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Research Article

Temperature-induced physiological and phytochemical changes in *Ziziphora clinopodioides* during the growing season

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Abstract

Temperature has a profound effect on plant growth, development, and phytochemical production. The aim of this study was to evaluate the physiological and phytochemical responses of *Ziziphora clinopodioides* L. to decreasing temperatures throughout the growing season, focusing on nitrogen levels, chlorophyll content, and essential oil composition. By identifying these changes, we sought to determine the optimal growing conditions to improve the quality and industrial applications of this medicinal plant. The investigation was conducted through a field experiment in 2022 at the Faculty of Agriculture, Azarbaijan Shahid Madani University. The experiment followed a randomized complete block design. The results showed a significant relationship between decreasing ambient temperatures and the phytochemical components of the plant. As the temperature decreased, there was a corresponding reduction in the levels of chlorophyll, nitrogen, and essential oils. The results showed an inverse relationship between essential oil content and pulegone with decreasing air temperature. In contrast, menthone, menthol, and piperitone showed a positive correlation with cooler temperatures. Pulegone content decreased by 81.75% from the first sampling at 22.5°C to the sixth sampling at 7°C, with the highest concentration observed at the highest temperature (22.5°C) and the lowest at the lowest temperature (-1°C). Conversely, menthone and menthol concentrations were minimal at the highest temperature (22.5°C) during the first stage and peaked at the seventh stage when temperatures reached 5.5°C. These results provided valuable insights into the biochemical responses of *Z. clinopodioides* to temperature variation, enhancing our understanding of its essential oil quality and potential industrial applications.

Keywords: Essential oil, Leaf temperature, Pulegone, Temperature stress

Introduction

Ziziphora clinopodioides L., commonly known as kakuti, is an aromatic and perennial plant of the family Lamiaceae (Satil and Selvi, 2020). This plant is highly valued for its essential oil and extracts, which have multiple applications in the cosmetic, sanitary, food, and pharmaceutical industries. The essential oil of *Z. clinopodioides* is a colorless or pale-yellow liquid, the yield and composition of which are strongly influenced by the genotype of the plant and the environmental conditions in which it grows (Mehalaine and Chenchouni, 2021).

The essential oil contains a variety of phenolic, flavonoid, and oxygenated monoterpene compounds, including pulegone, piperitone, and thymol. In traditional medicine, *Z. clinopodioides* has been used to treat colds, inflammation, menstrual pain, and digestive disorders (Amiri *et al*., 2019; Hazrati *et al*., 2020a). The growth, yield, and phytochemical composition of medicinal plants are shaped by both genetic and environmental factors, with environmental conditions exerting a profound influence. Understanding these ecological requirements is essential for optimizing the selection, propagation, cultivation, and domestication of such plants and has practical applications for native species (Hazrati *et al*., 2024).

Environmental factors, such as temperature, light, moisture, and nutrients, play a critical role in plant growth and physiology. These factors vary across seasons and locations, leading to significant changes in plant metabolites during different stages of development (Amiri *et al*., 2019; Li *et al*., 2020). In particular, temperature is a major determinant of plant growth and physiological processes, especially photosynthesis. Changes in temperature have profound effects on the morphological, physiological, and phytochemical characteristics of medicinal plants (Hatfield and Prueger, 2015; Chen *et al*., 2018). Cold stress occurs at

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low temperatures ranging from 20°C to below 0°C, while freezing stress refers to damage caused by temperatures below 0°C (Dumlupınar *et al*., 2019). In natural environments, extreme temperatures, whether low or high, negatively affect plant growth, performance, and photosynthesis. These conditions reduce photosynthetic efficiency, CO_2 uptake, and photosystem II activity. Low temperatures disrupt key components of photosynthesis, including thylakoid electron transport, the carbon reduction cycle, and stomatal regulation, often leading to photoinhibition (Bhattacharya, 2022). The impact of low temperatures on photosynthetic efficiency is especially pronounced under high irradiance (Hou *et al*., 2016).

Although photoinhibition reduces the efficiency of photosynthetic electron transport, it can also act as a protective mechanism for the photosynthetic apparatus under environmental stress. Despite the reduction in photosynthetic capacity, plants often exhibit increased sugar accumulation as part of their stress response. This energy dissipation as heat or fluorescence helps to mitigate the damage caused by stress (Ouellet and Charron, 2013; Hajihashemi *et al*., 2018). Cold stress limits the full expression of a plant’s genetic potential by directly inhibiting metabolic reactions and indirectly affecting water uptake and causing cellular dehydration (Ouellet and Charron, 2013; Zhang *et al*., 2020). Exposure to low temperatures induces various physiological, biochemical, and molecular changes in plants that increasetheir resistance to cold stress through a process known as cold acclimation, the ability to survive and mitigate damage under low-temperature conditions. Cold stress increases leaf apoplastic content and total soluble protein while decreasing chlorophyll a and total chlorophyll content. Studies have shown that cold stress significantly affects the diversity of secondary metabolites (Verma and Shukla, 2015; Bhattacharya, 2022).

The synthesis and accumulation of active compounds in medicinal plants vary across temperature ranges and are strongly influenced by both low and high temperatures (Wang *et al*., 2020; Mehalaine and Chenchouni, 2021). Photosynthetic pigments such as chlorophylls and carotenoids, which are crucial for converting light energy into chemical energy, also undergo changes under different temperature conditions. These changes often limit photosynthesis by reducing chlorophyll concentrations at various stages of plant development (Ouellet and Charron, 2013; Zhang *et al*., 2020). Under low-temperature conditions, plant growth slows, and transpiration rates decrease. This reduced transpiration affects the translocation of water-soluble nutrients, such as nitrogen, from the roots to the aerial parts of the plant (Jiao *et al*., 2022). Plants employ several strategies to cope with cold stress, with cold acclimation serving as a critical mechanism to stabilize cellular membranes against freezing damage (Cao *et al*., 2024).

The content and composition of essential oils in aromatic plants are closely linked to their growth conditions and are significantly influenced by environmental factors (Hazrati *et al*., 2024). Recent studies have highlighted the importance of metabolic responses to cold stress, as secondary metabolites play a pivotal role in cold and freezing tolerance (Singh *et al*., 2020). During cold stress adaptation, plants produce secondary metabolites as defensive compounds that act as inducers of stress responses (Lee *et al*., 2019). Consequently, changes in metabolite composition are considered key indicators of a plant’s ultimate response to abiotic stress (Ma *et al*., 2016). The assessment of secondary metabolites provides a powerful tool for detecting and analyzing plant responses under cold stress. This approach provides valuable insights into their mechanisms of adaptation to low temperature environments (Clemente-Moreno *et al*., 2020).

The aim of this research was to evaluate how different temperatures affect the chlorophyll content, nitrogen content, and essential oil composition of *Z. clinopodioides*. The novelty of our study lies in the comprehensive investigation of how reduced temperatures affect the essential oil profile of this important aromatic plant. Given the significant influence of temperature on plant physiology and secondary metabolites, understanding how *Z. clinopodioides* responds to temperature variation during the growing season may guide optimal selection, domestication, and cultivation strategies in specific climatic regions, benefiting the pharmaceutical, cosmetic, and food industries.

Materials and methods

This study was conducted on *Z. clinopodioides* at the research field of Azarbaijan Shahid Madani University in East Azarbaijan, Tabriz, Iran (35° 84' N, 51° 81' E, 1468 m a.s.l.) during the years 2022–2023. The region is characterized as semi-arid, with an annual rainfall of 298 mm, mainly during the autumn and winter seasons. The Sahand Mountains, located to the east, contribute to the region’s cold winters, while the proximity to Lake Urmia results in higher temperatures during the summer months.

The study employed a randomized complete block design with five replications and eight treatments, which included sampling at different temperatures (22.5, 17.5, 15, 10.5, 9, 7, 5.5, and -1°C). The aim was to investigate the effect of decreasing temperature on nitrogen content, chlorophyll levels, and the phytochemical compounds in the essential oil of *Z. clinopodioides*, and to explore the relationship between the content of these compounds and temperature reduction. Temperature and light intensity were measured at different sampling times (Figure 1).

The seeds of *Z. clinopodioides* were collected the previous year from field-grown mother plants at the main research field of Azarbaijan Shahid Madani University. To break the dormancy, the seeds were cold stratified in a freezer at -19°C for one week before

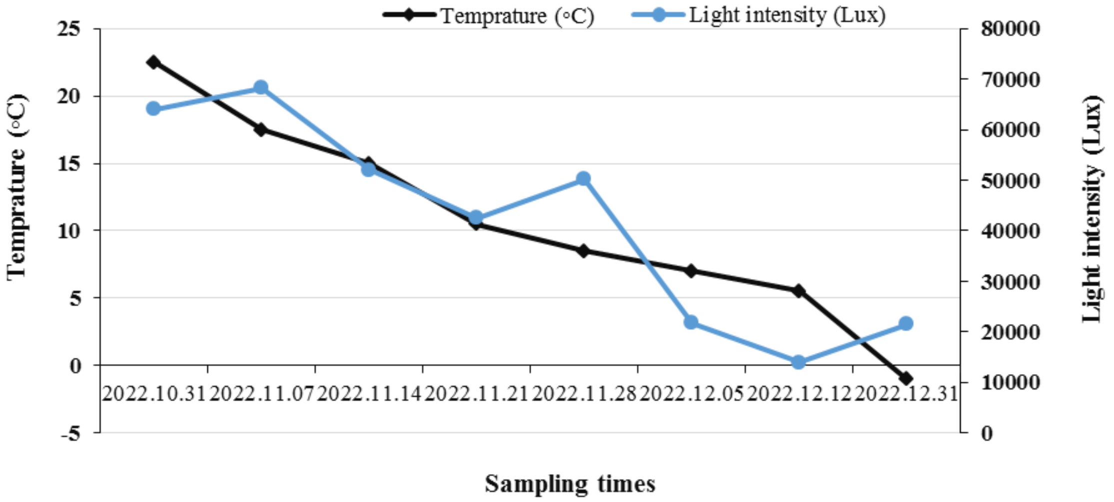

Figure 1. Temperature and light intensity on different sampling dates of *Ziziphora clinopodioides* L. in the experimental field.

sowing (Ezzatmand, 2024). On 10 March, the seeds were planted in special trays filled with peat moss substrate at a depth of 2 mm. The trays were watered daily using overhead irrigation, and germination occurred within 7–10 days. After germination, the seedlings were maintained in a greenhouse until they were ready for transplantation to the main field. Before planting, on 10 May 2022, the field was plowed and leveled. Plots of 2×1.5 m were prepared, each containing four ridges. On 12 May 2022, seedlings were transplanted a spacing of 35×40 cm. Each plot accommodated 32 plants, and the experiment was conducted in three replications. The first harvest took place on 31 November 2022, with 10 plants selected from each plot for analysis. Leaf chlorophyll content, leaf nitrogen levels, and temperature were measured using a Plant Nutrition Analyser (model TYS-4N, China) device. Samples were selected from the middle leaves of the plants at a standard height above the ground. This instrument is equipped with a non-contact infrared sensor that measures leaf surface temperature with an accuracy of ±0.5°C. For each treatment, 10 plants were randomly selected, and the temperature of a fully developed leaf in the upper canopy was measured for each plant. Measurements were taken between 11 am and 2 pm under clear, sunny conditions to ensure uniform environmental conditions for all samples. For each plant, the average of three measurements from different points on the leaf was recorded, and then an overall average was calculated for all 10 plants. Light intensity was also recorded at each sampling time using a lux meter (model WT81). This measurement process was repeated on seven additional dates (Figure 1). At each sampling stage, the plants were harvested 5 cm above the soil surface, and essential oil was extracted from the fresh aerial parts for further analysis.

Essential oil content and composition: To quantify the essential oil content, 50 g of fresh *Z. clinopodioides* leaves were combined with 500 ml of distilled water in a 1-liter glass flask connected to a Clevenger apparatus. The essential oil was extracted over a period of two hours. The remaining aqueous solution was then removed using sodium sulfate, following the procedure described in the British Pharmacopoeia (2023). The extracted oil from each sample was weighed, and the essential oil percentage was calculated. Gas chromatography analysis was conducted using an Agilent Technologies 7890A system equipped with a mass selective detector and an HP-5 fused silica capillary column (30 m × 0.32 mm ID, 0.25 µm FT). The oven temperature program began at 60°C, increased to 210°C at a rate of 3°C min^{-1}, and then to 240°C at 20°C min^{-1}. The final temperature of 240°C was held constant for 8.5 min. The mass spectrometry parameters included an ionization voltage of 70 eV and an ion source temperature of 200°C.

The GC-MS analysis was performed using an Agilent Technologies -5975C-MS and 7890A-GC system with a HP-5MS fused silica capillary column (30 m × 0.25 mm ID, 0.25 µm coating thickness). The oven temperature program was identical to the GC analysis, with a total run time of 60 minutes. The MS system was operated at an electron ionization (EI) energy of 70 eV, an ion source temperature of 230°C, and an interface line temperature of 280°C. The injection port temperature was set at 280°C, with a split ratio of 1:50. Helium was used as the carrier gas at a flow rate of 1 ml min^{-1}, and a mass range of 50–480 amu was used for compound detection. Compounds were identified by comparing their retention times and indices with those in the National Institute of Standards and Technology (NIST 11.0) mass spectra library, the Wiley MS data system library (Wiley, Chichester, UK), and relevant literature. Retention indices were calculated using the C_5–C_{24} n-alkane series under similar thermal conditions. Further identification was achieved by matching mass spectral fragmentation patterns with data from the Adams and Wiley 7.0 library and other published spectra (Adams, 2007). The relative abundance of each essential oil constituent was determined by calculating the peak area of each compound relative to the total chromatographic peak area.

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Statistical analysis: A randomized complete block design (RCBD) with eight treatments was used. Each treatment was replicated five times for the evaluation of physiological traits and essential oil content, while two replications were used for the analysis of essential oil compounds. Data from the study were statistically analyzed using SAS software version 9.2. Microsoft Excel 2016 was used to generate the figures. Comparisons of means were made using Tukey's test at the 5% probability level to determine significant differences between treatments.

Results and discussion

Leaf chlorophyll content (SPAD Index): Analysis of variance revealed a significant effect of decreasing temperature on the chlorophyll content of *Z. clinopodioides* (Table 1). Using Tukey's test (P ≤0.05), the mean total leaf chlorophyll content was compared among eight sampling stages. The highest chlorophyll content was observed at the first sampling stage (22.5°C) (54.90), closely followed by the second stage (17.5°C) (52.20). In contrast, the lowest chlorophyll content was recorded at the fourth stage (10.5°C) (33.83). Notably, no significant differences were found between the 15°C and -1°C. These results suggest that chlorophyll content decreases significantly under conditions of reduced temperature and light intensity. The results indicated a direct relationship between temperature reduction and chlorophyll concentration in *Z. clinopodioides*, with lower temperatures correlating with reduced chlorophyll levels (Figure 2).

Chlorophyll content varies significantly widely between species and cultivars, and stress conditions have a profound effect on these levels. Cold stress is a critical factor influencing chlorophyll concentration, which isused by researchers as an indicator of plant cold tolerance (Wang *et al*., 2008; Bano *et al*., 2015). Under low temperature conditions, leaf chlorophyll content typically declines due to reduced activity of enzymes involved in chlorophyll synthesis and potential damage to chloroplasts, i.e., the cellular organelles that house chlorophyll. This decrease reflects the complex physiological mechanisms underlying plant responses to cold stress.

Cold stress disrupts the electron transport chain, leading to reduced photosynthetic enzyme activity and stomatal conductance. These effects inhibit both the light and dark reactions of photosynthesis while accelerating chlorophyll degradation through multiple pathways. -Reactive oxygen species play a significant role in this process by directly damaging chloroplast structures (Ouellet and Charron, 2013; Zhang *et al*., 2020). In addition, lipid peroxidation, caused by cold-induced oxidative stress, contributes to chlorophyll reduction by degrading cell membranes, further impairing photosynthetic efficiency (Kuk *et al*., 2003; Tian *et al*., 2020). Chlorophyll fluorescence serves as a sensitive indicator of photosynthetic performance under cold stress. Increased fluorescence typically reflects a reduction in photosynthetic enzyme activity and changes in molecular responses (Khanizadeh and Deell, 2001; Miralles-Crespo *et al*., 2011). Numerous studies have documented changes in chlorophyll content in different plant species in response to cold stress. For example, low temperatures significantly reduce chlorophyll content in oilseed rape, affecting key physiological processes (Jiaojiao *et al*., 2019). Wheat cultivars exhibit variable responses, with some showing significant decreases in chlorophyll content and others displaying increases following cold acclimation (Alisoltani *et al*., 2012). In *Ligustrum lucidum*, cold stress leads to reduced leaf chlorophyll content (Yang *et al*., 2019), while *Camellia sinensis* experiences significant chlorophyll depletion (Li *et al*., 2018). Broader studies have consistently reported reductions in chlorophyll levels under cold stress in species such as Bermuda grass, cucumber, tomato, pepper, marigold, and celery (Anwar *et al*., 2018; Elkelish *et al*., 2020; Bhandari *et al*., 2018; Cheng *et al*., 2018; Huang *et al*., 2017).

Leaf nitrogen content: Analysis of variance revealed significant differences in the leaf nitrogen content of *Z. clinopodioides* across temperatures (Table 1). Tukey's test revealed detectable variations in nitrogen content among the eight temperatures (P ≤0.05). The highest nitrogen content was recorded during the first two sampling stages: 20.08 mg/g at the highest ambient temperature (22.5°C) and 19.18 mg/g at the second-highest temperature (17.5°C). Notably, no statistically significant differences were observed between these two temperatures. Conversely, the lowest nitrogen content was recorded at -1°C, with an average of 13.33 mg/g, with no significant differences observed between the samples taken from 17.5°C to -1°C. Figure 3 shows a clear correlation between environmental conditions and leaf nitrogen content. The maximum nitrogen levels coincided with the highest temperature and light intensity during the first (22.5°C) and second (17.5°C) sampling periods. As temperature and light intensity declined, a corresponding reduction in nitrogen content was observed, with the lowest levels recorded during the eighth sampling under conditions of minimum temperature and light intensity. These results highlight an inverse relationship between temperature reduction and leaf nitrogen content in *Z. clinopodioides*, indicating that environmental stress has a significant impact on the nutrient composition of the plant. Leaf nitrogen is a critical component of plant physiology, playing a fundamental role in growth and development. Its content is highly sensitive to environmental conditions, particularly temperature, with cold stress causing significant declines in nitrogen levels. The relationship between nitrogen and plant water dynamics is complex and involves three primary mechanisms: increased root activity for simultaneous nitrogen and water uptake, an accelerated growth rate demanding greater water resources, and the essential role of nitrogen in the production of photosynthetic compounds

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Table 1. Effects of cold stress (low temperature) on physiological parameters of *Ziziphora clinopodioides* L.

Source of variation	df	Chlorophyll index	Nitrogen content	Leaf temperature	Essential oil content
Temperature	7	357.09^{**}	36.23^{**}	104.01^{**}	0.030^{**}
Replication	4	12.45^{ns}	3.71^{ns}	4.59^{**}	0.001^{ns}
Error	28	22.57	1.65	0.51	0.003
Coefficient of variation (%)	-	11.76	8.64	3.90	14.48

ns, * and ** indicate non-significant and significant differences at 5% and 1% probability levels, respectively

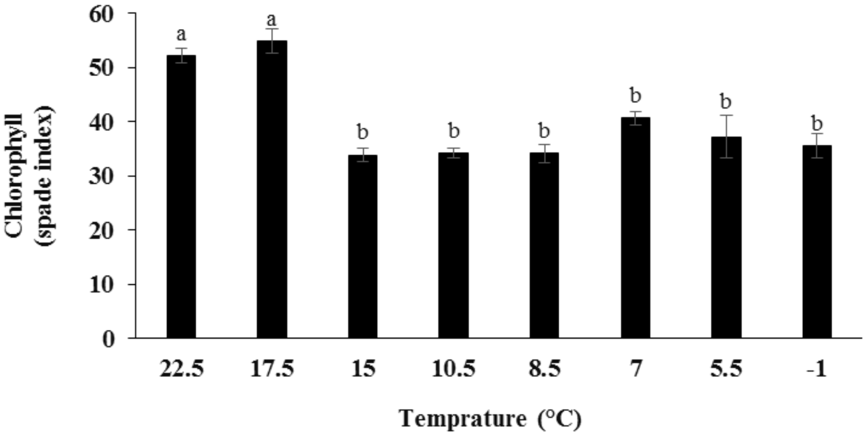

Figure 2. Effect of cold stress (low temperature) on total chlorophyll content of Ziziphora clinopodioides. Different letters within the same column indicate significant differences between mean values according to Tukey's honest significant difference (HSD) test (P ≤ 0.05). Each column represents the mean ± standard error.

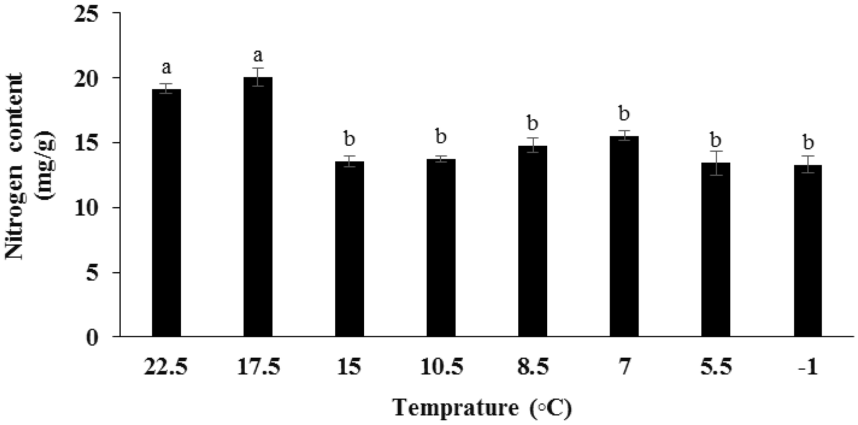

Figure 3. Effect of cold stress (low temperature) on total nitrogen content of Ziziphora clinopodioides. Different letters within the same column indicate significant differences among mean values according to Tukey's honest significant difference (HSD) test (P ≤ 0.05). Each column represents the mean ± standard error.

such as chlorophyll and chromophore molecules. Adequate nitrogen availability increases photosynthetic efficiency, thereby increasing water demand for energy production and metabolic processes (Fageria *et al.*, 2008; Taiz and Zeiger, 2010; Marschner, 2012).

Cold stress profoundly disrupts plant physiological systems through its interactions with nitrogen and chlorophyll content. Key physiological changes include impaired stomatal function, leading to significantly reduced photosynthetic efficiency, and increased intercellular CO_2 concentrations. In addition, cold stress triggers the excessive production of ROS, which initiates a cascade of detrimental cellular effects. These reactive molecules can peroxidize membrane lipids, fragment DNA, and inactivate critical enzymatic processes, ultimately challenging the plant's ability to maintain metabolic homeostasis (Wu *et al.*, 2015; Jiaojiao *et al*., 2019; Dumlupınar *et al*., 2019).

Low temperatures induce complex physiological adaptations, mainly through changes in enzyme activity and nitrogen metabolism. Cold stress can reduce the activity of enzymes critical for the synthesis of nitrogenous compounds, potentially due to diminished chlorophyll synthesis enzymes or limited availability of essential precursors such as amino acids and nitrogen. As part of a strategic response, plants actively redistribute nitrogen from leaves to stems and roots to protect against leaf desiccation and frost damage. This adaptive mechanism, while conserving resources, disrupts photosynthetic capacity by reducing leaf

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chlorophyll and nitrogen content. At the same time, reduced root activity further compromises nitrogen uptake, creating a cascading effect that limits nitrogen availability in leaf tissues. These physiological changes represent a sophisticated survival strategy that allows plants to mitigate cold stress through metabolic reconfiguration (Jiao *et al*., 2022; Xiong and Zhu, 2001; Yamori *et al*., 2011; Schulz *et al*., 2013). The resulting reduction in leaf chlorophyll and nitrogen content is a critical acclimation response that allows plants to conserve resources and improve cold tolerance.

A study was conducted to investigate the effects of cold stress on the yield and quality of *Oryza sativa* L., focusing on leaf conditions during the ripening stage. The aim was to analyze changes in leaf characteristics in response to low temperatures. The results showed a continuous decrease in photosynthetic activity, which was exacerbated under cold conditions. Similarly, leaf chlorophyll content decreased in both control and cold-treated plants after treatment. Under controlled conditions, a significant correlation was observed between chlorophyll content and leaf nitrogen content (Hwang *et al*., 2019). Similar results have been reported in studies on tomato (Khan *et al*., 2019), cucumber (Anwar *et al*., 2018), maize (Jie *et al*., 2016), and eggplant (Wu *et al*., 2015), which consistently showed reduced photosynthetic activity and slower growth under cold stress. These reductions appear to be closely linked to reduced nitrogen levels, suggesting that nitrogen stress plays a key role in the physiological response of plants to cold stress.

Leaf temperature: Analysis of variance revealed significant variation in leaf temperature across different growth stages of *Z. clinopodioides* (Table 1). Tukey's test at the 5% significance level identified notable fluctuations in leaf temperature during plant development. The highest leaf temperature, 24.51°C, was observed during the second sampling stage (17.5°C), while the lowest, 12.81°C, was recorded during the sixth stage (7°C). A significant difference was particularly evident between the third and eighth sampling stages (Figure 4). As depicted in Figure 4, leaf temperature exhibited a gradual decline corresponding to the decrease in ambient air temperature.

Cold-tolerant plants have developed sophisticated adaptation mechanisms to withstand low-temperature conditions. These mechanisms involve a range of physiological responses, including the production of cold-resistant proteins, metabolic regulation of thermogenesis (internal heat production), and increased oxidation of nutrients such as fats and carbohydrates. Plant hormones, particularly salicylic acid, play a key role in regulating thermogenesis under cold stress. Elevated levels of this hormone act as a key regulatory mechanism, enabling plants to adapt to environmental challenges by increasing heat production. This complex process not only helps plants maintain optimal internal temperatures but also protects against cold-induced cellular damage. Insights into these hormonal and physiological mechanisms could inform the development of crop varieties with enhanced cold tolerance, with the potential to improve in agricultural resilience in cold climates (Khan *et al*., 2017; Saleem *et al*., 2021; Vergata *et al*., 2022; Devi *et al*., 2023).

Essential oil content: Statistical analysis revealed significant variations in the percentage of essential oil at different temperatures of the *Z. clinopodioides* plant (Table 1). According to Tukey's test ($P \leq 0.05$), the highest essential oil content (0.55%) was recorded during the second sampling stage (17.5°C). Notably, no statistically significant differences were observed between the samples taken at 22.5, 17.5, 15, and 10.5°C. On the other hand, the lowest essential oil content (0.10%) was observed at -1°C, with no significant differences between the samples collected at 8.5, 7, 5.5, and -1°C. Figure 5 clearly illustrates the progressive decline in essential oil content with decreasing temperature, highlighting the direct correlation between lower temperatures and decreased essential oil production.

The reduction in essential oil production can be attributed to complex physiological responses to temperature stress. According to the Stefan-Boltzmann law, air temperature directly influences the intensity of solar radiation (Gim and Kim, 2016; Tang *et al*., 2020), which triggers cascading effects on plant metabolic processes. Lower temperatures lead to reduced light intensity, critically impacting physiological mechanisms such as decreased enzyme activity involved in secondary metabolite biosynthesis and reduced photosynthetic efficiency (Ouellet and Charron, 2013; Qaderi *et al*., 2023). Temperature stress also disrupts essential oil biosynthesis by suppressing key enzymatic activities (Ouellet and Charron, 2013), impairing nutrient uptake, and compromising cellular health (Ouellet and Charron, 2013; Jiao *et al*., 2022). Additionally, low temperatures alter gene expression (Jurczyk *et al*., 2012; Li *et al*., 2020) and reduce photosynthetic rates (Tian *et al*., 2020), further affecting the metabolic pathways required for essential oil production. These interrelated physiological disruptions ultimately lead to reduced production and accumulation of essential oil components, significantly decreasing essential oil content under cold stress conditions (Qaderi *et al*., 2023).

Essential oil components: Using gas chromatography-mass spectrometry (GC-MS), this study comprehensively analyzed the essential oil composition of *Z. clinopodioides* and identified eight distinct compounds, categorized into hydrocarbon and oxygenated monoterpenes. The major essential oil components, characterized by significant concentration variations, included pulegone (75.35–81.75%), menthone (7.15–15.50%), menthol (0.75–3.35%), and piperitone (3.60–7.65%) (Figure 6). The analysis of variance revealed that sampling temperatures significantly influenced the concentrations of essential oil components, including α-pinene, limonene, 1,8-

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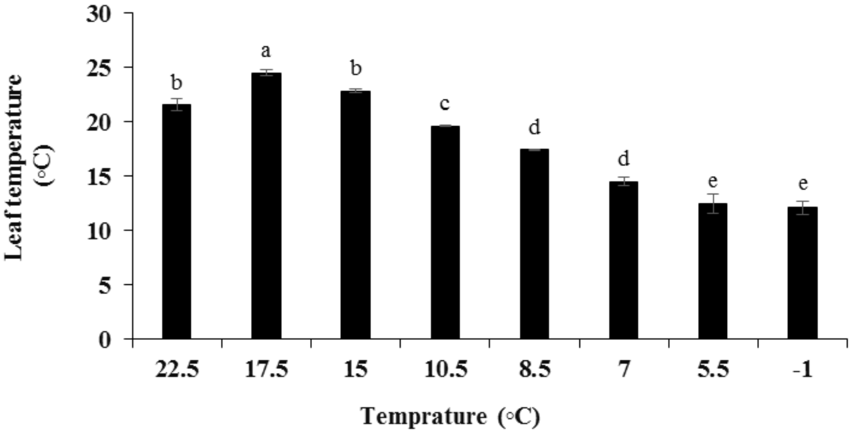

Figure 4. Effect of cold stress (low temperature) on leaf temperature of Ziziphora clinopodioides. Different letters within the same column indicate significant differences among mean values according to Tukey's honest significant difference (HSD) test (P ≤ 0.05). Each column represents the mean ± standard error.

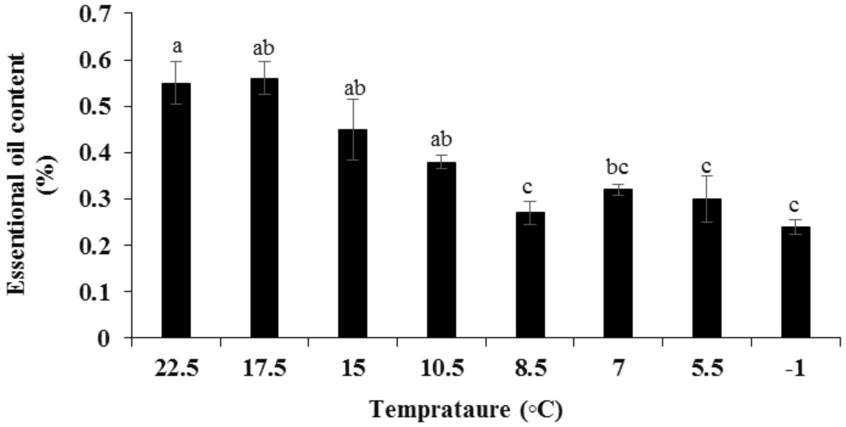

Figure 5. Effect of cold stress (low temperature) on the essential oil content of Ziziphora clinopodioides. Different letters within the same column indicate significant differences among mean values according to Tukey's honest significant difference (HSD) test (p ≤ 0.05). Each column represents the mean ± standard error.

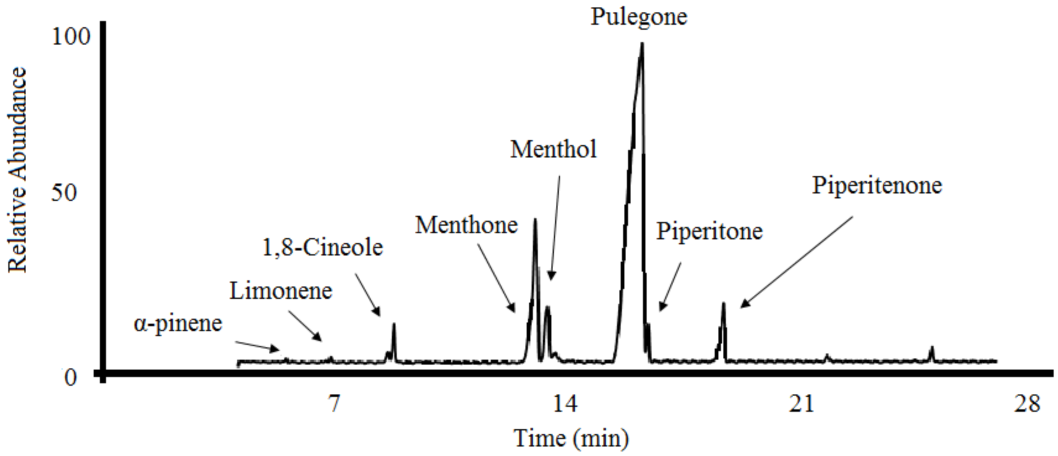

Figure 6. Gas chromatography-mass spectrometry (GC-MS) chromatogram of Ziziphora clinopodioides essential oil.

cineole, menthone, menthol, pulegone, piperitone, and piperitenone (Table 2). Notable differences were observed across varying environmental temperatures. Specifically, α-pinene concentration peaked at 0.27% at 10.5°C, with no statistically significant differences among the 22.5, 17.5, 15, 10.5, 5.5, and -1 °C samples. Conversely, the lowest α-pinene concentration (0.10%) was recorded at 8.5°C, with no significant variation

Table 2. Analysis of variance (mean squares) of the effect of cold stress (low temperature) on the essential oil composition of *Ziziphora clinopodioides*.

Source of variation	df	α-pinene	Limonene	1,8-cineole	Menthone	Menthol	Pulegone	Piperitone	Piperitenone
Temperature	7	0.008**	0.004**	0.646**	15.976**	2.050**	8.922**	4.995**	8.92**
Replication	1	0.001^{ns}	0.001^{ns}	0.016^{ns}	0.003^{ns}	0.008^{ns}	0.490^{ns}	0.331^{ns}	0.49^{ns}
Error	7	3.787	1.541	3231.696	1597691.455	20505.134	14870.833	4995.410	0.008
Cv (%)	-	15.1	10.34	9.18	3.33	8.60	0.92	5.61	16.04

^{ns}, * and ** indicate non-significant and significant differences at 5% and 1% probability levels, respectively.

between the 10.5 and 7°C samples. These results emphasize the complex interactions between environmental conditions and essential oil composition in medicinal plants. They highlight the intricate biochemical dynamics of *Z. clinopodioides* throughout its developmental cycle and provide valuable insights into how temperature affects the biosynthesis of key essential oil components.

Comparative mean analysis revealed statistically significant differences in limonene levels between the different temperature sampling stages. The highest limonene concentration (0.23%) was recorded at 10.5°C, with no significant differences observed among the stages at 22.5, 17.5, 15, 10.5, 7, and -1°C. In contrast, the lowest limonene concentration (0.11%) was observed at 7°C, with no significant differences between 8.5 and 7°C. Statistical analysis also revealed significant differences in 1,8-cineole concentrations across temperatures. The highest 1,8-cineole content (2.70%) was detected at 22.5°C, with no significant difference between 22.5 and 17.5°C. Conversely, the lowest concentration (1.05%) occurred at 7°C, with no statistically significant differences observed among 10.5, 8.5, 7, 5.5, and -1°C. Table 3 illustrates a clear inverse relationship between temperature and limonene concentration, indicating a progressive decline in limonene levels with increased cold exposure. This trend underscores the temperature-dependent metabolic adaptations in the essential oil composition of *Z. clinopodioides*. Analysis of variance revealed statistically significant differences in menthone levels across sampling stages with varying air temperatures. The maximum menthone concentration (15.50%) was recorded at 10.5°C, with no significant differences among the 10.5, 8.5, and 7°C stages. Conversely, the minimum menthone content (7.15%) was observed at 22.5°C, highlighting the dynamic changes in this essential oil component during plant development under different temperature conditions. Similarly, menthol concentrations exhibited significant variation across sampling stages. The highest menthol content (3.35-%) was recorded at 5.5°C, while the lowest concentration (0.45-%) was observed at 7°C. Notably, no statistically significant differences were identified among the 22.5, 17.5, and 15°C stages. Pulegone content also varied across growth stages of *Z. clinopodioides* under different temperatures. The highest concentration (81.35%) was observed at 22.5°C, with no significant differences between 22.5 and 17.5°C. Pulegone content progressively decreased, reaching its lowest level (74.95%) at 5.5°C. No statistically significant differences were found between the stages from 15 to -1°C.

The statistical analysis revealed significant variations in piperitone content across the different temperature treatments. The highest piperitone concentration (7.65%) was recorded at 22.5°C, with no statistically significant difference between 22.5 and 17.5°C. Conversely, the lowest concentration (3.60%) was observed at 10.5°C, with no significant differences among 10.5, 8.5, 5.5, and -1°C. The data suggest an inverse relationship between temperature and piperitone levels, with concentrations declining from the early sampling stages to the later ones.

This observation is consistent with broader research on plant responses to cold stress, which induce complex biochemical changes that significantly alter phytochemical concentrations. Cold temperatures elicit diverse physiological responses, including intricate metabolic adaptations that can either increase or decrease the production of secondary metabolites. For instance, cold stress often triggers enzymatic and metabolic adjustments that maintain cellular integrity under adverse conditions. When exposed to low temperatures, plants often show an increased production of protective antioxidants such as flavonoids, carotenoids, and anthocyanins. This response is commonly attributed to increased enzyme activity and the synthesis of protective compounds (Yamori *et al.*, 2011; Schulz *et al.*, 2013). However, cold stress can also lead to cellular damage, including water infiltration and structural disruption, which can reduce phytochemical content (Promyou *et al.*, 2012; Ashraf *et al.*, 2018).

Plants employ sophisticated physiological mechanisms to mitigate cold stress, including the regulation of enzyme functions, the production of antifreeze proteins and antioxidant metabolites, and the modulation of growth-related metabolites. These adaptive strategies result in dynamic changes in phytochemical levels, reflecting the plant's capacity to survive under adverse environmental conditions. The remarkable ability of plants to adjust their biochemical composition under stress highlights the importance of these changes as survival mechanisms (Jeong and Sung, 2011; Hossain *et al.*, 2019; Samec *et al.*, 2022). Cold stress also modulates secondary metabolite accumulation through complex mechanisms involving phytohormones, transcriptional regulation, enzymatic activity, and epigenetic processes (Qaderi *et al.*, 2023;

Table 3. Essential oil composition (%) when exposing *Ziziphora clinopodioides* to low temperatures.

Different time points (°C)	Piperitenone	Piperitone	Pulegone	Menthol	Menthone	1,8-cineole	Limonene	α-pinene
22.5	7.65±0.15^{a}	0.15±0.05^{d}	81.35±0.15^{a}	0.45±0.05^{e}	0.45±0.05^{e}	2.70±0.20^{a}	0.20±0.01^{a}	0.27±0.02^{a}
17.5	7.45±0.15^{a}	0.25±0.02^{d}	79.05±0.15^{ab}	0.67±0.02^{de}	0.67±0.10^{de}	2.45±0.15^{ab}	0.21±0.01^{a}	0.19±0.01^{abc}
15	4.85±0.35^{bc}	0.28±0.02^{d}	77.75±0.25^{bc}	1.15±0.05^{cd}	1.15±0.45^{cd}	1.85±0.15^{bc}	0.22±0.02^{a}	0.21±0.01^{ab}
10.5	3.60±0.10^{d}	0.39±0.01^{cd}	76.70±0.50^{bc}	2.50±0.00^{b}	2.50±0.50^{b}	1.55±0.05^{cd}	0.32±0.02^{a}	0.21±0.01^{ab}
9.2	3.70±0.50^{cd}	0.75±0.05^{bc}	77.60±0.10^{bc}	1.65±0.05^{c}	1.65±0.20^{c}	1.25±0.05^{cd}	0.12±0.02^{bc}	0.10±0.11^{c}
7	5.55±0.05^{b}	0.60±0.00^{bcd}	75.00±0.50^{c}	2.60±0.20^{b}	2.60±0.25^{b}	1.05±0.05^{d}	0.11±0.01^{c}	0.14±0.04^{bc}
5.5	4.35±0.15^{cd}	1.45±0.016^{a}	75.95±0.45^{c}	3.35±0.15^{a}	3.35±0.20^{a}	1.55±0.05^{cd}	0.19±0.01^{ab}	0.11±0.01^{bc}
-1	4.40±0.10^{bcd}	0.77±0.07^{b}	76.70±0.10^{bc}	1.40±0.10^{c}	1.40±0.05^{c}	1.55±0.05^{cd}	0.19±0.01^{ab}	0.11±0.01^{bc}

Different letters within the same column indicate significant differences among mean values according to Tukey's honest significant difference (HSD) test ($P \leq 0.05$). Each column represents the mean ± standard error.

He *et al*., 2022). These metabolic and physiological adjustments emphasize the intricate nature of plant responses to low temperatures, orchestrating significant changes in secondary metabolite production across various species.

Different plant species exhibit unique metabolic responses to temperature changes. In *Hordeum vulgare* L. (barley), exposure to low temperatures (5°C) triggers significant metabolic adjustments. Labate and Leegood (1989) reported a rapid decline in leaf respiration rates accompanied by a substantial increase in phosphorylated metabolites, with hexose phosphates accounting for about two-thirds of the observed metabolic changes. These results highlight the remarkable ability of plants to regulate respiration and carbohydrate metabolism under cold stress. Research on various plant species reveals diverse physiological and metabolic adaptations to low-temperature stress. For example, *Brassica oleracea* exhibits significant alterations in physiological status and phytochemical composition, characterized by decreased carotenoid and flavonoid levels and increased proline accumulation (Samec *et al*., 2022). Similarly, *Withania somnifera* shows increased production of secondary metabolites during seasonal low temperatures, with implications for optimizing harvesting strategies (Kumar *et al*., 2012). In a comprehensive study on mango cultivars, prolonged cold stress was shown to induce the accumulation of flavonoids, terpenoids, lignans, coumarins, and alkaloids, with the extent of accumulation closely correlated with the duration of exposure (Kong *et al*., 2024). Molecular studies have also revealed that low-temperature stress can negatively regulate specific biosynthetic pathways. For instance, in *Catharanthus roseus*, the indole alkaloid terpenoid pathway is downregulated under cold conditions (Dutta *et al*., 2007).

Cluster analysis and principal component analysis (PCA): The measured characteristics of *Z. clinopodioides* at different temperatures were analyzed using cluster analysis, which illustrated the similarities and dissimilarities between temperature treatments based on branch lengths (Figure 8). The most similar temperature treatments were 22.5°C and 17.5°C, with almost identical physiological characteristics and essential oil composition. The next similar treatment was 9°C. Another close cluster was formed by the 15°C and 10.5°C treatments. Conversely, the most distinct temperature treatments were 7°C, 5.5°C, and -1°C, which showed the greatest divergence in measured characteristics (Figure 8).

PCA was used to analyze the physiological and essential oil characteristics of Z. *clinopodioides* in response to different temperatures to provide a comprehensive overview and interpretation of the results obtained. In the principal component analysis across temperatures, PC1 explained 70.43% of the cumulative variance, and PC2 explained 10.84% of the total variance (Figure 7). Temperature treatments of 22.5°C, 17.5°C, and 15°C, positioned on the positive side of PC1 in the upper and lower right quadrants, were associated with plants having higher concentrations of limonene, leaf temperature, pulegone, α-pinene, essential oil content, 1,8-cineole, piperitenone, nitrogen content, and SPAD value. In contrast, treatments at 9°C, 7°C, 5.5°C, and -1°C on the negative side of PC2 in the lower left quadrant produced plants with elevated levels of menthol and piperitone, while 10. °C on the negative side of PC2 in the upper left quadrant was characterized by higher menthone content.

Correlation analysis of plant traits and chemical composition: Linear correlation analysis revealed intricate interactions between environmental conditions and plant physiological traits, as well as essential oil composition (Figure 9). The chlorophyll index exhibited significant positive correlations with ambient temperature, light intensity, nitrogen content, essential oil content, and compounds such as α-pinene, pulegone, and piperitone, while displaying negative correlations with menthol and menthone. Similarly, leaf nitrogen content demonstrated complex relationships, positively correlating with several environmental and phytochemical parameters, including ambient temperature, chlorophyll content, essential oil content, and essential oil compounds such as α-pinene, 1,8-cineole, pulegone, and piperitone.

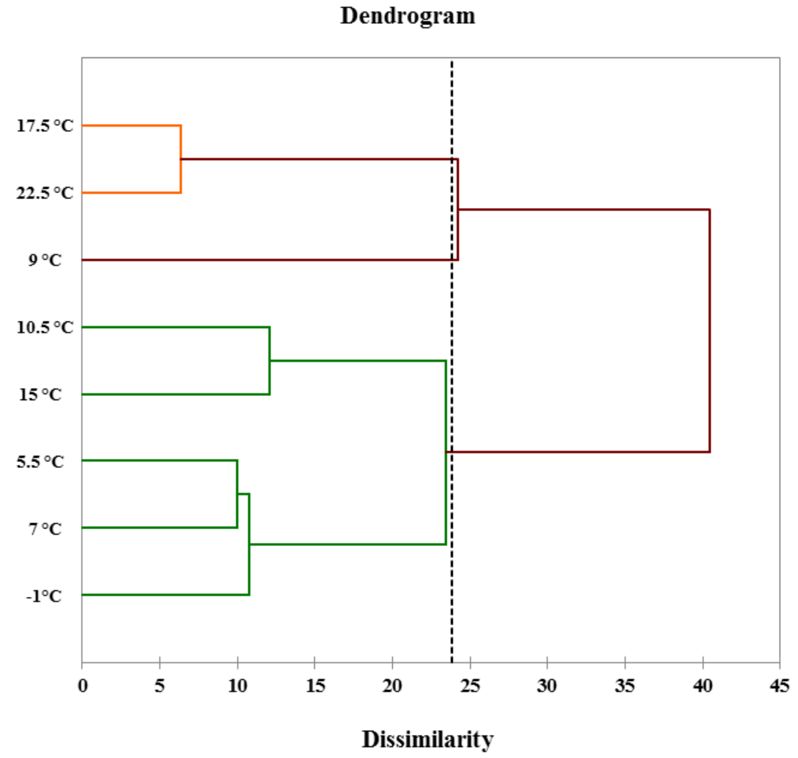

Figure 7. Hierarchical cluster analysis based on all studied traits at different temperatures in *Z. clinopodioides*.

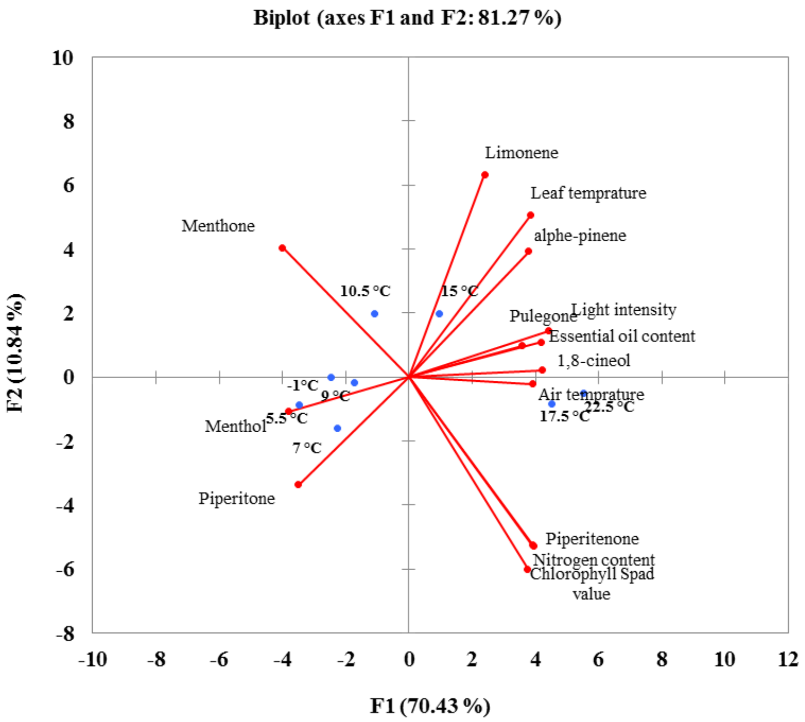

Figure 8. Principal component analysis (PCA) of physiological and essential oil traits of *Z. clinopodioides* under different temperatures.

Leaf temperature correlated positively with ambient temperature, light intensity, nitrogen content, essential oil content, and several major compounds. However, it showed negative correlations with menthol, menthone, and piperitone (Dutta *et al*., 2007; Kumar *et al*., 2012; Samec *et al*., 2022; Kong *et al*., 2024). Essential oil content and its individual components exhibited detectable correlation patterns. Essential oil content was positively associated with numerous environmental and physiological traits, including the chlorophyll index,

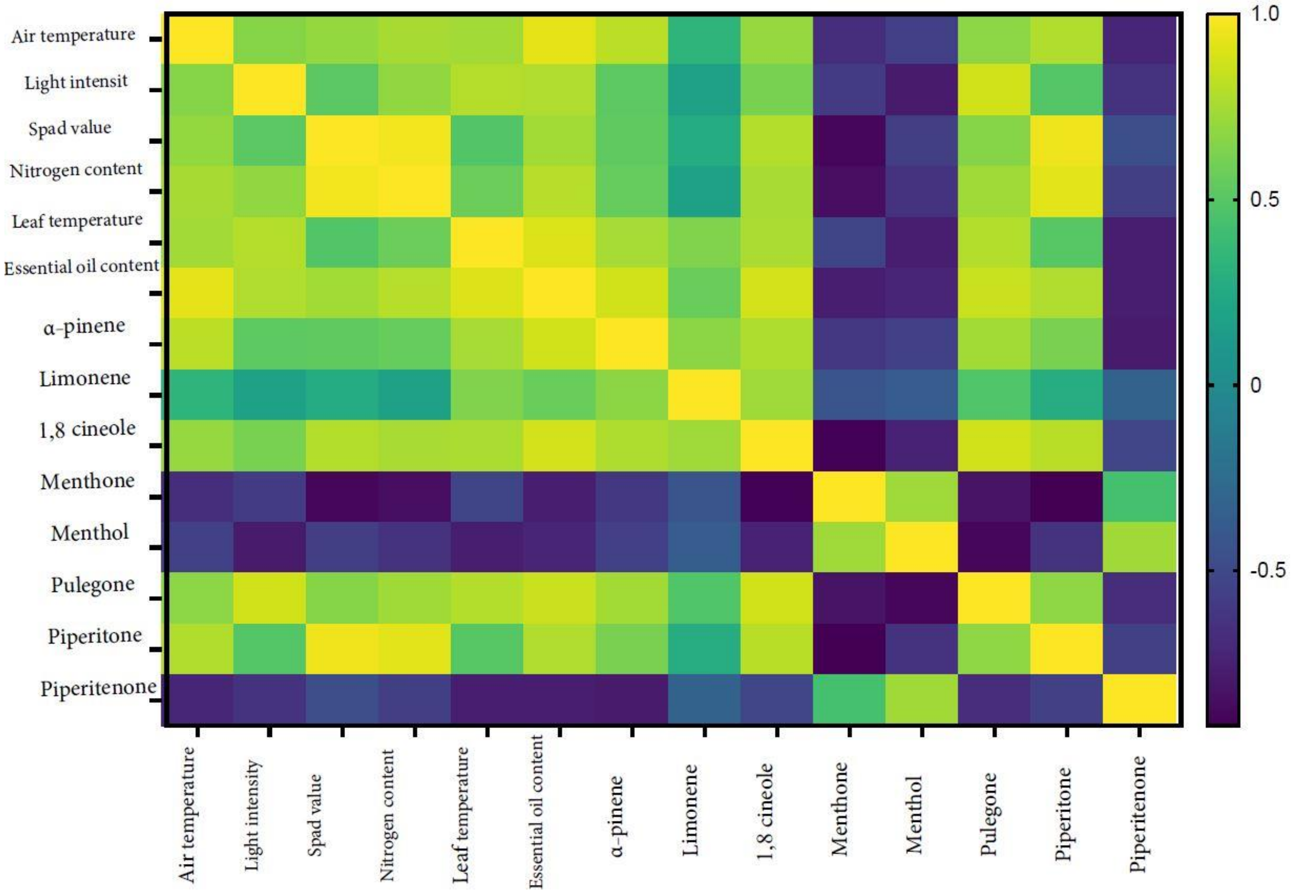

Figure 9. Correlation analysis of the studied traits under the influence of different temperature conditions (decreasing temperatures).

ambient temperature, light intensity, nitrogen content, and essential oil compounds such as α-pinene, limonene, pulegone, and 1,8-cineole. Additionally, α-pinene displayed similarly widespread positive correlations, while limonene showed more limited correlational relationships. Interestingly, 1,8-cineole emerged as a notable compound, demonstrating positive correlations with nearly all traits studied, contrasting with the predominantly negative correlations observed for menthone and menthol. Pulegone displayed consistent positive correlations with most traits, whereas piperitenone exhibited mixed correlations. The comprehensive correlation analysis highlights the dynamic and interconnected nature of plant metabolic processes, reflecting the intricate interplay between environmental factors (low temperature), physiological responses, and secondary metabolite production in this plant species.

Conclusion

Z. clinopodioides L. is a valuable medicinal plant with significant pharmacological potential, distinguished by its evergreen and perennial flowering characteristics. This comprehensive study systematically evaluated the effects of decreasing ambient temperature on plant physiological traits and essential oil composition, revealing complex metabolic responses to temperature variation. The results showed a clear relationship between ambient temperature and plant biochemistry. Key physiological parameters, including chlorophyll content, nitrogen levels, and essential oil percentages, showed significant decreases with decreasing temperatures. In particular, pulegone concentration showed a negative correlation with decreasing temperatures, whereas menthone, menthol, and piperitone showed opposite trends, with concentrations increasing in colder conditions. These results highlight the plant’s sophisticated adaptive mechanisms and the dynamic nature of the biochemical composition in response to changing environmental conditions. This study provides valuable insights into the seasonal variability of *Z. clinopodioides* and offers a comprehensive understanding of how temperature fluctuations influence its phytochemical profile. By establishing a robust framework for evaluating essential oil quality and potential industrial applications, this research makes a significant contribution to the broader field of medicinal plant biochemistry. Future studies should aim to build on these findings by exploring the effects of different habitat conditions and low temperature thresholds on plant performance, chemical composition, and potential antibacterial properties across different seasonal contexts.

References

Adams, R. P. (2007). Identification of essential oil components by gas chromatography/uadrupole mass spectrometry. 4th Ed. Carol Stream: Allured Publishing.

Alisoltani, A., Alizadeh, H., Mahfoozi, S., & Khayalparast, F. (2012). Effect of short and long terms cold acclimation on biochemical characteristics of spring and winter wheat (*Triricum aestivum* L.) cultivars. *Iranian Journal of Crop Sciences*, *14*, 108-120.

Amiri, F., Gholipouri, A., Kheirkhah, M., & Mirjalili, M. H. (2019). Eco-Physiology of *Ziziphora clinopodioides* LAM. (Lamiaceae) from Iran. *Annali di Botanica*, *9*, 63-72.

Anwar, A., Yan, Y., Liu, Y., Li, Y., & Yu, X. (2018). 5-Aminolevulinic acid improves nutrient uptake and endogenous hormone accumulation, enhancing low-temperature stress tolerance in cucumbers. *International Journal of Molecular Sciences*, *19*(11), 3379.

Ashraf, M. A., Iqbal, M., Rasheed, R., Hussain, I., Riaz, M., & Arif, M. S. (2018). Environmental stress and secondary metabolites in plants: An overview. *Plant Metabolites and Regulation under Environmental Stress*, 153-167.

Bano, S., Aslam, M., Saleem, M., Basra, S., & Aziz, K. (2015). Evaluation of maize accessions under low temperature stress at early growth stages. *Journal of Animal and Plant Sciences*, *25*, 392-400.

Bhattacharya, A. (2022). Physiological Processes in Plants under Low Temperature Stress. Springer, Singapore. https://doi.org/10.1007/978-981-16-9037-2_1

Bhandari, S. R., Kim, Y. H., & Lee, J. G. (2018). Detection of temperature stress using chlorophyll fluorescence parameters and stress-related chlorophyll and proline content in paprika (*Capsicum annuum* L.) seedlings. *Horticultural Science and Technology*, *36*(5), 619-629.

British Pharmacopoeia. (2023). Published on the Recommendation of the Medicines Commission pursuant to the Medicines Act, Her Majesty’s Stationary Office. London, UK.

Cao, J., Bao, J., Lan, S., Qin, X., Ma, S., & Li, S. (2024). Research progress on low-temperature stress response mechanisms and mitigation strategies in plants. *Plant Growth Regulation*, *2024*, 1-22.

Cheng, X., Wang, L., Nie, L., & Li, Y. (2018). Chlorophyll fluorescence characteristics and antioxidant enzyme activities of chrysanthemum leaves under low temperature stress. *Journal of Henan Agricultural Sciences*, (4), 20.

Clemente-Moreno, M. J., Omranian, N., Saez, P. L., Figueroa, C. M., Del-Saz, N., Elso, M., et al. (2020). Low-temperature tolerance of the Antarctic species *Deschampsia antarctica*: A complex metabolic response associated with nutrient remobilization. *Plant, Cell and Environment*, *43*, 1376-1393. https://doi: 10.1111/pce.13737

Anket, Sh., Sangeeta, P., Renu, B., Bingsong, Zh., & Durgesh Kumar, T. (2023). Role of salicylic acid in the regulation of physiological and molecular aspects of plants under abiotic stress. In: the Role of Growth Regulators and Phytohormones in Overcoming Environmental Stress (eds. Devi, K., Bakshi, P., Kour, J., Dhiman, S., Ibrahim, M., Bhardwaj, T., and Bhardwaj, R.) Pp. 175-196. Academic Press.

Didaran, F., Kordrostami, M., Ghasemi-Soloklui, A. A., Pashkovskiy, P., Kreslavski, V., Kuznetsov, V., & Allakhverdiev, S. I. (2024). The mechanisms of photoinhibition and repair in plants under high light conditions and interplay with abiotic stressors. *Journal of Photochemistry and Photobiology B: Biology*, *259*, 15.

Dumlupınar, R., Akbulut, N., Turk, H., & Sheikhi Didani, B. (2019). Comparison of cold-hardiness levels of wheat cultivars based on alterations in alternative oxidase protein level. *Eastern Anatolian Journal of Science*, *5*(2), 10-15.

Dutta, A., Sen, J., & Deswal, R. (2007). Downregulation of terpenoid indole alkaloid biosynthetic pathway by low temperature and cloning of a AP2 type C-repeat binding factor (CBF) from *Catharanthus roseus* (L). G. Don. *Plant Cell Reports*, *26*, 1869-1878.

Elkelish, A., Qari, S. H., Mazrou, Y. S., Abdelaal, K. A., Hafez, Y. M., Abu-Elsaoud, A. M., Batiha, G. E. S., El-Esawi, M. A., & El Nahhas, N. (2020). Exogenous ascorbic acid induced chilling tolerance in tomato plants through modulating metabolism, osmolytes, antioxidants, and transcriptional regulation of catalase and heat shock proteins. *Plants*, 9(4), 1-21.

Ezzatmand, I. (2024). The effects of different light intensities and plant propagation methods on the growth, yield and phytochemical compounds of *Ziziphora clinopodioides*. Master thesis. Azarbaijan Shahid Madani University.

Fageria, N. K., Baligar, V. C., & Li, Y. C. (2008). The role of nutrient-efficient plants in improving crop yields in the twenty-first century. *Journal of Plant Nutrition*, *31*(6), 1121-1157.

Gim, Y, & Kim, W. (2016). On the thermodynamic origin of the initial radiation energy density in warm inflation. *Journal of Cosmology and Astroparticle Physics*, *2016*, 022-022. https://doi.org/10.1088/1475-7516/2016/11/022

Hajihashemi, S., Noedoost, F., Geuns, J. M., Djalovic, I., & Siddique, K. H. (2018). Effect of cold stress on photosynthetic traits, carbohydrates, morphology, and anatomy in nine cultivars of *Stevia rebaudiana*. *Frontiers in Plant Science*, *9*, 1-12.

Hatfield, J. L, & Prueger, J. H. (2015). Temperature extremes: Effect on plant growth and development. *Weather Clim Extrem*, *10*, 4-10.

Hazrati, S., Govahi, M., Sedaghat, M., & Kashkooli, A. B. (2020A). A comparative study of essential oil profile, antibacterial and antioxidant activities of two cultivated Ziziphora species (*Z. clinopodioides* and *Z. tenuior*). *Industrial Crops and Products*, *157*, 112942.

Hazrati, S., Tahmasebi-Sarvestani, Z., Nicola, S., Kashkooli, A. B., Habibzadeh, F., Mohammadi, H., & Mokhtassi-Bidgoli, A. (2020b). Effect of light and water deficiency on growth and concentration of various primary and secondary metabolites of *Aloe vera*. *Journal of Agricultural Science and Technology*, *22*(5), 1343-1358.

Hazrati, S., Mousavi, Z., & Nicola, S. (2024). Harvest time optimization for medicinal and aromatic plant secondary metabolites. *Plant Physiology and Biochemistry*, 108735.

He, J., Yao, L., Pecoraro, L., Liu, C., Wang, J., Huang, L., & Gao, W. (2022). Cold stress regulates accumulation of flavonoids and terpenoids in plants by phytohormone, transcription process, functional enzyme, and epigenetics. *Critical Reviews in Biotechnology, 43*, 680-697.

Hossain, M. A., Kumar, V., Burritt, D., Fujita, M., & Makela, P. (2019). Osmoprotectant-mediated abiotic stress tolerance: Recent Advances and Future Perspectives. Springer Cham.

Hou, W., Sun, A. H., Chen, H. L., Yang, F. S., Pan, J. L., & Guan, M. Y. (2016). Effects of chilling and high temperatures on photosynthesis and chlorophyll fluorescence in leaves of watermelon seedlings. *Biologia Plantarum, 60,* 148-154.

Huang, W., Ma, H. Y., Huang, Y., Li, Y., Wang, G. L., Jiang, Q., Wang, F., & Xiong, A. S. (2017). Comparative proteomic analysis provides novel insights into chlorophyll biosynthesis in celery under temperature stress. *Physiologia Plantarum*, *161*(4), 468-485.

Hwang, W. H., Lee, C. G., Jeong, J. H., Lee, H. S., & Choi, K. J. (2019). Leaf Condition of Rice (*Oryza sativa* L.) in response to cold stress during ripening stage. *Journal of Crop Science and Biotechnology*, *22*, 489-494.

Jeong, H. J, & Sung, S. J. (2011). Barley DNA-binding methionine aminopeptidase, which changes the localization from the nucleus to the cytoplasm by low temperature, is involve in freezing tolerance. *Plant Science*, *180*, 53-60.

Jiao, X., Yu, X., Ding, J., Du, Q., Zhang, J., Song, X., & Li, J. (2022). Effects of rising VPD on the nutrient uptake, water status and photosynthetic system of tomato plants at different nitrogen applications under low temperature. *Scientia Horticulturae, 304*, 111335.

Jiaojiao, J., Zigang, L., Wenbo, M., Wancang, S., Junyan, W., Yan, F., Xuecai, L., Lijun, L., Ya, Z., Mingxia, X., & Chunmei, X. (2019). Transcriptome analysis of photosynthetic characteristics was induced by low temperature stress in *Brassica napus* L. *Research Square*, 1-36.

Jie, L. I., Jun-Gui, X. U., Lin, C., Guan, Y. J., Jin, H. U., & Center, S. S. (2016). Effect of priming on germination and physiological characteristics of different types of corn seeds under low-temperature stress. *Plant Physiology Journal*, *52*, 157-166.

Jurczyk, B., Rapacz, M., Budzisz, K., Barcik, W., & Sasal, M. (2012). The effects of cold, light and time of day during low-temperature shift on the expression of CBF6, FpCor14b and LOS2 in *Festuca pratensis*. *Plant Science*, *183*, 143-148.

Khan, T. A., Fariduddin, Q., & Yusuf, M. (2017). Low-temperature stress: Is phytohormones application a remedy?. *Environmental Science and Pollution Research*, *24*, 21574-21590.

Khan, T. A., Yusuf, M., Ahmad, A., Bashir, Z., Saeed, T., Fariduddin, Q., Hayat, S., Mock, H. P., & Wu, T. (2019). Proteomic and physiological assessment of stress sensitive and tolerant variety of tomato treated with brassinosteroids and hydrogen peroxide under low-temperature stress. *Food Chemistry*, *289*, 500-511.

Khanizadeh, S, & Deell, J. (2001). Chlorophyll fluorescence: A new technique to screen for tolerance of strawberry flowers to spring frost. *Small Fruits Review, 1*(3), 61-67.

Kong, Y., Hou, X., Liu, Z., & Li, Y. (2024). Cold-stress induced metabolomic and transcriptomic changes in leaves of three mango varieties with different cold tolerance. *BMC Plant Biolog, 24*(1), 1-17.

Kuk, Y. I., Shin, J. S., Burgos, N. R., Hwang, T. E., Han, O., Cho, B. H., Jung, S., & Guh, J. O. (2003). Antioxidative enzymes offer protection from chilling damage in rice plants. *Crop Science*, *43*(6), 2109-2117.

Kumar, A., Abrol, E., Koul, S., & Vyas, D. (2012). Seasonal low temperature plays an important role in increasing metabolic content of secondary metabolites in (*Withania somnifera* L.) Dunal and affects the time of harvesting. *Acta Physiologiae Plantarum*, *34*, 2027-2031.

Labate, C, & Leegood, R. (1989). Influence of low temperature on respiration and contents of phosphorylated intermediates in darkened barley leaves. *Plant Physiology*, *91*(3), 905-10. https://doi.org/10.1104/PP.91.3.905

Lee, J. H., Kwon, M. C., Jung, E. S., Lee, C. H., & Oh, M. M. (2019). Physiological and metabolomic responses of kale to combined chilling and UV-A treatment. *International Journal of Molecular Sciences*, *20*(19), 1-17.

Li, Y., Kong, D., Fu, Y., Sussman, M. R., & Wu, H. (2020). The effect of developmental and environmental factors on secondary metabolites in medicinal plants. *Plant Physiology and Biochemistry*, *148*, 80-89.

Li, Y. Q., Kong, D. X., Liang, H. L., & Wu, H. (2018). Alkaloid content and essential oil composition of (*Mahonia breviracema*) cultivated under different light environments. *Journal of Applied Botany and Food Quality*, *91*, 171-179.

Ma, X., Xia, H., Liu, Y., Wei, H., Zheng, X., Song, C., & Luo, L. (2016). Transcriptomic and metabolomic studies disclose key metabolism pathways contributing to well-maintained photosynthesis under the drought and the consequent drought-tolerance in rice. *Frontiers in Plant Science*, *7*, 1886.

Marschner, P. (2012). Marschner's Mineral Nutrition of Higher Plants. Academic Press, London.

Mehalaine, S, & Chenchouni, H. (2021). New insights for the production of medicinal plant materials: Ex vitro and in vitro propagation of valuable Lamiaceae species from northern Africa. *Current Plant Biology*, *27*, 100216.

Miralles-Crespo, J., Martinez-Lopez, J. A., Franco-Leemhuis, J. A., & Banon-Arias, S. (2011). Determining freezing injury from changes in chlorophyll fluorescence in potted oleander plants. *Hort Science*, *46*(6), 895-900.

Ouellet, F, & Charron, J. B. (2013). Cold acclimation and freezing tolerance in plants. *Encyclopedia of Life Sciences*, *10*(9780470015902), a0020093.

Promyou, S., Ketsa, S., & van Doorn, W. G. (2012). Salicylic acid alleviates chilling injury in anthurium (*Anthurium andraeanum* L.) flowers. *Postharvest Biology and Technology*, *64*(1), 104-110.

Qaderi, M. M., Martel, A. B., Strugnell, C. A. (2023). Environmental factors regulate plant secondary metabolites. *Plants 12*, 447.

Saleem, M., Fariduddin, Q., & Janda, T. (2021). Multifaceted role of salicylic acid in combating cold stress in plants: A review. *Journal of Plant Growth Regulation*, *40*(2), 464-485.

Samec, D., Ljubej, V., Redovnikovic, I. R., Fistanic, S., & Salopek-Sondi, B. (2022). Low temperatures affect the physiological status and phytochemical content of flat leaf kale (*Brassica oleracea* var. acephala) sprouts. *Foods, 11*, 264.

Satıl, F, & Selvi, S. (2020). Ethnobotanical features of *Ziziphora* L. (Lamiaceae) taxa in Turkey. *International Journal of Nature and Life Sciences*, *4*(1), 56-65.

Schulz, P., Herde, M., & Romeis, T. (2013). Calcium-dependent protein kinases: Hubs in plant stress signaling and development. *Plant Physiology*, *163*(2), 523-530.

Singh, A. K., Dhanapal, S., & Yadav, B. S. (2020). The dynamic responses of plant physiology and metabolism during environmental stress progression. *Molecular Biology Reports*, *47*(2), 1459-1470.

Tang, K., Wang, X., Dong, K., Li, Y., Li, J., Sun, B., & Wu, J. (2020). A thermal radiation modulation platform by emissivity engineering with graded metal–insulator transition. *Advanced Materials*, *32*(36), 1907071.

Taiz, L, & Zeiger, E. (2010). Plant Physiology. Inc., Publishers, Sinauer Associates.

Tian, X., Xie, J., & Yu, J. (2020). Physiological and transcriptomic responses of Lanzhou Lily (*Lilium davidii*, var. unicolor) to cold stress. *PloS One*, *15*(1), e0227921.

Vergata, C., Yousefi, S., Buti, M., Vestrucci, F., Gholami, M., Sarikhani, H., & Martinelli, F. (2022). Meta-analysis of transcriptomic responses to cold stress in plants. *Functional Plant Biology*, *49*(8), 704-724.

Verma, N, & Shukla, S. (2015). Impact of various factors responsible for fluctuation in plant secondary metabolites. *Journal of Applied Research on Medicinal and Aromatic Plants, 2*, 105-113.

Wang, F., Wang, G., Li, X., Huang, J., & Zheng, J. (2008). Heredity, physiology and mapping of a chlorophyll content gene of rice (*Oryza sativa* L.). *Journal of Plant Physiology, 165*, 324-330.

Wang, Y., Ji, S., Dai, H., Kong, X., Hao, J., Wang Singh, A. K., Dhanapal, S., & Yadav, B. S. (2020). The dynamic responses of plant physiology and metabolism during environmental stress progression. *Molecular Biology Reports, 47*, 1459-1470. https://doi: 10.1007/s11033-019-05198-4

Wu, X. X., Ding, H. D., Chen, J. L., Zhu, Z. W., & Zha, D. S. (2015). Exogenous spray application of 24-epibrassinolide induced changes in photosynthesis and anti-oxidant defences against chilling stress in eggplant (*Solanum melongena* L.) seedlings. *The Journal of Horticultural Science and Biotechnology*, *90*(2), 217-225.

Xiong, L, & Zhu, J. K. (2001). Abiotic stress signal transduction in plants: molecular and genetic perspectives. *Physiologia Plantarum*, *112*(2), 152-166.

Yamori, W., Noguchi, K., Hikosaka, K., & Terashima, I. (2011). Phenotypic plasticity in photosynthetic temperature acclimation among crop species with different cold tolerances. *Plant Physiology*, *152*(1), 388-399.

Yang, Y., Zhang, R., Duan, X., Hu, Z., Shen, M., & Leng, P. (2019). Natural cold acclimation of *Ligustrum lucidum* in response to exogenous application of paclobutrazol in Beijing. *Acta Physiologiae Plantarum*, *41*(1), 15.

Zhang, Z., Wu, P., Zhang, W., Yang, Z., Liu, H., Ahammed, G. J., & Cui, J. (2020). Calcium is involved in exogenous NO-induced enhancement of photosynthesis in cucumber (*Cucumis sativus* L.) seedlings under low temperature. *Scientia Horticulturae, 261*, 108953.

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