

EFFECTS OF WATER DEFICIT ON THE GROWTH AND CHLOROPHYLL CONTENT OF *Capsicum Frutescens*

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Abstract: Water stress can have major impacts on plant growth through the unbalanced formation of reactive oxygen species (ROS). High production of ROS during water stress can reduce crop yield, thus affecting productivity. Thus, this study determines the effect of water deficit on growth and chlorophyll content of *Capsicum frutescens*. The *Capsicum frutescens* were treated with 0, 20, 40, 60, 80 and 100 mL of water for 20 days. The growth and chlorophyll content were measured at 0, 5, 10, 15, and 20 days of treatment periods. Water stress did not significantly affect the plant height and stem diameter of the *C. frutescens* subjected to different volumes of water. The total number of leaves, leaf area, as well as fresh and dry weights were unaffected at early stages. However, all parameters increased significantly at the end of the treatment, especially in plants treated with 80 and 100 mL of water. The chlorophyll content was unchanged at earlier stages of the experiment. However, the chlorophyll content reduced drastically at later stages of the experiment period. The results reveal that different volumes of water did not significantly affect the growth of *C. frutescens*. In contrast, the chlorophyll content of *C. frutescens* was significantly decreased by water stress.

Keywords: Water stress, reactive oxygen species, *Capsicum frutescens*, growth, chlorophyll content.

Introduction

Reactive oxygen species (ROS) are considered as by-products of plant aerobic metabolism and are generated in several cellular compartments, especially the chloroplast, mitochondria and peroxisomes (Dietz *et al.*, 2016). Under normal conditions, ROS are present in small amounts and well-ordered levels. However, under unfavourable biotic and abiotic circumstances, plants generate huge numbers of ROS, resulting in profound or irreversible effects on the cellular redox state, which can eventually lead to oxidative stress (Nita & Grzybowski, 2016). Oxidative stress is a common phenomenon in organisms exposed to water stress, as well as many other abiotic stresses, thus adversely limiting the development of tissues and organs, often leading to abnormal plant growth, subsequently affecting the productivity of crop plants. It is a universal problem, restricting the global crop production seriously, and recent global climate change has made this situation more serious (Hammad & Ali, 2014). Currently,

it is documented that drought have significantly reduced the yield of soybean (33.1–12.2%), maize (11.6%) and wheat (9.2%) (Matiu *et al.*, 2017).

Plant encounters drought stress when water lost exceeds the water uptake or when the water supply to the roots is disturbed. Plants have developed a wide range of mechanisms to adapt and maintain survival and productivity under water stress. These responses comprise complex mechanisms, inducing various morphological, biochemical, physiological, and molecular aspects resulting in either drought avoidance, escape or drought tolerance, and the mechanism is highly varied among plant species (Lee *et al.*, 2005).

Growth of plants that reflects water stress can be measured through many different parameters, such as fresh weight, stem diameter and leaf area. A previous study by Chartzoulakis *et al.* (2002) demonstrated that the total plant leaf area and its dry weight were reduced by more

than 50% when the plants experienced moderate water stress. Changes in the leaf morphological and anatomical characteristics induced by water deficit can also be observed in avocado plants. Water stress can also influence the chlorophyll content of the plants, as reported in *Glycine max* (Masoumi *et al.*, 2011) and *Triticum aestivum* (Moaveni, 2011).

Chilli pepper (*Capsicum frutescens*) from the family Solanaceae is one of the important commercial crops in Malaysia, with a total production of 35,695 mt in 2018 (DOA, 2018). Approximately 20 wild chilli species have been documented (Nadeem *et al.*, 2011) to be highly in demand and consumed by the worldwide community in terms of daily cooking, either for fresh consumption or processed into pickles, spice or sauce. It has valuable source of antioxidants, as well as vitamins, especially vitamins A, B and C. They even contain potassium, iron, magnesium and folic acid, which are good for health, and are low in calories, sodium, protein and carbohydrate (Sahitya *et al.*, 2018). The pungency of chilli is caused by capsinoids, including major amounts of capsaicin and dehydrocapsaicin. On top of that, it's also equipped with wide arrays of compounds exhibiting anti-inflammatory, anti-

allergic, anti-carcinogenic and antioxidative properties (Lee *et al.*, 2005).

The aim of this study is to determine the effect of water deficit on the growth and chlorophyll content of *C. frutescens*. Base on previous studies, it is hypothesised that both the growth and chlorophyll content of *C. frutescens* will decrease as the water treatments decrease. Therefore, this study will help scientists and farmers increase crop productivity under adverse conditions by providing new information on the effect of water deficit on *C. frutescens*. A better understanding of plant adaptation to water stress may help enhance irrigation management practices.

Materials and Methods

Experimental Design and Field Layout

Experimental design and field layout were set up based on the method by Owusu-Sekyere *et al.* (2010). The Randomised Complete Block Design (RCBD) was used, with six treatments, labelled T1 to T6. One hundred fifty (150) polybags were filled with sandy loam soil (500 g). A total of 30 boxes (0.45m x 0.45m) were used. Each box contains 5 polybags. Figure 1 shows the experimental design and field



Figure 1: The experimental design and field layout of the *C. frutescens* study

layout of the *C. frutescens* using RCBD in a greenhouse. Five replicates from each treatment were chosen using a random table to assay the chlorophyll content and growth measurements.

Plant Materials

Seeds of *C. frutescens* were obtained from Pertubuhan Peladang Kuala Terengganu, Terengganu, Malaysia. A total of 200 seeds of *C. frutescens* were germinated under the greenhouse. Each polybag (17 cm x 13 cm x 12.5 cm) contained a single seed and was watered with a constant volume of 100 mL each morning, every day for a month.

Plant Treatments

A total of 150 two-month-old plants with fully expanded young leaves were treated with 6 different volumes of water: 100, 80, 60, 40, 20 and 0 mL. An amount of 100 mL water was used for the control group. Five replicates of the plants were randomly selected for 5 different assessment times for each water treatment. The chlorophyll content and growth parameters were measured at 0, 5, 10, 15, and 20 days of the experiment period.

Determination of Chlorophyll Content

The chlorophyll content was determined based on the method by Harbone (1984). Under dim light and over ice, 0.15g of *C. frutescens* leaf was grounded in a mortar and pestle with 3.0 mL of 80% v/v acetone. The homogenate was centrifuged at 10,000 rpm for 10 minutes. Then, the absorbance of the supernatant was measured at 663 nm and 645 nm using a spectrophotometer (Shimadzu UV-1601). The total chlorophyll content was calculated using the following formula:

$$\text{Total chlorophyll} = \frac{20.4 A_{645} + 8.67 A_{633} \times v}{\alpha \times 1000 \text{ mL} \times w}$$

where: w = leaves weight (g); v = acetone volume (mL) and $\alpha = 1.0$

Growth measurements

Growth was represented by plant height, stem diameter, number of leaves, and leaf area, as well as fresh and dry weight measurements. The growth of *C. frutescens* were determined at 0, 5, 10, 15, and 20 days of treatment.

Plant Height and Stem Diameter

The plant height was measured from the soil surface to the top of the plant using a measuring tape. The point of measurement for the stem diameter was at the middle part from the base of the main stem. The stem diameter was measured using an electronic digital caliper.

Number of Leaves

The number of leaves was determined by counting the number of total leaves on each of the plants for each treatment.

Leaf Area

The leaf area was measured using five randomly selected leaves from each plant. The length, or the longest part along the petiole line of the leaf (Figure 2a), and the width (widest breath across the leaf) (Figure 2b) were measured using a 30 cm ruler (Owusu-Sekyere *et al.*, 1990). The leaf area was calculated using the following formula:

$$\text{Leaf Area (LA)} = \alpha \times L \times W$$

Where: L = leaf length (cm); W=leaves and $\alpha = 0.75$ (factor used for determination of leaf area in *Capsicum*)

Fresh and Dry Weights of Leaves

Five leaves from each plant were selected from each treatment. The leaves were immediately weighed, and the data was recorded. Then, the samples were dried in the oven at 80°C for 2 days or until their final weight was constant to determine their dry weight.

Statistical Analysis

The data was analysed using the SPSS software (Version 25) and the data was expressed as mean

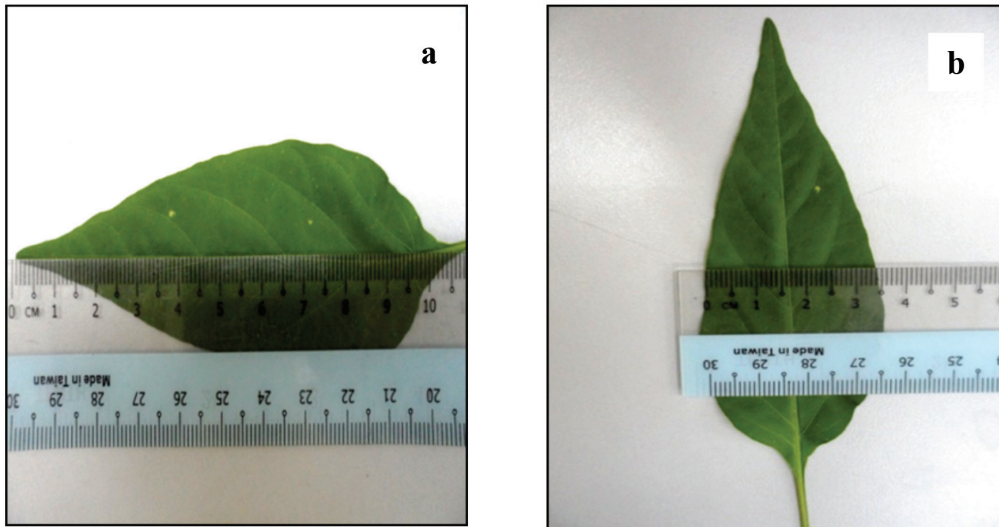


Figure 2: Length (a) and width (b) of the leaf

± standard error. Comparison of the growth and chlorophyll content of *C. frutescens* among the different water treatments and days of treatments were tested using two-way ANOVA at $\alpha=0.05$ as the significant level.

Results

Effect of different volumes of water on the growth of *C. frutescens*

Plant Height

Figure 3 shows that there was no significant difference ($p>0.05$) in plant height between treated and control plants at early stages of the treatment period. On day 10, plants treated with 0, 20 and 40 mL of water were significantly higher ($p<0.05$) in terms of plant height compared with other treated plants. However, after 10 days, the plant height in plants treated with 0 and 20 mL of water was reduced significantly ($p<0.05$) and was unaffected at 20 days.

Stem Diameter

There was no significant difference ($p>0.05$) observed in all treated and control plants throughout the treatment period (Figure 4). The mean for stem diameter ranged from 4.7 ± 0.33 to 6.3 ± 0.88 mm.

Number of Leaf

The total number of *C. frutescens* leaves were not significantly affected ($p>0.05$) in the treated and control plants at the early stages of treatment period. The number of leaves increased significantly ($p<0.05$) after 10 days, especially in plants treated with 100 mL of water with values of 16.7 ± 0.88 . The leaf number doubled up to 32.3 ± 1.76 at 20 days of treatment period (Figure 5).

Leaf Area

A similar pattern was observed in leaf area, where it was unchanged at the early stages of the treatment period. However, after 15 days, the leaf area of the control plants increased significantly ($p<0.05$) with a maximum value of 158.84 ± 10.32 cm², followed by plants treated with 80 mL of water, with a value of 140.85 ± 14.01 cm² (Figure 6).

Fresh and Dry Weights

No significant difference ($p>0.05$) was observed in fresh and dry weights of *C. frutescens* in the treated and control plants up to 15 days of treatment. After 15 days, the fresh weight shot up to 2.56 ± 0.08 g in the control plants and 2.30 ± 0.12 g in the plants treated with 80 mL of water (Figure 7). Meanwhile, dry weight at

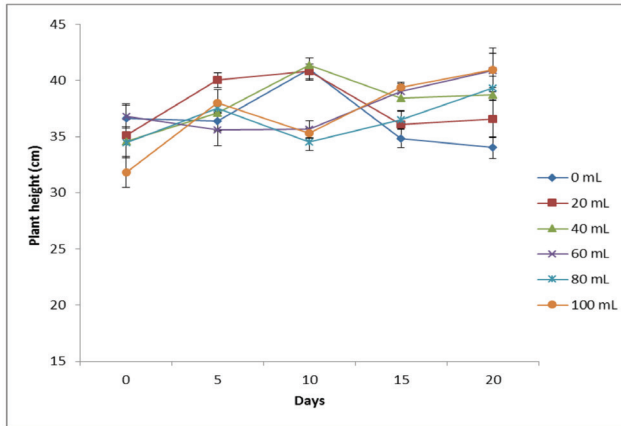


Figure 3: Changes in plant height of *C. frutescens* treated with different volumes of water. Data are presented as mean \pm standard error (n=5)

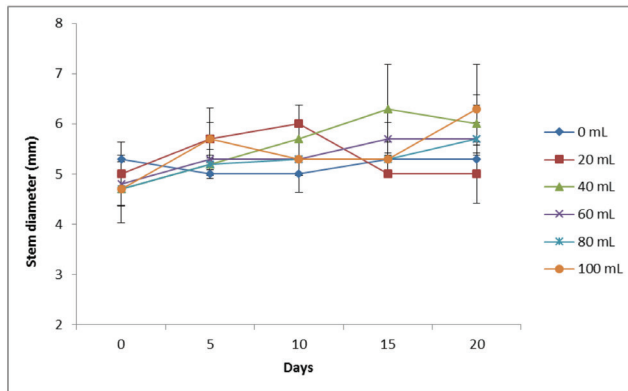


Figure 4: Changes in stem diameter of *C. frutescens* treated with different volumes of water. Data are presented as mean \pm standard error (n=5)

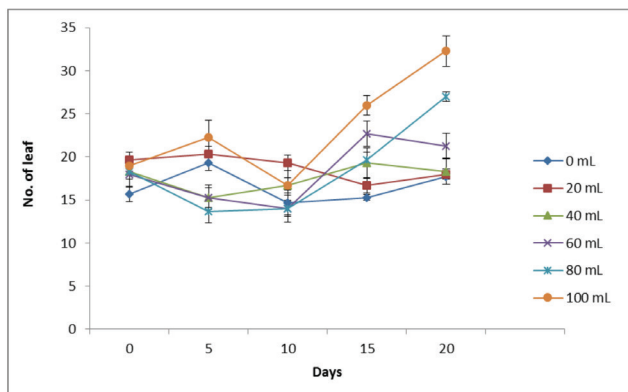


Figure 5: Changes in number of leaves of *C. frutescens* treated with different volumes of water. Data are presented as mean \pm standard error (n=5)

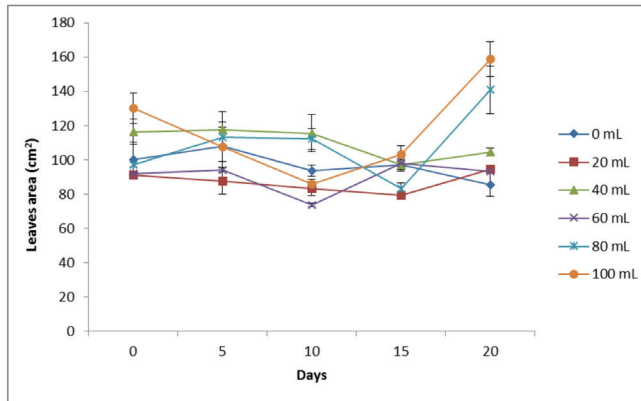


Figure 6: Changes in leaf area (cm²) of *C. frutescens* treated with different volumes of water. Data are presented as mean \pm standard error (n=5)

later stages of the experiment was the highest in plant treated with 80 mL of water with a value of 0.55 ± 0.05 g, followed by plants treated with 60 mL of water with a value of 0.32 ± 0.06 g. No significant difference ($p > 0.05$) was observed in the other treated plants (Figure 8).

Effect of Different Volumes of Water on the Chlorophyll Content of *Capsicum frutescens*

The results show that there was no significant difference ($p > 0.05$) in the chlorophyll contents of the *C. frutescens* in treated and control groups up to 15 days of experiment. At the end of the treatment period, the chlorophyll content increased significantly ($p < 0.05$) in plants treated with 100 and 80 mL of water. The chlorophyll

contents in the other treated plants reduced significantly by almost 80% at 20 days of treatments (Figure 9).

Discussion

Effect of Different Volumes of Water on the Growth of *C. frutescens*

In this study, the growth of *C. frutescens* was assessed by measuring plant height, stem diameter, number of leaves, leaf area, as well as fresh and dry weights. The height and stem diameters of *C. frutescens* did not show much difference throughout the experimental period. However, lower volumes of water inhibit the plant height at later stages of the experiment.

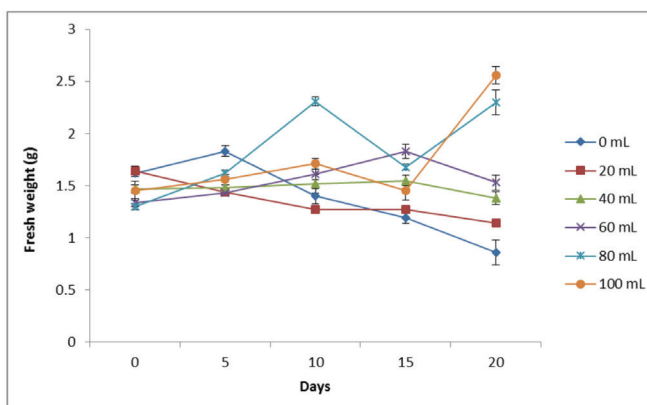


Figure 7: Changes in fresh weight (g) of *C. frutescens* treated with different volumes of water. Data are presented as mean \pm standard error (n=5)

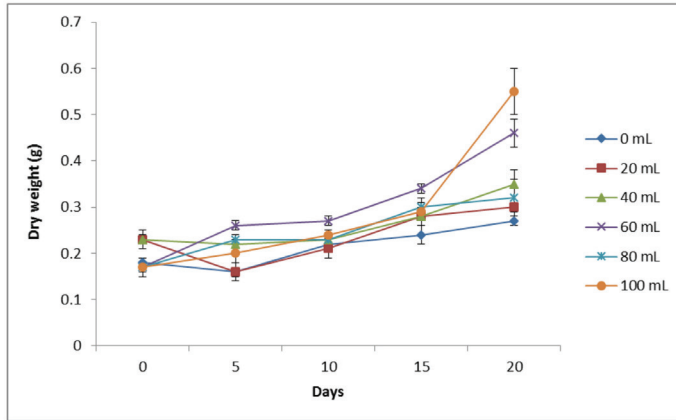


Figure 8: Changes in dry weight (g) of *C. frutescens* treated with different volumes of water. Data are presented as mean \pm standard error (n=5)

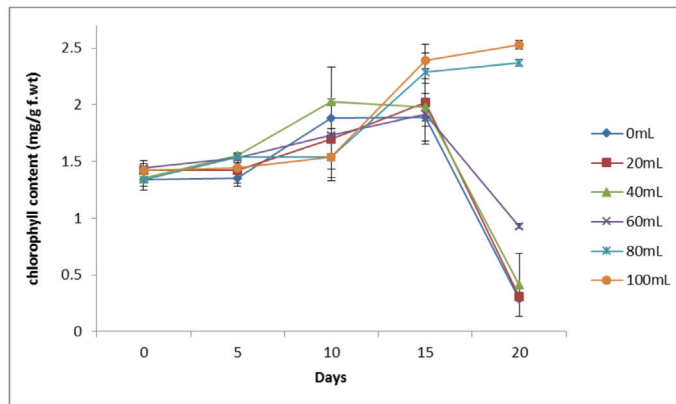


Figure 9: Changes in chlorophyll content (mg/g fwt) of *C. frutescens* treated with different volumes of water. Data are presented as mean \pm standard error (n=5)

As stated by Din *et al.* (2011), plant growth is controlled by several factors, but water plays a major role, in which a small decrease in water availability to growing plants immediately reduces its metabolic and physiological functions. Variations in plant height among genotypes might also be a sign that different genotypes have different water requirements for their physiological and biological processes (Zubaer *et al.*, 2007). Aliche *et al.* (2020) postulated that stem growth reduction during water stress may also serve an advantageous purpose for the plants in order to reduce transport distance. This suggests that stem growth reduction under limited water availability can

facilitate efficient water, nutrient and assimilate transports in plants.

On the other hand, in this recent study, stem diameter was similar to each other during the period of treatment. As proposed by Brian (1988), the effect of water deficit on plant growth can have both short- and long-term effects. Processes, such as stomata opening or closure, can be observed within minutes, whereas leaf expansion (leaf area) may be affected over a period of months. Stem diameter was also included as long-term effect of water deficit. The growth of the stem diameter was strongly modulated by both cell division and cell expansion (Skirycz & Inze, 2010). The results

obtained in this study were in accordance with the results of Perrier *et al.* (2017) and Fracasso *et al.* (2016). They found that water deficit did not significantly affect the diameter of Sorghum internodes. The present study is not in line with some other studies of Zheng *et al.* (2016), who reported that stem diameter in rice plant was reduced proportionally to drought intensity and contributed to the reduction of stem biomass.

The number and total leaf area was not totally diverse in range, but the control plants and plants treated with 80 mL of water showed an increase in both parameters at the end of the experimental period. Water deficit induced a reduction in leaf area expansion by leaf wilting and rolling. This occurs as a result of cell losing its rigidity due to insufficient soil moisture, less growth and health (Zobayed *et al.*, 2007). Reduced turgor pressure in the leaves is associated with the interruption in water flow from the xylem to other elongating cells. As a result, the decreased rate of both cell division and elongation may possibly cause a smaller leaf area, while slow leaf initiation rate leads to reduced number of leaves. These phenomena were also spotted in other crop plants as a response to chilling and drought stress (Taiz & Zeiger, 2006; Lukatkin *et al.*, 2012; Jouyban *et al.*, 2013).

Leaves constitute the most important part of the plant body, as it is where photosynthesis takes place. The photosynthates produced are subsequently translocated to all different parts of a plant. Thus, the growth and development, as well as the productivity, of a plant are greatly determined by the active growth of the leaves (Hussain & Ali, 2015). The leaf growth of *C. frutescens* was related to the water received as the fresh and dry weights of the leaves decreased with the treatment of 0 mL. Water deficit inhibited the uptake of nitrogen, phosphorus and potassium within the plant (Kirnak *et al.*, 2001), which contributed to the changes in the fresh weight of the plant. Compared with other treated plants, leaves in the control plants had increases in both fresh and dry weights. Decreases in leaves fresh and dry weights might be initial

evidence of water stress symptoms as observed in Superior Seedless and Razaki grape cultivars (Kamiloglu *et al.*, 2014). Similarly, Hussain and Ali (2015) proved that the effect of water stress is initially reflected in the leaves, thus affecting the overall growth and development of a plant. Abdalla and El-Khoshiban (2007) also reported that two *Triticum aestivum* cultivars were found to be affected by water stress due to the reduction in the fresh and dry weights of the shoots. They attributed the decline in fresh weight to the decrease in the water contents of stressed plant cells and tissues, which lose their turgor and thus shrink. In addition, Cakir (2004) also reported that there was a significant dry weight loss in plants under water stress due to stem internodes elongation, delayed ear and ovule development, which reduced the leaf area, grain yield and starch accumulation in endosperm. Water stress did not significantly affect the fresh and dry weights of *C. frutescens* in the earlier treatment period, owing to the irrigation at the beginning of the intensive growth stage, thus exhibiting an increase in plant weight that resulted from a rapid process of biomass accumulation.

Effect of Different Volumes of Water on the Chlorophyll Content of *Capsicum frutescens*

Our study also discovered a positive effect between water treatment and chlorophyll content, especially in 40 to 100 mL treated plants. Chlorophyll content is a good indicator in determining the drought tolerance trait, which specifies photosynthetic efficiency under water stress. Guo *et al.* (2016) proposed that the decrease in photosynthetic activity is related to the decrease in chlorophyll content of *Lycium ruthenicum* Murr. seedlings subjected to drought stress. Pirzad *et al.* (2011) also reported that increasing intensity of drought stress managed to reduce the chlorophyll concentration in seedlings of *Matricaria chamomilla*. Similar to other plants, severe stress limit the photosynthesis rate of *C. frutescens*. At the same time, starch begins to accumulate, leading to a loss in enzyme activities involved in photosynthesis. Besides, water stress also directly inhibits metabolism

by limiting the CO₂ entry into the leaf, therefore slowing down the carbon fixation rate (Farooq *et al.*, 2009). Hence, a decrease in Rubisco activity, alterations in the photosynthetic pigments, reduced photosynthetic electron transport components that can potentially reduce the molecular oxygen, resulting in the production of ROS, which are deleterious to photosynthetic apparatus (Reddy *et al.*, 2004; Basu *et al.*, 2016).

Water stress is the common ruthless abiotic factor that usually happens in tropical regions, which have high humidity and receive heavy rain throughout the year. Water stress can be induced in two ways, either by water deficit or excess water logging. For example, severe floods may occur during the rainy season, which would generate high concentrations of ROS in plants, which can bring a huge impact on the economic and agricultural sector due to crop production being limited. Thus, plants that live in tropical regions like *C. frutescens* need to develop their own defense mechanism against ROS action (Alsher *et al.*, 2002). Water stress contributes to the excess formation of ROS in treated plants. The imbalanced production of ROS, which is highly reactive and toxic, may lead to oxidative damage, universally known as oxidative stress. The impacts of oxidative stress to plants vary. Oxidative stress usually can decrease plant growth and the chlorophyll content of affected plants.

Conclusions

The above results indicated that treatment with different volumes of water did not significantly affect the growth of *C. frutescens*. In contrast, the chlorophyll content of *C. frutescens* was notably lowered by water stress, especially at the later stages of the treatment period. The data obtained demonstrated that a deficit in irrigation or water supply is lowered below the maximum levels and mild stress is permitted with nominal effects on the growth of *C. frutescens*. It is suggested that such practice is cost-effective, allowing an optimal use of allocated water, helping farmers to maximise the yields. Further research on the molecular aspects of the response

of *C. frutescens* to water stress should be done to increase the understanding of the plant metabolism in water stress as there are other factors affecting the growth and photosynthesis activities of this plant.

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