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ARTICLE

Effects of Water Stress on Growth and Chlorophyll Contents of *Ocimum gratissimum* L. (Basil) [Lamiaceae]

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ABSTRACT

Ocimum gratissimum is an essential plant because of its wide food and medicinal usage. Despite its relevance, its morpho-physiological compositions are influenced by several abiotic stresses. Hence, this study examined the effects of water stress on the growth and chlorophyll contents of *O. gratissimum*. Seedlings of *O. gratissimum* were grown in twenty-four pots, two per pot and were arranged using a complete randomized design with four groups: Very Wet *O. gratissimum* (VWO), Moderately Water Stress *O. gratissimum* (MWSO), Strongly Water Stress *O. gratissimum* (SWSO) and Adequately Watered *O. gratissimum* (AWO) as control. Fifty centiliters of water was applied in AWO once daily, VWO twice daily, MSWO once in three days and SWSO once a week. Growth parameters: Stem height, number of leaves, leaf area, stem girth and petiole length were determined one week after treatment for six weeks. Chlorophyll contents were determined at two weeks intervals after treatment for eight weeks. Descriptive statistics such as mean ± standard deviation and one-way Analysis of Variance ($p < 0.05$) were done using SAS software. Results obtained showed the highest mean stem height (27.50 ± 0.29 cm), number of leaves (37.00 ± 9.0), leaf area (735.7 ± 4.12 cm^2), stem girth (0.40 ± 0.00 cm) and petiole length (7.20 ± 0.40 cm) in VWO. Similar results were obtained for chlorophyll (56.70 ± 0.65 mg^{-1}). It could be concluded that regular watering of *O. gratissimum* could promote growth and increase chlorophyll contents of the plant.

Keywords: Water deficit; Pigmentation; Medicinal plant; *Ocimum gratissimum*; Growth

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1. Introduction

Water is essential for the survival of all known forms of life. Obviously, vegetation is not an outlier. As the primary component of all living things, water plays a crucial role in a wide variety of biochemical processes, from the production of glucose during photosynthesis to the transport of nutrients and minerals [1,2]. Numerous responses from flora and fauna have been documented in response to climatic change-induced shifts in rainfall patterns and the unpredictable flooding and drought conditions they bring [3]. Water shortages or floods are common causes of plant stress. Reduced stomatal conductance, leaf area, number of leaves, and fresh weight are all results of water stress [4]. Hartmann et al. [5] reported that water stress due to drought is the most significant abiotic factor limiting plant growth and development. Hsiao and Xu [6] documented that a plant's growth is easily slowed down by water stress, and the root system is given more attention than the leaves. To conserve moisture, the leaves of plants in arid regions tend to be few and small.

Pigmentation in plants can be directly related to their stress physiology, as concentrations of carotenoid increase, there is an associated decrease in concentrations of chlorophyll under flooding conditions and the senescence process [7]. A decrease in the photosynthetic capacity of mesophyll cells during continued flooding leads to a further reduction of photosynthesis [8]. Ekanayake et al. [9] reported that moderate water stress slows chlorophyll formation while severe dehydration can be detrimental to the process of chlorophyll formation.

Ocimum gratissimum is an herbaceous plant that is a member of the Lamiaceae family [10]. The leaves, which can reach 10 × 5 cm in size, are ovate to ovate-lanceolate in shape, sub-acuminate to acuminate at the apex, cuneate and decuneate at the base, with a coarsely crenate, serrate margin, pubescent and dotted on both sides, and up to 1.9 m in height [11]. The plant's native range includes India and West Africa and other tropical regions. In Nigeria, it is found in Savannah and coastal areas, and it is known by its several vernacular names. It is called "Efrinrin-nla" for the Yorubas, "Ahuji" by the Abakaliki Igbos, "Nchuanwu" by other Igbos, "Daidoya" by the Hausas [12,13] and Sanmadido by Ogu (Egun) people of Lagos and Ogun States. Thus, this study aimed at assessing the effects of water stress on the growth and chlorophyll contents *Ocimum gratissimum*.

2. Materials and methods

2.1 Collection of materials

Experiments were conducted in a screen house located at the Botanical Garden of the Department of Botany, Faculty of Science, Lagos State University, Ojo, Lagos, Nigeria for a period of 3 months. Forty-eight (48) seedlings of *O. gratissimum* L. about a month old were obtained from the vegetable farm at the Post Service of Ojo Military Cantonment, along LASU-Igando road, Lagos. Loamy soil was obtained from ETF Research Laboratory Complex, Faculty of Science, LASU, Ojo, Lagos.

2.2 Soil preparation

Loamy soil of 8 kg was measured using a weighing balance into equally perforated plastic pots of 22 cm in depth and 16.5 cm in diameter. A total of twenty-four (24) pots were prepared and watered lightly to ease the transplanting of the seedlings the following day.

2.3 Seedling transplant and establishment

The forty-eight (48) seedlings obtained were transplanted, two per pot at 1 cm depth. The seedlings were allowed to establish themselves by providing them with 50 cL of tap water twice daily for four weeks.

2.4 Treatment inducement

The established seedlings of *O. gratissimum* were divided into four (4) groups namely, Very Wet *O. gratissimum* (VWO), Adequately Watered *O. gratissimum* (AWO) (Control), Moderately Water Stressed *O. gratissimum* (MWSO) and Strongly Water Stressed *O. gratissimum* (SWSO) arranged in completely randomized design. Each of the treatments has six replicates. The

VWO was supplied with 50 cL of tap water two (2) times daily. The AWO was supplied with 50 cL of tap water once daily. This was taken as the control experiment. The MWSO were supplied with 50 cL of tap water once in 3 days while SWSO were given 50 cL of tap water once a week.

2.5 Morphological data collection

The collection of morphological data started a week after the inducement of treatments. The morphological character measured were stem height (SH), number of leaves (NL), leaf area (LA), petiole length (PL) and stem girth (SG). Stem height and petiole length were measured and recorded in centimeters using the meter rule. Number of leaves were counted and recorded. Leaf area was gotten by multiplying leaf length by leaf breadth in square centimeters (cm^2) and was calculated using the formula of Okubena-Dipeolu et al. [14].

$$\text{Leaf area } (cm^2) = 0.853 + (\text{leaf blade length} \times \text{leaf blade breadth}) \times 8.7440$$

The stem girth was measured in centimeters using Vernier Caliper.

The morphological characters were collected weekly for six (6) consecutive weeks.

2.6 Chlorophyll contents determination

The chlorophyll contents of the leaf were determined using Dunn et al. [15] and Sumanta et al. [16] methods with slight modifications. Freshly excised 0.5 g leaves of *O. gratissimum* L. was collected from the upper, middle, and lower parts of the plant and weighed. The sample from the three (3) parts for each treatment was chopped and transferred into 20 mL of 98% Di-methyl sulfoxide (DMSO).

The mixture was stored in the refrigerator (10 °C) for about eight (8) weeks after the last chlorophyll extraction for one-time analysis. The absorbance of the extracts was then read in a visible spectrophotometer (Model V5000) at 665 and 649 wavelengths. The measurement was replicated thrice using leaves from 3 different parts of different plants for each treatment. The chlorophyll contents ($mg \cdot L^{-1}$) were calculated using the following equations according to Ekanayake et al. [9] and Sumanta et al. [16].

$$\text{Chlorophyll a (Chl.a)} = 12.47*A_{665} - 3.62*A_{649}$$

$$\text{Chlorophyll b (Chl.b)} = 25.06*A_{649} - 6.5*A_{665}$$

$$\text{Total Chlorophyll (Chl.)} = 20.2*A_{649} + 8.02*A_{665}$$

where A is Absorbance.

Extraction of leaf chlorophyll contents for each of the four treatments was carried out once in two (2) weeks for eight (8) weeks.

2.7 Statistical analysis

Data obtained were analyzed using Statistical Analysis System. One-way Analysis of Variance (ANOVA) was conducted to determine significant differences between the parameters. Means were separated using Duncan's Multiple Range Test (DMRT) at $p < 0.05$.

3. Results

3.1 Effects of water stress on growth characters

Stem height

The result on the effects of water stress on stem height of *O. gratissimum* showed significant differences at $p < 0.05$ among Control Plants (Adequately Watered *O. gratissimum*) (AWO), Very Wet *O. gratissimum* (VWO), Moderately Water Stressed *O. gratissimum* (MWSO) and Strongly Water Stressed *O. gratissimum* (SWSO) from the first week after treatment to the end of the experiment **(Figure 1**). The result however revealed that VWO showed significantly greater stem heights ($p < 0.05$) throughout the period of the experiment except for 1st and 2nd WAT while SWSO showed significantly least stem heights $p < 0.05$ throughout the period of the experiment (**Figure 1**).

Number of Leaves

The result on the effects of water stress on the number of leaves of *O. gratissimum* showed a significant difference at $p < 0.05$ among AWO, VWO, MWSO and SWSO from the 1st week after treatment to the end of the experiment (**Figure 2**). The result however revealed that VWO showed a significantly higher number of leaves ($p < 0.05$) from the 3rd week after treatment to the end of the experiment. While SWSO showed a significantly least number of leaves from the first week after treatment to the end of the experiment (**Figure 2**).

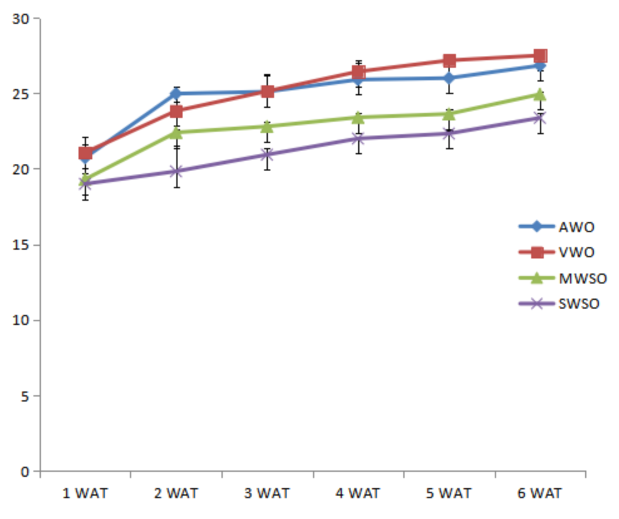

Figure 1. Effects of water stress on stem height of *O. gratissimum.*

AWO: Adequately Watered *O. gratissimum* (Control), VWO: Very Wet *O. gratissimum*, MSWO: Moderately Water Stressed *O. gratissimum*, SWSO: Strongly Water Stressed *O. gratissimum,* WAT: Week(s) After Treatment.

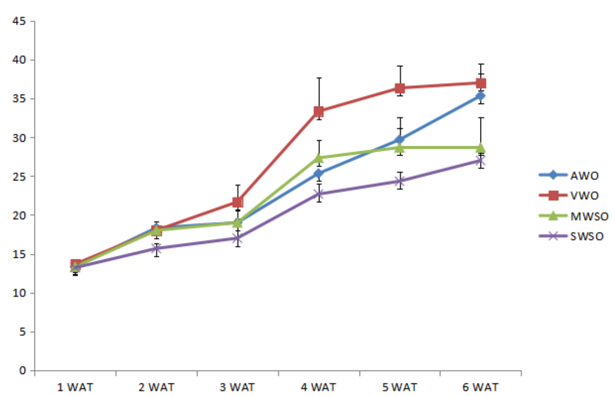

Figure 2. Effects of water stress on number of leaves of *O. gratissimum.*

AWO: Adequately Watered *O. gratissimum* (Control), VWO: Very Wet *O. gratissimum*, MSWO: Moderately Water Stressed *O. gratissimum*, SWSO: Strongly Water Stressed *O. gratissimum,* WAT: Week(s) After Treatment.

Leaf area

The result on the effects of water stress on the leaf area of *O. gratissimum* showed a significant difference at $p < 0.05$ among AWO, VWO, MWSO and SWSO respectively throughout the period of the experiment (**Figure 3**). The results however revealed the highest leaf area in VWO from the 1st week after treatment to the 6th week after treatment and the least leaf area is recorded for SWSO from the 1st week to the end of the experimental period. It can also be seen that the highest leaf area is found in very wet plants in the 6th week of the experiment (**Figure 3**).

Stem girth

The result on the effects of water stress on the stem girth of *O. gratissimum* showed significant differences at $p < 0.05$ among AWO, VWO, MWSO and SWSO respectively (**Table 1**). The result however revealed a significant increase in the stem girth of AWO and VWO from the 1st week after treatment to the end of the experiment while a significant decrease in the stem girth of MWSO and SWSO were observed in the 5th and 6th week after treatment respectively (**Table 1**).

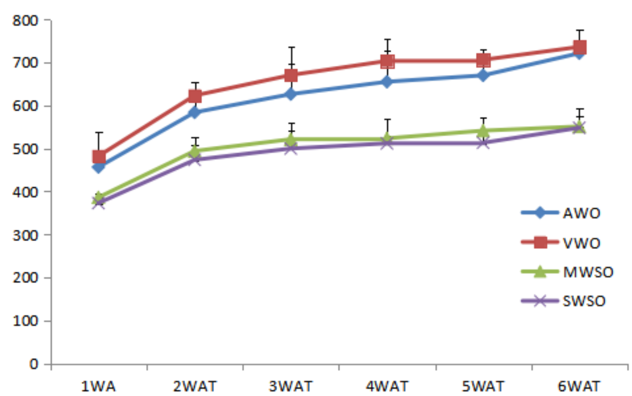

Figure 3. Effects of water stress on leaf area of *O. gratissimum.*

AWO: Adequately Watered *O. gratissimum* (Control), VWO: Very Wet *O. gratissimum*, MSWO: Moderately Water Stressed *O. gratissimum*, SWSO: Strongly Water Stressed *O. gratissimum,* WAT: Week(s) After Treatment.

Petiole length

The result on the effect of water stress on the petiole length of *O. gratissimum* showed significant differences at $p < 0.05$ among AWO, VWO, MWSO and SWSO (**Table 2**). The result however revealed a significant increase in the petiole length of AWO, VWO and MWSO throughout the experimental period while a significant decrease in the petiole length of SWSO from the 5th week after treatment was observed (**Table 2**). The highest petiole length is recorded for VWO in the 6th week after treatment.

3.2 Effects of water stress on chlorophyll contents of *O. gratissimum*

The result on the effects of water stress on chlorophyll contents of *O. gratissimum* showed significant differences at $p < 0.05$ among AWO, VWO, MWSO and SWSO respectively (**Table 3**).

The result revealed a significant increase in the chlorophyll content of AWO while a significant decrease in the chlorophyll content of plants in other treatments (VWO, MWSO and SWSO) from 2nd week after treatment to the 8th week after treatment (**Table 3**). Also, a significant increase in chlorophyll b, and total chlorophyll content was observed throughout the experiment irrespective of the treatment the plants were exposed to. However, the highest total chlorophyll content was observed in VWO in the 8th week after treatment (**Table 3**).

Table 1. Effects of water stress on stem girth of *O. gratissimum*.

Treatment	1WAT (cm)	2WAT (cm)	3WAT (cm)	4WAT (cm)	5WAT (cm)	6WAT (cm)
AWO	$0.2±0.00^{a}$	$0.27±0.03^{b}$	$0.30±0.06^{b}$	$0.37±0.02^{a}$	$0.37±0.03^{b}$	$0.40±0.03^{a}$
VWO	$0.23±0.03^{a}$	$0.30±0.00^{a}$	0.33±.0.03a	$0.37±0.03^{a}$	$0.40±0.00^{a}$	$0.40±0.00^{a}$
MWSO	$0.2±0.00^{a}$	$0.27±0.03^{b}$	0.30±0.00b	$0.32±0.02^{b}$	$0.30±0.00^{c}$	$0.30±0.00^{b}$
SWSO	$0.2±0.00^{a}$	$0.27±0.03^{b}$	$0.30±0.00^{b}$	$0.30±0.03^{c}$	$0.30±0.00^{c}$	$0.25±0.03^{c}$

Means in the same column that do not have similar superscripts are significantly different at $P < 0.05$.AWO: Adequately Watered *O. gratissimum* (Control), VWO: Very Wet *O. gratissimum,* MSWO: Moderately Water Stressed *O. gratissimum,* SWSO: Strongly Water Stressed *O. gratissimum*, WAT: Week(s) After Treatment.

Table 2. Effects of water stress on the petiole length of *O. gratissimum*.

Treatment	1WAT (cm)	2WAT (cm)	3WAT (cm)	4WAT (cm)	5WAT (cm)	6WAT (cm)
AWO	$4.40±0.31^{ab}$	$5.20±0.4^{a}$	$5.40±0.31^{b}$	$6.07±0.30^{b}$	$6.27±0.23^{b}$	$7.00±0.35^{ab}$
VWO	$4.57±0.23^{a}$	$5.20±0.25^{a}$	$6.20±.0.4^{a}$	$6.93±0.54^{a}$	$7.0±0.40^{a}$	$7.20±0.40^{a}$
MWSO	$4.0±0.12^{cd}$	$4.23±0.12^{c}$	$4.83±0.33^{d}$	$5.03±0.23^{c}$	$5.30±0.44^{c}$	$5.47±0.35^{c}$
SWSO	$4.17±0.17^{bc}$	$4.80±0.35^{ab}$	$5.0±0.50^{c}$	$5.43±0.29^{d}$	$4.63±0.19^{d}$	$4.43±0.27^{d}$

Means in the same column that do not have similar superscripts are significantly different at $p < 0.05$. AWO: Adequately Watered *O. gratissimum* (Control), VWO: Very Wet *O. gratissimum,* MSWO: Moderately Water Stressed *O. gratissimum,* SWSO: Strongly Water Stressed *O. gratissimum*, WAT: Week(s) After Treatment.

Table 3. Effects of water stress on chlorophyll contents of *O. gratissimum*.

	Chlorophyll a (mg·L^{-1})				**Chlorophyll b (mg·L^{-1})**				**Total chlorophyll (mg·L^{-1})**			
Trmt.	**2WAT**	**4WAT**	**6WAT**	**8WAT**	**2WAT**	**4WAT**	**6WAT**	**8WAT**	**2WAT**	**4WAT**	**6WAT**	**8WAT**
AWO	19.74±0.35^{a}	20.08±0.26^{b}	20.54±0.17^{a}	20.66±0.61^{a}	18.17±3.19^{a}	19.95±3.68^{c}	22.78±2.82^{b}	25.14±2.16^{cd}	41.42±3.80^{a}	43.02±3.74^{c}	44.23±2.4c	47.14±2.31^{c}
VWO	20.92±0.23^{a}	20.55±0.27^{a}	19.92±0.56^{b}	18.22±0.26^{d}	16.54±5.00^{b}	24.60±1.09^{a}	25.64±2.07^{a}	36.77±0.59^{a}	39.01±5.37^{ab}	46.32±2.29^{ab}	48.55±1.92^{a}	56.70±0.65^{a}
MWSO	20.98±0.17^{a}	20.84±0.16^{a}	20.65±0.12^{a}	19.35±0.38^{b}	16.83±0.11^{b}	22.99±2.63^{bc}	25.79±2.14^{a}	26.91±1.68^{c}	40.49±0.30^{a}	46.31±2.38^{ab}	47.22±1.74^{ab}	49.90±1.54^{ab}
SWSO	20.59±0.42^{a}	20.56±0.89^{a}	19.45±0.60^{b}	18.78±0.17^{c}	16.95±2.41^{b}	24.03±3.45^{b}	26.92±1.51^{a}	30.55±0.70^{b}	40.12±3.07^{a}	47.35±2.77^{a}	48.54±0.81^{a}	51.29±0.50^{b}

Means in the same column that do not have similar superscripts are significantly different at $p < 0.05$. AWO: Adequately Watered *O. gratissimum* (Control), VWO: Very Wet *O. gratissimum,* MSWO: Moderately Water Stressed *O. gratissimum,* SWSO: Strongly Water Stressed *O. gratissimum*, WAT: Week(s) After Treatment.

4. Discussion

4.1 Effects of water stress on growth characters of *O. gratissimum*

Plants' growth and survival are a function of the amount of water readily available for uptake within the reach of the plant's roots. Hence, plants suffer hardship under water stress conditions. While struggling to survive, the majority of them have demonstrated adjustment in their morphological characteristics such as stem height, leaf area, root length and so on [17].

Very Wet *O. gratissimum* (VWO) was found with the highest mean stem height throughout the experimental period except at 2 WAT while Strongly Water Stressed *O. gratissimum* (SWSO) had the least mean stem height from the first week after treatment to the sixth week after treatment (**Figure 1**). This is probably due to the very high-water requirement at the meristematic region of the plant's shoot for cell division which results in growth. Hence reduced stem height of Strongly Water Stressed *O. gratissimum* (SWSO) could be attributed to reduced growth activities caused by severe water stress. The findings of the present study corroborate the reports of those authors [1,3,6,18,19] who submitted significant mean stem height in plants subjected to regular watering. Ayeni et al. [20] found that daily-watered *Abelmoschus esculentus* seedlings had the shortest mature plants, but these results contradict that conclusion. It's possible that the crop was grown in nutrient-depleted soil, which could explain the result. The number of leaves differed significantly ($p < 0.05$) among Adequately Watered Plants *O. gratissimum* (AWO), Very Wet *O. gratissimum* (VWO), Moderately Water Stressed *O. gratissimum* (MWSO) and Strongly Water Stressed *O. gratissimum* (**Figure 2**). The highest number of leaves (37.00 ± 9.0) was obtained in plants watered twice daily compared to other treatments (**Figure 2**). This could infer that *O. gratissimum* tends to produce more leaves during water excess conditions than during water deficit conditions. The significant number of leaves recorded in VWO could be needed to give off excess water into the atmosphere as the plant has no place for storage of excess water. This agrees with the submissions of Adelani [21] and Dauda et al. [22] who reported a significant number of leaves in *Citrus tangelo*, *Abelmoschus esculentus*, *Chocorus olitorus*, *Telferia* spp and *Amaranthus* spp watered daily. Also, various investigators [23-28] have reported the highest number of leaves in plants exposed to regular watering and decreased leaf number under water stress. The significant number of leaves recorded in VWO followed by AWO could also be an indication that *O. gratissimum* requires enough water for proper growth and development and could be attributed to available nitrogen in the soil which was aided by its dissolution potential in soil watered daily and this facilitates its uptake. This agrees with the findings of Olubode et al. [29] who found that as applied moisture content increased, plant nutrient uptake did as well.

A significant leaf area was recorded in VWO. This could be ascribed to the fact that a large leaf area is needed by plants under excess water conditions to aid transpiration and photosynthetic activities. When plants are under extreme water stress, they often reduce their leaf area, stop producing new leaves, or drop their older leaves to conserve water [3]. Results of the present study are in line with the findings of Gonzales et al. [30], Oboho and Igharo [26], Olubode et al. [29], Ogunrotimi and Kayode [28], Adelani [31] who reported that regular watering enhanced leaf area and photosynthesis in plants.

A significant stem girth was also recorded in VWO. This could infer that the stem guith of *O. gratissimum* is a function of water available for uptake. However, the reduced girth found in water deficit *O. gratissimum* could be because of dehydration of the plant which must have caused reduced photosynthetic activities of the plants [32]. The findings of this study corroborate that of Olajide et al. [33] who reported a significant difference in *Dialium guineense* seedling girth due to the application of different watering regimes. However, a contrasting result was submitted by Gbadamosi [3] who reported that girth of seedlings was not significantly ($p < 0.05$) different under watering regimes and water quantity.

The findings of this study also showed significant petiole length in VWO. The length of the petiole has an influence on the leaf lamina and exposes it to the sun for effective photosynthesis. This could indicate that a long petiole is needed for effective and efficient food production and to act as a storage organ. This study agrees with the reports of Turner [34], Ohashi et al. [35] and Durigon et al. [36] who indicated a significant effect of irrigation deficit in the length of petioles and petiolules. In contrast, petiole length was not significantly influenced by soil water stress but there was a reduction in growth between weeks 3 and 6 because of other weather conditions [37].

4.2 Effects of water stress on chlorophyll contents of *O. gratissimum.*

Because water is essential to all living things and plays a role in crucial biochemical processes like photosynthesis, the productivity of plant species is affected by whether water is available in sufficient quantity and at a biologically tolerable interval [3]. Plants rely on readily available water for a wide range of essential processes, such as cell division, elongation, stem height, leaf expansion, and chlorophyll formation [38].

Results regarding chlorophyll content shown in **Table 3**, indicated a significantly different mean chlorophyll content (56.70 ± 0.65 mg·L^{-1}) in VWO followed by SWSO (51.29 ± 0.50 mg·L^{-1}). As a result, chlorophyll formation in *O. gratissimum* cannot be said to depend solely on water availability as chlorophyll-b and total chlorophyll content of the plant increased across the treatment all through the experiment period. This result could indicate that electron transport activity and the photosynthesis apparatus of *O. gratissimum* are not damaged by water stress. This study's findings are consistent with those of Dzomeku et al. [37], who found no correlation between soil moisture stress and chlorophyll contents and found that chlorophyll content exhibited undulating characteristics in response to the watering regime. While previous research has shown that water stress reduces wet CO_2 and photosynthesis [35,39-44], the current study finds the opposite to be true for a wheat crop under water stress conditions.

5. Conclusions and recommendation

The results of this study suggested that *O. gratissimum* benefited from an abundance of water, as it expanded and contained more chlorophyll overall. As a result, it might be recommended that this plant be given consistent watering to achieve the best possible growth performance, which in turn typically increases yield and income.

Author Contributions

Oluwole S.O and Asokere, S.Y. conceived the idea, Ogun M.L., Asokere S.Y., Ewekeye T.S. and Ojewumi A.W. designed it, Asokere S.Y., Ogun M.L. and Ojewumi A.W executed it, Oluwole S.O, Asokere S.Y., Ewekeye T.S and Ogun M.L. interpreted the data and Ogun M.L., Ewekeye T.S. and Asokere S.Y. wrote the manuscript.

Conflict of Interest

All authors declare no conflict of interest.

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