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Physiological and Phytochemical Responses of *Foeniculum vulgare* var. *vulgare* Mill. and *Foeniculum vulgare* var. *azoricum* Mill. to Bio-Organic Manure as partial or full Substitute for Inorganic Amendment

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Abstract: A field experiment was conducted to study the physiological and phytochemical responses of two fennel varieties (*Vulgar* and *Azoricum*) to bio-organic fertilization separately or combined with chemical NPK treatments. This experiment was carried out on the sandy soil of experimental farm, Fac. of Agric., Al-Azhar Univ. Cairo branch, during 2010/ 2011 and 2011/ 2012. The results indicated that there are significant effects of fertilization treatments (control, conventional NPK, bio-organic and 50% NPK combined with bio-organic) and fennel varieties (*Vulgar* and *Azoricum*) on plant growth (plant height, number of branches/plant and number of umbel/plant), yield of fruits and essential oil yield per plant. Conventional NPK treatment and bio-organic + 50% NPK treatment gave the best results. Besides, *Foeniculum vulgare* var. *vulgare* overcame *Foeniculum vulgare* var. *Azoricum* in the same parameters. The interaction treatments indicated that, the combination of organic (cattle manure) and Bio (*Azotobacter Croccuccum* + *Bassills megatherium*) fertilizers with half of the conventional NPK dose was almost as effective as the conventional NPK alone on growth and yield parameters. Using 50% NPK + bio-organic with *Foeniculum vulgare* var. *Vulgar* gave the highest values of oxygenated compounds. In the public health point of view, the best treatment was bio-organic with *Foeniculum vulgare* var. *vulgare* that achieved high anethole (62.1 and 62.3%) and low estragole (3.9 and 4.9%) contents in the first and the second seasons, respectively.

Key words: *Foeniculum vulgare* Mill, fennel, bio-organic, fertilization, essential oil, anithole, estragole.

INTRODUCTION

Fennel (*Foeniculum vulgare* Mill.) which belongs to the family of Apiaceae (Umbelliferae) is one of the most important medicinal and aromatic plants. Essential oil of fennel is used as flavoring agents in food products such as beverages, bread, pickles, pastries, and cheese. It is also used as a constituent of cosmetic and pharmaceutical products (Piccaglia *et al*., 2001).

Herbal drugs and essential oils of fennel have hepatoprotective effects (Ozbek *et al*., 2003). Traditionally in Europe and Mediterranean areas fennel is used as antispasmodic, diuretic, anti-inflammatory, analgesic, secretomotor, secretolytic, eye lotion, and antioxidant remedy and integrator (Gori *et al*., 2012). Moreover, fennel fruits possess anticancer activity (Anand *et al.,* 2008). Recently it was shown that fennel essential oil possesses emmenagogue and galactagogue properties (Babu *et al*., 2010) and is a cure for pediatric colic and respiratory disorders due to its antispasmodic effect (Liu *et al*., 2010 and Sajomsang *et al*., 2009). In addition, the volatile oils (1-3%) of fennel are used to control flatulent dyspepsia and colic in children (Mahfouz and Sharaf-Eldin, 2007). Antioxidant and antimicrobial activity of fennel has also been reported (Ruberto *et al.,* 2000).

Essential oil is a rich source of anethole, limonene, fenchone, estragole which plays an important role in the determination of the quality of the essential oil of seeds (Gross *et al.,* 2002). These oil components depend upon internal and external factors affecting the plant such as genetic structures and ecological conditions (Telci *et al.,* 2009). Many phytochemical researches have been conducted so far to investigate the chemical composition of fennel essential oil with different results: depending on the time of harvests, conservation, region, and area of cultivation.

The major components of fennel are phenylpropanoid derivatives: *trans*-anethole and estragole (methyl chavicol), and then alpha-phellandrene, limonene, fenchone, and alpha-pinene (Dıaz *et al*., 2006). British Pharmacopoeia (2009) reported that, grade bitter fennel oil must be composed anethole (55 – 75%), fenchone (12 – 25%), estragole not more than 6.0%, limonene (0.9 – 5.0%), anisaldehyde not more than 2.0% and α-pinene (1.0 – 10.0%). Like other crops, the increase in fruits (seed) and active ingredient yield of medicinal herbs is influenced by genetic and environmental parameters (Akbarinia *et al*., 2006). Chemical fertilizers are key components for providing crop nutrient that are needed in recent years, but many researchers have started to

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give attention to the negative effects of using chemical NPK fertilizers in agriculture, both in agriculture itself and on human beings. So, the intensive and continuous use of chemical fertilizers has side effects in the polluting of the underground water, destroying microorganisms and insects, making plants more susceptible to the attack of diseases and reducing soil fertility, which increase environmental production cost (Abdelaziz *et al*., 2007).

To avoid the risk of these negative effects of chemical fertilizers, it is necessary to use organic or biological fertilizers which provide plant nutrients and also increase the long term sustainability of agro-ecosystems (Murty and Ladha, 1988; Mehnaz and Lazarovits, 2006). The using of biofertilizers that contain different microbial strains has led to a decrease in the use of chemical fertilizers and has provided high quality products free of harmful Agrochemicals for human safety (Mahfouz and Sharaf-Eldin, 2007). It is also, suggested that there should be a complete or partial substitution of mineral fertilization (NPK) by using of organic and bio-fertilizers which are sure and economical for farmers (Dahmardeh, 2012). Free-living nitrogen-fixing bacteria *eg Azotobacter chroococcum* and *Azospirillum lipoferum,* were found to have not only the ability to fix nitrogen but also the ability to release phytohormones similar to gibberellic acid and indole acetic acid, which could stimulate plant growth, absorption of nutrients, and photosynthesis (Fayez *et al.,*1985). However, an organic farming approach with or without chemical fertilizers seems to be a possible solution for these problems (Abass *et al*., 2012). Application of organic and bio fertilizers as a substitute for inorganic fertilizers in order to grow the medicinal and aromatic plants, should not be considered as a simple objective and short term benefits, but as a mean to improve environmental conditions and human health (Shahram and Ordookhani, 2011).

The biological manure supplies a wide variety of nutrients along with organic matters that improves the physical characteristics of soil. Its beneficial effects on plant growth are sometimes difficult to duplicate with other materials. There is also a positive interaction between the combination of organic manures and NPK (Pong and Laty, 2000). Organic and biological (N fixing and P dissolving bacteria) fertilizers can be considered as a suitable substitution for chemical fertilizers in developing sustainable medicinal plant production systems (Moradi *et al*., 2011; Badran and Safwat, 2004). Many investigators have pointed out the influence of organic manure by increasing the growth, yield and essential oil production of Fennel (Badran and Safwat, 2004) and Anise (Safwat *et al*., 2001), Cumin (Safwat and Badran, 2002). Several studies have reported that cattle manure can increase the growth and yield of some medicinal plants such as coriander (Salem and Awad, 2005; Carrubba, 2009 and Darzi, 2012), dill (Khalid and Shafei, 2005), basil (Biasi *et al*., 2009).

This experiment was conducted to study the response (physiological and phytochemical) of two fennel varieties (*vulgare* and *azoricum*) to fertilization with bio-organic manure separately or in combination with 50% of conventional NPK compared to conventional NPK fertilizer under sandy soil conditions.

MATERIALS AND METHODS

A field experiment was carried out during two consecutive seasons of 2010/2011 and 2011/2012 in a sandy soil of experimental farm, Fac. of Agric., Al-Azhar Univ. Cairo branch. Physical and chemical properties of the experimental soil are shown in Table 1. Fennel varieties seeds were obtained from Agricultural Research Center, Horticulture Institute, Department of vegetable crops, to be used as plant material in this study and sown in a well-prepared soil on 1 November of both years. Seedlings were thinned to two plants per hill and irrigated 21 days after sowing. The experimental unit area was 3.9 m^2 (2.25 × 1.75 m) with 75 cm between rows and a distance of 40 cm between plants in the row.

Experimental Design:

The experiment was arranged in a split plot design with 3 replicates, where the two fennel varieties were arranged within the main plots, while the four amendments were randomly distributed in the subplots.

Main plots (fennel varieties) were:

V1: local (*Foeniculum vulgare* var. *vulgare*) and

V2: Germany (*Foeniculum vulgare* var. *azoricum*)

Subplots (fertilization types) were:

F1: Untreated plants (control).

F2: Conventional NPK (calcium super phosphate 250 kg/fed, ammonium sulphate 250 kg/fed and potassium sulphate 150 kg/feddan.

F3: Bio-Organic (Azotobacter Croccuccum + Bassills megatherium + 25 m^3/fed cattle manure), chemical components of decomposed cattle manure was obtained from a local farm are shown in Table 2

F4: 50% NPK + Bio-Organic.

The bacterial species; Azotobacter Croccuccum and Bassills megatherium were obtained from the Microbiological department of Agricultural Research Center. At harvesting time the plant density was 26500 plants per feddan. The following data were recorded; Plant growth (plant height: cm, number of branches/plant and number of umbel/plant), Fruits yield, g/plant, Essential oil yield, ml/plant and ingredient components of essential oil, %.

Table 1: Physical and chemical properties of the experimental soil.

Characteristics	First season	Second season
Mechanical analysis		
Coarse sand %	18.68	18.29
Fine sand	66.46	68.11
Silt %	5.11	4.47
Clay %	9.75	9.13
Soil texture	Sandy	Sandy
Chemical analysis		
pH	6.99	7.08
EC m. mohs/cm	0.63	0.74
$CaCO_3$ %	3.27	4.30
Soluble cations (m.equ/L)		
Ca^{+}	1.55	1.10
Mg^{++}	0.96	0.74
Na^{+}	1.65	1.81
K^{+}	1.49	0.89
Soluble anion (m.equ/L)		
HCO^{-}_{3}	0.79	0.85
Cl^{-}	1.59	1.50
SO^{-}_{4}	3.34	2.11
Macro element (ppm)		
Total N	9.87	11.02
P_2O_5	5.22	6.00
K_2O	368.03	244.00
Micro elements (ppm)		
Fe	3.12	2.87
Cu	0.31	0.39
Za	0.87	0.76
Mn	4.75	3.40

Table 2: Chemical analysis of cattle manure.

PH	E.C mmhos	Soluble cations meq/L.				Soluble anions meq/L		
		Ca++	Mg+'	Na+	K+	HCO_3	Cl^-	SO_4^-
7.21	1.34	13.00	41.00	92.00	32.00	134.0	10.20	137.50
Humidity		Ash	O.M.	Total N%		Available		
						P%		K%
58%		6.6%	42%	1.8		1 .2		1.2

Essential Oil Extraction:

Essential oil was obtained from dried aerial parts from Foeniculum vulgare Mill. by steam distillation using Clevenger system during 3 h. The extracted essential oil was dried with anhydrous sodium sulfate. Then, the oil was weighed and stored in sealed amber flasks at 4°C until analysis.

Essential Oil Analysis:

Essential oil constituents were analyzed using GC-MS (Gas Chromatography Mass Spectrometry). GC-MS analysis were performed on an HP 6890 Series gas chromatograph coupled to an HP 5973 mass selective detector (HP Technologies, USA). The GC analysis was performed on a HP-5MS (30 m x 0.25 mm I.D., 0.25 μm film thickness) capillary column (5% diphenyl, 95 dimethylpolysiloxane; HP, USA). The oven temperature was programmed from 40°C (3 min) to 60°C at a rate of 2°C/min and from 60 to 120 at a rate of 4 °C/min and from 120 to 240 at a rate of 6 °C/min, final temperature held for 10 min. The MS was operated in the full-scan mode from 35 to 550 m/z. The injector and detector temperatures were 240 and 250ºC, respectively. Helium used as the carrier gas was adjusted to a linear velocity of 32 cm/s. The identification of essential oil components was on the basis of comparison of their retention times with standard components and mass spectra with published data (Adams, 2007). Quantitative data were obtained from electronic integration of peck areas. The instrument control was performed by the HP software (HP Technologies, USA).

Statistical Analysis:

The obtained data were subjected to statistical analysis (Two-way ANOVA) using SAS software (SAS Institute, version 8, 2001). Differences between the treatments were performed by Tukey's Honestly Significant Difference (HSD) test at the 5% level at 5% confidence interval.

RESULTS AND DISSECTIONS

1. Physiological Responses:

1.1. Plant Growth:

Significant effects on plant growth characters (plant height, number of branches/plant and number of umbels/plant) were detected for the fertilization (non-fertilized, conventional NPK, bio-organic and 50% NPK + bio-organic) treatments and fennel varieties; *Vulgar* and *Azoricum* (Table 3). There were also significant differences in plant growth parameters among fertilization (F_1, F_2, F_3 and F_4) and among two fennel varieties (V_1 and V_2) treatments. It is also clear that, the use of chemical N, P, and K (F_2: conventional NPK) treatment enhanced the vegetative growth characters of fennel plants than the other treatments in both seasons followed by the treatment of the combination of bio (*Azotobacter Croccuccum* and *Bassills megatherium*), organic (cattle manure) and half dose of conventional chemical NPK (F_4: 50% NPK + Bio-Organic). On the other hand, there is no significant difference in the number of branches/plant and number of umbel/plant between the treatments of F_2 and F_4 during first and second seasons. The lowest values of plant growth characters in both seasons were obtained with control (F_1: unfertilized plants) treatment. Data in Table 3 also indicate that there is a significant difference in the number of branches between F_3 and F_4 treatments in the second season but not in the first season. From the result obtained in the same table, it can be arranged the fertilization treatments according to plant growth parameters (plant height, number of branches / plant and number of umbels / plant) as follows: F_2 (conventional NPK) > F_4 (50% NPK + Bio-Organic) > F_3 (Bio-Organic) > F1 (control). Similar finding was gained by Hassan *et al*., (2012) who found that, the highest vegetative growth parameters (plant height, branch number and fruit number/plant) of coriander plants were obtained by applying NPK following by organic (compost) and bio (mixture of bacterial strains) fertilizers in the two experimental seasons. The result also obtained is harmony of those obtained by Abou El-Magd *et al.*, (2008) who reported that organic manure treatment increased all the vegetative growth parameters of sweet fennel expressed. These results also are in agreement with El-Hifny (2010) who found that organic manure application enhanced plant growth in addition to mineral content, and are in agreement with Hellal *et al*., (2011) who indicated that applying biofertilizer treatment alone or in combination with chemical N fertilizer increased the growth, yield and chemical constituents of the dill plant compared to the untreated control.

Foeniculum vulgare var. *vulgare* was significantly better than *Foeniculum vulgare* var. *Azoricum* by 54.28, 24.57 and 15.16% of plant height, number of branches/plant and number of umbel/plant respectively at the first season. This trend also was obtained in the second season. This finding is in agreement to Osman (2009) who found that, local or bitter variety (*Foeniculum vulgare* var. *vulgare* Mill.) was superior to sweet or German one (*Foeniculum vulgare* var. *azoricum* Mill.) in most cases of vegetative growth. The results recorded in Table 3 show a significant effect of the interaction between fertilization (F1, F2, F3 and F4) treatments and two fennel varieties (V_1 and V_2).

Data in Table 3 also clear that a significant difference in vegetative growth of fennel plants among the interaction treatments. The best results of plant height, number of branches/plant and number of umbel/plant were obtained by the combined between conventional NPK and *Foeniculum vulgare* var. *vulgare* (F_2V_1). On the other hand, there are no significant differences in plant growth parameters between F_2V_1 and F_4V_1 (50% NPK + bio-organic × *Foeniculum vulgare* var. *vulgare*) treatments in two successive seasons, except the plant height of the second season. The interaction between non-fertilized plants (control) and *Foeniculum vulgare* var. *Azoricum* (F_1V_2) recorded the lowest values of the vegetative growth characters. Statistical analysis data of Table 3 indicate that, there are no significant differences in the number of branches/plant between F_1V_2 and both F_1V_1, F_3V_1 and F_4V_1 treatments at first season. While during the second season, there were no significant differences in the number of umbel/plant between F_1V_2 and both F_1V_1 and F_3V_2 treatments. The results obtained of fennel growth characters are in agreement with Kandil (2002) who found that the fertilization with chemical fertilizer in both seasons gave the tallest fennel plants, highest numbers of branches per plant followed by fertilization with compost/*Azotobacter*, compost and chicken manure gave the shortest. These results also in harmony with those reported by Azzaz *et al.* (2009) on *Foeniculum vulgare* Mill, who found that the differences between NPK and NPK + bio-fertilizers were not significant in plant height parameter.

1.2. Fruits Yield:

Data concerning total fruit yield/plant as affected by different amendments and two fennel varieties treatments are shown in Table 4. It is observed that, there is a significant effect of the amendments and fennel varieties treatments on fruit yield (g/plant). Furthermore, the treatment of F_2 (conventional NPK) gave the best results of fruits yield (35.38 and 44.26 g/plant) in the first and second seasons, respectively. This result is in a harmony with obtaining by Hassan *et al*., (2012) who found that, the maximum fruit yield/plant was obtained by using an NPK followed by organic and bio fertilizers. On the other hand, there is not a significant difference in fruits yield/plant between this treatment (F_2) and the treatment of F_4 (50% NPK + Bio-Organic). Similar trend was observed in the second season. Abo-Baker and Mostafa (2011) revealed that the inoculation with the mixture of biofertilizers combined with 50 or 100% chemical fertilizers improved, in most cases, growth characters of *Hibiscus sabdariffa* and increased sepal yield or at least did not differ significantly from the control (full recommended dose of NPK alone).

Table 3: Effect of fertilization and fennel varieties on plant growth parameters at 2010-2011 and 2011 - 2012 seasons.

Variety (V)	Fertilization (F)				Mean
	F_1	F_2	F_3	F_4	
First season 2010 - 2011					
Plant height, cm					
V_1	68.50	88.77	83.30	87.30	81.97
V_2	40.40	64.87	51.43	55.80	53.13
Mean	54.45	76.82	67.37	71.55	
HSD test at 5%: F: 1.84 V: 0.96 F × V: 3.15					
Number of branches per plant					
V_1	*4.50*	7.00	5.57	6.27	5.83
V_2	*4.00*	5.43	*4.53*	*4.77*	4.68
Mean	4.25	6.22	5.05	5.52	
HSD test at 5%: F: 0.66 V: 0.35 F × V: 1.13					
Number of umbel per plant					
V_1	15.63	26.30	21.60	25.50	22.26
V_2	11.80	24.40	17.90	23.20	19.33
Mean	13.72	25.35	19.75	24.35	
HSD test at 5%: F: 1.71 V: 0.89 F × V: 2.92					
Second season 2011 - 2012					
Plant height, cm					
V_1	74.30	101.13	95.90	98.30	92.41
V_2	52.00	77.88	65.00	71.30	66.55
Mean	63.15	89.51	80.45	84.80	
HSD test at 5%: F: 1.27 V: 0.66 F × V: 2.17					
Number of branches per plant					
V_1	6.07	8.77	7.00	8.57	7.60
V_2	4.03	7.53	5.80	7.00	6.09
Mean	5.05	8.15	6.40	7.78	
HSD test at 5%: F: 0.94 V: 0.49 F × V: 1.61					
Number of umbel per plant					
V_1	*22.13*	31.27	27.43	30.73	27.89
V_2	*19.60*	29.30	*21.77*	27.30	24.49
Mean	20.87	30.28	24.60	29.02	
HSD test at 5%: F: 1.50 V: 0.79 F × V: 2.57					

So, applying 50% of the recommended dose of NPK plus mixture of biofertilizer can save half of the quantity of chemical fertilizer, decrease the production cost and obtain high quality product. The results presented in Table 4 have demonstrated that, there is a significant difference between two fennel varieties (*vulgare* and *azoricum*) in fruits yield per plant. It was also noticed that the *vulgare* variety shows higher value of fruits yield/plant than the *azoricum* variety by 14.83 and 3.89% in the first and second seasons respectively. These results are in agreement with that obtained by Osman (2009) who noticed that the German (*azoricum*) variety shows lower value of fruits yield/plant than the local (*vulgare*) variety. The obtained results in Table 4 revealed that fruits yield (g/plant) was statistically affected by the interaction between fertilization treatments (control, conventional NPK, bio-organic and 50% NPK + bio-organic) and fennel variety (*vulgare* and *azoricum*). The results also indicated that the treatment of F_2V_1 gave the highest fruits yield (36.47 and 45.26g/plant) in the first and the second seasons, respectively. On the other hand, there was no significant difference between this treatment (F_2V_1) and both F_2V_2 and F_4V_2 treatments in both seasons. The lowest fruits yield (16.87 and 34.46 g/plant) was obtained with the treatment of F_1V_2 (control with *Foeniculum vulgare* var. *azoricum*) in the first and the second experimental seasons, respectively.

The positive physiological response of fennel (*Foeniculum vulgare* Mill.) to different amendments might be due to the direct effect of chemical NPK amendment or indirect of bio and organic amendments. The direct effect might be due to the role of NPK fertilization treatments in improving growth, yield, essential oil synthesis and chemical composition of fennel plants that could be explained by recognizing the fundamental involvement of N, P and K nutrients in very large number of enzymatic, energy transfer and biological processes (Farouk *et al*., 2007).

Table 4: Effect of fertilization and fennel varieties on fruits yield per plant at 2010-2011 and 2011 – 2012 seasons.

Varity (V)	Fertilization (F)				Mean
	F_1	F_2	F_3	F_4	
First season 2010-2011					
Fruits yield, g/plant					
V_1	25.33	36.47	30.23	34.63	31.67
V_2	16.87	35.27	24.67	33.53	27.58
Mean	21.10	35.87	27.45	34.08	
HSD test at 5%: F: 2.49 V: 1.30 F × V: 4.26					
Fruits season 2011-2012					
Seed yield, g/plant					
V_1	34.46	45.26	41.43	44.23	41.35
V_2	38.31	43.25	35.48	42.17	39.80
Mean	36.38	44.26	38.45	43.20	
HSD test at 5%: F: 2.06 V: 1.08 F × V: 3.53					

Indirect effect of bio amendment might be due to the microbial propagation activation since the free living nitrogen fixing bacteria (*Azotobacter croccocum*) have the ability not only to fix nitrogen but also to release certain phytohormons of GA_3 and IAA nature which could stimulate plant growth, absorption of nutrients and photosynthesis process (Fayez *et al*., 1985 and Abdel-Latif *et al*., 2001). The phosphate-dissolving bacteria (*Bacillus megatherium)* also may have increased the availability of phosphorus, and it may have increasing the uptake of trace elements (El-Haddad et a1., 1993). It increased the available phosphorus in plant's tissues which affects a large number of enzymatic reactions that depend on phosphorylation (Follet *et al*., 1981) and converted the insoluble forms into soluble ones through the releasing of organic and inorganic acids and CO_2 (Pamela and Hayasaka, 1982). On the other hand, the use of biofertilizers on Egyptian soils has decreased the pH, which had led to increased availability of trace elements that enhance plant growth (Mahfouz and Sharaf-Eldin, 2007). These findings could be ascribed to the increases in root surface area, root hairs and root elongation as affected by *Azotobacter* as mentioned by Sundaravelu and Muthukrishinan (1993). It can be said that, these results also might be due to the effect of cattle manure on enhancement physical criteria of the soil including better aeration, better water holding capacity, better nutrient availability and good balance between nutrients in the soil solution (Zebarth *et al*., 1999), improvement of biological activities (Salem and Awad, 2005), increased the presence of P, K and Mg in the soil beside the solubility of Ca, Mg and NO as a result of the continuous lowering of pH by manure application. In addition, organic matter may affect plant growth as a source of growth promoters, auxins, vitamins, amino acids (Melo and De- Oliveira, 1999), improve possibility of seedbed preparation for root growth (Mosaddeghi *et al*., 2000). Slow release of nutrients from cattle manure during growth period and hence low leaching of the nutrients could also be other criteria for cattle manures. These conditions lead to improve vegetative growth of fennel plants, flower induction, increasing umbel number per plant by enhancing the nitrogen content and the rate of photosynthesis (Badran and Safwat, 2004) which related to the fruits yield and quality of the plant.

2. Phytochemical Responses:

2.1. Essential Oil Yield:

The yield of essential oil of fennel fruits was significantly affected by different treatments of amendments and by fennel varieties in both seasons (Table 5). Data in the same table clear that, fertilization with conventional mineral NPK (F_2) or with the combination of 50% NPK and bio-organic (F_4) enhanced oil yield (ml/plant) more than others fertilization treatments. In first season, it is clear that, fertilization with conventional NPK (F_2) or with the combination of 50% NPK and bio-organic (F_4) treatments gave the greatest volatile oil yield by 263.33 and 213.33% over control (F_1), respectively. This trend also obtained in second season. According to the results obtained in Table 7, the yield of essential oil (ml/plant) can be arranged as follow: F2 > F4 > F3 > F1.

The obtained results in Table 5 cleared that, there is a significantly difference in oil yield per plant between two fennel verities. It is also clear that, *Foeniculum vulgare* var. *vulgare* was superior to *Foeniculum vulgare* var. *azoricum* in essential oil yield (ml/plant). The yield of volatile of *Foeniculum vulgare* var. *vulgare* increased by 28.13% and by 11.76% over *Foeniculum vulgare* var. *azoricum* in the first and the second experimental seasons, respectively.

Tabulated data in Table 5 refer to, the interaction between the treatments of amendments and fennel varieties achieved a significantly increasing in essential oil yield (ml/plant). Results also cleared that, the highest values of essential oil yield (1.14 and 1.29 ml/plant) were obtained by the treatment of F_2V_1 (conventional NPK × *Foeniculum vulgare* var. *vulgare*) in the first and the second seasons respectively compared to the others interaction treatments. On the other side, there is no significant difference between these treatments (F_2V_1) and F_4V_1 (50% NPK + bio-organic combined with *Foeniculum vulgare* var. *vulgare*) in the first season but was a significant in the second one. The lowest oil yield (0.19 and 0.46 ml/plant) was resulted with the F_1V_2 (non-fertilized plants × *Foeniculum vulgare* var. *azoricum*) treatment in the first and the second seasons, respectively.

These findings are in accordance with the observations of Mahfouz and Sharaf Eldin (2007) on *Foeniculum vulgare* Mill, who found that the highest yield of volatile oil resulted from inoculating the plants with *Azotobacter* + *Azospirilium* in the presence of a half dose of NPK. These results also are confirmed by Patel *et al*., (2003) on *Foeniculum vulgare* who improved the oil content of fennel by application of different organic manures alone or mix with inorganic fertilizers significantly. Osman (2009) reported that, chemical fertilization combined with both bio and organic fertilizers increase essential oil and chemical constituents in fennel plants. Azzaz *et al*., (2009) also found that, the essential oil of fennel was augmented when applied different mineral and organic fertilizers. According to the present analysis, increased essential oil yield can be owing to the improvement of oil yield components such as; essential oil content and fruits yield (Darzi, 2012).

Table 5: Effect of fertilization and fennel varieties on essential oil yield per plant at 2010-2011 and 2011 – 2012 seasons.

Variety (V)	Fertilization (F)				mean
	F_1	F_2	F_3	F_4	
First season					
V1	0.42	1.14	0.70	1.04	0.82
V2	0.19	1.05	0.48	0.84	0.64
Mean	0.30	1.09	0.59	0.94	
HSD test at 5%: F= 0.07 V= 0.04 F × V = 0.12					
Second season					
V1	0.62	1.29	0.79	1.09	0.95
V2	0.46	1.08	0.85	1.02	0.85
Mean	0.54	1.19	0.82	1.06	
HSD test at 5%: F= 0.04 V= 0.02 F × V = 0.07					

2.2. Chemical Constituents of Essential Oil (Quality of Oil):

Components which were identified in fennel essential oil of different treatments were divided into three main groups (Table 6) namely, hydrocarbons, oxygenated and unidentified ones. The oxygenated compounds (Fig 1) are grouped as: esters, aldehydes, ketones, alcohols, phenols and oxides. The pharmaceutical value of medicinal plants depends on their secondary metabolite components (Gross *et al*., 2002), on the other words, the pharmaceutical effects of the essential oil are mainly due to its content of oxygenated compounds (Osman, 2009). The hydrocarbons (Fig 2) are organic compounds consisting entirely of hydrogen and carbon atoms arranged in chains that form the family of terpenes as monoterpenes, sesquiterpenes and diterpenes.

The analysis of the essential oil in fennel (Table 8) showed that, seven ingredients identified, the main ingredient was *trans*-anethole (56.8 - 64.3%). Other major components were fenchone (12.1 - 14.9%), estragol (1.6 – 7.0%) and limonene (1.1 - 3.2%). Minor components of the essential oil were anisaldehyd (0.7 – 1.3%), – αpinine (1.2 – 1.8%), comphene (1.6 – 3.0%). These results were in agreement with Shahat *et al*., (2011) who found that, *trans*-anethole, estragole, fenchone and limonene were highly abundant in all of the examined oils of *Foeniculum vulgare* var. *vulgare* and *Foeniculum vulgare* var. *azoricum*, and with Khalil *et al*., (2008) who reported that, the major components in fennel seed oil are anethole, more than 45% and limonene, more than 12%. These results are in agreement with the British Pharmacopoeia monograph (2009).

Data in Table 7 indicated that, all fertilization treatments increased the chemical ingredients of essential oil compared to unfertilized plants with both varieties. This trend was also observed with two seasons. According to British Pharmacopeia (BP, 2009) and the results obtained in Table 7 it is clear that, the treatment of F_4V_1 (50% NPK + Bio-Organic with *Foeniculum vulgare* var. *vulgare*) gave the highest content of anethole (64.2 and 64.3%) in the first and the second seasons, respectively. On the other hand, *Foeniculum vulgare* var. *vulgare* gave the content of anethole more than *Foeniculum vulgare* var. *azoricum* by 3.9 and 3.5% in the first and the second seasons, respectively. Also, it can be arranged the interaction treatments according to anethol content in the essential oil on both seasons as follows: $F_4V_1 > F_3V_1 > F_2V_1 > F_4V_2 > F_2V_2 > F_1V_1 > F_1V_2$.

The results obtained in Table 7 indicated that, estragole (1-Allyl-4-methoxybenzene: $C_{10}H_{12}O$) content was at the limit of British Pharmacopiea (BP, 2009) (less than 6.0%) with all different treatments in both experimental seasons except the treatments of F_2V_1 (7.0%), F_4V_1 (6.8%) and F_4V_2 (6.1%) in the second season. In general, the increasing of methyl chavicol (estragole) means low quality of the essential oil (Kandil *et al*., 2009).

Foeniculum vulgare var. *azoricum* achieved the best results (3.2 and 4.6%) of estragol in fennel essential oil for the first and the second seasons, respectively. This result was less than those obtained in case of *Foeniculum vulgare* var. *vulgare* (4.1 and 5.5%) in the first and the second seasons, respectively. This trend was achieved with fenchone, anisaldehyde, limonene, α-pinene and comphene in both the experimental seasons. This result was harmony with shahat *et al*. (2011) who found that, *Foeniculum vulgare* var. *vulgare* overcame *Foeniculum vulgare* var. *azoricum* in the content of estragole, anisaldehyde, limonene, α-pinene and comphene. Estragole or

4-allylanisole, methyl chavicol ($C_{10}H_{12}O$) is an oxygenated aromatic, and although it has 10 carbon atoms, it is not a terpenoid compound. From data in Table 7 it is also observed that, the lowest estragole content was achieved with control (F_1) followed by Bio-Organic (F_3) in both seasons and with both varieties treatments. This trend also was observed with fenchone, anisaldehyde, limonene, α-pinene and comphene in both the experimental seasons with both varieties treatments. These results of estragole content are in agreement with Moradi *et al*. (2011) who reported that, the using of organic and biofertilizers lead to a change in the composition of essential oil. They also found that, estragole content was significantly decreased in organic and biological fertilizer treatments.

The lowest content of fenchone (12.1 and 12.1%), limonene (1.1 and 1.7%), anisaldehyd (0.7 and 0.8%), α-pinene (1.2 and 1.3%) and camphene (1.6 and 1.9%) were obtained with the interaction between control plants and *Foeniculum vulgare* var. *azoricum* (F_1V_2) in the first and the second seasons, respectively. On the other hand, the highest content of the same chemical ingredients was obtained with the treatments of F_4V_1 and F_2V_1 in the first season. Our present results on fennel are in support of the findings of Tallat (2005) on *Pelargonium graveolens* L. According to Anand *et al*., (2008) anethole, which is the principal active constituent of fennel seeds, showed an anticancer activity. The result of present work also was in agreement with the reports of Akgül and Bayrak (1988) who reported that the major component of essential oil of bitter fennel (*Foeniculum vulgare* var. *vulgare*) was *trans*-anethole (64.7% in fruits). The obtained results also in agreement with Moradi *et al*. (2011) who found that the application of different organic and biological fertilizers increased the anethole content which is the most important components of the sweet fennel essence and consequently improved the quality and with Anwar *et al*., (2005) who reported that the use of vermicompost on basil (*Ocimum basilicum*) was led to increase in limonene and methyl chavicol content which improve the quality of essential oil. The positive phytochemical response of fennel (*Foeniculum vulgare* Mill.) to different amendments might be due to that; the biosynthesis of secondary metabolites in medicinal and aromatic plants are strongly influenced by environmental factors (Stutte, 2006), as well as, genetic factor. So, the use of chemical N, P and K enhance the growth and essential oil synthesis in fennel plants, also organic manure and bio fertilizers improve the levels of enzymes that are very important in the biosynthesis of essential oil (Sell, 2003) and accelerated metabolism reactions, as well as, stimulated enzymes of important to photosynthesis and metabolism (El-Sherbeny *et al*., 2007). On the other hand, the use of chemical NPK with conventional dose alone or the use of half conventional dose (50% NPK) with cattle manures lead to the increasing of estragol (methyl chavicol) content of fennel essential oil. This result was obtained with the two seasons and with the two varieties.

Table 6: Chemical constituents of fennel essential oil as affecting by fertilization and fennel varieties.

Fertilization	Ingredients of essential oil, %																			
	Oxygenated compounds										Hydrocarbons								Unidentified	
	Anethole		Fenchone		Anisaldehyde		Estragole		Total		α-Pinine		Limonene		Comphene		Total			
	V_1	V_2	V_1	V_2	V_1	V_2	V_1	V_2	V_1	V_2	V_1	V_2	V_1	V_2	V_1	V_2	V_1	V_2	V_1	V_2
First season																				
F_1	58.8	56.8	12.5	12.1	0.8	0.7	3.0	1.6	75.1	71.2	1.3	1.2	2.0	1.1	1.8	1.6	5.1	3.9	19.8	24.9
F_2	61.3	60.6	14.2	13.8	1.3	1.1	4.8	4.0	81.6	79.5	1.6	1.5	3.3	3.2	2.4	2.2	7.3	6.9	11.1	13.6
F_3	62.1	59.0	13.2	13.0	1.1	0.9	3.9	3.0	80.3	75.9	1.5	1.4	2.4	2.6	2.0	1.8	5.9	5.8	13.8	18.3
F_4	64.2	60.7	14.4	13.9	1.2	1.0	4.7	4.2	84.5	79.8	1.7	1.6	3.2	3.1	2.2	2.0	7.1	6.7	8.4	13.5
mean	61.6	59.3	13.6	13.2	1.1	0.9	4.1	3.2	80.4	76.6	1.5	1.4	2.7	2.5	2.1	1.9	6.3	5.8	13.3	17.6
Second season																				
F_1	59.1	57.8	12.7	12.1	0.8	0.8	3.4	2.6	76.0	73.3	1.4	1.3	2.2	1.7	2.2	1.9	5.8	4.9	18.2	21.8
F_2	61.9	60.6	14.5	13.2	1.3	1.2	7.0	5.5	84.7	80.5	1.8	1.7	4.0	3.5	3.2	2.4	9.0	7.6	6.3	11.9
F_3	62.3	59.4	13.4	12.6	1.1	0.9	4.9	4.2	81.7	77.1	1.5	1.5	3.0	2.9	2.7	2.1	7.2	6.5	11.1	16.4
F_4	64.3	61.5	14.9	13.2	1.3	1.1	6.8	6.1	87.3	81.9	1.8	1.7	3.8	3.3	3.0	2.4	8.6	7.4	4.1	10.7
mean	61.9	59.8	13.9	1`2.8	1.1	1.0	5.5	4.6	82.4	78.2	1.6	1.5	3.3	2.8	2.8	2.2	7.7	6.5	9.9	15.3

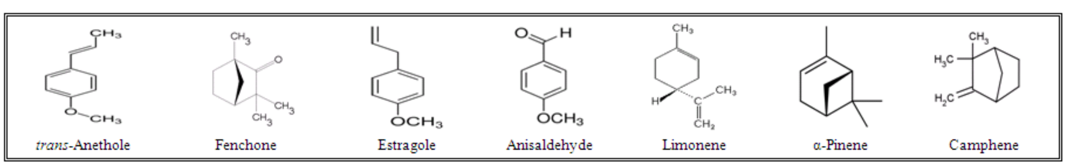

Fig. 1: Oxygenated compounds and Hydrocarbons of fennel essential oil.

This result may be due to the direct role of nitrogen and phosphorus elements in the biosynthesis of methyl chavicol or estragol. Plants synthesize this compound, which smells like licorice, from the amino acid phenylalanine via the shikimate pathway presented by (Yoshida, 1969 and Sangwan *et al*., 2001) as Figure 2. In this pathway, amino acid (phenylalanin), enzymes (as hexokinase, enolase, Chorismate synthase, *O*-methyltransferase, PAL and SAM enzymes) and co-enzymes (as ATP, NADPH) involve in methyl chavicol (estragole) synthesis. These components (amino acid, enzymes and co-enzymes) consist of nitrogen (N) and phosphorus (P) (as well as hydrogen, carbon and oxygen) which obtain in adequate and excess amounts of chemical NPK alone or combined with Bio-Organic amendments. These conditions lead to more synthesis and production of methyl chavicol than pharmacopeia limits.

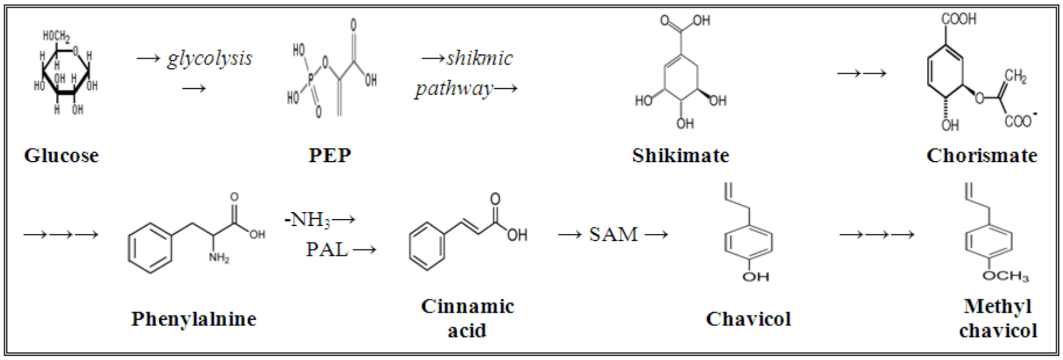

Fig. 2: Schematic of methyl chavicol synthesis modified from the glucose →→ phenylalanine and phenylalanine →→ methyl chavicol mechanisms presented by Yoshida (1969) and Sangwan *et al*. (2001), respectively.

Conclusion:

All fertilization treatments significantly improved plant growth, fruits yield, essential oil yield compared to untreated plants (control). There were no significantly differences between the treatment of F_2 and F_4 in the term of plant growth (number of branches/plant and number of umbels/plant) except plant height. This trend also obtained in case of fruits yield (g/plant). The treatment of F_2 gave the best results of oil yield (ml/plant) followed by F_3. The highest anethole content was obtained F_4 followed by F_3 and F_2, respectively. The lowest estragole (methyl chavicole) content was resulted with control followed by F_3, F_4 and F_2, respectively. *Foeniculum vulgare* var. *vulgare* overcame *Foeniculum vulgare* var. *azoricum* in all parameters in this article. Effective results in fruit and oil yield per plant point of view were obtained after planting *Foeniculum vulgare* var. *vulgare* and application of conventional NPK or 50% NPK + Bio-Organic. According to British Pharmacopeia (BP, 2009) the application of Bio-Organic alone combined with *Foeniculum vulgare* var. *vulgare* achieved good quality and healthy fennel essential oil.

In order to obtain the fennel essential oil with high quality and healthy, it can be recommended that, sawing *Foeniculum vulgare* var. *vulgare* with the application of Bio-Organic (*Azotobacter Croccuccum* + *Bassills megatherium* + 25 m^3/fed cattle manure).

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