BUKTI KORESPONDENSI RESPONDENSI DAN SUBSTANSI ISI ARTIKEL JURNAL INTERNASIONAL BEREPUTASI

Judul : Growth and yield of Brazilian spinach under different shading intensities and harvesting periods in a tropical lowland urban ecosystem

Jurnal : Revista de Agricultura Neotropical-(Scopus Q4)

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Kontribusi : Co-Author

No	Perihal	Tanggal
1.	Submission	18 Januari 2024
2.	Pre-review	31 Januari 2024
3.	Review	07 Mei 2024
4.	Editor Decision: Accepted	22 Mei 2024
5.	Proofing	03 Juni 2024
6.	Final Paper	13 Juni 2024

1. Submission (18 Januari 2024)

BRAZILIAN SPINACH GROWTH UNDER DIFFERENT SHADING INTENSITIES AND HARVESTING PERIODS IN LOWLAND TROPICAL URBAN ECOSYSTEM

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ABSTRACT

Brazilian spinach, a lesser-known leafy vegetable, has a high nutritional value and is essential for human health. A study was conducted to evaluate the growth of Brazilian spinach in tropical lowland urban ecosystems under different levels of shade intensity and harvest periods. The research used a split-plot design, assigning different levels of shading intensity as the main plot and harvesting periods as sub-plots. The results showed that Brazilian spinach growth was more favourable when exposed to treatment without shade compared to shaded conditions. The impact of shading on plant growth was observed during the early stages of growth, as indicated by alterations in canopy parameters and SPAD values. After productivity assessment, the impact of shading was assessed by branch elongation, yield, fresh weight, and dry weight of each organ. Shading increased the carbon content of Brazilian spinach leaf, while reducing the nitrogen content. More frequent harvesting resulted in an increase in yield components but suppressed the growth of stems and branches. Therefore, it is recommended to cultivate Brazilian spinach in an unshaded area with a biweekly harvesting routine.

KEYWORDS

Harvest time, leafy green, lesser-known vegetable, plant acclimatization, solar irradiation intensity.

INTRODUCTION

Brazilian spinach (*Alternanthera sissoo*) is a leafy vegetable originating from Brazil. As reported by Ikram et al. (2021), Brazilian spinach is rich in flavonoids, vitamins, minerals, and other antioxidants, which have been found to have positive effects on human health. The cultivation and use of this particular plant by the Indonesian population are infrequent, leading to its classification as a rather rare plant species. Indonesia's agroclimatology exhibits similarities to its indigenous location, hence indicating the potential for cultivating this plant within the country.

Urban cultivation faces several challenges, especially in regard to the availability of light for plants. Shaded areas in urban environments tend to prevail, impeding the penetration of light into plant development. Consequently, the amount of light received by plants decreases, leading to disruptions in certain aspects of plant metabolism. This phenomenon is particularly observed in horticultural crops characterized by compact growth, such as Brazilian spinach. Based on Shafiq et al. (2021), regulating alterations occur in plants as a means to enhance the efficiency of photosynthesis. The tolerance of plants to the intensity of light they receive varies depending on the specific plant species. Certain vegetable crops have been reported as being capable of growing under shaded conditions. In this regard, Sifuentes-Pallaoro et al. (2020) revealed that *Lactuca canadensis* exhibits favourable growth under shading, while Lakitan et al. (2021) found that celery also demonstrates similar adaptability.

Brazilian spinach is a perennial leafy vegetable, enabling its continuous growth throughout the year. Additionally, this suggests that regular harvesting is required. Annual plants undergo periodic harvesting that involves a defoliation mechanism. According to the findings of Raza et al. (2021), the implementation of a defoliation treatment on plants has been observed to enhance overall plant growth, particularly in terms of leaf growth, especially during the vegetative phase. Further experimentation is required to enhance the output of Brazilian spinach, a plant species characterised by its commercially valuable leaf organs.

The cultivation of Brazilian spinach is characterized by its simplicity, since it may be easily grown. Muda et al. (2022) reported that the propagation of Brazilian spinach can be achieved via stem cuttings. There is an insufficient amount of research pertaining to the adaptability of Brazilian spinach to shading environments. The capacity of Brazilian spinach to acclimatise to shading environments for a specific duration will ensure the availability of sustainable vegetable nutrition. The study was aimed to evaluating the adaptability of Brazilian spinach to shading conditions via various harvesting periods.

MATERIALS AND METHODS

Research site and agroclimatic characteristic

The research was carried out in the Jakabaring research facility located in Palembang (104º46'44"E, 3º01'35"S), South Sumatra, Indonesia. This research began with initiating the propagation of stem cuttings on 30 January 2023, and concluded the data collection on 02 May 2023. The research site is situated in a tropical urban lowland area with an elevation of 8 meters above sea level (masl). The agroclimatic characteristics of the area are shown in Figure 1.

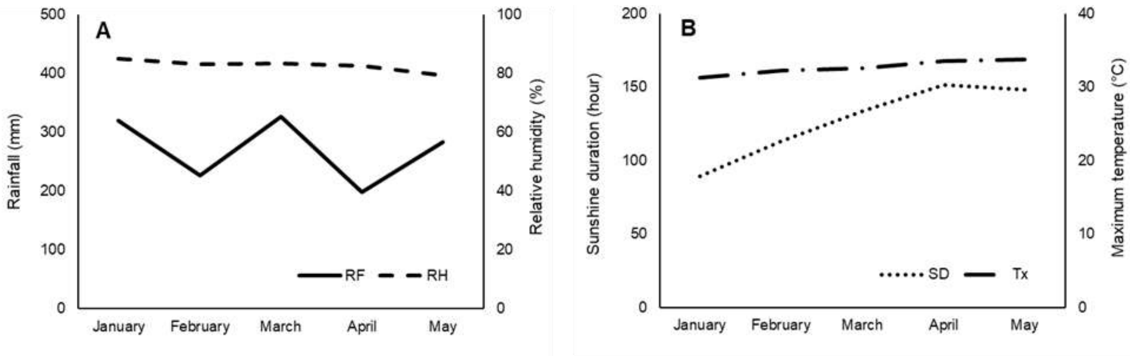

Figure 1. Agroclimatic characteristics in research location as indicated by total monthly rainfall (RF) and average humidity (RH) (A), and total monthly sunshine duration (SD) and average maximum temperature (Tx) (B). (Source: Indonesian Agency for Meteorology, Climatology and Geophysics).

Cultivation and treatment procedures

The propagation material used was stem cuttings with two leaves that were taken from healthy mother plant. The planting materials were planted in pots with dimensions of 27.5 cm in diameter and 20 cm in height. The pots were filled with a growing medium that was a mixture of top soil and chicken manure (3:1 v/v). Prior to planting, the growing medium had a bio-sterilization treatment (2 g/l), with the addition of live microorganisms including *Streptomyces thermovulgaris*, *Thricoderma virens*, and *Geobacillus thermocatenulatus*. The growing medium was subsequently subjected to a one-week incubation period before planting.

The growing medium that had been incubated was used for the planting of Brazilian spinach cuttings, which were subsequently arranged in accordance with the principles of a split-plot design. The main plot of the study focused on the intensity of shading, whereas the subplot examined the harvest period. The treatment involved selecting shading intensity at three separate levels, namely no-shading (S0), 45% shading (S45), 55% shading (S55), and 85% shading (S80). Furthermore, treatment for the harvest period started after the simultaneous harvesting, which was carried out at 5 weeks after planting (WAP). The designated harvest period has three different intervals, namely appearing per 2 weeks, per 3 weeks, and per 4 weeks, symbolised as H2, H3, and H4, respectively.

The plants were systematically positioned within shadow houses measuring 4 meters in length, 2 meters in width, and 2 meters in height. These shadow houses are constructed using knockdown frames made of 1.5-inch PVC pipes. The entire perimeter of the shadow house is enveloped with a shade material, specifically a black polyethylene net, which has been tested for its density to ensure optimal shading.

The leaves of the Brazilian spinach cuttings get trimmed when it reaches one week after planting (WAP) in order to maintain uniformity in the planting material. Meanwhile, fertilization was conducted using NPK fertilizer (16:16:16) at 1 week after planting (WAP) and 5 WAP. The watering of each plant was normally carried out around 08:00 a.m.

Data collection

The data collection covered Brazilian spinach growth and yield data. The growth data that was obtained is categorised into two categories of measurements, such as non-destructive and destructive. The dataset for non-destructive growth measurement includes several variables, including SPAD values, canopy width, canopy diameter, canopy index, branch length, and stem diameter. In addition, the destructive measurements included the stem fresh weight, branch fresh weight, root fresh weight, stem dry weight, branch dry weight, and root dry weight. The collected data concerning Brazilian spinach yield covers several parameters, including the fresh weight of marketable leaf, fresh weight of non-marketable leaf, dry weight of marketable leaf, dry weight of non-marketable leaf, the carbon content of marketable leaf, nitrogen content of marketable leaf, and the C:N ratio of marketable leaf. The moisture content of the planting medium was also examined in order to determine the water content of the substrate.

The SPAD value was monitored using chlorophyll meters (SPAD-502 Plus, Konica-Minolta Optics, Inc., Osaka, Japan). Canopy area was calculated using a digital image scanner for Android (Easy Leaf Area software, developed by Easlon & Bloom

2014). Substrate moisture (SM) was measured using a soil moisture meter (PMS-714, Lutron Electronics Canada, Inc., Pennsylvania, USA). The carbon, nitrogen, and C:N ratios were examined using Kjedahl-Titrimetry in the Integrated Laboratory of Sampoerna Agro. Tbk.

The dry weight of each plant organ was determined by treating it to a drying process in an oven set at a temperature of 100°C for a duration of 24 hours. Prior to being placed in the oven, the plant's organs are trimmed to a reduced thickness to accelerate the drying process.

Data analysis

All data collected was analysed using the RStudio software version 1.14.1717 for Windows (developed by RStudio team, PBC, Boston, MA). Significant differences among treatments were tested using the least significant difference (LSD) procedure at $p<0.05$.

RESULTS AND DISCUSSION

The Brazilian spinach growth during early vegetative growth before harvested

The early vegetative growth of Brazilian spinach is analysed by considering its unique characteristics, such as canopy growth and SPAD value. This approach involves non-destructive observation, allowing plants to grow naturally. The canopy characteristics selected were: canopy area, canopy diameter, and canopy index.

Brazilian spinach grown in unshaded conditions (S0) had a higher leaf initiation and larger individual leaf area compared to in shading conditions. More and larger leaves contribute to the increase in canopy area. This leads to a broader canopy compared to those grown under shade. The Brazilian spinach canopy area growth increased significantly in the S0 condition, particularly 2 to 5 weeks after planting, compared to shade conditions (S45, S55, and S80). However, no significant leaf growth was observed in the S45 and S55 shades. The shading with the highest density (S80) showed suppressed growth, starting 2 weeks after planting (Figure 2).

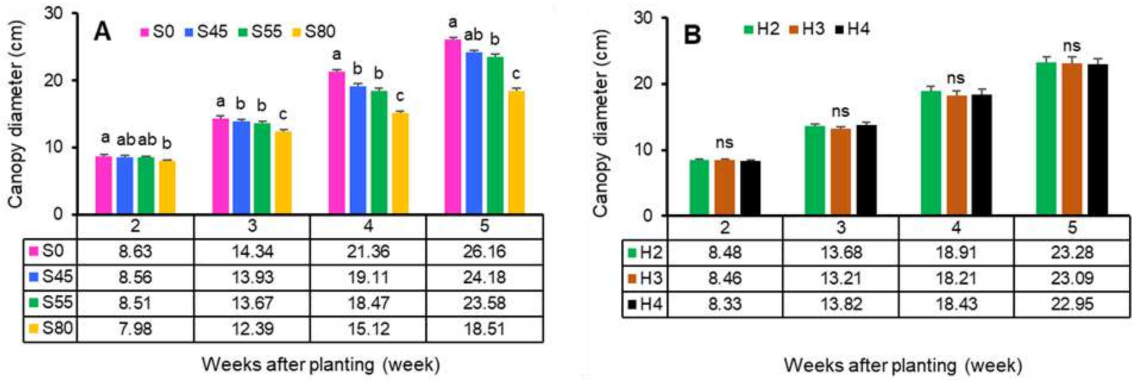

Figure 2. Brazilian spinach canopy area on early vegetative growth on different shading (A) and harvest period (B) treatment. The shading consists of no shading (S0), 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent harvest period per 2 weeks, 3 weeks, and 4 weeks, respectively. The ns mean non-significant difference at $p<0.05$.

Brazilian spinach branches significantly influence canopy diameter, with elongation affecting canopy expansion. Shading treatment inhibits branch growth. Full sunlight cultivation leads to a wider canopy than canopies grown under different levels of shading (S45, S55, and S80) (Figure 3).

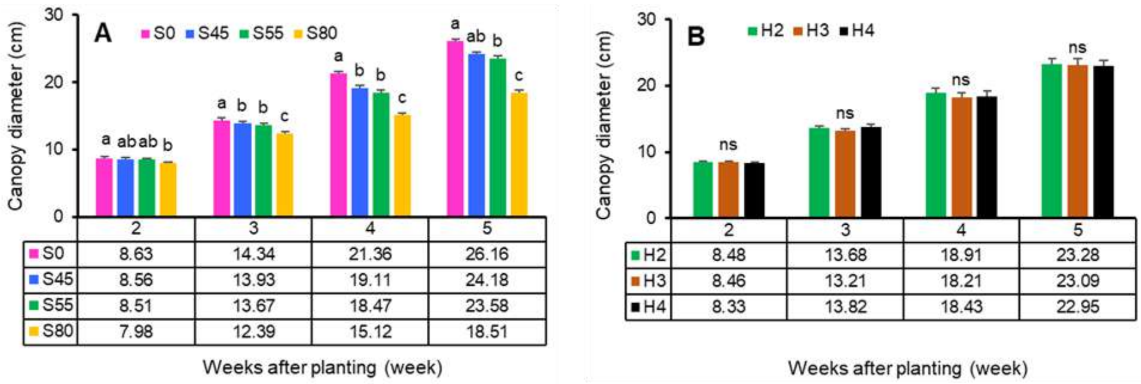

Figure 3. Brazilian spinach canopy diameter on early vegetative growth on different shading (A) and harvest period (B) treatment. The shading consists of no shading (S0), 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent harvest period per 2 weeks, 3 weeks, and 4 weeks, respectively. The ns mean non-significant difference at $p<0.05$.

The growth of leaf and branch significantly affects canopy density, with dominant growth resulting in a denser canopy as represented by canopy density. The effect is most noticeable between 4 and 5 weeks after planting. Brazilian spinach grown under shading conditions, especially S80, showed reduced leaf size and branch elongation, resulting in lower canopy density (Figure 4).

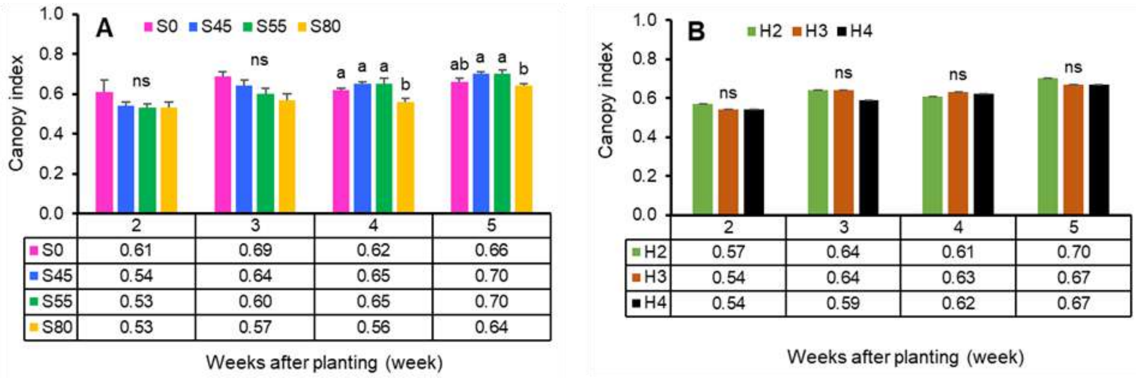

Figure 4. Canopy index on early vegetative growth on different shading and harvest period as treatment. The shading consists of no shading (S0), 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent harvest period per 2 weeks, 3 weeks, and 4 weeks, respectively. The ns mean non-significant difference at $p<0.05$.

According to this research's findings, Brazilian spinach's canopy growth was more hindered under greater shading (S80) than it was unshaded. The constituent organs of the canopy, such as the leaves and branches, endure stunted growth, which prevents the canopy from growing. According to Fadilah et al. (2022), denser shading intensity was shown to inhibit purple pakchoy leaf growth. According to the findings of Wan et al. (2020), plants planted in the shading area produce less photosynthetic performance than plants grown in full sunlight. Moreover, Liang et al. (2020) have underscored the significance of shading for plants, noting that it leads to a decrease in photosynthesis, resulting in a reduction in carbon flow. The inhibition of vegetative organ growth, particularly the canopy, in Brazilian spinach is attributed to the decreased carbon flow. This reduction in carbon flow occurs throughout the early growth cycle. The phenomenon of reduced vegetative organ development due to shading during the early growth phase has been documented in various vegetable crops, including chili (Kesumawati et al., 2020).

The SPAD value is a method for evaluating leaf nitrogen and chlorophyll content, with a positive relationship between these factors. Brazilian spinach leaf's SPAD value was affected by shading treatments, with differences observed within each shading treatment from the early growth 2 weeks after planting (WAP) (Figure 5).

Brazilian spinach grown without shading (S0) showed a higher SPAD value compared to under different shading levels, with a notable rise starting 4 weeks after planting. This trend was also observed in S45 and S55. On the other hand, Brazilian spinach grown at S80 showed a stagnation trend, persisting until the end of the early growth, specifically 2 to 5 weeks after planting.

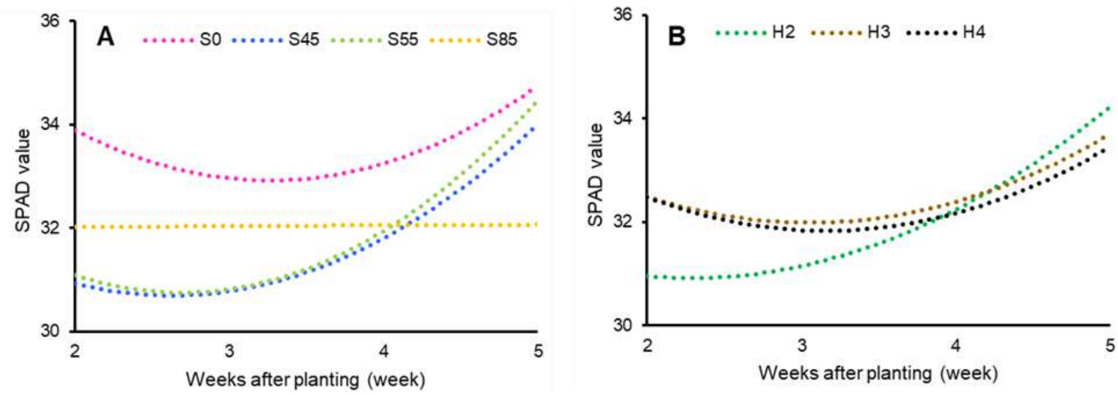

Figure 5. The SPAD value on early vegetative growth on different shading (A) and harvest period (A) treatment. The shading consists of no shading (S0), 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent harvest period per 2 weeks, 3 weeks.

The SPAD value is a widely used method for assessing leaf chlorophyll and nitrogen content, with its reliability well established. It has been found to have a positive correlation with both chlorophyll content and leaf nitrogen content (Song et al., 2021; Farnisa et al., 2023). Prior research had provided confirmation on the capacity of specific leafy vegetables, namely *Talinum paniculatum* (Lakitan et al., 2021) and spinach (Mendoza-Tafolla et al., 2019), to proficiently evaluate and track the quantities of leaf chlorophyll and nitrogen content.

Brazilian spinach grown under full sun (S0) has a higher SPAD value than that grown under shading, indicating that shading reduces the solubility of chlorophyll and nitrogen. Wang et al. (2020a) found that shading affects the solubility of nitrogen, leading to a decrease in leaf nitrogen content. Li et al. (2020) highlighted the biochemical alterations caused by shading stress. This condition is due to Brazilian spinach, particularly in plants subjected to the 80% shading treatment (S80).

Brazilian spinach growth after harvested

The vegetative growth of Brazilian spinach after harvest was conducted at 5 weeks after planting. The growth of branch was compared under different shading conditions, harvest periods, and their interaction effects. Brazil spinach grown under S80 exhibited shorter branches as early as 11 weeks after planting. However, different shading treatments (S0, S45, and S55) showed comparable levels of branch elongation until 9 weeks after planting. Brazilian spinach grown in S45 had an increased rate of branch elongation, particularly at 10 and 11 weeks after planting.

The elongation of the Brazilian spinach branch was influenced by the harvesting period. Less frequent harvesting leads to the highest branch elongation, especially from 7 to 11 weeks after planting. An interaction between shading level and harvesting period treatment was observed, starting within 9 weeks after planting. This highlights the importance of harvesting frequency in influencing Brazilian spinach growth.

The study revealed a decrease in branch elongation in Brazilian spinach at S80, indicating a decrease in the allocation of photosynthetic products. This is due to reduced levels of non-structural carbohydrates, which are essential for growth (Yamashita et al., 2020). However, in the S0 treatment, photosynthesis is optimised, leading to increased branch growth.

The increased frequency of harvesting inhibits branch growth, and it is possible that the distribution of photosynthetic products changes, potentially causing a heightened initiation process of new leaves (Oliveira et al., 2021). Additionally, Raza et al. (2021) reported that maize plants with a higher number of eliminated leaves have an increased allocation of photosynthetic resources towards expanded leaves, as evidenced by an enhanced leaf area.

Table 1. Branch elongation of Brazilian spinach after harvested on different shading, harvest period, and their interaction.

Treatment	Weeks after planting (week)						
	5	6	7	8	9	10	11
				Shading			
S0	11.98 ± 0.16 a	15.89 ± 0.17 a	19.18 ± 0.43 a	22.31 ± 0.38 a	24.16 ± 0.34 a	25.22 ± 0.33 b	26.17 ± 0.53 b
S45	11.63 ± 0.33 ab	15.11 ± 0.39 ab	19.00 ± 0.55 a	22.02 ± 0.76 a	24.30 ± 0.70 a	28.23 ± 0.95 a	29.02 ± 1.20 a
S55	11.03 ± 0.17 b	14.00 ± 0.22 b	18.03 ± 0.57 a	20.74 ± 0.80 a	22.32 ± 0.85 a	25.91 ± 0.95 b	26.74 ± 1.07 ab
S80	8.68 ± 0.13 c	10.25 ± 0.17 c	12.66 ± 0.36 b	14.47 ± 0.51 b	14.93 ± 0.54 b	15.97 ± 0.76 c	16.46 ± 0.75 c
Probability	***	***	***	***	***	***	***
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
$LSD_{0.05}$	0.897	1.491	1.744	2.357	2.192	2.283	2.829
			Harvest period				
H2	11.06 ± 0.41	14.03 ± 0.69	15.80 ± 0.80 b	18.51 ± 1.09 c	19.66 ± 1.21 c	21.59 ± 1.41 c	21.90 ± 1.36 c
H3	10.72 ± 0.42	13.80 ± 0.71	18.07 ± 0.90 a	19.54 ± 1.02 b	21.75 ± 1.26 b	24.31 ± 1.58 b	24.95 ± 1.61 b
H4	10.70 ± 0.44	13.60 ± 0.66	17.78 ± 0.85 a	21.61 ± 0.98 a	22.88 ± 1.17 a	25.60 ± 1.45 a	26.94 ± 1.61 a
Probability	ns	ns	***	***	***	***	***
P-value	0.186	0.198	<0.001	<0.001	<0.001	<0.001	<0.001
$LSD_{0.05}$	0.462	0.507	0.607	0.878	0.708	0.922	0.876
			Shading x harvest period				
S0H2	12.23 ± 0.12	16.05 ± 0.34	18.55 ± 0.54	21.47 ± 0.44	23.22 ± 0.49 cd	24.24 ± 0.58 de	24.57 ± 0.62 ef
S0H3	11.93 ± 0.41	16.20 ± 0.20	20.31 ± 0.72	22.40 ± 0.85	24.85 ± 0.40 b	25.68 ± 0.39 cd	26.91 ± 0.63 cd
S0H4	11.77 ± 0.27	15.41 ± 0.14	19.08 ± 0.44	23.08 ± 0.41	24.41 ± 0.53 bc	25.74 ± 0.34 cd	27.04 ± 0.78 bcd
S45H2	11.99 ± 0.62	15.51 ± 1.10	17.45 ± 0.72	20.97 ± 1.52	22.53 ± 0.85 d	25.43 ± 1.20 cd	25.43 ± 0.91 de
S45H3	10.89 ± 0.71	14.68 ± 0.52	19.57 ± 0.59	20.87 ± 0.95	23.77 ± 0.60 bcd	28.07 ± 0.83 b	28.54 ± 0.87 bc
S45H4	12.01 ± 0.19	15.14 ± 0.41	19.99 ± 0.95	24.24 ± 0.20	26.59 ± 0.68 a	31.19 ± 0.67 b	33.08 ± 0.99 a
S55H2	11.08 ± 0.11	13.97 ± 0.22	16.00 ± 0.56	18.64 ± 0.81	19.53 ± 0.71 d	22.70 ± 0.97 e	23.19 ± 0.82 f
S55H3	11.37 ± 0.44	14.21 ± 0.67	19.19 ± 0.59	20.58 ± 1.41	23.50 ± 1.48 bcd	27.88 ± 1.25 b	28.27 ± 1.61 bc
S55H4	10.64 ± 0.03	13.81 ± 0.21	18.90 ± 0.28	23.00 ± 0.45	23.92 ± 0.49 bcd	27.15 ± 0.70 b	28.77 ± 1.02 b
S80H2	8.95 ± 0.06	10.58 ± 0.13	11.61 ± 0.27	12.97 ± 0.37	13.32 ± 0.40 h	13.98 ± 0.60 g	14.41 ± 0.53 h
S80H3	8.70 ± 0.32	10.13 ± 0.37	13.22 ± 0.70	14.33 ± 0.60	14.89 ± 0.22 g	15.62 ± 0.39 g	16.09 ± 0.29 h
S80H4	8.38 ± 0.11	10.04 ± 0.32	13.14 ± 0.38	16.11 ± 0.38	16.59 ± 0.75 f	18.32 ± 1.27f	18.87 ± 1.17 g
Probability	ns	ns	ns	ns	**	**	**
P-value	0.237	0.89	0.211	0.383	0.009	0.008	0.003
$LSD_{0.05}$	0.920	1.014	1.214	1.756	1.416	1.844	1.751

The ns mean non-significant difference at $p<0.05$.

Brazilian spinach showed significant differences in leaf growth when treated with different shading and harvesting periods. Cultivated without shade (S0), it tends to dominate leaf growth compared to cultivated under different levels of shading (S45, S55, and S80) (Table 2). However, this method also demonstrated a significant proportion of non-marketable leaves. This indicates that early leaf growth is achieved without shading, but it also leads to accelerated leaf aging and increased pest susceptibility, resulting in a higher proportion of non-marketable leaves compared to those cultivated under shading.

The frequent harvesting of Brazilian spinach leads to the initiation of young leaves, resulting in more marketable leaves. This is evident in the yield of commercially viable leaves throughout the H2 and H3 periods. However, during the H3 harvesting period, a significant proportion of non-marketable leaves are produced due to leaf aging. In contrast, extended harvesting periods (H4) often lack the capacity for leaf initiation, resulting in decreased yields of both marketable and non-marketable leaves. The interaction impact of shading and harvesting period significantly showed on leaf growth, with the most significant impact observed under 80% shade, especially during the longer harvesting period (H4).

Brazilian spinach's leaf initiation is higher in conditions without shade compared to shading conditions, affecting both marketable and non-marketable leaves. This is due to reduced carbohydrate accumulation and allocation (Hussain et al., 2019). Shading conditions inhibited plant growth, while without shade, leaf senescence accelerated due to enhanced photosynthesis. Meanwhile, direct sunlight exposure accelerates ageing processes in plants, similar to sweet basil (Castronuovo et al., 2019). However, without shade, spinach is more susceptible to pest infestation, leading to an increased prevalence of non-marketable leaves. Implementing shading at a specific density is a viable pest control strategy.

Brazilian spinach harvesting, similar to leaf and shoot pruning, has been found to increase yield. Dheeraj et al. (2022) found that pruning at the apical meristem increased growth-promoting hormones, specifically cytokinin. Xu et al. (2020a) reported that pruning tomato plants also increases cytokinin hormone levels. Cytokinin hormones influence cell division processes, including those during leaf cell development. Harvesting Brazilian spinach at H2 and H3 treatment results in elevated levels of cytokinin hormones, enhancing leaf initiation and resulting in a greater marketable yield.

Tabel 2. Brazilian spinach yield on different shading, harvest period, and their interaction.

Treatment	Marketable yield		Non-marketable yield	
	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
Shading				
S0	109.07 ± 8.55 a	13.16 ± 0.91 a	46.99 ± 2 .32 a	6.85 ± 0.41 a
S45	60.04 ± 3.57 b	5.80 ± 0.33 b	33.40 ± 2.74 b	3.44 ± 0.57 b
S55	52.63 ± 3.19 b	5.14 ± 0.37 b	32.92 ± 3.18 b	3.53 ± 0.35 b
S80	14.76 ± 1.04 c	1.22 ± 0.10 c	4.68 ± 1.00 c	0.49 ± 0.10 c
Probability	***	***	***	***
P-value	<0.001	<0.001	<0.001	<0.001
$LSD_{0.05}$	12.754	1.21	6.526	0.403
Harvest period				
H2	67.22 ± 12.80 a	6.58 ± 1.47 a	28.90 ± 5.03 b	3.57 ± 0.71 b
H3	60.95 ± 11.05 a	7.05 ± 1.56 a	33.69 ± 5.76 a	4.41 ± 0.84 a
H4	49.20 ± 7.66 b	5.37 ± 0.97 b	25.91 ± 4.06 c	2.75 ± 0.62 c
Probability	***	**	**	**
P-value	<0.001	0.001	0.008	0.001
$LSD_{0.05}$	7.391	0.793	4.546	0.793
Shading x harvest period				
S0H2	130.79 ±7.94 a	14.25 ± 0.64 a	48.17± 1.71 a	6.85 ± 0.57 ab
S0H3	117.30 ± 7.13 a	15.44 ± 0.36 a	47.58± 7.46 a	7.73 ± 0.93 a
S0H4	79.10 ± 6.74 b	9.78 ± 0.77 b	45.22± 1.88 a	5.98 ± 0.19 bc
S45H2	69.51 ± 5.90 bc	6.12 ± 0.76 c	35.36± 3.37 bc	3.88 ± 0.40 def
S45H3	51.03 ± 3.93 d	5.40 ± 0.38 c	40.14± 0.18 ab	4.97 ± 0.14 cd
S45H4	59.57 ± 4.14 cd	5.90 ± 0.66 c	24.70± 4.02 d	1.48 ± 0.74 gh
S55H2	53.05 ± 6.86 d	4.76 ± 0.80 c	28.81± 2.70cd	3.21 ± 0.36 ef
S55H3	58.36 ± 4.61cd	5.88 ± 0.59 c	44.67± 2.41 a	4.69 ± 0.48 cde
S55H4	46.47 ± 4.24d	4.78 ± 0.52 c	25.30± 1.38 d	2.69 ± 0.15 fg
S80H2	15.54 ± 1.30e	1.17 ± 0.14 d	3.27± 0.19 e	0.37 ± 0.02 h
S80H3	17.09 ± 1.32e	1.48 ± 0.13 d	2.37± 1.07 e	0.28 ± 0.18 h
S80H4	11.63 ± 1.28e	1.01 ± 0.14 d	8.41±0.52 e	0.83 ± 0.06 h
Probability	***	***	**	*
P-value	<0.001	<0.001	0.008	0.05
$LSD_{0.05}$	14.781	1.587	9.092	1.587

The metabolism of Brazilian spinach was influenced by shading and harvesting periods. Brazilian spinach grown without shade (S0) increased metabolism activity compared to the shading areas (S45, S55, and S80). This is represented by the carbon and nitrogen levels (Table 3). Higher metabolism correlates with enhanced nitrogen usage, which is crucial for plant metabolic processes. Therefore, increasing fertilization frequency is necessary for plants exposed to sunlight. Leaf nitrogen concentration remains consistent across harvesting periods, suggesting no significant differences in nitrogen across different harvesting periods.

The carbon-nitrogen ratio calculation can be used to determine leaf hardness in Brazilian spinach leaves. The study showed that unshaded (S0) areas yield more tough leaves, decreasing with increased shading levels. Despite this, Brazilian spinach consistently showed comparable levels of leaf hardness across harvesting periods.

Shading significantly impacts the carbon reduction and nitrogen enrichment of leaves, affecting the process of photosynthesis. Light intensity and photosynthesis are linked, with studies showing a reduction in carbon and nitrogen buildup in plants exposed to shading. Tang et al. (2022) found that plants exposed to modest levels of irradiation exhibit reduced concentrations of leaf non-structural carbohydrates, leading to a reduction in carbon content. Nitrogen accumulation in shaded Brazilian spinach leaves is due to limited light availability, hindering the conversion of nitrogen into organic nitrogen compounds essential for plant metabolic processes. Gao et al. (2020) found that prolonged shading reduces nitrogen utilization efficiency in plant. Wang et al. (2020b) demonstrated that the procedure of removing leaves of plants results in an increase in non-structural carbohydrates in leaf. On the other hand, an increased frequency of harvesting triggers the growth of new leaves, leading the movement of nitrogen toward younger leaves. Jasinski et al. (2021) reported that nitrogen mobilization occurs from older to younger leaves.

Table 3. Carbon, Nitrogen and C-N ratio of Brazilian spinach leaf on different shading, harvest period, and their interaction.

Treatment	Carbon (%)	Nitrogen (%)	C-N ratio
Shading			
S0	34.64	2.83	12.28
S45	32.75	4.56	7.20
S55	34.21	4.77	7.19
S80	34.32	4.99	6.84
Harvest period			
H2	35.85	4.38	8.74
H3	33.90	4.42	8.10
H4	32.20	4.07	8.30
Shading x harvest periode			
S0H2	34.23	2.63	13.00
S0H3	34.28	2.90	11.83
S0H4	35.42	2.95	12.01
S45H2	32.02	4.70	6.81
S45H3	33.89	4.77	7.10
S45H4	32.34	4.20	7.70
S55H2	36.50	5.01	7.29
S55H3	32.14	4.94	6.50
S55H4	34.00	4.36	7.79
S80H2	40.66	5.16	7.88
S80H3	35.30	5.07	6.96
S80H4	27.01	4.76	5.68

The presence of shading in Brazilian spinach is linked to biomass production. Under unshaded conditions, it enhances photosynthesis, leading to increased biomass production. However, under intense shading conditions, it reduces biomass in various parts of the plant. Harvesting over extended periods (H3 and H4) results in increased biomass accumulation, particularly in the stem and branch in the final observation.

Brazilian spinach, when grown under shading conditions and extended harvesting, showed inhibited growth due to restricted photosynthetic activity. This caused the restricted allocation of photosynthetic products to individual plant organs. Studies have shown that shading reduces biomass accumulation and alterations in plant morphological traits (Xu et al., 2020b). Additionally, there is a distribution of photosynthetic activity that utilizes plant organs beyond just leaves. This aligns with Yu et al. (2019) finding that when plants age and their organs undergo senescence, photosynthetic flux redirects towards the stem. This highlights the importance of considering the allocation of photosynthetic products to plant growth through periodic harvesting.

Table 4. Dry weight of Brazilian spinach organs on different shading, harvest period, and their interaction at 13 weeks after planting (WAP).

Treatment	Stem dry weight (g)	Branch dry weight (g)	Leaf dry weight (g)	Root dry weight (g)
		Shading		
S0	2.35 ± 0.19 a	14.24 ± 0.98 a	7.92 ± 0.88 a	5.28 ± 1.20 a
S45	1.36 ± 0.20 b	5.39 ± 0.98 b	3.70 ± 0.79 b	2.07 ± 0.81 ab
S55	1.26 ± 0.13 b	5.21 ± 0.79 b	4.58 ± 0.69 b	1.13 ± 0.19 b
S80	0.25 ± 0.04 c	0.40 ± 0.05 c	0.85 ± 0.62 c	0.44 ± 0.20 b
Probability	***	***	***	*
P-value	< 0.001	< 0.001	< 0.001	0.046
$LSD_{0.05}$	0.479	1.755	2.037	3.325
		Harvest period		
H2	1.05 ± 0.27 b	4.39 ± 1.28 c	3.63 ± 0.68 b	1.73 ± 0.48 a
H3	1.25 ± 0.20 b	6.12 ± 1.49 b	2.88 ± 0.65 b	2.19 ± 0.86 a
H4	1.62 ± 0.27 a	8.42 ± 1.90 a	6.28 ± 1.22 a	2.77 ± 1.05 a
Probability	**	***	***	ns
P-value	0.002	< 0.001	< 0.001	0.517
$LSD_{0.05}$	0.286	1.228	1.117	1.872
		Shading x harvest period		
S0H2	2.35 ± 0.53 ab	11.26 ± 0.97 c	6.43 ± 0.44 b	4.27 ± 0.49 a
S0H3	2.03 ± 0.04 bc	13.92 ± 0.61 b	5.98 ± 0.36 b	4.20 ± 2.25 ab
S0H4	2.66 ± 0.25 a	17.54 ± 0.62 a	11.36 ± 0.28 a	7.36 ± 2.97 ab
S45H2	0.78 ± 0.13 fg	3.26 ± 0.70 fg	2.49 ± 0.43 cd	0.88 ± 0.26 bc
S45H3	1.41 ± 0.06 de	4.63 ± 0.33 fg	2.34 ± 0.22 cd	3.30 ± 2.41 bc
S45H4	1.89 ± 0.38 bcd	8.29 ± 2.05 d	6.28 ± 1.52 b	2.04 ± 0.76 bc
S55H2	0.87 ± 0.12 ef	2.80 ± 0.67 gh	3.59 ± 0.81 c	1.06 ± 0.37 bc
S55H3	1.35 ± 0.17 de	5.51 ± 0.62 ef	3.18 ± 0.34 c	1.00 ± 0.09 bc
S55H4	1. 57 ± 0.14 cd	7.32 ± 1.23 de	6.99 ± 0.77 b	1.32 ± 0.52 bc
S80H2	0.19 ± 0.01 h	0.24 ± 0.04 i	2.00 ± 1.84 cde	0.73 ± 0.64 bc
S80H3	0.22 ± 0.03 gh	0.41 ± 0.05 hi	0.03 ± 0.03 e	0.26 ± 0.16 c
S80H4	0.35 ± 0.09 fgh	0.53 ± 0.11 hi	0.50 ± 0.33 de	0.34 ± 0.05 c
Probability	ns	*	*	ns
P-value	0.134	0.049	0.013	0.584
$LSD_{0.05}$	0.572	2.457	2.234	3.744

The ns mean non-significant difference at $p<0.05$.

Visual appearance of Brazilian spinach on different treatment

The study analysed the shoot appearance of Brazilian spinach under different shading conditions and harvesting periods. Unshaded areas had a denser appearance, while based on the harvesting period, treatments tend to show similarities with each other (Figure 6). Furthermore, Brazilian spinach grown unshaded (S0) showed greater root growth and a higher density of root hairs than other shading, while samples subjected to varying harvesting periods (H2, H3, and H4) showed similar root morphology without any significant differences (Figure 7).

Brazilian spinach showed varying morphological traits under different treatments. Shading causes alterations in plant organs, as shown on soybean stems, which experience inhibited growth (Castronuovo et al., 2019). Cao et al. (2022) reported that *Cynodon dactylon* shoot organs also experience alterations. Root development also shows a distinct reaction to shading, with a decline in root growth under shading stress. Fu et al. (2020) found reductions in root volume and length, indicating a decline in root growth under these conditions.

Brazilian spinach with a longer harvesting period (H4) showed a rise in branches and stems, with a higher presence of mature leaves. Pruning at longer intervals increased plant height and branches in *Talinum Paniculatum*, while extending harvesting intervals hindered fresh leaf commencement (Purbajanti et al., 2019). Bessonova et al. (2023) found that removing leaves and branches from plants led to the development of shoot features with a greater number and area of leaves.

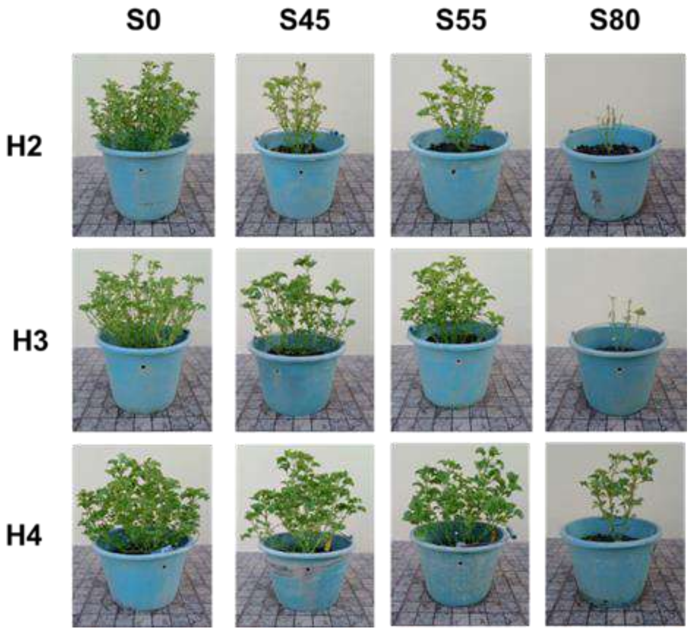

Figure 6. Visualization of Brazilian spinach shoot on different shading and harvest period at 13 weeks after planting (WAP). Shading treatment consist of S0: no-shading, S45: 45% shading, S55: 55% shading, and S80: 80% shading. Meanwhile, the harvest period consists of H2: per 2 weeks, H3: per 3 weeks, and H4: per 4 weeks. Photos: Strayker Ali Muda.

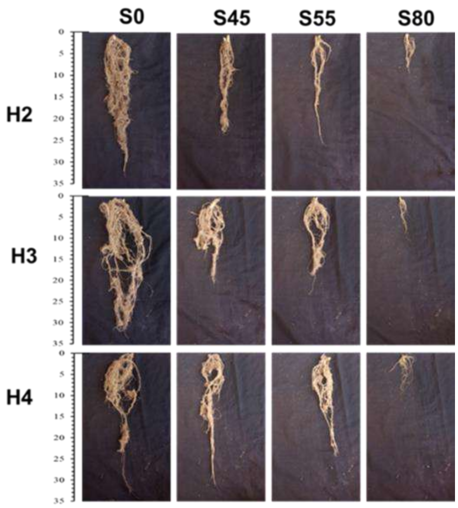

Figure 7. Visualization of Brazilian spinach root on different shading and harvest period at 13 weeks after planting (WAP). Shading treatment consist of S0: no-shading, S45: 45% shading, S55: 55% shading, and S80: 80% shading. Meanwhile, the harvest period consists of H2: per 2 weeks, H3: per 3 weeks, and H4: per 4 weeks. Photos: Strayker Ali Muda.

Water status on different treatment

The water availability for Brazilian spinach growth was represented by substrate moisture. Increased shading intensity (S80) leads to higher moisture content, reducing direct sunlight exposure and reducing evaporation, resulting in reduced water loss. Conversely, Brazilian spinach grown in areas with lower shading or without shading showed higher evaporation rates, indicating more water loss, as shown by substrate moisture levels (Figure 8). The use of shading can effectively adjust microclimate conditions, such as substrate moisture levels (Bollman et al., 2021). The study found that shaded growing media had higher humidity levels than unshaded media, and the addition of shading reduced evaporation rate, as confirmed by Khawam et al. (2019).

The more frequently Brazil spinach is harvested, the wider the substrate surface is not covered by the canopy, causing higher evaporation rates and reduced water availability. This phenomenon aligns with the findings of Huang et al. (2020), who provided empirical evidence that plants with lower canopy density exhibit higher rates of water loss via evaporation.

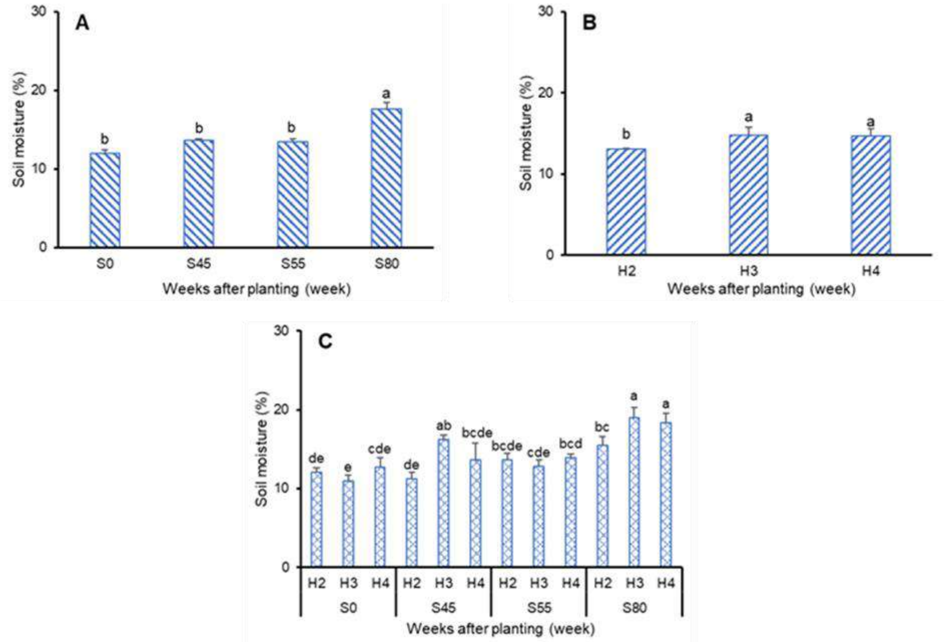

Figure 8. Substrate moisture on different shading (A), harvest period (B), and their interaction (C). Shading treatment consist of S0: no-shading, S45: shading 45%, S55: shading 55%, and S80: shading 80%. Meanwhile, the harvest period consists of H2: every 2 weeks, H3: every 3 weeks, and H4: every 4 weeks.

CONCLUSION

The adoption of shading led to a decrease in the growth and yield of Brazilian spinach through an alteration in the morphological traits of its root, stem, branch, and leaf. In addition, the implementation of a 2-week harvesting period led to increased Brazilian spinach growth and yield. Interactions between shading and harvest periods primarily pertain to the length of branches, yields (both marketable and non-marketable), dry weight of organs (namely branch and leaf), and substrate moisture.

ACKNOWLEDGMENT

We are very grateful for the suggestions, input and corrections from anonymous reviewers and the editors of the *Revista de Agricultura Neotropical* journal.

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2. Pre-review (31 Januari 2024)

33718	8464_BRAZILIAN SPINACH GROWTH UNDER DIFFERENT SHADING INTENSITIES AND HARVESTING PERIODS IN LOWLAND TROPICAL URBAN ECOSYSTEM_reviewed.docx	enero 31, 2024	Article Text

BRAZILIAN SPINACH GROWTH UNDER DIFFERENT SHADING INTENSITIES AND HARVESTING PERIODS IN LOWLAND TROPICAL URBAN ECOSYSTEM

ABSTRACT

Brazilian spinach, a lesser-known leafy vegetable, has a high nutritional value and is essential for human health. A study was conducted to evaluate the growth of Brazilian spinach in tropical lowland urban ecosystems under different levels of shade intensity and harvest periods. The research used a split-plot design, assigning different levels of shading intensity as the main plot and harvesting periods as sub-plots. The results showed that Brazilian spinach growth was more favourable when exposed to treatment without shade compared to shaded conditions. The impact of shading on plant growth was observed during the early stages of growth, as indicated by alterations in canopy parameters and SPAD values. After productivity assessment, the impact of shading was assessed by branch elongation, yield, fresh weight, and dry weight of each organ. Shading increased the carbon content of Brazilian spinach leaf, while reducing the nitrogen content. More frequent harvesting resulted in an increase in yield components but suppressed the growth of stems and branches. Therefore, it is recommended to cultivate Brazilian spinach in an unshaded area with a biweekly harvesting routine.

KEYWORDS

Harvest time, leafy green, lesser-known vegetable, plant acclimatization, solar irradiation intensity.

INTRODUCTION

Brazilian spinach (*Alternanthera sissoo*) is a leafy vegetable originating from Brazil. As reported by Ikram et al. (2022), Brazilian spinach is rich in flavonoids, vitamins, minerals, and other antioxidants, which have been found to have positive effects on human health. The cultivation and use of this particular plant by the Indonesian population are infrequent, leading to its classification as a rather rare plant species. Indonesia's agroclimatology exhibits similarities to its indigenous location, hence indicating the potential for cultivating this plant within the country.

Urban cultivation faces several challenges, especially in regard to the availability of light for plants. Shaded areas in urban environments tend to prevail, impeding the penetration of light into plant development. Consequently, the amount of light received by plants decreases, leading to disruptions in certain aspects of plant metabolism. This phenomenon is particularly observed in horticultural crops characterized by compact growth, such as Brazilian spinach. Based on Shafiq et al. (2021), regulating alterations occur in plants as a means to enhance the efficiency of photosynthesis. The tolerance of plants to the intensity of light they receive varies depending on the specific plant species. Certain vegetable crops have been reported as being capable of growing under shaded conditions. In this regard, Sifuentes-Pallaoro et al. (2020) revealed that *Lactuca canadensis* exhibits favourable growth under shading, while Lakitan et al. (2021a) found that celery also demonstrates similar adaptability.

Brazilian spinach is a perennial leafy vegetable, enabling its continuous growth throughout the year. Additionally, this suggests that regular harvesting is required. Annual plants undergo periodic harvesting that involves a defoliation mechanism. According to the findings of Raza et al. (2019), the implementation of a defoliation treatment on plants has been observed to enhance overall plant growth, particularly in terms of leaf growth, especially during the vegetative phase. Further experimentation is required to enhance the output of Brazilian spinach, a plant species characterised by its commercially valuable leaf organs.

The cultivation of Brazilian spinach is characterized by its simplicity, since it may be easily grown. Muda et al. (2022) reported that the propagation of Brazilian spinach can be achieved via stem cuttings. There is an insufficient amount of research pertaining to the adaptability of Brazilian spinach to shading environments. The capacity of Brazilian spinach to acclimatise to shading environments for a specific duration will ensure the availability of sustainable vegetable nutrition. The study was aimed to evaluating the adaptability of Brazilian spinach to shading conditions via various harvesting periods.

MATERIALS AND METHODS

Research site and agroclimatic characteristic

The research was carried out in the Jakabaring research facility located in Palembang (104º46'44"E, 3º01'35"S), South Sumatra, Indonesia. This research began

with initiating the propagation of stem cuttings on 30 January 2023, and concluded the data collection on 02 May 2023. The research site is situated in a tropical urban lowland area with an elevation of 8 meters above sea level (masl). The agroclimatic characteristics of the area are shown in Figure 1.

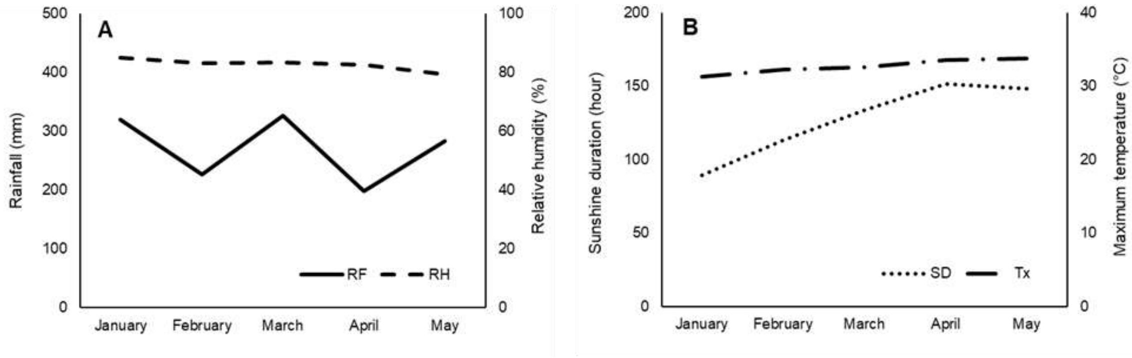


Figure 1. Agroclimatic characteristics in research location as indicated by total monthly rainfall (RF) and average humidity (RH) (A), and total monthly sunshine duration (SD) and average maximum temperature (Tx) (B). (Source: Indonesian Agency for Meteorology, Climatology and Geophysics).

Cultivation and treatment procedures

The propagation material used was stem cuttings with two leaves that were taken from healthy mother plant. The planting materials were planted in pots with dimensions of 27.5 cm in diameter and 20 cm in height. The pots were filled with a growing medium that was a mixture of top soil and chicken manure (3:1 v/v). Prior to planting, the growing medium had a bio-sterilization treatment (2 g/l), with the addition of live microorganisms including *Streptomyces thermovulgaris, Thricoderma virens,* and *Geobacillus thermocatenulatus*. The growing medium was subsequently subjected to a one-week incubation period before planting.

The growing medium that had been incubated was used for the planting of Brazilian spinach cuttings, which were subsequently arranged in accordance with the principles of a split-plot design. The main plot of the study focused on the intensity of shading, whereas the subplot examined the harvest period. The treatment involved selecting shading intensity at three separate levels, namely no-shading (S0), 45% shading (S45), 55% shading (S55), and 85% shading (S80). Furthermore, treatment for the harvest period started after the simultaneous harvesting, which was carried out at 5 weeks after planting (WAP). The designated harvest period has three different intervals, namely

appearing per 2 weeks, per 3 weeks, and per 4 weeks, symbolised as H2, H3, and H4, respectively.

The plants were systematically positioned within shadow houses measuring 4 meters in length, 2 meters in width, and 2 meters in height. These shadow houses are constructed using knockdown frames made of 1.5-inch PVC pipes. The entire perimeter of the shadow house is enveloped with a shade material, specifically a black polyethylene net, which has been tested for its density to ensure optimal shading.

The leaves of the Brazilian spinach cuttings get trimmed when it reaches one week after planting (WAP) in order to maintain uniformity in the planting material. Meanwhile, fertilization was conducted using NPK fertilizer (16:16:16) at 1 week after planting (WAP) and 5 WAP. The watering of each plant was normally carried out around 08:00 a.m.

Data collection

The data collection covered Brazilian spinach growth and yield data. The growth data that was obtained is categorised into two categories of measurements, such as non-destructive and destructive. The dataset for non-destructive growth measurement includes several variables, including SPAD values, canopy width, canopy diameter, canopy index, branch length, and stem diameter. In addition, the destructive measurements included the stem fresh weight, branch fresh weight, root fresh weight, stem dry weight, branch dry weight, and root dry weight. The collected data concerning Brazilian spinach yield covers several parameters, including the fresh weight of marketable leaf, fresh weight of non-marketable leaf, dry weight of marketable leaf, dry weight of non-marketable leaf, the carbon content of marketable leaf, nitrogen content of marketable leaf, and the C:N ratio of marketable leaf. The moisture content of the planting medium was also examined in order to determine the water content of the substrate.

The SPAD value was monitored using chlorophyll meters (SPAD-502 Plus, Konica-Minolta Optics, Inc., Osaka, Japan). Canopy area was calculated using a digital image scanner for Android (Easy Leaf Area software, developed by Easlon & Bloom 2014). Substrate moisture (SM) was measured using a soil moisture meter (PMS-714, Lutron Electronics Canada, Inc., Pennsylvania, USA). The carbon, nitrogen, and C:N ratios were examined using Kjedahl-Titrimetry in the Integrated Laboratory of Sampoerna Agro. Tbk.

The dry weight of each plant organ was determined by treating it to a drying process in an oven set at a temperature of 100°C for a duration of 24 hours. Prior to being placed in the oven, the plant's organs are trimmed to a reduced thickness to accelerate the drying process.

Data analysis

All data collected was analysed using the RStudio software version 1.14.1717 for Windows (developed by RStudio team, PBC, Boston, MA). Significant differences among treatments were tested using the least significant difference (LSD) procedure at $p<0.05$.

RESULTS AND DISCUSSION

The Brazilian spinach growth during early vegetative growth before harvested

The early vegetative growth of Brazilian spinach is analysed by considering its unique characteristics, such as canopy growth and SPAD value. This approach involves non-destructive observation, allowing plants to grow naturally. The canopy characteristics selected were: canopy area, canopy diameter, and canopy index.

Brazilian spinach grown in unshaded conditions (S0) had a higher leaf initiation and larger individual leaf area compared to in shading conditions. More and larger leaves contribute to the increase in canopy area. This leads to a broader canopy compared to those grown under shade. The Brazilian spinach canopy area growth increased significantly in the S0 condition, particularly 2 to 5 weeks after planting, compared to shade conditions (S45, S55, and S80). However, no significant leaf growth was observed in the S45 and S55 shades. The shading with the highest density (S80) showed suppressed growth, starting 2 weeks after planting (Figure 2).

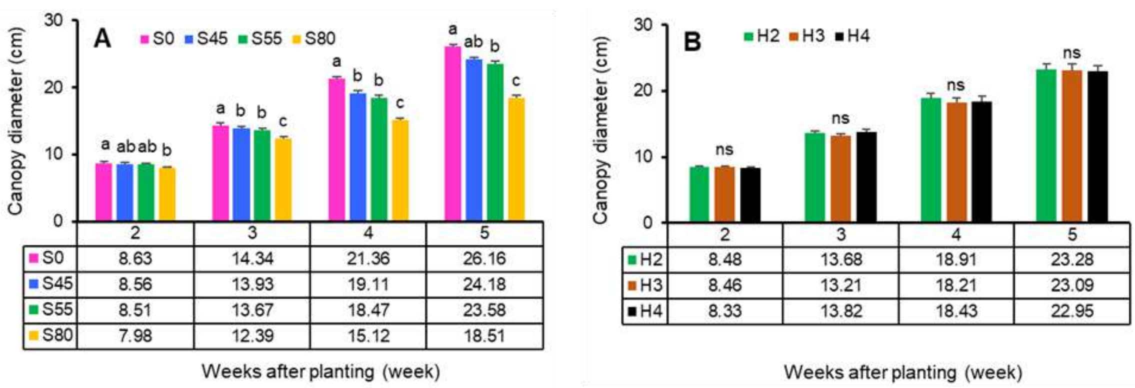
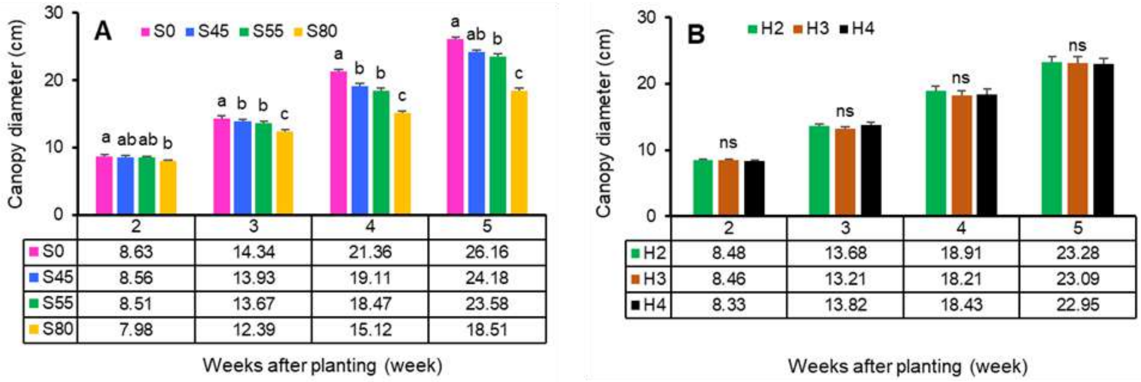


Figure 2. Brazilian spinach canopy area on early vegetative growth on different shading (A) and harvest period (B) treatment. The shading consists of no shading (S0), 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent harvest period per 2 weeks, 3 weeks, and 4 weeks, respectively. The ns mean non-significant difference at p<0.05.

Brazilian spinach branches significantly influence canopy diameter, with elongation affecting canopy expansion. Shading treatment inhibits branch growth. Full sunlight cultivation leads to a wider canopy than canopies grown under different levels of shading (S45, S55, and S80) (Figure 3).



	2	3	4	5
S0	8.63	14.34	21.36	26.16
S45	8.56	13.93	19.11	24.18
S55	8.51	13.67	18.47	23.58
S80	7.98	12.39	15.12	18.51

	2	3	4	5
H2	8.48	13.68	18.91	23.28
H3	8.46	13.21	18.21	23.09
H4	8.33	13.82	18.43	22.95

Figure 3. Brazilian spinach canopy diameter on early vegetative growth on different shading (A) and harvest period (B) treatment. The shading consists of no shading (S0), 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent harvest period per 2 weeks, 3 weeks, and 4 weeks, respectively. The ns mean non-significant difference at p<0.05.

The growth of leaf and branch significantly affects canopy density, with dominant growth resulting in a denser canopy as represented by canopy density. The effect is most noticeable between 4 and 5 weeks after planting. Brazilian spinach grown under shading conditions, especially S80, showed reduced leaf size and branch elongation, resulting in lower canopy density (Figure 4).

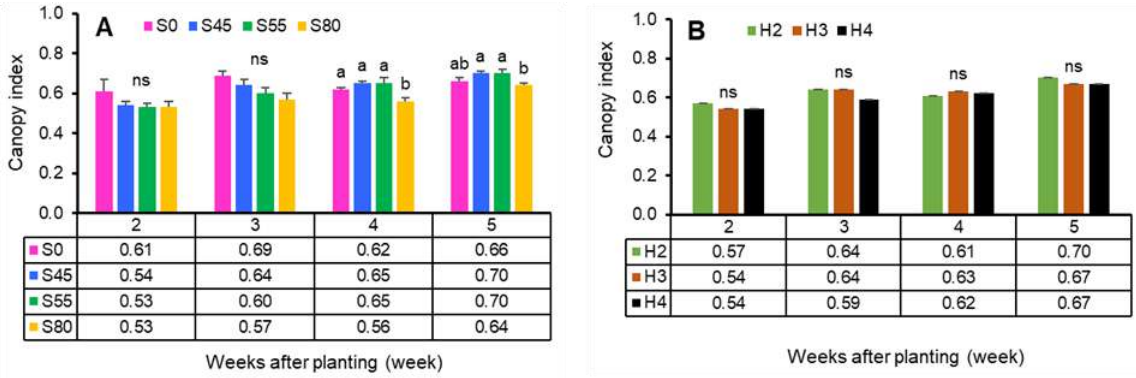


Figure 4. Canopy index on early vegetative growth on different shading and harvest period as treatment. The shading consists of no shading (S0), 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent harvest period per 2 weeks, 3 weeks, and 4 weeks, respectively. The ns mean non-significant difference at $p<0.05$.

According to this research's findings, Brazilian spinach's canopy growth was more hindered under greater shading (S80) than it was unshaded. The constituent organs of the canopy, such as the leaves and branches, endure stunted growth, which prevents the canopy from growing. According to Fadilah et al. (2022), denser shading intensity was shown to inhibit purple pakchoy leaf growth. According to the findings of Wan et al. (2020), plants planted in the shading area produce less photosynthetic performance than plants grown in full sunlight. Moreover, Liang et al. (2020) have underscored the significance of shading for plants, noting that it leads to a decrease in photosynthesis, resulting in a reduction in carbon flow. The inhibition of vegetative organ growth, particularly the canopy, in Brazilian spinach is attributed to the decreased carbon flow. This reduction in carbon flow occurs throughout the early growth cycle. The phenomenon of reduced vegetative organ development due to shading during the early growth phase has been documented in various vegetable crops, including chili (Kesumawati et al., 2020).

The SPAD value is a method for evaluating leaf nitrogen and chlorophyll content, with a positive relationship between these factors. Brazilian spinach leaf's SPAD value was affected by shading treatments, with differences observed within each shading treatment from the early growth 2 weeks after planting (WAP) (Figure 5).

Brazilian spinach grown without shading (S0) showed a higher SPAD value compared to under different shading levels, with a notable rise starting 4 weeks after

planting. This trend was also observed in S45 and S55. On the other hand, Brazilian spinach grown at S80 showed a stagnation trend, persisting until the end of the early growth, specifically 2 to 5 weeks after planting.

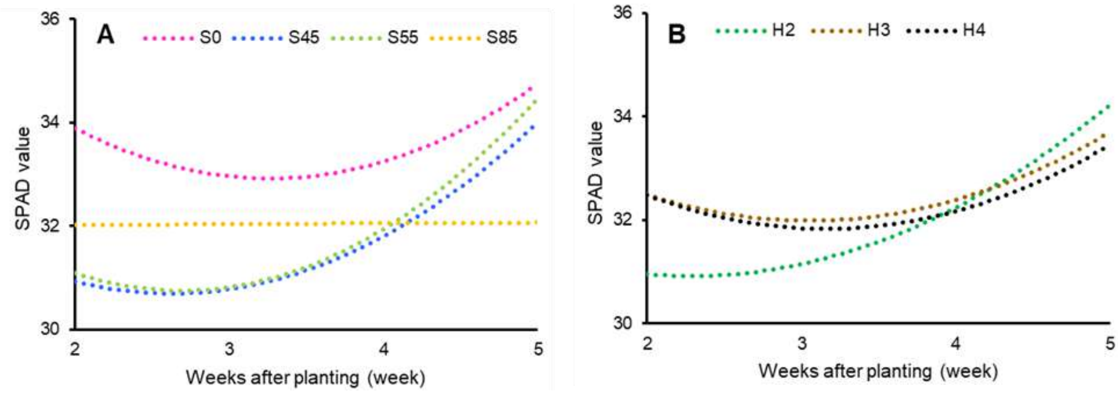


Figure 5. The SPAD value on early vegetative growth on different shading (A) and harvest period (A) treatment. The shading consists of no shading (S0), 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent harvest period per 2 weeks, 3 weeks.

The SPAD value is a widely used method for assessing leaf chlorophyll and nitrogen content, with its reliability well established. It has been found to have a positive correlation with both chlorophyll content and leaf nitrogen content (Song et al., 2021; Farnisa et al., 2023). Prior research had provided confirmation on the capacity of specific leafy vegetables, namely *Talinum paniculatum* (Lakitan et al., 2021b) and spinach (Mendoza-Tafolla et al., 2019), to proficiently evaluate and track the quantities of leaf chlorophyll and nitrogen content.

Brazilian spinach grown under full sun (S0) has a higher SPAD value than that grown under shading, indicating that shading reduces the solubility of chlorophyll and nitrogen. Wang et al. (2020a) found that shading affects the solubility of nitrogen, leading to a decrease in leaf nitrogen content. Li et al. (2020) highlighted the biochemical alterations caused by shading stress. This condition is due to Brazilian spinach, particularly in plants subjected to the 80% shading treatment (S80).

Brazilian spinach growth after harvested

The vegetative growth of Brazilian spinach after harvest was conducted at 5 weeks after planting. The growth of branch was compared under different shading conditions, harvest periods, and their interaction effects. Brazil spinach grown under S80 exhibited shorter branches as early as 11 weeks after planting. However, different shading

treatments (S0, S45, and S55) showed comparable levels of branch elongation until 9 weeks after planting. Brazilian spinach grown in S45 had an increased rate of branch elongation, particularly at 10 and 11 weeks after planting (Table 1).

The elongation of the Brazilian spinach branch was influenced by the harvesting period. Less frequent harvesting leads to the highest branch elongation, especially from 7 to 11 weeks after planting. An interaction between shading level and harvesting period treatment was observed, starting within 9 weeks after planting. This highlights the importance of harvesting frequency in influencing Brazilian spinach growth.

The study revealed a decrease in branch elongation in Brazilian spinach at S80, indicating a decrease in the allocation of photosynthetic products. This is due to reduced levels of non-structural carbohydrates, which are essential for growth (Yamashita et al., 2020). However, in the S0 treatment, photosynthesis is optimised, leading to increased branch growth.

The increased frequency of harvesting inhibits branch growth, and it is possible that the distribution of photosynthetic products changes, potentially causing a heightened initiation process of new leaves (Oliveira et al., 2021). Additionally, Raza et al. (2019) reported that maize plants with a higher number of eliminated leaves have an increased allocation of photosynthetic resources towards expanded leaves, as evidenced by an enhanced leaf area.

Table 1. Branch elongation of Brazilian spinach after harvested on different shading, harvest period, and their interaction.

Treatment	Weeks after planting (week)						
	5	6	7	8	9	10	11
				Shading			
S0	11.98 ± 0.16 a	15.89 ± 0.17 a	19.18 ± 0.43 a	22.31 ± 0.38 a	24.16 ± 0.34 a	25.22 ± 0.33 b	26.17 ± 0.53 b
S45	11.63 ± 0.33 ab	15.11 ± 0.39 ab	19.00 ± 0.55 a	22.02 ± 0.76 a	24.30 ± 0.70 a	28.23 ± 0.95 a	29.02 ± 1.20 a
S55	11.03 ± 0.17 b	14.00 ± 0.22 b	18.03 ± 0.57 a	20.74 ± 0.80 a	22.32 ± 0.85 a	25.91 ± 0.95 b	26.74 ± 1.07 ab
S80	8.68 ± 0.13 c	10.25 ± 0.17 c	12.66 ± 0.36 b	14.47 ± 0.51 b	14.93 ± 0.54 b	15.97 ± 0.76 c	16.46 ± 0.75 c
Probability	***	***	***	***	***	***	***
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
$LSD_{0.05}$	0.897	1.491	1.744	2.357	2.192	2.283	2.829
			Harvest period				
H2	11.06 ± 0.41	14.03 ± 0.69	15.80 ± 0.80 b	18.51 ± 1.09 c	19.66 ± 1.21 c	21.59 ± 1.41 c	21.90 ± 1.36 c
H3	10.72 ± 0.42	13.80 ± 0.71	18.07 ± 0.90 a	19.54 ± 1.02 b	21.75 ± 1.26 b	24.31 ± 1.58 b	24.95 ± 1.61 b
H4	10.70 ± 0.44	13.60 ± 0.66	17.78 ± 0.85 a	21.61 ± 0.98 a	22.88 ± 1.17 a	25.60 ± 1.45 a	26.94 ± 1.61 a
Probability	ns	ns	***	***	***	***	***
P-value	0.186	0.198	<0.001	<0.001	<0.001	<0.001	<0.001
$LSD_{0.05}$	0.462	0.507	0.607	0.878	0.708	0.922	0.876
			Shading x harvest period				
S0H2	12.23 ± 0.12	16.05 ± 0.34	18.55 ± 0.54	21.47 ± 0.44	23.22 ± 0.49 cd	24.24 ± 0.58 de	24.57 ± 0.62 ef
S0H3	11.93 ± 0.41	16.20 ± 0.20	20.31 ± 0.72	22.40 ± 0.85	24.85 ± 0.40 b	25.68 ± 0.39 cd	26.91 ± 0.63 cd
S0H4	11.77 ± 0.27	15.41 ± 0.14	19.08 ± 0.44	23.08 ± 0.41	24.41 ± 0.53 bc	25.74 ± 0.34 cd	27.04 ± 0.78 bcd
S45H2	11.99 ± 0.62	15.51 ± 1.10	17.45 ± 0.72	20.97 ± 1.52	22.53 ± 0.85 d	25.43 ± 1.20 cd	25.43 ± 0.91 de
S45H3	10.89 ± 0.71	14.68 ± 0.52	19.57 ± 0.59	20.87 ± 0.95	23.77 ± 0.60 bcd	28.07 ± 0.83 b	28.54 ± 0.87 bc
S45H4	12.01 ± 0.19	15.14 ± 0.41	19.99 ± 0.95	24.24 ± 0.20	26.59 ± 0.68 a	31.19 ± 0.67 b	33.08 ± 0.99 a
S55H2	11.08 ± 0.11	13.97 ± 0.22	16.00 ± 0.56	18.64 ± 0.81	19.53 ± 0.71 d	22.70 ± 0.97 e	23.19 ± 0.82 f
S55H3	11.37 ± 0.44	14.21 ± 0.67	19.19 ± 0.59	20.58 ± 1.41	23.50 ± 1.48 bcd	27.88 ± 1.25 b	28.27 ± 1.61 bc
S55H4	10.64 ± 0.03	13.81 ± 0.21	18.90 ± 0.28	23.00 ± 0.45	23.92 ± 0.49 bcd	27.15 ± 0.70 b	28.77 ± 1.02 b
S80H2	8.95 ± 0.06	10.58 ± 0.13	11.61 ± 0.27	12.97 ± 0.37	13.32 ± 0.40 h	13.98 ± 0.60 g	14.41 ± 0.53 h
S80H3	8.70 ± 0.32	10.13 ± 0.37	13.22 ± 0.70	14.33 ± 0.60	14.89 ± 0.22 g	15.62 ± 0.39 g	16.09 ± 0.29 h
S80H4	8.38 ± 0.11	10.04 ± 0.32	13.14 ± 0.38	16.11 ± 0.38	16.59 ± 0.75 f	18.32 ± 1.27f	18.87 ± 1.17 g
Probability	ns	ns	ns	ns	**	**	**
P-value	0.237	0.89	0.211	0.383	0.009	0.008	0.003
$LSD_{0.05}$	0.920	1.014	1.214	1.756	1.416	1.844	1.751

Remark: the ns mean non-significant difference at $p<0.05$.

Brazilian spinach showed significant differences in leaf growth when treated with different shading and harvesting periods. Cultivated without shade (S0), it tends to dominate leaf growth compared to cultivated under different levels of shading (S45, S55, and S80) (Table 2). However, this method also demonstrated a significant proportion of non-marketable leaves. This indicates that early leaf growth is achieved without shading, but it also leads to accelerated leaf aging and increased pest susceptibility, resulting in a higher proportion of non-marketable leaves compared to those cultivated under shading.

The frequent harvesting of Brazilian spinach leads to the initiation of young leaves, resulting in more marketable leaves. This is evident in the yield of commercially viable leaves throughout the H2 and H3 periods. However, during the H3 harvesting period, a significant proportion of non-marketable leaves are produced due to leaf aging. In contrast, extended harvesting periods (H4) often lack the capacity for leaf initiation, resulting in decreased yields of both marketable and non-marketable leaves. The interaction impact of shading and harvesting period significantly showed on leaf growth, with the most significant impact observed under 80% shade, especially during the longer harvesting period (H4).

Brazilian spinach's leaf initiation is higher in conditions without shade compared to shading conditions, affecting both marketable and non-marketable leaves. This is due to reduced carbohydrate accumulation and allocation (Hussain et al., 2020). Shading conditions inhibited plant growth, while without shade, leaf senescence accelerated due to enhanced photosynthesis. Meanwhile, direct sunlight exposure accelerates ageing processes in plants, similar to sweet basil (Castronuovo et al., 2019). However, without shade, spinach is more susceptible to pest infestation, leading to an increased prevalence of non-marketable leaves. Implementing shading at a specific density is a viable pest control strategy.

Brazilian spinach harvesting, similar to leaf and shoot pruning, has been found to increase yield. Dheeraj et al. (2022) found that pruning at the apical meristem increased growth-promoting hormones, specifically cytokinin. Xu et al. (2020a) reported that pruning tomato plants also increases cytokinin hormone levels. Cytokinin hormones influence cell division processes, including those during leaf cell development. Harvesting Brazilian spinach at H2 and H3 treatment results in elevated levels of cytokinin hormones, enhancing leaf initiation and resulting in a greater marketable yield.

Tabel 2. Brazilian spinach yield on different shading, harvest period, and their interaction.

Treatment	Marketable yield		Non-marketable yield	
	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
Shading				
S0	109.07 ± 8.55 a	13.16 ± 0.91 a	46.99 ± 2 .32 a	6.85 ± 0.41 a
S45	60.04 ± 3.57 b	5.80 ± 0.33 b	33.40 ± 2.74 b	3.44 ± 0.57 b
S55	52.63 ± 3.19 b	5.14 ± 0.37 b	32.92 ± 3.18 b	3.53 ± 0.35 b
S80	14.76 ± 1.04 c	1.22 ± 0.10 c	4.68 ± 1.00 c	0.49 ± 0.10 c
Probability	***	***	***	***
P-value	<0.001	<0.001	<0.001	<0.001
LSD$_{0.05}$	12.754	1.21	6.526	0.403
Harvest period				
H2	67.22 ± 12.80 a	6.58 ± 1.47 a	28.90 ± 5.03 b	3.57 ± 0.71 b
H3	60.95 ± 11.05 a	7.05 ± 1.56 a	33.69 ± 5.76 a	4.41 ± 0.84 a
H4	49.20 ± 7.66 b	5.37 ± 0.97 b	25.91 ± 4.06 c	2.75 ± 0.62 c
Probability	***	**	**	**
P-value	<0.001	0.001	0.008	0.001
LSD$_{0.05}$	7.391	0.793	4.546	0.793
Shading x harvest period				
S0H2	130.79 ±7.94 a	14.25 ± 0.64 a	48.17± 1.71 a	6.85 ± 0.57 ab
S0H3	117.30 ± 7.13 a	15.44 ± 0.36 a	47.58± 7.46 a	7.73 ± 0.93 a
S0H4	79.10 ± 6.74 b	9.78 ± 0.77 b	45.22± 1.88 a	5.98 ± 0.19 bc
S45H2	69.51 ± 5.90 bc	6.12 ± 0.76 c	35.36± 3.37 bc	3.88 ± 0.40 def
S45H3	51.03 ± 3.93 d	5.40 ± 0.38 c	40.14± 0.18 ab	4.97 ± 0.14 cd
S45H4	59.57 ± 4.14 cd	5.90 ± 0.66 c	24.70± 4.02 d	1.48 ± 0.74 gh
S55H2	53.05 ± 6.86 d	4.76 ± 0.80 c	28.81± 2.70cd	3.21 ± 0.36 ef
S55H3	58.36 ± 4.61cd	5.88 ± 0.59 c	44.67± 2.41 a	4.69 ± 0.48 cde
S55H4	46.47 ± 4.24d	4.78 ± 0.52 c	25.30± 1.38 d	2.69 ± 0.15 fg
S80H2	15.54 ± 1.30e	1.17 ± 0.14 d	3.27± 0.19 e	0.37 ± 0.02 h
S80H3	17.09 ± 1.32e	1.48 ± 0.13 d	2.37± 1.07 e	0.28 ± 0.18 h
S80H4	11.63 ± 1.28e	1.01 ± 0.14 d	8.41±0.52 e	0.83 ± 0.06 h
Probability	***	***	**	*
P-value	<0.001	<0.001	0.008	0.05
LSD$_{0.05}$	14.781	1.587	9.092	1.587

Remark: the ns mean non-significant difference at $p<0.05$.

The metabolism of Brazilian spinach was influenced by shading and harvesting periods. Brazilian spinach grown without shade (S0) increased metabolism activity compared to the shading areas (S45, S55, and S80). This is represented by the carbon and nitrogen levels (Table 3). Higher metabolism correlates with enhanced nitrogen usage, which is crucial for plant metabolic processes. Therefore, increasing fertilization frequency is necessary for plants exposed to sunlight. Leaf nitrogen concentration remains consistent across harvesting periods, suggesting no significant differences in nitrogen across different harvesting periods.

The carbon-nitrogen ratio calculation can be used to determine leaf hardness in Brazilian spinach leaves. The study showed that unshaded (S0) areas yield more tough leaves, decreasing with increased shading levels. Despite this, Brazilian spinach consistently showed comparable levels of leaf hardness across harvesting periods.

Shading significantly impacts the carbon reduction and nitrogen enrichment of leaves, affecting the process of photosynthesis. Light intensity and photosynthesis are linked, with studies showing a reduction in carbon and nitrogen buildup in plants exposed to shading. Tang et al. (2022) found that plants exposed to modest levels of irradiation exhibit reduced concentrations of leaf non-structural carbohydrates, leading to a reduction in carbon content. Nitrogen accumulation in shaded Brazilian spinach leaves is due to limited light availability, hindering the conversion of nitrogen into organic nitrogen compounds essential for plant metabolic processes. Gao et al. (2020) found that prolonged shading reduces nitrogen utilization efficiency in plant. Wang et al. (2020b) demonstrated that the procedure of removing leaves of plants results in an increase in non-structural carbohydrates in leaf. On the other hand, an increased frequency of harvesting triggers the growth of new leaves, leading the movement of nitrogen toward younger leaves. Jasinski et al. (2021) reported that nitrogen mobilization occurs from older to younger leaves.

Table 3. Carbon, Nitrogen and C-N ratio of Brazilian spinach leaf on different shading, harvest period, and their interaction.

Treatment	Carbon (%)	Nitrogen (%)	C-N ratio
Shading			
S0	34.64	2.83	12.28
S45	32.75	4.56	7.20
S55	34.21	4.77	7.19
S80	34.32	4.99	6.84
Harvest period			
H2	35.85	4.38	8.74
H3	33.90	4.42	8.10
H4	32.20	4.07	8.30
Shading x harvest periode			
S0H2	34.23	2.63	13.00
S0H3	34.28	2.90	11.83
S0H4	35.42	2.95	12.01
S45H2	32.02	4.70	6.81
S45H3	33.89	4.77	7.10
S45H4	32.34	4.20	7.70
S55H2	36.50	5.01	7.29
S55H3	32.14	4.94	6.50
S55H4	34.00	4.36	7.79
S80H2	40.66	5.16	7.88
S80H3	35.30	5.07	6.96
S80H4	27.01	4.76	5.68

Remark: the ns mean non-significant difference at $p<0.05$.

The presence of shading in Brazilian spinach is linked to biomass production. Under unshaded conditions, it enhances photosynthesis, leading to increased biomass production. However, under intense shading conditions, it reduces biomass in various parts of the plant. Harvesting over extended periods (H3 and H4) results in increased biomass accumulation, particularly in the stem and branch in the final observation.

Brazilian spinach, when grown under shading conditions and extended harvesting, showed inhibited growth due to restricted photosynthetic activity. This caused the restricted allocation of photosynthetic products to individual plant organs. Studies have shown that shading reduces biomass accumulation and alterations in plant morphological traits (Xu et al., 2020b). Additionally, there is a distribution of photosynthetic activity that utilizes plant organs beyond just leaves. This aligns with Yu et al. (2019) finding that when plants age and their organs undergo senescence, photosynthetic flux redirects towards the stem. This highlights the importance of considering the allocation of photosynthetic products to plant growth through periodic harvesting.

Table 4. Dry weight of Brazilian spinach organs on different shading, harvest period, and their interaction at 13 weeks after planting (WAP).

Treatment	Stem dry weight (g)	Branch dry weight (g)	Leaf dry weight (g)	Root dry weight (g)
		Shading		
S0	2.35 ± 0.19 a	14.24 ± 0.98 a	7.92 ± 0.88 a	5.28 ± 1.20 a
S45	1.36 ± 0.20 b	5.39 ± 0.98 b	3.70 ± 0.79 b	2.07 ± 0.81 ab
S55	1.26 ± 0.13 b	5.21 ± 0.79 b	4.58 ± 0.69 b	1.13 ± 0.19 b
S80	0.25 ± 0.04 c	0.40 ± 0.05 c	0.85 ± 0.62 c	0.44 ± 0.20 b
Probability	***	***	***	*
P-value	< 0.001	< 0.001	< 0.001	0.046
$LSD_{0.05}$	0.479	1.755	2.037	3.325
		Harvest period		
H2	1.05 ± 0.27 b	4.39 ± 1.28 c	3.63 ± 0.68 b	1.73 ± 0.48 a
H3	1.25 ± 0.20 b	6.12 ± 1.49 b	2.88 ± 0.65 b	2.19 ± 0.86 a
H4	1.62 ± 0.27 a	8.42 ± 1.90 a	6.28 ± 1.22 a	2.77 ± 1.05 a
Probability	**	***	***	ns
P-value	0.002	< 0.001	< 0.001	0.517
$LSD_{0.05}$	0.286	1.228	1.117	1.872
		Shading x harvest period		
S0H2	2.35 ± 0.53 ab	11.26 ± 0.97 c	6.43 ± 0.44 b	4.27 ± 0.49 a
S0H3	2.03 ± 0.04 bc	13.92 ± 0.61 b	5.98 ± 0.36 b	4.20 ± 2.25 ab
S0H4	2.66 ± 0.25 a	17.54 ± 0.62 a	11.36 ± 0.28 a	7.36 ± 2.97 ab
S45H2	0.78 ± 0.13 fg	3.26 ± 0.70 fg	2.49 ± 0.43 cd	0.88 ± 0.26 bc
S45H3	1.41 ± 0.06 de	4.63 ± 0.33 fg	2.34 ± 0.22 cd	3.30 ± 2.41 bc
S45H4	1.89 ± 0.38 bcd	8.29 ± 2.05 d	6.28 ± 1.52 b	2.04 ± 0.76 bc
S55H2	0.87 ± 0.12 ef	2.80 ± 0.67 gh	3.59 ± 0.81 c	1.06 ± 0.37 bc
S55H3	1.35 ± 0.17 de	5.51 ± 0.62 ef	3.18 ± 0.34 c	1.00 ± 0.09 bc
S55H4	1. 57 ± 0.14 cd	7.32 ± 1.23 de	6.99 ± 0.77 b	1.32 ± 0.52 bc
S80H2	0.19 ± 0.01 h	0.24 ± 0.04 i	2.00 ± 1.84 cde	0.73 ± 0.64 bc
S80H3	0.22 ± 0.03 gh	0.41 ± 0.05 hi	0.03 ± 0.03 e	0.26 ± 0.16 c
S80H4	0.35 ± 0.09 fgh	0.53 ± 0.11 hi	0.50 ± 0.33 de	0.34 ± 0.05 c
Probability	ns	*	*	ns
P-value	0.134	0.049	0.013	0.584
$LSD_{0.05}$	0.572	2.457	2.234	3.744

Remark: the ns mean non-significant difference at $p<0.05$.

Visual appearance of Brazilian spinach on different treatment

The study analysed the shoot appearance of Brazilian spinach under different shading conditions and harvesting periods. Unshaded areas had a denser appearance, while based on the harvesting period, treatments tend to show similarities with each other (Figure 6). Furthermore, Brazilian spinach grown unshaded (S0) showed greater root growth and a higher density of root hairs than other shading, while samples subjected to varying harvesting periods (H2, H3, and H4) showed similar root morphology without any significant differences (Figure 7).

Brazilian spinach showed varying morphological traits under different treatments. Shading causes alterations in plant organs, as shown on soybean stems, which experience inhibited growth (Castronuovo et al., 2019). Cao et al. (2022) reported that *Cynodon dactylon* shoot organs also experience alterations. Root development also shows a distinct reaction to shading, with a decline in root growth under shading stress. Fu et al. (2020) found reductions in root volume and length, indicating a decline in root growth under these conditions.

Brazilian spinach with a longer harvesting period (H4) showed a rise in branches and stems, with a higher presence of mature leaves. Pruning at longer intervals increased plant height and branches in *Talinum Paniculatum*, while extending harvesting intervals hindered fresh leaf commencement (Purbajanti et al., 2019). Bessonova et al. (2023) found that removing leaves and branches from plants led to the development of shoot features with a greater number and area of leaves.

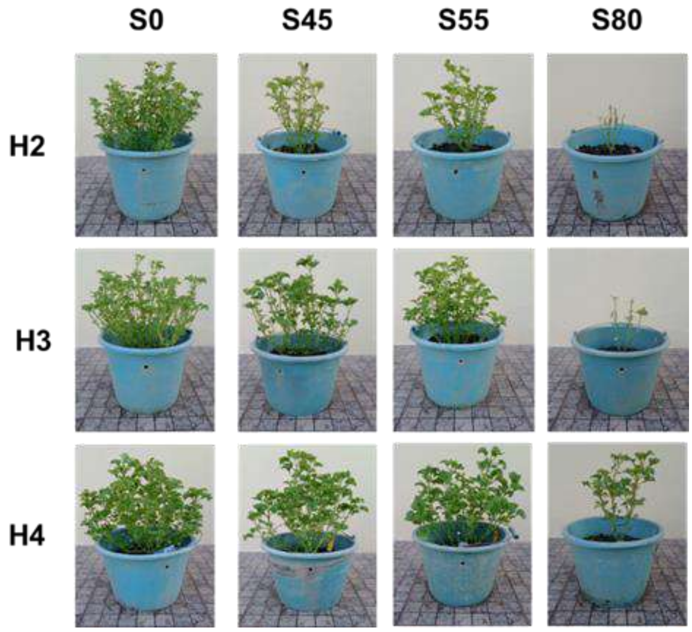


Figure 6. Visualization of Brazilian spinach shoot on different shading and harvest period at 13 weeks after planting (WAP). Shading treatment consist of S0: no-shading, S45: 45% shading, S55: 55% shading, and S80: 80% shading. Meanwhile, the harvest period consists of H2: per 2 weeks, H3: per 3 weeks, and H4: per 4 weeks. Photos: Strayker Ali Muda.

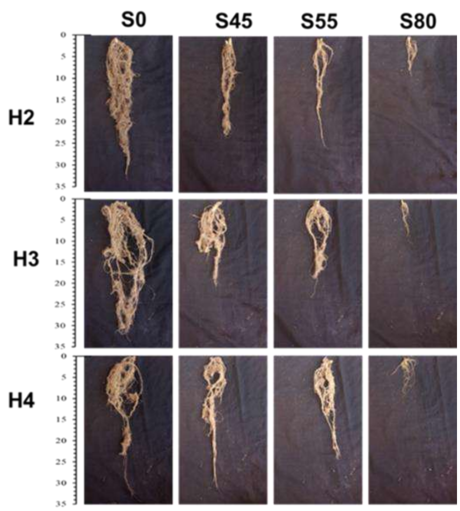


Figure 7. Visualization of Brazilian spinach root on different shading and harvest period at 13 weeks after planting (WAP). Shading treatment consist of S0: no-shading, S45: 45% shading, S55: 55% shading, and S80: 80% shading. Meanwhile, the harvest period consists of H2: per 2 weeks, H3: per 3 weeks, and H4: per 4 weeks. Photos: Strayker Ali Muda.

Water status on different treatment

The water availability for Brazilian spinach growth was represented by substrate moisture. Increased shading intensity (S80) leads to higher moisture content, reducing direct sunlight exposure and reducing evaporation, resulting in reduced water loss. Conversely, Brazilian spinach grown in areas with lower shading or without shading showed higher evaporation rates, indicating more water loss, as shown by substrate moisture levels (Figure 8). The use of shading can effectively adjust microclimate conditions, such as substrate moisture levels (Bollman et al., 2021). The study found that shaded growing media had higher humidity levels than unshaded media, and the addition of shading reduced evaporation rate, as confirmed by Khawam et al. (2019).

The more frequently Brazil spinach is harvested, the wider the substrate surface is not covered by the canopy, causing higher evaporation rates and reduced water availability. This phenomenon aligns with the findings of Huang et al. (2020), who provided empirical evidence that plants with lower canopy density exhibit higher rates of water loss via evaporation.

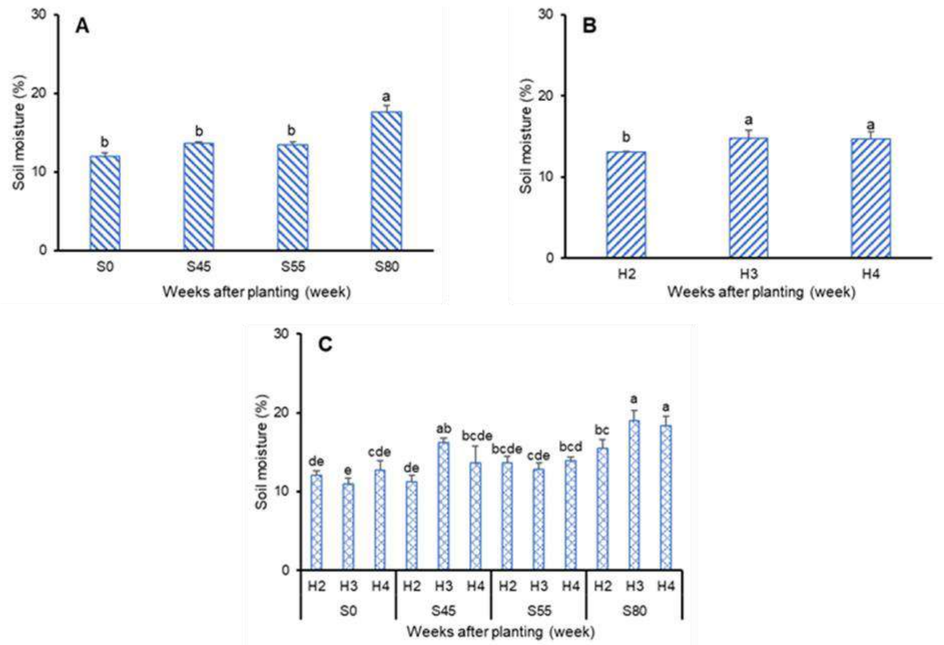


Figure 8. Substrate moisture on different shading (A), harvest period (B), and their interaction (C). Shading treatment consist of S0: no-shading, S45: shading 45%, S55: shading 55%, and S80: shading 80%. Meanwhile, the harvest period consists of H2: every 2 weeks, H3: every 3 weeks, and H4: every 4 weeks.

CONCLUSION

The adoption of shading led to a decrease in the growth and yield of Brazilian spinach through an alteration in the morphological traits of its root, stem, branch, and leaf. In addition, the implementation of a 2-week harvesting period led to increased Brazilian spinach growth and yield. Interactions between shading and harvest periods primarily pertain to the length of branches, yields (both marketable and non-marketable), dry weight of organs (namely branch and leaf), and substrate moisture.

ACKNOWLEDGMENT

We are very grateful for the suggestions, input and corrections from anonymous reviewers and the editors of the *Revista de Agricultura Neotropical* journal.

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3. Review (07 Mei 2024)

Notifications

×

[REAN] Editor Decision

2024-05-07 05:48 PM

Strayker Muda, Benyamin Lakitan, Andi Wijaya, Susilawati Susilawati, Zaidan Zaidan, Yakup Yakup:

We have reached a decision regarding your submission to REVISTA DE AGRICULTURA NEOTROPICAL, "BRAZILIAN SPINACH GROWTH UNDER DIFFERENT SHADING INTENSITIES AND HARVESTING PERIODS IN LOWLAND TROPICAL URBAN ECOSYSTEM".

Our decision is: Revisions Required

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Reviewer 1

BRAZILIAN SPINACH GROWTH UNDER DIFFERENT SHADING INTENSITIES AND HARVESTING PERIODS IN LOWLAND TROPICAL URBAN ECOSYSTEM

ABSTRACT

Brazilian spinach, a lesser-known leafy vegetable, has a high nutritional value and is essential for human health. A study was conducted to evaluate the growth of Brazilian spinach in tropical lowland urban ecosystems under different levels of shade intensity and harvest periods. The research used a split-plot design, assigning different levels of shading intensity as the main plot and harvesting periods as sub-plots. The results showed that Brazilian spinach growth was more favourable when exposed to treatment without shade compared to shaded conditions. The impact of shading on plant growth was observed during the early stages of growth, as indicated by alterations in canopy parameters and SPAD values. After productivity assessment, the impact of shading was assessed by branch elongation, yield, fresh weight, and dry weight of each organ. Shading increased the carbon content of Brazilian spinach leaf, while reducing the nitrogen content. More frequent harvesting resulted in an increase in yield components but suppressed the growth of stems and branches. Therefore, it is recommended to cultivate Brazilian spinach in an unshaded area with a biweekly harvesting routine.

KEYWORDS

Harvest time, leafy green, lesser-known vegetable, plant acclimatization, solar irradiation intensity.

INTRODUCTION

Brazilian spinach (*Alternanthera sissoo*) is a leafy vegetable originating from Brazil. As reported by Ikram et al. (2022), Brazilian spinach is rich in flavonoids, vitamins, minerals, and other antioxidants, which have been found to have positive effects on human health. The cultivation and use of this particular plant by the Indonesian population are infrequent, leading to its classification as a rather rare plant species. Indonesia's agroclimatology exhibits similarities to its indigenous location, hence indicating the potential for cultivating this plant within the country.

Urban cultivation faces several challenges, especially in regard to the availability of light for plants. Shaded areas in urban environments tend to prevail, impeding the penetration of light into plant development. Consequently, the amount of light received by plants decreases, leading to disruptions in certain aspects of plant metabolism. This phenomenon is particularly observed in horticultural crops characterized by compact growth, such as Brazilian spinach. Based on Shafiq et al. (2021), regulating alterations occur in plants as a means to enhance the efficiency of photosynthesis. The tolerance of plants to the intensity of light they receive varies depending on the specific plant species. Certain vegetable crops have been reported as being capable of growing under shaded conditions. In this regard, Sifuentes-Pallaoro et al. (2020) revealed that *Lactuca canadensis* exhibits favourable growth under shading, while Lakitan et al. (2021a) found that celery also demonstrates similar adaptability.

Brazilian spinach is a perennial leafy vegetable, enabling its continuous growth throughout the year. Additionally, this suggests that regular harvesting is required. Annual plants undergo periodic harvesting that involves a defoliation mechanism. According to the findings of Raza et al. (2019), the implementation of a defoliation treatment on plants has been observed to enhance overall plant growth, particularly in terms of leaf growth, especially during the vegetative phase. Further experimentation is required to enhance the output of Brazilian spinach, a plant species characterised by its commercially valuable leaf organs.

The cultivation of Brazilian spinach is characterized by its simplicity, since it may be easily grown. Muda et al. (2022) reported that the propagation of Brazilian spinach can be achieved via stem cuttings. There is an insufficient amount of research pertaining to the adaptability of Brazilian spinach to shading environments. The capacity of Brazilian spinach to acclimatise to shading environments for a specific duration will ensure the availability of sustainable vegetable nutrition. The study was aimed to evaluating the adaptability of Brazilian spinach to shading conditions via various harvesting periods.

MATERIALS AND METHODS

Research site and agroclimatic characteristic

The research was carried out in the Jakabaring research facility located in Palembang (104º46'44"E, 3º01'35"S), South Sumatra, Indonesia. This research began

with initiating the propagation of stem cuttings on 30 January 2023, and concluded the data collection on 02 May 2023. The research site is situated in a tropical urban lowland area with an elevation of 8 meters above sea level (masl). The agroclimatic characteristics of the area are shown in Figure 1.

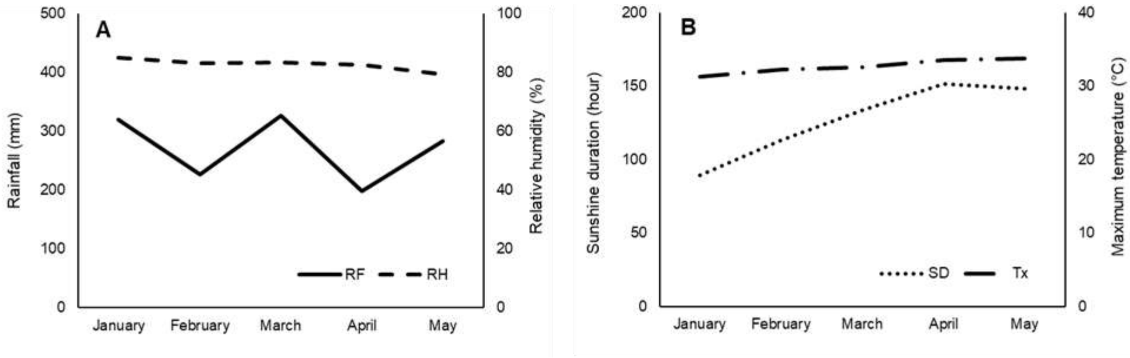

Figure 1. Agroclimatic characteristics in research location as indicated by total monthly rainfall (RF) and average humidity (RH) (A), and total monthly sunshine duration (SD) and average maximum temperature (Tx) (B). (Source: Indonesian Agency for Meteorology, Climatology and Geophysics).

Cultivation and treatment procedures

The propagation material used was stem cuttings with two leaves that were taken from healthy mother plant. The planting materials were planted in pots with dimensions of 27.5 cm in diameter and 20 cm in height. The pots were filled with a growing medium that was a mixture of top soil and chicken manure (3:1 v/v). Prior to planting, the growing medium had a bio-sterilization treatment (2 g/l), with the addition of live microorganisms including *Streptomyces thermovulgaris*, *Thricoderma virens*, and *Geobacillus thermocatenulatus*. The growing medium was subsequently subjected to a one-week incubation period before planting.

The growing medium that had been incubated was used for the planting of Brazilian spinach cuttings, which were subsequently arranged in accordance with the principles of a split-plot design. The main plot of the study focused on the intensity of shading, whereas the subplot examined the harvest period. The treatment involved selecting shading intensity at three separate levels, namely no-shading (S0), 45% shading (S45), 55% shading (S55), and 85% shading (S80). Furthermore, treatment for the harvest period started after the simultaneous harvesting, which was carried out at 5 weeks after planting (WAP). The designated harvest period has three different intervals, namely

appearing per 2 weeks, per 3 weeks, and per 4 weeks, symbolised as H2, H3, and H4, respectively.

The plants were systematically positioned within shadow houses measuring 4 meters in length, 2 meters in width, and 2 meters in height. These shadow houses are constructed using knockdown frames made of 1.5-inch PVC pipes. The entire perimeter of the shadow house is enveloped with a shade material, specifically a black polyethylene net, which has been tested for its density to ensure optimal shading.

The leaves of the Brazilian spinach cuttings get trimmed when it reaches one week after planting (WAP) in order to maintain uniformity in the planting material. Meanwhile, fertilization was conducted using NPK fertilizer (16:16:16) at 1 week after planting (WAP) and 5 WAP. The watering of each plant was normally carried out around 08:00 a.m.

Data collection

The data collection covered Brazilian spinach growth and yield data. The growth data that was obtained is categorised into two categories of measurements, such as non-destructive and destructive. The dataset for non-destructive growth measurement includes several variables, including SPAD values, canopy width, canopy diameter, canopy index, branch length, and stem diameter. In addition, the destructive measurements included the stem fresh weight, branch fresh weight, root fresh weight, stem dry weight, branch dry weight, and root dry weight. The collected data concerning Brazilian spinach yield covers several parameters, including the fresh weight of marketable leaf, fresh weight of non-marketable leaf, dry weight of marketable leaf, dry weight of non-marketable leaf, the carbon content of marketable leaf, nitrogen content of marketable leaf, and the C:N ratio of marketable leaf. The moisture content of the planting medium was also examined in order to determine the water content of the substrate.

The SPAD value was monitored using chlorophyll meters (SPAD-502 Plus, Konica-Minolta Optics, Inc., Osaka, Japan). Canopy area was calculated using a digital image scanner for Android (Easy Leaf Area software, developed by Easlon & Bloom 2014). Substrate moisture (SM) was measured using a soil moisture meter (PMS-714, Lutron Electronics Canada, Inc., Pennsylvania, USA). The carbon, nitrogen, and C:N ratios were examined using Kjedahl-Titrimetry

The dry weight of each plant organ was determined by treating it to a drying process in an oven set at a temperature of 100°C for a duration of 24 hours. Prior to being placed in the oven, the plant's organs are trimmed to a reduced thickness to accelerate the drying process.

Data analysis

All data collected was analysed using the RStudio software version 1.14.1717 for Windows (developed by RStudio team, PBC, Boston, MA). Significant differences among treatments were tested using the least significant difference (LSD) procedure at $p<0.05$.

RESULTS AND DISCUSSION

The Brazilian spinach growth during early vegetative growth before harvested

The early vegetative growth of Brazilian spinach is analysed by considering its unique characteristics, such as canopy growth and SPAD value. This approach involves non-destructive observation, allowing plants to grow naturally. The canopy characteristics selected were: canopy area, canopy diameter, and canopy index.

Brazilian spinach grown in unshaded conditions (S0) had a higher leaf initiation and larger individual leaf area compared to in shading conditions. More and larger leaves contribute to the increase in canopy area. This leads to a broader canopy compared to those grown under shade. The Brazilian spinach canopy area growth increased significantly in the S0 condition, particularly 2 to 5 weeks after planting, compared to shade conditions (S45, S55, and S80). However, no significant leaf growth was observed in the S45 and S55 shades. The shading with the highest density (S80) showed suppressed growth, starting 2 weeks after planting (Figure 2).

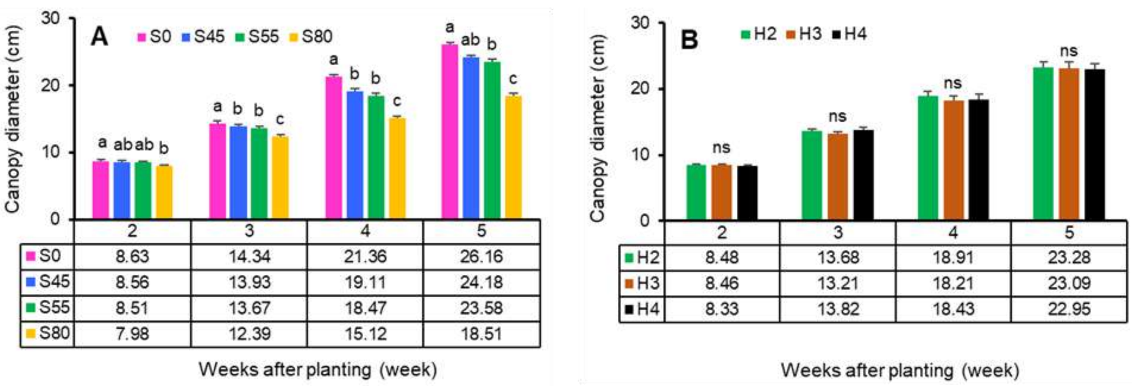

Figure 2. Brazilian spinach canopy area on early vegetative growth on different shading (A) and harvest period (B) treatment. The shading consists of no shading (S0), 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent harvest period per 2 weeks, 3 weeks, and 4 weeks, respectively. The ns mean non-significant difference at p<0.05.

Brazilian spinach branches significantly influence canopy diameter, with elongation affecting canopy expansion. Shading treatment inhibits branch growth. Full sunlight cultivation leads to a wider canopy than canopies grown under different levels of shading (S45, S55, and S80) (Figure 3).

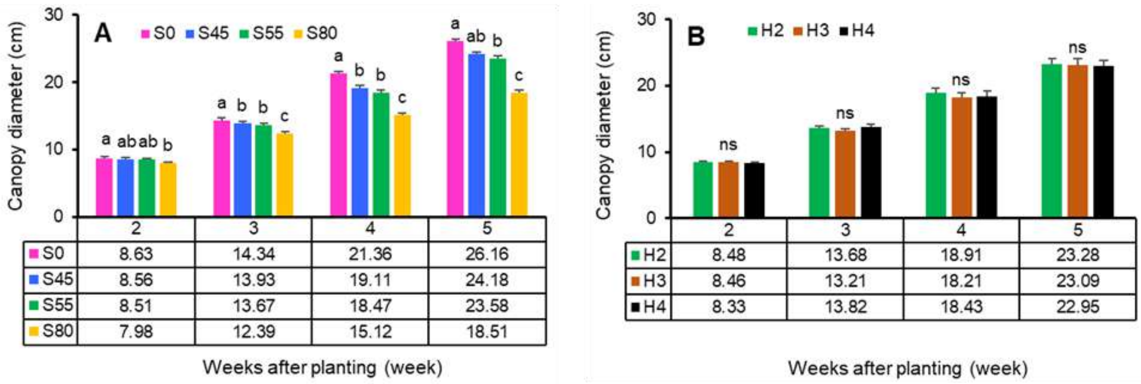

Figure 3. Brazilian spinach canopy diameter on early vegetative growth on different shading (A) and harvest period (B) treatment. The shading consists of no shading (S0), 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent harvest period per 2 weeks, 3 weeks, and 4 weeks, respectively. The ns mean non-significant difference at p<0.05.

The growth of leaf and branch significantly affects canopy density, with dominant growth resulting in a denser canopy as represented by canopy density. The effect is most noticeable between 4 and 5 weeks after planting. Brazilian spinach grown under shading conditions, especially S80, showed reduced leaf size and branch elongation, resulting in lower canopy density (Figure 4).

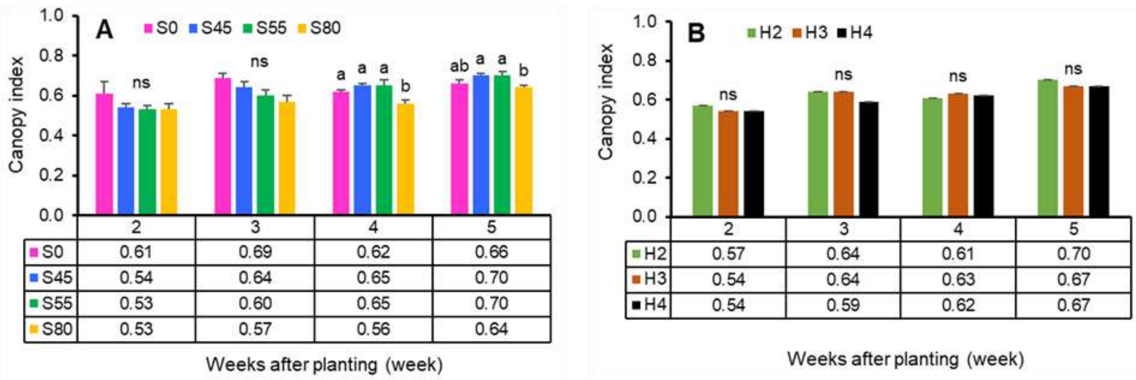

Figure 4. Canopy index on early vegetative growth on different shading and harvest period as treatment. The shading consists of no shading (S0), 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent harvest period per 2 weeks, 3 weeks, and 4 weeks, respectively. The ns mean non-significant difference at $p<0.05$.

According to this research's findings, Brazilian spinach's canopy growth was more hindered under greater shading (S80) than it was unshaded. The constituent organs of the canopy, such as the leaves and branches, endure stunted growth, which prevents the canopy from growing. According to Fadilah et al. (2022), denser shading intensity was shown to inhibit purple pakchoy leaf growth. According to the findings of Wan et al. (2020), plants planted in the shading area produce less photosynthetic performance than plants grown in full sunlight. Moreover, Liang et al. (2020) have underscored the significance of shading for plants, noting that it leads to a decrease in photosynthesis, resulting in a reduction in carbon flow. The inhibition of vegetative organ growth, particularly the canopy, in Brazilian spinach is attributed to the decreased carbon flow. This reduction in carbon flow occurs throughout the early growth cycle. The phenomenon of reduced vegetative organ development due to shading during the early growth phase has been documented in various vegetable crops, including chili (Kesumawati et al., 2020).

The SPAD value is a method for evaluating leaf nitrogen and chlorophyll content, with a positive relationship between these factors. Brazilian spinach leaf's SPAD value was affected by shading treatments, with differences observed within each shading treatment from the early growth 2 weeks after planting (WAP) (Figure 5).

Brazilian spinach grown without shading (S0) showed a higher SPAD value compared to under different shading levels, with a notable rise starting 4 weeks after

planting. This trend was also observed in S45 and S55. On the other hand, Brazilian spinach grown at S80 showed a stagnation trend, persisting until the end of the early growth, specifically 2 to 5 weeks after planting.

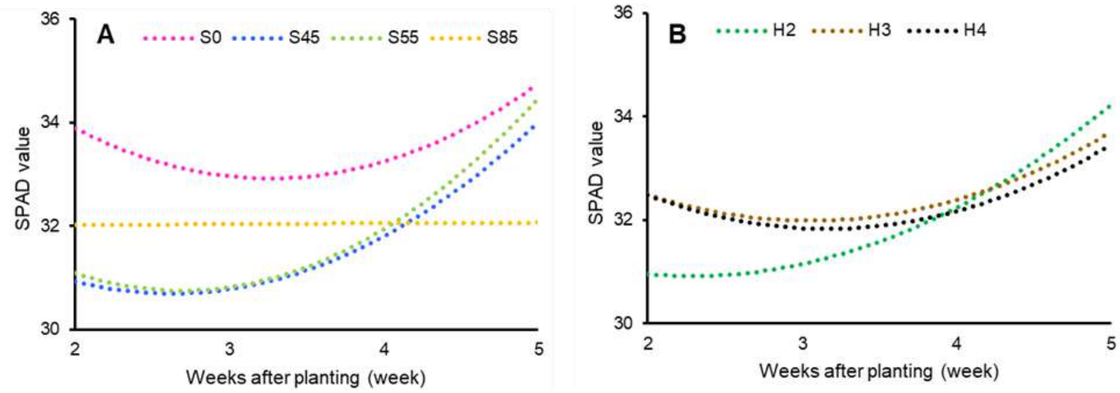

Figure 5. The SPAD value on early vegetative growth on different shading (A) and harvest period (A) treatment. The shading consists of no shading (S0), 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent harvest period per 2 weeks, 3 weeks.

The SPAD value is a widely used method for assessing leaf chlorophyll and nitrogen content, with its reliability well established. It has been found to have a positive correlation with both chlorophyll content and leaf nitrogen content (Song et al., 2021; Farnisa et al., 2023). Prior research had provided confirmation on the capacity of specific leafy vegetables, namely *Talinum paniculatum* (Lakitan et al., 2021b) and spinach (Mendoza-Tafolla et al., 2019), to proficiently evaluate and track the quantities of leaf chlorophyll and nitrogen content.

Brazilian spinach grown under full sun (S0) has a higher SPAD value than that grown under shading, indicating that shading reduces the solubility of chlorophyll and nitrogen. Wang et al. (2020a) found that shading affects the solubility of nitrogen, leading to a decrease in leaf nitrogen content. Li et al. (2020) highlighted the biochemical alterations caused by shading stress. This condition is due to Brazilian spinach, particularly in plants subjected to the 80% shading treatment (S80).

Brazilian spinach growth after harvested

The vegetative growth of Brazilian spinach after harvest was conducted at 5 weeks after planting. The growth of branch was compared under different shading conditions, harvest periods, and their interaction effects. Brazil spinach grown under S80 exhibited shorter branches as early as 11 weeks after planting. However, different shading

treatments (S0, S45, and S55) showed comparable levels of branch elongation until 9 weeks after planting. Brazilian spinach grown in S45 had an increased rate of branch elongation, particularly at 10 and 11 weeks after planting (Table 1).

The elongation of the Brazilian spinach branch was influenced by the harvesting period. Less frequent harvesting leads to the highest branch elongation, especially from 7 to 11 weeks after planting. An interaction between shading level and harvesting period treatment was observed, starting within 9 weeks after planting. This highlights the importance of harvesting frequency in influencing Brazilian spinach growth.

The study revealed a decrease in branch elongation in Brazilian spinach at S80, indicating a decrease in the allocation of photosynthetic products. This is due to reduced levels of non-structural carbohydrates, which are essential for growth (Yamashita et al., 2020). However, in the S0 treatment, photosynthesis is optimised, leading to increased branch growth.

The increased frequency of harvesting inhibits branch growth, and it is possible that the distribution of photosynthetic products changes, potentially causing a heightened initiation process of new leaves (Oliveira et al., 2021). Additionally, Raza et al. (2019) reported that maize plants with a higher number of eliminated leaves have an increased allocation of photosynthetic resources towards expanded leaves, as evidenced by an enhanced leaf area.

Table 1. Branch elongation of Brazilian spinach after harvested on different shading, harvest period, and their interaction.

Treatment	Weeks after planting (week)						
	5	6	7	8	9	10	11
				Shading			
S0	11.98 ± 0.16 a	15.89 ± 0.17 a	19.18 ± 0.43 a	22.31 ± 0.38 a	24.16 ± 0.34 a	25.22 ± 0.33 b	26.17 ± 0.53 b
S45	11.63 ± 0.33 ab	15.11 ± 0.39 ab	19.00 ± 0.55 a	22.02 ± 0.76 a	24.30 ± 0.70 a	28.23 ± 0.95 a	29.02 ± 1.20 a
S55	11.03 ± 0.17 b	14.00 ± 0.22 b	18.03 ± 0.57 a	20.74 ± 0.80 a	22.32 ± 0.85 a	25.91 ± 0.95 b	26.74 ± 1.07 ab
S80	8.68 ± 0.13 c	10.25 ± 0.17 c	12.66 ± 0.36 b	14.47 ± 0.51 b	14.93 ± 0.54 b	15.97 ± 0.76 c	16.46 ± 0.75 c
Probability	***	***	***	***	***	***	***
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
$LSD_{0.05}$	0.897	1.491	1.744	2.357	2.192	2.283	2.829
			Harvest period				
H2	11.06 ± 0.41	14.03 ± 0.69	15.80 ± 0.80 b	18.51 ± 1.09 c	19.66 ± 1.21 c	21.59 ± 1.41 c	21.90 ± 1.36 c
H3	10.72 ± 0.42	13.80 ± 0.71	18.07 ± 0.90 a	19.54 ± 1.02 b	21.75 ± 1.26 b	24.31 ± 1.58 b	24.95 ± 1.61 b
H4	10.70 ± 0.44	13.60 ± 0.66	17.78 ± 0.85 a	21.61 ± 0.98 a	22.88 ± 1.17 a	25.60 ± 1.45 a	26.94 ± 1.61 a
Probability	ns	ns	***	***	***	***	***
P-value	0.186	0.198	<0.001	<0.001	<0.001	<0.001	<0.001
$LSD_{0.05}$	0.462	0.507	0.607	0.878	0.708	0.922	0.876
			Shading x harvest period				
S0H2	12.23 ± 0.12	16.05 ± 0.34	18.55 ± 0.54	21.47 ± 0.44	23.22 ± 0.49 cd	24.24 ± 0.58 de	24.57 ± 0.62 ef
S0H3	11.93 ± 0.41	16.20 ± 0.20	20.31 ± 0.72	22.40 ± 0.85	24.85 ± 0.40 b	25.68 ± 0.39 cd	26.91 ± 0.63 cd
S0H4	11.77 ± 0.27	15.41 ± 0.14	19.08 ± 0.44	23.08 ± 0.41	24.41 ± 0.53 bc	25.74 ± 0.34 cd	27.04 ± 0.78 bcd
S45H2	11.99 ± 0.62	15.51 ± 1.10	17.45 ± 0.72	20.97 ± 1.52	22.53 ± 0.85 d	25.43 ± 1.20 cd	25.43 ± 0.91 de
S45H3	10.89 ± 0.71	14.68 ± 0.52	19.57 ± 0.59	20.87 ± 0.95	23.77 ± 0.60 bcd	28.07 ± 0.83 b	28.54 ± 0.87 bc
S45H4	12.01 ± 0.19	15.14 ± 0.41	19.99 ± 0.95	24.24 ± 0.20	26.59 ± 0.68 a	31.19 ± 0.67 b	33.08 ± 0.99 a
S55H2	11.08 ± 0.11	13.97 ± 0.22	16.00 ± 0.56	18.64 ± 0.81	19.53 ± 0.71 d	22.70 ± 0.97 e	23.19 ± 0.82 f
S55H3	11.37 ± 0.44	14.21 ± 0.67	19.19 ± 0.59	20.58 ± 1.41	23.50 ± 1.48 bcd	27.88 ± 1.25 b	28.27 ± 1.61 bc
S55H4	10.64 ± 0.03	13.81 ± 0.21	18.90 ± 0.28	23.00 ± 0.45	23.92 ± 0.49 bcd	27.15 ± 0.70 b	28.77 ± 1.02 b
S80H2	8.95 ± 0.06	10.58 ± 0.13	11.61 ± 0.27	12.97 ± 0.37	13.32 ± 0.40 h	13.98 ± 0.60 g	14.41 ± 0.53 h
S80H3	8.70 ± 0.32	10.13 ± 0.37	13.22 ± 0.70	14.33 ± 0.60	14.89 ± 0.22 g	15.62 ± 0.39 g	16.09 ± 0.29 h
S80H4	8.38 ± 0.11	10.04 ± 0.32	13.14 ± 0.38	16.11 ± 0.38	16.59 ± 0.75 f	18.32 ± 1.27f	18.87 ± 1.17 g
Probability	ns	ns	ns	ns	**	**	**
P-value	0.237	0.89	0.211	0.383	0.009	0.008	0.003
$LSD_{0.05}$	0.920	1.014	1.214	1.756	1.416	1.844	1.751

Remark: the ns mean non-significant difference at $p<0.05$.

Brazilian spinach showed significant differences in leaf growth when treated with different shading and harvesting periods. Cultivated without shade (S0), it tends to dominate leaf growth compared to cultivated under different levels of shading (S45, S55, and S80) (Table 2). However, this method also demonstrated a significant proportion of non-marketable leaves. This indicates that early leaf growth is achieved without shading, but it also leads to accelerated leaf aging and increased pest susceptibility, resulting in a higher proportion of non-marketable leaves compared to those cultivated under shading.

The frequent harvesting of Brazilian spinach leads to the initiation of young leaves, resulting in more marketable leaves. This is evident in the yield of commercially viable leaves throughout the H2 and H3 periods. However, during the H3 harvesting period, a significant proportion of non-marketable leaves are produced due to leaf aging. In contrast, extended harvesting periods (H4) often lack the capacity for leaf initiation, resulting in decreased yields of both marketable and non-marketable leaves. The interaction impact of shading and harvesting period significantly showed on leaf growth, with the most significant impact observed under 80% shade, especially during the longer harvesting period (H4).

Brazilian spinach's leaf initiation is higher in conditions without shade compared to shading conditions, affecting both marketable and non-marketable leaves. This is due to reduced carbohydrate accumulation and allocation (Hussain et al., 2020). Shading conditions inhibited plant growth, while without shade, leaf senescence accelerated due to enhanced photosynthesis. Meanwhile, direct sunlight exposure accelerates ageing processes in plants, similar to sweet basil (Castronuovo et al., 2019). However, without shade, spinach is more susceptible to pest infestation, leading to an increased prevalence of non-marketable leaves. Implementing shading at a specific density is a viable pest control strategy.

Brazilian spinach harvesting, similar to leaf and shoot pruning, has been found to increase yield. Dheeraj et al. (2022) found that pruning at the apical meristem increased growth-promoting hormones, specifically cytokinin. Xu et al. (2020a) reported that pruning tomato plants also increases cytokinin hormone levels. Cytokinin hormones influence cell division processes, including those during leaf cell development. Harvesting Brazilian spinach at H2 and H3 treatment results in elevated levels of cytokinin hormones, enhancing leaf initiation and resulting in a greater marketable yield.

Tabel 2. Brazilian spinach yield on different shading, harvest period, and their interaction.

Treatment	Marketable yield		Non-marketable yield	
	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
Shading				
S0	109.07 ± 8.55 a	13.16 ± 0.91 a	46.99 ± 2 .32 a	6.85 ± 0.41 a
S45	60.04 ± 3.57 b	5.80 ± 0.33 b	33.40 ± 2.74 b	3.44 ± 0.57 b
S55	52.63 ± 3.19 b	5.14 ± 0.37 b	32.92 ± 3.18 b	3.53 ± 0.35 b
S80	14.76 ± 1.04 c	1.22 ± 0.10 c	4.68 ± 1.00 c	0.49 ± 0.10 c
Probability	***	***	***	***
P-value	<0.001	<0.001	<0.001	<0.001
$LSD_{0.05}$	12.754	1.21	6.526	0.403
Harvest period				
H2	67.22 ± 12.80 a	6.58 ± 1.47 a	28.90 ± 5.03 b	3.57 ± 0.71 b
H3	60.95 ± 11.05 a	7.05 ± 1.56 a	33.69 ± 5.76 a	4.41 ± 0.84 a
H4	49.20 ± 7.66 b	5.37 ± 0.97 b	25.91 ± 4.06 c	2.75 ± 0.62 c
Probability	***	**	**	**
P-value	<0.001	0.001	0.008	0.001
$LSD_{0.05}$	7.391	0.793	4.546	0.793
Shading x harvest period				
S0H2	130.79 ±7.94 a	14.25 ± 0.64 a	48.17± 1.71 a	6.85 ± 0.57 ab
S0H3	117.30 ± 7.13 a	15.44 ± 0.36 a	47.58± 7.46 a	7.73 ± 0.93 a
S0H4	79.10 ± 6.74 b	9.78 ± 0.77 b	45.22± 1.88 a	5.98 ± 0.19 bc
S45H2	69.51 ± 5.90 bc	6.12 ± 0.76 c	35.36± 3.37 bc	3.88 ± 0.40 def
S45H3	51.03 ± 3.93 d	5.40 ± 0.38 c	40.14± 0.18 ab	4.97 ± 0.14 cd
S45H4	59.57 ± 4.14 cd	5.90 ± 0.66 c	24.70± 4.02 d	1.48 ± 0.74 gh
S55H2	53.05 ± 6.86 d	4.76 ± 0.80 c	28.81± 2.70cd	3.21 ± 0.36 ef
S55H3	58.36 ± 4.61cd	5.88 ± 0.59 c	44.67± 2.41 a	4.69 ± 0.48 cde
S55H4	46.47 ± 4.24d	4.78 ± 0.52 c	25.30± 1.38 d	2.69 ± 0.15 fg
S80H2	15.54 ± 1.30e	1.17 ± 0.14 d	3.27± 0.19 e	0.37 ± 0.02 h
S80H3	17.09 ± 1.32e	1.48 ± 0.13 d	2.37± 1.07 e	0.28 ± 0.18 h
S80H4	11.63 ± 1.28e	1.01 ± 0.14 d	8.41±0.52 e	0.83 ± 0.06 h
Probability	***	***	**	*
P-value	<0.001	<0.001	0.008	0.05
$LSD_{0.05}$	14.781	1.587	9.092	1.587

Remark: the ns mean non-significant difference at $p<0.05$.

The metabolism of Brazilian spinach was influenced by shading and harvesting periods. Brazilian spinach grown without shade (S0) increased metabolism activity compared to the shading areas (S45, S55, and S80). This is represented by the carbon and nitrogen levels (Table 3). Higher metabolism correlates with enhanced nitrogen usage, which is crucial for plant metabolic processes. Therefore, increasing fertilization frequency is necessary for plants exposed to sunlight. Leaf nitrogen concentration remains consistent across harvesting periods, suggesting no significant differences in nitrogen across different harvesting periods.

The carbon-nitrogen ratio calculation can be used to determine leaf hardness in Brazilian spinach leaves. The study showed that unshaded (S0) areas yield more tough leaves, decreasing with increased shading levels. Despite this, Brazilian spinach consistently showed comparable levels of leaf hardness across harvesting periods.

Shading significantly impacts the carbon reduction and nitrogen enrichment of leaves, affecting the process of photosynthesis. Light intensity and photosynthesis are linked, with studies showing a reduction in carbon and nitrogen buildup in plants exposed to shading. Tang et al. (2022) found that plants exposed to modest levels of irradiation exhibit reduced concentrations of leaf non-structural carbohydrates, leading to a reduction in carbon content. Nitrogen accumulation in shaded Brazilian spinach leaves is due to limited light availability, hindering the conversion of nitrogen into organic nitrogen compounds essential for plant metabolic processes. Gao et al. (2020) found that prolonged shading reduces nitrogen utilization efficiency in plant. Wang et al. (2020b) demonstrated that the procedure of removing leaves of plants results in an increase in non-structural carbohydrates in leaf. On the other hand, an increased frequency of harvesting triggers the growth of new leaves, leading the movement of nitrogen toward younger leaves. Jasinski et al. (2021) reported that nitrogen mobilization occurs from older to younger leaves.

Table 3. Carbon, Nitrogen and C-N ratio of Brazilian spinach leaf on different shading, harvest period, and their interaction.

Treatment	Carbon (%)	Nitrogen (%)	C-N ratio
Shading			
S0	34.64	2.83	12.28
S45	32.75	4.56	7.20
S55	34.21	4.77	7.19
S80	34.32	4.99	6.84
Harvest period			
H2	35.85	4.38	8.74
H3	33.90	4.42	8.10
H4	32.20	4.07	8.30
Shading x harvest periode			
S0H2	34.23	2.63	13.00
S0H3	34.28	2.90	11.83
S0H4	35.42	2.95	12.01
S45H2	32.02	4.70	6.81
S45H3	33.89	4.77	7.10
S45H4	32.34	4.20	7.70
S55H2	36.50	5.01	7.29
S55H3	32.14	4.94	6.50
S55H4	34.00	4.36	7.79
S80H2	40.66	5.16	7.88
S80H3	35.30	5.07	6.96
S80H4	27.01	4.76	5.68

Remark: the ns mean non-significant difference at $p<0.05$.

The presence of shading in Brazilian spinach is linked to biomass production. Under unshaded conditions, it enhances photosynthesis, leading to increased biomass production. However, under intense shading conditions, it reduces biomass in various parts of the plant. Harvesting over extended periods (H3 and H4) results in increased biomass accumulation, particularly in the stem and branch in the final observation.

Brazilian spinach, when grown under shading conditions and extended harvesting, showed inhibited growth due to restricted photosynthetic activity. This caused the restricted allocation of photosynthetic products to individual plant organs. Studies have shown that shading reduces biomass accumulation and alterations in plant morphological traits (Xu et al., 2020b). Additionally, there is a distribution of photosynthetic activity that utilizes plant organs beyond just leaves. This aligns with Yu et al. (2019) finding that when plants age and their organs undergo senescence, photosynthetic flux redirects towards the stem. This highlights the importance of considering the allocation of photosynthetic products to plant growth through periodic harvesting.

Table 4. Dry weight of Brazilian spinach organs on different shading, harvest period, and their interaction at 13 weeks after planting (WAP).

Treatment	Stem dry weight (g)	Branch dry weight (g)	Leaf dry weight (g)	Root dry weight (g)
Shading				
S0	2.35 ± 0.19 a	14.24 ± 0.98 a	7.92 ± 0.88 a	5.28 ± 1.20 a
S45	1.36 ± 0.20 b	5.39 ± 0.98 b	3.70 ± 0.79 b	2.07 ± 0.81 ab
S55	1.26 ± 0.13 b	5.21 ± 0.79 b	4.58 ± 0.69 b	1.13 ± 0.19 b
S80	0.25 ± 0.04 c	0.40 ± 0.05 c	0.85 ± 0.62 c	0.44 ± 0.20 b
Probability	***	***	***	*
P-value	< 0.001	< 0.001	< 0.001	0.046
$LSD_{0.05}$	0.479	1.755	2.037	3.325
Harvest period				
H2	1.05 ± 0.27 b	4.39 ± 1.28 c	3.63 ± 0.68 b	1.73 ± 0.48 a
H3	1.25 ± 0.20 b	6.12 ± 1.49 b	2.88 ± 0.65 b	2.19 ± 0.86 a
H4	1.62 ± 0.27 a	8.42 ± 1.90 a	6.28 ± 1.22 a	2.77 ± 1.05 a
Probability	**	***	***	ns
P-value	0.002	< 0.001	< 0.001	0.517
$LSD_{0.05}$	0.286	1.228	1.117	1.872
Shading x harvest period				
S0H2	2.35 ± 0.53 ab	11.26 ± 0.97 c	6.43 ± 0.44 b	4.27 ± 0.49 a
S0H3	2.03 ± 0.04 bc	13.92 ± 0.61 b	5.98 ± 0.36 b	4.20 ± 2.25 ab
S0H4	2.66 ± 0.25 a	17.54 ± 0.62 a	11.36 ± 0.28 a	7.36 ± 2.97 ab
S45H2	0.78 ± 0.13 fg	3.26 ± 0.70 fg	2.49 ± 0.43 cd	0.88 ± 0.26 bc
S45H3	1.41 ± 0.06 de	4.63 ± 0.33 fg	2.34 ± 0.22 cd	3.30 ± 2.41 bc
S45H4	1.89 ± 0.38 bcd	8.29 ± 2.05 d	6.28 ± 1.52 b	2.04 ± 0.76 bc
S55H2	0.87 ± 0.12 ef	2.80 ± 0.67 gh	3.59 ± 0.81 c	1.06 ± 0.37 bc
S55H3	1.35 ± 0.17 de	5.51 ± 0.62 ef	3.18 ± 0.34 c	1.00 ± 0.09 bc
S55H4	1. 57 ± 0.14 cd	7.32 ± 1.23 de	6.99 ± 0.77 b	1.32 ± 0.52 bc
S80H2	0.19 ± 0.01 h	0.24 ± 0.04 i	2.00 ± 1.84 cde	0.73 ± 0.64 bc
S80H3	0.22 ± 0.03 gh	0.41 ± 0.05 hi	0.03 ± 0.03 e	0.26 ± 0.16 c
S80H4	0.35 ± 0.09 fgh	0.53 ± 0.11 hi	0.50 ± 0.33 de	0.34 ± 0.05 c
Probability	ns	*	*	ns
P-value	0.134	0.049	0.013	0.584
$LSD_{0.05}$	0.572	2.457	2.234	3.744

Remark: the ns mean non-significant difference at $p<0.05$.

Visual appearance of Brazilian spinach on different treatment

The study analysed the shoot appearance of Brazilian spinach under different shading conditions and harvesting periods. Unshaded areas had a denser appearance, while based on the harvesting period, treatments tend to show similarities with each other (Figure 6). Furthermore, Brazilian spinach grown unshaded (S0) showed greater root growth and a higher density of root hairs than other shading, while samples subjected to varying harvesting periods (H2, H3, and H4) showed similar root morphology without any significant differences (Figure 7).

Brazilian spinach showed varying morphological traits under different treatments. Shading causes alterations in plant organs, as shown on soybean stems, which experience inhibited growth (Castronuovo et al., 2019). Cao et al. (2022) reported that *Cynodon dactylon* shoot organs also experience alterations. Root development also shows a distinct reaction to shading, with a decline in root growth under shading stress. Fu et al. (2020) found reductions in root volume and length, indicating a decline in root growth under these conditions.

Brazilian spinach with a longer harvesting period (H4) showed a rise in branches and stems, with a higher presence of mature leaves. Pruning at longer intervals increased plant height and branches in *Talinum Paniculatum*, while extending harvesting intervals hindered fresh leaf commencement (Purbajanti et al., 2019). Bessonova et al. (2023) found that removing leaves and branches from plants led to the development of shoot features with a greater number and area of leaves.

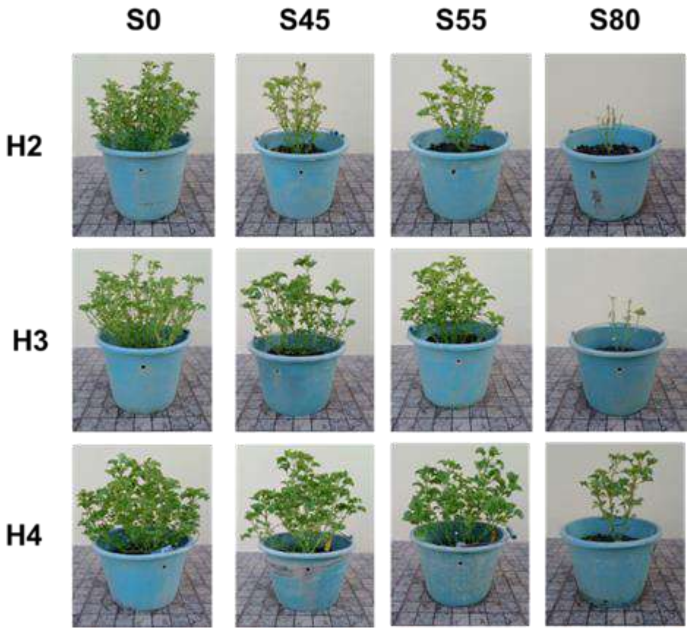

Figure 6. Visualization of Brazilian spinach shoot on different shading and harvest period at 13 weeks after planting (WAP). Shading treatment consist of S0: no-shading, S45: 45% shading, S55: 55% shading, and S80: 80% shading. Meanwhile, the harvest period consists of H2: per 2 weeks, H3: per 3 weeks, and H4: per 4 weeks. Photos: Strayker Ali Muda.

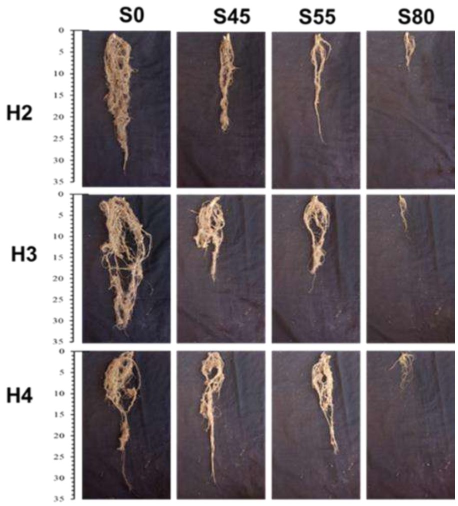

Figure 7. Visualization of Brazilian spinach root on different shading and harvest period at 13 weeks after planting (WAP). Shading treatment consist of S0: no-shading, S45: 45% shading, S55: 55% shading, and S80: 80% shading. Meanwhile, the harvest period consists of H2: per 2 weeks, H3: per 3 weeks, and H4: per 4 weeks. Photos: Strayker Ali Muda.

Water status on different treatment

The water availability for Brazilian spinach growth was represented by substrate moisture. Increased shading intensity (S80) leads to higher moisture content, reducing direct sunlight exposure and reducing evaporation, resulting in reduced water loss. Conversely, Brazilian spinach grown in areas with lower shading or without shading showed higher evaporation rates, indicating more water loss, as shown by substrate moisture levels (Figure 8). The use of shading can effectively adjust microclimate conditions, such as substrate moisture levels (Bollman et al., 2021). The study found that shaded growing media had higher humidity levels than unshaded media, and the addition of shading reduced evaporation rate, as confirmed by Khawam et al. (2019).

The more frequently Brazil spinach is harvested, the wider the substrate surface is not covered by the canopy, causing higher evaporation rates and reduced water availability. This phenomenon aligns with the findings of Huang et al. (2020), who provided empirical evidence that plants with lower canopy density exhibit higher rates of water loss via evaporation.

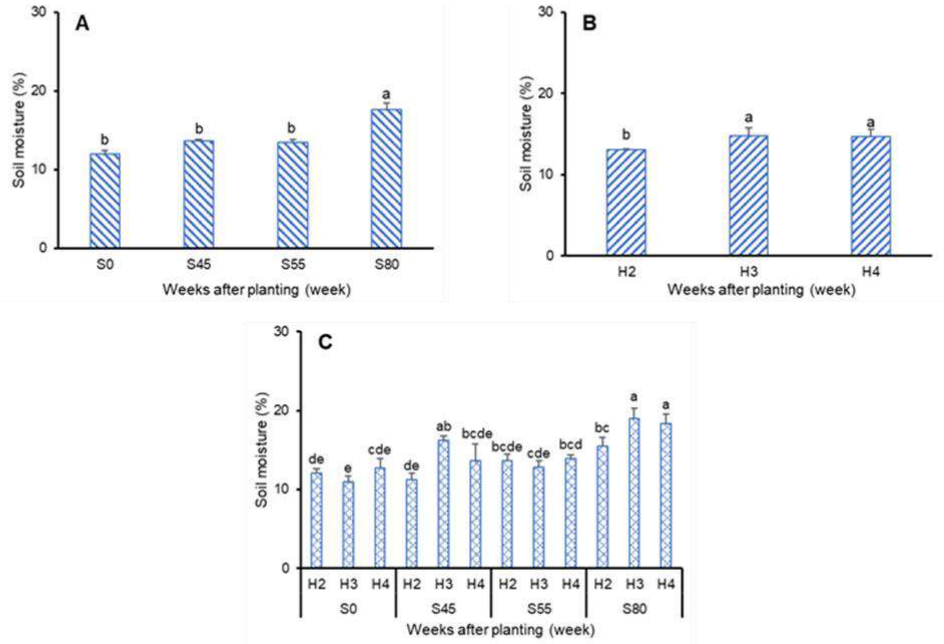

Figure 8. Substrate moisture on different shading (A), harvest period (B), and their interaction (C). Shading treatment consist of S0: no-shading, S45: shading 45%, S55: shading 55%, and S80: shading 80%. Meanwhile, the harvest period consists of H2: every 2 weeks, H3: every 3 weeks, and H4: every 4 weeks.

CONCLUSION

The adoption of shading led to a decrease in the growth and yield of Brazilian spinach through an alteration in the morphological traits of its root, stem, branch, and leaf. In addition, the implementation of a 2-week harvesting period led to increased Brazilian spinach growth and yield. Interactions between shading and harvest periods primarily pertain to the length of branches, yields (both marketable and non-marketable), dry weight of organs (namely branch and leaf), and substrate moisture.

ACKNOWLEDGMENT

We are very grateful for the suggestions, input and corrections from anonymous reviewers and the editors of the *Revista de Agricultura Neotropical* journal.

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Reviewer 2

BRAZILIAN SPINACH GROWTH UNDER DIFFERENT SHADING INTENSITIES AND HARVESTING PERIODS IN LOWLAND TROPICAL URBAN ECOSYSTEM

ABSTRACT

Brazilian spinach, a lesser-known leafy vegetable, has a high nutritional value and is essential for human health. A study was conducted to evaluate the growth of Brazilian spinach in tropical lowland urban ecosystems under different levels of shade intensity and harvest periods. The research used a split-plot design, assigning different levels of shading intensity as the main plot and harvesting periods as sub-plots. The results showed that Brazilian spinach growth was more favourable when exposed to treatment without shade compared to shaded conditions. The impact of shading on plant growth was observed during the early stages of growth, as indicated by alterations in canopy parameters and SPAD values. After productivity assessment, the impact of shading was assessed by branch elongation, yield, fresh weight, and dry weight of each organ. Shading increased the carbon content of Brazilian spinach leaf, while reducing the nitrogen content. More frequent harvesting resulted in an increase in yield components but suppressed the growth of stems and branches. Therefore, it is recommended to cultivate Brazilian spinach in an unshaded area with a biweekly harvesting routine.

KEYWORDS

Harvest time, leafy green, lesser-known vegetable, plant acclimatization, solar irradiation intensity.

INTRODUCTION

Brazilian spinach (*Alternanthera sissoo*) is a leafy vegetable originating from Brazil. As reported by Ikram et al. (2022), Brazilian spinach is rich in flavonoids, vitamins, minerals, and other antioxidants, which have been found to have positive effects on human health. The cultivation and use of this particular plant by the Indonesian population are infrequent, leading to its classification as a rather rare plant species. Indonesia's agroclimatology exhibits similarities to its indigenous location, hence indicating the potential for cultivating this plant within the country.

Urban cultivation faces several challenges, especially in regard to the availability of light for plants. Shaded areas in urban environments tend to prevail, impeding the penetration of light into plant development. Consequently, the amount of light received by plants decreases, leading to disruptions in certain aspects of plant metabolism. This phenomenon is particularly observed in horticultural crops characterized by compact growth, such as Brazilian spinach. Based on Shafiq et al. (2021), regulating alterations occur in plants as a means to enhance the efficiency of photosynthesis. The tolerance of plants to the intensity of light they receive varies depending on the specific plant species. Certain vegetable crops have been reported as being capable of growing under shaded conditions. In this regard, Sifuentes-Pallaoro et al. (2020) revealed that *Lactuca canadensis* exhibits favourable growth under shading, while Lakitan et al. (2021a) found that celery also demonstrates similar adaptability.

Brazilian spinach is a perennial leafy vegetable, enabling its continuous growth throughout the year. Additionally, this suggests that regular harvesting is required. Annual plants undergo periodic harvesting that involves a defoliation mechanism. According to the findings of Raza et al. (2019), the implementation of a defoliation treatment on plants has been observed to enhance overall plant growth, particularly in terms of leaf growth, especially during the vegetative phase. Further experimentation is required to enhance the output of Brazilian spinach, a plant species characterised by its commercially valuable leaf organs.

The cultivation of Brazilian spinach is characterized by its simplicity, since it may be easily grown. Muda et al. (2022) reported that the propagation of Brazilian spinach can be achieved via stem cuttings. There is an insufficient amount of research pertaining to the adaptability of Brazilian spinach to shading environments. The capacity of Brazilian spinach to acclimatise to shading environments for a specific duration will ensure the availability of sustainable vegetable nutrition. The study was aimed to evaluating the adaptability of Brazilian spinach to shading conditions via various harvesting periods.

MATERIALS AND METHODS

Research site and agroclimatic characteristic

The research was carried out in the Jakabaring research facility located in Palembang (104º46'44"E, 3º01'35"S), South Sumatra, Indonesia. This research began

with initiating the propagation of stem cuttings on 30 January 2023, and concluded the data collection on 02 May 2023. The research site is situated in a tropical urban lowland area with an elevation of 8 meters above sea level (masl). The agroclimatic characteristics of the area are shown in Figure 1.

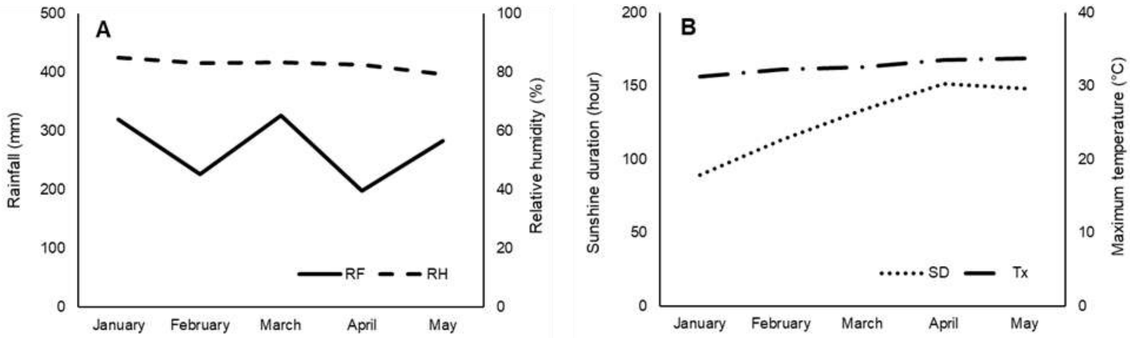

Figure 1. Agroclimatic characteristics in research location as indicated by total monthly rainfall (RF) and average humidity (RH) (A), and total monthly sunshine duration (SD) and average maximum temperature (Tx) (B). (Source: Indonesian Agency for Meteorology, Climatology and Geophysics).

Cultivation and treatment procedures

The propagation material used was stem cuttings with two leaves that were taken from healthy mother plant. The planting materials were planted in pots with dimensions of 27.5 cm in diameter and 20 cm in height. The pots were filled with a growing medium that was a mixture of top soil and chicken manure (3:1 v/v). Prior to planting, the growing medium had a bio-sterilization treatment (2 g/l), with the addition of live microorganisms including *Streptomyces thermovulgaris*, *Thricoderma virens*, and *Geobacillus thermocatenulatus*. The growing medium was subsequently subjected to a one-week incubation period before planting.

The growing medium that had been incubated was used for the planting of Brazilian spinach cuttings, which were subsequently arranged in accordance with the principles of a split-plot design. The main plot of the study focused on the intensity of shading, whereas the subplot examined the harvest period. The treatment involved selecting shading intensity at three separate levels, namely no-shading (S0), 45% shading (S45), 55% shading (S55), and 85% shading (S80). Furthermore, treatment for the harvest period started after the simultaneous harvesting, which was carried out at 5 weeks after planting (WAP). The designated harvest period has three different intervals, namely

appearing per 2 weeks, per 3 weeks, and per 4 weeks, symbolised as H2, H3, and H4, respectively.

The plants were systematically positioned within shadow houses measuring 4 meters in length, 2 meters in width, and 2 meters in height. These shadow houses are constructed using knockdown frames made of 1.5-inch PVC pipes. The entire perimeter of the shadow house is enveloped with a shade material, specifically a black polyethylene net, which has been tested for its density to ensure optimal shading.

The leaves of the Brazilian spinach cuttings get trimmed when it reaches one week after planting (WAP) in order to maintain uniformity in the planting material. Meanwhile, fertilization was conducted using NPK fertilizer (16:16:16) at 1 week after planting (WAP) and 5 WAP. The watering of each plant was normally carried out around 08:00 a.m.

Data collection

The data collection covered Brazilian spinach growth and yield data. The growth data that was obtained is categorised into two categories of measurements, such as non-destructive and destructive. The data set for non-destructive growth measurement includes several variables, including SPAD values, canopy width, canopy diameter, canopy index, branch length, and stem diameter. In addition, the destructive measurements included the stem fresh weight, branch fresh weight, root fresh weight, stem dry weight, branch dry weight, and root dry weight. The collected data concerning Brazilian spinach yield covers several parameters, including the fresh weight of marketable leaf, fresh weight of non-marketable leaf, dry weight of marketable leaf, dry weight of non-marketable leaf, the carbon content of marketable leaf, nitrogen content of marketable leaf, and the C:N ratio of marketable leaf. The moisture content of the planting medium was also examined in order to determine the water content of the substrate.

The SPAD value was monitored using chlorophyll meters (SPAD-502 Plus, Konica-Minolta, Osaka, Japan). Canopy area was calculated using a digital image scanner for Android (Easy Leaf Area software, developed by Easlon & Bloom 2014). Substrate moisture (SM) was measured using a soil moisture meter (PMS-714, Lutron Electronics Canada, Inc., Pennsylvania, USA). The carbon, nitrogen, and C:N ratios were examined using Kjedahl-Titrimetry in the Integrated Laboratory of Sampoerna Agro. Tbk.

The dry weight of each plant organ was determined by treating it to a drying process in an oven set at a temperature of 100°C for a duration of 24 hours. Prior to being placed in the oven, the plant's organs are trimmed to a reduced thickness to accelerate the drying process.

Data analysis

All data collected was analysed using the RStudio software version 1.14.1717 for Windows (developed by RStudio team, PBC, Boston, MA). Significant differences among treatments were tested using the least significant difference (LSD) procedure at $p<0.05$.

RESULTS AND DISCUSSION

The Brazilian spinach growth during early vegetative growth before harvested

The early vegetative growth of Brazilian spinach is analysed by considering its unique characteristics, such as canopy growth and SPAD value. This approach involves non-destructive observation, allowing plants to grow naturally. The canopy characteristics selected were: canopy area, canopy diameter, and canopy index.

Brazilian spinach grown in unshaded conditions (S0) had a higher leaf initiation and larger individual leaf area compared to in shading conditions. More and larger leaves contribute to the increase in canopy area. This leads to a broader canopy compared to those grown under shade. The Brazilian spinach canopy area growth increased significantly in the S0 condition, particularly 2 to 5 weeks after planting, compared to shade conditions (S45, S55, and S80). However, no significant leaf growth was observed in the S45 and S55 shades. The shading with the highest density (S80) showed suppressed growth, starting 2 weeks after planting (Figure 2).

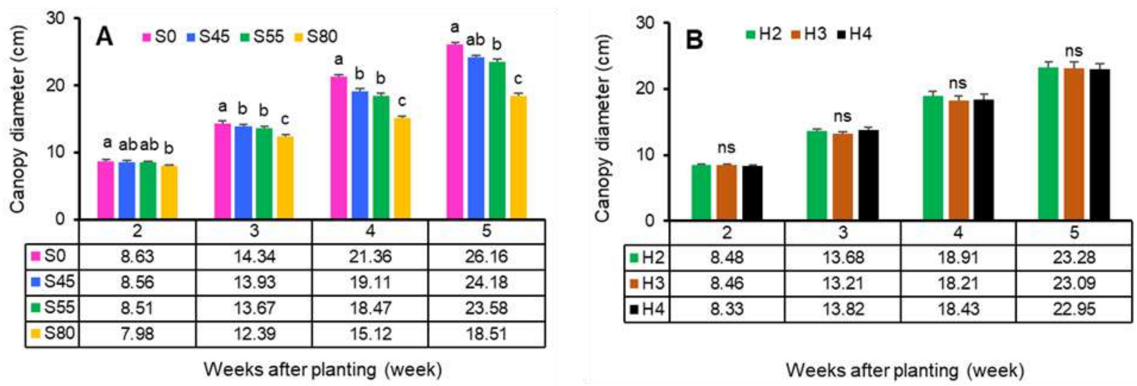

Figure 2. Brazilian spinach canopy area on early vegetative growth on different shading (A) and harvest period (B) treatment. The shading consists of no shading (S0), 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent harvest period per 2 weeks, 3 weeks, and 4 weeks, respectively. The ns mean non-significant difference at $p<0.05$.

Brazilian spinach branches significantly influence canopy diameter, with elongation affecting canopy expansion. Shading treatment inhibits branch growth. Full sunlight cultivation leads to a wider canopy than canopies grown under different levels of shading (S45, S55, and S80) (Figure 3).

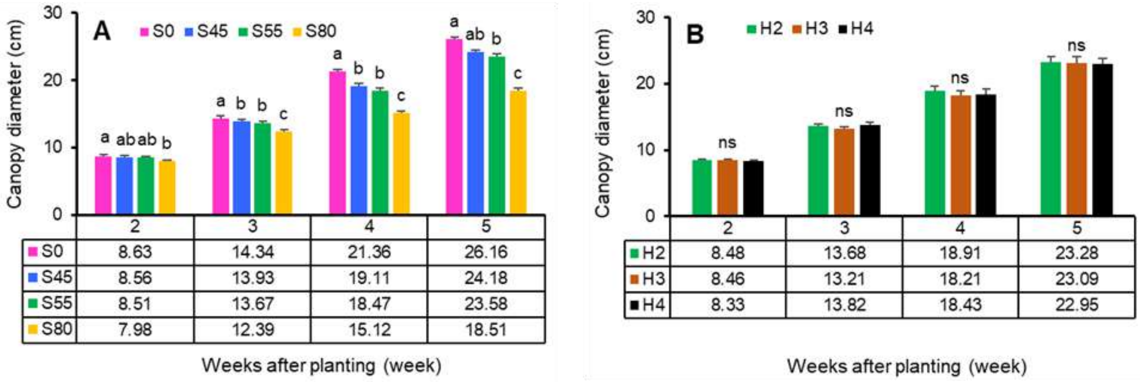

Figure 3. Brazilian spinach canopy diameter on early vegetative growth on different shading (A) and harvest period (B) treatment. The shading consists of no shading (S0), 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent harvest period per 2 weeks, 3 weeks, and 4 weeks, respectively. The ns mean non-significant difference at $p<0.05$.

The growth of leaf and branch significantly affects canopy density, with dominant growth resulting in a denser canopy as represented by canopy density. The effect is most noticeable between 4 and 5 weeks after planting. Brazilian spinach grown under shading conditions, especially S80, showed reduced leaf size and branch elongation, resulting in lower canopy density (Figure 4).

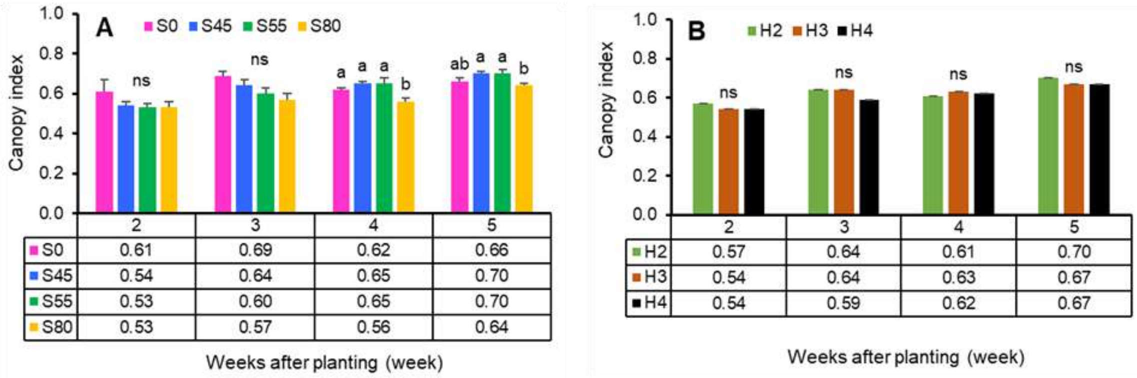

Figure 4. Canopy index on early vegetative growth on different shading and harvest period as treatment. The shading consists of no shading (S0), 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent harvest period per 2 weeks, 3 weeks, and 4 weeks, respectively. The ns mean non-significant difference at $p<0.05$.

According to this research's findings, Brazilian spinach's canopy growth was more hindered under greater shading (S80) than it was unshaded. The constituent organs of the canopy, such as the leaves and branches, endure stunted growth, which prevents the canopy from growing. According to Fadilah et al. (2022), denser shading intensity was shown to inhibit purple pakchoy leaf growth. According to the findings of Wan et al. (2020), plants planted in the shading area produce less photosynthetic performance than plants grown in full sunlight. Moreover, Liang et al. (2020) have underscored the significance of shading for plants, noting that it leads to a decrease in photosynthesis, resulting in a reduction in carbon flow. The inhibition of vegetative organ growth, particularly the canopy, in Brazilian spinach is attributed to the decreased carbon flow. This reduction in carbon flow occurs throughout the early growth cycle. The phenomenon of reduced vegetative organ development due to shading during the early growth phase has been documented in various vegetable crops, including chili (Kesumawati et al., 2020).

The SPAD value is a method for evaluating leaf nitrogen and chlorophyll content, with a positive relationship between these factors. Brazilian spinach leaf's SPAD value was affected by shading treatments, with differences observed within each shading treatment from the early growth 2 weeks after planting (WAP) (Figure 5).

Brazilian spinach grown without shading (S0) showed a higher SPAD value compared to under different shading levels, with a notable rise starting 4 weeks after

planting. This trend was also observed in S45 and S55. On the other hand, Brazilian spinach grown at S80 showed a stagnation trend, persisting until the end of the early growth, specifically 2 to 5 weeks after planting.

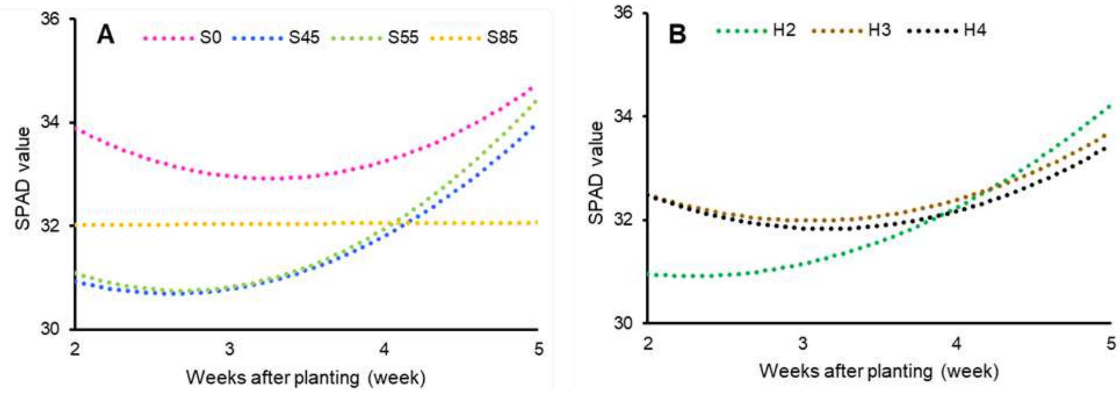

Figure 5. The SPAD value on early vegetative growth on different shading (A) and harvest period (A) treatment. The shading consists of no shading (S0), 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent harvest period per 2 weeks, 3 weeks.

The SPAD value is a widely used method for assessing leaf chlorophyll and nitrogen content, with its reliability well established. It has been found to have a positive correlation with both chlorophyll content and leaf nitrogen content (Song et al., 2021; Farnisa et al., 2023). Prior research had provided confirmation on the capacity of specific leafy vegetables, namely *Talinum paniculatum* (Lakitan et al., 2021b) and spinach (Mendoza-Tafolla et al., 2019), to proficiently evaluate and track the quantities of leaf chlorophyll and nitrogen content.

Brazilian spinach grown under full sun (S0) has a higher SPAD value than that grown under shading, indicating that shading reduces the solubility of chlorophyll and nitrogen. Wang et al. (2020a) found that shading affects the solubility of nitrogen, leading to a decrease in leaf nitrogen content. Li et al. (2020) highlighted the biochemical alterations caused by shading stress. This condition is due to Brazilian spinach, particularly in plants subjected to the 80% shading treatment (S80).

Brazilian spinach growth after harvested

The vegetative growth of Brazilian spinach after harvest was conducted at 5 weeks after planting. The growth of branch was compared under different shading conditions, harvest periods, and their interaction effects. Brazil spinach grown under S80 exhibited shorter branches as early as 11 weeks after planting. However, different shading

treatments (S0, S45, and S55) showed comparable levels of branch elongation until 9 weeks after planting. Brazilian spinach grown in S45 had an increased rate of branch elongation, particularly at 10 and 11 weeks after planting (Table 1).

The elongation of the Brazilian spinach branch was influenced by the harvesting period. Less frequent harvesting leads to the highest branch elongation, especially from 7 to 11 weeks after planting. An interaction between shading level and harvesting period treatment was observed, starting within 9 weeks after planting. This highlights the importance of harvesting frequency in influencing Brazilian spinach growth.

The study revealed a decrease in branch elongation in Brazilian spinach at S80, indicating a decrease in the allocation of photosynthetic products. This is due to reduced levels of non-structural carbohydrates, which are essential for growth (Yamashita et al., 2020). However, in the S0 treatment, photosynthesis is optimised, leading to increased branch growth.

The increased frequency of harvesting inhibits branch growth, and it is possible that the distribution of photosynthetic products changes, potentially causing a heightened initiation process of new leaves (Oliveira et al., 2021). Additionally, Raza et al. (2019) reported that maize plants with a higher number of eliminated leaves have an increased allocation of photosynthetic resources towards expanded leaves, as evidenced by an enhanced leaf area.

Table 1. Branch elongation of Brazilian spinach after harvested on different shading, harvest period, and their interaction.

Treatment	Weeks after planting (week)						
	5	6	7	8	9	10	11
				Shading			
S0	11.98 ± 0.16 a	15.89 ± 0.17 a	19.18 ± 0.43 a	22.31 ± 0.38 a	24.16 ± 0.34 a	25.22 ± 0.33 b	26.17 ± 0.53 b
S45	11.63 ± 0.33 ab	15.11 ± 0.39 ab	19.00 ± 0.55 a	22.02 ± 0.76 a	24.30 ± 0.70 a	28.23 ± 0.95 a	29.02 ± 1.20 a
S55	11.03 ± 0.17 b	14.00 ± 0.22 b	18.03 ± 0.57 a	20.74 ± 0.80 a	22.32 ± 0.85 a	25.91 ± 0.95 b	26.74 ± 1.07 ab
S80	8.68 ± 0.13 c	10.25 ± 0.17 c	12.66 ± 0.36 b	14.47 ± 0.51 b	14.93 ± 0.54 b	15.97 ± 0.76 c	16.46 ± 0.75 c
Probability	***	***	***	***	***	***	***
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
$LSD_{0.05}$	0.897	1.491	1.744	2.357	2.192	2.283	2.829
			Harvest period				
H2	11.06 ± 0.41	14.03 ± 0.69	15.80 ± 0.80 b	18.51 ± 1.09 c	19.66 ± 1.21 c	21.59 ± 1.41 c	21.90 ± 1.36 c
H3	10.72 ± 0.42	13.80 ± 0.71	18.07 ± 0.90 a	19.54 ± 1.02 b	21.75 ± 1.26 b	24.31 ± 1.58 b	24.95 ± 1.61 b
H4	10.70 ± 0.44	13.60 ± 0.66	17.78 ± 0.85 a	21.61 ± 0.98 a	22.88 ± 1.17 a	25.60 ± 1.45 a	26.94 ± 1.61 a
Probability	ns	ns	***	***	***	***	***
P-value	0.186	0.198	<0.001	<0.001	<0.001	<0.001	<0.001
$LSD_{0.05}$	0.462	0.507	0.607	0.878	0.708	0.922	0.876
			Shading x harvest period				
S0H2	12.23 ± 0.12	16.05 ± 0.34	18.55 ± 0.54	21.47 ± 0.44	23.22 ± 0.49 cd	24.24 ± 0.58 de	24.57 ± 0.62 ef
S0H3	11.93 ± 0.41	16.20 ± 0.20	20.31 ± 0.72	22.40 ± 0.85	24.85 ± 0.40 b	25.68 ± 0.39 cd	26.91 ± 0.63 cd
S0H4	11.77 ± 0.27	15.41 ± 0.14	19.08 ± 0.44	23.08 ± 0.41	24.41 ± 0.53 bc	25.74 ± 0.34 cd	27.04 ± 0.78 bcd
S45H2	11.99 ± 0.62	15.51 ± 1.10	17.45 ± 0.72	20.97 ± 1.52	22.53 ± 0.85 d	25.43 ± 1.20 cd	25.43 ± 0.91 de
S45H3	10.89 ± 0.71	14.68 ± 0.52	19.57 ± 0.59	20.87 ± 0.95	23.77 ± 0.60 bcd	28.07 ± 0.83 b	28.54 ± 0.87 bc
S45H4	12.01 ± 0.19	15.14 ± 0.41	19.99 ± 0.95	24.24 ± 0.20	26.59 ± 0.68 a	31.19 ± 0.67 b	33.08 ± 0.99 a
S55H2	11.08 ± 0.11	13.97 ± 0.22	16.00 ± 0.56	18.64 ± 0.81	19.53 ± 0.71 d	22.70 ± 0.97 e	23.19 ± 0.82 f
S55H3	11.37 ± 0.44	14.21 ± 0.67	19.19 ± 0.59	20.58 ± 1.41	23.50 ± 1.48 bcd	27.88 ± 1.25 b	28.27 ± 1.61 bc
S55H4	10.64 ± 0.03	13.81 ± 0.21	18.90 ± 0.28	23.00 ± 0.45	23.92 ± 0.49 bcd	27.15 ± 0.70 b	28.77 ± 1.02 b
S80H2	8.95 ± 0.06	10.58 ± 0.13	11.61 ± 0.27	12.97 ± 0.37	13.32 ± 0.40 h	13.98 ± 0.60 g	14.41 ± 0.53 h
S80H3	8.70 ± 0.32	10.13 ± 0.37	13.22 ± 0.70	14.33 ± 0.60	14.89 ± 0.22 g	15.62 ± 0.39 g	16.09 ± 0.29 h
S80H4	8.38 ± 0.11	10.04 ± 0.32	13.14 ± 0.38	16.11 ± 0.38	16.59 ± 0.75 f	18.32 ± 1.27f	18.87 ± 1.17 g
Probability	ns	ns	ns	ns	**	**	**
P-value	0.237	0.89	0.211	0.383	0.009	0.008	0.003
$LSD_{0.05}$	0.920	1.014	1.214	1.756	1.416	1.844	1.751

Remark: the ns mean non-significant difference at $p<0.05$.

Brazilian spinach showed significant differences in leaf growth when treated with different shading and harvesting periods. Cultivated without shade (S0), it tends to dominate leaf growth compared to cultivated under different levels of shading (S45, S55, and S80) (Table 2). However, this method also demonstrated a significant proportion of non-marketable leaves. This indicates that early leaf growth is achieved without shading, but it also leads to accelerated leaf aging and increased pest susceptibility, resulting in a higher proportion of non-marketable leaves compared to those cultivated under shading.

The frequent harvesting of Brazilian spinach leads to the initiation of young leaves, resulting in more marketable leaves. This is evident in the yield of commercially viable leaves throughout the H2 and H3 periods. However, during the H3 harvesting period, a significant proportion of non-marketable leaves are produced due to leaf aging. In contrast, extended harvesting periods (H4) often lack the capacity for leaf initiation, resulting in decreased yields of both marketable and non-marketable leaves. The interaction impact of shading and harvesting period significantly showed on leaf growth, with the most significant impact observed under 80% shade, especially during the longer harvesting period (H4).

Brazilian spinach's leaf initiation is higher in conditions without shade compared to shading conditions, affecting both marketable and non-marketable leaves. This is due to reduced carbohydrate accumulation and allocation (Hussain et al., 2020). Shading conditions inhibited plant growth, while without shade, leaf senescence accelerated due to enhanced photosynthesis. Meanwhile, direct sunlight exposure accelerates ageing processes in plants, similar to sweet basil (Castronuovo et al., 2019). However, without shade, spinach is more susceptible to pest infestation, leading to an increased prevalence of non-marketable leaves. Implementing shading at a specific density is a viable pest control strategy.

Brazilian spinach harvesting, similar to leaf and shoot pruning, has been found to increase yield. Dheeraj et al. (2022) found that pruning at the apical meristem increased growth-promoting hormones, specifically cytokinin. Xu et al. (2020a) reported that pruning tomato plants also increases cytokinin hormone levels. Cytokinin hormones influence cell division processes, including those during leaf cell development. Harvesting Brazilian spinach at H2 and H3 treatment results in elevated levels of cytokinin hormones, enhancing leaf initiation and resulting in a greater marketable yield.

Tabel 2. Brazilian spinach yield on different shading, harvest period, and their interaction.

Treatment	Marketable yield		Non-marketable yield	
	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
Shading				
S0	109.07 ± 8.55 a	13.16 ± 0.91 a	46.99 ± 2 .32 a	6.85 ± 0.41 a
S45	60.04 ± 3.57 b	5.80 ± 0.33 b	33.40 ± 2.74 b	3.44 ± 0.57 b
S55	52.63 ± 3.19 b	5.14 ± 0.37 b	32.92 ± 3.18 b	3.53 ± 0.35 b
S80	14.76 ± 1.04 c	1.22 ± 0.10 c	4.68 ± 1.00 c	0.49 ± 0.10 c
Probability	***	***	***	***
P-value	<0.001	<0.001	<0.001	<0.001
$LSD_{0.05}$	12.754	1.21	6.526	0.403
Harvest period				
H2	67.22 ± 12.80 a	6.58 ± 1.47 a	28.90 ± 5.03 b	3.57 ± 0.71 b
H3	60.95 ± 11.05 a	7.05 ± 1.56 a	33.69 ± 5.76 a	4.41 ± 0.84 a
H4	49.20 ± 7.66 b	5.37 ± 0.97 b	25.91 ± 4.06 c	2.75 ± 0.62 c
Probability	***	**	**	**
P-value	<0.001	0.001	0.008	0.001
$LSD_{0.05}$	7.391	0.793	4.546	0.793
Shading x harvest period				
S0H2	130.79 ±7.94 a	14.25 ± 0.64 a	48.17± 1.71 a	6.85 ± 0.57 ab
S0H3	117.30 ± 7.13 a	15.44 ± 0.36 a	47.58± 7.46 a	7.73 ± 0.93 a
S0H4	79.10 ± 6.74 b	9.78 ± 0.77 b	45.22± 1.88 a	5.98 ± 0.19 bc
S45H2	69.51 ± 5.90 bc	6.12 ± 0.76 c	35.36± 3.37 bc	3.88 ± 0.40 def
S45H3	51.03 ± 3.93 d	5.40 ± 0.38 c	40.14± 0.18 ab	4.97 ± 0.14 cd
S45H4	59.57 ± 4.14 cd	5.90 ± 0.66 c	24.70± 4.02 d	1.48 ± 0.74 gh
S55H2	53.05 ± 6.86 d	4.76 ± 0.80 c	28.81± 2.70cd	3.21 ± 0.36 ef
S55H3	58.36 ± 4.61cd	5.88 ± 0.59 c	44.67± 2.41 a	4.69 ± 0.48 cde
S55H4	46.47 ± 4.24d	4.78 ± 0.52 c	25.30± 1.38 d	2.69 ± 0.15 fg
S80H2	15.54 ± 1.30e	1.17 ± 0.14 d	3.27± 0.19 e	0.37 ± 0.02 h
S80H3	17.09 ± 1.32e	1.48 ± 0.13 d	2.37± 1.07 e	0.28 ± 0.18 h
S80H4	11.63 ± 1.28e	1.01 ± 0.14 d	8.41±0.52 e	0.83 ± 0.06 h
Probability	***	***	**	*
P-value	<0.001	<0.001	0.008	0.05
$LSD_{0.05}$	14.781	1.587	9.092	1.587

Remark: the ns mean non-significant difference at $p<0.05$.

The metabolism of Brazilian spinach was influenced by shading and harvesting periods. Brazilian spinach grown without shade (S0) increased metabolism activity compared to the shading areas (S45, S55, and S80). This is represented by the carbon and nitrogen levels (Table 3). Higher metabolism correlates with enhanced nitrogen usage, which is crucial for plant metabolic processes. Therefore, increasing fertilization frequency is necessary for plants exposed to sunlight. Leaf nitrogen concentration remains consistent across harvesting periods, suggesting no significant differences in nitrogen across different harvesting periods.

The carbon-nitrogen ratio calculation can be used to determine leaf hardness in Brazilian spinach leaves. The study showed that unshaded (S0) areas yield more tough leaves, decreasing with increased shading levels. Despite this, Brazilian spinach consistently showed comparable levels of leaf hardness across harvesting periods.

Shading significantly impacts the carbon reduction and nitrogen enrichment of leaves, affecting the process of photosynthesis. Light intensity and photosynthesis are linked, with studies showing a reduction in carbon and nitrogen buildup in plants exposed to shading. Tang et al. (2022) found that plants exposed to modest levels of irradiation exhibit reduced concentrations of leaf non-structural carbohydrates, leading to a reduction in carbon content. Nitrogen accumulation in shaded Brazilian spinach leaves is due to limited light availability, hindering the conversion of nitrogen into organic nitrogen compounds essential for plant metabolic processes. Gao et al. (2020) found that prolonged shading reduces nitrogen utilization efficiency in plant. Wang et al. (2020b) demonstrated that the procedure of removing leaves of plants results in an increase in non-structural carbohydrates in leaf. On the other hand, an increased frequency of harvesting triggers the growth of new leaves, leading the movement of nitrogen toward younger leaves. Jasinski et al. (2021) reported that nitrogen mobilization occurs from older to younger leaves.

Table 3. Carbon, Nitrogen and C-N ratio of Brazilian spinach leaf on different shading, harvest period, and their interaction.

Treatment	Carbon (%)	Nitrogen (%)	C-N ratio
Shading			
S0	34.64	2.83	12.28
S45	32.75	4.56	7.20
S55	34.21	4.77	7.19
S80	34.32	4.99	6.84
Harvest period			
H2	35.85	4.38	8.74
H3	33.90	4.42	8.10
H4	32.20	4.07	8.30
Shading x harvest periode			
S0H2	34.23	2.63	13.00
S0H3	34.28	2.90	11.83
S0H4	35.42	2.95	12.01
S45H2	32.02	4.70	6.81
S45H3	33.89	4.77	7.10
S45H4	32.34	4.20	7.70
S55H2	36.50	5.01	7.29
S55H3	32.14	4.94	6.50
S55H4	34.00	4.36	7.79
S80H2	40.66	5.16	7.88
S80H3	35.30	5.07	6.96
S80H4	27.01	4.76	5.68

Remark: the ns mean non-significant difference at $p<0.05$.

The presence of shading in Brazilian spinach is linked to biomass production. Under unshaded conditions, it enhances photosynthesis, leading to increased biomass production. However, under intense shading conditions, it reduces biomass in various parts of the plant. Harvesting over extended periods (H3 and H4) results in increased biomass accumulation, particularly in the stem and branch in the final observation.

Brazilian spinach, when grown under shading conditions and extended harvesting, showed inhibited growth due to restricted photosynthetic activity. This caused the restricted allocation of photosynthetic products to individual plant organs. Studies have shown that shading reduces biomass accumulation and alterations in plant morphological traits (Xu et al., 2020b). Additionally, there is a distribution of photosynthetic activity that utilizes plant organs beyond just leaves. This aligns with Yu et al. (2019) finding that when plants age and their organs undergo senescence, photosynthetic flux redirects towards the stem. This highlights the importance of considering the allocation of photosynthetic products to plant growth through periodic harvesting.

Table 4. Dry weight of Brazilian spinach organs on different shading, harvest period, and their interaction at 13 weeks after planting (WAP).

Treatment	Stem dry weight (g)	Branch dry weight (g)	Leaf dry weight (g)	Root dry weight (g)
		Shading		
S0	2.35 ± 0.19 a	14.24 ± 0.98 a	7.92 ± 0.88 a	5.28 ± 1.20 a
S45	1.36 ± 0.20 b	5.39 ± 0.98 b	3.70 ± 0.79 b	2.07 ± 0.81 ab
S55	1.26 ± 0.13 b	5.21 ± 0.79 b	4.58 ± 0.69 b	1.13 ± 0.19 b
S80	0.25 ± 0.04 c	0.40 ± 0.05 c	0.85 ± 0.62 c	0.44 ± 0.20 b
Probability	***	***	***	*
P-value	< 0.001	< 0.001	< 0.001	0.046
LSD$_{0.05}$	0.479	1.755	2.037	3.325
		Harvest period		
H2	1.05 ± 0.27 b	4.39 ± 1.28 c	3.63 ± 0.68 b	1.73 ± 0.48 a
H3	1.25 ± 0.20 b	6.12 ± 1.49 b	2.88 ± 0.65 b	2.19 ± 0.86 a
H4	1.62 ± 0.27 a	8.42 ± 1.90 a	6.28 ± 1.22 a	2.77 ± 1.05 a
Probability	**	***	***	ns
P-value	0.002	< 0.001	< 0.001	0.517
LSD$_{0.05}$	0.286	1.228	1.117	1.872
		Shading x harvest period		
S0H2	2.35 ± 0.53 ab	11.26 ± 0.97 c	6.43 ± 0.44 b	4.27 ± 0.49 a
S0H3	2.03 ± 0.04 bc	13.92 ± 0.61 b	5.98 ± 0.36 b	4.20 ± 2.25 ab
S0H4	2.66 ± 0.25 a	17.54 ± 0.62 a	11.36 ± 0.28 a	7.36 ± 2.97 ab
S45H2	0.78 ± 0.13 fg	3.26 ± 0.70 fg	2.49 ± 0.43 cd	0.88 ± 0.26 bc
S45H3	1.41 ± 0.06 de	4.63 ± 0.33 fg	2.34 ± 0.22 cd	3.30 ± 2.41 bc
S45H4	1.89 ± 0.38 bcd	8.29 ± 2.05 d	6.28 ± 1.52 b	2.04 ± 0.76 bc
S55H2	0.87 ± 0.12 ef	2.80 ± 0.67 gh	3.59 ± 0.81 c	1.06 ± 0.37 bc
S55H3	1.35 ± 0.17 de	5.51 ± 0.62 ef	3.18 ± 0.34 c	1.00 ± 0.09 bc
S55H4	1. 57 ± 0.14 cd	7.32 ± 1.23 de	6.99 ± 0.77 b	1.32 ± 0.52 bc
S80H2	0.19 ± 0.01 h	0.24 ± 0.04 i	2.00 ± 1.84 cde	0.73 ± 0.64 bc
S80H3	0.22 ± 0.03 gh	0.41 ± 0.05 hi	0.03 ± 0.03 e	0.26 ± 0.16 c
S80H4	0.35 ± 0.09 fgh	0.53 ± 0.11 hi	0.50 ± 0.33 de	0.34 ± 0.05 c
Probability	ns	*	*	ns
P-value	0.134	0.049	0.013	0.584
LSD$_{0.05}$	0.572	2.457	2.234	3.744

Remark: the ns mean non-significant difference at $p<0.05$.

Visual appearance of Brazilian spinach on different treatment

The study analysed the shoot appearance of Brazilian spinach under different shading conditions and harvesting periods. Unshaded areas had a denser appearance, while based on the harvesting period, treatments tend to show similarities with each other (Figure 6). Furthermore, Brazilian spinach grown unshaded (S0) showed greater root growth and a higher density of root hairs than other shading, while samples subjected to varying harvesting periods (H2, H3, and H4) showed similar root morphology without any significant differences (Figure 7).

Brazilian spinach showed varying morphological traits under different treatments. Shading causes alterations in plant organs, as shown on soybean stems, which experience inhibited growth (Castronuovo et al., 2019). Cao et al. (2022) reported that *Cynodon dactylon* shoot organs also experience alterations. Root development also shows a distinct reaction to shading, with a decline in root growth under shading stress. Fu et al. (2020) found reductions in root volume and length, indicating a decline in root growth under these conditions.

Brazilian spinach with a longer harvesting period (H4) showed a rise in branches and stems, with a higher presence of mature leaves. Pruning at longer intervals increased plant height and branches in *Talinum Paniculatum*, while extending harvesting intervals hindered fresh leaf commencement (Purbajanti et al., 2019). Bessonova et al. (2023) found that removing leaves and branches from plants led to the development of shoot features with a greater number and area of leaves.

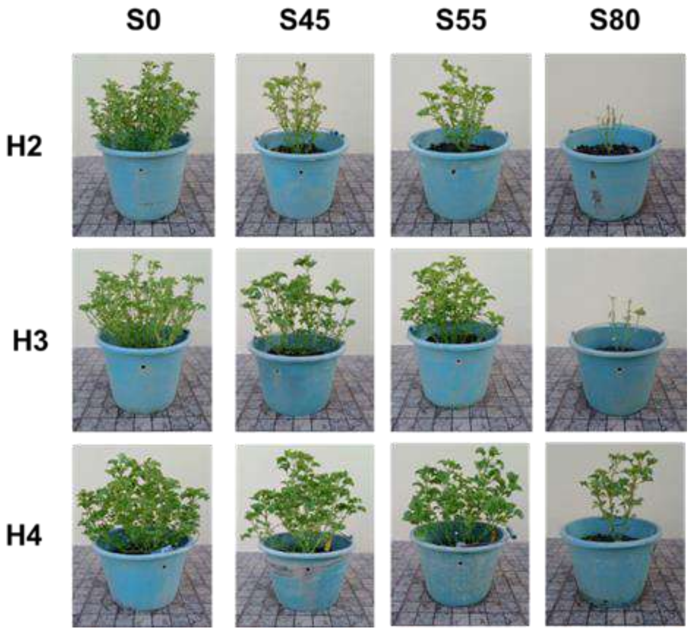

Figure 6. Visualization of Brazilian spinach shoot on different shading and harvest period at 13 weeks after planting (WAP). Shading treatment consist of S0: no-shading, S45: 45% shading, S55: 55% shading, and S80: 80% shading. Meanwhile, the harvest period consists of H2: per 2 weeks, H3: per 3 weeks, and H4: per 4 weeks. Photos: Strayker Ali Muda.

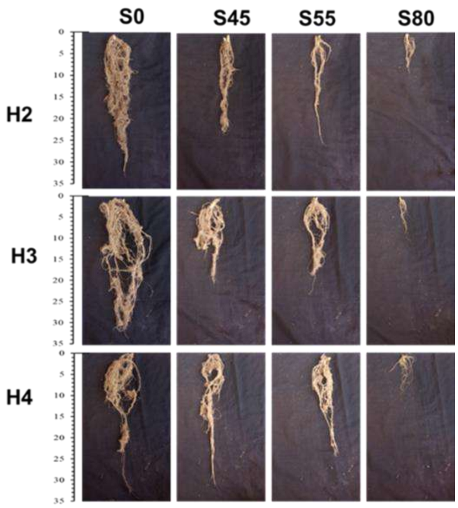

Figure 7. Visualization of Brazilian spinach root on different shading and harvest period at 13 weeks after planting (WAP). Shading treatment consist of S0: no-shading, S45: 45% shading, S55: 55% shading, and S80: 80% shading. Meanwhile, the harvest period consists of H2: per 2 weeks, H3: per 3 weeks, and H4: per 4 weeks. Photos: Strayker Ali Muda.

Water status on different treatment

The water availability for Brazilian spinach growth was represented by substrate moisture. Increased shading intensity (S80) leads to higher moisture content, reducing direct sunlight exposure and reducing evaporation, resulting in reduced water loss. Conversely, Brazilian spinach grown in areas with lower shading or without shading showed higher evaporation rates, indicating more water loss, as shown by substrate moisture levels (Figure 8). The use of shading can effectively adjust microclimate conditions, such as substrate moisture levels (Bollman et al., 2021). The study found that shaded growing media had higher humidity levels than unshaded media, and the addition of shading reduced evaporation rate, as confirmed by Khawam et al. (2019).

The more frequently Brazil spinach is harvested, the wider the substrate surface is not covered by the canopy, causing higher evaporation rates and reduced water availability. This phenomenon aligns with the findings of Huang et al. (2020), who provided empirical evidence that plants with lower canopy density exhibit higher rates of water loss via evaporation.

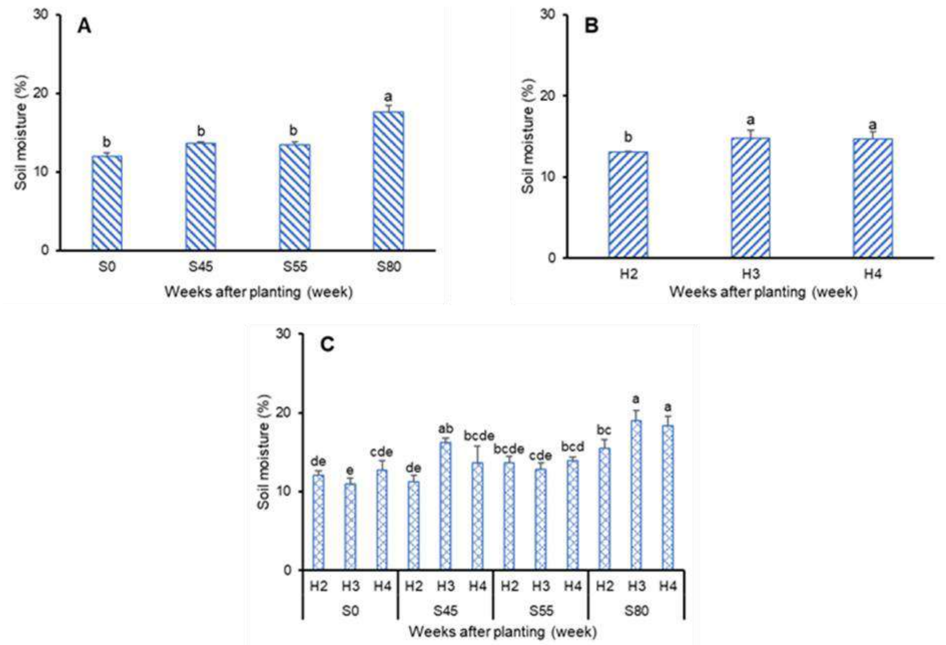

Figure 8. Substrate moisture on different shading (A), harvest period (B), and their interaction (C). Shading treatment consist of S0: no-shading, S45: shading 45%, S55: shading 55%, and S80: shading 80%. Meanwhile, the harvest period consists of H2: every 2 weeks, H3: every 3 weeks, and H4: every 4 weeks.

CONCLUSION

The adoption of shading led to a decrease in the growth and yield of Brazilian spinach through an alteration in the morphological traits of its root, stem, branch, and leaf. In addition, the implementation of a 2-week harvesting period led to increased Brazilian spinach growth and yield. Interactions between shading and harvest periods primarily pertain to the length of branches, yields (both marketable and non-marketable), dry weight of organs (namely branch and leaf), and substrate moisture.

ACKNOWLEDGMENT

We are very grateful for the suggestions, input and corrections from anonymous reviewers and the editors of the *Revista de Agricultura Neotropical* journal.

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Revised version

BRAZILIAN SPINACH GROWTH UNDER DIFFERENT SHADING INTENSITIES AND HARVESTING PERIODS IN LOWLAND TROPICAL URBAN ECOSYSTEM

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ABSTRACT

Brazilian spinach, a lesser-known perennial leafy vegetable, growing in the tropical ecosystem. The study was conducted to evaluate the growth of Brazilian spinach in tropical lowland urban ecosystem under different levels of shading intensities and harvesting periods. The research used a split-plot design, with different levels of shading intensities (no-shading, shading 45%, shading 55%, and shading 80%) as the main plot and harvesting periods (per 2 weeks, per 3 weeks, and per 4 weeks) as the sub-plot. The results showed that Brazilian spinach growth was more favourable when exposed to treatment without shading compared to shading conditions. Shading treatment, especially at shading 80%, had a negative impact on plant growth was observed during the early stages of growth, as indicated by alterations in canopy parameters (canopy area (26,47 cm2), canopy diameter (7.98 cm), and canopy index (0.52)) and SPAD values trend. Shading 80% has reduced branch elongation, yield (marketable (14.76 g) and non-marketable (4.68)), stem dry weight (0.25 g), branch dry weight (0.40 g), leaf dry weight (0.85 g), and root dry weight (0.44 g). Conversely, Brazilian spinach grown on no-shading increased the carbon content (34.64 %) and reduced nitrogen content (2.83 %) of marketable leaves. More frequent harvesting (per 2 weeks) increased in marketable yield (67.22 g), but suppressed the growth of stem (1.05 g), branches (4.39 g), and root (1.73). Therefore, it is recommended to cultivate Brazilian spinach in an unshaded area with a biweekly harvesting routine.

KEYWORDS

Harvest time, leafy green, lesser-known vegetable, plant acclimatization, solar irradiation intensity.

INTRODUCTION

Brazilian spinach (*Alternanthera sissoo*) is a leafy vegetable originating from Brazil. As reported by Ikram et al. (2022), Brazilian spinach is rich in flavonoids, vitamins, minerals, and other antioxidants, which have been found to have positive effects on human health. The cultivation and use of this particular plant by the Indonesian population are infrequent, leading to its classification as a rather rare plant species. Indonesia's ecosystem exhibits similarities to its indigenous location, hence indicating the potential for cultivating this plant within the country.

Urban cultivation faces several challenges, especially in regard to the availability of light for plants. Shaded areas in urban environments tend to prevail, impeding the penetration of light into plant development. Consequently, the amount of light received by plants decreases, leading to disruptions in certain aspects of plant metabolism. This phenomenon is particularly observed in horticultural crops characterized by compact growth, such as Brazilian spinach. Based on Shafiq et al. (2021), regulating alterations occur in plants as a means to enhance the efficiency of photosynthesis. The tolerance of plants to the intensity of light they receive varies depending on the specific plant species. Certain vegetable crops have been reported as being capable of growing under shaded conditions. In this regard, Sifuentes-Pallaoro et al. (2020) revealed that *Lactuca canadensis* exhibits favourable growth under shading, while Lakitan et al. (2021a) found that celery also demonstrates similar adaptability. Furthermore, Gomes et al. (2023) reported that in the Brazilian ecosystem cultivated plants will grow well at full or at less 70% light intensity.

Brazilian spinach is a perennial leafy vegetable, enabling its continuous growth throughout the year. Additionally, this suggests that regular harvesting is required. Annual plants undergo periodic harvesting that involves a defoliation mechanism. According to the findings of Raza et al. (2019), the implementation of a defoliation treatment on plants has been observed to enhance overall plant growth, particularly in terms of leaf growth, especially during the vegetative phase. Further experimentation is required to enhance the output of Brazilian spinach, a plant species characterised by its commercially valuable leaf organs, which are described as young and acceptable damage by pests and diseases.

The cultivation of Brazilian spinach is characterized by its simplicity, since it may be easily grown. Muda et al. (2022) reported that the propagation of Brazilian spinach can be achieved via stem cuttings. There is an insufficient amount of research pertaining to the adaptability of Brazilian spinach to shading environments. The capacity of Brazilian spinach to acclimatise to shading environments for a specific duration will ensure the availability of sustainable vegetable nutrition. The study was aimed to evaluating the adaptability of Brazilian spinach to shading conditions via various harvesting periods.

MATERIALS AND METHODS

Research site and agroclimatic characteristic

The research was carried out in the Jakabaring research facility located in Palembang (104º46'44"E, 3º01'35"S), South Sumatra, Indonesia. This research began with initiating the propagation of stem cuttings on 30 January 2023, and concluded the data collection on 02 May 2023. The research site is situated in a tropical urban lowland area with an elevation of 8 meters above sea level (masl). The agroclimatic characteristics of the area are shown in Figure 1.

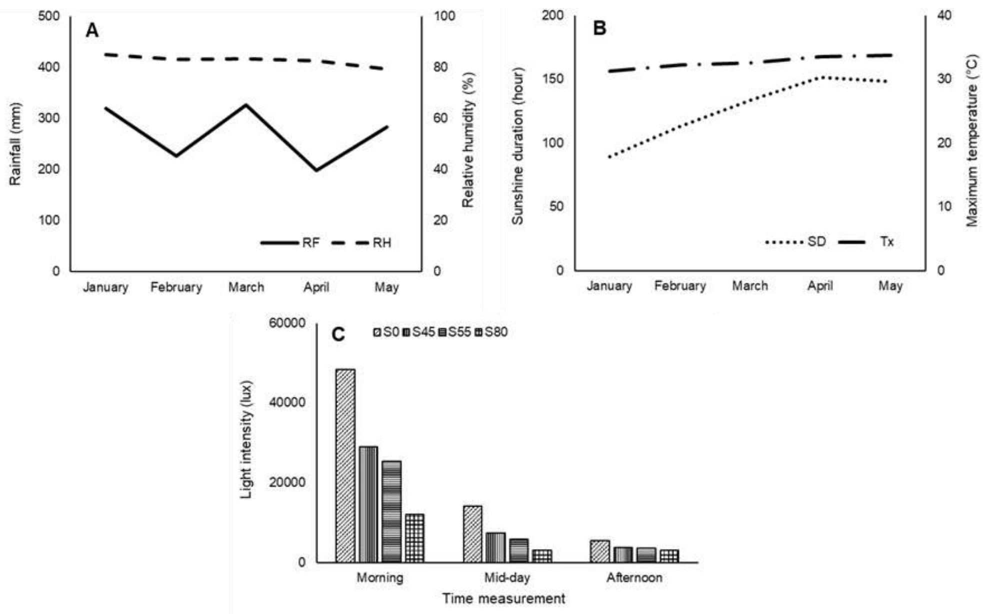

Figure 1. Agroclimatic characteristics in research location as indicated by total monthly rainfall (RF) and average humidity (RH) (A), sunshine duration (SD) and average

maximum temperature (Tx) (B), and average light intensity of each treatment (C). (Source: Indonesian Agency for Meteorology, Climatology and Geophysics). S0= no-shading; S45= shading 45%; S55= shading 55%; S80= shading 80%.

Cultivation and treatment procedures

The propagation material used was stem cuttings with two leaves that were taken from healthy mother plant. Mother plant used as a source for stem cuttings was a 3-month-old plant and in healthy condition. The planting materials were planted in pots with dimensions of 27.5 cm in diameter and 20 cm in height. The pots were filled with a growing medium that was a mixture of top soil and chicken manure (3:1 v/v). Prior to planting, the growing medium had a bio-sterilization treatment (2 g/l), with the addition of live microorganisms including *Streptomyces thermovulgaris*, *Thricoderma virens*, and *Geobacillus thermocatenulatus*. The growing medium was subsequently subjected to a one-week incubation period before planting. The bio-sterilization application aims to prevent the pathogen infestation from substrate to cultivated plants.

The growing medium that had been incubated was used for the planting of Brazilian spinach cuttings, which were subsequently arranged in accordance with the principles of a split-plot design. The main plot of the study focused on the intensity of shading, whereas the subplot examined the harvest period. The treatment involved selecting shading intensity at three separate levels, namely no-shading (S0), 45% shading (S45), 55% shading (S55), and 85% shading (S80). Furthermore, treatment for the harvest period started after the simultaneous harvesting, which was carried out at 5 weeks after planting (WAP). The designated harvest period has three different intervals, namely appearing per 2 weeks, per 3 weeks, and per 4 weeks, symbolised as I2, I3, and I4, respectively. The first harvest was carried out at five week after planting (WAP).

The plants were systematically positioned within shadow houses measuring 4 meters in length, 2 meters in width, and 2 meters in height. These shadow houses are constructed using knockdown frames made of 1.5-inch PVC pipes. The entire perimeter of the shadow house is enveloped with a shade material, specifically a black polyethylene net, which has been tested for its density to ensure optimal shading.

The leaves of the Brazilian spinach cuttings get trimmed when it reaches one week after planting (WAP) in order to maintain uniformity in the planting material. Meanwhile, fertilization was applied using compound NPK fertilizers (16:16:16) at 1 and 5 WAP at a dose of 3 g/plant. The watering of each plant was normally carried out every day around 08:00 a.m and 05:00 p.m.

Data collection

The data collection covered Brazilian spinach growth and yield data. The growth data that was obtained is categorised into two categories of measurements, such as non-destructive and destructive. The dataset for non-destructive growth measurement includes several variables, including SPAD values, canopy width, canopy diameter, canopy index, branch length, and stem diameter. In addition, the destructive measurements included the stem fresh weight, branch fresh weight, root fresh weight, stem dry weight, branch dry weight, and root dry weight. Destructive observation was carried out at 13 weeks after planting. The collected data concerning Brazilian spinach yield covers several parameters, including the fresh weight of marketable leaf, fresh weight of non-marketable leaf, dry weight of marketable leaf, dry weight of non-marketable leaf, the carbon content of marketable leaf, nitrogen content of marketable leaf, and the C:N ratio of marketable leaf. Marketable leaf is young, healthy, and easily-breakable. Meanwhile, non-marketable leaf is aged, damaged by pests and/or diseases, and high fiber content. The moisture content of the planting medium was also examined in order to determine the water content of the substrate.

The SPAD value was monitored using chlorophyll meters (SPAD-502 Plus, Konica-Minolta Optics, Inc., Osaka, Japan). Canopy area was measured using a digital image scanner for Android (Easy Leaf Area software, developed by Easlon & Bloom 2014). Canopy diameter was measured using a measuring tape on the widest side of the canopy. Canopy index was the ratio of the measured canopy area to circular area with widest diameter. Meanwhile, substrate moisture (SM) was measured using a soil moisture meter (PMS-714, Lutron Electronics Canada, Inc., Pennsylvania, USA). Light intensity was measured using a lux meter (GM1030, Benetech, Inc., Illinois, USA). Carbon content was analysed using the furnace method, while nitrogen content was analysed using the Kjedahl-Titrimetry method.

The dry weight of each plant organ was determined by treating it to a drying process in an oven set at a temperature of 100°C for a duration of 24 hours. Prior to being placed in the oven, the plant's organs are trimmed to a reduced thickness to accelerate the drying process.

Data analysis

The effect of shading intensities and harvest periods were revealed by analysis of variance (ANOVA). Significant differences among treatments were tested using the Tukey's honestly significant difference (HSD) procedure at P<0.05. All data was analysed using the RStudio software version 1.14.1717 for Windows (developed by RStudio team, PBC, Boston, MA).

RESULTS AND DISCUSSION

The Brazilian spinach growth during early vegetative growth before harvested

The early vegetative growth of Brazilian spinach is analysed by considering its unique characteristics, such as canopy growth and SPAD value. This approach involves non-destructive observation, allowing plants to grow naturally. The canopy characteristics selected were: canopy area, canopy diameter, and canopy index.

Brazilian spinach grown in unshaded conditions (S0) had a higher leaf initiation and larger individual leaf area compared to in shading conditions. More and larger leaves contribute to the increase in canopy area. This leads to a broader canopy compared to those grown under shade. The Brazilian spinach canopy area growth increased significantly in the S0 condition, particularly 2 to 5 weeks after planting, compared to shade conditions (S45, S55, and S80). However, no significant leaf growth was observed in the S45 and S55 shades. The shading with the highest density (S80) showed suppressed growth, starting 2 weeks after planting (Figure 2).

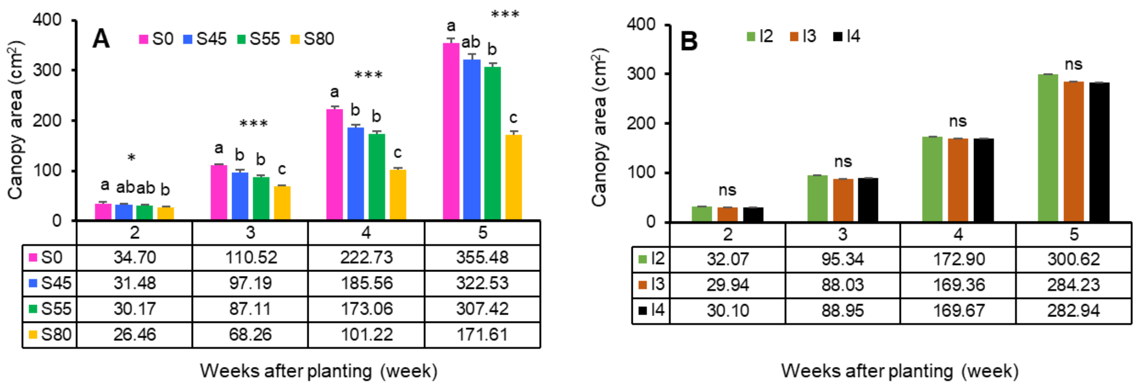

Figure 2. Brazilian spinach canopy area on early vegetative growth on different shading (A) and harvest period (B) treatment. The shading consists of no-shading (S0), 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, I2, I3, and I4 represent harvest period per 2 weeks, 3 weeks, and 4 weeks, respectively. The ns= non-

significant difference at P<0.05; *= significant difference at P<0.05; ***= significant difference at P<0.001.

Brazilian spinach branches significantly influence canopy diameter, with elongation affecting canopy expansion. Shading treatment inhibits branch growth. Full sunlight cultivation leads to a wider canopy than canopies grown under different levels of shading (S45, S55, and S80) (Figure 3).

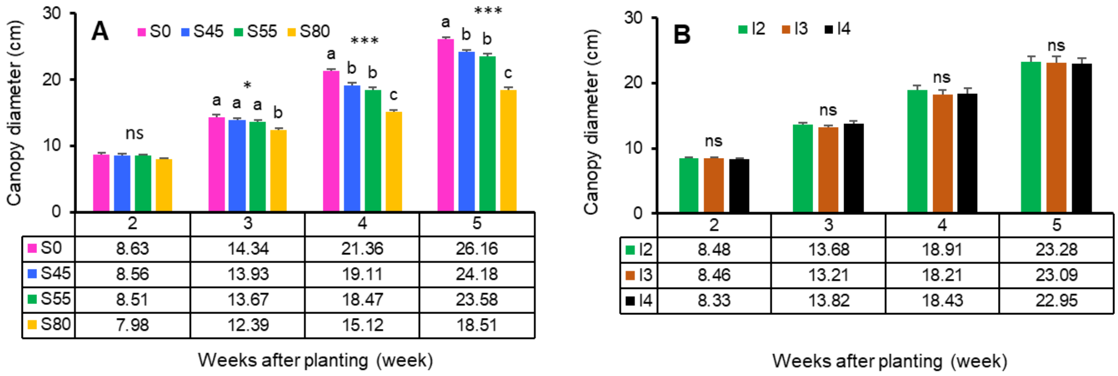

Figure 3. Brazilian spinach canopy diameter on early vegetative growth on different shading (A) and harvest period (B) treatment. The shading consists of no-shading (S0), 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, I2, I3, and I4 represent harvest period per 2 weeks, 3 weeks, and 4 weeks, respectively. The ns= non-significant difference at P<0.05; *= significant difference at P<0.05; ***= significant difference at P<0.001.

The growth of leaf and branch significantly affects canopy density, with dominant growth resulting in a denser canopy as represented by canopy density. The effect is most noticeable between 4 and 5 weeks after planting. Brazilian spinach grown under shading conditions, especially S80, showed reduced leaf size and branch elongation, resulting in lower canopy density (Figure 4).

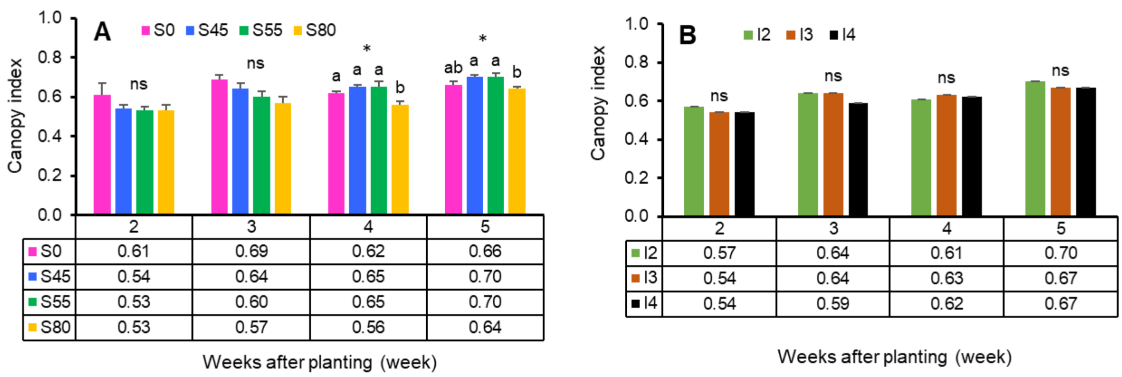

Figure 4. Canopy index on early vegetative growth on different shading and harvest period as treatment. The shading consists of no-shading (S0), 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, I2, I3, and I4 represent harvest period per 2 weeks, 3 weeks, and 4 weeks, respectively. The ns= non-significant difference at $P<0.05$; *= significant difference at $P<0.05$.

According to this research's findings, Brazilian spinach's canopy growth was more hindered under greater shading (S80) than it was unshaded. The constituent organs of the canopy, such as the leaves and branches, endure stunted growth, which prevents the canopy from growing. According to Fadilah et al. (2022), denser shading intensity was shown to inhibit purple pakchoy leaf growth. According to the findings of Wan et al. (2020), plants planted in the shading area produce less photosynthetic performance than plants grown in full sunlight. Moreover, Liang et al. (2020) have underscored the significance of shading for plants, noting that it leads to a decrease in photosynthesis, resulting in a reduction in carbon flow. The inhibition of vegetative organ growth, particularly the canopy, in Brazilian spinach is attributed to the decreased carbon flow. This reduction in carbon flow occurs throughout the early growth cycle. The phenomenon of reduced vegetative organ development due to shading during the early growth phase has been documented in various vegetable crops, including chili (Kesumawati et al., 2020).

The SPAD value is a method for evaluating leaf nitrogen and chlorophyll content, with a positive relationship between these factors. Brazilian spinach leaf's SPAD value was affected by shading treatments, with differences observed within each shading treatment from the early growth 2 weeks after planting (WAP). The Brazilian spinach's leaf grown no-shading (S0) showed a higher SPAD value compared to under different shading levels, with a notable rise starting 4 weeks after planting. This trend was also observed in S45 and S55. On the other hand, Brazilian spinach grown at S80 showed a stagnation trend, persisting until the end of the early growth, specifically 2 to 5 weeks after planting (Figure 5).

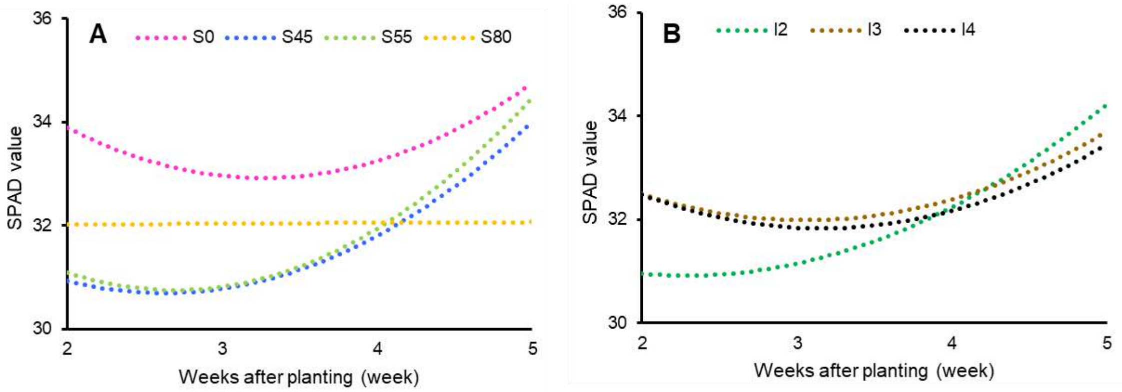

Figure 5. The Brazilian spinach's leaf SPAD value on early vegetative growth on different shading (A) and harvest period (A) treatment. The shading consists of no-shading (S0), 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, I2, I3, and I4 represent harvest period per 2 weeks, 3 weeks.

The SPAD value is a widely used method for assessing leaf chlorophyll and nitrogen content, with its reliability well established. It has been found to have a positive correlation with both chlorophyll content and leaf nitrogen content (Song et al., 2021; Farnisa et al., 2023). Prior research had provided confirmation on the capacity of specific leafy vegetables, namely *Talinum paniculatum* (Lakitan et al., 2021b) and spinach (Mendoza-Tafolla et al., 2019), to proficiently evaluate and track the quantities of leaf chlorophyll and nitrogen content.

Brazilian spinach grown under no-shading (S0) has a higher SPAD value than that grown under shading, indicating that shading reduces the solubility of chlorophyll and nitrogen. Wang et al. (2020a) found that shading affects the solubility of nitrogen, leading to a decrease in leaf nitrogen content. Li et al. (2020) highlighted the biochemical alterations caused by shading stress. This condition is due to Brazilian spinach, particularly in plants subjected to the 80% shading treatment (S80).

Brazilian spinach growth after harvested

The vegetative growth of Brazilian spinach after harvest was conducted at 5 weeks after planting. The growth of branch was compared under different shading conditions, harvest periods, and their interaction effects. Brazil spinach grown under S80 exhibited shorter branches as early as 11 weeks after planting. However, different shading treatments (S0, S45, and S55) showed comparable levels of branch elongation until 9 weeks after planting. Brazilian spinach grown in S45 had an increased rate of branch elongation, particularly at 10 and 11 weeks after planting (Table 1).

The elongation of the Brazilian spinach branch was influenced by the harvesting period. Less frequent harvesting leads to the highest branch elongation, especially from 7 to 11 weeks after planting. An interaction between shading level and harvesting period treatment was observed, starting within 9 weeks after planting. This highlights the importance of harvesting frequency in influencing Brazilian spinach growth.

The study revealed a decrease in branch elongation in Brazilian spinach at S80, indicating a decrease in the allocation of photosynthetic products. This is due to reduced levels of non-structural carbohydrates, which are essential for growth (Yamashita et al., 2020). However, in the S0 treatment, photosynthesis is optimised, leading to increased branch growth.

The increased frequency of harvesting inhibits branch growth, and it is possible that the distribution of photosynthetic products changes, potentially causing a heightened initiation process of new leaves (Oliveira et al., 2021). Additionally, Raza et al. (2019) reported that maize plants with a higher number of eliminated leaves have an increased allocation of photosynthetic resources towards expanded leaves, as evidenced by an enhanced leaf area.

Table 1. Branch elongation of Brazilian spinach after harvested on different shading, harvest period, and their interaction.

Treatment	Weeks after planting (week)						
	5	6	7	8	9	10	11
Shading							
S0	11.98 ± 0.16 a	15.89 ± 0.17 a	19.18 ± 0.43 a	22.31 ± 0.38 a	24.16 ± 0.34 a	25.22 ± 0.33 b	26.17 ± 0.53 a
S45	11.63 ± 0.33 ab	15.11 ± 0.39 ab	19.00 ± 0.55 a	22.02 ± 0.76 a	24.30 ± 0.70 a	28.23 ± 0.95 a	29.02 ± 1.20 a
S55	11.03 ± 0.17 b	14.00 ± 0.22 b	18.03 ± 0.57 a	20.74 ± 0.80 a	22.32 ± 0.85 a	25.91 ± 0.95 b	26.74 ± 1.07 a
S80	8.68 ± 0.13 c	10.25 ± 0.17 c	12.66 ± 0.36 b	14.47 ± 0.51 b	14.93 ± 0.54 b	15.97 ± 0.76 c	16.46 ± 0.75 b
Probability	***	***	***	***	***	***	***
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Harvest period							
I2	11.06 ± 0.41	14.03 ± 0.69	15.80 ± 0.80 b	18.51 ± 1.09 c	19.66 ± 1.21 c	21.59 ± 1.41 c	21.90 ± 1.36 c
I3	10.72 ± 0.42	13.80 ± 0.71	18.07 ± 0.90 a	19.54 ± 1.02 b	21.75 ± 1.26 b	24.31 ± 1.58 b	24.95 ± 1.61 b
I4	10.70 ± 0.44	13.60 ± 0.66	17.78 ± 0.85 a	21.61 ± 0.98 a	22.88 ± 1.17 a	25.60 ± 1.45 a	26.94 ± 1.61 a
Probability	ns	ns	***	***	***	***	***
P-value	0.186	0.198	<0.001	<0.001	<0.001	<0.001	<0.001
Shading x harvest period							
S0I2	12.23 ± 0.12	16.05 ± 0.34	18.55 ± 0.54	21.47 ± 0.44	23.22 ± 0.49 cd	24.24 ± 0.58 de	24.57 ± 0.62 de
S0I3	11.93 ± 0.41	16.20 ± 0.20	20.31 ± 0.72	22.40 ± 0.85	24.85 ± 0.40 b	25.68 ± 0.39 cd	26.91 ± 0.63 bc
S0I4	11.77 ± 0.27	15.41 ± 0.14	19.08 ± 0.44	23.08 ± 0.41	24.41 ± 0.53 bc	25.74 ± 0.34 cd	27.04 ± 0.78 bc
S45I2	11.99 ± 0.62	15.51 ± 1.10	17.45 ± 0.72	20.97 ± 1.52	22.53 ± 0.85 d	25.43 ± 1.20 cd	25.43 ± 0.91 cd
S45I3	10.89 ± 0.71	14.68 ± 0.52	19.57 ± 0.59	20.87 ± 0.95	23.77 ± 0.60 bcd	28.07 ± 0.83 b	28.54 ± 0.87 b
S45I4	12.01 ± 0.19	15.14 ± 0.41	19.99 ± 0.95	24.24 ± 0.20	26.59 ± 0.68 a	31.19 ± 0.67 b	33.08 ± 0.99 a
S55I2	11.08 ± 0.11	13.97 ± 0.22	16.00 ± 0.56	18.64 ± 0.81	19.53 ± 0.71 d	22.70 ± 0.97 e	23.19 ± 0.82 e
S55I3	11.37 ± 0.44	14.21 ± 0.67	19.19 ± 0.59	20.58 ± 1.41	23.50 ± 1.48 bcd	27.88 ± 1.25 b	28.27 ± 1.61 b
S55I4	10.64 ± 0.03	13.81 ± 0.21	18.90 ± 0.28	23.00 ± 0.45	23.92 ± 0.49 bcd	27.15 ± 0.70 b	28.77 ± 1.02 b
S80I2	8.95 ± 0.06	10.58 ± 0.13	11.61 ± 0.27	12.97 ± 0.37	13.32 ± 0.40 h	13.98 ± 0.60 g	14.41 ± 0.53 g
S80I3	8.70 ± 0.32	10.13 ± 0.37	13.22 ± 0.70	14.33 ± 0.60	14.89 ± 0.22 g	15.62 ± 0.39 g	16.09 ± 0.29 g
S80I4	8.38 ± 0.11	10.04 ± 0.32	13.14 ± 0.38	16.11 ± 0.38	16.59 ± 0.75 f	18.32 ± 1.27f	18.87 ± 1.17 f
Probability	ns	ns	ns	ns	**	**	**
P-value	0.237	0.89	0.211	0.383	0.009	0.008	0.003

Remark: The ns= non-significant difference at P<0.05; **= significant difference at P<0.01; ***= significant difference at P<0.001.

Brazilian spinach showed significant differences in leaf growth when treated with different shading and harvesting periods. Cultivated no-shading (S0), it tends to dominate leaf growth compared to cultivated under different levels of shading (S45, S55, and S80) (Table 2). However, this method also demonstrated a significant proportion of non-marketable leaves. This indicates that early leaf growth is achieved without shading, but it also leads to accelerated leaf aging and increased pest susceptibility, resulting in a higher proportion of non-marketable leaves compared to those cultivated under shading.

The frequent harvesting of Brazilian spinach leads to the initiation of young leaves, resulting in more marketable leaves. This is evident in the yield of commercially viable leaves throughout the I2 and I3 periods. However, during the I3 harvesting period, a significant proportion of non-marketable leaves are produced due to leaf aging. In contrast, extended harvesting periods (I4) often lack the capacity for leaf initiation, resulting in decreased yields of both marketable and non-marketable leaves. The interaction impact of shading and harvesting period significantly showed on leaf growth, with the most significant impact observed under 80% shade, especially during the longer harvesting period (I4).

Brazilian spinach's leaf initiation is higher in conditions without shade compared to shading conditions, affecting both marketable and non-marketable leaves. This is due to reduced carbohydrate accumulation and allocation (Hussain et al., 2020). Shading conditions inhibited plant growth, while without shade, leaf senescence accelerated due to enhanced photosynthesis. Meanwhile, direct sunlight exposure accelerates ageing processes in plants, similar to sweet basil (Castronuovo et al., 2019). However, without shade, spinach is more susceptible to pest infestation, leading to an increased prevalence of non-marketable leaves. Implementing shading at a specific density is a viable pest control strategy.

Brazilian spinach harvesting, similar to leaf and shoot pruning, has been found to increase yield. Dheeraj et al. (2022) found that pruning at the apical meristem increased growth-promoting hormones, specifically cytokinin. Xu et al. (2020a) reported that pruning tomato plants also increases cytokinin hormone levels. Cytokinin hormones influence cell division processes, including those during leaf cell development. Harvesting Brazilian spinach at I2 and I3 treatment results in elevated levels of cytokinin hormones, enhancing leaf initiation and resulting in a greater marketable yield.

Tabel 2. Brazilian spinach yield on different shading, harvest period, and their interaction.

Treatment	Marketable yield		Non-marketable yield	
	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
		Shading		
S0	109.07 ± 8.55 a	13.16 ± 0.91 a	46.99 ± 2 .32 a	6.85 ± 0.41 a
S45	60.04 ± 3.57 b	5.80 ± 0.33 b	33.40 ± 2.74 b	3.44 ± 0.57 b
S55	52.63 ± 3.19 b	5.14 ± 0.37 b	32.92 ± 3.18 b	3.53 ± 0.35 b
S80	14.76 ± 1.04 c	1.22 ± 0.10 c	4.68 ± 1.00 c	0.49 ± 0.10 c
Probability	***	***	***	***
P-value	<0.001	<0.001	<0.001	<0.001
		Harvest period		
I2	67.22 ± 12.80 a	6.58 ± 1.47 a	28.90 ± 5.03 b	3.57 ± 0.71 b
I3	60.95 ± 11.05 a	7.05 ± 1.56 a	33.69 ± 5.76 a	4.41 ± 0.84 a
I4	49.20 ± 7.66 b	5.37 ± 0.97 b	25.91 ± 4.06 b	2.75 ± 0.62 c
Probability	***	**	**	**
P-value	<0.001	0.001	0.008	0.002
		Shading x harvest period		
S0I2	130.79 ±7.94 a	14.25 ± 0.64 a	48.17± 1.71 a	6.85 ± 0.57 ab
S0I3	117.30 ± 7.13 a	15.44 ± 0.36 a	47.58± 7.46 a	7.73 ± 0.93 a
S0I4	79.10 ± 6.74 b	9.78 ± 0.77 b	45.22± 1.88 a	5.98 ± 0.19 bc
S45I2	69.51 ± 5.90 bc	6.12 ± 0.76 c	35.36± 3.37 bc	3.88 ± 0.40 def
S45I3	51.03 ± 3.93 d	5.40 ± 0.38 c	40.14± 0.18 ab	4.97 ± 0.14 cd
S45I4	59.57 ± 4.14 cd	5.90 ± 0.66 c	24.70± 4.02 d	1.48 ± 0.74 gh
S55I2	53.05 ± 6.86 d	4.76 ± 0.80 c	28.81± 2.70cd	3.21 ± 0.36 ef
S55I3	58.36 ± 4.61cd	5.88 ± 0.59 c	44.67± 2.41 ab	4.69 ± 0.48 cde
S55I4	46.47 ± 4.24d	4.78 ± 0.52 c	25.30± 1.38 d	2.69 ± 0.15 fg
S80I2	15.54 ± 1.30e	1.17 ± 0.14 d	3.27± 0.19 e	0.37 ± 0.02 h
S80I3	17.09 ± 1.32e	1.48 ± 0.13 d	2.37± 1.07 e	0.28 ± 0.18 h
S80I4	11.63 ± 1.28e	1.01 ± 0.14 d	8.41±0.52 e	0.83 ± 0.06 h
Probability	***	***	**	*
P-value	<0.001	<0.001	0.008	0.05

Remark: *= significant difference at P<0.05; **= significant difference at P<0.01; ***= significant difference at P<0.001.

The metabolism of Brazilian spinach was influenced by shading and harvesting periods. Brazilian spinach grown no-shading (S0) increased metabolism activity compared to the shading areas (S45, S55, and S80). This is represented by the carbon and nitrogen levels (Table 3). Higher metabolism correlates with enhanced nitrogen usage, which is crucial for plant metabolic processes. Therefore, increasing fertilization frequency is necessary for plants exposed to sunlight. Leaf nitrogen concentration remains consistent across harvesting periods, suggesting no significant differences in nitrogen across different harvesting periods.

The carbon-nitrogen ratio calculation can be used to determine leaf hardness in Brazilian spinach leaves. The study showed that no-shading (S0) areas yield more tough leaves, decreasing with increased shading levels. Despite this, Brazilian spinach consistently showed comparable levels of leaf hardness across harvesting periods.

Shading significantly impacts the carbon reduction and nitrogen enrichment of leaves, affecting the process of photosynthesis. Light intensity and photosynthesis are linked, with studies showing a reduction in carbon and nitrogen buildup in plants exposed to shading. Tang et al. (2022) found that plants exposed to modest levels of irradiation exhibit reduced concentrations of leaf non-structural carbohydrates, leading to a reduction in carbon content. Nitrogen accumulation in shaded Brazilian spinach leaves is due to limited light availability, hindering the conversion of nitrogen into organic nitrogen compounds essential for plant metabolic processes. Gao et al. (2020) found that prolonged shading reduces nitrogen utilization efficiency in plant. Wang et al. (2020b) demonstrated that the procedure of removing leaves of plants results in an increase in non-structural carbohydrates in leaf. On the other hand, an increased frequency of harvesting triggers the growth of new leaves, leading the movement of nitrogen toward younger leaves. Jasinski et al. (2021) reported that nitrogen mobilization occurs from older to younger leaves.

Table 3. Carbon, Nitrogen, and C-N ratio of Brazilian spinach leaf on different shading, harvest period, and their interaction.

Treatment	Carbon (%)	Nitrogen (%)	C-N ratio
		Shading	
S0	34.64	2.83	12.28
S45	32.75	4.56	7.20
S55	34.21	4.77	7.19
S80	34.32	4.99	6.84
		Harvest period	
I2	35.85	4.38	8.74
I3	33.90	4.42	8.10
I4	32.20	4.07	8.30
		Shading x harvest period	
S0I2	34.23	2.63	13.00
S0I3	34.28	2.90	11.83
S0I4	35.42	2.95	12.01
S45I2	32.02	4.70	6.81
S45I3	33.89	4.77	7.10
S45I4	32.34	4.20	7.70
S55I2	36.50	5.01	7.29
S55I3	32.14	4.94	6.50
S55I4	34.00	4.36	7.79
S80I2	40.66	5.16	7.88
S80I3	35.30	5.07	6.96
S80I4	27.01	4.76	5.68

The presence of shading in Brazilian spinach is linked to biomass production. Under unshaded conditions, it enhances photosynthesis, leading to increased biomass production. However, under intense shading conditions, it reduces biomass in various parts of the plant. Harvesting over extended periods (I3 and I4) results in increased biomass accumulation, particularly in the stem and branch in the final observation.

Brazilian spinach, when grown under shading conditions and extended harvesting, showed inhibited growth due to restricted photosynthetic activity. This caused the restricted allocation of photosynthetic products to individual plant organs. Studies have shown that shading reduces biomass accumulation and alterations in plant morphological traits (Xu et al., 2020b). Additionally, there is a distribution of photosynthetic activity that utilizes plant organs beyond just leaves. This aligns with Yu et al. (2019) finding that when plants age and their organs undergo senescence, photosynthetic flux redirects towards the stem. This highlights the importance of considering the allocation of photosynthetic products to plant growth through periodic harvesting.

Table 4. Dry weight of Brazilian spinach organs on different shading, harvest period, and their interaction at 13 weeks after planting (WAP).

Treatment	Stem dry weight (g)	Branch dry weight (g)	Leaf dry weight (g)	Root dry weight (g)	Total dry weight (g)
			Shading		
S0	2.35 ± 0.19 a	14.24 ± 0.98 a	7.92 ± 0.88 a	5.28 ± 1.20 a	29.79 ± 2.58 a
S45	1.36 ± 0.20 b	5.39 ± 0.98 b	3.70 ± 0.79 b	2.07 ± 0.81 ab	12.53 ± 2.28 b
S55	1.26 ± 0.13 b	5.21 ± 0.79 b	4.58 ± 0.69 b	1.13 ± 0.19 b	12.19 ± 1.48 b
S80	0.25 ± 0.04 c	0.40 ± 0.05 c	0.85 ± 0.62 c	0.44 ± 0.20 b	1.94 ± 0.79 c
Probability	***	***	***	*	***
P-value	< 0.001	< 0.001	< 0.001	0.046	< 0.001
			Harvest period		
I2	1.05 ± 0.27 b	4.39 ± 1.28 c	3.63 ± 0.68 b	1.73 ± 0.48	10.80 ± 2.53 b
I3	1.25 ± 0.20 b	6.12 ± 1.49 b	2.88 ± 0.65 b	2.19 ± 0.86	12.44 ± 2.80 b
I4	1.62 ± 0.27 a	8.42 ± 1.90 a	6.28 ± 1.22 a	2.77 ± 1.05	19.09 ± 4.19 a
Probability	**	***	***	ns	***
P-value	0.002	< 0.001	< 0.001	0.517	< 0.001
			Shading x harvest period		
S0I2	2.35 ± 0.53	11.26 ± 0.97 c	6.43 ± 0.44 b	4.27 ± 0.49	24.31 ± 1.12 bc
S0I3	2.03 ± 0.04	13.92 ± 0.61 b	5.98 ± 0.36 b	4.20 ± 2.25	26.14 ± 1.73 b
S0I4	2.66 ± 0.25	17.54 ± 0.62 a	11.36 ± 0.28 a	7.36 ± 2.97	38.92 ± 3.54 a
S45I2	0.78 ± 0.13	3.26 ± 0.70 fg	2.49 ± 0.43 cd	0.88 ± 0.26	7.41 ± 1.49 fgh
S45I3	1.41 ± 0.06	4.63 ± 0.33 fg	2.34 ± 0.22 cd	3.30 ± 2.41	11.68 ± 2.69 ef
S45I4	1.89 ± 0.38	8.29 ± 2.05 d	6.28 ± 1.52 b	2.04 ± 0.76	18.50 ± 4.66 cd
S55I2	0.87 ± 0.12	2.80 ± 0.67 gh	3.59 ± 0.81 c	1.06 ± 0.37	8.31 ± 1.41 fg
S55I3	1.35 ± 0.17	5.51 ± 0.62 ef	3.18 ± 0.34 c	1.00 ± 0.09	11.04 ± 0.58 ef
S55I4	1. 57 ± 0.14	7.32 ± 1.23 de	6.99 ± 0.77 b	1.32 ± 0.52	17.21 ± 1.74 de
S80I2	0.19 ± 0.01	0.24 ± 0.04 h	2.00 ± 1.84 cd	0.73 ± 0.64	3.17 ± 2.43 gh
S80I3	0.22 ± 0.03	0.41 ± 0.05 h	0.03 ± 0.03 d	0.26 ± 0.16	0.92 ± 0.27 h
S80I4	0.35 ± 0.09	0.53 ± 0.11 h	0.50 ± 0.33 d	0.34 ± 0.05	1.71 ± 0.49 gh
Probability	ns	*	*	ns	*
P-value	0.013	0.049	0.013	0.584	0.034

Remark: The ns= non-significant difference at $P<0.05$; *= significant difference at $P<0.05$; **= significant difference at $P<0.01$; ***= significant difference at $P<0.001$.

Visual appearance of Brazilian spinach on different treatment

The study analysed the shoot appearance of Brazilian spinach under different shading conditions and harvesting periods. Unshaded areas had a denser appearance, while based on the harvesting period, treatments tend to show similarities with each other (Figure 6). Furthermore, Brazilian spinach grown no-shading (S0) showed greater root growth and a higher density of root hairs than other shading, while samples subjected to varying harvesting periods (I2, I3, and I4) showed similar root morphology without any significant differences (Figure 7).

Brazilian spinach showed varying morphological traits under different treatments. Shading causes alterations in plant organs, as shown on soybean stems, which experience inhibited growth (Castronuovo et al., 2019). Cao et al. (2022) reported that *Cynodon dactylon* shoot organs also experience alterations. Root development also shows a distinct reaction to shading, with a decline in root growth under shading stress. Fu et al. (2020) found reductions in root volume and length, indicating a decline in root growth under these conditions.

Brazilian spinach with a longer harvesting period (I4) showed a rise in branches and stems, with a higher presence of mature leaves. Pruning at longer intervals increased plant height and branches in *Talinum Paniculatum*, while extending harvesting intervals hindered fresh leaf commencement (Purbajanti et al., 2019). Bessonova et al. (2023) found that removing leaves and branches from plants led to the development of shoot features with a greater number and area of leaves.

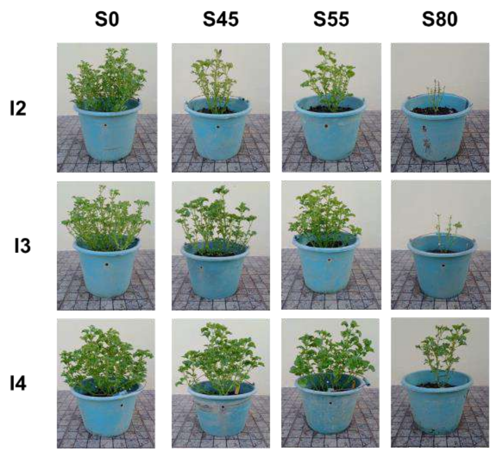

Figure 6. Visualization of Brazilian spinach shoot on different shading and harvest period at 13 weeks after planting (WAP). Shading treatment consist of S0: no-shading, S45: 45% shading, S55: 55% shading, and S80: 80% shading. Meanwhile, the harvest period consists of I2: per 2 weeks, I3: per 3 weeks, and I4: per 4 weeks. Photos: Strayker Ali Muda.

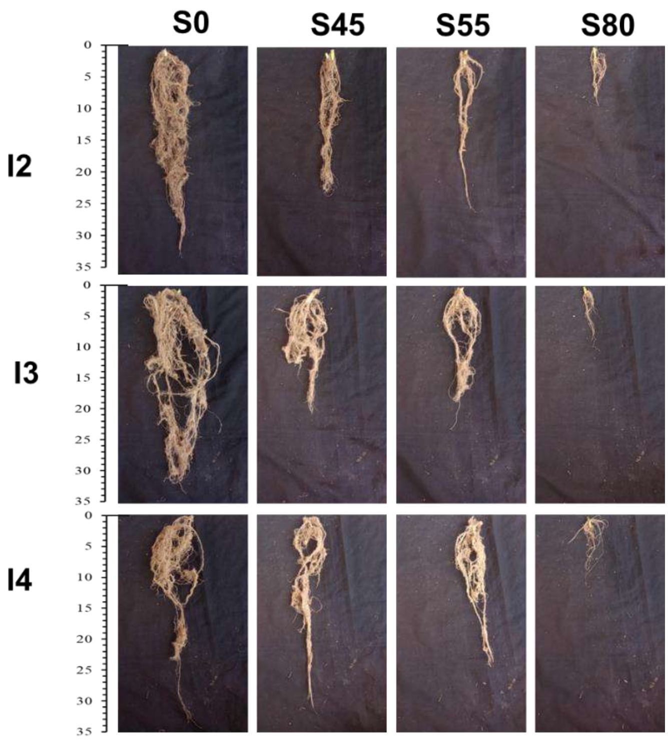

Figure 7. Visualization of Brazilian spinach root on different shading and harvest period at 13 weeks after planting (WAP). Shading treatment consist of S0: no-shading, S45: 45% shading, S55: 55% shading, and S80: 80% shading. Meanwhile, the harvest period consists of I2: per 2 weeks, I3: per 3 weeks, and I4: per 4 weeks. Photos: Strayker Ali Muda.

Water status on different treatment

The water availability for Brazilian spinach growth was represented by substrate moisture. Increased shading intensity (S80) leads to higher moisture content, reducing direct sunlight exposure and reducing evaporation, resulting in reduced water loss. Conversely, Brazilian spinach grown in areas with lower shading or without shading showed higher evaporation rates, indicating more water loss, as shown by substrate moisture levels (Figure 8). The use of shading can effectively adjust microclimate conditions, such as substrate moisture levels (Bollman et al., 2021). The study found that shaded growing media had higher moisture levels than unshaded media, and the addition of shading reduced evaporation rate, as confirmed by Khawam et al. (2019).

The more frequently Brazil spinach is harvested, the wider the substrate surface is not covered by the canopy, causing higher evaporation rates and reduced water availability. This phenomenon aligns with the findings of Huang et al. (2020), who provided empirical evidence that plants with lower canopy density exhibit higher rates of water loss via evaporation.

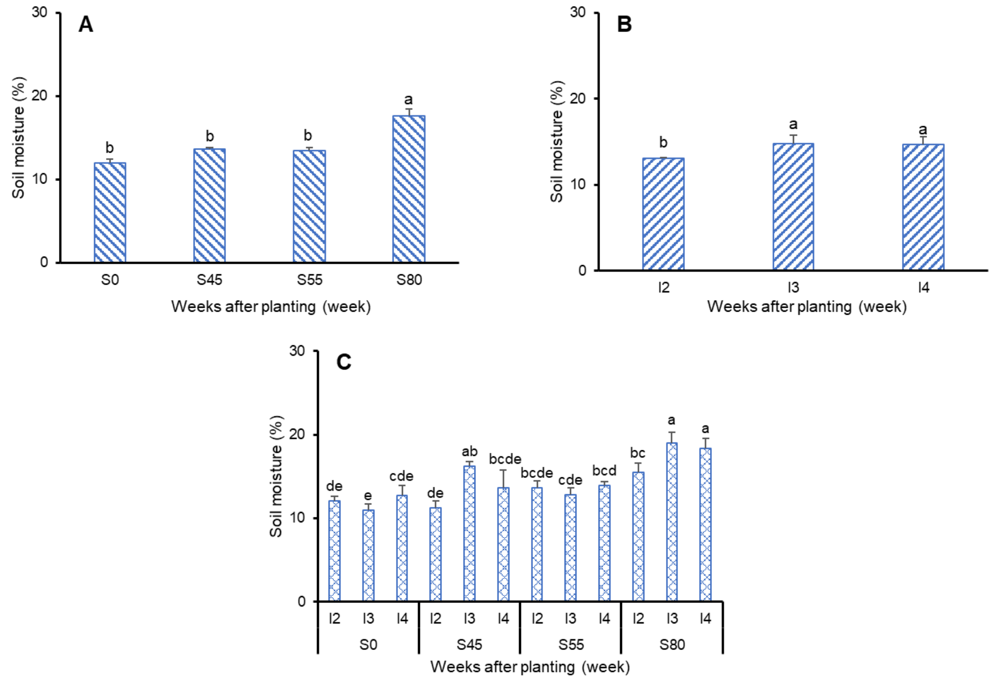

Figure 8. Substrate moisture on different shading (A), harvest period (B), and their interaction (C). Shading treatment consist of S0: no-shading, S45: shading 45%, S55: shading 55%, and S80: shading 80%. Meanwhile, the harvest period consists of I2: per 2 weeks, I3: per 3 weeks, and I4: per 4 weeks.

CONCLUSION

The adoption of shading led to a decrease in the growth and yield of Brazilian spinach through an alteration in the morphological traits of its root, stem, branch, and leaf. In addition, the implementation of a 2-week harvesting period led to increased Brazilian spinach marketable yield (67.22 g). Interactions between shading and harvest periods primarily pertain to the length of branches, yields (both marketable and non-marketable), dry weight of organs (namely branch and leaf), and substrate moisture. Therefore, the recommendation for Brazilian spinach cultivation in Indonesia is to be planted under direct sunlight and harvested two time per weeks.

ACKNOWLEDGMENT

We are very grateful for the suggestions, input and corrections from anonymous reviewers and the editors of the *Revista de Agricultura Neotropical* journal. This research was funded by the "Penelitian Profesi" Program, Universitas Sriwijaya 2024.

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4. Editor Decision: Accepted (22 Mei 2024)

Notifications ×

[REAN] Editor Decision

2024-05-22 02:40 PM

Strayker Muda, Benyamin Lakitan, Andi Wijaya, Susilawati Susilawati, Zaidan Zaidan, Yakup Yakup:

We have reached a decision regarding your submission to REVISTA DE AGRICULTURA NEOTROPICAL, "BRAZILIAN SPINACH GROWTH UNDER DIFFERENT SHADING INTENSITIES AND HARVESTING PERIODS IN LOWLAND TROPICAL URBAN ECOSYSTEM".

Our decision is to: Accept Submission

____ REVISTA DE AGRICULTURA NEOTROPICAL http://periodicosonline.uems.br/index.php/agrineo

5. Proofing (03 Juni 2024)

EST. 2013

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Manuscript Title

Growth and Yield of Brazilian Spinach Under Different Shading Intensities and Harvesting Periods in a Tropical Lowland Urban Ecosystem

Author(s)

Strayker Ali Muda, Benyamin Lakitan, Andi Wijaya, Susilawati Susilawati, Zaidan, Yakup

Date Issued

June 3, 2024

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GROWTH AND YIELD OF BRAZILIAN SPINACH UNDER DIFFERENT SHADING INTENSITIES AND HARVESTING PERIODS IN A TROPICAL LOWLAND URBAN ECOSYSTEM

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ABSTRACT

Brazilian spinach (*Alternanthera sissoo*) is a less prominent perennial leafy vegetable growing in the tropical ecosystem. Therefore, this research aimed to investigate the effects of different shading intensities and harvesting periods on the growth and yield of Brazilian spinach in a tropical lowland urban ecosystem. The investigation used a split-plot design, with different shading intensities (no-shading, 45%, 55%, and 80% shading) as the main plot and harvesting periods (every 2, 3, and 4 weeks) as the subplot. The results showed that Brazilian spinach growth was significantly enhanced under no-shading conditions compared to shading treatments. Furthermore, 80% shading negatively impacted plant growth during early stages, as evidenced by alterations in parameters including canopy area (26.47 cm^2), diameter (7.98 cm), and index (0.52), as well as Soil Plant Analysis Development (SPAD) values. This led to reduced branch elongation, marketable yield (14.76 g), and non-marketable yield (4.68), along with a decreased dry weight of stems (0.25 g), branches (0.40 g), leaves (0.85 g), and roots (0.44 g). However, marketable leaves from unshaded plants had higher carbon content (34.64 %) and lower nitrogen content (2.83 %). More frequent harvesting every 2 weeks elevated marketable yield (67.22 g) but suppressed the growth of stems (1.05 g), branches (4.39 g), and roots (1.73). Based on these observations, the cultivation of Brazilian spinach in unshaded areas with a biweekly harvesting routine was recommended.

KEYWORDS

Harvest time, Leafy green, Less prominent vegetable, Plant acclimatization, Solar irradiation intensity.

RESUMO

O espinafre brasileiro, uma hortaliça folhosa perene pouco conhecida, cresce em um ecossistema tropical. O estudo foi conduzido para avaliar o crescimento do espinafre brasileiro no ecossistema urbano de planície tropical sob diferentes níveis de intensidades de sombreamento e períodos de colheita. A pesquisa utilizou um desenho de parcela dividida, com diferentes níveis de intensidades de sombreamento (sem sombreamento, sombreamento de 45%, sombreamento de 55% e sombreamento de 80%) como parcela principal e períodos de colheita (a cada 2 semanas, a cada 3 semanas e a cada 4 semanas) como subparcela. Os resultados mostraram que o crescimento do espinafre brasileiro foi mais favorável quando exposto ao tratamento sem sombreamento em comparação com as condições de sombreamento. O tratamento com sombreamento, especialmente o sombreamento de 80%, teve um impacto negativo sobre o crescimento da planta observado durante os estágios iniciais de crescimento, conforme indicado pelas alterações nos parâmetros da copa (área da copa (26,47 cm2), diâmetro da copa (7,98 cm) e índice da copa (0,52)) e tendência dos valores SPAD. O sombreamento de 80% reduziu o alongamento dos ramos, a produção (comercializável (14,76 g) e não comercializável (4,68)), o peso seco do caule (0,25 g), o peso seco do ramo (0,40 g), o peso seco da folha (0,85 g) e o peso seco da raiz (0,44 g). Por outro lado, o espinafre brasileiro cultivado sem sombreamento aumentou o teor de carbono (34,64%) e reduziu o teor de nitrogênio (2,83%) das folhas comercializáveis. A colheita mais frequente (a cada 2 semanas) aumentou o rendimento comercializável (67,22 g), mas suprimiu o crescimento do caule (1,05 g), dos ramos (4,39 g) e da raiz (1,73). Portanto, recomenda-se cultivar o espinafre brasileiro em uma área não sombreada com uma rotina de colheita quinzenal.

PALAVRAS-CHAVE

Tempo de colheita, folhas verdes, hortaliças menos conhecidas, aclimatação da planta, intensidade da irradiação solar.

INTRODUCTION

Brazilian spinach (*Alternanthera sissoo*) is a leafy vegetable originating from Brazil and has been reported by Ikram et al. (2022) to richly contain flavonoids, vitamins, minerals, and other antioxidants, with beneficial effects on human health. Limited cultivation and utilization of this particular plant leads to being classified as a rare species. The similarity of Indonesia's ecosystem to the native environment of Brazilian spinach suggests the potential for cultivating this plant in the country.

Urban cultivation faces several challenges, specifically regarding the availability of light for plants. Shaded areas in urban environments often limit light penetration, which can hinder plant development. Consequently, reduced light availability disrupts certain metabolism aspects, particularly in horticultural crops with compact growth, such as Brazilian spinach. Shafiq et al. (2021) state that plants regulate growth to enhance photosynthesis efficiency. The tolerance of plants to different light intensities varies based on species, and some vegetables can grow under shaded conditions. For instance, Sifuentes-Pallaoro et al. (2020) found that shaded *Lactuca canadensis* had excellent growth, while Lakitan et al. (2021a) observed similar adaptability in celery. Gomes et al. (2023) reported that certain plants in the Brazilian ecosystem thrive under full or 70% light intensity.

Brazilian spinach grows continuously across the year due to being a perennial leafy vegetable, necessitating regular harvesting. Similarly, annual plants are subjected to a defoliation mechanism to enable periodic harvesting. Raza et al. (2019) found that defoliation treatments enhance overall plant growth, particularly the leaf parts, during the vegetative phase. Further experimentation is required to optimize the yield of Brazilian spinach with commercially valuable leaf organs which are vulnerable, particularly at young age, to damage from pests and diseases.

The cultivation of Brazilian spinach is relatively simple because it can be easily grown. Muda et al. (2022) reported that this plant can be successfully propagated through stem cuttings. However, the exploration of Brazilian spinach adaptability to shading environments is limited. The capacity to acclimatize to shading environments over specific durations will ensure the availability of sustainable vegetable nutrition. Therefore, this research aimed to investigate the adaptability of Brazilian spinach to shading conditions during various harvesting periods.

MATERIAL AND METHOD

Research site and agroclimatic characteristics

The investigation process was conducted at the Jakabaring research facility in Palembang (104º46'44"E, 3º01'35"S), South Sumatra, Indonesia, starting with stem cuttings propagation on January 30, 2023, while data collection was completed on May 2, 2023. The research site is located in a tropical lowland urban area with an elevation of 8 masl and several agroclimatic characteristics presented in Figure 1.

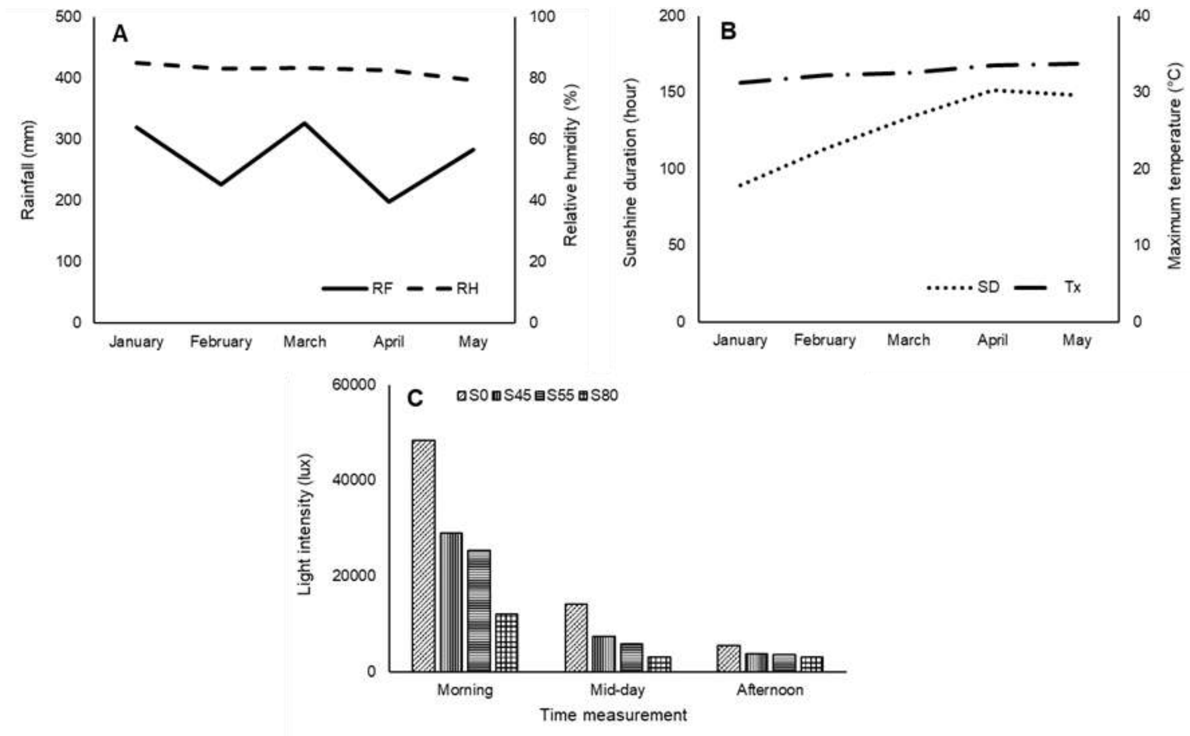

Figure 1. Agroclimatic characteristics of the research site include total monthly rainfall (RF) and average humidity (RH) (A), sunshine duration (SD) and average maximum temperature (Tx) (B), and average light intensity of each treatment (C). (Source: Indonesian Agency for Meteorology, Climatology, and Geophysics). S0= no-shading; S45= 45% shading; S55= 55% shading; and S80= 80% shading.

Cultivation and treatment procedures

The propagation materials used were stem cuttings with two leaves obtained from healthy 3-month-old parent plants. The cuttings were planted in pots (comprising 27.5 cm diameter and 20 cm height) filled with a growing medium that consists of a 3:1 mixture (v/v) of topsoil and chicken manure. Initially, the growing medium was subjected to bio-sterilization (2 g/l) with the addition of live microorganisms, including *Streptomyces thermovulgaris*, *Thricoderma virens*, and *Geobacillus thermocatenulatus*. This was subsequently incubated for one week to prevent infestation of the plants by pathogens.

The incubated growing medium was used to cultivate Brazilian spinach cuttings, which were arranged according to a split-plot design. The main plot in this research focused on shading intensities, while the subplot examined the harvest periods. Applied treatments comprised four levels of shading intensities, including S0, S45, S55, and S80. Additionally, the designated harvest periods had three different intervals of 2 (I2), 3 (I3), and 4 weeks (I4) following an initial harvest at five weeks after planting (WAP).

Brazilian spinach plants were systematically positioned in shadow houses measuring 4 m in length, 2 m in width, and 2 m in height. These houses were constructed using knockdown frames made of 1.5-inch PVC pipes and entirely covered with a black polyethylene net, which has been tested for appropriate density to provide optimal shading.

The leaves of all cultivated cuttings were trimmed 1 WAP to maintain uniformity in size. Additionally, a 3 g/plant dose of NPK fertilizer (16:16:16) was applied at 1 and 5 WAP, while watering was conducted daily at around 08:00 a.m. and 05:00 p.m.

Data collection

Growth and yield data were collected from Brazilian spinach cultivated during this research. All growth data were categorized into non-destructive and destructive measurements. Furthermore, non-destructive measurements conducted at 13 WAP covered SPAD values, branch length, stem diameter, as well as canopy width, diameter, and index. Destructive measurements comprised fresh and dry weight of branches, roots, and stems. Yield data included fresh and dry weight of both marketable and non-marketable leaves, as well as carbon content, nitrogen content, and the carbon-nitrogen (C:N) ratio of marketable leaves. In this context, marketable leaves were young, healthy, and easily breakable, while non-marketable leaves were aged, damaged by pests or diseases, and high in fiber content. During the investigation process, the moisture content of the planting medium was examined to determine the water level present in the used substrate.

SPAD values were monitored using chlorophyll meters (SPAD-502 Plus, Konica-Minolta Optics, Inc., Osaka, Japan), while canopy area was measured with a digital image scanner for Android (Easy Leaf Area software, developed by Easlon & Bloom 2014). Canopy diameter was estimated with a measuring tape on the widest part, while the index was evaluated as the ratio of the measured canopy area to the circular area with the widest diameter. Additionally, substrate moisture (SM) was calculated with a soil moisture meter (PMS-714, Lutron Electronics Canada, Inc., Pennsylvania, USA). Light intensity was evaluated with a lux meter (GM1030, Benetech, Inc., Illinois, USA). Carbon and nitrogen content was analyzed using the furnace and Kjedahl-Titrimetry methods, respectively.

The dry weight of each plant organ was determined by drying in an oven at 100°C for 24 hours. All plant organs were initially trimmed to reduce thickness and accelerate the drying process.

Data analysis

The effects of shading intensities and harvest periods were determined through analysis of variance (ANOVA). Disparities among these treatments were evaluated with Tukey's honestly significant difference (HSD) procedure at $P<0.05$. Subsequently, all data were analyzed using the RStudio software version 1.14.1717 for Windows (developed by the RStudio team, PBC, Boston, MA).

RESULT AND DISCUSSION

Brazilian spinach growth during early vegetative stages before harvesting

The early vegetative growth of Brazilian spinach was assessed during the period preceding harvesting by considering unique characteristics, such as canopy growth and SPAD values. This non-destructive method enabled plants to grow naturally, allowing for the evaluation of canopy characteristics including area, diameter, and index.

Brazilian spinach grown in unshaded conditions (S0) had a higher leaf initiation and larger individual leaf area compared to shading conditions. More and larger leaves contributed to the increase in canopy area to attain a broader size, compared to those grown under shade. The canopy area increased significantly in S0, particularly 2 to 5 WAP, compared to under S45, S55, and S80. However, no significant leaf growth was observed in S45 and S55, with S80 showing suppressed growth starting at 2 WAP (Figure 2).

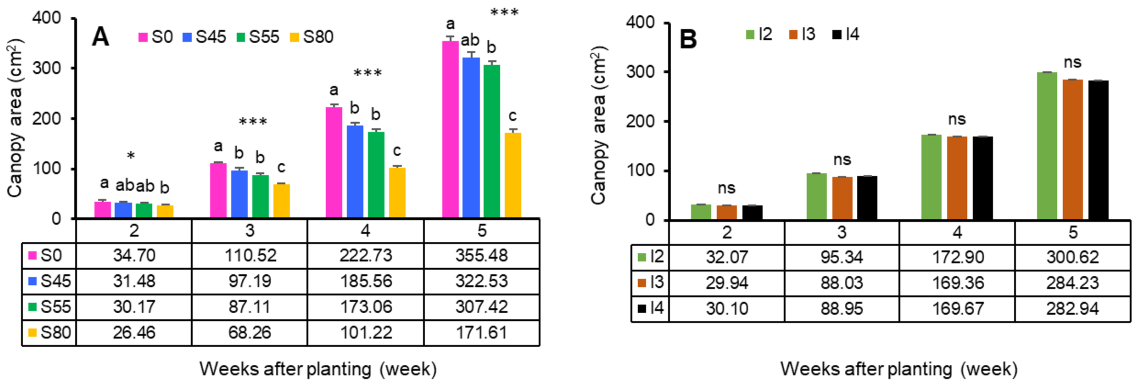

Figure 2. Brazilian spinach canopy area during early vegetative growth under different shading (A) and harvest period (B) treatments. The shading intensities consist of S0, S45, S55, and S80, while harvest periods include I2, I3, and I4. The ns= non-significant difference at $P<0.05$; *= significant difference at $P<0.05$; ***= significant difference at $P<0.001$.

Branches of Brazilian spinach significantly influenced canopy diameter, with elongation affecting canopy expansion. Shading conditions inhibited branch growth, while full sunlight cultivation led to a wider canopy than under S45, S55, and S80 (Figure 3).

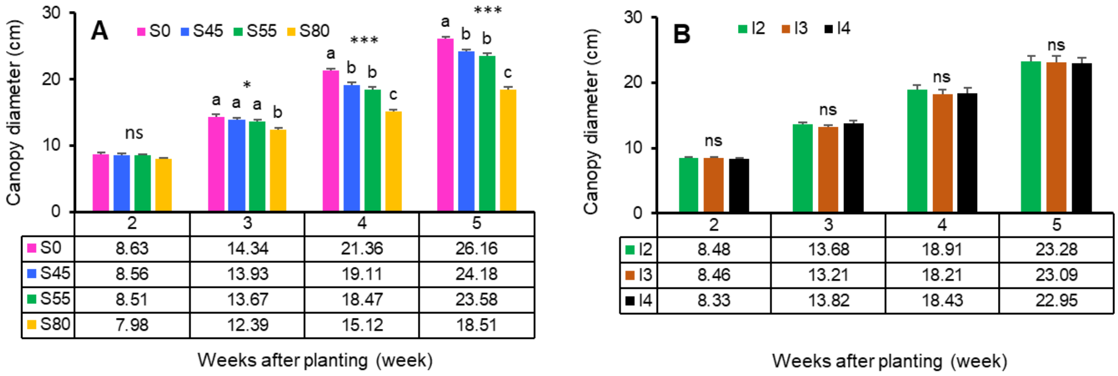

Figure 3. Brazilian spinach canopy diameter during early vegetative growth under different shading (A) and harvest period (B) treatments. Shading intensities consist of S0, S45, S55, and S80, while harvest periods include I2, I3, and I4. The ns= non-significant difference at P<0.05; *= significant difference at P<0.05; ***= significant difference at P<0.001.

Leaf and branch growth significantly affected canopy density, with dominant growth resulting in a denser canopy, most detectable between 4 and 5 WAP. Brazilian spinach cultivated under shading conditions, specifically S80, showed reduced leaf size and branch elongation, leading to lower canopy density (Figure 4).

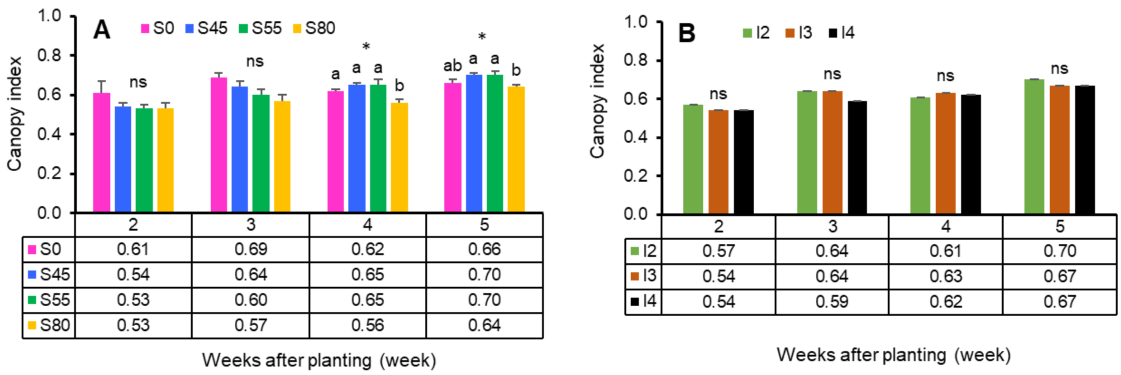

Figure 4. Canopy index for early vegetative growth at different shading and harvest period treatments. The shading intensities consist of S0, S45, S55, and S80, while harvest periods include I2, I3, and I4. The ns= non-significant difference at P<0.05; *= significant difference at P<0.05.

The results showed that the canopy growth of Brazilian spinach was more hindered at S80 than when unshaded. The constituent organs of the canopy, such as leaves and branches, endured stunting, which prevented the canopy from growing. According to Fadilah et al. (2022), denser shading intensity inhibited the growth of purple Pak Choi leaves. Wan et al. (2020) reported that plants cultivated in the shading areas produced less photosynthetic performance than those exposed to full sunlight. Moreover, Liang et al. (2020) reiterated the significance of shading for plants, which led to photosynthesis decline, resulting in reduced carbon flow. The inhibition of vegetative organ growth, particularly the canopy in Brazilian spinach, was attributed to decreased carbon flow, which occurred all through the early growth cycle. The phenomenon of reduced vegetative organ development due to shading during the early growth stage has been identified in various vegetable crops, including chili (Kesumawati et al., 2020).

The SPAD value is a method for evaluating leaf nitrogen and chlorophyll content, with a positive relationship between these factors. Brazilian spinach leaf's SPAD value was affected by shading treatments, with differences observed in each treatment from the early growth stage at 2 WAP. Furthermore, leaves grown at S0 showed a higher SPAD value compared to under different shading intensities, with a significant rise starting at 4 WAP. This trend was similarly observed in S45 and S55, but Brazilian spinach cultivated at S80 showed a stagnation trend, persisting until the completion of the 2 to 5 WAP early growth stage (Figure 5).

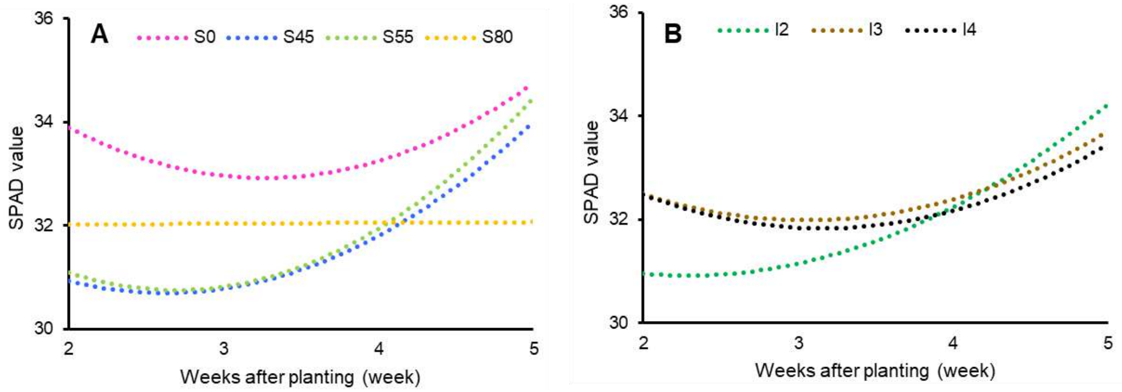

Figure 5. The SPAD value of Brazilian spinach leaves during early vegetative growth under different shading (A) and harvest period (A) treatments. The shading intensities consist of S0, S45, S55, and S80, while harvest periods include I2, I3, and I4.

The SPAD value is a very reliable method widely used for assessing leaf chlorophyll and nitrogen content due to the positive correlation of SPAD with these two parameters (Song et al., 2021; Farnisa et al., 2023). Prior research confirmed the capacity of specific leafy vegetables, including *Talinum paniculatum* (Lakitan et al., 2021b) and spinach (Mendoza-Tafolla et al., 2019), to proficiently evaluate and track the quantities of leaf chlorophyll and nitrogen content.

Brazilian spinach grown under S0 had a higher SPAD value than the shaded counterparts, showing that shading reduced the solubility of chlorophyll and nitrogen. Wang et al. (2020a) found that shading affected nitrogen solubility, leading to a decrease in the content found in leaves. Li et al. (2020) identified the biochemical alterations caused by shading stress, particularly in plants subjected to S80.

Brazilian spinach growth after harvested

The vegetative growth of Brazilian spinach after harvest was examined at 5 WAP, where branch growth was compared under different shading conditions, harvest periods, and interaction effects. Cuttings cultivated under S80 produced shorter branches at 11 WAP, but S0, S45, and S55 treatments generated comparable levels of branch elongation until 9 WAP. Those grown at S45 had an increased rate of branch elongation, particularly at 10 and 11 WAP (Table 1).

The elongation of Brazilian spinach branches was influenced by harvesting periods, where less frequent harvesting led to the highest elongation, specifically from 7 to 11 WAP. Interactions between shading intensities and harvesting periods were observed, starting at 9 WAP, with the results showing the importance of harvesting frequency in influencing growth.

Reduced elongation of Brazilian spinach branches at S80 was observed in this research, showing a decrease in the allocation of photosynthetic products. This is due to reduced levels of non-structural carbohydrates, which are essential for growth (Yamashita et al., 2020), but photosynthesis was optimized at S0, leading to increased branch growth.

The increased frequency of harvesting inhibits branch growth, potentially altering the distribution of photosynthetic products and triggering a more rapid initiation of new leaves (Oliveira et al., 2021). Additionally, Raza et al. (2019) reported that maize plants with a higher number of removed leaves allocated more photosynthetic resources toward the remaining leaves, as evidenced by area enhancement.

Table 1. Elongation of Brazilian spinach branches after harvesting at different shading intensities, harvest periods, and the interactions between both treatments.

Treatment	Weeks after planting (week)						
	5	6	7	8	9	10	11
Shading							
S0	11.98 ± 0.16 a	15.89 ± 0.17 a	19.18 ± 0.43 a	22.31 ± 0.38 a	24.16 ± 0.34 a	25.22 ± 0.33 b	26.17 ± 0.53 a
S45	11.63 ± 0.33 ab	15.11 ± 0.39 ab	19.00 ± 0.55 a	22.02 ± 0.76 a	24.30 ± 0.70 a	28.23 ± 0.95 a	29.02 ± 1.20 a
S55	11.03 ± 0.17 b	14.00 ± 0.22 b	18.03 ± 0.57 a	20.74 ± 0.80 a	22.32 ± 0.85 a	25.91 ± 0.95 b	26.74 ± 1.07 a
S80	8.68 ± 0.13 c	10.25 ± 0.17 c	12.66 ± 0.36 b	14.47 ± 0.51 b	14.93 ± 0.54 b	15.97 ± 0.76 c	16.46 ± 0.75 b
Probability	***	***	***	***	***	***	***
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Harvest period							
I2	11.06 ± 0.41	14.03 ± 0.69	15.80 ± 0.80 b	18.51 ± 1.09 c	19.66 ± 1.21 c	21.59 ± 1.41 c	21.90 ± 1.36 c
I3	10.72 ± 0.42	13.80 ± 0.71	18.07 ± 0.90 a	19.54 ± 1.02 b	21.75 ± 1.26 b	24.31 ± 1.58 b	24.95 ± 1.61 b
I4	10.70 ± 0.44	13.60 ± 0.66	17.78 ± 0.85 a	21.61 ± 0.98 a	22.88 ± 1.17 a	25.60 ± 1.45 a	26.94 ± 1.61 a
Probability	ns	Ns	***	***	***	***	***
P-value	0.186	0.198	<0.001	<0.001	<0.001	<0.001	<0.001
Shading x harvest period							
S0I2	12.23 ± 0.12	16.05 ± 0.34	18.55 ± 0.54	21.47 ± 0.44	23.22 ± 0.49 cd	24.24 ± 0.58 de	24.57 ± 0.62 de
S0I3	11.93 ± 0.41	16.20 ± 0.20	20.31 ± 0.72	22.40 ± 0.85	24.85 ± 0.40 b	25.68 ± 0.39 cd	26.91 ± 0.63 bc
S0I4	11.77 ± 0.27	15.41 ± 0.14	19.08 ± 0.44	23.08 ± 0.41	24.41 ± 0.53 bc	25.74 ± 0.34 cd	27.04 ± 0.78 bc
S45I2	11.99 ± 0.62	15.51 ± 1.10	17.45 ± 0.72	20.97 ± 1.52	22.53 ± 0.85 d	25.43 ± 1.20 cd	25.43 ± 0.91 cd
S45I3	10.89 ± 0.71	14.68 ± 0.52	19.57 ± 0.59	20.87 ± 0.95	23.77 ± 0.60 bcd	28.07 ± 0.83 b	28.54 ± 0.87 b
S45I4	12.01 ± 0.19	15.14 ± 0.41	19.99 ± 0.95	24.24 ± 0.20	26.59 ± 0.68 a	31.19 ± 0.67 b	33.08 ± 0.99 a
S55I2	11.08 ± 0.11	13.97 ± 0.22	16.00 ± 0.56	18.64 ± 0.81	19.53 ± 0.71 d	22.70 ± 0.97 e	23.19 ± 0.82 e
S55I3	11.37 ± 0.44	14.21 ± 0.67	19.19 ± 0.59	20.58 ± 1.41	23.50 ± 1.48 bcd	27.88 ± 1.25 b	28.27 ± 1.61 b
S55I4	10.64 ± 0.03	13.81 ± 0.21	18.90 ± 0.28	23.00 ± 0.45	23.92 ± 0.49 bcd	27.15 ± 0.70 b	28.77 ± 1.02 b
S80I2	8.95 ± 0.06	10.58 ± 0.13	11.61 ± 0.27	12.97 ± 0.37	13.32 ± 0.40 h	13.98 ± 0.60 g	14.41 ± 0.53 g
S80I3	8.70 ± 0.32	10.13 ± 0.37	13.22 ± 0.70	14.33 ± 0.60	14.89 ± 0.22 g	15.62 ± 0.39 g	16.09 ± 0.29 g
S80I4	8.38 ± 0.11	10.04 ± 0.32	13.14 ± 0.38	16.11 ± 0.38	16.59 ± 0.75 f	18.32 ± 1.27f	18.87 ± 1.17 f
Probability	ns	Ns	ns	ns	**	**	**
P-value	0.237	0.89	0.211	0.383	0.009	0.008	0.003

Remark: The ns= non-significant difference at P<0.05; **= significant difference at P<0.01; ***= significant difference at P<0.001.

Brazilian spinach showed significant differences in leaf growth when treated with different shading intensities and harvesting periods. Leaf growth was more dominant at S0 compared to at S45, S55, and S80, as presented in (Table 2). This showed early leaf growth at S0 with accelerated aging and increased pest susceptibility, resulting in a higher proportion of non-marketable leaves compared to those cultivated under shading.

Frequent harvesting of Brazilian spinach led to the initiation of young leaves, producing more marketable types. This was evident in the yield of commercially viable leaves all through the I2 and I3 periods. However, during I3, a significant proportion of non-marketable leaves were produced due to aging. Extended periods such as I4 often lack the capacity for leaf initiation, resulting in a decreased yield of both marketable and non-marketable leaves. The interaction of shading intensities and harvesting periods affected leaf growth, with the most significant impact observed at S80, specifically during I4.

Leaf initiation in Brazilian spinach was higher at S0 compared to S45-S80, affecting both marketable and non-marketable leaves due to reduced carbohydrate accumulation and allocation (Hussain et al., 2020). Shading conditions inhibited plant growth, while lack of shading accelerated leaf senescence due to enhanced photosynthesis. Direct sunlight exposure accelerates aging processes in plants, such as sweet basil (Castronuovo et al., 2019). However, without shade, spinach is more susceptible to pest infestation, leading to an increased prevalence of non-marketable leaves. The implementation of shading at a specific density is a viable strategy for controlling pests.

Brazilian spinach harvesting, similar to leaf and shoot pruning, has been found to increase yield. Dheeraj et al. (2022) found that pruning at the apical meristem increased growth-promoting hormones, specifically cytokinin. Additionally, Xu et al. (2020a) reported that pruning tomato plants elevated cytokinin hormone levels. Regarding this aspect, cytokinin influences cell division processes, such as during leaf development. Harvesting Brazilian spinach at I2 and I3 periods resulted in elevated cytokinin levels, enhancing leaf initiation and generating a greater marketable yield.

Table 2. Brazilian spinach yield at different shading intensities, harvest periods, and interactions.

Treatment	Marketable yield		Non-marketable yield	
	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
Shading				
S0	109.07 ± 8.55 a	13.16 ± 0.91 a	46.99 ± 2 .32 a	6.85 ± 0.41 a
S45	60.04 ± 3.57 b	5.80 ± 0.33 b	33.40 ± 2.74 b	3.44 ± 0.57 b
S55	52.63 ± 3.19 b	5.14 ± 0.37 b	32.92 ± 3.18 b	3.53 ± 0.35 b
S80	14.76 ± 1.04 c	1.22 ± 0.10 c	4.68 ± 1.00 c	0.49 ± 0.10 c
Probability	***	***	***	***
P-value	<0.001	<0.001	<0.001	<0.001
Harvest period				
I2	67.22 ± 12.80 a	6.58 ± 1.47 a	28.90 ± 5.03 b	3.57 ± 0.71 b
I3	60.95 ± 11.05 a	7.05 ± 1.56 a	33.69 ± 5.76 a	4.41 ± 0.84 a
I4	49.20 ± 7.66 b	5.37 ± 0.97 b	25.91 ± 4.06 b	2.75 ± 0.62 c
Probability	***	**	**	**
P-value	<0.001	0.001	0.008	0.002
Shading x harvest period				
S0I2	130.79 ±7.94 a	14.25 ± 0.64 a	48.17± 1.71 a	6.85 ± 0.57 ab
S0I3	117.30 ± 7.13 a	15.44 ± 0.36 a	47.58± 7.46 a	7.73 ± 0.93 a
S0I4	79.10 ± 6.74 b	9.78 ± 0.77 b	45.22± 1.88 a	5.98 ± 0.19 bc
S45I2	69.51 ± 5.90 bc	6.12 ± 0.76 c	35.36± 3.37 bc	3.88 ± 0.40 def
S45I3	51.03 ± 3.93 d	5.40 ± 0.38 c	40.14± 0.18 ab	4.97 ± 0.14 cd
S45I4	59.57 ± 4.14 cd	5.90 ± 0.66 c	24.70± 4.02 d	1.48 ± 0.74 gh
S55I2	53.05 ± 6.86 d	4.76 ± 0.80 c	28.81± 2.70cd	3.21 ± 0.36 ef
S55I3	58.36 ± 4.61cd	5.88 ± 0.59 c	44.67± 2.41 ab	4.69 ± 0.48 cde
S55I4	46.47 ± 4.24d	4.78 ± 0.52 c	25.30± 1.38 d	2.69 ± 0.15 fg
S80I2	15.54 ± 1.30e	1.17 ± 0.14 d	3.27± 0.19 e	0.37 ± 0.02 h
S80I3	17.09 ± 1.32e	1.48 ± 0.13 d	2.37± 1.07 e	0.28 ± 0.18 h
S80I4	11.63 ± 1.28e	1.01 ± 0.14 d	8.41±0.52 e	0.83 ± 0.06 h
Probability	***	***	**	*
P-value	<0.001	<0.001	0.008	0.05

Remark: *= significant difference at P<0.05; **= significant difference at P<0.01; ***= significant difference at P<0.001.

The metabolism of Brazilian spinach was influenced by shading intensities and harvesting periods. Increased metabolism activity was observed at S0 compared to S45, S55, and S80, evidenced by the carbon and nitrogen levels (Table 3). Higher metabolism correlates with enhanced nitrogen usage, which is crucial for plant metabolic processes. Therefore, increasing fertilization frequency is necessary for plants exposed to sunlight. In this research, leaf nitrogen concentration remained consistent across different harvesting periods, suggesting no significant differences in nitrogen content.

The C:N ratio calculation can be used to determine leaf hardness in Brazilian spinach. This research showed that lack of shading produced tougher leaves, decreasing with increased shading levels. However, comparable levels of leaf hardness were observed across different harvesting periods.

Shading significantly impacts the carbon reduction and nitrogen enrichment of leaves, affecting the process of photosynthesis. Light intensity and photosynthesis are connected, as investigations show reduced carbon and nitrogen content in shaded plants. For instance, Tang et al. (2022) found that plants exposed to modest levels of irradiation had reduced concentrations of leaf non-structural carbohydrates, leading to a reduction in carbon content. Nitrogen accumulation in shaded Brazilian spinach leaves was due to limited light availability, hindering conversion into organic nitrogen compounds essential for plant metabolic processes. Gao et al. (2020) identified that prolonged shading reduced nitrogen utilization efficiency in plants. Wang et al. (2020b) observed increased content of non-structural carbohydrates resulting from the procedure of removing plant leaves. However, elevated harvesting frequency triggers the growth of new leaves, driving the movement of nitrogen toward younger leaves. Jasinski et al. (2021) stated that nitrogen mobilization occurs from older to younger leaves.

Table 3. Carbon, Nitrogen, and C-N ratio of Brazilian spinach leaves at different shading intensities, harvest periods, and interactions.

Treatment	Carbon (%)	Nitrogen (%)	C-N ratio
	Shading		
S0	34.64	2.83	12.28
S45	32.75	4.56	7.20
S55	34.21	4.77	7.19
S80	34.32	4.99	6.84
	Harvest period		
I2	35.85	4.38	8.74
I3	33.90	4.42	8.10
I4	32.20	4.07	8.30
	Shading x harvest period		
S0I2	34.23	2.63	13.00
S0I3	34.28	2.90	11.83
S0I4	35.42	2.95	12.01
S45I2	32.02	4.70	6.81
S45I3	33.89	4.77	7.10
S45I4	32.34	4.20	7.70
S55I2	36.50	5.01	7.29
S55I3	32.14	4.94	6.50
S55I4	34.00	4.36	7.79
S80I2	40.66	5.16	7.88
S80I3	35.30	5.07	6.96
S80I4	27.01	4.76	5.68

The presence of shading in Brazilian spinach is connected to biomass production, as unshaded conditions enhance photosynthesis, leading to increased biomass production. However, under intense shading conditions, it reduces biomass in various plant parts. Harvesting over extended periods such as I3 and I4 resulted in elevated biomass accumulation, particularly in the stems and branches.

Brazilian spinach subjected to shading conditions and extended harvesting periods showed inhibited growth due to restricted photosynthetic activity. This caused the restricted allocation of photosynthetic products to individual plant organs. Previous research has shown that shading reduced biomass accumulation and caused alterations in plant morphological characteristics (Xu et al., 2020b). Additionally, photosynthetic activity in certain cases is redistributed to use other organs apart from the leaves. This corresponds with the report by Yu et al. (2019) that when plants age and the organs enter senescence, photosynthetic flux redirects toward the stem, suggesting the importance of allocating photosynthetic products to support plant growth through periodic harvesting.

Table 4. The dry weight of Brazilian spinach organs at different shading intensities, harvest periods, and interactions at 13 WAP

Treatment	Stem dry weight (g)	Branch dry weight (g)	Leaf dry weight (g)	Root dry weight (g)	Total dry weight (g)
			Shading		
S0	2.35 ± 0.19 a	14.24 ± 0.98 a	7.92 ± 0.88 a	5.28 ± 1.20 a	29.79 ± 2.58 a
S45	1.36 ± 0.20 b	5.39 ± 0.98 b	3.70 ± 0.79 b	2.07 ± 0.81 ab	12.53 ± 2.28 b
S55	1.26 ± 0.13 b	5.21 ± 0.79 b	4.58 ± 0.69 b	1.13 ± 0.19 b	12.19 ± 1.48 b
S80	0.25 ± 0.04 c	0.40 ± 0.05 c	0.85 ± 0.62 c	0.44 ± 0.20 b	1.94 ± 0.79 c
Probability	***	***	***	*	***
P-value	< 0.001	< 0.001	< 0.001	0.046	< 0.001
			Harvest period		
I2	1.05 ± 0.27 b	4.39 ± 1.28 c	3.63 ± 0.68 b	1.73 ± 0.48	10.80 ± 2.53 b
I3	1.25 ± 0.20 b	6.12 ± 1.49 b	2.88 ± 0.65 b	2.19 ± 0.86	12.44 ± 2.80 b
I4	1.62 ± 0.27 a	8.42 ± 1.90 a	6.28 ± 1.22 a	2.77 ± 1.05	19.09 ± 4.19 a
Probability	**	***	***	ns	***
P-value	0.002	< 0.001	< 0.001	0.517	< 0.001
			Shading x harvest period		
S0I2	2.35 ± 0.53	11.26 ± 0.97 c	6.43 ± 0.44 b	4.27 ± 0.49	24.31 ± 1.12 bc
S0I3	2.03 ± 0.04	13.92 ± 0.61 b	5.98 ± 0.36 b	4.20 ± 2.25	26.14 ± 1.73 b
S0I4	2.66 ± 0.25	17.54 ± 0.62 a	11.36 ± 0.28 a	7.36 ± 2.97	38.92 ± 3.54 a
S45I2	0.78 ± 0.13	3.26 ± 0.70 fg	2.49 ± 0.43 cd	0.88 ± 0.26	7.41 ± 1.49 fgh
S45I3	1.41 ± 0.06	4.63 ± 0.33 fg	2.34 ± 0.22 cd	3.30 ± 2.41	11.68 ± 2.69 ef
S45I4	1.89 ± 0.38	8.29 ± 2.05 d	6.28 ± 1.52 b	2.04 ± 0.76	18.50 ± 4.66 cd
S55I2	0.87 ± 0.12	2.80 ± 0.67 gh	3.59 ± 0.81 c	1.06 ± 0.37	8.31 ± 1.41 fg
S55I3	1.35 ± 0.17	5.51 ± 0.62 ef	3.18 ± 0.34 c	1.00 ± 0.09	11.04 ± 0.58 ef
S55I4	1. 57 ± 0.14	7.32 ± 1.23 de	6.99 ± 0.77 b	1.32 ± 0.52	17.21 ± 1.74 de
S80I2	0.19 ± 0.01	0.24 ± 0.04 h	2.00 ± 1.84 cd	0.73 ± 0.64	3.17 ± 2.43 gh
S80I3	0.22 ± 0.03	0.41 ± 0.05 h	0.03 ± 0.03 d	0.26 ± 0.16	0.92 ± 0.27 h
S80I4	0.35 ± 0.09	0.53 ± 0.11 h	0.50 ± 0.33 d	0.34 ± 0.05	1.71 ± 0.49 gh
Probability	ns	*	*	ns	*
P-value	0.013	0.049	0.013	0.584	0.034

Remark: The ns= non-significant difference at P<0.05; *= significant difference at P<0.05; **= significant difference at P<0.01; ***= significant difference at P<0.001.

The visual appearance of Brazilian spinach at different treatments

The shoot appearance of Brazilian spinach under different shading conditions and harvesting periods was examined in this research. Unshaded areas had a denser appearance, while different harvesting periods tended to produce related results (Figure 6). Cuttings cultivated at S0 had greater root growth and a higher density of root hairs than under shading, while samples subjected to varying harvesting periods of I2, I3, and I4 showed similar root morphology without any significant differences (Figure 7).

Varying morphological characteristics were identified in Brazilian spinach under different treatments. Shading causes alterations in plant organs, as observed on soybean stems, which experience inhibited growth (Castronuovo et al., 2019). Similarly, Cao et al. (2022) reported that *Cynodon dactylon* shoot experienced alterations and root development showed a distinct reaction of declined growth when exposed to shading stress. Fu et al. (2020) found reductions in root volume and length, showing decreased root growth under these conditions.

Brazilian spinach with a longer harvesting period (I4) showed an increase in branches and stems, with a higher presence of mature leaves. Pruning at longer intervals increased plant height and branches in *Talinum Paniculatum*, while extending harvesting intervals hindered fresh leaf commencement (Purbajanti et al., 2019). Bessonova et al. (2023) found that removing leaves and branches led to the development of shoot features with a greater number and area of leaves.

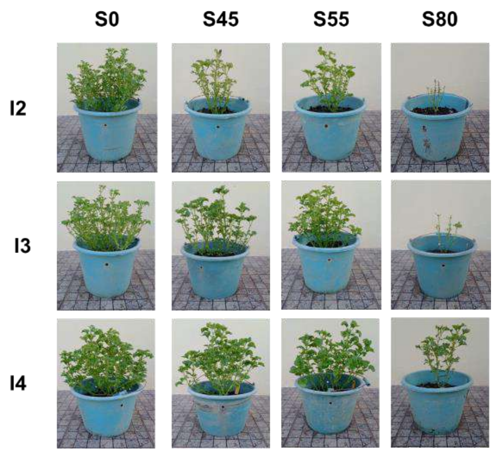

Figure 6. Visualization of Brazilian spinach shoots under different shading and harvest period treatments at 13 WAP. Shading intensities consist of S0, S45, S55, and S80, while the harvest periods include I2, I3, and I4. Photos: Strayker Ali Muda.

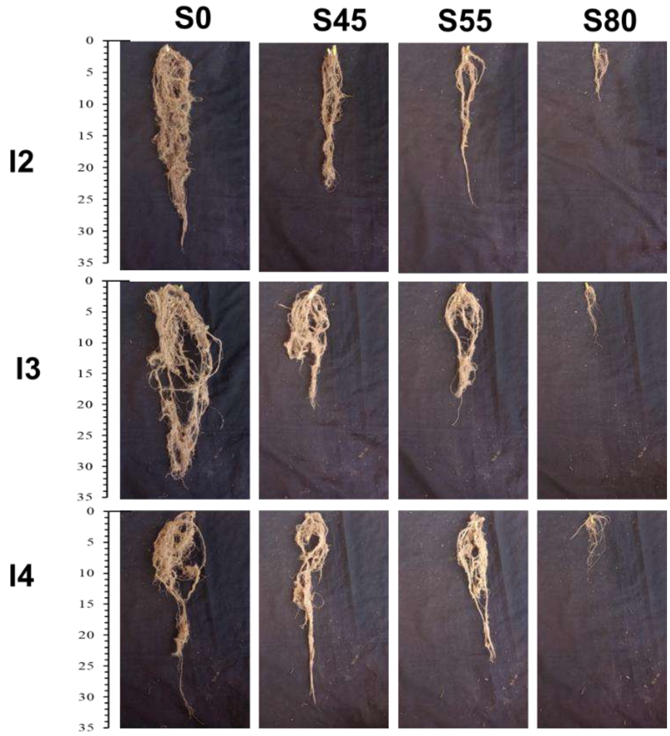

Figure 7. Visualization of Brazilian spinach roots under different shading and harvest period treatments at 13 WAP. Shading intensities consist of S0, S45, S55, and S80, while the harvest periods include I2, I3, and I4. Photos: Strayker Ali Muda.

Water content at different treatments

The water availability for Brazilian spinach growth was represented by substrate moisture (SM). Increased shading intensity (S80) leads to higher moisture content, decreasing direct sunlight exposure and evaporation, which results in reduced water loss. However, Brazilian spinach grown in areas with lower or total absence of shading showed higher evaporation rates, signifying more water loss, as evidenced by SM levels (Figure 8). The use of shading can effectively adjust microclimate conditions, such as SM levels (Bollman et al., 2021). This research found that shaded growing media had higher moisture levels than the unshaded counterparts, and the addition of shading reduced evaporation rate, as confirmed by Khawam et al. (2019).

Frequent harvesting of Brazil spinach reduces the coverage of the substrate surface by the canopy, causing higher evaporation rates and reduced water availability. This phenomenon correlates with the results of Huang et al. (2020) who provided empirical evidence regarding plants with lower canopy density experiencing higher rates of water loss through evaporation.

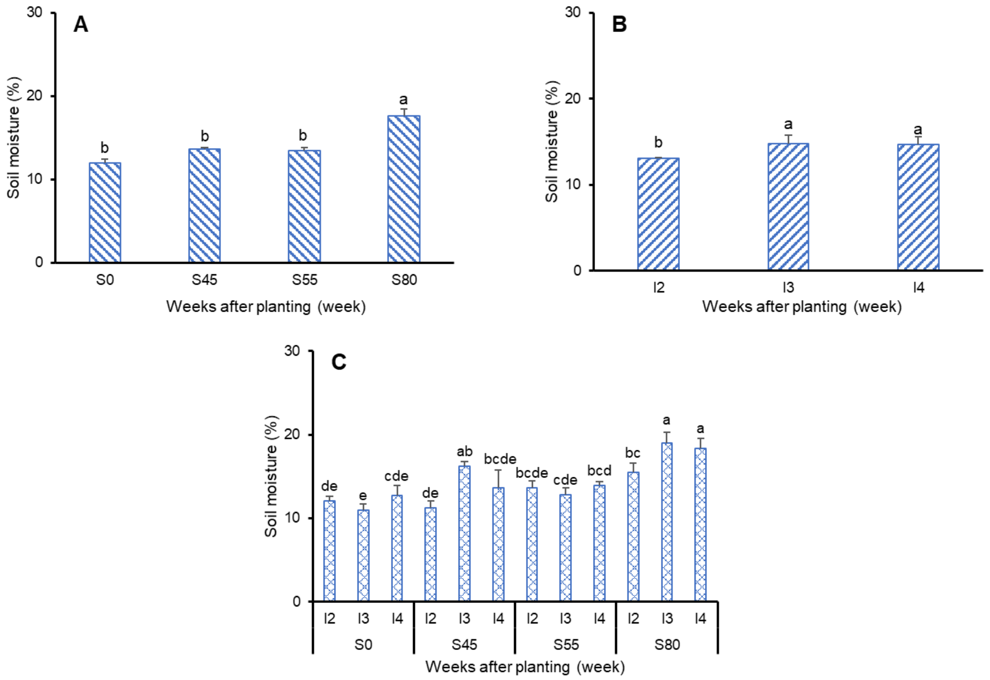

Figure 8. Substrate moisture at different shading intensities (A), harvest periods (B), and the interactions between both treatments (C). Shading intensities consist of S0, S45, S55, and S80, while the harvest periods include I2, I3, and I4.

CONCLUSION

In conclusion, the results showed that the adoption of shading led to a decrease in the growth and yield of Brazilian spinach through alterations in root, stem, branch, and leaf morphological characteristics. Additionally, the implementation of 2 WAP significantly increased marketable yield to 67.22 g. Interactions between shading intensities and harvest periods primarily influenced SM, the length of branches, yield, as well as dry weight of branches and leaves. Therefore, Brazilian spinach was recommended to be cultivated in Indonesia under direct sunlight and harvested every two weeks.

AUTHORS' CONTRIBUTION

Strayker Ali Muda: Research execution, data collection, data analysis, drawing graph, and writing original manuscript. Benyamin Lakitan: Corresponding author, idea conception, methodology, data interpretation, manuscript revision, and supervision. Andi Wijaya, Susilawati Susilawati: Experimental design, methodology, data analysis, manuscript revision, and supervision. Yakup Yakup, Zaidan Zaidan: data interpretation, methodology, and manuscript revision.

ACKNOWLEDGMENT

The authors are grateful for the suggestions, input, and corrections provided by anonymous reviewers and the editors of the *Revista de Agricultura Neotropical* journal. The authors are also grateful for the funding assistance obtained through the “Penelitian Profesi” Program, Universitas Sriwijaya 2024.

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6. Final (13 Juni 2024)

GROWTH AND YIELD OF BRAZILIAN SPINACH UNDER DIFFERENT SHADING INTENSITIES AND HARVESTING PERIODS IN A TROPICAL LOWLAND URBAN ECOSYSTEM

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ABSTRACT

Brazilian spinach (*Alternanthera sissoo*) is a less prominent perennial leafy vegetable growing in the tropical ecosystem. Therefore, this research aimed to investigate the effects of different shading intensities and harvesting periods on the growth and yield of Brazilian spinach in a tropical lowland urban ecosystem. The investigation used a split-plot design, with different shading intensities (no-shading, 45%, 55%, and 80% shading) as the main plot and harvesting periods (every 2, 3, and 4 weeks) as the subplot. The results showed that Brazilian spinach growth was significantly enhanced under no-shading conditions compared to shading treatments. Furthermore, 80% shading negatively impacted plant growth during early stages, as evidenced by alterations in parameters including canopy area (26.47 cm^2), diameter (7.98 cm), and index (0.52), as well as Soil Plant Analysis Development (SPAD) values. This led to reduced branch elongation, marketable yield (14.76 g), and non-marketable yield (4.68), along with a decreased dry weight of stems (0.25 g), branches (0.40 g), leaves (0.85 g), and roots (0.44 g). However, marketable leaves from unshaded plants had higher carbon content (34.64 %) and lower nitrogen content (2.83 %). More frequent harvesting every 2 weeks elevated marketable yield (67.22 g) but suppressed the growth of stems (1.05 g), branches (4.39 g), and roots (1.73). Based on these observations, the cultivation of Brazilian spinach in unshaded areas with a biweekly harvesting routine was recommended.

KEYWORDS

Harvest time, Leafy green, Less prominent vegetable, Plant acclimatization, Solar irradiation intensity.

RESUMO

O espinafre brasileiro, uma hortaliça folhosa perene pouco conhecida, cresce em um ecossistema tropical. O estudo foi conduzido para avaliar o crescimento do espinafre brasileiro no ecossistema urbano de planície tropical sob diferentes níveis de intensidades de sombreamento e períodos de colheita. A pesquisa utilizou um desenho de parcela dividida, com diferentes níveis de intensidades de sombreamento (sem sombreamento, sombreamento de 45%, sombreamento de 55% e sombreamento de 80%) como parcela principal e períodos de colheita (a cada 2 semanas, a cada 3 semanas e a cada 4 semanas) como subparcela. Os resultados mostraram que o crescimento do espinafre brasileiro foi mais favorável quando exposto ao tratamento sem sombreamento em comparação com as condições de sombreamento. O tratamento com sombreamento, especialmente o sombreamento de 80%, teve um impacto negativo sobre o crescimento da planta observado durante os estágios iniciais de crescimento, conforme indicado pelas alterações nos parâmetros da copa (área da copa (26,47 cm2), diâmetro da copa (7,98 cm) e índice da copa (0,52)) e tendência dos valores SPAD. O sombreamento de 80% reduziu o alongamento dos ramos, a produção (comercializável (14,76 g) e não comercializável (4,68)), o peso seco do caule (0,25 g), o peso seco do ramo (0,40 g), o peso seco da folha (0,85 g) e o peso seco da raiz (0,44 g). Por outro lado, o espinafre brasileiro cultivado sem sombreamento aumentou o teor de carbono (34,64%) e reduziu o teor de nitrogênio (2,83%) das folhas comercializáveis. A colheita mais frequente (a cada 2 semanas) aumentou o rendimento comercializável (67,22 g), mas suprimiu o crescimento do caule (1,05 g), dos ramos (4,39 g) e da raiz (1,73). Portanto, recomenda-se cultivar o espinafre brasileiro em uma área não sombreada com uma rotina de colheita quinzenal.

PALAVRAS-CHAVE

Tempo de colheita, folhas verdes, hortaliças menos conhecidas, aclimatação da planta, intensidade da irradiação solar.

INTRODUCTION

Brazilian spinach (*Alternanthera sissoo*) is a leafy vegetable originating from Brazil and has been reported by Ikram et al. (2022) to richly contain flavonoids, vitamins, minerals, and other antioxidants, with beneficial effects on human health. Limited cultivation and utilization of this particular plant leads to being classified as a rare species. The similarity of Indonesia's ecosystem to the native environment of Brazilian spinach suggests the potential for cultivating this plant in the country.

Urban cultivation faces several challenges, specifically regarding the availability of light for plants. Shaded areas in urban environments often limit light penetration, which can hinder plant development. Consequently, reduced light availability disrupts certain metabolism aspects, particularly in horticultural crops with compact growth, such as Brazilian spinach. Shafiq et al. (2021) state that plants regulate growth to enhance photosynthesis efficiency. The tolerance of plants to different light intensities varies based on species, and some vegetables can grow under shaded conditions. For instance, Sifuentes-Pallaoro et al. (2020) found that shaded *Lactuca canadensis* had excellent growth, while Lakitan et al. (2021a) observed similar adaptability in celery. Gomes et al. (2023) reported that certain plants in the Brazilian ecosystem thrive under full or 70% light intensity.

Brazilian spinach grows continuously across the year due to being a perennial leafy vegetable, necessitating regular harvesting. Similarly, annual plants are subjected to a defoliation mechanism to enable periodic harvesting. Raza et al. (2019) found that defoliation treatments enhance overall plant growth, particularly the leaf parts, during the vegetative phase. Further experimentation is required to optimize the yield of Brazilian spinach with commercially valuable leaf organs which are vulnerable, particularly at young age, to damage from pests and diseases.

The cultivation of Brazilian spinach is relatively simple because it can be easily grown. Muda et al. (2022) reported that this plant can be successfully propagated through stem cuttings. However, the exploration of Brazilian spinach adaptability to shading environments is limited. The capacity to acclimatize to shading environments over specific durations will ensure the availability of sustainable vegetable nutrition. Therefore, this research aimed to investigate the adaptability of Brazilian spinach to shading conditions during various harvesting periods.

MATERIAL AND METHOD

Research site and agroclimatic characteristics

The investigation process was conducted at the Jakabaring research facility in Palembang (104º46'44"E, 3º01'35"S), South Sumatra, Indonesia, starting with stem cuttings propagation on January 30, 2023, while data collection was completed on May 2, 2023. The research site is located in a tropical lowland urban area with an elevation of 8 masl and several agroclimatic characteristics presented in Figure 1.

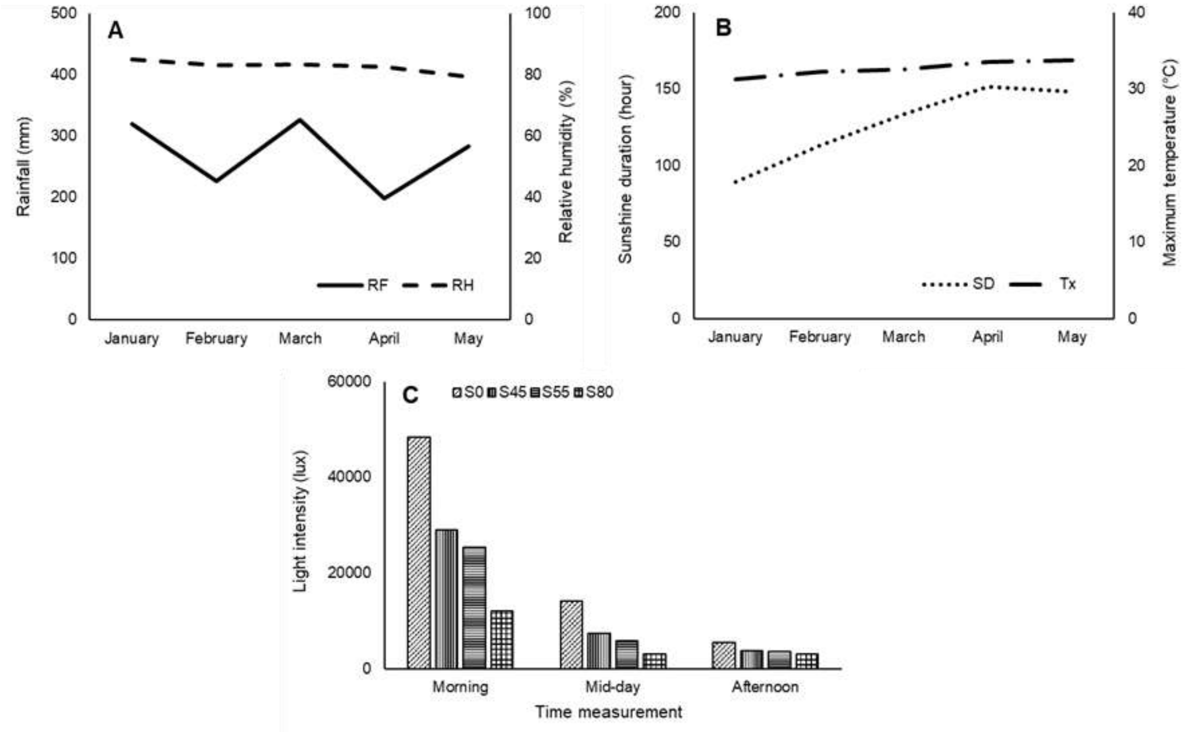


Figure 1. Agroclimatic characteristics of the research site include total monthly rainfall (RF) and average humidity (RH) (A), sunshine duration (SD) and average maximum temperature (Tx) (B), and average light intensity of each treatment (C). (Source: Indonesian Agency for Meteorology, Climatology, and Geophysics). S0= no-shading; S45= 45% shading; S55= 55% shading; and S80= 80% shading.

Cultivation and treatment procedures

The propagation materials used were stem cuttings with two leaves obtained from healthy 3-month-old parent plants. The cuttings were planted in pots (comprising 27.5 cm diameter and 20 cm height) filled with a growing medium that consists of a 3:1 mixture (v/v) of topsoil and chicken manure. Initially, the growing medium was subjected to bio-sterilization (2 g/l) with the addition of live microorganisms, including *Streptomyces thermovulgaris*, *Thricoderma virens*, and *Geobacillus thermocatenulatus*. This was subsequently incubated for one week to prevent infestation of the plants by pathogens.

The incubated growing medium was used to cultivate Brazilian spinach cuttings, which were arranged according to a split-plot design. The main plot in this research focused on shading intensities, while the subplot examined the harvest periods. Applied treatments comprised four levels of shading intensities, including S0, S45, S55, and S80. Additionally, the designated harvest periods had three different intervals of 2 (I2), 3 (I3), and 4 weeks (I4) following an initial harvest at five weeks after planting (WAP).

Brazilian spinach plants were systematically positioned in shadow houses measuring 4 m in length, 2 m in width, and 2 m in height. These houses were constructed using knockdown frames made of 1.5-inch PVC pipes and entirely covered with a black polyethylene net, which has been tested for appropriate density to provide optimal shading.

The leaves of all cultivated cuttings were trimmed 1 WAP to maintain uniformity in size. Additionally, a 3 g/plant dose of NPK fertilizer (16:16:16) was applied at 1 and 5 WAP, while watering was conducted daily at around 08:00 a.m. and 05:00 p.m.

Data collection

Growth and yield data were collected from Brazilian spinach cultivated during this research. All growth data were categorized into non-destructive and destructive measurements. Furthermore, non-destructive measurements conducted at 13 WAP covered SPAD values, branch length, stem diameter, as well as canopy width, diameter, and index. Destructive measurements comprised fresh and dry weight of branches, roots, and stems. Yield data included fresh and dry weight of both marketable and non-marketable leaves, as well as carbon content, nitrogen content, and the carbon-nitrogen (C:N) ratio of marketable leaves. In this context, marketable leaves were young, healthy, and easily breakable, while non-marketable leaves were aged, damaged by pests or diseases, and high in fiber content. During the investigation process, the moisture content of the planting medium was examined to determine the water level present in the used substrate.

SPAD values were monitored using chlorophyll meters (SPAD-502 Plus, Konica-Minolta Optics, Inc., Osaka, Japan), while canopy area was measured with a digital image scanner for Android (Easy Leaf Area software, developed by Easlon & Bloom 2014). Canopy diameter was estimated with a measuring tape on the widest part, while the index was evaluated as the ratio of the measured canopy area to the circular area with the widest diameter. Additionally, substrate moisture (SM) was calculated with a soil moisture meter (PMS-714, Lutron Electronics Canada, Inc., Pennsylvania, USA). Light intensity was evaluated with a lux meter (GM1030, Benetech, Inc., Illinois, USA). Carbon and nitrogen content was analyzed using the furnace and Kjedahl-Titrimetry methods, respectively.

The dry weight of each plant organ was determined by drying in an oven at 100°C for 24 hours. All plant organs were initially trimmed to reduce thickness and accelerate the drying process.

Data analysis

The effects of shading intensities and harvest periods were determined through analysis of variance (ANOVA). Disparities among these treatments were evaluated with Tukey's honestly significant difference (HSD) procedure at $P<0.05$. Subsequently, all data were analyzed using the RStudio software version 1.14.1717 for Windows (developed by the RStudio team, PBC, Boston, MA).

RESULT AND DISCUSSION

Brazilian spinach growth during early vegetative stages before harvesting

The early vegetative growth of Brazilian spinach was assessed during the period preceding harvesting by considering unique characteristics, such as canopy growth and SPAD values. This non-destructive method enabled plants to grow naturally, allowing for the evaluation of canopy characteristics including area, diameter, and index.

Brazilian spinach grown in unshaded conditions (S0) had a higher leaf initiation and larger individual leaf area compared to shading conditions. More and larger leaves contributed to the increase in canopy area to attain a broader size, compared to those grown under shade. The canopy area increased significantly in S0, particularly 2 to 5 WAP, compared to under S45, S55, and S80. However, no significant leaf growth was observed in S45 and S55, with S80 showing suppressed growth starting at 2 WAP (Figure 2).

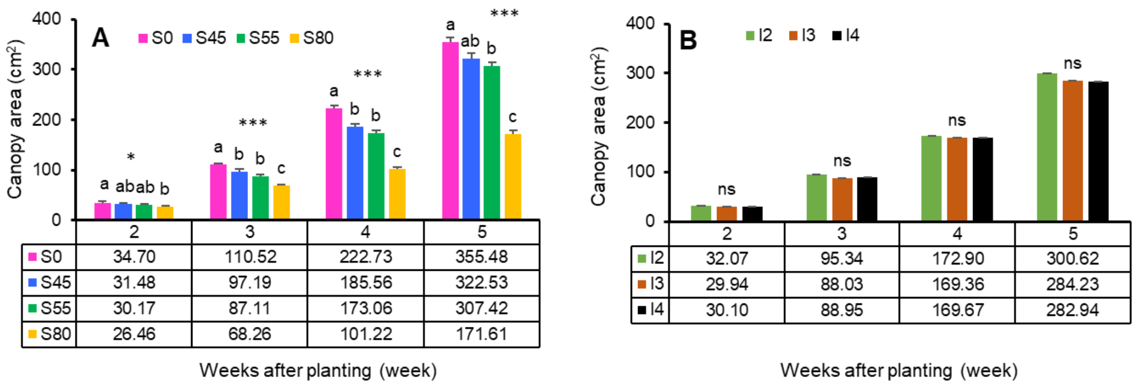
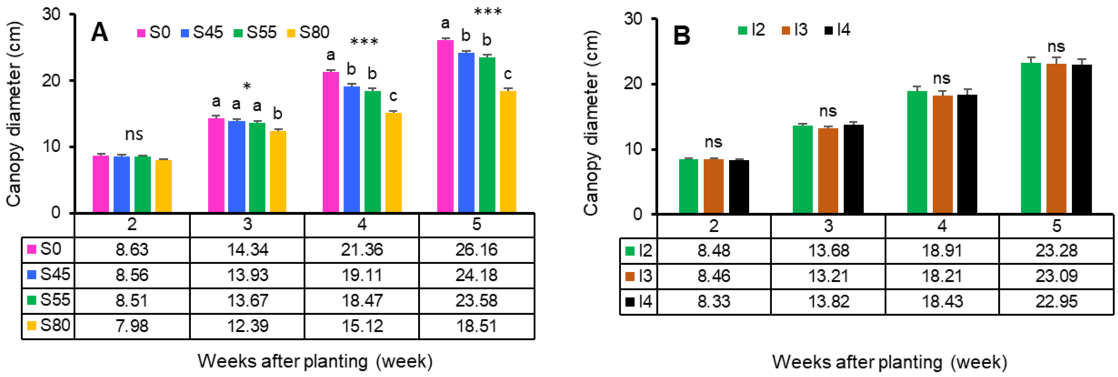


Figure 2. Brazilian spinach canopy area during early vegetative growth under different shading (A) and harvest period (B) treatments. The shading intensities consist of S0, S45, S55, and S80, while harvest periods include I2, I3, and I4. The ns= non-significant difference at P<0.05; *= significant difference at P<0.05; ***= significant difference at P<0.001.

Branches of Brazilian spinach significantly influenced canopy diameter, with elongation affecting canopy expansion. Shading conditions inhibited branch growth, while full sunlight cultivation led to a wider canopy than under S45, S55, and S80 (Figure 3).

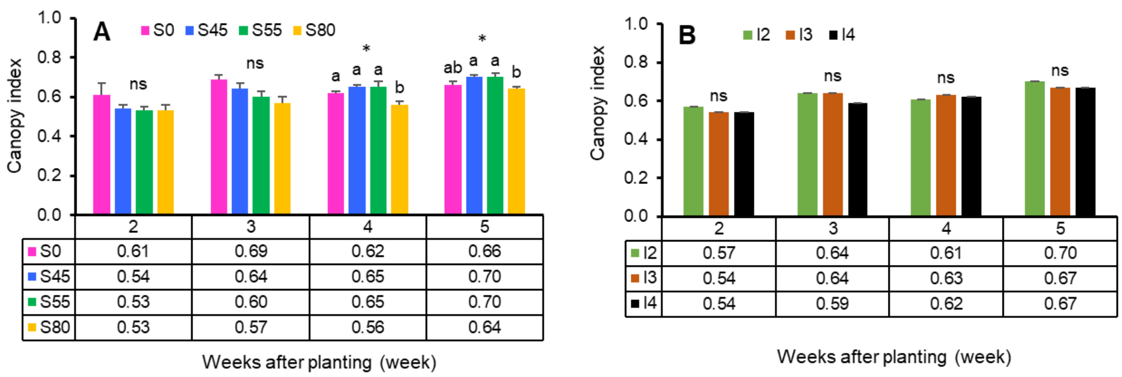


	2	3	4	5
S0	8.63	14.34	21.36	26.16
S45	8.56	13.93	19.11	24.18
S55	8.51	13.67	18.47	23.58
S80	7.98	12.39	15.12	18.51

	2	3	4	5
I2	8.48	13.68	18.91	23.28
I3	8.46	13.21	18.21	23.09
I4	8.33	13.82	18.43	22.95

Figure 3. Brazilian spinach canopy diameter during early vegetative growth under different shading (A) and harvest period (B) treatments. Shading intensities consist of S0, S45, S55, and S80, while harvest periods include I2, I3, and I4. The ns= non-significant difference at P<0.05; *= significant difference at P<0.05; ***= significant difference at P<0.001.

Leaf and branch growth significantly affected canopy density, with dominant growth resulting in a denser canopy, most detectable between 4 and 5 WAP. Brazilian spinach cultivated under shading conditions, specifically S80, showed reduced leaf size and branch elongation, leading to lower canopy density (Figure 4).



	2	3	4	5
S0	0.61	0.69	0.62	0.66
S45	0.54	0.64	0.65	0.70
S55	0.53	0.60	0.65	0.70
S80	0.53	0.57	0.56	0.64

	2	3	4	5
I2	0.57	0.64	0.61	0.70
I3	0.54	0.64	0.63	0.67
I4	0.54	0.59	0.62	0.67

Figure 4. Canopy index for early vegetative growth at different shading and harvest period treatments. The shading intensities consist of S0, S45, S55, and S80, while harvest periods

include I2, I3, and I4. The ns= non-significant difference at P<0.05; *= significant difference at P<0.05.

The results showed that the canopy growth of Brazilian spinach was more hindered at S80 than when unshaded. The constituent organs of the canopy, such as leaves and branches, endured stunting, which prevented the canopy from growing. According to Fadilah et al. (2022), denser shading intensity inhibited the growth of purple Pak Choi leaves. Wan et al. (2020) reported that plants cultivated in the shading areas produced less photosynthetic performance than those exposed to full sunlight. Moreover, Liang et al. (2020) reiterated the significance of shading for plants, which led to photosynthesis decline, resulting in reduced carbon flow. The inhibition of vegetative organ growth, particularly the canopy in Brazilian spinach, was attributed to decreased carbon flow, which occurred all through the early growth cycle. The phenomenon of reduced vegetative organ development due to shading during the early growth stage has been identified in various vegetable crops, including chili (Kesumawati et al., 2020).

The SPAD value is a method for evaluating leaf nitrogen and chlorophyll content, with a positive relationship between these factors. Brazilian spinach leaf's SPAD value was affected by shading treatments, with differences observed in each treatment from the early growth stage at 2 WAP. Furthermore, leaves grown at S0 showed a higher SPAD value compared to under different shading intensities, with a significant rise starting at 4 WAP. This trend was similarly observed in S45 and S55, but Brazilian spinach cultivated at S80 showed a stagnation trend, persisting until the completion of the 2 to 5 WAP early growth stage (Figure 5).

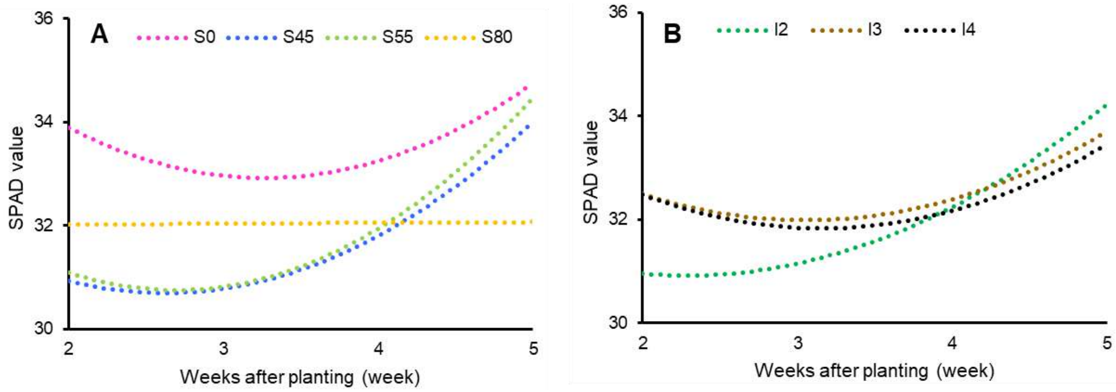


Figure 5. The SPAD value of Brazilian spinach leaves during early vegetative growth under different shading (A) and harvest period (A) treatments. The shading intensities consist of S0, S45, S55, and S80, while harvest periods include I2, I3, and I4.

The SPAD value is a very reliable method widely used for assessing leaf chlorophyll and nitrogen content due to the positive correlation of SPAD with these two parameters (Song et al., 2021; Farnisa et al., 2023). Prior research confirmed the capacity of specific leafy vegetables, including *Talinum paniculatum* (Lakitan et al., 2021b) and spinach (Mendoza-Tafolla et al., 2019), to proficiently evaluate and track the quantities of leaf chlorophyll and nitrogen content.

Brazilian spinach grown under S0 had a higher SPAD value than the shaded counterparts, showing that shading reduced the solubility of chlorophyll and nitrogen. Wang et al. (2020a) found that shading affected nitrogen solubility, leading to a decrease in the content found in leaves. Li et al. (2020) identified the biochemical alterations caused by shading stress, particularly in plants subjected to S80.

Brazilian spinach growth after harvested

The vegetative growth of Brazilian spinach after harvest was examined at 5 WAP, where branch growth was compared under different shading conditions, harvest periods, and interaction effects. Cuttings cultivated under S80 produced shorter branches at 11 WAP, but S0, S45, and S55 treatments generated comparable levels of branch elongation until 9 WAP. Those grown at S45 had an increased rate of branch elongation, particularly at 10 and 11 WAP (Table 1).

The elongation of Brazilian spinach branches was influenced by harvesting periods, where less frequent harvesting led to the highest elongation, specifically from 7 to 11 WAP. Interactions between shading intensities and harvesting periods were observed, starting at 9 WAP, with the results showing the importance of harvesting frequency in influencing growth.

Reduced elongation of Brazilian spinach branches at S80 was observed in this research, showing a decrease in the allocation of photosynthetic products. This is due to reduced levels of non-structural carbohydrates, which are essential for growth (Yamashita et al., 2020), but photosynthesis was optimized at S0, leading to increased branch growth.

The increased frequency of harvesting inhibits branch growth, potentially altering the distribution of photosynthetic products and triggering a more rapid initiation of new leaves (Oliveira et al., 2021). Additionally, Raza et al. (2019) reported that maize plants with a higher number of removed leaves allocated more photosynthetic resources toward the remaining leaves, as evidenced by area enhancement.

Table 1. Elongation of Brazilian spinach branches after harvesting at different shading intensities, harvest periods, and the interactions between both treatments.

Treatment	Weeks after planting (week)						
	5	6	7	8	9	10	11
Shading							
S0	11.98 ± 0.16 a	15.89 ± 0.17 a	19.18 ± 0.43 a	22.31 ± 0.38 a	24.16 ± 0.34 a	25.22 ± 0.33 b	26.17 ± 0.53 a
S45	11.63 ± 0.33 ab	15.11 ± 0.39 ab	19.00 ± 0.55 a	22.02 ± 0.76 a	24.30 ± 0.70 a	28.23 ± 0.95 a	29.02 ± 1.20 a
S55	11.03 ± 0.17 b	14.00 ± 0.22 b	18.03 ± 0.57 a	20.74 ± 0.80 a	22.32 ± 0.85 a	25.91 ± 0.95 b	26.74 ± 1.07 a
S80	8.68 ± 0.13 c	10.25 ± 0.17 c	12.66 ± 0.36 b	14.47 ± 0.51 b	14.93 ± 0.54 b	15.97 ± 0.76 c	16.46 ± 0.75 b
Probability	***	***	***	***	***	***	***
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Harvest period							
I2	11.06 ± 0.41	14.03 ± 0.69	15.80 ± 0.80 b	18.51 ± 1.09 c	19.66 ± 1.21 c	21.59 ± 1.41 c	21.90 ± 1.36 c
I3	10.72 ± 0.42	13.80 ± 0.71	18.07 ± 0.90 a	19.54 ± 1.02 b	21.75 ± 1.26 b	24.31 ± 1.58 b	24.95 ± 1.61 b
I4	10.70 ± 0.44	13.60 ± 0.66	17.78 ± 0.85 a	21.61 ± 0.98 a	22.88 ± 1.17 a	25.60 ± 1.45 a	26.94 ± 1.61 a
Probability	ns	Ns	***	***	***	***	***
P-value	0.186	0.198	<0.001	<0.001	<0.001	<0.001	<0.001
Shading x harvest period							
S0I2	12.23 ± 0.12	16.05 ± 0.34	18.55 ± 0.54	21.47 ± 0.44	23.22 ± 0.49 cd	24.24 ± 0.58 de	24.57 ± 0.62 de
S0I3	11.93 ± 0.41	16.20 ± 0.20	20.31 ± 0.72	22.40 ± 0.85	24.85 ± 0.40 b	25.68 ± 0.39 cd	26.91 ± 0.63 bc
S0I4	11.77 ± 0.27	15.41 ± 0.14	19.08 ± 0.44	23.08 ± 0.41	24.41 ± 0.53 bc	25.74 ± 0.34 cd	27.04 ± 0.78 bc
S45I2	11.99 ± 0.62	15.51 ± 1.10	17.45 ± 0.72	20.97 ± 1.52	22.53 ± 0.85 d	25.43 ± 1.20 cd	25.43 ± 0.91 cd
S45I3	10.89 ± 0.71	14.68 ± 0.52	19.57 ± 0.59	20.87 ± 0.95	23.77 ± 0.60 bcd	28.07 ± 0.83 b	28.54 ± 0.87 b
S45I4	12.01 ± 0.19	15.14 ± 0.41	19.99 ± 0.95	24.24 ± 0.20	26.59 ± 0.68 a	31.19 ± 0.67 b	33.08 ± 0.99 a
S55I2	11.08 ± 0.11	13.97 ± 0.22	16.00 ± 0.56	18.64 ± 0.81	19.53 ± 0.71 d	22.70 ± 0.97 e	23.19 ± 0.82 e
S55I3	11.37 ± 0.44	14.21 ± 0.67	19.19 ± 0.59	20.58 ± 1.41	23.50 ± 1.48 bcd	27.88 ± 1.25 b	28.27 ± 1.61 b
S55I4	10.64 ± 0.03	13.81 ± 0.21	18.90 ± 0.28	23.00 ± 0.45	23.92 ± 0.49 bcd	27.15 ± 0.70 b	28.77 ± 1.02 b
S80I2	8.95 ± 0.06	10.58 ± 0.13	11.61 ± 0.27	12.97 ± 0.37	13.32 ± 0.40 h	13.98 ± 0.60 g	14.41 ± 0.53 g
S80I3	8.70 ± 0.32	10.13 ± 0.37	13.22 ± 0.70	14.33 ± 0.60	14.89 ± 0.22 g	15.62 ± 0.39 g	16.09 ± 0.29 g
S80I4	8.38 ± 0.11	10.04 ± 0.32	13.14 ± 0.38	16.11 ± 0.38	16.59 ± 0.75 f	18.32 ± 1.27f	18.87 ± 1.17 f
Probability	ns	Ns	ns	ns	**	**	**
P-value	0.237	0.89	0.211	0.383	0.009	0.008	0.003

Remark: The ns= non-significant difference at P<0.05; **= significant difference at P<0.01; ***= significant difference at P<0.001.

Brazilian spinach showed significant differences in leaf growth when treated with different shading intensities and harvesting periods. Leaf growth was more dominant at S0 compared to at S45, S55, and S80, as presented in (Table 2). This showed early leaf growth at S0 with accelerated aging and increased pest susceptibility, resulting in a higher proportion of non-marketable leaves compared to those cultivated under shading.

Frequent harvesting of Brazilian spinach led to the initiation of young leaves, producing more marketable types. This was evident in the yield of commercially viable leaves all through the I2 and I3 periods. However, during I3, a significant proportion of non-marketable leaves were produced due to aging. Extended periods such as I4 often lack the capacity for leaf initiation, resulting in a decreased yield of both marketable and non-marketable leaves. The interaction of shading intensities and harvesting periods affected leaf growth, with the most significant impact observed at S80, specifically during I4.

Leaf initiation in Brazilian spinach was higher at S0 compared to S45-S80, affecting both marketable and non-marketable leaves due to reduced carbohydrate accumulation and allocation (Hussain et al., 2020). Shading conditions inhibited plant growth, while lack of shading accelerated leaf senescence due to enhanced photosynthesis. Direct sunlight exposure accelerates aging processes in plants, such as sweet basil (Castronuovo et al., 2019). However, without shade, spinach is more susceptible to pest infestation, leading to an increased prevalence of non-marketable leaves. The implementation of shading at a specific density is a viable strategy for controlling pests.

Brazilian spinach harvesting, similar to leaf and shoot pruning, has been found to increase yield. Dheeraj et al. (2022) found that pruning at the apical meristem increased growth-promoting hormones, specifically cytokinin. Additionally, Xu et al. (2020a) reported that pruning tomato plants elevated cytokinin hormone levels. Regarding this aspect, cytokinin influences cell division processes, such as during leaf development. Harvesting Brazilian spinach at I2 and I3 periods resulted in elevated cytokinin levels, enhancing leaf initiation and generating a greater marketable yield.

Table 2. Brazilian spinach yield at different shading intensities, harvest periods, and interactions.

Treatment	Marketable yield		Non-marketable yield	
	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
Shading				
S0	109.07 ± 8.55 a	13.16 ± 0.91 a	46.99 ± 2 .32 a	6.85 ± 0.41 a
S45	60.04 ± 3.57 b	5.80 ± 0.33 b	33.40 ± 2.74 b	3.44 ± 0.57 b
S55	52.63 ± 3.19 b	5.14 ± 0.37 b	32.92 ± 3.18 b	3.53 ± 0.35 b
S80	14.76 ± 1.04 c	1.22 ± 0.10 c	4.68 ± 1.00 c	0.49 ± 0.10 c
Probability	***	***	***	***
P-value	<0.001	<0.001	<0.001	<0.001
Harvest period				
I2	67.22 ± 12.80 a	6.58 ± 1.47 a	28.90 ± 5.03 b	3.57 ± 0.71 b
I3	60.95 ± 11.05 a	7.05 ± 1.56 a	33.69 ± 5.76 a	4.41 ± 0.84 a
I4	49.20 ± 7.66 b	5.37 ± 0.97 b	25.91 ± 4.06 b	2.75 ± 0.62 c
Probability	***	**	**	**
P-value	<0.001	0.001	0.008	0.002
Shading x harvest period				
S0I2	130.79 ±7.94 a	14.25 ± 0.64 a	48.17± 1.71 a	6.85 ± 0.57 ab
S0I3	117.30 ± 7.13 a	15.44 ± 0.36 a	47.58± 7.46 a	7.73 ± 0.93 a
S0I4	79.10 ± 6.74 b	9.78 ± 0.77 b	45.22± 1.88 a	5.98 ± 0.19 bc
S45I2	69.51 ± 5.90 bc	6.12 ± 0.76 c	35.36± 3.37 bc	3.88 ± 0.40 def
S45I3	51.03 ± 3.93 d	5.40 ± 0.38 c	40.14± 0.18 ab	4.97 ± 0.14 cd
S45I4	59.57 ± 4.14 cd	5.90 ± 0.66 c	24.70± 4.02 d	1.48 ± 0.74 gh
S55I2	53.05 ± 6.86 d	4.76 ± 0.80 c	28.81± 2.70cd	3.21 ± 0.36 ef
S55I3	58.36 ± 4.61cd	5.88 ± 0.59 c	44.67± 2.41 ab	4.69 ± 0.48 cde
S55I4	46.47 ± 4.24d	4.78 ± 0.52 c	25.30± 1.38 d	2.69 ± 0.15 fg
S80I2	15.54 ± 1.30e	1.17 ± 0.14 d	3.27± 0.19 e	0.37 ± 0.02 h
S80I3	17.09 ± 1.32e	1.48 ± 0.13 d	2.37± 1.07 e	0.28 ± 0.18 h
S80I4	11.63 ± 1.28e	1.01 ± 0.14 d	8.41±0.52 e	0.83 ± 0.06 h
Probability	***	***	**	*
P-value	<0.001	<0.001	0.008	0.05

Remark: *= significant difference at $P<0.05$; **= significant difference at $P<0.01$; ***= significant difference at $P<0.001$.

The metabolism of Brazilian spinach was influenced by shading intensities and harvesting periods. Increased metabolism activity was observed at S0 compared to S45, S55, and S80, evidenced by the carbon and nitrogen levels (Table 3). Higher metabolism correlates with enhanced nitrogen usage, which is crucial for plant metabolic processes. Therefore, increasing fertilization frequency is necessary for plants exposed to sunlight. In this research, leaf nitrogen concentration remained consistent across different harvesting periods, suggesting no significant differences in nitrogen content.

The C:N ratio calculation can be used to determine leaf hardness in Brazilian spinach. This research showed that lack of shading produced tougher leaves, decreasing with increased shading levels. However, comparable levels of leaf hardness were observed across different harvesting periods.

Shading significantly impacts the carbon reduction and nitrogen enrichment of leaves, affecting the process of photosynthesis. Light intensity and photosynthesis are connected, as investigations show reduced carbon and nitrogen content in shaded plants. For instance, Tang et al. (2022) found that plants exposed to modest levels of irradiation had reduced concentrations of leaf non-structural carbohydrates, leading to a reduction in carbon content. Nitrogen accumulation in shaded Brazilian spinach leaves was due to limited light availability, hindering conversion into organic nitrogen compounds essential for plant metabolic processes. Gao et al. (2020) identified that prolonged shading reduced nitrogen utilization efficiency in plants. Wang et al. (2020b) observed increased content of non-structural carbohydrates resulting from the procedure of removing plant leaves. However, elevated harvesting frequency triggers the growth of new leaves, driving the movement of nitrogen toward younger leaves. Jasinski et al. (2021) stated that nitrogen mobilization occurs from older to younger leaves.

Table 3. Carbon, Nitrogen, and C-N ratio of Brazilian spinach leaves at different shading intensities, harvest periods, and interactions.

Treatment	Carbon (%)	Nitrogen (%)	C-N ratio
	Shading		
S0	34.64	2.83	12.28
S45	32.75	4.56	7.20
S55	34.21	4.77	7.19
S80	34.32	4.99	6.84
	Harvest period		
I2	35.85	4.38	8.74
I3	33.90	4.42	8.10
I4	32.20	4.07	8.30
	Shading x harvest period		
S0I2	34.23	2.63	13.00
S0I3	34.28	2.90	11.83
S0I4	35.42	2.95	12.01
S45I2	32.02	4.70	6.81
S45I3	33.89	4.77	7.10
S45I4	32.34	4.20	7.70
S55I2	36.50	5.01	7.29
S55I3	32.14	4.94	6.50
S55I4	34.00	4.36	7.79
S80I2	40.66	5.16	7.88
S80I3	35.30	5.07	6.96
S80I4	27.01	4.76	5.68

The presence of shading in Brazilian spinach is connected to biomass production, as unshaded conditions enhance photosynthesis, leading to increased biomass production. However, under intense shading conditions, it reduces biomass in various plant parts. Harvesting over extended periods such as I3 and I4 resulted in elevated biomass accumulation, particularly in the stems and branches.

Brazilian spinach subjected to shading conditions and extended harvesting periods showed inhibited growth due to restricted photosynthetic activity. This caused the restricted allocation of photosynthetic products to individual plant organs. Previous research has shown that shading reduced biomass accumulation and caused alterations in plant morphological characteristics (Xu et al., 2020b). Additionally, photosynthetic activity in certain cases is redistributed to use other organs apart from the leaves. This corresponds with the report by Yu et al. (2019) that when plants age and the organs enter senescence, photosynthetic flux redirects toward the stem, suggesting the importance of allocating photosynthetic products to support plant growth through periodic harvesting.

Table 4. The dry weight of Brazilian spinach organs at different shading intensities, harvest periods, and interactions at 13 WAP

Treatment	Stem dry weight (g)	Branch dry weight (g)	Leaf dry weight (g)	Root dry weight (g)	Total dry weight (g)
			Shading		
S0	2.35 ± 0.19 a	14.24 ± 0.98 a	7.92 ± 0.88 a	5.28 ± 1.20 a	29.79 ± 2.58 a
S45	1.36 ± 0.20 b	5.39 ± 0.98 b	3.70 ± 0.79 b	2.07 ± 0.81 ab	12.53 ± 2.28 b
S55	1.26 ± 0.13 b	5.21 ± 0.79 b	4.58 ± 0.69 b	1.13 ± 0.19 b	12.19 ± 1.48 b
S80	0.25 ± 0.04 c	0.40 ± 0.05 c	0.85 ± 0.62 c	0.44 ± 0.20 b	1.94 ± 0.79 c
Probability	***	***	***	*	***
P-value	< 0.001	< 0.001	< 0.001	0.046	< 0.001
			Harvest period		
I2	1.05 ± 0.27 b	4.39 ± 1.28 c	3.63 ± 0.68 b	1.73 ± 0.48	10.80 ± 2.53 b
I3	1.25 ± 0.20 b	6.12 ± 1.49 b	2.88 ± 0.65 b	2.19 ± 0.86	12.44 ± 2.80 b
I4	1.62 ± 0.27 a	8.42 ± 1.90 a	6.28 ± 1.22 a	2.77 ± 1.05	19.09 ± 4.19 a
Probability	**	***	***	ns	***
P-value	0.002	< 0.001	< 0.001	0.517	< 0.001
			Shading x harvest period		
S0I2	2.35 ± 0.53	11.26 ± 0.97 c	6.43 ± 0.44 b	4.27 ± 0.49	24.31 ± 1.12 bc
S0I3	2.03 ± 0.04	13.92 ± 0.61 b	5.98 ± 0.36 b	4.20 ± 2.25	26.14 ± 1.73 b
S0I4	2.66 ± 0.25	17.54 ± 0.62 a	11.36 ± 0.28 a	7.36 ± 2.97	38.92 ± 3.54 a
S45I2	0.78 ± 0.13	3.26 ± 0.70 fg	2.49 ± 0.43 cd	0.88 ± 0.26	7.41 ± 1.49 fgh
S45I3	1.41 ± 0.06	4.63 ± 0.33 fg	2.34 ± 0.22 cd	3.30 ± 2.41	11.68 ± 2.69 ef
S45I4	1.89 ± 0.38	8.29 ± 2.05 d	6.28 ± 1.52 b	2.04 ± 0.76	18.50 ± 4.66 cd
S55I2	0.87 ± 0.12	2.80 ± 0.67 gh	3.59 ± 0.81 c	1.06 ± 0.37	8.31 ± 1.41 fg
S55I3	1.35 ± 0.17	5.51 ± 0.62 ef	3.18 ± 0.34 c	1.00 ± 0.09	11.04 ± 0.58 ef
S55I4	1. 57 ± 0.14	7.32 ± 1.23 de	6.99 ± 0.77 b	1.32 ± 0.52	17.21 ± 1.74 de
S80I2	0.19 ± 0.01	0.24 ± 0.04 h	2.00 ± 1.84 cd	0.73 ± 0.64	3.17 ± 2.43 gh
S80I3	0.22 ± 0.03	0.41 ± 0.05 h	0.03 ± 0.03 d	0.26 ± 0.16	0.92 ± 0.27 h
S80I4	0.35 ± 0.09	0.53 ± 0.11 h	0.50 ± 0.33 d	0.34 ± 0.05	1.71 ± 0.49 gh
Probability	ns	*	*	ns	*
P-value	0.013	0.049	0.013	0.584	0.034

Remark: The ns= non-significant difference at P<0.05; *= significant difference at P<0.05; **= significant difference at P<0.01; ***= significant difference at P<0.001.

The visual appearance of Brazilian spinach at different treatments

The shoot appearance of Brazilian spinach under different shading conditions and harvesting periods was examined in this research. Unshaded areas had a denser appearance, while different harvesting periods tended to produce related results (Figure 6). Cuttings cultivated at S0 had greater root growth and a higher density of root hairs than under shading, while samples subjected to varying harvesting periods of I2, I3, and I4 showed similar root morphology without any significant differences (Figure 7).

Varying morphological characteristics were identified in Brazilian spinach under different treatments. Shading causes alterations in plant organs, as observed on soybean stems, which experience inhibited growth (Castronuovo et al., 2019). Similarly, Cao et al. (2022) reported that *Cynodon dactylon* shoot experienced alterations and root development showed a distinct reaction of declined growth when exposed to shading stress. Fu et al. (2020) found reductions in root volume and length, showing decreased root growth under these conditions.

Brazilian spinach with a longer harvesting period (I4) showed an increase in branches and stems, with a higher presence of mature leaves. Pruning at longer intervals increased plant height and branches in *Talinum Paniculatum*, while extending harvesting intervals hindered fresh leaf commencement (Purbajanti et al., 2019). Bessonova et al. (2023) found that removing leaves and branches led to the development of shoot features with a greater number and area of leaves.

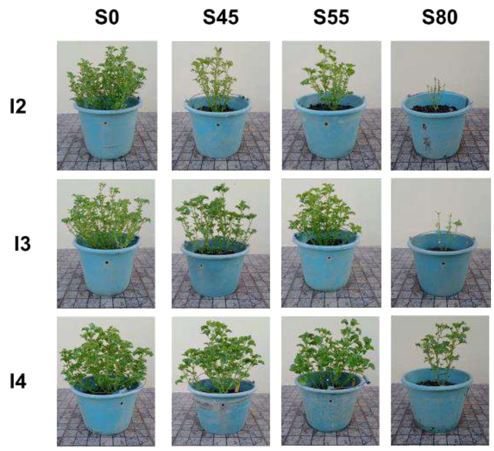


Figure 6. Visualization of Brazilian spinach shoots under different shading and harvest period treatments at 13 WAP. Shading intensities consist of S0, S45, S55, and S80, while the harvest periods include I2, I3, and I4. Photos: Strayker Ali Muda.

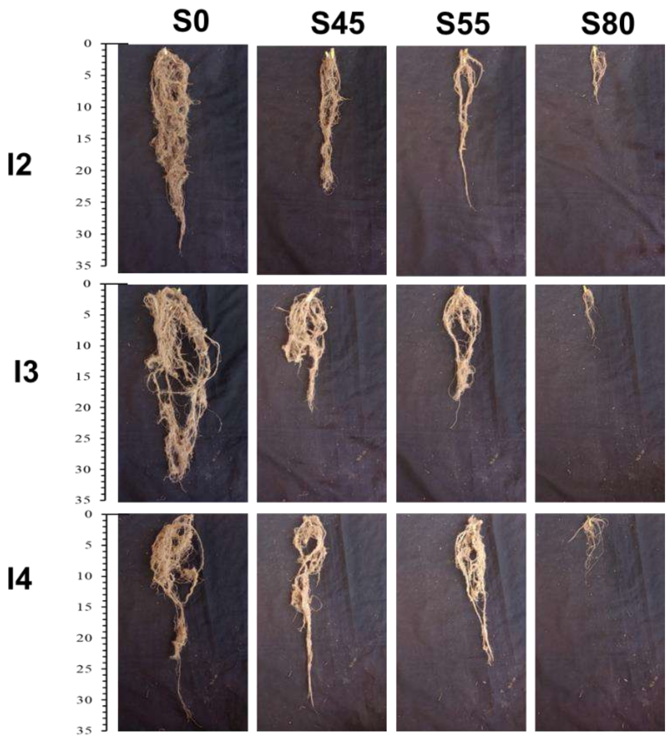


Figure 7. Visualization of Brazilian spinach roots under different shading and harvest period treatments at 13 WAP. Shading intensities consist of S0, S45, S55, and S80, while the harvest periods include I2, I3, and I4. Photos: Strayker Ali Muda.

Water content at different treatments

The water availability for Brazilian spinach growth was represented by substrate moisture (SM). Increased shading intensity (S80) leads to higher moisture content, decreasing direct sunlight exposure and evaporation, which results in reduced water loss. However, Brazilian spinach grown in areas with lower or total absence of shading showed higher evaporation rates, signifying more water loss, as evidenced by SM levels (Figure 8). The use of shading can effectively adjust microclimate conditions, such as SM levels (Bollman et al., 2021). This research found that shaded growing media had higher moisture levels than the unshaded counterparts, and the addition of shading reduced evaporation rate, as confirmed by Khawam et al. (2019).

Frequent harvesting of Brazil spinach reduces the coverage of the substrate surface by the canopy, causing higher evaporation rates and reduced water availability. This phenomenon correlates with the results of Huang et al. (2020) who provided empirical evidence regarding plants with lower canopy density experiencing higher rates of water loss through evaporation.

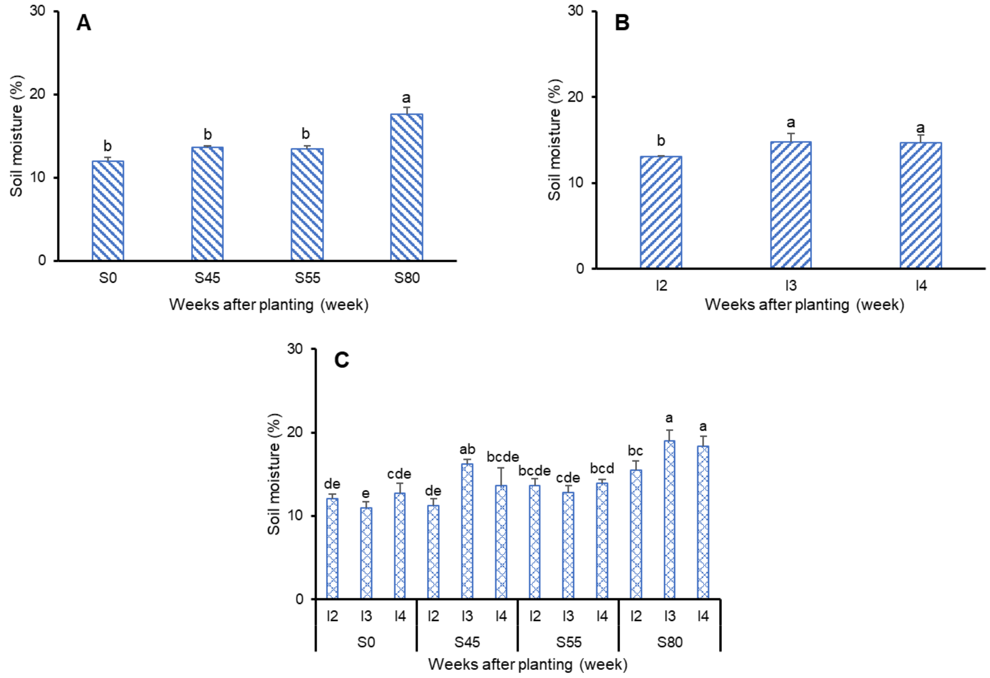


Figure 8. Substrate moisture at different shading intensities (A), harvest periods (B), and the interactions between both treatments (C). Shading intensities consist of S0, S45, S55, and S80, while the harvest periods include I2, I3, and I4.

CONCLUSION

In conclusion, the results showed that the adoption of shading led to a decrease in the growth and yield of Brazilian spinach through alterations in root, stem, branch, and leaf morphological characteristics. Additionally, the implementation of 2 WAP significantly increased marketable yield to 67.22 g. Interactions between shading intensities and harvest periods primarily influenced SM, the length of branches, yield, as well as dry weight of branches and leaves. Therefore, Brazilian spinach was recommended to be cultivated in Indonesia under direct sunlight and harvested every two weeks.

AUTHORS' CONTRIBUTION

Strayker Ali Muda: Research execution, data collection, data analysis, drawing graph, and writing original manuscript. Benyamin Lakitan: Corresponding author, idea conception, methodology, data interpretation, manuscript revision, and supervision. Andi Wijaya, Susilawati Susilawati: Experimental design, methodology, data analysis, manuscript revision, and supervision. Yakup Yakup, Zaidan Zaidan: data interpretation, methodology, and manuscript revision.

ACKNOWLEDGMENT

The authors are grateful for the suggestions, input, and corrections provided by anonymous reviewers and the editors of the *Revista de Agricultura Neotropical* journal. The authors are also grateful for the funding assistance obtained through the "Penelitian Profesi" Program, Universitas Sriwijaya 2024.

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