International Journal of Medicinal Plants and Natural Products (IJMPNP)
Volume 12, Issue 1, 2026, PP 1-10
ISSN (Online) 2454-7999
https://doi.org/10.20431/2454-7999.1201001
www.arcjournals.org

Iron-Deficiency–Induced Alterations in Phenolic Composition, Antibacterial and Antifungal Activities of Tunisian Dill (*Anethum Graveolens*)

Hanen Wasli[1*], Maroua Oueslati[2], Nahida Jelali[3], Riadh Ksouri[4], Najla Sadfi-Zouaoui[2]

[1] Laboratory of Biotechnology and Biomonitoring of the Environment and Oasis Ecosystems (LBBEOE), Université de Tunis El-Manar, Faculté des Sciences de Tunis (FST), Tunisia

[2]Laboratoire de Mycologie, Pathologies et Biomarqueurs, Département de Biologie

[3]Laboratoire des Plantes Extrêmophiles, Centre de Biotechnologie de Borj-Cédria, Hammam Lif, Tunisie

[4]Laboratoire des Plantes Aromatiques et Médicinales, Centre de Biotechnologie de Borj-Cédria, 2050 Hammam-Lif, P.O.B. 901, Tunisia

Corresponding Author:** ***Hanen Wasli, *Laboratoire des Plantes Aromatiques et Médicinales, Centre de Biotechnologie de Borj-Cédria, Hammam Lif, Tunisie, • Université de Tunis El-Manar, Faculté des Sciences de Tunis (FST), Tunisia*

Abstract: *Iron (Fe) deficiency strongly influences plant secondary metabolism and bioactivity. Anethum graveolens L. (dill) is rich in phenolic compounds with notable antimicrobial potential, yet the effect of Fe scarcity on these activities remains unclear. In this study, dill plants were grown hydroponically under Fe-sufficient (+Fe) and Fe-deficient (–Fe) conditions. Fe deficiency significantly enhanced phenolic and flavonoid contents in roots (total phenolics increased by 35% and flavonoids by 20% compared to control), whereas leaves showed minimal changes. Root extracts from Fe-deficient plants exhibited markedly improved antimicrobial activity: antibacterial activity increased against Micrococcus luteus and Staphylococcus warneri (up to 40% larger inhibition zones), and antifungal activity was enhanced against Colletotrichum spp. (up to 45% increase) and Penicillium expansum (30% increase) relative to Fe-sufficient roots. Correlation analyses indicated that phenolic compounds were the main contributors to the enhanced bioactivities. These results demonstrate that Fe deficiency triggers root-specific metabolic reprogramming, boosting phenolic-mediated antimicrobial defenses. This study highlights the potential of cultivating dill under Fe-limited conditions to enhance its antimicrobial properties, with implications for medicinal plant production and bioactive metabolite optimization.t*

Keywords: *Direct Fe deficiency, Anethum graveolens, Antibacterial activity, Antifungal activity, Phenolic compounds.*

1. Introduction

Iron (Fe) plays a central role in sustaining plant growth, photosynthetic efficiency, and redox homeostasis. However, in calcareous and bicarbonate-rich soils—widely distributed in Northern and Northwestern Tunisia—Fe becomes poorly available, causing Fe-deficiency chlorosis and triggering major physiological and metabolic perturbations [1]. Beyond impairing primary metabolism, Fe scarcity reshapes the synthesis of secondary metabolites, particularly phenolic compounds, which are deeply implicated in plant defense mechanisms [2]. These metabolites not only act as antioxidants but also contribute substantially to the plant's antimicrobial arsenal [3] .

Anethum graveolens L. (dill) is well recognized as a rich reservoir of bioactive phytochemicals with strong antimicrobial and antifungal potential. Numerous studies have shown that its essential oils, phenolic acids, flavonoids, and furanocoumarins exhibit potent inhibitory activities against a wide range of microorganisms. The antibacterial spectrum includes Gram-positive species such as Staphylococcus aureus and Listeria monocytogenes, and Gram-negative bacteria including Escherichia coli and Salmonella spp. [4,5]. These effects are attributed mainly to monoterpenes such as D-carvone, D-limonene and α-phellandrene, which disrupt cell membrane integrity, cause leakage of cellular contents, and inhibit nucleic acid synthesis [6].

Likewise, the antifungal activity of A. graveolens is well documented. Its essential oils demonstrate strong fungistatic and fungicidal effects against Candida albicans, Aspergillus niger, Aspergillus flavus and Penicillium islandicum [5]. These compounds can modify fungal membrane permeability, inhibit spore germination, and interfere with ergosterol biosynthesis, thereby suppressing fungal growth and pathogenicity [4,6].

Despite this remarkable antimicrobial potential, the impact of Fe deficiency on these activities remains poorly understood. Since phenolic biosynthesis, terpenoid production and stress-response pathways are strongly influenced by Fe nutritional status, fluctuations in Fe availability could significantly alter the quantity and type of antimicrobial metabolites produced [7,8]. Fe deficiency is also known to induce oxidative stress, which may stimulate or suppress the synthesis of defense-related secondary metabolites depending on stress severity and plant adaptive capacity [9].

Thus, exploring whether Fe deficiency enhances, reduces, or reprograms the antimicrobial and antifungal properties of A. graveolens is of great scientific and agronomic interest. Understanding these interactions could clarify whether Fe stress stimulates the accumulation of antimicrobial phenolics as a protective strategy, or whether nutrient shortage compromises the plant's ability to synthesize bioactive defense compounds.

The present study therefore aims to characterize how direct Fe deficiency alters the phenolic profile of A. graveolens and to determine the resulting effects on its antioxidant, antibacterial and antifungal activities. By establishing this relationship, the study provides new insights into how medicinal plants modulate their chemical defense networks under micronutrient stress, and highlights the potential of cultivating bioactive species in Fe-poor soils while preserving or enhancing their antimicrobial performance.

2. MATERIAL AND METHODS

2.1. Chemicals

Iron(II)sulfate, potassium hexacyanoferrate (III), iron chloride(III), diammonium salt, 2,3-terc-butil-4-hidroxianisol(BHT), 2,2-diphenyl-1-picrylhydrazyl and gallic acid were obtained from Sigma-Aldrich (St.Louis, MO, USA), while thiobarbituric acid (TBA) were purchased from Acros Organics (Geel, Belgium). Ascorbic acid, Folin–Ciocalteu reagent, sodium carbonate, sodium phosphate, potassium hydroxide and ethylenediamine tetraacetic acid (EDTA) were purchased from Panreac (Barcelona, Spain). Hydrogen peroxide and sodium hydroxide were purchased from Fisher Scientific (Hampton, USA). Methanol was purchased from Lab-Scan (Lisbon, Portugal). TSB and TSA were employed throughout the experiments with bacteria.Potato Dextrose Agar (PDA) was used for experiments with fungi.

2.2. Plant Material and Growth Conditions

Seeds of Anethum graveolens were collected from Nefza province , Tunisia 36° 58′ 31″ N, 9° 04′ 51″ E, and surface-sterilized in a 30% saturated calcium hypochlorite solution for 2 min, followed by thorough rinsing with distilled water. After a 4-hour imbibition, seeds were germinated on filter paper in Petri dishes moistened with 0.1 mM $CaSO_4$ at 19 °C for 10 days. Uniform seedlings were then transferred to a half-strength aerated nutrient solution for 20 days to ensure early growth establishment. Subsequently, ten uniform seedlings per group were transferred to 10 L of full-strength aerated nutrient solution containing 1.25 mM $Ca(NO_3)_2$, 1.25 mM KNO_3, 0.5 mM $MgSO_4$, 0.25 mM KH_2PO_4, 10 µM H_3BO_3, 1 µM $MnSO_4$, 0.5 µM $ZnSO_4$, 0.05 µM $(NH_4)_6Mo_7O_{24}$, and 0.4 µM $CuSO_4$. Two experimental treatments were established: a control supplied with 30 µM Fe(III)-EDTA (+Fe) and a Fe-deficient treatment without Fe(III)-EDTA (–Fe). The pH of both solutions was adjusted to 6.0 with NaOH. Hydroponic cultures were maintained in a controlled growth chamber under a 16/8 h light/dark cycle, day/night temperatures of 24/18 °C, relative humidity of 60/80%, and a PPFD of 200 µmol m^{-2} s^{-1} at the plant canopy.

2.3. Preparation of Extracts

Using a magnetic stirrer plate, 1 g of dry A. graveolens powder of the organs were mixed with 10 mL of 80% methanol for 30 minutes at 120 rpm to create extracts. After being kept at 4°C for a full day, the extracts were filtered through Whatman No. 4 filter paper and vacuum-evaporated until they were completely dry. After that, they were kept at 4°C until they were examined [10]. Folin-Ciocalteau's method [11] was used to identify phenolic chemicals (Wasli et al. 2025). To put it briefly, 0.5 mL of distilled water and 0.125 mL of Folin-Ciocalteu reagent were combined with a series of dilutions of hydro-methanolic extracts (0.125 mL). Following a three-minute incubation period, 1.25 mL of a 7% sodium carbonate solution was added to the mixture, and distilled water was used to get the final volume down to 3 ml. For an additional two hours, the reaction mixtures were incubated at room temperature. After then, absorbance was measured at 760 nm in comparison to an extract-free blank. Using a calibration curve with gallic acid, the total phenolic content of the organ extracts (i.e., three replicates per treatment) was expressed as milligrams gallic acid equivalent (GAE) per gram of dry weight (mg GAE/g DW). The range of the calibration curve was 50–400 mg/mL (R2=0.99).

2.4. Total flavonoid Content (TFC)

A colorimetric technique created by Wasli et al. [11] was used to determine the total flavonoids of the hydro-methanolic extracts. 75 µL of 5% NaNO2 solutions were added to 0.25 mL of the appropriate diluted extracts. After shaking the mixture for six minutes, 0.15 ml of a newly made 10% AlCl3 solution was added. 0.5 mL of 1 M NaOH was added after 5 minutes at room temperature. Using pure water, the final volume was adjusted to 2.5 mL and well mixed. The mixture's absorbance at 510 nm was measured in comparison to a blank mixture that did not include the sample. The standard (+)-catechin graph was used to compute the amounts of flavonoid molecules, which were then represented as mg catechin equiv. g-1DW (mg CE g-1DW). The range of the calibration curve was 50–500 mg/mL.

2.5. Scavenging ability on the 2,2-diphenyl-1- picrylhydrazyl radical

The ability of scavenging DPPH was performed following the procedure described by Rguez et al. [12]. Briefly, 1 mL of six different concentrations () of dill extracts was prepared and added to 0.25 mL of methanolic solution of DPPH (0.2 mmol/L) in a test tube. After 30 min of incubation in the dark, the absorbance of the mixtures was measured at 517 nm. The antiradical activity was expressed as IC50 (mg/mL), the extract dose required to cause a 50% inhibition. Inhibition of DPPH radical was calculated as follows: DPPH scavenging effect (%) = [(Ac-As)/Ac]*100. where Ac and As are the absorbance at 30 min of the control and the sample, respectively. Ascorbic acid was used as the positive control.

2.6. Ferrous Chelating Activity

This activity was assessed as described by Zhao et al. [13]. Different concentrations of extracts were added to 0.05 mL of FeCl2, 4H2O solution (2 mM) and left for incubation at room temperature for 5 min. Then, the reaction was initiated by adding 0.1 mL of ferrozine (5 mM), and the mixture was adjusted to 3 ml with distilled water, shaken vigorously, and left at room temperature for 10 min. Absorbance of the solution was then measured at 562 nm. The inhibition of ferrozine-Fe2+ complex formation was calculated using the formula (1). Results are expressed as EC50, efficient concentration corresponding to 50% ferrous iron chelating.

2.7. Screening of the Antimicrobial Activity

2.7.1. Antibacterial Activity

To check the presence of antibacterial activity of A. graveolens extracts, tests were performed following the agar well diffusion method against human pathogenic bacteria. Gram negative: Enterobacter Kobei (WE37 K), Enterobacter cloacae (WE91 B), and Gram positive Staphylococcus warneri (KC61) and Micrococcus luteus (MG91). Strains were identified by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (WC MALDI-TOF MS). An indoor database was used to compare the protein profiles. LB medium was poured into each Sterile Petri dish. One milliliter of Bacterial suspension (108 CFU/mL-1) of pathogenic strains was mixed with LB medium at 45°C. The mixture was immediately overlain on plates containing LB medium. Wells of 6 mm diameter were formed with a sterile steel borer and punched in the agar. Aseptically, 50 µL of natural products were added into prepared wells. The Plates were then incubated for 48 h at 25°C [14]. Tests were carried out in triplicate (mean value n = 3) and results were recorded by measuring the zone of growth inhibition around the well. Negative control was treated by SDW.

2.7.2. Antifungal activity on PDA plates

The efficiency of extracts on growth inhibition of fungi was assayed by applying a dual culture technique on PDA plates. Phytopathogenic fungi used are: Penicillium expansum and Aspergillus niger isolated from blue rots of apples in refrigeration stations. Alternaria citri responsible for decay isolated from field infection. Geotrichum candidum which induces one of the most important and nauseating rots of citrus called sour rot. Fusarium and Colletotrichum causal agents respectively of Fusarium wilt and anthracnose. The fungal disc was placed in the center of the plate. At 2.5 cm from the center, wells were formed and punched in the agar, and 50 µL of extracts were added. The control positive consists of a fungal culture without extracts. Plates were incubated for 5 days at 25°C. The zones of inhibition with 0.1 mm diameter and over are considered [15]. Results presented are the mean of two assays. The effect of the different extracts on fungal mycelia was examined by light microscopy (LEICA DM 2000 LED).

2.8. 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 $p < 0.05$. Multivariate data analysis was carried out using principal component analysis (PCA). The PCA type used is Pearson's correlation and it was done using XLSTAT, considering variables centered on their means and normalized with a standard deviation of 1.

3. Results

3.1. Total Polyphenol, Flavonoid, and Condensed Tannin Content

Phenolic contents showed clear organ-dependent variation, with control leaves accumulating substantially higher levels of total phenolics (6.27 mg GAE g^{-1} DW) compared to control roots (1.52 mg GAE g^{-1} DW). Under deficiency conditions, both organs exhibited an overall increase in phenolic compounds relative to their respective controls; however, the response was considerably more pronounced in roots, where TPC rose to 2.03 mg GAE g^{-1} DW, indicating a stronger metabolic adjustment in below-ground tissues (Table 1). In contrast, flavonoid accumulation displayed a more restricted pattern: a significant increase was observed only in deficient roots, which showed a 24% rise compared to control roots, while leaf flavonoid levels remained statistically unchanged between control and deficient plants (Table 1). This pattern suggests that roots are more responsive than leaves to deficiency stress in terms of phenolic and flavonoid biosynthesis

3.2. Evaluation of Total Antioxidant Capacity and Scavenging Ability on DPPH Radical, And Ferrous Ion Ability

Several methods are commonly used to measure the antioxidant capacity of extracts. Each method results in the generation of or uses a different radical that is directly involved in the oxidative process through a variety of mechanisms. No single assay can represent the total antioxidant capacity, and for this reason four different and complementary assays were used to evaluate the extract antioxidant activities: DPPH free radical scavenging and chelating effect on ferrous ions. As shown in Table 2 under Fe-sufficient condition, DPPH scavenging capacity was more marked in leaves than in roots (matching with the lowest values of IC50). Direct Fe deficiency had no significant effect against this radical in leaf organs (Table 2), while a prominent increase was depicted in root tissues (+71% in respect to control). In addition, polyphenolic extracts were showed to be able to chelate ferrous ions mostly in deficient roots.

3.3. Evaluation of Antimicrobial Activity

The effect of direct Fe deficiency on the antimicrobial activity of A. graveolens leaf and root extracts was investigated (Table 3). Methanolic extracts of different organs have been tested against two gram-positive bacteria (Micrococcus luteus and Staphylococcus warneri), two gram-negative bacteria (Enterobacter cloacae and Enterobacter Kobei) and six pathogenic fungi (Alternaria citri, Aspergillus niger, Colletotrichum spp, Fusarium spp, Geotrichum candidum, and Penicillium expansum) using agar-well diffusion method. Clearly, the antibacterial activity differs according to the plant organs. Indeed, under Fe-sufficient condition, results showed that leaf extracts were shown to be not efficient in fighting any bacteria, contrariwise root tissues promoted an interesting activity against Micrococcus luteus and Staphylococcus warneri strains (Figure 2).

3.4. Evaluation of Antifungal Activity

As for antifungal capacity, efficient leaf organs had a strong activity against Aspergillus niger, while control root tissues were particularly effective against Colletotrichum sp and Penicillium expansum fungi with a diameters of inhibition varying between 4 and 15 mm (Table 4; Figure 4). A significant increase in antibacterial activity was induced in deficient root organs against Micrococcus luteus. In the same trend, antifungal activity was found to be enhanced in treated roots particularly against Colletotrichum sp fungi (Figure 3).

3.5. Correlation

The correlation matrix shows that polyphenols are strongly correlated with flavonoids (r = 0.97) and display strong negative correlations with DPPH (r = –0.80), CA (r = –0.50), Gram-positive bacteria (r = –0.98), and fungi (r = –0.91). Similarly, flavonoids show strong negative correlations with DPPH (r = –0.81), CA (r = –0.44), Gram-positive bacteria (r = –0.99), and fungi (r = –0.84). DPPH is moderately and negatively correlated with CA (r = –0.11) and strongly positively correlated with Gram-positive bacteria (r = 0.88) and fungi (r = 0.67). CA shows a weak positive correlation with Gram-positive bacteria (r = 0.35) and a moderate positive correlation with fungi (r = 0.57). Gram-positive activity is strongly correlated with fungal inhibition (r = 0.85). No correlation values were reported for Gram-negative bacteria (Figure 4).

Figure1. *Aspect of dill plants cultivated under Fe-sufficient (+Fe) or Fe-deficient (−Fe) conditions during the treatment period (12 days).*

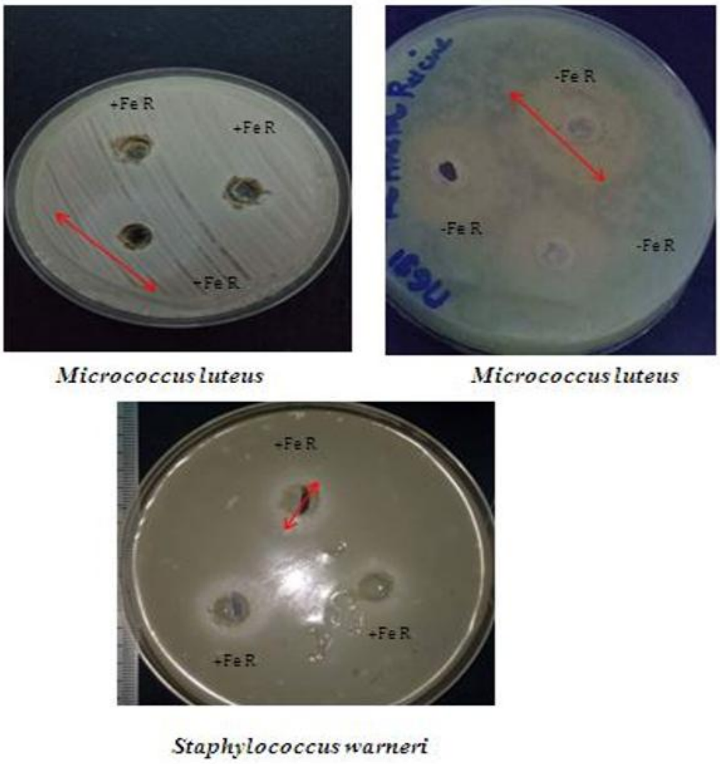

Figure2. *Example of inhibition zones induced by root (R) organs under Fe-sufficient (+Fe) or Fe-deficient (−Fe) conditions against Micrococcus luteus or Staphylococcus warneri.*

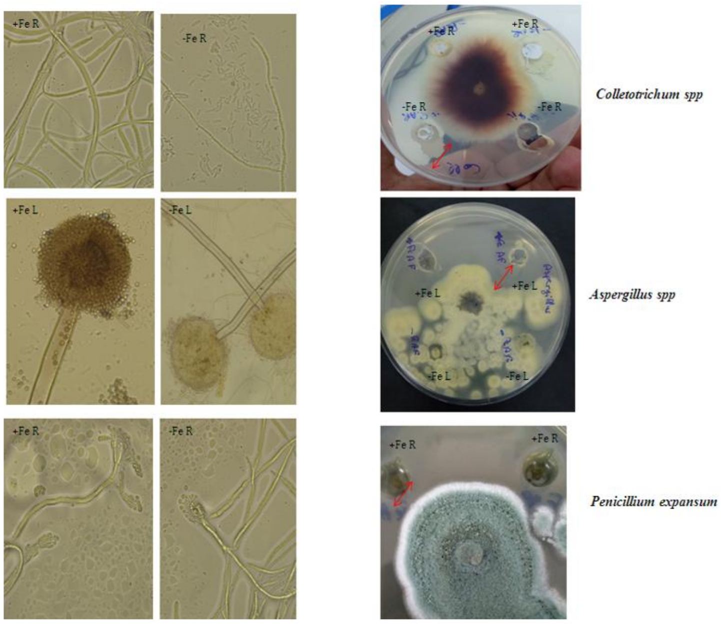

Figure3. *Antimycelial activity induced by leaf (L) or root (R) organs under Fe-sufficient (+Fe) or Fe-deficient (−Fe) conditions correlated with light microscope observation.*

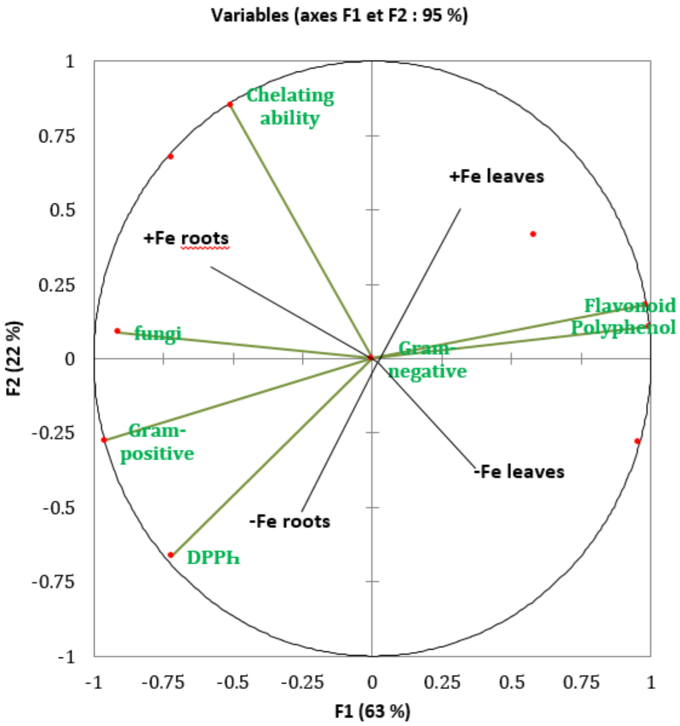

Figure4. *Principle component analysis of oxidative markers, enzymatic activities, phenolic compounds and antioxidant activities of Anethum graveolens subjected to direct Fe deficiency. * In bold, significant values at the level of significant alpha 0.005.*

Table1. *Changes in total polyphenol and flavonoïd content in leaves and roots of A. graveolens plants grown for 12 days under iron-sufficient (+Fe) or iron-deficient medium (-Fe). Values are means of three replicates and standard deviation. Values with different superscripts (a-d) are significantly different at p < 0.05.*

	Polyphenol content (mg GAE/g DW)	Flavonoid content (mg CE/g DW)
+Fe L	$6.27{\pm}0.04^b$	$3.44{\pm}0.1^a$
-Fe L	$7.33{\pm}0.01^a$	$3.55{\pm}0.2^a$
+Fe R	$1.52{\pm}0.02^d$	$0.72{\pm}0.03^c$
-Fe R	$2.03{\pm}0.08^c$	$0.91{\pm}0.05^b$

Table2. *Changes DPPH● scavenging and chelating ability, in leaves and roots of A. graveolens grown for two weeks under Fe-sufficient (+Fe) or Fe-deficient (−Fe) conditions. IC50: the concentration of the extract generating 50% inhibition; EC50: the effective concentration at which the absorbance was 0.5. Values are means of three replicates and standard deviation. Values with different superscripts (a-d) are significantly different at p < 0.05.*

	DPPH (IC_{50}, µg/mL)	Chelating ability (EC_{50}, µg/mL)
+Fe L	54 ± 2.6^{c}	310 ± 11^{b}
-Fe L	56 ± 1.2^{c}	270 ± 14^{d}
+Fe R	712 ± 5^{a}	410 ± 0.5^{a}
-Fe R	308 ± 0.2^{b}	250 ± 3^{c}
Ascorbic acid	7±0.01 -	- -
BHT	-	-
EDTA		30±0.00
Gallic acid	-	-

Table3. *Changes in antibacterial and antifungal activities in leaves and roots of of A. graveolens plants grown for two weeks under Fe-sufficient (+Fe) or Fe-deficient (−Fe) conditions. SD: standard deviation. IZ: inhibition zone. The diameter of disc was 6 mm. No antimicrobial activity (–), inhibition zone <1 mm. Weak inhibition zone, inhibition zone 1 mm. Slight antimicrobial activity, inhibition zone 2 to 3 mm. Moderate antimicrobial activity, inhibition zone 4 to 5 mm. High antimicrobial activity, inhibition zone 6 to 9 mm. Strong antimicrobial activity, inhibition zone >9 mm. Values are means of three or two replicates and standard deviation. Values with different superscripts (a-d) are significantly different at p< 0.05.*

	Diameter of inhibition zone (mm)			
	+Fe L	-Fe L	+Fe R	-Fe R
Bacteria				
Micrococcus luteus	ND	ND	21 ± 0.02^{b}	26.0 ± 0.03^{a}
Enterobacter cloacae	ND	ND	ND	ND
Staphylococcus warneri	ND	ND	1.66±0.04	ND
Enterobacter Kobei	ND	ND	ND	ND
Fungi	ND	ND	ND	ND
Alternaria citri	ND	ND	ND	ND
Fusarium spp	ND	ND	ND	ND
Penicillium expansum,	ND	ND	4.5±0.01	ND
Aspergillus niger	10.5±0.01	ND	ND	ND
Colletotrichum spp	ND	ND	15.5 ± 0.01^{a}	17.5 ± 0.01^{b}
Geotrichum candidum	ND	ND	ND	ND

Table4. *r values of Pearson correlation's test among phenolic compounds and biological activities of Anethum graveolens subjected to direct Fe deficiency*

Variables	**Polyphenol**	**Flavonoid**	**DPPH**	**CA**	**Gram-positive**	**Gram-negative**	**Fungi**
Polyphenol	1	0,97	-0,80	-0,50	-0,98	-	-0,91
Flavonoid	0,97	1	-0,81	-0,44	-0,99	-	-0,84
DPPH	-0,80	-0,81	1	-0,11	0,88	-	0,67
CA	-0,50	-0,44	-0,11	1	0,35	-	0,57
Gram-positive	-0,98	-0,99	0,88	0,35	1	-	0,85
Gram-negative					1		
fungi		-0,91	-0,84	0,67	0,57	0,85	1

4. Discussion

Chlorosis is a characteristic symptom of Fe deficiency in young developing leaves and has been widely documented in several crop species, including chia [11], fennel [16], and Medicago [17]. In the present study, dill plants subjected to Fe starvation exhibited pronounced interveinal chlorosis in their emerging leaves (Figure 1). This visual symptom is consistent with the sharp decline in SPAD values, which reflect reduced chlorophyll content under Fe deprivation [18]. The onset of iron chlorosis generally results from a dilution of chlorophyll as leaves maintain normal expansion despite Fe limitations, combined with the impaired ability of cells to synthesize or stabilize newly formed chlorophyll molecules within thylakoid membranes. Beyond affecting chlorophyll, Fe deficiency also disrupts carotenoid metabolism. As reported by Jin et al. [19], genes involved in lycopene biosynthesis (ZDS and lcyB) are downregulated under Fe deficiency, whereas the beta-carotene 3-hydroxylase gene (crtZ), responsible for lutein and zeaxanthin formation, increases dramatically, suggesting that the characteristic chlorosis observed in young leaves may partly result from a relative enrichment of carotenoids.

Such disturbances in pigment metabolism are often accompanied by an increased generation of reactive oxygen species (ROS). Indeed, unfavorable environmental conditions such as Fe deprivation can trigger excessive production of singlet oxygen ($^1O_2$), superoxide radicals ($O_2^{\bullet-}$), hydroxyl radicals (OH•), and hydrogen peroxide (H_2O_2) [10]. The accumulation of these reactive molecules disrupts redox homeostasis and leads to oxidative injuries affecting lipids, proteins, and nucleic acids [20]. To limit oxidative stress, plants activate several antioxidant mechanisms, including the synthesis of phenolic compounds. These metabolites play pivotal protective and regulatory roles, reflecting a high degree of metabolic plasticity since their biosynthesis is rapidly and reversibly enhanced under stress conditions [21]. In this study, total phenolic and flavonoid contents were measured to evaluate the antioxidant response of dill under Fe deficiency. Under Fe-sufficient conditions, both classes of metabolites accumulated preferentially in leaves compared with roots. Under Fe deprivation, however, their levels increased significantly relative to the control, with a markedly stronger induction in roots, while leaves displayed no substantial change between Fe-deficient and Fe-sufficient plants. Regarding condensed tannins, Fe deficiency induced a substantial reduction in leaf tissues, whereas tannins were completely absent in roots irrespective of Fe availability, suggesting a region-specific biosynthesis and a strong dependency on Fe nutritional status.

The consistent elevation of phenolic compounds under Fe deficiency reflects a finely coordinated metabolic reprogramming that integrates redox signaling, nutrient acquisition, and secondary metabolism. Iron scarcity perturbs electron transport chains in chloroplasts and mitochondria, enhancing electron leakage and generating an oxidative signature [10], which acts as a potent signal activating the phenylpropanoid pathway. This activation involves the transcriptional upregulation of key enzymes such as PAL, C4H, and CHS, leading to enhanced biosynthesis of flavonoids and phenolic acids [19]; similar transcriptomic stimulation of secondary metabolism under Fe deficiency has also been reported in roots [22]. In parallel, Strategy I species rely on phenolic exudation as a central component of Fe acquisition, since root-derived phenolics solubilize and reduce Fe^{3+}, improving its mobilization and uptake in alkaline and calcareous soils [19]. Furthermore, the suppression of chlorophyll biosynthesis and photosynthetic activity under Fe deficiency causes a metabolic overflow of carbon that is redirected from primary metabolism toward the synthesis of carbon-rich secondary metabolites such as phenolics [23], a response consistent with the metabolic plasticity described under abiotic constraints [3]. These compounds additionally provide structural and biochemical protection by stabilizing membranes and mitigating ROS-induced lipid and protein oxidation [24] . Collectively, these mechanisms explain why phenolic accumulation is a hallmark of Fe-limited plants, integrating redox-mediated signaling, enhanced Fe-mobilization strategies, and stress-induced metabolic rerouting to optimize plant resilience under micronutrient stress.

The assessment of antioxidant capacity in plant extracts requires the application of multiple complementary methods, as each assay targets distinct reactive species and operates through specific mechanistic pathways. Since no single assay can comprehensively reflect the total antioxidant potential [25], we employed DPPH radical scavenging, ferrous ion chelation, and reducing power assays to evaluate the antioxidant activities of dill extracts. As shown in Table 3, root extracts from Fe-deficient plants exhibited significantly enhanced DPPH-scavenging activity, whereas leaf extracts remained largely unchanged relative to the control. Similarly, the ferrous ion chelating capacity was markedly increased in roots under Fe deprivation, while leaf extracts displayed more variable responses. These organ-specific differences are consistent with previous observations indicating that plant antioxidant responses are highly dependent on both the type of abiotic stress and the tissue considered [11]. Correlation analyses further revealed that antioxidant activities were strongly associated with total phenolic and flavonoid contents: DPPH scavenging ($r = 0.792$; $r = 0.814$), and ferrous ion chelating ability ($r = 0.503$; $r = 0.546$) (Table 5; Figure 5). These findings corroborate the well-established role of phenolic compounds as major contributors to antioxidant capacity [25].

In parallel, dill extracts were investigated for their antimicrobial abilities through antibacterial and antifungal activities. Noting and to the best of our knowledge, this is the first report studying the effect of direct Fe deficiency on the antimicrobial activity. Our results showed a strong antibacterial and antifungal activity especially in deficient root organs. The efficiency of root extracts against the bacteria could in part be because of their phenolic composition (r= -0,9846). In fact, several studies attributed the inhibitory effect of plant extracts against bacterial pathogens to the phenolic composition [26]. The inhibitory effect of phenolic compounds could be explained by adsorption to cell membranes, interaction with enzymes, substrate and metal ion deprivation. Moreover, comparison of the antibacterial ability between organs revealed that the activity was strain- and origin-depend [26]. Fungi and bacterial inocula interact with plant roots to recover nutrient availability in soil for plant uptake [27]. According to Rashid et al. [28] mycelium of AM fungi release carbon compounds, which will act as energy source for soil microorganisms in the mycorrhizosphere, nonetheless the carbon products, are in small amount than already present in rhizosphere. Likewise, bacteria exude carbon compounds, which increase the AM fungi hyphal growth and its root colonization [29]. During the interaction of the complex system: rhizobia, AM fungi and legume, AM fungi enhance the yield of legume by providing water and nutrients, especially P which enhances rhizobium N2 fixation through influencing energy production pathways [30,31]. Bacteria improve phosphate accessibility for uptake of AM fungi and plant through phosphatase enzyme and organic acid production in the soil [32]. Both fungi and bacterial inocula increase the nutrient availability in the soil solution through organic matter decomposition, N fixation, P, K and Fe mobilization.

5. CONCLUSION

Direct Fe deficiency in Anethum graveolens induces organ-specific metabolic reprogramming, particularly in roots, leading to enhanced accumulation of phenolic and flavonoid compounds. This biochemical shift is closely associated with significantly improved antimicrobial performance: antibacterial activity against Micrococcus luteus and Staphylococcus warneri increased by up to 40%, while antifungal activity against Colletotrichum spp. and Penicillium expansum rose by 45% and 30%, respectively, compared with Fe-sufficient roots. These findings demonstrate that Fe scarcity can stimulate the plant's chemical defense network rather than merely impair growth, enhancing its capacity to produce bioactive metabolites that inhibit microbial growth. Understanding these organ-specific responses provides critical insights into the adaptive strategies of medicinal plants under micronutrient stress and suggests that cultivating A. graveolens in Fe-limited soils may optimize its antimicrobial potential, offering practical applications for natural product development and plant-based pathogen control.

REFERENCES

[1] Jelali, N., Donnini, S., Dell'Orto, M., Abdelly, C., Gharsalli, M., Zocchi, G. 2017. Implication of antioxidant defence in tolerance to Fe deficiency of Pisum sativum leaves. J Appl Biotechnol Bioeng. 2, 1–9.

[2] Zocchi, G. 2006. Metabolic changes in iron-stressed dicotyledonous plants. In: Abadía, J. (Ed.), Iron Nutrition in Plants and Rhizospheric Microorganisms. Springer, Netherlands, 359–370.

[3] Wasli, H., Jelali, N., Silva, A.M.S., Ksouri, R., Cardoso, S.M. 2018. Variation of polyphenolic composition, antioxidants and physiological characteristics of dill under bicarbonate-induced Fe deficiency. Ind Crops Prod. 126, 466–467.

[4] Jana, S., Shekhawat, G.S. 2010. Anethum graveolens: An Indian traditional medicinal herb and spice. Phcog Rev. 4, 179–184.

[5] Oshaghi, E.A., Khodadadi, I., Mirzaei, F., Khazaei, M., Tavilani, H., Goodarzi, M.T. 2017. Methanolic extract of dill leaves inhibits AGEs formation and shows hepatoprotective effects. J Pharm. 2017, 1–9.

[6] Vagi, E., Simandi, B., Suhadja, A., Hethelyi, E. 2005. Essential oil composition and antimicrobial activity of Origanum majorana extracts. Food Res Int. 38, 51–57.

[7] Nikolic, M., Pavlovic, J. 2018. Responses to iron deficiency and toxicity and iron use efficiency in plants. In: Plant Micronutrient Use Efficiency. 55–69.

[8] Briat, J.F., Dubos, C., Gaymard, F. 2015. Iron nutrition, biomass production and plant product quality. Trends Plant Sci. 20, 33–40.

[9] Jelali, N., Donnini, S., Dell'Orto, M., Abdelly, C., Gharsalli, M., Zocchi, G. 2014. Root antioxidant responses of Pisum sativum to Fe deficiency. Plant Biol. 16, 607–614.

[10] Hafsi, C., Falleh, H., Saada, M., Ksouri, R., Abdelly, C. 2017. Potassium deficiency alters growth, photosynthetic performance and secondary metabolites of Sulla carnosa. Plant Physiol Biochem. 118, 609–617.

[11] Wasli, H., Boukari, N., Jelali, N., Borgi, M.A. 2025. Physiological and biochemical responses of Fe-deficient Salvia hispanica to salicylic acid. Russ J Plant Physiol. 72, 243.

[12] Rguez, S., Msaada, K., Daami-Remadi, M., Chayeb, I., Bettaieb-Rebey, I., et al. 2018. Chemical composition and biological activities of S. officinalis essential oils. Plant Biosyst. 153, 264–272.

[13] Zhao, H., Dong, J., Lu, J., Chen, J., Li, Y., et al. 2006. Effect of solvent mixtures on antioxidant evaluation in barley. J Agric Food Chem. 54, 7277–7286.

[14] Mougou, I., Boughalleb-M'Hamdi, N. 2016. Epidemiology of Pseudomonas syringae pv. syringae in citrus. Br Microbiol Res J. 16, 1–10.

[15] Essghaier, B., Naouar, A., Abdelhak, J., Zid, M.F., Sadfi-Zouaoui, N. 2014. Antimicrobial activity of di(4-sulfamoyl-phenyl-ammonium) sulphate. Microbiol Res. 169, 504–510.

[16] Jelali, N., Youssef, R.B., Boukari, N., Abdelly, C. 2022. Adaptation of Sulla carnosa to Fe deficiency and salicylic acid priming. Russ J Plant Physiol. 69, 76.

[17] Wasli, H., Ben Mansour, R., Hessini, K., Abid, C., Herchi, W., Cardoso, S.M., et al. 2024. Effects of salinity and iron on physiological traits of Foeniculum vulgare. Russ J Plant Physiol. 71, 1–14.

[18] Boukari, N., Jelali, N., Abdelly, C., Hannoufa, A. 2023. SA priming modulates antioxidant defense in Medicago sativa under Fe deficiency and salinity. Physiol Plant. 175, e14026.

[19] Jin, L.F., Liu, Y.Z., Du, W., Fu, L.N., Hussain, S.B., Peng, S.A. 2017. Transcriptional pathways involved in Fe-deficiency chlorosis in citrus. Tree Genet Genomes. 85, 38–49.

[20] Jelali, N., Smiri, M., Dhifi, W., Mnif, W., Abdelly, C., Gharsalli, M. 2012. Secondary metabolism responses in Pisum sativum under Fe deficiency. Afr J Biotechnol. 11, 14828–14836.

[21] Rodríguez-Celma, J., Pan, I.C., Li, W., Lan, P., Buckhout, T.J., Schmidt, W. 2013. Molecular responses to Fe deficiency. Front Plant Sci. 4, 276.

[22] Wasli, H., Ben Mansour, R., Saada, M., Chebbi, M., Jelali, N., Ksouri, R. 2021. Effects of Fe depletion on photosynthesis and antioxidant capacity in A. graveolens. Agrochimica. 65, 317.

[23] Pérez-Pérez, J., Minguillón, S., Kabbas-Piñango, E., Payá, C., Campos, L., et al. 2024. Metabolic crosstalk between monoterpenes and salicylic acid in tomato defense. Plant Physiol. 195, 2323–2338.

[24] Michalak, A. 2006. Phenolic compounds and antioxidant activity in plants under heavy-metal stress. Pol J Environ Stud. 15, 523–530.

[25] Bettaieb-Rebey, I., Bourgou, S., Rahali, F.Z., Msaada, K., Ksouri, R., Marzouk, B. 2016. Relation between salt tolerance and biochemical changes in cumin. J Food Drug Anal. 25, 391–402.

[26] Ouerghemmi, I., Bettaieb-Rebey, I., Rahali, F.Z., Bourgou, S., Pistelli, L., et al. 2017. Antioxidant and antimicrobial phenolics from Ruta chalepensis. J Food Drug Anal. 25, 350–359.

[27] Prakamhang, J., Tittabutr, P., Boonkerd, N., Teamtisong, K., Uchiumi, T., Abe, M., Teaumroong, N. 2015. PGPR interactions with Bradyrhizobium in soybean symbiosis. Appl Soil Ecol. 85, 38–49.

[28] Rashid, M.I., Mujawar, L.H., Shahzad, T., Almeelbi, T., Ismail, I.M.I., Oves, M. 2016. Contribution of bacteria and fungi to nutrient bioavailability in degraded soils. Microbiol Res. 183, 26–41.

[29] Barea, J.M., Pozo, M.J., Azcón, R., Azcón-Aguilar, C. 2005. Microbial cooperation in the rhizosphere. J Exp Bot. 56, 1761–1778.

[30] Minerdi, D., Fani, R., Gallo, R., Boarino, A., Bonfante, P. 2001. Nitrogen-fixation genes in endosymbiotic Burkholderia. Appl Environ Microbiol. 67, 725–732.

[31] Mortimer, P., Pérez-Fernández, M., Valentine, A. 2008. Role of arbuscular mycorrhizae in carbon and nutrient economy in Phaseolus vulgaris. Soil Biol Biochem. 40, 1019–1037.

[32] Owen, D., Williams, A.P., Griffith, G.W., Withers, P.J.A. 2015. Bio-inoculants to improve phosphorus acquisition in agriculture. Appl Soil Ecol. 86, 41–54.

Citation: *Hanen Wasli, et.al., " Iron-Deficiency–Induced Alterations in Phenolic Composition, Antibacterial and Antifungal Activities of Tunisian Dill (Anethum Graveolens)" International Journal of Medicinal Plants and Natural Products (IJMPNP), vol 12, no. 1, 2026, pp. 1-10 DOI: https://doi.org/10.20431/2454- 7999.1201001.*