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The Impact of Copper Chlorophyllin on the Growth, Yield, and Physiological Characteristics of Spinach Plants under Drought Stress

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ONE of the major factors significantly modifying plant physiology and ultimately causing a loss in crop productivity is water deficiency stress. Plant-based pigments are highly effective in enhancing growth and reducing the consequences of water stress. The present study aimed to identify the effects of copper chlorophyllin (Cu-Chl) at rates of 0, 1, and 2% on the growth, yield, and physiological traits of spinach plants of the variety Viroflay grown under three irrigation levels of 100%, 75%, and 50% crop evapotranspiration rates (ETc) in the 2020 and 2021 growing seasons. In comparison with full irrigation (100% ETc), deficit irrigation with 50% ETc drastically reduced plant growth parameters (height, leaf number, leaf area, and fresh and dry weight), yield, relative water content (RWC), leaf membrane stability index (MSI), electrolyte leakage (EL), chlorophyll a, chlorophyll b, carotenoids, Fe, and Cu content. However, the nitrate and oxalate content increased in the plants. All studied values were significantly higher except nitrate and oxalate content which were lower with Cu-Chl application, in comparison with those without Cu-Chl application. The present work demonstrated that the adverse effects of water deficit on growth, yield, plant water status, and photosynthetic pigments of spinach plants could be alleviated when using Cu-Chl application.

Keywords: Chlorophyll, Copper chlorophyllin, Plant water status, Spinach, Water deficit.

Introduction

Drought is one of the most serious environmental stresses to which plants are frequently subjected. It has significant effects on how plant species are distributed among ecological niches and restricts crop species yields (Kapoor et al., 2020). Leaf wilting, a reduction in plant height, and a disruption in the creation of buds and flowers are all noticeable indicators of a plant experiencing water scarcity during its early vegetative stage (Bhatt & Rao, 2005). As a result of low soil moisture during a drought, plants cannot absorb as many nutrients (Razmjoo et al., 2008). Moreover, reduced leaf area per shoot due to water shortage changes the canopy's design, which in turn impacts gas exchange, water relations, and vegetative growth and development (Rahmati et al., 2018). Plant morphology, physiological, biochemical, and metabolic processes are all impacted by drought stress, consequently negatively affecting plant productivity (Kapoor et al., 2020; Badran, 2022; Gaafar et al., 2022; Loutfy et al., 2022). In order to maintain cell turgor and absorb water through their roots, plants must stimulate various methods that help in avoiding water loss (Geilfus, 2019). The major effects of drought on plants include reduced frequency of cell division and cell enlargement, root differentiation, foliage dimensions, shoot length, changed stomatal motions, water and mineral nutrition associated with lower plant production, and ineffective water use (Kumawat & Sharma, 2018).

The natural green pigment chlorophyll is saponified in alkaline media, including methanolic sodium hydroxide, resulting in isocyclic ring opening and phytyl group removal to produce copper chlorophyllin (Cu-Chl), a semi-synthetic derivative. Cu-Chl has some technological advantages over chlorophyll, such as better hydrophilicity and tinctorial power as well as

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strong stability towards acid and light, when Mg is substituted with a Cu atom in an acid solution (Tumolo & Lanfer-Marquez, 2012). Previous studies have demonstrated that Cu-Chl enhances the potential for antioxidant defense, osmotic adjustment, photosynthetic activity, and root growth in plants cultivated under drought stress (Zhang et al., 2019). It has been demonstrated that applying Cu-Chl increases plant growth and yield (Merghany et al., 2019). The aim of the current study was to investigate the effects of Cu-Chl foliar spray on the growth, yield, and physiological responses to drought stress of spinach plants.

Materials and Methods

Location and growth conditions

The field experiment was conducted at the Baloza Research Station in Egypt's North Sinai Governorate, located at latitude 31°3′N and longitude 32°36′E. The soil of the experimental site was sand (according to international textural grade) in texture, composed of 89.12% sand, 6.34% silt, and 4.54% clay with pH of 8.14, EC of 1.37 mS/cm, and available nutrients (25mg N kg^{-1}, 1.47mg P kg^{-1}, and 32 mg K kg^{-1}) in the upper 0–30cm soil layer. The spinach cv. Viroflay seeds were planted on the 2nd of October (2020 and 2021) in rows with a distance of 40 cm between them, and they were harvested 70 days later. Each experimental plot was 9.6 m^2 in size and had six 2.4m wide by 4m long rows. Using a drip irrigation system, the plants were watered with As-Salam conduit water (pH 7.45, EC 2.5mS/cm).

Experimental design and treatments

The experiment was arranged based on a strip plot design with three replicates comprising combinations of three irrigation treatments (W) and three copper chlorophyllin levels (Cu-Chl). The horizontal plots were allocated to the three irrigation water levels (W): 100% (control), 75%, and 50% evapotranspiration rates (ETc). Meanwhile, the vertical plots were devoted to the three copper chlorophyllin (Cu-Chl) foliar application rates: control (water), 0.1%, and 0.2%.

Based on daily climate data from the Central Laboratory for Agricultural Climate, Egypt, irrigation water requirements for spinach plants were determined (Table 1). The FAO-56 approach was utilized for calculating the water requirements daily (Allen et al., 1998). The crop coefficient (k_c) values were obtained from the regional agriculture extension services, whereas the reference evapotranspiration (ET0) values were determined daily through the use of recorded climatic data. The k_c values for the initial, middle, and late stages were 0.70, 1.00, and 0.95, respectively. According to the recommendations of Nakayama & Bucks (2012), the seasonal irrigation demand was 1152.50m^3 ha^{-1} due to minimal active rain in the research location as a result of its scarcity (Table 2).

The irrigation has three to four weekly irrigations and lasts from the 2nd of October to the 10th of December. The irrigation period is determined by the irrigation method, the deficit degree, and the computed average ETc for this week (combining weather conditions and phenological stage).

Regarding copper chlorophyllin application, Cu-Chl was obtained from Innovative Research & Development (InRaD) Company, Egypt. Using a handheld sprayer at sundown, treatments were evenly administered to the foliage till runoff. The first foliar application to treatments took place 15 days after sowing, the second foliar application 25 days after sowing, and the third foliar application 35 days after sowing.

TABLE 1. Average monthly meteorological data (October, November, and December) for about 10 years (2010–2019) of Baloza region, North Sinai, Egypt

	October	November	December
Max. temperature (˚C)	28.7	24.4	20.4
Min. temperature (˚C)	18.1	14	9.8
Relative humidity (%)	63	62	61
Wind speed (Km/hour)	12.37	11.90	13.65
Sunshine hours (hour)	8.8	8.1	7.7
Rainfall (mm)	5	6	7
ET0 (mm/day)	4.71	3.28	2.34

ET0 = reference evapotranspiration.

TABLE 2. Seasonal water requirements of spinach plants

Growth stage	Month	ET0	Kc	ETc	Water requirements (m^3/ha/Day)		
					100% ETc	75% ETc	50% ETc
Initial	02–21 Oct.	4.71	0.70	3.30	16.50	12.38	8.25
Development	22–31 Oct.	4.71	1.00	4.71	23.55	17.66	11.78
	01–10 Nov.	3.28	1.00	3.28	16.40	12.30	8.20
Middle	11–30 Nov.	3.28	0.95	3.12	15.60	11.70	7.80
Late	01–10 Dec.	2.34	0.95	2.22	11.10	8.33	5.55
Total water requirements (m^3/ha/season)					1152.50	864.50	576.30

ET0 = reference evapotranspiration, Kc = crop coefficient, and ETc = crop evapotranspiration.

Sampling and measurements

Plant growth and yield

Plants were harvested 70 days after sowing. Ten plants per treatment were sampled randomly to identify the plant growth parameters (height, leaf number, leaf area, and fresh and dry weight). In order to record the total yield, plants from an area of 2m^2 on each plot were harvested and weighed to determine the fresh weight, which was expressed as tons per hectare.

Relative water content (RWC)

The method outlined by Zhang et al. (2015) was utilized for calculating the RWC. In order to determine the fresh weight, leaf samples (100mg) were promptly gathered and weighed (FW). After being divided into 5mm slices, the leaf sample was put in a 2mL microcentrifuge tube with 1.8 mL of deionized (d.i.) H_2O. The leaf sample was dried after around 15h at 4 degrees Celsius and weighed immediately to measure the turgid weight (TW). In order to determine the dry weight, the leaf tissue was then dried at 70°C for 72h (DW). The following formula was used to calculate the RWC:

RWC (%) = [(FW - DW) / (TW - DW)] × 100.

Leaf membrane stability index (MSI)

Two identical 0.2g leaf tissue samples were used and put in test tubes with 10 ml of double-distilled water (Rady, 2011). A conductivity bridge was utilized for measuring the electrical conductivity of a solution after heating one sample to 40°C in a water bath for 30min (EC1). The conductivity of the second sample was assessed after 10 minutes of boiling at 100°C (EC2). The following formula was used to determine the MSI%:

MSI (%) = [1 − (EC1/ EC2)] × 100.

Electrolyte leakage (EL)

The electrical conductivity (EC1) of twenty leaf discs was calculated in a boiling tube containing 10mL deionized water (Sullivan & Ross, 1979). The electrical conductivity (EC2) was then measured after heating the contents to a range of 45–55 degrees Celsius for 30min each. After 10 minutes of boiling at 100°C, the electrical conductivity (EC3) of the sample was measured. The following formula was used to determine the EL%:

EL (%) = [(EC2− EC1) / EC3] × 100.

Photosynthetic pigments

Fresh spinach leaves were extracted in 85% acetone under dark conditions in order to extract chlorophyll a, chlorophyll b, and carotenoids. A spectrophotometer (model Unico UV/ VIS- SQ2800) was utilized for measuring the chlorophyll content by taking readings at 663, 644, and 452nm (Lichtenthaler, 1987).

Nitrate concentration in plants

Using the technique defined by Wang & Li (1996), nitrate concentration was determined. Distilled water was utilized to extract the nitrate. A specific amount of distilled water was added to a centrifuge tube along with 2g of fresh sample and 0.50g of arenaceous quartz after being pounded in a pestle and mortar. The supernatant was utilized for measuring the nitrate concentration with flow injection analysis following Cd-catalyzed reduction to NO2- after centrifugation (FIASTAR 5000). Using the concentrations in the spinach leaves, the nitrate concentration in shoots was estimated.

Oxalates content

These steps were utilized for determining oxalate content (Wang & Du, 1989). 20mL of distilled water was added after 2.0g of frozen spinach shoot samples was finely homogenized in a mortar and pestle. After filtering, the homogenate was washed three times with 50mL of distilled water. A 200mL volumetric flask was utilized for holding the entire filtrate. A centrifuge tube containing 2mL of 2mol L^{-1} $CaCl_2$ and 0.5mL of 2mol L^{-1} NaOH was filled with 5mL of the filtrate. The flocky yellow-green precipitate was removed by centrifuging at 4,000rpm min^{-1} for 5min, and the supernatant was removed and diluted with 5mL of distilled water. Afterwards, this emulsion was exposed to a uviol lamp at 365nm for 5h in order to produce a powder white precipitate. Separating the white powder precipitate, it was then dissolved in 5mL of 1.00mol L^{-1} perchloric acid. This solution was carefully transferred into a 25mL color comparison tube filled with 6mL of 10.0mmol/L $KMnO_4$ and diluted with deionized water to 25mL. The absorbance of the solution was measured at 520nm after 5min (756 spectrophotometer).

Fe and Cu contents

The plant material was dried at 60 °C, ground, and ashed in a furnace (CZYLOK, FCF5SH) at 450°C for 6h. 5mL of 6 mol•dm^3 HCl was used to dissolve the ash, and then distilled water was used to dilute it to a constant volume (Ostrowska et al., 1991). Using an Atomic Absorption Spectrophotometry (ASA) device from Varian Spectra AA 220 FS, the obtained extracts were examined to identify their Fe and Cu levels.

Statistical analysis

Data are expressed as mean ±SD (standard deviation). Using SPSS version 19, a two-way analysis of variance was conducted (SPSS Inc., Chicago, IL, USA). Moreover, a Tukey›s test was run to see if there was a significant difference ($P< 0.05$) between the mean values.

Results and Discussion

Growth parameters and yield

The spinach growth and yield were affected by irrigation levels based on ETc and foliar application of Cu-Chl (Table 3). In both seasons, deficit irrigation at 50% ETc restricted plant height, leaf number, leaf area, and fresh and dry weight, as well as the total yield. On the contrary, 100% ETc encouraged these plant growth parameters and the total yield. There were significant differences between the three irrigation levels in both seasons. In this context, our results are close to those of studies conducted by Leskovar & Piccinni (2005), Leskovar et al. (2012), and Semida et al. (2017). These results, which are most probably the results of adequate irrigation, may have increased the levels of auxins and gibberellins within biological concentrations, encouraging cell division and cell size enlargement and thereby increasing vegetative growth (El-saeid, 1981), which directly impacts plant productivity (Doro, 2012). Conversely, when under water stress, plants close their stomata, reducing the carbon amount they can take in through their leaves and their ability to photosynthesize. Consequently, plant development and biomass buildup are reduced (De Souza et al., 2005; Banon et al., 2006; Osakabe et al., 2014; Mannan et al., 2016; Yan et al., 2016).

Cu-Chl application significantly affects plant height, leaf number, leaf area, and fresh and dry weight, as well as total yield. These characteristics considerably improved as the Cu-Chl application increased. The highest values were found with 2% Cu-Chl in both growing seasons (Table 3). These results agree with those demonstrated by Merghany et al. (2019). Cu-Chl application may enhance plant root growth, photosynthetic function, osmotic regulation, antioxidant defense capacity, and defense against microbial attack, all of which contribute to improved plant growth and productivity (Tumolo & Lanfer-Marquez, 2012; Zhang et al., 2019). Exogenous Cu-Chl application causes various classes of ROS detoxifying genes and genes previously implicated in stress protection to be increased. Furthermore, it results in reduced leaf growth inhibition under stress, indicating that Cu-Chl can enhance plant growth and yield (Islam et al., 2021). Interaction between irrigation levels and foliar Cu-Chl treatment significantly affects all spinach plant growth indices and total yield in both seasons (Table 3). The highest values were obtained from irrigation with 100% ETc level combined with foliar application of 2% Cu-Chl. Meanwhile, the lowest values were obtained from irrigation with 50% ETc level combined with those untreated by Cu-Chl.

TABLE 3. Effect of irrigation water levels based on percent evapotranspiration rates (ETc) and copper chlorophyllin (Cu-Chl) on growth parameters and yield of spinach plant in the 2020 and 2021 growing seasons

	Irrigation (% ETc)	2020				2021			
		Cu-Chl (%)							
		0	1	2	Mean	0	1	2	Mean
Plant height (cm)	100	27.43^{c}	29.34^{b}	31.28^{a}	29.35^{a}	29.51^{c}	31.83^{b}	34.80^{a}	32.05^{a}
	75	24.12^{e}	25.90d	28.05c	26.02^{b}	23.78^{f}	26.05^{e}	27.84^{d}	25.89^{b}
	50	19.20^{g}	20.07^{g}	22.20^{f}	20.49^{c}	20.05^{h}	22.00^{g}	23.35^{f}	21.80^{c}
	Mean	23.58^{c}	25.10^{b}	27.18^{a}		24.45^{c}	26.63^{b}	28.66^{a}	
Plant leaf number	100	15.67^{b}	16.67^{a}	17.33^{a}	16.56^{a}	16.00^{a}	16.33^{a}	17.00^{a}	16.44^{a}
	75	12.33^{d}	14.00^{c}	15.33^{b}	13.89^{b}	11.33^{cd}	12.00^{c}	13.00^{b}	12.11^{b}
	50	9.33^{f}	10.67^{e}	11.33^{e}	10.44^{c}	9.67^{e}	10.00^{e}	10.67^{de}	10.11^{c}
	Mean	12.44^{c}	13.78^{b}	14.67^{a}		12.33^{b}	12.78^{b}	13.56^{a}	
Leaf area (cm^2)	100	149.67^{c}	158.60^{b}	165.19^{a}	157.82^{a}	150.08^{c}	168.16^{b}	171.40^{a}	163.21^{a}
	75	126.14^{f}	130.80^{e}	146.05^{d}	134.33^{b}	132.70^{e}	140.15^{d}	149.79^{c}	140.88^{b}
	50	102.60^{i}	112.59^{h}	120.93^{g}	112.04^{c}	115.02^{h}	119.59^{g}	124.77^{f}	119.79^{c}
	Mean	126.14^{c}	134.00^{b}	144.05^{a}		132.60^{c}	142.63^{b}	148.65^{a}	
Plant fresh weight (g)	100	41.45^{c}	49.20^{b}	54.25^{a}	48.30^{a}	44.48^{c}	47.81^{b}	50.63^{a}	47.64^{a}
	75	35.11^{e}	39.14^{d}	41.43^{c}	38.56^{b}	37.88^{e}	42.04^{d}	44.08^{c}	41.33^{b}
	50	23.34^{h}	27.56^{g}	29.67^{f}	26.86^{c}	25.87^{h}	29.75^{g}	30.96^{f}	28.86^{c}
	Mean	33.30^{c}	38.63^{b}	41.78^{a}		36.08^{c}	39.87^{b}	41.89^{a}	
Plant dry weight (g)	100	5.89^{c}	6.27^{b}	7.00^{a}	6.39^{a}	6.02^{c}	6.42^{b}	6.83^{a}	6.43^{a}
	75	4.85^{e}	5.51^{d}	5.70^{cd}	5.35^{b}	4.93^{e}	5.43^{d}	5.92^{c}	5.43^{b}
	50	2.97^{h}	3.75^{g}	4.11^{f}	3.61^{c}	3.37^{h}	4.11^{g}	4.38^{f}	3.95^{c}
	Mean	4.57^{c}	5.17^{b}	5.60^{a}		4.77^{c}	5.32^{b}	5.71^{a}	
Total yield (ton ha^{-1})	100	14.30^{c}	16.04^{b}	18.67^{a}	16.34^{a}	15.13^{c}	17.71^{b}	19.02^{a}	17.29^{a}
	75	12.54^{e}	13.13^{d}	14.06^{c}	13.24^{b}	12.68^{e}	14.02^{d}	14.72^{cd}	13.81^{b}
	50	9.73^{g}	10.15^{g}	10.94^{f}	10.27^{c}	10.89^{f}	11.22^{f}	11.66^{f}	11.26^{c}
	Mean	12.19^{c}	13.11^{b}	14.56^{a}		12.90^{c}	14.32^{b}	15.14^{a}	

RWC, MSI, and EL%

The leaf RWC, MSI, and EL percent of each treatment are presented in Fig. 1 (A, B, and C). The leaf RWC, MSI, and EL percent showed highly significant differences with irrigation levels and Cu-Chl treatments. In addition, all irrigation levels x Cu-Chl interactions were highly significant (Fig. 1). Increasing irrigation rates resulted in increasing leaf RWC, MSI, and EL percent, and the values were higher under irrigation with 100% ETc level compared to other irrigation treatments (75% and 50% ETc level) in both seasons. The results agree with those of Vasques-Tello et al. (1990), Semida et al. (2017), Abdelkhalik et al. (2019), and Zhang et al. (2019). An adequate supply of water may increase water availability in the soil inducing improvement in the plant water status, resulting in the enhancement of RWC% in the plant cells (Farooq et al., 2009). The protoplasm dehydration under a water deficit may be the reason for the reduction in RWC%. Reactive oxygen species (ROS) are produced as a result of water stress, which damages membranes and oxidizes lipids. Consequently, MSI% (membrane stability index) of cells decreases (Sibel & Birol, 2007; Abd El-Mageed et al., 2021)

The exogenous application of Cu-Chl resulted in a significant increase in RWC, MSI, and EL. The application of Cu-Chl (2%) exhibited the highest values followed by Cu-Chl (1%) in comparison with untreated plants in both seasons. Osmotic stress negatively impacts plant water status under water deficiency stress, resulting in a reduction in RWC and the potential for accumulating harmful ROS that damage cell membrane lipid and lead to a fall in MSI and EL% (Abd El-Hady et al., 2018). Cu-Chl enhances osmotic adjustment during drought stress (Zhang et al., 2019) and has antioxidant activity, which may shield plants from damage caused by ROS, as demonstrated by Tumolo & Lanfer-Marquez (2012). This suggests that the Cu-Chl may improve the water status of plants, hence causing an increase in RWC, MSI, and EL% in plant leaves. Our study revealed that under irrigation with 100% ETc level, the application of 2% Cu-Chl indicated the highest RWC, MSI, and El% in leaves of spinach plants in comparison with untreated plants with Cu-Chl under irrigation with 50% ETc level in both seasons.

Photosynthetic Pigments

Irrigation water treatments significantly impacted chlorophyll a, chlorophyll b, and carotenoids that make up the leaf's photosynthetic pigments (Fig. 2: A, B, and C). At the lowest irrigation water (50% ETc), chlorophyll a, chlorophyll b, and carotenoids contents decreased, whereas the highest values were detected in the leaves of plants under the 100% ETc irrigation in both seasons. These results agreed with those reported by Abd El-Mageed et al. (2018), who noted that leaf photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids) had significant reductions under irrigation water rates at 60% ETc in comparison with those noted under irrigation water rates at 100% and 80% Etc. Additionally, another study that investigated the effects of various irrigation water rates based on Etc on the content of chlorophyll a, chlorophyll b, and carotenoids discovered that the highest levels of photosynthetic pigments were recorded with irrigation water rate at 100% Etc, while the lowest levels were recorded with irrigation water rate at 55% Etc (Mahmoud et al., 2021). The reduction in chlorophyll content may have been caused by increased ethylene production (Matile et al., 1997). Drought stress improved the ethylene generation causing stress and degrading cell membrane integrity through lipid breakdown. Ethylene coming into touch with the chloroplast directly as a result of lipid degradation activates the chlorophyllase (chlase) gene, severely damaging the chlorophyll (Matile et al., 1997; Karimpour, 2019).

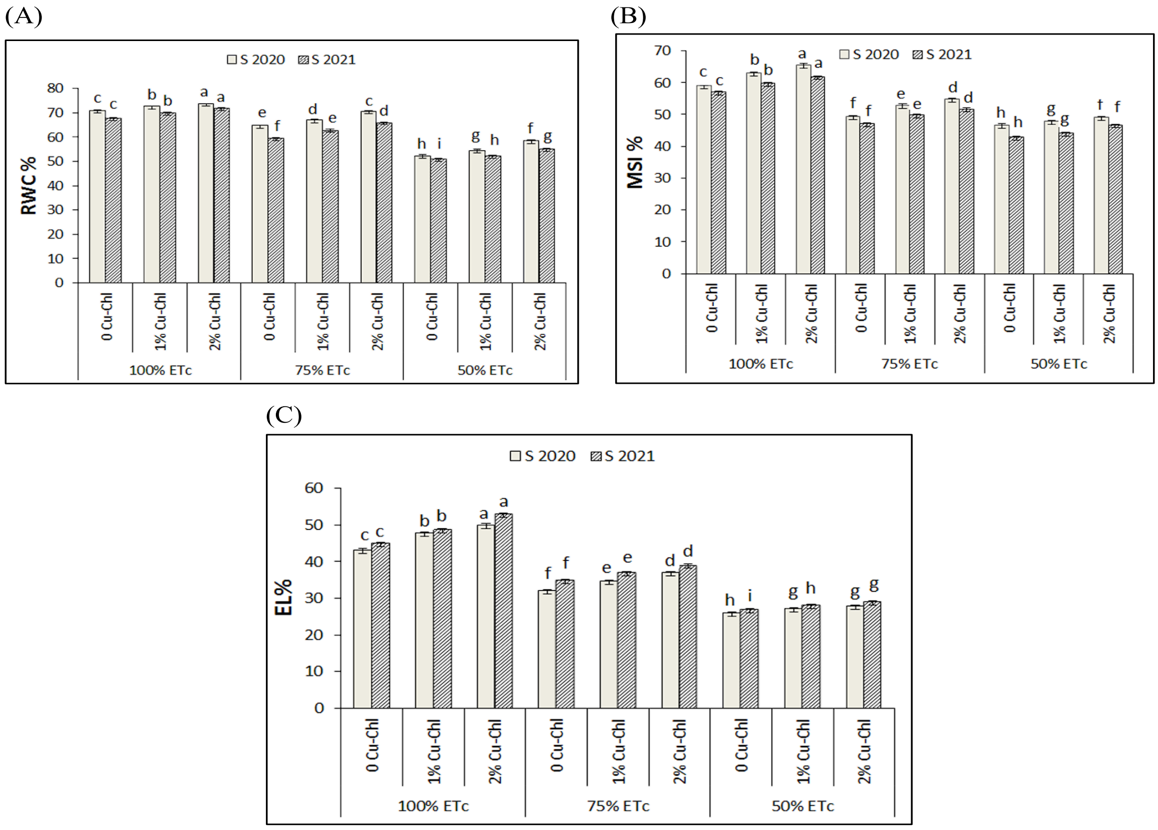

Fig.1. Effect of irrigation water levels based on percent evapotranspiration rates (ETc) and copper chlorophyllin (Cu-Chl) on RWC, MSI, and EL% of spinach plant in the 2020 and 2021 growing seasons

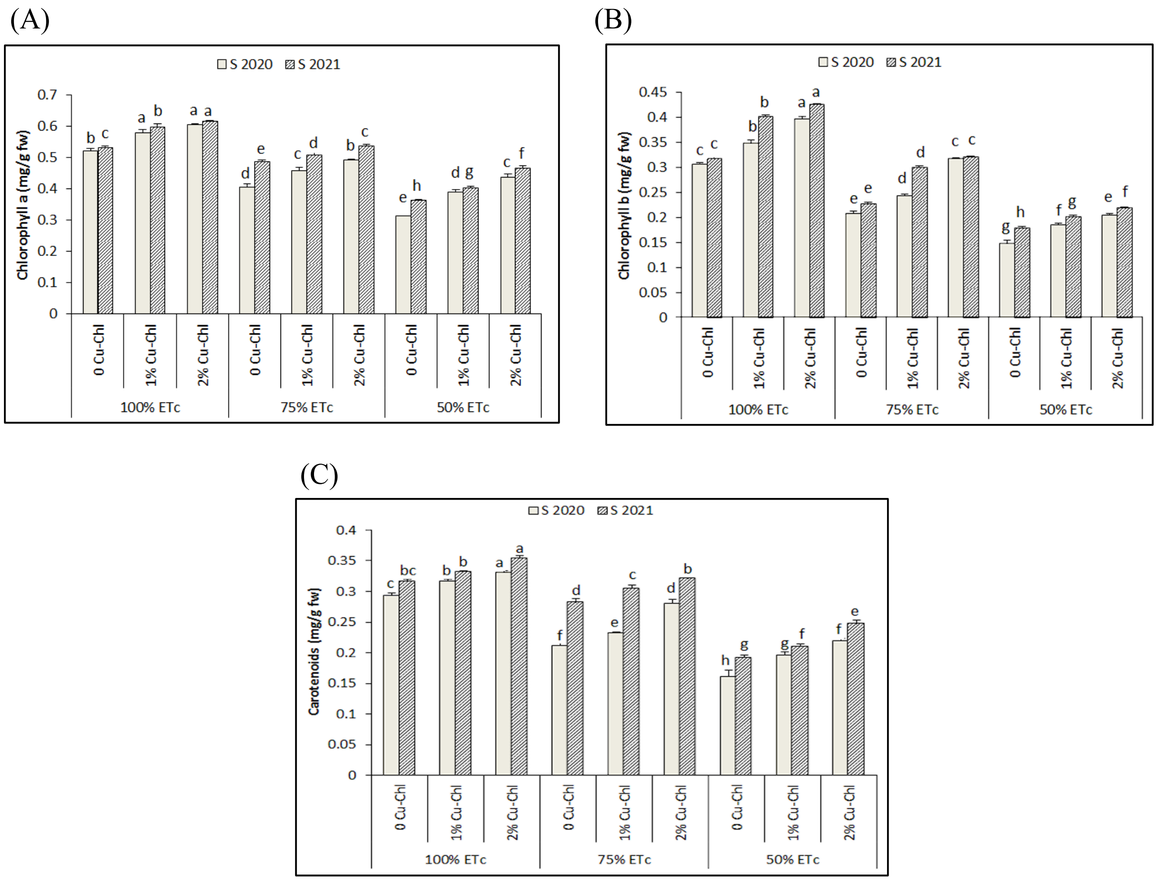

Fig. 2. Effect of irrigation water levels based on percent evapotranspiration rates (ETc) and copper chlorophyllin (Cu-Chl) on chlorophyll a, chlorophyll b, and carotenoids in spinach plant in the 2020 and 2021 growing seasons

Significant differences were observed in chlorophyll a, chlorophyll b, and carotenoids contents in leaves of spinach plants in response to treatment with Cu-Chl concentrations (Fig. 2). The contents of chlorophyll a, chlorophyll b, and carotenoids in spinach plants trended upward with an increase in Cu-Chl concentrations. Chlorophyll a, chlorophyll b, and carotenoids contents increased to a maximum value at the highest Cu-Chl concentration (2%), with increases of 24.27 and 17.14%, 38.46 and 33.06%, and 24.22 and 16.29% in the first and second seasons, respectively, in comparison with those in the control group. These findings agreed with those reported by Zhang et al. (2019), who reported that, under drought stress conditions, foliar application of Cu-Chl increased the concentration of chlorophyll a, chlorophyll b, and carotenoids in tomato plants. Chlorophyll content in onion increased with foliar application of Cu-Chl (Merghany et al., 2019). The ability of synthetic pigments to shield chlorophyll from ultraviolet B radiation (Schmidt & Zhang, 2001) and to shield plants from ROS-induced damage to photosynthetic (Tumolo & Lanfer-Marquez, 2012) apparatus may be a contributing factor to improving photosynthetic pigments achieved by foliar application of Cu-Chl. Our findings revealed that the highest values of chlorophyll a, chlorophyll b, and carotenoids contents were determined in the 2% Cu-Chl foliar spraying under irrigation with 100% ETc treatment, while the lowest values of chlorophyll a, chlorophyll b, and carotenoids contents were observed in the no Cu-Chl application with irrigation at 50% Etc treatment in both seasons.

Nitrate, oxalates, Fe, and Cu contents

Our findings are presented in Table 4, demonstrating that with rising irrigation levels, the contents of nitrate and oxalates in the leaves of spinach plants dramatically dropped while the contents of Fe and Cu significantly increased. The lowest values of nitrate and oxalates contents and the highest values of Fe and Cu contents were recorded with irrigation with 100% ETc level treatment in both seasons. Water deficit of 50% ETc showed the maximum of nitrate and oxalates contents and the minimum Fe and Cu contents in the leaves of spinach plants in both seasons. Similar findings were reported by Schiattone et al. (2018), who noticed that deficit irrigation led to excessive nitrate accumulation in rocket plants. This may be attributed to the negative effects of deficit irrigation on the activity of nitrate reductase (NR), the enzyme governing the reduction of nitrate in plant organs. It may also be attributed to reduced absorption of nitrogen by roots under water stress (Correia et al., 2005). Under water deficit stress generally increased oxalate content of plants (Gouveia et al., 2020). Tadayyon et al. (2018) and Aqaei et al. (2020) observed a reduction in Fe and Cu contents of plant under water deficit, which is consistent with the results of the present study. Irrigation water deficit can potentially reduce nutrient-uptake kinetics per unit root, such as by decreasing the activity of enzymes involved in nutrient assimilation, which might then slow nutrient uptake (Robredo et al., 2011). Furthermore, less transpiration due to drought results in a reduction in volume flow in the xylem, which then restricts transport and decreases mass flow and diffusivity of nutrients between the roots and stems (Liang et al., 2018).

TABLE 4. Effect of irrigation water levels based on percent evapotranspiration rates (ETc) and copper chlorophyllin (Cu-Chl) on nitrate, oxalates, Fe, and Cu contents of spinach plant in the 2020 and 2021 growing seasons.

	Irrigation (% ETc)	2020				2021			
		Cu-Chl (%)							
		0	1	2	Mean	0	1	2	Mean
Nitrate (mg/kg)	100	494.66^{f}	440.03^{g}	402.95^{h}	445.88^{c}	572.17^{g}	537.70^{h}	509.51^{i}	539.80^{c}
	75	572.67^{c}	544.19^{d}	522.85^{e}	546.57^{b}	667.97^{d}	629.05^{e}	609.29^{f}	635.44^{b}
	50	631.81^{a}	616.90^{a}	595.72^{b}	614.81^{a}	734.32^{a}	714.77^{b}	695.88^{c}	714.99^{a}
	Mean	566.38^{a}	533.71^{b}	507.17^{c}		658.16a	627.17^{b}	604.89^{c}	
Oxalates (mg/kg)	100	773.02^{f}	745.25^{g}	715.34^{h}	744.54^{c}	723.10^{f}	687.85^{g}	635.99^{h}	682.32^{c}
	75	818.15^{d}	796.55^{e}	775.73^{f}	796.81^{b}	794.52^{d}	756.54^{e}	728.31^{f}	759.79^{b}
	50	866.35^{a}	842.29^{b}	825.10^{c}	844.58^{a}	837.87^{a}	820.71^{b}	805.52^{c}	821.37^{a}
	Mean	819.18^{a}	794.69^{b}	772.06^{c}		785.17^{a}	755.03^{b}	723.27^{c}	
Fe (mg/kg)	100	40.82^{c}	47.24^{b}	51.01^{a}	46.36^{a}	47.75^{c}	50.83^{b}	55.85^{a}	51.48^{a}
	75	35.81^{e}	37.85^{d}	40.76^{c}	38.14^{b}	39.50^{e}	44.00^{d}	47.07^{c}	43.52^{b}
	50	30.93^{h}	31.96^{g}	33.50^{f}	32.13^{c}	33.85^{h}	36.15^{g}	37.98^{f}	35.99^{c}
	Mean	35.85^{c}	39.02^{b}	41.76^{a}		40.37^{c}	43.66^{b}	46.97^{a}	
Cu (mg/kg)	100	29.98^{f}	50.31^{c}	70.12^{a}	50.14^{a}	35.22^{f}	51.53^{c}	67.89^{a}	51.55^{a}
	75	23.07^{h}	39.75^{e}	52.88^{b}	38.57^{b}	30.02^{g}	46.81^{e}	58.00^{b}	44.94^{b}
	50	14.53^{i}	26.20^{g}	44.56^{d}	28.43^{c}	18.91^{h}	30.26^{g}	49.37^{d}	32.85^{c}
	Mean	22.52^{c}	38.75^{b}	55.85^{a}		28.05^{c}	42.87^{b}	58.42^{a}	

As given in Table 4, the nitrate, oxalates, Fe, and Cu contents in spinach plants were significantly influenced by Cu-Chl treatments. Cu-Chl application induced a reduction in the nitrate and oxalates contents, whereas it induced an increase in the Fe and Cu contents in both seasons. Treatment of Cu-Chl by 2% gave the minimum values of the nitrate and oxalates, whereas it gave the maximum values of Fe and Cu content in spinach plants in comparison with untreated plants in both seasons. The effect of interaction between the irrigation levels based on ETc and foliar application of Cu-Chl on nitrate, oxalates, Fe, and Cu content was observed. Irrigation at 100% ETc level with 2% Cu-Chl treatment gave the lowest values of nitrate and oxalates content (402.95, 509.51 and 715.34, 635.99mg/kg) and the highest values of Fe and Cu content (51.01, 55.85 and 70.12, 67.89mg/kg) in leaves of spinach plants in the first and second seasons, respectively. Meanwhile, deficit irrigation (50% ETc) without Cu-Chl application gave the highest values of nitrate and oxalates content (631.81, 734.32 and 866.35, 837.87mg/kg) and the lowest values of Fe and Cu content (30.93, 33.85 and 14.53, 18.91mg/kg) in leaves of spinach plants in the first and second seasons, respectively. The differences among treatments were significant in both seasons.

Ethics approval: Not applicable.

Conclusions

As a result of water deficit stress, we found a significant reduction in spinach plant growth parameters (height, leaf number, leaf area, and fresh and dry weight), yield, plant water status (RWC, MSI, and EL%), leaf photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids), and Fe and Cu contents. Conversely, a significant increase was found in the concentrations of nitrate and oxalates. According to the findings, the foliar application of Cu-Chl (2%) was a successful strategy for preventing the damaging effects of water deficiency stress on spinach plants.

References

Abd El-Hady, M.A., Fergani, M.A., El-Temsah, M.E., Abdelkader, M.A. (2018) Physiological response of some wheat cultivars to antitranspirant under different irrigation treatments. *Middle East Journal of Agriculture*, **7**, 100-109.

Abdelkhalik, A., Pascual-Seva, N., Najera, I., Domene, M.A., Baixauli, C., Pascual, B. (2019) Effect of deficit irrigation on the productive response of drip-irrigated onion (*Allium cepa* L.) in Mediterranean conditions. *The Horticulture Journal*, **88**, 488-498.

Abd El-Mageed, T.A., Semida, W.M., Taha, R.S., Rady, M.M. (2018) Effect of summer-fall deficit irrigation on morpho-physiological, anatomical responses, fruit yield and water use efficiency of cucumber under salt affected soil. *Scientia Horticulturae*, **237**, 148-155.

Abd El-Mageed, T.A. , Rady, M.O.A., Abd El-Wahed, M.H., Abd El-Mageed, S.A., Omran, W.M., Aljuaid, B.S., et al. (2021) Consecutive seasonal effect on yield and water productivity of drip deficit irrigated sorghum in saline soils. *Saudi Journal of Biological Sciences*. doi:https://doi.org/10.1016/j.sjbs.2021.12.045

Allen, R.G., Pereira, L.S., Raes, D., Smith, M. (1998) Crop evapotranspiration - Guidelines for computing crop water requirements - FAO Irrigation and drainage paper 56, FAO Irrigation and Drainage Papers. Food and Agriculture Organization of the United Nations, Rome.

Aqaei, P., Weisany, W., Diyanat, M., Razmi, J., Struik, P.C. (2020) Response of maize (*Zea mays* L.) to potassium nano-silica application under drought stress, *Journal of Plant Nutrition*, doi: 10.1080/01904167.2020.1727508

Badran, A.E. (2022) Assessment of variation and stability parameters of five quinoa genotypes under drought stress conditions. *Egyptian Journal of Botany,* **62**(1), 21-30.

Banon, S., Ochoa, J., Franco, J., Alarcon, J., Sánchez-Blanco, M.J. (2006) Hardening of oleander seedlings by deficit irrigation and low air humidity. *Environmental and Experimental Botany,* **56**, 36–43.

Bhatt, R.M., Rao, N.K.S. (2005) Influence of pod load on response of okra to water stress. *Indian Journal of Plant Physiology*, **10**, 54–59.

Correia, M.J., Fonseca, F., Azedo-Silva, J., Dias, C., David, M.M., Barrote, I., Osorio, M.L., Osorio, J. (2005) Effects of water deficit on the activity of nitrate reductase and content of sugars: nitrate and free amino acids in the leaves and roots of sunflower and white lupin plants growing under two nutrient supply regimes. *Physiologia Plantarum,* **124**, 61–70.

De Souza, C.R., Maroco, J.P., Dos Santos, T.P., Rodrigues, M.L., Lopes, C., Pereira, J.S., Chaves, M.M. (2005) Control of stomatal aperture and carbon uptake by deficit irrigation in two grapevine cultivars. *Agriculture, Ecosystems & Environment,* **106**, 261–274.

Doro, A.K. (2012) Effect of irrigation interval on the yield of garlic (*Allium sativum* L.) at Ajiwa irrigation site of katsina State-Nigeria. *Journal of Research in National Development*, **10**(2), ,30-33.

El-Saeid, H.M. (1981) Effect of water imbalance on growth in *Phaseolus vulgaris*. *M. Sc. Thesis*, Fac. Agric. Ain-Shams Univ., Cairo, Egypt.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S.M.A. (2009) Plant drought stress: effects, mechanisms and management. *Agronomy for Sustainable Development,* **29**, 185–212.

Gaafar, R.M., Seyam, M.M., El-Shanshory, A.R. (2022) Expression patterns of drought-related miRNAs in Chickpea (*Cicer arietinum* L.) under drought stress. *Egyptian Journal of Botany*, **62**(1), 227-240.

Geilfus, C.M. (2019) Drought Stress. In: "*Controlled Environment Horticulture*"; Springer: Cham, Switzerland, pp. 81–97.

Gouveia, C.S.S., Gananca, J.F.T., Lebot, V., Pinheiro de Carvalho, M.A.A. (2020) Changes in oxalate composition and other nutritive traits in root tubers and shoots of sweet potato (*Ipomoea batatas* L. [Lam.]) under water stress. *Journal of the Science of Food and Agriculture*, **100**, 1702-1710.

Islam, M.T., Ckurshumova, W., Fefer, M., Liu, J., Uddin, W., Rosa, C.A. (2021) Plant based modified biostimulant (copper chlorophyllin), mediates defense response in *Arabidopsis thaliana* under salinity stress. *Plants*, **10**, 625.

Kapoor, D., Bhardwaj, S., Landi, M., Sharma, A., Ramakrishnan, M., Sharma, A. (2020) The impact of drought in plant metabolism: How to exploit tolerance mechanisms to increase crop production. *Applied Sciences*, **10**, 5692, doi:10.3390/app10165692

Karimpour, M. (2019) Effect of drought stress on RWC and chlorophyll content on wheat (*Triticum durum* L.) Genotypes. *World Essays Journal*, **7**, 52–56.

Kumawat, K.R., Sharma, N.K. (2018) Effect of drought stress on plants growth. *Popular Kheti*, **6**, 239–241.

Leskovar, D.I., Piccinni, G. (2005) Yield and leaf quality of processing spinach under deficit irrigation. *HortScience*, **40**, 1868-1870.

Leskovar, D.I., Agehara, S., Yoo, K., Pascual-Seva, N. (2012) Crop coefficient-based deficit irrigation and planting density for onion: growth, yield and bulb quality. *HortScience*, **47**, 31-37.

Liang, B., Gao, T., Zhao, Q., Ma, C., Chen, Q., Wei, Z., Li, C., Li, C., Ma, F. (2018) Effects of exogenous dopamine on the uptake, transport, and resorption of apple ionome under moderate drought. *Frontiers in Plant Science*, **9**, 755. doi: 10.3389/fpls.2018.00755.

Lichtenthaler, H.K. (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods in Enzymology*, **148**, 350–382.

Loutfy, N., Hassanein, A.M., Inouhe, M., Salem, J.M. (2022) Biological aspects and proline metabolism genes influenced by polyethylene glycol and salicylic acid in two wheat cultivars. *Egyptian Journal of Botany*, **62**(3), 671-685.

Mahmoud, I.M., AbouEl-Kasem, S.A.A., El-Kassas, M.S.A. (2021) Response of water stressed tomato plants to soil organic to soil organic amendments. *SINAI Journal if Applied Sciences*, **10**, 67-88.

Mannan, M.A., Halder, E., Karim, M.A., Ahmed, J.U. (2016) Alleviation of adverse effect of drought stress on soybean (*Glycine max*. L.) by using poultry biochar. *Bangladesh Agronomy Journal,* **19**, 61–69.

Matile, P., Schellenberg, M., Vicentini, F. (1997) Planta localization of chlorophyllase in the chloroplast envelope. *Planta*, **201**, 96-99.

Merghany, M.M., Abdelgawad, K.F., Tawfic, G.A., Ahmed, S.S. (2019) Yield, quality and leaves anatomy structure of spring onion sprayed by nanocomposite to control Thrips tabaci. *Plant Archives,* **19**, 1839-1849

Nakayama, F.S., Bucks, D.A. (2012) "*Trickle Irrigation for Crop Production: Design, Operation and Management*", Elsevier, , 394p.

Ostrowska, A., Gawlinski S., Szczubialka, Z. (1991) "*Methods for Analysis and Evaluation of Soil and Plant Properties*",1st ed.; IOS Warszawa: Warszawa, Poland, pp. 158–167, In Polish.

Osakabe, Y., Osakabe, K., Shinozaki, K., Tran, L.S.P. (2014) Response of plants to water stress. *Frontiers in Plant Science*, **5**, 86. doi:org/10.3389/fpls.2014.00086

Rady, M.M. (2011) Effect of 24-epibrassinolide on growth, yield, antioxidant system and cadmium content of bean (*Phaseolus vulgaris* L.) plants under salinity and cadmium stress. *Scientia Horticulturae,* **129**, 232–237.

Rahmati, M., Miras-Avalos, J.M., Valsesia, P., Lescourret, F., Genard, M., Davarynejad, G.H., Bannayan, M., Azizi, M., Vercambre, G. (2018) Disentangling the effects of water stress on carbon acquisition, vegetative growth, and fruit quality of peach trees by means of the QualiTree model. *Frontiers in Plant Science*, **9**, 3. doi.org/10.3389/fpls.2018.00003.

Razmjoo, K., Heydarizadeh, P., Sabzalian, M.R. (2008) Effect of salinity and drought stresses on growth parameters and essential oil content of *Matricaria chamomile. International Journal of Agriculture and Biology*, **10**, 451–454.

Robredo, A., Perez-Lopez, U., Miranda-Apodaca, J., Lacuesta, M., Mena-Petite, A., Munoz-Rueda, A. (2011) Elevated CO reduces the drought effect on nitrogen metabolism in barley plants during drought and subsequent recovery. *Environmental and Excremental Botany,* **71**, 399–408.

Schiattone, M.I., Viggiani, R., Di Venere, D., Sergio, L., Cantore, V., Todorovic, M., Perniola, M., Candido, V. (2018) Impact of irrigation regime and nitrogen rate on yield, quality and water use efficiency of wild rocket under greenhouse conditions. *Scientia Horticulturae*, **229**, 182–192

Schmidt, R.E., Zhang, X. (2001) Alleviation of photochemical activity decline of turfgrasses exposed to soil moisture or UV radiation stress. *International Turfgrass Society Research Journal,* **9**, 340–346.

Semida, W.M., Abd El-Mageed, T.A., Mohamed, S.E., El-Sawah, N.A. (2017) Combined effect of deficit irrigation and foliar-applied salicylic acid on physiological responses, yield, and water-use efficiency of onion plants in saline calcareous soil. *Archives of Agronomy and Soil Science,* **63**, 1227–1239.

Sibel, T., Birol, T. (2007) Some physiological responses of drought stress in wheat genotypes with different ploidity in Turkiye. *World Journal of Agricultural Sciences,* **3**, 178-183

Sullivan, C.Y., Ross, W.M. (1979) Selecting the drought and heat resistance in grain sorghum. In: "*Stress Physiology in Crop Plants*", Mussel, H., Staples, R.C. (Ed.), pp. 263–281. John Wiley & Sons, New York, USA.

Tadayyon, A., Nikneshan, P., Pessarakli, M. (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.

Tumolo, T., Lanfer-Marquez, U. (2012) Copper chlorophyllin: A food colorant with bioactive properties? *Food Research International*, **46**, 451–459.

Vasques-Tello, A., ZuilyFodil, Y., Phamthi, A.T., Vieira, D.A., Silva, J.B. (1990) Electrolyte and Pi leakage and soluble sugar content as physiological tests for screening resistance to water stress in Phaseolus and Vigna species. *Journal of Experimental Botany,* **41**, 827-832.

Wang, S.Y., Du, M. (1989) Determination of total oxalate in spinach by spectrophotometry. *Acta Nutrimenta Sinica, (in Chinese)*, **11**, 71- 76.

Wang, Z.H., Li, S.X. (1996) Relationships between nitrate contents and water, total N as well as total P in different organs of vegetable plants. *Plant Nutrition and Fertilizer Science,* **2**, 144-152.

Yan, W., Zhong, Y., Shangguan, Z.A. (2016) A meta-analysis of leaf gas exchange and water status responses to drought. *Scientific Reports,* **6**, 20917. doi: 10.1038/srep20917

Zhang, X., Ervin, E.H., Liu, Y., Hu, G., Shang, C., Fukao, T., Alpuerto, J. (2015) Differential responses of antioxidants, abscisic acid, and auxin to deficit irrigation in two perennial ryegrass cultivars contrasting in drought tolerance. *Journal of the American Society for Horticultural Science,* **140**, 562–572.

Zhang, X., Goatley, M., Conner, J., Wilkins, M., Teshler, I., Liu, J., Fefer, M., Ckurshumova, W. (2019) Copper chlorophyllin impacts on growth and drought stress tolerance of tomato plants. *HortScience*, **54**, 2195-2201.

تأثير كلوروفللين النحاس على النمو والمحصول والصفات الفسيولوجية للسبانخ تحت إجهاد الجفاف

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إجهاد الجفاف أحد أكثر العوامل التى تؤثربشكل كبيرفي نمو و فسيولوجيا النبات، مما يؤدي في النهاية إلى إنخفاض إنتاجية المحاصيل. الأصباغ النباتية تتمتع بقدرة ملحوظة على تحسين النمو وتقليل آثار إجهاد نقص المياه في النباتات. الهدف من هذه الدراسة هو دراسة تأثير كلوروفيللين النحاس (Cu-Chl) بمعدلات 0، 1 و2٪ على النمو، المحصول والخصائص الفسيولوجية لنبات السبانخ صنف فيروفلاى تحت ثلاث معدلات للرى بنسبة 100٪، 75٪ و50٪ من ETc خلال موسمين الزراعة 2020 و2021. أظهرت النتائج أنه مقارنةً بالرى الكامل (100٪ ETc)، أدى نقص الري بنسبة 50٪ ETc إلى انخفاض كبير في صفات نمو النبات (الارتفاع، عدد الأوراق، مساحة الورقة، الوزن الطازج والجاف)، المحصول، محتوى الماء النسبي (RWC)، مؤشر ثبات غشاء الأوراق (MSI)، الإرتشاح الإلكتروليتى (EL)، الكلوروفيل أ، ب، الكاروتينات، محتوى الحديد ومحتوى النحاس، وفى المقابل زاد محتوى النباتات من النترات والأكسالات. كانت جميع القيم المدروسة أعلى بشكل معنوى باستثناء محتوى النترات والأكسالات كان أقل عند المعاملة بكلوروفللين النحاس. أظهرت الدراسة الحالية أنه من الممكن التخفيف من التأثير السلبي لنقص المياه على النمو، المحصول وحالة النبات المائية لنبات السبانخ عند استخدام كلوروفللين النحاس.