3 Biotech (2023) 13:412
https://doi.org/10.1007/s13205-023-03836-0

ORIGINAL ARTICLE

Biochemical and pharmaceutical traits of *Marrubium vulgare* L. plants treated with plant growth-promoting bacteria and elevated CO_2

Hamada AbdElgawad[1,2] · Shereen Magdy Korany[3] · Nashwa Hagagy[4] · Mohammad Yaghoubi Khanghahi[5] · Ahmed Mohamed Reyad[2] · Carmine Crecchio[5] · Adel M. Zakri[6] · Emad A. Alsherif[2] · Marwa Reda Bakkar[3]

Received: 24 May 2023 / Accepted: 27 October 2023 / Published online: 22 November 2023
© King Abdulaziz City for Science and Technology 2023

Abstract

The present research aimed to understand the influence of plant growth-promoting bacteria (PGPB) on various biochemical, nutritional, and pharmaceutical characteristics of *Marrubium vulgare* plants grown under elevated carbon dioxide (eCO_2). To achieve this objective, a pot experiment was carried out, consisting of two treatments, namely: (i) biofertilization (Bf) by a PGPB strain (*Micromonospora* sp.) and (ii) two different air CO_2 levels, including ambient CO_2 (aCO_2) and eCO_2 concentrations (410 and 710 µmol CO_2 mol^{-1}, respectively). The improvement in the photosynthesis rate of eCO_2 and Bf-treated plants can explain the increase in the production of carbohydrate. This is evidenced by a substantial rise, reaching up to + 75% and 25% in the total sugar and starch content in plants subjected to eCO_2 treatment, respectively. Additionally, eCO_2-treated plants exhibited a remarkable 102% increase in soluble sugar synthesis, while plants subjected to Bf treatment showed a notable increase of 66%. Such modifications could be the main factor affecting plants carbon and nitrogen metabolism. Although the level of certain amino acids (such as glycine, tyrosine, and phenylalanine) in plants exhibited significant increases in response to eCO_2 and Bf, the levels of other amino acids demonstrated enhancements in plants grown under eCO_2 (e.g., histidine) or under treatments containing Bf (e.g., alanine and ornithine). Improvements in primary metabolites led to more benefits in plants treated with Bf and CO_2 by boosting secondary metabolites accumulation, including phenolics (+ 27–100%), flavonoids (+ 30–92%), and essential oils (up to + 296%), as well as improved antioxidant capacity (FRAP). This remarkable effectiveness was evident in the significant increase in the biomass production, highlighting the synergistic impact of the treatments. Therefore, the interaction of Bf and eCO_2 not only induced plant biomass accumulation but also improved the nutritional and pharmaceutical value of *M. vulgare* plants.

Keywords Actinobacteria · Elevated CO_2 · Essential oils · Flavonoids · *Marrubium vulgare* · Metabolites · Phenolic acids · Plant growth-promoting bacteria

Introduction

The high demand for bioactive metabolites such as plants' essential oils has received considerable critical attention due to the increasing appeal of natural components in industrial sections, such as food flavoring, fragrances' production, cosmetics, and pharmaceuticals (Guedri Mkaddem et al. 2022). Among medicinal and industrial plants, *Marrubium vulgare* L. has been the subject of phytochemical and pharmacological research. It is used in food and pharmaceutical industry,

✉ Hamada AbdElgawad
hamada.abdelgawad@uantwerpen.be

✉ Emad A. Alsherif
eaalsherif@uj.edu.sa

[1] Integrated Molecular Plant Physiology Research (IMPRES), Department of Biology, University of Antwerp, Antwerp, Belgium

[2] Botany and Microbiology Department, Faculty of Science, Beni-Suef University, Beni Suef 62521, Egypt

[3] Botany and Microbiology Department, Faculty of Science, Helwan University, Cairo 11795, Egypt

[4] Botany and Microbiology Department, Faculty of Science, Suez Canal University, Ismailia, Egypt

[5] Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy

[6] Plant Production Department, College of Food and Agricultural Sciences, King Saud University, Riyadh, Saudi Arabia

being rich in a vast array of bioactive metabolites (e.g., phenolic acids, phenylethanoids, flavonoids, and essential oils) (Rached et al. 2022) and biological activities, such as antimicrobial (Ortega-Ramirez et al. 2014) and antioxidant (Edziri et al. 2012). The main factors affecting the biochemical composition of the above compounds in *M. vulgare* plants are the environmental conditions, such as bioclimates and edaphic factors (Demiroz Akbulut et al. 2023).

The raised atmospheric level of carbon dioxide (eCO_2) is one of the aspects responsible for global warming and the consequent irreversible climate change. Alterations in land use and management (e.*g*., agrochemicals' application, biomass burning, deforestation, etc.), burning of fossil fuels, and industrial activities result in continuously higher CO_2 emissions (Shabbaj et al. 2022). It has been reported that such human activities will raise the atmospheric CO_2 levels from the current 414 μmol CO_2 mol^{-1} to greater than 700 μmol CO_2 mol^{-1} at the end of this century (Fang et al. 2022). The increase in CO_2 in turn influences plants' growth, productivity, and some physiological and biochemical traits (Silva et al. 2020). This effect is more pronounced, especially when nutrients and water are sufficiently available to the plant (AbdElgawad et al. 2014). Exposure to eCO_2 improved photosynthesis in plants, increased the allocation of photosynthetic carbon to roots, and increased the demand for nutrients uptake from soil. Consequentially, it improved the carbon and nitrogen metabolisms (Fang et al. 2022; Sekhar et al. 2023). In fact, due to the extensive development of root systems in eCO_2 conditions, roots are unable to fulfill plants' needs for nutrients' uptake. Hence, fertilizer application can furnish more benefits to plant roots under eCO_2, which supply more nutrients for plant growth (Du et al. 2023).

The application of microbial-based fertilizations has been considered a sustainable strategy for providing macro- and micronutrients, One of these strategies involves employing plant growth-promoting bacteria (PGPB) as biofertilizers and biostimulants in plant cultivation (Yaghoubi et al. 2019). PGPB have great potential in stimulating plant growth and health through symbiotic biological fixation of nitrogen, siderophores' production, and solubilizing phosphorus, potassium, and other minerals (Yaghoubi et al. 2021; Cao et al. 2023). Moreover, the application of PGPB as bio-inoculants improved phytohormones (e.g., gibberellins, auxins, and cytokinin) biosynthesis declined the ethylene levels in plants and induces plant resistance to unfavorable environmental conditions (Yaghoubi et al. 2021; Strafella et al. 2021). Among these beneficial bacteria, actinobacteria (e.g., *Micromonospora*) have been suggested as bio-inoculants because of their great potential in biocontrol, plant growth-promoting and bioremediation activities, which are due mainly to the biosynthesis of secondary metabolites, pigment and spore production, and unique lifestyles present in these actinobacteria (Trujillo et al. 2015; Faddetta et al. 2023). Despite considerable reports concerning the favorable consequences of PGPB on agronomic and physiological traits of plants (Bakhshandeh et al. 2020), little is known about the interacting effects of PGPB and eCO_2 on the metabolites' composition of plants.

Therefore, the aim of the current study was to shed new insights on some biochemical, pharmaceutical, and antioxidant parameters of *M. vulgare* plants biofertilized by PGPB. Attempts were also made to provide more information on how such traits are affected by eCO_2 for safeguarding plants from potential adverse impacts of future increasing CO_2. We hypothesized that PGPB application can positively affect secondary metabolites and nutritional values of *M. vulgare* plants, particularly under eCO_2 treatment conditions.

Materials and methods

Bacterial isolation and purification

Twenty-four actinobacterial species were obtained after applying a moist heat treatment (water bath at 50 °C for 5–6 min) to eliminate fast-growing bacteria. A gram of soil was serially diluted in sterile saline solution at a ratio of 1:10–1:1000. Actinobacteria were isolated using starch-casein agar medium (Küster and Williams 1964) and soil extract media (Barakate et al. 2002) with glycerol, as carbon source. Cycloheximide and nystatin (0.05 mg mL^{-1}) were added to the medium as antifungal agents, and the pH of the media was set to 7.2 (Porter et al. 1980; Phillips and Hanel 1950). Nalidixic acid (10 mg L^{-1}) was also utilized to inhibit bacteria capable of overcrowding, without inhibiting the growth of actinobacteria (Araragi 1979; You and Park 1996). After 7–14 days of incubation at 28 °C, the number of colonies on the plates was determined. The purified colonies were stored on starch-casein slants in 20% glycerol at − 20 °C, aAs stock cultures.

Morphological identification of bacterial isolates

The purified isolates were identified to belong to the genus Actinobacteria following direct microscopic observation at 1000 × magnification for the aerial and substrate mycelial growth on coverslips inserted in starch-casein agar medium (Kawato and Shinobu 1959), in accordance with Bergey's Manual of Determinative Bacteriology (Holt et al. 1994). Also, the colors of the produced diffusible pigments, as well as the aerial and substrate mycelia, were visually identified.

Molecular identification of bacterial isolates

Genomic DNA was extracted from the selected potential actinobacterial strains according to the technique outlined by Hong et al. (2009). The PCR amplification of the 16S rRNA gene was performed by the universal primers 27F (5-GAGTTTGATCCTGGCTCA-3) and 1498R (5-ACGGCT ACCTTGTTACGACTT-3), which are complementary to the conserved regions at the 5- and 3-end of the whole gene sequence of *Escherichia coli* (Reddy et al. 2000). All PCR amplifications were carried out in 25 µL reactions containing 50 ng of total DNA, 5 U of Taq DNA polymerase (EuroTaq; EuroClone), 3 mM of each 2′-deoxynucleoside 5′-triphosphate (dNTP), and 3 mM $MgCl_2$ using a MyCycler™ thermal cycler (Bio-Rad Laboratories Inc.). The PCR conditions were set on an initial denaturation (94 °C for 5 min) and 35 cycles of amplification (94 °C for 1 min, 54 °C for 1 min, and 72 °C for 2 min), followed by an extension step (72 °C for 5 min). The amplicons were sequenced from both ends by MacroGen Company (South Korea; http://www.dna.macrogen.com). The sequences were aligned by the BLASTn tool against the NCBI database (www.ncbi.nlm.nih.gov) to identify the bacterial strains. The MEGAX software program was used to carry out the cluster analysis.

Determination of biological and phytochemical activities of the selected isolate

The medium was inoculated with a 24-h culture and then placed in incubation at 37 °C for 7 days under dark conditions, followed by centrifugation at 6000 rpm for 10 min. 1N HCl was added to the resulting supernatant, and the pH was adjusted to 2.8. The entire acidified supernatant was transferred to a 250 mL conical flask; an equal volume of diethyl ether was added, and the mixture was incubated in the dark for 4 h. Subsequently, the samples were stored overnight at 4 °C in a separating funnel. The solvent phase (upper layer) was carefully collected, and the organic phase (lower layer) was then discarded. The upper layer was then allowed to evaporate, and finally, 2–3 mL of high-performance liquid chromatography (HPLC) grade methanol was introduced. The quantification of indole-3-acetic acid (IAA) and gibberellic acid (GA3) was performed using HPLC. To assess cytokinin (CK) production by actinobacteria, a cucumber cotyledon greening bioassay was used, as described by Fletcher et al. (1982).

The actinobacteria were also tested for the production of ammonia using the method described by Cappuccino and Sherman (1992). Phosphate solubilization capacity was measured by the Olsen and Sommers method (1982). The antioxidant activity of the selected isolate was measured by the ferric reducing antioxidant power (FRAP) assays (Schlesier et al. 2002), reading the absorbance of the extracted samples at 590 nm. The analysis of the total phenolic and flavonoid content of the bacterial isolates was done according to the method described by Holder and Boyce (1994).

Plant materials and experimental setup

A pot experiment was arranged based on a completely randomized design with two treatments and three replications. The first treatment included two levels of biofertilization, including no biofertilization (Co) and biofertilization by the *Micromonospora* sp. strain (Bf). The second treatment consisted of two levels of air CO_2 concentrations, namely ambient CO_2 (aCO_2, 410 µmol CO_2 mol^{-1}) and elevated CO_2 (eCO_2, 710 µmol CO_2 mol^{-1}). In this regard, the target CO_2 emissions were injected into the airflow of the chamber, and their levels were monitored using a CO_2 analyzer (WMA-4, PP Systems, Hitchin, UK). *M. vulgare* seeds were sterilized in a Clorox solution (20% v/v) for 10 min (Abdallah et al. 2018) and planted in pots filled with a mixture of loamy soil and organic compost (1:1, v/v) (potting mix, Tref EGO substrates, Moerdijk, The Netherlands). Plants were kept in a controlled-environment chamber for 10 weeks, with a constant regime of 25 °C and 16/8 h day/night photoperiod. Plant leaves were sampled 10 weeks after planting; a part of them was used to measure biomass production and the remaining for subsequent biochemical analysis.

To apply the biofertilization treatment, the bacterial strain was grown in Nutrient Broth medium at 29 °C for 24 h and then was concentrated by centrifugation (at 5000 rpm for 15 min), and the obtained pellet was washed and re-suspended in a sterile potassium chloride solution (0.9%, w/v) (Yaghoubi et al. 2020). The density of this bacterial suspension was adjusted to 10^{-6} CFU mL^{-1}, approximating an optical density at 600 nm equivalent to 0.6–0.7, and used for seeds and soil inoculation before the initial planting and for subsequent additions to pots every 2 weeks (Yaghoubi et al. 2021). Control (No biofertilization) seeds and pots were also treated with the sterile potassium chloride solution.

Measurements

Plant biomass and photosynthesis rate

Plant shoot tissues were collected 10 weeks after planting and a part of them was used to measure the fresh and dry weights of the shoot. The photosynthesis rate of the last developed leaves was estimated using the LI-COR portable photosynthesis system (LI-COR 6400/XT, USA).

Determination of total and individual carbohydrates

Total and soluble sugar content was measured using Nelson's colorimetric method adopted by Clark and Switzer (1977). Starch content was determined by the anthrone method (Yemm and Willis 1954). The content of individual sugars was assessed by high-performance liquid chromatography (HPLC; Shimadzu, Hertogenbosch, The Netherlands) as fully described by Hamad et al. (2015).

Measurement of amino acid profile

Quantitative determination of amino acids was carried out using a Waters Acquity UPLC-tqd system (Milford, Worcester County MA, USA), equipped with a BEH amide 2.1 × 50 column (Yaghoubi et al. 2022). Briefly, samples were homogenized in ethanol–water (80:20, v/v), spiked with norvaline, and centrifuged at 14,000 rpm for 20 min. The supernatant was re-suspended in chloroform and HPLC-grade deionized water, followed by filtering using a Millipore microfilter (0.2 µM pore size).

Determination of phenolic and flavonoid compounds

The quantification of individual phenolic and flavonoids was done using the HPLC method fully described by Al Jaouni et al. (2018). Briefly, samples were extracted in an acetone–water solution (4:1, v/v) and then centrifuged. The supernatant was evaporated under vacuum, and the residue was re-suspended in HPLC-grade methanol. The compounds were determined using an HPLC equipped with a Lichrosorb Si-60, 7 µm, 3 × 150 mm column and a diode-array detector; the mobile phase contained water-formic acid (90:10, v/v) and acetonitrile/water/formic acid (85:10:5, v/v/v). The content of each compound was calculated using a calibration curve of the corresponding standard.

Essential oils' analysis

The composition of essential oils was determined by a Varian system (Varian Star 3400 CX gas chromatograph; Les Ulis, France) equipped with a fused silica capillary DB-5MS (5% phenylmethyl polysyloxane; 30-m/0.25-mm; film thickness, 0.25 mm) column, as fully explained by Mkaddem Mounira et al. (2022). Quantitative data were obtained electronically from the FID area percent data without the use of correction factors. Peak integration and quantification were performed automatically with Saturn 2100 Workstation software. A check of the integration of each peak was carried out and corrected manually if necessary. Essential oils' quantification was also carried out using a gas chromatography–mass spectrometer (GC–MS) equipped with a Varian Saturn GC/MS/MS 4D mass selective detector in the electron impact mode (70 eV). These components were identified based on the comparison of their Kovats indices (KI), co-injection of standards, and MS experimental data, with those contained in commercial or literature libraries (NIST 02 version 2.62). Alkanes (C5–C24) were used as reference points in the calculation of KI (Guedri Mkaddem et al. 2022).

Measurement of antioxidant capacity and low-density lipoproteins (LDL) oxidation

The total antioxidant capacity (FRAP) was determined in *M. vulgare* plants using Trolox as a reference base and reading the absorbance at 517 nm (Yaghoubi et al. 2022). Oxygen-radical absorbance capacity (ORAC) of plant extracts was also assessed, based on the method of Kassim et al. (2013). Accordingly, two wavelengths, 485 and 520 nm, were used as excitation and emission wavelengths, respectively. The ORAC value was referred to as the net protective area under the quenching curve of fluorescein in the presence of an antioxidant.

Inhibition of LDL oxidation (TBARS and conjugated dienes) was measured based on the method of Chen et al. (2016). Accordingly, dialyzed LDL (100 µg protein mL^{-1}) were diluted in 10 mM PBS (phosphate-buffered saline containing 0.01 M phosphate buffer and 0.15 M NaCl, pH 7.4). After incubation, lipid peroxidation of the LDL was measured. Thiobarbituric acid reactive substances (TBARS) content was determined at 532 nm/600 nm, using 1,1,3,3-tetramethoxypropane as standard for calibration curve, while conjugated diene formation of LDL solution in PBS, incubated with $CuSO_4$ (10 µM) in the absence or presence of various concentrations of bovine colostrum protein (Habeeb et al. 2020), was measured at 232 nm. Inhibition of hemolysis was measured based on the protocol described by Ishnava and Shah (2014). Accordingly, a suspension of human erythrocyte was washed with a phosphate-buffered saline, centrifuged, and incubated with crude extract for 60 min at 37 °C. After incubation, samples were centrifuged, and the absorbance of the released hemoglobin was measured at 540 nm.

Statistical analysis

All statistical analyses, including a two-way analysis of variance (ANOVA) and Tukey HSD (Honestly significant difference) test, as well as graphs drawing, were performed using the SigmaPlot software. The results were expressed as mean ± standard deviation.

Results

Bacterial identification

The best isolate was selected for further investigation based on the colony structure and their biological ability in vitro conditions (Data not shown). The results of 16S rRNA gene sequencing were compared to those of known 16S rRNA sequences using BLAST and the GenBank database (Fig. 1). Accordingly, the selected isolate was closely related to unidentified species with the same genus and associated within the genus *Micromonospora* (sequence identity > 99%). The NCBI and GenBank nucleotide sequence databases received the 16S rRNA gene data under the accession number (OQ918014) for deposit.

Phytochemical and biological activity of *Micromonospora* sp. strain

High amounts of the total phenolic and flavonoid content of *Micromonospora* sp. extracts were observed, as shown in Table 1. Regarding FRAP analyses, the scavenging activity of *Micromonospora* sp. was also high (Table 1). This actinobacteria strain also showed potential in phytohormones' production such as indole acetic acid (IAA), gibberellic acid (GA), and cytokinin (CK) (Table 1). The results of further examination of the potentially beneficial bacterial solubilization and fixation activity in the corresponding liquid medium are presented in Table 1. Accordingly, the amounts of solubilized phosphate and ammonia were about 150 and 6 mg L^{-1}, respectively.

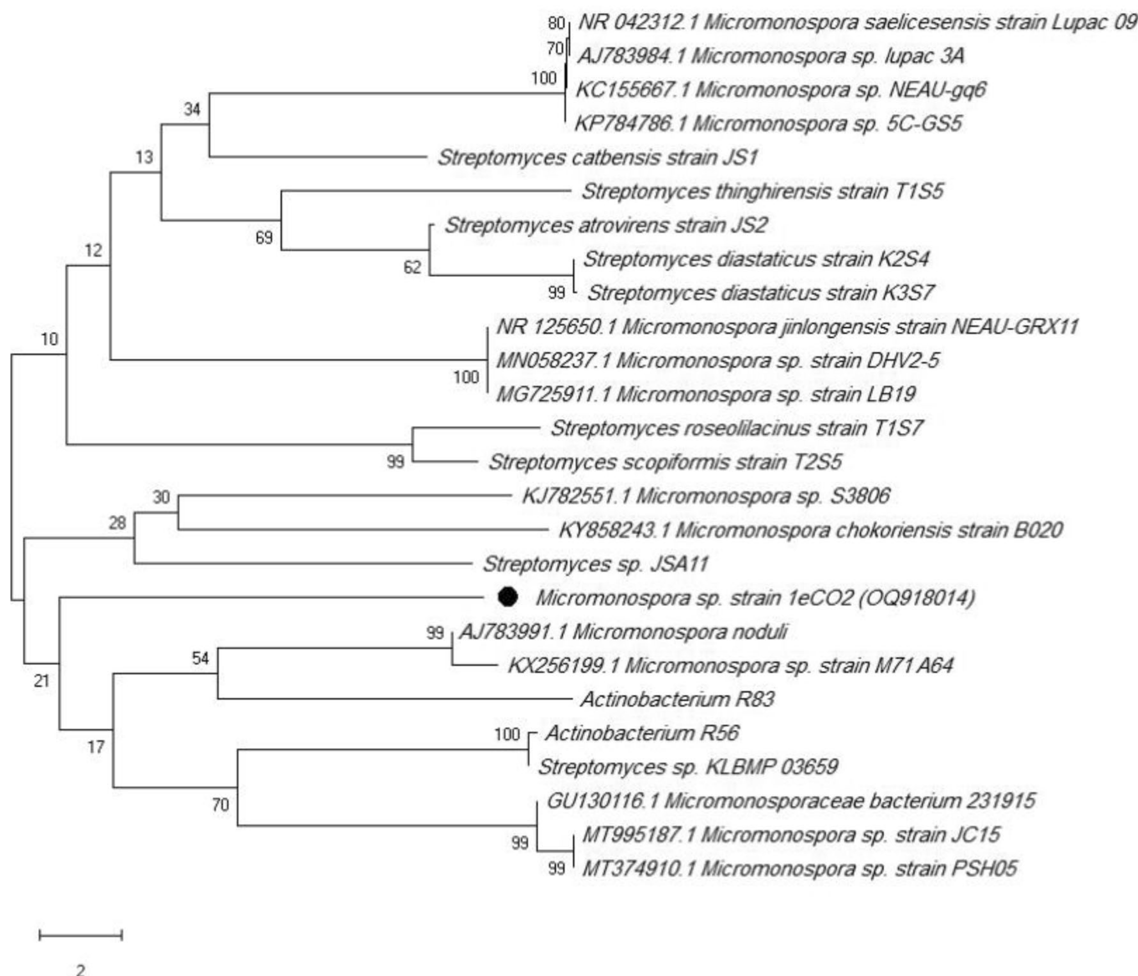

Fig. 1 Neighbor-joining tree (partial sequences ~ 1400 bp) showing the phylogenetic relationships of Actinobacterial 16S rRNA gene sequences of the potential strain to closely related ($S \geq 99\%$) sequences from the GenBank database

Table 1 The biological ability of Micromonospora sp. As indicated by flavonoids and phenolics levels, total antioxidant capacity measured by ferric reducing antioxidant power (FRAP) assays, production of plant growth regulators, and nutrient solubilization and fixation in vitro

Antioxidant activity (FRAP) (μmole Trolox g^{-g} DW)	63.0 ± 4.3
Total flavonoids (mg quercetin equivalent (QE) g^{-1} DW)	11.7 ± 0.6
Total phenols (mg gallic acid equivalent (GAE) g^{-1} DW)	36.1 ± 2.9
IAA (mg g^{-1} DW)	3.8 ± 0.2
GA (mg g^{-1} DW)	1.0 ± 0.0
CK (mg g^{-1} DW)	0.9 ± 0.0
Ammonia (mg L^{-1})	5.8 ± 0.4
Phosphate (mg L^{-1})	150.0 ± 1.1

Values are represented by means ± standard deviations of three independent replicates

Carbohydrates' metabolism

To explore carbon metabolism in *M. vulgare* plants, the adaptation and modification in sugar content were investigated under eCO_2 and biofertilization treatments (Fig. 2). In this regard, eCO_2 significantly ($p < 0.05$) increased total sugar and starch content in plants up to 75% and 25% higher than the control treatment, respectively. Moreover, both eCO_2 and Bf treatments significantly ($p < 0.05$) enhanced the soluble sugar content by 102% and 66%, respectively, compared to the control treatment (Fig. 2a). Interestingly, by focusing on the three key forms of soluble sugars, a different response of fructose, glucose, and sucrose was observed (Fig. 2b). Accordingly, the highest accumulation of glucose was recorded in plants grown under eCO_2 conditions, which was about + 107% higher than that recorded for in Bf-treated plants grown under aCO_2. In contrast, only combined treatment (eCO_2 + Bf) significantly affected the fructose and sucrose contents and increased them up to + 60% and + 27%, respectively, compared to those in those in the unfertilized plants cultivated under aCO_2 conditions (Fig. 2b). In confirmation of these findings, the analysis of enzymes involved in sugar metabolism revealed that the activity of sucrose synthase and starch synthase reached the highest values in plants grown under eCO_2 and eCO_2 + Bf treatments, being

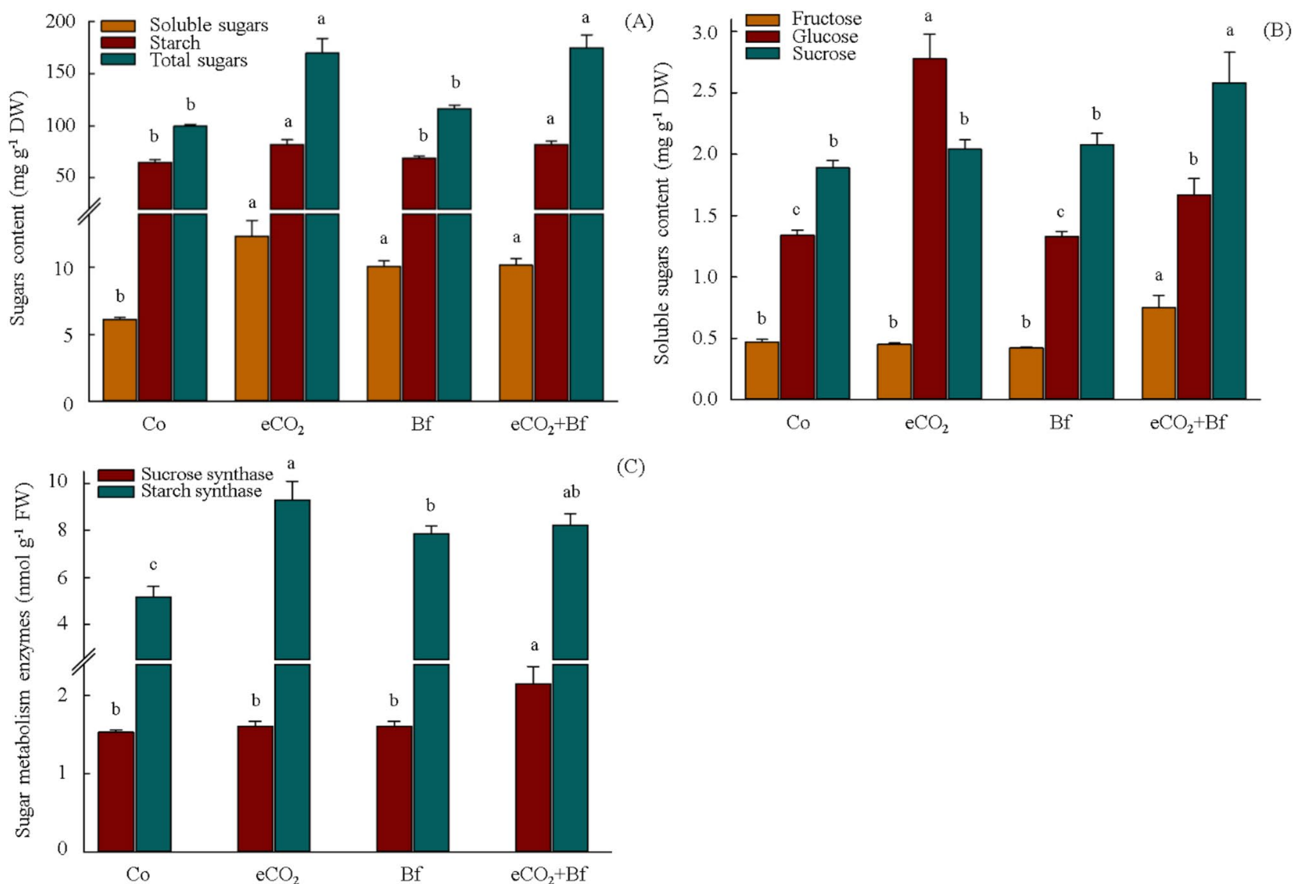

Fig. 2 The effect of plant growth-promoting *Micromonospora* sp (Bf) and elevated CO_2 (eCO_2) on sugars content (**A**), individual soluble sugars (**B**), and sugar metabolism enzymes (**C**). Means in each parameter followed by similar letter(s) are not significantly different at 5% probability level ($p \leq 0.05$, Tukey test)

these increases 41% and 80% higher than those in unfertilized plants, respectively (Fig. 2c).

Amino acids and essential oils' composition

To discover how eCO_2 and biofertilization influenced the nitrogen metabolism in *M. vulgare* plants, the amino acid composition was measured. Accordingly, alanine, lysine, and histidine were the most abundant detected amino acids in plants, which respectively varied from 17.3, 3.2, and 2.1 mg g^{-1} of protein in unfertilized plants to 28.1, 6.7, and 2.9 mg g^{-1} of protein in plants treated with eCO_2 + Bf (Table 2). Although the content of some amino acids (e.g., lysine, glycine, asparagine, tyrosine, phenylalanine, cysteine, and aspartate) in *M. vulgare* plants was significantly increased in response to both eCO_2 and Bf treatments, the content of others was increased only in plants grown under eCO_2 condition (e.g., histidine) or treated with Bf-containing treatments (e.g., alanine, valine, serine, ornithine, and threonine), compared to those in unfertilized plants (Table 2).

The composition of essential oils was investigated to reveal the shifts in secondary metabolites of *M. vulgare* plants in response to the treatments (Table 3). Sixteen compounds of essential oils were detected in leaf samples, the most concentrated of which were β-caryophyllene, γ-cadinene, and α-himachalane. The data reported here appear to support the assumption that biofertilization had a great impact on most of these detected oils (10 out of 16), including β-caryophyllene, α-himachalene, thymol methyl ether, γ-terpinene, camphene, pinene, α-terpinene, cis-α-bisabolene, linalyl anthranilate, and α-phellandrene, that were significantly ($p < 0.05$) more accumulated in Bf-containing treatments. Moreover, the content of γ-cadinene, δ-cadinene, thymol, and β-myrcene under both eCO_2 and Bf treatments was significantly ($p < 0.05$) higher than in the control treatment, while carvacrol concentration was significantly higher only under eCO_2 growth condition (Table 3).

Flavonoids and phenolic acids' compounds

The effect of eCO_2 and BF on flavonoids and phenolic acids in plants was analyzed (Fig. 3). All flavonoid compounds were significantly affected by the applied treatments. Accordingly, the content of apigenin, rutin, and naringin reached the highest values in the combined treatment (eCO_2 + Bf), equal, respectively, to 180, 25, and 30 μg g^{-1} dry weight, which were 50%, 92%, and 30% higher than those in unfertilized control plants. Although quercetin and naringenin were not affected by eCO_2, their content significantly increased in Bf-containing treatments (Bf and eCO_2 + Bf) up to + 70% compared to the control treatment (Fig. 3). In contrast, luteolin level was significantly increased in eCO_2 and eCO_2 + Bf-treated plants. This increase was 57–71% higher than in unfertilized plants (Fig. 3).

Further analysis revealed the responses of five detected phenolic compounds in plants (Fig. 4). Compelling evidence

Table 2 Amino acids (mg g^{-1} dry weight) composition in leaves of *M. vulgare* in response to biofertilization (Bf) and elevated CO_2 (eCO_2) growth conditions

	No fertilization		Biofertilization	
	Co	eCO_2	Bf	Bf + eCO_2
Alanine	17.31 ± 1.07 b	18.88 ± 1.79 b	25.09 ± 1.01 a	28.06 ± 0.97 a
Lysine	3.20 ± 0.21 c	4.89 ± 0.03 b	4.97 ± 0.15 b	6.67 ± 0.29 a
Histidine	2.15 ± 0.22 b	2.75 ± 0.17 a	2.53 ± 0.28 b	2.95 ± 0.22 a
Arginie	1.64 ± 0.18 a	2.02 ± 0.10 a	2.14 ± 0.29 a	1.76 ± 0.10 a
Glycine	1.18 ± 0.07 b	2.03 ± 0.13 a	2.21 ± 0.14 a	2.01 ± 0.26 a
Asparagine	1.03 ± 0.06 b	1.81 ± 0.11 a	1.84 ± 0.08 a	2.05 ± 0.05 a
Tyrosine	0.99 ± 0.06 c	1.32 ± 0.08 b	1.64 ± 0.11 a	1.41 ± 0.09 ab
Valine	0.48 ± 0.03 c	0.58 ± 0.04 c	0.78 ± 0.05 b	1.38 ± 0.12 a
Phenylalanine	0.33 ± 0.02 c	0.76 ± 0.06 b	0.76 ± 0.06 b	0.87 ± 0.03 a
Serine	0.35 ± 0.02 b	0.59 ± 0.04 ab	0.64 ± 0.04 a	0.78 ± 0.05 a
Ornithine	0.21 ± 0.03 b	0.24 ± 0.02 ab	0.27 ± 0.02 a	0.32 ± 0.01 a
Threonine	0.11 ± 0.01 b	0.16 ± 0.01 ab	0.19 ± 0.01 a	0.21 ± 0.01 a
Cysteine	0.03 ± 0.00 c	0.20 ± 0.01 b	0.12 ± 0.01 bc	0.52 ± 0.05 a
Aspartate	0.03 ± 0.00 b	0.05 ± 0.00 a	0.06 ± 0.00 a	0.05 ± 0.01 a
Methionine	0.02 ± 0.00 b	0.02 ± 0.00 b	0.03 ± 0.00 ab	0.05 ± 0.00 a
Leucine	0.02 ± 0.00 b	0.03 ± 0.00 ab	0.03 ± 0.00 ab	0.04 ± 0.00 a

Means in each row followed by similar letter(s) are not significantly different at 5% probability level (Tukey test). Co: Control; eCO_2: Elevated CO_2: Bf: biofertilization by the *Micromonospora* sp. strain

Means in each parameter followed by similar letter(s) are not significantly different at 5% probability level ($p \leq 0.05$, Tukey test)

Table 3 Essential oils (μg 100-mg^{-1} dry weight) composition in leaves of *M. vulgare* in response to biofertilization (Bf) and elevated CO_2 (eCO_2)

	No fertilization		Biofertilization	
	Co	eCO_2	Bf	Bf + eCO_2
β-Caryophyllene	15.43 ± 1.92 c	14.21 ± 1.79 c	95.59 ± 9.40 a	53.96 ± 6.14 b
γ-Cadinene	30.80 ± 2.07 c	38.63 ± 2.77 b	47.11 ± 8.41 a	42.30 ± 3.51 ab
α-Himachalene	27.42 ± 1.37 c	27.27 ± 2.21 c	34.81 ± 0.11 b	48.34 ± 2.21 a
δ-Cadinene	12.54 ± 0.62 c	44.47 ± 2.31 a	24.12 ± 2.32 b	49.71 ± 3.89 a
Carvacrol	23.84 ± 1.81 c	28.21 ± 0.41 b	22.63 ± 2.64 c	43.39 ± 2.37 a
Thymol methyl ether	17.81 ± 0.89 b	17.70 ± 1.43 b	20.57 ± 1.92 a	22.29 ± 1.45 a
γ-Terpinene	10.13 ± 0.51 c	10.07 ± 0.81 c	12.15 ± 0.25 b	17.78 ± 0.97 a
Camphene	8.28 ± 0.72 c	6.39 ± 0.52 c	13.32 ± 1.61 b	18.71 ± 2.97 a
Pinene	8.73 ± 0.43 c	8.68 ± 0.71 c	11.08 ± 0.03 b	15.39 ± 0.72 a
Isoborneol	13.08 ± 0.81 a	13.75 ± 1.12 a	10.82 ± 2.31 b	14.72 ± 2.11 a
α-Terpinene	7.16 ± 0.36 c	7.12 ± 0.57 c	14.78 ± 0.92 a	10.05 ± 0.71 b
Thymol	7.89 ± 0.61 d	12.82 ± 1.03 b	14.42 ± 1.16 a	10.76 ± 0.74 c
cis-α-Bisabolene	6.75 ± 0.79 b	6.71 ± 0.54 b	8.08 ± 0.19 a	7.19 ± 0.31 ab
Linalyl anthranilate	6.21 ± 0.31 c	6.18 ± 0.50 c	7.49 ± 0.23 b	10.16 ± 0.25 a
β-Myrcene	4.85 ± 0.39 c	7.81 ± 1.37 b	12.22 ± 2.81 a	11.59 ± 1.01 a
α-Phellandrene	5.10 ± 0.13 b	5.87 ± 0.21 ab	6.22 ± 0.39 a	5.08 ± 0.14 b

Means in each row followed by similar letter(s) are not significantly different at 5% probability level (Tukey test). Co: Control; eCO_2: Elevated CO_2: Bf: biofertilization by the *Micromonospora* sp. strain. *D* Means in each parameter followed by similar letter(s) are not significantly different at 5% probability level ($p \leq 0.05$, Tukey test)

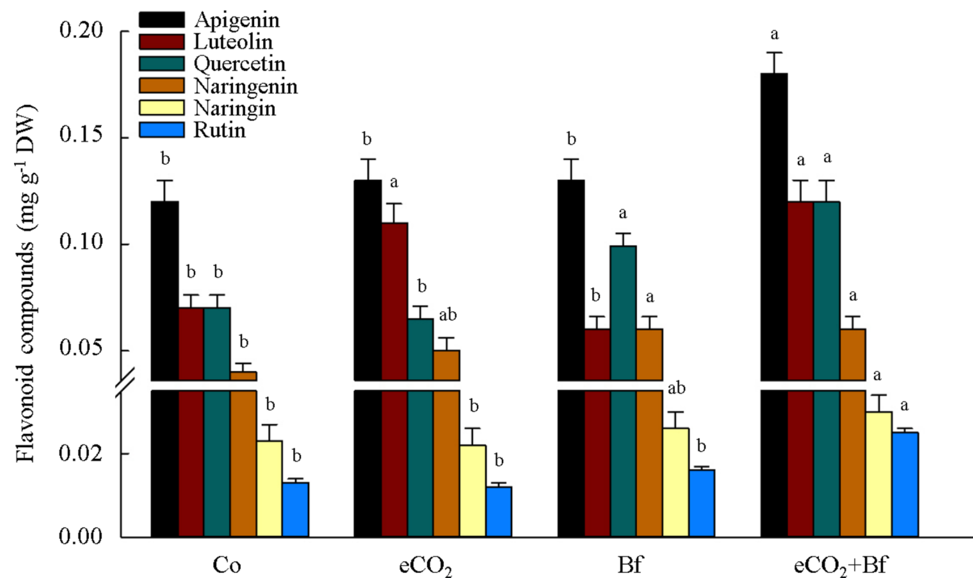

Fig. 3 The effect of plant growth-promoting *Micromonospora* sp (Bf) and elevated CO_2 (eCO_2) on flavonoid compounds. Means in each parameter followed by similar letter(s) are not significantly different at 5% probability level ($p \leq 0.05$, Tukey test)

of a substantial increase in non-enzymatic antioxidant compounds was uncovered when the combined treatment of eCO_2 and Bf was applied. Accordingly, the content of chlorogenic acid, *p*-coumaric acid, rosmarinic acid, chicoric acid, caffeic acid, and gallic acid was significantly increased in eCO_2 + Bf-treated plants, i.e., 20.1, 3.9, 2.6, 1.6, 0.7, and 0.5 μg g^{-1} dry weight, which were 100%, 50%, 52%, 27%, 72%, and 57% higher than those in unfertilized control plants, respectively. Among these phenolic compounds, only caffeic acid was significantly influenced by the individual application of eCO_2 and Bf treatments (+ 45% and + 55%, respectively) compared to the control treatment (Fig. 4).

Antioxidant activity

The antioxidant potential of natural compounds extracted from *M. vulgare* plants was analyzed based on the free radical scavenging and low-density lipoprotein (LDL)

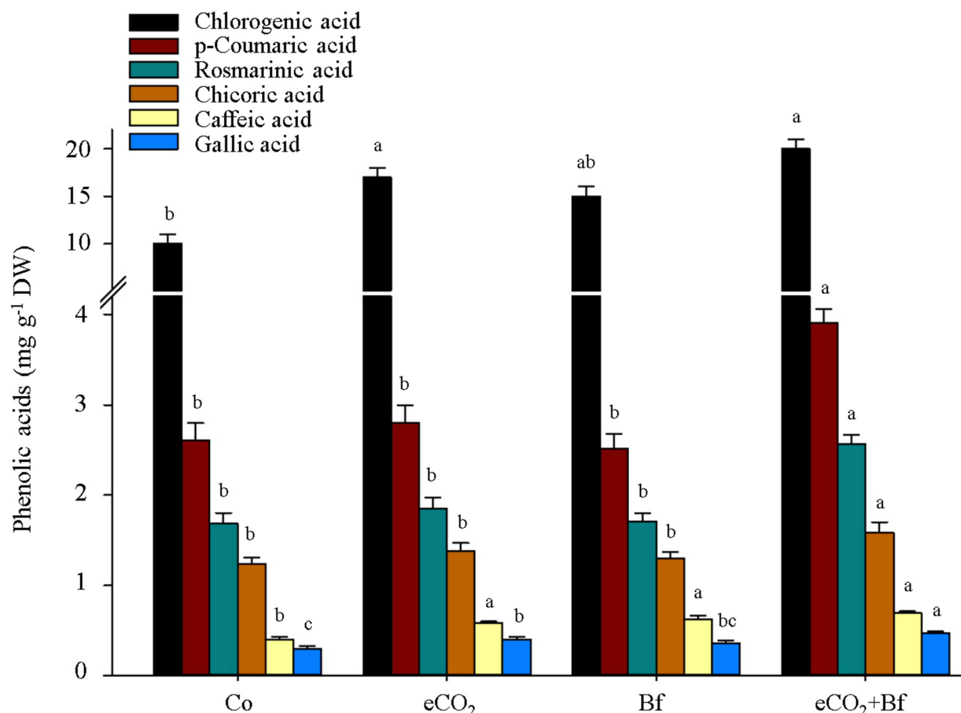

Fig. 4 The effect of plant growth-promoting *Micromonospora* sp (Bf) and elevated CO_2 (eCO_2) on phenolic acids' content. Means in each parameter followed by similar letter(s) are not significantly different at 5% probability level ($p \leq 0.05$, Tukey test)

modifying properties. In this regard, the two most common antioxidant parameters, oxygen-radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP), were significantly improved in response to biofertilization and eCO_2 treatments (Fig. 5). Accordingly, combined treatment increased both ORAC and FRAP parameters by 214% and 245% compared to those in those unfertilized control plants. Although eCO_2 increased the levels of three other antioxidant parameters, including inhibition of hemolysis, inhibition of LDL oxidation (conjugated dienes), and inhibition of LDL oxidation (TBARS), biofertilization improved only the parameters of inhibition of LDL oxidation (+ 51% and + 65%, respectively; $p < 0.05$)

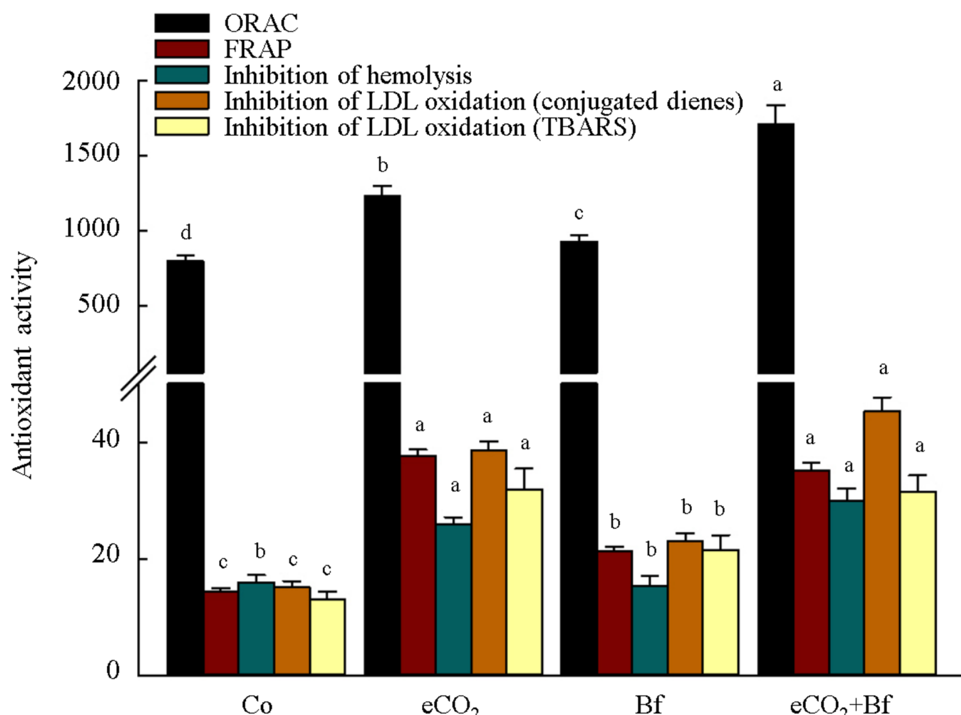

Fig. 5 The effect of plant growth-promoting *Micromonospora* sp (Bf) and elevated CO_2 (eCO_2) on antioxidant activity, including ferric reducing antioxidant power (FRAP; µmole Trolox g^{-1} DW), oxygen-radical absorbance capacity (ORAC; µmole TE g^{-1} DW), inhibition of LDL oxidation (TBARS and conjugated dienes; represented as %), and inhibition of hemolysis (%). Means in each parameter followed by similar letter(s) are not significantly different at 5% probability level ($p \leq 0.05$, Tukey test)

to a lesser extent than eCO_2 treatment (− 41% and − 33%, respectively; $p < 0.05$) (Fig. 5).

Plant biomass and photosynthesis capacity

It is apparent from Fig. 6 that eCO_2 conditions caused a sharp increase in the photosynthesis rate ($p < 0.05$), equal to 16.3 µmol CO_2 m^{-2} s^{-1} in eCO_2-treated plants and 17.5 µmol CO_2 m^{-2} s^{-1} in eCO_2 + Bf treatment, which was 37% and 47% higher than that in unfertilized control plants, respectively. This increment in the individual application of biofertilizer was also significantly (+ 20%; $p < 0.05$) greater than the control treatment (Fig. 6). These results mirror the improvements in plant biomass production (Fig. 6). Accordingly, plant fresh weight increased from 5.3 g p/plant in control treatment to 8.8 (+ 66%), 8.5 (+ 60%), and 10.9 (+ 106%) g/plant, in response to eCO_2, Bf, and eCO_2 + Bf treatments, respectively. Such improvement for dry weight was also significant in eCO_2- and Bf-treated plants, so that the dry weight increased in eCO_2, Bf, and eCO_2 + Bf treatments about 2.7, 2.2, and 3.2 times higher than that in the unfertilized control plants, respectively (Fig. 6).

Discussion

One of the aims of the current research was to assess the shifts in carbohydrate metabolism in *M. vulgare* plants in response to plant growth-promoting bacteria treatment and eCO_2. In this regard, the highest total sugar content was recorded under eCO_2 conditions. The improvement in photosynthesis rate in eCO_2-treated plants (Fig. 6) can be attributed as the primary reason for the increase in carbohydrate production and, as a result, the alterations in the plants' carbon and nitrogen metabolism (Thompson et al. 2017). It has been reported that the high photosynthesis capacity in plants is associated with the high activation of Rubisco, since this photosynthetic enzyme is not usually saturated under aCO_2, and thus, it can improve the carboxylation rate under eCO_2 (Alsherif et al. 2023b). Moreover, the changes in the production of certain sugars compared to others under eCO_2 growth conditions have been reported. In this context, Thompson et al.'s (2017) study provided insight into how different sugars can be utilized to handle many functions in plants and how the amount of carbon partitioned into either of those sugars may be in part determined by which sugar the plant demands to regulate specific genes.

Although the highest production of glucose was observed under eCO_2 conditions, only combined treatment (eCO_2 + Bf) significantly enhanced fructose and sucrose contents (Fig. 2b). In this regard, Rogers et al. (2004) reported higher content of hexose sugars (e.g., glucose) in response to eCO_2, compared to sucrose, and suggested various source and sink activities in treated plants during the developing stages. Glucose, sucrose, and fructose are produced in source tissues as three main soluble sugars; among these, sucrose is then transported to the sink tissues (Ma et al. 2021). Besides sucrose, starch is one of the major end products of photosynthesis in most plants and is also a main carbon source involved in synthesizing other essential

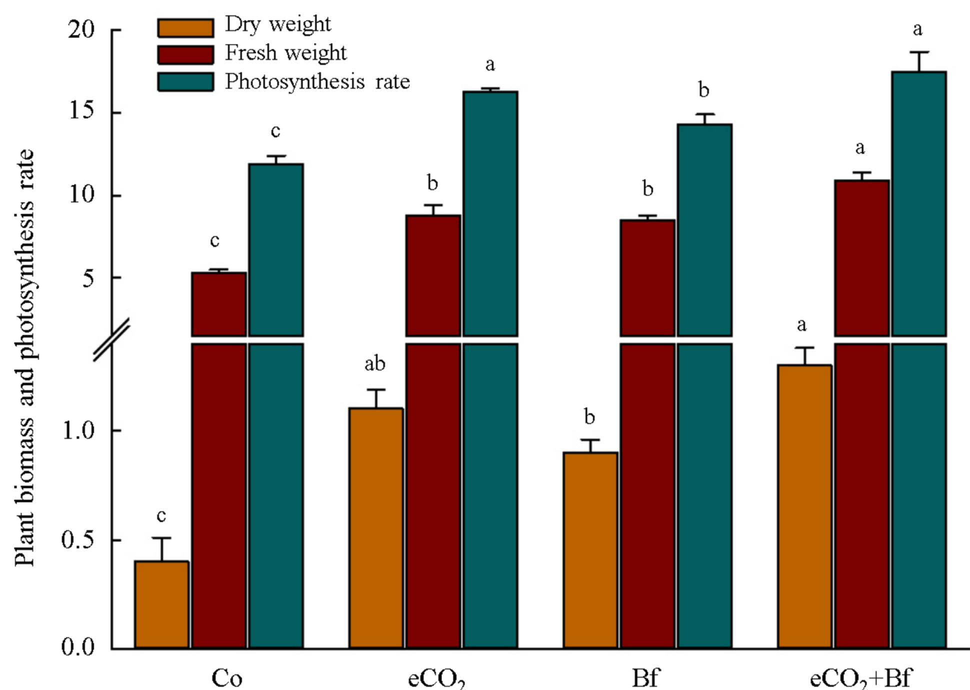

Fig. 6 The effect of plant growth-promoting *Micromonospora* sp (Bf) and elevated CO_2 (eCO_2) on plant biomass (g $plant^{-1}$) and photosynthesis rate (µmol CO_2 m^{-2} s^{-1}). Means in each parameter followed by similar letter(s) are not significantly different at 5% probability level ($p \leq 0.05$, Tukey test)

structural and metabolic compounds, such as proteins (Ma et al. 2019). An increment in starch content in plants grown under eCO_2 has already been reported by Aranjuelo et al. (2008), which is usually in a state of balance by overnight conversion to sucrose and storage during daylight hours (Smith et al. 2005). Nevertheless, Thompson et al. (2017) reported a gradual starch accumulation in eCO_2-treated plants over time because of uncomplete depletion of all of the plants' starch resources during the night. These results are consistent with those relative to enzyme activities involved in the assimilation and transport of carbohydrates in plants, sucrose synthase, and starch synthase enzymes (Fig. 2c). Likewise, it has been reported that enzyme activities are significantly influenced by eCO_2, which can therefore regulate many metabolic processes, such as photosynthesis, assimilation, and carbon partitioning. This process of carbon partitioning ultimately leads to increased plant biomass production (Hofmann et al. 2013; Ma et al. 2021).

It is evident that the content of nearly all detected amino acids in leaves was influenced by biofertilization (alanine, valine, serine, ornithine, and threonine), eCO_2 (histidine), and combined treatment (lysine, glycine, asparagine, tyrosine, phenylalanine, cysteine, aspartate, methionine, and leucine) (Table 2). This suggested the probability of their synthesis to acclimate to eCO_2 conditions, some of which were more accumulated in Bf-treated plants. Besides the involvement of essential amino acids in diverse physiological pathways in plants (e.g., protein biosynthesis and stimulation of stress tolerance in plants) (Rai 2002), these compounds are essential elements that contribute significantly to the nutritional value of the plant (e.g., histidine, methionine, lysine, valine, and threonine) (Alotaibi e al. 2021). In addition, some highly accumulated amino acids, detected in the current research, are known for their contribution to germination (e.g., methionine, phenylalanine, and threonine) and are considered natural plant growth stimulators (e.g., valine, serine, and leucine) (Paleckienė et al. 2007; Shen et al. 2021). Therefore, the high content of some relevant amino acids observed in the biofertilization and eCO_2 treatments could be assumed a fundamental advantage under such strategies and conditions. Similarly, recent research reported higher levels of amino acids content in response to microbial stimulants (Shen et al. 2021; Popkoet al. 2018) and eCO_2 (Saleh et al. 2018; Al Jaouni et al. 2018), which were associated with the improvement in plant growth and yield (Alotaibi et al. 2021; Shen et al. 2021). It seems that bio-inoculation and eCO_2 induced an up-regulation in photosynthesis. Consequently, it boosted the availability of non-structural carbohydrates, supplied C skeletons, and provided the required metabolic energy for the biosynthesis of amino acids (Shen et al. 2021; Nunes-Nesi et al. 2010).

Similar to amino acids, the content of almost all detected essential oils in plants showed an increase in PGPB grown under aCO_2 or eCO_2 conditions (Table 3), although the profile was similar to those found in unfertilized control plants. In line with the previous research, alternation in essential oils' composition in plants was observed in response to eCO_2 (Kumar et al. 2017) and PGPB (Araújo et al. 2020). It was suggested that the quantity and composition of essential oils could be changed depending on the growth conditions and environmental factors (Xiao et al. 2018). Araújo et al. (2020) proposed the potential of beneficial microorganisms in modifying the content of essential oils as a consequence of stimulating secondary metabolite production and accumulation. It appears that some essential oils were affected only by biofertilization treatment, such as β-caryophyllene and α-himachalene, a natural bicyclic sesquiterpene, which were among the most concentrated essential oils in plants. In contrast, another known terpenoid, carvacrol, was only affected by eCO_2 treatment. Although the reason for such differences is not clear, it is in accordance with the previous studies that demonstrated only PGPB seemed to induce the accumulation of some specific major compounds of the volatile fractions (Planchamp et al. 2014; Pace et al. 2020).

High CO_2 treatment improved the levels of some detected flavonoids and phenolic acids in *M. vulgare* plants, especially when they were treated with combined treatments (Figs. 3, 4). The improvement of some detected flavonoids (luteolin) and phenolic compounds (chlorogenic acid, caffeic acid, and gallic acid) in the current research was consistent with the previous reports under eCO_2 at 550 (Bindi et al. 2001), 750 (Zhang et al. 2021), and 800 µmol CO_2 mol^{-1} (Ghasemzadeh et al. 2010). This effect may be explained by the fact that eCO_2 can improve the synthesis of primary metabolites (e.g., soluble sugars), which can act as precursors of flavonoids and phenols synthesis (Zhang et al. 2021). Another plausible explanation for this phenomenon is the increase in sink capacity observed in plants grown under eCO_2. This, in turn, leads to a redirection of fixed carbon toward carbon-based secondary pathways, among which a prominent process involves the biosynthesis of phenolic compounds through the deamination of phenylalanine (Goufo et al. 2014). Likewise, observed increase in the content of phenylalanine amino acid in eCO_2-treated plants (Table 2) might indicate the availability of this precursor to being allocated to phenolic biosynthesis pathways.

Similarly, it has been reported that plant–PGPB interactions can affect plant growth by the mediation of the specialized host-derived secondary metabolites such as flavonoids under diverse environmental conditions (Wang et al. 2022). The evidence of positive effects of biofertilizer treatment can be seen in the case of higher content of some flavones (e.g., naringenin) (Fig. 3), which function as substrates for flavonoid biosynthetic enzymes, and consequently enhance synthesizing various arrays of flavonoid metabolites (Wang et al. 2022). Reciprocally, the production of some flavones (e.g., apigenin)

in plants can affect the beneficial microbes and improve their potential in root surface colonization by triggering biofilm formation (Yan et al. 2022), which might be one of the effective reasons for improving growth and biomass production in PGPB-treated plants. Also, the interaction between plants and beneficial microorganisms in higher production and utilization of some flavonols, such as quercetin, a plant pigment, and rutin, was previously reported (Zubek et al. 2015; Schütz et al. 2021). Moreover, an increase in the content of some amino acids (tyrosine and phenylalanine) by PGPB and eCO_2 treatments can be one of the reasons for the higher range of *p*-coumaric acid, because they are precursors in synthesizing *p*-coumaric acid through the shikimic acid pathway, which in turn may convert it into other phenolic acids and flavonoids (Roychoudhury et al. 2021). The results also indicated the effects of biofertilization and eCO_2 on the improvement of the level of chlorogenic acid, the most abundant form of phenolic acids in *M. vulgare* plants, which can be hydrolyzed and converted to caffeic acid and its esters like rosmarinic acid (Asther et al. 2005).

Such improvements in phenolic and flavonoid compounds in PGPB- and eCO_2-treated plants resulted in higher antioxidant activity (Fig. 5). A significant increment in the total antioxidant capacity was previously reported in response to the combined treatment of biofertilizer and eCO_2 even under non-stress conditions (Alsherif et al. 2023a). This is likely due to the elevated levels of carbon and nitrogen intermediates in the treated plants, which then contribute to the production of phytochemicals, such as flavonoids and phenols (AbdElgawad et al. 2021). Such antioxidant potential plays a vital role in acclimating the plant to climate change conditions (Oh et al. 2009), particularly by protecting the photosynthetic machine (AbdElgawad et al. 2014). Thus, this can be considered as one of the most important protection systems under eCO_2 conditions (Alsherif et al. 2023a). The potential of plant extracts to inhibit oxidation of low-density lipoprotein (LDL) was also investigated in plants treated with biofertilizer and eCO_2, based on the formation of conjugated dienes and thiobarbituric acid reagent substances (TBARS) (Fig. 5). Accordingly, the great ability of antioxidant activity in plant extracts was reported in association to up-regulating LDL receptor, due to the protective actions of plant phenols and flavonoids on lipoprotein metabolism (Salleh et al. 2020). Overall, this suggests that the synergy between PGPB and eCO_2 enhances the nutritional and pharmaceutical qualities of *M. vulgare* plants.

Conclusions

The current study was undertaken to assess the potential of plant growth-promoting Actinobacteria (*Micromonospora* sp.), as a biofertilizer, in improving some biochemical, nutritional quality, and pharmaceutical traits of *Marrubium vulgare* plants under ambient and eCO_2 conditions. It is clear that the *M. vulgare* plants have benefited from both biofertilizers and eCO_2, in which the synergistic consequence of applied treatments was remarkably more apparent than individual treatment application. Such strong potency of a *Micromonospora* sp. was reflected in heightened biomass production and photosynthetic capacity through improved biosynthesis of primary (+ 25–102%) and secondary metabolites (+ 27–296%) and promoting antioxidant capacity (+ 51–245%). Hence, the synergy between PGPB and eCO_2 not only offers a promising avenue for enhancing plant biomass and adaptability to complex climate changes but also enhances the nutritional and pharmaceutical qualities of *M. vulgare* plants.

Acknowledgements The authors would like to extend their sincere appreciation to the Researchers Supporting Project number (RSPD2023R1089), King Saud University, Riyadh, Saudi Arabia.

Author contributions All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by [HAE], [MYK], and [NH]. The first draft of the manuscript was written by [MRB, SMK, CC, EAA, AR, and AMZ] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding The authors would like to extend their sincere appreciation to the Researchers Supporting Project number (RSPD2023R1089), King Saud University, Riyadh, Saudi Arabia.

Data availability All data will be available after a reasonable request from the corresponding author.

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

Ethical approval This article is according to the international, national, and institutional rules considering biodiversity rights.

References

Abdallah SAS, Hassan HMS, El-Mekawy MA et al (2018) Application of tissue culture technique on *Marrubium vulgare* L. plant. Hortscience J Suez Canal Univ 7:1–10

AbdElgawad H, Peshev D, Zinta G et al (2014) Climate extreme effects on the chemical composition of temperate grassland species under ambient and elevated CO_2: a comparison of fructan and Non-fructan accumulators. PLoS ONE 9(3):e92044. https://doi.org/10.1371/journal.pone.0092044

AbdElgawad H, Okla MK, Al-amri SS et al (2021) Effect of elevated CO_2 on biomolecules' accumulation in caraway (*Carum carvi* L.) plants at different developmental stages. Plants 10:2434. https://doi.org/10.3390/plants10112434

Al Jaouni S, Saleh AM, Wadaan MAM et al (2018) Elevated CO_2 induces a global metabolic change in basil (*Ocimum basilicum* L.) and peppermint (*Mentha piperita* L.) and improves their

biological activity. J Plant Physiol 224–225:121–131. https://doi.org/10.1016/j.jplph.2018.03.016

Alotaibi MO, Khamis G, AbdElgawad H et al (2021) *Lepidium sativum* sprouts grown under elevated CO_2 hyper accumulate glucosinolates and antioxidants and exhibit enhanced biological and reduced anti nutritional properties. Biomolecules 11:1174. https://doi.org/10.3390/biom11081174

Alsherif EA, Yaghoubi Khanghahi M, Crecchio C et al (2023a) Understanding the active mechanisms of plant (*Sesuvium portulacastrum* L.) against heavy metal toxicity. Plants 12:676. https://doi.org/10.3390/plants12030676

Alsherif EA, Hajjar D, Aldilami M et al (2023b) Physiological and biochemical responses of wheat to synergistic effects of selenium nanoparticles and elevated CO_2 conditions. Front Plant Sci 14:1183185. https://doi.org/10.3389/fpls.2023.1183185

Aranjuelo I, Irigoyen JJ, Sánchez-Díaz M et al (2008) Carbon partitioning in N2 fixing *Medicago sativa* plants exposed to different CO_2 and temperature conditions. Funct Plant Biol 35:306–317. https://doi.org/10.1071/FP07296

Araragi M (1979) Actinomycete flora of tropical upland farm soils on the basis of genus composition and antagonistic property. Soil Sci Plant Nutr 25:513–521

Araújo NAF, Brandão RM, Barguil BM et al (2020) Plant growth-promoting bacteria improve growth and modify essential oil in rose (*Rosa hybrida* L.) cv. Black Prince Front Sustain Food Syst 4:606827. https://doi.org/10.3389/fsufs.2020.606827

Asther M, Estrada Alvarado MI, Haon M et al (2005) Purification and characterization of a chlorogenic acid hydrolase from Aspergillus niger catalysing the hydrolysis of chlorogenic acid. J Biotechnol 115(1):47–56. https://doi.org/10.1016/j.jbiotec.2004.07.009

Bakhshandeh E, Pirdashti H, Shahsavarpour Lendeh K et al (2020) Effects of plant growth promoting microorganisms inoculums on mineral nutrition, growth and productivity of rice (*Oryza sativa* L.). J Plant Nutr 43:1643–1660. https://doi.org/10.1080/01904167.2020.1739297

Barakate M, Ouhdouch Y, Oufdou K et al (2002) Characterization of rhizospheric soil streptomycetes from Moroccan habitatsand their antimicrobial activities. World J Microbiol Biotechnol 18:49–54

Bindi M, Fibbi L, Miglietta F (2001) Free air CO_2 enrichment (FACE) of grapevine (*Vitis Vinifera* L.): II. Growth and quality of grape and wine in response to elevated CO_2 concentrations. Eur J Agro 14:145–155

Cao M, Narayanan M, Shi X et al (2023) Optimistic contributions of plant growth-promoting bacteria for sustainable agriculture and climate stress alleviation. Environ Res 217:114924. https://doi.org/10.1016/j.envres.2022.114924

Cappuccino JC, Sherman N (1992) Microbiology: a laboratory manual. Benjamin Pub. Co., New York, pp 125–179

Chen C-W, Chang C-Y, Chiang S-H (2016) The inhibition effect of cell DNA oxidative damage and LDL oxidation by bovine colostrums. Molecules 21:1378

Clark JM, Switzer RL (1977) Experimental biochemistry, 2nd edn. W.H Freeman & Company, San Francisco

Demiroz Akbulut T, Aydin Kose F, Demirci B et al (2023) Chemical profile and cytotoxicity evaluation of aerial parts of *Marrubium vulgare* L. from different locations in Turkey Chem. Biodivers 2023:e202201188

Du B, Shukla MK, Yang X et al (2023) Enhanced fruit yield and quality of tomato by photosynthetic bacteria and CO_2 enrichment under reduced irrigation. Agric Water Manag 277:108106. https://doi.org/10.1016/j.agwat.2022.108106

Edziri H, Mastouri M, Aouni M et al (2012) Polyphenols content, antioxidant and antiviral activities of leaf extracts of *Marrubium deserti* growing in Tunisia. S Afr J Bot 80:104–109

Faddetta T, Polito G, Abbate L et al (2023) Bioactive metabolite survey of actinobacteria showing plant growth promoting traits to develop novel biofertilizers. Metabolites 13(3):374. https://doi.org/10.3390/metabo13030374

Fang R, Li Y, Yu Z et al (2022) Warming offsets the beneficial effect of elevated CO_2 on maize plant-carbon accumulation in particulate organic carbon pools in a Mollisol. CATENA 213:106219. https://doi.org/10.1016/j.catena.2022.106219

Fletcher RA, Kallumbil V, Steele P (1982) An improved bioassay for cytokinins using cucumber cotyledons. Plant Physiol 69:675–677

Ghasemzadeh A, Jaafar HZE, Rahmat A (2010) Elevated carbon dioxide increases contents of flavonoids and phenolic compounds, and antioxidant activities in Malaysian young ginger (*Zingiber officinale* Roscoe.) varieties. Molecules 15:7451–7466

Goufo P, Pereira J, Figueiredo N et al (2014) Effect of elevated carbon dioxide (CO_2) on phenolic acids, flavonoids, tocopherols, tocotrienols, γ-oryzanol and antioxidant capacities of rice (*Oryza sativa* L.). J Cereal Sci 59:15–24. https://doi.org/10.1016/j.jcs.2013.10.013

Guedri Mkaddem M, Zrig A, Ben Abdallah M et al (2022) Variation of the Chemical composition of essential oils and total phenols content in natural populations of *Marrubium vulgare* L. Plants 11:612. https://doi.org/10.3390/plants11050612

Habeeb TH, Abdel-Mawgoud M, Yehia RS et al (2020) Interactive impact of arbuscular mycorrhizal fungi and elevated CO_2 on growth and functional food value of *Thymus vulgare*. J Fungi 6:168. https://doi.org/10.3390/jof6030168

Hamad I, AbdElgawad H, Al Jaouni S et al (2015) Metabolic analysis of various date palm fruit (*Phoenix dactylifera* L.) cultivars from Saudi Arabia to assess their nutritional quality. Molecules 20:13620–13641. https://doi.org/10.3390/molecules200813620

Hofmann LC, Straub S, Bischof K (2013) Elevated CO_2 levels affect the activity of nitrate reductase and carbonic anhydrase in the calcifying rhodophyte *Corallina officinalis*. J Exp Bot 64:899–908. https://doi.org/10.1093/jxb/ers369

Holder I, Boyce S (1994) Agar well diffusion assay testing of bacterial susceptibility to various antimicrobials in concentrations non-toxic for human cells in culture. Burns 20:426–429

Holt JG, Krieg NR, Sneath PH et al (1994) Regular, nonsporing gram-positive rods. In: Bergey's manual of determinative bacteriology, 9th ed. Williams & Wilkins Co., Baltimore, MD, USA, p 566

Hong K, Gao AH, Xie QY et al (2009) Actinomycetes formarine drug discovery isolated from mangrove soils and plants in China. Mar Drugs 7:24–44

Ishnava KB, Shah PP (2014) Anticariogenic and hemolytic activity of selected seed protein extracts in vitro conditions. J Dent (tehran) 11(5):76–86

Kassim NK, Rahmani M, Ismail A et al (2013) Antioxidant activity-guided separation of coumarins and lignan from *Melicope glabra* (Rutaceae). Food Chem 139(1–4):87–92

Kawato M, Shinobu RA (1959) Simple technique for the microscopical observation, memoirs of the Osaka University Liberal Arts and Education. Nat Sci 8:114

Kumar R, Kaundal M, Sharma S et al (2017) Effect of elevated [CO_2] and temperature on growth, physiology and essential oil composition of *Salvia sclarea* L. in the western Himalayas. J Appl Res Med Aromat Plant 6:22–30. https://doi.org/10.1016/j.jarmap.2017.01.001

Küster E, Williams ST (1964) Selection of media for isolation of streptomycetes. Nature 202:928–929

Ma Y, Reddy VR, Devi MJ et al (2019) *De novo* characterization of the Goji berry (*Lycium barbarium* L.) fruit transcriptome and analysis of candidate genes involved in sugar metabolism under different CO_2 concentrations. Tree Physiol 39:1032–1045. https://doi.org/10.1093/treephys/tpz014

Ma Y, Xie Y, Ha R et al (2021) Effects of elevated CO_2 on photosynthetic accumulation, sucrose metabolism-related enzymes, and

genes identification in Goji Berry (*Lycium barbarum* L.). Front Plant Sci 12:643555. https://doi.org/10.3389/fpls.2021.643555
Mkaddem Mounira G, Ahlem Z, Abdallah Mariem B et al (2022) Essential oil composition and antioxidant and antifungal activities of two varieties of *Ocimum basilicum* L. (Lamiaceae) at two phenological stages. Agronomy 12:825. https://doi.org/10.3390/agronomy12040825
Nunes-Nesi A, Fernie AR, Stitt M (2010) Metabolic and signaling aspects underpinning the regulation of plant carbon nitrogen interactions. Mol Plant 3:973–996
Oh M-M, Trick HN, Rajashekar CB (2009) Secondary metabolism and antioxidants are involved in environmental adaptation and stress tolerance in lettuce. J Plant Physiol 166:180–191
Olsen S, Sommers LE (1982) Phosphorous. In: Methods of soil analysis. Part 2: CHEMICAL and microbiological properties. J Am Soc Agron 1982:403–427
Ortega-Ramirez LA, Rodriguez-Garcia I, Leyva JM et al (2014) Potential of medicinal plants as antimicrobial and antioxidant agents in food industry: a hypothesis. J Food Sci 79:129–137
Pace L, Pellegrini M, Palmieri S (2020) Plant growth-promoting rhizobacteria for *in vitro* and *ex vitro* performance enhancement of Apennines' Genepì (*Artemisia umbelliformis subsp. eriantha*), an endangered phytotherapeutic plant. In Vitro Cell Dev Biol Plant 56:134–142. https://doi.org/10.1007/s11627-019-10035-1
Paleckienė R, Sviklas A, Šlinkšienė R (2007) Physicochemical properties of a microelement fertilizer with amino acids. Russ J Appl Chem 80:352–357
Phillips GB, Hanel E (1950) Control of mold contaminants on solid media by the use of actidione. J Bacteriol 60:104–105
Planchamp C, Glauser G, Mauch-Mani B (2014) Root inoculation with *Pseudomonas putida* KT2440 induces transcriptional and metabolic changes and systemic resistance in maize plants. Front Plant Sci 5:719
Popko M, Michalak I, Wilk R et al (2018) Effect of the new plant growth biostimulants based on amino acids on yield and grain quality of winter wheat. Molecules 23:470
Porter JN, Wilhelm JJ, Tresner HD (1980) Method for the preferential isolation of actinomycetes from soils. Appl Microbiol 8:174–178
Rached S, Imatara H, Habsaoui A et al (2022) Characterization, Chemical Compounds and Biological Activities of *Marrubium vulgare* L. Essential Oil Process 10:2110. https://doi.org/10.3390/pr10102110
Rai V (2002) Role of amino acids in plant responses to stresses. Biol Plant 45:481–487
Reddy GS, Aggarwal RK, Matsumoto GI et al (2000) *Arthrobacter flavus* sp. nov., a psychrophilic bacterium isolated from apond in McMurdo Dry Valley. Antarctica Int JSyst Evol Microbiol 50:1553–1561
Rogers A, Allen DJ, Davey PA et al (2004) Leaf photosynthesis and carbohydrate dynamics of soybeans grown throughout their lifecycle under free-air carbon dioxide enrichment. Plant Cell Environ 27:449–458. https://doi.org/10.1111/j.1365-3040.2004.01163.x
Roychoudhury S, Sinha B, Choudhury BP et al (2021) Scavenging properties of plant-derived natural biomolecule para-coumaric acid in the prevention of oxidative stress-induced diseases. Antioxidants 10:1205. https://doi.org/10.3390/antiox10081205
Saleh AM, Selim S, Al Jaouni S et al (2018) CO_2 enrichment can enhance the nutritional and health benefits of parsley (*Petroselinum crispum* L.) and dill (*Anethum graveolens* L.). Food Chem 269:519–526
Salleh MZ, Runnie I, Roach PD et al (2020) Inhibition of low-density lipoprotein oxidation and up-regulation of low-density lipoprotein receptor in HepG2 cells by tropical plant extracts. J Agric Food Chem 50(13):3693–3697. https://doi.org/10.1021/jf011593f
Schlesier K, Harwat M, Böhm V et al (2002) Assessment of antioxidant activity by using different in vitro methods. Free Radical Res 36:177–187
Schütz V, Frindte K, Cui J et al (2021) Differential impact of plant secondary metabolites on the soil microbiota. Front Microbiol 12:666010
Sekhar KM, Reddy KSR, Reddy AR (2023) Photosynthesis and carbon sequestration efficacy of *Conocarpus erectus* L. (*Combretaceae*) grown under elevated CO_2 atmosphere. Plant Physiol Rep 28:113–123. https://doi.org/10.1007/s40502-022-00702-8
Shabbaj II, Madany MMY, Balkhyour MA et al (2022) CO_2 enrichment differentially upregulated sugar, proline, and polyamine metabolism in young and old leaves of wheat and sorghum to mitigate indium oxide nanoparticles toxicity. Front Plant Sci 13:843771. https://doi.org/10.3389/fpls.2022.843771
Shen M, Li J, Dong Y et al (2021) Profiling of plant growth-promoting metabolites by phosphate-solubilizing bacteria in maize rhizosphere. Plants 10:1071. https://doi.org/10.3390/plants10061071
Silva RG, Alves RC, Zingaretti SM (2020) Increased [CO_2] causes changes in physiological and genetic responses in C_4 crops: a brief review. Plants 9:1567. https://doi.org/10.3390/plants9111567
Smith AM, Zeeman SC, Smith SM (2005) Starch degradation. Annu Rev Plant Biol 56:73–89. https://doi.org/10.1146/annurev.arplant.56.032604.144257
Strafella S, Simpson DJ, Yaghoubi Khanghahi M et al (2021) Comparative genomics and in vitro plant growth promotion and biocontrol traits of lactic acid bacteria from the Wheat Rhizosphere. Microorganisms 9:78. https://doi.org/10.3390/microorganisms9010078
Thompson M, Gamage D, Hirotsu N et al (2017) Effects of elevated carbon dioxide on photosynthesis and carbon partitioning: a perspective on root sugar sensing and hormonal crosstalk. Front Physiol 8:578. https://doi.org/10.3389/fphys.2017.00578
Trujillo ME, Riesco R, Benito P et al (2015) Endophytic Actinobacteria and the interaction of *Micromonospora* and nitrogen fixing plants. Front Microbiol 6:1341. https://doi.org/10.3389/fmicb.2015.01341
Wang L, Chen M, Lam PY (2022) Multifaceted roles of flavonoids mediating plant-microbe interactions. Microbiome 10:233. https://doi.org/10.1186/s40168-022-01420-x
Xiao Z, Luo J, Niu Y et al (2018) Characterization of key aroma compounds from different rose essential oils using gas chromatography-mass spectrometry, gas chromatography-olfactometry and partial least squares regression. Nat Prod Res 32:1567–1572. https://doi.org/10.1080/14786419.2017.1389933
Yaghoubi Khanghahi M, Pirdashti H, Rahimian H et al (2019) The role of potassium solubilizing bacteria (KSB) inoculations on grain yield, dry matter remobilization and translocation in rice (*Oryza sativa* L.). J Plant Nutr 42:1165–1179. https://doi.org/10.1080/01904167.2019.1609511
Yaghoubi Khanghahi M, Strafella S, Crecchio C (2020) Changes in photo-protective energy dissipation of photosystem II in response to beneficial bacteria consortium in durum wheat under drought and salinity stresses. Appl Sci 10:5031. https://doi.org/10.3390/app10155031
Yaghoubi Khanghahi M, Leoni B, Crecchio C (2021) Photosynthetic responses of durum wheat to chemical/microbiological fertilization management under salt and drought stresses. Acta Physiol Plant 43:123. https://doi.org/10.1007/s11738-021-03289-z
Yaghoubi Khanghahi M, AbdElgawad H, Verbruggen E et al (2022) Biofertilisation with a consortium of growth-promoting bacterial strains improves the nutritional status of wheat grain under control, drought, and salinity stress conditions. Physiol Plant. https://doi.org/10.1111/ppl.13800
Yan D, Tajima H, Cline LC et al (2022) Genetic modification of flavone biosynthesis in rice enhances biofilm formation of soil

diazotrophic bacteria and biological nitrogen fixation. Plant Biotechnol J 20:2135–2148
Yemm E, Willis AJ (1954) The estimation of carbohydrate in plant extracts by Anthrone. Biochem J 57:508–514
You K, Park YA (1996) New method for the selective isolation of actinomycetes from soil. Biotechnol Tech 10:541–546
Zhang C, Jia X, Zhao Y et al (2021) The combined effects of elevated atmospheric CO_2 and cadmium exposure on flavonoids in the leaves of *Robinia pseudoacacia* L. seedlings. Ecotoxicol Environ Saf 210:111878. https://doi.org/10.1016/j.ecoenv.2020.111878
Zubek S, Rola K, Szewczyk A et al (2015) Enhanced concentrations of elements and secondary metabolites in *Viola tricolor* L. induced by arbuscular mycorrhizal fungi. Plant Soil 390(1):129–142

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.