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Essential Oil Production, Yield and Water Use Efficiency of Fennel (*Foeniculum vulgare* Mill.) as Affected by Sowing Date and Plant Density

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ABSTRACT

In order to study the effect of sowing date and plant density on essential oil, yield and water use efficiency (WUE) of fennel, an experiment was conducted in research field of Islamic Azad University, Birjand branch, Iran as a split-plot experiment based on a randomized complete block design with nine treatments and three replications. The main plot was sowing date at three levels of March 19, April 9 and April 20. The sub-plot was plant density at three levels of 6.7, 10 and 20 plants m^{-2}. According to the results of analysis of variance, seed, biological and essential oil yield and WUE were significantly affected by sowing date, plant density and their interaction at 1% probability level, but the interaction between sowing date and plant density did not significantly affect essential oil percentage. As means comparison showed, delay in sowing from March 19 to April 20 led to 85.6 and 85.6% loss of seed yield and biological yield, respectively. Despite higher essential oil percentage at delayed sowing, the highest oil yield was obtained at sowing date of March 19 with the average amount of 20.96 L ha^{-1} which was 5.82 times as high as that at the sowing date of April 20. The sowing date of March 19 had higher water use efficiency (WUE) for seed and oil production than the sowing date of April 20 by 5.5 and 3.67%, respectively. However, it increased biological yield and WUE for seed, biomass and essential oil production by 71.8, 64, 44.9 and 74.9%, respectively. The highest seed yield with the average amount of 726.90 kg ha^{-1} was obtained at the density of 20 plants m^{-2} which was 16 and 62% higher than that at the densities of 10 and 6.7 plants m^{-2}. Lower densities had lower essential oil yield despite higher oil percentage. In general, it is recommended to use sowing date of March 19 with the density of 20 plants m^{-2} for the cultivation of fennel because of having the highest seed and essential oil yield and WUE.

***Keywords**:* Fennel, Sowing date, Plant density, Water use efficiency, Yield, Essential oil.

INTRODUCTION

Fennel (*Foeniculum vulgare* Mill.) belongs to the family of Apiaceae. The essential oil percentage of fennel varies depending on the part of the plant and ecological factors. Mean essential oil content is 1-1.5% in leaves, 0.6-0.7% in roots, and 2-6% in fruits (Omidbeigi, 1997). Fennel is widely cultivated in Europe, Asia, the USA and many African countries as well as Brazil and Argentina for medicinal and feeding purposes (Kapoor *et al*., 2004).

One of the greatest challenges for agriculture in arid and semi- arid regions is to develop technology or agronomic options to improve water use efficiency (Turner, 2004)**.** The selection of sowing date and plant density are very important for obtaining maximum yield in plants. These factors play an essential role in achieving the proper conditions during growth period to achieve maximum performance and optimum water use efficiency in medicinal plants (Hassani, 2006).

There are various suggestions about fennel sowing date. Some researchers recommend autumn (October-November) as the suitable sowing date for fennel given the optimum temperature for its germination (6-8°C), although in the case of the occurrence of long chilling, the roots might be struck by hypothermia which may lead to desiccation of the plants (Ayub *et al*., 2008). In an experiment on sowing dates of fennel (May 10, 20 and 30), Ghanbari-odivi *et al.* (2013) indicated that the second sowing date had the highest seed, biomass and essential oil yield and essential oil percentage. In that research seed yield in the sowing dates were 811, 956 and 554 kg ha^{-1}, respectively. In a study on fennel sowing dates (September 4, October 4 and November 4), Ayub *et al*. (2008) reported that the sowing date of October 4 had the highest seed and biological yields. Delayed sowing in that experiment decreased seed and biological yield by 84.6 and 62.5% at the first years respectively. Seed essential oil percentage did not significantly affected by sowing date at both years. The results of Lotfi1 *et al.* (2013) in Ilam indicated that vegetative and reproductive growth of fennel was better in early planting date (March 5). Blazewicz and Sanjeet *et al.* (2010) showed that the early sowing of fennel had positive effect on plant growth, seed yield and oil production. In another experiment on planting dates of anise (*Pimpinella anisum* L.) from October 15 to December 15, Meena *et al.* (2012) indicated that the first sowing date had the highest seed yield (898 kg. ha^{-1}). Nowak and Szemplinski (2014) showed that essential oil percent of coriander (*Coriandrum sativum* L.) seed increased only very slightly (1.15 to 1.24%) with change of planting date. Hashemian Ahmadi and Hadipanah (2014) studied the effect of different sowing dates (June 12 and July 5 and 17) on *Dracocephalum moldavica* L. The results of the research indicated that sowing date had significant effect on oil percentage and oil yield. The highest oil yield (1.197 L ha^{-1}) was obtained at the second sowing date (July 5). Nasiri *et al.* (2014) reported declined biomass water use efficiency in ajowan in late planting dates. Although, in another experiment, corn WUE increased in late planting date (Feyzbakhsh *et al.*, 2015).

Khorshidi (2014) reported that the highest essential oil percentage (3.53%) of fennel obtained in the lowest plant density. Zand *et al.* (2013) studied densities of 67, 34, 23 and 17 plants m^{-2} in anise and reported that the highest seed yield and essential oil content of seed was related to density of 17 plants m^{-2}. Moreover, the highest essential oil percentage (6.22%) obtained in density of 17 plants m^{-2}. Density of 34 plants per m^{-2} had the lowest seed essential oil content (4.61%). In an experiment on ajowan (*Carum copticum*) Ghilavizadeh *et al.* (2013) studied the effect of plant densities (12.5, 16.6 and 25 plants per m^{2}). The highest biological, seed and essential oil yield obtained with density of 25 plants per m^{2}, but seed essential oil content did not significantly affected by the treatments. In another experiment, Moosavi *et al.* (2013) reported that fruit yield of coriander significantly increased by 12.4 and 31.4% as density increased from 30 to 40 and 50 plants m^{-2}, respectively. In this study, essential oil percentage of seed did not significantly affected by plant density. Ullah *et al.* (2014) showed that essential oil concentration of anise decreased as planting densities increased. Pirzad *et al*. (2008) reported that plant density significantly affected essential oil yield and percentage and WUE of chamomile. The highest essential oil percentage and yield and WUE were obtained from irrigation after 50 mm evaporation from evaporation pan and density of 33.33 plants m^{-2}. Sadeghi *et al*. (2009) studied the effect of sowing date and plant density on caraway (*Cuminum carvi*) yield and reported that as sowing was delayed from March 3 to March 13 and 23, seed and biomass yield significantly decreased. They stated that seed and biological yields were significantly affected by plant density and the interaction between sowing date and plant density.

Environmental factors, particularly sowing date and plant density deeply impact the quantity and quality of medicinal herbs. Thus, the objective of the current study was to examine the effect of these factors on seed yield, essential oil yield and water use efficiency of fennel in Birjand, Iran.

MATERIALS AND METHODS

The study was carried out in Hajiabad Research Station of Islamic Azad University, Birjand Branch, Iran (Long. 59°13´ E., Lat. 32°52´ N., Alt. 1400 m). The soil was loam-sandy with pH of 8.6, EC of 4.57 µmho.cm^{-1} and organic carbon content of 0.25% at the depth of 0-30 cm. The average long-time minimum and maximum temperature is 4.6 and 27.5°C with average annual precipitation of 169 mm and average minimum and maximum relative humidity of 23.5 and 59.6%, respectively. The regional climate is hot and arid.

The study was conducted as a split-plot experiment based on randomized complete block design with nine treatments and three replications. In this study, the effects of sowing date at three levels (March 19, April 9 and April 30) as the main plot and plant densities at three levels (6.7, 10 and 20 plants m^{-2}) as the sub-plot were examined. Each plot was 2×6 m with four 50 cm long planting rows. The field had been left fallow in the previous year. The field preparation operations, including tillage, was carried out in early-March. According to the results of soil test, 200 kg ha^{-1} ammonium phosphate was applied to the soil before final disking. Seeds disinfected by carboxinthiram fungicide (2:1000), were dry-sown at the depth of 2 cm. The desired plant densities were created by changing the spacing between plants at the emergence of the second filiform leaf. Irrigation was carried out once every 8-10 days and the weeds were removed 3-4 times at each sowing date. Pressurized system with hose and contour was used for irrigating the plots. To determine the yield, an area of 2 m^2 was harvested from the middle of each plot. Then, their seeds were winnowed in order to specify seed yield, biological yield. Seed essential oil percentage was determined by distillation with steam with the means of Clevenger extractor. Then, it was multiplied by seed yield to have essential oil yield. Also, essential oil, seed and biomass water use efficiencies were calculated by dividing each one's yield by applied water amount. Finally, the data for each trait were analyzed by MSTAT-C software and the means were compared by Duncan Multiple Range Test at 5% probability, level.

RESULTS AND DISCUSSION

Seed yield and biological yield

The results showed that sowing date, plant density and their interaction had significantly effects on seed and biological yields of fennel at 1% probability level (Table 1). With the delay in sowing from March 19 to April 30, fennel seed yield decreased from 1019.77 to 146.62 kg ha^{-1} (85.6%) and its biological yield decreased from 4045.74 to 581.32 kg ha^{-1} (85.6%) (Table 2).

The increase in plant density significantly increased seed yield, so that the highest mean seed yield (726.90 kg ha^{-1}) was obtained at the density of 20 plants m^{-2} which was 16 and 62% greater than that at the densities of 10 and 6.7 plants m^{-2}, respectively. Also, the increase in density from 6.7 plants m^{-2} to 10 and 20 plants m^{-2} increased biological yield by 37.4 and

71.8%, respectively (Table 3).

The highest seed yield (1161.30 kg ha^{-1}) was obtained at sowing date of March 19 with the density of 20 plants m^{-2} and the lowest one (97.93 kg ha^{-1}) was obtained at sowing date of April 30 with the density of 6.7 plants m^{-2} (Figure 1). The highest biological yield (4798.34 kg ha^{-1}) was obtained at sowing date of March 19 with the density of 20 plants m^{-2} and the lowest one (355.70 kg ha^{-1}) was obtained at sowing date of April 30 with the density of 6.7 plants m^{-2} (Figure 2).

Increasing plant density from 10 to 20 plants m^{-2} although did not have a significant effect on seed yield in first sowing date, but increased seed yield in the second sowing date (Figure 1). It seems that favorable environmental conditions especially light and temperature at the first sowing date improved resources use by fennel in treatment 10 plants m^{-2}. In the second sowing date (April 9) seed yield at densities of 6 and 10 plants per m^2 decreased significantly due to lower leaf area, branching and reproductive units. Moreover 40 days delay in planting from March 19 to April 30 severely declined seed yield in all planting densities (Figure 1). Probably this is related to shorter growing season and adverse environmental conditions especially light and temperature in sowing date of April 9. However, in all planting dates, increasing the density from 6.7 to 20 plants m^2 increased seed yield of fennel (Figure 1). It seem that although increase in plant density decreased seed yield of single plant because of severer inter-plant competition, but the increase in plant number per m^2 compensated the decrease in yield of single plant. In other words, increasing plant density significantly increased seed yield according to the increase in reproductive units per m^2 in all sowing dates. Al-Dalain *et al.* (2012) reported that early planting significantly increased the fruit yield combined with higher number of branches per plant, number of umbrella per plant, number of fruit per plant and plant height.

The comparison of means showed that although the changes of plant density had a significant effect on biological yield of fennel in the first and second sowing dates, but the increase in density in the third planting date had no significant effect on this trait (Figure 2). It can be said that in earlier sowing dates and more plant densities, plants can completely use environmental resources especially radiation because of higher leaf area index and the establishment of full plant coverage; therefore, biological yield per unit area increased. On the other hand, although higher densities in the first and second sowing dates, usually decrease single biomass yield as a result of inter-plant competition, but biological yield per unit area increases with plant density. In the third planting date (April 30) due to lack of sufficient opportunities for vegetative and reproductive growth and lower light and temperature, increasing plant density could not significantly improve biological yield. Since the variations of seed yield was highly correlated with biological (biomass) yield ($r = 0.994^{**}$ in Table 4), it seems that the high seed yield at the first sowing date with the density of 20 plants m^{-2} was brought about by its higher biological yield.

Soleimani *et al.* (2010) reported 29.9% decrease in fennel yield due to the delay in sowing date from March 25 to May 5. Moreover, in a study on the effects of sowing date and plant density on caraway, Sadeghi *et al.* (2009) reported that a 20-day delay in sowing date significantly decreased seed and biological yields. The highest seed and biological yields were observed at the first sowing date and the highest plant density that are according to results of this research.

Table 1. Results of analysis of variance for the effect of sowing date and plant density on yield, essential oil percentage and water use efficiency (WUE) of fennel

Sources of variation	df	Seed Yield	Biological yield	Essential oil percentage	Essential oil yield	Seed WUE	Biomass WUE	Essential oil WUE
Replication	2	18622.366^{ns}	418564.66^{ns}	0.011^{ns}	9.88^{ns}	374.384^{ns}	8316.238^{ns}	0.19^{ns}
Sowing date (A)	2	1723439.628**	27026042.437**	0.505**	682.244**	29817.83**	467235.319**	11.569**
Error a	4	7309.883	166843.97	0.027	8.032	129.999	2941.037	0.15
Plant density (B)	2	178710.695**	3417079.878**	0.23**	45.018**	3943.802**	76993.394**	1.012**
A × B	4	19296.692**	310875.85**	0.024^{ns}	4.14**	380.049**	5961.143**	0.083**
Error b	12	542.258	27206.247	0.014	0.273	11438	512.148	0.006
C.V. (%)	-	13.88	17.04	15.30	14.13	13.88	16.66	14.06

ns, * and ** : non-significance and significance at 5 and 1% probiability levels, respectively

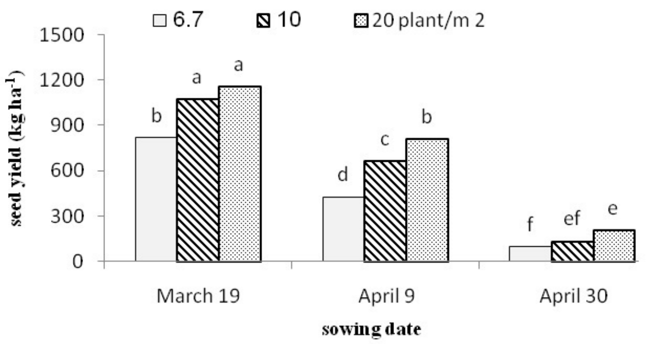

Figure 1. Interaction effects of sowing date and plant density on seed yield of fennel

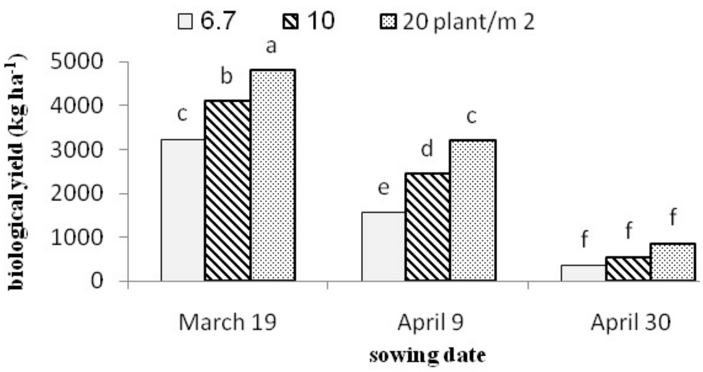

Figure 2. Interaction effects of sowing date and plant density on biologycal yield of fennel

Essential oil percentage and yield

As the results showed, sowing date and plant density had significant effect on essential oil percentage and yield at 1% probability level but their interaction significantly effected only essential oil yield but not essential oil percentage (Table 1). Delay in sowing date significantly increased seed essential oil percentage, so that the highest essential oil percentage (2.42%) was obtained at sowing date of April 30 and the lowest one (2.02%) was obtained at sowing date of March 19. Indeed, the sowing dates of March 19 and Aril 9 were ranked in the same statistical group for the essential oil percentage (Table 2). Probably_this result is related to optimum climate conditions (low temperature) in sowing date of April 30 for biosynthesis of essential oil. The study of Morteza *et al*. (2009) on *Valeriana officinalis* showed that delayed sowing date significantly increased essential oil that is in accordance to the results of this research.

Table 2. Mean comparisons of different sowing date on yield, essential oil percentage and water use efficiency (WUE)of fennel

Sowing date	Seed yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Essential oil percentage	Essential oil yield (L ha^{-1})	Seed WUE (g m^{-3})	Biomass WUE (g m^{-3})	Essential oil WUE (cc m^{-3})
March 19	1019.77 a	4045.74 a	2.02 b	20.96 a	138.74 a	552.44 a	2.85 a
April 9	635.63 b	2397.29 b	2.08 b	13.41 b	97.64 b	368.26 b	2.06 b
April 30	146.62 c	581.32 c	2.42 a	3.60 c	25.07 c	99.38 c	0.61 c

Means with the same letter(s) at each column had no significant difference at 5% probiability level

Despite higher essential oil percentage in delayed sowings date, the highest essential oil yield was produced at the sowing date of March 19 with average amount of 20.96 L ha^{-1} which was 5.81 times as great as that produced under the sowing date of April 30 (Table 2).This result is related to considerable decrease in seed yield because essential oil yield is a function of essential oil percentage and seed yield (Table 2). The study of Darzi *et al*. (2005) on fennel on cumin showed that delayed sowing significantly decreased essential oil yield.The highest essential oil percentage (2.29%) was obtained at the density of 6.7 plants m^{-2} and the lowest one (2.03%) was obtained at the density of 20 plants m^{-2}. Different plant densities were ranked in separate statistical groups with respect to essential oil percentage (Table 3). Given that inter-plant competition is less at lower densities than at higher ones and single plants have ample space, plants shading is lower and hence, canopy intercepts more light and so, essential oil percentage is higher at lower densities. Also, Letchamo *et al*. (1995) stated that the essential oil percentage was higher under excessive light conditions than under normal light and essential oil biosynthesis highly depended on light regimes (Naghdibadi *et al*., 2002). Also, Hajseyyedhadi *et al*. (2002) reported the increase in essential oil percentage of chamomile at lower densities which confirms the results of the current study.

As shown in Table 3, essential oil production per unit area was significantly higher at the density of 20 plants m^{-2} than at the lower densities because of the increase in seed yield at higher densities. Although increasing plant density from 10 to 20 plants m^{-2} did not have a significant effect on essential oil yield in first and second sowing dates, but increasing plant density from 6.7 to 10 or 20 plants m^{-2} significantly increased essential oil yield in both planting dates. However, all densities for essential oil yield trait in the third planting date were in the same statistical group (Figure 3).

Table 3. Mean comparison of different levels of plant density on yield, essential oil percentage and water use efficiency (WUE) of fennel

Density (plant m^{-2})	Seed yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Essential oil percentage	Essential oil yield (L ha^{-1})	Seed WUE (g m^{-3})	Biomass WUE (g m^{-3})	Essential oil WUE (cc m^{-3})
20	726.90 a	2948.49 a	2.03 b	14.56 a	106.08 a	431.77 a	2.13 a
10	626.44 b	2359.34 b	2.14 ab	13.21 b	90.71 b	341.51 b	1.92 b
6.7	448.63 c	1716.52 c	2.29 a	10.19 c	64.67 c	246.80 c	1.47 c

Means with the same letter(s) at each column had no significant difference at 5% probability level

However, in spite of higher essential oil percentage at lower densities (Table 3), essential oil production per hectare was significantly increased at the density of 20 plants m^{-2} in comparison with density of 6.7 plants m^{-2} as a result of increased (Figure 1). As shown in Figure 3, mean comparisons of essential oil yield indicated sowing date of March 19 with the density of 20 plants m^{-2} had the highest essential oil yield (23.27 L ha^{-1}) and sowing date of April 30 with the density of 6.7 plants m^{-2} had the lowest one (2.61 L ha^{-1}). The studies of Rahmati *et al*. (2009) on chamomile and Pirzad *et al*. (2007) on *Matricaria chamomilla* L. showed that the increase in plant density increased essential oil yield which confirmed the results of the current study.

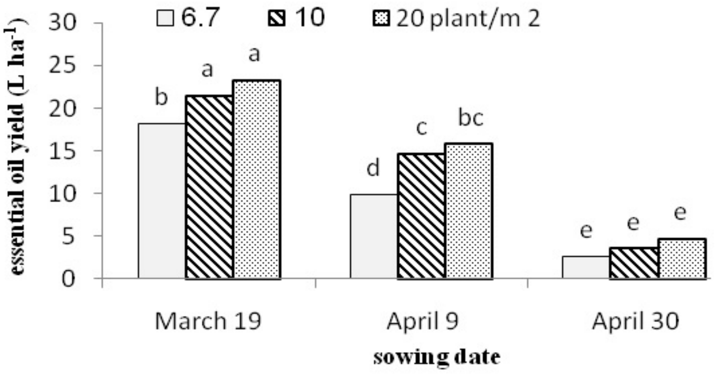

Figure 3. Interaction effects of sowing date and plant density on essential oil yield of fennel

Water use efficiency (WUE) for yields of seed, biomass and essential oil

According to the results of analysis of variance, the effects of sowing date, plant density and their interaction were significant on WUE of fennel essential oil, seed and biomass at 1% statistical level (Table 1).

Mean comparisons showed that delay in sowing date significantly decreased WUE, so that the water use efficiencies for seed, biomass and essential oil, production in the first planting date compared to the third planting date had superiority of 5.5, 5.6 and 4.7 times, respectively (Table 2). Moreover, the results indicated that with the increase in plant density, WUE significantly increased, so that WUE for essential oil production at the density of 20 plants m^{-2} (2.13 cc m^{-3}) was 10.9 and 44.9% greater than that at the densities of 10 and 6.7 plants m^{-2}, respectively. Also WUE for seed and biomass production was 64 and 74.9% greater at the density of 20 plants m^{-2} than at the density of 6.7 plants m^{-2}, respectively (Table 3).

Table 4. Correlation coefficients between different fennel traits

Traits	1	2	3	4	5	6	7
1.Seed yield	1						
2. Biological yield	0.994**	1	1				
3. Essential oil percentage	0.991**	-0.748**	1				
4. Essential oil yield	0.996**	0.986**	-0.742**	1			
5. Seed WUE	0.992**	0.988**	-0.825**	0.985**	1		
6. Biomass WUE	0.987**	0.996**	-0.812**	0.981**	0.993**	1	
7. Essential oil WUE	0.971**	0.979**	-0.764**	0.995**	0.989**	0.982**	1

ns, * and ** : non-significance and significance at 5 and 1% levels, probability respectively

Sowing date of March 19 with the density of 20 plants m^{-2} produced 3.17 cc essential oil, 158.03 g seed and 658.83 g biomass per m^3 applied water. Sowing date of April 30 with the density of 6.7 plants m^{-2} produced 0.44 cc essential oil, 16.74 g seed and 60.83 g biomass per m^3 applied water. Therefore, the former treatment had the highest water use potential and the latter had the lowest one (Figure 4-6). In addition, increasing plant density from 6.7 to 20 plants m^{-2} had a significant effect on WUE for seed and biomass yields in all sowing dates. But WUE for essential oil production in the third sowing date did not affected by changing in plant density (Figure 4-6). Although the used water for irrigation decreased in delayed sowing dates, as a result of shorter growth period, but higher seed, biomass and essential oil production at earlier sowing dates led to significant increase in WUE for seed, biomass and essential oil production. Also, significant increase in these traits with the increase in plant density could be related to the increase in seed, biomass and essential oil yields at higher densities (Figure 1-3). It has to be mentioned that WUE for seed, biomass and essential oil production was positively correlated to seed, biomass and essential oil yields (r =0.992** , 0.996** and r= .995**, respectively as shown in Table 4). Yadav and Dahama (2003) in a study on cumin (*Cuminum cyminum* L.) showed that delaying in sowing date declined WUE.

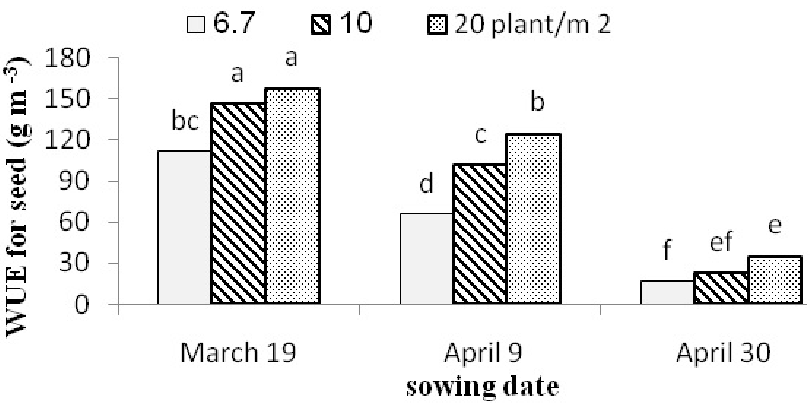

Figure 4. Interaction effects of sowing date and plant density on water use efficiency for seed of fennel

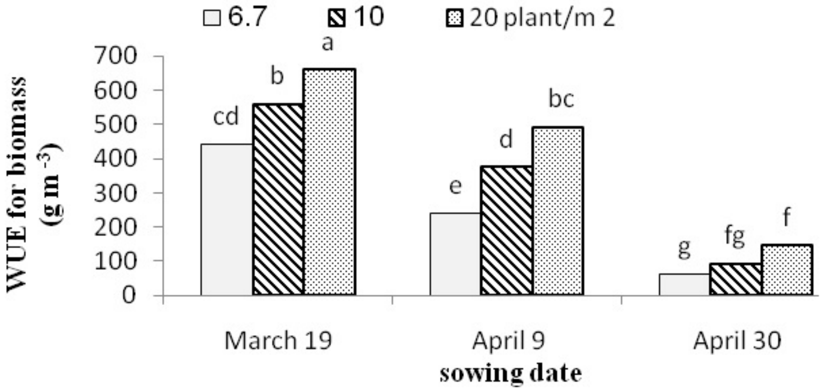

Figure 5. Interaction effects of sowing date and plant density on water use efficiency for biomass of fennel

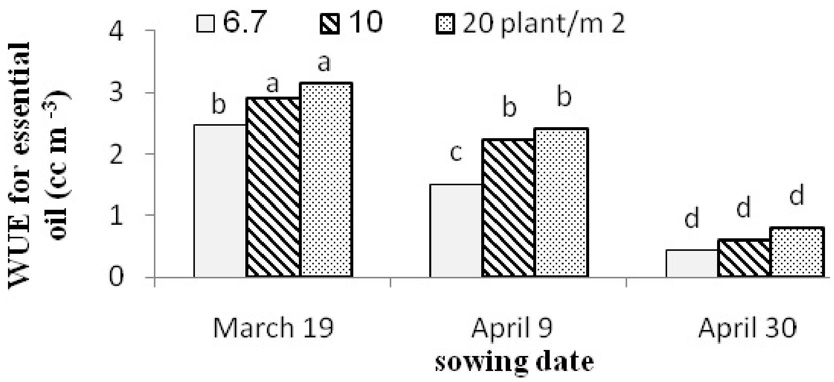

Figure 6. Interaction effects of sowing date and plant density on water use efficiency for essential oil of fennel

CONCLUSION

It can be said that earlier sowing date increased seed yield and biological yield per unit area through lengthening growth period, increasing leaf area, producing a plenty of auxiliary branches and number of reproductive units. It appears that favorable environmental conditions especially light and temperature at the first sowing date allowed the plants to better use of these conditions, to produce more assimilates and finally, to increase their seed and biological yields. Moreover, more density increased yield and WUE via increasing of light uptake and assimilates production. In total general, it is recommended to use sowing date of March 19 with the density of 20 plants m^{-2} for the cultivation of fennel because of having the highest seed and essential oil yields and WUE.

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