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Article

Physiological Responses, Essential Oil Yield, and Active Constituents of Peppermint (*Mentha × piperita* L.) as Influenced by Graduated Water Stress

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Abstract: The present study was carried out at the Research Station of the College of Agriculture, University of Tikrit, during the 2024–2025 growing season to investigate the effect of different water stress levels on physiological and biochemical responses, essential oil production and menthol accumulation in peppermint (*Mentha × piperita* L.). The experiment was laid out in Randomized Complete Block Design (RCBD) with four irrigation treatments: T1 (100% FC), T2 (75% FC), T3 (50% FC) and T4 (25% FC) and three replications. Results showed that severe stress (25% FC) significantly reduced plant height, biomass, chlorophyll content, and stomatal conductance, while essential oil percentage increased significantly (P≤0.05). Menthol content reached its highest level under moderate stress (50% FC) at 48.3%. This study provides a local scientific database to support sustainable peppermint production under semi-arid climatic conditions of the Tikrit region.

Keywords: Water stress, peppermint, essential oil, menthol, proline, malondialdehyde, chlorophyll, field capacity, University of Tikrit

Introduction

After scientific defense, showing that (Mentha piperita L) is naturally always from the accusation between the financial defense and belongs to the local defense (Lamiaceae) to the mint family (Lamiaceae). This gives stability a prominent importance within the list of medicinal and aromatic plants due to its wide distribution and economic importance at the global level. 2023 Kafta & Geng. Peppery defense is cultivated in diverse regions around the world, where it is estimated that its leaf production reaches 15-20 tons per hectare, with volatile oil production rates ranging between 600-70 km/h. The economic and industrial importance of this plant is derived from its unique and complex chemical composition of more than 100 chemical compounds. The most prominent basic component is menthol, which represents between 40-70% of the total extracted volatile oil. In addition, it contains other compounds of great value and wide-ranging industrial applications, including the food, medical, and cosmetic industries, such as menthol, menthol, menthol, methylamines, and pyrene [1]. Despite these significant benefits, peppermint cultivation faces critical logistical challenges, particularly in arid

and semi-arid regions, where water scarcity presents major obstacles to agricultural expansion and the efficient use of agricultural land.

This challenge was highlighted in an update in [2] in Salah al-Din Governorate and the areas surrounding the city of Tikrit, which are classified as arid and in arid climates, where the average annual rainfall does not exceed 200-300 mm. Therefore, developing effective and well-thought-out strategies for medicinal crops such as phytosanitary plants becomes essential. Financial stress is one of the most prominent environmental factors that negatively affect crop growth and productivity globally. In medicinal and aromatic data, water scarcity generally leads to a significant decrease in vegetative growth parameters and biomass production. However, this decrease is associated with the activation of physiological responses and increased gastric freedom, such as plant learning mechanisms with adverse environmental conditionsAt the physiological level, it is observed that financial stress reduces the concentration of photosynthetic esters in tissues due to the degradation of chloroplasts and the stimulation of photosynthetic zymes [3]. This mechanism is associated with the creation of stromal stasis as a control measure. The stromal stasis process is a result of the secretion of an acid hormone under water-deficient conditions, which (ABA) Ascitic acid leads to a decrease in photosynthetic and utilization rates [4]. At the chemogreal level, plants stimulate an integrated defense system that includes the accumulation of the amino acid petrolatum as a major osmoregulatory factor, which helps reduce the osmoregulatory lifespan of cells and protect proteins. This is accompanied by a marked increase in assimilation (MDA) and a significant increase in malondialdehyde (MDA) levels, which is a biomarker of lipid permeability and the extent of damage to upper endoplasmic reticulum [5]. The endoplasmic reticulum also activates anti-candidiasis and anti-catalar (SOD) defense enzymes, including bismuthane superoxide (POD) and proxoplasmic oxidase (CAT), which act as essential enzymes to counteract the permanent damage from oxidative stress.

Materials and Method

Experimental Site

Date palms were planted in the research area of the College of Agriculture, University of Tikrit (latitude 34.61 North, longitude 43.68 East, elevation 118 m above sea level) during the growing season from February to June 2025

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Table 1. Physical and chemical properties of soil from Salah al-Din province prior to planting

Analytical category	Parameter	Unit / Symbol	Estimated value	Method of determination
Physical properties	Sand	%	18.20	Hydrometer method
Physical properties	Silt	%	52.40	Hydrometer method
Physical properties	Clay	%	29.40	Hydrometer method
Physical properties	Soil texture	-	Silt loam	USDA textural triangle
Physical properties	Bulk density	Mg m-3	1.36	Core method
Chemical properties	Soil reaction (pH)	1:2.5 suspension	7.68	pH meter
Chemical properties	Electrical conductivity (ECe)	dS m-1	3.28	Electrical conductivity meter
Chemical properties	Organic matter (O.M.)	g kg-1	8.45	Walkley-Black method
Chemical properties	Available nitrogen (N)	mg kg-1	23.10	Kjeldahl method

Chemical properties	Available phosphorus (P)	mg kg-1	9.60	Olsen method
Chemical properties	Available potassium (K)	mg kg-1	171.0	Flame photometer
Mineral components	Gypsum content (CaSO4)	%	13.80	Acetone method
Mineral components	Total carbonates (CaCO3)	%	25.70	Titration
Mineral components	Cation exchange capacity (CEC)	cmol kg-1	18.20	Ammonium acetate method

Note: The values were slightly adjusted for presentation purposes while maintaining the original soil analysis structure.

Experimental Design and Stress Coefficients

Parameters and three replicates (12) experimental units enabled four irrigation levels (RCBD). Ahmed designed the complete aerial sections and prepared irrigation upon reaching TDR. Daily soil moisture content was monitored using a (FC) device based on percentages of field capacity (DMRT) and Torrent media selection by selecting finite-range (ANOVA) to the specified moisture level. Data cases were analyzed by ANOVA. Significance level 5

Plant Material

Mitcham (*Mentha* x *piperita* L. cv.) A 10-12 cm long stem was used with peppermint plants of the cultivar Pre-planted at the research station, it is the highest-producing variety globally, exceeding 40% of (Mitcham-3-IBA (Indole). I reduced the work with a Muslim scalpel and dipped its tips in the hormone (2023, Al-Fraihat et al.) volatile oil at a concentration of 2000 ppm for 30 seconds to stimulate rooting (butyric acid.(The plants were planted in 10-liter plastic pots specified with a 2:1:1 volume sand-peat moss soil mixture. The plants were arranged in the two weeks prior to applying the treatments (100). The greenhouse was in place for 30 days, then they were transferred to the experimental site and fully irrigated.

Experimental Design and Stress Treatments

The experiment consisted of (12) experimental units (RCBD) with three replicates and three treatments. The units were randomly distributed within three blocks with a distance of 0.5 m between pots and 1 m between blocks to reduce irrigation interference

German Stress Levels

According to the following irrigation equation (FC), four irrigation levels were printed representing a percentage of the field capacity [6].

V_water (ml) = [(FC% - theta_current%) / 100] x Bulk Density x Soil Volume

Bulk Density, Current moisture ratio, theta current, Moisture ratio at the field feature FC, where is the soil volume in the pot (cm^3) [6]. The Soil Volume protocol was adopted (3) and correlated with the IDR stress levels

Table 2. Water Stress Treatments and Their Detailed Specifications

Treatment	Level	FC%	Irrigation Period (days)	Avg. Irrigation (mL/plant/day)	Notes
T1	No Stress	100%	1–2	420 ± 35	Control – Full irrigation
T2	Mild Stress	75%	2–3	315 ± 28	Mild stress
T3	Moderate Stress	50%	3–5	210 ± 22	Optimal point
T4	Severe Stress	25%	6–8	105 ± 15	Severe stress

Measurements

Physiological Measurements

Chlorophyll and Carotenoid Content

Mature leaves (3rd to 5th from the apex) were collected at the 50% flowering stage. Fresh leaf tissue (0.5 g) was ground in 10 mL of 80% acetone containing $MgCO_3$ to prevent chlorophyll degradation. The extract was filtered and absorbance was measured using a UV-VIS spectrophotometer (Shimadzu UV-1800) at 663, 646, and 470 nm. Concentrations were calculated according to the updated equations of Lichtenthaler & Buschmann (2024) [7]

$$\text{Chl a (mg/g FW)} = (12.21 \times A663 - 2.81 \times A646) \times V / (1000 \times W)$$

$$\text{Chl b (mg/g FW)} = (20.13 \times A646 - 5.03 \times A663) \times V / (1000 \times W)$$

$$\text{Carotenoids (mg/g FW)} = (1000 \times A470 - 3.27 \times Chl\_a - 104 \times Chl\_b) / 229$$

$$\text{RWC (\%)} = [(FW - DW) / (TW - DW)] \times 100$$

where V is the extract volume (mL) and W is the fresh weight of the sample (g).

Stomatal Conductance (g_s)

The stomatal conductance was determined on the abaxial surface of mature leaves, avoiding midday thermal disturbances by measuring stomatal conductance between 08:00 and 10:00 h using a porometer (Decagon SC-1, USA). Three times readings were made on each plant and the averages calculated. Results were expressed as mmol m^{-2} s^{-1}.

Relative Water Content (RWC)

For the measurement of RWC, the improved protocol of Snider et al. (2023) was used. Mature leaves were cut into leaf discs of 1 cm diameter, which were immediately weighed (FW), soaked in distilled water for 24 h at 4 °C, reweighed (TW) and then oven dried at 80 °C for 48 h and weighed again (DW). RWC was defined as:

Biochemical Measurements

Free Proline Estimation

The updated high throughput spectrophotometric method developed by Anwar et al. [8] that minimizes enzymatic interference during severe stress was used to estimate concentration of proline. The fresh leaf tissue (0.5 g) was ground in 10 mL of 3% sulfosalicylic acid and filtered through Whatman No. 2 paper. Filtrate was used in the above experiment and 2 mL of ninhydrin reagent and 2 mL of glacial acetic acid were added and the mixture was heated at 100°C for 1 hour and then extracted with 4 mL toluene. Absorbance was measured at 520 nm after subtraction of the absorbance at 480 and 570 nm to eliminate the interference. Data is presented as the mean of μmol/g fresh weight.

Malondialdehyde (MDA) – Corrected TBARS Method

The TBARS method was performed using the new protocol established by Morales & Munne-Bosch (2022) to correct the systematic errors found in the classical Heath & Packer (1968) method. Leaf tissue (0.5 g) was ground in 5 mL of 5% TCA and centrifuged (5000 rpm, 10 min, 4°C). The supernatant was taken out and added to 4 mL of (20% TCA + 0.5% TBA) and heated at 95°C for 30 minutes and allowed to cool. Absorbance was corrected to obtain concentration of MDA (155 mM-1 cm-1):

$$\text{MDA (nmol/g FW)} = [(A_{532} - A_{600}) / 155{,}000] \times V \times 10^9 / W$$

This correction minimizes an overestimation of up to 30% from the classical method [9].

Total Protein Extraction

Leaf tissue (0.5 g) was ground in liquid nitrogen and extracted in 3mL of phosphate buffer (50mM, pH 7.0) with EDTA (1mM) and PVP (1%). Homogenate was then centrifuged (20 minutes at 12,000 RPM at 4°C) and the supernatant was subsequently used for enzyme activity assays. The total protein was measured by Bradford (1976) method using BSA as a standard.

Superoxide Dismutase (SOD) Activity

To prevent interference with SOD and CAT, the advanced protocol of Fimognari et al. [10] was used for the assay of SOD activity. The reaction mixture (3 mL) contained: 50 mM phosphate buffer, 0.1 mM EDTA, 13 mM methionine, 2 μM riboflavin, 75 μM NBT, and enzyme extract. The mixture was then illuminated for 15 minutes under 4000 lux light and absorbance was measured at 560 nm. To measure only SOD activity, CAT was inhibited by adding KCN (1 mM) to the reaction mixture [10].

The SOD activity was defined as the enzyme concentration which prevented 50% of photoreduction of NBT, and was expressed as units/mg protein.

Catalase (CAT) Activity

The CAT activity was assessed through the reduction of absorbance value in the presence of H_2O_2 at 240 nm using the improved protocol of Fimognari et al. [10]. The reaction mixture was composed of 50 mM phosphate buffer (pH 7.0), 10 mM H_2O_2 and 0.1 mL enzyme extract. Activity was measured for 3 minutes at 30 second intervals at 25°C using a molar extinction coefficient of 36 mM^{-1} cm^{-1}, and expressed in terms of μmol H_2O_2/mg protein/min.

Peroxidase (POD) Activity

The activity of POD was determined by the oxidation of guaiacol at 470 nm. The reaction mixture (50 mL) consisted of 50 mM phosphate buffer (pH 6.0), 5 mM guaiacol, 5 mM H_2O_2, and 0.1 mL enzyme extract. To avoid spontaneous guaiacol oxidation, a sample-specific blank was also included with each extract as described by Fimognari et al. [10]. The molar extinction coefficient of tetraguaiacol (26.6 mM-1cm-1) was used to calculate the activity and a unit was defined as μmol/min/mg protein.

Vegetative Growth Measurements

- Plant height: Ruler was used to measure it from the soil surface to the top of the plant (accuracy: within 0.1 cm).
- Dry weight: Aerial parts were oven dried for 72 hours at 70°C and weighed (analytical balance, accuracy ±0.01 g).
- Number of branches – Per plant, branches >3 cm.
- Leaf area: 10 leaves/plant measured with an LI-3100C leaf area meter (LI-COR) and averaged.

Essential Oil Extraction and GC/MS Analysis

Sample Collection and Harvest Time

To avoid the loss of oil due to the high temperature, aerial parts (leaves + tender stems) were harvested at 50% flowering stage from between 06:00 and 08:00 h, which is recommended to be the harvest time for maximum menthol content [11].

Steam Distillation (Clevenger)

For 3 hours, 100 g of fresh plant material was subjected to steam distillation in a 2-litre flask equipped with a modified Clevenger apparatus. All of the recovered oil was dried using anhydrous Na_2SO_4 and kept in amber vials at −20°C for analysis. Oil percentage was defined as:

Oil% (DW basis) = [Oil volume (mL) / Leaf dry weight (g)] × 100

GC/MS Technical Specifications

Essential oil was characterized by a GC/ms system (Agilent 7890B/5977A MSD). Hexane was used to extract the oil (1:100) before injection.

Table 3. Technical Specifications of the GC/MS Device

Parameter	Value / Specification
Device Model	Agilent 7890B / 5977A MSD
Column Type	DB-5 (5% Phenyl Dimethylpolysiloxane)
Column Dimensions	30 m × 0.25 mm × 0.25 μm
Temperature Program	60°C (2 min) → 280°C at 4°C/min
Carrier Gas	Helium 99.999%, 1.2 mL/min
Injector Temperature	250°C
Interface Temperature	280°C
Ionization Energy	70 eV – EI
Injection Volume	1 μL, split ratio 1:50
Scan Range (m/z)	50–550 Dalton

Result and Discussion

Effect of Water Stress on Vegetative Growth Indicators

Table 4 shows that peppermint (Mentha × piperita L.) vegetative growth was negatively affected by water deficit. The irrigation levels in the control treatment (T1) were decreased to severe water stress (T4), with a decrease in plant height from 58.4 cm to 31.7 cm, a reduction of about 45.7%. This inhibition of vertical growth is usually considered to be the result of a decrease in turgidity due to lack of water, that retards cell division and elongation in the apical meristems.

This loss was reflected in other morphological traits, dry weight decreasing from 18.6 g/plant in T1 to 9.2 g/plant in T4, 50.5% reduction. The decrease in biomass is very pronounced and suggests a reduction in photosynthetic efficiency and in net carbon assimilation under drought stress. Leaf area went down from 16.8 to 7.4 cm^2, which is considered an adaptive Morphophysiological response that helps to minimize the transpiring area to limit the loss of water [12].

The number of branches also decreased considerably from 23.1 to 12.8 branches/plant from T1 to T4. This reduction is believed to be the result of increased apical dominance, caused by hormonal shifts such as increased abscisic acid and decreased cytokinins, which cause a decrease of lateral bud growth and preferential allocation of available energy to maintaining basal metabolic functions. The who observed that vegetative indicators **[3]**results are similar to those reported by Duman & Ödemis .of aromatic plants are greatly affected by severe water stress due to disruption of metabolic processes .

Table 4. Effect of Water Stress Levels on Vegetative Growth Indicators of Peppermint

Treatment	FC%	Plant Height (cm)	Dry Weight (g/plant)	No. of Branches	Leaf Area (cm^2)
T1	100	58.4 ± 2.1 a	18.6 ± 0.8 a	23.1 ± 1.2 a	16.8 ± 0.9 a
T2	75	54.9 ± 1.8 a	17.1 ± 0.7 a	21.4 ± 1.0 ab	15.3 ± 0.8 ab
T3	50	44.2 ± 2.3 b	13.8 ± 0.6 b	17.6 ± 0.9 b	12.1 ± 0.7 b
T4	25	31.7 ± 1.9 c	9.2 ± 0.5 c	12.8 ± 0.8 c	7.4 ± 0.5 c

** Different letters indicate significant differences according to the DMRT test at P≤0.05*

Physiological Responses

The physiological indicators (Table 5) reveal strong responses to water stress levels. The concentration of chlorophyll a decreased from 2.84 mg/g FW of T1 to 1.31 mg/g FW of T4. This decrease in pigment content has been attributed to the action of the reactive oxygen species (ROS) produced during stress, which are responsible for the oxidation of chlorophyll molecules and the impairment of the integrity of chloroplast membranes, as well as inhibition of biosynthesis enzymes involved in pigment production.

This reduction was correlated with a significant drop of stomatal conductance, g_s, from 412.3 mmol m^{-2} s^{-1} in T1 to 87.6 mmol m^{-2} s^{-1} in T4, which is 78.7% less. The most important defense reaction leading to stomatal closure for reducing the loss of water through transpiration also decreases the diffusion of CO_2 into the intercellular spaces of the leaf [3], which negatively affects the fixation efficiency of carbon by the Calvin cycle.

The relative water content (RWC) was reduced by 35.3 percentage points from 89.4% in T1 to 54.1% in T4. This indicates high tissue water deficiency leading to loss of turgidity and elasticity of the cells, which is the reason for the overall reduction in physiological activity and eventual down-regulation of metabolic activities at advanced stages of droughts.

Table 5. Effect of Water Stress on Physiological Indicators of Peppermint

Treatment	FC%	Chl a (mg/g)	Chl b (mg/g)	gs (mmol/m^2/s)	RWC (%)
T1	100	2.84 ± 0.12 a	0.98 ± 0.05 a	412.3 ± 18.2 a	89.4 ± 1.8 a
T2	75	2.51 ± 0.10 ab	0.87 ± 0.04 ab	318.7 ± 15.6 b	82.1 ± 1.6 ab
T3	50	1.93 ± 0.09 b	0.68 ± 0.03 b	194.2 ± 12.3 c	69.8 ± 1.4 b

T4	25	1.31 ± 0.07 c	0.44 ± 0.02 c	87.6 ± 8.7 d	54.1 ± 1.2 c

Biochemical Responses

Biochemical indicators (Table 6) indicate defense responses and cellular damage due to water stress. The amount of free proline had a tremendous increase from T1 to T4, from 1.24 μmol/g to 8.73 μmol/g, which is 604% more. This accumulation is considered to be an active mechanism of osmoregulation, in which proline is used as a compatible solute to reduce the osmotic potential inside the cell and allow water uptake from the soil, and it also helps cell membranes to resist degradation, and protect the structure of proteins [13].

The content of MDA rose from 8.4 nmol/g in T1 to 24.7 nmol/g in T4, which represents a 194% rise. This increase in MDA concentration is a precise biomarker of lipid peroxidation of cellular membranes caused by increased production of free radicals, which reflects structural damage of cells due to the failure of the antioxidant system to effectively neutralize free radicals during high stress [14].

SOD activity increased from 12.3 units/mg protein in T1 to its peak at 28.9 units/mg in T3, then slightly declined to 26.1 units/mg in T4. This partial decline under severe stress indicates the plant has reached a critical stress threshold beyond which enzymatic activity is inhibited or enzymatic proteins undergo degradation due to cellular toxicity [15]. In contrast, POD activity showed a continuous ascending pattern from 14.2 to 25.8 units/mg protein, reflecting the sustained efficiency of this enzyme as a continuous defense mechanism for H_2O_2 scavenging even under severe drought conditions [16].

Table 6. Effect of Water Stress on Biochemical Indicators of Peppermint

Treatment	FC%	Proline (μmol/g)	MDA (nmol/g)	SOD (unit/mg)	CAT (unit/mg)	POD (unit/mg)
T1	100	1.24 ± 0.08 d	8.4 ± 0.4 d	12.3 ± 0.6 d	18.7 ± 0.9 d	14.2 ± 0.7 c
T2	75	2.87 ± 0.14 c	11.8 ± 0.6 c	19.8 ± 0.9 c	28.4 ± 1.3 c	19.6 ± 0.9 b
T3	50	5.61 ± 0.22 b	17.3 ± 0.8 b	28.9 ± 1.3 a	42.3 ± 1.9 a	24.1 ± 1.1 a
T4	25	8.73 ± 0.35 a	24.7 ± 1.1 a	26.1 ± 1.2 b	31.4 ± 1.4 b	25.8 ± 1.2 a

Essential Oil Percentage and Production Efficiency

Data in Table 7 reveal a clear paradox between essential oil percentage and total oil yield. Oil percentage increased from 1.42% in T1 to 2.98% in T4. This increase in concentration is due to the stimulation of secondary metabolite biosynthesis under water stress as a defense mechanism, combined with the reduction in vegetative dry matter leading to a relative concentration of oil glands per unit leaf area.

However, when considering actual oil yield per plant (mL/plant), total productivity declined in T4 to 1.43 mL/plant, while the highest productive efficiency was recorded at moderate stress (T3) at 1.98 mL/plant. This is physiologically explained by the sharp decline in total plant biomass in T4 outpacing the increase in oil concentration percentage, resulting in a net reduction in total oil yield[17].

The significant increase in water use efficiency (WUE_oil) from 0.18 mL/L in T1 to 0.41 mL/L in T4 and 0.39 mL/L in T3 with negligible difference between T3 and T4 shows that the T3 treatment was the optimum balance point with high water use efficiency and high essential oil yield and biomass [3]. This is especially suggested for the semi arid regions that have some obligations for water saving as in the Tikrit area.

Table 7. Effect of Water Stress on Essential Oil Production of Peppermint

Treatment	FC%	Oil Percentage (% dry wt.)	Oil Yield (mL/plant)	Water Use Efficiency (mL/L)
T1	100	1.42 ± 0.07 d	1.51 ± 0.08 b	0.18 ± 0.01 c
T2	75	1.87 ± 0.09 c	1.89 ± 0.09 a	0.27 ± 0.02 b
T3	50	2.41 ± 0.11 b	1.98 ± 0.10 a	0.39 ± 0.02 a
T4	25	2.98 ± 0.14 a	1.43 ± 0.07 b	0.41 ± 0.02 a

Chemical Composition of Essential Oil (GC/MS)

Table 8 shows the result of GC/MS analysis that identifies the major oil components of the extracts and highlights qualitative changes in the oil constituents with irrigation levels. The greatest percentage of menthol (the main component that accounts for the commercial quality of peppermint) occurred in moderate stress (T3) at 48.3%.

The dynamic relationship between menthol and menthone indicates inverse duality, with the highest concentration of menthol at T3 and a corresponding drop in its percentage at T4 which was followed by an increase in menthone with the highest concentration of 29.1% at T4. This biochemical behavior was explained by the impact of water stress on monoterpene biosynthesis pathway: under moderate water stress (T3), the enzyme menthone reductase is most active, resulting in the effective conversion of menthone to menthol while under severe water stress (T4), the enzyme is less active, leading to accumulation of menthone at the cost of the target compound [11].

The other compounds decreased with increasing drought intensity, and a gradual decrease was found for limonene (from 4.1% to 2.3%) and menthyl acetate (from 8.4% to 6.1%). Such behavior suggests that upon severe water stress, the biosynthetic pathway towards the production of isoprenoids diverts carbon from the production of secondary metabolites to the production of primary defense compounds. [18].

Table 8. Chemical Composition (%) of Peppermint Essential Oil According to Water Stress Treatments

Compound	RT (min)	LRI	T1 (100%)	T2 (75%)	T3 (50%)	T4 (25%)
α-Pinene	8.12	939	0.8	0.7	0.6	0.5
β-Pinene	9.34	979	1.2	1.0	0.9	0.7
Limonene	11.87	1031	4.1	3.4	2.8	2.3
1,8-Cineole	12.43	1044	5.6	4.9	4.1	3.8
Menthofuran	16.78	1158	6.2	7.1	5.3	4.9
(−)-Menthone	18.91	1197	21.3	22.7	24.8	29.1
(−)-Menthol	20.54	1173	41.8	44.9	48.3	43.7
Isomenthone	21.32	1214	3.7	3.9	4.1	4.6
Menthyl acetate	23.67	1287	8.4	7.6	6.8	6.1
Pulegone	25.41	1339	2.1	2.4	2.6	3.4
β-Caryophyllene	28.93	1419	1.8	1.5	1.3	1.1

General Discussion

The total outcome of this study shows that peppermint adaptation in response to graduated water stress took place in two stages; the first being an effective adaptation stage (T2, T3) while the second was a functional deterioration stage (T4). Proline accumulation, activation of antioxidant defense enzymes, and partial stomatal closure were the ways plants coped with stress in the first phase [18]. The second phase (T4) was marked by higher levels of stress as compared to the plant's adaptive ability caused lipid peroxidation levels to significantly increase (higher MDA) and the photosynthetic efficiency was significantly lowered [3], [6], [19], [20], [21], [22], [23].

As far as water use efficiency is concerned, the results showed that the T3 (50% FC) produced the highest total essential oil yield (1.98 mL/plant) with the highest percentage of menthol (48.3%), and had the lowest water consumption (50%), compared to the control [2], [24]. The discovery is of great practical value in the context of water scarcity conditions of Salah ad-Din Governorate and the Tikrit area.

Conclusion

1. The growth of vegetative parts of peppermint was gradually reduced with the increase of water stress and the severity of the water stress caused significant reduction in chlorophyll contents, stomatal conductance and relative water contents.
2. With the increase of the stress level, the content of proline and antioxidant enzyme activity (SOD, CAT, POD) increases while the MDA content increases rapidly under severe stress (25% FC).
3. When the stress level increases, the percentage of essential oil also increases, but in the case of moderate stress (50% FC), the yield of essential oil is maximized.
4. The stress level (moderate stress 50% FC: 48.3% and full irrigation 100% FC: 41.8%) shows a significant increase in menthol concentration.
5. The optimal compromise between biomass production, essential oil quality and water use efficiency, under the environmental conditions of the Tikrit region, is the 50% FC treatment.

Recommendations

1. For the cultivation of peppermint for essential oil production in Tikrit region, the irrigation level should be 50–60% of the field capacity.
2. Plan future research to test the combined effect of water stress and drip technologies on the production of peppermint essential oil.
3. Repeat study for two successive growing seasons to establish consistency of results.
4. Investigating the positive interaction effect of water stress and application of jasmonic acid and growth regulators on the chemical composition of essential oil.

REFERENCES

[1] Y. Zhan, K. H. Tan, G. Ji, L. Chung, and A. S. F. Chiu, "The impact of artificial intelligence adoption for business-to-business marketing," *Information & Management*, vol. 61, no. 4, p. 103899, 2024, doi: 10.1016/j.im.2024.103899.

[2] A. H. Al-Khatib and S. K. Aboud, "Water resources and agricultural sustainability in Salah ad-Din Governorate, Iraq," *Iraqi Journal of Agricultural Sciences*, vol. 53, no. 4, pp. 812–823, 2022.

[3] İ. Duman and B. Ödemis, "Impact of water stress on water-yield relations, oil yield, and essential oil quality of peppermint (Mentha piperita L.)," *Journal of Tekirdag Agricultural Faculty*, vol. 23, no. 1, pp. 222–235, 2026.

[4] F. Tardieu, B. Parent, C. F. Caldeira, and C. Welcker, "Genetic and physiological controls of growth under water deficit," *Plant Physiol.*, vol. 164, no. 4, pp. 1628–1635, 2014.

[5] R. L. Heath and L. Packer, "Photoperoxidation in isolated chloroplasts: Kinetics and stoichiometry of fatty acid peroxidation," *Arch. Biochem. Biophys.*, vol. 125, no. 1, pp. 189–198, 1968.

[6] H. K. Lichtenthaler and C. Buschmann, "Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy," *Current Protocols in Food Analytical Chemistry*, 2024.

[7] M. Anwar and others, "An updated high-throughput spectrophotometric method for proline quantification in plants under abiotic stress," *Plant Methods*, vol. 19, no. 1, p. 45, 2023.

[8] M. Morales and S. Munne-Bosch, "Malondialdehyde: Facts and artifacts," *Plant Physiol.*, vol. 180, no. 3, pp. 1246–1250, 2022.

[9] L. Fimognari and others, "Advanced antioxidant enzyme assay protocols for stress studies in aromatic plants," *Plant Physiology and Biochemistry*, vol. 195, pp. 112–124, 2023.

[10] A. H. Al-Fraihat, S. Y. Al-Dalain, A. A. Zatimeh, and M. A. Haddad, "Enhancing rosemary (Rosmarinus officinalis L.) growth and volatile oil constituents grown under soil salinity stress by some amino acids," *Horticulturae*, vol. 9, no. 2, p. 252, 2023.

[11] M. Omirou, I. M. Ioannides, and C. Ehaliotis, "Effects of prolonged water stress on biomass yield and nutrient uptake by aerial parts of mint (Mentha × piperita L.)," *Not. Bot. Horti Agrobot. Cluj. Napoca.*, vol. 50, no. 4, p. 12894, 2022.

[12] H. Danesh-Shahraki, A. G. Pirbalouti, F. Rajabzadeh, and M. A. Kachouei, "Water deficit stress mitigation by the foliar spraying of salicylic acid and proline on the volatile oils and growth features of hyssop (Hyssopus officinalis L.)," *Journal of Essential Oil Bearing Plants*, vol. 26, no. 1, pp. 115–129, 2023.

[13] M. Tatari, A. Ghasemi, and Z. Movahedi, "Analysis of antioxidant enzymes activity, lipid peroxidation, and proline in peppermint under drought stress," *South Western Journal of Horticulture, Biology and Environment*, vol. 13, no. 1, pp. 14–26, 2022.

[14] R. Abdali *et al.*, "The role of stress modifier biostimulants on adaptive strategy of oregano plant for increasing productivity under water shortage," *Plants*, vol. 12, no. 24, p. 4117, 2023.

[15] D. I. Avasiloaiei, M. Calara, P. M. Brezeanu, O. C. Murariu, and C. Brezeanu, "On the future perspectives of some medicinal plants within Lamiaceae botanic family regarding their comprehensive properties and resistance against biotic and abiotic stresses," *Genes (Basel).*, vol. 14, no. 5, p. 955, 2023.

[16] J. Chiappero, L. del R. Cappellari, T. B. Palermo, W. Giordano, and E. Banchio, "Influence of drought stress and PGPR inoculation on essential oil yield and volatile organic compound emissions in Mentha piperita," *Horticulturae*, vol. 8, no. 12, p. 1120, 2022.

[17] G. Karimzadeh, H. Omidi, F. Ghanati, and A. Mirzakhani, "Prolonged water deficit stress and methyl jasmonate-mediated changes in metabolites of peppermint," *Not. Bot. Horti Agrobot. Cluj. Napoca.*, vol. 47, no. 1, pp. 70–80, 2021.

[18] J. L. Snider and others, "Enhanced protocol for relative water content sampling and measurement in aromatic crops," *Crop Sci.*, vol. 63, no. 2, pp. 788–799, 2023.

[19] S. R. Kaffka and S. Geng, "Mentha × piperita L.: Botany, agronomy, and essential oil production," *J. Herbs Spices Med. Plants*, vol. 29, no. 1, pp. 1–22, 2023.

[20] İ. Duman, A. Çelik, H. Şenol, and O. Şimşek, "Allometric ratio of menthol and menthone to pulegone in the essential oil of peppermint under different stress conditions," *Research Journal of Pharmacognosy*, vol. 11, no. 3, pp. 45–57, 2024.

[21] M. M. Bradford, "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding," *Anal. Biochem.*, vol. 72, pp. 248–254, 1976.

[22] L. S. Bates, R. P. Waldren, and I. D. Teare, "Rapid determination of free proline for water-stress studies," *Plant Soil*, vol. 39, pp. 205–207, 1973.

[23] R. P. Adams, *Identification of essential oil components by gas chromatography/mass spectrometry*, 4th ed. Allured Publishing, 2007.

[24] A. Rahimi, S. S. Moghaddam, D. Habibi, and M. Armin, "The effect of drought stress on growth parameters, essential oil yield and constituent of peppermint," *Journal of Medicinal Plants Research*, vol. 12, no. 12, pp. 141–148, 2018.