


The Effect of Photoperiod and CO₂ Concentration on Growth, Phytochemical Characteristics and Essential Oil Content of Thyme

Ahmed Kareem Hussein Aljammali, Sahebali Bolandnazar*, Saeedeh Alizadeh-Salte and Jafar Hajiloo

Department of Horticultural Sciences, University of Tabriz, Iran

Article Info	ABSTRACT
<p>Article Type Original Article</p> <p>Article History Received: 24 April 2025 Accepted: 21 May 2025 © 2012 Iranian Society of Medicinal Plants. All rights reserved.</p> <p>*Corresponding author sbolandnazar@gmail.com</p>  <p>How to cite this paper Aljammali, A.K.H., Bolandnazar, S., Alizadeh-Salte, S., Hajiloo, J. The Effect of Photoperiod and CO₂ Concentration on Growth, Phytochemical Characteristics and Essential Oil Content of Thyme. Journal of Medicinal Plants and By-products, 2025; 14(6):607-613. doi: 10.22034/jmpb.2025.369084.1940</p>	<p><i>Thymus vulgaris</i> L., a member of the Lamiaceae family, is one of the most widely used medicinal and aromatic plants. This experiment aimed to investigate the effects of CO₂ enrichment at 350, 700, and 1000 mg/L (C1, C2, and C3) combined with supplementary lighting photoperiods of 12, 16, and 20 hours (L1, L2, and L3) on several attributes of <i>T. vulgaris</i>. Measurements included fresh and dry weights of plant organs, photosynthetic pigments, essential oil quantity and composition, as well as the activity of catalase, ascorbate peroxidase, and polyphenol oxidase. The antioxidant capacity, along with the assessment of phenolic compounds and flavonoid content, was also conducted. The L3C3 treatment significantly improved several parameters, including fresh weight, dry weight, plant height, chlorophyll a, chlorophyll b, essential oil content, carotenoids, phenolic compounds, flavonoids, and antioxidant activity. These showed respective increases of 110%, 276%, 82%, 63%, 63%, 160%, 45%, 68%, 13%, and 45%. Additionally, the L3C1 treatment significantly enhanced peroxidase activity by a factor of 108%. Furthermore, under the L3C1 treatment, catalase, superoxide dismutase, and polyphenol oxidase activities increased by 37%, 50%, and 73%, respectively.</p> <p>Keywords: CO₂, Photoperiod, Antioxidant activity, <i>Thymus vulgaris</i></p>

INTRODUCTION

The atmospheric concentration of CO₂ has risen from approximately 280 ppm before the Industrial Revolution to 408 ppm in recent years, primarily due to fossil fuel combustion and deforestation. Elevated CO₂ enhances net photosynthetic rates, thereby increasing plant productivity and yield [1]. It also promotes the accumulation of soluble sugars in the edible portions of vegetables. However, elevated CO₂ is associated with a 9.5% reduction in protein concentration in vegetables [2]. Furthermore, [3] (2003) reported that elevated CO₂ increased total antioxidant capacity, total phenols, total flavonoids, ascorbic acid, and chlorophyll b by 59.0%, 8.9%, 45.5%, 9.5%, and 42.5%, respectively, reflecting an improvement in beneficial phytochemicals within vegetables. Light represents a fundamental environmental factor influencing plant development and modulating physiological and biochemical processes, depending on its quantity, quality, direction, and duration [4]. The daily light integral, defined as the product of photosynthetic photon flux density (PPFD) and photoperiod, represents the total PPF emitted by a light source over 24 hours. This typically exhibits a linear relationship with crop yield and nutrient accumulation [5]. The intensity and duration of light also dictate energy allocation within plants, affecting carbohydrate partitioning and the synthesis of vital compounds such as chlorophyll, flavonoids, and carotenoids (Kaiser *et al.*, 2018). However, excessive light exposure, especially under high PPFD, may induce photoinhibition and oxidative stress, necessitating the activation of protective mechanisms like non-photochemical quenching and antioxidant enzyme systems [6]. Horticultural crops exhibit diverse responses to varying photoperiods, which have been extensively documented in the literature [7]. While plant responses to extended photoperiods are

largely governed by genetic factors, exceeding the photoperiod tolerance threshold can result in photooxidative damage, the generation of reactive oxygen species, and circadian rhythm mismatches. This can potentially lead to reduced yield and economic losses for producers [8]. Polyphenolic compounds, a vast class of secondary metabolites, are ubiquitous in plants and can be categorized into two primary subgroups: flavonoids and phenolic acids. Flavonoids, which constitute the most significant phenolic group in foods, comprise over 4000 aromatic compounds, including anthocyanins, proanthocyanidins, flavonols, and catechins. Phenolic acids include hydroxycinnamic acids (e.g., caffeic, ferulic, and sinapic acids) and hydroxybenzoic acids (e.g., benzoic, gentisic, and p-anisic acids) [9]. Flavonoids perform numerous functional roles in plants, including the production of yellow, red, or blue pigmentation. Subclasses of flavonoids exhibit protective functions in plant tissues, with their biosynthesis often stimulated by abiotic stressors such as drought, low temperatures, mechanical injury, or excessive UV radiation (Chalker-Scott, 1999; Winkel-Shirley, 2002). Beyond anthocyanin pigments, other flavonoids and phenolic acids, particularly sinapate esters and hydroxylated or methoxylated cinnamic acid derivatives, are implicated in photoprotection. These compounds predominantly accumulate in the epidermal cells of plant tissues following exposure to light stress [10]. Flavonoids are synthesized through the general phenylpropanoid pathway, and the key steps in their biosynthetic pathways are well-characterized (see reviews by [11, 12]).

Photoperiod has been shown to upregulate the expression of genes associated with polyphenol biosynthesis. Polyphenolic compounds, including rutin, increased substantially with extended illumination, with rutin demonstrating a remarkable 547%

increase. Light serves as a critical regulatory factor influencing polyphenol accumulation during the growth of mung bean sprouts. Prolonged illumination time not only increased polyphenol content but also significantly enhanced biosynthesis and antioxidant activity. Notably, constant light exposure induced the expression of at least eight key genes within the phenylpropanoid pathway, resulting in increased biosynthesis of polyphenols and enhanced antioxidant capacity [13].

Photoperiod influences not only polyphenol synthesis but also the vegetative and reproductive growth of plants. For instance, plants exposed to a 16-hour photoperiod exhibited significantly higher concentrations of total phenolics compared to those under an 8-hour photoperiod. Similarly, increasing light exposure from 16 to 22 hours enhanced chlorophyll content and antioxidant activity in wheatgrass [14]. Long-day photoperiods have been associated with the upregulation of flavonoid biosynthesis genes and a substantial increase in anthocyanin and flavonol content in sweet potato leaves [15]. Photoperiod also modulates the antioxidant potential of plants, particularly under biotic and abiotic stress conditions. Basil microgreens, for example, respond to the photooxidative stress induced by continuous light by producing higher levels of bioactive antioxidant compounds [14]. Prolonged photoperiods have been linked to increased sugar and carbohydrate metabolism, which correlates with improved plant height, total chlorophyll content, and antioxidant activity in pulse microgreens [16]. A 24-hour photoperiod provides uninterrupted photon energy for CO₂ fixation through the Calvin cycle, thereby promoting continuous growth and biomass production. [17] reported enhanced wheatgrass yield under prolonged photoperiod conditions. Under such conditions, the highest levels of vitamin C, flavonoids, anthocyanins, and antioxidant potential composite index (APCI) were observed. Additionally, chlorophyll content increased linearly with the transition from shorter to longer photoperiod cycles [18].

However, the effects of photoperiod are species-specific and dependent on various physiological factors. Research examining LED light exposure with 24-hour and 16-hour photoperiods on radish, mizuna, cabbage, broccoli, and arugula demonstrated that while leaf weight and area were greater under a 16-hour photoperiod, a 24-hour photoperiod enhanced the accumulation of anthocyanins (ACNs), total flavonoid content (TFC), and proline (Pro). Notably, nitrate content decreased only in arugula under continuous light conditions [19]. Despite its benefits, continuous light can impose light stress, leading to chlorophyll degradation. The reduction in chlorophyll levels under continuous light is attributed to the synthesis of chlorophyll-degrading enzymes, which are induced in young leaves under stress conditions [20]. While extended photoperiods may offer benefits such as increased yield and phytochemical content, careful management is required to mitigate potential light-induced stress and optimize plant health and productivity.

Despite the well-documented influence of elevated CO₂ concentrations and photoperiods on plant growth, yield, and secondary metabolite production, there is limited research on their interactive effects on *T. vulgaris*, a widely valued medicinal and aromatic plant. While elevated CO₂ levels have been shown to enhance photosynthetic rates, antioxidant activities, and the accumulation of bioactive compounds, the combined impact of varying CO₂ levels and photoperiod durations on the biosynthesis of essential oils, phenolic compounds, and antioxidant enzymes in *T. vulgaris* remains unexplored. Additionally, existing studies on photoperiod modulation have focused predominantly on microgreens, cereals, and vegetables, leaving a significant gap in

understanding how long photoperiods and continuous lighting influence the growth dynamics, phytochemical composition, and stress-response mechanisms in perennial aromatic herbs like *T. vulgaris*.

This study aims to investigate the synergistic effects of CO₂ enrichment and photoperiod extension on the physiological, biochemical, and phytochemical characteristics of *T. vulgaris*. The research will evaluate the impact of CO₂ enrichment (350, 700, and 1000 mg/L) on growth, biomass, and secondary metabolite production in *T. vulgaris*, as well as assess the influence of extended photoperiods (12, 16, and 20 hours) on photosynthetic pigments, essential oils, and antioxidant compounds. Furthermore, the study will explore the interactive effects of CO₂ enrichment and photoperiod extension on the activity of antioxidant enzymes, including catalase, ascorbate peroxidase, and polyphenol oxidase, in *T. vulgaris*. The goal is to identify optimal CO₂ and photoperiod conditions for maximizing yield and bioactive compound content, providing insights for sustainable agricultural practices in cultivating high-value medicinal plants.

MATERIALS AND METHODS

The current experiment was conducted in the Vegetable Physiology Laboratory of the Department of Horticultural Science and Engineering, Faculty of Agriculture, University of Tabriz. It was designed as a factorial experiment in a Completely Randomized Block Design with three replications.

Experimental Design

The first factor was carbon dioxide (CO₂) enrichment at three levels: ambient CO₂ (350 mg/L), 700 mg/L, and 1000 mg/L. A carbon dioxide cylinder was carefully prepared for injection. The injection process continued until the desired level of carbon dioxide was achieved. Subsequently, the regulator automatically halted any further injection to maintain the specified parameters. Carbon dioxide sensor manufactured by Taiwan's Unity Company. The CO₂ was supplied from a source of pure edible-grade (grade 3) carbon dioxide with a purity of 99.99%. The second factor was photoperiod, with plants grown under three separate conditions providing day lengths of 12, 16, and 20 hours. LED lamps with an intensity of 8000 lux were used for lighting in all treatments, with a fixed distance of 40 cm between the lamps and the plants.

Plant Cultivation

Thirty-day-old *Thymus vulgaris* seedlings, obtained from commercial seeds, were transplanted into 3-L plastic pots filled with a peat and perlite mixture (2:1 v/v). The plants were grown for seven weeks under controlled environmental conditions, including 65% relative humidity, a maximum daytime temperature of 26 °C, and a minimum nighttime temperature of 21 °C. Fertigation was performed every four days using Hoagland nutrient solution.

Biomass Measurement

When the plants had been grown for eight weeks, the plants were harvested. Randomly, from each CO₂ level, eight plants were selected for biomass measurement. The total biomass was separated into aerial parts and roots. The dry weight of these fractions was determined after drying at 85 °C for 48 hours. At the beginning of the flowering period, the fresh weight of the shoots was measured using a scale with an accuracy of 0.01 g. Plant organs were then dried in an oven at 72 °C, and their dry weights were recorded.

Chlorophyll Measurement

Chlorophyll content was quantified following the protocol outlined by [21], which provides a reliable method for assessing pigment levels in plant tissues. Leaf samples were homogenized, and the pigments were extracted using 80% acetone, a widely used solvent due to its efficiency in dissolving chlorophyll and other pigments while minimizing degradation. The extracted solution was clarified by centrifugation to remove particulate matter, ensuring precise absorbance readings. Absorbance was measured at specific wavelengths (typically 663 nm and 645 nm) using a spectrophotometer, enabling the calculation of chlorophyll *a* and *b* concentrations as well as total chlorophyll content based on standard equations. This method ensures reproducibility and accuracy, making it ideal for comparative studies of chlorophyll dynamics under different environmental conditions.

Catalase Activity Assay

Catalase activity was measured using a mixture of 200 μ L reaction buffer, 150 μ L methanol, and 30 μ L working stock H_2O_2 , gently shaken together. 50 μ L of sample (or standard solution) was added to each microtube and mixed for 20 minutes in the absence of light. For the control sample, 50 μ L of reaction buffer was used. The reaction was terminated by adding 150 μ L potassium hydroxide solution. Subsequently, 150 μ L chromogenic reagent was added to each microtube and vortexed. Further analytical steps for enzyme activity and other assays were conducted in accordance with established protocols. After a 10-minute incubation period, 150 microliters of potassium periodate were added to the samples. The samples were then centrifuged at $8,000 \times g$ for 10 minutes. Finally, the absorbance of the samples was measured at 550 nm using a spectrophotometer, following a method described by [22].

Measurement of Ascorbate Peroxidase Activity

The reaction mixture, with a final volume of one ml, comprised 250 microliters of 100 mM phosphate buffer (pH 7), 250 microliters of 1 mM ascorbate, 250 microliters of 0.4 mM EDTA, 190 microliters of double-distilled water, 10 microliters of 10 mM hydrogen peroxide, and 50 microliters of enzyme extract. Absorbance was recorded at 290 nm at the start of the reaction and one minute after initiation, based on a protocol established by [23].

Measurement of Polyphenol Oxidase Activity

Polyphenol oxidase activity was assessed using a method outlined by [24]. Test tubes were placed in a water bath maintained at 20 °C. Each tube received 1.2 ml of 0.2 M phosphate buffer (pH 6.8), followed by the addition of 0.2 ml of 0.02 M pyrogallol. Once the temperature stabilized at 20 °C, 2 ml of enzyme extract were added to each tube. Changes in absorbance at 290 nm were recorded over a 2-minute interval. Enzyme activity was expressed as the change in absorbance at 290 nm per minute per mg of protein.

Measurement of Antioxidant Activity

To evaluate antioxidant activity, 0.1 ml of fruit juice was combined with 0.9 ml of 100 mM Tris-HCl buffer (pH 7.5). Subsequently, 1 ml of DPPH solution (500 μ M in ethanol) was added, and the mixture was vigorously shaken. Afterward, the solution was incubated in darkness for 30 minutes. A control sample was prepared using distilled water instead of fruit juice. Absorbance was measured at 517 nm using a spectrophotometer, with the DPPH-free reaction mixture employed for background correction [25].

Measurement of Total Phenolic Content

Ten grams of each plant sample was weighed and homogenized with 10 ml of 50% ethanol. After 30 minutes, the mixture was filtered using Whatman No. 2 filter paper. The alcoholic extracts were further diluted with an additional 10 ml of 50% ethanol. A volume of 0.5 ml from each extract was combined with 2.5 ml of Folin-Ciocalteu reagent, diluted at a ratio of 1:10, along with 2 ml of a 7.5% sodium carbonate solution. The samples were incubated at 20 °C for two hours before measuring absorbance at 750 nm using a spectrophotometer [26].

Measurement of Total Flavonoid Content

Total flavonoid content was determined using an aluminum chloride colorimetric assay, a widely recognized and reliable spectrophotometric method. This procedure involved the reaction of flavonoids present in the sample with aluminum chloride, forming a stable complex that exhibited a characteristic absorbance measurable at a specific wavelength using a spectrophotometer. The intensity of the absorbance correlated directly with the flavonoid concentration. The results were quantified and expressed in terms of quercetin equivalents, a standard reference compound that facilitated comparison and standardization of the flavonoid content across different studies [27].

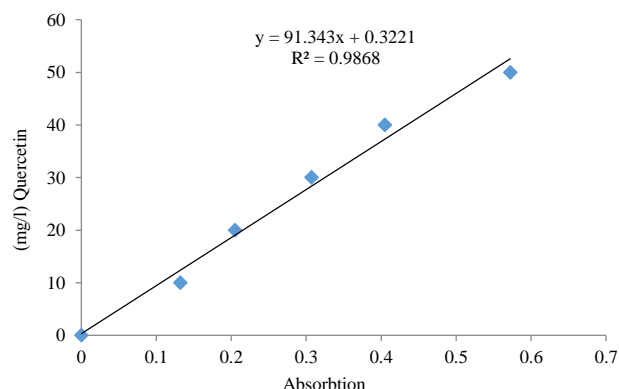


Fig. 1 Quercetin Standard Chart

Experimental Design and Data Analysis

The experiments were conducted following a factorial arrangement within a randomized complete block design (RCBD), a robust statistical framework commonly used to control variability and ensure reliable results in experimental studies. This design incorporated multiple factors, enabling the evaluation of both individual and interactive effects of treatments while minimizing confounding influences. Each treatment was replicated three times to enhance the statistical power and precision of the findings. Data analysis was performed using SPSS statistical software, a comprehensive tool widely recognized for its advanced analytical capabilities. Treatment means were statistically compared through Duncan's multiple range test (DMRT), a post-hoc method designed to identify significant differences between group means while controlling for type I error. This approach ensured that the comparative results were both rigorous and interpretable. Visual representation of the data was achieved using Microsoft Excel, which facilitated the creation of clear and informative graphs and charts. These graphical elements complemented the statistical analysis, providing an accessible means to interpret and communicate the findings effectively to a broader audience.

RESULTS

The results (Fig. 2–9) showed that the interaction between photoperiod and carbon dioxide (CO_2) injection significantly

enhanced all measured parameters at the 1% level. The direct effect of photoperiod alone was also significant at the 1% level, leading to increases across all parameters. CO₂ injection significantly affected essential oil content at the 5% level, but did not have a significant impact on carotenoids, chlorophyll a, flavonoids,

antioxidant activity, or antioxidant enzymes. However, CO₂ injection did significantly increase parameters such as dry weight, fresh weight, plant height, chlorophyll b, and phenolic compounds at the 1% level.

Table 1 The results of mean comparison of the effect of Photoperiod (L) and Carbondioxide on some morphological traits of *T. Vulgaris*

Treatment	Carotenoid	Ch b	Ch a	height	Dry weight	Fresh weight
L1c1	0.61 d	0.41 f	0.55 c	19 f	1.7 f	17.6 f
L1c2	0.62 cd	0.46 e	0.55 c	19.3 f	2.7e	18 f
L1c3	0.6 d	0.51 d	0.6 c	20 f	3.4 d	20.6 ef
L2c1	0.633 cd	0.58 bc	0.65 bc	23.3 e	3.8 cd	22.3 de
L2c2	0.66 cd	0.57 c	0.71 b	24.3 e	4.3 c	25 cd
L2c3	0.65 cd	0.623 b	0.72 b	27.3 d	4.9 bc	27.6 c
L3c1	0.72 bc	0.627 ab	0.84 a	31 c	5.4 b	30.6 b
L3c2	0.8 ab	0.62 ab	0.86 a	32.3 b	6.3 a	36.3 a
L3c3	0.89 a	0.67 a	0.9 a	34.6 a	6.4 a	37 a

C₁: (CO₂ 350 mg/l), C₂: (CO₂ 700 mg/l) and C₃: (CO₂ 1000 mg/l), (L₁: (lighting photoperiods of 12 hours) L₂: (lighting photoperiods of 16 hours) and L₃: (lighting photoperiods of 20 hours)

It appears that extended photoperiods positively influenced all parameters in this study. Similarly, increased CO₂ concentrations, likely by enhancing photosynthesis, contributed to greater fresh and dry weights, as well as plant height. Photoperiod seemed to exert a direct influence on these outcomes, while the effectiveness of CO₂ was modulated by other factors. The L3C3 treatment had a positive and significant effect on fresh weight, dry weight, plant height, chlorophyll a, chlorophyll b, essential oil content, carotenoids, phenolic compounds, flavonoids, and antioxidants, increasing these parameters by 110%, 276%, 82%, 63%, 63%, 160%, 45%, 68%, 13%, and 45%, respectively. The L3C1 treatment significantly enhanced peroxidase activity by a factor of 108%. Additionally, catalase, superoxide dismutase, and polyphenol oxidase activities increased by 37%, 50%, and 73%, respectively, under the L3C1 treatment. The results indicate that the levels of chlorophyll a and b, fresh and dry biomass, plant height, and carotenoid content in thyme increased with longer photoperiods and higher concentrations of carbon dioxide. Maximum values were observed under a 20-hour photoperiod and a carbon dioxide concentration of 1000 mg/L, while the lowest values were recorded under a 12-hour photoperiod and lower carbon dioxide concentrations.

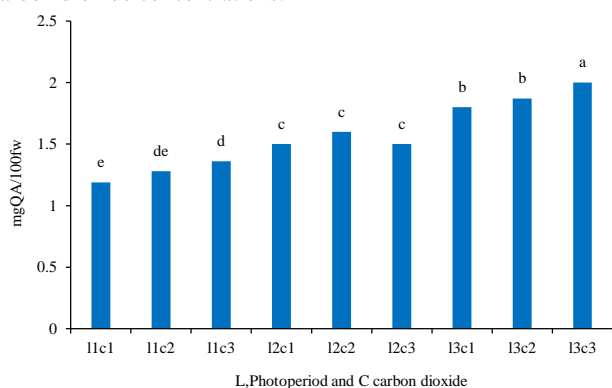


Fig. 2 effect of the interaction of photoperiod and carbon dioxide on phenolic compounds of *T. Vulgaris* (C₁: (CO₂ 350 mg/L), C₂: (CO₂ 700 mg/L) and C₃: (CO₂ 1000 mg/L), (L₁: (lighting photoperiods of 12 hours) L₂: (lighting photoperiods of 16 hours) and L₃: (lighting photoperiods of 20 hours)

The results (Fig. 2) indicated that an increase in both photoperiod and carbon dioxide concentration led to a rise in the amount of phenolic compounds in garden thyme. The highest concentration was observed at 20 hours during the day, when the carbon dioxide concentration was 1000 ppm, while the lowest concentration occurred at 12 hours during the day under the control carbon dioxide conditions.

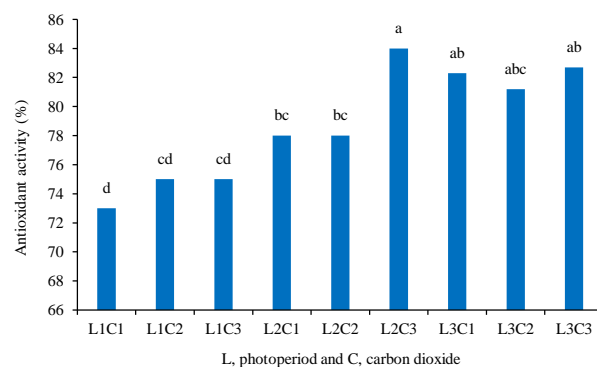


Fig. 3 effect of the interaction of photoperiod and carbon dioxide on Antioxidant activity of *Thymus Vulgaris* (C₁: (CO₂ 350 mg/L), C₂: (CO₂ 700 mg/L) and C₃: (CO₂ 1000 mg/L), (L₁: (lighting photoperiods of 12 hours) L₂: (lighting photoperiods of 16 hours) and L₃: (lighting photoperiods of 20 hours)

The results (Fig. 3) demonstrated that as both the photoperiod and carbon dioxide concentration increased, the antioxidant properties of garden thyme improved. The highest antioxidant value was observed at 16 hours during the day, when the carbon dioxide concentration was 1000 ppm, while the lowest value was recorded at 12 hours during the day under the control carbon dioxide conditions.

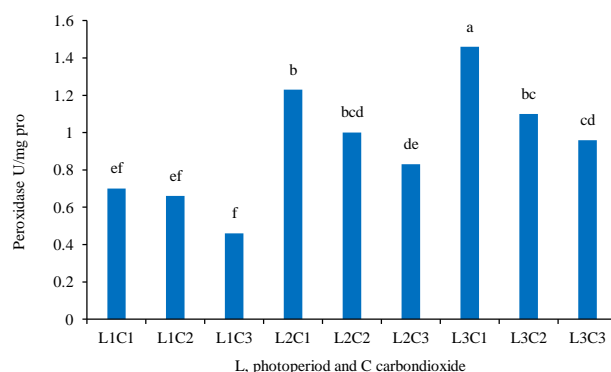


Fig. 4 effect of the interaction of photoperiod and carbon dioxide on Peroxidase of *T. Vulgaris*

(C₁: (CO₂ 350 mg/L), C₂: (CO₂ 700 mg/L) and C₃: (CO₂ 1000 mg/L), (L₁: (lighting photoperiods of 12 hours) L₂: (lighting photoperiods of 16 hours) and L₃: (lighting photoperiods of 20 hours)

The results (Fig. 4) indicated that the peroxidase activity in garden thyme increased with the extension of the photoperiod, while carbon dioxide had the opposite effect. The highest peroxidase activity was observed at 20 hours during the day under controlled carbon dioxide conditions, while the lowest activity was recorded

at 12 hours during the day with a carbon dioxide concentration of 1000 mg/liter.

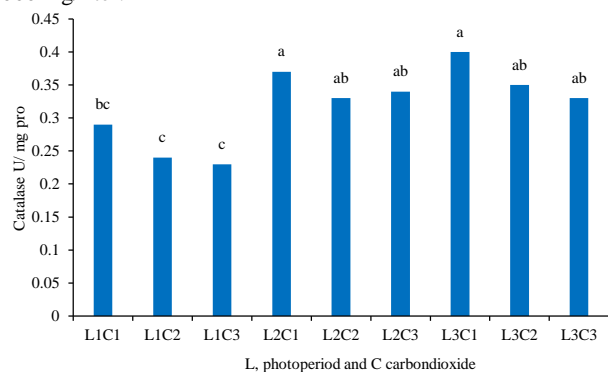


Fig. 5 effect of the interaction of photoperiod and carbon dioxide on Catalase of *T. Vulgaris*

(C₁: (CO₂ 350 mg/L), C₂: (CO₂ 700 mg/L) and C₃: (CO₂ 1000 mg/L), (L₁: (lighting photoperiods of 12 hours) L₂: (lighting photoperiods of 16 hours) and L₃: (lighting photoperiods of 20 hours)

The results (Fig. 5) showed that with the increase in photoperiod and carbon dioxide concentration, the catalase activity in garden thyme also increased. The maximum activity was observed at 20 hours during the day under control carbon dioxide conditions, while the lowest activity occurred at 12 hours during the day with a carbon dioxide concentration of 1000 mg/liter.

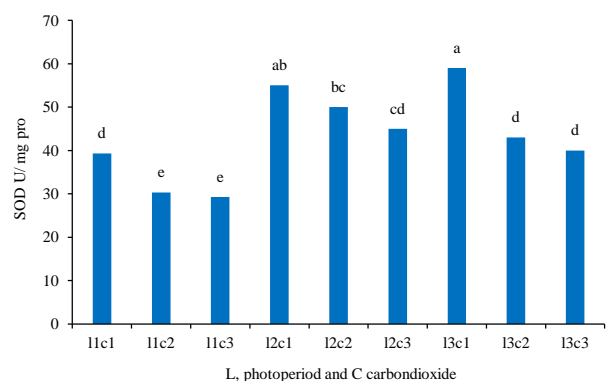


Fig. 6 effect of the interaction of photoperiod and carbon dioxide on Superoxide dismutase (SOD) of *Thymus Vulgaris*

(C₁: (CO₂ 350 mg/L), C₂: (CO₂ 700 mg/L) and C₃: (CO₂ 1000 mg/L), (L₁: (lighting photoperiods of 12 hours) L₂: (lighting photoperiods of 16 hours) and L₃: (lighting photoperiods of 20 hours)

The results (Fig. 6) showed that the amount of superoxide dismutase in garden thyme increased with the extension of the photoperiod. The maximum activity was observed at 20 hours during the day under control carbon dioxide conditions, while the lowest activity occurred at 12 hours during the day with a carbon dioxide concentration of 1000 mg/liter.

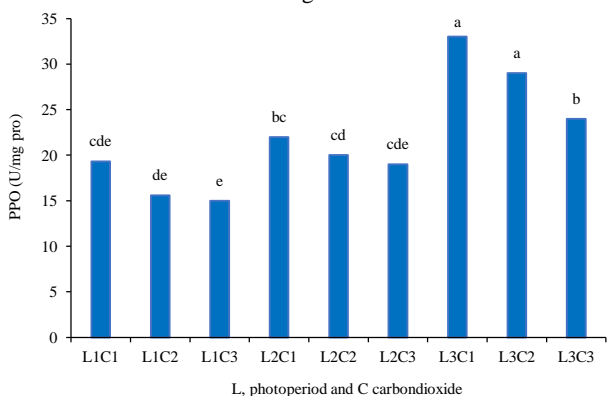


Fig. 7 effect of the interaction of photoperiod and carbon dioxide on Polyphenol oxidase (PPO) of *T. Vulgaris*

(C₁: (CO₂ 350 mg/L), C₂: (CO₂ 700 mg/L) and C₃: (CO₂ 1000 mg/L), (L₁: (lighting photoperiods of 12 hours) L₂: (lighting photoperiods of 16 hours) and L₃: (lighting photoperiods of 20 hours)

The results (Fig. 7) showed that the amount of polyphenol oxidase in garden thyme increased with the extension of the photoperiod. The maximum activity was observed at 20 hours during the day under control carbon dioxide conditions, while the lowest activity occurred at 12 hours during the day with a carbon dioxide concentration of 1000 mg/liter.

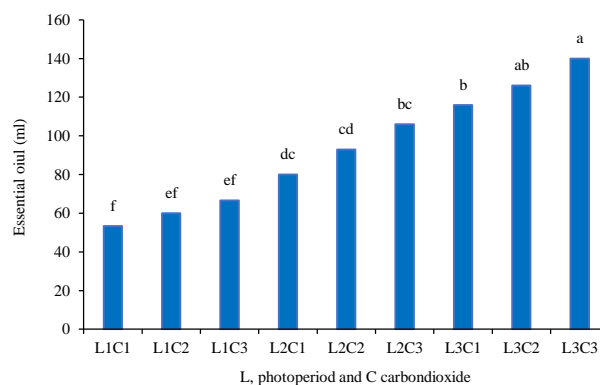


Fig. 8 effect of the interaction of photoperiod and carbon dioxide on Essential oil volume (per 15 gr dry matter) of *T. vulgaris*. (C₁: (CO₂ 350 mg/L), C₂: (CO₂ 700 mg/L) and C₃: (CO₂ 1000 mg/L), (L₁: (lighting photoperiods of 12 hours) L₂: (lighting photoperiods of 16 hours) and L₃: (lighting photoperiods of 20 hours)

The results (Fig. 8) showed that with the increase in photoperiod and carbon dioxide concentration, the amount of essential oil in garden thyme increased. The highest concentration was observed at 20 hours during the day with a carbon dioxide concentration of 1000 mg/liter, while the lowest concentration was recorded at 12 hours during the day under the controlled carbon dioxide conditions.

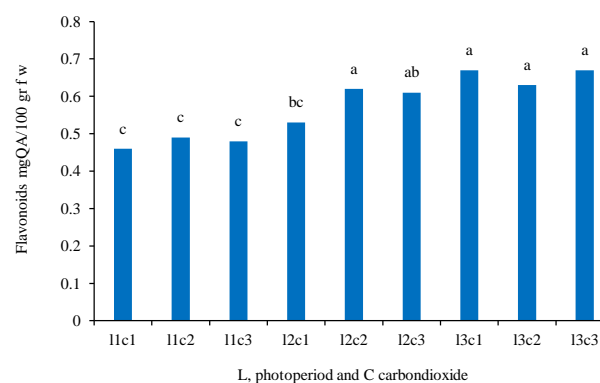


Fig. 9 effect of the interaction of photoperiod and carbon dioxide on Flavonoid of *T. Vulgaris*. (C₁: (CO₂ 350 mg/L), C₂: (CO₂ 700 mg/L) and C₃: (CO₂ 1000 mg/L), (L₁: (lighting photoperiods of 12 hours) L₂: (lighting photoperiods of 16 hours) and L₃: (lighting photoperiods of 20 hours)

The results (Figure 9) showed that the amount of flavonoids in garden thyme increased with the extension of the photoperiod. The highest concentration was observed at 20 hours during the day with a carbon dioxide concentration of 1000 mg/liter, while the lowest concentration occurred at 12 hours during the day under controlled carbon dioxide conditions.

DISCUSSION

Environmental factors are well-known to influence the metabolite profiles of plants. Specifically, variations in light conditions and CO₂ concentration can significantly increase the levels of functional phytochemicals in fruits and leafy vegetables [2]. For

example, light quality, photoperiod, and light intensity have all been reported to boost the antioxidant content in lettuce [28]. Polyphenols, such as chlorogenic acid (CGA), are markedly elevated under specific environmental conditions, including light quality and CO₂ enrichment [29]. Combinations of continuous blue light and high CO₂ concentration have been shown to considerably enhance CGA content in young-leaf lettuce. Furthermore, continuous light exposure increases antioxidant levels in lettuce [30]. Specific combinations of environmental stimuli can trigger a notable accumulation of CGA by activating genes within the phenylpropanoid pathway, which also impacts the flavonoid biosynthetic pathway [29].

Elevated CO₂ concentrations enhance photosynthesis in plants, leading to greater production of carbohydrates and overall biomass. The specific organ to which these extra carbohydrates are allocated can vary not only between different plant species but also within the same species. While these carbohydrates are a primary energy source for plant growth, they also function as signaling molecules and have various other uses beyond simply providing carbon and energy. The sugar signaling pathways in roots, in particular, are not fully understood, nor is how they are affected by elevated CO₂. Under elevated CO₂, some plants allocate more sugars to their roots, where these sugars likely influence gene regulation and, consequently, modify nutrient uptake and transport. Glucose and sucrose also promote root growth, an effect similar to what occurs under elevated CO₂. Sugars also interact with hormones to regulate root growth and impact hormone biosynthesis [30].

The current atmospheric CO₂ concentration often limits photosynthesis, growth, and productivity in many C3 plant species. In leaves, the photosynthetic rate is determined by the activity of ribulose biphosphate carboxylase-oxygenase (Rubisco), which is influenced by CO₂ levels, temperature, and light. Current atmospheric CO₂ levels are insufficient to fully saturate Rubisco activity, and short-term increases in CO₂ availability have been shown to enhance photosynthetic rates. This effect occurs partly because elevated CO₂ inhibits Rubisco's oxygenase activity, thereby reducing photorespiratory CO₂ loss [31].

Elevated CO₂ levels reduce the accumulation of reactive oxygen species (ROS), thereby decreasing the need for antioxidant enzyme activity. This phenomenon is evident in Figures 3–6, which illustrate reduced enzyme activity with increasing CO₂ concentrations. CO₂ enrichment promotes photosynthesis and water-use efficiency, ultimately enhancing overall plant growth [3]. It has also been demonstrated that CO₂ enrichment facilitates light energy capture and CO₂ fixation, leading to higher photosynthetic rates and capacities. For example, short-term exposure of C3 plants to elevated CO₂ concentrations can increase net photosynthetic rates by 10%–50% [32]. In the current research, CO₂ injection improved growth conditions, reduced plant stress, and decreased the activity of antioxidant enzymes. Under optimal cultivation conditions, CO₂ enrichment significantly enhances photosynthesis and associated plant growth processes. In tomatoes, combining artificial light with carbon dioxide enrichment resulted in a 50% increase in dry weight [33].

Similarly, in chrysanthemums, integrating artificial light with carbon dioxide supplementation significantly boosted growth rates [34]. For tomatoes, treatments involving light and carbon dioxide, both independently and in combination, positively influenced fresh and dry biomass. Notably, the combined treatment increased the plant's dry weight by up to nine-fold [35]. In asparagus seedling production, artificial light coupled with carbon dioxide supplementation doubled the shoot dry weight. Additionally, under

controlled laboratory conditions, artificial light optimizes the utilization of carbon dioxide by modifying leaf structure and increasing leaf surface area [36].

Increased supplementary lighting also positively impacts cucumber growth, improving biomass production, leaf chlorophyll content, and overall yield [37]. Changes in light conditions affect starch accumulation and thylakoid structure in tomatoes. In eggplant, carbon dioxide treatment at a concentration of 950 mg/L significantly enhanced chlorophyll content, whereas the same treatment had no measurable effect on chlorophyll levels in potatoes, onions, or cabbages [38]. Variability in responses to elevated carbon dioxide levels has been observed among plant species, particularly concerning changes in the nutritional value of carotenoids [39].

Plants regulate their molecular bioactivity in response to daily and seasonal environmental fluctuations in temperature, light, humidity, and precipitation [40]. An increase in carbon-based secondary metabolites often occurs when environmental conditions promote an accumulation of non-structural carbohydrates (TNC) in plants. Elevated atmospheric CO₂ frequently increases TNC concentrations in plants, potentially stimulating secondary metabolism, although experimental results have not always indicated a relationship as predictable as that seen with nutrient deficiency [41]. Light plays a crucial role in regulating the contents of glucose, fructose, and soluble sugars [42]. Long days increase soluble sugars in leaves [43]. Soluble sugars can be converted into phenylalanine, which is a precursor to phenolic compounds; thus, factors that increase the amount of soluble sugars can lead to increased phenolic compounds [44].

CONCLUSION

A 20-hour photoperiod combined with 350 mg/L of carbon dioxide yielded the best results for antioxidant enzymes and flavonoids. However, for phenolic compounds and essential oil content, the optimal results were achieved with a 20-hour photoperiod and 1000 mg/L of carbon dioxide. This indicates that while a 20-hour day length consistently produced superior outcomes, its interaction effects varied depending on the specific carbon dioxide concentration.

Acknowledgements

I would like to express my gratitude and appreciation to all those who collaborated in all stages of this research, especially Dr. Ghasem Shekari from the Fanavaran Kasht Zagros company for preparing the cultivation conditions.

REFERENCES

1. Dong J., Gruda N., Li X., Tang Y., Zhang P and Duan Z. Sustainable vegetable production under changing climate: The impact of elevated CO₂ on yield of vegetables and the interactions with environments-A review. *Journal of Cleaner Production*. 2020;253:119920. DOI: 10.1016/j.jclepro.2019.119920.
2. Dong J., Gruda N., Lam S.K., Li X., Duan Z. Effects of Elevated CO₂ on Nutritional Quality of Vegetables: A Review. *Frontiers in Plant Science*. 2018; 9:924. DOI: 10.3389/fpls.2018.00924.
3. Wang S.Y., Bunce J.A., Maas J.L. Elevated carbon dioxide increases contents of antioxidant compounds in field grown strawberries. *Journal of Agricultural and Food Chemistry*. 2003; 51(15): 4315–4320. DOI: 10.1021/jf021172d.
4. Fukuda A., Sato K., Fujimori C., Yamashita T., Takeuchi A., Ohuchi H., Umatani C., Kanda S. Direct photoreception by pituitary endocrine cells regulates hormone release and pigmentation. *Science*. 2025 3;387(6729):43-8. DOI: 10.1126/science.adj9687

5. Hernández K.V., Moreno-Romero J., Hernández de la Torre M., Pérez Manríquez C., Ríos Leal D., Martínez-García J.F. Effect of light intensity on steviol glycosides production in leaves of *Stevia rebaudiana* plants. *Phytochemistry*. 2022 Feb;194:113027. DOI:10.1016/j.phytochem.2021.113027
6. Song J., Chen Z., Zhang A., Wang M., Jahan M.S., Wen Y., Liu X. The Positive Effects of Increased Light Intensity on Growth and Photosynthetic Performance of Tomato Seedlings in Relation to Night Temperature Level. *Agronomy*. 2022;12(2):343. DOI:10.3390/agronomy12020343
7. Jackson S.D. Plant responses to photoperiod. *New Phytol.* 2009;181(3):517-531. DOI:10.1111/j.1469-8137.2008.02681.x
8. Hernández K.V., Moreno-Romero J., Hernández de la Torre M., Pérez Manríquez C., Ríos Leal D., Martínez-García J.F. Effect of light intensity on steviol glycosides production in leaves of *Stevia rebaudiana* plants. *Phytochemistry*. 2022 Feb;194:113027. DOI:10.1016/j.phytochem.2021.113027
9. Chalker-Scott L. Environmental significance of anthocyanins in plant stress responses. *Photochem Photobiol.* 1999;70(1):1-9. DOI:10.1111/j.1751-1097.1999.tb01944.x
10. Zhou B., Feng X., Huang W-H., Liu Q., Ibrahim S.A., Liu Y., *et al.* Effects of light intensity on the biosynthesis of glucosinolate in Chinese cabbage plantlets. *Scientia Horticulturae*. 2023;316:112036. DOI:10.1016/j.scienta.2023.112036
11. Winkel-Shirley B. Biosynthesis of flavonoids and effects of stress. *Current Opinion in Plant Biology*. 2002;5(3):218-223. DOI:10.1016/S1369-5266(02)00256-X
12. Heller W., Forkmann G. Biosynthesis of flavonoids, in *The Flavonoids Advances in Research since 1986*. 2017;Routledge. 499-535.
13. Cheng Y., Xiang N., Cheng X., Chen H., Guo X. Effect of photoperiod on polyphenol biosynthesis and cellular antioxidant capacity in mung bean (*Vigna radiata*) sprouts. *Food Research International*. 2022;159:111626. DOI:10.1016/j.foodres.2022.111626
14. Fayeizadeh M.R., Ansari N.A., Sourestani M.M., Hasanuzzaman M. Variations in photoperiods and their impact on yield, photosynthesis and secondary metabolite production in basil microgreens. *BMC Plant Biology*. 2024;26:24(1):712. DOI:10.1186/s12870-024-05448-z
15. Carvalho L.C., Grassi L.M., Braga G.D., Castro I.T., Barros R.P., Moreno D.A. Effect of photoperiod on flavonoid pathway activity in sweet potato (*Ipomoea batatas* L.) leaves. *Food Chemistry*. 2009 Aug;116(3):384-390. DOI:10.1016/j.foodchem.2009.05.005.
16. Kumar A., Singh N., Kaur A., Joshi R., *et al.* Sneak peek into the chlorophyll content, antioxidant activity, targeted and non targeted UHPLC QTOF LC/MS metabolomic fingerprints of pulse microgreens grown under different photoperiod regimes. *Food Bioscience*. 2023;52:102506. DOI:10.1016/j.fbio.2023.102506.
17. Grant N.P. Exploring the natural variation of photosynthesis and abiotic stress in wheat varieties and reduced height mutants. Washington State University. 2020.
18. Adams S., Langton F. Photoperiod and plant growth: a review. *The Journal of Horticultural Science and Biotechnology*. 2005; 80(1): 2-10.
19. Adams S.R., Langton F.A. Photoperiod and plant growth: a review. *The Journal of Horticultural Science and Biotechnology*. 2005;80(1):2-10. DOI:10.1080/14620316.2005.11511882
20. Shibaeva T.G., Sherudilo E.G., Rubaeva A.A., Titov A.F. Continuous LED Lighting Enhances Yield and Nutritional Value of Four Genotypes of Brassicaceae Microgreens. *Plants*. 2022;11(2):176. DOI:10.3390/plants11020176
21. Arnon D.I. Copper enzymes in isolated chloroplasts: polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*. 1949;24(1):1-15. DOI:10.1104/pp.24.1.1
22. Aebi H. Catalase in vitro. *Methods Enzymol.* 1984;105:121-126. DOI:10.1016/S0076-6879(84)05016-3.
23. Nakano Y., Asada K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and Cell Physiology*. 1981; 22(5): 867-880.
24. Mayer A.M., Harel E., Shaul R.B. Assay of catechol oxidase – a critical comparison of methods. *Phytochemistry*. 1966;5(4):783-789. DOI:10.1016/S0031-9422(00)83660-2
25. Brand-Williams W., Cuvelier M.E., Berset C. Use of a free radical method to evaluate antioxidant activity. *LWT- Journal of Food Science and Technology*. 1995;28(1):25-30. DOI:10.1016/S0023-6438(95)80008-5
26. Singleton, V.L., Rossi J.A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*. 1965;16(3): 144-158.
27. Chang C.-C., *et al.* Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of food and Drug Analysis*. 2002;34, 731-732.
28. Song J., Huang H., Song S., Zhang Y., Su W., Liu H. Effects of photoperiod interacted with nutrient solution concentration on nutritional quality and antioxidant and mineral content in lettuce. *Agronomy*. 2020;10(7):920. DOI:10.3390/agronomy10070920
29. Shimomura M., Yoshida H., Fujiuchi N., Ariizumi T., Ezura H., Fukuda N. Continuous blue lighting and elevated carbon dioxide concentration rapidly increase chlorogenic acid content in young lettuce plants. *Scientia Horticulturae*. 2020;272:109550. DOI:10.1016/j.scienta.2020.109550
30. Thompson M., Gamage D., Hirotsu N., Martin A., Seneweera S. Effects of elevated carbon dioxide on photosynthesis and carbon partitioning: a perspective on root sugar sensing and hormonal crosstalk. *Frontiers in Physiology*. 2017;8:578. DOI:10.3389/fphys.2017.00578
31. Bowes G. Facing the inevitable: plants and increasing atmospheric CO₂. *Annual Review of Plant Biology*. 1993; 44:309-332. <https://doi.org/10.1146/annurev.pp.44.060193.001521>
32. Long S.P., Drake B.G. Effect of the long-term elevation of CO₂ concentration in the field on the quantum yield of photosynthesis of the C₃ sedge *Scirpus olneyi*. *Plant Physiology*. 1991;96(1):221-226. DOI:10.1104/pp.96.1.221
33. Fierro A., Tremblay N., Gosselin A. Supplemental carbon dioxide and light improved tomato and pepper seedling growth and yield. *Hort Science*. 1994;29:152-154.
34. Hosseinzadeh M., Aliniaieifard S., Shomali A., Didaran F. Interaction of light intensity and CO₂ concentration alters biomass partitioning in chrysanthemum. *Journal of Horticultural Research*. 2021;29(2):45-56. DOI:10.2478/johr-2021-0015
35. Huber B.M., Louws F.J., Hernández R. Impact of different daily light integrals and carbon dioxide concentrations on the growth, morphology, and production efficiency of tomato seedlings. *Frontiers in Plant Science*. 2021;12:615853. DOI:10.3389/fpls.2021.615853
36. Guo J.M., Jermyn W.A., Turnbull M.H. Diurnal and seasonal photosynthesis in two asparagus cultivars with contrasting yield. *Crop Science*. 2002;42(2):399-405. DOI:10.2135/cropsci2002.3990
37. Hao X., Papadopoulos A.P., Brainard G.C., Bugbee B. Effects of supplemental lighting and cover materials on growth, photosynthesis, biomass partitioning, early yield and quality of greenhouse cucumber (*Cucumis sativus* L. cv. Flamingo). *Scientia Horticulturae*. 1999;80(1-2):1-18. DOI:10.1016/S0304-4238(98)00217-9
38. Sage R.F., Sharkey T.D., Seemann J.R. Acclimation of photosynthesis to elevated CO₂ in five C₃ species. *Plant Physiology*. 1989;89(2):590-596. DOI:10.1104/pp.89.2.590
39. Loladze I., Nolan J.M., Ziska L.H., Knobbe A.R. Rising atmospheric CO₂ lowers concentrations of plant carotenoids essential to human health: a meta-analysis. *Molecular Nutrition & Food Research*. 2019;63(15):e1801047. DOI:10.1002/mnfr.201801047
40. Liebelt D.J., Jordan J.T., Doherty C.J. Only a matter of time: the impact of daily and seasonal rhythms on phytochemicals. *Phytochemistry Reviews*. 2019;18(6):1409-1433. DOI:10.1007/s11101-019-09617-z
41. Booker F.L. Influence of carbon dioxide enrichment, ozone and nitrogen fertilization on cotton (*Gossypium hirsutum* L.) leaf and root composition. *Plant, Cell & Environment*. 2000;23(6):573-583. DOI:10.1046/j.1365-3040.2000.00576.x
42. Zhan L., Hu J., Ai Z., Pang L., Li Y., Zhu M. Light exposure during storage preserving soluble sugar and l-ascorbic acid content of minimally processed romaine lettuce (*Lactuca sativa* L. var. longifolia). *Food Chemistry*. 2013;136(1):273-278. DOI:10.1016/j.foodchem.2012.07.123
43. Ceunen S., Geuns J.M.C. Glucose, sucrose, and steviol glycoside accumulation in *Stevia rebaudiana* grown under different photoperiods. *Biologia Plantarum*. 2013;57(2):390-394. DOI:10.1007/s10535-012-0289-6
44. Shekari G., Javanmardi J. Effects of foliar application of pure amino acid and amino acid containing fertilizer on broccoli (*Brassica oleracea* L. var. italica) transplants. *Advances in Crop Science and Technology*. 2017;5(3):280. DOI:10.4172/2329-8863.1000280