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Phytochemical and morpho-physiological response of *Melissa officinalis* L. to different NH_4^+ to NO_3^- ratios under hydroponic cultivation

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Abstract

Background The utilization of nutrition management, has recently been developed as a means of improving the growth and production of phytochemical compounds in herbs. The present study aimed to improve the growth, physiological, and phytochemical characteristics of lemon balm (*Melissa officinalis* L.) using different NH_4^+ (ammonium) to NO_3^- (nitrate) ratios (0:100, 25:75, 50:50, 75:25 and 100:0) under floating culture system (FCS).

Results The treatment containing 0:100 - NH_4^+:NO_3^- ratio showed the most remarkable values for the growth and morpho-physiological characteristics of *M. officinalis*. The results demonstrated that maximum biomass (105.57 g) earned by using the ratio of 0:100 and minimum at 75:25 ratio of NH_4^+: NO_3^-. The plants treated with high nitrate ratio (0:100 - NH_4^+:NO_3^-) showed the greatest concentration of total phenolics (60.40 mg GAE/g DW), chlorophyll a (31.32 mg/100 g DW), flavonoids (12.97 mg QUE/g DW), and carotenoids (83.06 mg/100 g DW). Using the 75:25 - NH_4^+:NO_3^- ratio caused the highest dry matter (DM), N and K macronutrients in the leaves. The highest antioxidant activity by both DPPH (37.39 µg AAE/mL) and FRAP (69.55 mM Fe^{++}/g DW) methods was obtained in 75:25 - NH_4^+:NO_3^- treatment. The *p*-coumaric acid as a main abundant phenolic composition, was detected by HPLC analysis as the highest content in samples grown under 0:100 - NH_4^+:NO_3^- treatment. Also, the major compounds in *M. officinalis* essential oil were identified as geranial, neral, geranyl acetate and geraniol by GC analysis. With increasing NO_3^- application, geraniol and geranyl acetate contents were decreased.

Conclusions The findings of present study suggest that the management of $NH4^+$ to NO_3^- ratios in nutrient solutions could contribute to improving growth, physiological and phytochemical properties of *M. officinalis*. The plants treated with high nitrate ratio (especially 0:100 - NH_4^+:NO_3^-) showed the greatest effects on improving the growth and production of morpho-physiological and phytochemical compounds. By comprehensively understanding the intricate dynamics among nitrogen sources, plants, and their surroundings, researchers and practitioners can devise inventive approaches to optimize nitrogen management practices and foster sustainable agricultural frameworks.

Keywords Ammonium, Essential oil, Hydroponic cultivation, Nitrate, Phenolics

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Background

Medicinal and aromatic plants, in addition to their important role in medical uses, are widely used in many fields related to the food, cosmetics and spice industries [1]. Today, medicinal plants are valuable natural resource that is considered by developed countries as a raw material for the production of safe drugs for humans. *Melissa officinalis* L., commonly called lemon balm or bee balm, is a perennial herbaceous plant with alimentary and medicinal properties, belonging to the Lamiaceae family and the subfamily Nepetoideae [2, 3]. Likewise, lemon balm has been utilized in traditional medicine for treatment of diseases and fundamental health needs [2, 4]. In addition, the leaves of *M. officinalis* are used to remedy moderate abdominal disorders, rheumatism and biliary dyskinesia due to their digestive and antispasmodic properties [2, 5]. The essential oil of this plant is obtained from fresh main and secondary branches, flowers and leaves. According to previous studies [6, 7] geranial, linalool, citral and neral are main active constituents found in lemon balm essential oil, which are responsible for producing the distinct lemon scent. Lemon balm essential oil may also contain Herpes anti-virus compounds type 2 (HSV-2) [8, 9].

Certainly, Innovative methods for cultivating plants, such as hydroponics, aquaponics, and aeroponics, offer alternative approaches to traditional soil-based agriculture. These methods utilize controlled environments and specialized techniques to optimize plant growth and resource utilization. These innovative cultivation methods offer benefits such as increased crop yields, faster growth rates, and reduced resource consumption, making them valuable tools for sustainable agriculture in diverse settings [10–12]. Hydroponics is a revolutionary food production technology that is environmentally friendly, sustainable, reliable and flexible [13–16]. Moreover, hydroponic culture systems present higher yields, greater production of secondary metabolites, and less pests and diseases damage in compared with soil cultivation [17–19]. In hydroponic systems, pH can be checked and controlled in addition to nutrient management. The impacts of various nitrogen forms on morphological, physiological, and phytochemical plant parameters can be assessed by hydroponic systems due to nitrogen changes in the soil [20]. These techniques have already been used to evaluate nitrogen efficiency among plant species based on nitrogen forms (NO_3^- and NH_4^+) [21]. Nitrogen (N) is the key element in soil fertility and production of agricultural crops and compared to other nutrients, is more needed by the plants [22]. Plants rely on nitrogen as an essential nutrient for their development, growth, yield, and quality. It is also a key element of the chlorophyll molecule that plays an important role in photosynthesis [23]. Nitrogen is one of the most important influencing elements in the synthesis of phytochemicals and photosynthetic rates. There is often a positive relationship between the activity of key enzymes of important biosynthetic pathways (such as phenylpropanoid pathway), and the accumulation of carbon-based phytochemical compounds in medicinal plants. These pathways and enzymes are directly related to the availability and type of nitrogen in the plant culture medium [24]. Ammonium and nitrate are the two primary forms of nitrogen that are commonly utilized by plants to fulfil their nitrogen needs [25]. In different studies on medicinal plants such as *Echinacea purpurea* (L.) Moench [26], *Cannabis sativa* L [27]. , *Dracocephalum moldavica* L [28]. , and *Erythropalum scandens* Blume [29] have been proven different effects of ammonium and nitrate treatments in morphological traits and phytochemical compounds. Factors such as yield, quality, photosynthesis and plant growth are highly dependent on the form of nitrogen in soil or nutrient solution [30, 31]. It is more common for plants to use the NO_3^- form as a source of nitrogen. It is also possible for plant roots to absorb the NH_4^+ form when there is limited access to NO_3^-. When NH_4^+ is used as the sole source of nitrogen, most plant species show reduced growth, small leaves, smoothing of the root system, and in severe cases leads to ammonium toxicity and chlorination of leaves [32]. The nitrate to ammonium ratio required for the best plant development and growth depends on a number of factors, including environmental factors, growth stage, genotype, and the availability to nitrogen [33, 34]. Ammonium (NH_4^+) is a common nitrogen source, yet it can be toxic to plants at high concentrations, leading to various physiological and morphological disorders. Understanding the mechanisms of nitrogen tolerance and the strategies for mitigating nitrogen toxicity is essential for improving crop resilience and efficiency. One of the primary strategies plants employ to mitigate ammonium toxicity is the co-application of nitrate (NO_3^-). Research has shown that the presence of nitrate can alleviate the toxic effects of ammonium, allowing for improved growth and development even in high ammonium environments [35, 36]. The underlying mechanisms involve complex signaling pathways that regulate gene expression and physiological responses to different nitrogen sources. At the molecular level, plants respond to nitrogen stress through various signaling pathways and transcriptional changes. The expression of genes related to ammonium transport and assimilation is tightly regulated in response to nitrogen availability [37, 38]. The use of transcriptome analysis has revealed distinct expression patterns in plants subjected to low nitrogen stress, highlighting the adaptive responses that enable plants to cope with nitrogen deficiency or excess [39]. These molecular responses include the upregulation of specific transporters and enzymes that facilitate nitrogen uptake and assimilation, as well as the modulation of metabolic pathways to balance nitrogen levels within the plant. Furthermore, plant hormones are pivotal in overseeing diverse facets of nitrogen management within plants, impacting functions like nitrogen absorption, assimilation, redistribution, and distribution. The regulation of nitrogen tolerance in plants through hormones involves intricate interactions among diverse signaling pathways and molecular mechanisms. Nitrogen acquisition is controlled by adjusting nitrate uptake systems and promoting the growth of lateral roots. Generally, transporters encoded by the NRT1 and NRT2 families exhibit differing affinities for nitrate. Various signals influence the expression of NRT genes. The development of lateral roots is influenced by multiple signals, including nitrate, nitrogen assimilation products, and several hormones such as ABA, AUX, ETH, CKs, BR, SLs, JA, and SA [40–43]. The absorption of NH_4^+ by plant roots, leads to the rhizosphere pH reduction. To maintain an ionic balance in root zone, for every NH_4^+ ion that entering roots, a H^+ ion gets out from roots and can make a pH reduction in rhizosphere [44, 45]. In one study, the content and function of total rosmarinic acid and total flavonoids, as well as ursolic acid and oleanolic acid, decreased with NH_4^+ from 100 to 25% and peaked at 75:25 ratio of NH_4^+ to NO_3^- [46]. Chen et al. [47] showed that biomass production of *Prunella vulgaris* L. increased from 25 to 100% by increasing the ratio of NH_4^+, that the highest biomass production was observed in all organs in 75:25 (NH_4^+:NO_3^-) treatment. Some plant species, such as corn, beans, peas, and tomatoes prefer nitrate as dominant form of nitrogen in fertilizer recipes. However, some species, such as tea, prefer ammonium-rich nitrogen sources [48, 49].

The hydroponic systems such as floating culture (FCS), presents a high biomass, a superiority to the soil based growing systems. Nitrate and ammonium relations in hydroponic cultures is different from soil condition and complex somewhat. Additionally, nitrate to ammonium ratios were less studied on hydroponic condition. Essential oil, phenolic compounds and other products of lemon balm have various uses. *M. officinalis* is in addition very vital and important in medicine field. Hydroponic systems are very efficient and safe to plant growing [13, 15, 18]. The production of medicinal plants in hydroponic systems is a new challenge. Alongside the higher biomass of medicinal plants obtained in FCS hydroponic, the phytochemical contents should be reach to an acceptable range and to this, we need to manage the nutrient solution rightly, as an effective factor in plant metabolites biosynthesis. To improve the phytochemical traits of lemon balm cultivated in a greenhouse using FCS, this study aimed to investigate the impact of varying proportions of NH_4^+ to NO_3^- on the plant's morpho-physiological and phytochemical properties.

Methods

Plant materials

An experiment in the greenhouse at the Horticultural Sciences Department, Urmia University, Urmia, Iran (West Azerbaijan province) in 2021 was designed with completely randomized design (CRD) and four replications. In this research study, the effect of various ratios of NH_4^+ to NO_3^- (100:0, 75:25, 50:50, 25:75 and 0:100) on growth, yield, physiological and phytochemical properties and antioxidant activity of lemon balm in FCS was evaluated. Seeds are prepared from a reputable seed center and sown in seed trays with 2.5 centimeters of cell diameter. A mixture of fine peat moss and perlite (70:30) were used for seed sprouting and as supporting media. Well grown seedlings with 3 to 4 leaves, were transferred to floating culture system (FCS). The treatments used in this study were applied just after the transplanting in to the culture system. The day/night temperature of greenhouse was adjusted to 29/21°C during growth period and the relative humidity maintained at 55%. The light intensity was 560–640 µmol $m^{-2}s^{-1}$. Three-layered polyethylene containers with the 200 L capacity were used as FCS containers. The container dimensions were 100×100×20 cm. The aeration of nutrient solution well done by a 0.75 kW side channel blower and air bubble diffusers. 16 plants take place in every container and floated by a polystyrene sheet. The pH of nutrient solutions monitored every week and if needed, the pH adjustment was done with sulfuric acid (H_2SO_4) or potassium hydroxide (KOH). The inserted sulfur or potassium from H_2SO_4 or KOH, estimated and was added to other treatments also. When the nutrient solution level down to third, containers refilled with the fresh solution. The research steps were presented graphically in Fig. 1 (a-f).

The Sampling for traits measuring and essential oil extraction was done in flowering stage. Nutrition recipes with the same concentration of total N and other nutrients but different in NO_3^- to NH_4^+ ratios, are used as nutrient solution for every treatment (Table 1).

Growth and morphological traits

Precision measuring tools like scales and meter measuring tape were used to measure a variety of metrics, including plant height (in centimeters), the number of lateral branches, as well as the dry weights of the aerial parts and roots (in grams).

Nutrient concentration

The concentration of nitrogen in *M. officinalis* leaves was measured by Tedesco et al. [45] method and also other elements such as P, K, Ca and Mg were measured with using atomic spectroscopy absorption and colorimetry (AAS, Shimadzu AA-6300, Japan) as a result the nutrient

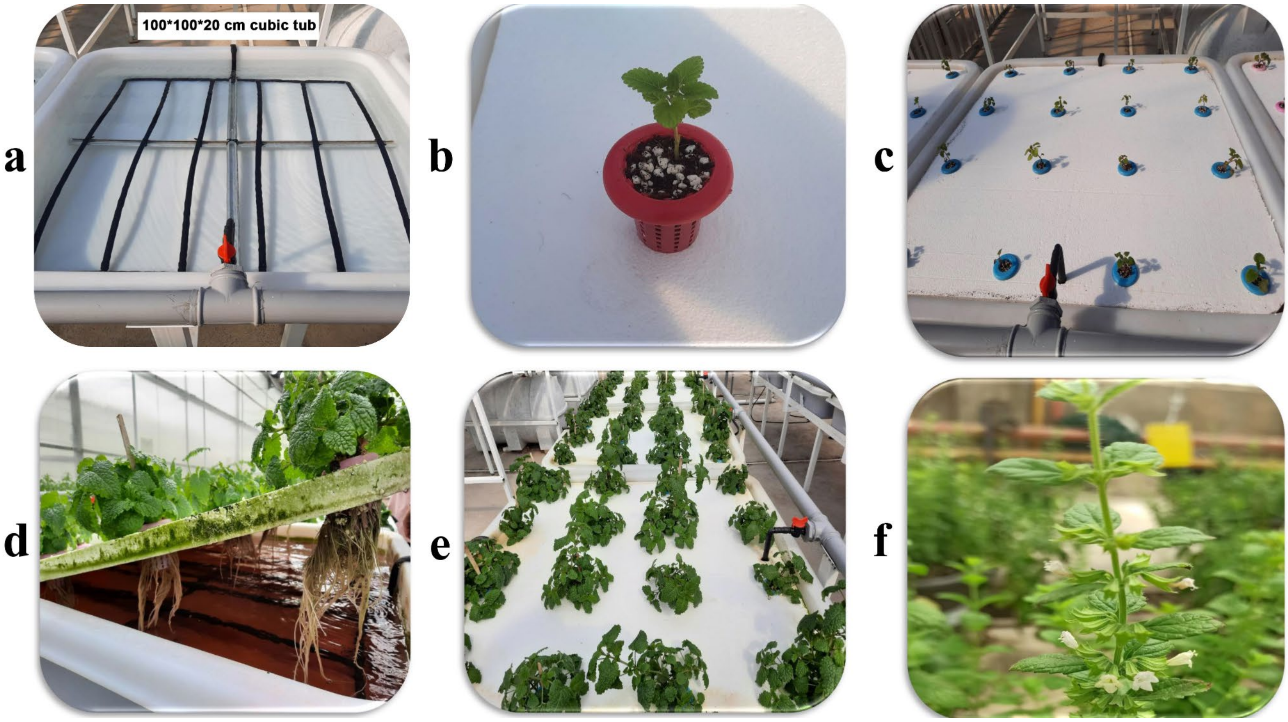


Fig. 1 Research steps (**a**-**f**) of lemon balm cultivation in a greenhouse using floating culture system (FCS)

Table 1 Nutrients content in hydroponic solution for two N-form treatments

Nutrient content (mM)	NH_4^+:NO_3^- ratios				
	100:0	75:25	50:50	25:75	0:100
N	15	11.25: 3.75	7.5: 7.5	3.75: 11.25	15
$H_2PO_4^-$	1.97	1.97	1.97	1.97	1.97
K^+	7.10	7.10	7.10	7.10	7.10
Ca^{2+}	4.41	4.41	4.41	4.41	4.41
Mg^{2+}	1.87	1.87	1.87	1.87	1.87

mM: millimolar; NH_4^+: ammonium; NO_3^-: nitrate, N: nitrogen, $H_2PO_4^-$: Dihydrogen phosphate; K^+: potassium; Ca^{2+}: calcium; Mg^{2+}: magnesium

concentration of lemon balm leaves was reported based on the percentage of their dry weight [50].

Photosynthetic pigments measurement

The chlorophyll *a*, chlorophyll *b* and carotenoid content was measured by Lichtenthaler [51] method. Half a gram of leaf sample was poured into a porcelain mortar and crushed using liquid nitrogen. Then, 10 mL of acetone (80% v/v) was added to the plant material and subjected to sonication for 30 min at a temperature of 25 °C. The resulting mixture was then filtered through a filter paper, followed by transfer into a test tube and centrifugation at 4000 rpm for 10 min. The samples were transferred to a spectrophotometer and exposed to wavelengths of 470, 646 and 663 nm (using 80% acetone as a blank control). The values of chlorophyll *a*, chlorophyll *b*, and total carotenoid were determined by applying the equations below:

$$
\begin{aligned}
\mathrm{Chlorophyll}_a(\mathrm{Chl}_a) &= 12.25\ \mathrm{A663} \\
&\quad - 2.79\ \mathrm{A646} \times \mathrm{V/W}. \\
\mathrm{Chlorophyll}_b(\mathrm{Chl}_b) &= 21.50\ \mathrm{A646} \\
&\quad - 5.1\ \mathrm{A663} \times \mathrm{V/W}. \\
\text{Total carotenoid (TCC)} &= (1000\mathrm{A470} \\
&\quad - 1.82\mathrm{Chla} - 85.02\mathrm{Chlb})/198 \times \mathrm{V/W}.
\end{aligned}
$$

Preparation of methanolic extract

For extraction, dried aerial parts of lemon balm (1 g) grounded and placed in apart vials containing 10 mL of CH_3OH 80% (v/v) and subjected to an ultra-sonication process (Elmasonic, Germany) at 25 °C with frequency of 40 Hz for 45 min. Briefly, the recovered extracts were centrifuged (4000 rpm, 5 min) and poured into separate vials. The mentioned vials were maintained at 4 °C and in a dark place for doing phytochemical tests and HPLC analysis [52].

Total phenolic content (TPC)

The total phenolic content (TPC) was assessed using the method of Rahimi et al. [53], which involved the use of the Folin-Ciocalteu reagent. To do this process, 5 µL of the methanolic extract was first mixed with 600 mL of pre-diluted folin-ciocalteu (1:10) and subsequently placed at ambient temperature for 5–10 min; in the next step, 480 µL of sodium carbonate (7.5 g per 100 mL) was added to the resulting solution. After about 30 min of keeping the samples in the dark and at ambient temperature, the absorbance of the samples was read by means of a spectrophotometer (UNICO, China) at the wavelength of 760 nm against a blank (the same solution but without methanolic extract). To measure absorption at this step, distilled water and gallic acid were employed as blank and standard, respectively, whose absorption was initially read. The results were placed in the standard curve of gallic acid that is equivalent to mg of gallic acid (GAE) per gram of dry plant weight (mg GAE/g DW).

Determination of total flavonoid content (TFC)

Measurement of TFC performed using the $AlCl_3$ reagent [54]. To measure the TFC, 15 µL extract was combined with 1.5 mL of methanol (80%), 100 µL of aluminum chloride solution (10%), 100 µl of potassium acetate solution (1 M) and 4.78 mL of distilled water. The absorbance of the mixture was read after 40 min at 415 nm against a blank (the same solution but without methanolic extract). The TFC of leaves is reported according to mg quercetin equivalent per g dry weight plant (mg QUE/g DW).

Phenolic compound analysis by HPLC

In the present study, the major phenolic composition of lemon balm samples containing caffeic acid, rosmarinic acid, chlorogenic acid, cinnamic acid, gallic acid, coumaric acid, quercetin, rutin and apigenin were evaluated with HPLC. The HPLC equipment (Wilmington, DE, USA) consisted of an integrated system with a Dionex UltiMate 3000, a 20 µL manual sample loop, a column oven, an ultrasound degasser (Hwashin, Korea), a quaternary pump (LPG-3400RS), and a photodiode array detector with detection wavelengths of 250, 272, 310, and 360 nm (DAD-3000RS). A filtered extract (20 µL) was separated using a ZORBAX Eclipse XDB column with a size of 4.6×250 mm and a pore size of 5 µm (produced by Dr. Mainsch, Germany) at a flow rate of 1.5 mL min^{-1}. Acetonitrile (solvent A) and acetic acid solution pH 3.0 (1.0% V/V in water) (solvent B) were used as eluent (mobile phases) with the gradient elution program of 10%A/90%B (0–5 min), 15%A/85%B (1.5 min), 20%A/80%B (1.5 min), 25%A/75%B (1.5 min), 25–65%A/75−35%B (increase in solvent A concentration on 5% from 25 to 65% and decrease in solvent B concentration on 5% from 75−35 during 10–20 min), remaining on 65%A/35%B (20–25 min) and 10%A/90%B (25–35 min). Peak identification of the studied phenolics were calculated using the retention time (RT) and photodiode array (PDA) spectra comparison of commercially standard with real samples [55].

Determination of antioxidant activity

DPPH assay

First, 2000 µL of DPPH solution (0.006 g DPPH in 150 mL methanol 80%) (2,2-diphenyl-1-picrylhydrazyl) was added to exactly amount of extract, then, the absorbance of the solution was measured (λ=517 nm) [56], after incubation (30 min) of solution at laboratory temperature. Moreover, the inhibition percentage of the DPPH radical was calculated using the following approach:

$$\mathrm{DPPHsc}\%x = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The DPPH scavenging activity of extracts was calculated as ascorbic acid equivalent (AAE): (µg AAE/mL).

FRAP assay

According to Žugić et al. [57] 250 µL of the extract and 3µL of fresh FRAP reagent (300 mM sodium acetate buffer (pH 3.6), ferric-tripyridyl-s-triazine and ferric chloride) were mixed together. The resulting mixture was placed in a hot water bath (temperature 37 °C) for 30 min and its absorbance was read at 593 nm by spectrophotometer. Different concentrations of $FeSO_4.7H_2O$ were employed for calibration, in order to draw a calibration curve; then it was exhibited as mM Fe^{++}/g DW.

Essential oil analysis

Extraction of essential oil (EO)

First, we crushed the aerial part of the plants with a mill, then to extraction of essential oil (EO), 40 g of dried aerial part was extracted by hydro-distillation method (40 g dry Plant tissue in 500 mL distilled water) for 3 h using a modified Clevenger-type apparatus. The obtained essential oil of *M. officinalis*, kept at 4 °C refrigerator until the chromatographic analysis time.

GC and GC/MS analysis

Essential oil was analyzed by using Agilent 6890 N gas chromatography (GC-FID) that has separated with a silica capillary column which filled with HP-5MS nonpolar fused (0.25 mm, film thickness: 0.25 µm length: 30 m) and also N_2 gases was used as a carrier gas which had flow rate of 1 mL per minute. The initial gas chromatography oven temperature was set with heating rate of 60 to 250 °C at 5 °C/ minutes and maintained for isothermal mode for 10 min and then FID temperature was programmed at 250 °C and 280 °C, respectively. Used electrospray ionization energy was 70 eV. The split injection into the column was done with a split ratio of 1:100. The volume of injected sample was 0.1 µL. Chromatographic (GC-MS) analysis was conducted with an Agilent technologies gas chromatograph model 5973, fitted with an HP-5MS capillary column (0.25 mm, film thickness: 0.25 µm length: 30 m). The GC-MS analysis was conducted on an Agilent 5973 instrument fitted with a HP-5MS column (30 m × 0.25 µm mm; 0.25 µm film thickness) programmed as above. the carrier gas was helium (He) with a constant flow of 1 mL/min and split proportion was 1:50. Also used mass range of compounds was 2-800 amu. The essential oil compounds were identified by calculation of their retention indices (RI) by using n-alkanes (C6–C24) with the same injection conditions. Identification of essential oil compositions was made by comparison of their mass spectra (obtained using X Calibur (2.07) with those of the internal reference mass spectra library [58, 59].

Statistical analysis

In this research study, the effect of various ratios of NH_4^+ to NO_3^- (100:0, 75:25, 50:50, 25:75 and 0:100) on growth, yield, physiological and phytochemical properties and antioxidant activity of lemon balm in floating culture system (FCS) was evaluated. The obtained data were analyzed based on one-way ANOVA in completely randomized design (CRD) and four replications (4 observations per replication) using SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA). Comparisons of means was done using Duncan's new multiple range test (DMRT) at $p \leq 0.01$ probability level. Figures were generated using GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). Correlation coefficients heatmap plot based on Pearson's method was created using corrplot R-package.

Results

Growth and morphological traits

In the present study, the significant increase of biomass was obtained with the decrease in $NH_4^+:NO_3^-$ ratios. The biomass, growth, morphological traits and number of lateral branches of *M. officinalis* in response to the various ratios of NH_4^+: NO_3^- were analyzed and the results demonstrated that maximum biomass (105.57 g), plant height (72.20 cm), and number of lateral branches (25.56) earned by using the ratio of 0:100 and minimum at 75:25 ratio of NH_4^+: NO_3^-. Intermediate values achieved under the 50:50 and 25:75 ratios of ammonium to nitrate (Fig. 2: a-d). Plants grown under 100:0 ratio of $NH_4^+:NO_3^-$ were destroyed in the early stages of growth, due to NH_4^+ toxicity.

Nutrient content

According to the results of this study, changes in $NH_4^+:NO_3^-$ ratio can affect absorption and accumulation process of some elements, significantly, so that the studied elements accumulation generally increases with decreasing in $NH_4^+:NO_3^-$ ratio (Table 2). The results revealed that the highest and lowest content of Mg and Ca were observed in plants treated with 0:100 and 75:25 ratios of $NH_4^+:NO_3^-$, respectively. Also, the highest amount of K (6.21%) and N (4.04%) earned by using the 75:25 ratio of $NH_4^+:NO_3^-$ and the lowest amounts was obtained in 0:100 and 50:50 ratios of $NH_4^+:NO_3^-$, respectively. The results of mean comparisons showed that there is no significant difference between 50:50 - $NH_4^+:NO_3^-$, and 25:75 - $NH_4^+:NO_3^-$ treatments in terms of N concentration in leaves. In addition, using the 25:75 and 50:50 ratios of $NH_4^+:NO_3^-$ leads to maximum (1.22%) and minimum (0.79%) levels of P, respectively. Also, in terms of phosphorus concentration, no significant difference was observed between 75:25 - $NH_4^+:NO_3^-$, 50:50 - $NH_4^+:NO_3^-$, and 0:100 - $NH_4^+:NO_3^-$ treatments. Eventually, plants treated with 75:25 ratio of $NH_4^+:NO_3^-$ had higher dry matter content while the $NH_4^+:NO_3^-$ ratio of 0:100 cause lower dry matter content.

Photosynthetic pigments (Chl *a*, Chl *b* and TCC)

The content of Chl *a*, Chl *b* and TCC of leaves in the different NH_4^+: NO_3^- ratios are presented in Fig. 3 (a-c). Plants fertilized with 0:100 - $NH_4^+:NO_3^-$ treatment had the highest TCC (83.06 mg/100 g DW) (Fig. 3c) and Chl *a* (31.32 mg/100 g DW) (Fig. 3a), but the highest (37.56 mg/100 g DW) and lowest (8.24 mg/100 g DW) amount of Chl *b* (Fig. 3b) was observed in 75:25 - $NH_4^+:NO_3^-$ and 50:50 - $NH_4^+:NO_3^-$ treatments, respectively. Also, no significant difference was observed between 50:50 - $NH_4^+:NO_3^-$, and 25:75 - $NH_4^+:NO_3^-$ treatments in the Chl *b* content.

Effect of the NH_4+:NO_3^- ratios on TPC and TFC

The various $NH_4^+:NO_3^-$ ratios in nutrient solution had an impressive impact on the TPC and TFC of lemon balm (Fig. 3: d-e). Our study showed that, the highest of total phenolic (60.40 mg GAE/g DW) and flavonoid (12.97 mg QUE /g DW) contents were observed in samples supplied with a 0:100 ratio and the lowest in plants of 75:25 ratio of $NH_4^+:NO_3^-$. Also, no significant difference was observed between 75:25 - $NH_4^+:NO_3^-$, 50:50 - $NH_4^+:NO_3^-$, and 25:75 - $NH_4^+:NO_3^-$ treatments in the amount of TPC. The results of mean comparisons showed that there is no significant difference between 75:25 - $NH_4^+:NO_3^-$, and 50:50 - $NH_4^+:NO_3^-$ as well as 25:75 - $NH_4^+:NO_3^-$, and 0:100 - $NH_4^+:NO_3^-$ treatments in terms of flavonoid content. In general, the biosynthesis of phenolic and flavonoid compounds is strongly influenced by form of fertilizer nitrogen. The TPC and TFC contents increased significantly with decreasing NH_4^+ from 75 to 0, and peaked in the 0:100 ($NH_4^+:NO_3^-$) treatment.

Phenolic compound analysis by HPLC

Chromatogram of standards used for analysis of phenolic compounds by HPLC are shown in Fig. 4. The amounts of phenolic compounds in the various ratios of $NH_4^+:NO_3^-$

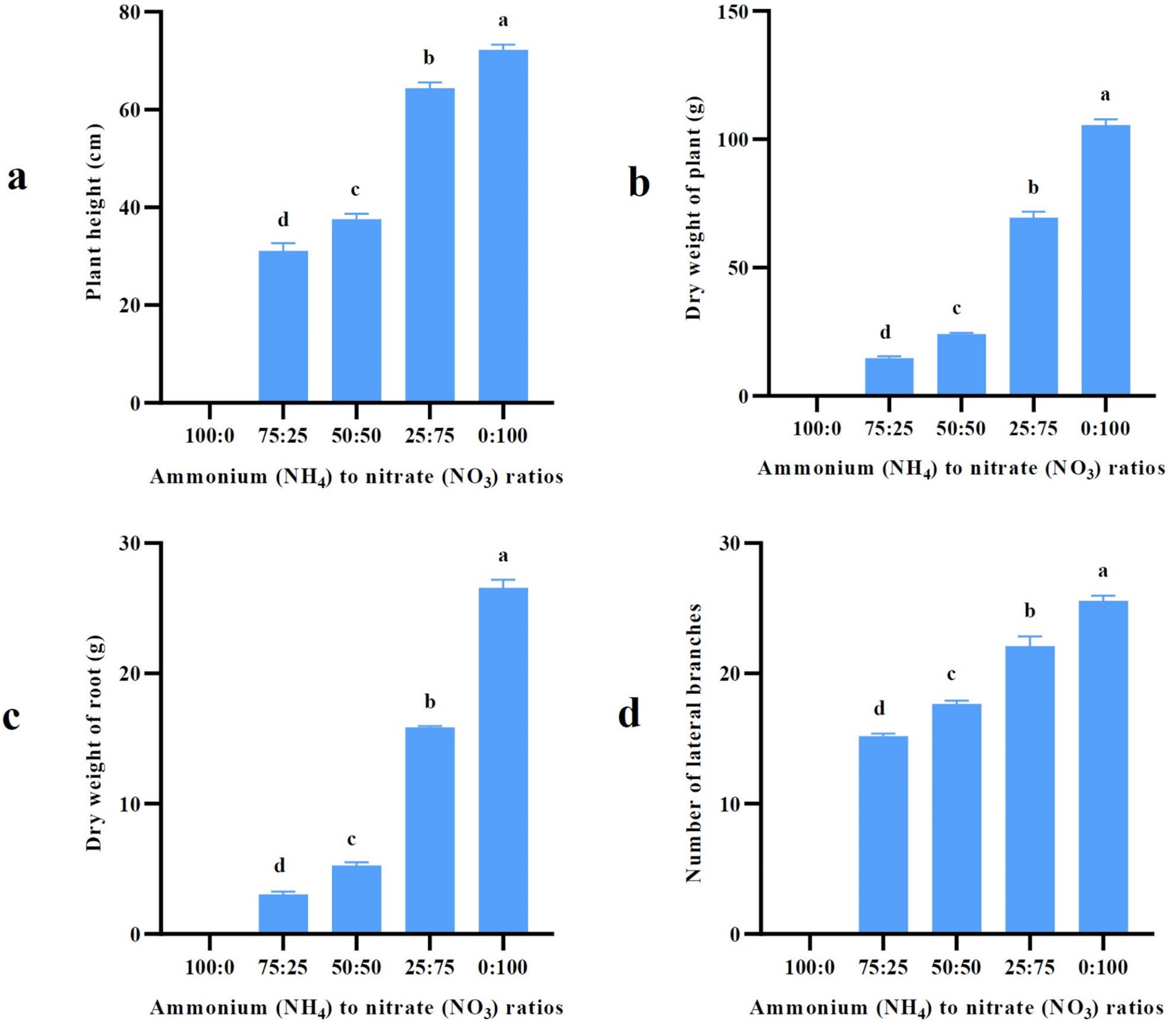


Fig. 2 The biomass, growth and morphological properties of *Melissa officinalis* in response to the various NH_4^+:NO_3^- ratios: **a**) plant height; **b**) dry weight of plant; **c**) dry weight of root; **d**) number of lateral branches. Various NH_4^+:NO_3^- ratios had significant effects on biomass, growth and morphological properties of *Melissa officinalis* ($p < 0.01$). Different lowercase letters above bars indicate highly significant ($p < 0.01$) difference. Error bars represent SD

Table 2 The nutrient content of *M. Officinalis* in response to the different NH_4^+:NO_3^- ratios

NH_4^+:NO_3^- ratios	**Nutrient content (%)**					
	DM	***N***	***P***	**K**	**Ca**	**Mg**
100:00*	-	-	-	-	-	-
75:25	99.0 ± 1.30a	4.04 ± 0.21a	0.87 ± 0.06b	6.21 ± 0.26a	0.32 ± 0.01d	0.62 ± 0.04c
50:50	98.3 ± 1.30ab	3.06 ± 0.16c	0.79 ± 0.01b	5.38 ± 0.20bc	0.36 ± 0.02c	0.91 ± 0.02b
25:75	98.1 ± 0.40ab	3.18 ± 0.12c	1.22 ± 0.11a	5.67 ± 0.30b	0.44 ± 0.02b	0.91 ± 0.03b
0:100	96.8 ± 0.70b	3.73 ± 0.15b	0.87 ± 0.03b	4.94 ± 0.17c	0.52 ± 0.04a	1.12 ± 0.07a
Significant levels	**	**	**	**	**	**

* The plants of 100 NH_4^+:0 NO_3^- treatment was destroyed in mid-growth stages, due to ammonium toxicity. ** indicate significance at the 1% level. Means with different letters are statistically significant at 1% level of probability. DM: Dry matter; N = nitrogen; P = phosphorus; K = potassium; Ca = calcium; Mg = magnesium

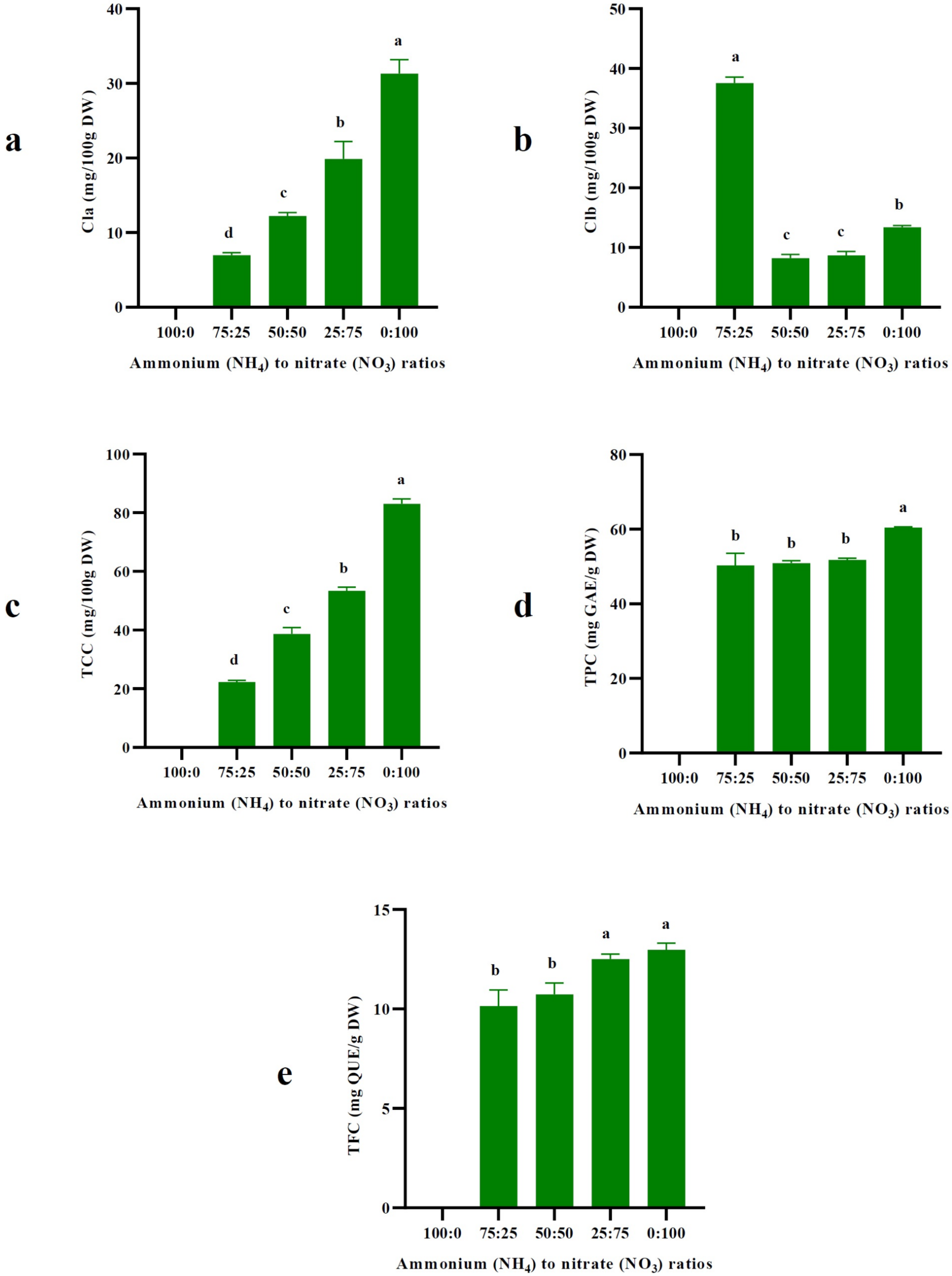


Fig. 3 The phytochemical content of *Melissa officinalis* in response to the different NH_4^+:NO_3^- ratios: (**a**) chlorophyll *a* (Chl *a*); (**b**) chlorophyll *b* (Chl *b*); (**c**) total carotenoid content (TCC); (**d**) total phenolic content (TPC); (**e**) total flavonoid content (TFC). Various NH_4^+:NO_3^- ratios had significant effects on phytochemical content of *Melissa officinalis* ($p < 0.01$). Different lowercase letters above bars indicate highly significant ($p < 0.01$) difference. Error bars represent SD

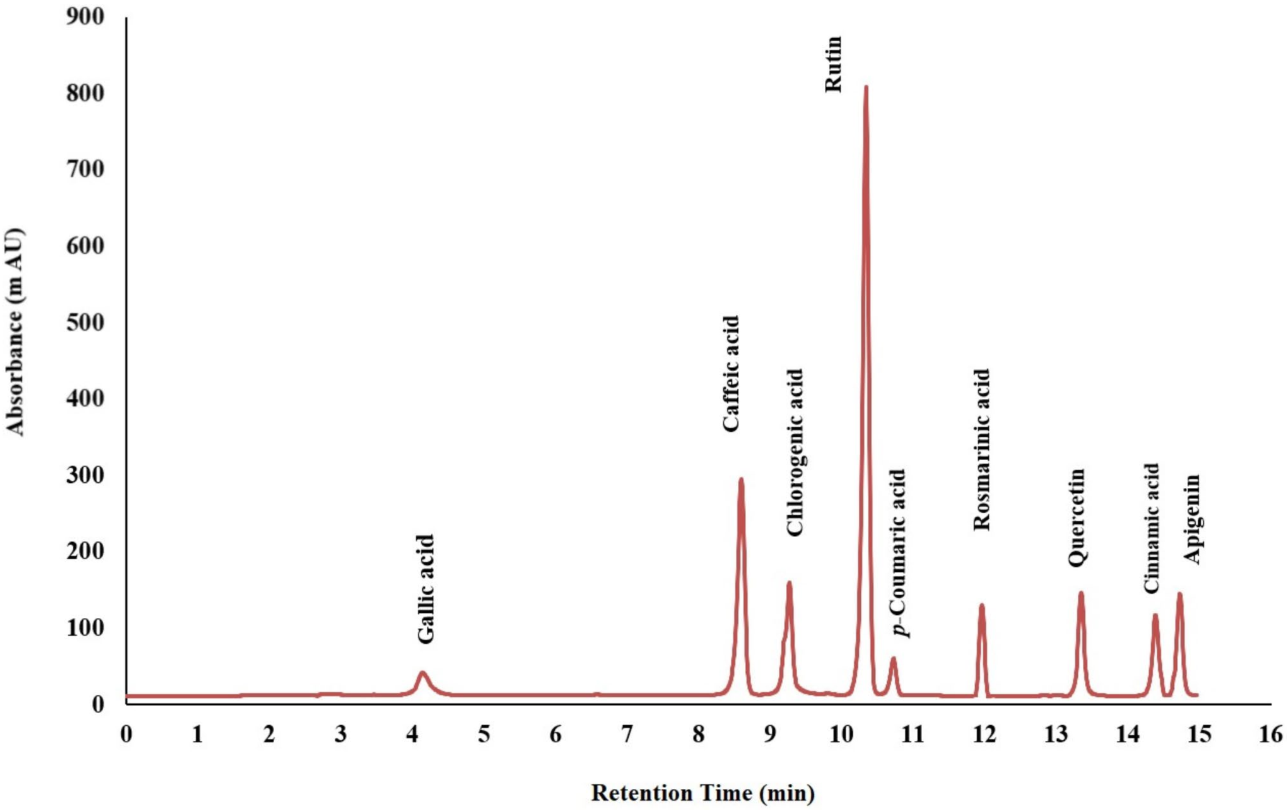


Fig. 4 Chromatogram of standards used for analysis of phenolic compounds by HPLC-DAD

Table 3 The phenolic compounds of *M. officinalis* in response to the different NH_4^+:NO_3^- ratios

NH_4^+:NO_3^- ratios	Phenolic compounds (mg/100 g)								
	Gallic acid	Caffeic acid	Chlorogenic acid	Rutin	*p*-Coumaric acid	Rosmarinic acid	Quercetin	Cinnamic acid	Apigenin
100:00*	-	-	-	-	-	-	-	-	-
75:25	8.90 ± 0.04b	83.2 ± 0.06a	11.34 ± 0.05b	46.68 ± 0.06a	1875.06 ± 0.06b	41.08 ± 0.02a	5.80 ± 0.06b	0.92 ± 0.02c	4.14 ± 0.05d
50:50	7.98 ± 0.03d	31.08 ± 0.04b	2.54 ± 0.02c	21.82 ± 0.03c	1420.32 ± 0.04d	28.96 ± 0.06d	2.34 ± 0.03c	1.80 ± 0.06a	7.68 ± 0.04a
25:75	8.38 ± 0.02c	6.14 ± 0.03c	23.20 ± 0.06a	23.68 ± 0.03b	1696.48 ± 0.05c	29.60 ± 0.03c	2.40 ± 0.05c	1.02 ± 0.03b	5.32 ± 0.06c
0:100	18.18 ± 0.05a	1.68 ± 0.04d	1.46 ± 0.04d	25.50 ± 0.06d	2286.94 ± 0.03a	36.68 ± 0.03b	9.82 ± 0.07a	0.98 ± 0.05bc	5.68 ± 0.04b
Significant levels	**	**	**	**	**	**	**	**	**

* The plants of 100 NH_4^+:0 NO_3^- treatment was destroyed in mid-growth stages, due to ammonium toxicity. ** indicate significance at the 1% level. Means with different letters are statistically significant at 1% level of probability

are shown in Table 3. Phenolic compositions in methanolic extracts of *M. officinalis* were shown that influenced by different ratio of NH_4^+:NO_3^- ($p < 0.01$). The *p*-coumaric acid (1420.32-2286.94 mg/100 g) was as most main composition in samples. The highest content of quercetin (9.82 mg/100 g), *p*-coumaric acid (2286.94 mg/100 g), and gallic acid (18.18 mg/100 g) compounds were obtained in 0:100 - NH_4^+:NO_3^- treatment. Furthermore, the highest contents of apigenin (7.68 mg/100 g) and cinnamic acid (1.80 mg/100 g) were identified in 50:50 - NH_4^+:NO_3^- treatment. Plants demonstrated higher levels of rosmarinic and caffeic acids and rutin when they were exposed to NH_4^+.

Antioxidant activity by DPPH and FRAP assays

The antioxidant capacity of the samples studied by two different assays including DPPH (Fig. 5a) and FRAP (Fig. 5b). According to the results, the highest and lowest antioxidant capacity on both assays was recorded in 75:25 - NH_4^+:NO_3^- and 0:100 - NH_4^+:NO_3^- treatments, respectively. The results of mean comparisons showed that there is no significant difference between 75:25 - NH_4^+:NO_3^-, and 50:50 - NH_4^+:NO_3^- treatments in terms of antioxidant capacity based on DPPH assay. In general, plants treated with higher NH_4^+ concentrations, had more antioxidant capacity as compared to plant's feds with higher NO_3^-.

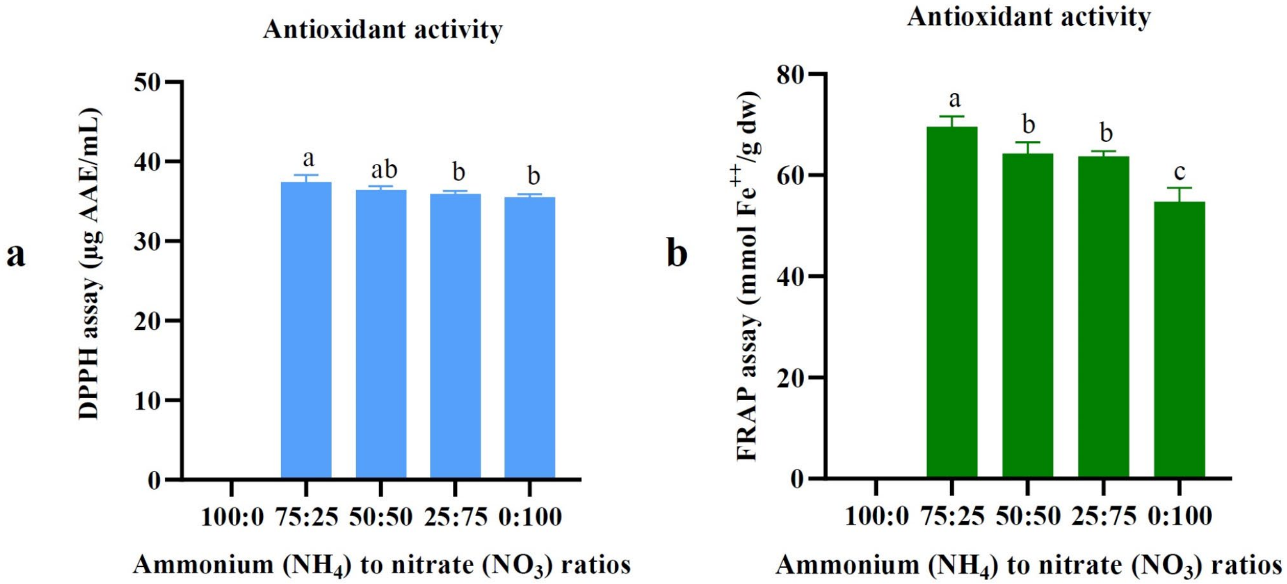


Fig. 5 The antioxidant capacity of *Melissa officinalis* in different ratios of NH_4^+:NO_3^- by DPPH (Fig. 5a) and FRAP (Fig. 5b) assays. DPPH = antioxidant activity based on 2,2-diphenyl-1-picrylhydrazyl assay; FRAP: antioxidant activity based on ferric reducing antioxidant power assay. Various NH_4^+:NO_3^- ratios had significant effects on antioxidant capacity of *Melissa officinalis* ($p < 0.01$). Different lowercase letters above bars indicate highly significant ($p < 0.01$) difference. Error bars represent SD

GC and GC-MS analysis of essential oil (EO)

The analysis of the volatile profile of essential oils, including both qualitative and quantitative aspects, is presented in Table 4. In general, 58 volatile compounds were identified in *M. officinalis* essential oils from the 4 treatments. The neral, geranial, geraniol, and geranyl acetate were the main compounds identified. Analysis of essential oils by GC indicated that NH_4^+:NO_3^- ratios had a significant effect on the essential oil compounds. With increasing NO_3^-, geranyl acetate and geraniol were decreased. On the other hand, the content of neral was increased and then decreased. Other constituents in essential oil were increased by increasing NO_3^- and some showed the opposite trend. According to the results of the research; the highest and the lowest amounts of geranyl acetate and geraniol were gained in 75:25 and 0:100 ratios of NH_4^+:NO_3^-, respectively. The maximum (47.30%) and minimum (21.42%) amounts of geranial were observed in 75:25 - NH_4^+:NO_3^- and 50:50 - NH_4^+:NO_3^- treatments, respectively. Moreover, the highest (25.92%) and lowest (16.63%) content of neral were observed in 25:75 and 75:25 ratios of ammonium to nitrate, respectively. Finally, the maximum (95.77%) and minimum (70.67%) of total major compounds (geranial, neral, geranyl acetate, geraniol, β-caryophyllene, caryophyllene oxide, citronellal, and farnesol) were recorded in 75:25 and 25:75 treatments of NH_4^+:NO_3^-, respectively. In general, the best ratio for main compositions was 75:25 ratio of NH_4^+:NO_3^-.

Correlation analysis

The simple correlation coefficients of the measured traits are shown in Fig. 6. Positive correlations are indicated in blue, while negative correlations are in red. Simple correlation coefficients between morpho-physiological traits and phytochemical compounds showed that some of them had a significant correlation ($P \leq 0.01$, $P \leq 0.05$), and in some cases no significant correlation was seen, as shown in the Fig. 6. In the present study, it was found a positive and remarkable correlation between antioxidant activity based on the DPPH method with TFC ($r = 0.92$), TPC ($r = 0.91$), element Mg ($r = 0.85$), gallic acid ($r = 0.86$), quercetin ($r = 0.64$), p-coumaric acid ($r = 0.55$) and with apigenin ($r = 0.42$). Also, it was obtained a positive and significant correlation between antioxidant activity based on the FRAP assay with element K ($r = 0.96$), geranyl acetate ($r = 0.85$), geraniol ($r = 0.8$), caffeic acid ($r = 0.8$), rutin ($r = 0.62$), Clb ($r = 0.58$) and with geranial ($r = 0.54$). Monoterpenes (geranyl acetate, geraniol and geranial) are obtained from lemon balm essential oils have shown antioxidant activity based on the FRAP assay. As regards the antioxidant properties are attributed to the presence of various phytochemical compounds such as phenolic compounds, and photosynthetic pigment; hence, the high antioxidant capacity of samples may be strongly correlated to their high phenolic and monoterpene compounds (Fig. 6). Other correlation coefficients between morpho-physiological traits and phytochemical compounds showed in the Fig. 6.

Table 4 The essential oil compositions of *M. Officinalis* in response to the different NH_4^+:NO_3^- ratios

Component	Essential oil composition (%) M. officinalis in different NH_4^+:NO_3^- ratios				
	100:0*	**75:25**	**50:50**	**25:75**	**0:100**
6-Methyl-5-hepten-2-one	-	1.09	1.97	2.28	3.72
Neral	-	**16.63**	**21.08**	**25.92**	**22.04**
Geranial	-	**47.30**	**21.42**	**23.69**	**27.86**
Geraniol	-	**9.05**	**2.55**	**1.59**	**0.78**
Geranyl acetate	-	**11.63**	**6.00**	**4.81**	**3.42**
Farnesyl acetate	-	0.09	0.64	0.14	0.26
Nerol	-	0.68	0.13	0.09	0.31
Farnesol	-	3.46	7.41	2.89	4.42
β -caryophyllene	-	**3.71**	**4.97**	**5.82**	**4.43**
Caryophyllene oxide	-	**2.62**	**4.12**	**4.69**	**7.78**
Citronellal	-	**1.37**	**4.91**	**2.65**	**2.02**
Geranyl linalool	-	1.63	5.25	3.79	2.18
6,11-Dimethyl-2,6,10-dodecatrien-1-ol	-	0.13	0.49	1.09	0.15
Geranyl acetone	-	0.54	-	-	-
α-Farnesene	-	-	0.43	0.91	0.29
β-farnesene	-	-	0.66	0.38	0.05
Linalool	-	-	1.03	1.27	1.71
trans-β-Ocimene	-	-	0.14	0.58	0.86
Rosefuran epoxide	-	-	0.66	0.83	0.23
β-Thujone	-	-	0.82	0.31	0.28
Methyl geranate	-	-	1.01	0.89	1.40
(E)-β-Ionone	-	-	0.56	0.69	1.17
α-Copaene	-	-	0.13	0.29	0.88
Fitone	-	-	0.84	0.65	0.55
β-Bisabolene	-	-	0.42	0.18	0.05
α- Caryophyllene	-	-	1.52	0.93	0.56
Neryl acetone	-	-	1.01	1.54	2.01
α-pinene	-	-	0.99	0.32	-
Camphor	-	-	0.43	1.60	-
γ-cadinene	-	-	0.30	0.20	-
(E)-Nerolidol	-	-	0.74	1.74	-
p-Menthane-3,8-diol	-	-	0.71	0.12	-
Myrcene	-	-	0.24	0.16	-
α-Terpinene	-		0.67	0.97	1.36
Bergamal	-	-	0.13	0.19	0.38
α -Cubebene	-		0.91	0.74	0.66
Camphene	-	-	0.26	-	-
Sabinene	-	-	0.33	-	-
1,8 Cineol	-	-	0.63	-	-
β-Pinene	-	-	0.96	-	-
Neryl acetate	-	-	0.21	-	-
Cis -Rose oxide	-	-	0.22	-	-
β-Cubebene	-	-	0.55	-	-
Bicyclogermacrene	-	-	0.53	0.21	-
Citronellyl acetate	-	-	0.81	0.18	0.08
Eugenol	-	-	0.09	0.17	0.86
Geranic acid	-	-	-	1.62	3.02
Thymol	-	-	-	0.08	0.29
allo-Aromadendrene	-	-	-	0.28	0.23
1 OCTEN 3 OL	-	-	-	0.85	0.36
neo-Isopulegol	-	-	-	0.59	-
trans- Carveol	-	-	-	0.57	-

Table 4 (continued)

Component	Essential oil composition (%) M. officinalis in different NH_4^+:NO_3^- ratios				
	100:0*	75:25	50:50	25:75	0:100
Piperitone	-	-	-	0.46	-
α-Humulene	-	-	-	0.55	0.81
α-Cadinol	-	-	-	0.27	0.44
Germacrene-D	-	-	-	0.35	0.45
β -Elemene	-	-	-	0.16	-
cis –Linalool oxide	-	-	-	-	0.18
Total amount of compounds	-	99.93	99.88	99.89	99.92

* The plants of 100 NH_4^+:0 NO_3^- treatment was destroyed in mid-growth stages, due to ammonium toxicity. Major components of the essential oils are highlighted in bold

Discussion

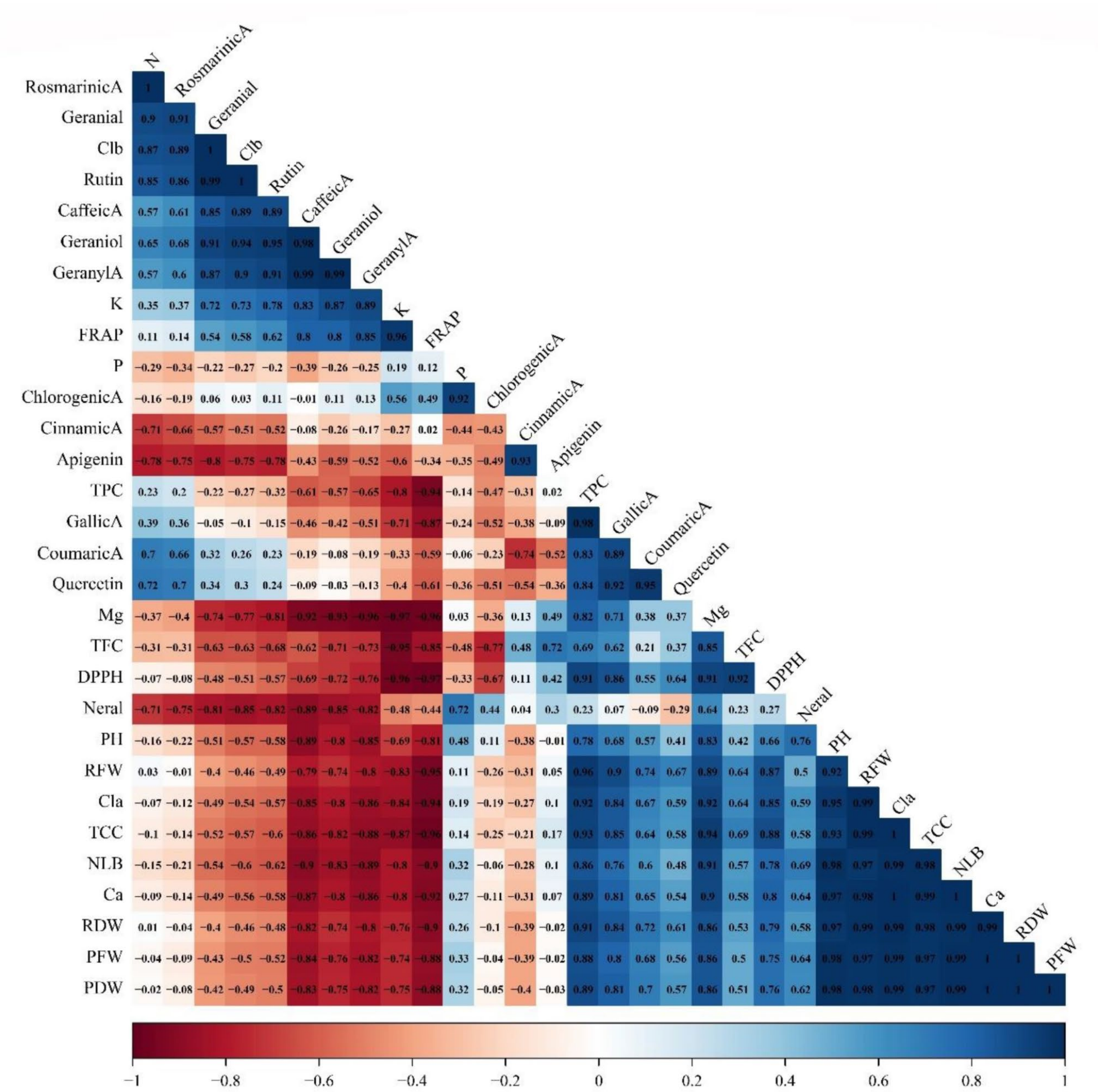
Growth and morphological traits

The highest plant dry mass and growth traits of *M. officinalis* in response to the different treatments of NH_4^+:NO_3^-, were gained at 0:100 - NH_4^+:NO_3^- treatment and the lowest values was obtained in the 75:25 - NH_4^+:NO_3^- treatment (Fig. 2: a-d). A similar result is reached in the study by Ahmadi et al. [60] and Schiefloe et al. [61], which decreasing ammonium to nitrate ratios (10 NH_4^+:90 NO_3^-) in the nutrient solution of purple coneflower (*Echinacea purpurea*) caused to improve in growth and morphological traits such as plant fresh biomass, plant height and root fresh weight significantly. Decreased plant dry mass in response to NH_4^+ nutrition may be associated to reduction in fresh weight of plant, number of lateral branches and root and plant height. Nitrogen is an essential nutrient for plants and, when limiting, can reduce crop growth and productivity [62]. Nitrogen deficiency reduces photosynthesis capacity and primary metabolism, thereby reducing biomass production [63, 64]. One of the major processes for growing of plant is photosynthesis. Plants treated with NH_4^+as main nitrogen source are suppressed by reduced net photosynthetic rate [65, 66]. Furthermore, root formation and growth of plants is sensitive to ammonium toxicity [67] and reduction of root mass and length may associated to ethylene signal transduction [68] or auxin transport [66, 69]. Different mechanisms and factors involved in ammonium-nitrogen toxicity in various plant species including reducing glycosylation of proteins, acid-base balance disturbances, energy lost due to exporting excess NH_4^+, and the external environment acidification [70]. In watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai.) increase of biomass and large root system were obtained with a reduce in the NH_4^+:NO_3^- ratio [66]. Also, accumulation of biomass and some elements of tomato plant decreased when NH_4^+ was predominant and the growth traits were negatively affected by high concentrations of NH_4^+ in the nutrient solution [71]. The N fertilization is a main factor effective on basil yield [72]. In *Ocimum basilicum* L., the supply of NH_4^+, even in the presence of NO_3^-, disturbed the plant growth, considerably [64]. The negative impact of NH_4^+ on photosynthetic rate, survival and growth has been attributed to high concentration of NH_4^+ in leaf organ, which can cause separation of the phosphorylation from electron transfer reactions in chloroplast [73]. Similar results were found by Saloner and Bernstein [27], which total plant biomass generally increased with the increase in nitrate supply (NO_3^-). Plants fertilized with 0:100 - NH_4^+:NO_3^- treatment had the highest fresh and dry biomass.

Plant hormones such as salicylic acid (SA) and cytokinin (CYT) play crucial roles in enhancing nitrogen tolerance in plants, particularly under varying environmental conditions. Their interactions with nitrogen signaling pathways and stress responses are vital for plant growth and resilience. Cytokinin mediates nitrogen signaling by regulating nitrogen partitioning and development, crucial for amino acid and nucleic acid synthesis [74]. Transgenic plants with increased cytokinin synthesis showed improved nitrogen-use efficiency, maintaining biomass under nitrogen deficiency [75]. Salicylic acid acts as a signaling molecule that helps plants cope with abiotic stresses, including those affecting nitrogen uptake [76]. Moreover, the interaction between cytokinins and other hormones, such as auxins, plays a significant role in regulating plant responses to nitrogen availability. The antagonistic relationship between auxins and cytokinins is crucial for maintaining the shoot/root growth ratio, which is essential for optimizing nutrient uptake and water use efficiency [77]. This hormonal balance allows plants to adapt their growth strategies in response to varying nitrogen and water availability in the soil, thereby enhancing their overall resilience to abiotic stresses.

Nutrient content

The results of this research nutrient content partially support the results of Roosta and Schjoerring [78] and Helali et al. [79] studies. They reported that the concentration of anionic elements such as P was higher than that of cationic elements such as Ca and Mg in plants whose main nitrogen source was ammonium, compared to plants



	N	RosmarinicA	Geranial	Clb	Rutin	CaffeicA	Geraniol	GeranylA	K	FRAP	P	ChlorogenicA	CinnamicA	Apigenin	TPC	GallicA	CoumaricA	Quercetin	Mg	TFC	DPPH	Neral	PH	RFW	Cla	TCC	NLB	Ca	RDW	PFW
RosmarinicA	1																													
Geranial	0.9	0.91																												
Clb	0.87	0.89	1																											
Rutin	0.85	0.86	0.99	1																										
CaffeicA	0.57	0.61	0.85	0.89	0.89																									
Geraniol	0.65	0.68	0.91	0.94	0.95	0.98																								
GeranylA	0.57	0.6	0.87	0.9	0.91	0.99	0.99																							
K	0.35	0.37	0.72	0.73	0.78	0.83	0.87	0.89																						
FRAP	0.11	0.14	0.54	0.58	0.62	0.8	0.8	0.85	0.96																					
P	−0.29	−0.34	−0.22	−0.27	−0.2	−0.39	−0.26	−0.25	0.19	0.12																				
ChlorogenicA	−0.16	−0.19	0.06	0.03	0.11	−0.01	0.11	0.13	0.56	0.49	0.92																			
CinnamicA	−0.71	−0.66	−0.57	−0.51	−0.52	−0.08	−0.26	−0.17	−0.27	0.02	−0.44	−0.43																		
Apigenin	−0.78	−0.75	−0.8	−0.75	−0.78	−0.43	−0.59	−0.52	−0.6	−0.34	−0.35	−0.49	0.93																	
TPC	0.23	0.2	−0.22	−0.27	−0.32	−0.61	−0.57	−0.65	−0.8	−0.94	−0.14	−0.47	−0.31	0.02																
GallicA	0.39	0.36	−0.05	−0.1	−0.15	−0.46	−0.42	−0.51	−0.71	−0.87	−0.24	−0.52	−0.38	−0.09	0.98															
CoumaricA	0.7	0.66	0.32	0.26	0.23	−0.19	−0.08	−0.19	−0.33	−0.59	−0.06	−0.23	−0.74	−0.52	0.83	0.89														
Quercetin	0.72	0.7	0.34	0.3	0.24	−0.09	−0.03	−0.13	−0.4	−0.61	−0.36	−0.51	−0.54	−0.36	0.84	0.92	0.95													
Mg	−0.37	−0.4	−0.74	−0.77	−0.81	−0.92	−0.93	−0.96	−0.97	−0.96	0.03	−0.36	0.13	0.49	0.82	0.71	0.38	0.37												
TFC	−0.31	−0.31	−0.63	−0.63	−0.68	−0.62	−0.71	−0.73	−0.95	−0.85	−0.48	−0.77	0.48	0.72	0.69	0.62	0.21	0.37	0.85											
DPPH	−0.07	−0.08	−0.48	−0.51	−0.57	−0.69	−0.72	−0.76	−0.96	−0.97	−0.33	−0.67	0.11	0.42	0.91	0.86	0.55	0.64	0.91	0.92										
Neral	−0.71	−0.75	−0.81	−0.85	−0.82	−0.89	−0.85	−0.82	−0.48	−0.44	0.72	0.44	0.04	0.3	0.23	0.07	−0.09	−0.29	0.64	0.23	0.27									
PH	−0.16	−0.22	−0.51	−0.57	−0.58	−0.89	−0.8	−0.85	−0.69	−0.81	0.48	0.11	−0.38	−0.01	0.78	0.68	0.57	0.41	0.83	0.42	0.66	0.76								
RFW	0.03	−0.01	−0.4	−0.46	−0.49	−0.79	−0.74	−0.8	−0.83	−0.95	0.11	−0.26	−0.31	0.05	0.96	0.9	0.74	0.67	0.89	0.64	0.87	0.5	0.92							
Cla	−0.07	−0.12	−0.49	−0.54	−0.57	−0.85	−0.8	−0.86	−0.84	−0.94	0.19	−0.19	−0.27	0.1	0.92	0.84	0.67	0.59	0.92	0.64	0.85	0.59	0.95	0.99						
TCC	−0.1	−0.14	−0.52	−0.57	−0.6	−0.86	−0.82	−0.88	−0.87	−0.96	0.14	−0.25	−0.21	0.17	0.93	0.85	0.64	0.58	0.94	0.69	0.88	0.58	0.93	0.99	1					
NLB	−0.15	−0.21	−0.54	−0.6	−0.62	−0.9	−0.83	−0.89	−0.8	−0.9	0.32	−0.06	−0.28	0.1	0.86	0.76	0.6	0.48	0.91	0.57	0.78	0.69	0.98	0.97	0.99	0.98				
Ca	−0.09	−0.14	−0.49	−0.56	−0.58	−0.87	−0.8	−0.86	−0.8	−0.92	0.27	−0.11	−0.31	0.07	0.89	0.81	0.65	0.54	0.9	0.58	0.8	0.64	0.97	0.98	1	0.99	1			
RDW	0.01	−0.04	−0.4	−0.46	−0.48	−0.82	−0.74	−0.8	−0.76	−0.9	0.26	−0.1	−0.39	−0.02	0.91	0.84	0.72	0.61	0.86	0.53	0.79	0.58	0.97	0.99	0.99	0.98	0.99	0.99		
PFW	−0.04	−0.09	−0.43	−0.5	−0.52	−0.84	−0.76	−0.82	−0.74	−0.88	0.33	−0.04	−0.39	−0.02	0.88	0.8	0.68	0.56	0.86	0.5	0.75	0.64	0.98	0.97	0.99	0.97	0.99	1	1	
PDW	−0.02	−0.08	−0.42	−0.49	−0.5	−0.83	−0.75	−0.82	−0.75	−0.88	0.32	−0.05	−0.4	−0.03	0.89	0.81	0.7	0.57	0.86	0.51	0.76	0.62	0.98	0.98	0.99	0.97	0.99	1	1	1

Fig. 6 Heatmap of the Pearson correlation coefficient matrix for the traits studied. The abbreviations used are as follows: N = element nitrogen; RosmarinicA = rosmarinic acid; Cla = chlorophyll *a*; Clb = chlorophyll *b*; CaffeicA = caffeic acid; GeranylA = geranyl acetate; K = element potassium; P = element phosphorus; Ca = element calcium; ChlorogenicA = chlorogenic acid; CinnamicA: cinnamic acid; GallicA = gallic acid; CoumaricA = *p*-coumaric acid; Mg = element magnesium; PH = plant height; RFW = fresh weights of roots; NLB = number of lateral branches; RDW = dry weights of roots; PFW = plant fresh weight; TPC = Total Phenol Content; TFC = Total Flavonoid Content; TTC = Total Tannin Content; DPPH = antioxidant activity based on 2,2-diphenyl-1-picrylhydrazyl assay; FRAP: antioxidant activity based on ferric reducing antioxidant power assay

whose source was nitrate. Toxicity of NH4$^+$ can improve by a high availability of K [78, 80, 81]. With increasing N-NH_4^+ supply, the potassium concentration of shoots decreased. Ammonium has been shown to affect the absorption and accumulation of K. Ammonium and K are highly similar regarding many characteristics such as size, hydration energy, and charge, that are important for membrane transport [81]. Generally, the potassium content (%) differences between treatments are not dramatic and all of them are in critical values range. However, the minor differences between potassium content may liaise with the dry matter content that higher potassium contents gained in such treatments led to higher dry matter content (Table 2). Phosphorus element plays a crucial role in maintaining the anion and ion balance of plants supplied with NH_4^+. Researchers have reported that, the amounts of elements in plant tissues were significantly influenced by various nitrogen forms. It has been reported that higher contents of some nutrients such as nitrogen and phosphorus were obtained in samples fed with a suitable mixture of NH_4^+ and NO_3^- as a source of nitrogen [80]. NH_4^+ is toxic if aggregate in plant organs while NO_3^-, can collect to high concentrations in plant organs without harmful sensible effects [82–84]. Compared with the sole NO_3^- treatment (0:100 - NH_4^+:NO_3^-), the addition of NH_4^+ affected the distribution of N, P, and K in plant tissues [85]. For instance, lisianthus plants (*Eustoma russellianum* (Hook.) G.Don) grown only in the NH_4^+ medium that shown a decrease in amount of magnesium (Mg) absorption and accumulation [86].

Photosynthetic pigments (Chl a, Chl b and TCC)

Nitrogen is essential in many macro-molecules, including amino acids, pigments, proteins, nucleic acids, chlorophyll, and secondary metabolites [87, 88]. Various N forms have a different effects on the content of chloroplast photosynthetic pigments and photosynthesis rate of plant species [89]. Due to accessibility of more nutrient such as nitrogen and magnesium (key components of photosynthetic pigments), plants fertilized with higher NO_3^- concentrations, prefer to present higher photosynthetic pigments level. Similar results were found by Ma et al. [29], which amount of chlorophyll b was highest under the 0 NO_3^-:100 NH_4^+ treatment. Also, our results approved the earlier results were obtained by Liu et al. [71] and Qadir et al. [90]. The influence of NH_4^+ and NO_3^- on photosynthesis in plants can vary based on factors like plant species, nitrogen availability, and environmental circumstances. NH_4^+ uptake by plants may sometimes lead to elevated photosynthetic rates compared to NO_3^- uptake. This is because NH_4^+ assimilation demands less energy than NO_3^- assimilation, enabling more efficient carbon allocation to photosynthesis. However, excessive NH_4^+ absorption can induce toxicity, disrupting cellular functions and hindering photosynthesis, which may manifest as chlorosis, reduced chlorophyll levels, and diminished photosynthetic capacity. NO_3^- is typically the preferred nitrogen source for many plants under normal conditions. Its conversion to NH_4^+ before assimilation into organic compounds necessitates energy, potentially affecting photosynthetic efficiency. Despite this energy requirement, NO_3^- assimilation provides an additional source of reducing power (NAD(P)H), which can enhance photosynthetic electron transport and ATP production [91, 92].

The interplay between ammonium (NH_4^+) and nitrate (NO_3^-) ratios significantly influences various physiological parameters in plants, particularly in relation to photosynthesis and growth. Understanding these dynamics is crucial for optimizing nutrient management in agricultural practices. Research indicates that higher ratios of NH_4^+ relative to NO_3^- can lead to reduced photosynthetic rates. A study on lettuce demonstrated that when the NH_4^+ concentration exceeded 30% of the total nitrogen source, photosynthesis and overall plant growth were adversely affected [93]. This decline in photosynthetic activity is often attributed to the inhibitory effects of NH_4^+ on the absorption of essential nutrients such as potassium (K), calcium (Ca), and magnesium (Mg), which are vital for chlorophyll synthesis and function [94, 95]. Additionally, the presence of NO_3^- has been linked to improved stomatal conductance, which enhances CO_2 uptake and subsequently boosts photosynthesis [96].

The sensitivity of various photosynthetic parameters to NH_4^+: NO_3^- ratios varies among plant species and environmental conditions. Parameters such as net photosynthesis rate, chlorophyll content, and stomatal conductance are particularly sensitive to changes in nitrogen source ratios. NH_4^+ is known to require more energy for assimilation compared to NO_3^-, as it necessitates the conversion to amino acids through the action of enzymes such as glutamine synthetase [97]. This increased energy demand can lead to a reduction in available energy for photosynthesis, particularly under conditions where NH_4^+ is the predominant nitrogen source [98]. Additionally, high NH_4^+ concentrations can induce oxidative stress, leading to the production of reactive oxygen species (ROS) that can damage chloroplasts and impair photosynthesis [99].

Phenolic compounds

Results of TPC and TFC are also consistent with other studies [46, 85]. Also, a similar result is reached in the study by Ahmadi et al. [26], which The TPC value was improved by increasing of NO_3^-:NH_4^+ ratio. The highest TPC was obtained with 10:90 - NH_4^+:NO_3^- ratio. The using of NH_4^+ as main nitrogen source, reduced the biosynthesis of phenolics in plant organs (roots and leaves) in several plant species such as *Pisum sativum* L [100]. , maize [101], and purple coneflower [102]. The availability and type of nitrogen can potentially impact the regulation of genes responsible for biosynthesizing and controlling phenolic compounds. Transcription factors and signaling pathways engaged in nitrogen detection and response might indirectly govern the activity of genes encoding enzymes responsible for phenolic compound metabolism [103]. There is limited and contradictory evidence available with respect to how the biosynthesis of phenolic compounds is affected by the form of N. The NH_4^+ may by changing intracellular pH that affects the synthesis of phytochemicals including phenylpropanoid metabolism [104]. However, the accurate mechanisms by which such metabolic changes occur are still unknown [105]. Phenolic compounds have critical roles as antioxidant and therapeutic potential in many illnesses such as anticancer, anti-inflammatory, anti-diabetic and anti-Alzheimer [106]. It seems plant stress can be caused by high NH_4^+ levels and have a positive impact on the production of phytochemicals in *M. officinalis.* In addition, Olsen et al. [107] suggested that high amount of ammonium could play an important role in increasing phenylalanine ammonia-lyase (PAL) activity and consequently increase in synthesis phytochemicals through the phenylpropanoid metabolism. In basil (*Ocimum basilicum* L.), the supply of NO_3^- form as source of nitrogen decreased the content of important phenolic acids such as rosmarinic acid, which was similar to the results of this research [108]. Nevertheless, other information is not accessible about of effect of various nitrogen forms on accumulation of phenolic acids and flavonoids. Current research suggests that the providing of NH_4^+ to *M. officinalis* in floating culture system could have lower effect than NO_3^- on the quantity of these components, indicating that this nutritional strategy could be desirable make better the medicinal and commercial values of *M. officinalis*. Plant roots absorb and integrate ammonium and nitrate into organic nitrogen compounds via distinct metabolic processes. These pathways play a role in modulating the production and buildup of phenolic compounds in plants. The assimilation of ammonium entails its conversion into glutamine and glutamate through the GS/GOGAT pathway. This process may indirectly impact phenolic compound synthesis by modifying the availability of precursor molecules. Nitrate assimilation involves the conversion of nitrate into nitrite and subsequently into ammonium, facilitated by nitrate reductase and nitrite reductase enzymes. The intermediates and by-products generated during nitrate reduction can directly influence the synthesis of phenolic compounds [109, 110].

Antioxidant activity

In the present study, it was found a positive correlation between antioxidant activity based on the DPPH method with phenolic compounds including TFC, TPC, and other phenolics such as gallic acid, quercetin, *p*-coumaric acid, and apigenin. Also, it was obtained a negative correlation between antioxidant activity based on the FRAP assay with TPC and TFC. According to the different nature of antioxidant evaluation methods, the results of this study showed that, DPPH assay would be an appropriate technique for determining antioxidant in *M. officinalis* extract. Our study results showed that, the antioxidant activity of the samples had a considerable positive correlation with major phenolic compositions which proves that great antioxidant capacity is a result of higher accumulation of these compounds. Therefore, NH_4^+ can plays a role in antioxidant capacity enhancement, indirectly. Phenolic compounds (such as *p*-coumaric acid, gallic acid, caffeic acid, and quercetin) generally act as electron donors and detoxify free radicals [111, 112]. Gallic acid and rosmarinic acid, have a role of antioxidant, that help to protect of cells against oxidative damages. Moreover has been proven to have many other important physiological functions, including anticancer, antiallergy and astringent [113, 114]. Also, it is known that quercetin and coumaric acid is an intense antioxidant with therapeutic efficacy potential [115, 116]. The result of present study, was in accordance with the findings of other researchers [108, 117, 118]. The study conducted by Naseri et al. [28] was also in agreement with the findings of this study that *Dracocephalum moldavica* L. species treated with high amount of NH_4^+, revealed considerably a greater amounts of antioxidant activity by DPPH assay. The measured phenolic acids including caffeic and gallic acids had strong correlation with antioxidant capacity. On a broader scale, there is a direct relation between higher levels of phenolic compounds and higher resistance to pathogens and herbivore bugs. Thus, agroecosystems can benefit by reducing pesticide use by adjusting nitrogen complements. Research indicates that the type of nitrogen source available to plants (ammonium or nitrate) can impact their responses to stress, including oxidative stress and defense mechanisms. Phenolic compounds like flavonoids are recognized for their involvement in safeguarding plants against oxidative stress and environmental adversities. Ammonium-based nutrition has been linked to heightened oxidative stress in plants when compared to nitrate-based nutrition. This heightened oxidative stress could prompt the production and buildup of phenolic compounds, serving as a component of the plant's defense mechanisms [28, 119]. The equilibrium between ammonium (NH_4^+) and nitrate (NO_3^-) concentrations in the nutrient solution has the potential to impact antioxidant activity within plants [120]. Several research findings propose that maintaining a balanced ratio of NH_4^+ to NO_3^- could enhance the activity of antioxidant enzymes. To sum up, NH_4^+ can trigger oxidative stress in plants, thereby affecting the antioxidant defense mechanisms and the synthesis of phenolic compounds [28, 119].

Essential oil (EO) composition

The levels of various volatile compounds found in the essential oil of *M. officinalis* can be affected by the ratio of ammonia to nitrate that is supplied to the plant. The importance of *M. officinalis* essential oil is shown by the large amount of published works, describing chemical compositions, biological attributes, and applications [121–124]. Nitrogen is a key factor in the production of phytochemicals such as essential oils and it also activate specific biosynthetic pathways for the producing of essential oils in medicinal plants [125]. In medicinal plants, N helps increase photosynthesis efficiency in leaves, chlorophyll content, and enzyme activity, which increases essential oil production [126]. Furthermore, N is the most valuable element in higher plants since it helps synthesize many organic structures containing enzymes, amino acids, etc., essential in producing essential oils [127].

Sustainable farming endeavors to maximize crop yield while minimizing negative environmental repercussions, notably nitrogen pollution. By carefully managing various nitrogen sources and adopting precise nutrient management techniques, agricultural sustainability can be bolstered, thereby curtailing nitrogen runoff into the environment, lessening greenhouse gas emissions, and safeguarding water quality. The incorporation of integrated methods such as crop rotation, cover cropping, and biological nitrogen fixation serves to amplify nitrogen cycling efficiency and bolster ecosystem resilience within agricultural settings. Ultimately, the ecological and physiological facets of employing ammonium and nitrate in agriculture involve a complex array of interconnected factors, encompassing plant nourishment, soil vitality, nitrogen circulation, stress reactions, and environmental stewardship. By comprehensively understanding the intricate dynamics among nitrogen sources, plants, and their surroundings, researchers and practitioners can devise inventive approaches to optimize nitrogen management practices and foster sustainable agricultural frameworks [128–130].

Conclusion

It has been proven that NO_3^- and NH_4^+ are main N-sources for higher plants for growth and development. In general, there is nothing information related to the impact of various N forms on lemon balm (*Melissa officinalis*) under FCS such as yield, growth, physiological and phytochemical traits. The results of the current research showed that studied traits of lemon balm were significantly affected by the different ammonium to nitrate ratios. Ammonium indicated toxic impacts on morphological properties (plant height, fresh and dry matter of plant and root and number of lateral branches) at higher concentrations and also when it was used as the only source of N. Also, in high concentrations of ammonium as nitrogen source, antioxidant activity was reported at the highest level. The plants treated with high nitrate ratio (especially 0:100 - NH_4^+:NO_3^-) showed the greatest concentration of total phenolics, chlorophyll a, total flavonoids, total carotenoids, and individual phenolics including *p*-coumaric acid, gallic acid, and quercetin. The findings of present study suggest that the management of $NH4^+$ to NO_3^- ratios in nutrient solutions could contribute to improve growth, physiological and phytochemical properties of *M. officinalis*. Considering the high demand of the pharmaceutical industry for the phytochemical compounds of medicinal plants (including *Melissa*) for the production of safe products, as well as the limitations of the production of medicinal plants in the field due to destructive environmental conditions such as temperature, salinity and drought stress, in the near future, it will be necessary to produce medicinal plants in hydroponic systems (due to its many advantages).

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Author contributions
A.A. contributed to the study conception and design. Data collection and analysis were performed F.S. and P.N. The first draft of the manuscript was written by F.S. and A.A. K.A. and P.N. prepared research material and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data availability
The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Declarations

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