



## Improved growth, yield of seeds and oil production of fennel (*Foeniculumvulgare* var. *vulgare*) plants

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- ✓ GC Mass.

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### Abstract

The present study was conducted to investigate the effect of nitrogen and biofertilizers (nitroben) as well as the irrigation intervals on fennel growth, oil yield and its quality. The results showed that, the increase in the nitrogen level added accompanied by a significant increase in plant growth and yield of seeds and oil. The treatment of the 100 % nitrogen of the recommended dose gave the highest seed yield and volatile oil. Biofertilizers increased the growth measurements and the yield of seeds (2.1kg/ha) and oil (35.2 l/ha). Also the short irrigation interval produced the maximum growth characteristics and the yield of seeds (1.36 kg/ha) and volatile oil (8.32l/ha) comparing to the long irrigation interval.

### 1. Introduction

Fennel (*Foeniculum vulgare*), Apiaceae, is an important aromatic herb due to its uses in medicine and foods. It is widely cultivated, throughout the temperate and tropical regions of the world due to the nutritional value of their seeds [1]. Which have emerged as a source of health beneficial compounds including minerals, vitamins and essential oils which explain their applications for pharmaceutical and food industries [2-3]. The cultivation of medicinal and aromatic plants has increased significantly in recent decades to meet the demand of the increased population and to avoid side effects of chemicals on human health. Moreover, medicinal plants are more profitable to growers as compared to many other traditional crops.

Because of its flavour, fennel is cultivated in Mediterranean countries, including Egypt, where it is cultivated mainly in the north upper governors such as Minya, Fayoum, BeniSuef, and Assiut. The essential oil is used as flavoring agent, carminative, diuretic, antispasmodic, stomachic, expectorant, and aromatic [4]. Also, it was reported that fennel seed possesses anticancer activity [5].

Moreover, essential oil of fennel has hepatoprotective effects [6], They are also known for their diuretic, anti-inflammatory, analgesic and antioxidant activities [4]. The constituents of *F. vulgare* oil were recorded by many workers. The major constituents of *F. vulgare* oil found to be estragole, anethole<trans>, limonene,  $\beta$ -terpinene, fenchone, and piperitone oxide [7-10]. Several studies have

been conducted in order to increase the production of medicinal and aromatic plants through the application of different fertilization treatments [11-14].

The important role of organic fertilizers for improving soil texture and nutrient supply to plants, organic acids and enhance nutrient absorption were emphasized by [15-17].

The effect of water supply on the growth and production of several medicinal and aromatic plants was observed by several investigators. Baher *et al.*, [18] found that greater soil, water stress decreased plant height, total fresh and dry weight of *Satureja hortensis*. Also in another research, on *Eragrostis curvula* plant showed that the stem number/plant and dry weight was negatively related to stress of water [19]. El Naimand Ahmed [20] recorded that frequent irrigation intervals 7 days improved vegetative growth, i.e. stem diameter, plant height; leaves number per plant, leaf area and shoot dry weight of sunflower. El-Mekawy [21] on *Achillea santolina* L. found that irrigation every 7 days, highly significant increased branches number/plant, height of plant, fresh and dry weight of herb/plant, compared to irrigation every 14 and 21 days. Also an experiment was carried out on *Curcuma spp* and proved that the irrigation intervals every one week improved growth characteristics and chemical composition [22]. The aim of this work was to improve the plant growth, increase the oil content and quality.

## 2. Material and Methods

### 2.1. Experimental

This experiment was carried out at the Experimental Farm, Faculty of Agriculture, Cairo University, Egypt, during the two successive seasons of 2013 and 2014 in clay soil. The laboratory studies were done in the Research of Medicinal and Aromatic plants Department, National Research Centre, Cairo - Egypt.

Seeds of fennel were kindly provided by the same Department. Samples from the soil were taken before cultivation from the depth 0-30 cm and were subjected to physical and chemical analysis in the Soil Science Department, National Research Centre according to the methods of [23] and tabulated in Table 1.

**Table 1:** Physical and chemical analysis of the experimental soil.

Physical analysis	Result	Chemical analysis	Result
Clay %	24.4	PH	8.1
Silt %	52.5	EC (dsm <sup>-1</sup> )	1.25
Sand %	25.0	Organic matter	0.55
Soil texture	Sandy loam	Available N%	1.25
		Available P%	0.77
		Available K%	0.25

#### 2.1.1. Fertilization experiment

Soil preparation involved ploughing twice and divided into plots (4m<sup>2</sup>). Each plot contained 3 rows with 15 plants. Organic manure 48 m<sup>3</sup>/ha and calcium super phosphate (360 Kg/ha) were added to all treatments during soil preparation. The seeds were sown at 40 × 60cm distances on the 1<sup>st</sup> of November during the two seasons.

#### 2.1.2. Fertilizers

The recommended dose of the chemical fertilizers were 480kg/ha ammonium sulphate (20.5% N), 360kg/ha calcium super phosphate (15.5% P<sub>2</sub>O<sub>5</sub>) and 125kg/ha potassium sulfate (48% K<sub>2</sub>O) The

nitrogen and potassium fertilizers were added in two doses during the growth season. The first dose after 50 days of transplanting, and the second after 75 of transplanting. 60 l/ha nitroben was the used biofertilizer which contained *Azotobacter chroococcum* (nitrogen fixing bacteria) were mixed with seeds during cultivation and they were obtained from the General Organization for Agriculture Equalization Fund (G.O.A.E.F.), Ministry of Agriculture, Egypt.

Fertilization treatments were as follows: T<sub>1</sub> (Control treatment without any fertilizers), T<sub>2</sub> (25% the recommended dose nitrogen fertilizer), T<sub>3</sub> (50% nitrogen fertilizer), T<sub>4</sub> (75% nitrogen fertilizer), T<sub>5</sub> (100% nitrogen fertilizer). T<sub>6</sub> (the biofertilizer only), T<sub>7</sub> (25% nitrogen fertilizer + the biofertilizer), T<sub>8</sub> (50% nitrogen fertilizer + the biofertilizer), T<sub>9</sub> (75 % nitrogen fertilizer + the biofertilizer) and T<sub>10</sub> (100 % nitrogen fertilizer + the biofertilizer).

### **2.1.3. Irrigation experiment**

The same steps of soil preparation and cultivation were applied as it were in the fertilization experiment, the recommended dose of chemical fertilizers was applied. Irrigation treatments were as follows: One week = irrigation every 7 days, Two weeks = irrigation every 15 days, Three weeks = irrigation every 21 days, Four weeks = irrigation every 30 days.

### **2.1.4. Measurement of growth parameters**

The following parameters were measured at the harvest time on the first of march: plant height cm, number of branches/plant, seeds yield per unit area, seed yield per ha, oil %, oil yield (ml) per unit area and oil yield per ha by liter.

### **2.2. Essential oil distillation**

The essential oils of the dried fennel seeds were extracted by hydro-distillation for 3 hr. according to the method of [24]. The essential oils were dehydrated over anhydrous sodium sulfate and subjected to GC/MS analysis. The essential oil yield (%) was calculated based on the dried weight using the following formula [25]:

$$\text{Essential oil yield \%} = \text{mass of essential oil obtained (g) / mass of dry matter (g)} \times 100$$

### **2.3. Essential oil analysis using gas chromatography-mass spectrometry (GC-MS)**

GC analysis was performed using a Shimadzu GC- 9A gas chromatograph equipped with a DB5 fused silica column (30 m x 0.25 mm i.d., film thickness 0.25 μ m). The oven temperature was held at 40°C for 5 min and then programmed until 250°C at a rate of 4°C/min. Injector and detector (FID) temperature were 260°C; helium was used as a carrier gas with a linear velocity of 32 cm/s. GC-MS analyses were carried out on a Varian 3400 system equipped with a DB-5 fused silica column (30 m x 0.25 mm i.d.); The oven temperature was 40 to 240°C at a rate of 4°C/min, transfer line temperature 260°C, injector temperature 250°C, carrier gas helium with a linear velocity of 31.5 cm/s, split ratio 1/60, flow rate at 1.1 ml/ min, Ionization energy 70 eV; scan time 1 s ; mass range 40-350 amu.

#### **2.3.1 Identification and quantitative analysis of essential oil**

Identifications were made by library searches [26] combining MS and retention data of authentic compounds by comparison of their GC retention indices (RI) with those of the literature or with those of standards available in our laboratories. The retention indices were determined in relation to a homologous series of n-alkanes (C<sub>8</sub>–C<sub>22</sub>) under the same operating conditions. Further identification

was made by comparison of their mass spectra with those stored in NIST 98 and Wiley5 Libraries or with mass spectra from literature. Component relative concentrations were calculated based on GC peak areas without using correction factors.

## 2.4. Experimental design and statistical analysis

The experimental design was a completely randomized block design with three replicates for each treatment. The data subjected to statistical analysis according to [27]. The means of the treatments were compared using the least significant difference (LSD) test at the 0.05 level.

## 3. Results and discussion

### 3.1. Effect of nitrogen and biofertilizer on growth, yield and essential oil production of fennel

The increase in the nitrogen amount added caused a significant increase in plant height, so the tallest height were that received higher amount of nitrogen in both treated or non-treated plants (Table 3). While the addition of biofertilizers slightly affected, so the treatment that received the same amount of nitrogen had approximately the same length in both treated and non-treated plants. For the effect of these treatments on the number of branches, it was clear that, the increase in nitrogen amount increased the branches number, so the highest number of branches was produced from plants fertilized by the highest amount of nitrogen. Also adding biofertilizers to the plants increased significantly the number of branches. The findings of this experiment was in close conformity with some investigators [28-29].

**Table 2:** Effect of some nitrogen levels and biofertilizers on the growth measurements of fennel plants (Mean of the seasons 2013 and 2014)

Treatments	Treated plants		Non treated plants	
	Plant height by cm	Number of branches	Plant height by cm	Number of branches
Control	1.18	5.30	1.17	5.00
25 % Nitrogen	1.23	5.70	1.17	5.30
50 % Nitrogen	1.27	5.80	1.23	5.30
75 % Nitrogen	1.28	6.00	1.28	5.70
100 % Nitrogen	1.27	5.90	1.28a	5.70
L. S. D 5 %	0.027	0.13	0.027	0.16

For the effect of fertilization on the yield of seeds, the data in Table (3) showed that, both nitrogen and biofertilizers had a significant effect in increasing the yield of seeds per unit area or ha. The treated plants gave higher yield of seeds than the non-treated plants for all treatments. Also the data indicated that the highest yield of seeds produced from plants received the highest amount of nitrogen 2.10 and 2.04 kg/ha for the treated and non -treated plants respectively, while the 25 % nitrogen treatment gave 616.8 and 581.7 for the treated and non-treated plants, respectively. The same results were obtained. Khalil et al. [12] and Ayub et al. [30].

Concerning the effect of nitrogen and biofertilizers on the volatile oil percentage and yield in fennel oil, the data in Table (4) indicated that, the plants received the highest amount of nitrogen produced higher oil percentage, while the biofertilizers showed a slight effect in some treatments. The differences were not significant in most cases. For the volatile oil yield per unit area, the plants showed a positive response to the increase in nitrogen level, so the treatments fertilized with the highest amount of nitrogen gave a higher amount of essential oil in comparing to other treatments. The treatment 100% N gave 29.25 ml of volatile oil per unit area, while the control gave 17.27 ml per unit area in the treated plants. The biofertilizers slightly affected the volatile oil production. The yield of volatile oil per ha had

the same manner as in the yield of volatile oil per unit area. So the increase in the yield of volatile oil of the 100 % N treatment than the control was about 41 %. It was proved that the higher rate of nitrogen increased the essential oil concentration in fennel by other authors [28].

**Table 3:** Effect of some nitrogen levels and biofertilizers on the yield of seeds (Mean of the seasons 2013 and 2014)

Treatments	Treated plants		Non treated plants	
	Seed yield kg/unit area	Seed yield ton/ha	Seed yield kg/unit area	Seed yield ton/ha
Control	1.23	1.48	1.18	1.39
25 % Nitrogen	1.26	1.51	1.23	1.47
50 % Nitrogen	1.55	1.86	1.45	1.74
75 % Nitrogen	1.73	2.08	1.65	1.98
100 % Nitrogen	1.75	2.10	1.70	2.04
L. S. D 5 %	0.027	5.75	0.027	12.25

**Table 4:** Effect of some nitrogen levels and biofertilizers on the volatile oil percentage and oil yield of seeds by ml/unit area and by liter /ha (Mean of the seasons 2013 and 2014)

Treatments	Oil %	Treated plants		Oil %	Non treated plants	
		Oil yield ml/unit area	Oil yield liter/ ha		Oil yield ml/unit area	Oil yield liter/ha
Control	1.38	17.27	20.7	1.40	16.51	19.8
25 % Nitrogen	1.43	18.1	21.7	1.45	17.84	21.4
50 % Nitrogen	1.50	23.05	27.6	1.55	22.48	27.1
75 % Nitrogen	1.57	27.60	33.0	1.58	26.44	31.7
100 % Nitrogen	1.65	29.25	35.2	1.65	28.31	34.0
L. S. D 5 %	0.027	0.027	0.13	0.027	0.35	0.27

### 3.2. Effect of irrigation intervals on growth, yield and essential oil production of fennel

For the effect of irrigation intervals on the growth and oil production of fennel plants, the data in Table (5) showed that the plants watered once a week were the tallest during the growth period, while those irrigated every 4 weeks were the shortest. For the effect of irrigation on the number of branches, the data showed that the same trend as in the plant height. The plants irrigated once a week produced the highest number of branches and decreased gradually with the long period of irrigation intervals. For the seeds yield per unit area, the data in the same Table showed that, the mean weight of seeds decreased with the longer irrigation intervals so that, the weight of seeds yield of the one week irrigated plants was more than twice as much as that of the yield of 4 weeks irrigated plants. The seed yield per ha in the same table, showed that, a high significant increases were noticed with the short irrigation intervals over the other treatments, the lowest yield was obtained from the four weeks treatment 0.54 kg/ha, while one week treatment gave 1.36 kg/ha. These results were in harmony with some researchers [31-32]. Regarding the essential oil percentage and yield, the data in Table (6) showed that, the highest percentage of volatile oil was obtained from the plants watered one time every two weeks followed by the plants watered every one week, while the lowest percentage was noticed with the plants watered every four weeks 0.97, while it was 1.49 and 1.46 % for the one week and two weeks, respectively. The one week irrigation treatment increased the oil yield ml/unit area and l/ha but the four weeks, irrigation treatment recorded the lowest oil yield ml/unit area and l/ha. The yield of the volatile oil was 20.0 liter/ha with one week treatment, while it was 5.23 liter/ha with four weeks treatment. The same results were observed by some authors [21 and 32].

**Table 5:** Effect of irrigation intervals on the growth measurements and seeds yield of fennel plants. (Mean of the seasons 2013 and 2014)

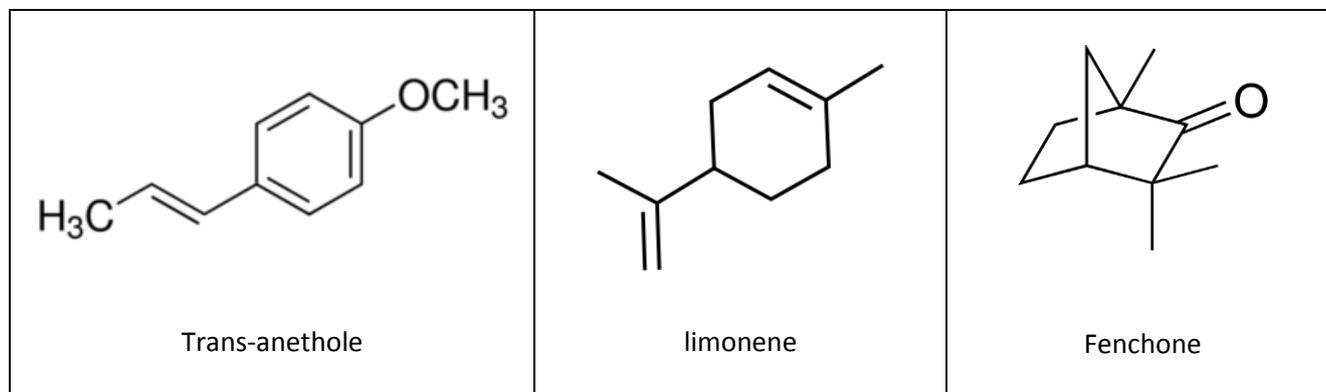
Treatments	Plant height by cm	Number of branches	Seed yield kg/unit area	Seed yield kg/ha
One week	1.38	5.67	1.14	1.36
Two weeks	1.17	5.00	0.75	0.90
Three weeks	0.95	5.23	0.70	0.84
Four weeks	0.85	4.70	0.45	0.54
L. S. D 5 %	0.032	0.21	0.32	0.28

**Table 6:** Effect of irrigation intervals on the oil production of fennel plant. (Mean of the seasons 2013 & 2014)

Treatments	Oil %	Oil yield ml/unit area	Oil yield liter/ha
One week	1.46	16.63	8.32
Two weeks	1.49	10.78	5.03
Three weeks	1.26	8.83	4.43
Four weeks	0.97	4.32	2.18
L. S. D 5 %	0.03	0.28	0.04

### 3.3. Fennel seed essential oil constituents:

Fourteen essential oil compounds were detected in fennel dry seed essential oil represented 84.42 % [Table \(7\)](#). The major constituents of fennel seed essential oil were *trans*-anethole (45.46%), Limonene (23.2%) and  $\alpha$ -Fenchone (8.49%) [Figure1](#). The molecular structure of the main constituents of fennel oil.



Oxygenated monoterpenes was found as the main group in the dry fennel seed oil, which recorded (54.36%) against 28.84 and 1.25% for monoterpeneshydrocarbon group and sesquiterpenes hydrocarbon group, respectively. Limonene was found as the major compounds of monoterpenes hydrocarbon group. At the same time  $\alpha$ -pinene, was found at a reasonable concentration in the same group which recorded 4.61 %, while camphene, sabinene, terpinene and terpinolene were found in minor concentrations in the same group. In addition to the presence of *trans*-anethole and  $\alpha$ -Fenchone at high percentage of oxygenated monoterpenes group also this group contains camphor and estragole in low content.  $\alpha$ -Curcumene,  $\beta$ -Caryophyllene,  $\gamma$ -Muurolene and  $\gamma$ -Cadinene were found in sesquiterpenes hydrocarbon group but in small quantities. Ibrahim [\[11\]](#); Khalil et al., [\[12\]](#) have reported similar results, they showed that the main compound in fennel seed essential oil was *trans*-anethole, limonene and  $\alpha$ -Fenchone.

**Table 7:** Essential oil constituents of fennel seeds volatile oil in the second season.

Compounds	KI	%
Monoterpene hydrocarbons		
$\alpha$ -pinene	939	4.61
Camphene	953	0.22
Sabinene	976	0.32
Limonene	1031	23.2
$\gamma$ -terpinene	1061	0.41
$\alpha$ -Terpinolene	1088	0.08
Total		28.84
Oxygenated monoterpene		
$\alpha$ -Fenchone	951	8.49
Trans-anethole	1283	45.46
Camphor	1151	0.21
Estragole	1195	0.20
Total		54.36
Sesquiterpenes hydrocarbons(SH)		
$\alpha$ -Curcumene	1483	0.75
Caryophyllene< beta>	1418	0.13
$\gamma$ -Muurolene	1477	0.26
$\gamma$ -Cadinene	1538	0.11
Total		1.25
Total identified		84.18

## Conclusion

The aim of this work was to maximize the use of crop fennel as an important group in the aromatic grains and plants suitable for export abroad, making it a source of hard currency to increase national income. Fertilizing is an important mean in increasing the yield of seeds and essential oil of fennel plant. Also the short irrigations intervals once a week gave the highest yield of seeds and volatile oil. Trans-anethole was found as the major constituents in most samples of fennel.

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