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

by Yakup Parto

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

Strayker Ali Muda[1], Benyamin Lakitan[1], Andi Wijaya[1], Susilawati Susilawati[1], Zaidan Zaidan[1], Yakup Yakup[1]

[1] Universitas Sriwijaya, College of Agriculture, Indralaya, South Sumatra, Indonesia. E-mail: straykerali@gmail.com, blakitan60@unsri.ac.id, andiwijayadani@yahoo.ac.id, susilawati@fp.unsri.ac.id, zaidanpnegara@fp.unsri.ac.id, yakup.parto@yahoo.com

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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 g). Based on these observations, the cultivation of Brazilian spinach in unshaded areas with a biweekly harvesting routine was recommended.

Keywords: Harvest time, Leafy green, Vegetables, Plant acclimatization, Solar irradiation.

Crescimento e rendimento do espinafre brasileiro sob diferentes intensidades de sombreamento e períodos de colheita em um ecossistema urbano de planície tropical

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 cm^2), 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 g). 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, Aclimatação da planta, Irradiação solar.

1. 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.

2. Material and Methods

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.

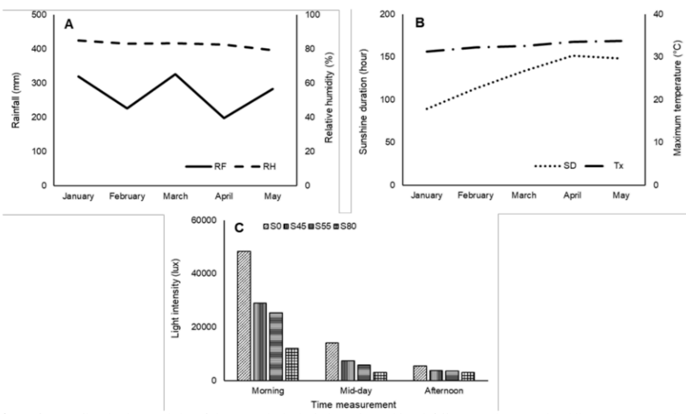

Figure 1. Agroclimatic characteristics of the research site include total monthly rainfall (RF) and average humidity (RH) (A), sunshine duration (SD) and average maximum temperature (Tx) (B), and average light intensity of each treatment (C). (Source: Indonesian Agency for Meteorology, Climatology, and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

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.

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 Kjeldahl-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. 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).

3. Results and Discussion

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). 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).

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).

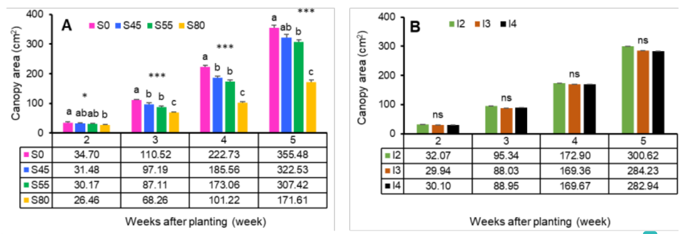

Figure 2. Brazilian spinach canopy area during early vegetative growth under different shading (A) and harvest period (B) treatments. The shading intensities consist of S0, S45, S55, and S80, while harvest periods include I2, I3, and I4. The ns= non-significant difference at P<0.05; *= significant difference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

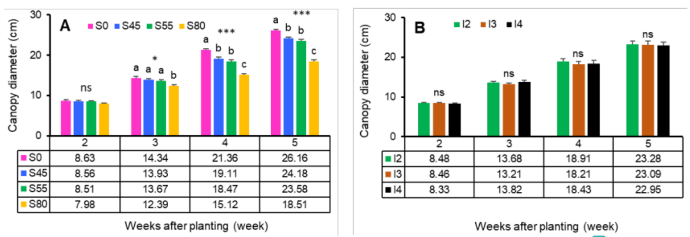

Figure 3. Brazilian spinach canopy diameter during early vegetative growth under different shading (A) and harvest period (B) treatments. Shading intensities consist of S0, S45, S55, and S80, while harvest periods include I2, I3, and I4. The ns= non-significant difference at P<0.05; *= significant difference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

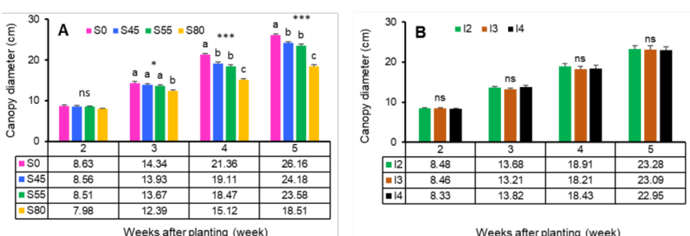

Figure 4. Canopy index for early vegetative growth at different shading and harvest period treatments. The shading intensities consist of S0, S45, S55, and S80, while harvest periods include I2, I3, and I4. The ns= non-significant difference at P<0.05; *= significant difference at P<0.05.

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).

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.

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.

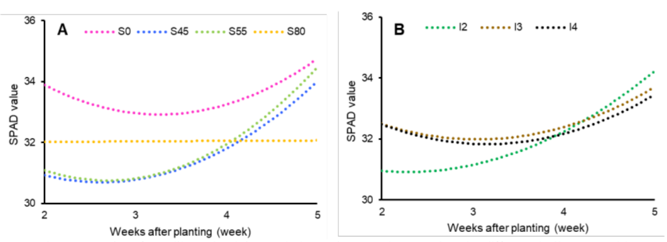

Figure 5. The SPAD value of Brazilian spinach leaves during early vegetative growth under different shading (A) and harvest period (A) treatments. The shading intensities consist of S0, S45, S55, and S80, while harvest periods include I2, I3, and I4 .

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.

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.

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.

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± 2.70 cd	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 ± 4.24 d	4.78 ± 0.52 c	25.30± 1.38 d	2.69 ± 0.15 fg
S80I2	15.54 ± 1.30 e	1.17 ± 0.14 d	3.27± 0.19 e	0.37 ± 0.02 h
S80I3	17.09 ± 1.32 e	1.48 ± 0.13 d	2.37± 1.07 e	0.28 ± 0.18 h
S80I4	11.63 ± 1.28 e	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.

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

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.

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 (Table 4).

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. 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.

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.

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.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.

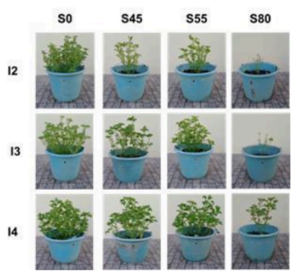

Figure 6. Brazilian spinach shoots under different shading and harvest period treatments at 13 WAP. Shading intensities consist of S0, S45, S55, and S80, while the harvest periods include I2, I3, and I4. Photos: Strayker Ali Muda

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 7. 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.

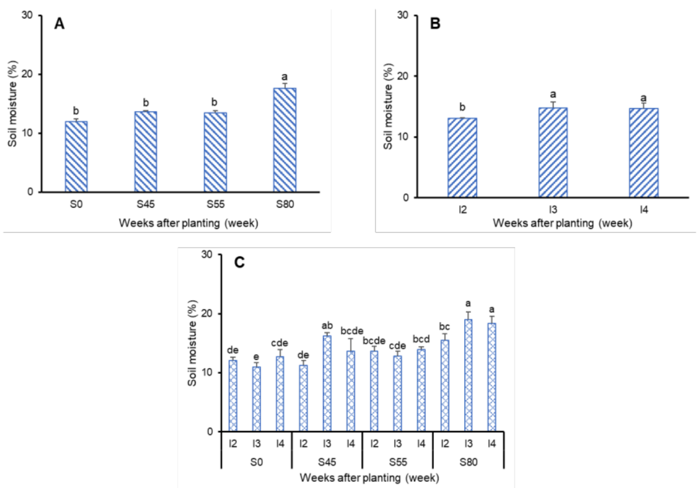

Figure 8. Substrate moisture at different shading intensities (A), harvest periods (B), and the interactions between both treatments (C). Shading intensities consist of S0, S45, S55, and S80, while the harvest periods include I2, I3, and I4.

4. Conclusions

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.

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