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Bicarbonate-indced Fe deficiency effects on essential oil composition of Tunisian aromatic plant: dill (*Anethum graveolens* L.)

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Abstract: *In the present report, A. graveolens shoots were subjected to Fe depeletion conditions and explored for their growth activity and essential oil (EO) composition. For this purpose, plants were cultivated for 14 days in hydroponic medium supplemented or not with 30 µM Fe + CaCO3+10 mM NaHCO3. Under lime Fe deficiency conditions, shoots fresh dry weight were significantly reduced. On the other hand, under the omni-presence of Fe (control conditions), A. graveolens shoots produced a medium essential oil yield. This latter decreased in yield by about 40% under induced Fe deficiency. In parallel, GC/MS analysis showed that, the major constituents under control conditions is camphor, whereas, the major volatile component under Fe depletion is dillapiole. Overall, the tolerance of dill is explained by its aptitude to reduce its growth activity to uphold the nutrient uptake of iron and by the enhancement of dillapiole secretion.*

Keywords: *Anethum graveolens ; Bicarbonate induced Fe deficiency ; Essential oil ; growth*

1. INTRODUCTION

Micronutrients are critical for plant growth owing to its engagement in several metabolic and cellular functions [1].When mineral deficiencies arise, the symptoms are more or less characteristic of each element. Either way, visual symptoms follow through metabolic disparity caused by scarce levels of micronutrients in the plant tissues. It can provoke alterations in the biosynthetic and/or catabolic capacity of a plant [2]. Plants are recurrently disputed by nutritional imbalances in soils micronutrients, trying to equalize the uptake, use, and storage of micronutrients. The distraction of this balance induces an overproduction of reactive oxygen species (ROS) in cell compartments. Plants are recurrently faced to Fe deficiency [3]. The prominent concentration of HCO3ions in calcareous soils; could upgrade Fe bioavailability [4], which lead to the appearance of chlorosis symptoms. On a metabolic scale, Fe shortage elicits oxidative stress, upsetting the expression and activity of diverse antioxidant enzymes. [5].

Dill is one of the most prevalent plants of the Apiaceae family. It is a renowned as Mediterranean aromatic plant [3]. In the folk medicine, such sweet herb is used for its miscellaneous therapeutic properties related to digestive, endocrine, reproductive and respiratory systems [7]. Biological activities of dill are related to its content of natural antioxidant [8] (Barros et al., 2009)

In Tunisia, calcareous soils are often encountered, thus cultivating dill under Fe deficiency is frequent. The deployement of secondary metabolites would occur, however, biomass production is restricted. Content variations in polyphenols within species may reveal different requirements to deal with Fe deficiency [3].

On the other hand, EO yield of some species can alter owing to multiple factors (age, growth cycle, climatic conditions, soil type and cropping pattern) [9]. Until now, there is no clear information on the possible influence of bicarbonate induced Fe deficiency on the culture conditions. Thus, the current research was aimed to scrutinize the difference in growth activity and chemical composition of essential oils obtained from shoots of A. graveolens subjected to induced Fe deficiency conditions.

2. MATERIAL AND METHODS

2.1. Plant Material and Growth Conditions

Dill seeds were collected from Manouba provenance (35° 18′ Nord, 10° 43′ Est Tunisia). Nine-day-old seedlings were transferred to a half strength aerated nutrient solution for 15 days and then cultured as groups of 10 plants in 10 L of full strength aerated nutrient solution containing Hoagland solution [3] and ferric ethylene diamine tetra-acetic acid (FeEDTA). Plants were either optimally supplied with iron (Fe) (+Fe ; 30µM, Fe) or subjected to induced Fe deficiency (+Fe +Bic, 30µM Fe+ 10mM HCO3). The pH either adjusted to 6 for both (C) treatments using a Hanna pH 210 pH meter. The culture was maintained under controlled climatic condition upheld in the growth chamber with a day/night photoperiod of 16/8h, temperature (day/night) of 24/18◦C, PPFD of 200 µmoL m−2 s −1 and a RH of 70%

2.2. Determination of Dry and Fresh Matter

At the end of the hydroponic culture (fourteen days), plants were seperated into shoots and roots,and fresh weights (FW) were instantlymeasured. Samples were then oven-dried for 48 h at 65 ◦C for dry weight (DW) determination [3]

2.3. Essential Oil (EO) Isolation

One hundred gram from shoots of each treatment (+Fe/+Fe+Bic) was submitted to hydrodistillation technique for 3 hours with 750 mL of dionized water with Clevenger-type apparatus. The distillate obtained oil was collected and dried over anhydrous sodium sulfate and stored in sealed glass vials in a refrigerator at -20°C prior to analysis [10] . Besides, Yield based on DW of the sample was calculated.

Yield of oil = [Weight of oil/Weight of dried shoots] × 100

2.4. Analyses of the Essential Oil (EO) by Gas Chromatographyemass Spectrometry (GC-MS)

The analysis of the volatile constituents was run on a Hewlett– Packard GC–MS system (GC: 5890-series II; MSD 5972). The fused-silica HP-5 MS capillary column (30 m × 0.25 mm ID, film thickness of 0.25 µm) was directly coupled to the MS. The carrier gas was helium, with a flow rate of 1.2 ml/mn. Oven temperature was programmed (50°C for 1 min, then 50–280 °C at 5 °C/ min) and subsequently, held isothermal for 2 min. Injector port: 250 °C, detector: 280 °C, split ratio 1:50. Volume injected, 1 µL of 1% solution (diluted in hexane): HP 5972 recording at 70 eV; scan time 1.5 s; mass range 40–300 amu. Software adopted to handle mass spectra and chromatograms was a Chem Station. The components of the oil were identified by comparison of their mass spectra with those in the Wiley 275 GC–MS library and those in the literature, as well as by comparison of their retention indices with literature data. Retention indices of the components were determined relative to the retention times of a series of n-alkanes (relative to C9–C28 on the HP5 and HP-20 M columns).

2.5. Statistical Analyses

Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test was performed. The statistical tests were applied using Graph Pad Prism, version 6 and the significance level was $p < 0.05$.

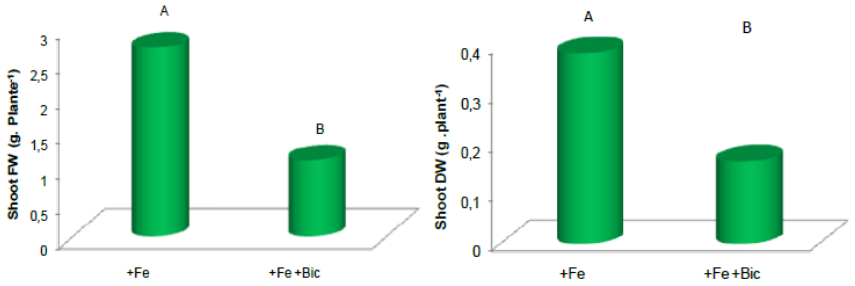
3. RESULTS AND DISCUSSION

3.1. Effect of Iron (Fe) Depletion on Plant Growth

As shown in Figure 1, bicarbonate induced Fe deficiency induced a significant decline in fresh matter of shoot dill plants (-49%). In the same line, such constraint provoked a noteworthy decline in shoot dry biomass (-53%) as compared to optimally sufficient Fe shoots.

As recorded in Pisum Sativum in plants subjected to calcareous medium, the decrease in shoot growth (SG) characterizes an adaptive strategy of the plant to maintain suitable levels of iron, additionally to uphold the photosynthetic activity [1]. The research of Ben Abdallah et al. [11], showed that Sulla plants, cultivated in a calcareous soil, declined their shoot growth, which fortify its strategy of iron uptake efficiency.

Figure 1 : Shoot fresh and dry matter of dill plants grown in the omni-presence of Fe (+Fe) or in the presence of Fe plus bicarbonate (+Fe + Bic).Means of six replicates.



3.2. Iron (Fe) Depetion Effect on EO Yield

As detected in Figure 2, in the optimally sufficient Fe shoots, EO yield was 2%, refering on their DW and was noticeably affected by Fe depletion conditions resulted in a decrease of EO yield to 1.2%. Thus, Fe deficiency reduced EO production of A. graveolens .

In parallel, GC/MS analysis of EO from dill shoots proved the omni-presnece of 18 compounds accounting for 89 % of the total EO (Table 1 , Figure 3). For shoots optimally supplied with Fe, the main compound was camphor (24.61%) followed by camphene (14.02 %), hexcosane (8.17 %) and α-pinene (7.30 %). Application of bicarbonate in hydroponic medium induced the abundance of dillapiole as major compound representing 29% from the total identified EO, then camphor (15.29%) and camphene (11.37%) (Table 2 , Figure 4).

Figure 2 : Effect of bicarbonate induced Fe deficiency on essential oil yield (%) of Anethum graveolens shoots. Values with different superscripts are significantly different at $p < 0.05$ (means of 3 replicates)

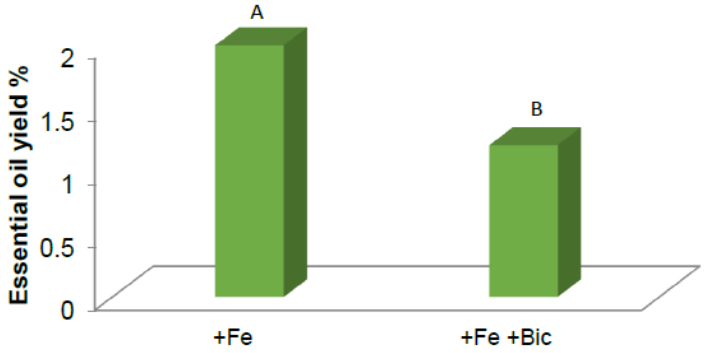


Figure 3 : Chromatogram of essential oil compounds from control shoots (+Fe) of Anethum graveolens. 1: α-pinene , 2: Camphene; 3: δ-3- Carene; 4: L-fenchone; 5: Camphor; 6: α -Fenchyl acetate; 7 : phenol ; 8: Myrtenyl acetate; 9: Phenol ; 10: γ-Cadinene ; 11 : Selina 3,7(11)-diene ; 12 : Naphthalene ; 13 : Dillapiole ; 14 : Oxabicyclo-heptane ; 15 : Carvone ; 16 : Octasone ; 17 : Octadecane ; 18 : Hexcosane

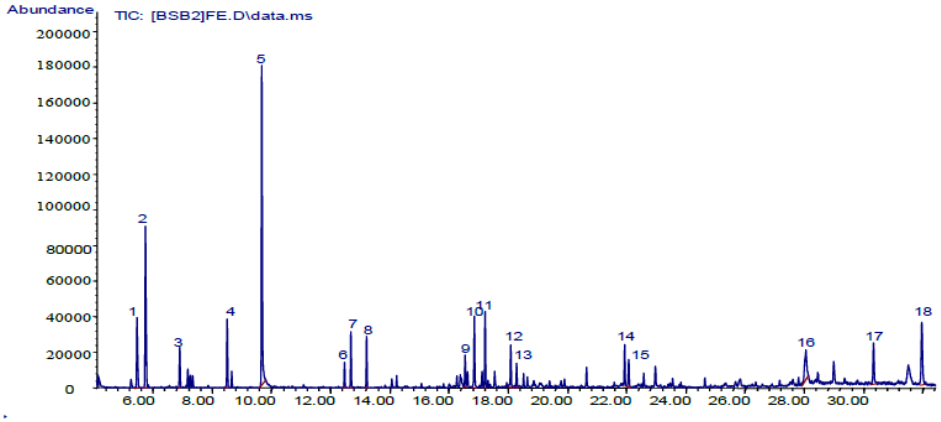


Table 1. Essential oil composition of *Anethum graveolens* cultured under control condition by GC-MS

Compound	*RT*	*Identified compound*	*Area*	*%*
1	5.4	*α-pinene*	951250	6.25
2	5.7	*Camphene*	2133035	14.02
3	6.8	*δ-3-Carene*	437485	2.87
4	8.4	*L-fenchone*	741712	4.87
5	9.6	*Camphor*	3744322	24.61
6	12.4	*α-Fenchyl acetate*	247849	1.62
7	12.6	*Phenol*	552661	3.63
8	13.2	*Myrtenyl Acetate*	480573	3.15
9	16.6	*Phenol*	324497	2.13
10	16.8	*γ-Cadinene*	822360	5.40
11	17.2	*Selina3,7 (11) diene*	781604	5.13
12	18.0	*Naphthalene*	479241	3.15
13	18.5	*Diallopiole*	239738	1.57
14	21.9	*Oxabicyclo-heptane*	450577	2.96
15	22.0	*Carvone*	326998	2.14
16	28.0	*Octasone*	741512	4.87
17	30.3	*Octadecane*	602299	3.95
18	31.9	*Hexcosane*	1154331	7.58
∑total			***15212044***	

Figure 4 : Chromatogram of essential oil compounds from bicarbonate induced Fe deficiency shoots (+Fe +Bic) of Anethum graveolens. 1: α-pinene , 2: Camphene; 3: δ-3-Carene; 4: L-fenchone; 5: Camphor; 6: a-fenchyl acetate; 7: Myrtenyl acetate ; 8: Phenol ; 9: γ-Cadinene : 10 : Selina 3,7 (11)-diene; 11: Naphthalene ; 12 : dillapiole ; 13 : Carvone ; 14 : Hexcosane

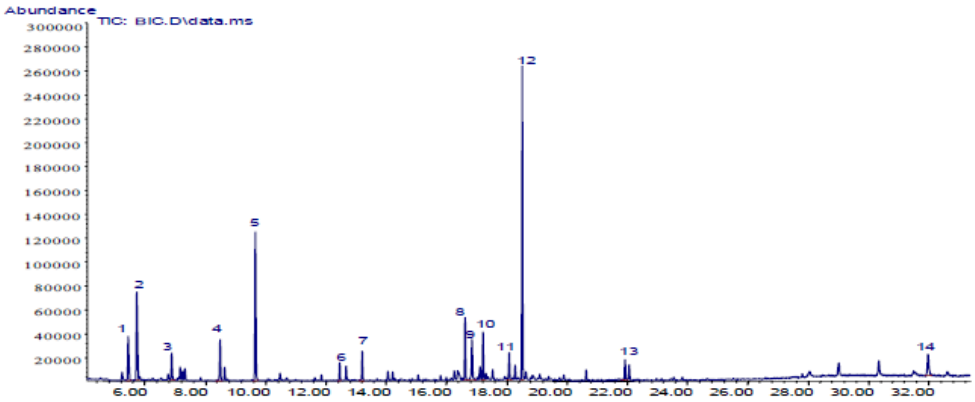


Table 2: Essential oil composition of *Anethum graveolens* cultured under control conditions by GC-MS

Compound	*RT*	*Identified compound*	*Area*	*%*
1	5.4	*α-Pinene*	934496	5.8
2	5.7	*Camphene*	1833146	11.37
3	6.8	*δ-3-Carene*	482098	2.99
4	8.5	*L-fenchone*	774794	4.80
5	9.6	*Camphor*	2464485	15.29
6	12.4	*α-Fenchyl acetate*	294282	1.82
7	13.2	*Myrtenyl Acetate*	473376	2.93
8	16.6	*Phenol*	993744	6.16
9	16.8	*γ-Cadinene*	867515	5.38
10	17.2	*Selina 3,7 (11) diene*	780297	4.81
11	18.09	*Naphtalene*	484298	3.00
12	18.5	*Diallopiole*	4681530	29.04
13	21.9	*Carvone*	371809	2.30
14	31.9	*Hexacosane*	680975	4.22
Total			***16 116 845***	

Although a decrease estimated; resulted from the decrease of α-pinene, camphene, camphor by about at 10 %, 19% and 36% as compared to the control. In turn, an significant increase was detected for other components.

The analysis of GC/MS profile of Indian dill plants, authenicate the existence of carvone (47.2%) as major component in seed essential oil, whereas dillapiole (90.2%) was the main component in leaves [7]. According to Bâatour et al. [9], the cultivation of salt stessed-Origanum majorana plants in greenhous, induced a decline in the content of EO especially for aliphatic hydrocarbons and terpenic alcohols groups. Although a stimulation of EO‘s production under salinity could be due be explainedby its higher oil gland density.

A numerous factors, including genetic, geo-climatic zones or growing conditions such as the dose of macro/micro-nutrients, temperature, soil nature, humidity, day length, altitude, and the amount of available water, influence the chemical composition of EO. It also depends on season or phenological stage of plant (i.e. vegetative, flowering and fruiting stages) [10]. Consistent with these factors, plant biosynthetic pathways can alter the relative proportions of the EO components. These disparities in chemical composition led to the concept of chemotypes, which are commonly defined as a distinct population within the same species that produces different chemical profiles for a particular class of secondary metabolites.

4. CONCLUSION

In conclusion, this work revealed a new insights about the tolerance of dill plants to Fe depletion conditions ; either by reducing their dry matter in order to preserve an adequate level of iron, or by enhancing the production of some essential oil compenets citing dillapiole (as major compenent under iron deficiency). Further applications of metabolic techniques could be useful to support the detected traits

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