

Effect of Phosphorous Fertilization on Anise, Coriander and Sweet Fennel Plants Growing under Arid Region Conditions

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ABSTRACT

Arid regions in Egypt are characterized by poor nutrients such as phosphorous (P) and unfavorable environmental conditions which negatively affect growth and productivity of medicinal and aromatic plants including anise (*Pimpinella anisum* L.), coriander (*Coriandrum sativum* L.) and sweet fennel (*Foeniculum vulgare* var. Dolce) plants. Thus, the main objective of the present investigation was to study the effect of different levels of P fertilizer as calcium superphosphate (15% P₂O₅) at 0 (control), 37.5, 56.3 and 75 kg ha⁻¹ P₂O₅ on the morphological and biochemical contents of these three plants under arid conditions over two successive seasons. The most effective rate was 56.3 kg ha⁻¹, resulting in a positive increase in vegetative growth characters [plant height (cm), leaf number (plant⁻¹), branch number (plant⁻¹), umbel number (plant⁻¹), herb fresh weight (plant⁻¹), herb dry weight (plant⁻¹) and fruit yield (plant⁻¹)]. The highest values of vegetative growth characters were 44.5, 35.6, 10.9, 31.2, 20.9, 6.8 and 6.9, respectively for anise; 83.0, 66.1, 7.8, 24.7 19.9, 8.7 and 6.9, respectively for coriander; 98.9, 32.8, 6.9, 16.8, 113.4, 85.8 and 24.7, respectively for sweet fennel. 75kg ha⁻¹ led to higher biochemical contents than the control. The increases were 1.2, 0.4 and 0.6% in essential oil; 3.7, 6.0 and 2.1% in fixed oil, 6.6, 11.0 and 9.0% in total carbohydrates; 0.6, 0.3 and 0.4% in soluble sugars; 3.7, 4.9 and 3.9% in crude protein; 1.4, 1.3 and 1.2% in nitrogen; 1.9, 0.8 and 1.5% in phosphorous; 1.5, 1.1 and 0.7% in potassium for anise, coriander and sweet fennel, respectively.

Keywords: essential oil, fixed oil, growth, nutrients, crude protein, soluble sugars, total carbohydrates

Abbreviations: CP, crude protein; EO, essential oil; FO, fixed oil; P, phosphorous; SS, soluble sugars; TC, total carbohydrates; VGC, vegetative growth characters

INTRODUCTION

Anise (*Pimpinella anisum* L., *Apiaceae*) has been used as an aromatic herb and spice since Egyptian times and antiquity and has been cultivated throughout Europe (Hänsel *et al.* 1999). In folk medicine, anise is used as an appetizer, tranquillizer and diuretic drug (Tyler *et al.* 1988; Lawless 1999). The traditional use of Pernod, Ouzo, Anisette, Raki, and many other anise-flavoured drinks after a heavy meal is a familiar example of its antispasmodic effect, especially in the digestive tract (Hänsel *et al.* 1999). Dried fruits of anise, commercially called aniseeds, contain the whole dry cremocarp of anise. For medical purposes, they are used to treat dyspeptic complaints and catarrh of the respiratory tract, and as mild expectorants. It was also reported that extracts from anise fruits have therapeutic effects on several conditions, such as gynaecological and neurological disorders (Czygan and Anis 1992; Lawless 1999). Ethanolic extract of anise-fruits contains *trans*-anethole, methylchavicol (estragole), eugenol, pseudoisoeugenol, anisaldehyde, coumarins (umbelliferon, scopoletin), caffeic acid derivatives (chlorogenic acid), flavonoids, fatty oil, proteins, minerals, polyenes and polyacetylenes as its major compounds (Hänsel *et al.* 1999). Due to their unique and preferred flavor and aroma, the swollen bases of sweet fennel (*Foeniculum vulgare* var. Dolce, *Apiaceae*) are freshly consumed in salads or cooked as a kitchen vegetable (Stuart 1982; Haupt 1986; Marotti *et al.* 1993). The major constituents of sweet fennel essential oil (EO) such as anethole and limonene are also used as essence in cosmetics and perfumes and for some medicinal purposes (Stuart 1982; Marotti *et al.* 1993). Coriander (*Coriandrum sativum* L.) is a culinary and medicinal plant that belongs to the *Apiacea* family. Coriander is of economic importance since it is used as a flavoring

agent in food products, perfumes and cosmetics. As a medicinal plant, *C. sativum* has been credited with a long list of medicinal uses. Powdered seeds or dry extract, tea, tincture, decoction or infusion have been recommended for dyspeptic complaints, loss of appetite, convulsion, insomnia and anxiety (Emamghoreishi 2005). Moreover, the EO and various extracts from coriander have been shown to possess antibacterial (Burt 2004), antioxidant (Wangensteen *et al.* 2004), anticancerous and antimutagenic (Chithra and Leelamma 2004) activities. Many phytochemical studies were investigated on the chemical composition of EO extracted from *C. sativum* fruits obtained from different origins (Steinegger and Hansel 1988). The composition of the EO extracted from leaves has also been evaluated (Eyres *et al.* 2005). The yield of EO extracted from *C. sativum* fruits showed a marked increase during maturation and linalool was the main compound at the fruiting stage (Kamel *et al.* 1994).

Plant nutrition is one of the most important factors that increase plant production. Phosphorous (P) plays an important role in various plant metabolic processes. It is a constituent of nucleic acid, phospholipids, the coenzymes, DNA and NADP, and most importantly ATP. It activates coenzymes for amino acid production used in protein synthesis; it decomposes carbohydrate production in photosynthesis, and is involved in many other metabolic processes required for normal growth such as photosynthesis, glycolysis, respiration, fatty acid synthesis. Moreover, P enhances seed germination and early growth, stimulates blooming, enhances bud set, aids in seed formation and hastens maturity (Espinosa *et al.* 1999). P leads to enhanced herb and EO yields of different mint species (Kothari *et al.* 1987). P is abundant in fruits and seeds of plants (Papadopoulos 1994). P fertilization increased the vegetative growth, EO, fixed oil

Table 1 Mechanical and chemical analysis of the soil.

Sand	Silt	Clay	Gravel	pH	EC		
					dS m ⁻¹		
%							
79.7	13.0	7.3	18.7	8.7	2.0		
Ca ⁺⁺	Mg ⁺⁺	Na ⁺⁺	K ⁺	CO ₃	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻
meq L ⁻¹							
4.9	5.6	11.9	0.6	1.8	1.9	18.6	1.2
K	Fe	Cu	Zn	Mn			
				mg L ⁻¹			
0.5	5.4	0.4	0.3	1.6			

(FO), total carbohydrates (TC), total soluble sugars (TSS) and NPK content of some *Apiaceae* (*Umbelliferaeae*) plants (Khalid 1996). It is widely found that increasing P as a fertilizer will promote reproductive yields (Egle *et al.* 1999) and inflorescence production (Besmer and Koide 1999), particularly when P is limiting in natural systems (Feller 1995). Conversely, limited P supply decreases the production of floral structures (Arnon and Hoagland 1943; Shamsi and Whitehead 1977; Ma *et al.* 2001). P concentrations were manipulated in order to maximize flower-head yield of *Calendula officinalis* L.; high P concentrations did not increase flower production; instead, they produced significantly more leaf biomass (Stewart and Lovett-Doust 2003). Three different concentrations of P (5, 30, and 60 mg L⁻¹) in nutrient solution were used to cultivate *Origanum dictamnus*, and significant differences (qualitative and quantitative) were observed between the EO samples (Economakis 2002). P had a stimulating effect on several growth parameters, TC, SS, mineral contents and EO production of chamomile flowers compared with the control (Nassar *et al.* 2004). Five levels of P (0, 30, 60, 90 and 120 kg P₂O₅ ha⁻¹) were evaluated; 90 kg P₂O₅ ha⁻¹ significantly increased fruit yield and TC of *Trichosanthes cucumerina* L. compared with other levels (Adebooye and Oloyede 2007). *T. cucumerina* plants treated with 150 and 200 kg P₂O₅ ha⁻¹ had the greatest plant height, highest number of leaves, root biomass and P content compared with the control (Karthikeyan 2008). 150 kg fed⁻¹ (1 feddan = 4200 m²) of superphosphate calcium produced maximum fresh leaves yield of artichoke (*Cynara cardunculus* var. 'Scolymus') plants (Ezz El-Din *et al.* 2010).

Arid regions in Egypt are characterized by poor nutrients such as P and unfavorable environmental conditions which negatively affect growth and productivity of medicinal and aromatic plants including anise, coriander and sweet fennel plants (Abd-Allah *et al.* 2001). The main objective of the present investigation was to study the effect of different levels of P fertilizer on the morphological and biochemical contents of anise, coriander and sweet fennel plants under these arid conditions.

MATERIALS AND METHODS

Experiments were carried out in an arid region at the Experimental Farm of the Desert Development Center (DDC), Sadat City, American University, Egypt, during two successive seasons in 1992/93 and 1993/94. The DDC area had been recently reclaimed and had not been cultivated before. Physical and chemical properties of the soil used in this study were determined according to Jackson (1973) and Cottenie *et al.* (1982) and are presented in **Table 1**. Coriander and anise seeds were kindly provided by the Department of Medicinal and Aromatic Plants, Ministry of Agriculture, Giza, Egypt whereas sweet fennel seeds (var. 'Dulce') were kindly obtained from France (Université d'Avignon). Sweet fennel seeds were sown in the third week of October during both seasons. Sweet fennel seedlings were transplanted into an open field 45 days after sowing. At the same time, coriander and anise seeds were sown directly in an open field. The experimental design was a complete randomized block with four replicates. The experimental area (plot) was 30 m² (4 m × 7.5 m) containing 15 rows; the distance between hills was 25 cm and 50 cm apart. Plants were thinned to two per hill 45 days after cultivating plants in the open

field. All agricultural practices other than experimental treatments were performed according to the recommendations of the Egyptian Ministry of Agriculture. Plots were divided into four groups subjected to soil application of P (P₂O₅) as calcium superphosphate (15% P₂O₅) at 4 rates: 0 (control), 37.5, 56.3 and 75 kg ha⁻¹.

Harvesting

At the fruiting stage, 20 plants from each replicate were harvested at the end of both first and second seasons. Vegetative growth characters (VGC) measurements [plant height (cm), leaf number (plant⁻¹), branch number (plant⁻¹), umbel number (plant⁻¹), herb fresh weight (plant⁻¹), herb dry weight (plant⁻¹) and fruit yield (plant⁻¹)] were recorded.

EO isolation

Ripened fruits were collected from each treatment at the end of the first and second season. 100 g from each replicate of all treatments was subjected to hydro-distillation for 3 h using a Clevenger-type apparatus (Clevenger 1928). The EO content was calculated as a percentage.

TC and TSS

TC and SS concentrations in leaves (collected at the end of the first and second season of each treatment) were determined according to Ciha and Brun (1978) with some modifications. Samples of 100 mg were homogenized with 10 mL of extracting solution [glacial acetic acid: methanol: water, 1:4:5, v/v/v for TSS or glacial acetic acid: H₂SO₄ (1n): water, 1:4:5, v/v/v for TC]. The homogenate was centrifuged for 10 min at 3,000 rpm and the supernatant was decanted. The residue was resuspended in 10 mL of extracting solution and centrifuged another 5 min at 3,000 rpm. The supernatant was decanted, combined with the original extract and made up to 50 mL with water. For measurement of TC and TSS, a phenol-sulfuric acid assay was used (Dubois *et al.* 1956). A volume of 0.5 mL of 5% (v/v) phenol solution and 2.5 mL of concentrated sulfuric acid were added to 0.5 mL aliquots. The mixture was shaken, heated in a boiling water-bath for 20 min and cooled to room temperature. The absorption was then determined by spectrophotometry at 490 nm.

FO, nutrients and CP determination

FO Extraction: 50 g of fruits were crushed to coarse powder and extracted with petroleum ether (40-60°C) in a Soxhlet apparatus (AOAC 1970).

N, protein, P and K (in the leaves) of both seasons of each treatment were determined using the methods described by the AOAC (1970) as follows:

The washed and dried materials were ground to fine powder with mortar and pestle and used for dried ashing.

For analysis of K the powdered plant material (0.2 g) was taken in pre-cleaned and constantly weighed silica crucible and heated in muffle furnace at 400°C till there was no evolution of smoke. The crucible was cooled in desiccator at room temperature. The ash totally free from carbon moistened with Conc. H₂SO₄ and heated on Hot plate till fumes of sulphuric acid get evolved the silica crucible with sulphated ash was again heated at 600°C in muffle furnace till weight of sample was constant (3-4 h) one gram sulphated ash were taken in beaker which dissolved in 100 ml 5% conc. HCl to obtain solution for determination of K through flame photometry, standard solution of each mineral was prepared and calibration curve drawn for K element using flame photometry.

For determination of protein and nitrogen using Micro Kjeldahl method, 1 g of plant sample taken in a Pyrex digestion tube and 30 ml of conc. H₂SO₄ carefully added, then 10 g potassium sulphate and 14 gm copper sulphate, mixture is placed on sand both on a low flame just to boil the solution, it was further heated till the solution becomes colorless and clear, allowed to cool, diluted with distilled water and transferred 800 ml Kjeldahl flask, washing the digestion flask, Three or four pieces of granulated zinc and 100 ml of 40% caustic soda were added and the flask was connected with the splash heads of the distillation apparatus. Next,

Table 2 Effect of P fertilization on the VGCs.

P ₂ O ₅ treatments (kg ha ⁻¹)	VGC						
	Plant height (cm)	Leaf number (plant ⁻¹)	Branch number (plant ⁻¹)	Umbel number (plant ⁻¹)	Plant fresh weight (plant ⁻¹)	plant dry weight (plant ⁻¹)	Fruit yield (plant ⁻¹)
Anise (<i>Pimpinella anisum</i>)							
0	24.5	21.6	7.7	16.8	11.9	2.8	2.8
37.5	41.3	32.6	8.4	203	18.6	4.9	5.9
56.3	44.5	35.6	10.9	31.2	20.9	6.8	6.9
75	41.3	33.1	7.9	25.3	18.5	4.9	4.3
LSD:							
0.05	3.5	3.8	0.5	2.8	2.3	0.2	0.7
0.01	4.2	4.7	0.9	3.1	3.4	1.1	1.1
Coriander (<i>Coriandrum sativum</i>)							
0	71.2	41.9	5.6	13.9	10.9	2.4	3.8
37.5	80.0	48.8	6.9	22.3	12.3	3.5	5.2
56.3	83.0	66.1	7.8	24.7	19.9	8.7	6.9
75	81.2	48.6	6.6	18.6	19.0	8.5	4.3
LSD:							
0.05	4.7	1.9	0.2	3.2	2.6	0.9	0.8
0.01	5.9	2.3	0.4	4.2	3.9	1.2	1.3
Sweet fennel (<i>Foeniculum vulgare</i> var. 'Dulce')							
0	75.1	21.3	4.2	9.4	88.9	34.6	15.9
37.5	87.8	22.8	5.1	12.6	100.4	71.8	21.3
56.3	98.9	32.8	6.9	16.8	113.4	85.8	24.7
75	82.2	23.3	5.1	13.1	110.9	75.3	18.6
LSD:							
0.05	6.4	2.1	0.3	2.1	5.9	9.8	3.5
0.01	7.6	2.8	0.5	3.8	8.7	11.2	4.4

25 ml of 0.1 N sulphuric acids was taken in the receiving flask and distilled; it was tested for completion of reaction. The flask was removed and titrated against 0.1 N caustic soda solution using Methyl Red indicator for determination of nitrogen, which in turn give the protein content.

For determination of phosphorous 2 g sample of plant material taken in 100 ml conical flask two spoons of Darco-G-60 is added followed by 50 ml of 0.5 M NaHCO₃ solution, next flask was corked, and allowed for shaking for 30 min on shaker. The content was filtered and filtrate was collected in flask from which 5 ml filtrate was taken in 25 ml volumetric flask to this 2 drops of 2,4-paranitrophenol and 5 N H₂SO₄ drop by drop was added with intermittent shaking till yellow color disappear, content was diluted about 20 ml with distilled water and then 4 ml ascorbic acid was added then the mixture was shaken well and the intensity of blue color at 660 nm on colorimeter was measured. The absorbances were compared and concentrations of phosphorous using standard value were calculated.

Statistical analysis

The averages of data from both seasons were statistically analyzed using analysis of variance (ANOVA) and least significant difference (LSD) values at 1 and 5% were calculated according to Snedecor and Cochran (1990).

RESULTS AND DISCUSSION

Effect of P fertilization on VGC

Data in **Table 2** shows the response of VGCs in anise, coriander and sweet fennel plants to the different rates of P. All P treatments were superior to the control treatment and significantly improved the VGCs. 56.3 kg ha⁻¹ resulted in the highest values of VGC compared with the control treatment. The highest values of VGC were 44.5, 35.6, 10.9, 31.2, 20.9, 6.8 and 6.9, respectively for anise; 83.0, 66.1, 7.8, 24.7, 19.9, 8.7 and 6.9, respectively for coriander; 98.9, 32.8, 6.9, 16.8, 113.4, 85.8 and 24.7, respectively for sweet fennel. The positive effects of P fertilization may be due to its important physiological roles: P enhances seed germination and early growth, stimulates blooming, enhances bud set, aids in seed formation and hastens maturity (Espinosa *et al.* 1999). Khalid (1996) and Nassar *et al.* (2004) found

that P had a stimulating effect on VGC parameters of *Apiaceae* plants and chamomile flowers, respectively compared with the control. P enhanced the VGC of bitter fennel (*Foeniculum vulgare* Mill.) (Tank *et al.* 2007; Osman 2009). P had a significant effect on the VGC of sweet fennel cultivars growing under arid regions in Egypt (Zaki *et al.* 2010, 2011).

Effect of P fertilization on EO

The effects of different treatments of P on the EO content extracted from anise, coriander and sweet fennel fruits are represented in **Table 3**. Generally, EO accumulated more in anise, coriander and sweet fennel plants as P levels increased, compared with the control. The highest level (75 kg ha⁻¹) of P seemed to be optimal for obtaining a higher concentration of EO than the control and other treatments: 1.2, 0.4 and 0.6% more than the control for anise, coriander and sweet fennel, respectively. The effect of different P treatments on peppermint EO may be due to its effect on enzyme activity and metabolism of EO production (Burbott and Loomis 1969). Khalid (1996) and Economakis (2002) showed that P significantly increased the EO content of *Apiaceae* and *Origanum dictamnus* plants. P enhanced EO extracted from bitter fennel (*Foeniculum vulgare* Mill.) (Tank *et al.* 2007; Osman 2009). P had a significant effect on the EO content isolated from sweet fennel cultivars growing under arid regions in Egypt (Zaki *et al.* 2010, 2011).

Effect of P fertilization on FO

Data presented in **Table 3** shows that the application of P fertilizer resulted in an increase in the accumulation of FO extracted from anise, coriander and sweet fennel fruits. The highest values of FO contents were obtained when P was applied at 75 kg ha⁻¹: 3.7, 6.0 and 2.1% higher than the control for anise, coriander and sweet fennel, respectively. Khalid (1996) found similar results for some *Apiaceae* plants while Espinosa *et al.* (1999) indicated that P plays an important role in various metabolic processes such as fatty acid (FO) synthesis. Phosphate caused a significant change in the fatty acid and lipid composition (fixed oil) of *Mono-dus subterraneus* (Khozin and Cohen 2006).

Table 3 Effect of P fertilization on chemical composition.

P ₂ O ₅ treatments (kg ha ⁻¹)	Chemical composition (%)							
	EO	FO	TC	SS	CP	N	P	K
Anise (<i>Pimpinella anisum</i>)								
0	2.1	5.1	12.1	2.1	5.7	2.2	0.6	1.2
37.5	2.2	6.4	12.8	2.3	7.0	2.7	0.9	1.6
56.3	2.8	7.8	14.6	2.6	7.3	2.8	1.5	2.2
75	3.3	8.8	18.7	2.7	9.4	3.6	1.9	2.4
LSD:								
0.05	0.1	0.9	1.5	NS	0.5	0.1	0.2	0.1
0.01	0.2	1.1	3.2	NS	0.6	0.3	0.3	0.2
Coriander (<i>Coriandrum sativum</i>)								
0	0.2	2.9	7.8	1.6	4.7	1.8	0.3	2.2
37.5	0.3	5.6	14.0	1.7	4.9	1.9	0.7	2.7
56.3	0.5	6.4	16.7	1.8	7.5	2.9	0.9	2.8
75	0.6	8.9	18.8	1.9	9.6	3.7	1.1	3.7
LSD:								
0.05	0.1	1.3	2.6	NS	0.3	0.2	0.2	0.2
0.01	0.2	2.4	3.8	NS	0.4	0.4	0.3	0.4
Sweet fennel (<i>Foeniculum vulgare</i> var. 'Dulce')								
0	1.4	1.5	9.5	1.8	4.2	1.6	0.7	2.1
37.5	1.5	1.9	11.7	1.9	4.9	1.9	0.9	2.3
56.3	1.9	2.9	15.5	2.1	7.0	2.7	1.6	2.5
75	2.0	3.6	18.5	2.2	8.1	3.1	1.8	2.8
LSD:								
0.05	0.1	0.5	2.3	NS	0.7	0.2	0.2	0.2
0.01	0.2	0.7	3.6	NS	0.9	0.3	0.3	0.3

Effect of P fertilization on TC and TSS

Table 3 shows that TC and TSS content of anise, coriander and sweet fennel increased with the application of P. However, the highest contents of TC and TSS were recorded when plants were treated with 75 kg ha⁻¹ compared with the control or other treatments: 6.6 and 0.6%, 11.0 and 0.3%, 9.0 and 0.4% higher TC and TSS than the control for anise, coriander and sweet fennel, respectively. P utilizes light energy in the presence of chlorophyll to combine carbon dioxide and water into simple sugars, with the energy being captured in ATP, which is then available as an energy source for the many other reactions that occur within the plant, while the sugars are used as building blocks to produce other cell structural and storage components (Espinosa *et al.* 1999). Adebooye and Oloyede (2007) noticed that P had a stimulating effect on the TSS content of *Trichosanthes cucumerina* L.

Effect of P fertilization on CP

CP contents were positively affected by soil application of P (**Table 3**). The 75 kg ha⁻¹ treatment recorded the highest CP content of anise, coriander and sweet fennel plants: 3.7, 4.9 and 3.9% higher than the control for anise, coriander and sweet fennel, respectively. Espinosa *et al.* (1999) claimed that P activates coenzymes for amino acid production used in protein synthesis. Accumulation of protein was depressed in lentil (*Lens culinaris* Medik. var. Barimasur) following P-deficiency (Sarker and Karmoker 2011).

Effect of P fertilization on the minerals

It is evident from **Table 3** that the contents of all minerals (NPK) under investigation gradually increased in all P treatments compared with the control treatment. Applying 75 kg ha⁻¹ P resulted in the highest mineral content in anise, coriander and sweet fennel plants: 1.4, 1.9 and 1.5%; 1.3, 0.8 and 1.1%; 1.2, 1.5, and 0.7% higher N, P and K than the control for anise, coriander and sweet fennel, respectively. The increase in essential minerals may be as a result of an increase in the dry matter of plant materials, as shown by El-Wahab and Mohamed (2007) in *Trachyspermum ammi* L. (ajowan) plants. *T. cucumerina* plants treated with 150 and

200 kg P₂O₅ ha⁻¹ had the greatest P content compared with the control (Karthikeyan 2008).

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