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Copper Chlorophyllin Impacts on Growth and Drought Stress Tolerance of Tomato Plants

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Abstract. **Plant-based pigments have been used as substances to improve crop yield and quality, but the mechanisms of their action on plant growth and stress tolerance are not well understood. The objective of this study was to investigate effects of two formulations of plant-based copper chlorophyllin (Cu-Chl) with and without synthetic paraffinic oil. These formulations, referred to as B18-0074 and B18-0075, were applied as a soil drench plus foliar or a foliar-only application. We investigated their impact on physiological responses of tomato plants under prolonged drought stress conditions. In addition, we examined photosynthetic impacts associated with the application of Cu-Chl formulations. B18-0074 increased leaf photosynthetic rate (Pn) by 8.8% with soil plus foliar application and 18.6% with foliar application relative to the control under drought stress at day 21. Similarly, B18-0075 increased Pn by 16.9% with soil plus foliar application and 24.6% with foliar application relative to the control under drought stress at day 21. The application of the two Cu-Chl–containing products increased leaf antioxidant enzyme catalase (CAT) and ascorbate peroxidase (APX) activity, as well as glutathione (GSH) content. The two products also increased leaf soluble sugars and proline content, indicating improvement of osmotic adjustment. Soil plus foliar and foliar application only of B18-0075 increased root biomass but did not consistently affect plant shoot growth. The results of this study suggest that application of Cu-Chl in combination with synthetic paraffinic oil may improve photosynthetic function, osmotic adjustment, antioxidant defense capacity, and root growth of tomato plant grown under drought stress conditions.**

Sustainable agriculture has the potential to provide for an ever-increasing human population (Mishra et al., 2012). Without intervention, the usual losses of crop yield due to biotic and abiotic stresses may, in a near future, be amplified by impacts of global warming, as well as erratic climate extremes and issues related to resistance (Mishra et al., 2012). In many regions of the world, drought is a major limiting factor for crop productivity (Huang et al., 2014). Climate change and the associated drought stress may have an increasingly negative impact on crop yields and quality (Zhang et al., 2012). Although plants have developed various defense systems to cope with abiotic stress, such as antioxidant defenses, hormonal regulation, osmotic adjustments, and saturation level of cell membrane lipids (Huang et al., 2014; Shinozaki and Yamaguchi-Shinozaki, 2007), these responses can be overwhelmed during climatic extremes.

Drought stress may inhibit photosynthesis and cause an energy imbalance so that the energy absorbed through the light-harvesting complex exceeds what can be dissipated or transduced by photosystem II (Reddy et al., 2004; Zhang et al., 2012). Excess energy may be directed to O_2 and result in accumulation of toxic reactive oxygen species (ROS) (Huang et al., 2014; Noctor et al., 2014; Zhang and Ervin, 2004). To limit oxidative damage under stress conditions, plants have developed a series of detoxification systems that break down the highly toxic ROS (Bian and Jiang, 2009; Wang et al., 2012; Zhang et al., 2012, 2015). Superoxide dismutase (SOD) has been considered as the first line of defense against ROS by dismutating the superoxide anion to hydrogen peroxide (H_2O_2), which is finely regulated by CAT and an array of peroxidases localized in almost all compartments of the plant cell, such as APX (Blokhina et al., 2003; Li and Luo, 2012). Cultivar variations in antioxidant enzyme activities were associated with differences in drought tolerance of grasses (Man et al., 2011; Xu et al., 2011) and other plant species (Türkan et al., 2005).

It has been reported that alteration in hormone metabolism is associated with plant tolerance to abiotic stress (Zhang et al., 2009, 2015). ABA and indole-3-acetic acid (IAA) are hormones that mediate signaling events involved in plant adaptation to stress and senescence. IAA is the primary auxin in the majority of plant species. ABA plays a regulatory role in controlling stomatal aperture under drought stress (Strivastava, 2002). Rapid ABA accumulation has been observed when plants are subjected to drought, salinity, and extreme temperatures (Xiong et al., 2002).

Plant growth regulating substances have been used to improve drought tolerance in various crop plants. Naturally derived organic substances or biostimulants have received increasingly attention in recent decade because these environmentally friendly substances can significantly improve crop stress tolerance by enhancing plant defense systems and protecting photosynthetic function, especially under stressful environments (Zhang and Schmidt, 1999). The use of synthetic pigment-containing products on golf-playing surfaces has increased dramatically by golf course superintendents to provide added green color and improve stress tolerance. Most turf colorants are synthesized from various phthalocyanine pigments. Certain Cu-, Zn-, and Ti-based phthalocyanine compounds are reported to reduce CO_2 exchange rate, evapotranspiration rate, chlorophyll fluorescence, and light transmission (McCarty et al., 2013, 2014). Some evidence suggests that a polychlorinated Cu II phthalocyanine compound induced a defense response to the dollar spot pathogen, although the mechanism was unrelated to systemic acquired resistance or induced systemic resistance (Hsiang et al., 2013).

Cu-Chl is a semisynthetic derivative of the natural green pigment chlorophyll and is used in food colorants, dietary supplements, and cosmetics and as an internal deodorant and an accelerant in wound healing. It displays some technological advantages over chlorophyll, such as greater hydrophilicity and tinctorial power and high stability toward acid and light (Tumolo and Lanfer-Marquez, 2012). The main raw material for preparation of Cu-Chl is natural chlorophyll, a macrocyclic molecule that consists of four pyrrole rings bonded by methylene bridges and coordinated to a magnesium atom. Cu-Chl is formed by the saponification of chlorophyll molecules in an alkaline medium containing methanolic sodium hydroxide, leading to isocyclic ring opening and phytyl group removal. Mg is replaced with a Cu atom in an acid medium, which gives Cu-Chl the desired chemical stability (Fig. 1). Previous research has shown that Cu-Chl possesses antioxidant activity against oxidative stress or radiation-generated reactive oxygen species (ROS). It also plays a positive role in protecting DNA against radiation-induced damage (Tumolo and Lanfer-Marquez, 2012). Although certain synthetic pigments have been used to improve quality and green color in plants such as turfgrasses, little is known on the effect of Cu-Chl on crop physiological responses.

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The objective of this study was to investigate effects of two formulations of Cu-Chl and with and without synthetic paraffinic oil (B18-0075 and B18-0074, respectively), applied as soil drench plus foliar spray or foliar spray only, on tomato plant physiological responses and yield under drought stress conditions and examine the mechanisms of Cu-Chl formulation's impact on tomato plants drought stress tolerance.

Materials and Methods

Plant materials and culture. Tomato 'Tiny Tim' seeds were planted in plastic tray with cells (2–3 seeds per cell) filled with potting mix, germinated, and thinned to one plant per cell at first true leaf plant stage. Seedlings were grown in tray cells placed in the Greenhouse for 2 weeks and transplanted into the 6-inch pots (15 cm diameter) filled with silt loam soil (fine-loamy, mixed mesic Typic Hapludult) mixed with a medium particle size sand (2:1, v/v) with equal amounts of soil per pot on 29 Mar. (Zhang et al., 2015). The soil moisture was determined by drying soil at 105 °C for 48 h. Soil moisture was 29.2% at 100% field capacity and 14.6% at 50% field capacity. Low-rate N fertilizer from complete fertilizer (28–8–18) with micronutrients was applied at the time of transplanting at 0.5 $g \cdot m^{-2}$ and biweekly thereafter.

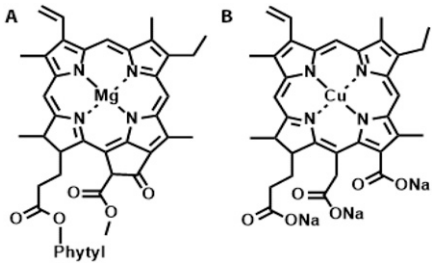

Fig. 1. Chemical structure of (**A**) chlorophyll a, and (**B**) copper chlorophyllin (Cu-Chl).

CU-CHL Application. Two Cu-Chl formulations (B18-0074 and B18-0075) were used for this study. B18-0074 solution was prepared by mixing 0.1% Cu-Chl (Organic Herb, China) with 0.04% emulsifier (B17-Emuls Green) and B18-0075 solution was made by mixing 0.1% Cu-Chl with 0.5% synthetic paraffinic oil and 0.04% emulsifier in distilled water.

The treatments included 1) well-watered control, 2) drought control, 3) B18-0074 applied as soil drench plus foliar application, 4) B18-0075 soil drench and foliar application, 5) B18-0074 foliar application, and 6) B18-0075 foliar application (Fig. 2). For treatments 3 and 4, 20 mL of the formulations were applied to each cell with a tomato seedling 24 h before transplanting and 20 mL water to the rest of the treatments. At the time of transplanting, 60 mL of formulation was applied to the soil in the pots for treatments 3 and 4 and 60 mL of water to the controls and treatments 5 and 6.

First foliar application to treatments 3, 4, 5, and 6 took place 7 d after transplanting, and second foliar application occurred 14 d after transplanting (7 d after first application). Treatments were applied to the foliage uniformly till runoff using a handheld sprayer (≈5 mL per pot).

Drought stress treatment. After transplanting, the plants were subjected to drought stress by deficit irrigation (50% capacity). The pots were dried down and rewatered to 50% capacity when the leave reached wilting point (lower leaves wilt, at ≈45% capacity), repeated. In this way, the plants experienced watering and wilting cycles. Rewatering was managed to take place at 6, 13, 20, and 27 d after transplanting, and samples were collected at 7, 14, and 21, and 28 d (24 h after rewatering). First sampling took place at transplanting as initial measurement. In a separate set of pots (six treatments and four replications), the treated plants were allowed to grow to maturity, and fruit yield was recorded. For the well-watered control, plants were irrigated daily and maintained at 100% field capacity. Soil moisture content was measured with a ThetaProbe soil moisture sensor (Delta-T Devices Ltd, Cambridge, UK) every other day. Moisture retention at –0.01 MPa and –1.5 MPa of the soil was estimated before treatment initiation with a standard pressure plate method (Klute, 1983). Three samples per matric potential were used.

Pressure plate estimates of volumetric soil moisture retention at –0.01 MPa were 21.1% ± 0.21% (standard error) and at –1.5 MPa were 6.2% ± 0.02%. The capacitance-probe measurements of in-pot volumetric moisture (0–8 cm depth) at day 0 averaged 34.2% ± 0.21%. The pots lost moisture uniformly and no significant differences in soil moisture content were measured between treatments (data not shown). Capacitance-probe estimates of in-pot volumetric water content declined during the dry-down cycles as follows: 34.2% ± 0.21% at day 0 and 7.7% ± 0.14% before rewatering.

Sampling and measurements. Normal function leaf blades (top three fully developed compound leaves) were sampled from each pot at transplanting before formulation application to soil, 7, 14, and 21, and 28 d after initial application. Fresh leaf samples were collected for leaf relative water content (RWC) measurement. Additional leaf samples (top three fully developed compound leaves) were collected, frozen with liquid nitrogen, and stored at –80 °C for metabolite analysis. The rest of each sample was dried at 70 °C for 3 d and used for analysis of sugars. Functional leaves from each treatment (four pots) were collected at each sampling date. In addition, plants of each treatment (four replications) were grown to maturity and root and shoot biomass was determined.

Leaf RWC. Leaf RWC was determined using the method described by Zhang et al. (2015). Leaf samples (≈100 mg) were collected and weighed immediately to determine fresh weight (FW). The leaf sample was cut into ≈5 mm sections and placed in a 2-mL microcentrifuge tube with 1.8 mL deionized (d.i.) H_2O. After ≈15 h at 4 °C, the leaf sample was blotted dry and weighed immediately to determine turgid weight (TW). The leaf tissue was then dried at 70 °C for 72 h to determine dry weight (DW). Leaf RWC was calculated following the equation: RWC (%) = [(FW – DW)/(TW – DW)] × 100.

Leaf Pn. Leaf Pn was measured using LI-6400XT portable photosynthetic system with temperature at 26 °C, CO_2 flow rate at 400, and light intensity at 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Chlorophyll A, B, and carotenoids. Chlorophyll from tomato leaf was extracted in acetone. Chlorophyll content was measured using a spectrophotometer according to Zhang et al. (2005).

Leaf malondialdehyde and antioxidants. Cell membrane lipid peroxidation was determined based on malondialdehyde (MDA) content. The MDA was measured according to Wu et al. (2017). Leaf samples (50 mg) were homogenized in 1.8 mL 10% trichloroacetic

Fig. 2. Representative tomato plants from each condition (day 28).

acid and centrifuged at 12,000 g_n for 20 min, then 1 mL 0.6% thiobarbituric acid in 10% trichloroacetic acid was added to 1 mL supernatant. The mixture was heated in boiling water for 30 min then quickly cooled in an ice bath. After centrifugation at 1600 g_n for 10 min, the absorbance of the mixture was determined at 532 and 600 nm. Nonspecific absorbance at 600 nm was subtracted from that at 532 nm. The concentration of MDA was calculated using this adjusted absorbance and MDA's extinction coefficient of 155 $mM^{-1} \cdot cm^{-1}$.

For antioxidant enzyme activity, frozen leaf samples (100 mg) were ground in liquid N2 and extracted in 1.8 mL of ice-cold 50 mmol sodium phosphate buffer (pH 7.0) containing 0.2 mM ethylenediaminetetraacetic acid (EDTA) and 1% polyvinylpyrrolidone in an ice-water bath. The homogenate was centrifuged at 12,000 g_n for 20 min at 4 °C. Supernatant was used to antioxidant enzyme activity.

Activity of CAT was determined using the method of Chance and Maehly (1955) with modifications. For CAT, the reaction solution (1 mL) contained 50 mM phosphate buffer (pH 7.0), 15 mM H_2O_2, and 30 µL of extract. The reaction was initiated by adding the enzyme extract. Changes in absorbance at 240 nm were read in 1 min using a spectrophotometer ($\varepsilon = 39.4\ M^{-1} \cdot cm^{-1}$).

The activity of APX was detected using the method of Zhang et al. (2015). The reaction solution (1 mL) contained 50 mM phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.1 mM EDTA, and 100 µL enzyme extract. The reaction was started with addition of 10 µL 10 mM H_2O_2. The absorbance of the solution was determined at 290 nm after 1 min ($\varepsilon = 2.8\ mM^{-1} \cdot cm^{-1}$).

GSH was analyzed according to Gossett et al. (1994) with some modifications. The supernatant for antioxidant enzyme assays was used for analysis of GSH. We used a microplate to measure GSH based on the change in absorbance during a 15-min reaction period.

Leaf sugar (sucrose and glucose) and proline content. The leaf sucrose and glucose were analyzed according to Zhang et al. (2006). Proline content was determined with the method of Bates et al. (1973) with some modifications. Briefly, leaves (50) were homogenized with 1.8 mL 3% sulfosalicylic acid and boiled at 100 °C for 10 min; 1 mL of the supernatant was mixed with 1 mL acetic acid and 1 mL acidic ninhydrin and heated at 100 °C for 40 min, the reaction mixture was extracted with 2 mL toluene after cooling and read the absorbance at 520 nm.

Leaf ABA and IAA content. Leaf hormones (IAA and ABA) were extracted according to Wu et al. (2017). Leaf tissue of each sample was ground with a mortar and pestle in liquid N_2 to powder, and the sample (50 mg) was extracted in 1.6 mL Na-phosphate buffer (0.05 M, pH 7.0) containing 0.02% sodium diethyldithiocarbamate as an antioxidant, and the hormones were extracted for 1 h at 4 °C with shaking. The C^{13}-IAA (50 ng) was added into each sample as an internal standard. The pH of the samples was adjusted to 2.6 with 1.0 M HCl. The sample was slurried with 150 mg Amberlite XAD-7 (Sigma-Aldrich, St. Louis, MO) for 30 min. After removal of the buffer, the XAD-7 was washed twice with 1 mL of 1% acetic acid before being slurried twice with 1.5 mL dichloromethane for 30 min each at 4 °C (Edlund et al., 1995). The combined dichloromethane fractions were reduced to dryness with nitrogen gas. Then samples were dissolved in 210 µL methanol and diluted with 490 µL d.i. H_2O containing 0.1% formic acid. The samples were filtered using an acrodisc 13-mm syringe filter with a 0.2-mm nylon membrane (Fisher Scientific Company, Pittsburgh, PA).

An Agilent tandem LC-MS/MS system with an ESI sample introduction interface (Agilent, Santa Clara, CA), consisting of 1290 UPLC and 6490 QQQ, was used for analyzing IAA and ABA in extracts. The high-performance liquid chromatography separation was performed on an Agilent Zorbax Extend-C18 analytical (4.6 × 50 mm, 5 µm) and guard (4.6 × 12 mm, 5 µm) columns. The analytes were eluted with water (mobile phase A) and methanol (B) in 0.1% formic acid in a gradient: 0 to 4.5 min B increasing from 30% to 80%, 4.5 to 5 min B increasing to 100%, 5 to 7 min B at 100%, and B decreasing to 30% at 7.5 min. The injecting volume was 10 µL and flow rate was 0.5 $mL \cdot min^{-1}$. The column temperature was 40 °C. The chromatography retention time, precursor ion, fragmental reactions monitored, ionization mode, and collision energies were set up according to Wu et al. (2017). The C^{13}-labeled IAA (IAA_{d5}) was used as an internal standard. The source parameters were: nebulizer pressure 310 kPa, dry gas temperature 250 °C, sheath gas temperature 200 °C, and gas flow 8 $mL \cdot min^{-1}$. The selected hormones were determined on the basis of retention times and ion products.

Twenty-eight days after transplanting plants were harvested. Roots were washed and cleaned, and roots and above-ground tissues were dried at 70 °C for 72 h. Above-ground biomass and root dry weight were determined.

Experimental design and statistical analysis. The six treatments were arranged in a completely randomized block design with four replications. The data were subjected to one-way analysis of variance using SAS software (v.9.3 for Windows; SAS Institute, 2010). The mean separations were performed using the Fisher's protected least significant difference test at the $P = 0.05$ level.

Results

Photosynthetic rate. Drought reduced Pn. Soil drench plus foliar application of B18-0074 and B18-0075 improved Pn at days 7, 14, and 21 (Table 1). Foliar application of the two products also improved Pn at days 14 and 21. The B18-0074 increased Pn by 8.8% with soil plus foliar application and 18.6% with foliar application relative to the control under drought stress at day 21. Similarly, the B18-0075 increased Pn by 16.9% with soil plus foliar application and 24.6% with foliar application relative to the control under drought stress.

Chlorophyll and carotenoids. Tomato plants subjected to drought stress had higher levels of chlorophyll and carotenoids in leaves than plants maintained in well-watered conditions. Foliar application of B18-0075 increased chlorophyll a, b, and a+b as measured at day 28 (Table 2). The treatments did not impact carotenoids content.

Leaf MDA and antioxidant enzyme activity. MDA has been used as a good indicator of lipid peroxidation. The greater MDA, the greater lipid peroxidation. Drought stress increased leaf MDA content. Soil plus foliar application or foliar application of B18-0075 and B18-0074 reduced MDA content as measured at day 28 (Table 3).

Treatments reduced CAT activity as measured at day 14 relative to the drought control (Table 3). Soil plus foliar application of B18-0075 and foliar application of B18-0074 increased CAT activity as measured at day 21 (Table 3). Soil plus foliar application of B18-0075 and B18-0074 increased APX activity relative to the control under drought at day 14. The two products increased GSH content relative to the control at days 14 and 21, regardless of application method (Table 3).

Leaf glucose, sucrose, proline content, and RWC. Drought stress induced accumulation of leaf glucose and sucrose. Soil plus foliar and foliar application of both treatments increased leaf glucose content as measured at days 7, 14, and 21 except for soil plus foliar application of B18-0075 at day 21 and foliar application of B18-0075 at day 7 compared with the control under the drought stress (Table 4). Two treatments increased leaf sucrose as measured at days 21 and 28 except for soil plus foliar application of B18-0075 at day 28 (Table 4). On day 21, treatment B18-0074 increased sucrose content by 90.0% with soil plus foliar application and 104.0% with foliar application compared with the control under drought. Treatment B18-0075 increased sucrose content by 21.2% with soil plus foliar application and 31.1% with foliar application compared with the untreated control under the drought stress.

Drought stress induced accumulation of proline in leaf tissues. Two treatments increased leaf proline content with foliar application but did not with soil plus foliar application relative to the control under drought stress at day 14, 21, and 21 (Table 4).

Drought stress treatment reduced leaf RWC, but the two products did not consistently impact RWC (Table 4).

Leaf ABA and IAA. Drought stress increased ABA and IAA content in leaf tissues. Soil application of the two products increased ABA content at day 7 but reduced ABA content at day 14, 21, and 28 (Table 5). Foliar application of the two formulations increased ABA content at day 21 but reduced ABA

Table 1. Leaf photosynthetic rate (Pn) in tomato plants under drought stress in response to B18-0074 and B18-0075 application.

		Time after initial treatment (day)				
		0	7	14	21	28
No.	Treatment	Pn ($\mu mol \cdot m^{-2} \cdot s^{-1}$)				
1	Untreated (W)	13.01	12.29 a	11.55 a	9.80 ab	16.40 a
2	Untreated (D)		4.83 c	4.51 d	8.34 c	2.52 bc
3	Soil/Foliar B18-0074 (D)		6.38 b	7.14 b	9.07 b	3.17 bc
4	Soil/Foliar B18-0075 (D)		6.38 b	6.85 b	9.75 ab	4.65 b
5	Foliar B18-0074 (D)		5.06 bc	5.62 c	9.89 a	1.44 c
6	Foliar B18-0075 (D)		6.34 bc	6.30 bc	10.39 a	2.72 bc

Means followed by same letters within same column for each data set are not significantly different at $P = 0.05$.
W = watered; D = drought.

Table 2. Leaf chlorophyll a, b, chlorophyll a+b, and carotenoids content in tomato plants under drought stress in response to B18-0074 and B18-0075 application.

		Time after initial treatment (day)				
		0	7	14	21	28
No.	Treatment	Chl a ($mg \cdot g^{-1}$ FW)				
1	Untreated (W)	1.312	1.35 a	1.30 b	1.05 b	0.84 c
2	Untreated (D)		1.46 a	1.63 a	1.52 a	1.26 b
3	Soil/foliar B18-0074 (D)		1.48 a	1.48 ab	1.57 a	1.22 b
4	Soil/foliar B18-0075 (D)		1.45 a	1.52 a	1.47 a	1.18 b
5	Foliar B18-0074 (D)		1.36 a	1.59 a	1.57 a	1.30 ab
6	Foliar B18-0075 (D)		1.43 a	1.66 a	1.52 a	1.48 a
		Chl b ($mg \cdot g^{-1}$ FW)				
1	Untreated (W)	0.476	0.52 b	0.48 b	0.39 b	0.31 c
2	Untreated (D)		0.56 ab	0.61 a	0.58 a	0.48 b
3	Soil/foliar B18-0074 (D)		0.59 a	0.57 a	0.61 a	0.49 b
4	Soil/foliar B18-0075 (D)		0.57 ab	0.58 a	0.56 a	0.46 b
5	Foliar B18-0074 (D)		0.53 b	0.61 a	0.62 a	0.51 ab
6	Foliar B18-0075 (D)		0.54 b	0.64 a	0.59 a	0.58 a
		Chl a+b ($mg \cdot g^{-1}$ FW)				
1	Untreated (W)	1.787	1.87 b	1.78 b	1.45 b	1.16 c
2	Untreated (D)		2.03 ab	2.25 a	2.10 a	1.74 b
3	Soil/foliar B18-0074 (D)		2.07 a	2.04 a	2.18 a	1.71 b
4	Soil/foliar B18-0075 (D)		2.02 ab	2.10 a	2.03 a	1.65 b
5	Foliar B18-0074 (D)		1.87 b	2.21 a	2.19 a	1.81 ab
6	Foliar B18-0075 (D)		1.97 ab	2.30 a	2.10 a	2.06 a
		Carotenoids ($mg \cdot g^{-1}$ FW)				
1	Untreated (W)	0.308	0.28 b	0.30 c	0.23 b	0.18 c
2	Untreated (D)		0.32 ab	0.42 a	0.35 a	0.28 ab
3	Soil/foliar B18-0074 (D)		0.32 ab	0.36 b	0.36 a	0.26 b
4	Soil/foliar B18-0075 (D)		0.33 a	0.37 ab	0.33 a	0.25 b
5	Foliar B18-0074 (D)		0.28 b	0.38 ab	0.36 a	0.29 ab
6	Foliar B18-0075 (D)		0.29 ab	0.41 a	0.34 a	0.33 a

Means followed by same letters within same column for each data set are not significantly different at $P = 0.05$.
W = watered; D = drought.

content at day 28 compared with drought control.

Soil plus foliar application of the two products reduced IAA content as measured at days 7, 14, 21, and 28, except for foliar B18-0075 at day 21 and soil plus foliar application of B18-0075.

Plant shoot and root biomass. Drought stress reduced shoot biomass but did not affect root biomass as measured at 28 d after transplanting (Fig. 2). Soil plus foliar and foliar application only of B18-0075 increased root biomass (Table 6). The two formulations did not significantly affect shoot biomass (Table 6).

Discussion

Petroleum-based agricultural oils have been applied as dilute sprays on plants to control insects, mites, and diseases for many years. They are also sometimes included in tank mixes as an adjuvant. The results of this study indicated that both formulations improved Pn, and the combination of Cu-Chl with synthetic paraffinic oil (B18-0075) had greater benefits. In addition, it also improved chlorophyll content under drought stress conditions. A previous study showed that other synthetic pigments could protect chlorophyll against ultraviolet B radiation and improve turfgrass quality (Schmidt and Zhang, 2001). Under drought stress, plants may accumulate toxic ROS, which damages cell membrane lipid, protein, and DNA, resulting in a decline in photosynthetic function and leaf senescence. Cu-Chl possesses antioxidant activity and may protect plants against ROS-induced injury to photosynthetic apparatus (Tumolo and Lanfer-Marquez. 2012). The results of this study also showed that Pn declined sharply from day 21 to day 28, and Cu-chl treatments had more consistent beneficial effect on Pn at day 14 and 21 relative to day 28 when plants experienced severe drought stress. This suggests that Cu-Chl treatments may have greater beneficial effects in alleviating Pn decline under relatively mild drought (days 14 and 21) relative to severe drought (day 28) when photosynthetic function was severely damaged.

The results of this study also showed that the two formulations improved antioxidant enzyme (CAT and APX) activity and metabolite GSH content and reduced lipid peroxidation (lower MDA) in tomato plants under drought stress. The increased antioxidant capacity by application of the two formulations may delay leaf senescence and improve photosynthetic function under drought stress conditions. Schmidt and Zhang (2001) noted that certain synthetic pigments increased photochemical efficiency and improved drought and ultraviolet B tolerance of Kentucky bluegrass. The results of this study also showed that Cu-Chl had greater effects in enhancing GSH content at day 14 and 21 relative to day 28. This suggests that Cu-Chl may have better effects on GSH under relatively mild drought (day 14 and day 21) than under severe drought (day 28) possibly due to

Table 3. Leaf catalase (CAT), ascorbate peroxidase (APX), glutathione (GSH), and malondialdehyde (MDA) of tomato plants in response to B18-0074 and B18-0075 application under drought.

		Time after initial treatment (day)				
		0	7	14	21	28
No.	Treatment	CAT (nmol·g^{-1} FW min^{-1})				
1	Untreated (W)	224.5 a	219.0 b	337.6 b	307.0 a	333.0 bc
2	Untreated (D)	214.5 a	267.8 ab	388.7 a	189.7 b	352.4 abc
3	Soil/foliar B18-0074 (D)	223.1 a	249.4 ab	347.3 b	249.4 ab	255.2 c
4	Soil/foliar B18-0075 (D)	226.7 a	254.7 ab	199.1 c	304.3 a	385.4 abc
5	Foliar B18-0074 (D)	215.7 a	275.5 a	234.5 c	300.0 a	489.6 a
6	Foliar B18-0075 (D)	221.4 a	293.7 a	228.9 c	264.2 ab	412.1 ab
		APX (μmol·g^{-1} FW min^{-1})				
1	Untreated (W)	10.5 a	9.9 b	13.5 b	12.2 a	11.0 ab
2	Untreated (D)	11.2 a	11.3 a	10.7 d	10.9 a	8.4 abc
3	Soil/foliar B18-0074 (D)	10.6 a	10.6 ab	10.7 d	12.0 a	7.7 bc
4	Soil/foliar B18-0075 (D)	12.1 a	10.0 b	15.0 a	11.8 a	12.0 a
5	Foliar B18-0074 (D)	10.6 a	11.5 a	11.9 c	11.2 a	7.6 bc
6	Foliar B18-0075 (D)	9.9 a	9.8 b	10.8 d	11.5 a	4.3 c
		GSH (nmol·g^{-1} FW)				
1	Untreated (W)	245.6 a	344.8 b	238.3 e	326.7 cd	245.3 d
2	Untreated (D)	224.5 a	535.8 a	349.1 d	305.1 e	312.6 b
3	Soil/foliar B18-0074 (D)	265.7 a	567.2 a	616.0 a	340.2 b	302.7 bc
4	Soil/foliar B18-0075 (D)	255.6 a	513.8 a	517.9 c	407.3 a	308.1 bc
5	Foliar B18-0074 (D)	251.3 a	528.3 a	621.0 a	331.5 bc	485.2 a
6	Foliar B18-0075 (D)	245.6 a	548.4 a	560.0 b	316.3 d	296.4 c
		MDA (nM·g^{-1} FW)				
1	Untreated (W)	12.1 a	12.7 a	13.4 c	14.6 b	14.6 e
2	Untreated (D)	11.9 a	12.5 a	19.0 a	24.5 a	35.1 a
3	Soil/foliar B18-0074 (D)	12.4 a	13.2 a	16.6 b	25.3 a	26.8 c
4	Soil/foliar B18-0075 (D)	12.0 a	13.5 a	12.5 c	25.6 a	24.1 d
5	Foliar B18-0074 (D)	11.5 a	12.9 a	18.6 a	23.3 a	29.0 b
6	Foliar B18-0075 (D)	10.9 a	12.8 a	12.9 c	23.5 a	28.4 b

Means followed by same letters within same column for each data set are not significantly different at $P = 0.05$.
W = watered; D = drought; FW = fresh weight.

Table 4. Leaf relative water content (RWC) and proline, glucose, and sucrose content of tomato plants in response to B18-0074 and B18-0075 application under drought.

		Time after initial treatment (day)				
		0	7	14	21	28
No.	Treatment	RWC (%)				
1	Untreated (W)	89.0 a	89.3 a	88.9 a	88.8 a	88.7 a
2	Untreated (D)	88.6 a	88.1 b	85.1 c	81.2 c	79.1 c
3	Soil/foliar B18-0074 (D)	89.5 a	89.0 ab	86.3 bc	82.9 bc	81.8 b
4	Soil/foliar B18-0075 (D)	89.2 a	88.2 b	85.6 c	82.4 bc	81.6 bc
5	Foliar B18-0074 (D)	88.5 a	88.9 ab	88.1 ab	82.9 bc	81.3 bc
6	Foliar B18-0075 (D)	90.5 a	88.9 ab	86.0 c	83.1 b	81.5 bc
		Proline (μg·g^{-1} FW)				
1	Untreated (W)	86.7 a	85.2 b	91.0 d	105.3d	110.1 c
2	Untreated (D)	87.1 a	101.6 ab	99.4 d	269.4c	228.4 b
3	Soil/foliar B18-0074 (D)	79.8 a	96.9 b	103.2 d	267.5c	239.1 b
4	Soil/foliar B18-0075 (D)	91.2 a	100.8 ab	148.0 c	254.4c	240.5 b
5	Foliar B18-0074 (D)	89.8 a	110.1 ab	335.0 b	484.0a	378.2 a
6	Foliar B18-0075 (D)	85.7 a	116.8 a	371.9 a	375.8b	378.4 a
		Glucose (mg·g^{-1} DW)				
1	Untreated (W)	12.1	12.2 c	12.6 c	12.5e	14.1 c
2	Untreated (D)		12.2 c	7.2 e	15.8d	40.5 a
3	Soil/foliar B18-0074 (D)		26.2 ab	14.1 b	18.7c	37.4 a
4	Soil/foliar B18-0075 (D)		23.4 b	25.1 a	16.7d	24.4 b
5	Foliar B18-0074 (D)		26.8 a	13.1 c	30.8a	41.5 a
6	Foliar B18-0075 (D)		11.9 c	10.0 d	24.7b	30.3 b
		Sucrose (mg·g^{-1} DW)				
1	Untreated (W)	10.7	11.3 a	2.6 e	2.8e	7.0 d
2	Untreated (D)		6.2 c	12.5 a	9.0d	13.1 c
3	Soil/foliar B18-0074 (D)		12.5 a	7.7 c	17.1b	21.6 a
4	Soil/foliar B18-0075 (D)		8.1 b	8.6 b	10.9c	11.5 c
5	Foliar B18-0074 (D)		2.6 d	7.6 c	18.4a	16.4 b
6	Foliar B18-0075 (D)		4.1 d	3.3 d	11.8c	22.1 a

Means followed by same letters within same column are not significantly different at $P = 0.05$.
W = watered; D = drought; FW = fresh weight; DW = dry weight.

severe damage of photosynthetic function and limitation of GSH biosynthesis at day 28.

Plants undergo osmotic adjustment to cope with drought stress and sustain growth. Soluble sugar and proline are two of the important metabolites for osmotic adjustment. The results of this study indicated that the two formulations increased leaf sugar (glucose and sucrose) and proline content regardless of application methods. This suggests that the Cu-Chl in combination with synthetic paraffinic oil may improve osmotic

Table 5. Tomato leaf abscisic acid (ABA) and indole-3-acetic acid (IAA) content as affected by application of B18-0074 and B18-0075 under drought.

		Time after initial treatment (day)				
		0	7	14	21	28
No.	Treatment	ABA (ng·g^{-1} FW)				
1	Untreated (W)	120.5	125.2 b	115.0 e	56.9 e	47.3 e
2	Untreated (D)		125.8 b	243.9 ab	454.1 b	264.7 a
3	Soil/foliar B18-0074 (D)		192.7 a	257.5 a	365.4 c	195.1 c
4	Soil/foliar B18-0075 (D)		173.9 a	187.2 d	181.5 d	196.3 c
5	Foliar B18-0074 (D)		91.9 b	220.3 c	541.3 a	220.1 b
6	Foliar B18-0075 (D)		104.0 b	230.4 bc	545.2 a	143.4 d
		IAA (ng·g^{-1} FW)				
1	Untreated (W)	3.3	3.1 a	3.8 d	5.6 e	4.7 d
2	Untreated (D)		3.2 a	7.2 a	17.4 b	12.6 a
3	Soil/foliar B18-0074 (D)		2.5 b	5.3 c	12.9 c	9.5 b
4	Soil/foliar B18-0075 (D)		2.4 b	5.4 c	13.1 c	12.1 a
5	Foliar B18-0074 (D)		1.1 d	3.5 d	10.9 d	8.6 bc
6	Foliar B18-0075 (D)		1.4 c	6.6 b	21.0 a	7.5 c

Means followed by same letters within same column are not significantly different at $P = 0.05$.
W = watered; D = drought.

Table 6. Tomato plant shoot and root biomass as affected by application of B18-0074 and B18-0075 under drought.

		Biomass (g)	
		Shoot	Root
No.	Treatment	Day 28	
1	Untreated (W)	5.56 a	0.827 ab
2	Untreated (D)	3.45 b	0.754 b
3	Soil/foliar B18-0074 (D)	3.20 b	0.836 ab
4	Soil/foliar B18-0075 (D)	3.26 b	0.848 a
5	Foliar B18-0074 (D)	3.13 b	0.790 ab
6	Foliar B18-0075 (D)	3.42 b	0.870 a

Means followed by same letters within each column are not significantly different at $P = 0.05$.
W = watered; D = drought.

adjustment by increasing soluble sugars and amino acids (e.g., proline) under drought stress. Zhang et al. (2015) reported that perennial ryegrass cultivar with higher proline had greater drought tolerance than that with lower proline under drought stress.

In addition, the two formulations increased ABA at certain sampling date but decreased ABA at late stage of drought (day 28). They also reduced IAA content. The treated plants with lower levels of ABA may have better stomatal conductance (stomata keeping partial opening), facilitating gas exchange and avoiding oxidative injury under prolonged drought. Zhang et al. (2009) noted that maize with lower ABA under drought had greater photochemical efficiency and yield under drought stress.

The results of this study indicated that soil plus foliar and foliar application only of B18-0075 increased root biomass. The two formulations did not affect shoot biomass. This suggests that application of B18-0075 may improve root growth, but both formulations did not consistently affect shoot growth of tomato plants. In all cases, addition of synthetic paraffinic oil provided greater protection. Synthetic oils may trigger induced systemic resistance responses, which may provide better protection against abiotic stresses (Hsiang et al., 2013).

In summary, this study demonstrates that application of Cu-Chl in combination with synthetic paraffinic oil may improve photosynthetic function, osmotic adjustment, and antioxidant defense capacity, as well as root growth of tomato plants grown under drought stress conditions.

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