Boosting Fennel Plant yield and Components using Combination of Manure, Compost and Biofertilizers.

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Field experiments were conducted during the two successive seasons of 2013/2014 and 2014/2015 to investigate the response of Fennel plants to manure, compost and biofertilizers. Thus, natural possible alternatives to highly soluble fertilizers had been used as manure and compost mixture with and without biofertilizers (two bacterial strains) inoculation on fennel plants yield, essential oil production and constituents in comparison with recommended dose of chemical fertilizers (NPK) as control.

The results revealed that manure and compost mixture with biofertilizers gave significant increases over control plants in all growth and chemical parameters. Where, the maximum value of fruit yield was recorded for plants received manure+compost +biofertilizers treatment in both seasons. The combined treatment of manure + compost + biofertilizers significantly increased the essential oil percentage as compared with the other treatments .The highest content of anethole as well as fenchone was obtained from NPK treatment. On the other hand, limonene resulted from the manure+ compost +biofertilizers treatment was higher than control. Myrcene compound was increased with manure + compost treatment in comparison with control. The highest value of estragole resulted from control plants, while the lowest amount of this compound was recorded from the treatment with manure+ compost +biofertilizers.

Key Words: Biofertilizers, Chemical fertilizers, Compost, Manure, essential oil, Fennel.

1. Introduction

Even though herbs had been priced for their medicinal, flavoring and aromatic qualities for centuries, the imitation products of the modern age surpassed their value for a while. However, the unsighted dependence on synthetics was trouncing all the barricades and incite people to come again to the naturals with optimism of safety and sanctuary.

The side effects of chemical treatments make medical plants more accepted among the other ways of treatment, therefore these herbs worth a lot (Sharifiashoriabadi et al., 2002 and Akbarinia et al., 2003). The cost of producing can be increased by using artificial fertilizers, however, they can cause some lethal ecological damages on the environment. For satisfying the current needs of herbs and steadiness of agriculture systems we must employ new input and sources (Rigby et al., 2001). The term "organic farming" at the present is well defined by the EU regulations 2092/91 (1991) and has been approved by the FAO and the WHO in 1999

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(Codex Alimentarius, 2001). Organic farming engages holistic production management systems (for crops and livestock) highlight the use of management practices in preference to the use of off-farm inputs. This is achieved by using, wherever possible, cultural, biological and mechanical systems in favorite to synthetic materials. Consequently to increase the crops quality particularly medicinal and aromatic plants, organic fertilization is more acceptable than chemical fertilizers and organic farming is a quality standard to be matched well by small farmers from all over the world (Abou El-Fadl *et al.* 1990).

Biofertilization including microbial inoculations is capable of improving the soil fertility and boost crop fertilizer use efficiency and consequently crop growth and yield (El-Naggar *et al.,* 2005) and (Badran 2009). This might be of economic value from the applied point of view of minimizing the applied dose of mineral fertilizers, thus reduce agricultural costs and soil pollution as well. The task of biofertilizers alone or in combination with organic or inorganic fertilizers has recently got appreciation in sustainable crop production (Kennedy *et al*., 2004; Bloemberg *et al*., 2000; Abdullahi and Sheriff, 2013). As a consequence several studies reported that, an increase in non-legume yields and its components due to inoculation with biofertilizer like *Azotobacter* and/or *Azospirillum* (Hamed, 1998; Said, 1998 Mohamed, 2000); (Ghallab and Salem, 2001); (Abd El -Maksoud, 2002; Khafagy, 2003 and Youssef *et al*., 2004). Quite a lot of experiments have shown that, the use of biological fertilizers make changes in essential oil's components (Darzi et al., 2008; Marotti et al., 1993 and Gross et al., 2002). Application of organic manures similarly, has affirmative effects on soil physical and biochemical properties. It lowers soil bulk density; increases water holding capacity, CEC, build up beneficial soil microbes, improve soil structure and aggregates as well (Doran, 1995; Drinkwater *et al*., 1995; Stamatiadis *et al*., 1999).

An important economic member of Apiaceae family is Fennel plants (Chiej, 1984), its perennial herb used to maintain health or treat symptoms of diseases such as digestive disorders, alleviating mild spasmodic, gastrointestinal ailments, relief of symptoms during inflammations of mucous membranes of the upper respiratory tract, mouth fresheners, treatment of anemia, constipation, increase immune system and brain function, and maintaining bone structure and decreases cancer tumor growth (EMA, 2008), and (medical news today). The pharmacopeia use concentrates on the fruits and the important ingredient represented in essential oil. According to the composition of the fruits oil the pharmacopeia differentiates between sweet fennel with about 2 % oil content with about 80 % anethole and bitter fennel with about 4 % oil content, 60% anethole and about 15 % fenchone (Wagner,

1999). There other components in the essential oil are Limonene, Estragole and Methyl Cavicol (Darzi et al., 2008).

Objectives

The present investigation was designed to study the effect of manure, compost and bio-fertilization on growth, productivity, essential oil yield and components of fennel plant in order to reduce and protect the environment against pollution caused by using extra chemical fertilizer applications, and to know which forms of organic fertilizers are the most appropriate for a good quality of fruits and essential oils.

MATERIAL AND METHODS

Site and soil

The present work was conducted at the Department of Ornamental Horticulture, Faculty of Agriculture, Cairo University, Giza, Egypt, during the two successive seasons of 2013/2014 and 2014/2015.Before planting, the soil was first mechanically ploughed and planked twice till the soil surface has been settled, some physical and chemical analysis of the soil was carried out as described by Page *et al*., (1982) represented in (Table 1).

Plant materials

Fennel (*Foeniculum vulgare* Mill) seeds (fruits) were obtained from the experimental Farm of Medicinal and Aromatic plants, Faculty of Pharmacy, Cairo Univ., Egypt**.** The experimental field was cropped with fennel seeds by broadcasting method on 13th Oct for the two seasons 2013/2014 and 2014/ 2015, the rows were at 40 cm abort, while it was 30 cm between the plants. The harvesting was done on 15th May for two years, No pests or diseases were found during the growing period in both seasons, hence no pesticide was used.

Table 1: Some soil physical and chemical properties of the experimental farm.

Parameter	2013/14	2014/15	Parameter	2013/14	2014/15
Physical characteristics			Chemical		
Texture	Clay	Clay	Soluble cations(meq/l)		
Clay (%)	37.1	40.5	Ca^{++}	7.00	7.22
Silt (%)	36.2	35.1	Mg^{++}	2.87	2.98
Fine sand (%)	22.9	21.0	K^{+}	0.27	0.33
Coarse sand (%)	3.80	3.40	Na^{+}	5.88	6.22
pH	8.03	7.88	Soluble anions (meq/l)		
EC dS/m	1.65	1.63	Cl^{-}	3.60	3.50
Organic matter%	1.50	1.70	SO_4^{-}	2.38	2.45
			Available N (ppm)	27.0	30.1
			Available P (ppm)	20.5	22.5

Treatments:

The fertilizer treatments were applied as follows:-

1. Full dose of NPK as recommended doses.
2. Compost + manure 1:1 (w/w).
3. Biofertilizers mixture. (*Azospirillum lipoferum* + *Bacillus megaterium*)
4. Compost + manure + biofertilizers.

NPK Treatment (control):

Recommended fertilizers doses were 125 kg / feddan ammaonium sulphate (20.5%N), 25 kg/feddan potassium sulphate (48% K_2O) and 20 kg / feddan mono-calcium super phosphate (15.5% P_2O_5). Super phosphate was added during soil preparation before sowing. Ammonium sulphate and potassium sulphate were added at twice equal dose. The first one was done after two weeks and the second after one month from sowing date.

Compost + manure mixture 1:1 (2.5 tons for each) was added to the soil 3 weeks before sowing. Analysis of the compost and manure components were determined according to Jackson (1973), shown in (Tables 2.and 3)

Biofertilizers

Two bacterial cultures (strains) containing 109-110 c.f.u. from *Bacillus megaterium* and *Azospirillum lipoferum* were prepared individually in Biofertilizer Unit, Soils Water and Environ. Res. Inst., Dept. of Microbiology, Agriculture Research Center (A. R. C.),Giza, Egypt, then, they were mixed well together by 1:1 (v/v). Inoculation was performed just before sowing: bacterium suspension was mixed with fennel seeds before planting, this mixture of bacteria was added to plant rhizosphere through a view holes already made in the soil surface for inoculated (4 holes/ row) at 30, 60 and 90 days after transplanting.

Table 2: Some chemical analysis of compost

Property	Value	Property	Value
pH (1:5)	7.5	N-NH^+_4 (ppm)	274.7
EC (1: 5 extract) ds/m	3.1	N-NO^-_3 (ppm)	33.1
Organic-C %	33.11	Ashes %	30
Organic matter %	70	Total content of Bacteria	2.5×10^7
Total-N %	1.82	Total content of Fungi	7×10^5
Total-K %	1.25	Weed seeds	0
C/N ratio	14:1	Nematode	0
Total-P %	1.29	Phosphate dissolving Bacteria	2.5×10^6
Fe-ppm	1019	Dehydrogenase activity (mg TPF/100g)	32.5
Mn-ppm	111	Nitrogenous activity (N mol C_2H_4/g/hr)	123.5
Cu-ppm	180	Seed germination after 48 hr. (%)	81.5
Zn-ppm	280		

Table3. Some chemical analysis of manure.

Property	Value	Property	Value
Macronutrients (%)		Micronutrients (ppm)	
N %	3.21	Fe	20.2
P%	0.71	Zn	31.2
K%	1.15	Mn	12.8
pH	7.50	Cu	25.0
C/N ratio	18.9	Organic matter (%)	63.6

Recorded parameters:

For interpretation of the growth characteristics the following parameters have been taken into account at harvest time:

-Plant height (cm).

-Number of branches per plant.

- Stem diameter (cm).

-No. of umbels/plant.

-Weight of fruits /plant (gm).

-Diameter of umbell(cm).

-Weight of 100 seeds / gm.

-Herb fresh weight (Ton / fed.).(20000 plant density).

-Herb dry weight (Ton / fed.).

Chemical analysis

Chemical composition of the fresh leaves:

Total chlorophyll contents were determined in fresh leaves samples (mg/g FW) according to (Saric*et. al*., 1967).

Chemical composition of the dry herb

The herb at cutting date was dried in an electric oven at 70C° for 24 hours according to (A.O.A.C.1970), then finely ground for chemical determination of macro-elements (N,P and K) The nitrogen percentage was determined after digestion according to the micro-kjeldhl method (Jackson, 1967). The phosphorus percentage was determined colorimetrically according to the method of (MurphyandReily 1962). Potassium percentage was determined with using the Atomic Absorption Spectrophotometer (3300) according to (Wilde *et al.* 1985). Total carbohydrates in the dry leaves were determined by using a colorimetric method (Herbert *et al*., 1971).

Volatile oil of fruits:

1-Volatile oil percentage

2-Volatile oil constituents.

Essential oil extraction

Essential oil was isolated using a Clevenger-type apparatus according to (Marotti and Piccaglia 1992). 100 g of fruits have been distilled for three hours in one liter water. The volatile oil obtained was analyzed using DsChrom 6200 Gas Chromatograph equipped with a flame ionization detector for separation of volatile oil constituents. The analysis conditions were as follows:-

-The chromatograph apparatus was fitted with capillary column BPX-5, 5% phenyl (equiv.) polysillphenylene-siloxane 30m x 0.25mm ID x 0.25µm film.

-Temperature program ramp increase with a rate of 1º C / min from 70º to 80º C, rate of 5º C / min from 80º to 120º C and rate of 10º C / min from 120º to 190º C

-Flow rates of gases: Nitrogen at 1 ml / min, hydrogen at 30 ml / min and 330 ml /min for air.

-Detector and injector temperatures were 300º C and 250º C, respectively. The obtained chromatogram and report of GC analysis for each sample were analyzed to calculate the percentage of the main components of the volatile oil.

Statistical Analysis

The experiment was designed at complete randomized block with 5 replicates in 4 treatments. Data on 5 plants were recorded for each treatment as replications, and analyzed using ANOVA at 5% significance level. The difference between treatments then analyzed using DMRT (Duncan Multiple Range Test) at 5%.

RESULTS AND DISCUSSION

Growth characters and yield:

Data in Tables (3 and 4) showed that growth characters of fennel plants were influenced by manure and compost mixture with biofertilizers treatments either individual or in combination in both growing seasons. Where, it was clear that, biofertilizers treatment alone resulted in significantly the lowest values as compared to NPK treatment regarding all growth parameters.

Table 3: Growth characters of fennel plants as affected by NPK, manure, compost, biofertilizers and their combination during the two growth seasons.(2013/2014 and 2014/2015).

Treatments		Plant height (cm)	No. of branches / plant	Stem diameter (cm)	Herb F.W (Ton / fed.)	Herb D.W (Ton / fed.)
NPK (Control)	F1	92.5b	12.2b	1.26b	4.560b	1.127b
	F2	94.4b	14.4b	1.33b	4.778b	1.221b
Compost+ manure	F1	78.2c	13.3b	1.19c	4.470b	1.015b
	F2	81.8c	13.4b	1.25b	4.580b	1.120b
Biofertilizers	F1	70.6d	9.5c	1.10c	3.421c	0.850c
	F2	73.3d	10.2c	1.14c	3.509c	1.002b
Compost +manure + biofertilizers	F1	118.3a	16.3a	1.52a	5.203a	1.700a
	F2	123.2a	18.1a	1.58a	5.807a	1.970a

Means with the same letter in a column are not significantly different by DMRT 5%
F1= first season F2= second season

Table 4: Yield characters of fennel plants as affected by NPK, manure, compost, biofertilizers and their combination during the two growth seasons.(2013/2014 and 2014/2015).

Treatments		No. of umbels /plant	Weight of fruits/plant /g	Diameter of umbel (cm)	Weight of 100 seed / g
NPK (Control)	F1	13.2b	31.29b	118.32c	0.60c
	F2	15.3b	33.95b	120.24c	0.69b
Compost+ manure	F1	12.4c	25.38c	115.97c	0.56c
	F2	14.6b	29.41b	120.11c	0.59c
Biofertilizers	F1	9.5c	15.21d	96.71d	0.38d
	F2	11.3c	18.97d	109.22d	0.45d
Compost +manure + biofertilizers	F1	16.2b	39.61a	130.51b	0.71b
	F2	21.3a	45.84a	150a	0.89a

Means with the same letter in a column are not significantly different by DMRT 5%
F1= first season F2= second season

It was clear that mixture of manure with compost inoculated with biofertilizers significantly increased all growth characters as compared to the control treatment (NPK) in both seasons.

The favorable effects of the combination between manure and compost and biofertilizers may be attributed to the effect of the beneficial bacteria on availability of the nutrient, vital enzymes, and hormonal stimulating effects on plant growth or increase of photosynthetic activity. Moreover, biofertilizers have several possible modes of action on plant growth and N_2 fixation, which contributes N to the plant, hormonal effects which alter plant metabolism and growth. General growth improvement in the entire root system resulted in enhanced mineral and water uptake. Similar results had been reported by (Bashan *et al*., 1989) who found that *Azospirillum* and *Pseudomonas* improved wheat growth through the significant increase in the

dry matter, which accumulated in both roots and shoots of the treated plants. Moreover (Valssak and Reynders 1980) found that *Azospirillum* produces IAA, Indol lactic acid, gibberellins by bacteria and degree of sensitivity of plants to phytohormones are being suggested as the main reason for this phenomenon (Jlik,1995). At the same time, the significant positive effect of manure and compost fertilizers on vegetable growth characters may be due to the improvement the soil physical and biological properties, and activates many beneficial species of living organisms due to luxury of organic matter availability, which release phytohormones and may stimulate the plant growth and absorption of nutrients, as well water use efficiency by different plants (Abd El-Moez*et al.*, 1999), and (Ghallab and El-Gahadban 2004).

Chemical analysis:

The data presented in (Table 5), showed that the effects of different treatments in Total chlorophyll, Total carbohydrates, and concentrations of N, P and K **%** in the shoots of fennel plants were similar to those recorded for growth characters.

Table 5. Total chlorophyll (mg/g fresh weight), total carbohydrates (%) and N, P, K (%)of fennel plants as affected by different fertilizers treatments during growth seasons, 2013/2014 and 2014/2015.

Treatments		Total chlorophyll mg/g FW	Total carbohydrates %DW	N %DW	P %DW	K % DW
NPK (Control)	F1	5.1b	18.45b	2.3a	0.27b	3.41b
	F2	5.7b	20.37b	2.5a	0.29b	3.63a
Manure + compost	F1	4.6c	17.89b	1.9b	0.26b	3.32b
	F2	5.3b	20.03b	2.2b	0.27b	3.56b
Biofertilizers	F1	3.2d	16.22b	1.2c	0.22c	3.04c
	F2	3.8d	18.21b	1.6c	0.23c	3.23b
Manure + compost + biofertilizers	F1	7.5a	26.32a	2.6a	0.32b	3.81a
	F2	8.7a	28.07a	2.9a	0.45a	3.88a

Means with the same letter in a column are not significantly different by DMRT 5%

The data (Table 5) evidently showed that, there were significant increases in total chlorophylls, total carbohydrates, N, P and K concentration obtained with the combination of manure, compost and biofertilizers treatments as compared to the control (NPK) treatment in both first and second season. On the other hand, application of biofertilizers treatment alone produced the lowest values of same previous parameters in comparison with either control plants or other treatments, although these diminishing were not significant with some parameters represented in total carbohydrates and K concentration . This escalating resulted

from manure and compost mixture with biofertilizers application might be due to the increase in the root surface per unit of soil volume as well as the high capacity of the plants supplied with compost fertilizer in building metabolites, which in turn contribute much to the increase of nutrient uptake. In this respect (Abd El-Moez*et al.*, 1999) on fennel and coriander plants and (Ghallab and El-Gahadban 2004) on marjoram plants, found that the macronutrient uptake by plant roots increased significantly by addition of organic compost to the prepared soil. They attributed the results to the effect of organic fertilizer on improving not only soil physical and biological properties but also chemical characteristics resulting in more release of available nutrient elements to be absorbed by plant roots and the water efficiency by different plants. These results are in agreement with those obtained by (Jacoub 1999) on *Ocimum basilicum* L. and *Thymus vulgaris* L. plants; (Mansour *et al.*, 1999) on spearmint and marjoram plants and (Badran and Safwat 2004) on fennel plants.

Regarding the promotion effects of biofertilizers mixture on macronutrients accumulation could be attributed to the inoculation of fennel plants, which resulted in a further effect on roots development and consequently their function in the uptake of the water and nutrients. Parallel results were reported by (Harridy*et al.*, 2001) who mentioned that, in soils deficient in P, mycorrhizal colonization supports plant development by supplying the plant with additional P and sometimes with N, K on lemongrass. More supportive evidences for the obtained results were found in the work of Ghallab and Salem 2001 on wheat, they found that the application of *Azospirllium* and *Serratia* mixture inoculation greatly enhanced the uptake of N, P, K as well as accumulation of these minerals in wheat plants. Also Omar *et al.,* 1991 reported that inoculation with *Azospirllium* cultures stimulated the uptake and accumulation of nutrients from the soil. Furthermore Dashti*et al.,* 1997 suggested that the promotive mechanism of growth and nitrogen fixation had been induced by plant growth-promoting rhizobacteria (PGPR) and beneficial fungus included both direct and indirect effects. The direct effects include an increase in the mobilization of insoluble nutrients followed by uptake enhancements by the plants (Lifshitz*et al.,* 1987) and production of plant growth regulators that stimulate plant pigments and growth (Gaskins *et al.,* 1985). The indirect effects include positive effects on symbiotic nitrogen fixation by enhancement of root nodule number or mass (Yahalom*et al.,* 1987).

Essential oil percentage.

The results showed that the combined treatment of manure + compost + biofertilizers significantly increased the essential oil percentage in the fruits, giving 1.25 and 1.51 % ,in both seasons respectively (Table 6) as compared with other treatments giving the lowest values 0.18 and 0.21 %,respectively of essential oil percentage for biofertilizers treatment. The increment of volatile oil productivity with combined treatment could be explain on the basis of available elements, vitamins, gibberellins, cytokines , hormone like substances, amino acids and sugars that lead to an increase in biochemical processes within the plant (luxury of metabolism) reflected on vegetative growth, in terms of the fresh yield consequently an increase in volatile oil percentage. Similar results were recorded by (Mohamed and Matter 2001) on marigold plants.

Chemical composition of oil

The components of fennel essential oil are qualitatively different between not only the different varieties of fennel: *vulgare* (bitter), and *dulce* (sweet) (Marotti*et al.* 1994) but also between the continental cultivars of fennel (Masada, 1976, Massoud, 1992 and Braun and Franz, 1999)**.** Therefore, in this research it was necessary to investigate the effect of different treatments on fennel essential oil main components.

The analysis of the essential oils in fennel fruits (Table 6) showed the presence of 07 compounds. The major compound was anethole, followed by limonene in all of the treatments. These results are in agreement with those obtained by (Embong*et al.* 1977) and (Braun and Franz 1999) who found that anethole, limonene, estragole and fenchone are the major constituents of fennel essential oil.

The highest contents of anethole 50.4 %, as well as fenchone0.60% were obtained from control plants (NPK) but the increment was not too high in comparison with data obtained from manure, compost and biofertilizers combination. On the other hand, limonene resulted from later treatment (40.5%) was higher than control (38.5 %). Also an augmentation was recorded in estragole resulted from control plants (4.9%), while the lowest value (3.8%) of this compound was obtained as a result of the mixture of manure, compost and biofertilizers. Myrcene compound (1.14) was increased with manure, compost and biofertilizers combination treatment in comparison with control.

Table 6. Percentage of fennel essential oil and its components as affected by different treatments during growth season

Treatments	NPK (Control)	Manure + compost	Biofertilizers	Manure + compost+ biofertilizers
Percentage of essential oil in fruits during growth seasons, 2013/2014 and 2014/2015.				
Oil%, first season , 2013/2014	0.63c	0.52c	0.18e	1.25a
Oil%, second season, 2014/2015	0.85b	0.81b	0.21d	1.51a
Oil components % , in the second season 2014 / 2015				
Anethole	50.4	40.9	46.2	49.1
Limonene	38.5	40.0	43.2	40.5
Fenchone	0.60	0.20	0.40	0.50
Estragole	4.90	4.00	4.70	3.80
Myrcene	0.97	1.14	0.55	0.68
α- pinene	0.64	0.71	0.45	0.69
B - pinene	0.73	0.82	0.92	0.89
Unknown	0.52	0.69	1.32	0.45

This finding strongly confirms the previous conclusion drawn about the essential oil percentage, and macronutrients (Table 6). In this respect (Bajaj, 1999) and (Nagata and Ebizuka, 2002) indicated that almost all oil components of fennel plants have medical useful effect; in the sense that any components having high concentration in oil and high medical effect if well purified and used alone, does not give the required medical effect unless it is found together with other oil components even with less percentage. This indicates the fact that all oil components rally together to induce the useful medical effect.

CONCLUSION

It could be concluded that, the combined treatment of manure, compost with biofertilizers increased the studied morphological characters, yield and different chemical parameters of fennel plants. This combination of organic and biological organisms resulted in considerable and even significant increases in the most studied parameters, over the control treatment (plants receiving only the recommended dose of NPK fertilizer). In addition to that, it was obvious that, manure and compost were more efficient when mixture with biofertilizers, due to its characters and enrichment of nutrients in comparison with biofertilizers mixture alone, as a result we can assess that obtained economic yield cannot be relay on application of biofertilizers alone. Also economic value should be taken into consideration, the production of safe plants without using high costs of chemical fertilizers which in turn minimize the agricultural costs as well as environmental pollution. The mixture of manure, compost and

biofertilizers reduced estragole from (4.9%) for the control plants (NPK) to the lowest amount (3.8%) for the combined treatment of manure, compost with biofertilizers.

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