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Effect of macro and micro nutrients on essential oil of coriander fruits

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Abstract
Coriander (*Coriandrum sativum* L.) from *Umberiferae* family (*Apiaceae*), rich in linalool, has potential using as source of essential oil and as a medicinal plant. Desert regions in Egypt are characterized by poor nutrients (macro and micro), limitation of water availability and unfavorable environmental conditions which negatively affect aromatic plants including coriander plants. The main objective of the present investigation was to study the effect of different levels of nitrogen & phosphorous fertilizers, trace elements and their interactions on the essential oil extracted from Egyptian coriander plants under these desert conditions. Application of nitrogen & phosphorous X micronutrients caused a pronounced increment in both essential oil content and yield of coriander compared with the treatments of nitrogen & phosphorous without micronutrients. Fifteen constituents were identified in essential oil extracted from coriander fruits, accounting for 99.1 – 99.7 % of total constituents, and belong to two chemical main classes. Oxygenated monoterpenes class was the major one (86.7% - 87.3%), the remaining fractions as monoterpene hydrocarbons formed the minor classes (11.9% - 12.9%). The main constituents of coriander fruits essential oil as detected by GC/MS were linalool (75.5% - 75.8%), limonene (6.8% - 7.3%) and camphor (3.7% - 4.3%) which increased by the levels of nitrogen & phosphorous increased. Nitrogen & phosphorous X micronutrients treatments resulted in higher values of main components compared with the treatments of nitrogen & phosphorous alone.

Key words: Coriander, nitrogen, phosphorous, fertilizer, macro, micro, nutrients, essential oil.

1. Introduction
Coriander (*Coriandrum sativum* L.) from *Umberiferae* family (*Apiaceae*) is an annual herbaceous plant originally from southern Europe, Asia and Caucasus. Being known as aromatic, medicinal and condimental plant, the dry fruits are rich in essential oil and have both odor and taste very pleasant. So, they have been widely used in food industry to prepare liqueur, sweets and condiment as well as perfume and cosmetics [1]. The essential oil of fruits is rich in linalool and it is used in pharmaceutical production to improve flavor and aroma of some medicines [1]. Ripe fruits of coriander are widely and popularly used in infusion preparation as analgesic, antispasmodic, febrifuge, carminative and diuretic agent. There are also reports on its home using against several respiratory and digestive affections [2]. Plant nutrition (both macro and micro) is one of the most important factors that affected the essential oil production. Some previous investigations on coriander plant were carried out by some authors[3-7]. High ratio of nitrogen resultant in a significant increase in essential oil (content and yield) of coriander fruits [3, 4]. Phosphorous fertilization also increased essential oil content extracted from coriander fruits [5, 6]. Nitrogen & phosphorous with micronutrients habited a significant increase in essential oil percent and yield of coriander fruits [5, 7]. Desert regions in Egypt are characterized by poor nutrients (macro and micro), limitation of water availability and unfavorable environmental conditions which

negatively affect aromatic plants including coriander plants [8]. The main objective of the present investigation was to study the effect of different levels of inorganic fertilizer in form of nitrogen & phosphorous fertilizers, trace elements and their interactions on the essential oil extracted from Egyptian coriander plants under these desert conditions.

2. Materials and methods

2.1. Experimental and Plant material

Experiments were carried out in arid region at the Experimental Farm of Desert Development Center (DDC) in Sadat City, American University, Egypt, during two successive seasons, 2005 - 06 and 2006 – 07 respectively. The area of DDC had been recently reclaimed and did not used ever before. Physico -chemical properties of the soil used in this study were determined according to Jackson [9, 10] and are presented in **Table 1**. Basic seeds of coriander were kindly provided by the Department of Medicinal and Aromatic Plants, Ministry of Agriculture, Giza, Egypt. Seeds were sown directly in the open field in the third week of October during both the seasons. The experimental area (plot) was kept 30 m² (4 m x 7.5m) containing 15 rows; the distance between hills was 25 cm and 50 cm apart. Post practice of thinning for two plants per hill was carried out 45 days after cultivation of plants in the open field. All cultural practices operations other than experimental treatments were performed in accordance with the recommendations of the Ministry of Agriculture, Egypt. Plots were divided into two main groups. The first group was subjected to different levels of nitrogen (N) & phosphorous (P) combinations: N_0P_0, N_1P_1, N_2P_2 and N_3P_3. The N and P amounts were described in **Table 2.** The second group was subjected to the same NP treatments while foliar spray (micro nutrients) was added at 1g L^{-1}. N source was ammonium sulphate (20% N). P_2O_5 source was calcium superphosphate (15% P_2O_5). Foliar spray source was commercial solution (Greenzite) which described in **Table 3**. Greenzite was added as foliar spray during 2 times, the first one after 2 weeks from thinning while the seconded one after 21 days from the first one.

Table 1. Mechanical and chemical analysis of the soil

Sand	79.7 %	P	0.1 mg g^{-1}	Zn	0.3 mg g^{-1}
Silt	13.0 %	K	0.6 mg g^{-1}	Mn	1.6 mg g^{-1}
Clay	7.3 %	Ca	4.9 mg g^{-1}	CO_3	1.8 mg g^{-1}
Gravel	18.7 %	Mg	5.6 mg g^{-1}	HCO_3	1.9 mg g^{-1}
Ph	8.7	Na	11.9 mg g^{-1}	Cl	18.6 mg g^{-1}
EC	2.0 dS m^{-1}	Fe	5.4 mg g^{-1}	SO_4	1.2 mg g^{-1}
N	0.1 mg g^{-1}	Cu	0.4 mg g^{-1}		

Table 2. Discretion of N and P amounts

N_0 = 0 kg N ha^{-1}		P_0 = 0 kg P_2O_5 ha^{-1}
N_1=100 kg N ha^{-1}		P_1 = 37.5 kg P_2O_5 ha^{-1}
N_2 = 150 kg N ha^{-1}		P_2 = 56.3 kg P_2O_5 ha^{-1}
N_3 = 200 kg N ha^{-1}		P_3 = 75 kg P_2O_5 ha^{-1}

Table 3. Discretion of Foliar spray solution

EDTA Na_2 Mn (40%)		Zn (570.27 mg L^{-1})
EDTA Na_2 Zn (48%)		Cu (0.054 mg L^{-1})
Fe (5.4 mg L^{-1})		Mo (0.027 mg L^{-1})
Mg (0.54 mg L^{-1})		Ni (0.005 mg L^{-1})
Mn (50.54 mg L^{-1})		Co (0.005 mg L^{-1})

2.2. Harvesting

At the end of fruiting stage of both the seasons plants were harvested and fruit yield (g $plant^{-1}$)] were recorded.

2.3. Essential oil isolation

Ripe fruits were collected from each treatment during the first and second seasons; air dried and weighed to extract the essential oil, then 100g from each replicate (04 replicates) of all treatments was subjected to hydro-distillation (HD) for 03 h using a Clevenger-type apparatus [11]. The essential oil content was calculated as a relative percentage (v/w). In addition, total essential oils (ml $plant^{-1}$) were calculated by using the dry fruits. The essential oils extracted from coriander were collected during the first and second seasons from each treatment, and then dried over anhydrous sodium sulphate to identify the chemical constituents.

2.4. GC/MS
The essential oil was analyzed on a VG analytical 70 - 250S sector field gas chromatography - mass spectrometry (GC/MS), 70 eV, using a SPsil5, 25 m x 30 m, 0.25 µm coating thickness, fused silica capillary column, injector 222°C, detector 240 °C, linear temperature 80–270°C at 10 °C/min. Diluted samples (1/100, v/v, in n - pentane) of 1 ml were injected, at 250 °C, manually and in the split less mode flame ionization detection (FID) using the HP Chem-station software on a HP 5980 gas chromatography (GC) with the same type column as used for GC/MS and same temperature program. Analyses were carried out using helium as the carrier gas, with the flow rate at 1.0 ml/min.
The libraries under GC/MS were NIST 98 and Wiley 5.

2.5. Qualitative and quantitative analyses
Identifications were made by library search[12] combining MS retention data of authentic compounds by comparison of their GC retention indices (RI) with those of the literature [12] or with those of standards available in our laboratories. The retention indices were determined in relation to a homologous series of n-alkanes (C_8–C_{22}) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST 98 and Wiley 5 Libraries or with mass spectra from literature. Component relative concentrations were calculated based on GC peak areas without using correction factors.

2.6. Statistical analysis
In this vary experiment, two factors were considered: NP treatments ($N_0 P_0$, $N_1 P_1$, $N_2 P_2$, and $N_3 P_3$) and trace elements. For each treatment there were 4 replicates, each of which had 3 plots. The number of experimental pots was 96 plots. The experimental design followed a complete random block design. According to Snedecor [13] the averages of data were statistically analyzed using 2-way analysis of variance (ANOVA-2). Significant values determined according to P values (P " 0.05 = significant, P < 0.01 = more significant and P < 0.001 = highly significant). The applications of that technique were according to the STAT-ITCF program [14].

3. Results

3.1. Effect of NP, micronutrients and their interactions on the essential oil
Data presented in **Table 4** indicated that NP, micronutrients and their interactions had a positive effect on essential oil content (%) and yield (ml plant^{-1}). Application of NP X micronutrients caused a pronounced increment in both essential oil content and yield compared with the treatments of NP without micronutrients. Fertilization with N_2P_2 X micronutrients or N_3P_3 X micronutrients resulted in the maximum mean values of essential oil content (0.5 %) and yields (0.1 ml plant^{-1}). The lowest essential oil content (0.1 %) and yield (tr. (< 0.1) ml plant^{-1}) were recorded at control treatments. ANOVA indicated that the increases in essential oil (%) were significant for NP treatments, highly significant for trace elements treatments and insignificant for NP X trace elements treatments. The increases of essential oil yield (ml plant^{-1}) were highly significant for NP, micronutrients and their interactions treatments.

3.2. Effect of NP and micronutrients interactions on the essential oil constituents
Fifteen constituents were identified in essential oil extracted from coriander fruits, accounting for 99.1 – 99.7 % of total constituents, and belong to two chemical main classes. Oxygenated monoterpenes class was the major one (86.7% - 87.3%), the remaining fractions as monoterpene hydrocarbons formed the minor classes (11.9% - 12.9%) **(Table 5)**. The main constituents of coriander fruits essential oil as detected by GC/MS were linalool (75.5% - 75.8%), limonene (6.8% - 7.3%) and camphor (3.7% - 4.3%) which increased as NP level increase. NP X micronutrients treatments resulted in higher values of main components compared with the treatments of NP alone. The highest amount of major compounds resulted from the N_3P_3 X micronutrients treatment compared with other treatments and control treatment. The chemical classes of coriander essential oil were changed in all treatments compared with control. The highest values of oxygenated monoterpenes and monoterpene hydrocarbons were produced from the control and N_3P_3 X micronutrients treatments respectively. ANOVA indicated that the changes in chemical classes and the most constituents were insignificant for NP X micronutrients treatments except the constituents of α-pinene, sabinene and tetradecane were significant **(Table 5)**.

Table 4. Effect of macro and micronutrients on essential oil content

Treatments		Essential oil content	
		%	ml plant $^{-1}$
Without micronutrients	N_0P_0	0.2	tr.
	N_1P_1	0.3	tr.
	N_2P_2	0.3	tr.
	N_3P_3	0.3	tr.
Overall Without micronutrients		0.3	tr.
With micronutrients	N_0P_0	0.3	tr.
	N_1P_1	0.4	tr.
	N_2P_2	0.4	0.1
	N_3P_3	0.5	0.1
Overall with micronutrients		0.4	0.1
Overall NP	N_0P_0	0.2	tr.
	N_1P_1	0.3	tr.
	N_2P_2	0.4	tr.
	N_3P_3	0.4	0.1
F value			
NP		3.4*	66.4***
Micronutrients		15.4***	194.4***
NP x micronutrients		0.6	40.8***
*P ≤ 0.05. ** P < 0.01.*** P < 0.001, tr. = < 0.05			

Table 5. Effect of macro and micronutrients on essential oil constituents (%).

No	Components (%)	RI*	Class	Treatments								F value
				NP				NP X trace elements				
				N_0P_0	N_1P_1	N_2P_2	N_3P_3	N_0P_0	N_1P_1	N_2P_2	N_3P_3	
1	α –Pinene	939	MH	1.3	1.4	1.7	1.8	1.6	1.4	1.2	1.5	3.4*
2	Camphene	953	MH	0.1	0.2	0.3	0.6	0.4	0.3	0.5	0.6	0.6
3	Sabinene	976	MH	0.5	0.4	0.4	0.2	0.5	0.7	0.4	0.6	3.4*
4	ß-Pinene	980	MH	0.2	0.3	0.2	0.3	0.4	0.3	0.5	0.5	1.2
5	Myrcene	991	MH	0.9	0.5	0.8	0.3	0.7	0.6	0.7	0.3	0.5
6	*P*-Cymene	1026	MH	0.5	0.9	0.7	0.9	0.3	0.7	0.5	0.7	0.0
7	Limonene	1031	MH	6.8	6.9	6.9	7.0	7.0	7.1	7.2	7.3	0.3
8	ɣ-Terpinene	1062	MH	1.9	1.3	1.5	1.4	1.8	1.5	1.5	1.4	0.4
9	Linalool	1098	OM	75.5	75.6	75.6	75.7	75.6	75.7	75.7	75.8	0.0
10	Camphor	1143	OM	3.7	3.8	3.9	3.9	4.0	4.1	4.1	4.3	0.1
11	Borneol	1165	OM	0.9	0.8	0.9	0.7	0.7	0.7	1.1	0.9	2.0
12	Terpine-4-ol	1177	OM	2.9	2.5	2.3	2.6	2.5	2.1	2.3	2.4	0.6
13	Geraniol	1255	OM	1.9	2.1	1.8	1.6	1.9	1.9	1.8	1.7	0.5
14	Geranyl acetate	1383	OM	1.7	1.6	1.7	1.5	1.7	1.5	1.7	1.4	0.1
15	Tetradecane	1399	OM	0.7	0.8	0.8	0.9	0.6	0.7	0.4	0.3	4.0*
MH = Monoterpene hydrocarbons				12.2	11.9	12.5	12.5	12.5	12.6	12.5	12.9	0.8
OM =Oxygenated monoterpenes				87.3	87.2	87.0	86.9	86.9	86.7	87.1	86.8	1.4
Total identified				99.5	99.1	99.5	99.4	99.4	99.3	99.6	99.7	
*RI = Confirmed by comparison with Retention indices on DB5 column [12].												
*P ≤ 0.05., ** P < 0.01., *** P < 0.001.												

4. Discussion

The variations in essential oil content and composition could be due to the effect of different N levels on enzymes activity and metabolism improvements [15]. Moreover, Masroor [16] found that the application of NP enhanced the contents of monocyclic constituents (anethole and methylchavicol) whereas; the foliar application of these elements the contents of bicyclic constituents. The specific effect of the mode of action of the nutrients on the production of these compounds could be explained on the basis of different primary metabolic pathways

of carbon. α-pinene, fenchone, and camphene are monoterpenes that are biosynthesized from acetyl-CoA via the mevalonate pathway [17]. α-Pinene and d-fenchone are biosynthesized from the pinyl branch of this pathway, while camphene is biosynthesized from the bornyl way of the pathway. This explains why changes in their content in the essential oil correlate positively. In contrast, anethole and methylchavicol are polyisoprenoids biosynthesised from phosphoenolpyruvate (PEP) and d-erythrose-4-phosphate via the shikimic acid pathway. They differ chemically only in the position of their instauration in the side chain. Thus, it can anticipate that changes in their concentrations might also be parallel. The common biosynthetic point of the two pathways is that PEP is a precursor via pyruvate for the synthesis of acetyl-CoA. This explains why increased production of the phenylpropanoids is at the expense of biosynthesis of the monoterpenes, since the precursor required for monoterpenes is being diverted into the shikimate pathway [17, 18]. Although there are a few reports on seed yield, essential oil content and yield, harvesting time, etc., information of the effect of N and P on their constituents is meager [19 - 23]. However, László [24] worked out that increasing doses of P increased carvone content (a monocyclic monoterpene) in *Anethum graveolens*. It is further concluded that basal as well as foliar fertilizer application of N and P could be employed to obtain the desired quality of sweet fennel essential oil. These results are in accordance with those obtained by previous studies such as Khalid [5] who reported that N fertilization increased the essential oil, of *apiaceae* plants. Hellal [25] indicated that N fertilizer increased the essential oil yield of dill (*Anethum graveolens* L.) plant. Sarab [26] obtained the essential oil concentration in the herb in the case of the application of the highest rate of nitrogen. Kandil [27] obtained the highest basil essential oil yield when the highest N rates were applied. The enhanced accumulation of essential oil under the conditions when plants are well supplied with nitrogen results from the increased production of biomass as well as from the direct impact on the biosynthesis of this substance [28]. The above cited studies and the present study prove the significant effect of an increased in amount of nitrogen on the concentration of linalool and chemical composition of the essential oil obtained from the basil herb [29, 30]. An increase in the amount of nitrogen in the nutritional environment of the plants resulted in the enhanced accumulation of essential oil, as well as in a rise in linalool and germacrene D concentration [31]. The data of this investigation essential oil were positively affected by phosphorus application. Phosphorus significantly increased essential oil concentration. The most effective P rate was 75 kg P ha^{-1}. These results are in agreement with the data of Nikolova [32] who showed P fertilization increased the essential oil concentration of chamomile. It is well documented that phosphorus is an essential element in reproduction of plants [32] and thus, the essential oil increased by applied P was expected in the present study. Phosphorus is also known to have multifarious cellular functions in plants [33], including signaling and trans membrane metabolic flux [33] and therefore, the secondary metabolism (essential oil) are modulated by these mechanisms [33]. Our results agree with those obtained by Kandeel [34] who reported that using micro elements as foliar application at 2000 mg L^{-1} X NP had a significant effect on essential oil content of parsley (*Petroselinum crispum* Mill). Previous results of Misra [35] on geranium (*Pelargonium graveolens* L. Her.' ex. Ait.) plants indicated that micronutrients affect the CO_2 assimilation rate, photosynthetic pigments content and ultimately the accumulation of geranium essential monoterpenes oil (s) and the production of biomolecule geraniol (main component of geranium essential oil). Essential oil constituents from *Coriandrum sativum* L. fruits were reported by some previous investigators. Msaada [36] indicated that geranyl acetate (46.27%), linalool (10.96%), nerol (1.53%) and neral (1.42%) were the main compounds at the first stage of maturity (immature fruits). At the middle stage, linalool (76.33%), cis-dihydrocarvone (3.21%) and geranyl acetate (2.85%) were reported as the main constituents. Essential oils at the final stage of maturity (mature fruits) consist mainly on linalool (87.54%) and cis-dihydrocarvone (2.36%). Additionally, accumulation of monoterpene alcohols and ketones was observed during maturation process of coriander fruit. The main components of essential oil extracted from *Coriandrum sativum* L. fruits grow in Brazil were linalool, γ -terpinene, α-pinene, limonene, geraniol and 2-decenal [37]. Linalool is the principal component extracted under supercritical CO_2 extraction of essential oil isolated from coriander fruits [38].

5. Conclusion

In conclusion, it appears that application of NP X micronutrients caused a pronounced increment in both essential oil [content (%) and yield (ml $plant^{-1}$)] of coriander. Therefore, it is strongly recommended that on arid region (characterized

by low amount of available nutrients), the crop must be supplied with adequate NP and macronutrients. Furthermore, the influence of NP and macronutrients addition on the essential oil of coriander is thoroughly studied on locations with wide range of climatology, physical and chemical properties and mineralogical characteristics.

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