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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Effects of Increased Carbon Dioxide and Temperature on Menthol Mint under Controlled Greenhouse Conditions: Yield, Essential Oil and Phenolic Composition*

Kontrollü Sera Koşullarında Artan Karbondioksit ve Sıcaklığın Mentollü Nane Üzerine Etkileri: Verim, Uçucu Yağ ve Fenolik Bileşimi

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Abstract

Climate change, characterized by rising atmospheric CO_2 concentrations and global temperatures, presents significant challenges and opportunities for agriculture, particularly for medicinal and aromatic plants. This study investigates the interactive effects of elevated CO_2 (400, 600, 800, and 1000 ppm) and temperature regimes (26/16°C and 29/19°C) on growth, essential oil composition, and phenolic profile of *Mentha arvensis* under controlled greenhouse conditions. A split-plot design with four replicates was used to assess plant growth parameters, essential oil yield and composition via GC-MS, and phenolic content through HPLC-PDA. The results revealed that elevated CO_2 significantly enhanced biomass accumulation in *M. arvensis*, with the highest yield recorded at 1000 ppm CO_2 under moderate temperature conditions. However, increases in temperature alone negatively affected leaf biomass, indicating a stress-induced shift in resource allocation. Essential oil content showed no statistically significant variation across treatments, and menthol, the major component, ranged between 77.2% and 82.5%. Among the phenolic compounds, rosmarinic acid was found to be particularly sensitive to the interaction between CO_2 and temperature. Under moderate temperatures, its concentration increased as CO_2 levels rose, whereas high-temperature conditions led to a decline, suggesting compromised secondary metabolism under heat stress. These findings indicate that secondary metabolites in *M. arvensis* exhibit species-specific and non-linear responses to climate variables, highlighting the potential of optimizing controlled environmental conditions to enhance the production of targeted bioactive compounds. This work contributes to understanding climate resilience in high-value crops such as *M. arvensis*, a natural source of crystalline menthol, and provides insights into sustainable phytochemical production strategies under changing environmental conditions.

Keywords: *Mentha arvensis*, Temperature, CO_2, Menthol, Rosmarinic acid

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Öz

İklim değişikliği, artan atmosferik CO_2 konsantrasyonları ve küresel sıcaklıklarla karakterize edilmekte olup, özellikle tıbbi ve aromatik bitkiler açısından tarım için önemli zorluklar ve fırsatlar sunmaktadır. Bu çalışma, kontrollü sera koşullarında, yükseltilmiş CO_2 seviyelerinin (400, 600, 800 ve 1000 ppm) ve sıcaklık rejimlerinin (26/16°C ve 29/19°C), *Mentha arvensis* bitkisinin büyümesi, uçucu yağ bileşimi ve fenolik profili üzerindeki etkileşimli etkilerini araştırmaktadır. Bitki büyüme parametrelerini, uçucu yağ verimini ve bileşimini GC-MS ile, fenolik içeriği ise HPLC-PDA yöntemiyle değerlendirmek amacıyla dört tekerrürlü bölünmüş parseller deneme deseni kullanılmıştır. Sonuçlar, *M. arvensis* türünde yükseltilmiş CO_2'nin biyokütle üretimini anlamlı düzeyde artırdığını göstermiştir; en yüksek verimler, orta sıcaklık koşullarında (26/16°C) 1000 ppm CO_2 seviyesinde elde edilmiştir. Ancak, sıcaklık artışının tek başına yaprak biyokütlesi üzerinde olumsuz etkileri olduğu ve bunun kaynakların stres nedeniyle farklı yönlere yönlendirilmesinden kaynaklanabileceği gözlemlenmiştir. Uçucu yağ içeriği, uygulamalar arasında istatistiksel olarak anlamlı bir değişiklik göstermemiştir; ana bileşen olan mentol oranı ise %77.2 ile %82.5 arasında bulunmuştur. Özellikle fenolik bileşikler içerisinde rozmarinik asit**,** CO_2 × sıcaklık etkileşimine oldukça duyarlı çıkmıştır. Orta sıcaklıkta, artan CO_2 seviyeleriyle birlikte rozmarinik asit konsantrasyonu yükselmiştir. Buna karşılık, yüksek sıcaklık koşulları altında bu bileşiğin düzeyinde düşüş gözlemlenmiş, bu da yüksek ısı stresinin sekonder metabolizmayı olumsuz etkilediğini düşündürmektedir. Bu bulgular, sekonder metabolitlerin iklim değişkenlerine karşı *M. arvensis*' te tür bazlı ve doğrusal olmayan tepkiler gösterdiğini ortaya koymakta ve kontrollü ortam koşullarının, hedeflenen biyoaktif bileşik üretimini artırmak için optimize edilebileceğini vurgulamaktadır. Bu çalışma, doğal kristal mentol kaynağı olan *M. arvensis* gibi yüksek değerli bitkilerin iklim direncine dair anlayışa katkı sağlamakta ve değişen çevresel koşullarda sürdürülebilir fitokimyasal üretim için geleceğe dönük stratejilere ışık tutmaktadır.

Anahtar Kelimeler: *Mentha arvensis*, Sıcaklık, CO_2, Mentol, Rozmarinik asit

1. Introduction

Global climate change, primarily driven by accelerated industrialization in recent decades, poses one of the most pressing challenges to ecosystems and future sustainability. Among the key drivers of climate change are elevated atmospheric carbon dioxide (CO_2) concentrations and rising temperatures, both of which have well-documented effects on agricultural productivity (Urkmez et al., 2024). CO_2 and temperature are fundamental environmental variables that significantly influence plant growth and development (Chang et al., 2016).

CO_2 enters plant leaves through stomata and diffuses into the intercellular spaces of mesophyll cells (Vince and Zoltan, 2011; Yavas and Unay, 2018). In the presence of water and light, CO_2 is assimilated through the process of photosynthesis facilitated by pigment molecules and converted into various organic compounds (Turk and Celik, 2006). As a result, elevated atmospheric CO_2 levels play a crucial role in enhancing photosynthetic activity, thereby promoting plant growth and biomass accumulation (Gamage et al., 2018).

Temperature, in conjunction with CO_2, further modulates plant physiological and biochemical processes. Several studies have demonstrated that elevated CO_2 and temperature conditions can stimulate biomass production in species such as *Gynostemma pentaphyllum* (Chang et al., 2016) and *Salvia sclarea* L. (Kumar et al., 2017). Similarly, enhanced growth responses under elevated CO_2 have been reported for various crops, including *Arabidopsis thaliana*, tobacco, tomato, wheat, and *Isatis indigotica* (Leakey et al., 2009; Aranjuelo et al., 2011; Li et al., 2013, 2017; Noguchi et al., 2015).

Increased CO_2 enhances photosynthetic rates, resulting in greater accumulation of non-structural carbohydrates such as starch and polysaccharides in the leaves. These are subsequently transported to other plant organs, contributing to increased overall biomass (Miglietta et al., 1998; Zhang et al., 2012). A wealth of literature supports the positive effects of elevated CO_2 on plant growth and yield, primarily attributed to improvements in photosynthetic efficiency (Miglietta et al., 1998; Morison and Lawlor, 1999; He et al., 2010; Sun et al., 2010; Vannette and Hunter, 2011; De la Mata et al., 2012; Zhang et al., 2012).

Beyond growth, elevated CO_2 and temperature can significantly affect plant physiology and alter both primary and secondary metabolic pathways (Chang et al., 2016). For instance, high CO_2 and temperature have been shown to reduce nitrogen content, total antioxidant capacity, and antioxidant compound levels in strawberry fruits (Sun et al., 2012). Similarly, reductions in specific secondary metabolites such as kaempferol and isorhamnetin aglycones in *Ginkgo biloba* (Huang et al., 2010), and cardenolides in *Asclepias syriaca* (Vannette and Hunter, 2011), have been reported under elevated CO_2 conditions.

Kumar et al. (2017) noted changes in the essential oil composition of *Salvia sclarea* L. in response to elevated CO_2 and temperature, while Jaafar et al. (2012) observed increased gallic acid synthesis in *Labisia pumila* varieties. However, the responses of secondary metabolites to environmental changes are highly species-specific. While some plant species exhibit increased secondary metabolite production under high-temperature conditions (Sallas et al., 2003; Loreto et al., 2006), others show reductions (Snow et al., 2003), highlighting the complexity of plant metabolic responses.

Among medicinal and aromatic plants, environmental factors play a pivotal role in shaping physiological and biochemical traits (Celik and Ayran, 2020). *Mentha* species are of considerable commercial importance due to their diverse bioactive compounds (Soltanbeigi and Ozguven, 2021). In particular, menthol mint (*Mentha arvensis*) is extensively cultivated for its high menthol content and broad applications across pharmaceutical, food, and cosmetic industries. It is also a rich source of flavonoids, phenolic acids, and essential oils.

M. arvensis contains flavonoids such as luteolin, mentoside, isorhoifolin, rutin, and hesperidin (Thawkar, 2016), as well as phenolic acids including caffeic, chlorogenic, and rosmarinic acids (Mahishi et al., 2005; Kumar and Chattopadhyay, 2007). Among these, rosmarinic acid is particularly significant due to its potent antioxidant properties and its abundance in the Lamiaceae family (Yanishlieva et al., 2006; Malayoglu, 2010; Yagcioglu, 2015; Celik and Ayran, 2020; Saglam et al., 2022). This compound is believed to contribute to plant defense against environmental stress and pathogens (Elmas, 2021).

Given the economic and medicinal significance of *M. arvensis*, and its sensitivity to environmental conditions, it serves as an ideal model to investigate plant responses to changing climate parameters. This study aims to assess

the impact of elevated CO_2 and temperature on the growth, development, essential oil composition, and phenolic acid profile particularly rosmarinic acid content of *M. arvensis* grown under controlled greenhouse conditions.

2. Materials and Methods

2.1. Plant material

This study utilized *Mentha arvensis* L. var. *piperascens* Holmes as the plant material. This taxon was selected based on prior assessments of mint genetic diversity, having been identified as a superior genotype due to its high essential oil content and elevated menthol concentration.

2.2. Greenhouse experiment

The experiment was conducted in a fully automated greenhouse equipped with four separate CO_2-regulated chambers (each 25 m²) at the Faculty of Agriculture, Malatya Turgut Ozal University. Two temperature regimes 26/16°C and 29/19°C (day/night) and four CO_2 concentrations (400, 600, 800, and 1000 ppm) were applied.

The study followed a split-plot experimental design with four replicates. Temperature served as the main plot factor, while CO_2 concentrations were assigned to sub-plots. Each chamber maintained a 14-hour light/10-hour dark photoperiod. Ambient CO_2 concentrations outside the greenhouse ranged from 370 to 430 ppm; thus, 400 ppm was used as the control.

Rooted cuttings from parent plants were transplanted into pots, with three seedlings per pot. Plants were grown under the specific treatment conditions assigned to each chamber. At full flowering, fresh herb biomass was harvested and weighed. Plant material was subsequently dried at 35°C until a constant weight was achieved, after which dry herb yield was recorded. Leaves were then separated from stems to determine dry leaf yield.

2.3. Essential oil distillation

Essential oils were extracted using a Clevenger-type apparatus. For each treatment group, 10 g of dried leaf material was distilled with 100 mL of water for 2 hours. The resulting oil volume was measured using a graduated burette and expressed as mL per 100 g of dry material (mL 100 g^{-1}). Extracted oils were stored at +4°C in amber vials until further analysis by GC-MS, following the method of Telci et al. (2011).

2.4. Essential oil component analysis

Essential oil composition was analyzed using gas chromatography-flame ionization detection/mass spectrometry (GC-FID/MS) with an Agilent 7890B GC System coupled to a 5977B Mass Selective Detector. Separation was performed using a HP-Innowax FSC column (60 m × 0.25 mm I.D., 0.25 µm film thickness). Helium was used as the carrier gas at a constant flow rate of 0.8 mL min^{-1}.

The oven temperature was programmed as follows: held at 60°C for 10 min, increased at 4°C min^{-1} to 220°C (held for 10 min), then increased at 1°C min^{-1} to a final temperature of 240°C. Injection and detector temperatures were set to 250°C and 300°C, respectively. The injection volume was 1 µL (10% v v^{-1} in hexane), applied with a split ratio of 1:30. Mass spectra were recorded over the range m/z 35–450 using an electron impact energy of 70 eV. Identifications were made by comparing retention times and mass spectra with commercial libraries and literature data.

2.5. Phenolic composition analysis

Quantitative analysis of phenolic compounds was conducted using high-performance liquid chromatography with a photodiode array detector (HPLC-PDA) on a Shimadzu Nexera-i LC-2040C 3D system. Separation was achieved using a phenyl-hexyl reversed-phase column (4.6 × 150 mm, 3 µm, GL Sciences Inter Sustain).

The mobile phase consisted of 0.1% formic acid in water (Solvent A) and acetonitrile (Solvent B), delivered at a flow rate of 1.0 mL min^{-1}. The gradient program was as follows: 95:5 (A:B) at 0 min, 90.5:9.5 at 7 min, 83:17 at 20 min, 40:60 at 35 min, and 100:0 at 40 min. Column temperature was maintained at 30°C. Injection volume was 10 µL for both standards and samples.

Standard stock solutions (1 mg mL^{-1}) were prepared and calibration curves were established for quantification. Fifteen phenolic compounds were targeted, including gallic acid, chlorogenic acid, epicatechin, ferulic acid, eriocitrin, rosmarinic acid, apigenin-7-O-glucoside, cinnamic acid, quercetin, and naringenin. Concentrations of

these compounds in the extracts were calculated and expressed as µg/g dry weight. Analytical performance parameters of the method are provided in *Table 1*.

Table 1. The analytical performance parameters of the method for the phenolic compounds

Compounds	RT (min)	Calibration Equation	R^2	LOD (µg/mL)	LOQ (µg/mL)
Gallic acid	4.352	y = 29799.9X + 6494.60	0.9995	0.7440	2.2547
Chlorogenic acid	12.073	y = 28066.0X + 25870.2	0.9990	1.3687	4.1476
Epicatechin	14.150	y = 4788.08X + 789.457	0.9986	8.4615	25.6410
Ferulic acid	20.971	y = 46665.0X - 14606.2	0.9928	1.3865	4.2016
Eriocitrin	21.437	y=4227.14X-161.184	0.9999	6.3218	19.1570
Rosmarinic acid	27.011	y=34304.9X-12772.0	0.9999	1.0053	3.0464
Apigenin 7-O-glucoside	27.574	y = 39321.9X + 1685.18	0.9993	0.7295	2.2107
Cinnamic acid	30.234	y=75026.0X-11276.0	0.9988	0.6776	2.0533
Quercetin	32.008	y = 26403.4X + 1558.71	0.9997	1.2724	3.8560
Naringenin	34.939	y = 24207.0X + 2212.93	0.9920	1.3147	3.9840

2.6. Statistical analysis

Data were analyzed using analysis of variance (ANOVA) based on a split-plot design. Temperature was treated as the main plot factor and CO_2 concentration as the sub-plot factor. Post-hoc comparisons were made using Tukey's HSD test to determine statistically significant differences between means ($p < 0.05$ and $p < 0.01$). Statistical analyses were conducted using SPSS software, version 28.

3. Results and Discussion

This study examined the effects of varying temperature and CO_2 concentrations on plant growth, yield, essential oil content and composition, and phenolic profile of *Mentha arvensis*.

3.1. Plant growth and yield responses

As shown in *Table 2*, both plant height and fresh and dry herb yields significantly decreased ($p < 0.01$) with rising temperatures. However, dry leaf yield did not follow this trend. While elevated temperatures significantly reduce other yield components, leaf yield within the same group appears to be modulated by variations in leaf-to-biomass ratio. Notably, an increase in total biomass may result in a relative decline in leaf proportion (Telci, 2001).

While the average essential oil content increased from 4.0% at 26/16°C to 5.2% at 29/19°C, this difference was not statistically significant. In contrast, CO_2 concentrations had a significant effect on all measured parameters (*Table 2*). Plant height and herb yields consistently increased with higher CO_2 levels, with the greatest values observed at 1000 ppm. These findings are consistent with previous studies emphasizing the pivotal role of CO_2 in photosynthesis and biomass accumulation through enhanced carbon assimilation (Lemon, 1983; Acock and Allen, 1985; Drake and Leadley, 1991; Gamage et al., 2018).

Although *M. piperita* and *M. spicata* prefer milder climates, *M. arvensis* exhibits a higher thermal tolerance (Farooqi and Sharma, 1988; Singh et al., 2005). However, elevated and constant greenhouse temperatures may have induced thermal stress, particularly in early growth stages, potentially impacting growth-related traits. The decrease in yields at 29°C suggests that stress responses were activated, modifying growth patterns controlled by multiple genes and influenced heavily by environmental cues.

Table 2. Effects of different temperatures and carbon dioxide concentrations on Mentha arvensis

Temperature (°C)	CO_2 (ppm)	Plant Height (cm)	Fresh Herba (g plant^{-1})	Dry Herba (g plant^{-1})	Dry Leaf (g plant^{-1})	Essential Oil (%)
26/16	400	59.3	93.4	16.7	10.3	4.3
	600	75.6	179.1	30.3	16.5	3.9
	800	73.8	130.3	22.6	12.7	3.5
	1000	77.8	197.5	42.3	21.8	4.1
29/19	400	42.6	80.0	15.3	14.3	5.0
	600	50.5	90.2	15.3	14.7	5.1
	800	70.0	134.5	25.4	20.7	5.5
	1000	68.2	133.0	25.1	19.8	5.2
MEAN VALUES						
CO_2 (ppm)	400	50.9b	86.7c	16.0c	12.8b	4.6
	600	63.0ab	133.6b	22.8b	15.6b	4.5
	800	71.9a	132.4b	24.0b	16.7ab	4.5
	1000	73.0a	165.2a	33.7a	20.8a	4.6
Temperature (°C)	26/16	71.6a	149.6a	27.0a	15.3	4.0
	29/19	57.8b	109.4b	20.3b	17.6	5.2
F VALUES						
	$F_{TemxCO2}$	1.1^{ns}	4.8^{*}	6.8^{**}	2.8^{ns}	2.3^{ns}
	F_{CO2}	7.6^{**}	8.03^{**}	12.6^{**}	4.8^{*}	0.2^{ns}
	F_{Tem}	11.2^{**}	15.4^{**}	15.5^{**}	2.5^{ns}	1.8^{ns}

*p<0.05; **p<0.01; ns: non-significant

Additionally, the interaction between CO_2 and temperature was statistically significant. While the highest fresh and dry herbage yields were achieved at 1000 ppm CO_2 under the 26/16°C regime, the highest values at 29/19°C were recorded at 800 ppm CO_2. This interaction suggests that elevated CO_2 cannot fully counteract the negative effects of higher temperatures, a trend also supported by prior studies (Balasooriya et al., 2018; Sun et al., 2012).

Elevated CO_2 is known to stimulate photosynthesis, promoting the synthesis and translocation of non-structural carbohydrates to various plant organs, thus increasing total biomass (Miglietta et al., 1998; Zhang et al., 2012; Chang et al., 2016). However, when combined with thermal stress, physiological constraints such as reduced nutrient uptake, increased water loss, and shorter internodes can reduce yield significantly (Tursun et al., 2018).

3.2. Essential oil content and composition

Essential oil content ranged from 3.5% to 5.5% depending on the applied treatments. Despite a general upward trend with increased CO_2 and temperature, the differences were not statistically significant. GC-MS analysis identified eight major compounds accounting for 92.5%–96.8% of total oil content: limonene, menthone, isomenthone, isopulegol, neomenthol, isoisopulegol, menthol, and piperitone (*Figure 1*). Menthol was the dominant constituent, comprising 77.20%–82.45% of the total essential oil, with limited variation across treatments.

Among the secondary components, isomenthone exhibited a consistent increase in response to elevated CO_2 concentrations, whereas menthone content increased up to 800 ppm CO_2 before subsequently declining. Both compounds were more abundant under the higher temperature regime (*Table 3*). These trends are consistent with previous studies reporting that elevated temperatures either enhanced menthol biosynthesis (Duriyaprapan et al., 1986; Singh et al., 1988) or led to increased menthone accumulation, potentially due to the inhibition of its conversion pathways under high temperatures or shortened photoperiods (Murray et al., 1986; Franz et al., 1984).

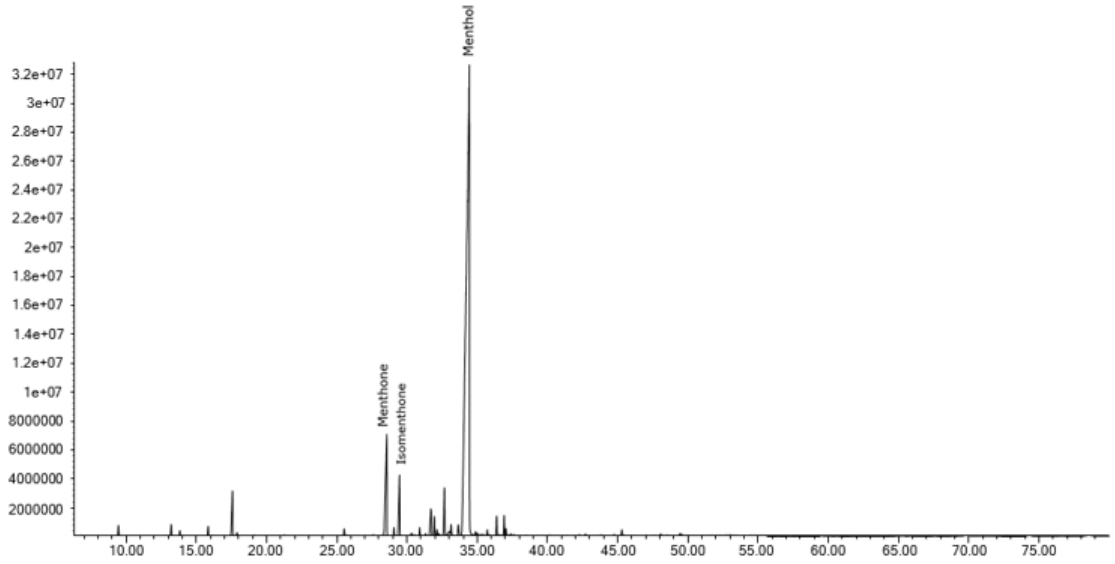


Figure 1. GC-MS chromatogram of Mentha arvensis essential oil

3.3. Phenolic composition

The effects of CO_2 and temperature on phenolic compounds were substantial and varied (*Table 4*). Among the 11 compounds analyzed, all except quercetin exhibited significant changes in response to CO_2. The most prominent findings were related to rosmarinic acid, a key phenolic compound in *Mentha* species.

Rosmarinic acid levels increased steadily with higher CO_2 concentrations at 26/16°C but declined at 29/19°C. This indicates a significant temperature × CO_2 interaction, suggesting that moderate temperatures combined with elevated CO_2 enhance secondary metabolism, while higher temperatures impair it.

This pattern is in line with previous reports highlighting the positive effects of elevated CO_2 on secondary metabolite production under optimal thermal conditions, likely due to increased carbon assimilation and substrate availability (Ainsworth and Long, 2005; Taub, 2010; Pant et al., 2021). However, at higher temperatures, the benefits of elevated CO_2 are reduced, potentially due to increased photorespiration, oxidative stress, and reduced CO_2 solubility, factors that can inhibit phenolic biosynthesis (Stiling and Cornelissen, 2007; Bita and Gerats, 2013).

Interestingly, the highest rosmarinic acid content was recorded at the lowest CO_2 concentration (400 ppm), with a decline observed at higher concentrations. This could be attributed to a metabolic shift favoring biomass over secondary metabolite production under high CO_2, a hypothesis supported by literature suggesting that excessive CO_2 can downregulate secondary metabolism in favor of primary growth (Al Jaouni et al., 2018; Isah, 2019).

3.4. Specific responses to CO_2 and temperature

The interaction between temperature and CO_2 underscores the complexity of plant responses to climate change variables. Similar variable outcomes have been observed in other species. For instance, terpene concentrations in *Pinus sylvestris* and *Picea abies* were found to decrease with CO_2 but increase with temperature (Sallas et al., 2003). Naghiloo et al. (2012) noted that plant age and temperature contribute to phenolic accumulation, while Snow et al. (2003) reported a 64% reduction in monoterpenes under combined high CO_2 and high temperature. These findings reinforce the concept that secondary metabolite responses are highly species- and condition-specific.

Table 3. Effects of different temperatures and carbon dioxide concentrations on essential oil components (%) of Mentha arvensis

Temperature (°C)	CO_2 (ppm)	Limonene	Menthone	Isomenthone	Isopulegol	Neomenthol	Isoisopulegol	Menthol	Piperitone	Σ
26/16	400	1.00	5.30	4.50	0.80	2.20	0.80	77.20	0.70	92.50
	600	0.65	5.30	4.15	0.70	1.95	0.55	80.50	0.70	94.50
	800	0.95	6.23	4.48	0.75	2.15	0.60	78.65	0.60	94.40
	1000	0.95	4.75	4.95	0.90	2.53	0.70	78.40	0.68	93.85
29/19	400	1.10	5.70	3.40	0.80	2.05	0.60	82.45	0.70	96.80
	600	0.75	5.05	4.70	0.90	2.30	0.55	79.45	0.70	94.40
	800	0.90	5.90	5.55	1.05	2.85	0.70	74.90	0.85	92.70
	1000	0.90	4.35	5.25	1.05	2.75	0.70	78.55	0.70	94.25
MEAN VALUES										
CO_2 (ppm)	400	1.03	5.43	3.95	0.80	2.13	0.73	79.83	0.70	94.60
	600	0.70	5.18	4.43	0.77	2.13	0.55	79.98	0.70	94.42
	800	0.93	6.06	5.01	0.90	2.50	0.65	76.78	0.73	93.55
	1000	0.93	4.55	5.10	1.00	2.64	0.70	78.48	0.69	94.08
Temperature (°C)	26/16	0.77	4.19	4.21	0.88	2.22	0.71	81.34	0.59	94.91
	29/19	1.00	6.26	5.04	0.85	2.48	0.59	76.19	0.81	93.21

Table 4. Effects of different temperatures and carbon dioxide concentrations on phenolic compounds (µg mg $^{-1}$) of Mentha arvensis

Temperature (°C)	CO_2 (ppm)	Gallic Acid	Chlorogenic Acid	epi-Catechin	Ferulic Acid	Eriocitrin	Rosmarinic Acid	Apigenin-7-glucoside	Cinnamic Acid	Quercetin	Naringenin
26/16	400	0.011	0.047	0.026	0.009	0.210	1.668	0.038	0.000	0.002	0.008
	600	0.026	0.029	0.003	0.000	0.031	1.556	0.049	0.001	0.002	0.000
	800	0.026	0.034	0.000	0.004	0.082	3.061	0.062	0.003	0.002	0.341
	1000	0.022	0.032	0.000	0.000	0.000	5.420	0.053	0.009	0.007	0.317
29/19	400	0.024	0.077	0.008	0.013	0.039	5.388	0.027	0.004	0.029	0.126
	600	0.017	0.031	0.000	0.002	0.035	0.698	0.038	0.003	0.027	0.273
	800	0.020	0.038	0.008	0.001	0.091	1.258	0.038	0.003	0.038	0.229
	1000	0.015	0.017	0.000	0.000	0.010	0.667	0.035	0.005	0.014	0.233
MEAN VALUES											
CO_2 (ppm)	400	0.018	0.062	0.017	0.011	0.124	3.528	0.033	0.002	0.015	0.067
	600	0.022	0.030	0.002	0.001	0.033	1.127	0.044	0.002	0.015	0.137
	800	0.023	0.036	0.004	0.003	0.087	2.160	0.050	0.003	0.020	0.285
	1000	0.019	0.024	0.000	0.000	0.005	3.044	0.044	0.007	0.010	0.275
Temperature (°C)	26/16	0.021	0.036	0.007	0.003	0.081	2.926	0.051	0.003	0.003	0.167
	29/19	0.019	0.041	0.004	0.004	0.044	2.003	0.035	0.004	0.027	0.215
F VALUES											
	F **Tem×CO_2**	8.20^{**}	10.50^{**}	5.4^{*}	1.33^{ns}	30.64^{***}	305.0^{**}	0.22^{ns}	1.33^{ns}	7.81^{**}	59.77^{**}
	F $_{CO2}$	3.40*	29.64**	12.45**	22.67**	43.75**	110.5**	4.39*	11.33**	1.77^{ns}	78.56*
	F $_{Tem}$	1.80^{ns}	3.67*	1.125^{ns}	0.00^{ns}	19.67**	84.64**	22.56**	0.00^{ns}	$1.6.7^{ns}$	16.73**

*p<0.05; **p<0.01; ns: non-significant

4. Conclusions

This study investigated the effects of elevated carbon dioxide and temperature on the yield, essential oil content, and phenolic composition of *Mentha arvensis* under controlled greenhouse conditions. Biomass yield increased with temperature; however, this was not accompanied by a corresponding rise in leaf yield, likely due to a disproportionate increase in stem and branch biomass. Elevated CO_2 concentrations significantly enhanced plant growth and total herb yield, yet had limited impact on essential oil and menthol content. Rosmarinic acid, a major phenolic compound, exhibited a more pronounced and temperature-dependent response to CO_2 enrichment than essential oil constituents. At 26/16°C, rosmarinic acid levels increased with rising CO_2, whereas this trend was reversed at 29/19°C.

These results underscore the species-specific and interactive effects of environmental factors on secondary metabolite biosynthesis. Optimizing greenhouse conditions could enhance the targeted production of high-value compounds such as menthol and rosmarinic acid. Future research should further investigate the physiological mechanisms underlying these responses and explore practical applications for controlled-environment agriculture and pharmaceutical production.

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Ethical Statement

There is no need to obtain permission from an ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Devrim, S.; Telci, I.; Tursun, A. O.; Design: Devrim, S.; Elmastas, M.; Tursun, A. O.; Ozek, T.; Ozek, G.; Telci, I.; Data Collection or Processing: Devrim, S.; Elmastas, M.; Tursun, A. O.; Ozek, T.; Ozek, G.; Telci, I.; Statistical Analyses: Telci, I.; Devrim, S.; Literature Search: Devrim, S.; Elmastas, M.; Tursun, A. O.; Telci, I.; Writing, Review and Editing: Devrim, S.; Tursun, A. O.; Telci, I.

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