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Growth, gas exchange and essential oil production of *Mentha spicata* L. under water deficiency¹

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ABSTRACT

Drought stress is a critical factor affecting plant growth and development. Understanding the effects of drought on the physiology of medicinal plants can contribute to the implementation of better water management techniques and promote a sustainable production. This study aimed to evaluate the influence of water stress on the growth and development of *Mentha spicata* L. plants. A completely randomized design was used, with two water conditions: irrigated and non-irrigated. The water deficit negatively affected the dry weight of roots and shoots, with a decrease in the transpiration rate and chlorophyll content, potentially impairing the photosynthetic activity. However, the essential oil production was not affected in plants subjected to water deficiency, indicating that the essential oil production may be more resilient to drought stress in this species.

KEYWORDS: Spearmint, medicinal plant, photosynthesis.

RESUMO

Crescimento, trocas gasosas e produção de óleo essencial de *Mentha spicata* L. sob deficiência hídrica

O estresse hídrico é um fator crítico que afeta o crescimento e desenvolvimento das plantas. Compreender os efeitos da seca na fisiologia de plantas medicinais pode contribuir para a implementação de melhores técnicas de manejo da água e promover uma produção sustentável. Objetivou-se avaliar a influência do estresse hídrico no crescimento e desenvolvimento de plantas de *Mentha spicata* L. Foi utilizado delineamento inteiramente casualizado, com dois sistemas: irrigado e não irrigado. O déficit hídrico afetou negativamente o peso seco das raízes e caules, com diminuição na taxa de transpiração e no teor de clorofila, prejudicando potencialmente a atividade fotossintética. No entanto, a produção de óleo essencial não foi afetada em plantas submetidas à deficiência hídrica, indicando que a produção de óleo essencial pode ser mais resiliente ao estresse de seca nessa espécie.

PALAVRAS-CHAVE: Hortelã, planta medicinal, fotossíntese.

INTRODUCTION

Mentha spicata L., also known as spearmint, is widely used in various medicinal and pharmaceutical applications due to its therapeutic properties. Spearmint has anti-inflammatory, analgesic, antioxidant and antimicrobial activities, among others (Miraj & Kiani 2016). Additionally, it is extensively used in the food industry as a natural flavoring agent (Miraj & Kiani 2016, Mahendran et al. 2021, Zhang et al. 2022).

Drought stress can adversely affect the growth and development of *Mentha* spp. plants, as well as their production of secondary metabolites, including phenolic compounds and essential oils (Matraka et

al. 2010, García-Caparrós et al. 2019, Marino et al. 2019, Brar et al. 2021, Singh et al. 2022).

Water deficiency stands out as one of the main limitations to global plant growth and development, particularly in arid and semi-arid regions. An inadequate soil moisture content can lead to a series of physiological and biochemical changes in plants, encompassed reduced growth rate, lower photosynthetic efficiency, decreased chlorophyll content and disruption of nutrient transport (Ingrao et al. 2023). Furthermore, water deficiency can lead to yield losses in crops, thereby impeding agricultural production and posing a substantial challenge to global food security (Trenberth et al. 2014, He & Rosa 2023, Ingrao et al. 2023).

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Studies demonstrate that drought stress can influence the quality and quantity of essential oil in secondary compounds, resulting in reduced biomass production, ultimately compromising the growth of medicinal plants (Gray et al. 2003, Abbaszadeh et al. 2009, Urban et al. 2017, Marino et al. 2019). For instance, a study conducted with lemon balm (*Melissa officinalis* L.) revealed that moderate drought stress enhances the production of essential oil and anxiolytic properties (Abbaszadeh et al. 2009). Furthermore, water deficiency can modify the chemical composition of bioactive compounds such as amentoflavone, a flavonoid found in *Hypericum perforatum* L. (Gray et al. 2003).

Understanding the impacts of water deficiency on the physiology of medicinal plants is crucial for implementing improved water management practices and fostering a sustainable production. Thus, this study aimed to assess the impact of water deficiency on biometric parameters, gas exchange and essential oil yield in *M. spicata* plants.

MATERIAL AND METHODS

The experiment was conducted at the Universidade Federal do Agreste de Pernambuco, in Garanhuns, Pernambuco state, Brazil (08°54'25"S, 36°29'36"W and altitude of 848 m). The seedlings, measuring 10-15 cm in length, were planted on February 23, 2022, in 5 L capacity pots, where they remained for 45 days in a greenhouse with constant irrigation. The soil used in the experiment was sieved through a 55-mesh screen, to provide a greater uniformity and reduce physical barriers to root growth, and presented the following characteristics: pH (in water: 1:2.5) = 6.5; H + Al = 1.48 cmol_c kg⁻¹; Al = 0.40 cmol_c kg⁻¹; Na = 0.47 cmol_c kg⁻¹; K = 0.77 cmol_c kg⁻¹; Ca = 2.90 cmol_c kg⁻¹; Mg = 2.60 cmol_c kg⁻¹; P = 64.6 mg kg⁻¹; base saturation = 82 %; field capacity = 0.110 g g⁻¹. Each 5 kg plastic pot was filled with air-dried soil.

A completely randomized design was used, with two water conditions: irrigated and non-irrigated. Each experimental unit consisted of 1 plant pot⁻¹, with 8 replicates, totaling 16 plants. After 45 days of planting, the treatments were differentiated, applying two periods of water stress to the plants (plants with three and six days of water deficit) and plants irrigated daily.

At 52 days of the experiment, the following variables were evaluated: plant height (cm), measured from the base to the apex of the tallest branch, using a ruler; shoot and root dry weight. Fresh plants (shoot and root) were placed in paper bags and dried at 65-70 °C, in a forced-air circulation oven, until a constant weight was achieved. The dried material was then weighed to obtain the dry weight of each plant part. The shoot/root ratio was calculated.

The gas exchange measurements were conducted using an infrared gas analyzer (IRGA), model LCPro SD (ADC-BioScientific Ltd., Hoddesdon, UK), from 9 to 11 a.m. The internal carbon concentration (*C_i*), stomatal conductance (*g_s*), transpiration (*E*), net photosynthesis rate (*A*), instantaneous water-use efficiency (WUE - *A/E*), calculated by relating net photosynthesis to transpiration, and instantaneous carboxylation efficiency (CE - *A/C_i*), calculated from the relation between net photosynthesis and internal carbon concentration, were also assessed. The temperature and humidity measurements were taken at 3 and 6 days after the suspension of irrigation using an IRGA. The chlorophyll index was estimated using a ChlorofiLOG® portable meter (Falker, Porto Alegre, RS, Brazil). The chlorophyll index was assessed at 3 and 6 days after the beginning of the water deficit.

For the essential oil extraction, the shoot dry plant material was crushed (particle size ≤ 2 mm) and 10 g were hydro-distilled with 330 mL of water in a modified Clevenger apparatus, for 120 min. Due to the low quantity of extracted oil, which was insufficient for manipulation, separating it by decantation was not feasible. Thus, the entire solution obtained from hydro-distillation was used, and later the oil was recovered. Three replicates of each treatment were performed, where the oil present in the obtained hydrolase (water-oil solution) was recovered with hexane and used as a solvent to separate the oil from the water, due to its low polarity. Subsequently, the hexane solution with oil was placed in a volumetric flask of known mass until all the solvents evaporated. After the solvent evaporation, the remaining oil was weighed to determine the oil mass by difference. The essential oil content was determined and expressed in mg 100 g⁻¹ of shoot dry matter, as well as the yield, which was expressed in g kg⁻¹ of shoot dry weight.

The data were submitted to analysis of variance with the Student's t-test, and means compared using the Tukey test at 5 % of probability. The statistical

analysis was performed with the Assistat statistical software.

RESULTS AND DISCUSSION

In conditions of water deficit, the plants developed mechanisms to adapt their growth and ensure survival. No differences were observed in the heights of irrigated and water-stressed spearmint plants (Figure 1A). However, the mint plants subjected to suspended irrigation for 6 days experienced a notable decrease of 37.75 % in shoot dry weight and 37.50 % in root dry weight (Figures 1B and 1C). These results align with those of Marino et al. (2019), who similarly observed a diminished dry weight. Specifically, they documented a 28 % decrease for plants under intermediate water deficit and a more substantial 40 % reduction under severe water stress, when compared to well-irrigated spearmint plants (Marino et al. 2019).

Delving deeper into the physiological and biochemical intricacies, the reduction in dry weight can be attributed to a cascade of responses. The limitation in water availability hinders cellular expansion and turgor pressure, contributing to the observed decrease in shoot and root biomass (Shao et al. 2008). The stomata closure, a response regulated by the plant hormone abscisic acid (ABA) under water stress, minimizes the water loss through

transpiration, but, simultaneously, restricts the uptake of the carbon dioxide essential for photosynthesis (Bharath et al. 2021). Consequently, this compromises the production of carbohydrates, which are crucial for growth and biomass accumulation (Khan et al. 2018).

The shoot/root ratio did not differ significantly, when compared to irrigated plants (Figure 1D). The shoot/root ratio serves as an indicator of the balance between root and shoot. This result indicates that, despite the reduced growth under water deficit conditions, the plants still maintain a balance between root and shoot.

The water deficiency significantly impacted the transpiration rate and photosynthesis in the non-irrigated mint plants, showing a statistical inferiority, if compared to the irrigated plants (Figures 2A and 2B). Similar outcomes were observed in plants in which the water scarcity led to substantial losses in the CO₂ exchange rate, total assimilative area, plant nutrient levels and overall biomass (Brar et al. 2021). The discussion of these results encompasses both descriptive and interpretive elements. Transpiration is a process regulated by water availability in plants (Cramer et al. 2008). Under water deficit conditions, a reduction in stomatal aperture occurs, diminishing the water loss through transpiration and, consequently, the loss of essential minerals for maintaining the cellular osmotic balance (Tombesi et al. 2015). The

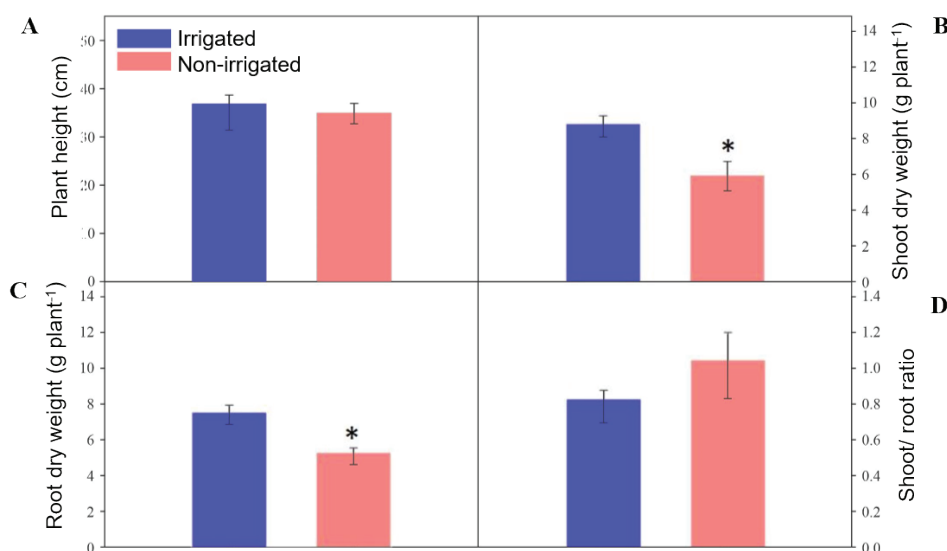


Figure 1. Plant height (A), shoot dry weight (B), root dry weight (C) and shoot/root ratio (D) of spearmint at 52 days of cultivation under irrigation and non-irrigation. * Significantly different ($p < 0.05$) between the treatments, after performing the Student's t-test.

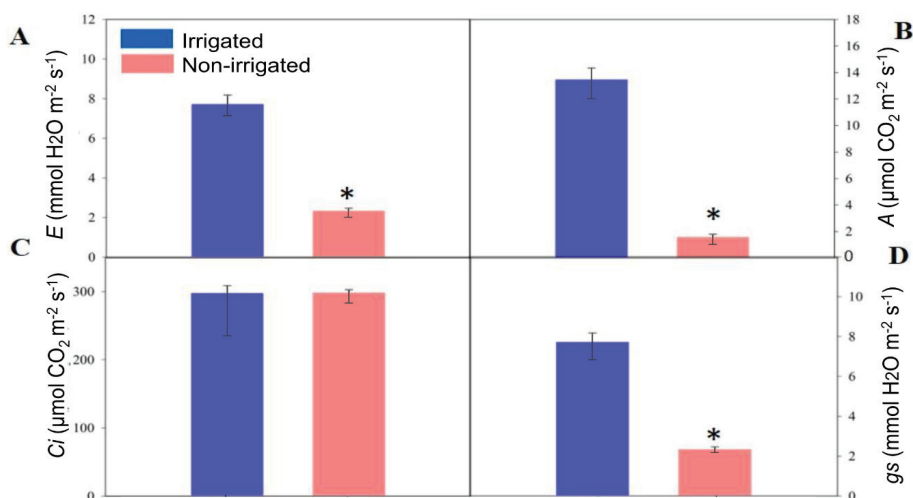


Figure 2. Evaluation of gas exchange parameters in irrigated and non-irrigated spearmint plants at 52 days after planting. *E*: transpiration (A); *A*: photosynthesis (B); *Ci*: internal CO₂ concentration (C); *gs*: stomatal conductance (D). * Significantly different ($p < 0.05$) between the treatments after performing the Student's t-test.

decrease in transpiration can result in a reduced CO₂ uptake by the stomata, negatively impacting the photosynthesis and, consequently, plant growth and yield (Ainsworth & Rogers 2007, Gago et al. 2016, Souza et al. 2020). This discussion integrates both the presentation of observed effects and the interpretation of the physiological implications, making it both descriptive and interpretive in nature. However, no significant changes were observed in the internal carbon concentration of the spearmint plants subjected to stress (Figure 2C). This could be indicative of a resilient response or an inherent adaptability of spearmint to the specific stress factors applied. It suggests that, under the given stress conditions, spearmint plants may have mechanisms in place to maintain relatively stable levels of internal carbon dioxide concentration, highlighting a potential resilience to the specific stressor employed.

In the non-irrigated spearmint plants, a reduction of 27.5 % in stomatal conductance (Figure 2D) was observed. Matraka et al. (2010) demonstrated that spearmint plants under water stress (40 % of soil water content) showed a significant reduction in stomatal conductance. This result indicates that the reduction in the photosynthetic rate is directly related to the decrease in the stomatal conductance, what is an adaptive response of plants to minimize water loss during water stress. Additionally, the reduction in stomatal conductance may also be related to the regulation of the RuBisCO enzyme activity, responsible for the CO₂ fixation during photosynthesis.

Under conditions of water deficiency, both the instantaneous water-use efficiency (WUE) and instantaneous carboxylation efficiency exhibited significant decreases (Figures 3A and 3B). These

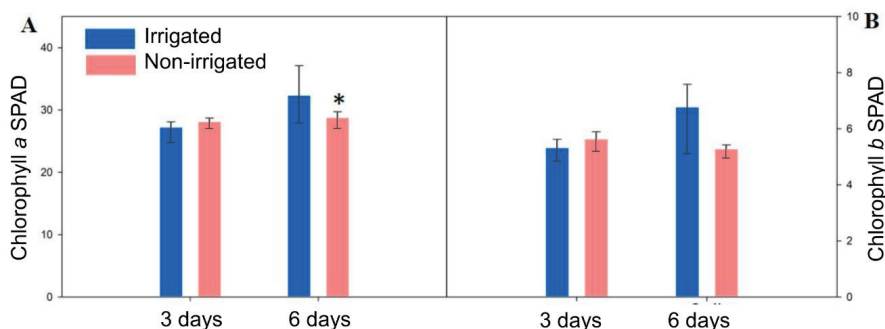


Figure 3. Water-use efficiency (A) and carboxylation efficiency (B) in irrigated and non-irrigated spearmint plants at 52 days after planting. * Significantly different ($p < 0.05$) between the treatments after performing the Student's t-test.

parameters are crucial physiological indicators of a plant's ability to use water and carbon dioxide (CO₂) during photosynthesis. A key factor influencing this dynamics is the impact of water stress on the RuBisCO enzyme, pivotal in the carbon fixation pathway, as highlighted by Parry et al. (2002). The present study supports the idea that reduced WUE and carboxylation efficiency are intricately linked to decreased RuBisCO activity (Hussain et al. 2022). This decline in enzyme activity may result from the constraints imposed by water stress on both the production and optimal functioning of RuBisCO. Consequently, the observed reductions in WUE and carboxylation efficiency indicate that mint plants under drought stress exhibit a diminished proficiency in using water and CO₂ resources. This inefficiency can potentially impact the long-term growth and development of the plant, underscoring the intricate physiological interplay between water availability and key enzymatic processes.

Water stress in plants can cause changes in leaf temperature and humidity, which are important indicators of the plant's hydration status. Leaf temperature can be influenced by the amount of water present in the leaves, as well as the rate of transpiration and evaporative cooling (Jackson et al. 1981, Urban et al. 2017). Leaf humidity, on the other hand, is directly related to water availability in the environment, and can be affected by the balance between transpiration and water absorption by roots (Kagawa 2022). No statistical differences were observed regarding leaf temperature between irrigated and non-irrigated spearmint plants (Figure 4A). However, a reduction in leaf humidity was observed in spearmint plants under water deficit

(Figure 4B), indicating a higher water loss through transpiration. This reduction in leaf humidity may be related to the plants' increased sensitivity to water stress, leading to a greater transpiration as an adaptive response to reduce leaf temperature and maintaining water homeostasis (Maseda & Fernández 2006). Additionally, the reduction in leaf humidity may also be related to a lower water availability in the soil, leading to reduced water absorption by roots and, consequently, lower leaf humidity.

The photosynthetic rate is directly related to the activity of the photosynthetic apparatus, which involves capturing light energy by photosynthetic pigments and converting it into chemical energy. The reduction in photosynthetic activity in plants subjected to water stress may result from a decrease in the activity of photosynthetic pigments, such as chlorophyll *a*, *b* and carotenoids, among others (Urban et al. 2017). A reduction in chlorophyll content was observed in the spearmint plants subjected to water restriction (Figure 5A). This data is consistent with Matraka et al. (2010), who observed a significant decrease in the chlorophyll *a* and *b* indices in *M. spicata* plants under water deficit. Furthermore, water deficit can lead to cell dehydration and a decrease in the activity of the enzymes involved in chlorophyll biosynthesis. According to Chng et al. (2022), dehydration can affect the structure of cell membranes and the integrity of chloroplasts, impairing the chlorophyll production. No statistical differences were observed in the chlorophyll *b* index (Figure 5B). Overall, impacts related to water-use efficiency, exchange parameters and chlorophyll *a* were observed in the spearmint plants under water deficit, resulting in growth parameter reductions (Figure 6).

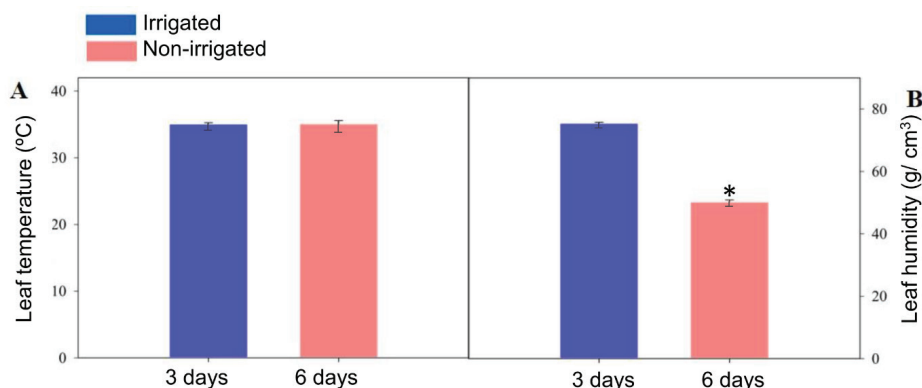


Figure 4. Leaf temperature (A) and humidity (B) in irrigated and non-irrigated spearmint plants at 52 days after planting. The analysis was assessed at 3 and 6 days after the beginning of the water deficit. * Significantly different ($p < 0.05$) between the treatments after performing the Student's t-test.

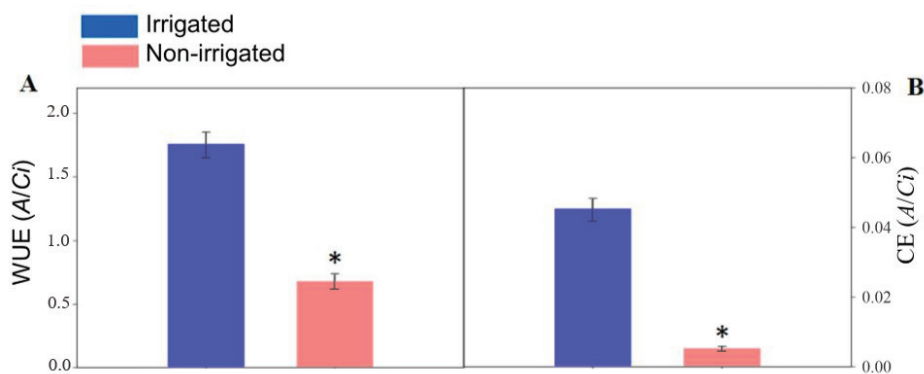


Figure 5. Assessment of photosynthetic pigments in spearmint plants at 3 and 6 days after the beginning of the irrigation suspension. A) Chlorophyll *a* index; B) chlorophyll *b* index. * Significantly different ($p < 0.05$) between the treatments after performing the Student's *t*-test. WUE: instantaneous water-use efficiency; CE: instantaneous carboxylation efficiency; *A*: photosynthesis rate; *C_i*: internal carbon concentration.

Regarding the essential oil content, no statistical differences were observed in the irrigated spearmint plants and those under water deficiency (Figure 7). This finding is consistent with the study by García-Caparrós et al. (2019), in which no reduction in essential oil content of *M. piperita* under water deficiency was observed. In contrast,

a reduction in essential oil content was observed in *M. piperita* (Khorasaninejad et al. 2011) under a limited water regime. Another study conducted with *M. piperita* showed that the water deficit led to an increase in essential oil content in plants subjected to mild stress, while a moderate water stress treatment caused a significant reduction in the essential oil content at different stages of plant growth (Abdi et al. 2019). In *M. longifolia* under water stress, there was an increase in essential oil yield and a reduction in the percentage of limonene, d-carvone and nutrient percentage (nitrogen and potassium) in the plants, with no significant change in the phosphorus concentration (Singh et al. 2022). Several factors can impact the essential oil content in plants, such as the physiological state of the plant, differences

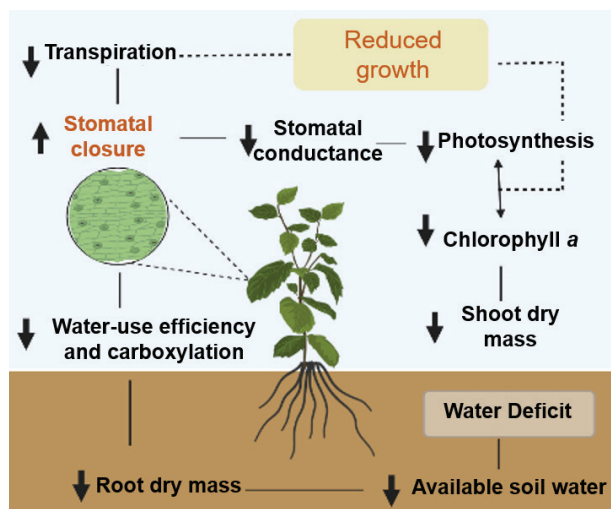


Figure 6. Schematic diagram of physiological responses of spearmint grown under water supply limits demonstrating that water deficiency reduces the water availability in the soil, what impacts the plant growth overall. In spearmint, it is observed that water deficiency promotes a reduction in transpiration, possibly due to a greater degree of stomatal closure. Additionally, there is a lower efficiency in water use and carboxylation of RuBisCo. Chlorophyll *a* and photosynthesis were also negatively affected, what may have contributed to the reduction of the dry weight of both the shoots and roots.

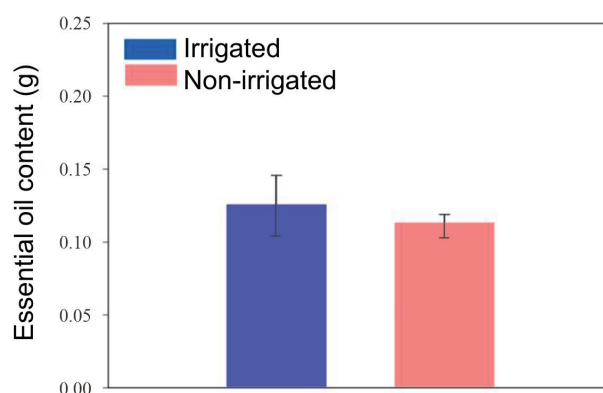


Figure 7. Evaluation of essential oil levels of *Mentha spicata* extracted at 52 days after cultivations in irrigated and non-irrigated plants. There were no significant differences ($p < 0.05$) between the treatments after the Student's *t*-test.

in the duration of drought and the plant species, what can lead to qualitatively and quantitatively different responses, in terms of essential oil content. In the present study, the essential oil production in spearmint may be more resilient to water stress than other physiological processes such as photosynthesis and transpiration.

CONCLUSIONS

1. Spearmint plants subjected to a 6-day water deficit with suspended irrigation showed a notable reduction in the root and shoot dry weight. Despite this reduction, a balance was maintained between root and shoot biomass;
2. Reductions in the chlorophyll content indicate a decreased photosynthetic activity, potentially due to dehydration impacting chlorophyll biosynthesis;
3. Both the instantaneous water-use efficiency and carboxylation efficiency decreased significantly under water deficiency, reflecting a compromised use of water and CO₂ during photosynthesis;
4. The essential oil content was not affected by water deficiency.

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