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Silicon Mediated Approach in Enhancing Water Stress Tolerance of *Alternanthera Sissoo*

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Abstract

Water stress causes various plant morphological, physiological, and biochemical responses. Silicon application may reduce the water stress tolerance in plants by increasing the physiology parameters and the crop's vegetative growth. Hence, the study aims to evaluate the effect of water stress levels on *Alternanthera sissoo*, known as Brazilian spinach, and determine which silicon (Si) level is the best under water scarcity. The method implied measuring the water stress by different levels of water level applied to the plant which are 100 % (well-watered), 75 % (moderate water deficit), 50 % (high water deficit), and 25 % (severe water deficit). The variation of silicon concentrations is S0 (control), S1, S2 and S3 (0, 0.6, 0.9 and 1.2 %v/v) arranged in the Randomized Complete Block Design (RCBD) in the greenhouse. Growth, physiological, and biochemical parameters were analyzed during the experiment. The research findings showed that water stress results were statistically reduce the leaf area, leaf number root length, fresh weight, chlorophyll content, relative water content (RWC), and malondialdehyde (MDA). The root length, leaf area, and malondialdehyde (MDA) show the interaction between water stress level and silicon concentration. The study proposed that the Si nutrient management strategy has the potential to minimize the impacts of drought stress in Brazilian spinach. Regarding research findings, it was determined that the optimal water requirement for *Alternanthera sissoo* is 50 % of the water treatments, with a silicon concentration of 1.2 %v/v. This is essential for better water management of the plant.

Keywords: *Alternanthera sissoo*, Spinach, Water stress, Silicon

Introduction

Alternanthera sissoo is known as Samba Lettuce, Poor Man's Spinach, or simply Sissoo or Sissoo Spinach. This plant is a member of the Amaranthaceae family, which is currently well-liked in Malaysia [1]. The essential elements needed for human health and welfare are found in green leafy vegetables. These consist of dietary fiber, vital fatty acids, vitamins, and amino acids [2]. Brazilian spinach is a green vegetable plant species native to Brazil and South America and is widely cultivated in tropical regions. It is rich in calcium, iron, folic acid, vitamin C, vitamin K, and carotenoids [1]. Brazilian spinach's nutritional value and phytochemical components can create an herbal-based immunostimulant [3]. Numerous economically significant plant species, such as ornamentals, weeds, protein-rich, and white-seeded grain species, are found in the Amaranthaceae family [4].

Brazilian spinach became a popular homegrown vegetable but was particularly susceptible to water scarcity, hindering its production and growth [5]. Climate change become a big issue in agriculture that affects the abiotic and biotic stress to maintain food security [6,7]. Increasing temperature and drought raises concerns not only for big-scale farmers, but small and homegrown farmers. Water stress may affect the growth and physiology of the *Alternanthera sissoo* and other vegetable crops [5]. For instance, Jabeen *et al.* [8] showed that the biochemical changes of *Spinacea oleracea* L increased under 40 % field capacity indicating that this plant was under stress. Another study proved that the yield of spinach was lower at lower water irrigation levels [9].

One of the ways to overcome water scarcity is by applying silicon fertilizer. Si has been widely reported for alleviating plant drought stress in silicon-accumulating such as barley, maize, rice, and wheat and non-accumulating plants such as tomato [10,11]. However, the effectiveness of silicon-mediated techniques in enhancing *Alternanthera sissoo* water stress tolerance and the underlying mechanisms are largely unknown. This project aims to understand better how silicon applications help to alleviate plant drought stress and optimize water usage. Less research has been done on Brazilian spinach and the effect of water stress and silicon approach on these plants has never been reported. This research aims to evaluate the impact of water stress on *Alternanthera sissoo*'s growth, physiological, and biochemical properties and to determine the optimum level of silicon under water stress of *Alternanthera sissoo*. This research aligns with SDG 13th and 2nd goals, which emphasize the need for immediate measures to address climate change.

Materials and methods

Experimental design

A Randomized Complete Block Design (RCBD) was set up for 4 water treatments; 100 % (well-watered), 75 % (moderate water deficit), 50 % (high water deficit), and 25 % (severe water deficit) with 4 concentrations of potassium silicate; S0 (control), S1, S2 and S3 (0, 0.6, 0.9 and 1.2 %v/v). Four replications were employed as the experimental design in this investigation. The Universiti Sultan Zainal Abidin Besut Campus greenhouse served as the study's location.

Plant preparation

The Sissoo spinach plants were obtained from a nearby nursery and subsequently utilized for stem cutting, which were then planted in polybags. A total of 64 stem cuttings were planted, with each stem placed in a separate polybag. The stem of spinach, around 6 - 8 cm long, with 3 - 4 nodes, was cut using a keen scalpel from the resources of mature plants. The stem cutting of Brazilian spinach was transplanted into the topsoil-filled polybags 15×16 inch^2 [1]. All plants were placed in the greenhouse with microclimate conditions, as shown in **Table 1**.

Table 1 The microclimate condition under the greenhouse during the experiment.

Microclimate parameters	Quantification
Relative humidity	60.5 - 92.2 %
Light intensity	7 - 2,126 lux
Day temperature	30 - 37 °C
Night temperature	20 - 24 °C
Ambient CO_2	400 ppm

Water and silicon treatments

The experiment was based on Najihah *et al.* [12] where the number of water treatments was identified using field capacity calculation. The 10 filled polybags were weighed first to determine the dry weight, and the average weight was stated. These polybags were watered until they were sufficiently moist to produce more water, and then they were re-weighted after all the extra water had been drained to obtain the turgid weight. The soil's field capacity will be assessed and found to have 100 % water treatment (well-watered). The remaining 75, 50 and 25 % of the water treatments were calculated based on the amount obtained and the field capacity. As a result, 4 different water treatments (100 % water as control, 75 % water, 50 % water and 25 % water) were used in the experiment. Then, each of these was replicated 4 times [12]. Based on the particular water needs of each replicate, irrigation was conducted once every 2 days. The 4 dosages of Si treatments S0 (control), S1, S2, and S3 (0, 0.6, 0.9 and 1.2 %v/v) were sprayed on day 15, day 25, day 35, day 45 and day 55 [13,14].

Vegetative measurement

Brazilian spinach grown with and without a water deficit had its vegetative characteristics measured, including the height of the plant, root length, and leaf area. The plant height was measured once a week using a measuring tape, following a modified version of Alam *et al.* [1] method. The measurement started at the bottom stem directly on the soil base and progressed to the topmost part of the plant for each replication of a different water treatment. All green leaves leaf area was quantified using a portable laser leaf area meter (CI-202, CID Bio-Science, USA). Plant roots were carefully removed, cleaned with tap water, and measured for length [15].

Physiology parameters

Leaf gas exchange

A closed infrared gas analyzer, the LI-6400XT Portable Photosynthesis System (Li-COR Inc., Nebraska, USA), was used to get the measurement. On completely grown young leaves number 2, the photosynthetic rate, stomatal conductance, and transpiration rate of Brazilian spinach were measured between 9:00 and 11:00 in the morning. The cuvette temperature was set to 30 °C, the flow rate was fixed at 500 µmol/s, the light was set at 800 µmol, and the instrument was set at 400 µmol mol^{-1} CO_2 [12].

Chlorophyll fluorescence

The data was taken using a portable PEA meter. The leaf clips that include allow access to dark-adapt samples before measuring them. Pieces are easily dark-adapted before measurement. The entire measurement process, from data collection to calculation and display of the essential f_v/f_m and Performance Index (PI) chlorophyll fluorescence metrics, is automated with a single button [16].

Chlorophyll content

The chlorophyll content index was obtained using the handy and portable SPAD-502 meter. The SPAD 502 Chlorophyll Metre was designed to determine the chlorophyll content of leaves [17]. The Brazilian spinach leaves are taken and repeated thrice to determine the average value [18].

Relative water content (RWC)

A puncher was used to take 3-disc samples of each Brazilian spinach variety, and a digital balance calculated the fresh weight (FW). Following 24 h in water to restore complete turgor, the samples are adequately dried using tissue paper and reweighed to determine the turgid weight (TW). The models are then oven-dried for 24 h at 75 °C and their dry weight (DW) is noted [12]. The RWC formula is as follows:

$$RWC = \frac{FW - DW}{TW - DW} \times 100$$

Biochemical properties

Malondialdehyde

Malondialdehyde (MDA) levels were determined using an Elabscience ® Metabolism Assay Kit. Fresh leaves were ground with mortar and pestle with liquid nitrogen. About 0.1 g of the leaves sample was added with 0.9 mL of 10× Concentrated extracting solution diluted 10 times with pure water. Then, the sample was homogenized in an ice bath. The homogenate tissues were centrifuged at 10000 rpm for 15 min. The working solution was prepared by adding the Clariant (Reagent 1), acid reagent (Reagent 2), and Chromogenic Agent (Reagent 3) in a ratio of 0.1:3:1. The standard curve was prepared by diluting the 200 nmol/mL standard solution with absolute ethanol to a serial concentration. The dilution gradient is 0, 5, 10, 15, 20, 30, 40 and 50 nmol/mL. For the standard tube, 100 µL of the standard solution was added with 600 µL of working solution, while in the sample tube, 100 µL sample was added with 600 µL of working solution. The tube was mixed entirely, tightened with preservative film, and then incubated in a 95 °C water bath for 40 min. The tube was cooled with a running water centrifuge at 2,000 rpm for 10 minutes, 250 µL supernatant was taken to a microplate and the OD value was measured at 523 nm with a microplate reader.

Statistical analysis

Two-way ANOVA statistical analysis was used to analyze the data with 2 independent variables as its targets (water treatment and silicon content) with SPSS statistical software (ver. 27.0 SPSS, Chicago, USA). Tukey was used for the post hoc analysis in this study.

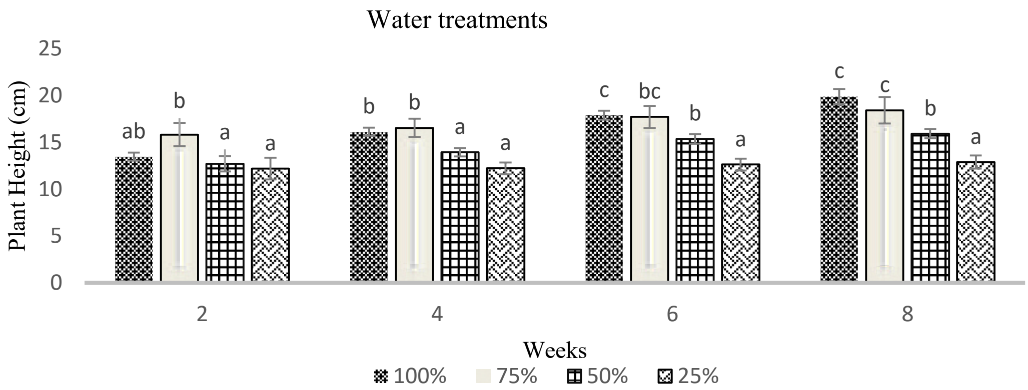
Result and discussion

Plant height

The plant height was observed for eight weeks, as shown in **Figure 1**. In general, the height of the Sissoo spinach plant was affected by the presence of water ($p \leq 0.05$). The 2nd and 4th weeks show that 75 % of water treatments give the highest rate of plant height compared to the 6th and 8th weeks. At the same time, 100 and 75 % water treatments were almost unchanged in week 6, which reveals statistically no significant difference. At week 8, 25 % of water treatments showed the lowest rate compared to other treatments, which indicates statistical significance. The end of the research offers 100 and 75 % not statistically significant.

Decreased water availability will result in shorter plants. Plants frequently respond to water stress by continuously reducing their height. Since cell growth is one of the physiological processes most vulnerable to water stress, a decline in height may be caused by either suppression of cell division or reduction of cell elongation due to a drop in turgor pressure [9]. According to a previous study the total plant height of rice under a severe drought was lower with varying water gradients than it was during mild and moderate droughts and under identical irrigation conditions [19]. From this, it can be concluded that water stress inhibits rice plant height more severely during the jointing and tillering stages of rice plant growth.

Silicon treatments were not statistically significant to the plant height ($p \geq 0.05$). The highest silicon level S3 (1.2 v/v%) gave the most significant reading for the eight-week observation, as shown in **Figure 1**. The concentration of silicon S0 (control) and S1 (0.6 v/v%) offers no significant different through all the weeks observed. At week 8, the concentration of S2 (0.9 v/v%) is significant to the concentration of S0 and S1. Silicon alleviates drought stress and enhances plant growth [20]. In the current study, the growth and development of soybean plants improved with the addition of silicon. Silicon application increased soybean plants' vegetative growth in water-limited and well-watered environments [21]. Potassium silicate fertilization is a potential approach since silicon is an elicitor that may cause plants to become resistant to abiotic stressors [22]. This was made abundantly evident when silicon was sprayed since it significantly reduced the acute decline brought on by inadequate irrigation in the study. The development of a silica double layer, which lowers stomata opening, leaf transpiration, and water losses without compromising plant growth in drought-stressed environments, may be impacted by silicon accumulation in transpiration organs [22,23].



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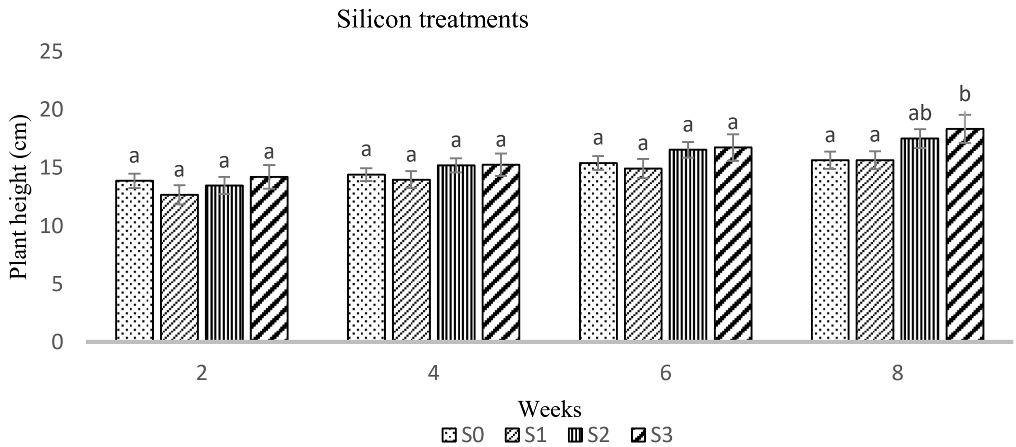

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Figure 1 Effects of different water treatments and silicon concentrations on the plant height of Sissoo spinach eight weeks period. Bars indicate the standard error of the mean (SEM).

Root length

Figure 2 indicates the interaction of different water treatment levels and the silicon fertilizer rate on Brazilian spinach's root length. The statistical significance of root length is related to silicone and water treatments ($p \leq 0.05$). **Figure 2** shows that severe water treatments (25 %) with 0 %v/v of Si fertilizer have the lowest value of root length compared to other treatment combinations. The treatments combination of 100 % water treatments and 1.2 %v/v Si fertilizer shows the highest root length. The study conducted by [24] demonstrated that root length decreased initially in exposure to mild to severe water stress, but it increased up to non-stress levels. While another study by Mishra *et al.* [15] stated that the root length is consistently greater in plants experiencing drought stress.

However, the root length increased under severe water stress due to applying Si. One of the probable causative mechanisms encouraging root elongation is the Si-enhanced cell wall flexibility in the root growth zone [25].

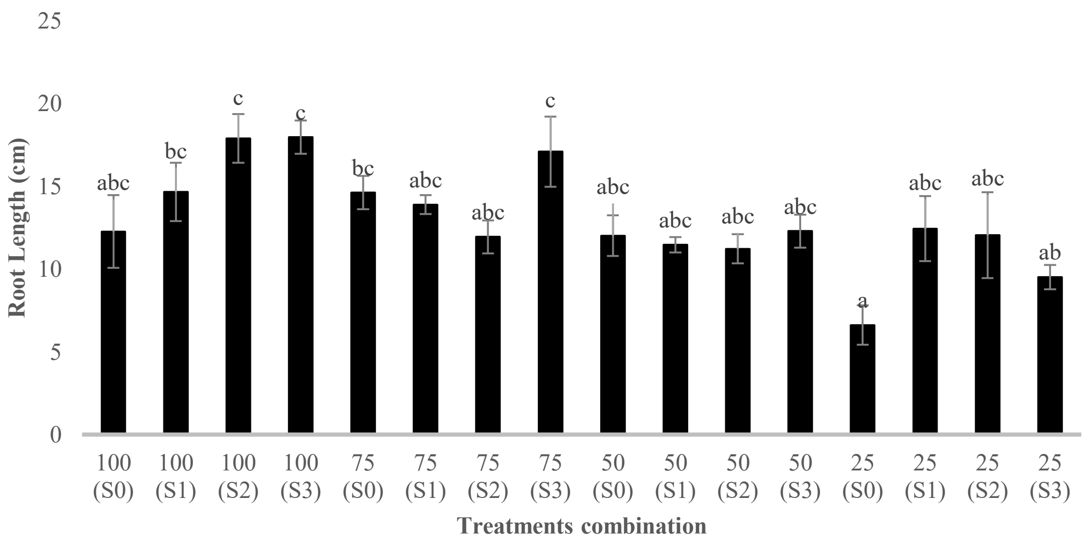


Figure 2 The combined effects of different water stress treatments and Si fertilizer rates on the root length of Sissoo spinach for week 8. Bars indicate the standard error of the mean (SEM). Note: (S0 = 0 %; S1 = 0.6 %; S2 = 0.9 %; S3 = 1.2 %) v/v%.

Leaf area

Figure 3 indicates the interaction of different water treatment levels and the silicon fertilizer rate in the Brazilian spinach leaf area. The treatment combination in **Figure 3** shows that severe water deficit (25 % water treatments) gives the lowest value of the leaf area of Brazilian spinach but is not statistically significant to most of the treatment combinations. Combining 100 % water treatments with 1.2 %v/v (S3) was statistically significant for most treatment combinations.

The findings demonstrated that a rise in water stress levels was associated with a drop in leaf area. These results agree with several studies, such as those conducted by Dehghanipoodeh *et al.* [26] in their experiment with strawberry plants. Water movement from the xylem to other elongating cells is disrupted, reducing turgor pressure in the leaves. A smaller leaf area could result from slower cell division and elongation [27]. Applying Si effectively raised *Glycyrrhiza uralensis* Fisch. and *Glycyrrhiza inflata* Bat leaf area, number and relative water content [28]. Following the application of Si to the leaf surface, Si was taken up by the leaves and stored in the cell wall of the inner leaf tissue as silica is hydrophilic, the treatment encouraged the build-up of water in the leaves, raised the water potential difference between the leaves and roots, enhanced the water pull, improved the roots' ability to absorb nutrients and water from the soil, and encouraged the growth and development of above-ground leaves [28].

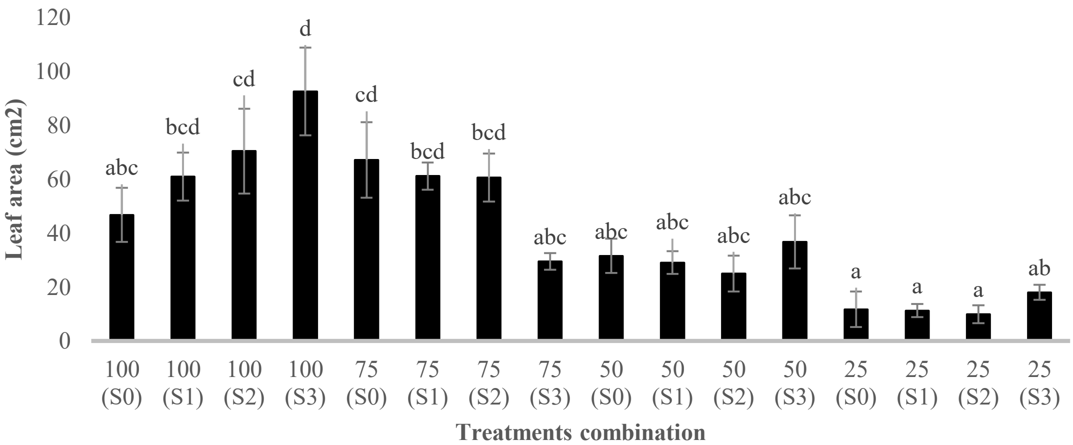


Figure 3 The combined effects of different water stress treatments and Si fertilizer rates on the leaf area of Sissoo spinach for week 8. Bars indicate the standard error of the mean (SEM).

Physiology parameters

Leaf gas exchange

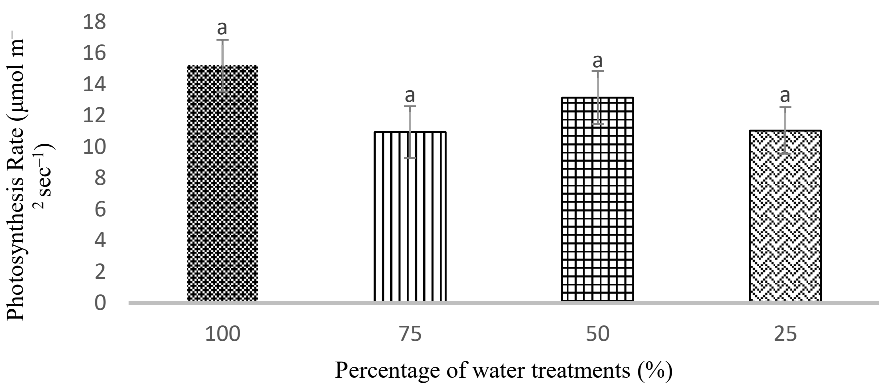
According to **Figure 4**, well-watered treatments (100 %) give a higher reading of photosynthesis rate. The result confirmed that water stress has affected the photosynthesis rates of Sissoo spinach cultivars, but not at the same level. There is no statistically significant between water treatments and photosynthesis rate. Photosynthesis rate decreases in water treatments by 75, 50 and 25 by 28.02, 13.49 and 27.37 %, respectively.

Photosynthesis is a vital process that plays a crucial role in the creation of biomass and the general growth and development of plants. Photosynthesis is a stress-sensitive physiological process that can be negatively affected by many stress conditions, leading to a decline in the functioning of the photosynthetic machinery [29,30]. Declines in photosynthesis rate with different water treatments have been studied in numerous plants, such as wheat [31], winter wheat [32], and maize [33].

Like the photosynthesis rate, stomatal conductance and transpiration rate are also influenced by water stress. The value of stomatal conductance increases as the water stress increases. In contrast, the value of the transpiration rate is not at the same level. The moderate water deficit gives a lower value than the high-water deficit. Both parameters in **Figure 4** illustrate that 25 % water treatments showed the lowest value while 100 % water treatments are the highest. The reduction of stomatal conductance from the control treatment is found to be higher at 42.29 % (25 % water), 14.38 % (50 % water), and 14.38% (75% water). The experiment showed a declination in the transpiration rate. The reduction of transpiration rate from the control treatment is found to be higher at 39.1 % (25 % water), 25.07 % (75 % water), and 16.12 % (50 % water).

Due to ongoing water stress, the stomata exhibit prolonged closure throughout the day. This causes a decrease in the rate at which carbon is absorbed and a decrease in water loss, leading to the prioritization of carbon assimilation above water availability. Stomatal closure can also lead to heightened vulnerability to photodamage. Stomata are the portals for carbon and water exchange [34]. Stomatal closure mitigates water loss resulting from transpiration, a process that can be regulated by signals transmitted from the roots in dehydrated soil - reductions in transpiration lead to elevated leaf temperature levels. The decrease in photosynthesis during water stress is primarily attributed to stomatal restriction [32]. It is essential to highlight that reducing stomatal conductance has protective consequences, as it helps plants save water and improves their water use efficiency [35]. Plants have developed several responses to water stress through morphological, anatomical, and cellular adaptations. These adaptations enable plants to survive in environments with persistent water stress. The process of stomatal closure serves as a physiological resistance mechanism. It happens when the mesophyll starts to experience dehydration, controlled by abscisic acid (ABA), increasing its concentration in the leaves [36].

Water shortage decreased soil moisture content and diminished water potential in above-ground plant structures, such as leaves and stems [37]. In dry situations, the rate of water loss through leaf transpiration exceeds the rate of water intake from roots. Roots absorb additional water through their growth, enabling plants to reduce water loss through stomata during water scarcity [38].



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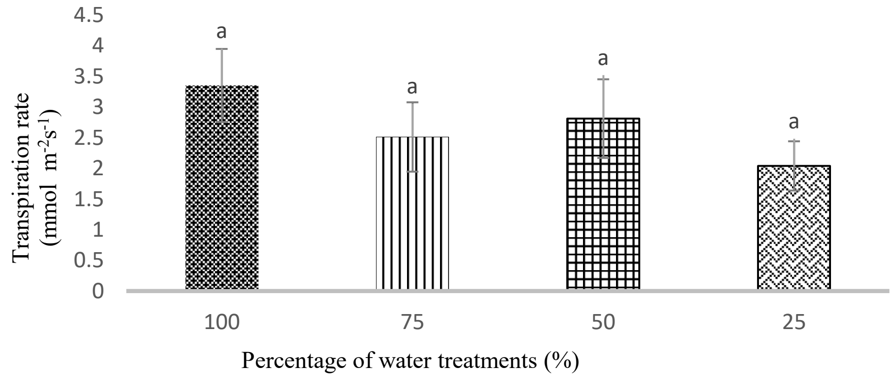

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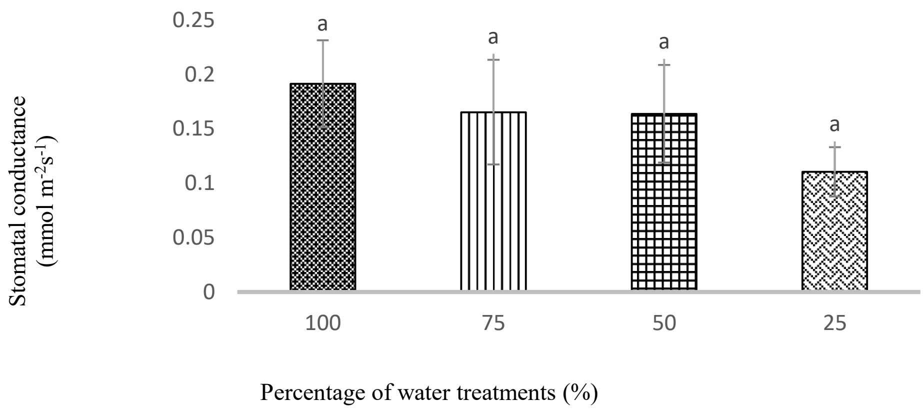


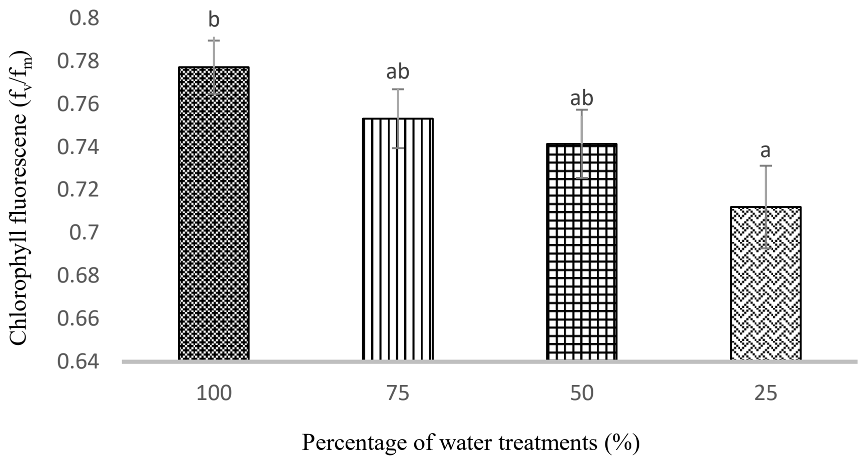
(c)

Figure 4 Effects of different water treatments on the photosynthesis rates, transpiration rate and stomatal conductance of Brazilian spinach on Week 8. Bars indicate the standard error of the mean (SEM).

Chlorophyll fluorescence

Figure 5 below shows the effects of different water treatments on the chlorophyll fluorescence of sissoo spinach for eight weeks. The decline of water levels from 100 > 75 > 50 > 25 % resulted in a markdown of chlorophyll fluorescence. The quantum efficiency of photosynthesis, or f_v/f_m, was measured in the eighth week of observation and was influenced by 4 distinct water concentrations: 100 % (control), 75, 50 and 25 %. The 25 and 50 % treatments showed the least quantity of f_v/f_m. Although chlorophyll fluorescence was higher in 100 % of the samples, there was no discernible difference between the 75 % and 50 % of water treatments.

Chlorophyll fluorescence is a valuable tool for monitoring the efficiency of photosynthesis in plants. As from this study, the values of chlorophyll fluorescence provided in **Figure 5** were affected by the water deficit. According to Zhang *et al.* [39] , the study found that dryness caused a significant decrease in tomato plants' chlorophyll fluorescence metric f_v/f_m. Chlorophyll fluorescence measurements offer valuable insights into photosystem II (PSII) activity and alterations in the photosynthetic metabolism of plants under stress. The f_v/f_m parameter has been extensively employed to identify stress-induced disturbances in the photosynthetic system. However, adding silicon greatly enhanced this value, improving the plants' photochemical efficiency. A similar result has also been observed in rice, a silicon accumulator under drought [40].



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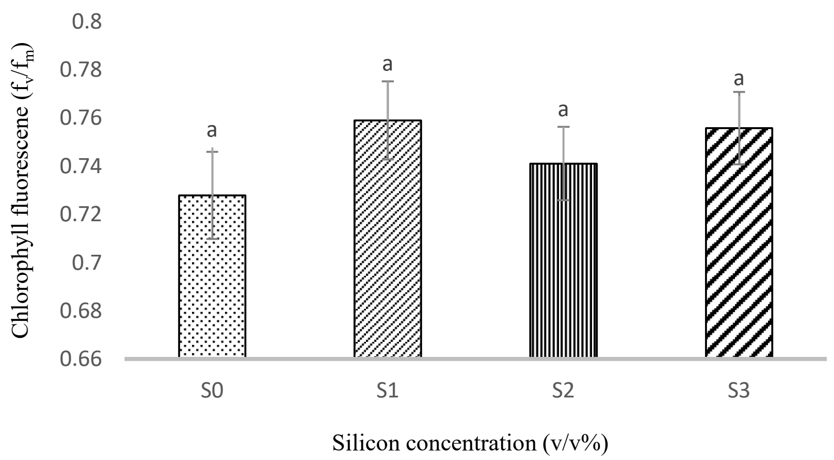

(b)

Figure 5 Effects of different water and silicon treatments on the chlorophyll fluorescence of Sissoo spinach on week 8. Bars indicate the standard error of the mean (SEM).

Table 2 Pearson correlation between measured parameters in week 8 of the experiment.

Parameters	1	2	3	4	5	6	7	8	9	10	11	12	13	14
FW	1													
DW	0.384^{**}	1												
RWC	0.636^{**}	–0.22	1											
Leaf area	0.331^{**}	0.052	0.367^{**}	1										
Root length	0.207	0.004	0	0.202	1									
Photosynthesis	0.076	–0.092	0.21	0.155	0.099	1								
Stomata	0.156	0.134	0.197	0.249^{*}	0.074	0.683^{**}	1							
Transpiration	0.104	0.034	0.245	0.154	–0.027	0.711^{**}	0.940^{**}	1						
Chlorophyll Fluorescence	0.279^{*}	–0.041	0.382^{**}	0.238	–0.173	0.033	0.098	0.162	1					
SPAD value	0.16	–0.079	0.265^{*}	0.343^{**}	0.083	0.133	0.135	0.039	0.021	1				
MDA	0.134	0.05	0.085	0.008	0.051	–0.101	0.098	0.028	–0.065	–0.006	1			
Leaves numbers	0.437^{**}	0.065	0.525^{**}	0.655^{**}	–0.005	0.238	0.227	0.147	0.186	0.430^{**}	0.055	1		
Stem diameter	–0.102	0.267^{*}	–0.19	–0.138	–0.238	–0.066	–0.043	–0.06	–0.155	$–0.373^{**}$	0.048	–0.022	1	
Height	0.118	0.314^{*}	–0.138	–0.013	–0.079	0.057	–0.062	–0.119	–0.112	–0.151	–0.019	0.175	0.588^{**}	1

Note: FW = Fresh weight; DW = Dry weight; RWC = Relative Water Content; Photosynthesis = photosynthesis rate; Stomata = stomatal conductance; Transpiration = transpiration rate; MDA = Malondialdehyde *and ** at $p \leq 0.05$ and $p \leq 0.01$ respectively.

Chlorophyll content

Figure 6 indicates the effects of different water treatments on the chlorophyll content of Sissoo spinach on week 8. The chlorophyll contents showed a significant flourishing value for the sissoo with a rise in water treatment levels. Among the water treatments, the 100 % water treatment was the highest when compared to the other water treatments. In contrast, the 25 % water treatment had the lowest chlorophyll content value and was statistically significantly different from the control. No statistically significant difference was observed across water treatments affecting chlorophyll content, falling between 75 and 50 %. Nevertheless, no substantial disparity was observed between the 75 and 100 % (control) water treatments.

The SPAD value can be an indicator for the chlorophyll content and leaf greenness [41]. According to Aslanpour and Omar [42], plants' chlorophyll content will decrease under drought stress conditions. The decline in chlorophyll levels during periods of stress is likely caused by an increase in the catalytic activity of chlorophylls and the degradation of photosynthetic pigments. This process also results from the absence of essential factors for chlorophyll synthesis and the disruption of its structure under stressful conditions [43].

The 75 and 50 % water treatments show no significant value, as shown in the study by Reyes *et al.* [36], stating that the plants were not under severe water stress. Hence, it is unlikely that the chlorophyll concentration was impacted. Despite treating plants to specific amounts of water stress, no changes in chlorophyll production were observed.

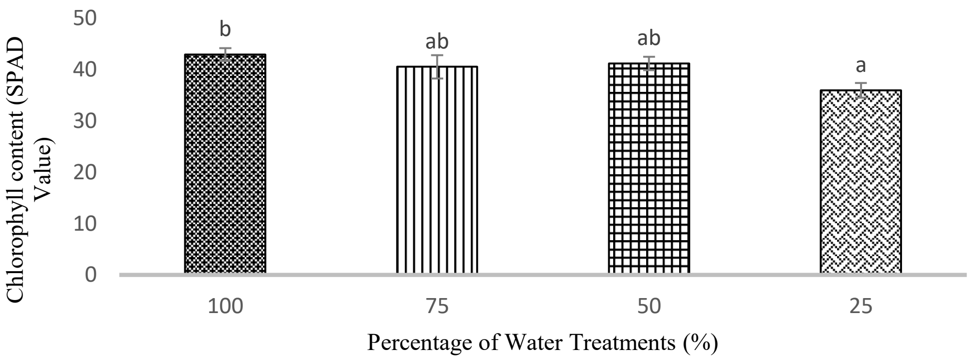

Figure 6 Effects of different water treatments on the chlorophyll content of Sissoo spinach on Week 8. Bars indicate the standard error of the mean (SEM).

Relative water content (RWC)

Figure 7 shows the effects of different water and silicon treatments on Sissoo spinach's relative water content (RWC) for 8 weeks. The decline of water levels from 100 % > 75 % > 50 % > 25 % resulted in a markdown of RWC. There is a significant difference between 100, 50 and 25 % of water treatments. However, 75 % of water treatments are not substantial to the 100 and 50 % of water treatments. Silicon fertilizers are statistically significant to the RWC. The silicon fertilizer of S3 (1.2 v/v%) gives a higher reading than the other and provides a significant difference with S0 (control).

RWC (relative water content) is a widely used indicator to exhibit the water stress of plants including spinach [44]. The reduction of RWC in plants experiencing drought stress has been seen in numerous plant species, such as in faba beans [43], sugarcane [45] and cowpea [46]. It may be influenced by the plant's overall health and vitality. In this context, the capacity to effectively regulate osmotic balance and maintain cell turgor is probably diminished. The concentration of organic solutes to sustain the membrane appears to be inadequate in this instance. It was noted that plants experiencing well-watered treatments maintained a consistently more outstanding relative water content (RWC) throughout the experiment than plants experiencing extreme or severe stress. These genotypes exhibited superior preservation of higher relative water content (RWC), resulting in improved hydration and more favorable internal water relations of the tissue. They potentially had an immense pressure potential and demonstrated a greater capacity for drought resistance [46].

The interaction between water stress levels and silicon rate did not significantly affect RWC. A study conducted by Mali and Aery [47] reported that the lack of water in the soil or the root systems' inability to replace water lost through transpiration by decreasing the absorbing surface could cause this decline in leaf RWC. Alternative moisture of plant water states, which reflect the metabolic activities in tissues, is defined as the relative water content in leaves. Silicon applied to tissue improved light-interception properties by maintaining the leaf blade's erect position and reducing transpiration, reducing water stress [48].

Based on **Table 2**, RWC had a significant positive correlation with leaf area ($R^2 = 0.367$; $p \leq 0.05$). This result was supported by Mishra *et al.* [15] where the results implied that greater leaf area under drought stress led to more transpiration, which in turn produced a drop in the RWC of leaves because, in lentils under drought stress, reduced leaf area is considered to be a favorable characteristic.

Biochemical properties

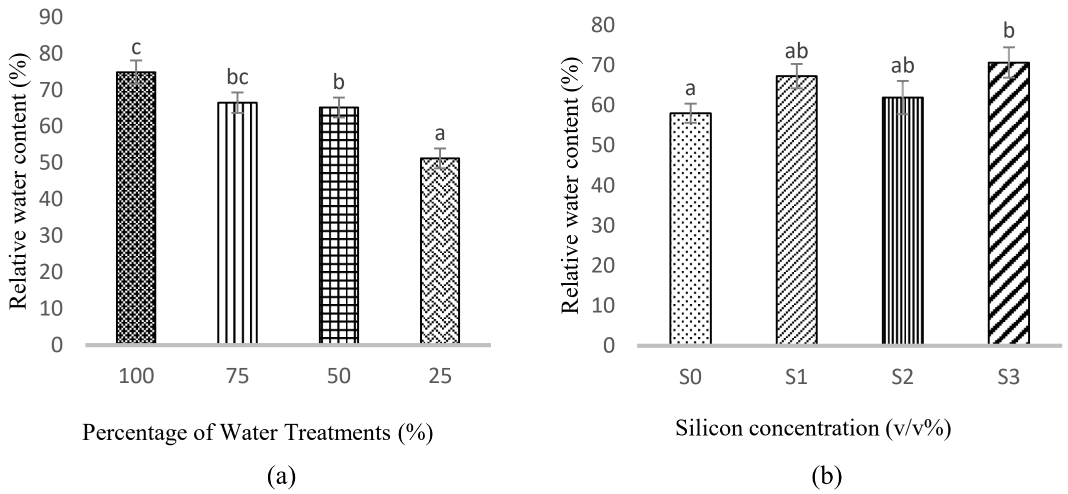

Figure 7 Effects of different water treatments and silicon concentration on Sissoo spinach's relative water content (RWC) on Week 8. Bars indicate the standard error of the mean (SEM).

Malondialdehyde

The biochemical properties of Sissoo spinach showed significant differences in water treatments in contrast with fertilizer treatments. Based on **Figure 8**, there is a substantial difference in the interaction between water treatments and Si fertilizer. Water treatments of 100 % give the lowest amount of MDA compared to other water treatments. Under 50 % water treatment, the MDA level increased by 26.52 % from the control compared to severe water treatment (25 %), where MDA rose to 91.36 %.

Malondialdehyde (MDA), a by-product generated by lipid membranes in response to reactive oxygen species (ROS), might serve as a drought indicator for assessing the extent of plasma membrane impairment and the capacity of plants to withstand drought-induced stress [49,50]. Silicon fertilizer was not significant to the MDA level. **Figure 8** illustrates the effects of different water stress treatments and Si fertilizer rates on the root length of Sissoo spinach on week 8. The combinations of 100 % water treatments with multiple Si concentrations give the lowest reading of the MDA level. According to Zhang *et al.* [50], the decrease in lipid peroxidation caused by silicon was linked to enhanced antioxidant defense and reduced generation of reactive oxygen species (ROS).

Based on **Figure 8**, the water treatments of 75 % give the highest value of MDA compared to 100 %, 75 and 25 %. The combination of 75 % water treatment with a concentration of Si and S2 (0.9 %v/v) gives the spike amount of MDA level. It's due to a pest attack during the experiment. Based on the study by Zhang *et al.* [51], high-density pest feeding increased the MDA content.

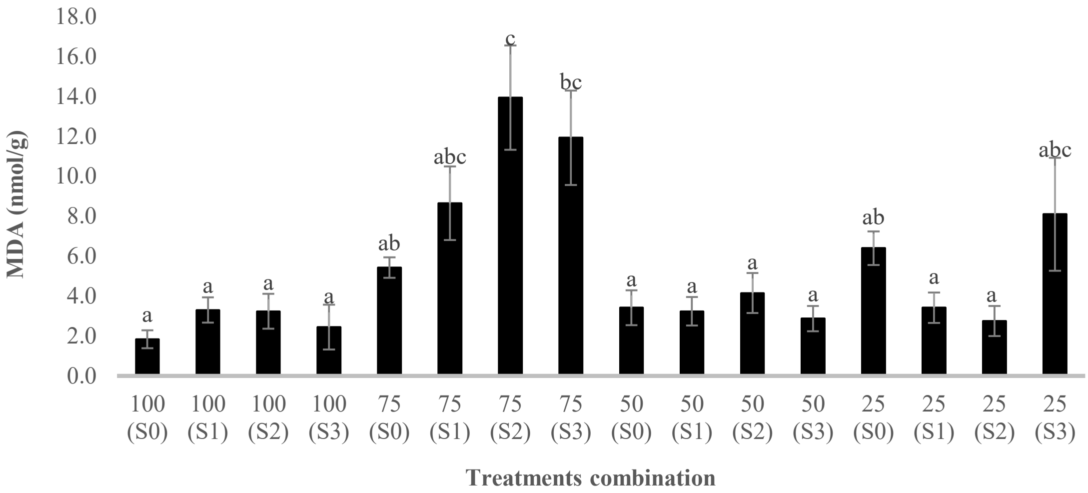

Figure 8 The combined effects of different water stress treatments and Si fertilizer rates on the root length of Sissoo spinach for week 8. Bars indicate the standard error of the mean (SEM).

Conclusions

The primary objective of this research study is to evaluate the effect of water stress on *Alternanthera sissoo*'s growth, physiological, and biochemical properties and to determine the optimum level of silicon under water stress of *Alternanthera sissoo*. This study also presents data regarding the optimal quantity of water necessary for cultivating Sissoo spinach and the optimum silicon level under water stress. Although Sissoo spinach has gained popularity and demand, limited study has been conducted to determine the optimal water requirement for its cultivation to maximize water efficiency and minimize unnecessary water usage. The growth parameters of *Alternanthera sissoo*, such as height, leaf area, root length and chlorophyll content, exhibited a significant drop in response to water stress. The drought also caused a decline in the plant's hydration status, chlorophyll fluorescence and leaf gas exchange. Applying silicon positively impacts the physiology and growth of Brazilian spinach. The root length, leaf area, and malondialdehyde (MDA) show the interaction between water stress level and silicon concentration. The study proposed that the Si nutrient management strategy has the potential to minimize the impacts of drought stress in Brazilian spinach. Regarding research findings, it was determined that the optimal water requirement for *Alternanthera sissoo* is 50 % of the water treatments, with a silicon concentration of 1.2 %v/v. This is crucial for more efficient water use without requiring a lot of water and better plant water management.

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