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Effect of mineral vs. biofertilizer on growth, yield, and essential oil content of fennel (*Foeniculum vulgare* Mill.)

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A b s t r a c t. In field experiments during two successive seasons (2003-2004 and 2004-2005), the effects of biofertilization on growth, fruit yield, and oil composition of fennel plants were investigated. Application of biofertilizer, which was a mixture of *Azotobacter chroococcum, Azospirillum liboferum,* and *Bacillus megatherium* applied with chemical fertilizers (only 50% of the recommended dosage of NPK) increased vegetative growth (plant height, number of branches, and herb fresh and dry weight per plant) compared to chemical fertilizer treatments only. The tallest plants, the highest number of branches per plant, and the highest fresh and dry weights of plants were obtained from the treatment of biofertilizer plus a half dose of chemical fertilizer (357 kg ammonium sulphate + 238 kg calcium super phosphate + 60 kg potassium sulphate ha^{-1}). The lowest fresh and dry weights of plants occurred with the 50% NPK. Also, addition of biofertilizer with the chemical fertilizer increased these characters more than the half dose of chemical fertilizer alone. Total carbohydrates in the dry plant material were influenced by the biofertilizer. The highest values of total carbohydrates were found in the treatment with biofertilizer plus a half dose of nitrogen and phosphorus. Nitrogen, phosphorus, and potassium levels in the plant tissue increased when soil was inoculated by nitrogen-fixing bacteria, phosphate-dissolving bacteria, and a mixture of all strains, respectively. The least amount of N, P and K in the plant tissue occurred with the half dose of chemical fertilizer. Essential oil content in the fennel fruits was increased due to inoculation compared to the half dose of chemical fertilizer. The highest oil yield per plant was observed with the treatment of biofertilizer plus a half dose of nitrogen and phosphorus. The lowest amount of essential oil yield was obtained with the half dose of chemical fertilizer. Oxygenated compounds were increased as a result of using biofertilizer. The highest anethol (*trans*-1-methoxy-4-(prop-1-enyl)benzeen; $C_{10}H_{12}O$)) in fennel essential oil occurred with the half dose of N, P, and K and inoculation with *Bacillus megatherium*.

K e y w o r d s: *Azotobacter chroococcum*, *Azospirillum lipoferum*, *Bacillus megatherium*, (*Foeniculum vulgare* Mill.)

INTRODUCTION

Fennel (*Foeniculum vulgare* Mill.; Fam. Umbelliferae) is one of the most important medicinal and aromatic plants due to its estrogenic activities and uses as a carminative, diuretic, anti-inflammatory, antimicrobial, and galactogogue; it is a substance which is used to increase the production of milk in humans and other animals. Also, it is given to infants in the treatment of flatulence. In addition, the volatile oils of fennel are used to control flatulent dyspepsia and colic in children. Using 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. Use of biofertilizers on Egyptian soils has decreased the pH, which had led to increased availability of trace elements that enhance plant growth. Yousry *et al.* (1978) found that inoculation of pea (*Pisum sativum*) plants with *Bacillus megatherium* increased plant dry matter by 10.9%, while the combined application of *B. megatherium* and P-fertilizer increased dry matter by 19.7%.

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). Maheshwari *et al.* (1991) showed that by using *Azotobacter chroococcum* alone on palmarosa (*Cymbopogon martinii* var. Motia), yield increased by 16% and when applied together with 80 kg nitrogen the yield increased by 29%. Lewis *et al.* (1995) used double applications of *Azotobacter*

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chroococcum (15l ha) on garlic (*Allium sativum* L.). They inoculated the soil with the bacteria after planting or immersed the cloves with a solution of the bacteria as a pre-planting treatment and then followed these treatments by a soil application 25 days later. They found that the highest profit was obtained by immersing the cloves in a solution with the bacteria as a pre-planting treatment followed by soil application.

Sharaf (1995) showed that inoculation with a mixture of *Azotobacter* and *Azospirillum* with full doses of rock phosphate and inorganic N-fertilizer, in combination with inoculation with vascular arbuscular mycorrhiza (VAM), improved growth of both datura (*Datura stramonium*) and ammi (*Ammi visnaga*: Fam. Umbelliferae) plants. Kandeel *et al.* (2002) found that dual inoculation with symbiotic N_2 fixers (*Azotobacter and Azospirillum*) with half or full doses of inorganic N fertilizer increased plant height, number of branches per plant, and fresh and dry weights of leaves and roots. Badran and Safwat (2004) and El-Ghadban *et al.* (2006) found that fennel responded to biofertilizer by increasing growth and oil yield and changing the chemical composition.

The objective of this study was to determine the growth, yield, and chemical composition of fennel plants as influenced by the application of biofertilizers. The goal was to minimize inorganic fertilize (NPK) usage.

MATERIALS AND METHODS

Field experiments were carried out during two successive seasons (2003-2004 and 2004-2005) at National Research Centre (NRC) Experimental Station (30°05´N, 31°22´E), located at about 22 m altitude, in the north of Egypt, Al-Gizah Governorate, to study the effect of biofertilization in combination with NPK fertilizer on growth and oil production of fennel plants. The main weather information for Cairo, Egypt concerning temperature (T), sunshine (SH) and rainfall (RF) is given in Table 1. Chemical properties of the soil used in this study are presented in Table 2. The strains of the biofertilizer used were: 1 – *Azotobacter chroococcum*, 2 – *Azospirillum lipoferum,* 3 – *Bacillus megatherium*. The biofertilizer (0.5 l from each strain + 9.5 l tap water) was added after one month of planting to plots according to the treatments shown below. These strains of the biofertilizer were mixed in equal parts and added to the clay soil during furrow irrigation. Ammonium sulphate (20.5% N) at the rate of 714 kg ha^{-1} and calcium super phosphate (15.5% P_2O_5) at the rate of 476 kg ha^{-1} were added during preparation of the soil. Potassium sulfate (48% K_2O) was added at the recommended dose (119 kg ha^{-1}).

Under laboratory conditions at the National Research Centre, distilled water was put in a flask, then peptone (5 g l^{-1}) and beef extract (3 g l^{-1}) were sterilized in an autoclave at 121ºC for 20 min. The flask was left at room temperature for 2 h until it cooled. After that, the strain was inoculated in a sterile room. The flask with the inoculant was incubated at 28ºC for 7-10 days to obtain the highest amount of growth. Finally, the strains were mixed and added to experimental soil. Treatments used in the field experiments were identified as follows: T1 (*Azospirillum lipoferum* + 50% NPK), T2 (*Azotobacter chroococcum* + 50% NPK), T3 (*Bacillus megatherium* + 50% NPK), T4 (mixture of biofertilizer T1, T2, and T3 + 50% NPK), T5 (50% NPK without inoculation), and T6 (100% NPK without inoculation)

The following data were recorded: plant height (cm), number of branches, plant fresh weigh (g), plant dry weight (g), fruit yield per plant (g), fruit yield (kg ha^{-1}), total carbohydrates (%), amount of NPK (%), oil yield per plant (ml), oil yield (l ha^{-1}), and oil constituents.

The experimental design was a completely randomized block design with three replications for each treatment. The area of each plot was 4 m^2 with three rows. The distance

T a b l e 1. Average values of main weather variables in Cairo*

Feature	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	
T (°C)	13.5	14.9	17.5	21.0	24.5	27.1	27.7	27.6	25.9	23.4	19.1	15.1	21.4[1]
SH (h)	213	222	243	279	315	372	357	333	309	297	228	201	3369[2]
RF (mm)	5.2	3.5	2.4	1.1	0.6	0.1	0.0	0.0	0.0	1.0	3.4	6.6	23.9[2]

*Source: Meteorological data of Cairo (CLAC, Egypt), average values from 2003 to 2005; SH – sunshine hours; RF – rainfall; [1] – average, [2] – total.

T a b l e 2. Properties of the soil used for growing fennel

Clay	Silt	Sand	$C_{org.}$	$M_{org.}$[1]	pH	EC (Sm^{-1})	N^2	P^3	K^3
		(%)						(ppm)	
55	25	20	0.16	0.48	8.03	0.63	490	39.8	280

[1] – organic matter, [2] – total, [3] – available.

between plants was 40 cm; the total number of plants in every plot was 15 plants. Seeds of fennel were sown in beds on the first of October during the two seasons. Growth, yield, and chemical composition during the two seasons were statistically analyzed according to the procedure of Snedecor and Cochran (1980). The means of the treatments were compared using the least significant difference test at the 0.05 level. Total carbohydrates in the dry leaves was determined by using a colorimetric method (Herbert *et al.*, 1971). Concentrations of nitrogen, phosphorus, and potassium in the dried leaves were determined using the method of Cottenie *et al*., 1982.

The volatile oil from fennel fruits was isolated by hydro distillation for 3 h in order to extract the essential oils using the method of Guenther (1961). The isolated volatile oil was dehydrated over anhydrous sodium sulphate and stored in a refrigerator until GC/MS (gas chromatography-mass spectrometry) analysis.

RESULTS AND DISCUSSION

Data in Table 3 show that fertilizing fennel plants using different strains of biofertilizers amended with a half dose of N, P, and K increased plant height, number of branches, plant fresh weight, plant dry weight, and fruit yield compared with the 50% and 100% NPK treatments. The largest amount of growth and yield occurred with 50% NPK and a mixture of strains *Azotobacter chroococcum*, *Azospirillum lipoferum*, and *Bacillus megatherium*, but the lowest amount of growth and yield occurred with the 50% NPK treatment. These results may be due to the role of *Azotobacter* and *Azospirillum* in nitrogen fixation. In addition, they provide growth promoting substances such as indole acetic acid and gibberellins (Fayez *et al.,* 1985). The phosphate-dissolving bacteria (PDB) *B. megatherium* also may have increased the availability of phosphorus, and it may have increasing the uptake of trace elements (El-Haddad *et a1.,* 1993).

These results are in agreement with those of Gad (2001) for fennel (*Foeniculum vulgare*) and dill (*Anethum graveolens*), who reported that biofertilizers on these plants increased growth and yield. Amin (1997), who studied coriander (*Coriandrum sativum*), fennel (*Feoniculum vulgare*), and caraway (*Carum carvi*), showed that the growth was influenced by seed inoculation (*Azotobacter* and *Azospirillum*) with a half dose of inorganic fertilizer. Plant growth was nearly equal to that obtained when the plants were fertilized with a full dose of inorganic fertilizer. Tehlan *et. al.* (2004) reported that plant growth and seed yield of fennel varied according to the strain applied.

T a b l e 3. Effect of biofertilizers on growth and fruit yield of fennel during the two seasons

Treatments	Plant height (cm)	Number of branches	Plant fresh weight (g)	Plant dry weight (g)	Fruit yield plant^{-1} (g)	Fruit yield ha^{-1} (t)
			First season			
Tr1	137.16	7.89	375.77	101.90	79.72	2.85
Tr2	133.99	7.11	350.08	99.00	76.56	2.73
Tr3	141.14	8.00	424.4	105.05	80.90	2.89
Tr4	151.44	8.11	469.82	113.10	90.32	3.23
Tr5	126.15	6.20	317.56	77.46	63.56	2.27
Tr6	131.70	6.77	335.88	81.54	67.67	2.42
LSD (5%)	14.83	NS	6.75	0.996	0.051	0.354
			Second season			
Tr1	140.19	8.00	384.66	103.00	80.00	2.86
Tr2	135.88	7.33	357.77	100.00	77.13	2.75
Tr3	140.99	8.89	430.00	106.33	81.66	2.92
Tr4	155.00	8.11	474.99	115.00	91.99	3.29
Tr5	129.11	6.77	318.00	77.77	64.00	2.28
Tr6	133.33	7.00	337.08	82.00	68.11	2.43
LSD (5%)	14.81	0.157	6.85	7.42	4.30	0.411

T1 (*Azospirillum lipoferum* + 50% NPK), T2 (*Azotobacter chroococcum* + 50% NPK), T3 (*Bacillus megatherium* + 50% NPK), T4 (mixture of biofertilizer T1, T2, and T3 + 50% NPK), T5 (50% NPK without inoculation), and T6 (100% NPK without inoculation); NS – not significant.

The highest oil yield (1.45 ml per plant or 51.8 l per ha) was obtained by a mixture of biofertilizer + 50% NPK (Table 4). The lowest oil yield (0.83 ml per plant and 29.6 l per ha) resulted with the 50% NPK treatment without inoculation. All treatments altered the composition of essential oils in fennel. These results agree with those of Kandeel *et al*. (2001) for *Foeniculum vulgar*, who found that the highest percentage of volatile oil resulted from inoculating the plants with *Azotobacter* + *Azospirilium* in the presence of a full dose of nitrogen, phosphorus, and potassium (714 kg ammonium sulphate + 714 kg calcium super phosphate + 190 kg potassium sulphate per ha). El-Sawy *et al.* (1998), who studied ammi (*Ammi visnaga*), reported that inoculation of the seeds with a mixture of *Azotobacter* and *Azospirillum* along with a full dose of rock phosphate and inorganic nitrogen fertilizers increased the content of khellin (4,9-dimethoxy-7-methyl-5H-furo(3,2-g) (1)-benzopyran, $C_{14}H_{12}O_5$)).

Table 4 also shows that inoculating fennel plants with *Azospirillum* + 50% NPK resulted in the highest N percentages. But the highest P and K percentages were obtained by fertilizing the plants with a mixture of the strains + 50% NPK. This is due to the effect of all strains. In addition, phosphate-dissolving bacteria secrete organic acids, which leads to a transfer of fixed phosphate to available phosphate. This may increase growth of roots in the soil that can take up phosphorus. Many investigators have explained the role of *Bacillus megatherium,* which increases the availability of phosphorus in the soil. Consequently there is an increase in phosphorus absorption as well as phosphorus accumulation in plant tissues**.** The lowest percentage of NPK occurred at half the recommended dose (50% NPK). T4 and T5 gave the highest and lowest total carbohydrates percentage. These results are in agreement with those of Gomaa and Abou-Aly (2001) on anise (*Pimpinella anisum*). They studied the effect of inoculation with non-symbiotic N_2-fixers (*Azotobacter chroococcum* and *Azospirillum brasilense*) in the presence of biogas manure or inorganic nitrogen fertilizer. The results showed that there was an increase in total nitrogen, phosphorus, and potassium of the anise plants due to inoculation with non-symbiotic N_2-fixers. Gad (2001) reported that nitrogen, phosphorus, and potassium in leaves of *Foeniculum vulgare* and *Anethum graveolens* were increased by applying biofertilizers.

The analysis of the essential oils in fennel (Table 5) showed the presence of 13 compounds. The major compound was anethol (*trans*-1-methoxy-4-(prop-1-enyl)benzeen; $C_{10}H_{12}O$)), followed by limonene (4-isopropenyl-1-methyl-cyclohexene; $C_{10}H_{16}$) in all the treatments. The minor compounds were α–pinene and β–pinene.

T a b l e 4. Chemical composition of fennel as affected by fertilizer treatments during the two seasons

Treatments	N	P	K	Total carbohydrates	Oil yield	
	(%)				(ml plant^{-1})	(l ha^{-1})
			First season			
Tr1	1.25	0.29	3.75	25.25	1.16	41.4
Tr2	1.19	0.27	3.70	24.90	1.10	39.3
Tr3	1.05	0.32	3.50	23.93	1.29	46.1
Tr4	1.20	0.37	4.00	29.60	1.45	51.8
Tr5	0.96	0.23	3.05	20.80	0.83	29.6
Tr6	1.04	0.23	3.35	22.60	0.95	33.9
LSD (5%)	0.18	0.037	0.095	0.77	0.038	4.3
			Second season			
Tr1	1.27	0.30	3.80	25.31	1.20	42.8
Tr2	1.19	0.29	3.75	24.96	1.13	40.3
Tr3	1.05	0.32	3.53	24.00	1.29	46.1
Tr4	1.22	0.38	3.91	31.11	1.47	52.5
Tr5	0.99	0.25	3.08	20.00	0.88	31.4
Tr6	1.11	0.23	3.35	21.9	0.97	34.6
LSD (5%)	NS	NS	NS	0.47	0.024	0.423

Explanations as in Table 3.

T a b l e 5. Chemical composition of fennel essential oil as affected by fertilizer treatments

Compounds	R.T.*	Concentration of compounds (%)					
		T1	T2	T3	T4	T5	T6
α - pinene	3.32	0.68	0.79	0.64	0.47	1.00	0.66
B - pinene	3.70	0.11	0.76	0.34	0.05	1.20	0.55
Unknown	4.20	0.36	-	0.33	-	-	0.55
Unknown	4.57	0.49	0.76	0.26	-	1.20	0.36
Unknown	5.53	0.35	-	-	-	-	0.23
Limonene	5.97	10.72	7.88	6.66	6.85	12.79	9.25
Unknown	6.26	0.47	-	-	-	0.49	-
Linalool	8.96	2.99	2.97	3.30	4.75	1.52	2.75
Anethol	15.75	79.35	82.42	86.16	85.76	80.28	83.36
Unknown	16.67	1.86	0.68	-	-	-	-
Methyl chavicol	19.35	2.34	2.43	2.09	1.93	1.66	1.90
Unknown	27.80	0.24	0.60	0.03	1.09	0.36	-
Unknown	29.00	0.01	-	-	-	-	-

Explanations as in Table 3. *R.T. – retention time.

CONCLUSION

Using biofertilizer (combined strains) plus half a dose of NPK have resulted in the greatest plant growth and yield.

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