Partial Substitution of Chemical Fertilization of Dutch Fennel Plant (*Foeniculum vulgare* Mill.) by Bio Fertilization

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Abstract

The present work was carried out on Dutch fennel plant strain during the two successive seasons of 2017/2018 and 2018/2019 at Baloza Research Station, North Sinai Governorate, Egypt. The aim of the work was to evaluate the effect of bio-fertilizer applications as partial replacement for chemical fertilization on vegetative growth, fruits yield, essential oil productivity and its main components as well as chemical constituents of Dutch fennel plants under Sinai conditions. The experiment was planned in a randomized complete blocks design (RCBD) with three replications for each treatment. Treatments were the combination of five fertilization levels (full recommended dose of NPK only, 75, 50, 25% of the recommended NPK plus bio-fertilizers and bio-fertilizers only). The results showed that application of T2 (75% of the recommended NPK plus bio-fertilizers) increased vegetative growth measurements (plant height, number of branches/plant, herb fresh weight/plant and herb dry weight/plant), yield parameters (number of umbels/plant, weight of 1000 seed, seed yield/plant and seed yield/feddan), maximized the essential oil percentage and yield compared to other treatments. However, the lowest values of vegetative growth measurements, yield parameters and essential oil productivity were obtained with plants treated by T5 (bio-fertilizers only). As general, the major chemical constituents of Dutch fennel essential oil were trans-anethole and estragole.

Keywords: *Foeniculum vulgare*, fertilization, growth, yield and volatile oil.

Introduction

Fennel (*Foeniculum vulgare* Mill.), Family Apiaceae, is an annual, biennial or perennial aromatic plant, depending on the variety **(Farrell 1988, Wichtl and Bisset, 1994)**. It is native to the Mediterranean and cultivated in Europe, Asia, Africa and some parts of South America **(Blumenthal *et al.*, 2000)**. It is ranked the first in the list of Egyptian exports of herbs and spices **(EMAP, 2011-2012)**. Fennel is used in many parts of the world for curing of many diseases, for example, abdominal pains, aperitif, cancer, colic in children, fever, flatulence, insomnia, kidney ailments, laxative, liver pain, and stomach ache **(Badgujar *et al.*, 2014; Rather *et al.*, 2012; Choi and Hwang 2004; Koppula and Kumar 2013)**. Egypt cultivates about 11000 feddans of fennel, mostly in Assiut and Qena Governorates as it is considered an important spice. But, the Egyptian fennel strain has a major drawback due to its high content of estragole which is among the substances banned for use in infant drugs and its low content of anethole; accordingly, exports of organic fennel from Egypt have been banned in some products in the EU markets **(Shalaby *et al.*, 2011 and Abd El-Aleem *et al.*, 2017)**. Therefore, there was an urgent need for introducing new fennel varieties for cultivation in our country. Dutch fennel (*Foeniculum vulgare* Mill. spp. vulgare) is a new strain of fennel imported from Holland by Sekem company in the last few years. It is characterized by higher yields of fruits and oil, a higher percentage of anethole and a lower percentage of estragole than the Egyptian fennel as reported by **(Shalaby *et al.*, 2011)**.

Many factors influence oil content in umbelliferous crops i.e. fertilization, salinity and irrigation **(Olle and Bender, 2010)**. Also, balanced mineral fertilization of aromatic plants is an important cultivation factor determining essential oil quantity and quality **(Renata, 2013)**. Chemical fertilization is used for increasing the productivity of medicinal and aromatic plants. However, excessive use of manufactured fertilizers raised production cost, caused environmental pollution and decreased the acceptance of the crops for export as well as affected the soil fertility **(Sherif and El-Naggar, 2005)**. For these reasons, it is recommend that completely or partial substitution of mineral fertilization (NPK) by using bio-fertilizers which are safe and economic to farmer. Bio-fertilizers are substances contain living microorganisms when added to seeds, plants, or soil colonize the rhizosphere and promote growth by increasing the supply or availability of primary nutrients to the host plant **(Vessey 2003)**. Recently, bio-fertilizers have been increasingly used in modern agriculture due to the wide knowledge in rhizospheric biology and the discovery of the promotive microorganism. Bio-fertilizers are environmentally friendly, economical and their continuous use improves soil fertility **(Mahdi *et al.*, 2010)** by fixing the atmospheric nitrogen and solubilizing insoluble phosphate and produce plant growth-promoting substances in the soil **(Mazid and Khan 2015)**. Also, **(Bhardwaj *et al.*, 2014)** reported that the use of bio-fertilizers improved crop yield by increasing contents of vitamins, essential amino acids, proteins and nitrogen fixation. *Azotobacter chroococcum* (nitrogen fixing bacteria), *Bacillus megaterium* var. *phosphaticum* (phosphate solubilizing bacteria) are known as plant growth promoting rhizobacteria **(Abdel Wahab and Hassan, 2013)**. *Bacillus*

megaterium dissolves phosphate in the soil into a soluble form by producing organic acids which lower the pH and cause the dissolution of bound forms of phosphate. Active dry yeasts (*Saccharomyces cerevisiae*) are natural safety bio-fertilizers cause various promotive effect on plants. They are natural source of cytokinin which simulates cell division and enlargement as well as the synthesis of protein, nucleic acid and vitamin B. Also, they release carbone dioxide which improves net photosynthesis **(Amer, 2004; Kurtzman and Fell, 2005)**.

The Egyptian government encourages cultivation of medicinal and aromatic plants in Sinai as the climate conditions are suitable for growing of these plants to increase exportation. Thus, the present research aimed to evaluate the effect of chemical and bio-fertilization on vegetative growth, seed yield, oil production and the main components of Dutch fennel plants under sandy soil conditions of Baloza Station, North Sinai, Egypt.

Materials and Methods

A two-year experiment was carried out during the two successive seasons of 2017/2018 and 2018/2019 in Agricultural Experimental Station of the Desert Research Center at Baloza village, North Sinai Governorate, Egypt. Seeds of Dutch fennel (*Foeniculum vulgare* Mill. spp. *vulgare*) strain were imported from Euro Herb company, Netherlands by Sekem company.

Soil:

A soil sample was randomly collected from the depth of 0 to 30 cm to determine the chemical and physical properties of the soil. The texture of experimental farm soil in Baloza Station was of sandy nature. This was demonstrated clearly from the mechanical and chemical analysis of the used soil before cultivation as shown in Tables (A, B).

Water irrigation:

Water sample was taken from the irrigation system of Baloza Experimental Station for analysis. The water analysis of the used irrigation water is shown in Table (C).

Compost:

Organic compost manure was added at 10 m^3/feddan throughout soil preparation before planting in each season. The chemical analysis of the used compost manure is shown in Table (D).

Table A. Mechanical analysis of the experimental soil area.

Depth (cm)	Sand (%)	Silt (%)	Clay (%)	Soil texture
0-30	90	5	5	Sandy

Table B. Chemical analysis of the experimental soil area.

pH	E.C. (ds/m)	O.M. (%)	Soluble anions (meq/l)				Soluble cations (meq/l)			
			CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+
8.17	5.99	0.17	-	2.46	40.71	17.17	19.53	13.25	26.91	0.65

Table C. The chemical analysis of irrigation water.

pH	E.C. (ppm)	Soluble anions (meq/l)				Soluble cations (meq/l)			
		CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+
7.76	2491	0.52	1.96	28.86	7.59	4.51	11.75	22.08	0.59

Table D. Chemical analysis of the used compost manure**.**

pH	EC (ds/m)	O.M. (%)	C/N ratio (%)	N (%)	P (%)	K (%)	Fe (%)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)
8.8	4.6	20.5	11.85	1.03	0.22	2.04	3.43	606.8	85.65	43.60

Seed Sowing:

Three to five seeds were sown into hills at 6th October in the first season and 8th October in the second season within the drippers of irrigation lines and irrigation was done immediately after sowing. The distance between rows was 75 cm and the distance between plants was 30 cm, drip irrigation lines with average (4 liter/hour) were used. Thinning was conducted one month after sowing leaving two plants per hill.

Chemical fertilizer sources

The sources of NPK chemical fertilizers were ammonium sulphate (20.5 % N), calcium superphosphate (15 % P_2O_5) and potassium sulphate (48 % K_2O).

Bio-fertilization Treatments:

Bio-fertilizers used were *Azotobacter chroococcum*, *Bacillus megaterium* var. phosphaticum, and Active dry yeast (*Saccharomyces cerevisiae*). A liquid bio-fertilizer from *Azotobacter chroococcum* and *Bacillus megaterium* var.

phosphaticum was obtained from soil fertility and Microbiology Department, Desert Research Center (DRC), Egypt. The liquid bio-fertilizer was added to the soil as a soil drench. Active dry yeast (*Saccharomyces cerevisiae*) was dissolved in water followed by adding sugar at the ratio of 1:1 (6g/l from active dry yeast and 6g/l from sugar) and kept overnight for activation and reproduction of yeast **(El-Tohamy *et al*., 2008)**. Active dry yeast was added as a foliar spray.

All horticultural practices including irrigation and pest and diseases control were done as used recommendation in this respect.

Treatments:

Basically, the experiment consisted of five fertilization levels, as follow:

1- Application of full recommended dose of NPK (300 kg ammonium sulphate, 300 kg calcium superphosphate and 80 kg potassium sulphate per feddan).
2- Application of 75 % from full recommended dose of NPK (225 kg ammonium sulphate, 225 kg calcium superphosphate and 60 kg potassium sulphate per feddan) + bio-fertilizers.
3- Application of 50 % from full recommended dose of NPK (150 kg ammonium sulphate, 150 kg calcium superphosphate and 40 kg potassium sulphate per feddan) + bio-fertilizers.
4- Application of 25 % from full recommended dose of NPK (75.5 kg ammonium sulphate, 75.5 kg calcium superphosphate and 20 kg potassium sulphate per feddan) + bio-fertilizers.
5- Application of bio-fertilizers only.

Application time of chemical fertilizers and bio-fertilizers:

Chemical fertilization with calcium superphosphate was conducted immediately before sowing in each season in only one dose during the preparation of the soil for cultivation. Nitrogen and potassium fertilizers were applied in three equal doses at 60, 90 and 120 days after seed sowing in both seasons. Bio-fertilizers were added at three times per season. The first one was after 60 days after seed sowing, followed by second and third one after 90 and 120 days from seed sowing in both seasons, respectively.

Growth evaluation:

Harvesting was carried out at 15th May in the first season and 19th May in the second season. Plant height (cm) and number of branches/plant were recorded before harvest. After harvest, data recorded were herb fresh and dry weighs/plant (g), number of umbels/plant, weight of 1000 seeds (g), fruit yield/plant (g) and fruit yield/feddan (kg). Essential oil percentage of Dutch fennel seeds was determined by hydro distillation for 3 h using the method of **British Pharmacopoea (1963)**, the oil percentage was used to calculate essential oil yield/plant (ml), and per feddan (l). The extracted volatile oil was dehydrated over anhydrous sodium sulphate and stored in a refrigerator until GC/MS analysis.

Chemical analysis:

Chlorophyll a, chlorophyll b, total chlorophyll and carotenoids (mg/g f.w.) were determined in leaf fresh samples at 90 days after seed sowing in both seasons as described by **(Saric *et al.*, 1967)**. N, P and K percentages as well as total carbohydrates in the dry herb were estimated at flowering time. Nitrogen was determined by modified micro Kjeldahle method as described by **(A.O.A.C. 1970)**. Phosphorus was colorimetrically determind using the method described by **(Murphy and Riley, 1962)** using spectrophotometer at 882 µv. Potassium percentage was estimated using flame photometry according to **(Cottenie *et al.*, 1982)**. Total carbohydrates in the dry leaves were determined by using a colorimetric method of **(Herbert *et al*., 1971)**.

Essential oil GC/MS analysis:

The GC-MS analysis of the essential oil samples was carried out using gas chromatography-mass spectrometry instrument stands at the Laboratory of Medicinal and Aromatic Plants Research, National Research Centre with the following specifications. Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with: a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GCMS system was equipped with a TR5 MS column (30 m x 0.32 mm i.d., 0.25 µm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL/min at a split ratio of 1:10 and the following temperature program: 60°C for 1 min; rising at 4.0 C/min to 240°C and held for 1 min. The injector and detector were held at 200 and 240°C, respectively. Diluted samples (1:10 hexane, v/v) of 1 µL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the compounds were identified using two different analytical methods: mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library).

Experiment layout and statistical analysis

This experiment was arranged in a complete randomized block design system, each treatment contained three replicates of 10 plants for each replicate. The obtained results were statistically analyzed by using MSTATC program **Bricker**, (**1991**). Analysis of variance was performed to determine significant differences. Means were compared using LSD test at 0.05 level according to **Snedecor and Cochran, (1967**).

Results and Discussion

Vegetative growth measurements:

1- Plant height: The results in table (1) revealed that, fertilization with T2 (75% NPK + bio-fertilizers) gave the highest values of plant height compared to other fertilization treatments as (78.33 and 87.73 cm) in the first and second seasons, respectively, and came in the second place the plants which were treated by T1 in both seasons with non-significant differences between them in the first season but there were significant differences between them in the second season. Meanwhile, T5 (bio-fertilizers only) produced the shortest plants compared to other treatments in both seasons and followed in ascending order by those received T4 in the first and second seasons, respectively.

2- Number of branches per plant: Data obtained on number of branches per plant as affected by different fertilization treatments shown in Table (1) indicated that, application of T2 (75% NPK + bio-fertilizers) produced the highest number of branches/plant as it recorded (13.80 and 13.27 branch/plant) in the first and second seasons, respectively. Whereas T1 gave the next lower values as it recorded (12.73 and 12.47 branch/plant) in the first and second seasons, respectively. The differences between the above-mentioned treatments were non-significant in the two seasons. While, the lowest number of branches per plant (7.20 and 6.33 branch/plant) were obtained from plants which were treated with T5 (bio-fertilizers only) in the first and second seasons, respectively followed in ascending order by T4 and T3 in the two seasons.

Table 1. Effect of bio and chemical fertilization on some vegetative growth parameters of Dutch fennel plants during the two seasons of 2017/2018 and 2018/2019.

Treatment	Plant height (cm)	Number of branches/plant	Fresh weight of herb/plant (g)	Dry weight of herb/plant (g)
		First season		
T1	75.80	12.73	348.52	138.86
T2	78.33	13.80	356.20	159.10
T3	74.67	11.40	336.90	111.42
T4	69.60	9.53	236.01	88.96
T5	55.78	7.20	114.36	41.56
LSD at p<0.05	3.47	1.84	52.13	17.15
		Second season		
T1	80.20	12.47	353.77	151.20
T2	87.73	13.27	375.28	162.93
T3	74.40	11.27	344.79	122.68
T4	70.33	10.67	257.93	95.42
T5	61.51	6.33	116.87	44.89
LSD at p<0.05	5.97	1.55	65.86	37.94

T1 (full recommended dose of NPK), T2 (75% NPK recommended dose plus bio-fertilizers), T3 (50% NPK recommended dose plus bio-fertilizers), T4 (25% NPK recommended dose plus bio-fertilizers), T5 (bio-fertilizers only).

3- Fresh weight of herb/plant (g): Data in Table (1) showed that, plants treated with T2 (75% NPK + bio-fertilizers) had the highest fresh weight of herb (356.20 and 375.28 g/plant) in the first and second seasons, respectively compared to other treatments and came in the second place T1 as (348.52 and 353.77 g/plant) in the first and second seasons, respectively with non-significant differences between them. Meanwhile, T3 scored the third place. Furthermore, the lowest values resulted from T5 (bio-fertilizers only) as (114.36 and 116.87 g/plant) in both seasons, respectively.

4- Dry weight of herb/plant (g): Table (1) revealed that, the heaviest dry weight of herb (159.10 and 162.93 g/plant) in both seasons, respectively, occurred with T2 (75% NPK + bio-fertilizers), followed in descending order by treated plants with T1 as its values were (138.86 and 151.20 g/plant), T3 and T4, respectively. The differences between T2 and T1 were significant in the first season and non-significant in the second season. Whereas, the lowest plant dry weight was obtained by T5 (bio-fertilizers only) as it recorded (41.56 and 44.89 g/plant) in both seasons, respectively, compared to other treatments in both seasons.

The aforementioned results of fertilization concerning vegetative growth are in parallel with those obtained by **Ali (2009)**; **Zaki *et al.*, (2010)** and **Abd-Allah (2012)** on fennel, **Abo-Baker and Mostafa (2011)** on *Hibiscus sabdariffa*, **Abd El-Wahab (2013)** on *Tanacetum vulgare*, **Abdel-Aziez *et al.*, (2014)** on black cumin, **Said-Al Ahl *et al.*, (2015)** on *Anethum graveolens* L., **Mahmoud *et al.*, (2017)** on caraway, **Gomaa *et al.*, (2018)** on roselle.

Yield Characters:

1- Number of umbels per plant: Results in Table (2) indicated that, treated plants by T2 (75% NPK + bio-

fertilizers) led to the highest values of number of umbels per plant as it recorded (54.40 and 56.27 umbel/plant) in the first and second seasons, respectively compared to other treatments. The next lower place was occupied by T1 as it scored (52.53 and 53.87 umbel/plant) in the first and second seasons, respectively with non-significant differences between T2 and T1 in the two seasons. Meanwhile T5 (bio-fertilizers only) led to the lowest values of number of umbels per plant as (18.87 and 17.93 umbel/plant) in the first and second seasons, respectively and followed in ascending order by T4 and T3 in the two seasons, respectively.

2- Weight of 1000 seeds (g): According to data in Table (2), the highest weight of 1000 seeds was obtained with T2 (75% NPK plus bio-fertilizers) as it scored (15.48 and 15.36 g) in the first and second seasons, respectively, followed by T1. The differences between the above-mentioned treatments were significant in the two seasons. However, the lowest values were recorded with T5 (bio-fertilizers only) as it scored (11.65 and 11.59 g) in first and second seasons, respectively and followed in ascending order by T4 and T3 in the two seasons, respectively.

Table 2. Effect of bio and chemical fertilization on yield characters of Dutch fennel plants during the two seasons of 2017/2018 and 2018/2019.

Treatment	Number of umbels/plant	Weight of 1000 seeds (g)	Seed yield/plant (g)	Seed yield/feddan (kg)
		First season		
T1	52.53	13.75	29.13	1087.41
T2	54.40	15.48	33.26	1241.88
T3	43.47	12.67	26.77	999.29
T4	33.60	12.63	23.20	866.39
T5	18.87	11.65	8.72	325.48
LSD at p<0.05	**4.35**	**0.49**	**10.61**	**396.10**
		Second season		
T1	53.87	14.05	32.87	1227.08
T2	56.27	15.36	37.03	1382.65
T3	45.80	13.44	32.00	1194.81
T4	36.20	12.94	22.02	822.21
T5	17.93	11.59	12.03	449.22
LSD at p<0.05	**3.80**	**0.78**	**9.06**	**338.23**

T1 (full recommended dose of NPK), T2 (75% NPK recommended dose plus bio-fertilizers), T3 (50% NPK recommended dose plus bio-fertilizers), T4 (25% NPK recommended dose plus bio-fertilizers), T5 (bio-fertilizers only).

3- Seed yield/plant (g): Table (2) revealed that, seed yield per plant takes the similar trend with number of umbels as the highest values were recorded with plants treated with T2 (75% NPK plus bio-fertilizers) as the values were (33.26 and 37.03 g/plant) in the first and second seasons, respectively, followed by T1 as (29.13 and 32.87 g/plant) in the first and second seasons, respectively with non-significant differences between them. Meanwhile, the lowest values were observed, in the fertilized plants with T5 (bio-fertilizers only) as (8.72 and 12.03 g/plant) in the first and second seasons, respectively, compared to other treatments in both seasons and the next higher values were obtained from application of T4 and T3 in the two seasons, respectively.

4- Seed yield/feddan (kg): Data in Table (2) showed that seed yield/feddan (kg), as well as seed yield/plant (g), gave the highest values with application of T2 (75% NPK + bio-fertilizers) as it scored (1241.88 and 1382.65 kg/feddan) in the first and second seasons, respectively and application of T1 recorded the next lower values (1087.41 and 1227.08 kg/feddan) in first and second seasons, respectively with non-significant differences between them. However, the lowest seed yield/feddan was recorded with T5 (bio-fertilizers only) as its values were (325.48 and 449.22 kg/feddan) in first and second seasons, respectively, compared to other treatments in the both seasons and followed in ascending order by T4 and T3 in both seasons, respectively.

These results are in close agreement with those reported by **Azzaz *et al.*, (2009); Abd-Allah (2012); Gamal and Ismail (2012); Kumar and Pramila (2014); Pariari *et al.*, (2015); Abdel Wahab *et al.*, (2016)** and **Badran *et al.*, (2017)** on fennel, **Khalil and Yousef (2005)** and **Gomaa and Youssef (2008)** on caraway, **Nabizadeh *et al.*, (2012)** on anise, **Hashemzadeh *et al.*, (2013); Tajpoor *et al.*, (2013)** on dill, **Sahu *et al.*, (2013); Patidar *et al.*, (2016)** on coriander, **Gholami *et al.*, (2014); Talaei *et al.*, (2014)** on cumin.

Oil Characters:

1- Essential oil percentage: As data in Table (3) indicated, the highest essential oil percentage was obtained with T2 (75% NPK + bio-fertilizers) as it scored (1.53 and 1.62 %) in the first and second seasons, respectively, and T3 came in the second place with non-significant differences between them in the two seasons. T1 occupied the third place in both

seasons. Meanwhile, the lowest values were recorded with T5 (bio-fertilizers only) as the values were (1.27 and 1.33 %) in the first and second seasons, respectively and the next higher values were obtained with T4 in the two seasons.

2- Oil yield/plant (ml): Data in Table (3) concerning oil yield per plant (ml) showed that, application of T2 (75% NPK recommended dose + bio-fertilizers) recorded the highest values as it scored (0.51 and 0.60 ml/plant) in the first and second seasons, respectively, followed by T1 as the values scored were (0.40 and 0.47 ml/plant) in the first and second seasons, respectively. While, the lowest values were obtained with plants treated by T5 (bio-fertilizers only) as it scored (0.11 and 0.16 ml/plant) in the first and second seasons, respectively, compared to the other treatments in both seasons and followed in ascending order by T4 and T3 in the two seasons, respectively.

Table 3. Effect of bio and chemical fertilization on essential oil productivity of Dutch fennel plants during the two seasons of 2017/2018 and 2018/2019**.**

Treatment	Essential oil percentage	Oil yield/plant (ml)	Oil yield/feddan (l)
		First season	
T1	1.40	0.40	15.10
T2	1.53	0.51	18.98
T3	1.43	0.38	14.28
T4	1.29	0.30	11.12
T5	1.27	0.11	4.14
LSD at p<0.05	**0.26**	**0.15**	**5.72**
		Second season	
T1	1.43	0.47	17.57
T2	1.62	0.60	22.38
T3	1.48	0.47	17.64
T4	1.35	0.30	11.08
T5	1.33	0.16	5.97
LSD at p<0.05	**0.06**	**0.14**	**5.23**

T1 (full recommended dose of NPK), T2 (75% NPK recommended dose plus bio-fertilizers), T3 (50% NPK recommended dose plus bio-fertilizers), T4 (25% NPK recommended dose plus bio-fertilizers), T5 (bio-fertilizers only).

3- Oil yield/feddan (liter): Table (3) revealed that, oil yield per feddan takes the same line with oil yield per plant as the highest values occurred with T2 (75% NPK recommended dose + bio-fertilizers) as its values were (18.98 and 22.38 L/feddan) in the first and second seasons, respectively. The next lower place was occupied by T1 as the values were (15.10 and 17.57 L/feddan) in the first and second seasons, respectively. The differences between the above-mentioned treatments were non-significant in both seasons. However, T5 (bio-fertilizers only) recorded the lowest values as (4.14 and 5.97 L/feddan) in the first and second season, respectively and the next higher values were recorded by T4 and T3, respectively in both seasons.

The results of oil parameters go on line with those obtained by **Mahfouz and Sharaf-Eldin (2007); Azzaz *et al*., (2009); Osman (2009); Gamal and Ismail (2012); Dadkhah (2014); Abdel Wahab *et al*., (2016)** on fennel, **Yeganehpoor *et al*., (2017)** on coriander, **Toaima (2005)** on *Achillea millefolium* L., **El-Shora (2009)** on *Mentha piperita*, **Hellal *et al*., (2011); Barandozi (2014); Singh and Singh (2016)** on dill, **Saker *et al*., (2012)** on marjoram, **Amran (2013)** on *Pelargonium graveolens*, **Sakr *et al*., (2014)** on sweet basil, **Talaei *et al*., (2014)** on cumin.

Photosynthetic Pigments:

1- Chlorophyll a: As indicated in Table (4), the highest values of Chlorophyll a (1.090 and 1.054 mg/g) in the first and second seasons, respectively were resulted from T2 treatment (75% NPK plus bio-fertilizers) and followed in descending order by T1 as it recorded (1.038 and 1.101 mg/g) in the first and second seasons, respectively with non-significant differences between them. While, the treated plants by T5 (bio-fertilizers only) recorded the lowest values as (0.753 and 0.758 mg/g) in the first and second seasons, respectively, compared to the other treatments in the both seasons.

2- Chlorophyll b: Data illustrated in Table (4) regarding Chlorophyll b content showed that, the highest values were recorded by T3 as it scored (0.517 and 0.525 mg/g) in the first and second seasons, respectively. The next lower place was occupied by T1 in the first season with significant differences between them, whereas, in the second season T2 (75% NPK recommended dose plus bio-fertilizers) scored the second place with significant differences between T2 and T3 Treatments. Meanwhile, the lowest values as (0.31 and 0.32 mg/g) were recorded by T5 treatment, in the first and second seasons, respectively.

3- **Total Chlorophyll:** Results shown in Table 4 revealed that, total chlorophyll in treated plants by T3 (50% NPK recommended dose plus bio-fertilizers) gave the highest values as (1.518 and 1.532 mg/g) in the first and second seasons, respectively and followed in descending order by T2 in the first season

with non-significant differences between them, whereas, in the second season T1 scored the second place with non-significant differences between T1 and T3 Treatments. While, the lowest values were recorded by T5 treatment (bio-fertilizers only) as it scored (1.068 and 1.077 mg/g) in the first and second seasons, respectively.

4- Carotenoids: It is obvious from data in Table (4) that, T2 (75% NPK recommended dose plus bio-fertilizers) recorded the highest values of carotenoids as (0.396 mg/g) in the first season, whereas, in the second season T1 gave the highest values as (0.407 mg/g). The differences between T2 and T1 were non-significant in both seasons. On the other hand, the lowest values were recorded by T5 treatment as (0.26 and 0.26 mg/g) for both seasons, respectively, compared to other treatments.

Table 4. Effect of bio and chemical fertilization on photosynthetic pigments of Dutch fennel plants during the two seasons of 2017/2018 and 2018/2019.

Treatment	Chl. A (mg/g)	Chl. B (mg/g)	Total chl.(mg/g)	Carotenoids (mg/g)
		First season		
T1	1.038	0.420	1.458	0.361
T2	1.090	0.412	1.502	0.396
T3	1.001	0.517	1.518	0.336
T4	0.797	0.321	1.118	0.293
T5	0.753	0.315	1.068	0.260
LSD at p<0.05	**0.059**	**0.059**	**0.059**	**0.059**
		Second season		
T1	1.101	0.416	1.517	0.407
T2	1.054	0.431	1.485	0.374
T3	1.007	0.525	1.532	0.340
T4	0.813	0.331	1.143	0.303
T5	0.758	0.319	1.077	0.264
LSD at p<0.05	**0.059**	**0.059**	**0.059**	**0.059**

T1 (full recommended dose of NPK), T2 (75% NPK recommended dose plus bio-fertilizers), T3 (50% NPK recommended dose plus bio-fertilizers), T4 (25% NPK recommended dose plus bio-fertilizers), T5 (bio-fertilizers only).

Chemical Composition:

Nitrogen percentage: As shown in Table (5), the highest values of nitrogen percentage were obtained by T2 (75% NPK recommended dose plus bio-fertilizers) as (1.702 %) in the first season while T1 (full NPK recommended dose) gave the highest values in the second season with significant differences between them in both seasons. On the opposite, the lowest values of nitrogen percentage were obtained by treating the plants with T4 treatment as the values were (1.176 and 1.155 %) in the first and second seasons, respectively. However, the rest treatments occupied intermediate place between the aforesaid treatments.

Phosphorus percentage: Data indicated in Table (5) revealed that, T2 (75% NPK recommended dose plus bio-fertilizers) recorded the highest value as (0.179 %) in the first season followed by T1 treatment with non-significant differences between them; whereas, in the second season T1 produced the highest value as it scored (0.217 %) and followed by T2 with significant differences between them. Meanwhile, the lowest values were recorded by T3 as the values were (0.064 and 0.075 %) in the first and second seasons, respectively.

Potassium percentage: Data of Table (5) indicated that, the highest values of potassium percentage were obtained as a result of using T5 treatment in the two seasons as it recorded (1.384 and 1.416 %), respectively followed by T1 treatment in the first season with non-significant differences between then and followed by T3 treatment in the second season with significant differences between them. On the opposite, the lowest percentage of potassium was obtained by treating the plants with T4 treatment as the values were (1.137 and 1.177 %) in the first and second seasons, respectively.

Total carbohydrates (%): It is obvious from Data of Table (5) that, the highest total carbohydrates percentage was obtained from the treatment of T3 (50% NPK recommended dose plus bio-fertilizers) as it recorded (17.83 %) in the first season only but T2 (75% NPK recommended dose plus bio-fertilizers) gave the highest value as (18.03 %) in the second season. The differences between the above-mentioned treatments were significant. While, the lowest values as (17.25 %) occurred with T5 (bio-fertilizers only) in the first season but T3 treatment gave the lowest values as (16.87 %) in the second season.

The aforementioned results of fertilization concerning chemical constituents are in parallel with those obtained by **Mahfouz and Sharaf-Eldin (2007); Osman (2009); Zaki *et al*., (2010); Abou El-Ghait *et al*., (2012); Dadkhah (2014)** and **Badran *et al*., (2017)** on fennel, **Hellal *et al*., (2011)** on dill, **Amran (2013)** on *Pelargonium graveolens*, **Mady and**

Youssef (2014) on dragonhead, **Ghatas and Abdallah (2016)** on *Echinacea purpurea*, **Mohamed and Ghatas (2016)** on violet, **Sakr (2017)** on *Calendula officinalis*, **Gomaa *et al*., (2018)** on roselle, **Hassanain *et al*., (2018)** on *Tageteserecta*.

Table 5. Effect of bio and chemical fertilization on herb chemical composition of Dutch fennel plants during the two seasons of 2017/2018 and 2018/2019.

Treatment	N percentage	P percentage	K percentage	Total Carbohydrates
First season				
T1	1.255	0.122	1.327	17.25
T2	1.702	0.179	1.311	17.51
T3	1.242	0.064	1.306	17.83
T4	1.176	0.083	1.137	17.38
T5	1.479	0.160	1.384	17.25
LSD at p<0.05	**0.157**	**0.059**	**0.059**	**0.059**
Second season				
T1	1.539	0.217	1.284	17.06
T2	1.232	0.142	1.267	18.03
T3	1.392	0.075	1.342	16.87
T4	1.155	0.087	1.177	17.52
T5	1.455	0.136	1.416	17.00
LSD at p<0.05	0.059	0.059	0.059	0.059

T1 (full recommended dose of NPK), T2 (75% NPK recommended dose plus bio-fertilizers), T3 (50% NPK recommended dose plus bio-fertilizers), T4 (25% NPK recommended dose plus bio-fertilizers), T5 (bio-fertilizers only).

GC/Mass analysis of Dutch fennel volatile oil:

With regard to volatile oil GC/Mass analysis, data in Table (6) showed that Gas chromatograms analysis of the volatile oil of Dutch fennel seeds revealed the presence of α-Pinene, Sabinene, ß-Pinene, Myrcene, l-Phellandrene, à-Terpinene, D-Limonene , 1,8 cineole, Terpinen-4-ol, Trans-Ocimene, ç-Terpinene, L-Fenchone, Camphor, Estragole, Trans-anethole and Tetracosamethyl-cyclododecasiloxane in most treatments. The main component of Dutch fennel seeds volatile oil was Anethole. However, the highest value of Anethole (66.23%) was observed by T4, followed by T1.

Table 6. Volatile oil GC/ Mass analysis as affected by some fertilization treatments during 2018/2019 season.

	Dutch Fennel				
	Concentration of compounds (%)				
Compounds	**T1**	**T2**	**T3**	**T4**	**T5**
α-Pinene	0.86	0.71	0.69	0.58	0.52
Sabinene	0.54	0.44	0.39	0.32	0.26
ß-Pinene	0.08	0.09	0.09	0.07	0.06
Myrcene	0.18	0.11	0.10	0.09	0.06
l-Phellandrene	0.09	–	0.07	0.07	–
à-Terpinene	0.13	–	–	–	–
D-Limonene	5.71	10.47	5.01	4.15	3.94
1,8-cineole	1.13	1.18	1.12	1.37	1.02
Trans-Ocimene	0.62	0.15	0.23	0.17	0.08
ç-Terpinene	0.64	0.26	0.43	0.30	0.17
L-Fenchone	3.59	4.64	4.66	5.87	3.90
Camphor	0.08	0.10	0.10	0.13	0.08
Terpinen-4-ol	0.29	0.11	–	–	–
Estragole	24.04	24.42	52.64	20.65	44.24
Trans-anethole	61.95	57.32	34.47	66.23	45.67
Tetracosamethyl-cyclododecasiloxane	0.07	–	–	–	–
Total	100	100	100	100	100

T1 (full recommended dose of NPK), T2 (75% NPK recommended dose plus bio-fertilizers), T3 (50% NPK recommended dose plus bio-fertilizers), T4 (25% NPK recommended dose plus bio-fertilizers), T5 (bio-fertilizers only).

The obtained results of this study may be due to the role of fertilization in growth and development of the plants; where the use of N-fixing bacteria e.g. Azotobacter and Azospirillum was found to have not only the ability to fix nitrogen but also to release certain phytohormones of cytokinins, gibberellins and

auxins which could enhance plant growth through absorption of nutrients and so on enhancing photosynthesis process (**Hegde *et al.*, 1999**). Microorganisms used as bio-fertilizers may affect the integrity of growing plants by one mechanism or more such as nitrogen fixation production of growth promoting substances or organic acids, enhancing nutrients uptake or protection against plant pathogens (**Hawaka, 2000**). Also, N-fixers synthesize stimulatory compounds such as, gibberellins, cytokinins and IAA. They act as growth regulators, which increased the surface area per unit of root length and were responsible for root hair branching with an eventual increase in the uptake of nutrients from the soil (**Sperenat, 1990 and Dadarwal *et al.*, 1997**). Besides, the use of Phosphate dissolving bacteria as a bio-fertilizer product containing very active phoshphate dissolving bactcria has proved its efficiency in enhancing different aspects of growth and development of many plant species including medicinal and aromatic ones. Establishment of a strong root system is related to the level of available phosphate in the soil. Phosphate dissolvers or vesicular arbuscular mycorrhizae and silica bacteria are capable of converting tricalcium phosphate to monocalcium phosphate ready for plant nutrition. Phosphate also increased mineral uptake and water use efficiency (**Hawaka, 2000**). Furthermore**,** to interpret and evaluate the effect of chemical fertilization concerned in this study, on augmenting the different tested vegetative growth parameters, yield component parameters and chemical constituents of Dutch fennel plants, it is important to refer to the physiological roles of nitrogen, phosphorus and potassium in plant growth and development. Such three macronutrient elements are the common elements usually included in fertilizers**.** Plant supplement with these macronutrients in form of fertilizers is necessary because the soil is usually in deficient of them due to plant removal leaching or they are not readily available for plants. Therefore, such addition of well-balanced NPK fertilization quantities insured production of high productivity and chemical constituents of Dutch fennel plants.

The role of NPK fertilization on promoting vegetative growth characters, enhancing yield component parameters and oil yield and as well as stimulating the chemical constituents content of Dutch fennel plants could be explained by recognizing their fundamental involvement in the very large number of enzymatic reaction that depend on NPK fertilization . NPK reflected directly on increasing the content of total carbohydrates, total sugars and total free amino acids as well as NPK % in the leaves were indirectly the cause for enhancing the augmenting of all other vegetative growth traits, oil yield and components of Dutch fennel plants **(Cooke, 1982)**.

Consequently, it is preferable from the previous results that treating Dutch fennel plants with the treatment of 75% of the recommended NPK plus bio-fertilizers for enhancing growth and oil productivity of this plant. Therefore, the present study strongly admit the use of such treatment to provide good and high exportation characteristics due to its safety role on human health.

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الاستبدال الجزئى للتسميد الكيماوى فى نبات الشمر الهولندى بواسطة التسميد الحيوى

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قسم البساتين – كلية الزراعة بمشتهر – جامعة بنها – مصر*

قسم النباتات الطبية والعطرية – مركز بحوث الصحراء – مصر**

تم إجراء الدراسة الحالية على سلالة نبات الشمر الهولندي خلال الموسمين المتتاليين 2018/2017 و 2019/2018 في محطة بحوث بالوظة ، محافظة شمال سيناء ، مصر. كان الهدف من هذه الدراسة هو تقييم تأثير إضافة الأسمدة الحيوية كبديل جزئي للتسميد الكيماوى على النمو الخضري ، محصول الثمار ، إنتاجية الزيت العطرى ومكوناته الرئيسية وكذلك المكونات الكيميائية لنبات الشمر الهولندي تحت ظروف سيناء. تم تصميم التجربة على شكل قطاعات كاملة العشوائية مع أخذ ثلاث مكررات لكل معاملة. كانت المعاملات عبارة عن مزيج من خمسة مستويات للتسميد (الجرعة الكاملة الموصى بها من النيتروجين والفوسفور والبوتاسيوم فقط ، 75 ، 50 ، 25 ٪ من الجرعة الموصى بها من النيتروجين والفوسفور والبوتاسيوم بالإضافة إلى الأسمدة الحيوية ، والأسمدة الحيوية فقط). أظهرت النتائج أن استخدام المعاملة الثانية (75% من الجرعة الموصى بها من التسميد الكيماوى بالنيتروجين والفوسفور والبوتاسيوم بالإضافة إلى الأسمدة الحيوية) أدى إلى زيادة قيم قياسات النمو الخضري (ارتفاع النبات ، عدد الأفرع لكل نبات ، الوزن الطازج للعشب لكل نبات ، الوزن الجاف للعشب لكل نبات) كما أدى إلى زيادة قيم صفات المحصول (عدد النورات لكل نبات ، وزن 1000 ثمرة ، محصول الثمار للنبات ، محصول الثمار للفدان) ، أيضا أدى استخدام المعاملة الثانية إلى زيادة قيم نسبة الزيت العطري ومحصول الزيت العطرى مقارنة بباقى المعاملات بينما أقل القيم فى قياسات النمو الخضرى وصفات المحصول وإنتاجية الزيت العطرى نتجت مع النباتات التي تم معاملتها بواسطة المعاملة الخامسة (الأسمدة الحيوية فقط). بشكل عام ، كانت المكونات الكيميائية الرئيسية للزيت العطرى لنبات الشمر الهولندي هي الأنيثول والإستراجول.