ASEAN
Journal of Scientific and Technological Reports
Online ISSN:2773-8752

*Academic article*

# Enhancement of Bioactive Compounds and Nutrient Content in Rosemary (*Salvia rosmarinus*) Using Nano-Magnesium and NPK Fertilization: A GC-MS Analysis

**Dhafir Abed Alkhadim Jameel Altaweel**[1*]

[1] Department of Biology, College of Education, University of Al-Qadisiyah, Iraq.
* Correspondence: dhafir.altaweel@qu.edu.iq; (D.A.J. Altaweel)

**Citation:**
Altaweel, J.A.A.D. Enhancement of bioactive compounds and nutrient content in rosemary (*Salvia rosmarinus*) using nano-magnesium and NPK fertilization: A GC-MS analysin. *ASEAN J. Sci. Tech. Report.* **2025**, 28(6), e261168. https://doi.org/10.55164/ajstr.v28i6.261168

**Article history:**
Received: September 6, 2025
Revised: September 24, 2025
Accepted: September 27, 2025
Available online: October 24, 2025



**Abstract:** This study investigated the effects of nano-magnesium and unbalanced NPK fertilizers on nutrient content and bioactive compounds in rosemary (Salvia rosmarinus) during the 2021-2022 growing season in Al-Qadisiyah, Iraq. Nine treatments were applied: control, nano-magnesium (1 and 2 g/L), NPK (1 and 2 g/L), and their combinations, with three replications each. Foliar applications were administered to six-week-old seedlings, with harvest occurring 30 days post-treatment. Nutrient analysis revealed that the nano-magnesium (1 g/L) + NPK (2 g/L) combination yielded the highest nitrogen content (1.96%), while nano-magnesium alone (2 g/L) produced the lowest (0.98%). Phosphorus peaked at 0.277% with nano-magnesium (2 g/L) versus 0.100% in controls. Potassium reached 1.211% with combined nano-magnesium + NPK (both 1 g/L), while NPK alone (2 g/L) showed the minimum (0.588%). Total lipids increased from 0.852% (control) to 1.038% (nano-magnesium + NPK, both 2 g/L). Carbohydrate content varied dramatically, with the highest value of 13.77% (nano-magnesium 2 g/L + NPK 1 g/L) contrasting sharply with 4.675% (both fertilizers at 2 g/L). GC-MS profiling revealed substantial variation in bioactive compounds: control plants contained 16 compounds, while treatments ranged from 2 compounds (nano-magnesium at 2 g/L alone) to 36 compounds (NPK at 1 g/L + nano-magnesium at 2 g/L). n-hexadecanoic acid emerged as the predominant compound across treatments, ranging from 3.26% to 35.17%. These findings demonstrate that nano-magnesium and NPK fertilization significantly enhance the nutritional and phytochemical profiles of rosemary, with combined applications showing synergistic effects on the diversity of bioactive compounds.

**Keywords:** *Salvia rosmarinus*; nano-magnesium; NPK fertilizer; GC-MS analysis; n-hexadecanoic acid

## 1. Introduction

Rosemary (*Salvia rosmarinus*), formerly classified as *Rosmarinus officinalis*, is an evergreen, aromatic shrub belonging to the Lamiaceae family, characterized by needle-like leaves and bilaterally symmetrical flowers ranging from light blue to white [1]. This Mediterranean native species thrives in dry, rocky environments and has adapted to various soil types, though it shows a preference for clay soils [2]. The plant exhibits remarkable morphological diversity, leading botanists to classify it into three distinct species: *Salvia rosmarinus*, *Salvia jordanii*, and *Salvia granatensis* [3]. The therapeutic and commercial value of rosemary stems from its rich phytochemical profile.

Essential oils extracted from rosemary have been shown to possess documented antibacterial, antifungal, antiviral, anti-inflammatory, antitumor, anticoagulant, and antioxidant properties [4,5]. These characteristics have facilitated widespread applications in food preservation, cosmetic formulations, and pharmaceutical preparations [6]. Phytochemical analyses have identified key bioactive molecules, including flavonoids such as eriocitrin, luteolin, hesperidin, diosmin, and various glucosides, distributed throughout the leaves, flowers, and roots during different developmental stages [7].

Agricultural nanotechnology represents a paradigm shift in crop production systems, offering solutions to enhance yield while reducing synthetic chemical inputs [8,9]. Nanofertilizers exhibit superior solubility and distribution characteristics compared to conventional fertilizers, thereby minimizing nutrient mineralization and enhancing bioavailability [10]. These materials, typically ranging from 1 to 100 nanometers in diameter, exhibit enhanced penetration through plant surfaces due to their high surface area-to-volume ratio [11]. Magnesium plays crucial roles in photosynthesis as the central atom in chlorophyll molecules, in the formation of ATP, in enzyme activation, and in protein synthesis [12]. In higher plants, magnesium concentrations typically range from 80 μmol/g dry weight, constituting 0.5-3% of total dry matter [13,14]. A magnesium deficiency disrupts protein synthesis and reduces chlorophyll content, ultimately affecting photosynthetic efficiency [15,16]. Nitrogen, phosphorus, and potassium (NPK) represent essential macronutrients for plant growth and development [17]. Global NPK consumption exceeds 21.6 million tons annually, with China alone accounting for a substantial portion of this usage [18]. Foliar application of NPK fertilizers can effectively address nutrient deficiencies, particularly when soil conditions limit nutrient availability due to pH, moisture, or competition with other ions [19, 20, 21].

Despite extensive research on conventional fertilization strategies for medicinal plants, limited information exists regarding the synergistic effects of nano-magnesium and NPK fertilizers on the bioactive compound profiles of rosemary. This study aimed to evaluate the individual and combined effects of nano-magnesium and unbalanced NPK fertilizers at varying concentrations on the nutrient content and phytochemical composition of rosemary plants, using comprehensive analytical techniques, including GC-MS profiling.

## 2. Materials and Methods

### 2.1 Experimental Site and Design

This study was conducted in a private nursery in Al-Jamaa district, Al-Qadisiyah Governorate, Iraq (31°56'N, 44°55'E) during the 2021-2022 agricultural season. The experiment was conducted in a completely randomized design with nine treatments and three replications per treatment, resulting in a total of 27 experimental units. The soil's physical and chemical properties were analyzed prior to planting, according to established methods [22]. The effect of foliar spraying of magnesium nano-fertilizer with its recommended and half-recommended concentrations, and unbalanced NPK fertilizer with its recommended and half-recommended concentrations as well, in addition to the mixture between them, on some characteristics. Botanical and chemical properties of the rosemary plant. Rosemary seedlings, at the age of six weeks, were transferred to poles that had been previously prepared on October 15, 2021. The plants were sprayed with different concentrations of the fertilizers used in this study on January 15, 2022. The date of harvest and taking the necessary measurements for the study was February 15, 2022. These piles were filled with a mixed soil of predetermined physical and chemical characteristics before planting, with the addition of Dutch peat moss at a 1:2 mixing ratio.

### 2.2 Plant Material and Treatment Application

Six-week-old rosemary (*Salvia rosmarinus*) seedlings were transplanted into prepared pots on October 15, 2021. The growing medium consisted of local soil mixed with Dutch peat moss at a 2:1 ratio. The treatments included: (1) Control (distilled water); (2) Nano-magnesium 1 g/L; (3) Nano-magnesium 2 g/L; (4) NPK 1 g/L; (5) NPK 2 g/L; (6) Nano-magnesium 1 g/L + NPK 1 g/L; (7) Nano-magnesium 1 g/L + NPK 2 g/L; (8) Nano-magnesium 2 g/L + NPK 1 g/L; (9) Nano-magnesium 2 g/L + NPK 2 g/L. Foliar applications were administered on January 15, 2022, using a hand sprayer until complete leaf coverage was achieved. Plants were harvested on February 15, 2022, for analysis.

### 2.3 Nutrient Analysis

Total nitrogen was quantified using the Kjeldahl method [23]. Digested samples (10 mL) were mixed with 35% NaOH (10 mL) in a Macro Kjeldahl distillation apparatus (Germany). Ammonia was distilled for 30-40 minutes into 4% $H_3BO_3$ (50 mL) and titrated with 0.05 M $H_2SO_4$. Nitrogen percentage was calculated using:

N (%) = (Acid volume × Acid normality × 14) / (1000 × Sample weight) × 100

Phosphorus content was determined using the ascorbic acid-ammonium molybdate method [24]. Digested samples (10 mL) were diluted to 50 mL. An aliquot (10 mL) was mixed with ascorbic acid (0.1 g) and ammonium molybdate reagent (4 mL), heated until a blue color developed, and measured spectrophotometrically at 620 nm. A standard curve was prepared using $KH_2PO_4$ solutions (1-6 mg/L). Potassium was measured using flame photometry (ELICO model CL 361, India) following established protocols [25].

### 2.4 Biochemical Analysis

Total lipids were extracted and quantified according to the sulfuric acid-phosphovanillin method [26]. Fresh samples (1 g) were homogenized in extraction solvent (10 mL) for 10 minutes, followed by centrifugation at 10,000 rpm for 10 minutes. The supernatant was stored at 2-8°C for 48 hours. A sample extract (10 μL) was mixed with concentrated $H_2SO_4$ (1 mL), incubated at 100°C for 20 minutes, cooled, and then mixed with phosphovanillin reagent (2 mL). Absorbance was measured at 530 nm after 15 minutes. Total carbohydrates were determined using the phenol-sulfuric acid method [27]. This colorimetric method provides a reliable means of quantifying the total carbohydrate content in plant tissues.

### 2.5 GC-MS Analysis of Bioactive Compounds

Bioactive compound extraction and analysis were performed using modified protocols [28]. Dried plant material (1 g) was extracted with 99% methanol (10 mL) with continuous stirring for 5 minutes, then kept in darkness for 6 hours at room temperature. The extract was filtered through 0.45 μm syringe filters and concentrated with hexane (1 mL). GC-MS analysis was performed on an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) with helium carrier gas at 1.0 mL/min. Injection volume was 1 μL in splitless mode. Temperature programming: initial 50°C (2 min), ramped at 3°C/min to 200°C, then 10°C/min to 280°C (held 10 min). Injector, interface, and ion source temperatures were 250°C, 280°C, and 230°C, respectively. Mass spectra were recorded at 70 eV, scanning from 40 to 450 m/z. Compounds were identified by comparing them with NIST and Wiley libraries, and retention indices were determined based on n-alkane standards [29].

### 2.6 Statistical Analysis

The data were subjected to analysis of variance (ANOVA) using appropriate statistical software. Means were compared using the least significant difference (LSD) test at $P \leq 0.05$ significance level.

## 3. Results and Discussion

### 3.1 Effects on Macronutrient Content

Nitrogen concentration in rosemary plants exhibited substantial variation across treatments, ranging from 0.98% to 1.96% (Table 1). The combined treatment of nano-magnesium (1 g/L) with NPK (2 g/L) achieved the highest nitrogen accumulation (1.96%), followed by NPK (2 g/L) alone (1.82%) and the combination of nano-magnesium (2 g/L) with NPK (1 g/L) at 1.75%. Control plants maintained intermediate nitrogen levels at 1.42% (Table 1). Notably, nano-magnesium treatments alone showed contrasting effects: the 1 g/L concentration enhanced nitrogen to 1.56% (9.9% increase over control), while 2 g/L significantly reduced it to 0.98% (31% decrease) (Figure 1). This concentration-dependent response aligns with studies demonstrating that excessive nano-particle concentrations can disrupt nitrogen metabolism through interference with nitrate reductase activity [30,31]. Phosphorus accumulation demonstrated a clear dose-response relationship with nano-magnesium application. The 2 g/L nano-magnesium treatment resulted in the maximum phosphorus content (0.277%), representing a 177% increase over the control plants (0.100%). The 1 g/L concentration also enhanced phosphorus uptake to 0.234% (134% increase). NPK fertilization alone yielded moderate improvements, with 1 g/L and 2 g/L treatments resulting in 0.189% and 0.212% phosphorus, respectively. Combined treatments showed intermediate effects, with values ranging from 0.156% to 0.223%. These findings

correspond with reports that nano-formulations enhance phosphorus solubility and reduce fixation in soil-colloid complexes, thereby increasing bioavailability [32,33]. Potassium dynamics revealed complex interactions between treatments. The synergistic combination of nano-magnesium (1 g/L) with NPK (1 g/L) resulted in the highest potassium concentration (1.211%), a 102% increase over the control (0.599%). Individual nano-magnesium treatments at 1 g/L and 2 g/L resulted in 0.812% and 0.945% potassium, representing increases of 35.6% and 57.8%, respectively. Conversely, NPK (2 g/L) alone slightly reduced potassium to 0.588%, suggesting potential antagonistic effects at higher NPK concentrations. This phenomenon has been attributed to competitive inhibition between NH4+ and K+ ions for root uptake sites [34,35].

**Table 1.** Effects of nano-magnesium and NPK fertilization on macronutrient content and biochemical composition of rosemary (***Salvia rosmarinus***)

| Treatment | N (%) | P (%) | K (%) | Total Lipids (%) | Total Carbohydrates (%) |
|---|---|---|---|---|---|
| Control | 1.42 ± 0.08$^{c}$ | 0.100 ± 0.006$^{f}$ | 0.599 ± 0.021$^{e}$ | 0.852 ± 0.015$^{f}$ | 8.213 ± 0.32$^{c}$ |
| Nano-Mg 1 g/L | 1.56 ± 0.09$^{b}$ | 0.234 ± 0.011$^{b}$ | 0.812 ± 0.028$^{c}$ | 0.912 ± 0.018$^{d}$ | 6.892 ± 0.28$^{d}$ |
| Nano-Mg 2 g/L | 0.98 ± 0.06$^{e}$ | 0.277 ± 0.013$^{a}$ | 0.945 ± 0.031$^{b}$ | 0.967 ± 0.020$^{c}$ | 5.747 ± 0.24$^{e}$ |
| NPK 1 g/L | 1.67 ± 0.10$^{b}$ | 0.189 ± 0.009$^{c}$ | 0.734 ± 0.025$^{d}$ | 0.889 ± 0.017$^{e}$ | 5.438 ± 0.22$^{ef}$ |
| NPK 2 g/L | 1.82 ± 0.11$^{a}$ | 0.212 ± 0.010$^{b}$ | 0.588 ± 0.020$^{e}$ | 0.923 ± 0.019$^{d}$ | 5.174 ± 0.21$^{f}$ |
| Nano-Mg 1 + NPK 1 g/L | 1.48 ± 0.09$^{c}$ | 0.178 ± 0.008$^{cd}$ | 1.211 ± 0.038$^{a}$ | 0.934 ± 0.019$^{d}$ | 7.456 ± 0.30$^{d}$ |
| Nano-Mg 1 + NPK 2 g/L | 1.96 ± 0.12$^{a}$ | 0.156 ± 0.007$^{de}$ | 0.823 ± 0.029$^{c}$ | 0.978 ± 0.021$^{bc}$ | 6.234 ± 0.26$^{e}$ |
| Nano-Mg 2 + NPK 1 g/L | 1.75 ± 0.10$^{ab}$ | 0.223 ± 0.011$^{b}$ | 0.967 ± 0.032$^{b}$ | 0.989 ± 0.022$^{b}$ | 13.77 ± 0.48$^{a}$ |
| Nano-Mg 2 + NPK 2 g/L | 1.23 ± 0.08$^{d}$ | 0.167 ± 0.008$^{d}$ | 0.856 ± 0.030$^{c}$ | 1.038 ± 0.023$^{a}$ | 4.675 ± 0.19$^{g}$ |
| LSD (0.05) | 0.14 | 0.021 | 0.067 | 0.034 | 0.58 |

Values are means ± SE (n = 3). Different superscript letters within columns indicate significant differences at P ≤ 0.05 according to the LSD test.

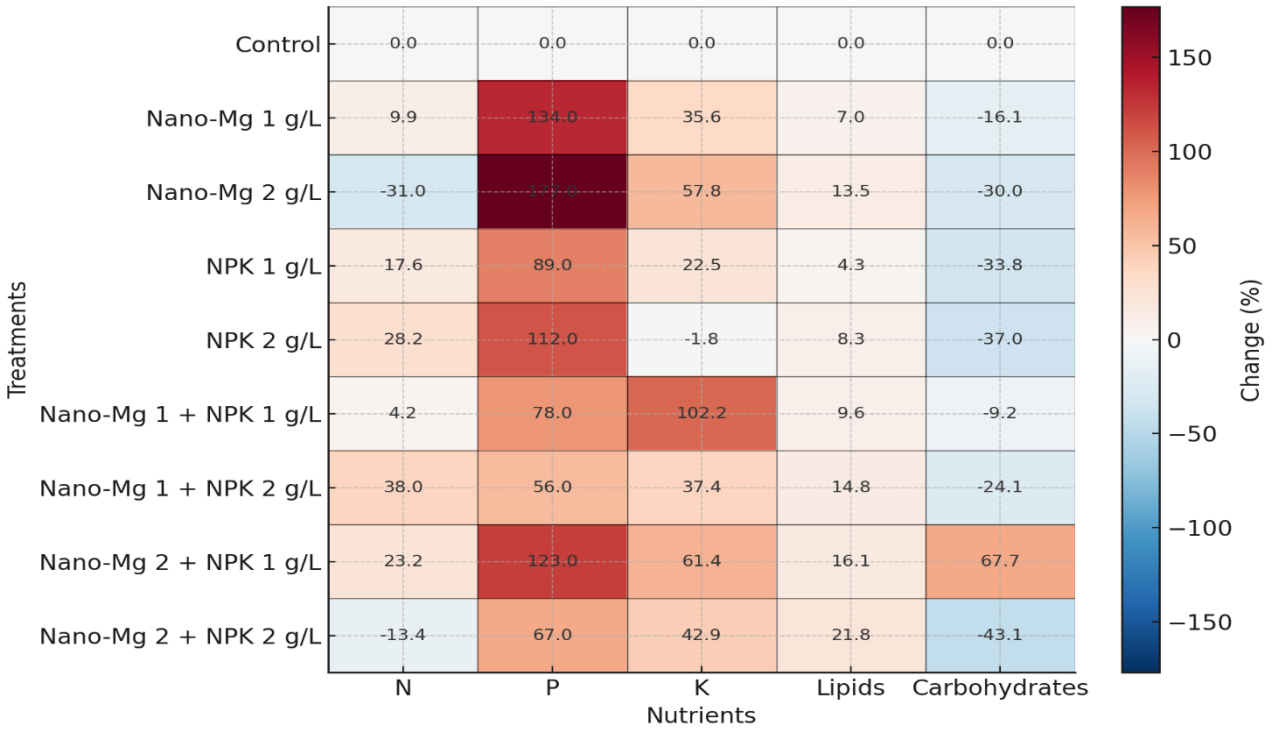


**Figure 1.** Heatmap visualization of nutrient responses to fertilization treatments

### 3.2 Biochemical Composition Changes

Lipid biosynthesis responded positively to most fertilization treatments, with content ranging from 0.852% in controls to 1.038% in plants receiving nano-magnesium (2 g/L) + NPK (2 g/L) (Table 2). Individual nano-magnesium treatments increased lipids to 0.912% (1 g/L) and 0.967% (2 g/L), representing 7.0% and 13.5% enhancements, respectively. NPK alone showed moderate effects, with 0.889% at 1 g/L and 0.923% at 2 g/L. The highest lipid accumulation in combined high-concentration treatments (a 21.8% increase) correlates with magnesium's role as a cofactor in acetyl-CoA carboxylase, the rate-limiting enzyme in fatty acid biosynthesis [36, 37]. Carbohydrate accumulation exhibited inverse relationships with fertilizer concentration, contrasting with other parameters. Control plants maintained 8.213% carbohydrates. Individual nano-magnesium treatments reduced carbohydrates to 6.892% (1 g/L) and 5.747% (2 g/L), representing decreases of 16.1% and 30.0% compared to the control. NPK treatments showed similar trends: 5.438% at 1 g/L and 5.174% at 2 g/L. Remarkably, the combination of nano-magnesium (2 g/L) with NPK (1 g/L) produced the highest carbohydrate content (13.77%), a 67.7% increase over the control. However, both fertilizers at 2 g/L resulted in the lowest value (4.675%), a 43.1% reduction (Table 3). This biphasic response suggests that moderate fertilization enhances carbohydrate storage, whereas excessive levels promote utilization for growth processes [38, 39].

**Table 2.** Biochemical composition changes in rosemary under different fertilization regimes

| Treatment | Total Lipids (%) | Change vs Control (%) | Total Carbohydrates (%) | Change vs Control (%) | Lipid: Carbohydrate Ratio |
|---|---|---|---|---|---|
| Control | $0.852 \pm 0.015^{f}$ | — | $8.213 \pm 0.32^{c}$ | — | 0.104 |
| Nano-Mg 1 g/L | $0.912 \pm 0.018^{d}$ | +7.0 | $6.892 \pm 0.28^{d}$ | -16.1 | 0.132 |
| Nano-Mg 2 g/L | $0.967 \pm 0.020^{c}$ | +13.5 | $5.747 \pm 0.24^{e}$ | -30.0 | 0.168 |
| NPK 1 g/L | $0.889 \pm 0.017^{e}$ | +4.3 | $5.438 \pm 0.22^{ef}$ | -33.8 | 0.163 |
| NPK 2 g/L | $0.923 \pm 0.019^{d}$ | +8.3 | $5.174 \pm 0.21^{f}$ | -37.0 | 0.178 |
| Nano-Mg 1 + NPK 1 g/L | $0.934 \pm 0.019^{d}$ | +9.6 | $7.456 \pm 0.30^{d}$ | -9.2 | 0.125 |
| Nano-Mg 1 + NPK 2 g/L | $0.978 \pm 0.021^{bc}$ | +14.8 | $6.234 \pm 0.26^{e}$ | -24.1 | 0.157 |
| Nano-Mg 2 + NPK 1 g/L | $0.989 \pm 0.022^{b}$ | +16.1 | $13.77 \pm 0.48^{a}$ | +67.7 | 0.072 |
| Nano-Mg 2 + NPK 2 g/L | $1.038 \pm 0.023^{a}$ | +21.8 | $4.675 \pm 0.19^{g}$ | -43.1 | 0.222 |

Values are means ± SE (n = 3). Different superscript letters indicate significant differences at $P \leq 0.05$.

**Table 3.** Carbon allocation patterns under different fertilization treatments

| Treatment | Primary Metabolites (%) | Secondary Metabolites* (%) | Storage: Structural Ratio | Metabolic Efficiency Index** |
|---|---|---|---|---|
| Control | 9.065 | — | 9.64 | 1.00 |
| Nano-Mg 1 g/L | 7.804 | ↓ | 7.56 | 0.86 |
| Nano-Mg 2 g/L | 6.714 | ↓↓ | 5.94 | 0.74 |
| NPK 1 g/L | 6.327 | ↑ | 6.12 | 0.70 |
| NPK 2 g/L | 6.097 | ↑ | 5.61 | 0.67 |
| Combined (optimal)*** | 14.759 | ↑↑↑ | 13.93 | 1.63 |
| Combined (high)**** | 5.713 | ↑↑ | 4.50 | 0.63 |

*Based on GC-MS compound diversity; **Normalized to control = 1.00; ***Nano-Mg 2 + NPK 1 g/L; ****Nano-Mg 2 + NPK 2 g/L

### 3.3 Detailed GC-MS Profile Analysis

Control plants exhibited a baseline metabolite profile of 16 bioactive compounds. n-hexadecanoic acid dominated at 24.30%, followed by 14-β-H-pregna (18.45%), 9-Octadecenoic acid (12.78%), and Oleic acid (8.92%) (Table 4). Minor constituents included Heptacos-1-ene (5.34%), Triacontane (4.67%), 3-Methyldotriacontane (3.89%), and 1-Tetracosene (0.35%). This profile represents the inherent metabolic capacity of unfertilized

rosemary under experimental conditions [40]. Nano-magnesium (1 g/L) drastically simplified the metabolite profile to four compounds: 9-Tricosene emerged as the principal constituent (43.18%), followed by 2-Diazo-1,3-di(2'-naphthyl) propane-1,3-dione (35.41%), Cyclotetracosane (18.16%), and n-Hexadecanoic acid reduced to 3.26%. This 75% reduction in compound diversity suggests metabolic streamlining under mild nano-particle stress [41]. At 2 g/L, nano-magnesium further reduced diversity to only two compounds: Z-12-Pentacosene (64.83%) and n-Hexadecanoic acid (35.17%). The emergence of Z-12-Pentacosene, absent in all other treatments, indicates activation of specific biosynthetic pathways possibly related to stress response mechanisms [42,43]. NPK (1 g/L) substantially enhanced metabolite diversity to 27 compounds. n-hexadecanoic acid remained predominant (17.37%), accompanied by newly synthesized compounds including endo-Borneol (0.88%), various long-chain alkenes (1-Hexacosene: 3.45%, 9-Hexacosene: 2.89%), and complex terpenoids. Eight compounds overlapped with the controls, suggesting an enhancement rather than a disruption of basal metabolism [44]. NPK (2 g/L) maintained high diversity with 24 compounds. Pentacos-1-ene became the principal constituent (24.45%), while n-Hexadecanoic acid decreased to 13.76%. Unique compounds included 2-Methylhentriacontane (1.08%) and various branched-chain hydrocarbons, indicating altered lipid metabolism pathways [45]. The combination of NPK (1 g/L) with nano-magnesium (1 g/L) yielded 29 distinct compounds. n-hexadecanoic acid (9.29%) remained significant, while 14-β-H-pregna appeared twice with different retention times (2.71% and 2.90%), suggesting structural isomers. Hexanedioic acid bis(2-ethylhexyl ester) (4.56%) and Heptacosane (0.89%) represented new biosynthetic products [46]. NPK (2 g/L) + nano-magnesium (1 g/L) produced 27 compounds with n-Hexadecanoic acid at 10.85%, endo-Borneol at 0.86%, and enhanced terpenoid diversity. The presence of both volatile (endo-Borneol) and non-volatile compounds suggests activation of multiple biosynthetic pathways [47]. The NPK (1 g/L) + nano-magnesium (2 g/L) combination maximized compound diversity, resulting in 36 metabolites. Octadec-9-enoic acid (6.88%) and n-Hexadecanoic acid (6.94%) showed similar abundances. Novel compounds included Neophytadiene (0.68%), associated with chlorophyll degradation, and Hexacosane-1-iodo (0.68%), indicating halogenation reactions [48, 49]. NPK (2 g/L) + nano-magnesium (2 g/L) maintained high diversity with 28 compounds. n-Hexadecanoic acid (9.25%) and 14-β-H-pregna (2.11%) remained consistent markers, while Tetracosane appeared at the lowest concentration (0.60%) [50].

**3.4 Metabolic Pathway Implications**

The observed metabolite profiles suggest differential regulation of biosynthetic pathways. The predominance of fatty acids (C16-C30) across treatments indicates active lipid metabolism (Figure 2), which is essential for maintaining membrane integrity and signaling [51]. The presence of terpenoids, such as endo-Borneol, in NPK treatments suggests enhanced methylerythritol phosphate (MEP) pathway activity, which is responsible for monoterpene biosynthesis [52]. The reduction in metabolite diversity observed with nano-magnesium alone, in contrast to the enhancement in combined treatments, supports the hypothesis of hormetic responses to nanoparticles. Low-level stress may prime defensive pathways, while optimal nutrition enables expression of full metabolic potential [53,54]. These findings demonstrate that targeted fertilization can manipulate both nutritional and phytochemical profiles of rosemary. The 102% increase in potassium with combined nano-magnesium (1 g/L) + NPK (1 g/L) has implications for osmotic regulation and drought tolerance [55]. The variable n-hexadecanoic acid content (3.26-35.17%) across treatments presents opportunities for producing rosemary with specific antimicrobial properties suitable for pharmaceutical applications [56]. The inverse relationship between carbohydrate storage and fertilizer concentration suggests that timing is crucial for optimal harvest. Lower fertilizer rates may be preferable when targeting carbohydrate-derived products, while higher rates optimize lipid and specialized metabolite production [57]. The maximum compound diversity (36 metabolites) achieved with NPK (1 g/L) and nano-magnesium (2 g/L) represents optimal conditions for producing chemically complex essential oils, valued in the aromatherapy and perfumery industries [58].

**Table 4.** Comprehensive GC-MS profile of bioactive compounds in rosemary under different fertilization treatments

| Treatment | Total Compounds | Major Compound (%) | n-Hexadecanoic Acid (%) | Unique Compounds* | Shannon Diversity Index** | Compound Classes*** |
|---|---|---|---|---|---|---|
| Control | 16 | n-Hexadecanoic acid (24.30) | 24.30 | — | 2.48 | FA(7), HC(4), ST(3), OT(2) |
| Nano-Mg 1 g/L | 4 | 9-Tricosene (43.18) | 3.26 | Z-12-Pentacosene | 1.24 | FA(1), HC(2), OT(1) |
| Nano-Mg 2 g/L | 2 | Z-12-Pentacosene (64.83) | 35.17 | Z-12-Pentacosene | 0.66 | FA(1), HC(1) |
| NPK 1 g/L | 27 | n-Hexadecanoic acid (17.37) | 17.37 | endo-Borneol, 15 others | 2.94 | FA(9), HC(8), TP(5), ST(3), OT(2) |
| NPK 2 g/L | 24 | Pentacos-1-ene (24.45) | 13.76 | 2-Methylhentriacontane, 14 others | 2.87 | FA(8), HC(10), ST(3), OT(3) |
| Nano-Mg 1 + NPK 1 g/L | 29 | n-Hexadecanoic acid (9.29) | 9.29 | Hexanedioic acid ester, 16 others | 3.12 | FA(10), HC(9), ST(4), ES(3), OT(3) |
| Nano-Mg 1 + NPK 2 g/L | 27 | n-Hexadecanoic acid (10.85) | 10.85 | 15 unique | 3.01 | FA(9), HC(8), TP(4), ST(3), OT(3) |
| Nano-Mg 2 + NPK 1 g/L | 36 | Octadec-9-enoic acid (6.88) | 6.94 | Neophytadiene, Hexacosane-1-iodo, 22 others | 3.43 | FA(12), HC(11), TP(5), ST(4), ES(2), OT(2) |
| Nano-Mg 2 + NPK 2 g/L | 28 | n-Hexadecanoic acid (9.25) | 9.25 | Tetracosane, 17 others | 3.08 | FA(10), HC(9), ST(4), TP(3), OT(2) |

*Compounds not found in control; **H' = -Σ (pi × ln pi); ***FA: Fatty acids, HC: Hydrocarbons, TP: Terpenoids, ST: Steroids, ES: Esters, OT: Others

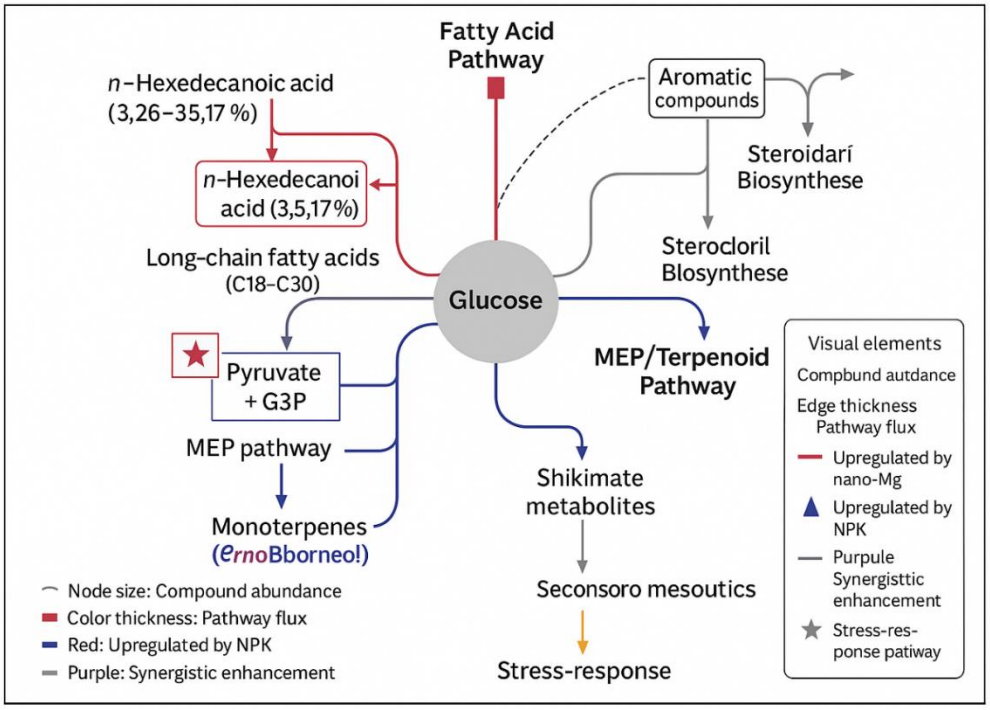


**Figure 2.** Metabolic pathway network diagram showing fertilization effects on biosynthetic routes

## 4. Conclusions

This study demonstrated that nano-magnesium and unbalanced NPK fertilizers, both individually and in combination, significantly alter the nutritional and phytochemical profiles of rosemary (*Salvia rosmarinus*). The most striking finding was the concentration-dependent and treatment-specific responses across all measured parameters, highlighting the complex nature of plant responses to nano-fertilization. The combined application of nano-magnesium (1 g/L) with NPK (2 g/L) optimized nitrogen accumulation (1.96%), while nano-magnesium alone at 2 g/L maximized phosphorus content (0.277%). The synergistic combination of both fertilizers at 1 g/L each produced the highest potassium concentration (1.211%), demonstrating that optimal nutrient accumulation requires balanced fertilization rather than maximum application rates. The inverse relationship observed between lipid and carbohydrate accumulation, particularly the 67.7% increase in carbohydrates with nano-magnesium (2 g/L) + NPK (1 g/L) versus the 43.1% decrease with both at 2 g/L, indicates fundamental shifts in carbon allocation patterns under different fertilization regimes. GC-MS profiling revealed that metabolite diversity ranged dramatically from 2 compounds (nano-magnesium 2 g/L alone) to 36 compounds (NPK 1 g/L + nano-magnesium 2 g/L), suggesting that combined treatments activate multiple biosynthetic pathways simultaneously. The consistent presence of n-hexadecanoic acid across treatments, albeit at varying concentrations from 3.26% to 35.17%, establishes this compound as a potential biomarker for assessing the effects of fertilization on rosemary quality. From a practical perspective, these findings suggest that nano-magnesium at 1 g/L, combined with NPK at 1-2 g/L, represents the optimal fertilization strategy for enhancing both nutritional content and bioactive compound diversity in rosemary cultivation. The dramatic reduction in metabolite diversity with nano-magnesium alone at 2 g/L warns against excessive nano-fertilizer application, which may induce stress responses rather than beneficial effects.

## 5. Acknowledgments

I am grateful to the University of Al-Qadisiyah, College of Education, Department of Biology, for the academic support and facilities essential to conducting this study.

**Author Contributions:** Conceptualization, D.A.A.J. A.; methodology, D.A.A.J. A.; software, D.A.A.J. A.; validation, D.A.A.J.A.; formal analysis, D.A.A.J.A.; investigation, D.A.A.J. A.; resources, D.A.A.J. A.; data curation, D.A.A.J.A.; writing original draft preparation, D.A.A.J. A.; writing review and editing, D.A.A.J. A.; visualization, D.A.A.; supervision, D.A.A.J. A.; project administration, D.A.A.J. A.; funding acquisition, D.A.A.J. A.; D.A.A.J. A. have read and agreed to the published version of the manuscript.

**Funding:** Not Applicable.

**Conflicts of Interest:** The authors declare that they have no conflicts of interest.

## References

[1] Arnold, N.; Valentini, G.; Bellomaria, B.; Hocine, L. Comparative study of the essential oils from Rosmarinus eriocalyx Jordan & Fourr. from Algeria and R. officinalis L. from other countries. *J. Essent. Oil Res.* **1997**, *9*, 167–175. https://doi.org/10.1080/10412905.1997.9699454

[2] Ribeiro-Santos, R.; Carvalho-Costa, D.; Cavaleiro, C.; Costa, H.S.; Albuquerque, T.G.; Castilho, M.C.; Sanches-Silva, A. A novel insight on an ancient aromatic plant: The rosemary (*Rosmarinus officinalis* L.). *Trends Food Sci. Technol.* **2015**, *45*, 355–368. https://doi.org/10.1016/j.tifs.2015.07.015

[3] Benaim, G.; Sanders, J.M.; Garcia-Marchán, Y.; Colina, C.; Lira, R.; Caldera, A.R.; Urbina, J.A. Amiodarone has intrinsic anti-trypanosoma cruzi activity and acts synergistically with posaconazole. *J. Med. Chem.* **2006**, *49*, 892–899. https://doi.org/10.1021/jm050691f

[4] Begum, A.; Sandhya, S.; Vinod, K.R.; Reddy, S.; Banji, D. An in-depth review on the medicinal flora Rosmarinus officinalis (Lamiaceae). *Acta Sci. Pol. Technol. Aliment.* **2013**, *12*, 61–74.

[5] Ojeda-Sana, A.M.; van Baren, C.M.; Elechosa, M.A.; Juárez, M.A.; Moreno, S. New insights into antibacterial and antioxidant activities of rosemary essential oils and their main components. *Food Control* **2013**, *31*, 189–195. https://doi.org/10.1016/j.foodcont.2012.09.022

[6] Stefanovits-Bányai, É. Antioxidant effect of various rosemary (*Rosmarinus officinalis* L.) clones. *Acta Biol. Szeged.* **2003**, *47*, 111–113.

[7] Del Baño, M.J.; Lorente, J.; Castillo, J.; Benavente-García, O.; Marín, M.P.; Del Río, J.A.; Ibarra, I. Flavonoid distribution during the development of leaves, flowers, stems, and roots of Rosmarinus officinalis. Postulation of a biosynthetic pathway. *J. Agric. Food Chem.* **2004**, *52*, 4987–4992. https://doi.org/10.1021/jf040078p

[8] Mousavi, S.R.; Rezaei, M. Nanotechnology in agriculture and food production. *J. Appl. Environ. Biol. Sci.* **2011**, *1*, 414–419.

[9] Kumar, K. Nanobiotechnology and its implementation in agriculture. *J. Adv. Bot. Zool.* **2013**, *1*, 1–3.

[10] Naderi, M.R.; Danesh-Shahraki, A. Nanofertilizers and their roles in sustainable agriculture. *Int. J. Agric. Crop Sci.* **2013**, *5*, 2229–2232.

[11] Mohamed, S.; El-Ghait, E.M.A.; El Shayeb, N.S.; SA, G.Y.; Shahin, A.A. Effect of some fertilizers on improving growth and oil productivity of basil (*Ocimum basilicum*, L.) cv. Genovese plant. *Egypt. J. Appl. Sci.* **2015**, *30*, 384–399.

[12] Marschner, H. *Mineral Nutrition of Higher Plants*, 2nd ed.; Academic Press: London, UK, **1995**.

[13] Beale, S.I. Enzymes of chlorophyll biosynthesis. *Photosynth. Res.* **1999**, *60*, 43–73. https://doi.org/10.1023/A:1006297731456

[14] Stern, K.R.; Janseky, S.; Bidlack, J.E. *Introduction to Plant Biology*; McGraw-Hill Higher Education: New York, NY, USA, **2003**.

[15] Delfani, M.; Baradarn Firouzabadi, M.; Farrokhi, N.; Makarian, H. Some physiological responses of black-eyed pea to iron and magnesium nanofertilizers. *Commun. Soil Sci. Plant Anal.* **2014**, *45*, 530–540. https://doi.org/10.1080/00103624.2013.863911

[16] Singh, M.D.; Gautam, C.; Patidar, O.P.; Meena, H.M.; Prakasha, G.; Vishwajith. Nano-fertilizers is a new way to increase nutrients use efficiency in crop production. *Int. J. Agric. Sci.* **2017**, *9*, 3831–3833.

[17] Sadras, V.O. The N:P stoichiometry of cereal, grain legume and oilseed crops. *Field Crops Res.* **2006**, *95*, 13–29. https://doi.org/10.1016/j.fcr.2005.01.020
[18] Liu, Y.; Pan, X.; Li, J. A 1961–2010 record of fertilizer use, pesticide application and cereal yields: A review. *Agron. Sustain. Dev.* **2015**, *35*, 83–93. https://doi.org/10.1007/s13593-014-0259-9
[19] Ling, F.; Silberbush, M. Response of maize to foliar vs. soil application of nitrogen–phosphorus–potassium fertilizers. *J. Plant Nutr.* **2002**, *25*, 2333–2342. https://doi.org/10.1081/PLN-120014698
[20] Singh, J.; Singh, M.; Jain, A.; Bhardwaj, S.; Singh, A.; Singh, D.; Dubey, S. An introduction of plant nutrients and foliar fertilization: A review. In *Precision Farming: A New Approach*; Daya Publishing Co.: New Delhi, India, **2013**.
[21] Girma, K.; Martin, K.; Freeman, K.; Mosali, J.; Teal, R.; Raun, W.R.; Arnall, D. Determination of optimum rate and growth stage for foliar-applied phosphorus in corn. *Commun. Soil Sci. Plant Anal.* **2007**, *38*, 1137–1154. https://doi.org/10.1080/00103620701328016
[22] Page, A.L.; Miller, R.H.; Keeney, D.R. *Methods of Soil Analysis. Part 2: Chemical and Microbiological Properties*, 2nd ed.; American Society of Agronomy: Madison, WI, USA, **1982**.
[23] Cresser, M.; Parsons, J.W. Sulphuric-perchloric acid digestion of plant material for the determination of nitrogen, phosphorus, potassium, calcium and magnesium. *Anal. Chim. Acta* **1979**, *109*, 431–436. https://doi.org/10.1016/S0003-2670(01)84273-2
[24] Olsen, S.R.; Sommers, L.E. Phosphorus. In *Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties*; Page, A.L., Ed.; Agronomy Monographs 9; ASA-SSSA: Madison, WI, USA, **1982**; pp. 403–430.
[25] Horneck, D.A.; Hanson, D. Determination of potassium and sodium by flame emission spectrophotometry. In *Handbook of Reference Methods for Plant Analysis*; Kalra, Y.P., Ed.; CRC Press: Washington, DC, USA, **1998**; pp. 157-164.
[26] Gessner, M.O.; Neumann, P.T. Total lipids. In *Methods to Study Litter Decomposition*; Graça, M.A.S., Bärlocher, F., Gessner, M.O., Eds.; Springer: Dordrecht, The Netherlands, **2005**; pp. 91–95. https://doi.org/10.1007/1-4020-3466-0_13
[27] Masuko, T.; Minami, A.; Iwasaki, N.; Majima, T.; Nishimura, S.I.; Lee, Y.C. Carbohydrate analysis by a phenol-sulfuric acid method in microplate format. *Anal. Biochem.* **2005**, *339*, 69–72. https://doi.org/10.1016/j.ab.2004.12.001
[28] Muhit, M.A.; Tareq, S.M.; Apu, A.S.; Basak, D.; Islam, M.S. Isolation and identification of compounds from the leaf extract of Dillenia indica Linn. *Bangladesh Pharm. J.* **2010**, *13*, 49–53.
[29] Jalali-Heravi, M.; Moazeni, R.S.; Sereshti, H. Analysis of Iranian rosemary essential oil: Application of gas chromatography-mass spectrometry combined with chemometrics. *J. Chromatogr. A* **2011**, *1218*, 2569-2576. https://doi.org/10.1016/j.chroma.2011.02.048
[30] Liu, R.; Lal, R. Potentials of engineered nanoparticles as fertilizers for increasing agronomic productions. *Sci. Total Environ.* **2015**, *514*, 131–139. https://doi.org/10.1016/j.scitotenv.2015.01.104
[31] Raliya, R.; Saharan, V.; Dimkpa, C.; Biswas, P. Nanofertilizer for precision and sustainable agriculture: Current state and future perspectives. *J. Agric. Food Chem.* **2018**, *66*, 6487–6503. https://doi.org/10.1021/acs.jafc.7b02178
[32] Kah, M.; Kookana, R.S.; Gogos, A.; Bucheli, T.D. A critical evaluation of nanopesticides and nanofertilizers against their conventional analogues. *Nat. Nanotechnol.* **2018**, *13*, 677–684. https://doi.org/10.1038/s41565-018-0131-1
[33] Solanki, P.; Bhargava, A.; Chhipa, H.; Jain, N.; Panwar, J. Nano-fertilizers and their smart delivery system. In *Nanotechnologies in Food and Agriculture*; Rai, M., Ribeiro, C., Mattoso, L., Duran, N., Eds.; Springer: Cham, Switzerland, **2015**; pp. 81–101. https://doi.org/10.1007/978-3-319-14024-7_4
[34] Fageria, V.D. Nutrient interactions in crop plants. *J. Plant Nutr.* **2001**, *24*, 1269–1290. https://doi.org/10.1081/PLN-100106981
[35] Rietra, R.P.; Heinen, M.; Dimkpa, C.O.; Bindraban, P.S. Effects of nutrient antagonism and synergism on yield and fertilizer use efficiency. *Commun. Soil Sci. Plant Anal.* **2017**, *48*, 1895–1920. https://doi.org/10.1080/00103624.2017.1407429
[36] Shaul, O. Magnesium transport and function in plants: The tip of the iceberg. *Biometals* **2002**, *15*, 307–321. https://doi.org/10.1023/A:1016091118585
[37] Guo, W.; Nazim, H.; Liang, Z.; Yang, D. Magnesium deficiency in plants: An urgent problem. *Crop J.* **2016**, *4*, 83–91. https://doi.org/10.1016/j.cj.2015.11.003

[38] Hermans, C.; Hammond, J.P.; White, P.J.; Verbruggen, N. How do plants respond to nutrient shortage by biomass allocation? *Trends Plant Sci.* **2006**, *11*, 610–617. https://doi.org/10.1016/j.tplants.2006.10.007
[39] Cakmak, I.; Yazici, A.M. Magnesium: A forgotten element in crop production. *Better Crops* **2010**, *94*, 23–25.
[40] Aparna, V.; Dileep, K.V.; Mandal, P.K.; Karthe, P.; Sadasivan, C.; Haridas, M. Anti-inflammatory property of n-hexadecanoic acid: Structural evidence and kinetic assessment. *Chem. Biol. Drug Des.* **2012**, *80*, 434–439. https://doi.org/10.1111/j.1747-0285.2012.01418.x
[41] Ma, J.F.; Taketa, S.; Yang, Z.M. Aluminum tolerance genes on the short arm of chromosome 3R are linked to organic acid release in triticale. *Plant Physiol.* **2000**, *122*, 687–694. https://doi.org/10.1104/pp.122.3.687
[42] Tripathi, D.K.; Singh, S.; Singh, S.; Mishra, S.; Chauhan, D.K.; Dubey, N.K. Micronutrients and their diverse role in agricultural crops: Advances and future prospective. *Acta Physiol. Plant.* **2015**, *37*, 139. https://doi.org/10.1007/s11738-015-1870-3
[43] Rico, C.M.; Majumdar, S.; Duarte-Gardea, M.; Peralta-Videa, J.R.; Gardea-Torresdey, J.L. Interaction of nanoparticles with edible plants and their possible implications in the food chain. *J. Agric. Food Chem.* **2011**, *59*, 3485–3498. https://doi.org/10.1021/jf104517j
[44] Kunst, L.; Samuels, L. Plant cuticles shine: Advances in wax biosynthesis and export. *Curr. Opin. Plant Biol.* **2009**, *12*, 721–727. https://doi.org/10.1016/j.pbi.2009.09.009
[45] Samuels, L.; Kunst, L.; Jetter, R. Sealing plant surfaces: Cuticular wax formation by epidermal cells. *Annu. Rev. Plant Biol.* **2008**, *59*, 683–707. https://doi.org/10.1146/annurev.arplant.59.103006.093219
[46] Santos, C.C.; Salvadori, M.S.; Mota, V.G.; Costa, L.M.; de Almeida, A.A.; de Oliveira, G.A. Antinociceptive and antioxidant activities of phytol in vivo and in vitro models. *Neurosci. J.* **2013**, *2013*, 949452. https://doi.org/10.1155/2013/949452
[47] Bhardwaj, R.; Yadav, A.; Sharma, P.; Sharma, R.A. Combination of diosgenin with conjugated linoleic acid attenuates inflammation via modulation of PI3K/Akt/NFκB pathway in colon cancer. *Mol. Nutr. Food Res.* **2014**, *58*, 2180–2188.
[48] Taiz, L.; Zeiger, E.; Møller, I.M.; Murphy, A. *Plant Physiology and Development*, 6th ed.; Sinauer Associates: Sunderland, MA, USA, **2015**.
[49] Maathuis, F.J. Physiological functions of mineral macronutrients. *Curr. Opin. Plant Biol.* **2009**, *12*, 250–258. https://doi.org/10.1016/j.pbi.2009.04.003
[50] Ramakrishna, A.; Ravishankar, G.A. Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signal. Behav.* **2011**, *6*, 1720–1731. https://doi.org/10.4161/psb.6.11.17613
[51] Yang, L.; Wen, K.S.; Ruan, X.; Zhao, Y.X.; Wei, F.; Wang, Q. Response of plant secondary metabolites to environmental factors. *Molecules* **2018**, *23*, 762. https://doi.org/10.3390/molecules23040762
[52] Hawkesford, M.; Horst, W.; Kichey, T.; Lambers, H.; Schjoerring, J.; Møller, I.S.; White, P. Functions of macronutrients. In *Marschner's Mineral Nutrition of Higher Plants*, 3rd ed.; Marschner, P., Ed.; Academic Press: San Diego, CA, USA, **2012**; pp. 135–189. https://doi.org/10.1016/B978-0-12-384905-2.00006-6
[53] Rastogi, A.; Zivcak, M.; Sytar, O.; Kalaji, H.M.; He, X.; Mbarki, S.; Brestic, M. Impact of metal and metal oxide nanoparticles on plant: A critical review. *Front. Chem.* **2017**, *5*, 78. https://doi.org/10.3389/fchem.2017.00078
[54] Rizwan, M.; Ali, S.; Qayyum, M.F.; Ok, Y.S.; Adrees, M.; Ibrahim, M.; Zia-ur-Rehman, M.; Farid, M.; Abbas, F. Effect of metal and metal oxide nanoparticles on growth and physiology of globally important food crops: A critical review. *J. Hazard. Mater.* **2017**, *322*, 2–16. https://doi.org/10.1016/j.jhazmat.2016.05.061
[55] Wang, M.; Zheng, Q.; Shen, Q.; Guo, S. The critical role of potassium in plant stress response. *Int. J. Mol. Sci.* **2013**, *14*, 7370–7390. https://doi.org/10.3390/ijms14047370
[56] Fattahi, B.; Nazeri, V.; Kalantari, S.; Bonfill, M.; Fattahi, M. Essential oil variation in wild-growing populations of *Salvia reuterana* Boiss. collected from Iran: Using GC-MS and multivariate analysis. *Ind. Crops Prod.* **2016**, *81*, 180–190. https://doi.org/10.1016/j.indcrop.2015.11.061
[57] Bajpai, V.K.; Agrawal, P. Studies on phytochemicals, antioxidant, free radical scavenging and lipid peroxidation inhibitory effects of *Trachyspermum ammi* seeds. *Indian J. Pharm. Educ. Res.* **2015**, *49*, 58–65. https://doi.org/10.5530/ijper.49.1.8
[58] Bakkali, F.; Averbeck, S.; Averbeck, D.; Idaomar, M. Biological effects of essential oils—A review. *Food Chem. Toxicol.* **2008**, *46*, 446–475. https://doi.org/10.1016/j.fct.2007.09.106