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

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ABSTRACT

Thymus vulgaris 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 *T. vulgaris*. 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.

Keywords: CO_2, Photoperiod, Antioxidant activity, *Thymus vulgaris*

INTRODUCTION

The atmospheric concentration of CO_2 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 CO_2 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 CO_2 is associated with a 9.5% reduction in protein concentration in vegetables [2]. Furthermore, [3] (2003) reported that elevated CO_2 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 CO_2 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 CO_2 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 CO_2 levels have been shown to enhance photosynthetic rates, antioxidant activities, and the accumulation of bioactive compounds, the combined impact of varying CO_2 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 CO_2 enrichment and photoperiod extension on the physiological, biochemical, and phytochemical characteristics of T. vulgaris. The research will evaluate the impact of CO_2 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 CO_2 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 CO_2 and photoperiod conditions for maximizing yield and bioactive compound content, providing insights for sustainable agricultural practices in cultivating high-value medicinal plants.

MATERIA 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 (CO_2) enrichment at three levels: ambient CO_2 (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 CO_2 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 µL reaction buffer, 150 µL methanol, and 30 µL working stock H_2O_2, gently shaken together. 50 µL 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 µL of reaction buffer was used. The reaction was terminated by adding 150 µL potassium hydroxide solution. Subsequently, 150 µL 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 × 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 µM 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].

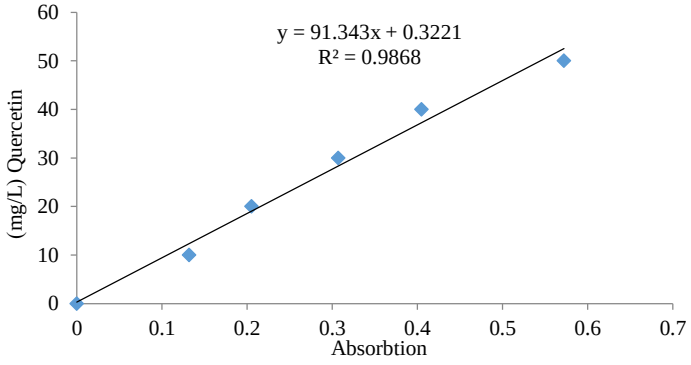

Fig. 1 Quercetin Standard Chart

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 (Figures 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. CO_2 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, CO_2 injection did significantly increase parameters such as dry weight, fresh weight, plant height, chlorophyll b, and phenolic compounds at the 1% level.

It appears that extended photoperiods positively influenced all parameters in this study. Similarly, increased CO_2 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 CO_2 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.

Table 1 The results of mean comparision of the effect of Photoperid (L) and Carbondioxide on some morphological traits of *Thymus 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

C_1: (CO_2 350 mg/L), C_2: (CO_2 700 mg/L) and C_3: (CO_2 1000 mg/L), (L_1: (lighting photoperiods of 12 hours) L_2: (lighting photoperiods of 16 hours) and L_3: (lighting photoperiods of 20 hours)

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.

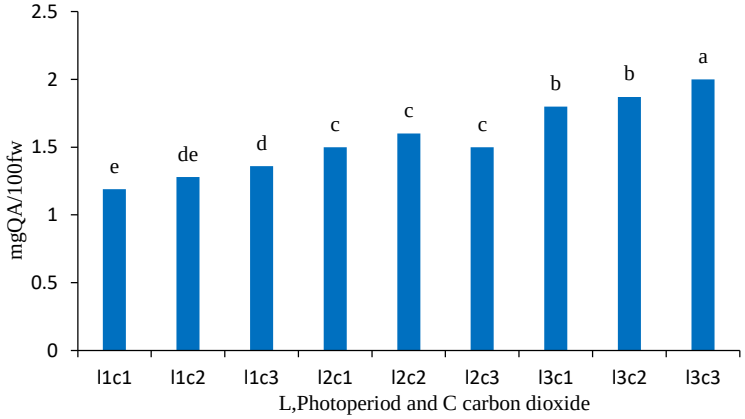

Fig. 2 effect of the interaction of photoperiod and carbon dioxide on phenolic compounds of *Thymus Vulgaris* (C_1: (CO_2 350 mg/L), C_2: (CO_2 700 mg/L) and C_3: (CO_2 1000 mg/L), (L_1: (lighting photoperiods of 12 hours) L_2: (lighting photoperiods of 16 hours) and L_3: (lighting photoperiods of 20 hours)

The results (Figure 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.

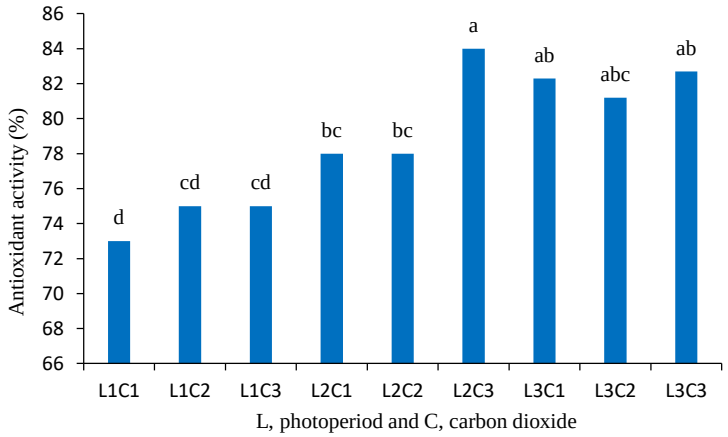

Fig. 3 effect of the interavtion of photoperiod and carbon dioxide on Antioxidant activity of *Thymus Vulgaris* (C_1: (CO_2 350 mg/L), C_2: (CO_2 700 mg/L) and C_3: (CO_2 1000 mg/L), (L_1: (lighting photoperiods of 12 hours) L_2: (lighting photoperiods of 16 hours) and L_3: (lighting photoperiods of 20 hours)

The results (Figure 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.

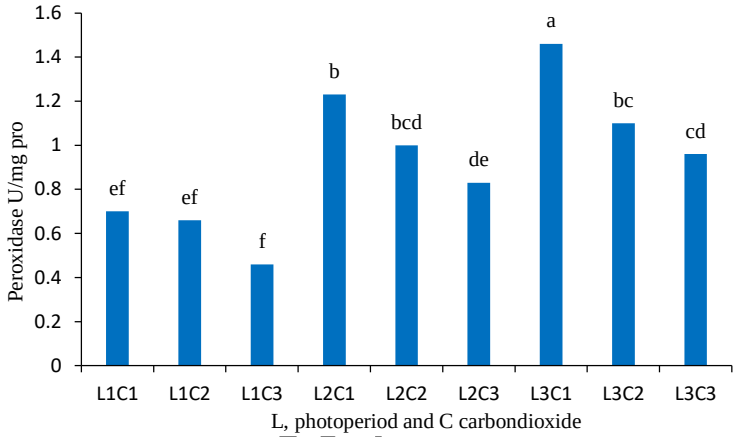

Fig. 4 effect of the interaction of photoperiod and carbon dioxide on Peroxidase of *Thymus Vulgaris*
(C_1: (CO_2 350 mg/L), C_2: (CO_2 700 mg/L) and C_3: (CO_2 1000 mg/L), (L_1: (lighting photoperiods of 12 hours) L_2: (lighting photoperiods of 16 hours) and L_3: (lighting photoperiods of 20 hours)

The results (Figure 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.

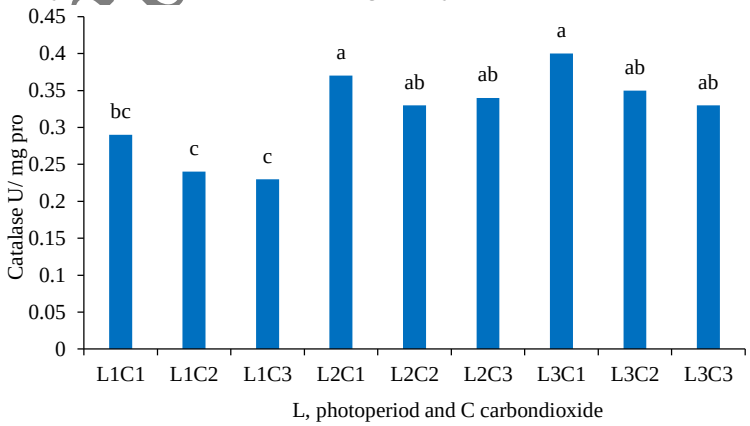

Fig. 5 effect of the interaction of photoperiod and carbon dioxide on Catalase of *Thymus Vulgaris*
(C_1: (CO_2 350 mg/L), C_2: (CO_2 700 mg/L) and C_3: (CO_2 1000 mg/L), (L_1: (lighting photoperiods of 12 hours) L_2: (lighting photoperiods of 16 hours) and L_3: (lighting photoperiods of 20 hours)

The results (Figure 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.

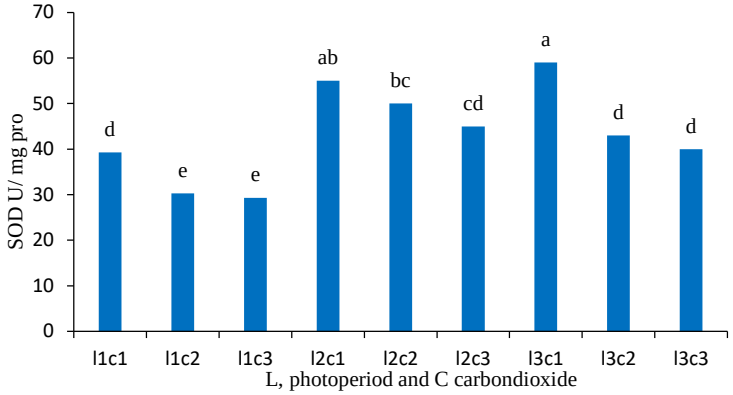

Fig. 6 effect of the interaction of photoperiod and carbon dioxide on Superoxide dismutase (SOD) of *Thymus Vulgaris* **(**C_1: (CO_2 350 mg/L), C_2: (CO_2 700 mg/L) and C_3: (CO_2 1000 mg/L), (L_1: (lighting photoperiods of 12 hours) L_2: (lighting photoperiods of 16 hours) and L_3: (lighting photoperiods of 20 hours)

The results (Figure 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.

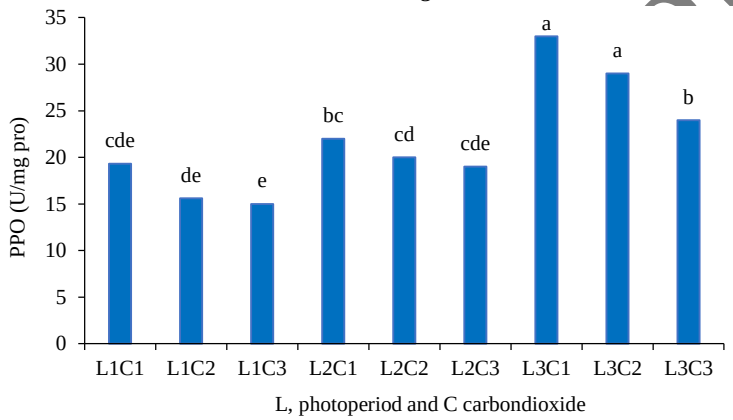

Fig. 7 effect of the interaction of photoperiod and carbon dioxide on Polyphenol oxidase (PPO) of *Thymus Vulgaris* **(**C_1: (CO_2 350 mg/L), C_2: (CO_2 700 mg/L) and C_3: (CO_2 1000 mg/L), (L_1: (lighting photoperiods of 12 hours) L_2: (lighting photoperiods of 16 hours) and L_3: (lighting photoperiods of 20 hours)

The results (Figure 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.

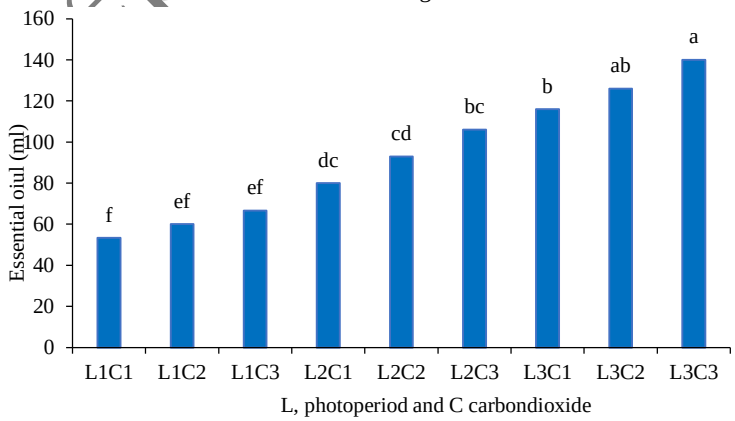

Fig. 8 effect of the interaction of photoperiod and carbon dioxide on Essential oil volume (per 15 gr dry matter) of *Thymus vulgaris.* **(**C_1: (CO_2 350 mg/L), C_2: (CO_2 700 mg/L) and C_3: (CO_2 1000 mg/L), (L_1: (lighting photoperiods of 12 hours) L_2: (lighting photoperiods of 16 hours) and L_3: (lighting photoperiods of 20 hours)

The results (Figure 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.

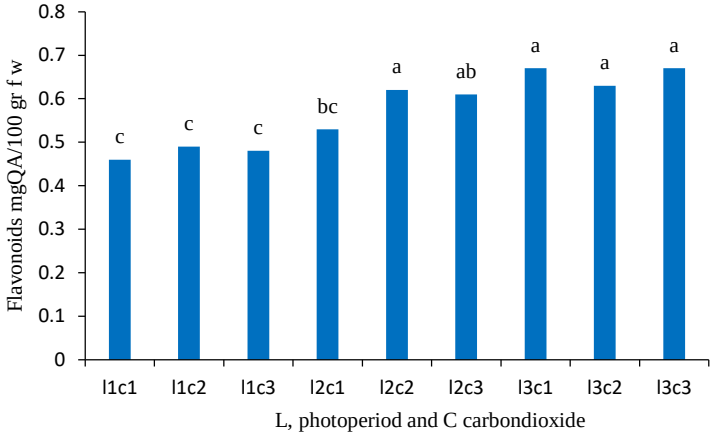

Fig. 9 effect of the interaction of photoperiod and carbon dioxide on Flavonoid of *Thymus Vulgaris.* **(**C_1: (CO_2 350 mg/L), C_2: (CO_2 700 mg/L) and C_3: (CO_2 1000 mg/L), (L_1: (lighting photoperiods of 12 hours) L_2: (lighting photoperiods of 16 hours) and L_3: (lighting photoperiods of 20 hours)

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 CO_2 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 CO_2 enrichment [29]. Combinations of continuous blue light and high CO_2 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 CO_2 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 CO_2. Under elevated CO_2, 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 CO_2. Sugars also interact with hormones to regulate root growth and impact hormone biosynthesis [30].

The current atmospheric CO_2 concentration often limits photosynthesis, growth, and productivity in many C3 plant species. In leaves, the photosynthetic rate is determined by the activity of ribulose bisphosphate carboxylase-oxygenase (Rubisco), which is influenced by CO_2 levels, temperature, and light. Current atmospheric CO_2 levels are insufficient to fully saturate Rubisco activity, and short-term increases in CO_2 availability have been shown to enhance photosynthetic rates. This effect occurs partly because elevated CO_2 inhibits Rubisco's oxygenase activity, thereby reducing photorespiratory CO_2 loss [31].

Elevated CO_2 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 CO_2 concentrations. CO_2 enrichment promotes photosynthesis and water-use efficiency, ultimately enhancing overall plant growth [3]. It has also been demonstrated that CO_2 enrichment facilitates light energy capture and CO_2 fixation, leading to higher photosynthetic rates and capacities. For example, short-term exposure of C3 plants to elevated CO_2 concentrations can increase net photosynthetic rates by 10%–50% [32]. In the current research, CO_2 injection improved growth conditions, reduced plant stress, and decreased the activity of antioxidant enzymes. Under optimal cultivation conditions, CO_2 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 CO_2 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.

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