



Effect of Copper Stress and Foliar Iron Application on the Growth and Quality of *Sideritis cypria* Plants Grown in Cocopeat Under Different Potassium Levels

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Abstract

The integration of medicinal and aromatic plants (MAP) in conventional cultivation is hindered by a limited understanding of appropriate cultivation practices and nutritional requirements, particularly for under-evaluated MAP such as the *Sideritis cypria* Post. species. Furthermore, the development of comprehensive nutrient management is essential, with potassium and iron to have a fundamental role in plant growth and mitigate potential adverse effects of heavy metal toxicity. The current study assessed the effect of nutrient solutions (NS) with different potassium (K; 150, 350, 550 mg L⁻¹) and copper (Cu; 5, 100 µM) levels, in combination with foliar iron (Fe) applications, in the soilless cultivation of *S. cypria* plants. The lowest K application (150 mg L⁻¹) was associated with increased fresh biomass production, while the highest K application (550 mg L⁻¹) revealed improved chlorophyll and carotenoid contents. Foliar Fe modulated these responses, and was associated with growth inhibition, oxidative stress and Fe accumulation in leaves. It also increased the accumulation of N and Cu. However, copper accumulation was well below the maximum residue levels of Cu in herbal infusions. *S. cypria* antioxidant activity and stress response to excess Cu was altered under the different K levels and foliar Fe application, highlighting the interplay between heavy metal toxicity and K levels. Moreover, treatments with intermediate K levels produced plants with high antioxidant activity and moderate antibacterial activity. Low to moderate K levels in the NS can be deployed when in excess Cu, without compromising *S. cypria* production and safety. Moreover, foliar Fe applications may be applied for biofortification, upon consideration of potential toxicity risks.

Keywords Heavy metals · Copper toxicity · Biofortification · Soilless cultivation · Nutrient solution

1 Introduction

Medicinal and aromatic plants (MAP) are sources of specialty materials such as essential oils, pharmaceuticals and cosmetics (Lubbe and Verpoorte 2011). Their growing demand, however, has led to significant challenges, including overharvesting and misidentification of wild plant species. These practices jeopardize the conservation of biodiversity and undermine the efficacy and safety of MAP-derived products (Atherton and Li 2023). To attain sustained

productivity via standardized production, the application of cultivation methods such as soilless culture systems (SCS) offer a promising solution (Mehdizadeh and Moghaddam 2023). The efficacy of SCS lies primarily in their precise control over plant mineral nutrition, via the nutrient solution (NS), which can be tailored to meet the specific demands of a plant, while optimizing the production of secondary metabolites (Maggini et al. 2022). However, the lack of knowledge regarding the cultivation practices and nutritional requirements of MAP presents a significant constraint, hindering their sustainable exploitation for meeting the increasing demands of the industry (Chrysargyris and Tzortzakis 2025).

A prime example of an under-evaluated MAP species is *Sideritis cypria* Post., belonging to the *Sideritis* L. genus, which includes of more than 150 species found in temperate and tropical regions (González-Burgos et al. 2011). *S. cypria*

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is endemic to Cyprus and is locally used in traditional medicine for ailments such as stomach and respiratory disorders, headaches and the common cold. This species is characterized by a rich nutritional and phytochemical profile, along with strong antioxidant and antimicrobial properties (Tomou et al. 2021; Xylia et al. 2024). Although the species has been introduced in conventional (Lytra et al. 2020) and intensive production systems, including hydroponics (Chrysargyris and Tzortzakis 2025), further studies are needed to optimize its cultivation, especially in terms of secondary metabolites production.

Mineral nutrition has a diverse role in the metabolism of plants (Alneyadi et al. 2024; da Silva et al. 2024). Macronutrients such as nitrogen (N), phosphorus (P) and potassium (K) have prime functions in metabolic and regulatory processes of plants (Singh et al. 2017, 2024). The interaction among the minerals is a very complex subject with several reports to that direction (Singh et al. 2024). For instance, some examples are the phosphorus and iron (Fe) interaction has been demonstrated to control plant growth, nitrogen increases phosphorus uptake by stimulating root growth, balanced nitrogen improved potassium absorption (Singh et al. 2024), while zinc (Zn) accumulated more in tomatoes under phosphorus deficient (Demirer et al. 2023). A synergistic effect on nitrates and potassium has been demonstrated, while antagonistic effects of potassium and ammonium nitrogen have been stated (Fang et al. 2021). Potassium has a direct influence in the activation of various enzymes, in the protection of electrical potential gradients in cell membranes and in the preservation of the anion-cation balance (Çalışkan and Çalışkan 2018). It also has a very precise role in the metabolic activities of plant cells, while it influences the plant secondary metabolism, affecting metabolites such as phenolics, flavonoids, carotenoids, and organic acids (Mardanluo et al. 2018; Saloner and Bernstein 2022). In K deficiency, plants exhibit a low-energy status, decreased yield and quality (Barker and Pilbeam 2016; Jaiswal et al. 2016). It has also been documented that K deficiency impacts photosynthesis and the production of reactive oxygen species (ROS) (Hafsi et al. 2014). However, K sufficiency is linked with the synthesis of secondary metabolites, the strengthening of cell walls and membrane integrity, as well as the increase of enzyme functions and improvement ROS balance and antioxidation (Jaiswal et al. 2016; Wang et al. 2013).

Copper (Cu) is a naturally occurring mineral in soils, that is essential micronutrient for plants, influencing a number of metabolic functions, including photosynthesis, respiration, enzyme production, and nitrogen assimilation (Cruz et al. 2022; Rai et al. 2021). However, anthropogenic processes, including mining and smelting, and the excessive

application of Cu-containing fertilizers and agrochemicals have caused Cu pollution in the natural environment (Amin et al. 2021). Its excess by even a small amount can be toxic for plants, with negative impacts on germination, plant growth and morphology (Adrees et al. 2015; Rob et al. 2024). Some responses include poor biomass production and root growth, chlorosis and necrosis, due to the increased production of ROS and oxidative stress (Kumar et al. 2021). Copper toxicity can also impair the uptake of other minerals, including K, calcium (Ca) and Fe (Adrees et al. 2015).

Iron, a vital micronutrient for plants, absorbed as Fe^{2+} and Fe^{3+} , is associated with various physiological processes including respiration and redox reactions (Awad-Allah and Elsokkary 2020; Mahmoud et al. 2022). It is also a critical component of electron chains, required for photosynthesis and chlorophyll synthesis (Schmidt et al. 2020). In addition, Fe is a constituent of key antioxidant enzymes, such as catalase, ascorbate peroxidase and superoxide dismutase (Rai et al. 2021). Iron deficiency impairs a plants' normal growth and development (Mahmoud et al. 2022), and can also induce the accumulation of Cu, as both nutrients compete with for reduction sites (Bosnić et al. 2019; Yruela 2009). However, excessive Fe, can be toxic for plants, particularly through the iron-dependent conversion of superoxide ion (O_2^-) and hydrogen peroxide (H_2O_2) into highly reactive hydrogen oxide (OH), although it can also protect from oxidative damage by scavenging ROS (Kumar et al. 2008). In addition, increased Cu can reduce the demand for Fe, due to the rapid synthesis of Cu proteins which replace Fe proteins, and the suppression of ferric-chelate reductase activity (Rai et al. 2021). However, these effects depend on the plant species, as they adopt different tolerance strategies (Yruela 2009). It is evident that low Fe availability can exacerbate Cu toxicity, contingent on plant species and soil conditions. Thus, Fe fertilization via root or foliar applications is often recommended (De Conti et al. 2020). Interestingly, Fe can modulate the tolerance to heavy metal stress due the Fe plaque formation, which acts a buffer for metals such as Cu, Zn and manganese (Mn) (Asle-Mohammadi et al. 2024).

As both Cu and Fe are transition metals involved in redox reaction, and are toxic in high concentrations, their uptake and utilization should be tightly regulated (Franco et al. 2023). Furthermore, K is a macronutrient that affects the anion-cation balance, and the presence of the K^+ ion influences the uptake of the free copper ion, as they compete for binding sites, potentially alleviating Cu toxicity (Fageria 2015; Lock et al. 2007). In addition, adequate supply of K is essential for optimizing Fe utilization within plant metabolic processes (Ahmed et al. 2023; Awad-Allah and Elsokkary 2020; de Morais et al. 2023). The hypothesis of this

study was that K in different levels in the NS and/or foliar application with Fe can mitigate any induced stress occurred in *S. cypria* plants by the increased Cu levels. These parameters highlight the need for a balanced nutrient management strategy, particularly under conditions of heavy metal toxicity. Thus, the objective of the current study was to evaluate the effect of varying K levels in the hydroponic NS, on the growth and physiological performance of hydroponically cultivated *S. cypria*. Additionally, the study aimed to assess the simultaneous effects of excessive Cu on plant health, and to evaluate the role of foliar Fe in enhancing productivity and mitigating Cu-induced toxicity.

2 Materials and Methods

2.1 Plant Materials and Experimental Site

The experimental study was carried out under protected cultivation at the greenhouse located in the experimental farm of the Cyprus University of Technology in Limassol, Cyprus. *S. cypria* seedlings were provided by the Department of Aromatic Plants, Ministry of Agriculture, Cyprus, and were transplanted in 2.3 L pots using a substrate media consisting of cocopeat and perlite (4:1 v/v), in November 2022. Each pot was accompanied with plates to collect run-off. Plants were initially supplemented by a starter NS (sNS) for 28 days after transplanting (DAT). Following an acclimation period, plants were subjected to the main NS under examination, with varying K levels: (i) K150 (150 mg K L⁻¹), (ii) K350 (350 mg K L⁻¹), and (iii) K550 (550 mg K L⁻¹). All other nutrients remained constant. The NS had an electrical conductivity (EC) of 2.57 mS cm⁻¹, and a pH of 6.0 which was regulated using diluted (5% v/v) nitric acid. The NS was applied once or twice weekly, based on real-time plant and environment monitoring, using 50–100 mL NS pot⁻¹. Treatments were divided, considering the foliar applications; (i) No foliar Fe (pure distilled water - dH₂O), and (ii) Foliar Fe with iron (II) sulfate heptahydrate (dH₂O with 0.5 mg L⁻¹ FeSO₄·7H₂O or 1.8 mM Fe). Foliar spraying was applied three times during the experiment, beginning at 58 DAT. Concentrations and spraying intervals were based on previous findings (Chrysargyris et al. 2018). At 95 DAT, plants were subjected to different Cu levels in the NS, namely (i) 5 µM Cu (control), and (ii) 100 µM Cu, with the addition of copper sulphate. Plants were grown under these different Cu levels, with periodical fertigation applications for an additional 30 days, resulting in a total growth duration of 125 DAT. During cultivation, common agricultural practices were followed. The average day and night temperature was 19.5 °C and 13.6 °C, respectively, while the average day and night humidity was 60.4% and 71.5%, respectively.

2.2 Agronomic Parameters

The study included 48 plants, which were separated per each of the three different K levels, two Cu levels, and two Fe foliar applications. Thus, the study had a total of 12 treatments (3 K levels x 2 Cu levels x 2 Fe foliar applications) with each one accommodating 3–5 plants (replicates). Once cultivation ended, all plants for each treatment were used to measure plant fresh (g) and dry weight (g), and dry matter content (%).

2.3 Leaf Phytochemistry Features

Shortly before harvesting, leaf phytochemistry features were determined. SPAD (Soil Plant Analysis Development) was determined using the SPAD-502 Plus chlorophyll meter (Minolta, Osaka, Japan). Chlorophyll fluorescence (Fv/Fm) was measured with the OptiSci OS-30p chlorophyll fluorometer (Opti-Sciences, Hertfordshire, UK), following leaf incubation in the dark (15 min). Finally chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophylls (Total Chl) and total carotenoids (Total Car) contents were evaluated as previously described (Richardson et al. 2002; Wellburn 1994). Leaf tissue (0.2 g) was extracted with 7 mL methanol 100% and incubated in a heat bath at 65 °C for 30 min in the dark. Afterwards, the absorbance was measured at 470, 653 and 666 nm and photosynthetic pigments, i.e., chlorophylls (Chl a, Chl b, Total Chl) and carotenoids (Total Car) contents were calculated and expressed as milligrams per gram of fresh weight (mg g⁻¹ FW).

2.4 Plant Tissue Ion Concentration Analysis

Leaf tissue samples were collected from a pool of plants per treatment, were dried at 42 °C, and ash-burned at 550 °C for 270 min. Following acid digestion (2 M HCl), K and sodium (Na) were determined by flame photometry (Lasany Model 1832, Lasany International, Panchkula, India), P was determined by the molybdate/vanadate method (yellow method) using a spectrophotometer (Multiskan GO, Thermo Fischer Scientific, Waltham, USA), and N was determined by the Kjeldahl method (BUCHI, Digest automat K-439 and Distillation Kjelflex K-360, Flawil, Switzerland) as described by Chrysargyris et al. (2019). Magnesium (Mg) and Ca, and micronutrients, including Fe, Zn, and Cu, were determined by an atomic absorption spectrophotometer (PG Instruments AA500FG, Leicestershire, UK) by using flame and graphite, respectively.

2.5 Total Phenols, Total Flavonoids, Antioxidants and Stress Indicators

Three leaf tissue replicates per treatment were used to obtain the methanolic (50% v/v) extracts for the quantification of

total phenols and flavonoids. Methanolic extracts were homogenized (ULTRA-TURRAX T 25, IKA-Werke, Germany), sonicated, followed shaking (200 rpm), and centrifuged (5000 rpm) at 4 °C. Finally, supernatant collected and stored for analysis. Total phenols content was determined by the employment of the Folin–Ciocalteu assay, with measurements at 755 nm. Results were expressed in gallic acid equivalents (mg GAE g⁻¹ FW). Antioxidant activity was evaluated with the following assays: (i) 2,2-diphenyl-1-picrylhydrazyl (DPPH), (ii) ferric reducing antioxidant power (FRAP), and (iii) 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay, as previously described (Chrysargyris et al. 2016). For FRAP, samples were measured at 593 nm, and the absorbance was converted in a FRAP value via the change of the absorbance against a standard. For DPPH, samples were measured at 517 nm. ABTS was measured as described previously (Xylia et al. 2022), with measurements at 734 nm against a blank sample. Results were expressed as Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) equivalents (mg of Trolox g⁻¹ FW). Finally, flavonoids were determined using the aluminium chloride (AlCl₃) colorimetric assay (Chrysargyris et al. 2016). Briefly, extracts and 0.75 mL of 5% NaNO₂ (sodium nitrite) were incubated for 6 min. Subsequently, 0.15 mL of 10% AlCl₃ and 0.5 mL 1 M NaOH (sodium hydroxide) were added at 5 min intervals while the final volume was brought to 2.5 mL using distilled water. Measurements were conducted at 510 nm and expressed as Rutin equivalents (mg Rutin g⁻¹ FW).

The stress response of *S. cypria* plants was evaluated by the H₂O₂ and malondialdehyde (MDA) content, as described by Chrysargyris et al. (2020). In brief, H₂O₂ was determined based on a standard calibration curve ranging from 5 to 1000 µM H₂O₂, by measuring the absorbance at 390 nm, and results are expressed as µmol of H₂O₂ per gram of fresh weight (µmol H₂O₂ g⁻¹ FW). MDA was determined by measuring the absorbance of reaction mixtures at 532 nm (corrected at 600 nm). MDA was calculated using the extinction coefficient of 155 mM⁻¹ cm⁻¹, and results are expressed as nmol of MDA per gram of fresh weight (nmol MDA g⁻¹ FW).

2.6 Biological Properties of *S. cypria* Ethanolic Extracts

2.6.1 Preparation of Ethanolic Extracts

The ethanolic extracts were prepared from dried plant material (air-drying at 42 °C) following a procedure previously described (Lall et al. 2019). Briefly, appropriate amount of tissue was macerated for 72 h (at 160 rpm) with pure ethanol (Merck, Darmstadt, Germany) in a 1:2.5 v/v ratio. After vacuum filtration (Whatman No. 1, Merck, Darmstadt,

Germany), the ethanol was removed with a rotary evaporator (Laborota 4011 digital, Heidolph Instruments, Schwabach, Germany) until dryness. Stock solutions (8 mg mL⁻¹) were prepared in 30% ethanol (this concentration did not interfere with the bacterial growth), while two-fold dilutions were prepared in sterile dH₂O.

2.6.2 Total Phenols and Antioxidant Activity

The determination of the ethanolic extract's total phenols was performed using the Folin–Ciocalteu's phenol reagent according to the methodology described by Chrysargyris et al. (2023) and results were expressed as mg GAE per gram of dry extract weight (mg GAE g⁻¹ DW). The DPPH and FRAP assays were used for the estimation of the antioxidant activity of the obtained *S. cypria* ethanolic extracts following the methodologies reported by Chrysargyris et al. (2016). A third protocol (ABTS) was implemented to further assess the antioxidant activity of the obtained ethanolic extracts following the protocol described by Wojdyło et al. (2007). The results for the antioxidant activity were presented as mg of Trolox per gram of dry extract weight (mg Trolox g⁻¹ DW).

2.6.3 Antibacterial Activity

Bacterial Inoculum Preparation Fresh bacterial cultures (108 colony forming units per mL; 108 cfu mL⁻¹) were prepared for the following bacteria: *Staphylococcus aureus* (ATCC 11632), *Listeria monocytogenes* (ATCC 19111), *Escherichia coli* (ATCC 25922), and *Salmonella enterica* subsp. *enterica* (ATCC 51741). The inoculum was prepared in Brain heart infusion broth (BHI broth, Biokar, Beauvais Cedex, France) after overnight (16–18 h) incubation at 37 °C (Chrysargyris et al. 2017).

Disc Diffusion and Broth Microdilution Assay The disc diffusion assay was implemented as previously reported (Chrysargyris et al. 2017). In brief, a sterile filter paper disc (6 mm diameter) supplemented with 10 µL of ethanolic extract (4 mg mL⁻¹) was placed on the inoculated surface of solidified BHI agar medium. Following 24 h incubation at 37 °C, the diameter of the inhibition zone (DIZ) was recorded (in mm). Ampicillin (Sigma-Aldrich, Taufkirchen, Germany) was used as standard antibiotic (100 µg mL⁻¹).

The broth microdilution assay was implemented according to Chrysargyris et al. (2017). Briefly, 50 µL of BHI broth, 45 µL of ethanolic extract dilution (concentrations: 0.03–4 mg mL⁻¹), and 5 µL of bacterial inoculum (106 cfu mL⁻¹ per well) were added to each well of a sterile 96-well plate. After sealing, the plate was incubated for 24 h at 37 °C and the optical density was read every 30 min at 600 nm

(Multiskan GO, Thermo Fischer Scientific, Waltham, USA). The minimum inhibitory concentration (MIC) and the concentration inhibiting the bacterial growth by 50% (IC₅₀) were calculated and presented as mg mL⁻¹. A standard antibiotic (i.e. ampicillin) was used as reference (concentrations: 0.78–100 µg mL⁻¹). This procedure was performed under sterile conditions ensured using both negative (BHI broth alone and BHI broth with ethanolic extract dilution) and positive controls (BHI broth with bacterial inoculum).

2.7 Statistical Methods

Experimental data statistical analysis was conducted by applying three-way Analysis of variance (ANOVA) using SPSS v.21 (IBM, New York, USA). Main effects [K levels (K and Cu application (Cu), and Fe foliar application (Fe)], three first-order interactions (K × Cu, K × Fe, Cu × Fe) and a second-order interaction (K × Cu × Fe) were evaluated. Results are expressed as mean ± standard error (SE). When ANOVA adjudicated a significant treatment impact at $p \leq 0.05$, Duncan's multiple range tests we employed.

3 Results

3.1 Effect of K levels, Cu applications, Foliar Fe, and their Interactions

The effects of the different K levels, Cu applications, and Fe foliar applications, and their first- and second-order on *S. cypria* plant growth and physiology are examined in Table 1. Plant biomass and leaf pigments, including chlorophylls and carotenoids, were mainly affected by the K levels in the NS and foliar applications with Fe. Mineral accumulation in leaves and roots was mainly affected by the K levels, followed by Cu and Fe, and last by their interactions. The total phenols, flavonoids, H₂O₂, and MDA content as well as antioxidant capacity of the plants were affected mainly by K, followed by Fe and then by Cu, with notable effects from their interaction at first- and second-order.

3.2 Plant Growth Parameters

The impact of the evaluated parameters on *S. cypria* plant growth (fresh weight; FW, dry matter content; DM) is presented in Table 2. The highest *S. cypria* plant FW was observed under the application of K150, 100 µM Cu and no foliar Fe, while the lowest were attained under the application of K550, 100 µM Cu and foliar Fe. Regarding DM, plants exhibited higher values with K350, 5 µM Cu and no foliar Fe, compared to treatments involving K150 with 5 µM Cu, K550 with 5 µM Cu and foliar Fe, and K550 with 100 µM Cu.

3.3 Leaf Photochemistry Features

The effect of the examined parameters on leaf photochemistry features, is presented in Table 3. The highest SPAD was recorded with the application K350 with 100 µM Cu and no foliar Fe, while the lowest was recorded with the application K550, 5 µM Cu and foliar Fe. Chlorophyll fluorescence (Fv/Fm) was the highest with the application of K350, 5 µM Cu, and foliar Fe, while the lowest was with the application of K550, 5 µM Cu and no foliar Fe. The highest contents of Chl a and Total Chl were observed under the application of K550, 5 µM Cu and foliar Fe. However, these values did not differ significantly from other treatments that included either K550, or foliar Fe. The lowest values were recorded for plants treated with K150, 100 µM Cu and no foliar Fe. Similarly, Total Car were the highest with the application of K550, 5 µM Cu and foliar Fe, with improved values being observed across the K550 treatment. In contrast, low values of Total Car were observed under both K150 and K350 applications, with increases being observed with the co-application of foliar Fe, depending on the application of Cu.

3.4 Plant Nutrient Content

Leaf and root mineral content, as affected by the examined parameters, is detailed in Fig. 1. Leaf N was generally increased with the application of foliar Fe, and with the application of Cu in K350 and K550 treatments. However, K levels did not affect the content of N in leaves (Fig. 1, A1). In roots, however, the influence of foliar Fe deviated among treatments, depending on the level of K, while with the application of excess Cu, decreases were observed with ≥ 350 mg K L⁻¹ (Fig. 1, A2). In leaves, the lowest accumulation of P was observed with the application of K350, regardless of Cu or Fe foliar application, while in roots, the highest P contents were observed with the applications of K150 with 5 µM Cu, and K550 with 100 µM Cu, both with no foliar Fe (Fig. 1, B). Leaf K contents remained largely unaffected by the different K levels; however, excess Cu enhanced K accumulation under the K550 treatment (Fig. 1, C1). Additionally, foliar Fe applications resulted in increased root K contents under K350, and decreased root K contents under K150 and K550 (Fig. 1, C2). In leaves, Na was mostly increased with excess Cu at K150 (Fig. 1, D1). However, in roots, Na was mostly decreased with the increasing K levels in the NS (Fig. 1, D2). The lowest leaf Ca was observed with the application of K550 with no foliar Fe, with both Cu levels, while the highest content was observed with the application of K150, 100 µM Cu and foliar Fe, although the foliar application did not affect this treatment (Fig. 1, E1). Root Ca was sharply decreased with the application of K150, irrespective of Cu

Table 1 The effect of K levels (K), Cu application (Cu), and Fe foliar application (Fe) and their first-order interactions (K × Cu, K × Fe, Cu × Fe), and their second-order interaction (K × Cu × Fe)

FW (g)	K	Cu	Fe	K × Cu	K × Fe	Cu × Fe	K × Cu × Fe
	*	ns	*	ns	ns	ns	ns
DM (%)	*	ns	ns	*	ns	ns	ns
SPAD	*	ns	ns	ns	ns	ns	ns
Fv/Fm	*	ns	ns	***	ns	ns	ns
Chl a (mg g ⁻¹)	**	ns	**	ns	ns	ns	*
Chl b (mg g ⁻¹)	ns	ns	*	ns	ns	ns	ns
Total Chl (mg g ⁻¹)	*	ns	**	ns	ns	ns	ns
Total Car (mg g ⁻¹)	***	ns	ns	ns	ns	ns	**
Leaves N (g kg ⁻¹)	***	***	***	**	***	ns	ns
Leaves P (g kg ⁻¹)	***	ns	*	**	**	ns	ns
Leaves K (g kg ⁻¹)	*	**	ns	ns	ns	ns	ns
Leaves Na (g kg ⁻¹)	***	***	ns	***	ns	ns	***
Leaves Ca (g kg ⁻¹)	***	***	ns	*	***	ns	*
Leaves Mg (g kg ⁻¹)	ns	ns	ns	ns	ns	ns	ns
Leaves Fe (mg kg ⁻¹)	***	ns	***	ns	ns	*	ns
Leaves Cu (mg kg ⁻¹)	***	***	***	***	***	***	***
Leaves Zn (mg kg ⁻¹)	ns	ns	ns	ns	ns	ns	ns
Roots N (g kg ⁻¹)	***	**	***	***	ns	***	***
Roots P (g kg ⁻¹)	*	ns	ns	***	*	*	*
Roots Na (g kg ⁻¹)	***	ns	***	**	ns	***	***
Roots K (g kg ⁻¹)	***	***	ns	***	***	ns	***
Roots Ca (g kg ⁻¹)	***	**	**	***	***	***	***
Roots Mg (g kg ⁻¹)	*	ns	ns	ns	ns	ns	ns
Roots Fe (mg kg ⁻¹)	***	*	**	***	***	ns	*
Roots Cu (mg kg ⁻¹)	***	***	*	ns	*	ns	ns
Root Zn (mg kg ⁻¹)	ns	***	*	ns	*	ns	ns
Phenols (mg GAE g ⁻¹)	***	ns	*	ns	**	***	*
DPPH (mg Trolox g ⁻¹)	***	***	ns	ns	***	***	***
FRAP (mg Trolox g ⁻¹)	***	***	*	***	***	***	***
ABTS (mg Trolox g ⁻¹)	***	ns	**	***	***	***	***
Flavonoids (mg Rutin g ⁻¹)	***	**	***	***	***	ns	***
H ₂ O ₂ (μmol g ⁻¹)	***	ns	***	ns	***	*	***
MDA (nmol g ⁻¹)	*	ns	ns	*	***	ns	***

***: significant at $p \leq 0.001$; **: significant at $p \leq 0.01$; *: significant at $p \leq 0.05$; ns: not significant, according to three-way ANOVA. fresh weight (FW); dry matter (DM); chlorophyll fluorescence (Fv/Fm) chlorophyll a (Chl a); Chlorophyll b (Chl b); total chlorophylls (Total Chl); total carotenoids (Total Car); 2,2-diphenyl-1-picrylhydrazyl (DPPH); ferric reducing antioxidant power (FRAP); 2,20-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS); hydrogen peroxide (H₂O₂); malondialdehyde (MDA)

or foliar Fe application, compared to the rest of the treatments. In contrast, the highest values were observed with the application of K550, apart from the treatment involving the co-application with 5 μM Cu and no foliar Fe (Fig. 1, E2). Finally, leaf and root Mg uptake was slightly affected by the examined treatments (Fig. 1, F1, F2).

The micronutrient content of *S. cypria* was also affected by the treatments under examination (Fig. 2). Leaf Cu was positively correlated with the application of foliar Fe, while increases were observed with the application of excess Cu only when K550 was applied (Fig. 2, A1). However, root Cu was increased under excess Cu in the NS (Fig. 2, A2). Foliar Fe applications positively affected the accumulation of Fe in leaf tissues under 5 μM Cu, while no significant differences

were observed among treatments with excess Cu in the NS (Fig. 2, B1). However, root Fe was mostly influenced by K levels in the NS, with K550 resulting in decreased root Fe accumulation (Fig. 2, B2). Finally, most of the Zn was accumulated in the root part of *S. cypria* plants, while increases occurred with the application of excess Cu under K150 in the NS (Fig. 2, C2).

3.5. Total phenols, Total flavonoids, Antioxidant Capacity

Total phenols, antioxidants and flavonoids of *S. cypria* plants, as affected by the examined parameters, are presented in Fig. 3. Total phenols were the lowest with the application

Table 2 The effect of K levels (K150: 150 mg L⁻¹, K350: 350 mg L⁻¹, K550: 550 mg L⁻¹), Fe foliar application (0 and 1.8 mM Fe), and Cu application (5 and 100 µM Cu) on the fresh weight (FW; g) and dry matter (DM; %) of *Sideritis cypria* plants grown in soilless cultivation

K levels	Cu application	Foliar Fe	FW	DM
K150	5 µM	0 mM	42.48±6.31ab	18.44±0.04bc
		1.8 mM	33.33±2.16bc	17.87±0.35c
	100 µM	0 mM	48.70±0.56a	20.11±0.55ab
		1.8 mM	35.48±2.37bc	19.64±1.39abc
K350	5 µM	0 mM	36.53±4.84abc	20.73±0.70a
		1.8 mM	34.00±4.02bc	19.78±0.18abc
	100 µM	0 mM	36.56±5.22abc	19.78±0.19abc
		1.8 mM	31.83±3.45bc	19.38±0.58abc
K550	5 µM	0 mM	36.32±2.89abc	19.54±0.22abc
		1.8 mM	32.70±3.89bc	18.11±0.50bc
	100 µM	0 mM	31.16±4.04bc	17.79±1.08c
		1.8 mM	27.63±2.62c	18.15±0.14bc

Significant differences ($p \leq 0.05$) among foliar applications are indicated by different letters

of K350, 5 µM Cu and no foliar Fe (Fig. 3, A). In contrast, the highest phenols were obtained with the application of K550, 100 µM Cu and no foliar Fe, and with the application of K350, 5 µM Cu and foliar Fe, similar to the antioxidant capacity of plants, as assayed by DPPH (Fig. 3, B), FRAP (Fig. 3, C) and ABTS (Fig. 3, D). Flavonoids accumulation was primarily enhanced by Fe foliar in the presence of 5 µM Cu in all K levels, as well as under the K550 treatment irrespective of Fe foliar application (Fig. 3, E).

3.6 Stress Indicators

The stress response of *S. cypria* under the different treatment applications is presented in Fig. 4. In the case of H₂O₂ accumulation, increased levels were observed with K350 in the absence of foliar Fe, as well as with K550, regardless of foliar application. This was independent of

Cu concentrations in the NS, although excess Cu resulted in increased H₂O₂ in plants grown under K350 and K550 treatments, contingent on foliar Fe. Notably, while foliar Fe alleviated the plant's H₂O₂ increase under excess Cu in the K550 treatment, it had the opposite effect in the K350 treatment (Fig. 4, A). The MDA content was the highest under K550 with 100 µM Cu and no foliar Fe, whereas foliar Fe application mitigated this effect. However, an inverse trend was observed under K150, where foliar Fe resulted in increased MDA levels (Fig. 4, B).

3.7 Biological Properties of *S. Cypria* Ethanolic Extracts

3.7.1 Total Phenols and Antioxidant Activity

The total phenolic content and the antioxidant activity of the obtained *S. cypria* ethanolic extracts as affected by the investigated treatments are presented in Table 4. Total phenols were found increased on extracts from plants treated with K350 (highest value 35.95 mg GAE g⁻¹) compared to other treatments, whilst K150 (with 5 and 100 µM Cu) and K350 (with 100 µM Cu) were the treatments whose ethanolic extracts presented the lowest phenolic content. Similar, high antioxidant activity (FRAP and ABTS) was observed with extracts obtained from plants treated with K350 compared to the ones from K150 (with 100 µM Cu). In addition, extracts from K350 and K550 (with 100 µM Cu and foliar Fe) treatments also presented higher antioxidant activity (DPPH and ABTS) compared to K150 with 100 µM Cu (with and without Fe foliar application).

3.7.2 Antibacterial Activity

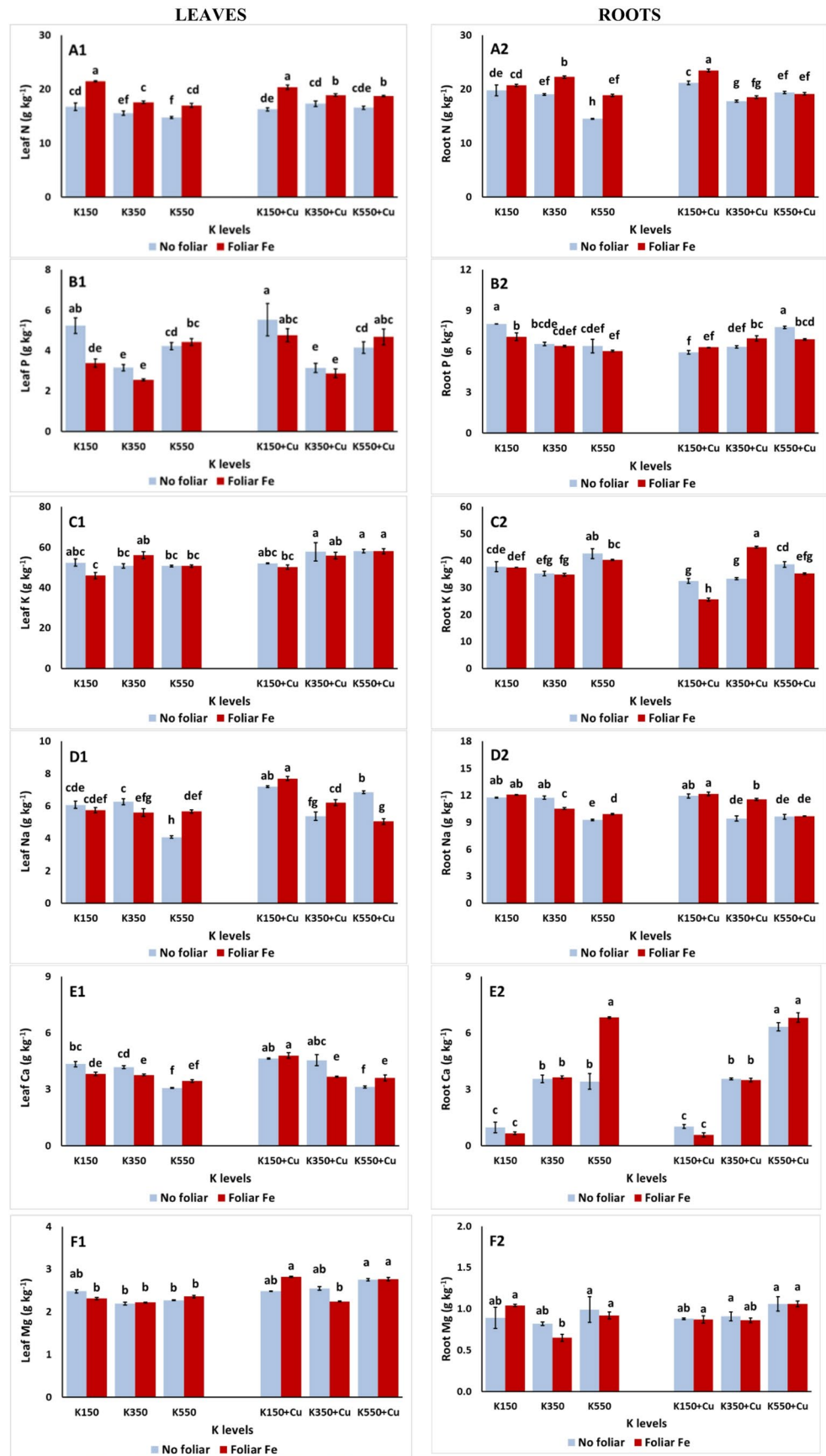
Table 5 presents the effects of the applied treatments on the antimicrobial activity of the ethanolic extracts obtained

Table 3 The effect of K levels (K150: 150 mg L⁻¹, K350: 350 mg L⁻¹, K550: 550 mg L⁻¹), Fe foliar application (0 and 1.8 mM Fe), and Cu application (5 and 100 µM Cu) on leaf SPAD, chlorophyll fluorescence (Fv/Fm), chlorophyll a (Chl a; mg g⁻¹), chlorophyll b (Chl b; mg g⁻¹), total chlorophylls (Total Chl; mg g⁻¹), total carotenoids (Total Car; mg g⁻¹) of *Sideritis cypria* plants grown in soilless cultivation

K levels	Cu application	Foliar Fe	SPAD	Fv/Fm	Chl a	Chl b	Total Chl	Total Car
K150	5 µM	0 mM	45.47±2.35abc	0.811±0.003abc	0.57±0.01 cd	0.21±0.01	0.78±0.01cde	0.12±0.02bc
		1.8 mM	47.08±0.71abc	0.810±0.003abc	0.63±0.07abcd	0.30±0.03	0.93±0.09abc	0.06±0.01d
	100 µM	0 mM	41.97±2.62c	0.799±0.005bcd	0.44±0.02e	0.23±0.06	0.67±0.05e	0.09±0.01 cd
		1.8 mM	46.88±2.97abc	0.808±0.001abc	0.69±0.05ab	0.29±0.03	0.99±0.08ab	0.13±0.01ab
K350	5 µM	0 mM	49.17±0.94abc	0.825±0.006ab	0.60±0.04bcd	0.23±0.01	0.83±0.05bcde	0.11±0.01bc
		1.8 mM	50.31±2.27ab	0.835±0.006a	0.67±0.04abc	0.27±0.02	0.94±0.06abc	0.13±0.01ab
	100 µM	0 mM	52.27±4.55a	0.812±0.010abc	0.53±0.02de	0.21±0.02	0.74±0.04de	0.10±0.01c
		1.8 mM	49.36±2.20abc	0.828±0.012ab	0.58±0.02bcd	0.27±0.05	0.85±0.05abcd	0.12±0.01bc
K550	5 µM	0 mM	48.13±1.27abc	0.779±0.006d	0.66±0.04abc	0.26±0.02	0.92±0.06abcd	0.13±0.01ab
		1.8 mM	42.27±2.69c	0.787±0.020 cd	0.74±0.04a	0.28±0.02	1.02±0.05a	0.16±0.02a
	100 µM	0 mM	44.33±1.61bc	0.827±0.007ab	0.64±0.02abcd	0.25±0.01	0.89±0.02abcd	0.13±0.01ab
		1.8 mM	47.43±1.08abc	0.801±0.004bcd	0.64±0.04abcd	0.26±0.02	0.89±0.06abcd	0.13±0.01ab

Significant differences ($p \leq 0.05$) among foliar applications are indicated by different letters

Fig. 1 The effect of K levels (K150: 150 mg L⁻¹, K350: 350 mg L⁻¹, K550: 550 mg L⁻¹), Fe foliar application (No foliar; 0, and Foliar Fe; 1.8 mM Fe), and Cu application (No additional Cu; 5, and additional Cu; 100 μ M Cu) on leaf and root N (A1, A2), P (B1, B2), K (C1, C2), and Na (D1, D2), Ca (E1, E2) and Mg (F1, F2), respectively, of *Sideritis cypria* plants grown in soilless cultivation. Significant differences ($p \leq 0.05$) among the different applications are indicated by different letters



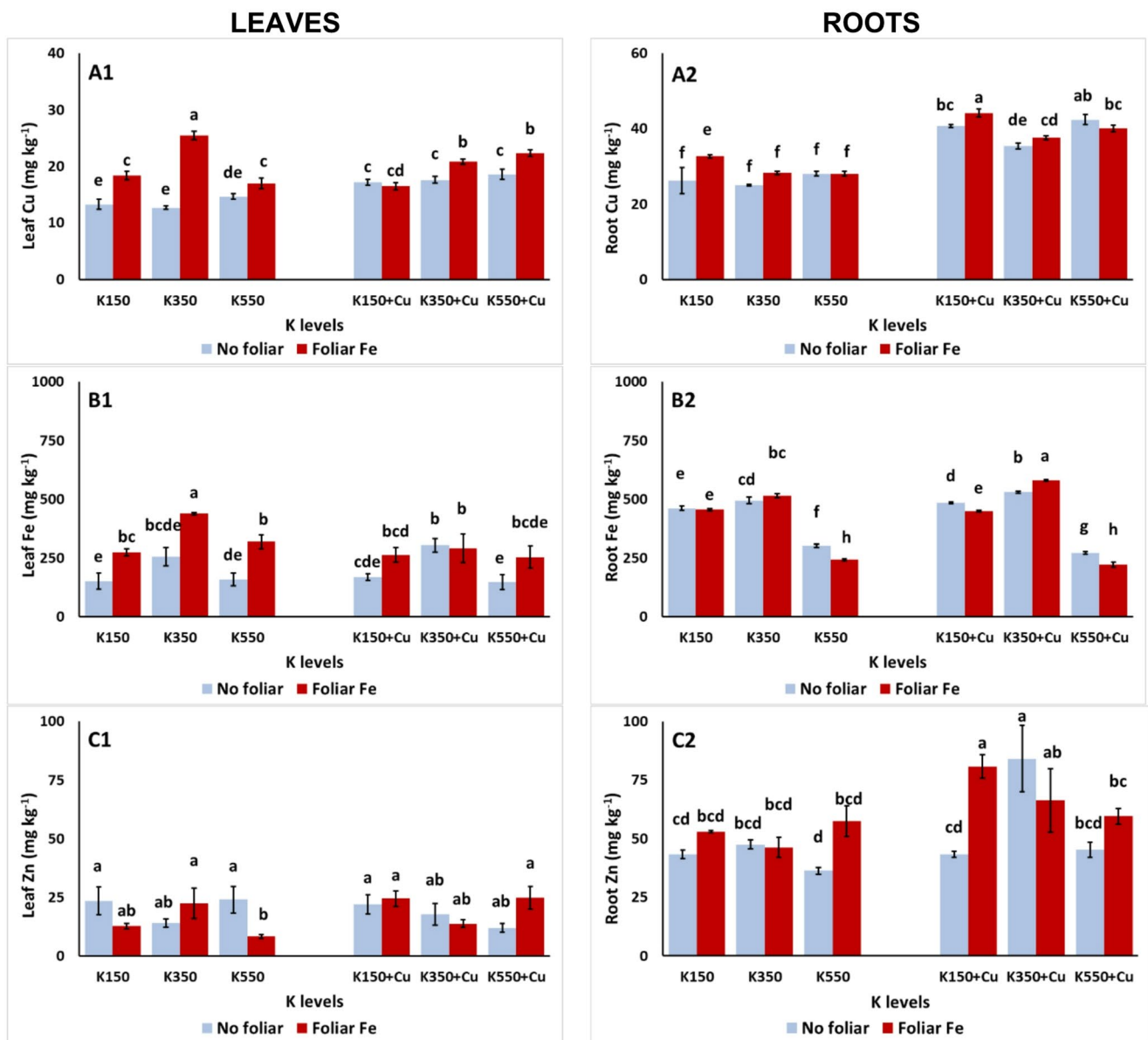


Fig. 2 The effect of K levels (K150: 150 mg L⁻¹, K350: 350 mg L⁻¹, K550: 550 mg L⁻¹), Fe foliar application (No foliar; 0, and Foliar Fe; 1.8 mM Fe), and Cu application (No additional Cu; 5, and additional Cu; 100 µM Cu) on leaf and root Cu (A1, A2), Fe (B1, B2), and Zn

(C1, C2), respectively, of *Sideritis cypria* plants grown in soilless cultivation. Significant differences ($p \leq 0.05$) among the different applications are indicated by different letters

from the treated plants. The overall DIZ values measured for all bacteria were found to be smaller than the ones of ampicillin, indicating lower antibacterial activity. However, extracts from plants treated with K350 presented DIZ similar to ampicillin when tested against *S. aureus*. The DIZ of plant extracts obtained from K150 (5 and 100 µM Cu with foliar Fe) were analogous to that of ampicillin against *S. enterica*.

The overall MIC and IC50 values recorded from the investigated extracts were higher than the values of ampicillin indicating lower antibacterial activity. Treatment with

K150 with 100 µM Cu (with and without foliar Fe) resulted in the plant extract with the highest MIC value (i.e., lowest antibacterial activity) against *S. aureus* compared to others. The extract obtained after treating *S. cypria* with K350 (5 µM Cu) presented an IC50 value comparable to the one of ampicillin against *S. aureus*. Extracts from *S. cypria* treated with K350, K550, K150 (5 µM Cu with foliar Fe), K150 (100 µM Cu), and K350 (100 µM Cu with foliar Fe) showed the lowest antibacterial activity against *L. monocytogenes*. The IC50 of the extracts obtained from K550 (5 µM Cu) were less effective (higher values) towards *L. monocytogenes*

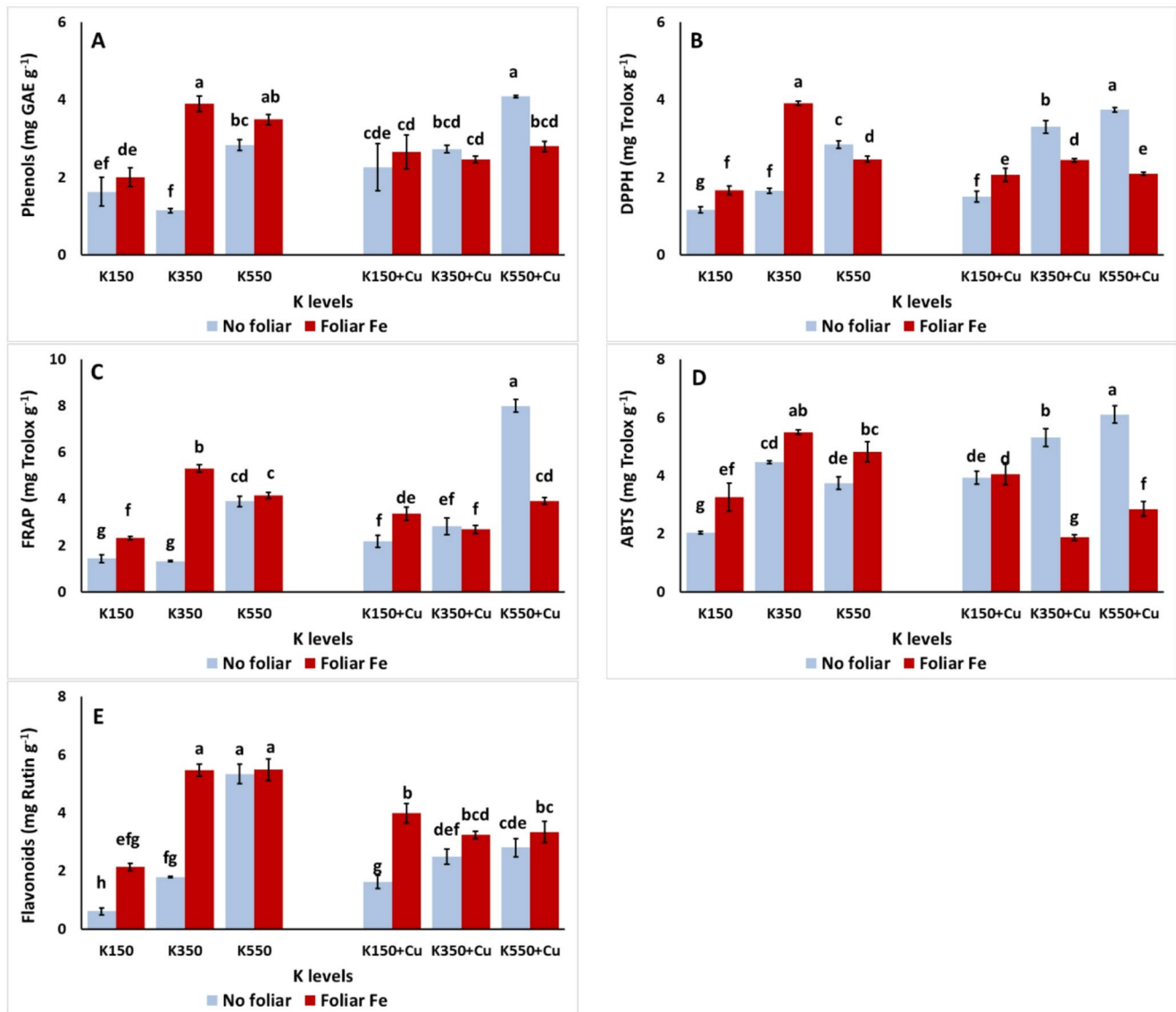


Fig. 3 The effect of K levels (K150: 150 mg L⁻¹, K350: 350 mg L⁻¹, K550: 550 mg L⁻¹), Fe foliar application (No foliar; 0, and Foliar Fe; 1.8 mM Fe), and Cu application (No additional Cu; 5, and additional Cu; 100 μM Cu) on (A) phenols, (B) DPPH, (C) FRAP, (D) ABTS,

and (E) flavonoids of *Sideritis cypria* plants grown in soilless cultivation. Significant differences ($p \leq 0.05$) among the different applications are indicated by different letters

compared to all other treatments. *E. coli* was found to be less susceptible (higher MIC values) to extracts from plants treated with K150 (100 μM Cu), K550 with 100 μM Cu (with and without foliar Fe). The IC₅₀ values from extracts obtained from treatments K350, K550 (5 μM Cu with foliar Fe) and K150 (5 μM Cu with foliar Fe), against *E. coli* did not significantly differ from the one from ampicillin. Treatment with K350 (5 and 100 μM Cu) resulted in plants which gave extracts that were less effective (higher MIC values) against *S. enterica*. All K550 treatments presented lower antibacterial activity (higher IC₅₀ values) against *S. enterica* compared to other treatments.

4 Discussion

The present study investigated the effects of varying K levels in the hydroponic NS, excess Cu and foliar Fe, on the growth, physiological responses and stress tolerance of *S. cypria*. These nutrients have critical and interrelated roles in various physiological processes of plants, as well their uptake mechanisms and behavior under sub-optimal concentrations. Several studies were conducted to evaluate the impact of Cu phytotoxicity (Lamb et al. 2012) on plant growth, the alleviation strategies by manipulating the nutrition of plants (Wang et al. 2015), as well as by applying

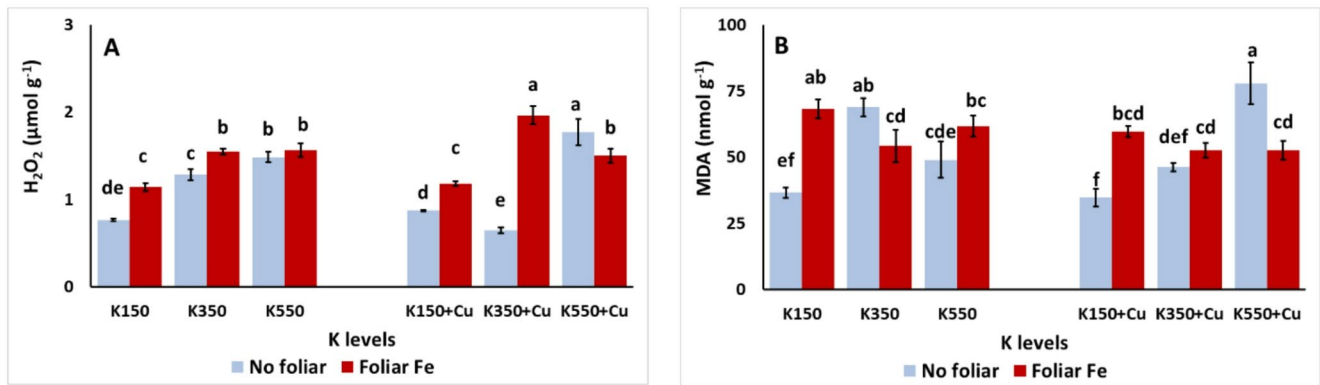


Fig. 4 The effect of K levels (K150: 150 mg L⁻¹, K350: 350 mg L⁻¹, K550: 550 mg L⁻¹), Fe foliar application (No foliar; 0, and Foliar Fe; 1.8 mM Fe), and Cu application (No additional Cu; 5, and additional

Cu; 100 μM Cu) on (A) H₂O₂, and (B) MDA of *Sideritis cyprica* plants grown in soilless cultivation. Significant differences ($p \leq 0.05$) among the different applications are indicated by different letters

Table 4 The effect of K levels (K150: 150 mg L⁻¹, K350: 350 mg L⁻¹, K550: 550 mg L⁻¹), Fe foliar application (0 and 1.8 mM Fe), and Cu application (5 and 100 μM Cu) on the total phenols and antioxidant activity of ethanolic extracts from *Sideritis cyprica* plants grown in soilless cultivation

K levels	Cu application	Foliar Fe	Phenols (mg GAE g ⁻¹)	DPPH (mg Trolox g ⁻¹)	FRAP (mg Trolox g ⁻¹)	ABTS (mg Trolox g ⁻¹)
K150	5 μM	0 mM	24.23±0.21 fg	12.50±0.13e	19.84±0.14e	14.88±0.33 cd
		1.8 mM	29.59±0.44c	16.57±0.22b	20.15±0.18e	16.08±0.29bc
	100 μM	0 mM	23.04±0.18 g	11.12±0.31f	18.29±0.15f	14.23±0.52d
		1.8 mM	27.38±0.62d	11.09±0.12f	20.13±0.19e	14.61±0.45d
K350	5 μM	0 mM	35.95±0.72a	16.78±0.23b	28.32±0.16a	19.38±0.10a
		1.8 mM	30.14±0.32c	12.58±0.26e	23.55±0.18c	15.34±0.53 cd
	100 μM	0 mM	24.92±0.42f	13.76±0.47 cd	19.89±0.28e	15.28±0.59 cd
		1.8 mM	34.43±0.51b	19.22±0.38a	27.44±0.17b	19.27±0.41a
K550	5 μM	0 mM	27.38±0.32d	14.41±0.23c	21.75±0.25d	16.86±0.45b
		1.8 mM	26.27±0.36de	12.97±0.16de	20.16±0.29e	15.57±0.64 cd
	100 μM	0 mM	25.52±0.45ef	12.27±0.06c	20.43±0.33e	15.15±0.10 cd
		1.8 mM	33.84±0.42b	19.38±0.41a	27.15±0.26b	20.45±0.14a

Significant differences ($p \leq 0.05$) among foliar applications are indicated by different letters

foliar Fe applications (Bosnić et al. 2019). However, given that these nutrient interactions can either exacerbate or alleviate stress conditions, and affect the plants' growth, physiology and nutritional status, elucidating these relationships is crucial for optimizing fertilization strategies and improving plant growth and quality under suboptimal environmental conditions.

Previous research on the hydroponic cultivation of *S. cyprica* suggest that plant growth in terms of biomass production is largely independent by the K concentration in the NS, as no significant differences are observed among plants treated with 150, 350 and 550 mg K L⁻¹ (Chrysargyris and Tzortzakakis 2025). The influence of K supply is inherently species-specific, as nutrient requirements vary among plant species. For instance, in *Ranunculus asiaticus* L., increased K fertilization caused a reduction in the plants' growth, which was independent of EC levels and osmotic response (Bernstein et al. 2011). However, overall

plant health and growth can be influenced by the N/K balance. K, in particular, functions as a counterion for nitrate (NO₃⁻) during its transport from roots to shoots (Alvarado-Camarillo et al. 2018). In the current study, the highest fresh biomass production was observed in the application of the lowest K (150 mg L⁻¹), although differences among the different treatments were mostly related to the foliar Fe application. Foliar Fe applications are commonly utilized to alleviate deficiencies when Fe availability in soils is limited (Mahmoud et al. 2022). However, in soilless cultivation, where Fe is supplied at optimal concentrations through the NS, additional foliar Fe can cause excessive Fe accumulation. This imbalance can lead to oxidative stress, disrupting cellular processes and inhibiting plant growth (Zaid et al. 2020). Regarding dry matter content, higher values were recorded at intermediate K application in the NS, coupled with typical Cu levels and no foliar Fe application. This suggests that balanced K levels, as established in previous

Table 5 The effect of K levels (K150: 150 mg L⁻¹, K350: 350 mg L⁻¹, K550: 550 mg L⁻¹), Fe foliar application (0 and 1.8 mM Fe), and Cu application (5 and 100 µM Cu) on the antibacterial activity of ethanolic extracts from *Sideritis cypria* plants grown in soilless cultivation

K levels	K150				K350				K550				Ampicillin			
	5 µM	1.8 mM	100 µM	0 mM	5 µM	1.8 mM	100 µM	0 mM	5 µM	1.8 mM	100 µM	0 mM	1.8 mM	100 µM	0 mM	1.8 mM
Cu levels																
Foliar Fe																
<i>S. aureus</i>	DIZ	6.00±0.00b	6.00±0.00b	6.00±0.00b	6.00±0.00b	6.50±0.50b	9.00±1.00a	6.00±0.00b	6.00±0.00b	6.00±0.00b	6.00±0.00b	6.00±0.00b	6.00±0.00b	6.00±0.00b	6.00±0.00b	9.50±0.50a
	MIC	0.12±0.00c	0.25±0.00b	0.50±0.00a	0.50±0.00a	0.50±0.00a	0.06±0.00d	0.25±0.00b	0.25±0.00b	0.25±0.00b	0.25±0.00b	0.25±0.00b	0.06±0.00d	0.25±0.00b	0.25±0.00b	0.0016±0.0000e
<i>L. monocytogenes</i>	IC50	1.06±0.12ef	2.62±0.27b	1.77±0.34cd	1.89±0.15c	6.00±0.00	0.50±0.04fg	1.27±0.18cde	1.03±0.10ef	1.31±0.20cde	1.60±0.22cde	3.36±0.39a	1.64±0.16cde	1.12±0.15def	3.36±0.39a	0.0156±0.0003g
	DIZ	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	7.00±0.00
<i>E. coli</i>	MIC	0.25±0.00b	0.50±0.00a	0.50±0.00a	0.25±0.00b	0.25±0.00b	0.50±0.00a	0.06±0.00c	0.25±0.00b	0.50±0.00a	0.50±0.00a	0.25±0.00b	0.25±0.00b	0.25±0.00b	0.25±0.00b	0.0008±0.0000d
	IC50	0.41±0.01f	0.59±0.02d	1.05±0.03b	0.26±0.01g	0.26±0.01g	0.52±0.00e	0.30±0.02g	0.31±0.00g	0.60±0.01d	1.20±0.01a	0.89±0.03c	0.56±0.01de	0.89±0.03c	0.30±0.01g	0.0008±0.0001h
<i>S. enterica</i>	DIZ	6.00±0.00b	6.00±0.00b	6.00±0.00b	6.00±0.00b	6.00±0.00b	6.00±0.00b	6.00±0.00b	6.00±0.00b	6.00±0.00b	6.00±0.00b	6.00±0.00b	6.00±0.00b	6.00±0.00b	6.00±0.00b	7.50±0.50a
	MIC	0.25±0.00b	0.25±0.00b	0.50±0.00a	0.25±0.00b	0.25±0.00b	0.12±0.00c	0.25±0.00b	0.25±0.00b	0.25±0.00b	0.25±0.00b	0.25±0.00b	0.25±0.00b	0.25±0.00b	0.25±0.00b	0.05±0.00d
	IC50	4.58±0.97bc	1.95±0.25cd	12.83±1.03a	12.61±1.16a	3.36±0.60cd	7.00±0.00cd	7.91±1.27b	13.62±2.97a	4.76±1.01bc	4.79±1.69bc	5.21±0.63bc	2.77±0.46cd	5.21±0.63bc	4.46±1.17bc	0.11±0.01d
	DIZ	7.00±0.00cd	8.00±0.00ab	7.00±0.00cd	8.00±0.00ab	7.00±0.00cd	7.00±0.00cd	7.00±0.00cd	7.00±0.00cd	7.00±0.00cd	7.50±0.50bc	7.50±0.50bc	6.50±0.50cd	7.50±0.50bc	7.00±0.00cd	8.50±0.50a
	MIC	0.06±0.00c	0.06±0.00c	0.06±0.00c	0.12±0.00b	0.12±0.00b	0.25±0.00a	0.12±0.00b	0.12±0.00b	0.12±0.00b	0.12±0.00b	0.12±0.00b	0.06±0.00c	0.12±0.00b	0.12±0.00b	0.0063±0.0000d
	IC50	0.95±0.04d	1.79±0.42bc	1.20±0.15cd	1.75±0.25bc	1.55±0.40bcd	1.34±0.12cd	1.78±0.05bc	1.18±0.07cd	1.18±0.07cd	2.60±0.05a	2.22±0.35ab	2.18±0.06ab	2.22±0.35ab	2.17±0.20ab	0.0574±0.0014e

Significant differences ($p \leq 0.05$) among foliar applications are indicated by different letters

findings (Chrysargyris and Tzortzakis 2025) on cultivated *S. cypria*, supports optimal dry matter accumulation. This parameter is particularly significant, given that *S. cypria* is primarily sold as dried tea leaves.

Plant physiology attributes were influenced by the examined parameters. Relative chlorophyll content as assessed by SPAD, and chlorophyll fluorescence as assessed by Fv/Fm were generally higher with the application of low to intermediate levels of K (150–350 mg L⁻¹), compared to the application of high K levels (550 mg L⁻¹). For Fv/Fm specifically, levels remained within established physiological thresholds for healthy plant growth at 0.75–0.85, although Cu has been associated with a decline in Fv/Fm (Chrysargyris et al. 2021). K deficiency is correlated with a decline in leaf photosynthesis, mainly due to a reduction in chlorophyll biosynthesis (Hafsi et al. 2014). In the current study, the influence of K levels was contingent on the application of excessive Cu and foliar Fe. However, chlorophyll (Chl a, Chl b, Total Chl) and carotenoids (Total Car) contents were generally increased with the application of high K level (550 mg L⁻¹) and/or the application of foliar Fe. Similarly, in melon (*Cucumis melo* L.), leaf chlorophylls were positively related to higher K levels in the NS, while a 50% reduction of K supply reduced leaf chlorophyll content (Asaduzzaman et al. 2018). However, Chrysargyris and Tzortzakis (2025) observed that chlorophyll contents in *S. cypria* cultivated hydroponically, exhibited a stronger correlation with N and P levels in the NS, than with K. In addition, Fe may effectively improve chlorophyll content and photosynthetic performance, by facilitating the activation of key enzymatic and biochemical processes involved in chlorophyll biosynthesis and photosynthesis (Ning et al. 2023).

Nutrient uptake is influenced by several factors, including key nutrients in the NS, such as K, the presence of toxic elements like Cu, and the application of micronutrient supplements such as foliar Fe applications (Asle-Mohammadi et al. 2024; Çalışkan and Çalışkan 2018; Feil et al. 2020). Interestingly, Fe is a crucial cofactor in nitrogen metabolism, facilitating more efficient nitrogen assimilation (Marschner 2011). Consistent with this, the current study observed a general increase in the leaf N content, following the application of foliar Fe, across the examined treatments. Furthermore, a lack of K accumulation in *S. cypria* plants is observed, despite higher K levels in the NS. These results are in contrast with those of Chrysargyris and Tzortzakis (2025), as they reported a slight increase of N accumulated in *S. cypria* leaves when exposed to 550 mg K L⁻¹ in a hydroponic NS. This discrepancy may be related to the shorter cultivation period (59 DAT) and cultivation technique used (Nutrient Film Technique) of the aforementioned experiment compared

to the cultivation period in the present study (125 DAT) and plants growing in substrate-cocopeat, as differences in K uptake and translocation may occur during later stages of plant development (Hawkesford et al. 2023). Increased K in the NS was also associated with increased root Ca and leaf Mg contents. It is well established that increased K supplement may reduce the uptake of other cations, including Mg and Ca, due to their competitive nature. In addition, the decrease of K may stimulate the uptake of other cationic species (Papadakis et al. 2023). However, Cu toxicity effects include the disruption of metabolic activities such as the uptake of minerals (Faizan et al. 2021). However, Fe can mitigate potential disruptions of Cu accumulation, by outcompeting Cu uptake (Bosnić et al. 2019). Nonetheless, in the present study, the application of foliar Fe was mostly associated with increased Cu in leaf tissues. It has been reported that accumulation of transition metals can cause moderate to strong deficiency of Fe in plants, thus decreasing plant growth. However, as no significant differences are observed on the produced biomass, foliar Fe may have alleviated the deficiency caused by excess Cu (Ullah et al. 2018). In addition, supplementary Fe may impact the transport mechanisms within the plant, and by extension the translocation of Cu from roots to leaves (Wairich et al. 2022). Ultimately, Cu accumulation in *S. cypria* leaves ranged from 12.67 to 25.44 mg kg⁻¹ DW, remaining well below the maximum residue levels (MRLs) of 100 mg kg⁻¹ for herbal leaf infusions, established by the European Union (European Food Safety Authority (EFSA) 2018). Moreover, the application of foliar Fe, primarily without excess Cu, caused a significant increase in Fe accumulation in leaves, with contents ranging from 253.25 to 438.00 mg kg⁻¹ DW, compared to the treatments without foliar Fe, ranging from 152.44 to 303.55 mg kg⁻¹ DW. These values under foliar Fe were notably higher than those previously reported for *S. cypria* and *S. perfoliata*, which ranged from 70 to 110 mg kg⁻¹ DW (Chrysargyris and Tzortzakis 2025), and 123.60 to 149.08 mg kg⁻¹ DW respectively (Dursun et al. 2016; Lall et al. 2019). Biofortification of Fe presents a sustainable option for alleviating the inadequate dietary intake of Fe (Clemens 2014).

It is acknowledged that excess Cu concentrations can cause oxidative stress in plants, prompting increased antioxidant activity (Adrees et al. 2015; Kumar et al. 2021). However, in the current study, the influence of Cu was modulated by the K levels in the NS and the application of foliar Fe. For instance, increased phenolics and antioxidant activity (as assayed by DPPH, FRAP, and ABTS) occurred under excess Cu during the application of K550 and no foliar Fe. As Cu stress generates ROS, it is possible that the biosynthesis of these secondary metabolites is increased in response

(Fortis-Hernández et al. 2024). However, the application of foliar Fe mediated these responses, contingent on K levels. Interestingly, foliar Fe coupled with increased accumulation of Fe led to an increase in H₂O₂ levels, indicating oxidative stress, while excess Cu also facilitated increased H₂O₂ production at certain K levels. Both Cu and Fe over strict concentrations can lead to the generation of ROS, including H₂O₂, via the Haber-Weis and Fenton reactions (Li et al. 2012). Finally, the influence of both Cu and Fe was contingent on the K rate applied. For instance, foliar Fe increased MDA under low and high K (150, 550 mg L⁻¹) without excess Cu. With excess Cu, this effect persisted under low K but had the opposite effect at high K levels. This may be due to the impact of K in the plants' antioxidant defense mechanisms, mitigating oxidative stress from heavy metals and toxicity, derived from Cu and Fe, thereby reducing MDA accumulation. Conversely, the combined K and Cu excess may have weakened defense mechanisms, increasing ROS production and resulting in higher lipid peroxidation under stress conditions (Ashley et al. 2006; Wang et al. 2013).

Macronutrients such as K play an important role in plant's growth as well as metabolism contributing to its persistence under abiotic and biotic stress (Wang et al. 2013). Under stress conditions, enzymatic and non-enzymatic antioxidant mechanisms are activated in an attempt of the plant to fight the occurring oxidative stress (Li et al. 2019). In addition to this, supply of sufficient K levels has been linked with increased polyphenolic content and activation of other antioxidant defense mechanisms (Wang et al. 2013). The results from the current study show that ethanolic extracts obtained from *S. cypria* plants treated with foliar Fe presented greater phenolic content and antioxidant activity. Iron is another crucial element of plant's metabolism and its antioxidant defense system. This element acts in various ways against oxidative stress including the breakdown of lipid peroxidation and its role as a cofactor in enzymes with antioxidant activity (i.e., catalase) (Kaur et al. 2024). However, excessive accumulation of Fe can result in oxidative stress due to the generation of free radicals inside the plant cells (Zaid et al. 2020). In the current study, treatment with low K levels plus excess of Cu (with or without foliar Fe) resulted in extracts with decreased phenolic content and antioxidant activity. This could be attributed to the potential stress caused by these treatments as observed by the increased H₂O₂ and MDA levels. Copper is a vital component for plant growth and is implicated on the activity of numerous enzymes such as polyphenol oxidase (PPO), cytochrome c oxidase, superoxide dismutase (SOD), and amine oxidase (AO), among others (Perfileva et al. 2025). The enzymatic antioxidant defense system (including enzymes such as SOD and peroxidase-POD) is the plant's main first response combating stress caused by free radicals

(Mu et al. 2024). In addition, this element is implicated on the metabolism of polyphenols, carbohydrates, and N (Perfileva et al. 2025). On the other hand, excessive Cu levels could cause oxidative stress in plants, which could result in the increase of antioxidant activity (Kumar et al. 2021). In addition to their antioxidant activity, plant extracts (including *S. cypria*) present antimicrobial, anti-inflammatory, and anticancer/anti-proliferative activities (Chrysargyris et al. 2024; Panayi et al. 2022).

The antimicrobial (antibacterial and antifungal) activity of plant extracts is mostly ascribed to their phytochemical constitution and especially their content in polyphenols and flavonoids (Fassinou Hotegni et al. 2024). In addition, the composition of the outer membrane of the bacterial cells plays an important role in their susceptibility to antimicrobial means. The cell wall of Gram-negative bacteria has an outer lipid bilayer membrane allowing selective permeability and thus, protecting the cells from xenobiotics (Lahmar et al. 2017). The results from the current study revealed a moderate antibacterial activity ($0.10 < \text{MIC} < 0.63 \text{ mg mL}^{-1}$) (Djappa et al. 2024) against the tested bacteria. In addition, a variation in the antibacterial activity of the plant extracts was observed against both Gram-negative (*E. coli*, *S. enterica*) and Gram-positive bacteria (*S. aureus*, *L. monocytogenes*). Treatments with K350 showed slightly higher antibacterial activity compared to other treatments. Our results are in line with previous reports where *S. cypria* ethanolic extracts also presented low to moderate antibacterial activity. Indeed, in a previous study it was shown that ethanolic extracts from *S. cypria* presented low effect on the biofilm formation of *E. coli*, while no activity was reported on the bacterium's swarming and swimming motility (Panayi et al. 2022). This could also be attributed to the increased phenolic content and antioxidant activity caused by specific treatments of the present study. Furthermore, numerous compounds from plant extracts and essential oils presenting anti-quorum sensing activity share structural similarities with molecules mediating in the communication of the quorum sensing process (Panayi et al. 2022). In addition, the variation between the observations of the two methods employed could also be attributed to the different diffusion of the plant extracts to the growth medium used (liquid versus solid) (Xylia et al. 2024). The outcomes of the current study could be used for future research for feasible combination with currently used antibiotics. During recent years, there is an urgent need to minimize the extended use of antibiotics due to the unnervingly high antibiotic resistance levels reported. When plant extracts and essential oils are combined with lower antibiotic doses, the effect could be similar and/or greater than when used alone. With such combinations, one should be cautious when investigating as antagonistic effects might also arise (Lahmar et al. 2017).

5 Conclusions

The current study was commissioned to assess the impact of varying potassium (K) levels (150, 350, 550 mg L^{-1}) in the hydroponic nutrient solution of *Sideritis cypria*, in the presence of common or excess copper (Cu) levels (5, 100 μM), and the deployment of foliar iron (Fe), with a particular emphasis on the interactions between these variables. Fresh biomass was improved with the application of the lowest K application (150 mg L^{-1}), while the employment of the highest K application (550 mg L^{-1}) was positively associated with chlorophyll and carotenoid contents. However, variations occurred depending on the application of foliar Fe. Supplementary Fe application may have disrupted the balance of Fe fertilization, particularly under hydroponic conditions, potentially inducing oxidative stress and growth inhibition. This is supported by the increased Fe accumulation in leaf tissues, along with elevated hydrogen peroxide levels under foliar Fe applications. Foliar Fe was also found to have a positive impact on nitrogen and Cu accumulation, mainly due to its influences on nitrogen metabolism and the uptake mechanisms of Cu. Copper accumulation was minimally affected by the elevated Cu in the nutrient solution, as leaf Cu contents remained below the maximum residue levels across all treatments. Ultimately, *S. cypria* antioxidant activity and stress response to excess Cu were contingent on the K levels and foliar Fe application. This highlights the interplay between heavy metal toxicity, and the modulation of plant stress response, with K having a crucial role in oxidative stress response and the production of antioxidants. In addition, high antioxidant activity and moderate antibacterial activity were observed by extracts obtained from plants treated with intermediate K levels. Thus, low to moderate K levels in the nutrient solution can be deployed under excess Cu conditions, without compromising *S. cypria* production and safety. Moreover, foliar Fe applications can be effective in the context of biofortification, provided that potential toxicity risks are considered.

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Data Availability Data will be available upon request to the authors.

Code Availability Not applicable

Declarations

Ethics approval Not applicable.

Consent To Participate Not applicable.

Consent for Publication Not applicable.

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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