JEE Journal of Ecological Engineering

Journal of Ecological Engineering 2022, 23(5), 149–164
https://doi.org/10.12911/22998993/147252
ISSN 2299–8993, License CC-BY 4.0

Received: 2022.02.14
Accepted: 2022.03.14
Published: 2022.04.01

Effect of Organic Manure and Plant Growth Promoting Microbes on Yield, Quality and Essential Oil Constituents of Fennel Bulb (*Foeniculum vulgare* Mill.)

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ABSTRACT
Bulb fennel (*Foeniculum vulgare* Mill.) has gained importance for its high-value bulb production. A field experiment was conducted in a farm in El-Santa, Gharbia, Egypt, to enhance productivity and quality attributes of *F. vulgare* bulbs using different fertilizers: biofertilizer, organic fertilizer (rabbit manure), and mineral fertilizer [nitrogen (N), phosphorus (P), and potassium (K)]. The biofertilizers included nitrogen fixer bacteria (Azos), phosphate solubilizing bacteria (Bm), and potassium solubilizing bacteria (Bc) with/without vesicular arbuscular mycorrhizal (VAM) fungi. Application of NPK at 150% of the RD and rabbit manure at 60 m^3/fed resulted in the highest values of branch number, bulb weight, bulb yield, percentages of total carbohydrates, N, P, and K, as well as features of marketable bulbs including firmness, total soluble solids, titratable acidity, vitamin C, and bulb essential oil. Moreover, the GC/MS analyses of bulb essential oil of the organically and chemically fertilized plants showed the increase of *trans*-anethole, the predominant constituent responsible for bulb's flavor. However, the highest proportion of estragole (9.65%), an undesirable compound, was recorded with 150% of recommended NPK. In comparison, the lowest estragole content (4.09% and 5.64%) was obtained by organic fertilizer (rabbit manure at 60 m^3/fed) and biofertilizer (Azos+Bm+Bc+VAM), respectively. The increase in bulb yield (11.76–11.99 ton/fed) and essential oil content (0.076–0.080%) of bulbs obtained with organic manure (rabbit manure at 60 m^3/fed) was accompanied by a marked decrease in estragole and an increase in the most important constituents, α-pinene, ß-pinene, limonene, *trans*-anethole, and anisaldehyde. Hence, the organic fennel bulb can be produced with an abundant and high-quality crop which consolidates the concept of ecological and organic farming for this important crop.

Keywords: *Foeniculum vulgare*, fennel bulb, organic fertilizer, biofertilizer, anethole, estragole.

INTRODUCTION

Foeniculum vulgare Mill. (bulb fennel), an aromatic herbaceous Mediterranean plant in the Apiaceae family. Bulb fennel is traditionally used as a medicinal herb and vegetable plant due to the swelling of leaf bases that form a bulb-like structure (pseudobulb) (Abdrabbo et al., 2019). The bulb of fennel (also known as fennel head) is an edible organ with rich phytonutrient contents such as phenols, flavonoids, vitamin C, phosphorus, potassium, calcium, and essential oils (Abou El-Magd et al., 2008; Salama et al., 2015; Barzegar et al., 2020). These bulbs are characterised by a rounded and poorly fibrous bulb and enjoyed raw where the licorice-like and aniseed flavor is more pronounced and cooked for spicier and sweeter form (Cucci et al., 2014). Among the biological and pharmacological activities of phytochemicals contained in different parts of *F. vulgare*; oestrogenic, hepatoprotective, antithrombotic, antioxidant, anticancer, chemopreventive, antibacterial, antistress, memory-enhancing, antiaging, acaricidal, and insecticidal properties (Badgujar et al., 2014). Furthermore, bulb fennel has stomachic, galactagogue, diuretic (Barros et al., 2010), and antiviral effects (Ibrahim and Moussa, 2021). Currently, bulb fennel is being cultivated as a promising nontraditional medicinal vegetable crop in Egypt for export and local consumption. The Italian Sicily Island is the largest producer of fennel bulbs in Europe, and the price of organic fennel bulbs is high, from $0.91 to $1.45 per pound (Miles et al., 2019).

The supply of essential nutrients for plants is one of the most important factors that increase plant productivity and its content of medicinal nutritional active substances. Moreover, plant secondary metabolites are highly responsive to soil fertility (Mosa et al., 2022). For instance, the yield and constituents of the bioactive volatile oils rely on several factors including ecotype, environmental condition and agricultural practices (Nada, 2019; Elyemni et al., 2022). Several reports have indicated that nitrogen (N), phosphorus (P), and potassium (K) fertilization enhanced the vegetative productivity and the accumulation of total carbohydrates, soluble sugars, vitamin C, total flavonoids, total phenols, and essential oil in apiaceous plants (Khalid, 2013; Barzegar et al., 2020). Chemical fertilizers are considered as one of the primary sources and quick-release fertilizers for providing the nutritional needs of crops. However, mineral fertilization has various adverse environmental impacts such as soil, water, and air pollution, which increases the cost of environmental protection (Randhir and Lee, 2000; Savci, 2012) and percise evaluation of nutrient uptake and recovery is critical in terms of crop productivity and environmental aspects (Farsad et al., 2011). Liu et al. (2014) showed that increasing N fertilizer in lettuce increased the content of total N in the soil and the accumulation of nitrate in plants. Therefore, rationalizing the application of chemical fertilizers is considered one of the critical issues for sustainable agriculture in reducing the harmful effects of farming on the environment (Wu et al., 2021).

Alternatively, organic or biofertilizers can be used to provide plant nutrients and also increase the long-term sustainability of agricultural ecosystems (Mącik et al., 2020; Shaji et al., 2021). Organic fertilizer is a natural source of essential plant nutrients. Both natural organic fertilizers, such as manure and processed ones like compost and humic acid, have several benefits. These include mitigating the risks of groundwater pollution (Moghaddam et al., 2021) and excessive fertilization, improving soil properties and quality, maintaining soil fertility as they help to compensate for the loss of organic matter in the short and long term, reducing environmental damage without reducing crop productivity and achieving sustainability of agriculture production (Shaji et al., 2021). Biological fertilizer (biofertilizer) is a substance containing microorganisms that when applied to plants, can enhance nutrient uptake and improve soil fertility and crop yield through several mechanisms (Mącik et al., 2020; Shaji et al., 2021). These mechanisms include N fixation, P and K solubilization, production of plant growth-promoting molecules, detoxification of soil contaminants, and protection of plants against pathogens, biotic and abiotic stresses (Mącik et al., 2020; Viji et al., 2021). Research into the widespread use of microbial inoculants (biofertilization) and organic additives (organic fertilization) in soil is one of the dominant areas of applied scientific research for sustainable agriculture development.

Although there were previous studies on the effect of different factors on the growth and production of fennel bulbs (Abou El-Magd et al., 2008; El-Seifi et al., 2015; Eisa, 2016; Abou El-Magd et al., 2017; Abdrabbo et al., 2019; Zaki et al., 2019), few have focused on the effect of different fertilizers on the quality of bulbs for essential oil content (Abou El-Magd et al., 2017; Zaki et al., 2018) and the components of essential oil in bulbs (Atta-Aly, 2001). The volatile oil in fennel is mainly located in fruits, leaves, and bulbs. The volatile components present in the essential oil of *F. vulgare* are more than 80 compounds (Badgujar et al., 2014). However, the major components are *trans*-anethole (40–95%), fenchone (0.60–28%), limonene (0.90–18%), estragole (2–15%), α-pinene (0.83–10%) and *p*-anisaldehyde (0.97–1.63%) (Bernáth and Németh, 2007; Coşge et al., 2008a; 2008b; Shalaby et al., 2011; Hammouda et al., 2014; Açıkgöz and Kara, 2020; Hong et al., 2021; Ibrahim and Moussa, 2021). Among them, terpenoid anethole is the most important component involved in determining the quality of the essential oil (Moradi et al., 2011).

Trans-anethole inhibits carcinogenesis and inflammation through inhibition of TNF-induced cellular responses (Chainy et al., 2000). On the contrary, the higher the percentage of estragole (methyl chavicol) in the oil, the lower the quality of the oil, fruits, and bulbs (El-Serafy and El-Sheshtawy, 2020) due to the genotoxic and carcinogenic properties of estragole (Bristol, 2011; Suzuki et al., 2012). An increase in the percentage of estragole in the oil led to a decline in Egyptian exports of fennel to the European Union (Shalaby et al., 2011; Abu El-Leel and Yousef, 2017). Reducing estragole ratio in the essential oil is so vital as no fennel population is free from it due to its association with the biosynthesis of anethole (Bernáth and Németh, 2007).

There is a need for obtaining edible bulbs with high market quality characteristics, a high proportion of anethole, and low in estragole. Hence, the objective of this study is to compare the productivity and quality of fennel bulbs and the change in the content and components of the essential oil under mineral, organic and bio-fertilization systems.

MATERIALS AND METHODS

Experimental site and soil properties

The experiments were carried out in a private farm (30°44'23.3"N 31°09'02.1"E) in El-Santa, Gharbia, Egypt, during the two successive seasons 2019/2020 and 2020/2021. The soil used was loamy soil. The chemical and physical characteristics of soil are presented in Table 1.

Plant material and experimental design

Bulb fennel seeds of cultivar "Florence" were obtained from Horticulture Research Institute, Agricultural Research Center (ARC), Giza, Egypt. The seeds were sown in nursery beds on November 6th in both seasons. Seedlings were transplanted after 45 days on December 20th to the field. The experiment was done in plots (1.5×2 m); each plot consisted of 3 rows at 50 cm apart and a distance of 50 cm between plants. The layout of the experiment was a complete randomized blocks design during the two seasons. Each treatment contained three replicates (plots), and each replicate consisted of 12 plants.

Chemical, organic and bio-fertilizers

The mineral sources of N, P, and K fertilizers were ammonium sulphate (20.6% N) 100 kg/fed (Feddan is a common land unit in the region and is equal to 0.42 Hectare), calcium superphosphate (15.5% P_2O_5) 50 kg/fed, and potassium sulphate (48% K_2O) 50 kg/fed, respectively, as recommended dose (RD). NPK was applied at two levels of 100 and 150 % of the RD. The fertilizers application for N and K were made three times after transplanting, 15 days interval, while calcium super phosphate was added as one dose during the soil preparation.

Organic fertilization, like rabbit manure, was provided from a private farm in El-Santa, Gharbia, Egypt. The chemical analysis of the rabbit manure is presented in Table 2. Organic manure was applied at two levels of 30 and 60 m^3/fed before planting while preparing the soil.

For bio-fertilizers, nitrogen fixer bacteria (*Azospirillum brasilense*) (Azos) along with phosphate and potassium solubilizing bacteria (*Bacillus megaterium* var. *phosphaticum* and *B. circulans*) (Bm and Bc, respectively) were obtained from the cultural collection of Soil Microbiology Laboratory, Agricultural Microbiology Department, National Research Center, Giza, Egypt. These bacterial cultures were grown as detailed in Abd-el-Malek and Ishac (1968) and Dobereiner et al. (1976). Vesicular arbuscular mycorrhizal (VAM) fungi, which contained three effective strains (*Glomus etunicatum*, *G. intraradices*, and *G. fasciculatum*) was used for soil inoculation after transplanting containing about 200 VAM spores/plant. After transplanting, each

Table 1. Physical and chemical properties of the experimental soil

Physical properties									
Clay %		Silt %		Fine sand %		Coarse sand %			
58.50		16.45		9.10		13.84			
Chemical properties									
K	Na	Mg	Ca	SO_4	Cl	HCO_3	SP	pH	E.C.
5.40	1.50	2.20	4.30	3.20	4.55	3.30	20.00	8.10	6.25

Table 2. Chemical analysis of rabbit manure used in the experiments

pH	E.C. mM	N %	P %	K %	C/N ratio	Fe ppm	Mn ppm	Cu ppm	Zn ppm
5.45	6.15	9.1	2.21	1.19	8.3/1	130	151	85	95

plant was inoculated with 3 ml of a mixture of bacterial suspension of the above three strains of bacteria alone (Azos+Bm+Bc) or with fungi strains (Azos+Bm+Bc+VAM).

The soil moisture was kept at 50–75 % of field capacity. In addition, the recommended practices for controlling pests and diseases were followed.

Measurements

At 90 days after transplanting, the maturity stage and suitable harvesting age (Haggag et al., 2019), the following physical and chemical characteristics were recorded. The recorded characters included the number of branches per plant, fresh and dry weights of the entire plant, including the aerial parts, bulbs and roots (g/plant), fresh and dry weights of the bulb (g) (roots and damaged outer leaves were excised, and vegetative parts at approximately 5 cm above bulb were removed). Finally, the bulb yield was calculated and expressed in ton/fed.

Chlorophyll-a, -b and total chlorophyll (mg/g FW) were determined spectrophotometrically in the fresh leaves according to the methods of Dere et al. (1998). Total carbohydrates (%) in dry leaves were determined using the phenol-sulphuric acid method, according to Dubois et al. (1956). Nitrogen (%) in dry leaves was determined according to the method described in AOAC (1995). Potassium and phosphorus (%) were also determined in dry leaves, according to Cottenie et al. (1982). Total Soluble Solids (TSS) (%) in bulbs were measured using a refractometer (Carl Zeiss, Jena, Germany) calibrated with distilled water. The firmness of bulbs was measured in kg/cm^2 by Magness, Ballauf pressure tester (Moto Meter, Germany). Titratable acidity was determined in bulbs as mg/100g FW using a standard solution of sodium hydroxide (0.01 N) and phenolphthalein indicator as described in AOAC (2000). Ascorbic acid (vitamin C) content in fresh bulbs (mg/100g FW) was assayed by titration of bulb juices with the 2,6-dichlorophenol indophenol dye method (AOAC, 2000).

Essential oil (%) in bulbs

The essential oil was extracted from bulbs by hydro-distillation with Clevenger-type apparatus. Air-dried bulb (100 g DW) was divided and placed in 1000 ml of distilled water in a 2000 ml round bottom flask at boiling temperature for three hours until no further oil can be extracted. The essential oil was chemically dried through anhydrous sodium sulfate and kept at 4–5°C until analysis. The essential oil content was expressed as a relative percentage (v/w).

Essential oil analysis

Gas chromatography condition

The Gas chromatography (GC) analysis was performed with a Shimadzu GC-9 gas chromatograph coupled with Flame Ionization Detector (FID) (Shimadzu Corporation, Kyoto, Japan), equipped with a dimethylsiloxane, 5% phenyl (DB-5)-fused silica column (60 m x 0.25 mm x 0.25 μm) (J & W Scientific Corporation, Folsom, CA, USA). Oven temperature was 50°C for 5 min and raised to 240°C at a rate of 3°C/min. Oil samples were diluted (1/100 v/v in n-pentane) and 1μl was injected. The temperature of the injector and flame ionization detector was 250°C and 265°C, respectively. The carrier gas was helium at a linear velocity of 32 cm/s. The percentages of oil constituents were calculated by the area normalization without considering response factors.

Gas chromatography-mass spectrometry (GC-MS) condition

The analysis was performed in a Varian 3400 GC-MS equipped with a DB-5-fused silica column (60 m x 0.25 mm x 0.25 μm). Oven temperature was held at 50–240°C at a rate of 4°C/min. Temperature of transfer line was 260°C. Linear velocity of the carrier gas (helium) was 31.5 cm/s, split ratio 1:60, ionization energy 70 eV, scan time 1 s, and at a mass range of 40–300 amu.

Identification of essential oil components

Identification of volatile components in bulb essential oil was carried out by comparing the mass spectra of the components with those in the mass spectral library or with authentic compounds. The identification was confirmed by comparing the retention indices (RIs) of components with RIs of authentic compounds or with published data (Adams, 1995). Identification of essential oil constituents was done by comparing the retention times (RTs) of constituents with standard substances and matching of mass spectral data with MS libraries (NIST/NBS and Wiley 275.1). Additionally, the authentic sample available for the identified compounds in GC or GC-MS was co-injected to confirm the assignment. Quantitative analysis was conducted by external standard method by calibration curves obtained by running GC for representative compounds. RIs were also detected by injecting a homologous series of n-alkanes for all constituents, C8–C22, into chromatographic column, then compared with literature values (Adams, 1995) to confirm identification. Computer matching was performed with commercially available data (Wiley GC-MS Library, Mass Finder 3 Library) (Adams, 1995).

The statistical analysis

All experiments were conducted in a randomized block complete design. The statistical analysis of data was subjected to Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) (Duncan, 1955) at $p<0.05$ using COSTAT package ver. 6.4 (CoHort software Monterey, USA) according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Number of branches per plant

The results of the growing seasons, 2019/2020 and 2020/2021, indicated that different types and concentrations of fertilizers had a significant effect on the branching of fennel plants (Table 3).

In both seasons, control plants recorded the lowest number of branches. The organic and inorganic nutrients positively affected the branching, superior to the biofertilizer. Youssef et al. (2020) also recorded a lower number of branches in the caraway plants due to the individual application of the biofertilizer (*Azotobacter chroococcum*, *B. megaterium* var. *phosphaticum*, and *Saccharomyces cerevisiae*). The highest significant number of branches per plant (19.69 and 21.03 branches) was counted for plants fertilized with NPK at 150% of RD followed by rabbit manure at 60 m^3/fed, which recorded 17.80 and 19.10 branch/plant in the first and second season, respectively. In the study by Abd El-Salam (1999), plant height and branches number of sweet fennel were enhanced by increasing rates of phosphorus fertilization. In the current study, application of biofertilizers (Azos+Bm+Bc and Azos+Bm+Bc+VAM) also improved fennel branching when compared with control. Other reports have indicated that phosphate solubilizing *B. megaterium* has a slightly stimulating effect on the growth and productivity of sweet fennel when compared to the biofertilizer phosphorine and mineral phosphorus (Zaki et al., 2019). El-Serafy and El-Sheshtawy (2020) also noticed high branching in *F. vulgare* spp. *vulgare* with the application of nitrogen-fixing bacteria (*B. polymyxa, A. chroococcum,* and *A. lipoferum*). The promoting effect of biological, organic, and mineral fertilization on fennel branching was also reported by Azzaz et al. (2009).

Fresh and dry weights of total plant

The results presented in Table 3 show that different fertilizers significantly affected fresh and dry weights of total plants compared to control plants. In general, statistically significant differences were recorded between all treatments in both seasons. In the first season, the highest

Table 3. Branches number, fresh and dry weights of total plants, fresh and dry weights of bulbs and bulb yield in fennel plants as affected by fertilization during the growing seasons (2019/2020 and 2020/2021)

Treatments	Branches No/plant		Total plant FW (g/plant)		Total plant DW (g/plant)		Bulb FW (g/bulb)		Bulb DW (g/bulb)		Bulb yield (ton/fed)	
	First season	Second season	First season	Second season	First season	Second season	First season	Second season	First season	Second season	First season	Second season
Control	9.44 f	10.38 e	308.75 g	401.06 d	93.20 f	91.35 e	197.50 f	211.13 e	38.32 e	44.19 d	3.22 f	3.44 e
Azos+Bm+Bc	11.54 e	12.33 d	478.17 f	500.83 d	98.68 e	100.81 de	244.08 e	259.16 d	55.14 d	50.05 cd	3.98 e	4.22 d
Azos+Bm+Bc+VAM	12.57 d	12.99 d	702.28 e	738.33 c	103.72 d	109.69 d	303.31 d	288.87 d	62.91 c	55.86 bc	4.94 d	4.71 d
Rabbit manure at 30 m^3/fed	13.49 c	12.63 d	840.50 d	913.12 b	117.47 c	112.84 cd	532.48 c	494.47 c	69.91 b	61.04 b	8.68 c	8.06 c
Rabbit manure at 60 m^3/fed	17.80 b	19.10 b	1187.90 b	1227.18 a	130.51 a	140.41 a	721.45 a	735.96 a	81.64 a	77.95 a	11.76 a	11.99 a
NPK at 100% of recommended dose	14.14 c	15.31 c	1019.71 c	1115.24 a	123.50 b	129.51 ab	690.41 b	682.80 b	79.24 a	81.69 a	11.25 b	11.13 b
NPK at 150% of recommended dose	19.69 a	21.03 a	1278.27 a	1230.70 a	133.58 a	125.80 bc	724.45 a	691.06 ab	83.18 a	85.38 a	11.81 a	11.26 ab

Mean values with different letters in the column are statistically different according to DMRT ($p<0.05$)

significant fresh weight of total plant (1278.27 g) was noticed for plants that received 150% of recommended NPK followed by rabbit manure at 60 m^3/fed (1187.90 g) and 100% of recommended NPK (1019.71 g); however, in the second season, statistical equivalence was recorded between these three treatments. Application of NPK at 150% of the RD and rabbit manure at 60 m^3/fed recorded the highest significant dry weights of total plant in the first season (133.58 and 130.51 g/plant, respectively). Also, Abd El-Salam (1999) found that dry weights of various organs of the fennel plant were increased by increasing phosphorus doses. However, organic fertilizer (rabbit manure at 60 m^3/fed) was more effective than mineral nutrients (NPK) in the second season. A similar observation was also recorded by Azzaz et al. (2009) in fennel plants.

The activating effect of the organic fertilizer on vegetative growth, branching and total plant weight may be an indirect result of improving the soil structure by increasing the water holding capacity of the soil resulting in good aeration and drainage that encourage better root growth and absorption of nutrients (Abou El-Magd et al., 2008). Potassium plays essential roles in N metabolism, photosynthesis, carbohydrate formation, stomata conduction and increasing the transport of photoassimilates to different parts of the plant and the absorption of other nutrients, as well as its important role in the activation of many enzymes in the plant cell (Pandey and Mahiwal, 2020).

Application of biofertilizers, especially Azos+Bm+Bc+VAM, also increased the total plant FW and DW compared to the control but was lower when compared with the mineral and organic fertilizers. Similar findings were also reported by Toaima et al. (2014) on *Calendula officinalis* and Youssef et al. (2020) on *Carum carvi*. In contrast, Zaki et al. (2019) reported a positive effect of *B. megaterium* or phosphorine on total fresh and dry weights of sweet fennel plant (leaves + bulbs) cv. De Florence.

Fresh and dry weights of bulb

From the results displayed in Table 3, it is clear that fresh and dry biomass weights of fennel bulbs responded significantly to the addition of fertilizers, especially the forms of mineral NPK and organic rabbit manure. In both growing seasons, higher dosages of mineral NPK (150% of RD) and organic rabbit manure (60 m^3/fed) recorded the highest biomass FW of bulbs, which reached 724.45 and 721.45 g/bulb in the first season, and 691.06 and 735.96 g/bulb in the second season, respectively, compared to non-fertilized plants (197.50 and 211.13 g/bulb). This increase represents 3.27 to 3.66-fold higher than control plants. In line with our findings, applying N and K increased the fresh weights of fennel bulbs (Barzegar et al., 2020). Additionally, Abou El-Magd et al. (2008) found that applying organic manure (poultry manure) increased the fresh and dry weights of sweet fennel cv. Dolce from 160.88 and 11.71 g/plant for control to 265.13 and 30.60 g/plant, respectively, after 120 days of transplanting. Therefore, bulb weights harvested here are considered to be higher than those obtained in previous studies by Abou El-Magd et al. (2008), Błażewicz-Woźniak (2010a), Cucci et al. (2014), Salama et al. (2015), Eisa (2016), Slatnar et al. (2017), Zaki et al. (2018), Abdrabbo et al. (2019), Miles et al. (2019), Zaki et al. (2019), Abd El-Rheem et al. (2019), and Marian et al. (2020).

About 1.76 to 2.17-fold increase in bulb dry weight than control was achieved for the treatments of mineral NPK and organic rabbit manure. Herein, rabbit manure at 60 m^3/fed, NPK at 100% or 150% of the RD recorded the highest significant weight of bulb dry matter without significant differences. The applied biofertilizers also enhanced the fresh weight of bulbs (1.24- 1.37-fold increase) and dry weight (1.25-1.64-fold gain) compared to control ones in both seasons.

Bulb yield

Results regarding the bulb yield were similar to results for the bulb fresh weight. The yield of fennel bulbs was highly enhanced due to applying bio, organic and inorganic fertilizers (Table 3). In general, the bulb yield of fertilized plants was about 1.23 – 3.67-fold higher than non-fertilized plants during the two seasons. Application of biofertilizers (Azos+Bm+Bc or Azos+Bm+Bc+VAM) significantly increased the yield of bulbs to 3.98 and 4.94 ton/fed, respectively, in the first season compared to control treatment (3.22 ton/fed). In addition, organic manure and mineral NPK exhibited a significant increase in the bulb's yield. The highest significant yield of bulbs was achieved in plants that received NPK at 150% of the RD (11.81 and 11.26 ton/fed) and rabbit manure at 60 m^3/fed (11.76 and 11.99 ton/fed) in the first and second season, respectively, followed by 100% of NPK RD. In this regard, the bulb yield was increased by 3.65 and 3.67–fold in the first season, and by 3.49 and 3.27-fold in the second season by the application of organic and mineral fertilizers, respectively. The total green yield in organic fennel (received compost and/or vermicompost) ranged 9.30 – 11.03 ton/fed (Abd El-Rheem et al., 2019). In this regard, crop yield is the function of photosynthesis activity, plant growth, phytochemical content and accumulation of biomass fresh and dry matter (Salama et al., 2015).

Many reports have indicated that adding N, P and K to fennel plants is important to enhance the plant growth and development, seed and bulb yield (Rai et al., 2002; EL-Sayed et al., 2009; Ehsanipour et al., 2012; Zaki et al., 2019). It is recognized that plant productivity fluctuates according to many factors, including the cultivated cultivar, weather conditions, soil fertility and irrigation water quality. On sweet fennel cv. Boelli RZ F_1, the bulb fresh yield reached 18.84 ton/ha with the application of 150 kg/ha N combined with 100 kg/ha K (Barzegar et al., 2020). Zaki et al. (2019) reported that high quality and total green yield (7.50 ton/fed) of sweet fennel cv. De Florence were gained with the application of mineral P at 75 kg P_2O_5/fed, and the total green yield was increased to 9.49 ton/fed when treated with a combination of this mineral P and the biofertilizer phosphorine. Moreover, a combination of 50% NPK and bio-organic fertilizer enhanced the productivity of fennel bulb cultivar Dolce (8.85 ton/fed) compared to 100% NPK (5.90 ton/fed) (Salama et al., 2015). Although N is an essential element for the growth and development of plants, environmental pollution causes the accumulation of high concentrations of nitrates in the edible parts of vegetable and medicinal plants, primarily if excessive N fertilizer is used (Liu et al., 2014). In this concern, Atta-Aly (2001) achieved 28.3 ton/ha of fennel swollen base (bulb) when var. *dolce* was treated with 50 m^3/ha of cattle manure only. This yield was increased insignificantly to 29.8 ton/ha when ammonium sulfate (20% N) was combined with cattle manure; however, the nitrate content in bulbs was increased.

Chlorophyll

Accurate quantification of leaf photopigments is an essential factor in monitoring fertilizer use and managing the overall health of plants, as yield levels are directly correlated with plant status and growth conditions. Chlorophyll is one of the most important photosynthetic pigments in plants that control photosynthetic potential by capturing energy from sunlight (Shah et al., 2017). In the current study, fennel plants that received NPK and rabbit manure exhibited a significantly higher content of chlorophyll-a, chlorophyll-b, and total chlorophyll in both seasons (Figure 1 A, B and C).

A higher dosage of organic manure (60 m^3/fed of rabbit manure) and NPK (150% of the RD) resulted in the highest statistical values of chlorophyll-a, chlorophyll-b and total chlorophyll. However, NPK fertilizer showed higher efficiency than organic manure. Other reports have shown a positive correlation between the doses of each N, P, and K application and the contents of chlorophyll and carotenoids in basil (Politycka and Golcz, 2004) and fennel (Barzegar et al., 2020). The chlorophyll-a, -b and total chlorophyll was increased in the first season from 2.54, 0.62 and 3.16 mg/g FW for control treatment to 3.52, 1.00 and 4.51 mg/g FW for 60 m^3/fed rabbit manure, and to 3.59, 1.03 and 4.62 mg/g FW when plants received 150% of NPK RD, respectively. A slight increase in chlorophyll-a and total chlorophyll were recorded for biofertilizer application compared to control, while no significant differences were observed in the case of chlorophyll-b. Nada (2014) also recorded an increase in the content of chlorophyll-a, -b, and carotenoids in biofertilized calendula plants (Azos+Bm+Bc+VAM or Azos+VAM) when compared with control.

Total carbohydrates

Total carbohydrates (%) in fennel leaves responded significantly to fertilization (Table 4). The highest total carbohydrates percentage was obtained by the treatments of NPK (150% of RD) and rabbit manure (60 m^3/fed rabbit manure) in both seasons, as it recorded 61.53 and 59.29% in the first season, and 70.31 and 68.48% in the second season, respectively, with non-significant differences between them. These values represent a 1.74–1.82-fold increase compared to control. Marschner (1995) mentioned that K plays a major role in increasing the

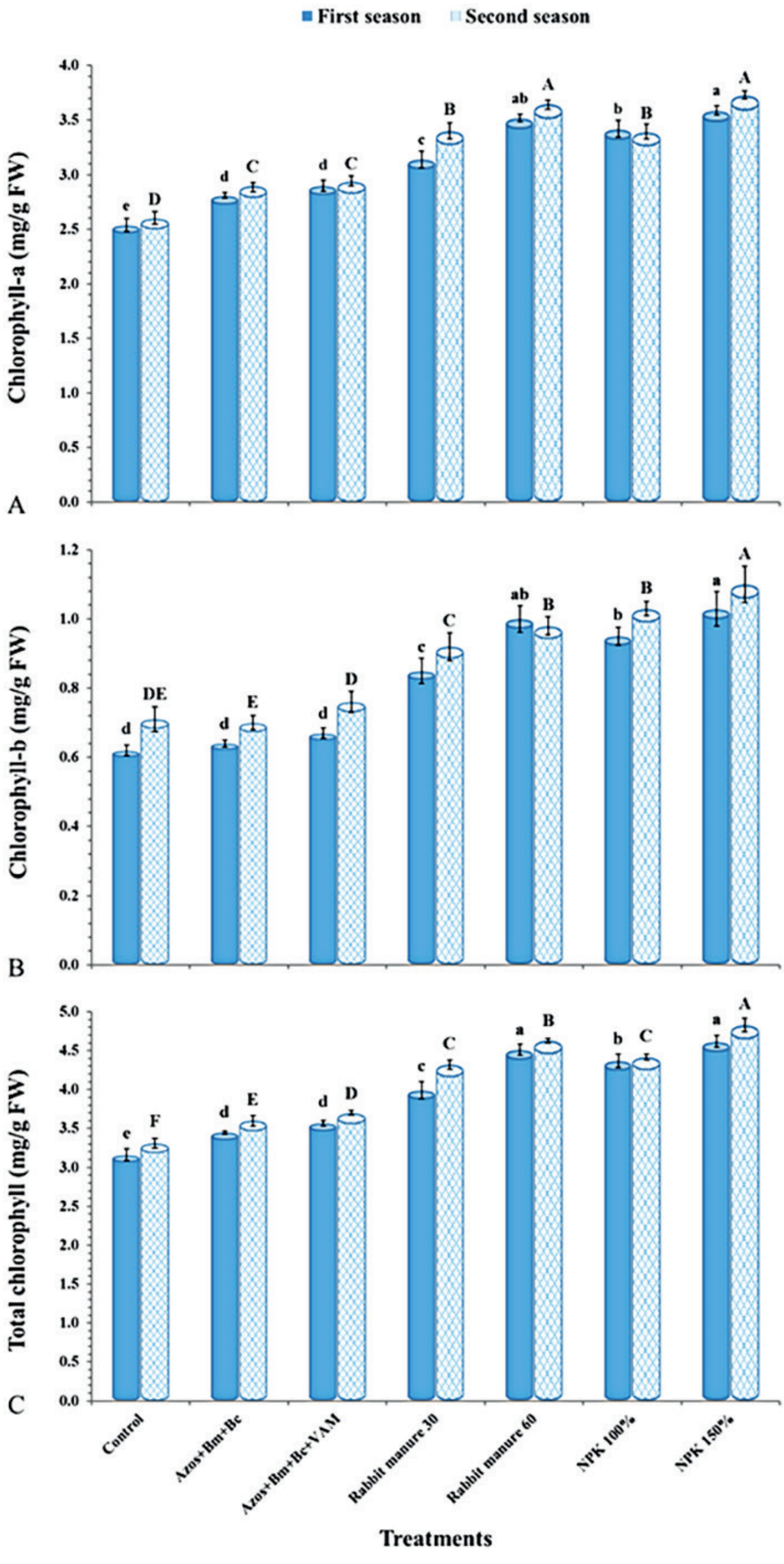

Figure 1. Effect of different fertilizers on chlorophyll-a (A), chlorophyll-b (B) and total chlorophyll (C) content in sweet fennel during seasons of 2019/2020 and 2020/2021. Bars represent ±SD (n=3). Columns with different letters are statistically different according to DMRT (p<0.05)

chlorophyll content in plants, thus increasing photosynthesis and accumulation of carbohydrates. Moreover, Deeken et al. (2002) found a positive correlation between sugar and K in *Arabidopsis* leaves. Using the biofertilizer Azos+Bm+Bc+VAM was more effective than Azos+Bm+Bc, as the total carbohydrates were 1.46 and 1.42-fold higher than control plants when applying Azos+Bm+Bc+VAM. Nada (2014) confirmed this, as a combination of VAM with Azos+Bm+Bc was superior to Azos+Bm+Bc and Azos+VAM in accumulating total carbohydrates in *Calendula officinalis*. Increasing the percentage of carbohydrates in the aerial parts of fennel plant had a parallel effect on the increase in bulb weight and yield as a final outcome.

Nitrogen, potassium and phosphorus

Data of Table 4 indicate that application of NPK at 150% of RD resulted in the highest significant values of N, P, and K in fennel leaves in the second season. However, in the first season, the organic fertilizer (rabbit manure at 60 m^3/fed) recorded percentages of N, P and K statistically similar to those recorded for chemical fertilizers. In the first season, the highest content of N, P and K (2.95, 0.63 and 3.63 %, respectively) was measured in plants enriched with 150% of recommended NPK followed by rabbit manure at 60 m^3/fed (2.87, 0.61 and 3.60 %, respectively). Zaki et al. (2019) found that the highest N, P and K contents in fennel leaves and bulbs were achieved in plants treated with mineral P at 75 kg P_2O_5/fed. Also, Eisa (2016) indicated that high mineral (NPK at 50 and 100% of RD) and organic fertilizers (farmyard and chicken manure) gave the highest content of N, P and K in fennel herb. Additionally, Abd El-Rheem *et al*. (2019) found a significant increase in nutrients content in shoot and bulb of organic fennel (compost and vermicompost). The present study combines VAM (vesicular arbuscular mycorrhizal fungi) with Azos+Bm+Bc (*Azospirillum brasilense*, *Bacillus megaterium* var. *phosphaticum*, and *B. circulans*), and this combined treatment was superior to Azos+Bm+Bc alone, and both treatments were more effective than the control treatment. The accumulation of N, P, and K in plants as a result of applying mineral, organic and bio-fertilizations was also proved in chamomile (Nada, 2019), collard (Helaly et al., 2020), caraway (Youssef et al., 2020), garlic (Jiku et al., 2020), moringa (Viji et al., 2021), coriander (Machado et al., 2022), and kale (Helaly et al., in press).

Firmness of bulbs

Firmness is an important characteristic of the fennel bulb, as it helps to store the bulbs for an extended shelf life and facilitates the harvest and handling of the bulbs until they reach the consumer without causing damage (Haggag et al., 2019). Bulb firmness can be affected by many factors and agricultural practices such as cultivar, seeding date, maturity stage, and balanced nutrition (Haggag et al., 2019; Khokhar, 2019; Miles et al., 2019). In the current experiment, bulbs firmness reached the highest value (7.19 and 7.38 kg/cm^2) by the application of 150% of recommended NPK, followed by100% of recommended NPK (6.67 and 6.51 kg/cm^2) and 60 m^3/fed of rabbit manure (6.16 and 6.72 kg/cm^2), in the first and second seasons, respectively. Close to our results, Haggag et al. (2019) found that the bulbs of sweet fennel cv. Florence harvested 130 days after sowing recorded around

Table 4. Percentages of total carbohydrates, nitrogen, phosphorus and potassium in leaves of fennel plants as affected by fertilization during the growing seasons (2019/2020 and 2020/2021)

Treatments	Total carbohydrates (%)		Nitrogen (%)		Phosphorus (%)		Potassium (%)	
	First season	Second season	First season	Second season	First season	Second season	First season	Second season
Control	32.18 e	40.39 d	1.92 f	2.03 f	0.24 d	0.30 e	2.22 f	2.15 g
Azos+Bm+Bc	43.21 d	41.01 d	2.08 e	2.13 e	0.33 c	0.29 e	2.42 e	2.38 f
Azos+Bm+Bc+VAM	47.04 c	57.49 c	2.21 d	2.25 d	0.37 c	0.35 de	2.62 d	2.57 e
Rabbit manure at 30 m^3/fed	49.31 c	60.56 bc	2.34 c	2.29 d	0.43 b	0.40 cd	2.82 c	2.91 d
Rabbit manure at 60 m^3/fed	59.29 a	68.48 ab	2.87 a	2.95 b	0.61 a	0.52 b	3.60 a	3.49 b
NPK at 100% of recommended dose	53.36 b	61.78 bc	2.47 b	2.53 c	0.47 b	0.41 c	3.09 b	3.19 c
NPK at 150% of recommended dose	61.53 a	70.31 a	2.95 a	3.06 a	0.63 a	0.70 a	3.63 a	3.70 a

Mean values with different letters in the column are statistically different according to DMRT (p<0.05)

6 kg/cm² firmness. These data suggest that the nutrient available to the plant due to the application of chemical and organic fertilization was greater than that available as a result of applying biofertilization because the bulbs firmness was lower when using biofertilizers.

Total soluble solids (TSS)

The TSS content increased significantly in all fennel bulbs that were fertilized compared to the control, achieving the highest percentage for organic and mineral fertilization (Table 5). NPK at 150% of the RD represented the most effective treatment in increasing bulbs TSS content (7.04%), followed by treatment NPK at 100% of RD (6.70%) and rabbit manure at 60 m^3/fed (6.22%) in the first season, representing 1.64, 1.56 and 1.45-fold higher than control, respectively. In the second season, the TSS contents were significantly equal in the bulbs produced by plants fertilized with any of these three fertilizers. Another study on sweet fennel bulbs, the highest content of TSS (6.60–6.80%) was recorded after 130 days of sowing, marked as the most appropriate age for harvesting these bulbs (Haggag et al., 2019). However, Zaki et al. (2018) reported a high TSS content (around 10.74 %) in organic fennel bulb var. Zefa fino. This may be related to different species, the variety grown, type of fertilizer, agricultural practices as well as climatic conditions.

These results are in harmony with those obtained by Barzegar et al. (2020) on fennel bulb, Khalid (2013) on fennel, anise, and coriander, and El-Nemr et al. (2012) on tomato plants that TSS, soluble sugars, and total carbohydrates were increased by increasing N and K dosages. The increase in TSS may be correlated with the increased photosynthesis efficiency due to enhanced chlorophyll biosynthesis (Barzegar et al., 2020).

Titratable acidity

Titratable acidity deals with measuring the concentration of total acid in fennel bulbs and is an indicator of bulb maturity and quality standards (Escalona et al., 2004; Amodio et al., 2017). Non-fertilized fennel plants produced bulbs with low titratable acidity (70.88 and 69.64 mg/100 g FW) in the first and second seasons, respectively, followed by the biofertilizer Azos+Bm+Bc and Azos+Bm+Bc+VAM (Table 5). The titratable acidity was increased significantly by applying organic and mineral fertilizers. For instance, in the first season, the values of titratable acidity were 1.16, 1.25, 1.20 and 1.43-fold higher than the control for plants that received rabbit manure at 30 and 60 m^3/fed and NPK at 100% and 150% of RD, respectively. Using the poultry manure to fertilize fennel bulbs, the total acidity content values recorded around 0.32 g/100 g FW (Zaki *et al*., 2018).

Ascorbic acid (vitamin C)

Obtaining fennel bulbs with high content of ascorbic acid (vitamin C) is an important issue. Biofertilizers (Azos+Bm+Bc or Azos+Bm+Bc+VAM) slightly enhanced the vitamin C content. A statistical increase in ascorbic acid was noticed due to applying organic and inorganic fertilization (Table 5). The highest significant content of ascorbic acid was determined in bulbs produced from plants receiving 60 m^3/fed of rabbit manure and 150% of

Table 5. Features of marketable bulbs of fennel plants as affected by fertilization during the growing seasons (2019/2020 and 2020/2021)

Treatments	Firmness (kg/cm^2)		TSS (%)		Titratable acidity (mg/100 g FW)		Ascorbic acid (mg/100 g FW)		Essential oil in bulbs (%)	
	First season	Second season	First season	Second season	First season	Second season	First season	Second season	First season	Second season
Control	4.25 f	4.58 e	4.30 g	4.22 d	70.88 f	69.64 e	22.80 e	28.41 c	0.034 e	0.040 c
Azos+Bm+Bc	5.08 e	4.99 de	4.53 f	4.83 c	74.24 e	79.36 d	25.84 de	30.95 c	0.044 d	0.042 c
Azos+Bm+Bc+VAM	5.52 d	5.47 cd	5.50 e	5.20 c	78.75 d	87.55 cd	28.82 cd	30.48 c	0.048 d	0.043 c
Rabbit manure at 30 m^3/fed	5.80 cd	5.66 c	5.98 d	5.70 b	82.53 c	88.52 c	31.03 cd	36.40 b	0.062 c	0.056 b
Rabbit manure at 60 m^3/fed	6.16 c	6.72 b	6.22 c	6.85 a	88.94 b	101.22 ab	36.99 ab	42.10 a	0.080 b	0.076 a
NPK at 100% of recommended dose	6.67 b	6.51 b	6.70 b	6.63 a	85.04 c	93.14 bc	32.81 bc	36.71 b	0.082 ab	0.079 a
NPK at 150% of recommended dose	7.19 a	7.38 a	7.04 a	6.90 a	101.64 a	104.61 a	42.14 a	40.53 ab	0.087 a	0.081 a

Mean values with different letters in the column are statistically different according to DMRT (p<0.05) the RD of NPK, without significant differences between the two treatments in both seasons. The vitamin C was raised in bulbs from 22.80 mg/100 g FW (control) to 36.99 and 42.14 mg/100 g FW (1.62 and 1.85-fold) by applying 60 m^3/fed rabbit manure and 150% of recommended NPK, respectively, in the first season. However, excessive application of manufactured NPK fertilizers will increase production cost, reduce bulbs export, pollute the environment and affect soil fertility (Sherif and El-Naggar, 2005; Abdrabbo et al., 2019). Values of vitamin C content obtained here were also observed in the study conducted by Badawi et al. (2005), where vitamin C content in bulbs ranged from 21.83 to 48.04 mg/100 g FW when fennel plants were fertilized by organic additives from different sources. Moreover, Salama et al. (2015) concluded that organic and bio-organic fertilization enhanced vitamin C content (32.88–34.00 mg/100 g FW), antioxidant activity, and accumulation of total phenolics and total flavonoids in fennel bulbs. In the study conducted by Błażewicz-Woźniak (2010b), the bulbs of cultivars Rudy F_1 and Zefa Fino contained about 8.71 mg/100 g FW of vitamin C.

Essential oil content in bulbs

The content of volatile oil and its components is one of the quality standards for fennel bulbs, due to the distinctive aromatic smell and the high medicinal value of the oil and its components. Data presented in Table 5 indicate that essential oil content in fennel bulbs significantly responded to organic and mineral fertilizers. The bulbs produced by plants fed with NPK at 150% and 100% of RD contained the maximum significant percentage of the essential oil, followed by rabbit manure at 60 m^3/fed in the first season (0.087%, 0.082%, and 0.080%, representing 2.56, 2.41, and 2.35-fold increase over the control). However, excessive application of mineral/inorganic fertilizers causes problems for human health and surrounded environment (Liu et al., 2014). The essential oil content of the organically fertilized fennel bulbs in the Zaki *et al*. (2018) study (0.05–0.08%) is close to the isolated oil in the current study. Regarding the effect of biofertilization, the essential oil content improved significantly only in the first season. Non-significant stimulation of biofertilizer on volatile oil content was also observed in Youssef et al. (2020)'s study on *Carum carvi* plant when they used biofertilizer alone.

The increase in the essential oil content in bulbs due to organic manure amounted to 2.35 and 1.90-fold higher than control in the first and second season, respectively. Atta-Aly (2001) observed that essential oil content in the fennel swollen base (bulb) var. *dolce* reached 1.24 ml/kg upon fertilization with 50 m^3/ha of cattle manure, and the addition of mineral N did not significantly affect the oil content. The positive effects of organic fertilizers on plant growth, yield quality and productivity are related to many factors, including the improvement of the plant nutritional status (Pavla and Pokluda, 2008).

On a study for the determination of the maturity stage of bulbs in sweet fennel (*F. vulgare* var. *dulce*) cv. Florence, Haggag et al. (2019) concluded that the bulbs harvested 130 days after sowing, as the most suitable age for harvesting, were characterized in both seasons of the experiment by the content of 0.0137 to 0.0157% of the essential oil; however, the essential oil contents during the deferent development stages of bulbs ranged between 0.012 and 0.026%. Abou El-Magd et al. (2017) indicated that the essential oil content in fennel bulbs was 0.05–0.06, 0.04–0.05, and 0.06–0.07% (V/W) in Dulce, Zefa fino and Selma cultivars, respectively.

Essential oil constituents of bulbs

In addition to the apparent quality and weight of the fennel bulbs, the market value of the bulbs is also supported by increasing their health benefits and reducing potentially harmful substances. The unique flavor of the fennel bulb is due to the anethole component in the essential oil (Badgujar et al., 2014). *trans*-anethole – the most common constituent in fennel – possesses potent aldose reductase and antioxidant properties (Dongare et al., 2012). The GC-MS analysis of essential oil isolated from the fennel bulb revealed the presence of 20 compounds (Table 6). *trans*-anethole was detected as the main component. The highest percentage of *trans*-anethole (42.25 and 41.25%) was recorded by applying NPK at 150% of the RD and rabbit manure at 60 m^3/fed, respectively. The use of bio-fertilizer in the form of Azos+Bm+Bc+VAM achieved a percentage of *trans*-anethole close to that recorded for the control (39.46% and 39.57%, respectively). Anisaldehyde compound was higher with all types of fertilizers than in control plants. Atta-Aly (2001) found that *trans*-anethole content in fennel bulbs var. *dolce* increased when manure was combined with ammonium sulfate or ammonium nitrate. On the contrary, any conjunction of cattle manure with ammonium sulfate, ammonium nitrate, or urea resulted in a statistical decrease in *para*-anisaldehyde in var. *dolce* and limonene in var. *azoricum*.

The limonene content was highest in the bulbs of the biologically fertilized plants, followed by the organically and then chemically fertilized plants, while the lowest content of limonene was in the unfertilized plants (3.05%, 2.97%, 2.39% and 2.21%, respectively). α-pinene and β-pinene were higher in fertilized plants than control ones, and higher in organic and biofertilizer, respectively, than NPK fertilizer. The highest proportion of α-pinene (11.05%) was observed in the treatment of rabbit manure at 60 m^3/fed, while β-pinene reached the highest percent with the biofertilizer Azos+Bm+Bc+VAM (0.38%).

Although NPK markedly increased the bulb yield and *trans*-anethole content, previous reports have indicated that nitrate content in fennel bulbs received synthetic N fertilizers (ammonium sulfate, ammonium nitrate, or urea) was higher than that of organic additives only (Atta-Aly, 2001). High content of nitrate in the diet is considered dangerous to human health (Ali et al., 2021). On the other hand, increasing the proportion of estragole (methyl chavicol) in the bulb oil, reduces the quality of bulbs required for export and marketing (El-Serafy and El-Sheshtawy, 2020), as estragole is genotoxic and carcinogenic (Bristol, 2011; Suzuki et al., 2012). In the current study, the highest percentage of estragole (9.65%) was recorded in the treatments of NPK at 150% of RD followed by control bulbs (9.84%). In comparison, the lowest content of estragole (4.09% and 5.64%) was obtained by the organic fertilizer (rabbit manure at 60 m^3/fed) and the biofertilizer (Azos+Bm+Bc+VAM), respectively. A higher dosage of N increased estragole content and decreased linalool levels in basil oil (Nurzyńska-Wierdak and Borowski, 2011). This may be related to the stimulating effect of N on the increase of amino acids in plant, including phenylalanine, the main amino acid in the biosynthesis of estragole (Yoshida, 1969; Bernáth and Németh, 2007; El-Serafy and El-Sheshtawy, 2020). Lewinsohn *et al*. (2000) reported that the difference in methyl chavicol level in response to various treatments was related to the activity of O-methyltransferase that catalyzes the biosynthesis of methyl chavicol. Similarly, Atta-Aly (2001) obtained fennel bulbs with low content of methyl chavicol (estragole) when *F. vulgare* var. *dulce* and var. *azoricum* were treated with cattle manure. However, further additives of synthetic N

Table 6. Essential oil components (%) of fennel bulb as influenced by fertilization

No	Component	RT (min)	RI	Control	Azos+Bm+Bc+VAM	Rabbit manure at 60 m^3/fed	NPK at 150% of recommended dose
1	α-Pinene	12.57	939	10.13	10.98	11.05	9.21
2	β-Pinene	13.09	982	0.24	0.38	0.35	0.29
3	Sabinene	13.54	984	0.15	0.20	0.19	0.18
4	Myrcene	14.56	1003	0.39	0.38	0.28	0.45
5	Phellndrene	12.53	1005	6.28	6.24	6.89	5.94
6	p-Cymene	15.46	1038	2.95	3.84	3.64	2.21
7	Limonene	15.70	1031	2.21	3.05	2.97	2.39
8	Z-β-Ocimene	16.15	959	0.19	0.18	0.21	0.16
9	α-Terpinene	15.45	1035	6.95	7.63	7.58	6.19
10	γ-Terpinene	16.63	1062	5.42	5.93	5.84	5.12
11	Fenchone	12.24	1077	6.34	6.84	6.94	6.51
12	6- Camphenol	18.91	1112	0.18	0.22	0.19	0.16
13	Camphor	19.41	1164	0.42	0.49	0.57	0.46
14	Terpinen-4-ol	20.34	1177	0.21	0.25	0.28	0.23
15	α-Terpineol	10.68	1187	0.29	0.30	0.31	0.38
16	Estragole	21.10	1195	9.84	5.64	4.09	9.65
17	Fenchyl acetate	19.84	1237	3.89	3.56	2.54	3.2
18	Trans-anethole	21.75	1240	39.57	39.46	41.25	42.25
19	Anisaldehyde	22.95	1280	0.08	0.14	0.12	0.16
20	Germacrene D	21.65	1490	0.09	0.11	0.14	0.15
Total identified				95.82	95.82	95.43	95.29

fertilizers markedly increased the undesirable compound methyl chavicol. This interesting reduction in the proportion of estragole was also achieved by El-Serafy and El-Sheshtawy (2020) in the essential oil isolated from fennel fruits upon biofertilization with N fixing bacteria (*B. polymyxa, A. chroococcum,* and *A. lipoferum*).

The increase in the essential oil content of the bulbs obtained with organic manure (rabbit manure at 60 m^3/fed) was accompanied by a marked decrease in the undesirable estragole compound and an increase in the most important constituents, α-pinene, ß-pinene, limonene, *trans*-anethole, and anisaldehyde. This indicates that using organic fertilizer on bulb fennel plants and avoiding mineral fertilizers can produce bulbs with a favorable aroma that provides the desired health benefits.

CONCLUSIONS

In the current study, fennel growth, bulb yield, and bulb essential oil content were responded significantly to biofertilizer, organic additive and synthetic fertilizer. The proportions of the essential oil components of fennel bulbs differed markedly according to the type of fertilizer. The high levels of synthetic NPK and rabbit manure maximized growth parameters, bulb yield, bulb quality characteristics, and bulb essential oil. *trans*-anethole, the compound that characterizes fennel oil, had a high share in the bulbs harvested from chemically and organically fertilized plants. However, the undesirable constituent, methyl chavicol (estragole), was high with chemical (NPK) fertilization. Hence, it can be concluded that using organic fertilization for bulb fennel plants and the avoidance of synthetic mineral fertilizers ensures satisfactory productivity of bulbs with excellent health benefits and favorable taste without polluting the surrounding environment. Further experiments should be carried out to fertilize bulb fennel with a combination of organic and biological sources and chemical fertilizers should be reduced or excluded to obtain bulbs with high yield, acceptable market quality, sufficient nutraceuticals content, and little harmful estragole compound. Similar studies should also be conducted on the yield and essential oil content of fennel fruits, the most oil-producing plant organ in fennel, and reduce the proportion of estragole.

Acknowledgments

The authors extend their sincere thanks and appreciation to Prof. Dr. Magdi Attia, Soil Microbiology Laboratory, Agric. Microbiol. Dept., National Research Center, Giza, Egypt for providing the microbial strains used in this study. Also, the authors are grateful to Dr. Hossam Metwally, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt, for his assistance in analyzing essential oil from fennel bulbs.

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