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Effect of water deficit on growth and concentration of secondary metabolites of *Thymus vulgaris*

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Abstract

The aim of the experiment was to investigate the effect of the level and timing of watering on the productivity and chemical constituents of garden thyme (*Thymus vulgaris* L., cultivar 'Varico 3') at growth stage (GS) 202 based on the extended BBCH scale in growth chamber for 13 weeks. Different irrigation regimes have been applied based on the soil water content (SWC%) as follows: 1) control (C: 1–13th weeks – 70% SWC), 2) mild drought stress (S1: 1–4th weeks – 70% SWC, from the 6th week, continuously decreasing drought stress to 40% DVD for 10–13th weeks), 3) sudden drought stress (S2: 1–9th weeks – 70% SWC, 10–13th weeks – 40% DVD), and 4) constant drought stress (S3: 1–4th weeks – 70% DVK, 5–13th weeks – 40% SWC). At the end of the experiment, it was determined that water deficiency induced no direct impact on the width and height of garden thyme plants, while internode and shoot lengths were affected significantly. The plants grown under constant drought stress (S3) conditions showed the minimum values concerning lengths (internode 1.30 cm, shoot 7.12 cm), while the highest ones (internode 2.50 cm, shoot 22.08 cm) were found in the control treatment. However, water supply had no significant effect on the shoot number and chlorophyll content of the leaves. The impact of drought stress both on rosmarinic acid and total phenolic content (TPC) was significant and showed the highest values in the S2 and S3 treatments: S2 had the highest rosmarinic acid concentration of 4.61%, and S3 had the highest total phenolic concentration of 0.75 mg GAE ml^{-1}. A similar result was found for antioxidant capacity in the S3 treatment, where plants reached the highest values (275.44 mg AAE g^{-1} dry weight), while the total flavonoid content was not significantly affected by different water supply levels.
The results show that not only the level of water deficiency but also the change in water supply and the timing of the drought may have a significant effect on the quantity and quality of garden thyme production.

Keywords: garden thyme, drought stress, total flavonoid content, rosmarinic acid, total phenolic content.

Introduction

Garden thyme (*Thymus vulgaris* L.) is a well-known medicinal and aromatic plant belonging to the Lamiaceae family. It is a woody perennial subshrub of 20–50 cm height native to the Northwest Mediterranean region and worldwide cultivated in many countries with increasing economic importance (Stahl-Biskup, Venskutonis, 2012). Several thyme species had already been applied since the ancient Egypt and Greece for various purposes (Taher et al., 2021). Thyme has been found to have antimicrobial, antioxidant, antifungal, and antiseptic effect (Stahl-Biskup, Venskutonis, 2012; Aljabeili et al., 2018; Moghaddam, Mehdizadeh, 2020; Paulus et al., 2020). Drugs of *Thymi* (*T. herba* and *T. aetheroleum*) are included in the WHO (1999) monographs, the European Pharmacopoeia (2015) as well as in the European Union herbal monograph (2016). *T. herba* contains essential oil (0.5–4.0 ml 100 g^{-1}) with main monoterpenes of thymol, p-cymene, and γ-terpinene, while the most important phenolic compounds are rosmarinic, caffeic, and ferulic acids and derivatives (Stahl-Biskup, Venskutonis, 2012; Martines et al., 2015).

Increase of temperature has been predicted in global climatic models (IPCC, 2014). The changing climatic and environmental conditions such as water supply, temperature, and vegetation coverage in the area affect the life cycle as well as the quantity, quality, and composition of active ingredients in medicinal plants (Chaudhry, Sidhu, 2022). Nowadays, one of the most limiting factors in agricultural production is drought. The importance of irrigation is becoming more and more noticeable because of climate change (Nikolaou et al., 2020). Water deficiency has a significant influence on the content and composition of secondary metabolites in medicinal plants, as it was also demonstrated in the case of garden thyme (Szabó et al., 2016b). Moradi et al. (2018) found that all physiological parameters that

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changed significantly corresponded to the decrease of the soil moisture. Alavi-Samani et al. (2015) also reported that irrigation levels had a significant effect on the plant height, number of branches, and leaf area index. Drought stress has been found to affect the essential oil composition of aromatic plants and to decrease drug and biomass production (Alavi-Samani et al., 2013; Szabó et al., 2016a). A great number of research concerning the species belonging to the Lamiaceae family have already been conducted in connection with the effect of climate change and reduced water supply. Soltanbeigi et al. (2021) described the decreasing effect of drought stress for growth, water potential, plant biomass, and fatty acid content of common sage (*Salvia officinalis* L.). In the case of sweet basil (*Ocimum basilicum* L.), the drought stress induced a decrease in drug yield and in essential oil content (Khalid, 2006; Radácsi et al., 2020).

In the case of garden thyme, warm and dry climatic conditions have influence on the accumulation of volatile phenolic compounds (Aziz et al., 2008; Pluhár et al., 2016). In recent years, several attempts have been made, where the responses of garden thyme for the drought stress have been investigated focusing mainly on the active compounds and morphological properties. Kleinwächter et al. (2015) described the increasing effect of a short period of moderate stress on concentrations and overall contents of terpenes, oxidized glutathione, and monoterpene concentration as well as the enhancement of biosynthetic activity in greenhouse trials. However, the results of field experiment in Iran have shown that the plant responds well to drought stress by increasing the length and weight of roots (Kavian, Akhzari, 2021). Focusing on semi-arid regions, Khazie et al. (2008) investigated the effect of plant spacing and irrigation periods to optimise the drug yield and quality, while Al-Ramamneh (2009) aimed at finding the optimal plant density under non-irrigated conditions. Khosh-Khui et al. (2012) detected a slight loss of the total phenolic content and a decreased antioxidant activity in garden thyme, when ten days of irrigation intervals were applied for inducing intensive drought stress.

According to the above-mentioned predictions of global warming influencing agricultural production, the study was aimed at optimising the water supply in garden thyme production. Investigating the range of drought tolerance of garden thyme as a Mediterranean species exposed to arid conditions in the original habitats was found to be essential for future growing.

The aim of the current research was to investigate how drought stress affects the growth and dry weight, chlorophyll content, and secondary metabolites of *Thymus vulgaris* under the climatic conditions of Hungary, which may be an important part of determining the suitability and economics of cultivation. As a first step, morphological and physiological changes as well as yield parameters and different active compounds have been analysed as supposed indicators of drought stress induced by different water supply regimes applied among controlled conditions.

Material and methods

Seedlings of the garden thyme (*Thymus vulgaris* L.) hybrid cultivar 'Varico 3' belonging to the thymol chemotype (seed supplier: mediSeeds sàrl, Switzerland) were planted into pots at growth stage (GS) 202, based on the extended BBCH scale (Hack et al., 1992), and placed into a Weiss-Gallenkamp SGC 120 type growth chamber (Weiss Technik UK Ltd.) at the Research Station of the Department of Medicinal and Aromatic Plants, Institute of Horticultural Science, Hungarian University of Agriculture and Life Sciences in 2020. The medium was a commercially available soil mixture (Florasca Bio B type) consisting of 10% sand, 65% peat, and 25% composted cattle manure. At the beginning of the experiment, the pots were filled with the same volume (3900 g pot^{-1}) of soil.

Table 1. Climatic programmes applied for all treatments during the drought stress experiment on *Thymus vulgaris* plants

Duration of the treatment	Weeks	Temperature °C, day/night	Light intensity lux / day length, h
Acclimatisation period	0–1st	12/8	15.000 / 12
	2–4th	15/9	15.000 / 12
Drought stress experiment	1–5th	16/10	20.000 / 12
	6–9th	20/14	20.000 / 14
	10–13th	22/16	20.000 / 14

Light and temperature conditions were regulated in the growth chamber, where the parameters simulated the general Central European springtime weather with increasing temperature, day length, and light intensity. Relative air humidity was kept at 50% during the whole experiment lasting for 17 weeks (Table 1).

Different irrigation regimes have been initiated after an acclimatisation period of four weeks based on the soil water content (SWC%) determined by a gravimetric method. Water was supplied three times a week keeping the soil water levels specified for different treatments (Table 2). Beside the control (C) treatment with normal (70%) SWC (1), modelling a continuously decreasing water availability and mild drought stress three different regimes were applied: 2) mild drought stress (S1: 1–4th weeks – 70% SWC, from the 6th week, continuously decreasing drought stress to 40% DVD for 10–13th weeks), 3) sudden drought stress (S2: 1–9th weeks – 70% SWC, 10–13th weeks – 40% DVD), and 4) constant drought stress (S3: 1–4th weeks – 70% DVK, 5–13th weeks – 40% SWC).

During the experiment, morphological properties, relative water content (RWC) of the leaves, and changes in active compounds were recorded three times as responses of drought stress treatments on the 4th week of the acclimatisation period (C, S1, and S2: SWC 70%; S3: SWC 40%), on the 9th week of the drought stress treatment (C, S2: SWC 70%; S1: SWC 60%; S3: SWC

Table 2. Different soil water capacity (SWC) (%) treatments applied on *Thymus vulgaris* plants

Duration of the treatment	Weeks	Treatment			
		C (control)	S1	S2	S3
		70% SWC (normal)	70→40% SWC (mild)	70→40% SWC (sudden)	40% SWC (constant)
Acclimatisation period	1 (0–1st) week	70	70	70	70
	3 (2–4th) weeks	70	70	70	70
Drought stress experiment	5 (1–5th) weeks	70	70	70	
	4 (6–9th) weeks	70	60	70	40
	4 (10–13th) weeks	70	40	40	40

40%), and on the 13th week at the end of the experiment (C: SWC 70%; S1, S2, and S3: SWC 40%). However, biomass production, yield, and chlorophyll content (SPAD) were evaluated only at the end of the experiment. The plant height and width, the length of internodes, and the number of shoots had been detected in three plants per treatment. The plant height was determined on the tallest stem of each plant. Leaf parameters were recorded on the leaves found at the central part of the shoots. The chlorophyll content was determined in the leaves on the 13th week at the end of the experiment by a chlorophyll meter SPAD-502 Plus (Konica Minolta Inc.) in three replications per treatment. The results were expressed in SPAD units.

At the end of the experiment, the aerial parts and roots were separated and weighted for determining the fresh mass as well as the shoot to root ratio in four replications. Plant material was then subjected to natural air drying at room (22–24°C) temperature prior to dry weigh measurements.

For the determination of the total phenolic content (TPC), 1 g of dried and powdered garden thyme leaves was measured and extracted by 100 ml boiling distilled water and then kept at room temperature for 24 h. After the filtration, the extracts were stored in a freezer prior to the TPC determination. A modified method of Singleton and Rossi (1965) was used, where 0.5 ml of the sample solution was placed into a test tube and then mixed with 2.5 ml of a Folin-Ciocalteau's reagent (10 v/v %). After 1 min of incubation, 2 ml of sodium carbonate (0.7 M) was also added. The absorbance of the solution was measured at the wavelength of $\lambda = 760$ nm in a spectrophotometer Evolution 201/220 UV-Visible (Thermo Fisher Scientific, USA) after a 5 min incubation period in hot water (50°C) in comparison with the blank of distilled water. As a chemical standard for calibration, gallic acid (0.3 M) was used. The TPC of the samples was expressed as gallic acid equivalent (GAE) calculated on the dry weight basis of the GAE extract (mg GAE g^{-1} DW). The measurements were carried out in three replications.

The total flavonoid content (%) was determined according to the method specified for *Equiseti herba* in the Hungarian Pharmacopoeia (2004) with the modification of the measurable plant material. 0.4 g of the dried and powdered garden thyme leaves was extracted by the mixture of 1 ml hexametylene tetramine, 20 ml acetone, and 2 ml hydrogen chloride (HCl) for 30 min, then the extracts were filtered. Afterwards, the extraction was repeated twice by 20 ml acetone and diluted by a water and ethyl-acetate mixture. The absorbance was recorded at $\lambda = 760$ nm with the spectrophotometer after an incubation period of 30 min in comparison with a blank prepared by acetic acid and methanol. The total flavonoid content (%) was expressed as isoquercitroside equivalent calculated in the dry weight basis of the measured plant material (mg GAE ml^{-1} DW). The measurements were carried out in three replications.

For the determination of the polyphenols (rosmarinic acid) content, 500 g powdered dry plant material was suspended in 45 ml methanol. The suspension was heated for 30 min in a water bath, cooled, and then filtered (by filter of 45µm) into a 100 ml flask. The filtrate was refilled by methanol to a volume of 50 ml. The rosmarinic acid content was determined by a high-performance liquid chromatography (HPLC) method in three replications. The Waters HPLC system (Waters Corporation, USA) consisted of a 1525 binary pump with a 717plus autosampler, a Jetstream column thermostat, and a 2998 PDA detector, controlled by the software Empower2. A Kinetex C-18 column was used, 100 mm L 4.6 mm, internal diameter, 2.6 µm particle size. All solvents were HPLC grade. For the elution, 1:19:80 phosphoric acid:acetonitrile:water (mobile phase A) and 1:40:59 phosphoric acid:methanol:acetonitrile (mobile phase B) were used as solvents at a flow rate of 1 ml min^{-1} based on the European Pharmacopoeia (2015) specifications for *Melissae folium*. The gradient elution program started with 100% of the solvent A, then the ratio of solvent B was increased linearly and reached 35% in 10 min, then 100% in 2 min. Finally, the 100% solvent ratio of A was reached in 2 min. For the equilibration of the initial solvent composition, 8 min post-time was set. In all treatments, the column temperature was maintained at 35°C temperature, and the injection volume of 5 µL was used.

The antioxidant capacity was determined by the ferric reducing antioxidant power (FRAP) assay according to the method developed by Benzie and Strain (1996) with slight modifications. 1 g of dried and powdered garden thyme leaves was extracted with 100 ml of boiling distilled water and was kept at room temperature for 24 h. Then the extracts were filtered and stored in a freezer until the measurements. The FRAP reagent was prepared prior to the measurements to contain sodium acetate buffer (pH = 3.6), TPZ (2,4,6-tripiridil-s-triazine) in hydrogen chloride (HCl), and iron (III) chloride hexahydrate ($FeCl_3 \times 6H_2O$) solution (20 mmol l^{-1}) in a proportion of 10:1:1 (v/v/v). 10 µl of the test sample was added to 1.5 ml of the acting FRAP reagent and 40 µl distilled water, then the absorbance was recorded at $\lambda = 593$ nm after 5 min using the spectrophotometer. The blank of distilled water was applied. The FRAP values of samples were calculated from a standard curve equation and expressed as ascorbic acid equivalent based on the dry weight (mg AAE g^{-1} DW).

One-way analysis of variance (ANOVA) was applied for the evaluation of the effect of treatments on the morphological and physiological properties as well as on the biomass production, biologically active compounds, and changes in antioxidant capacity of garden thyme plants. ANOVA was performed by using the software package SPSS Statistics, version 25.0 (IBM Corp.).

Results and discussion

Drought stress is considered as one of the most important growth-limiting factors that decreases plant growth. Ability of plants to survive during arid periods depends on the plant species, growth stage, and the intensity of drought stress. Based on the results of our experiment, it was found that garden thyme plants were sensitive to any changes of water availability. Different water supply regimes initiated considerable changes in morphological traits, chlorophyll content, yield, active compounds, and FRAP antioxidant capacity.

Concerning morphological parameters, yield, active substances, and antioxidant capacity, data were obtained at the end of the experiment because of different treatments to evaluate the effect of water supply (Table 3).

Regarding the morphological traits, the analysis of variance showed no significant differences among the treatments in plant heights. However, the plants of the control treatment represented the highest values (31.80 cm), while comparing the water shortage treatments a decreasing tendency was found: S1: 31.30 cm, S2: 29.90 cm, and S3: 25.50 cm. Several studies have already shown that plant height together with other morphological traits were greatly affected by water deficiency. Askary et al. (2021) concluded that, among other parameters, drought stress decreased plant height, number of secondary branches, dry weight, and shoot number; meanwhile, another study found that the increasing period between the irrigations had a significant effect on plant height, fresh and dry weight of herb (Aziz et al., 2008). According to research data (Alavi-Samani et al., 2015; Szabó et al., 2017), water deficiency and its influence on growth, especially growth reduction of garden thyme plants, has been widely reported, which is similar to the results of our experiment (Table 3); they also correspond to those of Sanam et al. (2012) on *Salvia* species. Water deficiency also reduced assimilates and metabolites required for cell division and cell elongation thereby reducing plant height and other growth parameters in *Thymus citriodorus* (Tátrai et al., 2016). The water deficiency study of lemon balm (*Mellissa officinalis* L.) showed that the effect of drought stress on shoot yield was significant (Ardekani et al., 2007).

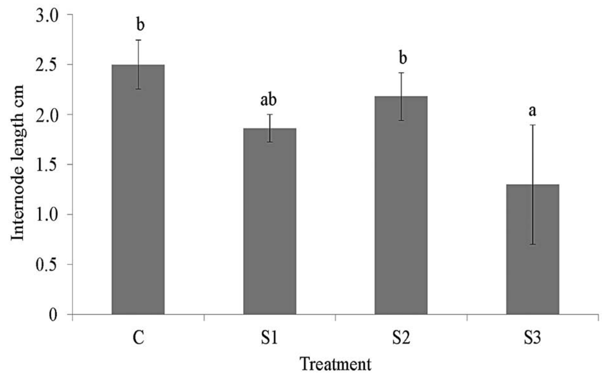
During our experiment, the plant width in diameter has changed because of different water supply regimes: in the control treatment at 70% SWC, the highest mean value (17.00 cm) was reached, while in the S3 treatment (40% SWC), it showed the lowest one (25.50 cm). However, these differences were not significant (Table 3). However, there were significant differences between the treatments in the internode length, where the plants of control and S2 treatments showed the longest (2.50 and 2.20 cm) internodes (Figure 1). The plants of S1 treatment ((70→40% SWC) represented an intermediate value (1.90 cm), while the ones of the S3 treatment (40% SWC) resulted in the shortest (1.30 cm) internodes.

In the case of shoot length values, significant differences have also been found because of different treatments. The plants of the control treatment developed the longest shoots (22.08 cm) followed by the S2 treatment (13.94 cm), while the S1 and S3 ones showed considerably shorter shoots: 11.58 and 7.12 cm, respectively (Figure 2). According to this result, it can be concluded that water shortage induces a decline in shoot elongation.

Table 3. Results of different water supply treatments applied on two-year-old *Thymus vulgaris* plants

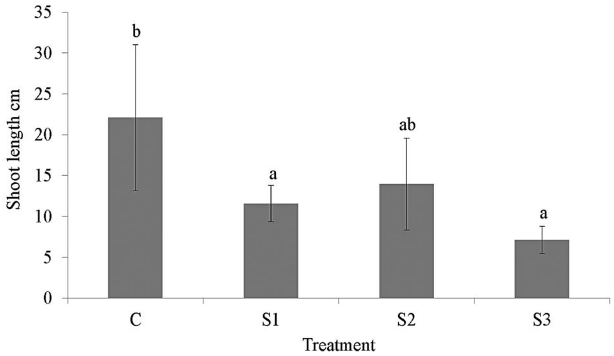
Parameters measured	Treatments			
	C (control)	S1	S2	S3
	70% SWC (normal)	70→40% SWC (mild)	70→40% SWC (sudden)	40% SWC (constant)
Plant height cm	**31.80 ± 4.90** a	31.30 ± 2.10 a	29.90 ± 2.60 a	25.50 ± 2.00 a
Plant width cm	**17.00 ± 4.70** a	15.00 ± 3.20 a	14.50 ± 2.20 a	11.40 ± 1.95 a
Shoot number, pieces	**70.50 ± 24.60** b	59.80 ± 17.60 a	66.00 ± 32.30 ab	28.80 ± 6.65 a
Chlorophyll content, SPAD units	**32.20 ± 10.40** a	20.10 ± 5.00 a	30.70 ± 10.70 a	25.26 ± 9.12 a
Fresh mass g $plant^{-1}$	**5.70 ± 2.25** a	4.55 ±6.31 a	5.62 ± 2.77 a	2.60 ± 0.69 a
Dry mass g $plant^{-1}$	**2.27 ± 0.82** a	1.75 ± 0.43 a	**2.27 ± 1.20** a	0.79 ± 0.18 a
Flavonoid content %	1.48 ± 0.06 a	1.65 ± 0.02 a	1.37 ± 0.01 a	**1.72 ± 0.02** a

Note. Mean ± SD; different letters indicate significant differences ($p < 0.05$) among treatments.



Note. C – control (70% SWC); drought stress: S1 – mild (70→40% SWC), S2 – sudden (70→40% SWC), S3 – constant (40% SWC); mean ± SD; different letters indicate significant differences ($p < 0.05$) among treatments.

Figure 1. Effect of different irrigation regimes on the internode length of *Thymus vulgaris* plants



Explanation under Figure 1.

Figure 2. Effect of different irrigation regimes on the shoot length of *Thymus vulgaris* plants

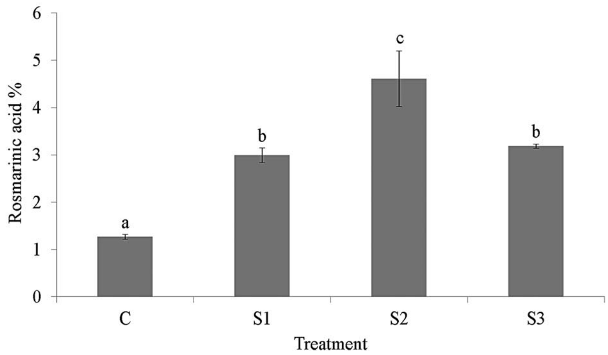
Plant shoots show numerous adaptive changes in response to drought stress. The decrease in shoot length induced by drought reduces the water loss of the plant. Plant cell growth is one of the most important factors affected by water deficiency initiating the closure of stomata and the decrease in the growth of cell leading to the decrease in shoot length. Kavian and Akhzari (2021) found that the shoot length significantly decreased as drought stress increased. Another study (Szabó et al., 2017) found similar results on drought stress and its influence on growth, especially shoot reduction has been reported.

Constant drought stress (S3; 40% SWC) reduced severely the number of shoots; however, this change was not significant. Control plants possessed the highest number (70.50) of shoots, while in the case of lower water supply levels these values were subjected to a decline: S1: 59.80, S2: 66.00, and S3: 28.80 shoots (Table 3). According to the reference data, it can be concluded that plants conserve available nutrients by limiting growth of new organs and tissues, as a high shoot number under drought stress conditions is considered an undesirable trait, because it ensures an unnecessary consumption and waste of soil moisture.

Concerning physiological plant responses to water shortage, the chlorophyll content of leaves was evaluated and expressed in SPAD units. The treatments showed no significant effect on the chlorophyll content of the plants. The average SPAD values of the treatments are shown in Table 3. In another experiment on garden thyme, it was found that water deficiency had a negative effect on the chlorophyll content of the leaves (Mohammadzadeh, Pirzad, 2020); however, it has not been manifested obviously in our experiment. Nevertheless, some authors have found opposite reactions, as the chlorophyll content increases with the increased drought stress in garden thyme (Ashrafi et al., 2018); however, in basil, the chlorophyll content decreased with the reduced soil water content (Damalas, 2019).

In our experiment, the effect of different water supply levels on fresh and dry weigh of garden thyme plants was not significant according to ANOVA; however, in the case of both fresh (2.60 g plant^{-1}) and dry (0.79 plant^{-1}) weigh values, constant drought stress (S3: 40% SWC) resulted in the lowest shoot mass (Table 3). The fresh shoot mass of the control treatment was the highest (5.70 g plant^{-1}) followed by S2 (5.62 g plant^{-1}) and S1 (4.55 g plant^{-1}) ones, respectively. This tendency was also observed in the case of dry weigh, where the plants of the control treatment represented again the highest value (2.27 ± 0.82 g plant^{-1}) followed by S2 (2.27 g plant^{-1}) and S1 (1.75 g plant^{-1}) ones, respectively.

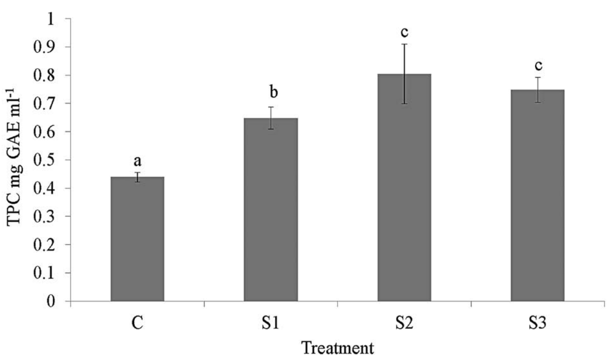
Drought generally increased the rosmarinic acid content, especially in the plants, where water deficiency changed suddenly from 70% to 40% SWC (S2). These plants accumulated the highest percentage of rosmarinic acid (4.61%) by the end of the experiment. The plants, where water supply decreased successively from 70% to 40% (S1: 2.99%), and the plants under constant drought stress (S3: 3.19%) produced relatively the same amount of rosmarinic acid values, while the lowest levels were detected in the plants (1.27%) of control treatment (Figure 3). It can be concluded that the garden thyme plants under drought stress conditions produced a higher amount of rosmarinic acid, and the results imply that longer drought periods could lead to an elevated rosmarinic acid biosynthesis.



Explanation under Figure 1.

Figure 3. Effect of different irrigation regimes on the rosmarinic acid content of dried and powdered *Thymus vulgaris* leaves

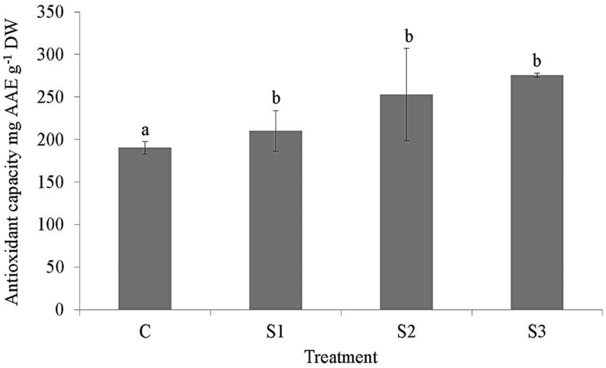
The total phenolic content (TPC) was significantly affected by water deficiency induced in garden thyme plants. TPC has been substantially increased in the S2 treatment as a result of abrupt water shortage (0.81 mg GAE ml^{-1}) and in the S3 one with the most severe drought stress (0.75 mg GAE ml^{-1}), while the lowest values (0.44 mg GAE ml^{-1}) were shown by the control treatment (Figure 4). This result demonstrated that the TPC of garden thyme plants is a drought-dependent variable, especially at higher levels of drought. The experiment of Askary et al. (2021) showed similar results: they found that drought stress increased the content of phenol.



Explanation under Figure 1.

Figure 4. Effect of different irrigation regimes on the total phenolic content (TPC) of dried and powdered *Thymus vulgaris* leaves

Similar results were found concerning antioxidant capacity. The highest values were detected in the plants of S2 and S3 treatments: 254.51 and 283.40 mg AAE g^{-1} DW, respectively. However, there was no significant difference between the plants of C (control) and S1 treatments with a lower antioxidant capacity: 191.67 and 233.17 mg AAE g^{-1} DW, respectively (Figure 5).



Explanation under Figure 1.

Figure 5. Effect of different irrigation regimes on the antioxidant capacity of dried and powdered *Thymus vulgaris* leaves

During our experiment, the flavonoid content was not significantly affected by different soil water levels. The highest values were found at constant drought stress (S3: 1.72 ± 0.02%) followed by the S1 (1.65%) and control (C: 1.48%) treatments, while the S2 (1.37%) one accumulated the lowest amount (Table 3). However, another study (Khalil et al., 2018) found opposite reactions, as drought significantly increased the flavonoid content.

Conclusions

During our experiment, various plant responses to different water supply regimes have been evaluated, analysing changes in morphology, yield, and active compounds of *Thymus vulgaris* plants in phytotron chambers.

1. The largest significant differences in the shoot and internode lengths of garden thyme generated the most severe constant drought stress (S3: 40% SWC). The statistical analysis showed that the shoot elongation is restricted to optimise the water use efficiency of the plant.

2. As with the previous results, the chlorophyll content of leaves was not significantly affected by water supply regimes. The SPAD units result showed that the highest chlorophyll content was obtained from the plants without drought stress (control (C) treatment) and those initially under no stress conditions before being suddenly subjected to drought stress (S2: 70→40% SWC). Although the results of our experiment did not show significant differences in the chlorophyll content, they suggest that the topic needs further investigation, as other studies have shown conflicting results.

3. During the experiment, the effect of drought stress on some active compounds of garden thyme was also investigated and significant differences were recorded. Rosmarinic acid, total phenolic content (TPC), and antioxidant capacity were highly affected by water supply levels. Normal water supply in the control (C: 70% SWC) treatment yielded the lowest values, while the highest ones were shown by the plants under continuously decreasing drought stress (S3: 40% SWC). This result demonstrated that the antioxidant capacity and TPC of garden thyme plants are drought-dependent variables, especially under constant drought stress.

4. Regarding rosmarinic acid, it can be concluded that the plants under drought stress produced a higher content of rosmarinic acid, and these results imply that longer drought periods may promote rosmarinic acid biosynthesis. As rosmarinic acid is the most relevant phenolic compound in garden thyme extracts, a close correlation among rosmarinic acid, TPC, and antioxidant capacity values can be found.

It can be concluded that garden thyme, which is generally considered as a drought tolerant species, has different adaptation mechanisms and is able to respond to the changing environmental conditions including different water availability. The results of our experiment suggest that garden thyme plants tolerate water deficiency; however, occasional irrigation is suggested in arid periods to avoid yield losses. Nevertheless, the determination of the optimal amount and distribution of water supply requires further studies.

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Vandens trūkumo įtaka vaistinių čiobrelių augimui ir antrinių metabolitų koncentracijai

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Santrauka

Tyrimo tikslas – nustatyti sausros atsiradimo laipsniškumo ir sausros trukmės įtaką 13 savaičių kamerose auginto vaistinio čiobrelio (*Thymus vulgaris* L., 'Varico 3') produktyvumui ir cheminėms savybėms nuo 202-ojo vystymosi tarpsnio, nustatyto pagal BBCH skalę. Taikyti skirtingi drėkinimo režimai, įvertinti pagal dirvožemio vandens kiekį (DVK %): 1) kontrolinis variantas (C: 1–13 savaitės – 70 % DVK), 2) nedidelė sausra (S1: 1–4 savaites – 70 % DVK, laipsniškai stiprėjanti sausra nuo 6 savaitės iki 40 % DVK 10–13 savaites), 3) staigi sausra (S2: 1–9 savaitės – 70 % DVK, 10–13 savaitės – 40 % DVK) ir 4) nuolatinė sausra (S3: 1–4 savaitė – 70 % DVK, 5–13 savaitės – 40 % DVK). Tyrimo pabaigoje nustatyta, kad vandens trūkumas neturėjo reikšmingos įtakos vaistinių čiobrelių aukščiui ir plotui, bet labai paveikė tarpubamblių ir ūglių ilgius. Ilgiausiai trunkant sausrai (S3) auginti augalai buvo žemiausi: tarpubambliai – 1,30 cm, ūgliai –7,12 cm, taikant kontrolinį režimą, augalai buvo aukščiausi: tarpubambliai – 2,50 cm, ūgliai – 22,08 cm. Tačiau vandens tiekimas neturėjo didelės įtakos nei ūglių skaičiui, nei lapų chlorofilo koncentracijai. Nustatyta reikšminga sausros įtaka ir rozmarino rūgščiai, ir suminei fenolių koncentracijai taikant S2 ir S3 režimus: S2 atveju nustatyta didžiausia rozmarino rūgšties koncentracija – 4,61 %, S3 atveju buvo didžiausia suminė fenolių koncentracija – 0,75 mg galo rūgšties ekvivalento (GRE) ml^{-1}. Taikant S3 režimą, augalų antioksidacinė geba buvo didžiausia (275,44 mg askorbo rūgšties ekvivalento (ARE) g^{-1} sausosios masės), o suminė flavonoidų koncentracija reikšmingai nesiskyrė esant skirtingiems vandens režimams.

Tyrimo duomenys parodė, kad ne tik vandens trūkumo lygmuo, bet ir vandens tiekimo kaitos ir sausros atsiradimo metas gali būti esmingai svarbūs vaistinių čiobrelių derliaus kiekiui bei kokybei.

Reikšminiai žodžiai: *Thymus vulgaris*, sausros sukelta įtampa, suminė flavonoidų koncentracija, rozmarino rūgštis, suminė fenolių koncentracija.