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Unveiling Sulfur Deficiency-Induced Biochemical Alterations in Medicinal Plants: A Comparative Hydroponic Study on Metabolic Responses in *Ocimum citriodorum* Vis. and *Mentha suaveolens* Ehrh.

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Abstract: Sulfur plays a central role in plant metabolism, participating in the formation of numerous essential compounds such as cysteine, methionine, proteins, coenzymes, vitamins, secondary metabolites, and various pigments. In this study, we examined how sulfur limitation affects two Lamiaceae species: lemon basil (*Ocimum citriodorum* Vis.) and apple mint (*Mentha suaveolens* Ehrh.). Plants were grown hydroponically under controlled greenhouse conditions and supplied with three sulfur concentrations (1.0, 0.18, and 0.06 mM) for 8 weeks. Our results revealed that sulfur deficiency caused a significant decrease in protein content (up to 80%) while soluble sugars increased by nearly 74%. We also noted a clear reduction in chlorophyll content and biomass. These findings highlight the strong effect of sulfur availability on primary metabolism and confirm its essential role in maintaining the biochemical functions that ensure the health and quality of aromatic and medicinal plants. Further studies should explore secondary metabolite profiles and recovery responses to sulfur resupply.

Keywords: sulfur deprivation; *Ocimum citriodorum*; *Mentha suaveolens*; biochemical responses; hydroponics; chlorophyll.

1. Introduction

Sulfur (S) is an essential macronutrient that plays multiple roles in plant development and metabolism. Beyond its inclusion in the amino acids cysteine and methionine [1], it is required for the synthesis of key cellular compounds, including glutathione, coenzymes, and several vitamins, all of which contribute to photosynthetic activity, redox balance, and detoxification mechanisms [2–4]. Despite this fundamental role, the effects of sulfur deficiency on wild and medicinal plants, which often accumulate sulfur-rich secondary metabolites, are poorly characterized.

Over the past decade, soil sulfur levels have declined, leading to an increasing incidence of sulfur deficiency in plants [5,6]. This decrease is generally due to the significant decline in anthropogenic SO_2 emissions, driven by stricter environmental legislation and the widespread use of highly purified fertilizers that contain little or no sulfur [7]. Numerous studies have reported that sulfur deficiency causes various biochemical and physiological changes in plants, notably reductions in metabolism and growth [8–10]. Its effects have been observed across many crop species, including cereals, legumes, and oilseeds [11–20], in which sulfur limitation disrupts nitrogen assimilation, reduces photosynthetic capacity, and alters growth and metabolic processes [8–10].

Although medicinal plants often accumulate high levels of sulfur-dependent secondary metabolites, their responses to sulfur limitation remain poorly studied. This study focuses on two economically and medicinally important Lamiaceae species: lemon basil (*Ocimum citriodorum* Vis.) and apple mint (*Mentha suaveolens* Ehrh.). These species were selected as study models because the biosynthesis of their essential oils, which are crucial to their medicinal value, depends on sulfur precursors such as cysteine, methionine, and S-adenosylmethionine (SAM) [2,21,22]. We suggested that their distinct metabolisms would yield different responses to sulfur deficiency across plant species. In order to ensure precise nutrient supply, the plants were grown hydroponically under controlled greenhouse conditions. This study provides a comparative assessment of the impact of gradual sulfur deficiency on the growth and major biochemical traits of these two important medicinal species.

2. Materials and Methods

2.1. Plant material and growth conditions.

The experiments were carried out using two plants belonging to the Lamiaceae family: lemon basil (*Ocimum citriodorum* Vis.) and apple mint (*Mentha suaveolens* Ehrh.). Lemon basil seeds were sterilized using 10% sodium hypochlorite and subsequently rinsed with distilled water. The seeds were then placed on moistened Whatman filter paper in Petri dishes and incubated at 25°C [23]. For apple mint, cuttings of similar size taken from wild plants were rooted before being transferred to the hydroponic system. This experiment was conducted using a completely randomized design. Nine independent 20-liter hydroponic tanks were used (three per sulfur treatment) and randomly arranged in the greenhouse to minimize potential environmental biases. Each tank, containing several uniform plants, served as a single biological replicate, while individual plants within the same tank were considered technical subsamples. All plants were cultivated in a modified Hoagland nutrient solution [24], continuously aerated, and renewed weekly. The plants were grown in a greenhouse under the following conditions: 25°C during the day and 18°C at night, 75% relative humidity, and a 16-hour photoperiod of light followed by 8 hours of darkness. The light intensity, measured as photosynthetic photon flux density (PPFD), was maintained at 300 µmol photons m^{-2} s^{-1}.

Plants were exposed for 8 weeks to three sulfur (S) concentrations in the nutrient solution: a control at 1.0 mM S, and two deficiency levels at 0.18 mM S (moderate) and 0.06 mM S (severe). Complete nutritional composition is detailed in Table 1. We used three replicates per treatment (n=3), in accordance with standard practices for hydroponic studies, which allowed for a solid assessment of the effects of the treatments [25].

After eight weeks of sulfur deficiency, leaves were harvested and immediately stored at -20°C for subsequent analyses. Measurements of fresh and dry biomass for both shoots and roots were conducted immediately after harvest.

Table 1. Concentrations (mM) of macro- and micro-elements in the nutrient solutions. Treatments: C (control, 1.0 mM S), S1 (moderate sulfur deficiency, 0.18 mM S), S2 (severe sulfur deficiency, 0.06 mM S).

	Nutrient elements (mM)												
	P	**N**	**K**	**S**	**Mg**	**Mn**	**Cu**	**Zn**	**Cl**	**Mo**	**Ca**	**Fe**	**B**
C	1	3	4	**1**	0.5	0.002	0.0003	0.0016	2	0.0003	2	0.05	0.007
S1	1	3	4	**0.18**	0.5	0.002	0.0003	0.0016	2	0.0003	2	0.05	0.007
S2	1	3	4	**0.06**	0.5	0.002	0.0003	0.0016	2	0.0003	2	0.05	0.007

2.2. Analytical methods.

2.2.1. Determination of shoot and root dry weight (SDW, RDW):

The shoot and root dry weights of lemon basil and apple mint were determined by drying harvested samples at 60°C for 72 hours. The shoot dry weight (SDW) and root dry weight (RDW) were recorded when mass values were constant [26].

2.2.2. Total sulfur content.

Total sulfur content was determined by digesting 50 mg of leaf dry matter in a mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$) in a ratio of 85:15, at 120°C for 4 hours. After digestion, the resulting solution was filtered, and the final volume was adjusted to 10 mL with distilled water [27]. The sulfate concentration in the extracts was then determined turbidimetrically, in accordance with the methods described by Astolfi & Zuchi [28] and Bardsley & Lancaster [29]. Sulfate concentration in the extracts was determined turbidimetrically. This was achieved by precipitating the sulfate ions as barium sulfate and measuring the suspension's optical density at 430 nm. A calibration curve was prepared using magnesium sulfate standards across a concentration range of 0-50 µg/mL SO_4^{2-}. All samples were measured within this linear range. All samples were measured in triplicate. Results were expressed as mg sulfur per g dry weight (mg·g^{-1} DW).

2.2.3. Chlorophyll content.

The chlorophyll content in leaves was extracted using the Dimethyl sulfoxide (DMSO) method as described by Hiscox & Israelstam, 1979 [30]. Leaf tissue was placed in test tubes containing DMSO, and the mixture was incubated at 65°C for 1 hour until the chlorophyll was totally dissolved. After filtration, the absorbance of the extract was measured using a spectrophotometer at 663 nm and 645 nm. The content of chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll (Chl T) was calculated according to Arnon [31] using the following equations:

$$Chl\ a = (12.7 \times A_{663}) - (2.69 \times A_{645}) \quad (1)$$

$$Chl\ b = (22.9 \times A_{645}) - (4.68 \times A_{663}) \quad (2)$$

$$Chl\ T = (20.2 \times A_{645}) + (8.02 \times A_{663}) \quad (3)$$

The calculated concentrations were expressed as mg·g^{-1} fresh weight (FW). The spectrophotometer was calibrated with a DMSO blank before measurements.

2.2.4. Total soluble sugar content (TSS).

The total soluble sugars (TSS) were measured according to the procedure described by Erice [32]. We homogenized 100 mg of fresh leaf tissue in 5 mL of potassium phosphate buffer (50 mM, pH 7.5). The mixture was then centrifuged at 10,000 × g for 15 minutes. The resulting supernatant was used as the extract for total soluble sugars (TSS). The soluble sugars were measured according to the protocol established by Yemm & Willis [33]. Then, 0.1 mL of supernatant was mixed with anthrone reagent at 90°C for 10 minutes, and the absorbance was measured at 625 nm (JASCO V-730 spectrophotometer). A calibration curve was established with D-glucose solutions at concentrations ranging from 0 to 200 µg/mL. Each sample was analyzed in three technical replicates. All measurements were within the linearity range of the curve. The results are expressed in mg of glucose equivalent per gram of fresh weight ($mg \cdot g^{-1}$ FW).

2.2.5. Soluble protein content.

For the determination of soluble protein content, 200 mg of a fresh leaf sample was used. Leaves were homogenized in 2 mL of sodium phosphate buffer (100 mM, pH 7.5) on ice. After centrifugation at 8,000 x g for 15 minutes, the supernatant was considered the protein extract. Soluble protein content was determined photometrically at 750 nm according to the method of Lowry [34]. A standard curve was prepared using bovine serum albumin (BSA) across a concentration range of 0-100 µg/ml. For each biological replicate, the protein content was determined from three replicate measurements. All sample measurements were performed within this validated linear range. Results were expressed as mg protein per g fresh weight ($mg \cdot g^{-1}$ FW).

3.3. Statistical analysis.

Data were analyzed using a combined analysis of variance (ANOVA). Before ANOVA, data assumptions were verified: normality was assessed using the Shapiro-Wilk test ($p > 0.05$), and homogeneity of variances was assessed using Levene's test ($p > 0.05$). Mean comparisons were performed using Tukey's HSD test at $\alpha = 0.05$. Pearson correlation coefficients and principal component analysis (PCA) were conducted using mean values on centered and scaled data. All analyses were performed with Minitab 18 and XLSTAT (Version 2016.02.28).

3. Results and Discussion

3.1. Analysis of variances (ANOVA).

A combined analysis of variance (ANOVA) indicated significant influences ($p \leq 0.05$) of plant species (P), sulfur treatment (T), and their interaction (P×T) on all parameters measured (Table 2). The sulfur treatment was the most significant factor, accounting for between 46% and 96% of the total variance observed. Its effect was most pronounced on total chlorophyll (Chl T, 96%), shoot dry weight (SDW, 95%), and soluble proteins (89%), and substantial on total soluble sugars (TSS, 77%), total sulfur (TS, 66%), root dry weight (RDW, 46%), and chlorophyll b (Chl b, 48%). In contrast, the plant species factor was the main source of variation for chlorophyll a (Chl a, 76%), accounting for 34% of the variance in Chl b, but contributed less than 20% to the variance of all other parameters. The P×T interaction was

significant for RDW (51% of variance), TS, TSS, and Chl a, but non-significant for other parameters.

Table 2. Mean square values from the combined analysis of variance for biomass and biochemical parameters in lemon basil and apple mint subjected to three sulfur concentrations (1.0, 0.18, and 0.06 mM S).

Source	Df	RDW	SDW	TS	Chl a	Chl b	Chl T	TSS	Proteins
Plant	1	0.00299	0.07960	0.42627***	0.568477***	0.011476	0.033153*	2.5052	0.1197
Trt	2	1.05903***	4.14936*	1.28816***	0.141576***	0.016039*	0.461553***	33.4012***	18.7805***
Rep	2	0.02934	0.02946	0.03536	0.000671	0.000382	0.000523	0.4739	0.1228
Plant*Trt	2	1.19712***	0.02257	0.20116**	0.038578**	0.002856	0.016553	5.6526*	0.3478
Error	10	0.02101	0.06605	0.01253	0.002468	0.00255	0.007222	1.3358	0.1516
Total	17	0.00299	0.07960	0.42627	0.568477	0.011476	0.033153	2.5052	0.1197

Df: Degree of freedom *Significant at $p < 0.05$ probability level; **Significant at $p < 0.01$ probability level; ***Significant at $p < 0.001$ probability level. RDW, root dry weight; SDW, shoot dry weight; TS, total sulfur content; Chl a, chlorophyll a; Chl b, chlorophyll b; Chl T, total chlorophyll content; TSS, total soluble sugar.

3.2. Biomass and biochemical parameters.

Significant differences in biomass and biochemical parameters were observed between sulfur treatments in both plant species (Figures 1 and 2).

Biomass Production: Root dry weight (RDW) decreased under sulfur deficiency. In lemon basil, RDW decreased by 5% under severe stress. In apple mint, decreases were 48% (moderate stress) and 63% (severe stress) (Figure 1A). Shoot dry weight (SDW) also decreased: in lemon basil by 25% (moderate) and 45% (severe); in apple mint by 26% (moderate) and 38% (severe) (Figure 1B).

Leaf Sulfur Content: Under control conditions, leaf sulfur content was 2.17 $mg.g^{-1}$ DW in lemon basil and 2.39 $mg.g^{-1}$ DW in apple mint. Under sulfur deficiency, concentrations decreased by 76% (moderate) and 83% (severe) in lemon basil, and by 48% (moderate) and 79% (severe) in apple mint (Figure 2A).

Chlorophyll Pigments: Chlorophyll a content decreased by 30% (moderate) and 52% (severe) in lemon basil, and by 1% (moderate) and 48% (severe) in apple mint (Figure 2B). Chlorophyll b decreased by 6% and 31% in lemon basil, and 16% and 52% in apple mint under the same conditions (Figure 2C). Total chlorophyll decreased by 25% and 47% in lemon basil, and 17% and 55% in apple mint (Figure 2D).

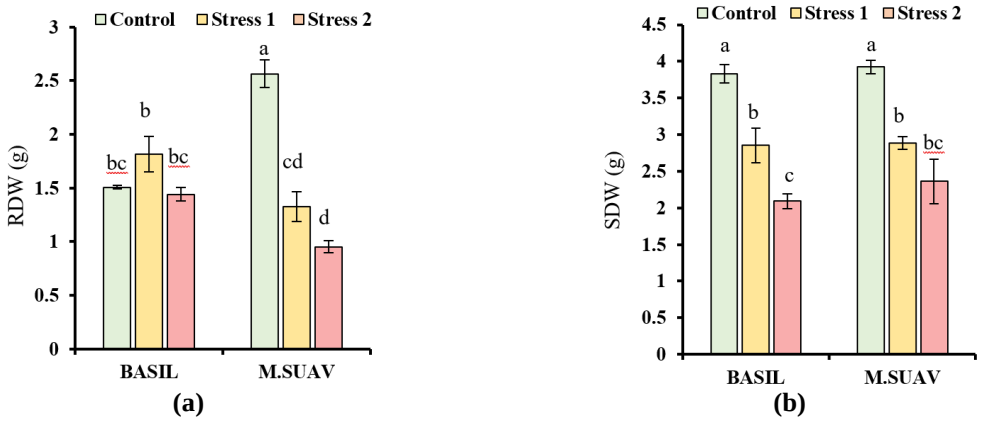

Figure 1. Effects of sulfur deficiency on biomass production in lemon basil and Apple mint (M.SUAV). Plants were exposed to three sulfur concentrations (1, 0.18, and 0.06 mM S). **(a)** Root dry weight (RDW); **(b)** Shoot dry weight (SDW). Values are means ± SE (n=3). Different letters indicate significant differences (Tukey's HSD test, $p \leq 0.05$).

Total Soluble Sugars and Proteins: Total soluble sugars (TSS) increased by 50% and 74% in lemon basil and 34% and 50% in apple mint under moderate and severe stress,

respectively (Figure 2E). Soluble protein content decreased by 43% and 68% in lemon basil, and 56% and 80% in apple mint under the same conditions (Figure 2F).

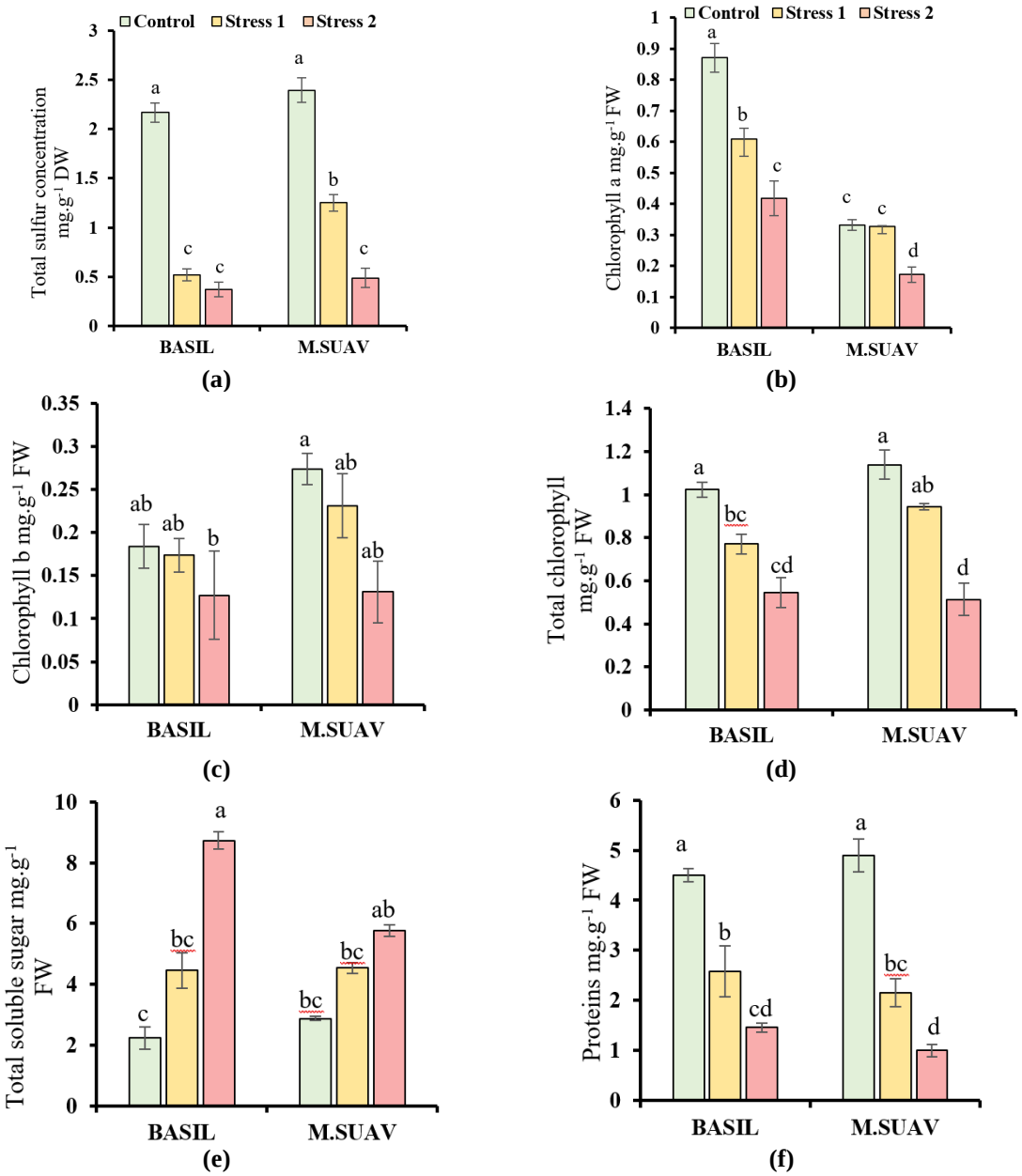

Figure 2. Biochemical responses to sulfur deficiency in lemon basil and Apple mint (M.SUAV). **(a)** Total sulfur content; **(b)** Chlorophyll a; **(c)** Chlorophyll b; **(d)** Total chlorophyll; **(e)** Total soluble sugars (TSS); **(f)** Soluble protein content. Values are means ± SE (n=3). Different letters indicate significant differences (Tukey's HSD test, $p \leq 0.05$).

3.3. Correlation analysis.

Pearson correlation analysis revealed significant relationships among the measured parameters under sulfur deficiency (Table 3). Root dry weight (RDW) showed significant positive correlations with shoot dry weight (SDW) (r = 0.597*), chlorophyll b (r = 0.522*), total chlorophyll (r = 0.614*), and soluble proteins (r = 0.695**). SDW correlated positively with total sulfur content (TS) (r = 0.790***), total chlorophyll (r = 0.913***), and soluble proteins (r = 0.928***), and negatively with total soluble sugars (TSS) (r = -0.771***). TS showed positive correlations with total chlorophyll (r = 0.858***) and soluble proteins (r = 0.760***), and a negative correlation with TSS (r = -0.734**).

Table 3. Matrix of Pearson correlation coefficients between growth and biochemical parameters under sulfur deficiency.

Variables	RDW	SDW	TS	Chl a	Chl b	Chl T	TSS	Protein
RDW	**1**	**0.597***	0.458	0.143	**0.522***	**0.614***	-0.408	**0.695****
SDW		**1**	**0.790****	0.448	**0.684****	**0.913*****	**-0.771*****	**0.928*****
TS			**1**	0.234	**0.652****	**0.858*****	**-0.734****	**0.760*****
Chl a				**1**	-0.023	0.367	-0.404	**0.519**
Chl b					**1**	**0.809*****	-0.343	**0.620***
Chl T						**1**	**-0.718****	**0.884*****
TSS							**1**	**-0.736****
Protein								**1**

RDW, root dry weight; SDW, shoot dry weight; TS, total sulfur; Chl a, chlorophyll a; Chl b, chlorophyll b; Chl T, total chlorophyll; TSS, total soluble sugars. *p < 0.05, **p < 0.01, ***p < 0.001. Values in bold are significantly different from zero at α = 0.05*.

3.4. Principal component analysis (PCA).

Principal component analysis (PCA) was employed as a multivariate statistical method to analyze the variability in biochemical profiles. The first two principal axes explained 88.2% of the total variability, with F1 accounting for 73.92% and F2 for 14.28% of the variance. F1 separated plants not subjected to stress (control) from those exposed to S deficiency treatments (0.18 mM and 0.06 mM). Control samples were associated with elevated levels of RDW, SDW, chlorophyll a, chlorophyll b, total chlorophyll, total sulfur content (TS), and protein content. Conversely, stressed samples were characterized by higher levels of total soluble sugars (TSS). F2 differentiated between lemon basil and apple mint. Lemon basil was associated with high levels of SDW, chlorophyll a, and soluble proteins, while apple mint was associated with high values of RDW, total chlorophyll, chlorophyll b, TS, and TSS (Figure 3).

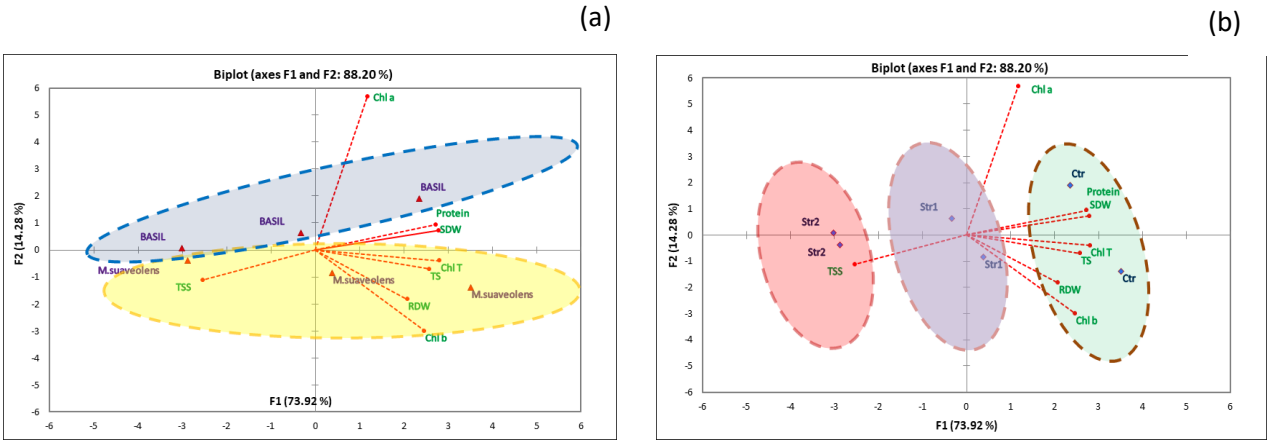

Figure 3. Principal component analysis (PCA) of biochemical traits under control and sulfur-deficient conditions. The first two components explain 88.2% of total variance (F1: 73.92%; F2: 14.28%). Analysis was performed on standardized data. Plots show **(a)** species grouping; **(b)** treatment separation along F1 and F2 axes.

3.5. Discussion.

Sulfur deficiency has become increasingly prevalent in agricultural systems due to reduced atmospheric deposition and the use of low-sulfur fertilizers [35], negatively impacting crop growth and nutritional quality. This deficiency disrupts key physiological parameters, including biomass, sulfur content, proteins, soluble sugars, and chlorophyll [36]. Our research focuses on these impacts in two medicinal species, *Ocimum citriodorum* and *Mentha suaveolens*, grown hydroponically under three sulfur concentrations (1.0, 0.18, and 0.06 mM

S). Sulfur plays an essential role in protein synthesis, chloroplast development, and enzyme activation [1,10,37]. Its deficiency therefore, impairs essential metabolic functions, resulting in reduced growth and compromised physiological processes.

The observed reductions in root and shoot dry weight under sulfur deficiency align with findings in other species [1,2,8], confirming the fundamental role of sulfur in plant growth. However, our study reveals notable interspecific differences: apple mint exhibited a more severe reduction in root biomass (-63%) than lemon basil (-5%), suggesting distinct allocation strategies under sulfur stress. These growth reductions appear to stem from impaired synthesis of sulfur-containing amino acids [38], potentially disrupting protein production and enzymatic activities essential for cell development [1]. Additionally, the parallel decline in chlorophyll content (Figures 2B-D) indicates compromised photosynthetic capacity, which may contribute to limiting carbon assimilation and energy availability for growth [7]. The stronger impact on apple mint's root system could reflect its different investment strategy in root versus shoot organs under nutrient stress.

Our results showed a clear and pronounced decline in leaf total sulfur concentration in both lemon basil and apple mint when sulfur availability was reduced. Under control conditions, sulfur levels reached 2.17 mg g^{-1} DW in lemon basil and 2.39 mg g^{-1} DW in apple mint. However, sulfur deficiency caused these concentrations to fall sharply to 0.37 mg g^{-1} DW (an 83% decrease) in lemon basil and 0.48 mg g^{-1} DW (a 79% decrease) in apple mint. Literature indicates that the critical sulfur concentration for deficiency is generally below 1.7 mg.g-1 DW [39], confirming that our treatments induced severe sulfur deficiency. These results are consistent with studies showing that reduced sulfur availability decreases internal sulfur content in plant leaves [8,40,41]. The reduction observed here likely reflects disruptions in sulfur metabolism, potentially affecting both sulfate uptake and its incorporation into organic compounds [1]. In addition, sulfur-stressed plants may favor storage in the roots rather than transport to the shoots, thereby contributing to the lower leaf sulfur concentrations recorded under deficiency [42].

Sulfur limitation also significantly affected chlorophyll pigments, with notable differences between the two species. In lemon basil, chlorophyll a decreased progressively as sulfur levels declined (-30% under moderate stress and -52% under severe stress). Apple mint, by contrast, maintained chlorophyll a under moderate deficiency (-1%) before exhibiting a substantial drop under severe stress (-48%). For chlorophyll b and total chlorophyll, apple mint displayed larger reductions (-52% and -55%) than lemon basil (-31% and -47%) under severe sulfur limitation. These contrasting trends suggest species-specific acclimation mechanisms in the photosynthetic apparatus. The observed chlorophyll reduction aligns with previous reports across multiple species, including *Morus alba* [43], *Medicago truncatula* [7], *Lycopersicum esculentum* [9], *Eruca sativa* L [8], and *Glycine max* (L.) [1]. The response is consistent with sulfur's central function in chlorophyll biosynthesis and in the maintenance of photosynthetic structures [44]. Sulfur deficiency likely limited chlorophyll production by restricting the availability of key sulfur-containing amino acids (cysteine and methionine), which are crucial precursors for chlorophyll-binding proteins [45]. Sulfur also contributes to redox regulation in the chloroplast, particularly through sulfur-rich proteins involved in ROS detoxification and electron transport [35], while components of the thioredoxin and ferredoxin systems help coordinate light reactions with carbon fixation [8].

Total soluble sugars (TSS) showed significant accumulation in both lemon basil and apple mint under sulfur-deficient conditions. Lemon basil exhibited a 50-74% increase in TSS,

while apple mint demonstrated a 34-50% rise. This pattern of sugar accumulation aligns with previous observations in sulfur-stressed plants [46]. Soluble sugars commonly act as osmoprotectants and regulators of stress signaling, but under sulfur deficiency, their accumulation may also reflect broader metabolic constraints [47].

Leaf protein content was strongly affected by sulfur restriction, decreasing by 43–68% in lemon basil and 56–80% in apple mint. This agrees with established knowledge that sulfur deprivation impairs protein biosynthesis due to the restricted availability of cysteine and methionine [1,40]. The reduction appears to stem from a decreased pool of sulfur-containing amino acids (cysteine and methionine), which are essential for protein assembly [48]. Given the central importance of proteins in plant metabolism, their synthesis, particularly for chlorophyll-associated proteins, appears strongly dependent on adequate sulfur supply. Our results align with previous studies demonstrating that sulfur deficiency can compromise the activity of key photosynthetic enzymes, such as Rubisco, thereby contributing to a reduction in overall protein content [1,48].

Correlation analyses highlighted coordinated physiological adjustments under sulfur limitation. Strong positive correlations among biomass parameters (RDW and SDW), chlorophyll pigments, and protein content (Table 3) indicate that growth and primary metabolism remain tightly linked under nutrient stress [1,38], underscoring sulfur's fundamental role in plant physiology. Conversely, the strong negative correlation between total sulfur (TS) and total soluble sugars (TSS) suggests a marked shift in carbon allocation. Reduced sulfur assimilation may limit glycolytic activity and sink strength, leading to the accumulation of soluble sugars [46]. Collectively, these correlation patterns illustrate a systemic metabolic reprogramming in which sulfur availability directly influences carbon utilization efficiency. It should be noted that these correlation patterns, while strong and consistent with established physiological knowledge, are based on a limited sample size (n=3 per treatment) and warrant verification in larger-scale studies.

Principal component analysis (PCA) effectively captured the systemic metabolic shifts induced by sulfur deficiency, with the first two principal components (PC1 and PC2) accounting for 88.2% of the total variance. The biplot revealed a clear separation along PC1, where control plants characterized by higher levels of total sulfur, proteins, and chlorophyll clustered distinctly from sulfur-deficient plants, which were associated with elevated soluble sugars. PC2 revealed clear differences between the species. Lemon basil was linked to higher shoot dry weight, chlorophyll a, and soluble proteins, while apple mint showed greater root dry weight, total chlorophyll, chlorophyll b, total sulfur, and soluble sugars. This confirms that, beyond sulfur effects, the two species have distinct physiological and metabolic traits. This clear segregation demonstrates the profound impact of sulfur availability on primary metabolism [21,22]. The effectiveness of PCA in discriminating between sulfur-sufficient and sulfur-deprived plants based on their biochemical profiles is consistent with its application in other studies investigating nutrient stress [48] and supports the major metabolic alterations reported in our study, and also emphasizes the species-specific metabolic signatures of lemon basil and apple mint. However, the small sample size necessitates cautious interpretation of the multivariate space, and these patterns should be viewed as indicative rather than definitive.

This study presents some limitations. The sample size (n = 3), although appropriate for hydroponic experiments, should be expanded in future work. The hydroponic setup provided precise nutrient control, but does not fully reflect soil conditions. Moreover, evaluating secondary metabolites would provide deeper insight into the consequences of sulfur deficiency

on the medicinal quality of these species. Future studies should assess essential oil production and examine plant recovery following sulfur resupply to understand metabolic plasticity better.

4. Conclusions

This study clearly shows that sulfur deficiency has a strong impact on both the growth and primary metabolism of *Ocimum citriodorum* (lemon basil) and *Mentha suaveolens* (apple mint). The noticeable decreases in shoot and root biomass, as well as reductions in chlorophyll, protein, and total sulfur content, underscore the vital role of sulfur in healthy plant development and essential physiological processes. At the same time, the build-up of soluble sugars under sulfur shortage suggests a disruption in how plants manage carbon and maintain metabolic balance.

Several limitations should be acknowledged. The experiment was conducted with a relatively small sample size (n = 3) and under hydroponic conditions, which may not fully mirror plant behavior in soil-based systems. In addition, the study focused exclusively on primary metabolism and covered only a limited growth period, limiting broader conclusions about long-term metabolic adjustments.

Despite these constraints, the findings provide valuable insight into how aromatic and medicinal plants respond to sulfur limitation. Future research should investigate the underlying molecular mechanisms governing sulfur-dependent metabolic pathways and evaluate the long-term effects of sulfur deficiency on secondary metabolism, including the quantity and composition of essential oils. Such studies will help clarify whether prolonged sulfur stress influences the biochemical quality and therapeutic value of these species.

Author Contributions

Conceptualization, D.B. and S.L.; methodology, D.B.; validation, D.B., K.H., and S.L.; formal analysis, D.B. and S.B.; investigation, D.B. and N.S.; resources, S.L.; data curation, D.B.; writing—original draft preparation, D.B.; writing—review and editing, M.F. and S.B.; visualization, D.B.; supervision, S.L. and K.H.; project administration, S.L. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

Abbreviation	Full Meaning
ANOVA	Analysis of Variance
BSA	Bovine Serum Albumin
C	Control Treatment (1.0 mM S)
S1	Moderate Sulfur Deficiency Treatment (0.18 mM S)
S2	Severe Sulfur Deficiency Treatment (0.06 mM S)
Chl a	Chlorophyll a
Chl b	Chlorophyll b
Chl T	Total Chlorophyll
DF	Degrees of Freedom
DMSO	Dimethyl Sulfoxide
DW	Dry Weight
FW	Fresh Weight
g	Gravitational Force (Centrifugation Unit)
HSD	Honest Significant Difference
mM	Millimolar
PCM	Protein Competition Model
PCA	Principal Component Analysis
PPFD	Photosynthetic Photon Flux Density
ROS	Reactive Oxygen Species
RDW	Root Dry Weight
Rubisco	Ribulose-1,5-bisphosphate carboxylase/oxygenase
S	Sulfur
SAM	S-adenosylmethionine
SDW	Shoot Dry Weight
SE	Standard Error
SO_2	Sulfur Dioxide
TS	Total Sulfur
TSS	Total Soluble Sugars
v/v	Volume by Volume

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