

## CU-CHLOROPHYLLIN AND IRRIGATION INTERVALS IMPACTS ON GROWTH AND PRODUCTIVITY OF *MENTHA VIRIDIS* PLANT UNDER SOUTH SINAI CONDITIONS

**Hanan A.E.A. Hashem**

Department of Medicinal and Aromatic Plants, Desert Research Center, Cairo, Egypt

E-mail: drhanan\_h@yahoo.com

This study was conducted on a private farm in the Tour Sinai region of South Sinai Governorate during the 2022 and 2023 growing seasons. The research aimed to explore how different concentrations of copper chlorophyllin formula (Cu-Chl) (0, 0.5, and 1 g L<sup>-1</sup>) affect the growth, productivity, and physiological traits of Spearmint (*Mentha viridis*) plant under three different irrigation intervals (every 2, 4, and 6 days). The findings revealed that extending the irrigation interval to 6 days, compared to watering every 2 days, significantly reduced plant growth parameters, including herb fresh weight per square meter, herb fresh yield per feddan, herb dry weight per square meter, herb dry yield per feddan, and volatile oil yield. But total chlorophyll, copper content, and proline content as well as the Water Use Efficiency (WUE) of the yield were increased under this treatment. However, the application of Cu-Chl notably improved all these traits compared to plants that did not receive Cu-Chl. The most effective treatment in this study was foliar spraying of plants with Cu-Chl at 1 g L<sup>-1</sup> combined with irrigation every 4 days. The study concluded that using Cu-Chl can overcome the negative effects of water deficit on the growth, yield, and physiological traits of Spearmint plant, as well as improve the Water Use Efficiency.

**Keywords:** *Mentha viridis*, copper chlorophyllin, irrigation intervals, volatile oil

### INTRODUCTION

Aromatic and medicinal plants have gained significant attention in various industries, including natural cosmetics, food production, fragrances, and pharmaceuticals (Olfa et al., 2009). While the production of secondary metabolites in these plants is primarily determined by their genetic makeup, environmental factors also play a crucial role in their biosynthesis (Yazdani et al., 2002). This means that growth parameters, essential oil yield, and composition are influenced by both biotic and abiotic environmental

conditions (Aziz et al., 2008 and Clark and Menary, 2008). Among these, abiotic stressors such as salinity and drought have the most profound impact on medicinal plants (Heidari et al., 2008).

Spearmint (*Mentha viridis* L.) is one of the most important aromatic plants, with a growing global demand for its essential oils in recent years. In Egypt, Spearmint has been cultivated for centuries, making it a significant herb in the region. In 2019, over 2,622 feddans were planted with mint, producing approximately 6,000 tons of herbs. That year, around 1,250 tons of mint were exported, generating \$1.25 million in revenue (Ministry of Agriculture report - export sector, 2018). Spearmint is primarily grown for its leaves and essential oil, which are widely used in the food processing, confectionery, and cosmetic industries (Scherer et al., 2013 and Igoumenidis et al., 2016). Additionally, Spearmint is valued for its antioxidant properties and its antibacterial, antifungal, and insect-repelling qualities (Charles, 2013), largely due to the presence of limonene and carvone.

Drought is one of the most challenging environmental conditions that plants face, significantly limiting crop yields and influencing the distribution of plant species within ecological niches. For medicinal plants, drought is the most critical abiotic stressor (Heidari et al., 2008). Studies have shown that Spearmint is particularly sensitive to water stress, requiring consistent and adequate irrigation for optimal biomass production (Okwany et al., 2009).

Given the current global climate change crisis and its adverse effects on agriculture, there is a growing focus on enhancing the quantity and quality of Egyptian cultivars of aromatic and medicinal plants. In this context, improving Spearmint productivity is crucial, and further research is needed to boost this plant's potential for export markets.

Copper chlorophyllin (Cu-Chl) is a semi-synthetic derivative formed through the saponification of natural chlorophyll in alkaline conditions, such as with methanolic sodium hydroxide. This process results in the opening of the isocyclic ring and the removal of the phytol group. When the magnesium atom in chlorophyll is replaced with a copper atom in an acid solution, Cu-Chl exhibits several technological advantages over natural chlorophyll. These include greater hydrophilicity, enhanced tinctorial power, and increased stability against acid and light (Tumolo and Lanfer-Marquez, 2012).

In 2019, El-Tayeb developed an innovative technology utilizing a copper chlorophyllin formula designed to significantly enhance plant growth and improve overall plant health. This breakthrough plays a crucial role in helping plants resist various stress factors, including insect attacks and environmental changes such as drought and extreme temperatures. By strengthening the plant's natural defenses, this technology not only promotes healthier and more robust plants but also addresses critical challenges in agriculture, particularly in regions facing harsh environmental conditions. El-Tayeb's invention represents a major advancement in agricultural practices, offering a sustainable solution to improve crop resilience and productivity,

which is increasingly vital in the face of global climate change and the growing demand for food security (El-Tayeb, 2019).

The other studies following the revolving this novel technology have shown that Cu-Chl enhances antioxidant defense, osmotic regulation, photosynthesis, and root development in in tomatoes plants experiencing drought stress (Zhang et al., 2019). Additionally, the application of Cu-Chl has been found to significantly improve plant growth and yield in drought conditions (Merghany et al., 2019).

Due to the eco-geographic isolation of the Tour Sinai region, implementing "Good Agricultural Practices" (GAP), including the use of diverse irrigation techniques and natural growth stimulants, is essential for sustaining agricultural production in the area. Mints are perennial plants, and their herbs are harvested multiple times a year, making proper nutrition critical for achieving optimal yields. However, a review of existing literature on *Mentha viridis* in the Tour Sinai region revealed a gap in research, with no studies found on its response to different irrigation intervals or the application of copper chlorophyllin.

Therefore, this study aimed to determine the optimal irrigation intervals and Cu-chlorophyllin concentrations to enhance herb yield, essential oil yield, and composition under the unique conditions of the Tour Sinai region. Addressing these factors is crucial for improving agricultural productivity and ensuring sustainable cultivation practices in this isolated area.

**MATERIALS AND METHODS**

This study was conducted over two consecutive seasons, 2022 and 2023, at a private farm located in the Tour Sinai region (28°17'56" N, 33°37'45" E) in South Sinai Governorate. The soil and irrigation water analyses for the experimental site are detailed in Tables 1 and 2 as recorded by Hashem et al. (2022). Before planting in each season, 20 m<sup>3</sup> feddan<sup>-1</sup> of organic compost manure was incorporated during soil preparation. The analysis of the organic manure is provided in Table (3). Additionally, meteorological data for the El-Tour area, averaged over the past 10 years, are presented in Table (4).

**Table (1).** Physical and chemical properties of the experimental soil site.

Soil depth (cm)	Soluble anions (me L <sup>-1</sup> )			pH of soil paste	EC (ds m <sup>-1</sup> )	Soluble cations (me L <sup>-1</sup> )			
	HCO <sub>3</sub> <sup>-</sup>	SO <sub>4</sub> <sup>2-</sup>	Cl <sup>-</sup>			Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>
0-30	2.10	17.65	23.64	7.24	4.36	8.23	12.56	20.20	2.40
<b>Clay (%)</b>			<b>Slit (%)</b>		<b>Sand (%)</b>		<b>Texture</b>		
11.35			24.15		64.50		Sandy loam		

pH: Acidity, E.C.: Electrical conductivity, me L<sup>-1</sup>: milli equivalent per liter

**Table (2).** Chemical analysis of the irrigation water.

pH	EC (dSm <sup>-1</sup> )	Soluble anions (me L <sup>-1</sup> )			Soluble cations (me L <sup>-1</sup> )			
		HCO <sub>3</sub> <sup>-</sup>	SO <sub>4</sub> <sup>2-</sup>	Cl <sup>-</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>
7.88	0.77	1.44	1.89	4.37	2.60	3.86	0.78	0.46

pH: Acidity, E.C.: Electrical conductivity, dSm<sup>-1</sup>: decseime per meter

**Table (3).** Analysis of organic manure used.

Moisture (%)	Organic matter (%)	Total C%	C/N ratio	Total N%	Total P%	Total K%
11.00	21.36	13.86	10.10	1.38	0.86	1.40

**Table (4).** Meteorological data of El-Tour area as average of 10 years ago.

Month	Prc. (mm m <sup>-1</sup> )	Temp. mean (C°)	Tmp. Max. (C°)	Tmp. min. (C°)	Rel. humidity %	Sunshine %	Wind m <sup>2</sup> (m s <sup>-1</sup> )	ETo (mm m <sup>-1</sup> )	ETo (mm d <sup>-1</sup> )
Jan.	10	14.8	18.7	10.8	42.0	79.6	3.6	95	3.1
Feb.	7	16.5	21.1	11.9	38.8	81.5	4.1	111	4.0
March	45	19.5	24.5	14.6	37.2	78.6	4.6	164	5.3
April	0	23.3	28.4	18.2	32.1	78.9	5.3	219	7.3
May	0	28.0	33.5	22.5	27.1	83.0	5.4	273	8.8
June	0	30.4	35.9	25.0	26.8	90.7	6.2	311	10.4
July	0	32.1	37.1	27.0	30.5	91.7	6.5	326	10.5
Aug.	0	32.2	37.3	27.2	31.0	91.3	5.6	299	9.6
Sept.	0	31.5	37.0	26.1	37.9	89.0	5.1	240	8.0
Oct.	0	28.1	33.6	22.6	39.0	88.3	4.0	185	6.0
Nov.	0	21.5	25.7	17.4	41.1	84.8	3.9	122	4.1
Dec.	0	18.9	23.6	14.2	93.2	78.3	3.1	99	3.2
<b>Total</b>	<b>62</b>							<b>2444</b>	

ET0 = reference evapotranspiration

Spearmint rhizomes, each 20 cm in length, were planted in the field on March 1<sup>st</sup> in both seasons, using a drip irrigation system with a flow rate of 4 L h<sup>-1</sup>. The rows were spaced 75 cm apart, with 30 cm between plants within each row. The experiment was arranged in a split-plot design with three replicates. The main plots were assigned to three different irrigation intervals (2, 4, and 6 days), while the sub-plots included varying concentrations of Cu-chlorophyllin (Cu-Chl) at 0, 0.5, and 1 g L<sup>-1</sup>. The Cu-Chl was provided by Prof. Tarek A. El-Tayeb, the inventor of chlorophyllin use as a foliar fertilizer and plant growth enhancer.

To ensure seedling survival, irrigation was maintained every two days at the beginning of the planting process. The treatments for irrigation intervals of 2, 4, and 6 days began 15 days after planting. The irrigation water was delivered through a trickle irrigation system with a 95% application efficiency. The number of irrigation events and the water quantities used are detailed in Table (5).

**Table (5).** Number of irrigations and water quantities ( $\text{m}^3 \text{ feddan}^{-1}$ ) during the two seasons (2022 and 2023).

Character Treatment	Number of irrigations		Water quantities ( $\text{m}^3 \text{ feddan}^{-1}$ )		Total number of irrigation	Total water quantities ( $\text{m}^3 \text{ feddan}^{-1}$ )
	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	1 <sup>st</sup> cut	2 <sup>nd</sup> cut		
<b>First season</b>						
2 days	69	61	4416	3904	130	8320
4 days	46	33	2944	2112	79	5056
6 days	37	25	2368	1600	62	3968
<b>Second season</b>						
2 days	69	61	4416	3904	130	8320
4 days	46	34	2944	2176	80	5120
6 days	34	26	2368	1664	63	4032

Cu-Chl treatments were evenly applied to the plants using a handheld sprayer until runoff occurred. The plants were treated with Cu-Chl at 3, 6, and 9 weeks after sowing, and the treatments were repeated at the same intervals 3, 6, and 9 weeks after the first harvest. Additionally, the recommended chemical fertilizers were applied according to the guidelines provided by Swaefy et al. (2007).

Two harvests were conducted per season, on July 10<sup>th</sup> and November 5<sup>th</sup>. The plants were harvested by cutting the herbs 10 cm above the soil surface to assess the following parameters:

### 1. Yield Parameters

- Herb fresh weight per square meter (g)
- Herb fresh yield per feddan (kg)
- Herb dry weight per square meter (g)
- Herb dry yield per feddan (kg)

### 2. Volatile Oil Parameters

- The volatile oil percentage was determined from air-dried herbs using hydrodistillation for 3 hours with a Clevenger-type apparatus (British Pharmacopoeia, 1963). The volatile oil yield was measured in milliliters per square meter ( $\text{ml m}^{-2}$ ).  
This can be calculated as follows: Oil % \* herb dry weight ( $\text{g m}^{-2}$ ).
- Volatile oil yield per feddan (L): This was calculated using the formula: Volatile oil yield per square meter  $\times$  4000  $\text{m}^2$ .
- Volatile oil composition: Gas Chromatography-Mass Spectrometry (GC-MS) analyses of the extracted volatile oil from the second season were conducted using a GC-MS instrument at the Laboratory of Medicinal and Aromatic Plants, National Research Center, Egypt.

### 3. Chemical Constituents

- Total chlorophyll (SPAD) in leaves: The total chlorophyll content in plant leaves was measured in SPAD units using a Minolta chlorophyll

meter (model SPAD 502). Chlorophyll measurements were taken from the most recently fully expanded leaf, with 10 readings averaged per experimental unit, following the method described by Markwell et al. (1995).

- **Cu Content in dry herb ( $\text{mg kg}^{-1}$ ):** The copper content in the dry herb was measured in milligrams per kilogram ( $\text{mg kg}^{-1}$ ) using an Atomic Absorption Spectrophotometry (AAS) device (Varian Spectra AA 220 FS) according to the method of Ostrowska et al., (1991). The plant material was ground and ashed in a furnace (CZYLOK, FCF5SH) at  $450^\circ\text{C}$  for six hours after being dried at  $60^\circ\text{C}$ . The ash was then dissolved in 5 ml of 6 mol HCl, diluted with distilled water to a consistent volume, and analyzed to determine the copper content.
- **Free proline content (ppm):** Free proline in fresh herb was measured as ppm according to Chinard (1952). Fresh plant sample (0.5 g) in mortar + 10 ml salphosalysilic acid 3% add during grinding. Filter the sample and take 2 ml from filtrate in test tube + 2 ml of acid ninhydrin + 2 ml of glacial acetic acid. Put in boiling water bath for 1h then cooling. Add 4ml toluene then separate the upper layer (proline) and measured at 520nm. This can be calculated as follows:

$$X = (Y - 0.173) / 0.065$$

X =concentration (ppm), Y = absorbance.

#### 4. Water Use Efficiency (WUE), $\text{kg m}^{-3}$

Crop Water Use Efficiency (WUE) was calculated by dividing the crop yield by the total amount of applied irrigation water, according to Talha and Aziz (1979).

#### Statistical analysis

The means of the treatments were compared using the Least Significant Difference (LSD) test at  $P \leq 0.05$ . All data were subjected to analysis of variance, and the statistical calculations were performed using Statistic software version 9 (Analytical Software, 2008).

## RESULTS AND DISCUSSION

### 1. Yield Parameters

The data presented in Table (6) demonstrate that irrigation intervals had significant effects on herb fresh and dry yields per square meter and per feddan in both seasons. Specifically, irrigating plants every 4 days resulted in significantly higher herb fresh and dry yields compared to irrigation every 2 or 6 days in both cuts of the second season. However, in the second cut of the first season, the increase in herb fresh yield between the 2-day and 4-day irrigation intervals was not statistically significant. Similarly, there were no significant differences in herb dry yield between the 2-day and 4-day intervals

compared to the 6-day interval in the first cut of the first season. These findings align with previous studies by Khorasaninejad et al. (2011) on mint, Bahreininejad et al. (2013) on *Thymus* sp., Abdel-Kader et al. (2014) on lemongrass, Farzad et al. (2016) on oregano and Hanafy et al. (2018) on rosemary.

The reduction in herb fresh and dry yields at longer irrigation intervals may be due to changes in canopy structure and reduced photosynthesis, or to decreased turgor pressure, which limits cell enlargement and increases leaf senescence (Shao et al., 2008 and Farooq et al., 2009). Leithy et al. (2006) also suggested that reduced plant growth is associated with a lower photosynthesis rate due to decreased stomatal conductance. Leaf area development is essential for photosynthesis and dry herb production (Jaleel et al., 2009) because a smaller leaf area reduces the ability to capture light, thereby lowering the photosynthesis rate (Khalid, 2006).

Additionally, reduced moisture availability in the rhizosphere and decreased nutrient absorption may also impact plant growth under limited irrigation (Singh et al., 1997). Under water deficit conditions, plants typically produce less biomass and allocate more to root growth to enhance water absorption (Albouchi et al., 2003).

The data in Table (6) also show that the application of Cu-chlorophyllin (Cu-Chl) had a significant positive impact on herb fresh weight per square meter, herb fresh yield per feddan, herb dry weight per square meter, and herb dry yield per feddan. These parameters improved as the concentration of Cu-Chl increased, with the highest values observed when plants were treated with  $1 \text{ g L}^{-1}$  of Cu-Chl compared to  $0.5 \text{ g L}^{-1}$  and the control treatments in both cuts of the two growing seasons. These results are consistent with those reported by Merghany et al. (2019) and Ramadan (2023) support the data from El-Tayeb's patent (2019). The addition of Cu-Chl to plants may enhance root growth, osmotic regulation, photosynthetic function, antioxidant defense capacity, and resistance to microbial attack, all of which contribute to improved plant growth and productivity (Tumolo and Lanfer-Marquez, 2012 and Zhang et al., 2019). Exogenous Cu-Chl treatment also increases the expression of genes associated with stress protection and various classes of ROS detoxifying genes. Moreover, under stress conditions, Cu-Chl reduces the restriction of leaf growth, indicating its potential to improve plant growth and yield (Islam et al., 2021).

The interaction between irrigation intervals and foliar spraying with Cu-Chl treatments had a significant impact on both the fresh and dry yield of Spearmint herb per square meter and per feddan across both cuts in the two seasons (Table 6). The highest yields were achieved with irrigation every 4 days in combination with foliar application of Cu-Chl at  $1 \text{ g L}^{-1}$ . In contrast, the lowest yields were observed with irrigation every 6 days and no Cu-Chl treatment.

**Table (6).** Effect of Irrigation Intervals, Cu-chlorophyllin and their interaction treatments on herb fresh and dry yield of *Mentha viridis* plant during two cuts in the two seasons (2022 and 2023).

Treatment	Character	Herb fresh yield m <sup>-2</sup> (g)		Herb fresh yield feddan <sup>-1</sup> (kg)		Herb dry yield m <sup>-2</sup> (g)		Herb dry yield feddan <sup>-1</sup> (kg)	
		1 <sup>st</sup> cut	2 <sup>nd</sup> cut	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	1 <sup>st</sup> cut	2 <sup>nd</sup> cut
<b>First season</b>									
Every 2 days		906.3 <sup>B</sup>	1108.4 <sup>A</sup>	3625.1 <sup>B</sup>	4433.8 <sup>A</sup>	312.4 <sup>A</sup>	340.7 <sup>A</sup>	1249.6 <sup>A</sup>	1362.8 <sup>A</sup>
Every 4 days		1009.9 <sup>A</sup>	1119.9 <sup>A</sup>	4039.5 <sup>A</sup>	4479.6 <sup>A</sup>	334.4 <sup>A</sup>	327.4 <sup>B</sup>	1337.7 <sup>A</sup>	1309.7 <sup>B</sup>
Every 6 days		728.4 <sup>C</sup>	858.1 <sup>B</sup>	2913.5 <sup>C</sup>	3432.5 <sup>B</sup>	266.5 <sup>B</sup>	286.5 <sup>C</sup>	1066.0 <sup>B</sup>	1146.0 <sup>C</sup>
<b>Second season</b>									
Every 2 days		592.7 <sup>B</sup>	833.5 <sup>B</sup>	2370.9 <sup>B</sup>	3334.0 <sup>B</sup>	144.9 <sup>B</sup>	172.2 <sup>B</sup>	579.6 <sup>B</sup>	688.8 <sup>B</sup>
Every 4 days		646.8 <sup>A</sup>	874.9 <sup>A</sup>	2587.4 <sup>A</sup>	3499.6 <sup>A</sup>	157.3 <sup>A</sup>	184.9 <sup>A</sup>	629.0 <sup>A</sup>	739.5 <sup>A</sup>
Every 6 days		553.6 <sup>C</sup>	733.6 <sup>C</sup>	2214.4 <sup>C</sup>	2934.4 <sup>C</sup>	125.8 <sup>C</sup>	158.3 <sup>C</sup>	503.4 <sup>C</sup>	633.2 <sup>C</sup>
<b>First season</b>									
Control		625.7 <sup>C</sup>	762.7 <sup>C</sup>	2502.8 <sup>C</sup>	3050.8 <sup>C</sup>	224.8 <sup>C</sup>	240.1 <sup>C</sup>	899.3 <sup>C</sup>	960.6 <sup>C</sup>
Cu-chl at 0.5 g L <sup>-1</sup>		947.4 <sup>B</sup>	1084.1 <sup>B</sup>	3789.8 <sup>B</sup>	4336.2 <sup>B</sup>	314.2 <sup>B</sup>	346.1 <sup>B</sup>	1257.0 <sup>B</sup>	1384.4 <sup>B</sup>
Cu-chl at 1 g L <sup>-1</sup>		1071.4 <sup>A</sup>	1239.7 <sup>A</sup>	4285.6 <sup>A</sup>	4958.9 <sup>A</sup>	374.2 <sup>A</sup>	368.38 <sup>A</sup>	1497.0 <sup>A</sup>	1473.5 <sup>A</sup>
<b>Second season</b>									
Control		420.3 <sup>C</sup>	645.4 <sup>C</sup>	1681.4 <sup>C</sup>	2581.5 <sup>C</sup>	103.6 <sup>C</sup>	145.2 <sup>C</sup>	414.2 <sup>C</sup>	580.6 <sup>C</sup>
Cu-chl at 0.5 g L <sup>-1</sup>		637.3 <sup>B</sup>	847.5 <sup>B</sup>	2549.2 <sup>B</sup>	3389.9 <sup>B</sup>	151.4 <sup>B</sup>	177.4 <sup>B</sup>	605.5 <sup>B</sup>	709.6 <sup>B</sup>
Cu-chl at 1 g L <sup>-1</sup>		735.5 <sup>A</sup>	949.1 <sup>A</sup>	2942.1 <sup>A</sup>	3796.6 <sup>A</sup>	173.1 <sup>A</sup>	192.8 <sup>A</sup>	692.3 <sup>A</sup>	771.2 <sup>A</sup>
<b>First season</b>									
Every 2 days	Control	735.4 <sup>f</sup>	969.2 <sup>e</sup>	2941.7 <sup>f</sup>	3876.7 <sup>e</sup>	259.6 <sup>d</sup>	282.8 <sup>e</sup>	1038.4 <sup>d</sup>	1131.1 <sup>e</sup>
	Cu-chl at 0.5 g L <sup>-1</sup>	951.5 <sup>d</sup>	1040.4 <sup>d</sup>	3806.1 <sup>d</sup>	4161.7 <sup>d</sup>	334.7 <sup>bc</sup>	358.5 <sup>b</sup>	1338.9 <sup>bc</sup>	1433.9 <sup>b</sup>
	Cu-chl at 1 g L <sup>-1</sup>	1031.9 <sup>c</sup>	1315.8 <sup>a</sup>	4127.4 <sup>c</sup>	5263.0 <sup>a</sup>	342.9 <sup>bc</sup>	380.8 <sup>a</sup>	1371.4 <sup>bc</sup>	1523.3 <sup>a</sup>
Every 4 days	Control	631.5 <sup>g</sup>	751.5 <sup>g</sup>	2526.0 <sup>g</sup>	3006.0 <sup>g</sup>	254.9 <sup>d</sup>	252.7 <sup>f</sup>	1019.7 <sup>d</sup>	1010.8 <sup>f</sup>
	Cu-chl at 0.5 g L <sup>-1</sup>	1134.4 <sup>b</sup>	1279.2 <sup>b</sup>	4537.6 <sup>b</sup>	5116.9 <sup>b</sup>	315.8 <sup>bc</sup>	352.5 <sup>bc</sup>	1263.2 <sup>bc</sup>	1410.1 <sup>bc</sup>
	Cu-chl at 1 g L <sup>-1</sup>	1263.7 <sup>a</sup>	1329.0 <sup>a</sup>	5054.9 <sup>a</sup>	5316.0 <sup>a</sup>	432.5 <sup>a</sup>	377.0 <sup>a</sup>	1730.0 <sup>a</sup>	1508.1 <sup>a</sup>
Every 6 days	Control	510.2 <sup>h</sup>	567.4 <sup>h</sup>	2040.6 <sup>h</sup>	2269.7 <sup>h</sup>	159.9 <sup>e</sup>	184.9 <sup>g</sup>	639.7 <sup>e</sup>	739.7 <sup>g</sup>
	Cu-chl at 0.5 g L <sup>-1</sup>	756.4 <sup>f</sup>	932.5 <sup>f</sup>	3025.7 <sup>f</sup>	3730.1 <sup>f</sup>	292.2 <sup>cd</sup>	327.3 <sup>d</sup>	1168.7 <sup>cd</sup>	1309.2 <sup>d</sup>
	Cu-chl at 1 g L <sup>-1</sup>	918.6 <sup>e</sup>	1074.4 <sup>c</sup>	3674.3 <sup>e</sup>	4297.8 <sup>c</sup>	347.4 <sup>b</sup>	347.3 <sup>c</sup>	1389.6 <sup>b</sup>	1389.2 <sup>c</sup>
<b>Second season</b>									
Every 2 days	Control	491.7 <sup>e</sup>	724.8 <sup>f</sup>	1967.0 <sup>e</sup>	2899.0 <sup>f</sup>	123.8 <sup>f</sup>	154.0 <sup>e</sup>	495.1 <sup>f</sup>	616.0 <sup>e</sup>
	Cu-chl at 0.5 g L <sup>-1</sup>	596.8 <sup>d</sup>	843.5 <sup>d</sup>	2387.3 <sup>d</sup>	3374.1 <sup>d</sup>	145.2 <sup>d</sup>	169.9 <sup>d</sup>	580.7 <sup>d</sup>	679.4 <sup>d</sup>
	Cu-chl at 1 g L <sup>-1</sup>	689.6 <sup>bc</sup>	932.2 <sup>b</sup>	2758.3 <sup>bc</sup>	3728.9 <sup>b</sup>	165.7 <sup>b</sup>	192.7 <sup>b</sup>	662.9 <sup>b</sup>	771.0 <sup>b</sup>
Every 4 days	Control	390.9 <sup>f</sup>	675.9 <sup>g</sup>	1563.8 <sup>f</sup>	2703.7 <sup>g</sup>	100.4 <sup>g</sup>	147.1 <sup>f</sup>	401.7 <sup>g</sup>	588.4 <sup>f</sup>
	Cu-chl at 0.5 g L <sup>-1</sup>	706.7 <sup>b</sup>	896.7 <sup>c</sup>	2826.7 <sup>b</sup>	3586.7 <sup>c</sup>	170.6 <sup>b</sup>	197.1 <sup>b</sup>	682.5 <sup>b</sup>	788.4 <sup>b</sup>
	Cu-chl at 1 g L <sup>-1</sup>	842.9 <sup>a</sup>	1052.1 <sup>a</sup>	3371.6 <sup>a</sup>	4208.3 <sup>a</sup>	200.7 <sup>a</sup>	210.4 <sup>a</sup>	802.8 <sup>a</sup>	841.7 <sup>a</sup>
Every 6 days	Control	378.3 <sup>f</sup>	535.5 <sup>h</sup>	1513.3 <sup>f</sup>	2141.8 <sup>h</sup>	86.5 <sup>h</sup>	134.4 <sup>g</sup>	345.9 <sup>h</sup>	537.5 <sup>g</sup>
	Cu-chl at 0.5 g L <sup>-1</sup>	608.4 <sup>d</sup>	802.3 <sup>e</sup>	2433.6 <sup>d</sup>	3209.0 <sup>e</sup>	138.3 <sup>e</sup>	165.3 <sup>d</sup>	553.2 <sup>e</sup>	661.0 <sup>d</sup>
	Cu-chl at 1 g L <sup>-1</sup>	674.1 <sup>c</sup>	863.1 <sup>d</sup>	2696.2 <sup>c</sup>	3452.5 <sup>d</sup>	152.8 <sup>c</sup>	175.2 <sup>c</sup>	611.1 <sup>c</sup>	701.0 <sup>c</sup>

Means having the same letter (s) within the same column are not significantly different according to LSD for all pairwise comparisons test at 5% level of probability.

## 2. Volatile Oil Production

The data in Table (7) show that irrigation intervals have a significant impact on volatile oil percentage, yield per square meter, and yield per feddan. The highest percentage of volatile oil was observed in plants irrigated every 6



days, compared to those irrigated every 2 or 4 days. However, in the first cut of the first season, there was no significant difference in volatile oil percentage between irrigation every 2 days and every 4 days. Similarly, in the second cut of the second season, no significant difference was found between irrigation intervals of 4 and 6 days. The highest volatile oil yield per square meter and per feddan was achieved with irrigation every 4 days, outperforming the yields from 2-day and 6-day irrigation intervals across both cuts in both seasons. Additionally, no significant difference in yield was noted between the 2-day and 4-day irrigation treatments during the first cut of the first season.

These findings align with previous research by Khorasaninejad et al. (2011) and Hanafy et al. (2018) on mint, Farzad et al. (2016) on oregano and Baher et al. (2002) on *Satureja hortensis*, all of which found that essential oil percentage increases under water deficit conditions.

The effect of irrigation intervals on essential oil percentage can be attributed to their influence on enzyme activity and the metabolism involved in essential oil production (Simon et al., 1992 and Khalid, 2006). Water stress may lead to an increase in essential oil content in many aromatic and medicinal plants due to the formation of more metabolites and the prevention of compound oxidation in stressed cells (Farahani et al., 2009). Additionally, Penka (1978) suggested that water deficit intensifies respiratory catabolic mechanisms that produce essential oils.

The data in Table (7) also indicate that foliar spraying with Cu-Chl treatments significantly affects volatile oil percentage and yield. The volatile oil yield increased progressively with higher Cu-Chl concentrations, with the highest values recorded in plants sprayed with Cu-Chl at  $1 \text{ g L}^{-1}$ , compared to other concentrations and the control treatment. This trend was consistent across both cuts in both growing seasons.

The substantial rise in volatile oil yield with Cu-Chl treatment is likely attributed to improvements in the plant's morphology and biochemical processes. These enhancements lead to more efficient oil production and increased stress tolerance, providing practical advantages for Spearmint cultivation and potentially for other essential oil crops. Manivasagaperumal et al. (2011) and Hasbullah and Taha (2023) also reported significant increases in essential oil yield in *Vigna radiata* and *Ocimum basilicum*, respectively, when treated with copper. This indicates that copper may be crucial in boosting essential oil production.

Moreover, the interaction between irrigation intervals and Cu-Chl concentrations showed significant effects on volatile oil percentage and yield per square meter and feddan. The highest volatile oil yield was observed in the treatment combining irrigation every 4 days with spraying Cu-Chl at  $1 \text{ g L}^{-1}$ , compared to all other interaction treatments. The lowest yields were recorded in the treatment with no Cu-Chl spraying and irrigation every 6 days in both seasons.

**Table (7).** Effect of Irrigation Intervals, Cu-chlorophyllin and their interaction treatments on volatile oil yield of *Mentha viridis* plant during two cuts in the two seasons (2022 and 2023).

Treatment	Character	Volatile oil (%)		Volatile oil yield m <sup>-3</sup> (ml)		Volatile oil yield feddan <sup>-1</sup> (L)	
		1 <sup>st</sup> cut	2 <sup>nd</sup> cut	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	1 <sup>st</sup> cut	2 <sup>nd</sup> cut
<b>First season</b>							
Every 2 days		3.48 <sup>B</sup>	3.33 <sup>C</sup>	11.05 <sup>A</sup>	11.28 <sup>B</sup>	44.18 <sup>A</sup>	45.11 <sup>B</sup>
Every 4 days		3.51 <sup>B</sup>	3.52 <sup>B</sup>	12.05 <sup>A</sup>	12.04 <sup>A</sup>	48.19 <sup>A</sup>	48.15 <sup>A</sup>
Every 6 days		3.66 <sup>A</sup>	3.72 <sup>A</sup>	9.91 <sup>B</sup>	10.96 <sup>B</sup>	39.64 <sup>B</sup>	43.86 <sup>B</sup>
<b>Second season</b>							
Every 2 days		3.16 <sup>C</sup>	3.17 <sup>C</sup>	4.66 <sup>B</sup>	5.53 <sup>B</sup>	18.65 <sup>B</sup>	22.13 <sup>B</sup>
Every 4 days		3.34 <sup>B</sup>	3.40 <sup>A</sup>	5.42 <sup>A</sup>	6.41 <sup>A</sup>	21.69 <sup>A</sup>	25.63 <sup>A</sup>
Every 6 days		3.48 <sup>A</sup>	3.46 <sup>A</sup>	4.50 <sup>C</sup>	5.53 <sup>B</sup>	17.99 <sup>C</sup>	22.11 <sup>B</sup>
<b>First season</b>							
Control		3.10 <sup>C</sup>	2.92 <sup>C</sup>	6.87 <sup>C</sup>	6.97 <sup>C</sup>	27.46 <sup>C</sup>	27.88 <sup>C</sup>
Cu-chl at 0.5 g L <sup>-1</sup>		3.62 <sup>B</sup>	3.62 <sup>B</sup>	11.39 <sup>B</sup>	12.51 <sup>B</sup>	45.56 <sup>B</sup>	50.05 <sup>B</sup>
Cu-chl at 1 g L <sup>-1</sup>		3.94 <sup>A</sup>	4.02 <sup>A</sup>	14.75 <sup>A</sup>	14.79 <sup>A</sup>	58.99 <sup>A</sup>	59.19 <sup>A</sup>
<b>Second season</b>							
Control		2.83 <sup>C</sup>	2.84 <sup>C</sup>	2.91 <sup>C</sup>	4.10 <sup>C</sup>	11.68 <sup>C</sup>	16.42 <sup>C</sup>
Cu-chl at 0.5 g L <sup>-1</sup>		3.36 <sup>B</sup>	3.46 <sup>B</sup>	5.09 <sup>B</sup>	6.14 <sup>B</sup>	20.35 <sup>B</sup>	24.57 <sup>B</sup>
Cu-chl at 1 g L <sup>-1</sup>		3.80 <sup>A</sup>	3.74 <sup>A</sup>	6.58 <sup>A</sup>	7.22 <sup>A</sup>	26.31 <sup>A</sup>	28.89 <sup>A</sup>
<b>First season</b>							
Every 2 days	Control	2.83 <sup>e</sup>	2.68 <sup>g</sup>	7.36 <sup>d</sup>	6.78 <sup>d</sup>	29.43 <sup>d</sup>	27.12 <sup>d</sup>
	Cu-chl at 0.5 g L <sup>-1</sup>	3.68 <sup>b</sup>	3.44 <sup>d</sup>	12.32 <sup>bc</sup>	12.32 <sup>b</sup>	49.28 <sup>bc</sup>	49.28 <sup>b</sup>
	Cu-chl at 1 g L <sup>-1</sup>	3.93 <sup>a</sup>	3.87 <sup>bc</sup>	13.46 <sup>b</sup>	14.73 <sup>a</sup>	53.84 <sup>b</sup>	58.94 <sup>a</sup>
Every 4 days	Control	3.06 <sup>d</sup>	2.93 <sup>f</sup>	7.80 <sup>d</sup>	8.29 <sup>c</sup>	31.22 <sup>d</sup>	33.18 <sup>c</sup>
	Cu-chl at 0.5 g L <sup>-1</sup>	3.48 <sup>c</sup>	3.60 <sup>d</sup>	11.05 <sup>c</sup>	12.68 <sup>b</sup>	44.19 <sup>c</sup>	50.70 <sup>b</sup>
	Cu-chl at 1 g L <sup>-1</sup>	4.00 <sup>a</sup>	4.02 <sup>ab</sup>	17.29 <sup>a</sup>	15.14 <sup>a</sup>	69.17 <sup>a</sup>	60.57 <sup>a</sup>
Every 6 days	Control	3.40 <sup>c</sup>	3.16 <sup>e</sup>	5.44 <sup>e</sup>	5.84 <sup>e</sup>	21.75 <sup>e</sup>	23.35 <sup>e</sup>
	Cu-chl at 0.5 g L <sup>-1</sup>	3.70 <sup>b</sup>	3.83 <sup>c</sup>	10.80 <sup>c</sup>	12.54 <sup>b</sup>	43.21 <sup>c</sup>	50.16 <sup>b</sup>
	Cu-chl at 1 g L <sup>-1</sup>	3.88 <sup>a</sup>	4.18 <sup>a</sup>	13.49 <sup>b</sup>	14.51 <sup>a</sup>	53.96 <sup>b</sup>	58.06 <sup>a</sup>
<b>Second season</b>							
Every 2 days	Control	2.67 <sup>f</sup>	2.55 <sup>f</sup>	3.31 <sup>d</sup>	3.93 <sup>e</sup>	13.25 <sup>d</sup>	15.71 <sup>e</sup>
	Cu-chl at 0.5 g L <sup>-1</sup>	3.16 <sup>d</sup>	3.35 <sup>c</sup>	4.59 <sup>c</sup>	5.70 <sup>d</sup>	18.34 <sup>c</sup>	22.79 <sup>d</sup>
	Cu-chl at 1 g L <sup>-1</sup>	3.67 <sup>b</sup>	3.62 <sup>b</sup>	6.09 <sup>b</sup>	6.97 <sup>b</sup>	24.37 <sup>b</sup>	27.90 <sup>b</sup>
Every 4 days	Control	2.84 <sup>ef</sup>	2.85 <sup>e</sup>	2.85 <sup>e</sup>	4.19 <sup>e</sup>	11.42 <sup>e</sup>	16.78 <sup>e</sup>
	Cu-chl at 0.5 g L <sup>-1</sup>	3.45 <sup>c</sup>	3.52 <sup>b</sup>	5.89 <sup>b</sup>	6.94 <sup>b</sup>	23.55 <sup>b</sup>	27.74 <sup>b</sup>
	Cu-chl at 1 g L <sup>-1</sup>	3.74 <sup>b</sup>	3.84 <sup>a</sup>	7.52 <sup>a</sup>	8.09 <sup>a</sup>	30.10 <sup>a</sup>	32.38 <sup>a</sup>
Every 6 days	Control	3.99 <sup>df</sup>	3.12 <sup>d</sup>	2.59 <sup>e</sup>	4.19 <sup>e</sup>	10.35 <sup>e</sup>	16.76 <sup>e</sup>
	Cu-chl at 0.5 g L <sup>-1</sup>	3.46 <sup>c</sup>	3.50 <sup>b</sup>	4.79 <sup>c</sup>	5.79 <sup>d</sup>	19.16 <sup>c</sup>	23.17 <sup>d</sup>
	Cu-chl at 1 g L <sup>-1</sup>	3.99 <sup>a</sup>	3.77 <sup>a</sup>	6.11 <sup>b</sup>	6.60 <sup>c</sup>	24.46 <sup>b</sup>	26.40 <sup>c</sup>

Means having the same letter (s) within the same column are not significantly different according to LSD for all pairwise comparisons test at 5% level of probability.

### 3. Volatile Oil Composition

Twenty-four compounds, representing more than 98% of the total volatile content in most Spearmint samples, were detected and identified (Table 8). The composition of the volatile oil varies depending on the irrigation intervals and/or Cu-Chl applications. The main compounds found across all treatments were Carvone (33.3-36.42%), 1,8-Cineole (17.66-19.12%), Caryophyllene (5.08-6.10%),  $\alpha$ -Pinene (5.25-6.10%), D-Limonene (2.13-3.39%), Sabinene (3.16-5.45%),  $\alpha$ -Bourbonene (3.04-3.84%), and 2-Cyclohexen-1-ol (3.05-3.88%). The content of other oil constituents varied without a consistent pattern.

**Table (8).** Effect of irrigation intervals, Cu-chlorophyllin and their interaction treatments on volatile oil components of *Mentha viridis* plant.

Compound (%)	RT	Treatments			
		T1	T2	T3	T4
1 $\alpha$ -Pinene	5.99	5.47	6.10	5.25	5.81
2 Sabinene	6.71	4.96	5.45	4.18	3.16
3 $\alpha$ -Myrcene	7.05	2.55	2.81	2.41	2.61
4 $\alpha$ -Terpinene	7.65	0.61	0.66	0.76	0.85
5 1,8-Cineole	7.92	19.12	17.66	17.70	18.45
6 D-Limonene	8.02	2.74	3.18	2.13	3.39
7 Terpinene	8.56	0.97	0.97	1.24	1.39
8 2-Cyclohexen-1-ol	9.92	3.05	3.88	3.46	3.39
9 Cyclohexanone	10.51	1.18	-	0.47	-
10 Endo-borneol	10.90	1.29	1.30	1.13	1.20
11 Dihydrocarvone	11.40	1.11	1.44	1.15	1.08
12 Trans-carveol	12.21	0.67	1.34	0.46	0.82
13 Carvone	12.51	36.42	33.31	36.18	34.79
14 Ethanone	14.03	0.42	0.47	0.45	0.52
15 2,4-Cycloheptadien-1-one	14.60	0.50	-	-	0.47
16 Cis-jasmone	15.87	0.41	0.48	-	0.42
17 $\alpha$ -Bourbonene	16.22	3.04	3.68	3.84	3.71
18 Elemene	16.31	1.74	2.63	2.75	3.08
19 Caryophyllene	16.95	5.08	5.98	6.10	5.57
20 Germacrene-D	17.13	4.78	5.93	6.19	4.99
21 1S, cis-calamenene	19.25	-	-	0.51	0.47
22 Spathulenol	19.94	1.02	0.76	1.14	1.09
23 Caryophyllene oxide	20.05	1.16	1.23	1.47	1.31
24 $\alpha$ -Cadinol	21.39	0.48	0.53	0.56	0.61
Total		98.77	99.79	99.53	99.18

\*T<sub>1</sub>= irrigation intervals every 2 days + Cu-chl at 2 g L<sup>-1</sup>, T<sub>2</sub>= irrigation intervals every 2 days + Cu-chl at 0 g L<sup>-1</sup>, T<sub>3</sub>= irrigation intervals every 4 days + Cu-chl at 2 g L<sup>-1</sup>, T<sub>4</sub> = irrigation intervals every 6 days + Cu-chl at 0 g L<sup>-1</sup>.

The interaction between irrigation intervals and Cu-Chl applications had varying effects on the concentrations of Carvone and 1,8-Cineole, the major compounds in Spearmint oil in this study. The highest concentrations of Carvone (36.42%) and 1,8-Cineole (19.12%) were achieved with irrigation every 2 days combined with Cu-Chl at 1 g L<sup>-1</sup>, followed by irrigation every 4 days with Cu-Chl at 1 g L<sup>-1</sup>. The lowest concentrations were recorded with irrigation every 2 days combined with no Cu-Chl application, resulting in Carvone at 33.31% and 1,8-Cineole at 17.66%.

#### 4. Total Chlorophyll and Copper Contents

In most cases across both seasons, irrigation intervals had an insignificant effect on total chlorophyll content. However, Table (9) shows that irrigation every 2 days significantly decreased total chlorophyll content in the first cut of the first season and the second cut of the second season compared to other irrigation treatments. Extending the irrigation interval to 6 days resulted in a slight, though not significant, increase in total chlorophyll content in both cuts of both seasons compared to other treatments. Generally, longer irrigation intervals tend to increase total chlorophyll content. These findings are consistent with those of El-Leithy et al. (2018) on rosemary and Shokhmgar et al. (2014) on fenugreek (*Trigonella foenum-gracum*), which reported that reduced water usage led to a decrease in chlorophyll content. Yazdiani and Taheri (2014) also found that drought stress had a significant positive effect on total chlorophyll content in marigold (*Calendula officinalis*).

The decrease in chlorophyll content may be attributed to increased ethylene synthesis under drought stress. This stress boosts ethylene production, leading to lipid breakdown and a subsequent loss of cell membrane integrity. When ethylene directly contacts the chloroplast due to lipid breakdown, it activates the chlorophyllase (chlase) gene, which significantly damages chlorophyll (Matile et al., 1997 and Karimpour, 2019). Significant differences in total chlorophyll content were observed in Spearmint plants treated with various concentrations of Cu-Chl (Table 9). Total chlorophyll content increased as Cu-Chl concentrations increased, reaching a maximum at the highest concentration (1 g L<sup>-1</sup>), with increases of 23.53% and 22.67%, and 20.42% and 17.89% in the first and second cuts of both seasons, respectively, compared to the control treatment. These results align with those reported by El-Tayeb (2019), Zhang et al. (2019) and Ramadan (2023), who found that foliar application of Cu-Chl under drought stress conditions increased chlorophyll a, chlorophyll b, and carotenoid concentrations in spinach and tomato plants. Merghany et al. (2019) also observed increased chlorophyll content in onions with Cu-Chl application. The improvement in photosynthetic pigments from Cu-Chl application may be due to synthetic pigments' ability to protect chlorophyll from UV-B

radiation (Schmidt and Zhang, 2001) and safeguard plants from ROS-induced damage to the photosynthetic apparatus (Tumolo and Lanfer-Marquez, 2012).

**Table (9).** Effect of irrigation intervals, Cu-chlorophyllin and their interaction treatments on total chlorophyll and Cu contents of *Mentha viridis* plant during two cuts in the two seasons (2022 and 2023).

Treatment \ Character		Total chlorophyll content (SPAD)		Cu (mg kg <sup>-1</sup> )	
		1 <sup>st</sup> cut	2 <sup>nd</sup> cut	1 <sup>st</sup> cut	2 <sup>nd</sup> cut
<b>First season</b>					
Every 2 days		35.24 <sup>B</sup>	40.12 <sup>A</sup>	49.36 <sup>A</sup>	50.12 <sup>A</sup>
Every 4 days		39.00 <sup>A</sup>	39.81 <sup>A</sup>	36.06 <sup>B</sup>	40.14 <sup>B</sup>
Every 6 days		39.75 <sup>A</sup>	40.16 <sup>A</sup>	29.56 <sup>C</sup>	30.70 <sup>C</sup>
<b>Second season</b>					
Every 2 days		35.22 <sup>A</sup>	38.81 <sup>A</sup>	50.89 <sup>A</sup>	50.51 <sup>A</sup>
Every 4 days		35.14 <sup>A</sup>	37.35 <sup>B</sup>	44.22 <sup>B</sup>	42.18 <sup>B</sup>
Every 6 days		35.47 <sup>A</sup>	37.04 <sup>B</sup>	31.84 <sup>C</sup>	31.27 <sup>C</sup>
<b>First season</b>					
Control		32.10 <sup>C</sup>	33.94 <sup>C</sup>	22.49 <sup>C</sup>	24.85 <sup>C</sup>
Cu-chl at 0.5 g L <sup>-1</sup>		39.92 <sup>B</sup>	42.25 <sup>B</sup>	37.73 <sup>B</sup>	39.66 <sup>B</sup>
Cu-chl at 1 g L <sup>-1</sup>		41.98 <sup>A</sup>	43.89 <sup>A</sup>	54.78 <sup>A</sup>	56.45 <sup>A</sup>
<b>Second season</b>					
Control		31.18 <sup>C</sup>	33.68 <sup>C</sup>	27.21 <sup>C</sup>	26.03 <sup>C</sup>
Cu-chl at 0.5 g L <sup>-1</sup>		35.48 <sup>B</sup>	38.51 <sup>B</sup>	41.60 <sup>B</sup>	40.63 <sup>B</sup>
Cu-chl at 1 g L <sup>-1</sup>		39.18 <sup>A</sup>	41.02 <sup>A</sup>	58.13 <sup>A</sup>	57.29 <sup>A</sup>
<b>First season</b>					
Every 2 days	Control	30.90 <sup>f</sup>	34.07 <sup>e</sup>	28.60 <sup>e</sup>	31.21 <sup>f</sup>
	Cu-chl at 0.5 g L <sup>-1</sup>	36.27 <sup>d</sup>	42.53 <sup>cd</sup>	49.33 <sup>b</sup>	49.90 <sup>c</sup>
	Cu-chl at 1 g L <sup>-1</sup>	38.55 <sup>c</sup>	43.77 <sup>ab</sup>	70.15 <sup>a</sup>	69.26 <sup>a</sup>
Every 4 days	Control	32.10 <sup>ef</sup>	33.55 <sup>e</sup>	22.01 <sup>f</sup>	25.69 <sup>h</sup>
	Cu-chl at 0.5 g L <sup>-1</sup>	42.32 <sup>b</sup>	42.55 <sup>bcd</sup>	35.65 <sup>d</sup>	40.62 <sup>e</sup>
	Cu-chl at 1 g L <sup>-1</sup>	42.60 <sup>b</sup>	43.32 <sup>abc</sup>	50.53 <sup>b</sup>	54.11 <sup>b</sup>
Every 6 days	Control	33.30 <sup>e</sup>	34.22 <sup>e</sup>	16.85 <sup>g</sup>	17.64 <sup>i</sup>
	Cu-chl at 0.5 g L <sup>-1</sup>	41.17 <sup>b</sup>	41.67 <sup>d</sup>	28.19 <sup>e</sup>	28.47 <sup>g</sup>
	Cu-chl at 1 g L <sup>-1</sup>	44.78 <sup>a</sup>	44.60 <sup>a</sup>	43.65 <sup>c</sup>	45.99 <sup>d</sup>
<b>Second season</b>					
Every 2 days	Control	31.90 <sup>e</sup>	34.73 <sup>d</sup>	33.82 <sup>f</sup>	32.52 <sup>f</sup>
	Cu-chl at 0.5 g L <sup>-1</sup>	35.33 <sup>d</sup>	39.80 <sup>b</sup>	50.48 <sup>c</sup>	50.19 <sup>c</sup>
	Cu-chl at 1 g L <sup>-1</sup>	38.43 <sup>b</sup>	41.90 <sup>a</sup>	68.36 <sup>a</sup>	68.81 <sup>a</sup>
Every 4 days	Control	31.03 <sup>ef</sup>	32.30 <sup>e</sup>	29.37 <sup>g</sup>	27.53 <sup>g</sup>
	Cu-chl at 0.5 g L <sup>-1</sup>	36.27 <sup>c</sup>	38.10 <sup>c</sup>	45.60 <sup>e</sup>	43.11 <sup>e</sup>
	Cu-chl at 1 g L <sup>-1</sup>	38.13 <sup>b</sup>	41.67 <sup>a</sup>	57.69 <sup>b</sup>	55.90 <sup>b</sup>
Every 6 days	Control	30.60 <sup>f</sup>	34.00 <sup>d</sup>	18.44 <sup>h</sup>	18.04 <sup>h</sup>
	Cu-chl at 0.5 g L <sup>-1</sup>	34.83 <sup>d</sup>	37.63 <sup>c</sup>	28.74 <sup>g</sup>	28.60 <sup>g</sup>
	Cu-chl at 1 g L <sup>-1</sup>	40.97 <sup>a</sup>	39.50 <sup>b</sup>	48.33 <sup>d</sup>	47.16 <sup>d</sup>

Means having the same letter (s) within the same column are not significantly different according to LSD for all pairwise comparisons test at 5% level of probability.

The results showed that the highest total chlorophyll content was found in plants treated with 1 g L<sup>-1</sup> Cu-Chl under irrigation every 6 or 4 days, while the lowest values were observed in plants without Cu-Chl application and irrigated every 2 or 4 days in most cases across both seasons.

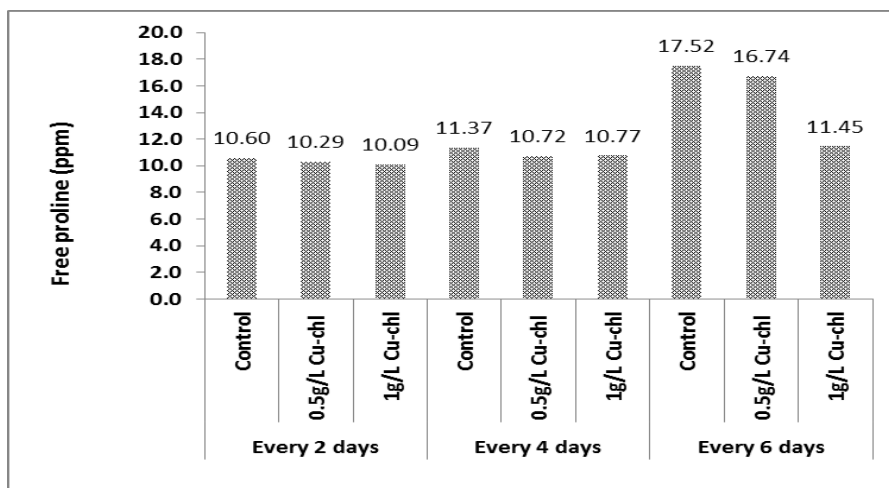
The data in Table (9) also suggests that irrigation intervals significantly influence Cu content in Spearmint plants. The highest Cu content was recorded with irrigation every 2 days across both cuts of both seasons. In contrast, irrigation every 6 days resulted in the lowest Cu content in Spearmint herb in both cuts of both seasons. These findings are consistent with those reported by Ramadan (2023) on spinach plants. Tadayyon et al. (2018) and Aqaei et al. (2020) found that water deficit conditions led to a decrease in plant Cu content, which aligns with the results of this study. A water deficit may reduce nutrient uptake by lowering enzyme activity involved in nutrient assimilation and reducing transpiration, which limits nutrient transport and uptake (Robredo et al., 2011 and Liang et al., 2018).

Table (9) also shows that Cu-Chl treatments significantly influenced Cu content in Spearmint herbs. Cu content increased with Cu-Chl treatment in both seasons, with the highest values recorded at 1 g L<sup>-1</sup> Cu-Chl, compared to untreated plants in both cuts of both seasons. These findings are consistent with those reported by Ramadan (2023) on spinach plants and Hasbullah and Taha (2023) on sweet basil plants.

The interaction between irrigation intervals and Cu-Chl foliar application significantly affected Cu content. Irrigation every 2 days with 1 g L<sup>-1</sup> Cu-Chl resulted in the highest Cu content (70.15, 69.26, 68.36, and 68.81 mg kg<sup>-1</sup>) in Spearmint herb in the first and second cuts of both seasons, respectively. In contrast, deficit irrigation (every 6 days) without Cu-Chl application resulted in the lowest Cu content (16.85, 17.64, 18.44, and 18.04 mg kg<sup>-1</sup>) in dry Spearmint herb in the first and second cuts of both seasons. The differences among treatments were significant in both seasons, consistent with the findings of Ramadan (2023) on spinach plants.

## 5. Free Proline Content

Fig. (1) shows that extending irrigation intervals to every 6 days led to an increase in free proline content in fresh herbs compared to irrigation every 2 days. Plants irrigated every 6 days accumulated significantly more proline, with increases of 30.8% and 29.5% over plants irrigated every 2 or 4 days, respectively. This suggests that plants subjected to drought stress tend to have higher levels of free proline than those grown under less stressful conditions (Bayer, 2007). Similarly, Simon-Sarkadi et al. (2006) found that drought stress elevated proline levels in coriander (*Coriandrum sativum* L.). Osmotic pressure regulators include amino acids, sugars, mineral ions, hormones, and proteins (Chun et al., 2018). As a soluble compound, proline plays a role in regulating osmotic pressure, reducing water loss from cells, and preserving cell integrity (Hayat et al., 2021).



**Fig. (1).** Effect of irrigation intervals, Cu-chlorophyllin and their interaction treatments on free proline contents of *Mentha viridis* plant during two cuts in the two seasons (2022 and 2023).

Additionally, free proline content decreased gradually with increasing Cu-Chl concentrations from 0.5 g L<sup>-1</sup> to 1 g L<sup>-1</sup>. The highest proline content (17.52 ppm) was observed in the treatment combining irrigation every 6 days with the control treatment (without Cu-Chl), compared to other treatments.

The application of Cu-Chl at a concentration of 1 g L<sup>-1</sup> appears to have a significant impact on the overall health of the plant, particularly in relation to its response to drought stress. The reduction in free proline content observed with increasing Cu-Chl concentration suggests that the plants are experiencing less drought stress when exposed to higher levels of Cu-Chl. Proline accumulation is a common response in plants under drought conditions, acting as an osmoprotectant to help maintain cell turgor and protect cellular functions. However, the decreased proline levels at 1 g L<sup>-1</sup> Cu-Chl indicate that the plants may be under less osmotic stress, possibly due to improved water retention, better root function, or enhanced stress tolerance provided by the Cu-Chl. This implies that at this concentration, Cu-Chl might be mitigating some of the negative effects of drought, thereby improving overall plant health and reducing the plant's need to produce and accumulate proline as a stress response.

**6. Water Use Efficiency (WUE)**

As shown in Table (10), irrigation intervals of every 6 days resulted in the highest water use efficiency (WUE) for fresh yield, reaching 2.145 kg m<sup>-3</sup> fresh yield m<sup>-3</sup> water in the second cut of the first season. In contrast, the lowest values of herb fresh yield, 0.821 and 1.136 kg m<sup>-3</sup>, were recorded when plants were irrigated every 2 days in both cuts of the first season. A similar trend was observed in the second season, where the maximum WUE values

were 0.935 and 1.763 kg m<sup>-3</sup> water, while the minimum values were 0.537 and 0.854 kg m<sup>-3</sup> for the same respective cuts.

**Table (10).** Effect of Irrigation Intervals, Cu-chlorophyllin and their interaction treatments on water use efficiency (WUE) of *Mentha viridis* plant during two cuts in the two seasons (2022 and 2023).

Character		Herb fresh yield WUE/FW (kg m <sup>-3</sup> )		Herb dry yield WUE (kg m <sup>-3</sup> )		Volatile oil yield WUE (L m <sup>-3</sup> )	
		1 <sup>st</sup> cut	2 <sup>nd</sup> cut	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	1 <sup>st</sup> cut	2 <sup>nd</sup> cut
<b>Treatment</b>							
<b>First season</b>							
Every 2 days		0.821 <sup>C</sup>	1.136 <sup>B</sup>	0.283 <sup>B</sup>	0.349 <sup>C</sup>	0.010 <sup>B</sup>	0.012 <sup>C</sup>
Every 4 days		1.372 <sup>A</sup>	2.121 <sup>A</sup>	0.454 <sup>A</sup>	0.620 <sup>B</sup>	0.016 <sup>A</sup>	0.023 <sup>B</sup>
Every 6 days		1.230 <sup>B</sup>	2.145 <sup>A</sup>	0.450 <sup>A</sup>	0.716 <sup>A</sup>	0.017 <sup>A</sup>	0.027 <sup>A</sup>
<b>Second season</b>							
Every 2 days		0.537 <sup>C</sup>	0.854 <sup>C</sup>	0.131 <sup>B</sup>	0.176 <sup>C</sup>	0.004 <sup>B</sup>	0.006 <sup>C</sup>
Every 4 days		0.879 <sup>B</sup>	1.608 <sup>B</sup>	0.214 <sup>A</sup>	0.340 <sup>B</sup>	0.007 <sup>A</sup>	0.012 <sup>B</sup>
Every 6 days		0.935 <sup>A</sup>	1.763 <sup>A</sup>	0.213 <sup>A</sup>	0.380 <sup>A</sup>	0.008 <sup>A</sup>	0.013 <sup>A</sup>
<b>First season</b>							
Control		0.796 <sup>C</sup>	1.278 <sup>C</sup>	0.284 <sup>C</sup>	0.410 <sup>C</sup>	0.009 <sup>C</sup>	0.012 <sup>C</sup>
Cu-chl at 0.5 g L <sup>-1</sup>		1.227 <sup>B</sup>	1.940 <sup>B</sup>	0.409 <sup>B</sup>	0.618 <sup>B</sup>	0.015 <sup>B</sup>	0.023 <sup>B</sup>
Cu-chl at 1 g L <sup>-1</sup>		1.401 <sup>A</sup>	2.184 <sup>A</sup>	0.495 <sup>A</sup>	0.658 <sup>A</sup>	0.019 <sup>A</sup>	0.026 <sup>A</sup>
<b>Second season</b>							
Control		0.539 <sup>C</sup>	1.091 <sup>C</sup>	0.132 <sup>C</sup>	0.250 <sup>C</sup>	0.004 <sup>C</sup>	0.007 <sup>C</sup>
Cu-chl at 0.5 g L <sup>-1</sup>		0.843 <sup>B</sup>	1.480 <sup>B</sup>	0.199 <sup>B</sup>	0.311 <sup>B</sup>	0.007 <sup>B</sup>	0.011 <sup>B</sup>
Cu-chl at 1 g L <sup>-1</sup>		0.970 <sup>A</sup>	1.655 <sup>A</sup>	0.227 <sup>A</sup>	0.335 <sup>A</sup>	0.009 <sup>A</sup>	0.013 <sup>A</sup>
<b>First season</b>							
Every 2 days	Control	0.667 <sup>f</sup>	0.993 <sup>h</sup>	0.235 <sup>d</sup>	0.290 <sup>h</sup>	0.007 <sup>f</sup>	0.010 <sup>g</sup>
	Cu-chl at 0.5 g L <sup>-1</sup>	0.863 <sup>e</sup>	1.066 <sup>g</sup>	0.303 <sup>cd</sup>	0.367 <sup>g</sup>	0.011 <sup>d</sup>	0.013 <sup>f</sup>
	Cu-chl at 1 g L <sup>-1</sup>	0.935 <sup>d</sup>	1.348 <sup>f</sup>	0.311 <sup>cd</sup>	0.390 <sup>f</sup>	0.012 <sup>cd</sup>	0.015 <sup>e</sup>
Every 4 days	Control	0.858 <sup>e</sup>	1.423 <sup>e</sup>	0.346 <sup>c</sup>	0.479 <sup>e</sup>	0.011 <sup>de</sup>	0.016 <sup>e</sup>
	Cu-chl at 0.5 g L <sup>-1</sup>	1.541 <sup>b</sup>	2.423 <sup>c</sup>	0.429 <sup>b</sup>	0.668 <sup>d</sup>	0.015 <sup>c</sup>	0.024 <sup>d</sup>
	Cu-chl at 1 g L <sup>-1</sup>	1.717 <sup>a</sup>	2.517 <sup>b</sup>	0.588 <sup>a</sup>	0.714 <sup>c</sup>	0.024 <sup>a</sup>	0.029 <sup>c</sup>
Every 6 days	Control	0.862 <sup>e</sup>	1.419 <sup>e</sup>	0.270 <sup>cd</sup>	0.462 <sup>e</sup>	0.009 <sup>ef</sup>	0.015 <sup>e</sup>
	Cu-chl at 0.5 g L <sup>-1</sup>	1.278 <sup>c</sup>	2.331 <sup>d</sup>	0.494 <sup>b</sup>	0.818 <sup>b</sup>	0.018 <sup>b</sup>	0.031 <sup>b</sup>
	Cu-chl at 1 g L <sup>-1</sup>	1.552 <sup>b</sup>	2.686 <sup>a</sup>	0.587 <sup>a</sup>	0.868 <sup>a</sup>	0.023 <sup>a</sup>	0.036 <sup>a</sup>
<b>Second season</b>							
Every 2 days	Control	0.446 <sup>f</sup>	0.743 <sup>g</sup>	0.112 <sup>f</sup>	0.158 <sup>i</sup>	0.003 <sup>e</sup>	0.004 <sup>h</sup>
	Cu-chl at 0.5 g L <sup>-1</sup>	0.541 <sup>e</sup>	0.864 <sup>f</sup>	0.132 <sup>e</sup>	0.174 <sup>h</sup>	0.004 <sup>d</sup>	0.006 <sup>g</sup>
	Cu-chl at 1 g L <sup>-1</sup>	0.625 <sup>d</sup>	0.955 <sup>e</sup>	0.150 <sup>d</sup>	0.197 <sup>g</sup>	0.005 <sup>c</sup>	0.007 <sup>f</sup>
Every 4 days	Control	0.531 <sup>e</sup>	0.242 <sup>d</sup>	0.136 <sup>e</sup>	0.270 <sup>f</sup>	0.004 <sup>d</sup>	0.008 <sup>f</sup>
	Cu-chl at 0.5 g L <sup>-1</sup>	0.960 <sup>c</sup>	1.648 <sup>c</sup>	0.232 <sup>c</sup>	0.362 <sup>d</sup>	0.008 <sup>b</sup>	0.013 <sup>d</sup>
	Cu-chl at 1 g L <sup>-1</sup>	1.145 <sup>a</sup>	1.934 <sup>b</sup>	0.273 <sup>a</sup>	0.387 <sup>c</sup>	0.010 <sup>a</sup>	0.015 <sup>b</sup>
Every 6 days	Control	0.639 <sup>d</sup>	1.287 <sup>d</sup>	0.146 <sup>d</sup>	0.323 <sup>e</sup>	0.004 <sup>d</sup>	0.010 <sup>e</sup>
	Cu-chl at 0.5 g L <sup>-1</sup>	1.028 <sup>b</sup>	1.929 <sup>b</sup>	0.234 <sup>c</sup>	0.397 <sup>b</sup>	0.008 <sup>b</sup>	0.014 <sup>c</sup>
	Cu-chl at 1 g L <sup>-1</sup>	1.139 <sup>a</sup>	2.075 <sup>a</sup>	0.258 <sup>b</sup>	0.421 <sup>a</sup>	0.010 <sup>a</sup>	0.016 <sup>a</sup>

Means having the same letter (s) within the same column are not significantly different according to LSD for all pairwise comparisons test at 5% level of probability.



The data also revealed that the WUE for dry herb yield increased gradually with longer irrigation intervals. The highest values were recorded from plants irrigated every 6 days, with 0.450 and 0.716 kg m<sup>-3</sup> in the first season and 0.213 and 0.380 kg m<sup>-3</sup> in the second season. On the other hand, the lowest WUE values for dry herb yield were observed when plants were irrigated every 2 days, with 0.283 and 0.349 kg m<sup>-3</sup> in the first season and 0.131 and 0.176 kg m<sup>-3</sup> in the second season, respectively. This trend was consistent with the results observed for volatile oil yield in this study.

The data from both seasons clearly demonstrated that decreasing irrigation intervals or increasing water application reduced WUE in both cuts. These findings are in line with those reported by Abd-Elghany et al. (2017) on Fenugreek plant, Okwany et al. (2011), Behera et al. (2014) and Serag El-Din and Mokhtar (2020), who stated that water-use efficiency in Spearmint crops significantly improved with increased water deficit.

The data in Table (10) also indicated that WUE for fresh herb yield, dry herb yield, and volatile oil yield significantly increased with higher Cu-Chl concentrations. The most effective treatment was the application of 1 g L<sup>-1</sup> Cu-Chl, while the lowest values were observed in the control treatment, where Spearmint plants were not sprayed with Cu-Chl in both seasons.

The interaction between irrigation intervals and Cu-Chl concentrations resulted in significant differences in WUE for fresh herb yield, dry herb yield, and volatile oil yield. The best interaction treatment was irrigation every 6 days combined with spraying plants with 1 g L<sup>-1</sup> Cu-Chl, which recorded 2.686, 1.139, and 2.075 kg of fresh herb m<sup>-3</sup>; 0.587, 0.868, 0.258, and 0.424 kg of dry herb m<sup>-3</sup>; and 0.023, 0.036, 0.010, and 0.016 L of volatile oil m<sup>-3</sup> in both cuts of the two seasons, respectively. The lowest WUE values for fresh herb yield, dry herb yield, and volatile oil yield were observed in plants irrigated every 2 days without Cu-Chl application, compared to other interaction treatments.

### CONCLUSIONS

It is recommended to cultivate Spearmint in the Tour Sinai region of South Sinai using an irrigation interval of every 4 days combined with foliar spraying of Cu-Chl at 1 g L<sup>-1</sup>. This approach resulted in the highest productivity, saved 34.8% of water when cultivating new areas and increased productivity by 62.81% compared to irrigation every 2 days with the same Cu-Chl treatment. In situations of severe water shortage, an irrigation interval of every 6 days with Cu-Chl at 1 g L<sup>-1</sup> can be used, as the reduction in productivity under these conditions was minimal.

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## REFERENCES

- Abd-Elghany, G.G., M.M. El-Shazly and H.A.E.A. Hashem (2017). Water management for the fenugreek plant and its response to bio-fertilization in north Sinai. *Egyptian Journal of Applied Science*, 32 (12 B): 494 – 515.
- Abdel-Kader, H.H., S.M.A. El-Gamal, M.H. Ali, Y.M. Hekmat and F.K. Yousef (2014). Effect of irrigation intervals and foliar fertilization on lemongrass (*Cymbopogon citratus*) (DC.) Stapf plant: A- Effects on yield and essential oil production and constituents. *Journal of Plant Production, Ramadana Univ.*, 5 (9): 1505-1522.
- Albouchi, A., Z. Bejaoui and M.H. EL-Aouni (2003). Influence of moderate or severe water stress on the growth of *Casuarina glauca* Sieb. Seedlings. *Secheresse*, 14: 137-142.
- Analytical software (2008). Statistix Version 9, Analytical Software, Tallahassee, Florida, USA.
- Aqaei, P., W. Weisany, M. Diyanat, J. Razmi and P.C. Struik (2020). Response of maize (*Zea mays* L.) to potassium nano-silica application under drought stress. *Journal of Plant Nutrition*, 43 (9): 1205-1216.
- Aziz, E.A., S.F. Hendawi, E.ED. Azza and E.A. Omer (2008). Effect of soil type and irrigation intervals on plant growth, essential oil and constituents of *Thymus vulgaris* plant. *American-Eurasian Journal of Agricultural and Environmental Sciences*, 4 (4): 443-450.
- Baher, Z.F., M. Mirza, M. Ghorbanli and M.B. Rezaii (2002). The influence of water stress on plant height, herbal and essential oil yield and composition in *Satureja hortensis* L. *Flavour and Fragrance Journal*, 17: 275-277.
- Bahreinejad, B., J. Razmjoo and M. Mirza (2013). Influence of water stress on morpho-physiological and phytochemical traits in *Thymus daenensis*. *International Journal of Plant Production*, 7: 151-166.
- Bayer, C. (2007). Proper proline management needed for effective results. *Journal of Medicinal Chemistry*, 18: 10–25.
- Behera, M.S., P.K. Mahapatra, R.B. Singandhupe, D.K. Kundu, K. Kannan, K.G. Mandal and S. Amarpreet (2014). Effect of drip fertigation on yield, water use efficiency and water productivity of mint (*Mentha arvensis* L.). *Journal of Agricultural Physics*, 14 (1): 37-43.
- British Pharmacopoeia (1963). In: 'Determination of Volatile Oil in Drugs'. The Pharmaceutical Press, London.
- Charles, D.J. (2013). *Antioxidant Properties of Spices, Herbs and other Source*; Frontier Natural Products Co-op: Norway, MI, USA.

- Chinard, F.P. (1952). Photometric estimation of proline and ornithine. *Journal of Biological Chemistry*, 199: 91–95.
- Chun, S.C., M. Paramasivan and M. Chandrasekaran (2018). Proline accumulation influenced by osmotic stress in arbuscular mycorrhizal symbiotic plants. *Frontiers in Microbiology*, 29: 2525.
- Clark, R.J. and R.C. Menary (2008). Environmental effects on peppermint (*Mentha piperita* L.). II. effects of temperature on photosynthesis, photorespiration and dark respiration in peppermint with reference to oil composition. *Australian Journal of Plant Physiology*, 7 (6): 693-697.
- El-Leithy, A.S., M.S. Hanafy and G.A.M. Anaam (2018). Effect of irrigation intervals, Cyto flow amin-50 and their interaction on rosemary (*Rosmarinus officinalis* L.). *Middle East Journal of Agriculture Research*, 7(3): 768 -781.
- El-Tayeb T.A. (2019). Natural formula for preparation of foliar fertilizer to improve plant growth. Patent No. 30364, (April, 2019) Egyptian Patent Office (EGPO), Academy of Scientific Research and Technology, Egypt.
- Farahani, H.A., S.A. Valadabadi, J. Daneshian, A.H. Shiranirad and M.A. Khalvati (2009). Medicinal and aromatic plants farming under drought conditions. *Journal of Horticulture and Forestry*, 1 (6): 86-92.
- Farooq, M., A. Wahid, N. Kobayashi, D. Fujita and S.M.A. Basra (2009). Plant drought stress: effects, mechanisms and management. *Agronomy for Sustainable Development*, 29: 185-212.
- Farzad, G., R.M. Parviz, G. Reza and H. Abbas (2016). Effects of irrigation intervals and organic manure on morphological traits, essential oil content and yield of oregano (*Origanum vulgare* L.). *Anais da Academia Brasileira de Ciências*, 88 (4): 2375-2385.
- Hanafy, M.S., A.S. EL-Leithy, and G.A.M. Anaam (2018). Effect of irrigation intervals, Cyto flow Amin-50 and their interaction on rosemary (*Rosmarinus officinalis* L.) I- On growth, yield and oil production. *Middle East Journal of Agriculture Research*, 7(3): 752-767.
- Hasbullah, R.M. and S.A. Taha (2023). Effect of spraying with some plant extracts and copper oxide nanoparticles and their interactions in vegetative growth, mineral content and volatile oils of basil plant *Ocimum basilicum* L. *Proceedings of the 4<sup>th</sup> International Agricultural Conference, IOP Conf. Series: Earth and Environmental Science*, 1213: 012068.
- Hashem, H.A.E.A., W.H. Abd-Allah and M.R. Khater (2022). Effect of some safe agricultural treatments on growth and productivity of *Nigella sativa* L. Plants under south Sinai conditions, *Egyptian Journal of Desert Research*, 72 (2): 315-334.

- Hayat, K., J. Khan, A. Khan, S. Ullah, S. Ali, S. Salahuddin and Y. Fu (2021). Ameliorative effects of exogenous proline on photosynthetic attributes, Nutrients uptake, and oxidative stresses under cadmium in pigeon pea (*Cajanus cajan* L.). *Plants*, 10: 796.
- Heidari, F., S.Z. Salmasi, A. Javanshir, H. Aliari and M.R. Dadpoor (2008). The effects of application microelements and plant density on yield and essential oil of Peppermint (*Mentha piperita* L.). *Iranian Journal of Medicinal And Aromatic Plants*, 24: 1-9.
- Igoumenidis, P.E., E.G. Lekka and V.T. Karathanos (2016). Fortification of white milled rice with phytochemicals during cooking in aqueous extract of *Mentha spicata* leaves. An adsorption studies. *LWT-Food Science and Technology*, 65: 589-596.
- Islam, M.T., W. Ckurshumova, M. Fefer, J. Liu, W. Uddin and C.A. Rosa (2021). Plant based modified biostimulant (copper chlorophyllin), mediates defense response in *Arabidopsis thaliana* under salinity stress. *Plants*, 10, 625.
- Jaleel, C.A., P. Manivannan, A. Wahid, M. Farooq, H.J. Al-Juburi, R. Somasundaram and V.A.M.R. Panneersel (2009). Drought stress in plants: a review on morphological characteristics and pigments composition. *International Journal of Agriculture and Biology*, 11 (1): 100-105.
- Karimpour, M. (2019). Effect of drought stress on RWC and chlorophyll content on wheat (*Triticum durum* L.) Genotypes. *World Essays Journal*, 7: 52-56.
- Khalid, K.A. (2006). Influence of water stress on growth, essential oil, and chemical composition of herbs (*Ocimum* sp.). *International Agrophysics*, 20: 289-296.
- Khorasaninejad, S., A. Mousavi, H. Soltanloo, K. Hemmati and K. Ahmad (2011). The effect of drought stress on growth parameters, essential oil yield and constituent of Peppermint (*Mentha piperita* L.). *Journal of Medicinal Plants Research*, 5 (22): 5360-5365.
- Leithy, S., T.A. El-Meseiry and E.F. Abdallah, (2006). Effect of biofertilizer, cell stabilizer and irrigation regime on rosemary herbage oil quality. *Journal of Applied Sciences*, 2: 773-779.
- Liang, B., T. Gao, Q. Zhao, C. Ma, Q. Chen, Z. Wei, C. Li, C. Li and F. Ma (2018). Effects of exogenous dopamine on the uptake, transport, and resorption of apple ionome under moderate drought. *Frontiers in Plant Science*, 9: 755.
- Manivasagaperumal, R., P. Vijayarangan, S. Balamurugan and G. Thiyagarajan (2011). Effect of copper on growth, dry matter yield and nutrient content of *Vigna radiata* L. Wilczek. *Journal of Phytology*, 3 (3): 53-62.

- Markwell, J., J.C. Osterman and J.L. Mitchell (1995). Calibration of the Minolta SPAD-502 leaf chlorophyll meter. *Photosynthesis Research*, 46: 467-472.
- Matile, P., M. Schellenberg and F. Vicentini (1997). Planta localization of chlorophyllase in the chloroplast envelope. *Planta*, 201: 96-99.
- Merghany, M.M., K.F. Abdelgawad, G.A. Tawfic and S.S. Ahmed (2019). Yield, quality and leaves anatomy structure of spring onion sprayed by nanocomposite to control *Thrips tabaci*. *Plant Archives*, 19: 1839- 1849.
- Okwany, R.O., T.R. Peters and K.L. Ringer (2009). Effect of sustained deficit irrigation on hay and oil yield of native Spearmint (*Mentha spicata*). In Proceedings of the 5<sup>th</sup> International Conference on Irrigation and Drainage Irrigation for Food, Energy and the Environment, Salt Lake City, UT, USA, 4-7: 239-252.
- Okwany, R.O., T.R. Peters, K.L. Ringer, D.B. Walsh and M. Rubio, (2011). Impact of sustained deficit irrigation on Spearmint (*Mentha spicata* L.) biomass production, oil yield and oil quality. *Irrigation Science*, 30 (3): 213-219.
- Olfa, B.R., W. Kaddour, W.M. Aidi and M.B. Lachaal (2009). Salt effects on the growth, mineral nutrition, essential oil yield and composition of marjoram (*Origanum majorana*). *Acta Physiologiae Plantarum*, 10: 374.
- Penka, M. (1978). Influence of irrigation on the contents of effective substances in officinal plants. *Acta Horticulturae*, 73: 181- 197.
- Ramadan, M.E. (2023). The Impact of Copper Chlorophyllin on the Growth, Yield, and Physiological Characteristics of Spinach Plants under Drought Stress. *Egyptian Journal of Botany*, 63 (3): 899-910.
- Robredo, A., U. Perez-Lopez, J. Miranda-Apodaca, M. Lacuesta, A. Mena-Petite and A. Munoz-Rueda (2011). Elevated CO<sub>2</sub> reduces the drought effect on nitrogen metabolism in barley plants during drought and subsequent recovery. *Environmental and Excremental Botany*, 71: 399–408.
- Scherer, R., M.F. Lemos, G.C.; Martinelli, J.D.L. Martins and A.G. Da Silva (2013). Antioxidant and antibacterial activities and composition of Brazilian Spearmint (*Mentha spicata* L.). *Industrial Crops and Production*, 50: 408-413.
- Schmidt, R.E. and X. Zhang (2001). Alleviation of photochemical activity decline of turfgrasses exposed to soil moisture or UV radiation stress. *International Turfgrass Society Research Journal*, 9: 340–346.
- Serag El-Din, W.M. and N.A.Y.O. Mokhtar (2020). Effect of irrigation scheduling and some antitranspirants on water relations and productivity of *Mentha varidis* L. *Am-Euras. Journal of Agriculture and Environmental Sciences*, 20 (5): 367-390.

- Shao, H.B., L.Y. CHU, C.A. Jaleel and C.X. Zhao, (2008). Water-deficit stress-induced anatomical changes in higher plants. *Comptes Rendus Biologies*, 331: 215-225.
- Shokhmgar, M., R. Baradaran, G. Mosavi, M. Poyan and E. Arazmjo (2014). Effects of irrigation interval and nitrogen on seed yield and physiological characteristics of fenugreek (*Trigonella foenum-gracum* L.). *Iranian Journal of Medicinal and Aromatic Plants*, 29 (3): 527-537.
- Simon, J.E., D. Reiss-Buhenheinra, R.J. Joly and D.J. Charles (1992). Water stress induced alterations in essential oil content and composition of sweet basil. *Journal of Essential Oil Research*, 4: 71-75.
- Simon-Sarkadi, L., G. Kocsy, A. Varhegyi, G. Galiba and J.A. Deronde (2006). Effect of drought stress at supraoptimal temperature on polyamine concentrations in transgenic coriander with increased proline levels. *Indian Journal of Medical Research*, 61: 833-839.
- Singh, M., R.S. Ganesha-Rao and S. Ramesh (1997). Irrigation and nitrogen requirement of lemongrass (*Cymbopogon flexuosus* (Sleud) Wats) on a red sandy loam soil under semiarid tropical conditions. *Journal of Essent Oil Research*, 9: 569-574.
- Swaefy, H.M.F., W.R.A. Sakr, A.Z. Sabh and A.A. Ragab (2007). Effect of some chemical and bio-fertilizers on peppermint plants grown in sandy soil. *Annals of Agricultural Sciences*, 52 (2): 451-463.
- Tadayyon, A., P. Nikneshan and M. Pessarakli (2018). Effects of drought stress on concentration of macro-and micro-nutrients in Castor (*Ricinus communis* L.) plant. *Journal of Plant Nutrition*, 41: 304-310.
- Talha, M. and M.A. Aziz (1979). Effect of irrigation and fertilization on yield and water economy of potato plant. *Egyptian Journal of Soil Science*, 19 (2): 231-246.
- Tumolo, T. and U. Lanfer-Marquez (2012). Copper chlorophyllin: A food colorant with bioactive properties? *Food Research International*, 46: 451-459.
- Yazdani, D., H. Jamshidi and F. Mojab (2002). Compare of essential oil yield and menthol existent in Peppermint (*Mentha piperita* L.) planted in different origin of Iran. *Journal of Medicinal Plants*, 3: 73-78.
- Yazdiani, Z. and G. Taheri (2014). Effects of abscisic acid on physiological characteristics of marigold (*Calendula officinalis* L.) in drought stress condition. 3<sup>rd</sup> National Congress on Medicinal Plants, 14-15 May 2014 Mashhad-Iran 226.
- Zhang, X., M. Goatley, J. Conner, M. Wilkins, I. Teshler, J. Liu, M. Fefer and W. Ckurshumova (2019). Copper chlorophyllin impacts on growth and drought stress tolerance of tomato plants. *HortScience*, 54: 2195-2201.

## تأثيرات كلوروفيلين النحاس وفترات الري على نمو وإنتاجية نبات النعناع تحت ظروف جنوب سيناء

حنان علي السيد علي هاشم

مركز بحوث الصحراء، قسم النباتات الطبية والعطرية، القاهرة، مصر

أجريت هذه الدراسة في مزرعة خاصة بمنطقة طور سيناء بمحافظة جنوب سيناء خلال موسمي الزراعة ٢٠٢٢ و ٢٠٢٣. يهدف البحث إلى معرفة تأثير التركيزات المختلفة لكلوروفيلين النحاس (Cu-Chl) (صفر، ٠,٥ و ١ جرام لتر<sup>-١</sup>) على النمو والإنتاجية والصفات الفسيولوجية لنبات النعناع (*Mentha viridis*) تحت ثلاث فترات ري مختلفة (كل ٢، ٤ و ٦ أيام). كشفت النتائج أن إطالة فترة الري إلى ٦ أيام مقارنة بالري كل يومين أدى إلى انخفاض معنوي في صفات النمو، بما في ذلك وزن العشب الطازج لكل متر مربع، محصول العشب الطازج لكل فدان، وزن العشب الجاف لكل متر مربع، محصول العشب الجاف للفدان ومحصول الزيت الطيار. ولكن، تم زيادة الكلوروفيل ومحتوى النحاس والبرولين بالإضافة إلى كفاءة استخدام المياه (WUE) للمحصول في ظل هذه المعاملة. ومع ذلك، فإن تطبيق كلوروفيلين النحاس أدى إلى تحسين جميع هذه الصفات بشكل ملحوظ مقارنة بالنباتات التي لم تعامل بكلوروفيلين النحاس. كانت المعاملة الأكثر تأثيراً في هذه الدراسة هي الرش الورقي للنباتات بـ Cu-Chl بمعدل ١ جم لتر<sup>-١</sup> مع الري كل ٤ أيام. استنتجت الدراسة أن استخدام كلوروفيلين النحاس يمكن أن يتغلب على الآثار السلبية لنقص الماء على النمو والمحصول والصفات الفسيولوجية لنبات النعناع بالإضافة إلى تحسين كفاءة استخدام المياه.