant density and plant arrangement (Amarjit *et al.,* 1990). Light absorption by lower leaves of plants increase as planting rows distance increase.

The aim of this study was to investigate the effect of planting arrangement (square, lozenge and rectangular) and different concentrations of potassium sulfate fertilizer (0, 100, 200 and 300 kg/ha) on the growth, yield, quantity of flower's essential oil and carotenoids of *Calendula officinalis* L.

MATERIALS AND METHODS

Experiments were carried out at the Tonekabon city (N 36° 47 47 and S 50° 49 30; altitude, 40 m above sea level; mean annual rainfall, 1000 mm; mean annual temperature, 12.7°C; Max. and Min. RH; 97 and 66%, respectively), located in the northern part of Iran, during season 2010/2011. 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.

The experiment was done as a factorial in randomized completely blocks design (R.C.B.D.) with 3 replications. The first factor was planting arrangement in three levels (square, lozenge and rectangular) and the second factor was potassium sulfate (K) in four levels (0, 100, 200 and 300 kg/ha). Seeds were planted into square arrangement (A_1) with distances of 30 × 30 cm as opposite, lozenge arrangement (A_2) with distances of 30 × 30 cm as intermediate and rectangular arrangement (A_3) with distances of 45 × 20 cm as opposite. Thinning was performed two weeks after planting. Measured traits were plant height, the number of flower per square meter, flower dry weight per square meter, the content of carotenoids in petals, and the amount of potassium in aerial organs, and the content of essential oils.

For determination of plant height, five plants were selected from each plot, and their length was calculated by a ruler. Flower number per square meter was obtained by account of the number of flower per plant multiplication of 11.11 densities. For determination of flower dry weight, flowers of single plant was collected from center of each plot, dried at 45°C for 6 h and then weighted. Then, the weight of these flowers and 11.11 densities per plot was crossed. For determination of potassium content, samples were dried in Oven at 80°C for 24 h. Dried samples were diluted by distilled water and potassium content was obtained via flame photometry. Leaf carotenoids were determined using acetone as extracting solvent and the absorbance was measured at 440, 645 and 663 nm. Sample extract was prepared as follows: 0.5 g of dry sample was thoroughly crushed and homogenized in a mortar with a pestle using 20 ml of 80% acetone. Filtered extract was reached to 50 ml by adding of 80% acetone. Concentration of carotenoids was calculated by following formula: The amount of carotenoids (mg/L) = $4.69 \times A_{440} - 0.268 \times (20.2) A_{645} + (8.02) A_{663}$

Where; A is wave length. The mean of flower diameter in each plot was determined by measurement of the completely open flower 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.

In this experiment, two factors were considered: plant arrangement (square, lozenge and rectangular) and potassium sulfate (0, 100, 200 and 300 kg/ha). For each treatment there were three replicates, each of plot had 3 × 2 m surface; in each plot, seeds were planted in 11.11 plants per square meter. The number of experimental units was 36 plots. Analysis of variance (ANOVA) was done using MSTAT-C statistical software and means were compared using Duncan's test.

RESULTS AND DISCUSSION

According on analysis of variance (Table 2), the effect of plant arrangement (A) and K_2SO_4 (K) on the flower number per square meter was significant at 0.05 and 0.01 level of probability, respectively. Interaction effect of A and K on the flower number per square meter was significant at 0.01 level of probability (Table 2). Mean comparison obtained from the data showed that the highest flower number per square meter was obtained from square planting arrangement and concentration of 300 kg/ha of K_2SO_4 (727.93 flowers). In the meanwhile, 200 kg/ha of K_2SO_4, and lozenge planting arrangement with 671.08 and 623.47 flowers per square meter, respectively, had the high flower number (Table 3). Our findings showed that the maximum of flower was obtained in square and lozenge planting arrangements along with 300 and 200 kg/ha of K_2SO_4 fertilizer. Positive effect of lozenge and square arrangements on the flower number is due to decreasing the intra- and inter-specific competition and increasing the light dispensation. Uniform dispensation of plants in planting rows causes more effective usage from environmental resources and delay to start of the competition. Our results were confirmed by those of Milli and Suble (2003) who reported that the maximum of flower number per pot marigold plant was obtained in square planting arrangement (45 × 45 cm). In our work, minimum of flower number was observed in control plants (without any K_2SO_4 fertilizer). Similar results were observed by Moradinejad (1995) and Daneshkhah *et al.*, (2007) in rose. These researchers showed that the largest number of flower was obtained under 60 kg/ha of potassium fertilizer.

Only, the effect of K_2SO_4 fertilizer on the flower dry weight per square meter was significant at 0.01 level of probability (Table 2). The highest value (140.5 g dry flower weight per square meter) was obtained by using the 300 kg/ha of K_2SO_4 fertilizer (Fig. 1). Potassium insufficient in control plants caused the least production of flower dry weight. Also, the lowest value of flower dry weight (100.91 g dry flower weight per square meter) was obtained in control plants cultivated in rectangular arrangement. Negative effect of rectangular arrangement on the flower dry weight is due to increasing the intra- and inter-specific competition and decreasing the light dispensation. This result is in agreement with that obtained by Ganjali *et al.*, (2010). The study of Ashoori Latmahalleh *et al.,* (2011) on Echium amoenum Fisch and Mey showed that dried flower weight increased as the plant density decreased. Study of Pal and Ghosh (2010) on African marigold demonstrated that the yield of flower increased with increased quantity of potassium fertilization from zero to 200 kg/ha.

The effect of different concentrations of K_2SO_4 fertilizer, different plant arrangement and their interaction effect on the content of petal carotenoids was significant at 0.01, 0.05 and 0.01 level of probability, respectively (Table 2). Maximum contents of petal carotenoids were obtained by using square arrangement (6.68 mg/L), 300 kg/ha of K_2SO_4 (7.38 mg/L) and their interaction effect (8.24 mg/L) (Table 3). Square arrangement increased the content of carotenoids for 7% more than that of rectangular arrangement. Also, the use of 300 kg/ha of K_2SO_4 fertilizer increased the content of carotenoids for 27% more than that of control. The carotenoid pigments from pot marigold' inflorescence represent a fundamental component of medicines (Marta *et al*., 2008). Decreasing the content of petal carotenoid pigments in plants cultivated under rectangular arrangement is due to the light insufficient, especially in lower nodes, but consumption of fertilizers containing potassium had significant effect on increasing of petals carotenoid. This result is in agreement with that obtained by Seddighi *et al.*, (2011). These researchers showed that the best light efficiency has been obtained by pot marigold plants cultivated under 300 kg/ha of potassium sulfate fertilizer and square and lozenge planting arrangements. Marta *et al*., (2008) believed that the mineral fertilizers applied in pot marigold plants had positive effect on pigments and stimulated both the photosynthesis and the accumulation of carotenoid pigments, and increased mobilization of carotenoid and other pigments presented in leaf and flower. Pal and Ghosh (2010) demonstrated that anthocyanin content in African marigold petals were directly related to potassium level. Similar results were obtained by Hend (2002) and Shalan *et al*., (2001). Total chlorophyll content in leaf tissues at active vegetative growing stage showed a gradual increase (Pal and Ghosh, 2010). Potassium has been reported to be involved in maximum increase in nutrient uptake (Belorkar *et al.*, 1992).

K_2SO_4 fertilizer and its interaction with planting arrangement had significant effect on potassium content of aerial part ($p \leq 0.01$) (Table 2), and maximum content (0.87 and 0.90 mg/L) in treatments of 300 kg/ha of K_2SO_4 and interaction effect of 300 kg/ha of K_2SO_4 and square planting arrangement, respectively (Table 3). In most treatments, the plants treated with 200 and 300 kg/ha of K_2SO_4 fertilizer included more potassium in aerial part than the plants treated with 100 kg/ha of potassium sulfate fertilizer and control plants (Table 3). Study of Pal and Ghosh (2010) on African marigold showed that the content of nitrogen, phosphorus and potassium in petals were directly related to potassium level. Treatment with different levels of potassium showed gradual increase in potassium content of the leaf tissues of African marigold, irrespective of the sources of potassium. Lunt and Kofranek (1958) and Joiner and Smith (1961) reported similar findings in chrysanthemum. Moradinejad (1995) showed that nitrogen and potassium fertilizers altered the content of potassium in leaves of rose.

The effect of different concentrations of K_2SO_4 and different planting arrangement on the content of essential oils was significant at 0.01 level of probability (Table 2). Mean comparison obtained from the data showed that maximum of the content of essential oils per square meter (0.27 and 0.25 g) was obtained from plants cultivated in concentration of 300 kg/ha of K_2SO_4 fertilizer and lozenge planting arrangement, respectively (Fig. 2). Current study showed that the content of essential oils per square meter increased as the concentration of K_2SO_4 increased. No the interaction effect of potassium sulfate and planting arrangement on the amount of essential oil was significant (Table 2). Decreasing the content of essential oils in plants cultivated under rectangular arrangement with decreased planting rows distance is due to the light insufficient. The presence of sulfate in soils with high pH is suitable for iron uptake (Damke and Bhattacharjee, 1997). Similar results were reported by Daneshkhah *et al*., (2007) and Tavasoli *et al*., (2009) by study on Matricaria chamomile. Studies of Morteza *et al*., (2009) on Valeriana officinalis L. showed that planting density significantly affected essential oil yield and essential oil percentage. Ganjali *et al*., (2010) showed that plant density was effective in increasing the amount of essence of *Calendula officinalis* L. Study of Ezz El-Din *et al*., (2010) on Carum carvi L. indicated that applying nitrogen and potassium fertilizers led to the highest total oil yield.

The data presented in Table 2 indicated that, the effect of planting arrangement and its interaction effect with K_2SO_4 fertilizer on the plant height was significant at 0.05 and 0.01 level of probability, respectively. Maximum of plant height (54.18 and 49.58 cm) was calculated in plants cultivated under rectangular planting arrangement along with 200 kg/ha K_2SO_4 fertilizer and rectangular planting arrangement, respectively (Table 3). Distance between plants in rectangular planting arrangement is more than that of square and lozenge planting arrangements. Study of Pal and Ghosh (2010) on African marigold showed that the highest plant height was obtained in plants cultivated under 200 kg/ha of potassium fertilizer. Ramezani *et al.,* (2011) showed that the plant height of Zea mays L. increased in line with increasing the rows space.

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Tables

Table 1. The results of soil analysis

Iron (ppm)	Mg (ppm)	Zn (ppm)	N (%)	P (ppm)	K (ppm)	Organic matter (%)	Soil texture	Ec (mmhos .cm^{-1})	PH	Depth of sampling (cm)
2	0.4	5	0.07	12	130	1.18	Lomy-Sand	0.22	6.84	0-30

Table 2. Analysis of variance (ANOVA) of different treatments on traits.

	df	MS						
		Dry weight of flower per m^2	Number of flower per m^2	Number of stem per bush	Cartenoid	Amount of potassium in aerial parts	Essential oil content	Plant height
Plant arrangement	2	1.760ns	6723.011*	0.335^{ns}	0.766*	0.001 ns	0.011**	45.956*
K_2SO_4	3	23.882 **	57890.45**	0.222^{ns}	3.966**	0.016**	0.026**	22.473^{ns}
A×K	6	1.452 ^{ns}	8713.569**	0.243 ^{ns}	1.493**	0.011**	0.002^{ns}	54.486**
Error	22	-	1754.364^{ns}	-	-	-	-	-
cv		10.8	6.09	5.7	6	3.9	13.9	6.04

ns: Non significant
*: Significant at 5%
**: Significant at 1%

Table 3.Effect of plant arrangement, K2SO4 and interaction between plant arrangment and K2SO4 on traits.

Treatment	Number of flower per m^2	Cartenoid (mg L^{-1})	Amounts of potassium on aerial parts (mg L^{-1})	Plant height (cm)
(A1) Square arrangement	610.58 ab	6.68 a	0.80 a	45.73 b
(A2) Lozeng arrangement	623.47 a	6.58 ab	0.81 a	47.01 ab
(A3) Rectangular arrangement	577.57b	6.20 b	0.82 a	49.58 a
(K1) Control	499.13 c	5.79 c	0.77 b	45.80 a
(K2) 100kg ha-1	583.25 b	6.45 b	0.79 b	46.47 a
(K3) 200kg ha-1	671.08 a	6.31 b	0.81 ab	49.22 a
(K4) 300kg ha-1	662.03 ab	7.38 a	0.87 a	48.27 a
A1k1	496.40 c	5.27 c	0.71 b	47.30 b
A1k2	573.31 b	6.48 b	0.75b	47.35 b
A1k3	644.68 ab	6.71 b	0.83 ab	41.60 c
A1k4	727.93 a	8.24 a	0.90 a	46.68 b
A2k1	552.58 bc	6.13 ab	0.84 ab	45.17 bc
A2k2	631.90 ab	6.74 b	0.73 b	46.47 b
A2k3	717.41 a	5.71 c	0.81 ab	51.89 ab
A2k4	591.98 b	7.74 a	0.87 ab	44.53 bc
A3k1	448.40 c	5.97 c	0.77 b	44.93 bc
A3k2	544.54 bc	6.14 ab	0.87 ab	45.60 bc
A3k3	651.16ab	6.51 b	0.80 ab	54.18 a
A3k4	666.19 ab	6.17 ab	0.83 ab	53.60 a

In each column means followed by the same letters are not significantly different at 5 % level of probability using DMRT.

Figures

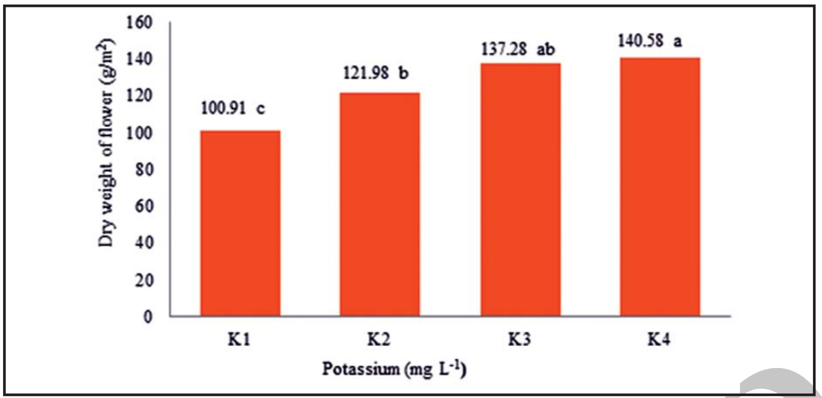

Fig. 1. Effect of different concentration of potassium on dry weight of flower.

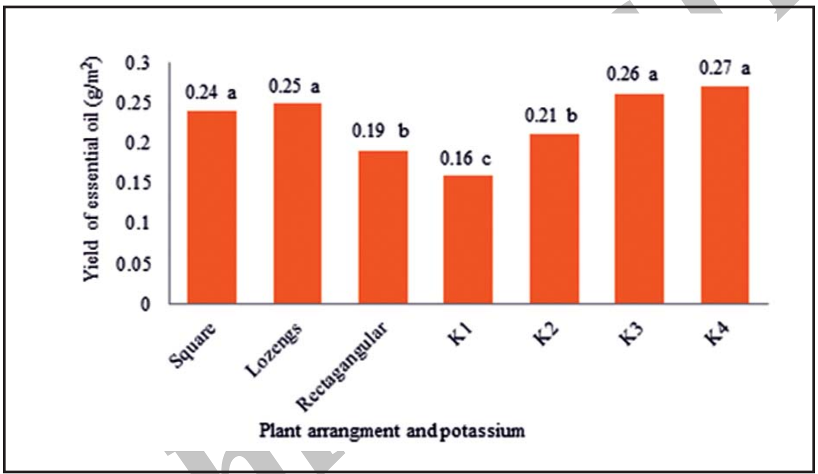

Fig. 2. Effect of plant arrangment and potassium on yield of essential oil.