Changes in yields and volatile oil composition of fennel (*Foeniculum vulgare* Mill.) in high plant populations

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Abstract

This research was managed to identify the effects of the different seed quantity (5, 10, 15 and 20 kg.ha^{-1}) and row interval (15 and 30 cm) on the yields and the volatile oil components of fennel (*Foeniculum vulgare* Mill.) under the Harran Plain conditions, during 2013 and 2014 years. The study was established according to the *split plot in randomised complete block des*ign. Seed and volatile oil yields, volatile oil ratio and volatile oil components were importantly affected seed quantity and row interval. The transaction of 15 cm × 10 kg.ha^{-1}, the highest seed and volatile oil yield were taken, was determined to be the most suitable. The *trans-anethole* was taken the primary component under all transactions and it was changed to depend on the row interval and seed quantities.

Introduction

The avoidance of the chemical medication habits has increased demand to medicinal and aromatic plants and its products. To turn towards traditional methods has given importance to the production and quality of aromatic and medicinal plants. Fennel is the one of these plants and native of the Mediterranean region belongs to the *Apiaceae* family (Khorshidi *et al.*, 2010).

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Fennel seeds could be used directly as grain or benefited from its essential oil, which extracted from seeds. Seeds of fennel contain precious essential oil is used in medicinal and food-confectionery and cosmetics industries. Lately performed researches have shown that the essential oil of fennel could be used as antioxidant, antifungal and antibacterial (Lucinewton *et al.*, 2005). Fennel essential oil content varies between 3.5-6% in seeds. In fennel essential oil, T-anethole, limonene, estragole and fenchone are the primary constituent that might be variable in different stages of development (Gross *et al.*, 2002; Koeduka *et al.*, 2009). Essential oil could be produced through the plant but 90% of the essential oil contained in seeds (Falzari *et al.*, 2006). Essential oil yield and its components are highly affected by genetic factors and growing conditions (Rahimmalek *et al.*, 2009). The yield and quality of the fennel plants have affected by cultivation techniques such as sowing date, plant density, harvesting date (Masood *et al.*, 2004; Arabacı and Bayram, 2005; Koocheki *et al.*, 2006; Khorshidi *et al.*, 2009a, 2009b; Moosavi, 2014). To enhance the fennel production ways are to increase planting areas and to increase yield on per unit area. It is the first principle of the successful growing, to establishment of optimum density of healthy plants in a field. The optimum plant density is among different factors that could increase the yield on per unit area. On the one hand, the lower number of plants in the unit area prevents the optimum use of the production components (fertiliser, water, light...) and on the other hand, an excessive number of plants in the unit area can be increasing plant competition while can reduce yield and quality an excessive number of plants in the unit area can increased plant competition while can reduced yield and quality. For this reason, the number of plants in the unit area was important in terms of better economic utilisation of all agricultural production inputs. Determination of chemical composition of essential oil in relation to cultivation factors might provide useful information for management.

Researches have shown that the plant density has different effects depends on growing conditions. Koocheki *et al.* (2006) reported that the fennel seed yield was increased by 71% when the number of plants per square meter was raised from 40 to 100. However, number of umbel in the plant, number of umbellet in umbel and seed number in umbellet were decreased. Also, Avcı (2013) reported that the tested seed quantities were significant effect on seed yield and yield component.

These studies were generally the results of studies conducted over a wide rows spacing. The possible effects of narrow row intervals and different seed quantities on the fennel plants will be vary. Fennel plant density important to plant development and plant characteristics, are known competitive effect. In the *Umbelliferae* family plants, the generative developing (flowering) firstly starts on the top of the main branch, and maintain towards side branches, related to plant growing. More auxiliary branching can increase the number of umbel per plant. Under these circumstances, the fennel plants have the seed various sizes, at different stages of ripening and maturity at various times. One of the very important agents determining the branching of the fennel plant is plant number in per area. The density of the plant in per area can vary depending on the seed quantity and row spacing.

This research was conducted to determine the effects of different amounts of seeds and row spacing on fennel (*Foeniculum vulgare* Mill.) yields and essential oil composition.

Materials and methods

Field studies

In the study, fennel seed (*Foeniculum vulgare* Mill.) Adana ecotype obtained from domestic growers was used as plant material. The study was carried out in the Agricultural Faculty of Harran University research area, located in the southeast region of Turkey (Sanliurfa), in 2013 and in 2014, for two years. The experiment area was semi-arid climate conditions.

Climate data demonstrate similarities for the experiment years. However, in 2013, more moderate climate conditions were detected. The average and minimum daily temperatures in the February and the March were higher than the same period in 2014. In both years, there was no precipitation during the seed-filling period of the fennel plant. The relative humidities were similar in the two growth years.

The research area belongs to the Harran I series, a profile of A, B and C horizons, flat and flattish slope with deep profile and alluvial main material. Analysis of soil before sowing showed that pH 7.84, 0.08% salt, 17% chalk, clay texture and 1.37% organic matter. The research was set up the *split plot in a randomised complete block design* with three replicates and the row intervals (15 and 30 cm) as main parcels and the four seed quantities (5, 10, 15 and 20 kg.ha^{-1}) as sub parcels were used. Each sub-plot consists of four rows with a length of 5 m. Seeds of fennel were sown on February 8, 2013 and February 1, 2014 with a single row of hand drill. Before each sowing in both years, 50 kg.ha^{-1} phosphorus and 50 kg.ha^{-1} nitrogen fertiliser were applied during soil preparation. In addition, 50 kg.ha^{-1} nitrogen fertiliser were applied at the beginning of the flowering stage. During the two vegetation periods, weed control was carried out by hoeing if necessary. The all other agricultural practises were applied as needed. All plants and umbels were turned yellow, and were harvested by hand in the first year of July 12, 2013 and in the second year of July 19, 2014. The harvested fennel plants were blended after being dried in the shade. Observations and samples were randomly taken for each plot on the basis of a unit area. Seed (kg.ha^{-1}) and essential oil yields (L.ha^{-1}), volatile oil ratio (%) and volatile oil components (%) were investigated as according to Özel *et al.* (2001).

Essential oil isolation

The plant materials obtained from each sub-plot were first dried in the shade and room temperature. Samples taken from blended and dried fennel seeds were ground. For each plot, 50 g of the grinded seed sample was subjected to hydrodistillation for 120 min to determine the volatile oil ratios. After that, volatile oils were measured and stored at 4°C for volatile oil components analysis.

GC-FID analysis

Gas chromatography (GC) analysis was achieved on a ThermoQuest-Finnigan Trace GC, accoutred a flame ionization detector (FID) and an auto sampler, AS 2000. Capillary column, a polyethylene glycol ZB-wax (film thickness, 30 m × 0.25 mm, 0.25 mm) was used. The carrier gas (He) flow was 1.5 mL.min^{-1}. The 60:1 split ratio was used. The analysis temperature program was performed as oven temperature at 70°C, from 70 to 220°C at the rate of 3°C.min^{-1} and from 220 to 240°C at the rate of 1°C.min^{-1}. Both of the injector and detector temperatures were held at 240°C. The 1 mL sample was injected.

Compounds identification

The determination of the volatile oil components was made according to the peak areas in the wax column. The identity of the volatile organic compounds was obtained by retention indices and peaks of the samples compared to standards [Limonene (Sigma-Aldrich, 62118), Fenchone (Sigma-Aldrich, 46210), Estragole (Sigma-Aldrich, 34098), Trans anethole (Sigma-Aldrich, 10368) and Anisaldehite (Sigma-Aldrich, 97063)].

Statistical analysis

All obtained data were subjected to analysis of the variance according to the *split plot in randomised complete blocks design*. The significant differences between the practices were grouped by the LSD (P<0.005) test.

Results

Seed yield (kg.ha^{-1})

In research the seed yield (kg.ha^{-1}) values that taken from different row spaces and seed quantities during growing seasons were presented at Table 1.

Row spaces, seed quantities and row space × seed quantity interaction were significant effected at P<0.01 level on seed yield in both growing seasons but, row space × seed quantity interaction was at P<0.05 level in the first growing season. The highest fennel seed yield was taken from 15 cm × 10 kg.ha^{-1} transaction and the lowest one from 30 cm × 15 kg.ha^{-1} transaction in the first growth season of the experiment. The 15 cm row space was determined significantly higher seed yield values than the 30 cm row space in both growing seasons (Table 1). According to mean values of row spaces, the seed quantities were positive effect on seed yield, seed yield was increased as seed quantity increased in the second growing season but were adverse effect in the first season and seed yield was decreased as seed quantity increased. In spite of the inconsistent effect of seed quantities on seed yield there was 10 kg.ha^{-1} seed quantity notable aspect of the combined values.

Essential oil yield (L.ha^{-1})

Volatile oil yield (L.ha^{-1}) values obtained from row spaces and seed quantities during growing seasons and its combined values were presented Table 2.

Row space was no significant effect on volatile oil yield in the first year but was in the second season at P<0.05 level. Seed quantity and row space × seed quantity interaction were significant effect on volatile oil yield in both growing seasons at P<0.01 level. In both seasons 15 cm row space was given higher oil yield than 30 cm row space. The highest volatile oil yield was been taken from 15 cm × 10 kg.ha^{-1} and the lowest from 30 cm × 15 kg.ha^{-1} in the first year. In both row spaces the lowest seed quantities (5 and 10 kg.ha^{-1}) have given higher (43.0 and 43.2 L.ha^{-1}) volatile oil yield than the highest (15 and 20 kg.ha^{-1}) seed quantities in the first year. In the second year the highest and lowest volatile oil yield were obtained from the same row space (30 cm) and that the lowest volatile oil yield was taken from 5 kg.ha^{-1} seed quantity and the highest was 10 kg.ha^{-1} seed quantity (Table 2). When we looked into combined values the 15 cm row space was given significantly higher volatile oil yield than 30 cm row space and the low seed quantities (5 and 10 kg.ha^{-1}) were given significantly higher volatile oil yield than high seed quantities (15 and 20 kg.ha^{-1}).

Essential oil content (%)

The volatile oil contents (%) taken row spaces and seed quantities, during growing seasons and its combined values were presented Table 3.

In both growing season, there was no any significant difference between 15 and 30 cm row spaces. Also, the seed quantities were no any significant effect on volatile oil content in the first year but were in the second growing season, at P<0.01 level. But row space × seed quantity interaction was important effect on volatile oil con-

Table 1. Average seed yield (kg.ha^{-1}) values that obtained from different row spaces and seed amounts during growing seasons and its combined values.

Row space	2013 Seed amounts (kg.ha^{-1}) 5	10	15	20	Mean
15 cm	1357.3^{abc}	1524.0^{a}	1298.7^{bcd}	1151.7^{def}	1332.9
30 cm	1442.3^{ab}	1224.7^{cde}	1001.3^{f}	1054.0^{ef}	1180.6
Mean	1399.8	1374.3	1150.0	1102.8	1256.8
LSD 5%	18.6 (row space × seed amount)				
2014					
15 cm	1814.0^{bc}	1808.7^{bc}	1951.3^{ab}	2001.0^{a}	1893.8
30 cm	1341.0^{d}	1761.0^{c}	1730.0^{c}	1705.3^{c}	1634.3
Mean	1577.5	1784.8	1840.7	1853.2	1764.0
LSD 5%	18.6 (row space × seed amount)				
Combined					
15 cm	1585.7^{ab}	1666.3^{a}	1625.0^{a}	1576.3^{ab}	1613.3
30 cm	1391.7^{c}	1492.8^{bc}	1365.7^{c}	1379.7^{c}	1407.5
Mean	1488.7	1579.6	1495.3	1478.0	1510.4
LSD 5%	13.15 (row space × seed amount)				

$^{a-f}$Values sharing same letters differ non-significantly (P>0.05).

Table 2. Essential oil yield (L.ha^{-1}) values that obtained from different row spaces and seed amounts during growing seasons and its combined values.

Row space	2013 Seed amounts (kg.ha^{-1}) 5	10	15	20	Mean
15 cm	40.6^{ab}	46.6^{a}	40.0^{a}	34.3^{bc}	40.4
30 cm	45.4^{a}	39.8^{ab}	28.6^{c}	30.8^{c}	36.2
Mean	43.0	43.2	34.3	32.6	38.3
LSD 5%	0.685 (row space × seed amount)				
2014					
15 cm	53.9^{a}	52.5^{a}	50.1^{a}	53.3^{a}	52.5
30 cm	35.4^{b}	54.6^{a}	52.4^{a}	50.6^{a}	48.2
Mean	44.6	53.6	51.2	52.0	50.3
LSD 5%	0.685 (row space × seed amount)				
Combined					
15 cm	47.2	49.6	45.1	43.8	46.4^{a}
30 cm	40.4	47.2	40.5	40.7	42.2^{b}
Mean	43.8^{b}	48.4^{a}	42.8^{b}	42.3^{b}	44.3
LSD 5%	0.32 (row space), 0.34 (seed amount)				

$^{a-c}$Values sharing same letters differ non-significantly (P>0.05).

tent in both growing seasons, in the first season at P<0.05 and in the second one at P<0.01 level.

In 2013 the highest volatile oil content was obtained from 30 cm × 10 kg.ha^{-1} seed quantity and the lowest one from 30 cm × 15 kg.ha^{-1}. But in 30cm row interval the low seed quantities (5 and 10 kg.ha^{-1}) were given the highest (3.25% and 3.15%) volatile oil contents. In 2014, the highest volatile oil content was determined from 30 cm × 10 kg.ha^{-1} seed quantity and the lowest one from 30 cm × 5 kg.ha^{-1} seed quantity. In both years there was no any trend in relation to essential oil content. According to combined values 10 kg.ha^{-1} seed quantity was significant effect on volatile oil content than others.

Essential oil constituents

Volatile oil constituents and its percentages (%) were presented at Table 4. The *trans*-anethole, limonene and estragole were the

Table 3. Essential oil content (%) were obtained from different row spaces and seed amounts during growing seasons and its combined values.

Row space	2013 Seed amounts (kg.ha^{-1}) 5	10	15	20	Mean
15 cm	2.98^{bc}	3.05^{abc}	3.08^{abc}	2.98^{bc}	3.03
30 cm	3.15^{ab}	3.25^{a}	2.85^{c}	2.92^{bc}	3.04
Mean	3.07	3.15	2.97	2.95	3.03
LSD 5%	0.25 (row space × seed amount)				
2014					
15 cm	2.97^{a}	2.90^{ab}	2.57^{c}	2.67^{bc}	2.78
30 cm	2.63^{c}	3.10^{a}	3.03^{a}	2.97^{a}	2.93
Mean	2.80	3.00	2.80	2.82	2.85
LSD 5%	0.25 (row space × seed amount)				
Combined					
15 cm	2.98	2.98	2.83	2.83	2.90
30 cm	2.89	3.18	2.94	2.94	2.98
Mean	2.93^{b}	3.08^{a}	2.88^{b}	2.88^{b}	2.94
LSD 5%	0.13 (seed amount)				

[a-c]Values sharing same letters differ non-significantly (P>0.05).

Table 4. Essential oil constituent values (%) were obtained from different row spaces and seed amounts during growing seasons.

Row space	Essential oil components (%), 2013 Limonene	Fenchone	Estragole	T-anethole	Anisaldehite	Total
15 cm	14.32^{a}	2.00^{a}	4.28^{a}	74.40	1.87^{a}	97.68
30 cm	10.80^{b}	1.50^{b}	3.82^{b}	77.63	1.24^{b}	95.83
Seed amounts						
5 kg.ha^{-1}	14.08^{a}	1.92^{a}	4.05^{b}	72.44^{c}	1.22^{d}	94.70
10 kg.ha^{-1}	12.91^{b}	1.95^{a}	4.06^{b}	75.76^{b}	1.68^{b}	97.20
15 kg.ha^{-1}	12.22^{b}	1.66^{b}	4.13^{a}	76.86^{b}	1.97^{a}	97.54
20 kg.ha^{-1}	11.03^{c}	1.47^{c}	3.96^{c}	78.97^{a}	1.36^{c}	97.57
Mean	12.56	1.75	4.05	76.01	1.56	96.76
LSD 5%	1.00	0.07	0.06	1.44	0.01	
Essential oil components (%), 2014						
15 cm	9.92	1.03^{a}	4.02^{b}	82.46^{a}	0.84^{b}	98.85
30 cm	9.57	0.96^{b}	4.09^{a}	79.45^{b}	1.44^{a}	95.88
Seed amounts						
5 kg.ha^{-1}	9.42^{b}	1.00^{b}	3.92^{c}	83.92^{a}	0.98^{d}	99.75
10 kg.ha^{-1}	10.20^{a}	1.13^{a}	4.10^{b}	80.78^{b}	1.34^{a}	98.05
15 kg.ha^{-1}	10.28^{a}	0.90^{c}	3.84^{d}	83.03^{a}	1.05^{c}	99.81
20 kg.ha^{-1}	9.08^{c}	0.96^{b}	4.36^{a}	75.10^{c}	1.21^{b}	90.91
Mean	9.74	1.00	4.06	80.79	1.14	97.38
LSD 5%	0.28	0.07	0.06	1.73	0.05	

[a-d]Values sharing same letters differ non-significantly (P>0.05).

major constituent of the fennel essential oil. The *trans*-anethole was significantly affected by row spaces in the first season and limonene in the second season. All constituents were affected by seed quantity in both seasons. Out of the *trans*-anethole almost all constituent values were higher in the first season than the second season (Table 4). As the primary constituent *trans*-anethole was not significantly affected by row space in the first season but affected in the second one. Though in the first season 15 cm row space was insignificantly lower *trans*-anethole value than 30 cm row space but significantly higher than other one in the second season. In the first season *trans*-anethole was increased as seed quantity increased but in the second season this trend was inconsistent. On the other hand the first season, *trans*-anethole mean value (76.01%) is lower than (80.79%) the second season. Limonene the second major constituent has similar trend like *trans*-anethole and limonene percentage was increased as seed quantity increased in the first season. But limonene was inconsistent trend in the second season like *trans*-anethole. The other major constituent estragole was significantly affected by row space in both seasons and 15 cm row space value was significantly higher than 30 cm row space value in the first season but lower than 30 cm row space value in the second season. Also seed quantities were significantly affected the estragole percentage in fennel volatile oil in both growing seasons. In the first season, the lowest estragole value obtained from 20 kg.ha^{-1} seed quantity while the highest estragole was obtained from the same seed quantity transaction in the second season. The fenchone was affected by row space and 15 cm row space was significantly higher values in both seasons than 30 cm row space. Fenchone and anisaldehit also was no remarkably consistent trend in both year related to seed quantities.

Discussion

The highest seed yield was taken from 15 cm × 10 kg.ha^{-1} seed quantity and 15 cm row space was determined significantly higher seed yield values than the 30 cm row space. Our findings supported by Arabacı and Bayram (2005) and Avcı (2013) reported that seed yield changed with plant density but not steadily and changed year to year and by Falzari *et al.* (2006) reported that yield of generative canopy appears to increase with density to a peak at 25 plant per m^2 and then to fall again. Khorshidi *et al.* (2009b) reported that generally seed yield increased as space decreased and achieved the highest yield from the highest plant density. Moosavi (2014) reported that seed yield increased as plant density increased. Masood *et al.*, (2004) studied with 40, 50, 60 and 70 cm row interval and reported that the highest seed yield per hectare has been obtained with the lowest row interval. There were different results of research probably due to different experimental conditions. The highest volatile oil yield was been taken from 15 cm × 10 kg.ha^{-1} and it was been most suits for the volatile oil yield. Parallel results reported by Khorshidi *et al.* (2010) that with increase spaces between plants the pattern of oil yield change was irregular. Seed yield and volatile oil content were two factors that contribute to final oil yield and oil yield in general follows the seed yield pattern.

The highest volatile oil content was determined from 30 cm × 10 kg.ha^{-1} seed quantity transaction. Similar results reported by Khorshidi *et al.* (2010) that in general with increase in spaces between plants the oil percentage increased significantly. Khorshidi *et al.* (2009a) reported that maximum oil percentage was obtained with the minimum plant density.

In general, the distribution of essential oil components differs from application to application. This result was consistent with the findings of some researchers; Falzari *et al.* (2006) reported that percentage of anethole in the oil at 4 plants per m^2 has higher value than other denser transactions. Khorshidi *et al.* (2009a) reported that the higher percentage of anethole (83.07%), estragol (3.47%) and fenchone (8.04%) were obtained with wide row interval between plants. And they said that different densities of planting were different effect on the volatile oil components. Rahimmalek *et al.* (2014) reported that the primary constituents were *trans*-anethole with a concentration ranging from 41.19 to 56.61%, fenchone ranged from 1.7 to 10.23% and limonene ranged from 11.5 to 31.7.

Conclusions

As a result of this study, it could be said that 15 cm row spacing and 10 kg.ha^{-1} seeds are suitable for fennel growing in the Southeast Anatolia of Turkey. Because, the highest seed and volatile oil yield were taken from this application. Also, *trans*-anethole was identified the primary component of fennel volatile oils under all applications and it was determined the highest values from the 15 cm row interval and 10 kg.ha^{-1} seed quantity transactions.

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