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Impact of Bio- and Chemical Fertilization on Growth, Yield, Essential Oil and Chemical Composition of Fennel (*Foeniculum vulgare* Mill.) Plant

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Abstract: A pot experiment was carried out in seventeen levels of fertilization to evaluate the effect of the bio-fertilizer mixture alone or in combination with chemical fertilizer (NPK) on Fennel (*Foeniculum vulgare* Mill). Bio-fertilizer was a mixture of *Azotobacter chroococcum*, *Bacillus circulans* and Vesicular-arbuscular mycorrhiza. Results indicated that the use of bio-fertilizer at 3.75 ml/pot four times with 25% of NPK gave the highest significant values of vegetative growth, yield parameters, and NPK uptake; however, fertilizing with bio-fertilizers without NPK application showed the lowest significant values in the two experimental seasons. The same addition of bio-fertilizer gave the highest significant values of total microbial count of soil and increased cumulative CO_2; whereas the addition of 100% NPK gave the lowest significant values in both seasons. The full dose of NPK significantly increased volatile oil percentage while the addition of bio-fertilizer only significantly decreased it in both seasons. GC analysis of essential oil showed that the highest percentage of anethol (12.77 %) was obtained by applying 3.75 ml/pot bio-fertilizer added one time + 25% NPK and decreased estragole (72.78%).

1 Introduction

Originally, the Mediterranean region was considered the native environment to the fennel (*Foeniculum vulgare* Mill.) which is an annual plant, belonging to the Apiaceae family, soon after, fennel was naturalized and cultivated worldwide (Lim 2013).

In this regard, Egypt is deemed one of the most important producers of aromatic and medicinal crops in the Middle East because it enjoys a suitable environment to cultivate these crops (Elsabawy 2012). Specifically, Egypt comes fifth among countries, producing fennel fruits as it produces 3.1% of the total amounts of world exports (Trade Map 2019). The Egyptian fennel is characterized by good yields (Massoud 1992) and superior to sweet or German varieties (Ghazal and Shahhat 2012). Moreover, the content of oxygenated compounds in the local variety is higher than the German one (Osman 2009). Fennel is utilized as an estrogenic, lactagogue, antioxidant, diuretic, immunological booster, and dyspepsia treatment in Egyptian traditional medicine (Ebeed et al 2010). Carminative, aromatic, antispasmodic, anti-inflammatory, and emmenagogue are all properties of fennel seed. It relieves gas and colic while also boosting digestion and appetite, it also increases the flow of milk in nursing mothers. The main constituents of fennel seed essential oil are trans-anethole, fenchone, estragol (methyl chavicol), and limonene. The oil can be used to treat flatulence or in toothpaste, soaps, air fresheners, etc. Moreover, if it is used externally, it can ease muscular and rheumatic pain (Lim 2013).

Being an important product with many uses, it is essential to try to increase the production of fennel in Egypt by using a good type and rationalizations of fertilizers that can help the plants absorb the available forms of nitrogen, phosphorus and potassium from soil, which are mostly exist in unattainable forms to the plants. These three elements are crucial to the growth and development of the plant; however, the use of chemical fertilizers is expensive and has a bad effect on the soil and plants. For example, high rates of N fertilizer applied to soils are harmful to any living creature (Barabasz et al 2002) and the high rates of P fertilizer that are added to the soil can precipitate and become fixed in the soil by 75–90% causing long-term impacts on the environment.

Bio-fertilizers can help the plants absorb the N, P and K elements more efficiently and safely. Bio-fertilizers are products containing living cells of diverse types of microorganisms, which can added to plant surfaces or seeds. They can help to colonize the rhizosphere or the interior of the plant promoting growth by converting nutritionally important elements from unavailable to available form. Bio-fertilizers also can protect the environment and be eco-friendly fertilizer because it increases the sustainability of soil, which ultimately leads to the reduction of damages caused by chemical fertilizer (Mohammadi and Sohrabi 2012). Furthermore, applying bio-fertilizers enhances the use efficiency of nutrients and plant growth in comparison to fully fertilized controls, while lowering fertilizer inputs by up to half dose without incurring yield loss (Da-Costa et al 2013, Good and Beatty 2011, Hayat et al 2010).

On the other hand, excessive use of chemical fertilizers can lead to many issues such as the huge waste of mineral resources. In addition, millions of tons of chemical nutrients that are added to soil continuously are not absorbed by plants. It is reported that up to 50% of N and 90% of P are not absorbed by the crops which can ultimately run away to the atmosphere or water sources, thus, causing global warming gases and salinization in soil (Da-Costa et al 2013, Simpson et al 2011).

Bio-fertilizers can be divided into many microorganisms such as nitrogen-fixing bacteria, phosphate-solubilizing microorganisms (PSMs) and potassium-solubilizing microbes (KSMs). First, the most common species of nitrogen-fixing bacteria inhabiting many soils around the world from the genus Azotobacter is *A. chroococcum* (Mahato et al 2009). It is the most common type of free-living heterotrophic and non-symbiotic nitrogen-fixing bacteria found in neutral or alkaline soils (Wani et al 2016). They provide large amounts of nitrogen to non-leguminous plants (Aasfar et al 2021) when nitrogen is converted to ammonia by them which is then absorbed by plants (Prajapati et al 2008). Second, the phosphate solubilizing microorganisms (PSMs) can be used as bio-fertilizers to enhance plant growth due to their capabilities of hydrolyzing organic and inorganic phosphorus compounds from insoluble compounds and providing eco-friendly and economical savings to overcome the low availability of P in the soil (Kalayu 2019, Sharma et al 2013). The Mycorrhizal symbiosis, a type of PSM, plays an effective role in promoting plant growth by obtaining Phosphorus and other mineral nutrients from the soil, bioremediation, and providing a defensive mechanism to the host plant against harmful pathogens (Aggarwal et al 2011). Third, Potassium is very tightly bound to the soil, so the use of potassium solubilizing microbes as bio-fertilizers will enhance potassium availability to plants and its absorption by plant roots and become environment friendly than the application of chemical K applied fertilizers (Padhan et al 2019). A type of KSMs used is *Bacillus circulans* of Bacillus species, which is a silicate solubilizing bacterium that enhances potassium availability to crops from insoluble K sources, thus, improving crop yield and decreasing chemical fertilizers additions (Padhan et al 2019).

This study aims to evaluate the ability of bio-fertilizer mixture and rationalization of mineral fertilization to enhance the production of fennel and reduce chemical fertilizer application, as well as, to increase anethole as an active ingredient instead of estragole (which is an undesirable component).

2 Materials and Methods

The pot experiment was carried out at the experimental farm of the Faculty of Agriculture, Ain Shams University, Cairo, Egypt during the two successive seasons of 2018/2019 and 2019/2020. Fennel (*Foeniculum vulgare* Mill) fruits were sown on the 28th Oct, of both seasons in 30 diameter plastic pots filled with clean and washed sand in combination with compost at a ratio of 3:1. Compost obtained from the Agricultural Botany Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt. The chemical analysis of the used compost is shown in **Table 1**.

2.1 Chemical fertilizers

The fully recommended dose of mineral fertilizers NPK were 300 Kg/ fed calcium-superphosphate (15.5 % P_2O_5) which was added during the preparation of the soil, 300 Kg/ fed ammonium nitrate (33.5 % NH_4NO_3) and 100 Kg/ fed potassium sulfate (48 % K_2O) were added in two equal doses. The first one was added 45 days starting from the appearance of the real leaves and the second was added 45 days after the first one (Osman 2009). The quantity per plant was calculated as follows: 16.66 g/plant calcium-superphosphate (15.5% P_2O_5), 16.66 g/ plant ammonium nitrate (33.5% NH_4NO_3) and 5.55 g/plant potassium sulfate (48% K_2O) and added at the same dates mentioned above.

2.2 Bio-fertilizer

The strains of bio-fertilizer used were *Azotobacter chroococcum* (10^8 CFU/ml.), *Bacillus circulans* (10^8 CFU/ml.) and (VAM) Vesicular-arbuscular mycorrhiza (200 spores/ml.). These strains were mixed in equal parts and added to the wet soil surface beside plants in liquid form once, twice, three, or four times. All inoculants were provided by the Bio-fertilizer Unit, Fac. of Agric., Ain Shams Univ., Cairo, Egypt.

2.3 Experimental Treatments

A mixture of three bio-fertilizer strains was added once (after one month of planting), twice (the first addition was a month after sowing and the second was a month after the first addition), three times or four times (the first addition was a month after sowing then a month between each other addition) in proportions as follow:
5 ml/pot bio-fertilizer only.
3.75 ml/pot bio-fertilizer with 25% of the recommended dose of NPK.
2.5 ml/pot bio-fertilizer with 50% of the recommended dose of NPK.
1.25 ml/pot bio-fertilizer with 75% of the recommended dose of NPK.
Control (Full dose of NPK as recommended doses).

The experiment was carried out in a completely randomized design with four replicates; each replicate contained 5 plants in 17 treatments (17×4×5=340 plants).

2.4 Data recorded

The number of main branches per plant and the number of umbels per plant were recorded after the full blooming. Fruit yield per plant (g) and seed index (weight of 100 fruits (g)) were recorded at the end of the experiment.

2.4.1 Percentage of essential oil

Hydro distillation is used to extract the essential oil from fruits according to the method described in British Pharmacopoeia (1963). Volatile oil percentage was calculated (v/w) and then kept at the refrigerator till GC analysis.

2.4.2 Determination of essential oil constituents by GC analysis

The volatile oil constituents were separated using a Ds Chrom 6200 Gas Chromatograph equipped with a flame ionization detector. The condition of analysis was as follows:

The chromatography apparatus was supplied with capillary column DB-WAX 122-7032 polysilyl-phenylene-siloxane 30 m ×0.25 mm ID × 0.25 µm film. The ramp of the temperature program increases with a rate of 13°C/ min from 60°C to 220°C. The rates of flow of gases were nitrogen at 1 ml/min, hydrogen at 3 ml/min, and 330 ml/min for air. The temperatures of the detector and injector were 280°C and 250°C, respectively.

Table 1. Chemical analysis of the used compost

P^H	EC (ds/m)	Density (Kg/m^3)	Moisture %	O.M %	O.C %	N%	P%	K%	C:N ratio
7	2.18	747	20.29	33.93	19.73	1.18	0.49	0.29	16.72

2.4.3 Determination of the uptake of nitrogen, phosphorus and potassium

Estimating the percentages of nitrogen, phosphorus, and potassium:

The chemical analysis was performed on dried herb samples at 70°C in a forced air oven. The three elements were determined in the acid-digested solution. Total nitrogen content was estimated by the micro-Kjeldahl method, phosphorous concentration was estimated by colorimeter method (ammonium molybdate) using spectrophotometer according to Okalebo et al (2002) and atomic absorption spectrophotometer was used for the determination of potassium concentration (Chapman and Pratt 1961).

The total uptake of nitrogen, phosphorous, and potassium was calculated according to (Sharma et al 2012) through the following equation:

Uptake of N/P/K (Mg g^{-1} dry weight) = nutrient percentage × dry matter

2.4.4 Microbial studies

Microbiological analysis of rhizosphere of fennel pot plants soil apart was done at the flowering stage and included the following measurements:

1- Total count of bacteria (TCB) was counted on Bunt and Rovira medium (Bunt and Rovira 1955).
2- Cumulative CO_2: in each incubation bottle which contained the soil we placed vials containing 5-ml of 1M NaOH for 24 hours and then we add 0.5M $BaCl_2$ solution to trapped the CO_2 in the NaOH and the total C of the trapped CO_2 was measured by titrating against 0.1M HCl using a phenolphthalein indicator and calculate the cumulative CO_2-C as the sum of CO_2–C effluxes (mg C/g) (Zibilske 1994).

2.5 Statistical analysis

The statistical analysis was performed using the CoStat Package Program (Version 6.303; CoHort Software, USA). The analysis of variance was used to examine the data (ANOVA). Duncan's Multiple Range Test was used to compare the discrepancies between data means**.** All statistical determinations were made at $P \leq 0.05$.

3 Results and Discussion

Data in **Table 2** showed that the addition of 3.75 ml. of bio-fertilization mixture four times + 25% NPK gave the best results for the number of branches per plant, and umbels number per plant in both seasons. However, adding the same treatment but three times, and adding 1.25 ml bio once with 75% NPK showed a non-significant difference between them for umbel number in the second season only. The addition of 5 ml. of bio-fertilizer alone regardless of the times number of addition gave the lowest value with non-significant differences between them in the first and the second seasons.

Data in **Table 3** showed that the inoculation with 3.75 ml. of bio-fertilizer added four times and applying 25% of NPK gave the highest fruit yield/plant, and highest seed index in both seasons. There was no statistically significant difference between this treatment and the addition of 2.5 ml. of bio added three or four times + 50% of NPK for fruit yield in the first season only. There wasn't a significant effect between this treatment and the treatments of 3.75 ml. bio-fertilizer was added three times + 25% NPK and 2.5 ml. of bio added three times + 50% of NPK in both seasons for seed index. In both seasons the addition of 5 ml. of bio-fertilizer only gave the lowest fruit yield/plant and seed index with non-significant differences between the numbers of additions.

Data in **Table 4** indicate that the various fertilization treatments had a significant effect on nitrogen and phosphorus uptake (Mg g^{-1}dry weight) in both seasons. The addition of 3.75 ml. bio added four times + 25% NPK leads to the highest nitrogen and phosphorous uptake rate for the first and the second season. The addition of 5 ml. of bio-fertilizer only leads to the lowest nitrogen and phosphorous uptake rate in both seasons with a non-significant difference between the numbers of additions.

The previous results indicated an increase in vegetative growth and fennel yield. This increment may be due to an increase in essential nutrient uptake by the plant. The nitrogen uptake increases because of *Azotobacter chroococcum* which improves the growth of roots and uptake of plant nutrients (Babalola 2010)**,** additionally due to better accelerated bacterial activity which leads to increased nitrogen fixation by converted atmospheric nitrogen to ammonia and the production of different growth hormones by them and their ability to produce a large amount of amino-acid like Glutamic acid and Lysine(González-López et al 2005) which leads to controlling the enhanced of root development and plant growth specifically vegetative

Table 2. Effect of inoculation with a mixture of *Azotobacter chroococcum*, *Bacillus circulans* and VAM per pot either separate or in combination with chemical fertilizer on the number of branches/plant and umbels number/plant of fennel, during 2018/2019 and 2019/2020 seasons

Treatments	Number of branches/plant		Umbels number/plant	
	2018/2019	2019/2020	2018/2019	2019/2020
Control (100%NPK)	4.66 e	5.8 abc	7.1 ab	7.39 ab
T1 (5 ml.bio*1)	3.97 f	3.18 d	4.07 f	4.30 d
T2 (5 ml.bio2)	3.41 fg	2.79 d	3.93 f	3.95 d
T3 (5 ml.bio3)	2.95 g	3.22 d	4.16 ef	4.58 d
T4 (5 ml.bio4)	3.5 fg	3.41 d	5.12 de	4.93 cd
T5 (3.75 ml. bio1+25%NPK)	5.1 cde	5.20 bc	5.8 cd	6.12 bc
T6 (3.75 ml. bio2+25%NPK)	5.06 de	5.05 c	6.1 bcd	6.74 ab
T7 (3.75 ml. bio3+25%NPK)	6 ab	5.72 abc	6.83 abc	7.75 a
T8 (3.75 ml.bio4+25%NPK)	6.41 a	6.38 a	7.62 a	8.01 a
T9 (2.5ml. bio1+50% NPK)	5.36 bcde	5.55 abc	7 ab	7.15 ab
T10 (2.5ml. bio2+50%NPK)	5.80 abc	5.88 abc	6.8 abc	7.08 ab
T11 (2.5ml. bio3+50%NPK)	5.87 ab	5.78 abc	6.66 abc	7.26 ab
T12 (2.5ml. bio4+50%NPK)	5.86 ab	5.66 abc	6.62 abc	7.61 ab
T13 (1.25 ml.bio1+75%NPK)	5.43 bcd	5.31 bc	6.61 abc	7.93 a
T14 (1.25 ml.bio2+75%NPK)	6 ab	6.13 ab	7.18 ab	6.80 ab
T15 (1.25 ml.bio3+75%NPK)	5.88 ab	6.15 ab	6.75 abc	7.08 ab
T16 (1.25 ml.bio4+75%NPk)	5.25 bcde	6.08 ab	6.5 abc	7.36 ab

1 once 2 twice 3 three times 4 four times first addition was a month after sowing, and a month separates the addition and another.

*bio-fertilizer.

Numbers followed by the same letter in the same columns are not significantly different at 5% DMRT.

Table 3. Effect of inoculation with a mixture of *Azotobacter chroococcum*, *Bacillus circulans* and VAM per pot either separate or in combination with chemical fertilizer on fruit yield/plant (g) and seed index of fennel, during 2018/2019 and 2019/2020 seasons

Treatments	fruit yield/plant (g)		Seed index	
	2018/2019	2019/2020	2018/2019	2019/2020
Control (100% NPK)	11.87 b	11.87 ab	1.4 abc	1.27 bcde
T1 (5 ml.bio*1)	5.22 cd	5.47 de	1.29 bcd	1.21 de
T2 (5 ml.bio2)	6.69 c	6.11 cde	1.23 cd	1.18 e
T3 (5ml.bio3)	4.62 cd	4.70 e	1.35 abcd	1.27 bcde
T4 (5ml.bio4)	2.20 d	4.13 e	1.22 d	1.25 cde
T5 (3.75 ml.bio1+25%NPK)	14.10 ab	10.56 ab	1.39 abcd	1.28 bcde
T6 (3.75 ml.bio2+25%NPK)	13.91 ab	10.03 ab	1.35 abcd	1.33 abc
T7 (3.75 ml.bio3+25%NPK)	16.55 ab	10.50 ab	1.48 a	1.36 ab
T8 (3.75 ml.bio4+25%NPK)	17.23 a	12.81 a	1.49 a	1.41 a
T9 (2.5 ml. bio1+50% NPK)	14.98 ab	9.13 abc	1.43 ab	1.23 cde
T10 (2.5 ml. bio2+50%NPK)	14.25 ab	12.49 ab	1.32 abcd	1.33 abc
T11 (2.5 ml. bio3+50%NPK)	17.10 a	12.51 ab	1.47 a	1.41 a
T12 (2.5 ml. bio4+50%NPK)	17.05 a	8.73 bcd	1.36 abcd	1.30 bcd
T13 (1.25 ml.bio1+75%NPK)	13.05 ab	11.39 ab	1.38 abcd	1.24 cde
T14 (1.25 ml.bio2+75%NPK)	14.79 ab	10.05 ab	1.41 abc	1.32 abc
T15 (1.25 ml.bio3+75%NPK)	16.30 ab	11.31 ab	1.42 ab	1.27 bcde
T16 (1.25 ml.bio4+75%NPk)	13.34 ab	11.73 ab	1.31 abcd	1.29 bcde

1 once 2 twice 3 three times 4 four times first addition was a month after sowing, and a month separates the addition and another.

*bio-fertilizer.

Numbers followed by the same letter in the same columns are not significantly different at 5% DMRT.

Table 4. Effect of inoculation with *Azotobacter chroococcum*, *Bacillus circulans* and VAM per pot either separate or in combination with chemical fertilizer on Nitrogen and Phosphorus uptake (Mg g^{-1}dry weight) of fennel, during 2018/2019 and 2019/2020 seasons

Treatments	Nitrogen uptake Mg g^{-1} dry weight		Phosphorus uptake Mg g^{-1} dry weight	
	2018/2019	2019/2020	2018/2019	2019/2020
Control (100% NPK)	0.159 abcd	0.264 bc	0.037 abc	0.031 abcde
T1 (5 ml.bio[*1])	0.101 cd	0.103i	0.026 c	0.016 g
T2 (5 ml.bio[2])	0.117 bcd	0.159 gh	0.033 abc	0.027 def
T3 (5 ml.bio[3])	0.138 bcd	0.117 hi	0.033 abc	0.021 fg
T4 (5 ml.bio[4])	0.080 d	0.095 i	0.029 bc	0.016 g
T5 (3.75 ml. bio[1]+25%NPK)	0.164 abcd	0.194 efg	0.044 abc	0.023 efg
T6 (3.75 ml. bio[2]+25% NPK)	0.142 abcd	0.217 cdef	0.037 abc	0.029 cdef
T7 (3.75 ml. bio[3]+25% NPK)	0.189 abc	0.172 fg	0.048 abc	0.029 cdef
T8 (3.75 ml.bio[4]+25%NPK)	0.234 a	0.317 a	0.058 a	0.039 a
T9 (2.5 ml. bio[1]+50% NPK)	0.187 abc	0.220 cdef	0.036 abc	0.029 bcdef
T10 (2.5 ml. bio[2]+50%NPK)	0.18 abc	0.206 defg	0.038 abc	0.036 abc
T11 (2.5 ml. bio[3]+50%NPK)	0.179 abc	0.253 bcd	0.053 ab	0.030 bcde
T12 (2.5 ml. bio[4]+50%NPK)	0.200 ab	0.274 bcde	0.039 abc	0.037 abc
T13 (1.25 ml.bio[1]+75%NPK)	0.166 abcd	0.255 bcd	0.044 abc	0.037 ab
T14 (1.25 ml.bio[2]+75%NPK)	0.199 ab	0.224 cdef	0.038 abc	0.029 bcdef
T15 (1.25 ml.bio[3]+75%NPK)	0.173 abc	0.292 ab	0.053 ab	0.034 abcd
T16 (1.25 ml.bio[4]+75%NPk)	0.199 ab	0.245 bcde	0.038 abc	0.036 abc

[1] once [2] twice [3] three times [4] four times first addition was a month after sowing, and a month separates the addition and another.

[*]bio-fertilizer.

Numbers followed by the same letter in the same columns are not significantly different at 5% DMRT.

growth (Wani et al 2016**)**. However, using *Azotobacter* alone with adding organic fertilizer gave the highest yield components and growth of fennel (Youssef et al 2020), and gave the highest seed yield on cumin (Mehta et al 2012). When added *Azotobacter* with other strains and using 50% chemical fertilizer enhanced plant growth and fruit yield of fennel (Ghazal and Shahhat 2012**,** Dadkhah 2014), and dill (Elsayed et al 2020)**.** Moreover, adding it with 75% NPK gave the same results on fennel, and using it with other strains enhanced vegetative growth and yield on fennel (Shahmohammadi et al 2014), dill (Hellal et al 2011), and cumin (Abdel-Kader et al 2016).

It is well known that phosphorus is essential for all cellular metabolic processes, and it is a basic structure component of numerous biomolecules like nucleic acids of genes and chromosomes and important in membrane structure, nucleotides, coenzymes, and phosphoproteins and high energy compounds such as ATP and NADPH (Mengel and Kirkby 2001, Barker and Pilbeam 2015), and by enhanced hormone activity like cytokinin and gibberellin which influence ion transport, leading to stomatal opening and regulating the chlorophyll (Allen et al 1982) and the increment of P content may be due to applying vesicular-arbuscular mycorrhiza (VAM) which making an extend hyphae into the soil and absorption phosphorus from soil then transfer it along the hyphae through arbuscular to the cortical of root cells (Barea 1991) and provides a large surface area and makes the plant able to reach a larger soil volume for nutrients acquisition and water to the external root surface. Moreover, inoculation plants with VAM elevate photosynthetic rates by enhancing the uptake of phosphorus (Aggarwal et al 2011). Increased the content of P by using VAM was mentioned by Kapoor et al (2004) in fennel shoots and fruits and combined them with chemical fertilizer gave the same result on fennel (Dadkhah 2012), coriander (Bastami and Majidian 2016) and Basil (Hasan and Rabie 2019).

Data in **Table 5** indicate that the addition of 3.75 ml. bio-fertilizer mixture added four times + 25% NPK leads to the highest Potassium uptake rate for the first and the second season and in the second season the addition of 2.5ml. Bio-fertilizer added three times+50%NPK showed a non-significant difference between them. The addition of 5 ml. of bio-fertilizer only leads to the lowest Potassium uptake rate in both seasons with a non-significant difference between the numbers of additions.

Table 5. Effect of inoculation with *Azotobacter chroococcum*, *Bacillus circulans* and VAM per pot either separate or in combination with chemical fertilizer on Potassium uptake (Mg g^{-1}dry) weight of fennel, during 2018/2019 and 2019/2020 seasons

Treatments	**Potassium uptake Mg g^{-1} dry weight**	
	2018/2019	**2019/2020**
Control (100% NPK)	0.238 defg	0.291 cde
T1 (5 ml.bio*1)	0.220 efg	0.197 f
T2 (5 ml.bio2)	0.186 g	0.243 ef
T3 (5 ml.bio3)	0.152 g	0.195 f
T4 (5 ml.bio4)	0.216 fg	0.182 f
T5 (3.75 ml. bio1+25% NPK)	0.355 abc	0.342 abcd
T6 (3.75 ml. bio2+25% NPK)	0.273 bcdef	0.280 bcde
T7 (3.75 ml. bio3+25% NPK)	0.376 abc	0.390 ab
T8 (3.75 ml. bio4+25% NPK)	0.408 a	0.412 a
T9 (2.5 ml. bio1+50% NPK)	0.342 abcd	0.314 bcde
T10 (2.5 ml. bio2+50%NPK)	0.337 abcd	0.390 ab
T11 (2.5 ml. bio3+50% NPK)	0.386 abc	0.407 a
T12 (2.5 ml. bio4+50% NPK)	0.363 abc	0.362 abcd
T13 (1.25 ml. bio1+75% NPK)	0.267 cdef	0.289 de
T14 (1.25 ml. bio2+75% NPK)	0.342 abcd	0.332 abcd
T15 (1.25 ml. bio3+75% NPK)	0.327 abcde	0.378 abc
T16 (1.25 ml. bio4+75% NPK)	0.345 abcd	0.329 abcd

[1] once [2] twice [3] three times [4] four times first addition was a month after sowing, and a month separates the addition and another.

*bio-fertilizer.

Numbers followed by the same letter in the same columns are not significantly different at 5% DMRT.

The obtained data could be explained by that the potassium uptake increasing maybe because of *Bacillus circulans* which is one of the Potassium-Solubilizing Microorganisms that increase the production of low molecular weight organic acids like citric, tartaric, and oxalic acids which leads to a decrease of p^H of the soil and providing protons and chelation the cation bound to potassium which increased the solubility of potassium compounds, also by complexion Ca^{2+} and other ions in silicate minerals of the soil. Moreover, mucilaginous capsule production by these microorganisms consists of exo-polysaccharides (Groudev 1987) with organic acid leading to illite and feldspar solubility to potassium release (Sheng and He 2006**).** Polysaccharides absorb these organic acids, causing them to attach to the surface of the mineral and as a result, the concentration of organic acids near the metal increases (Liu et al 2006)**.** Biofilms produced by bacteria are formed in the immediate vicinity of minerals and consist of organic acids, polysaccharides and protein thus, accelerating the process of weathering and forming a protective layer covering the mineral-water-hyphal/root hair that enhances the weathering of potassium-rich shale and the release of potassium, Si, and Aluminum in the rhizosphere when biofilm formation on the mineral surface (Man et al 2014). Increased seed yield when inoculation *Bacillus circulans* to anise plant reported by Darzi et al (2012) although this didn’t show a significant effect on plant height and 1000 seeds weight, and when Azzaz et al (2009) mixed *Bacillus circulans* with *Azotobacter* sp., and *Bacillus megatherium* enhanced plant growth, chemical composition, and volatile oil yield of fennel.

Data in **Table 6** indicated that the different fertilization treatments had a positive effect on essential oil % in the first and the second season, the addition of the full recommended dose of NPK gave the highest percentage of essential oil followed by the inoculation of 3.75 ml. bio added four times + 25% NPK with no significant difference between them in both seasons. The addition of 5 ml. of bio-fertilizer only gave the lowest percentage of essential oil in both seasons with no significant difference between the numbers of additions.

Table 6. Effect of inoculation with mixture of *Azotobacter chroococcum*, *Bacillus circulans* and VAM either separate or in combination with chemical fertilizer on essential oil percentage of fennel, during 2018/2019 and 2019/2020 seasons

Treatments	Essential oil percentage	
	2018/2019	**2019/2020**
Control (100%NPK)	1.48 a	1.43 a
T1(5ml.bio*[1])	0.97 ef	1.01 c
T2(5ml.bio[2])	1.04 cdef	1.03 c
T3(5ml.bio[3])	0.95 f	1.03 c
T4(5ml.bio[4])	1.01 def	1.03 c
T5(3.75ml. bio[1]+25%NPK)	1.14 cdef	1.09 bc
T6(3.75ml. bio[2]+25% NPK)	1.01 def	1.30 ab
T7(3.75ml. bio[3]+25% NPK)	1.11 cdef	1.23 abc
T8(3.75ml. bio[4]+25%NPK)	1.42 ab	1.30 ab
T9(2.5ml. bio[1]+50% NPK)	1.18 cde	1.16 bc
T10(2.5ml. bio[2]+50%NPK)	1.02 def	1.23 abc
T11(2.5ml. bio[3]+50%NPK)	1.06 cdef	1.16 bc
T12(2.5ml. bio[4]+50%NPK)	0.97 ef	1.18 abc
T13(1.25ml. bio[1]+75%NPK)	1.24 bc	1.27 abc
T14(1.25ml. bio[2]+75%NPK)	1.13 cdef	1.11 bc
T15(1.25ml. bio[3]+75%NPK)	1.12 cdef	1.05 bc
T16(1.25ml. bio[4]+75%NPk)	1.22 bcd	1.09 bc

[1] once [2] twice [3] three times [4] four times first addition was a month after sowing, and a month separates the addition and another.
*bio-fertilizer.
Numbers followed by the same letter in the same columns are not significantly different at 5% DMRT.

The GC analysis of fennel essential oil as indicated in **Table 7** showed the presence of 5 compounds in the first season. The highest percentage of Anethol (12.77 %) was obtained by 3.75ml. bio added one times + 25% NPK (T5) followed by (T12) the addition of 2.5 ml. bio added four times + 50% NPK (11.79 %), the addition of 100% NPK reached the lowest percentage of Estragole (68.61%) followed by (T12).

The formation of chemical compositions of the oils requires energy which is produced in the photosynthetic process (Dhifi et al 2016) and for that, the essential oil percentage and chemical composition are basically dependent on the rate of NPK (Nurzyńska 2013). Therefore, the importance of the three elements appears in influencing the amount of volatile oil and its components. From the obtained data we can conclude that the bio-fertilization enhanced the quantity and the quality of essential oil and this result agree with those obtained by Abdel Wahab et al (2016), Sajjadnia et al (2013), Dadkhah (2014), and El-Azim et al (2017) on fennel, Roshanpour et al (2014) on basil, Massoud et al (2019) on parsley. Although Hellal et al (2011) and Shahmohammadi et al (2014) reported that applying bio-fertilization not affected on the essential oil content and the percentage of the oil. The obtained data showed that applying bio-fertilization on fennel increased Anethol and decreased Estragole (which is an undesirable component) and this result agrees with Ghazal and Shahhat (2012) which showed a decrease in estragole content on fennel with applying organic and biological fertilizer treatments.

There was a significant effect of the fertilization treatments on total microbial count ($\times 10^4$ /g soil), and cumulative CO_2-C (mg C g^{-1} soil) of fennel during the 2018/2019 and 2019/2020 seasons as shown in **Table 8** which indicated that the inoculation of fennel plant with (3.75ml. bio added four times + 25% NPK) increased total microbial count and cumulative CO_2-C in the first and the second seasons, and the full addition of chemical fertilization gave the lowest number of total microbial count and reduced cumulative CO_2-C in both seasons, the addition of 1.25ml. bio added once or twice +75% NPK showed a non-significant difference with full addition of NPK.

Table 7. Effect of inoculation with *Azotobacter chroococcum, Bacillus circulans* and VAM per pot either separate or in combination with chemical fertilizer on the main constituents of essential oil of fennel, during the 2018/2019 season

Treatments	Concentration of compounds (%)				
	α- Pinene	Limonene	1.8 Cineol	Estragole	Anethol
Control (100% NPK)	2.90	18.59	10.60	68.61	1.77
T1 (5 ml.bio[*1])	2.01	16.10	6.19	73.20	1.74
T2 (5 ml.bio[2])	1.98	12.69	2.04	75.01	8.26
T3 (5 ml.bio[3])	0.78	14.78	1.87	81.40	0.66
T4 (5 ml.bio[4])	1.92	12.46	2.38	72.43	8.02
T5 (3.75 ml. bio[1]+25% NPK)	1.15	10.45	2.28	72.78	12.77
T6 (3.75 ml. bio[2]+25% NPK)	1.31	14.51	4.63	77.94	0.61
T7 (3.75 ml. bio[3]+25% NPK)	1.28	7.40	4.70	83.37	0.26
T8 (3.75 ml. bio[4]+25% NPK)	0.77	13.94	1.92	80.78	0.65
T9 (2.5 ml. bio[1]+50% NPK)	2.28	12.04	4.64	79.72	0.21
T10 (2.5 ml. bio[2]+50% NPK)	1.48	17.66	2.40	77.11	0.20
T11 (2.5 ml. bio[3]+50% NPK)	0.93	20.58	1.62	76.40	0.21
T12 (2.5 ml. bio[4]+50% NPK)	1.34	11.05	2.23	72.42	11.97
T13 (1.25 ml. bio[1]+75% NPK)	0.72	16.72	6.83	75.40	0.14
T14 (1.25 ml. bio[2]+75% NPK)	0.88	12.46	2.41	76.28	0.49
T15 (1.25 ml. bio[3]+75% NPK)	1.90	14.15	2.43	78.33	0.44
T16 (1.25 ml. bio[4]+75% NPK)	1.26	14.21	4.61	78.99	0.52

[1] once [2] twice [3] three times [4] four times first addition was a month after sowing, and a month separates the addition and another.
[*]bio-fertilizer.

Table 8. Effect of inoculation with *Azotobacter chroococcum, Bacillus circulans* and VAM per pot either separate or in combination with chemical fertilizer on Total microbial count ($\times 10^4$ /g soil) and cumulative CO_2-C (mg C g^{-1} soil) of fennel, during 2018/2019 and 2019/2020 seasons

Treatments	Total microbial count ($\times 10^4$ /g soil)		Cumulative CO_2-C (mg C g^{-1} soil)	
	2018/2019	2019/2020	2018/2019	2019/2020
Control (100% NPK)	21.5 m	24.75 m	0.002 m	0.003 i
T1 (5 ml.bio[*1])	54.5 i	51.25 i	0.0102 h	0.0105 f
T2 (5 ml.bio[2])	60.75 h	66.5 f	0.0106 gh	0.0108 f
T3 (5 ml.bio[3])	66.5 f	61 h	0.0125 e	0.0120 e
T4 (5 ml.bio[4])	86.5 c	73 e	0.0163 d	0.0192 c
T5 (3.75 ml.bio[1]+25% NPK)	65.5 fg	63.5 g	0.0175 c	0.0184 c
T6 (3.75 ml.bio[2]+25% NPK)	81.5 d	114 c	0.0198 b	0.0120 e
T7 (3.75 ml.bio[3]+25% NPK)	91.25 b	125.25 b	0.0203 b	0.0213 b
T8 (3.75 ml.bio[4]+25%NPK)	114.5 a	140.75 a	0.0224 a	0.02635 a
T9 (2.5 ml.bio[1]+50% NPK)	46.5 j	33.5 l	0.0107 gh	0.0108 f
T10 (2.5 ml.bio[2]+50% NPK)	63.25 gh	45.5 j	0.0113 fg	0.0112 ef
T11 (2.5 ml.bio[3]+50% NPK)	67 f	71.5 e	0.0117 ef	0.0132 d
T12 (2.5 ml.bio[4]+50% NPK)	72.5 e	83.5 d	0.0173 c	0.0136 d
T13 (1.25 ml.bio[1]+75% NPK)	31.75 l	26 m	0.0075 i	0.0035 i
T14 (1.25 ml.bio[2]+75% NPK)	31 l	26.5 m	0.0045 k	0.0065 h
T15 (1.25 ml.bio[3]+75% NPK)	32.5 l	34.5 l	0.0035 l	0.008 g
T16 (1.25 ml.bio[4]+75% NPK)	41.5 k	42.75 k	0.0057 j	0.0082 g

[1] once [2] twice [3] three times [4] four times first addition was a month after sowing, and a month separates the addition and another.
[*]bio-fertilizer.
Numbers followed by the same letter in the same columns are not significantly different at 5% DMRT.

According to the previous results, it appears that after the addition of bio-fertilizers to the soil, these strains have been able to adapt to the fennel plant rhizosphere and increase their reproduction and their vital activities and this may led to an increase in the microbial population in the rhizosphere and therefore total microbial count. This may be due to the production of melanin which is a dark-brown water-soluble produced by *Azotobacter chroococcum* on the processes of nitrogen fixation (Shivprasad and Page 1989) which is involved in the metabolism of microbial organisms and improved microbial activity in soils (Hajnal-Jafari et al 2012)**.** Furthermore, there is a close relationship between the biological activities of soil microorganisms and their number, so we can use the estimation of the value of emitted CO_2 during the respiration process of soil microorganisms as an index for the soil microbial respiration (Parastesh et al 2019**,** Smitha et al 2019).

Increased cumulative CO_2-C might be due to the Enhancement of microbial communities on the rhizosphere which stimulation microbial activities, and it agrees with (Awad et al 2013). Moreover, it enhances the physical properties of the soil, which leads to an increase in the rate of respiration of microbes (Jones et al 2011)**.** The increment of biomass carbon and soil microbial respiration in response to the application of biofertilizers reported by Parastesh et al (2019), and Dehsheikh et al (2020) and under sandy soil conditions Zaki et al (2010) indicate that the addition of bio-fertilizer increased total microbial count.

4 Conclusions

The addition of bio-fertilizers *Azotobacter chroococcum* (10^8 CFU), *Bacillus circulans* (10^8 CFU), and VAM (200 spores) enhanced plant rhizospher and microbial activity for the fennel root, which leads to increased nitrogen, phosphorus and potassium uptake and thus increased vegetative growth, enhanced the essential oil percentage and its component. From the overall results, we recommend that the addition of 3.75 ml. of bio-fertilizer mixture (*Azotobacter chroococcum*, *Bacillus circulans*, and VAM) four times with 25% of the recommended dosage of chemical fertilizer can enhance the productivity of fennel.

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