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Effect of Watering Regimen on Growth and Phytochemical Constituents of *Amaranthus cruentus*

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Abstract
The phytochemical constituents of plants may be affected by the growing condition of the plant. This study investigated the influence of different watering regimen (WR) on the phytochemical constituents of Amaranthus cruentus. The treatments were control, 2 day (2WR), 4 day (4WR), and 8 day (8WR). The vegetable was grown for 45 days and the plant parts were harvested for growth and phytochemicals analysis. The number of leaves was counted, the height was measured and the biomass was calculated. The flavonoids were determined using Aluminum chloride colorimetry method while the phenolics were determined using Folin chiocalteu method. The results indicated that 2WR significantly enhanced the accumulation of both flavonoids (51.03 ± 1.2 µg/g QE FW) and phenolics (84.11 ± 2.9 µg/g GAE FW) compared to other treatments. The biomass, plant height and number of leaves were higher in 2WR while 8WR have the lowest growth measurement. These findings highlight the importance of precise water management for optimizing the phytochemicals and growth of the plant.

Keywords: *Amaranthus cruentus*, flavonoids, phenolics, phytochemicals, watering regimen.

INTRODUCTION
Amaranthus cruentus (red amaranth or African spinach), a highly nutritious plant, has gained significant attention in recent years due to its rich content of bioactive compounds, including flavonoids and phenolics (Spórna-Kucab *et al*., 2023). These compounds have been linked to various health benefits, such as antioxidant (Spórna-Kucab *et al*., 2023), anti-malarial, antiviral (Engelhardt *et al*., 2023), antioxidant (Engelhardt *et al*., 2023; Madaram *et al*., 2023) and anti-

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cancer properties (Spórna-Kucab *et al*., 2023). The nutritional quality and phytochemical profile of amaranth are influenced by several factors, including soil type, fertilization, and most importantly, water availability (Netshimbupfe *et al*., 2023).

Water plays a crucial role in plant growth and development, affecting physiological processes such as photosynthesis, nutrient uptake, and secondary metabolite production (Bhatla & Lal *et al*., 2023). While optimal water conditions are essential for maximizing yield and quality, excessive or insufficient water supply can adversely impact plant growth and metabolite accumulation (Luo *et al*., 2023). Therefore, understanding the effect of watering regimens on the total flavonoid and phenolic contents of amaranth is crucial for optimizing its cultivation and maximizing its nutritional value.

Water availability is also a critical factor influencing the growth and development of plants. Previous studies have investigated the effects of different watering regimens on *Amaranthus* yield (Pulvento *et al*., 2022), biomass (Liu *et al*., 2004), and morphological characteristics (Sammour *et al*., 2012).

Moderate water stress can enhance amaranth growth and yield by improving root development and nutrient uptake (Fang *et al*., 2017). However, severe water stress can negatively impact plant growth and reduce yield (Song *et al*., 2019). Okunade *et al*. (2008) reported that irrigation frequency can significantly influence amaranth growth and productivity. Frequent irrigation can lead to waterlogging, which can hinder root growth and nutrient absorption. On the other hand, insufficient irrigation can result in water stress, affecting plant photosynthesis and overall development (Wagay *et al*., 2023).

Okunade *et al*. (2008) examined the effects of drip irrigation compared to conventional irrigation methods on amaranth. They found that drip irrigation can improve water use efficiency and enhance plant growth and yield. Ribeiro *et al*., (2017) investigated the interaction between nutrient availability and watering regimes on amaranth growth. They concluded that adequate nutrient supply is essential for maximizing amaranth growth under different water conditions.

Several studies have highlighted the significance of water availability in influencing the phytochemical profile of plants, including amaranth. For instance, (Lassouane *et al*., 2024) demonstrated that variations in watering regimes can lead to significant changes in the accumulation of flavonoids and phenolics.

Water stress, characterized by insufficient water supply, has been reported to induce the biosynthesis of secondary metabolites as a defense mechanism against environmental challenges (Wagay *et al*., 2023). Under such conditions, plants often accumulate higher levels of flavonoids and phenolics to protect themselves from oxidative damage. However, excessive waterlogging can also negatively impact plant growth and metabolism, potentially leading to reduced phytochemical production (Umicevic *et al*., 2024).

In addition to the effects of water stress, the timing and frequency of irrigation can also influence the accumulation of flavonoids and phenolics. Some studies have shown that moderate water deficit followed by rehydration can enhance the production of secondary metabolites (Lassouane *et al*., 2024). This phenomenon, known as priming, can induce plant stress responses without significantly compromising growth and yield.

Furthermore, the specific plant growth stage at which water availability is manipulated can impact the phytochemical profile. For example, Wu *et al*. (2023) and Qaderi *et al*. (2023) suggested that the timing of water stress or excess can differentially affect the accumulation of flavonoids and phenolics in different plant tissues.

While there is a growing body of research on the relationship between water management and phytochemical production in various plant species, studies specifically addressing amaranth are still relatively limited. Previous research on amaranth has primarily focused on the effects of different cultivation practices, such as fertilization (Shoniyozov & Ortikov 2023) and planting density (Gomez *et al*., 2024), on yield and nutritional quality. While these studies have provided valuable information, limited research has specifically investigated the impact of watering regimes on the total flavonoid and phenolic contents of amaranth.

Therefore, there is a clear need for further research to elucidate the complex relationship between water availability and the accumulation of flavonoids and phenolics in amaranth. This study aims to contribute to this knowledge gap by examining the effects of various watering regimes on the phytochemical profile of amaranth plants.

MATERIALS AND METHODS

Plant Material and Experimental Design

The study was conducted at Botanical garden of Federal University Dutse, Jigawa State Nigeria (11.7088° N, 9.3673° E). The seeds of *A. cruentus* were collected from National Horticultural Research institute, Nihort Bagauda sub-station Kano. The seeds were sown in poly bags using four watering regimen treatments which were 2 day (2WR), 4 day (4WR), 8 day (8WR) and control. Watering treatments were initiated at 2 weeks and continued until 45 days.

Data Collection

Plants were harvested at 6 weeks and divided into leaves, stems, and roots. Fresh plant samples were immediately transferred to lab for further analysis.

Growth Measurements

Plant height was measured; the number of leaves was counted while the biomass was calculated by measuring the dry weight of the plant (Kareem *et al*., 2022).

Determination of Total Flavonoid and Phenolic Contents

The total flavonoid content was determined using the Aluminum chloride colorimetric assay and the total phenolic content was determined using the Folin-Ciocalteu reagent (Bang *et al*., 2021). Results were expressed as mg of quercetin equivalent per gram dry weight (mg QE/g DW) for flavonoids and mg of gallic acid equivalent per gram dry weight (mg GAE/g DW) for phenolics. For the flavonoids, 100µl of 2% Aluminum chloride added to 100µl of plant extract and the absorbance was recorded at 430nm. For phenolics, 100µl of the reagent was added to 100µl of sample. After 3 minutes, 100µl of 2% sodium carbonate is then added and the absorbance was measured at 750nm after 30 minutes.

Statistical Analysis

Data were reported as mean ± standard deviation and subjected to ANOVA to determine significant differences among treatments.

RESULTS AND DISCUSSION

Effect of Watering Regimens on Growth of *Amaranthus cruentus*

Plants exposed to 2WR had the highest growth parameters while those exposed to 8WR had the lowest growth parameters. It was observed that 2WR plants had 19 number of leaves, a height of 45 ± 1.2 cm, and a dry weight of 4.02 ± 0.2 g while 8WR plants had 9 number of leaves, a height of 21 ± 0.9 cm, and a dry weight of 1.03 ± 0.2 g The differences observed on the growth parameters were statistically significant as shown in figure 1.

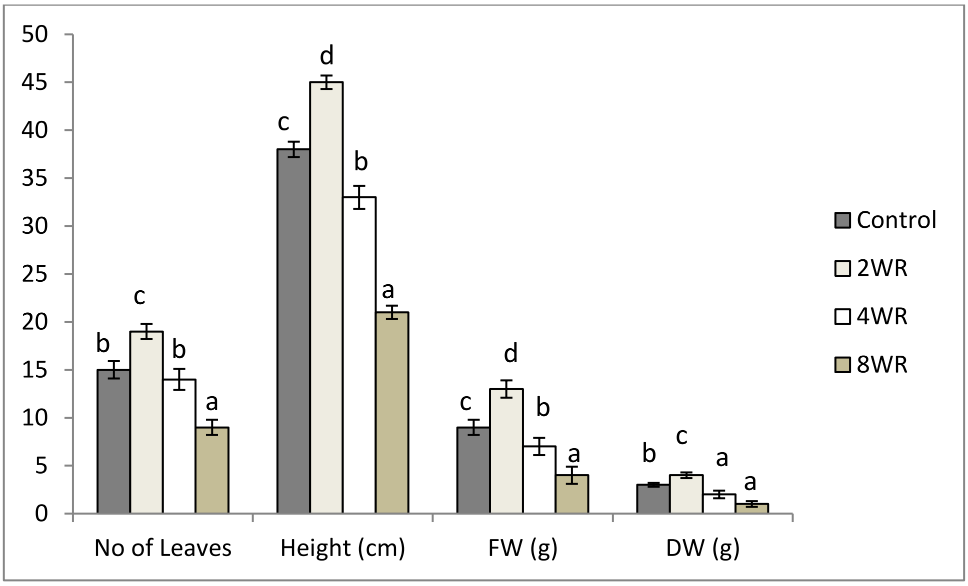

Figure 1: Effect of watering regimen on the growth of *A. cruentus.* (Different letters indicate significant difference among different growth parameter)

Plants under water stress often close their stomata to reduce water loss through transpiration. This can limit photosynthesis, leading to reduced growth and development (Yoo *et al*., 2009). Moreover, water stress can trigger hormonal responses in plants which can induce stomatal closure and alter growth processes (Osakabe *et al*., 2014). Plants subjected to water stress may invest more resources in root growth to improve water uptake (Fang *et al*., 2017). This can lead to increased root length and biomass, as observed in the mild water stress treatment. Under severe water stress, plants may prioritize survival over growth. They may allocate more resources to maintaining essential physiological functions, such as respiration and ion balance, at the expense of growth. Severe water stress can accelerate leaf senescence (Sade *et al*., 2018), leading to a decrease in leaf number and overall plant biomass.

Fang *et al*. (2017) found that moderate water stress can enhance root growth and improve nutrient uptake in *Amaranthus*, leading to increased plant height and biomass. Similarly, Song *et al*. (2019) reported that severe water stress can negatively impact plant growth, resulting in reduced leaf area, biomass, and overall productivity. However, the specific effects of water stress on *Amaranthus* growth can vary depending on the cultivar, environmental conditions, and the intensity of water stress. Sarker *et al*. (2018) found that some *Amaranthus* cultivars may be more tolerant to water stress than others, exhibiting higher resilience to drought conditions. Additionally, Chahal *et al*. (2018) and Ejieji *et al*. (2010) demonstrated that the timing and duration of water stress can influence the impact on *Amaranthus* growth, with short-term water deficits being less detrimental than prolonged periods of drought.

Effect of Watering Regimens on Total Flavonoids and Total Phenolics

The results of this study demonstrated a significant impact of watering regimes on the accumulation of total flavonoids and phenolics in amaranth plants. Plants subjected to 2WR water stress conditions exhibited the highest levels of both flavonoids (51.03 ± 1.2 µg/g QE FW) and phenolics (84.11 ± 2.9 µg/g GAE FW) while those exposed to 8WR had the lowest levels of flavonoids (37.04 ± 1.1 µg/g QE FW) and phenolics (62.15 ± 1.5 µg/g GAE FW) as shown in table 1

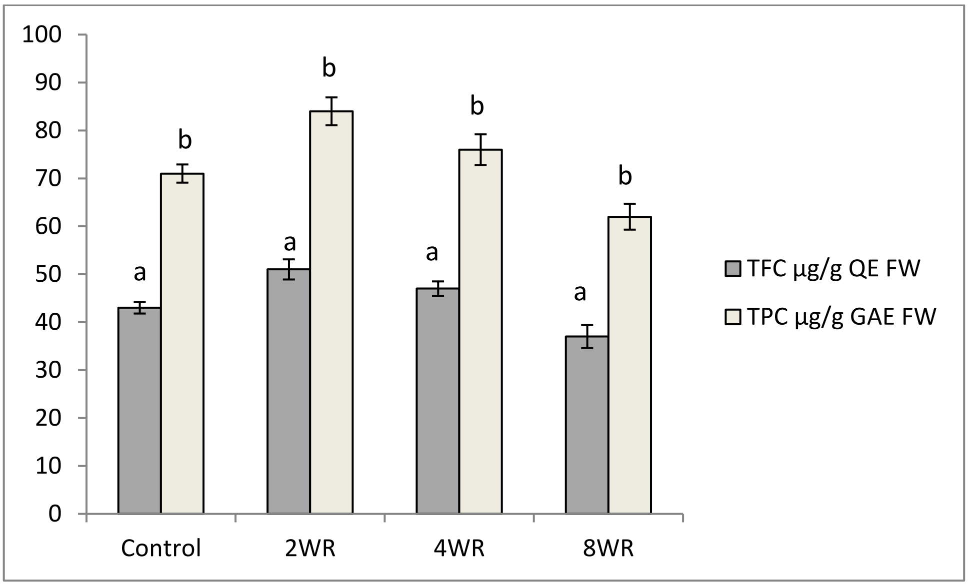

Figure 2: Total flavonoid and phenolic contents of *A. cruentus* under different watering regimes. (Different letters indicate significant difference among different growth parameter)

These findings indicate that a controlled water deficit can be an effective strategy to enhance the phytochemical profile of amaranth. However, excessive water stress, as represented by severe water deficit and waterlogging conditions, negatively impacted the accumulation of flavonoids and phenolics.

The findings of this study align with previous research that has demonstrated the positive impact of moderate water stress on the accumulation of flavonoids and phenolics in various plant species, including the research of Nina *et al*. (2023). These compounds are known to function as antioxidants, providing protection against oxidative damage induced by various environmental stresses, including water deficit (Gonzalez-Espindola *et al*., 2024).

The enhanced production of flavonoids and phenolics under moderate water stress conditions can be attributed to several factors. One possible explanation is the activation of plant defense mechanisms as a response to water scarcity (Jiang *et al*., 2024; Nicolas-Espinosa *et al*., 2023). The increased synthesis of secondary metabolites, such as flavonoids and phenolics, may serve as a protective strategy against oxidative damage caused by reactive oxygen species (ROS) generated during water stress.

However, excessive water stress, as represented by severe water deficit led to a decline in flavonoid and phenolic content. This finding suggests that there is an optimal level of water stress beyond which the negative effects on plant metabolism outweigh the stimulatory effects on secondary metabolite production. Waterlogging, on the other hand, is associated with oxygen deficiency in the root zone (Daniel & Hartman 2024), leading to impaired nutrient uptake and metabolic processes, which ultimately affects the biosynthesis of secondary metabolites.

It is important to note that the optimal watering regime for maximizing flavonoid and phenolic content may vary depending on the *Amaranthus* cultivar, environmental conditions, and specific growth stage. Therefore, further research is necessary to determine the precise water management strategies for different growing regions and cultivation practices.

CONCLUSION

In conclusion, the findings of this study support the notion that moderate water stress can be beneficial for *Amaranthus* growth, while severe water stress can have negative consequences. Further research is needed to elucidate the specific mechanisms underlying the effects of water stress on amaranth and to optimize irrigation practices for maximizing yield and quality in different environmental conditions.

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