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Influence of planting date and plant density on morphological characteristics, seed yield and essential oil percentage of oregano (*Origanum vulgare* L.)

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Abstract

Over utilization of medicinal plants from wild habitat is causing genetic diversity loss of these valuable plants. In order to determine the optimum planting date and density of oregano for successful production, a field experiment was conducted at the Faculty of Agriculture, Urmia University, Iran. The experimental design was split-plot based on randomized complete blocks with three planting dates (April 19, May 5 and May 20) as main plots and four plant densities (8.33, 10, 12.5 and 16.67 plants m^{-2}) were assigned as sub-plots, all of which replicated three times. Results showed that planting date had significant effects on all traits except the stem proportion and 1000 seed weight. The highest values for all traits were obtained from the first date of planting, whereas values decreased with later planting dates. Also, stem number, plant spread, fresh and dry herb yield, seed yield and essential oil yield significantly influenced by plant density. With increase in plant density, stem number in per plant and plant spread decreased. The maximum fresh and dry herb yield (10618.23 and 2934.36 kg ha^{-1}, respectively) and essential oil yield (42.786 kg ha^{-1}) were obtained at the highest plant density. In conclusion, first planting date with higher plant density caused the greater essential oil yield, fresh and dry herb yield.

Key words: Herb yield, height, flower, leaf, plant spread, sowing date, oregano, seed, morphology, essential oil, stem, medicinal plant.

Introductıon

Herbal ingredients are increasingly used in several fields including pharmaceutical products, food production or protection and cosmetics industry (Al-Kalaldeh *et al*., 2010; Sarikurkcu *et al*., 2015). Over the past few decades, interest in the biological activities of the plant products has increased, because these products are involved in human healthcare (Al-Kalaldeh *et al*., 2010). From this point, many medicinal plants have attracted scientists as natural sources that could be safer than synthetic ingredients (Sarikurkcu *et al*., 2015).

Unfortunately, over- utilization and exploitation of medicinal and aromatic plants from the wild habitat led to genetic diversity loss and habitat destruction of these valuable plants (Hoareau and DaSilva, 1999). If the overharvesting continues it is estimated that around 4000 and 10000 medicinal plant species might be endangered in the world (Edwards, 2004).

Domestication and cultivation of these species can reduce pressure on the wild habitat and thus avoid their extinction (Saglam *et al*., 2004). Culvation of medicinal plants is advisable because of the possibility to better control quality of the prospective bioactive components (Franz, 1983). Also, this process permits the production of uniform herb materials in the required quality and quantities (Mohammad *et al*., 2010).

Yield improvement of most crops especially medicinal plants can be achieved by adjusting components of the growing system such as planting date, plant density, etc (Deans and Svoboda, 1993). An alteration in planting date causes significant changes in weather parameters and thus the performance of the crop yield (Ramesh and Singh, 2008). Planting date governs the phenological development and biomass production of crop along with efficient conversion of biomass into economic yield (Khichar and Niwas, 2006). Yield of a crop can be taken as a product of biomass accumulation rate (solar radiation dependent) and the growth duration (ambient air temperature dependent) (Kaur and Hundal, 2006). Efficiency of conversion of heat energy into dry matter depends upon genetic factors, planting date and crop type (Rao *et al*., 1990).

The study of Mohammad *et al*. (2010) indicated that the highest morphological characteristics were obtained from the early planting date and decreased with later planting dates in German chamomile (*Matricaria chamomilla* L.). Changes in essential oil yield have been reported to be influenced by planting date (Rangappa *et al*., 1997). In other research, Habib-Ullah and Honermeier (2013) reported that delayed planting date caused significant decrease in yield contributing parameters such as branches/plant, umbels/plant and 1000 seed weight of three anise (*Pimpinella anisum* L.) cultivars.

On the other hand, plant density can alter the interception of photosynthetically active radiation (PAR) and the distribution of light within the canopy, and the amount of resources including water, nutrients, and soil volume available per plant, and thus affect canopy development (Heitholt and Sassenrath-Cole, 2010). Indeed, the optimization of plant density can lead to a higher yield in the plant by favorably use of nutrients and exposure of the plant to the light (Khorshidi *et al*., 2010). Ahmad *et al*. (2004) showed that plant height of fennel (*Foeniculum vulgare*) increased with increasing plant density. Also, Amarjit *et al*. (1992) found that, different plant densities of dill (*Anethum graveolens*) had no significant effect on essential oil content, but essential oil yield increased by increasing of plant density.

Origanum vulgare L. commonly known as 'oregano' is a well-known medicinal plant species belonging to the Lamiaceae family (Vokou *et al*., 1993; Spada and Perrino, 1996). This plant can be found abundantly on dry, rocky calcareous soils of mountain areas that grows throughout the Mediterranean and most part of Euro-Siberian and Irano-Turanian regions (Snogerup, 1971; Aligiannis *et al*., 2001) at a wide range of altitude from 0 to 4000 m (Snogerup, 1971). Essential oil of oregano has antimicrobial, antioxidant, cytotoxic, antiseptic and antifungal properties (Aligiannis *et al*., 2001). Moreover, in traditional medicine oregano is used to treat respiratory disorders, dyspepsia, painful menstruation, rheumatoid arthritis, scrofulosis and urinary tract disorders (Gruenwald *et al*., 2000; Aligiannis *et al*., 2001).

For more knowledge about oregano (*O. vulgare* L.) cultivation, this study was conducted to investigate the effects of planting date and plant density on morphological characteristics, essential oil content and seed yield of oregano.

Materıal and methods

Experimental site: The present experiment was conducted at the Research Farm of the College of Agriculture, Urmia University, Iran, located at 37˚32' N latitude, 45˚5' E longitudes and 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. Climate data including monthly average temperature and precipitation of the experiment site are shown in Fig. 1.

The soil type of experiment site was clay loam with pH 7.5 and consisted of 0.92 % organic matter. Soil sample analysis indicated that at the depth of 0-30 cm soil layers contained 0.16 % nitrogen, 12.1 ppm available phosphorus and 338 ppm available potassium. Also, the electrical conductivity (EC) was 0.65 $dS.m^{-1}$.

Experimental design and treatments: The experimental design was split-plot based on randomized complete blocks with three replications. The main plots were planting dates (April 19, May 5 and May 20) and subplots consisted of different plant densities

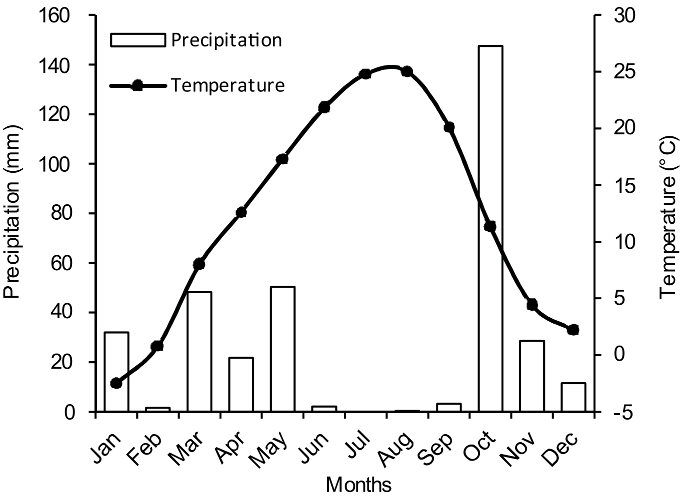

Fig. 1. 2014 monthly mean temperature (°C) and mean precipitation (in mm) in Urmia, Iran.

(16.67 (D_1), 12.5 (D_2), 10 (D_3) and 8.33 (D_4) plants m^{-2}). Each main plot area was 33.6 m^2 that divided to four sub plots (8.4 m^2). The distances between the blocks and sub plots were 2 and 0.5 m, respectively.

Plant material: Seeds of oregano were collected from wild habitat. Eight to ten seeds were sown in each small plastic 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) under greenhouse conditions. After four weeks, seedlings were thinned to two healthy seedlings per pot. Seedlings were transplanted after acclimation (5 days) when they were 70 days old in different planting dates mentioned above. The plots were immediately irrigated after transplanting for uniform establishment of seedlings and further irrigation was provided on requirement such that moisture was maintained at field capacity (irrigation intervals varied from 6 to 10 days).

Five years composted cattle manure were applied for all plots four months prior to transplanting. Weeds were controlled and removed by hand when needed. No synthetic pesticide and inorganic fertilizer was used during the study.

Morphological characteristics and yield: For morphological analysis, samples of oregano plants were taken from center rows of each plot. Plant height was measured from the soil surface to the tip of the tallest flowering stem with a yardstick. For each treatment, measurement of fresh and dry matter weights were evaluated by harvest of five randomly selected plants. The plants were harvested at a height of 5 cm above ground at full flowering stage 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 dry weight and proportion of leaves, stems and inflorescences were also 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 air-dried in the shade until it reached a constant weight (approximately one week) and weighted (dry herb yield).

Seed yield and 1000 seed weight: For each treatment, seed yield was measured at maturity stage by harvesting a 1 m^2 area from center rows of each plot. Seed yield and 1000 seed weight were determined after drying with digital balance.

Essential oil isolation: Plant materials were harvested at beginning of the flowering stage from each treatment and air-dried in the shade for two weeks. Dried and powdered leaves and flowers (50 g) were subjected to conventional hydro-distillation using Clevenger type apparatus for 2.5 h. The essential oil content of samples was determined on a percentage basis (v/w %). Also, essential oil yields as kg ha^{-1} were measured by using following formula:

EOY (kg ha^{-1}) = EO (%) × DW (kg ha^{-1})

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

Statistical analysis: Data were statically analyzed according to ANOVA by SAS 9.1 software, in order to test the main effects of planting dates, different densities and their interactions. Duncan's multiple range test at $P < 0.05$ level was used to compare the means when the ANOVA F-test indicated a significant effect of the treatments.

Results and dıscussion

Morphological characteristics and yield: Mean comparison of some morphological characteristics of oregano at different planting dates and densities were presented in Table 1 and 2. The results indicated that planting date had significant effect on all evaluated morphological traits (P < 0.05) except the stem proportion (Table 1 and 2). With the delay in planting date, the amounts of all traits were decreased (Table 1 and 2). Therefore, the highest and the lowest values for morphological traits obtained in P_1 and P_3, respectively. There were no significant differences between the planting dates for stem proportion (Table 1).

For each crop there is an optimum planting date and its delay can usually reduce the vegetative and reproductive yield (Ahmadi and Hadipanah, 2014). Therefore in early planting date due to longer growth season as well as less high temperature in early establishment period (early after transplanting), oregano had optimum growth and development which led to higher morphological traits. Although the decline in morphological traits may partially have resulted from development of the later planting date under warmer temperature and shortening the vegetative growth phase (Gesch, 2013). In other word, optimum temperature and relative humidity associated with early planting were suited for development and growth of early planted crops (Rao *et al*., 1999).

Plant density significantly affected stem number per plant, plant spread and fresh and dry herb yield (P < 0.05). With increase in plant density, stem number per plant and plant spread decreased, but fresh and dry herb yield increased (Table 2). Compared with D_1, D_4 showed 25.76 % more stem number per plant and 21.93 % wider plant spread (Table 1). However, D_1 produced 20.21 % and 17.66 % greater fresh and dry herb yield, respectively, as compared with D_4 (Table 2).

The decrease in morphological traits by increasing plant density can be due to inter- and intra-plant competition and resource limitations, specifically insufficient space for crops under higher densities (Moosavi, 2012). In other word, reduction in plant density provides more space for each plant and decreases inter-plant competition which increases the growth characteristics of crop (Gimplinger *et al*., 2008). Our results are in agreement with other reports which confirm the effect of planting date and plant density on growth of crops such as *Plantago ovata* (Moosavi *et al*., 2012), and *Brassica oleracea* var. *capitata* (Paranhos *et al*., 2016).

Also, there were interaction between planting date and plant density in the stem number per plant, plant spread, fresh and dry herb yield (Table 1 and 2). In other word, these mentioned traits were significantly affected by interaction of treatments (P < 0.01).

Seed yield and 1000 seed weight: Seed yield of oregano was significantly affected by planting date and density (P < 0.05). The early planting date (April 19) led to the highest seed yield (13.938 g m^{-2}) compared to the second (May 5) and third (May 20) date of planting. Of course, seed yield of oregano did not significantly differ between first and second planting dates. On the other hand, the highest plant density (D_1) produced the highest seed yield with

Table 1. Effect of planting date and plant density on plant height, stems number, plant spread, stem diameter, stem and leaf + flower proportion in oregano

Treatments		Plant height (cm)	Stem number	Plant spread (cm)	Stem diameter (mm)	Stem proportion (%)	Leaf + flower proportion (%)
Planting date		**	**	**	**	Ns	**
P_1 †		42.6 a	12.24 a	30.166 a	2.203 a	37.982 a	63.501 a
P_2		34.3 b	10.74 a	26.655 b	2.154 a	36.862 a	63.129 a
P_3		25.2 c	7.63 b	23.357 c	1.886 b	40.307 a	61.236 b
Plant density		Ns	**	**	Ns	Ns	Ns
D_1		34.5 a	9.12 c	24.422 d	2.063 a	36.993 b	62.993 a
D_2		33.2 a	9.95 bc	24.988 c	2.103 a	42.039 a	62.027 a
D_3		34.2 a	10.28 b	27.716 b	2.060 a	37.469 b	62.517 a
D_4		34.1a	11.47 a	29.778 a	2.098 a	37.032 b	62.951 a
P × D		Ns	*	**	Ns	Ns	Ns
P_1	D_1	42.1 ab	10.30 cd	26.410 f	2.177 ab	35.743 b	64.237 a
	D_2	41.8 ab	11.25 bc	27.047 e	2.217 a	43.107 ab	62.880 ab
	D_3	43.9 a	13.25 ab	31.133 b	2.193 ab	36.840 b	63.143 ab
	D_4	42.5 a	14.16 a	36.073 a	2.227 a	36.237 b	63.743 a
P_2	D_1	36.5 bc	9.70 cd	24.527 g	2.093 b	36.457 b	63.537 a
	D_2	34.2 c	11.23 bc	24.753 g	2.180 ab	37.040 b	62.957 ab
	D_3	33.1 c	10.40 cd	28.157 d	2.173 ab	37.810 ab	62.180 abc
	D_4	33.2 c	11.65 bc	29.183 c	2.170 ab	36.140 b	63.843 a
P_3	D_1	24.9 d	7.35 e	22.330 g	1.920 c	38.780 ab	61.207 bc
	D_2	23.6 d	7.38 e	23.163 i	1.913 c	45.970 a	60.243 c
	D_3	25.5 d	7.20 e	23.857 h	1.813 c	37.757 ab	62.227 abc
	D_4	26.6 d	8.60 de	24.077 h	1.897 c	38.720 ab	61.267 bc

† P = Planting date, D = Plant density. 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..**:P < 0.01; *:P < 0.05; Ns: non-significant.

14.927 g m^{-2}, representing 9.14 %, 16.37 % and 26.22 % more seed yield than D_2, D_3 and D_4, respectively (Table 1).

Planting date and plant density had no significant effects on 1000 seed weight. Also, the interactions between planting date and density for seed yield and 1000 seed weight were not significant (Table 2).

Date of planting is crucial for the vegetative growth and ultimate expressions of crop yield. Similar to morphological traits, late planting date may hamper the growth, quantity and quality of the crop yield. Also, date of planting controls the plant phenological development along with efficient conversion of biomass into economic yield (Khichar and Niwas, 2006). Early planting date induced a significantly higher oregano seed yield probably due to longer period of main and lateral stems and inflorescence development (Table 1). Therefore, similar to morphological traits, in early planting date (April 19), due to longer growth season and probably less heat stress, oregano had optimum growth and development which may result in higher seed yield. Our results are in accordance with the results of Foldesi and Barsi (1983) who found a decreasing trend in yield and yield components by delayed planting. The higher seed yield at higher densities may be due to the quick formation of plant canopy, the increase in leaf area index and better utilization of solar radiation and other resources (Moosavi *et al*., 2013). Nekonam and Razmjoo (2007) reported similar results for *Plantago ovata*.

Essential oil content and yield: The results showed that different planting dates had significant effects on essential oil content (EO) (Table 2) and yield (EOY) (Fig. 2). The highest values for EO and EOY were obtained in P_2 and P_1, respectively. Lower essential oil content in delayed planting date can be explained by slow flower development and lower leaves and flower production (as

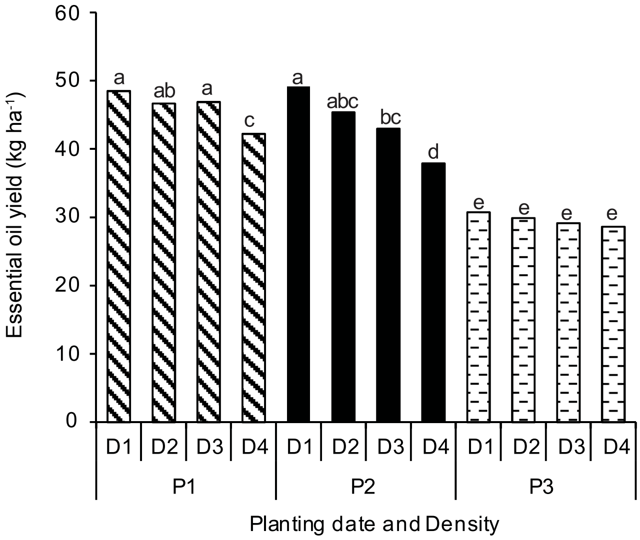

Fig. 2. Effect of planting date and density on essential oil yield (kg ha^{-1}) of oregano. Letters indicate significant differences by Duncan's multiple range test (P=0.05) and vertical bars indicate standard error.

Table 2. Effect of planting date and planting density on fresh and dry herb yield, seed yield, 1000 seed weight, EO and EOY in oregano

Treatments		Fresh herb yield (kg ha^{-1})	Dry herb yield (kg ha^{-1})	Seed yield (g m^{-2})	1000 seed weigh (g)	EO (%)
Planting date		**	**	**	Ns	**
P_1 †		10779.20 a	3062.25 a	13.938 a	0.243 a	1.506 a
P_2		10110.00 b	2845.03 b	13.807 a	0.245 a	1.540 a
P_3		8343.69 c	2254.11 c	11.244 b	0.249 a	1.314 b
Plant density		**	**	**	Ns	Ns
D_1		10618.23 a	2934.36 a	14.927 a	0.249 a	1.446 a
D_2		10078.72 b	2802.14 b	13.562 b	0.245 a	1.439 a
D_3		9447.42 c	2651.34 c	12.483 c	0.245 a	1.483 a
D_4		8832.81 d	2494.00 d	11.013 d	0.243 a	1.446 a
P × D		**	**	Ns	Ns	Ns
P_1	D_1	11840.70 a	3299.30 a	15.923 a	0.253 a	1.470 a
	D_2	11231.56 b	3147.50 b	14.420 b	0.236 b	1.483 a
	D_3	10621.10 c	3025.60 c	13.527 c	0.240 ab	1.550 a
	D_4	9423.43 d	2776.60 d	11.883 d	0.241 ab	1.520 a
P_2	D_1	11362.33 b	3168.80 b	15.767 a	0.245 ab	1.550 a
	D_2	10496.60 c	2945.57 c	14.603 b	0.247 ab	1.540 a
	D_3	9541.33 d	2743.80 d	13.330 c	0.246 ab	1.567 a
	D_4	9039.73 e	2521.93 e	11.527 d	0.243 ab	1.503 a
P_3	D_1	8651.67 f	2334.97 f	13.090 c	0.249 ab	1.317 b
	D_2	8508.00 f	2313.37 f	11.663 d	0.253 a	1.293 b
	D_3	8179.83 g	2184.63 g	10.593 e	0.248 ab	1.333 b
	D_4	8035.27 g	2183.47 g	9.630 f	0.245 ab	1.313 b

† P = Planting date, D = Plant density, EO: essential oil content, EOY: essential oil yield.
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.
**:P < 0.01; *:P < 0.05; Ns: non-significant.

main sources of essential oil synthesis) as compared with stems (Table 1). In other word, higher essential oil content or essential oil yield in early planting date might be due to the development of several branches with more leaves and flowers in the plant (Ramesh and Singh, 2008). Also, Kaul *et al*. (2000) reported that a high proportion of leaves and inflorescence to stem (similar to our finding in Table 1) is necessary for highest essential oil recovery.

Our results are in accordance with those of Hadj Seyed Hadi *et al*. (2004) who found decreasing trends in essential oil content and yield by delay in planting date. However, these results are in contrast to the results of Mohammad *et al*. (2010).

Plant density did not significantly affect the essential oil content (Table 2). Unlike essential oil content, essential oil yield significantly influenced by plant density ($P < 0.05$). With decrease in plant density essential oil yield decreased 4.99 %, 7.27 % and 15.24 % in D_2, D_3 and D_4, respectively, as compared with D_1 (Table 2). Higher essential oil yield in higher density could be due to the occurrence of higher dry herb yield as affected by more plant per unit area. Also, there were interactions between planting date and plant density for the essential oil yield ($P < 0.05$) (Fig. 2). In contrast, there were no significant interaction between planting date and plant density for essential oil content (Table 2). Within the P_1, P_2 and P_3 planting dates, all plant density treatments had a similar quantity of essential oil content (not significant difference).

With the delay of planting date, later planting dates led to decrease in the amount of all traits except the stem proportion and 1000 seed weight. Therefore, the highest and lowest values for all traits were obtained in April 19 and May 20, respectively. Contrarily, with increase in plant density, stem number per plant and plant spread decreased, but other measured traits were not significantly influenced by this treatment. In general, early and oil yield, fresh and dry herb yield.

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