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Effects of irrigation intervals and organic manure on morphological traits, essential oil content and yield of oregano (*Origanum vulgare* L.)

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ABSTRACT

In order to evaluate the effect of irrigation intervals and cattle manure levels on morphological traits, essential oil content and yield of oregano, an experiment was conducted at the experimental Farm of the Faculty of Agriculture, Urmia University, Iran. The experimental design was split-plots, arranged in randomized complete blocks with three replications. Main plots including irrigation intervals (1, 2 and 3 weeks) and four levels of cattle manure at 0, 10, 20 and 30 t ha^{-1} were allocated to sub plots. Our results showed that increasing irrigation intervals reduced values of all morphological traits except for proportion of stems. Also, values for stems number, plant spread, stem diameter, leaf area, fresh and dry herb yield increased by increasing cattle manure levels. On the other hand, morphological traits not influenced by interaction of treatments except for plant spread and leaf area. The highest essential oil content (2.07%) and yield (66.62 kg ha^{-1}) obtained in highest irrigation intervals and cattle manure levels. Whereas, 1 week irrigation interval without use of cattle manure produce lowest essential oil content (1.55%). For essential oil yield, the lowest value (46.37 kg ha^{-1}) was found in 2 weeks irrigation interval with application of 20 t ha^{-1} cattle manure.

Key words: cattle manure, essential oil, irrigation, *Origanum vulgare* L., yield.

INTRODUCTION

Nowadays, due to considerable effects of plants medication and their healthcare properties, in worldwide the consumption of medicinal plants and natural antioxidants are increasing continuously (Hecl and Sustrikova 2006, Liang et al. 2010, Raut and Karuppayil 2014). But, over utilization of medicinal and aromatic plants from natural habitats has damaged plant communication and caused a serious threat to these valuable herbs (Hoareau and Da Silva 1999). For this reason, global approach is to the production of medicinal plants in sustainable farming systems (Valiki et al. 2015).

For cultivation of medicinal and aromatic plants under field conditions, different management practices are require including awareness of ecological properties of plants, time of planting, plant density, nutrient and water requirements (Ozguven et al. 2008, Tabrizi et al. 2011). Among the different environmental constraints, water is one of the most important limiting factors for plant productivity (Bannayan et al. 2008, Laribi et al. 2009).

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Delfine et al. (2005) reported that the average of yield decrease on a world wide scale at more than 25% due to water deficit. Plant productivity and photosynthesis are adversely affected by water stress due to a series of morphological, physiological, biochemical and molecular changes (Tezara et al. 1999). Water deficit alters plant metabolism and severely affecting ecosystems and agriculture (Tezara et al. 1999, Wang et al. 2001). The optimization of irrigation are important in order to produce optimum fresh herbs and seed yield, because water is a major component of the fresh produce and effects both quantity and quality of crop yield (Jones and Tardien 1998, Ucan et al. 2007). In the case of medicinal plants, water deficit may cause changes in the biomass yield and composition of their essential oils. Effect of water stress on essential oil content and yield, secondary metabolites, morphological and physiological characteristic of different medicinal and aromatic plants has been reported in several studies. In *Thymus transcapicus* Klokov, increase of irrigation intervals caused significant reduction in dry weight, proportion of stem, leaf and flower in second year (Tabrizi 2011). Tucker and Maciarello (1994) found that water stress has negative effect on biomass and essential oil content in oregano (*Origanum vulgare* L.). Also, Hassani (2006) revealed that the plant height, stem diameter, number and length of auxiliary shoots, fresh and dry herb yield of *Dracocephalum moldavica* was declined with decreasing soil water content. In other hand, several study shown positive effect of water deficit on essential oil and alkaloids in medicinal plants (Bettaieb et al. 2009, Gholizadeh et al. 2010). In this respect, Khalid (2006) reported that severe water stress improved essential oil content more than moderate stress. Plant growth of *Ocimum americanum* L. decreased in response to water deficit, but essential oil and proline contents was increased with increasing water stress (Baeck et al. 2001).

Also, crop production is related to soil fertility. Fertile soil is fundamental resource for higher crop production, which supplies all the essential nutrients to the crop. Therefore, maintenance of soil fertility can be a pre-requisite for long term sustainable crop production (Schroth and Sinclair 2003). Long term use of chemical fertilizers depletes soil organic matter, causes soil degradation and environmental pollution problems (Rao 2001). Organic manures are carbon-based compounds that increase the productivity and improve quality of plants (Leu 2007). They have various benefits over chemical fertilizers. Organic fertilizers are low priced and eco-friendly inputs that have tremendous prospect of supplying nutrients which can reduce the over dependence on chemical fertilizer (Bajeli et al. 2016). The use of organic manure to soil amendments has been associated with desirable soil properties including higher plant available, water holding capacity, cation exchange capacity and lower bulk density as well as can foster beneficial microorganisms (Drinkwater et al. 1995). Previous studies have shown application of organic manures had progressive effects on seed yield, fresh weight, seed essential oil content and yield in *Coriandrum sativum* L. (Darzi 2012) and *Melisa officinalis* L. (Santos et al. 2009). Ram and Kumar (1997) found that utilization of organic manure in *Mentha piperita* L. was most effective in increasing the total biomass and essential oil yield. In other study, Kaplan et al.. (2009) showed that use of organic manure in *Salvia officinalis* improved fresh herb yield and essential oil content 43 and 41%, respectively.

Oregano (*Origanum vulgare* L.) belongs to the Lamiaceae family is a perennial herb (Vokou et al. 1993, Spada and Perrino 1996). This plant can be found abundantly on dry, rocky calcareous soils of mountain areas at a wide range of altitude from 0 to 4000 m (Snogerup 1971) that grows throughout the Mediterranean, Euro-Siberian and Irano-Turanian regions (Aligianis et al. 2001). Essential oil of oregano has antimicrobial, antioxidant, cytotoxic and antifungal properties (Adams et al. 1998). Also, this plant is popular culinary herbal crops that commonly used in the agriculture, pharmaceutical and food industry (Aligianis et al. 2001).

Limited information is available about the response of oregano to irrigation and nutrient supply. Therefore, the objective of this research was to investigate the performance of oregano under irrigation intervals and different cattle manure levels.

MATERIALS AND METHODS

EXPERIMENT SITE

This study was conducted at the research farm of Agriculture College of Urmia University, Iran (37°32› N latitude and 45°5› E longitudes) at an elevation of 1320 m above sea level during 2014 growing season. This site was characterized by semi-arid climate with a mean annual precipitation of 315.3 mm and average temperature of 13.48°C. The soil characteristics from the experimental field and organic manure (cattle manure) are given in Table I.

The experimental design was a split-plot randomized complete blocks with three replications in which irrigation intervals (1 (IR_1), 2 (IR_2) and 3 (IR_3) weeks) were allocated in main plots and cattle manure levels (0, 10, 20 and 30 t ha^{-1}) as sub plots.

Seeds of oregano were collected from natural habitat and sown under greenhouse conditions in small pots (8.2 cm height and 7 cm diameter) in a medium of mixed leaf mold, sand and loamy soil (1:2:3 v/v). Then 70 days old seedling after acclimation (5 days) were transferred outdoor in 5 May. They transplanted at 40 and 25 cm distance between rows and within rows, respectively. Each main plot size was 33.6 m^2 (12 × 2.8 m) that divided to four sub-plots (3 × 2.8 m). The first irrigation was conducted immediately after transplanting. Then two irrigation stages were also applied with intervals of 3 and 6 days for uniform establishment of seedlings.

Different quantities of five years composted cattle manure were broadcasted uniformly and incorporated to the soil four months prior to transplanting. Experimental plots were irrigated up to field capacity (22.5% θ_{fc}) with 330 liter in irrigation. Weeds were controlled by hand-hoeing when necessary.

MORPHOLOGICAL MEASUREMENT

Plant height was measured from the soil surface to the tip of the tallest flowering stem at full flowering stage. For each treatment, measurement of fresh and dry matter weights were evaluated by harvest of five randomly selected plants from the center rows of each plot. Plants were harvested at a height of 5 cm above ground and immediately weighted (fresh matter weight). After that plants were dried in a dry oven at 65°C for 48 h and re-weighted (dry matter weight). The proportion of leaves, stems and flower were separately determined by weighting of these segments with a digital balance. The fresh and dry herb yield (kg ha^{-1}) was determined by harvesting a 2 m^2 area from each plot. The collected plants were immediately weighted (fresh herb yield) by a digital balance, then dried in the shade until it reached a constant weight and weighted (dry herb yield).

TABLE I
Chemical and physical characteristics of the soil and cattle manure used in the field experiment.

	pH	OC (%)	EC (dS m^{-1})	Total N (%)	P (ppm)	K (ppm)	Texture
Soil	7.5	0.92	0.65	0.16	12.1	338	Clay-loam
Cattle manure	8.2	38.9	12.16	2.06	974	13700	-

SEED YIELD AND 1000 SEED WEIGHT

For each treatment, seed yield of oregano was measured at maturity stage by harvesting a 2 m^2 area from center rows of each plot. Then seeds were cleaned and seed yield were determined with digital balance (after drying). Also, for 1000 seed weight, 200 seeds were weighted.

ESSENTIAL OIL CONTENT AND YIELD

Fresh plants were cut at 5 cm above ground level at flowering stage from each treatment. Then, they were dried in the shadow for two weeks. Essential oils from shade dried parts of oregano plants were isolated by hydro-distillation for 2.5 h using Clevenger-type apparatus. Then essential oil content was quantified and stored at 4°C in a sterilized vial until required. Also, essential oil yields as kg ha^{-1} were measured by using following formula:

$$\text{EOY (kg ha}^{-1}\text{)} = \text{EO (\%)} \times \text{DW (kg ha}^{-1}\text{)}$$

Were EOY is essential oil yield, EO is essential oil and DW is dry herb yield.

STATISTICAL ANALYSIS

Data were statically processed by analysis of variance (ANOVA) with using of SAS (Version 9.1) software, followed by the Duncan's multiple range comparisons test with a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Data presented in Table II and III shows significant effects of irrigation intervals on proportion of leaf + flower and stem ($p < 0.05$) and other measured morphological traits ($p < 0.01$), while stems number and 1000 seed weight not influenced by irrigation intervals. Plant height, plant spread, stem diameter, leaf area, proportion of leaf + flower and stem, fresh and dry herb yield declined with increasing of irrigation intervals. In mentioned parameters, greatest values obtained under IR_1 and the minimum values for these traits observed under IR_2 or IR_3 (Table II and III). Unlike mentioned traits, the highest value for proportion of stem (42.71%) was obtained in IR_3 treatment and reduced to 40.27 and 38.85% in IR_2 and IR_1, respectively. Of course, there was no significant difference between IR_2 and IR_1 treatments (Table III).

The reductions in plant height, leaf area, fresh and dry herb yield and other traits in higher irrigation intervals may be due to the decrease in the cell enlargement and more leaf senescence resulting from reduced turgor pressure (Shao et al. 2008, Farooq et al. 2009) or it may be due to a decrease in photosynthesis and alter canopy structure during the higher irrigation intervals (Shao et al. 2008). Also, Leithy et al. (2006) believed that, decrease in plant growth has been associated with lower photosynthesis rate due to declined stomatal conductance. Development of optimal leaf area is important for photosynthesis and production of dry herb yield (Jaleel et al. 2009), because when leaf area is lower, the capacity to light trap decreases and this led to reduction in photosynthesis rate (Khalid 2006). Furthermore, the effect of reduced irrigation on growth of plant may be due to the lower availability of sufficient moisture in rhizosphere and the lower absorption of nutrients (Singh et al. 1997). Indeed, in order to increase absorption of water, plants in water deficit conditions often reduce their biomass production and contribution more biomass to roots (Albouchi et al. 2003). Our results are in agreement with other reports which confirm the effect of irrigation on growth of medicinal and aromatic plants such as *Lippia berlandieri* (Vazquez and Dunford 2005), *Hypericum brasiliense* (Zobayed et al. 2007), *Thymus transcapicus* (Tabrizi et al. 2011) and *Thymus daenansis* (Bahreininejad 2013).

In other hand, ANOVA results indicated that the plant spread, stem diameter, leaf area and fresh herb yield were significantly affected by

TABLE II
Effect of irrigation intervals, cattle manure levels and interaction of treatments on plant height, stems number, plant spread, stem diameter and leaf area in oregano.

Treatments		Plant height (cm)	Stems number	Plant spread (cm)	Stem diameter (mm)	Leaf area (cm^2)
Irrigation intervals						
IR_1 †		33.92 a	13.37 a	36.11 a	2.23 a	919.93 a
IR_2		27.98 b	11.37 a	29.33 b	2.11 a	877.95 a
IR_3		28.03 b	11.82 a	31.20 ab	1.50 b	672.69 b
Cattle manure						
CM_0		28.56 b	10.18 b	27.08 b	1.72 c	769.64 c
CM_{10}		29.22 ab	11.93 ab	33.07 a	1.92 b	825.08 b
CM_{20}		30.62 ab	13.67 a	35.11 a	2.04 a	841.31 b
CM_{30}		31.51 a	12.96 a	33.59 a	2.10 a	858.06 a
IR × CM						
IR_1	CM_0	30.20 b-d	10.47 bc	32.89 a-c	1.99 cd	897.40 a
	CM_{10}	33.20 a-c	13.53 a-c	36.22 ab	2.12 bc	919.19 a
	CM_{20}	35.27 ab	15.13 a	38.22 a	2.41 a	939.33 a
	CM_{30}	37.00 a	14.33 ab	37.11 ab	2.39 a	923.80 a
IR_2	CM_0	27.20 d	9.40 c	20.78 d	1.84 d	774.47 b
	CM_{10}	25.67 d	10.80 bc	25.78 cd	2.11 bc	891.54 a
	CM_{20}	28.53 cd	13.07 a-c	38.33 a	2.19 b	908.50 a
	CM_{30}	30.53 b-d	12.20 a-c	32.44 a-c	2.29 ab	937.30 a
IR_3	CM_0	28.27 cd	10.67 bc	27.56 b-d	1.34 f	637.07 d
	CM_{10}	28.80 cd	11.47 a-c	37.22 ab	1.53 e	664.51 cd
	CM_{20}	28.07 cd	12.80 a-c	28.78 a-d	1.52 e	676.10 cd
	CM_{30}	27.00 d	12.33 a-c	31.22 a-c	1.63 e	713.07 c
ANOVA						
IR		**	NS	**	**	**
CM		NS	*	**	**	**
IR × CM		NS	NS	*	NS	**

† IR = Irrigation intervals (weeks), CM = cattle manure levels (t ha^{-1}).
In each section, means followed by the same letter within columns are not significantly different (p0.05) according to Duncan's multiple range test.
* Significant at the 0.05 level of probability, ** Significant at the 0.01 level of probability, NS: no significant.

TABLE III
Effect of irrigation intervals, cattle manure levels and interaction of treatments on proportion of stem and leaf + flower, fresh and dry herb yield in oregano.

Treatments	Stem proportion (%)	Leaf + flower proportion (%)	Fresh herb yield (kg ha^{-1})	Dry herb yield (kg ha^{-1})	1000 seed weight (g)
Irrigation intervals					
IR_1 †	38.85 b	61.15 a	11704.6 a	3718.5 a	0.251 a
IR_2	40.27 b	59.73 a	10067.4 b	3026.5 b	0.244 a
IR_3	42.71 a	57.29 b	7951.2 c	2473.2 c	0.246 a
Cattle manure					
CM_0	41.15 a	58.85 a	9624.1 b	3009.6 b	0.245 a
CM_{10}	40.29 a	59.71 a	9795.6 b	3037.1 b	0.246 a
CM_{20}	39.55 a	60.45 a	10053.5 a	3097.7 ab	0.249 a
CM_{30}	41.44 a	58.56 a	10157.7 a	3146.5 a	0.251 a

TABLE III (continuation)

Treatments		**Stem proportion (%)**	**Leaf + flower proportion (%)**	**Fresh herb yield (kg ha^{-1})**	**Dry herb yield (kg ha^{-1})**	**1000 seed weight (g)**
IR × CM						
IR_1	CM_0	41.01 ab	58.99 ab	10144.4 a	3104.8 a	0.243 a
	CM_{10}	39.20 ab	60.80 ab	10132.0 a	3119.2 a	0.244 a
	CM_{20}	37.05 b	62.95 a	9848.4 a	3023.6 a	0.243 a
	CM_{30}	38.13 ab	61.87 ab	9761.2 a	3032.8 a	0.253 a
IR_2	CM_0	40.27 ab	59.73 ab	9723.2 a	2992.0 a	0.250 a
	CM_{10}	38.28 ab	61.72 ab	9814.4 a	3021.2 a	0.251 a
	CM_{20}	40.31 ab	59.69 ab	9744.0 a	2994.0 a	0.253 a
	CM_{30}	42.21 ab	57.79 ab	9852.0 a	3039.2 a	0.253 a
IR_3	CM_0	42.18 ab	57.82 ab	9900.8 a	3124.4 a	0.243 a
	CM_{10}	43.40 a	56.60 b	9932.4 a	3100.4 a	0.244 a
	CM_{20}	41.28 ab	58.72 ab	10076.0 a	3146.0 a	0.251 a
	CM_{30}	43.96 a	56.04 b	9964.0 a	3175.2 a	0.247 a
ANOVA						
IR		*	*	**	**	NS
CM		NS	NS	**	NS	NS
IR × CM		NS	NS	NS	NS	NS

† IR = Irrigation intervals (weeks), CM = cattle manure levels (t ha^{-1}).
In each section, means followed by the same letter within columns are not significantly different (*p*0.05) according to Duncan's multiple range test.
* Significant at the 0.05 level of probability, ** Significant at the 0.01 level of probability, NS: no significant.

cattle manure levels ($p < 0.01$). Also, significant differences observed between cattle manure levels ($p < 0.05$) for stems number, while other measured traits (i.e. dry weight, proportion of leaf + flower and stem and 1000 seed weight) not influenced by cattle manure levels (Table II and III). Our results showed that values for stems number and plant spread increased by increasing of cattle manure levels from 0 to 20 t ha^{-1}, while application of 30 t ha^{-1} of cattle manure had a slight negative effect on these traits (Table II). On the other hand, increasing the amount of applied cattle manure levels from 0 to 30 t ha^{-1} showed a progressive effect on stem diameter, leaf area, fresh and dry herb yield (Table II and III). Addition of cattle manure to the soil increase water holding capacity, improve soil physical and chemical properties, create a good condition for biological process in rhizosphere and provide nutrient availability for plants (Arancon et al. 2004, Valiki et al. 2015). Our results are in agreement with previously published reports on *Coriandrum sativum* (Darzi 2012) and *Salvia officinalis* L. (Kaplan et al. 2009).

Also, plant spread and leaf area significantly influenced by interaction of treatments (IR × CM) at $p < 0.05$ and $p < 0.01$, respectively. Although, interaction of treatments had no significant effects on other investigated morphological traits, seed yield and 1000 seed weight (Table II and III). Interaction of treatments did not show clear trend in plant spread (Table II). Higher values (38.33 and 38.22 cm) for this parameter obtained in IR_2 and IR_1 with using 20 t ha^{-1} cattle manure, respectively. In this trait, lowest value (20.78 cm) observed in IR_2 without utilization of cattle manure (Table II). The use of organic manure has been associated with higher plant available water, cation exchange capacity and lower bulk density as well as can increase beneficial micro-organisms (Drinkwater et al. 1995). Also, Arancon et al. (2004) reported that use of organic manures improve the growth rate in plant due to better availability of water and nutrient uptake that causes growth improvement.

For seed yield, ANOVA showed significant difference as effected by irrigation intervals ($p < 0.05$) (Figure 1), while this parameter not influenced by cattle manure levels and interaction of treatments (data not shown). The maximum and minimum values (14.6 and 12.64 g m^{-2}) for seed yield obtained under lowest and highest irrigation intervals (IR_1 and IR_3), respectively (Figure 1). Furthermore, 1000 seed weight not affected by irrigation intervals, cattle manure levels and interaction of treatments (Table III). Our results are consistent with research of Kim et al. (2007) who revealed that water stress did not affect the 1000 seed weight of sesame, showing that last responds to post flowering drought by reducing seed numbers, but not seed size.

ESSENTIAL OIL CONTENT AND YIELD

Interaction of irrigation intervals and cattle manure levels showed that essential oil ranged from 1.55 to 2.07% (Figure 2). The highest value for essential oil content of oregano obtained in IR_3 with application of 30 t ha^{-1} cattle manure. In other hand, IR_1 without use of cattle manure produce the lowest essential oil content (Figure 2). For essential oil yield, the lowest value (46.37 kg ha^{-1}) for essential oil yield was found in IR_2 with application of 20 t ha^{-1} cattle manure. Unlike, the maximum mean value (66.62 kg ha^{-1}) for essential oil yield was resulted in IR_3 by use of 30 t ha^{-1} cattle manure (Figure 3). This result may be due to increase in essential oil content via a higher density of oil glands as a result of reduction in leaf area. Also, the effect of irrigation intervals on essential oil percentage may be due to its effect on enzyme activity and metabolism of essential oil production (Simon et al. 1992, Khalid et al. 2006). Water stress maybe increased essential oil content of more medicinal and aromatic plants because

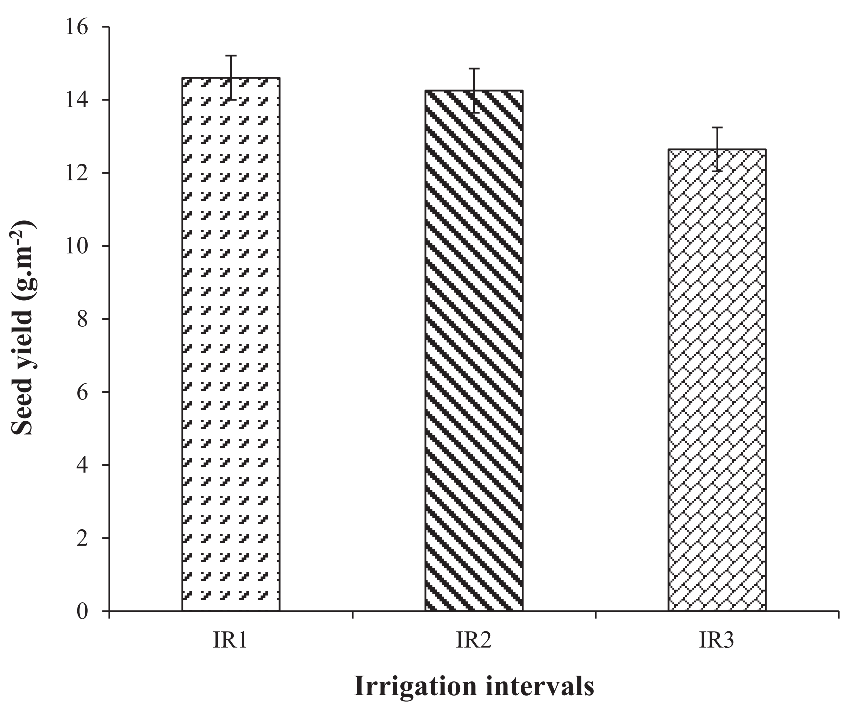

Figure 1 - Effect of irrigation intervals on seed yield of oregano. Vertical bars indicate standard errors.

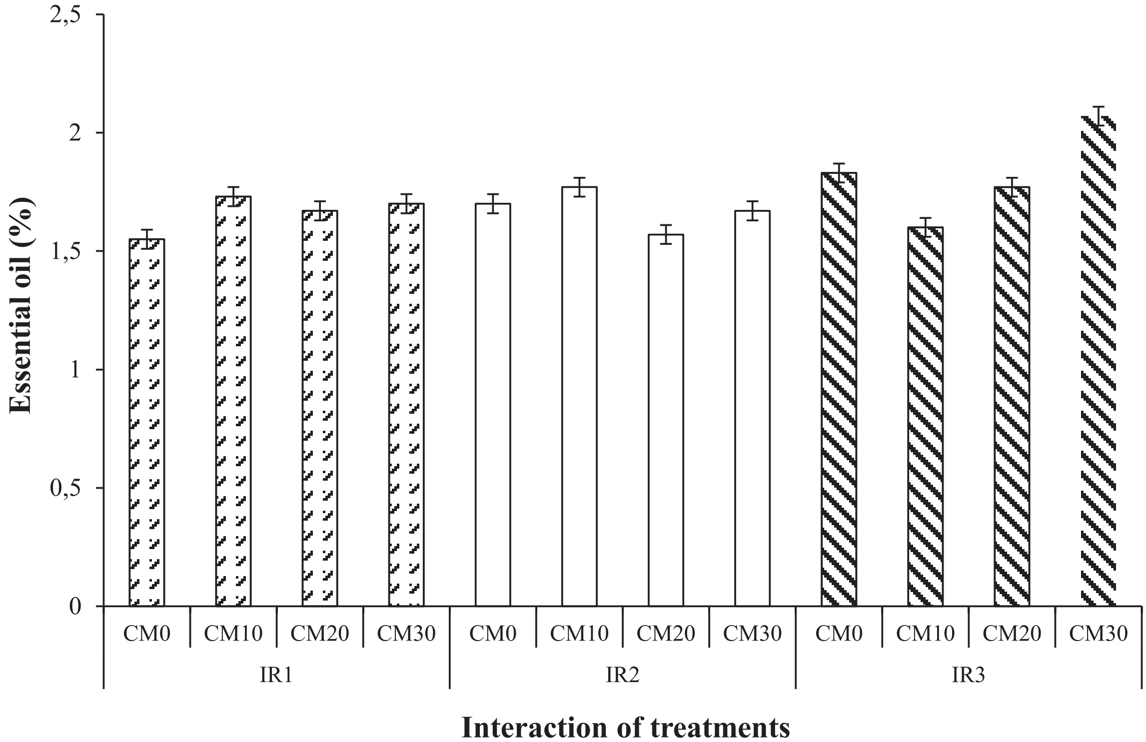

Figure 2 - Effect of irrigation intervals (IR) and cattle manure levels (CM) on essential oil content (%). vertical bars indicate standard errors.

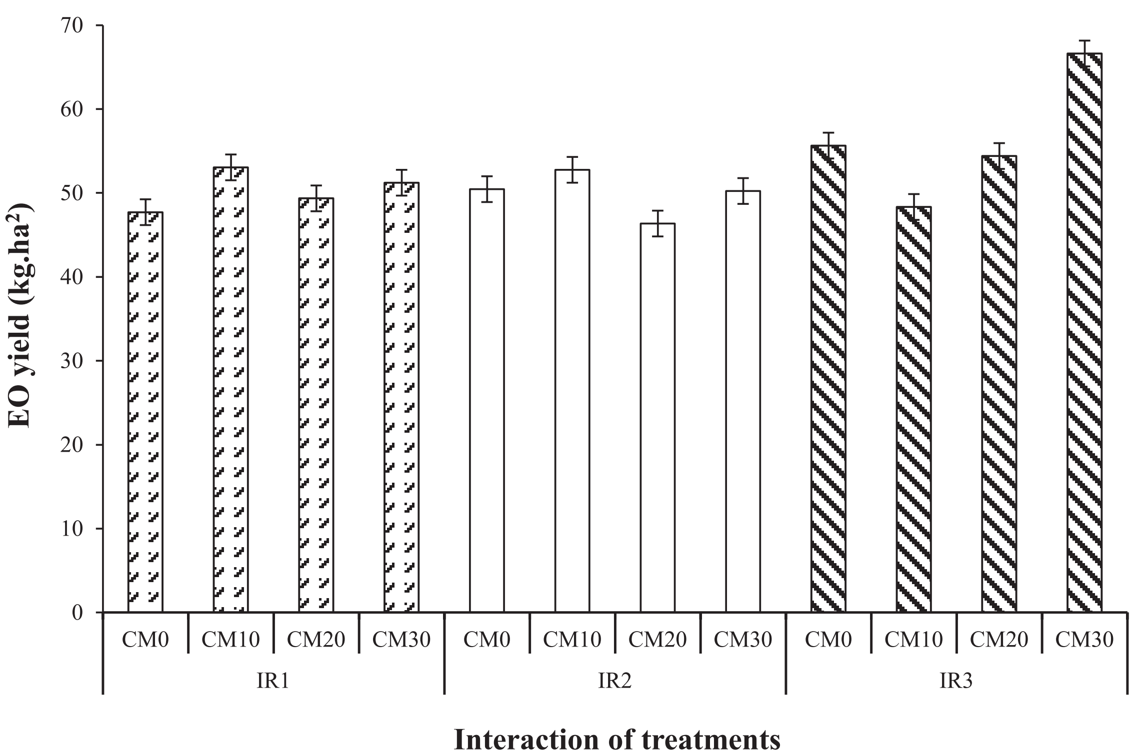

Figure 3 - Effect of irrigation intervals (IR) and cattle manure levels on (CM) essential oil yield (kg ha^{-1}). Vertical bars indicate standard errors.

in case of stress, more metabolites are produced in the plant cells and substances prevented from oxidization in these stressed cells (Farahani et al. 2009). Moreover, Penka (1978) mentioned that essential oils are the product of the respiratory catabolic processes which increase under water deficit. In agreement with our results, Baher et al. (2002) showed that essential oil percentage of *Satureja hortensis* increased under water deficit.

Also, the useful effect of cattle manure levels on essential oil percentage may be due to the influence of organic manure on accelerating metabolism reaction as well as stimulating enzymatic systems response for biosynthesis of essential oil content (Khalid and Hossein 2012). These results are contrast with those reported by Ram et al. (2006) and Tabrizi et al. (2011). Also, our results are in agreement with those reported on other plants such as *Pimpinella anisum* L. (Zehtab-Salmasi et al. 2001), *Petroelinum crispum* (Petropoulos et al. 2008), *Carum carvi* L. (Laribi et al. 2009) and *Apium graveolense* L. (Khalid and Hossein 2012).

CONCLUSIONS

The present study indicates that increasing of irrigation intervals have negative effect on morphological traits while, it not significant effect on essential oil content and yield. On the other hand, utilization of organic manure had positive effects on some parameters such as leaf area, fresh and dry herb yield, stems number, plant spread and stem diameter. In general, it seems that cultivation of oregano with 3 weeks irrigation intervals by use of 30 t ha^{-1} cattle manure is a good practice for achieving high essential oil content and yield in this region.

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