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17 **BRAZILIAN SPINACH GROWTH UNDER DIFFERENT SHADING**
18 **INTENSITIES AND HARVESTING PERIODS IN LOWLAND TROPICAL**
19 **URBAN ECOSYSTEM**

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25
26 **ABSTRACT**

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

41 **KEYWORDS**

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

44
45 **INTRODUCTION**

46 Brazilian spinach (*Alternanthera sissoo*) is a leafy vegetable originating from
47 Brazil. As reported by Ikram et al. (2021), Brazilian spinach is rich in flavonoids,
48 vitamins, minerals, and other antioxidants, which have been found to have positive effects

49 on human health. The cultivation and use of this particular plant by the Indonesian
50 population are infrequent, leading to its classification as a rather rare plant species.
51 Indonesia's agroclimatology exhibits similarities to its indigenous location, hence
52 indicating the potential for cultivating this plant within the country.

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

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

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

MATERIALS AND METHODS

Research site and agroclimatic characteristic

The research was carried out in the Jakabaring research facility located in Palembang (104°46'44"E, 3°01'35"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.

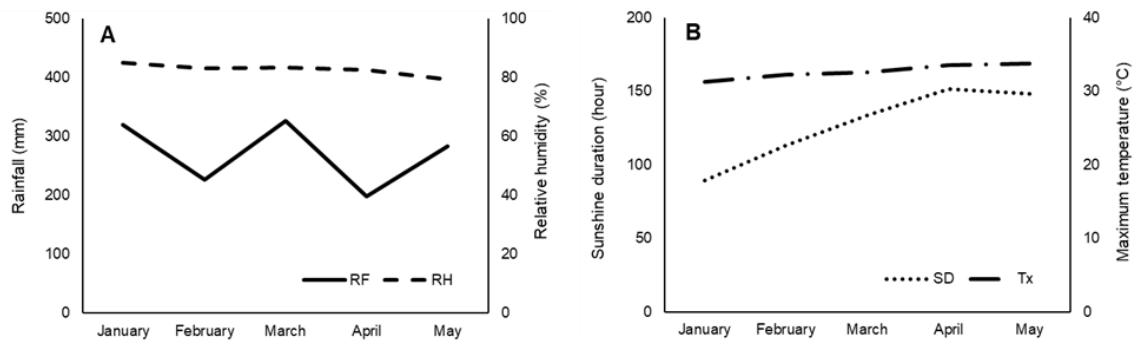


Figure 1. Agroclimatic characteristics in research location as indicated by total monthly rainfall (RF) and average humidity (RH) (A), and total monthly sunshine duration (SD) and average maximum temperature (Tx) (B). (Source: Indonesian Agency for Meteorology, Climatology and Geophysics).

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

107 selecting shading intensity at three separate levels, namely no-shading (S0), 45% shading
108 (S45), 55% shading (S55), and 85% shading (S80). Furthermore, treatment for the harvest
109 period started after the simultaneous harvesting, which was carried out at 5 weeks after
110 planting (WAP). The designated harvest period has three different intervals, namely
111 appearing per 2 weeks, per 3 weeks, and per 4 weeks, symbolised as H2, H3, and H4,
112 respectively.

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

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

123 *Data collection*

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

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

139 2014). Substrate moisture (SM) was measured using a soil moisture meter (PMS-714,
140 Lutron Electronics Canada, Inc., Pennsylvania, USA). The carbon, nitrogen, and C:N
141 ratios were examined using Kjeldahl-Titrimetry in the Integrated Laboratory of
142 Sampoerna Agro. Tbk.

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

147 *Data analysis*

148 All data collected was analysed using the RStudio software version 1.14.1717 for
149 Windows (developed by RStudio team, PBC, Boston, MA). Significant differences
150 among treatments were tested using the least significant difference (LSD) procedure at
151 $p < 0.05$.

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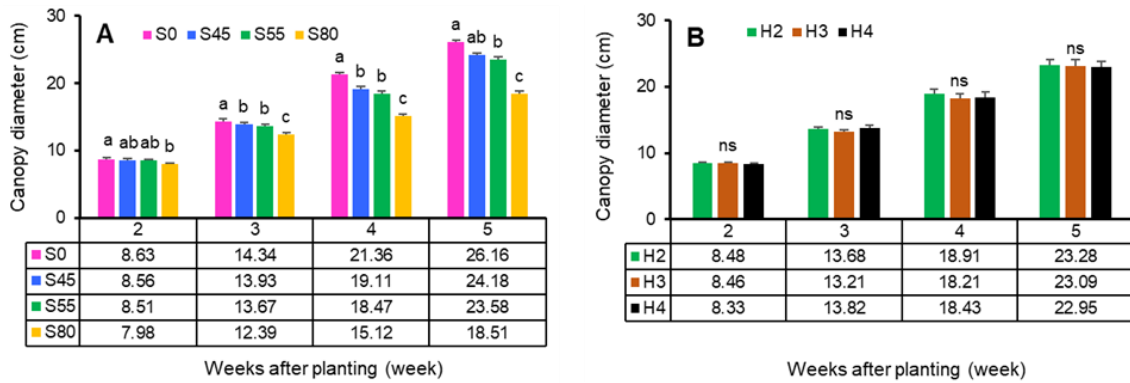
153 **RESULTS AND DISCUSSION**

154 *The Brazilian spinach growth during early vegetative growth before harvested*

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

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

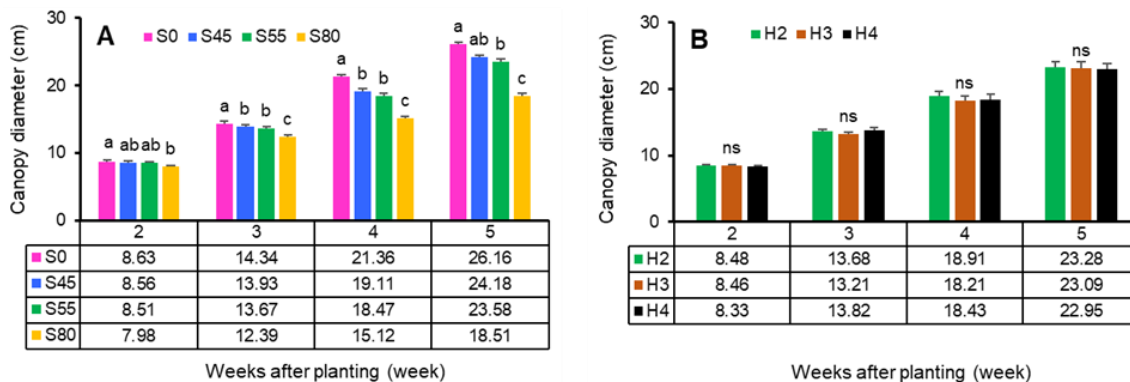
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168

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

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

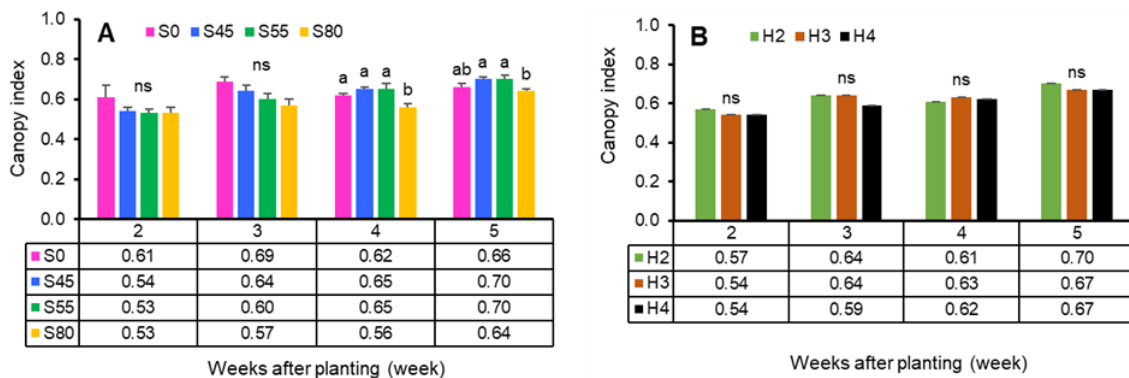


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179 Figure 3. Brazilian spinach canopy diameter on early vegetative growth on different
 180 shading (A) and harvest period (B) treatment. The shading consists of no shading (S0),
 181 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3,
 182 and H4 represent harvest period per 2 weeks, 3 weeks, and 4 weeks, respectively. The ns
 183 mean non-significant difference at $p < 0.05$.

184 The growth of leaf and branch significantly affects canopy density, with dominant
 185 growth resulting in a denser canopy as represented by canopy density. The effect is most
 186 noticeable between 4 and 5 weeks after planting. Brazilian spinach grown under shading

187 conditions, especially S80, showed reduced leaf size and branch elongation, resulting in
 188 lower canopy density (Figure 4).

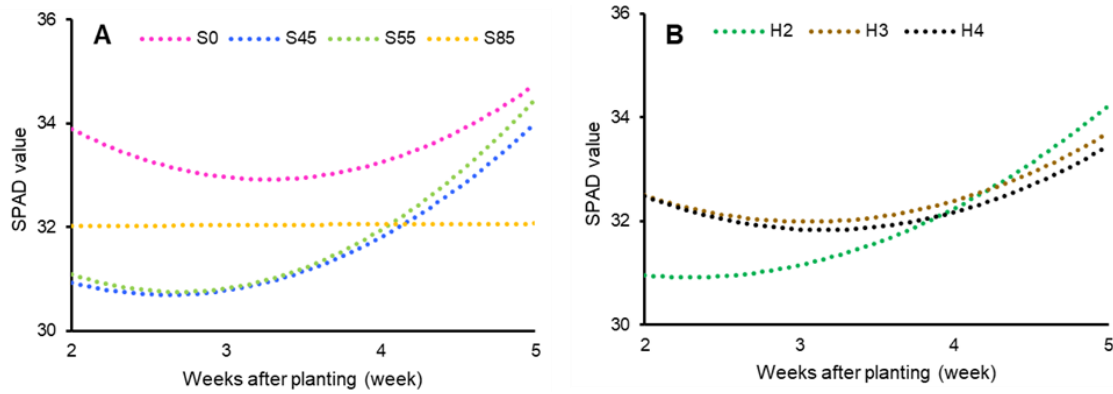


189
 190 Figure 4. Canopy index on early vegetative growth on different shading and harvest
 191 period as treatment. The shading consists of no shading (S0), 45% shading (S45), 55%
 192 shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent harvest
 193 period per 2 weeks, 3 weeks, and 4 weeks, respectively. The ns mean non-significant
 194 difference at $p < 0.05$.

195 According to this research's findings, Brazilian spinach's canopy growth was more
 196 hindered under greater shading (S80) than it was unshaded. The constituent organs of the
 197 canopy, such as the leaves and branches, endure stunted growth, which prevents the
 198 canopy from growing. According to Fadilah et al. (2022), denser shading intensity was
 199 shown to inhibit purple pakchoy leaf growth. According to the findings of Wan et al.
 200 (2020), plants planted in the shading area produce less photosynthetic performance than
 201 plants grown in full sunlight. Moreover, Liang et al. (2020) have underscored the
 202 significance of shading for plants, noting that it leads to a decrease in photosynthesis,
 203 resulting in a reduction in carbon flow. The inhibition of vegetative organ growth,
 204 particularly the canopy, in Brazilian spinach is attributed to the decreased carbon flow.
 205 This reduction in carbon flow occurs throughout the early growth cycle. The phenomenon
 206 of reduced vegetative organ development due to shading during the early growth phase
 207 has been documented in various vegetable crops, including chili (Kesumawati et al.,
 208 2020).

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

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



218 Figure 5. The SPAD value on early vegetative growth on different shading (A) and
 219 harvest period (A) treatment. The shading consists of no shading (S0), 45% shading
 220 (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent
 221 harvest period per 2 weeks, 3 weeks.
 222

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

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

236 *Brazilian spinach growth after harvested*

237 The vegetative growth of Brazilian spinach after harvest was conducted at 5 weeks
 238 after planting. The growth of branch was compared under different shading conditions,

239 harvest periods, and their interaction effects. Brazil spinach grown under S80 exhibited
240 shorter branches as early as 11 weeks after planting. However, different shading
241 treatments (S0, S45, and S55) showed comparable levels of branch elongation until 9
242 weeks after planting. Brazilian spinach grown in S45 had an increased rate of branch
243 elongation, particularly at 10 and 11 weeks after planting.

244 The elongation of the Brazilian spinach branch was influenced by the harvesting
245 period. Less frequent harvesting leads to the highest branch elongation, especially from
246 7 to 11 weeks after planting. An interaction between shading level and harvesting period
247 treatment was observed, starting within 9 weeks after planting. This highlights the
248 importance of harvesting frequency in influencing Brazilian spinach growth.

249 The study revealed a decrease in branch elongation in Brazilian spinach at S80,
250 indicating a decrease in the allocation of photosynthetic products. This is due to reduced
251 levels of non-structural carbohydrates, which are essential for growth (Yamashita et al.,
252 2020). However, in the S0 treatment, photosynthesis is optimised, leading to increased
253 branch growth.

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

260 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
<i>Shading</i>							
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
<i>Harvest period</i>							
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
<i>Shading x harvest period</i>							
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

261 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)
<i>Shading</i>				
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
<i>Harvest period</i>				
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
<i>Shading x harvest period</i>				
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 ± 2.70 cd	3.21 ± 0.36 ef
S55H3	58.36 ± 4.61 cd	5.88 ± 0.59 c	44.67 ± 2.41 a	4.69 ± 0.48 cde
S55H4	46.47 ± 4.24 d	4.78 ± 0.52 c	25.30 ± 1.38 d	2.69 ± 0.15 fg
S80H2	15.54 ± 1.30 e	1.17 ± 0.14 d	3.27 ± 0.19 e	0.37 ± 0.02 h
S80H3	17.09 ± 1.32 e	1.48 ± 0.13 d	2.37 ± 1.07 e	0.28 ± 0.18 h
S80H4	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
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
<i>Shading</i>			
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
<i>Harvest period</i>			
H2	35.85	4.38	8.74
H3	33.90	4.42	8.10
H4	32.20	4.07	8.30
<i>Shading x harvest periode</i>			
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)
<i>Shading</i>				
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
<i>Harvest period</i>				
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
<i>Shading x harvest period</i>				
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.

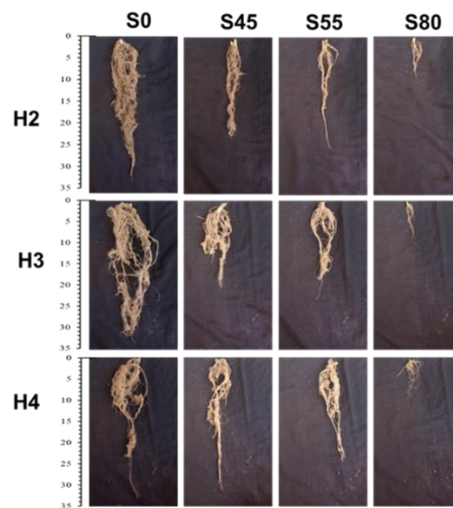


Figure 7. Visualization of Brazilian spinach root on different shading and harvest period at 13 weeks after planting (WAP). Shading treatment consist of S0: no-shading, S45: 45% shading, S55: 55% shading, and S80: 80% shading. Meanwhile, the harvest period consists of H2: per 2 weeks, H3: per 3 weeks,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

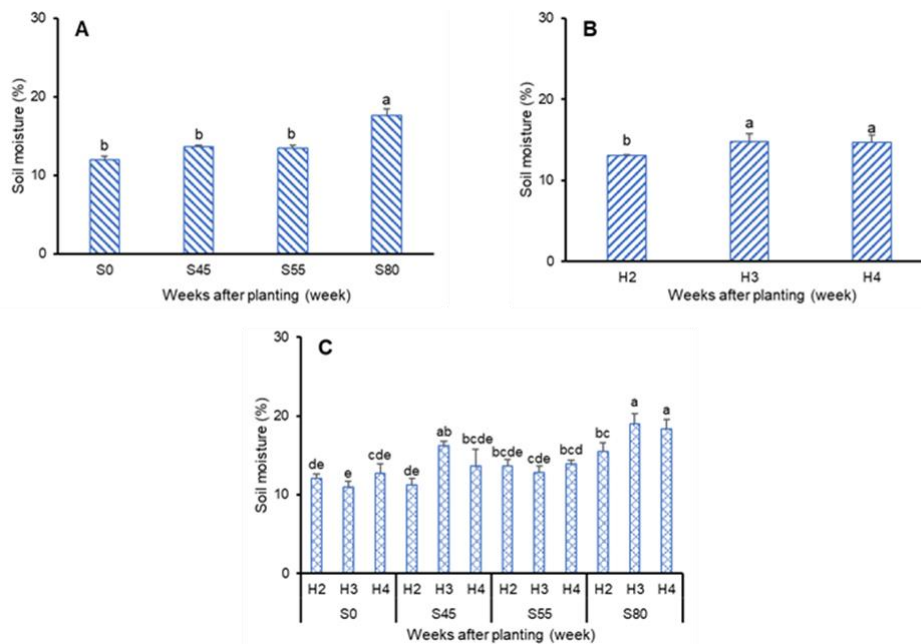


Figure 8. Substrate moisture on different shading (A), harvest period (B), and their interaction (C). Shading treatment consist of S0: no-shading, S45: shading 45%, S55: shading 55%, and S80: shading 80%. Meanwhile, the harvest period consists of H2: every 2 weeks, H3: every 3 weeks, and H4: every 4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

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
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2. Pre-review (31 Januari 2024)

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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 (104°46'44"E, 3°01'35"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.

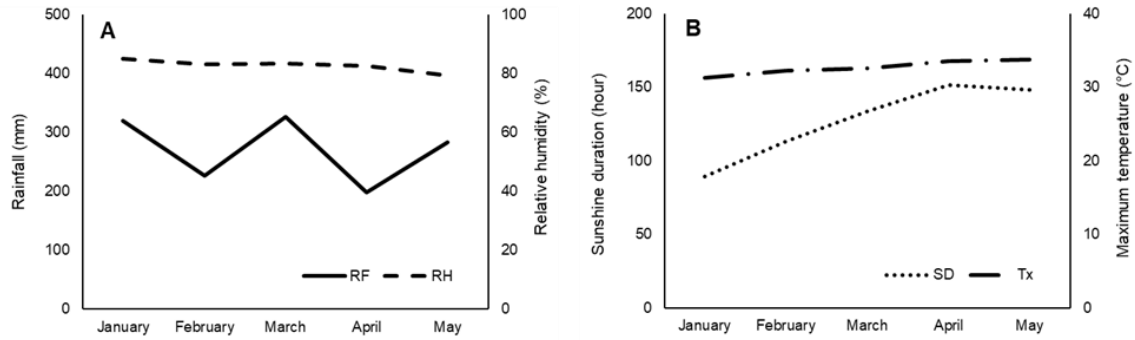


Figure 1. Agroclimatic characteristics in research location as indicated by total monthly rainfall (RF) and average humidity (RH) (A), and total monthly sunshine duration (SD) and average maximum temperature (Tx) (B). (Source: Indonesian Agency for Meteorology, Climatology and Geophysics).

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 Easlou & 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 Kjeldahl-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).

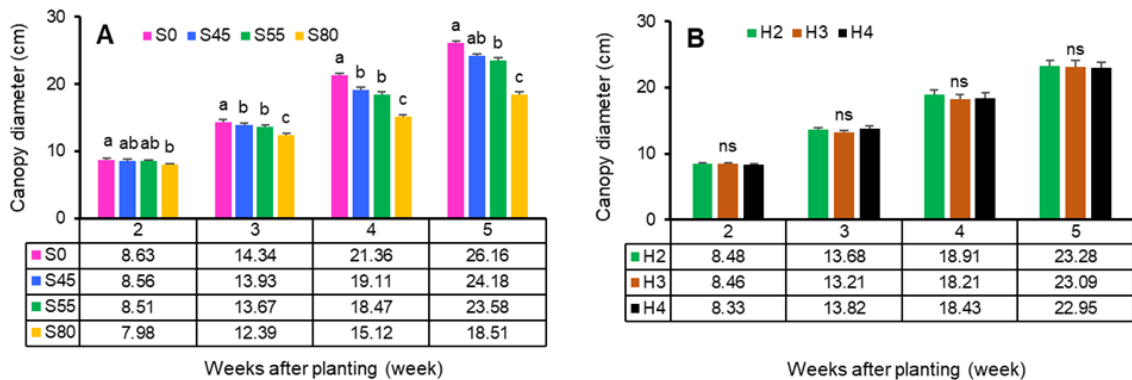


Figure 2. Brazilian spinach canopy area on early vegetative growth on different shading (A) and harvest period (B) treatment. The shading consists of no shading (S0), 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent harvest period per 2 weeks, 3 weeks,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

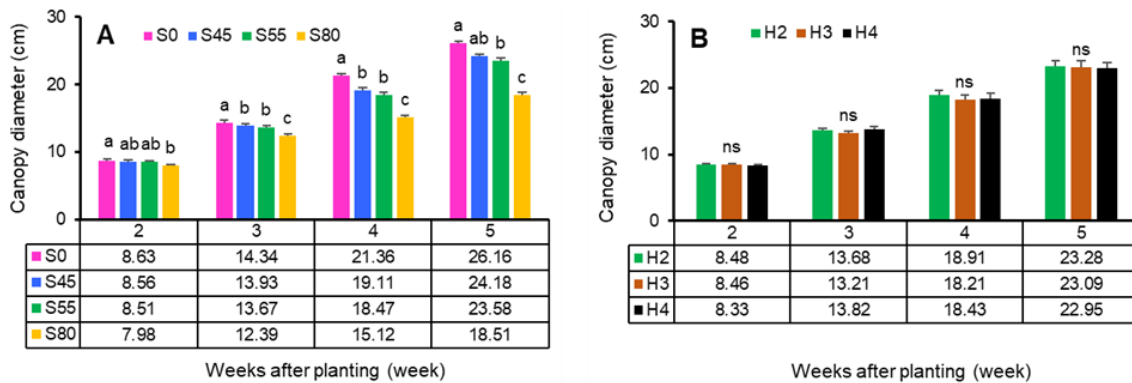


Figure 3. Brazilian spinach canopy diameter on early vegetative growth on different shading (A) and harvest period (B) treatment. The shading consists of no shading (S0), 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent harvest period per 2 weeks, 3 weeks,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

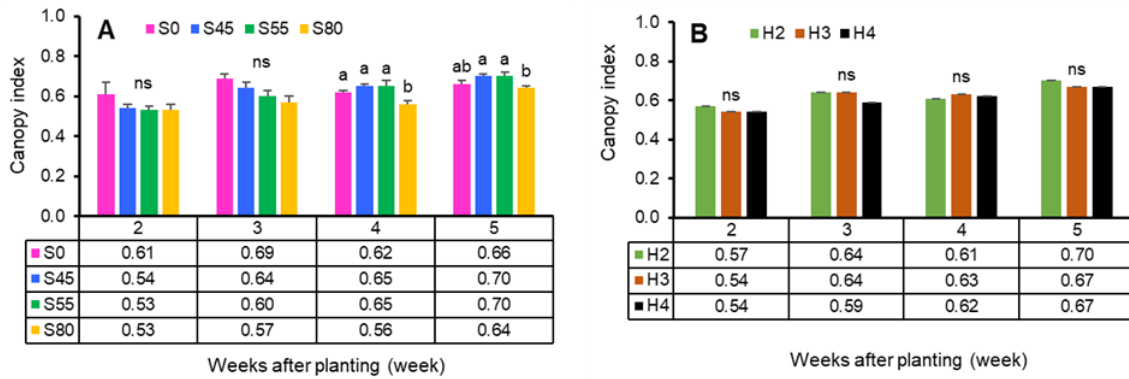


Figure 4. Canopy index on early vegetative growth on different shading and harvest period as treatment. The shading consists of no shading (S0), 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent harvest period per 2 weeks, 3 weeks, and 4 weeks, respectively. 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.

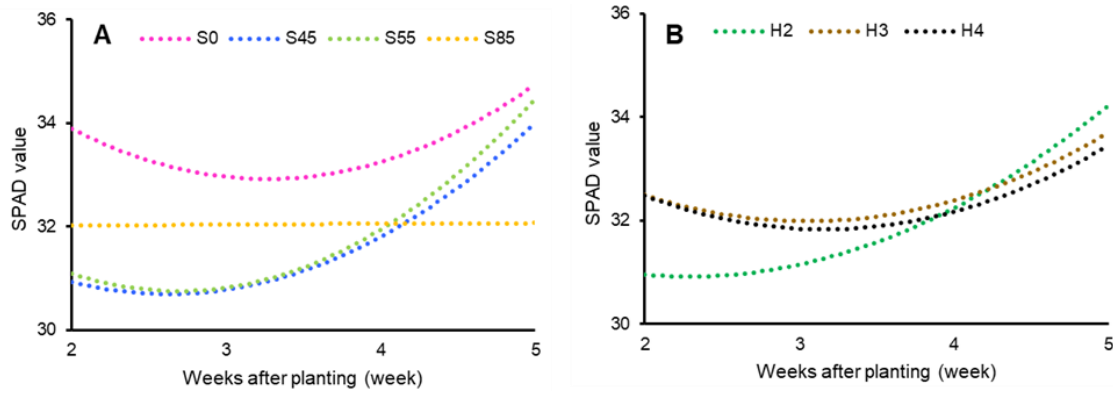


Figure 5. The SPAD value on early vegetative growth on different shading (A) and harvest period (A) treatment. The shading consists of no shading (S0), 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent harvest period per 2 weeks, 3 weeks.

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.

1 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
<i>Shading</i>							
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
<i>Harvest period</i>							
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
<i>Shading x harvest period</i>							
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

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

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

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

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

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

35 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)
<i>Shading</i>				
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
<i>Harvest period</i>				
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
<i>Shading x harvest period</i>				
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 ± 2.70 cd	3.21 ± 0.36 ef
S55H3	58.36 ± 4.61 cd	5.88 ± 0.59 c	44.67 ± 2.41 a	4.69 ± 0.48 cde
S55H4	46.47 ± 4.24 d	4.78 ± 0.52 c	25.30 ± 1.38 d	2.69 ± 0.15 fg
S80H2	15.54 ± 1.30 e	1.17 ± 0.14 d	3.27 ± 0.19 e	0.37 ± 0.02 h
S80H3	17.09 ± 1.32 e	1.48 ± 0.13 d	2.37 ± 1.07 e	0.28 ± 0.18 h
S80H4	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
LSD _{0.05}	14.781	1.587	9.092	1.587

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

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

45 The carbon-nitrogen ratio calculation can be used to determine leaf hardness in
 46 Brazilian spinach leaves. The study showed that unshaded (S0) areas yield more tough

47 leaves, decreasing with increased shading levels. Despite this, Brazilian spinach
48 consistently showed comparable levels of leaf hardness across harvesting periods.

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

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

Treatment	Carbon (%)	Nitrogen (%)	C-N ratio
<i>Shading</i>			
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
<i>Harvest period</i>			
H2	35.85	4.38	8.74
H3	33.90	4.42	8.10
H4	32.20	4.07	8.30
<i>Shading x harvest periode</i>			
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

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

66 The presence of shading in Brazilian spinach is linked to biomass production.
67 Under unshaded conditions, it enhances photosynthesis, leading to increased biomass
68 production. However, under intense shading conditions, it reduces biomass in various
69 parts of the plant. Harvesting over extended periods (H3 and H4) results in increased
70 biomass accumulation, particularly in the stem and branch in the final observation.
71 Brazilian spinach, when grown under shading conditions and extended harvesting,
72 showed inhibited growth due to restricted photosynthetic activity. This caused the
73 restricted allocation of photosynthetic products to individual plant organs. Studies have
74 shown that shading reduces biomass accumulation and alterations in plant morphological
75 traits (Xu et al., 2020b). Additionally, there is a distribution of photosynthetic activity that
76 utilizes plant organs beyond just leaves. This aligns with Yu et al. (2019) finding that
77 when plants age and their organs undergo senescence, photosynthetic flux redirects

78 towards the stem. This highlights the importance of considering the allocation of
 79 photosynthetic products to plant growth through periodic harvesting.

80 Table 4. Dry weight of Brazilian spinach organs on different shading, harvest period, and
 81 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)
<i>Shading</i>				
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
<i>Harvest period</i>				
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
<i>Shading x harvest period</i>				
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

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

83

84 *Visual appearance of Brazilian spinach on different treatment*

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

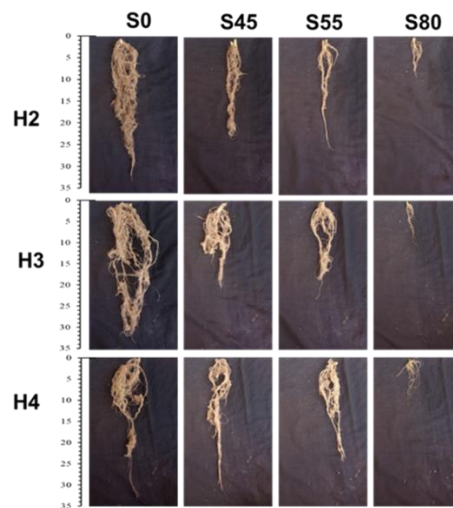
92 Brazilian spinach showed varying morphological traits under different treatments.
93 Shading causes alterations in plant organs, as shown on soybean stems, which experience
94 inhibited growth (Castronuovo et al., 2019). Cao et al. (2022) reported that *Cynodon*
95 *dactylon* shoot organs also experience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

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



105

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



111

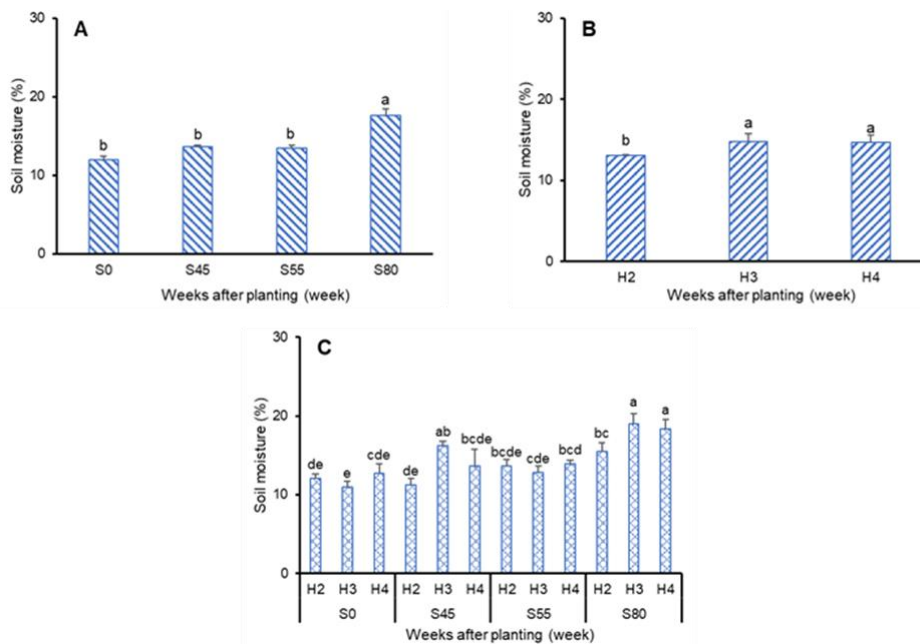
112 Figure 7. Visualization of Brazilian spinach root on different shading and harvest period
 113 at 13 weeks after planting (WAP). Shading treatment consist of S0: no-shading, S45: 45%
 114 shading, S55: 55% shading, and S80: 80% shading. Meanwhile, the harvest period
 115 consists of H2: per 2 weeks,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

117

118 *Water status on different treatment*

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

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



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

138

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.

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3. Review (07 Mei 2024)

Notifications

x

[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

REVISTA DE AGRICULTURA NEOTROPICAL

<http://periodicosonline.uems.br/index.php/agrineo>

287

288

289 **Reviewer 1**

290 **BRAZILIAN SPINACH GROWTH UNDER DIFFERENT SHADING**
291 **INTENSITIES AND HARVESTING PERIODS IN LOWLAND TROPICAL**
292 **URBAN ECOSYSTEM**

293
294 **ABSTRACT**

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

309 **KEYWORDS**

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

312
313 **INTRODUCTION**

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

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

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

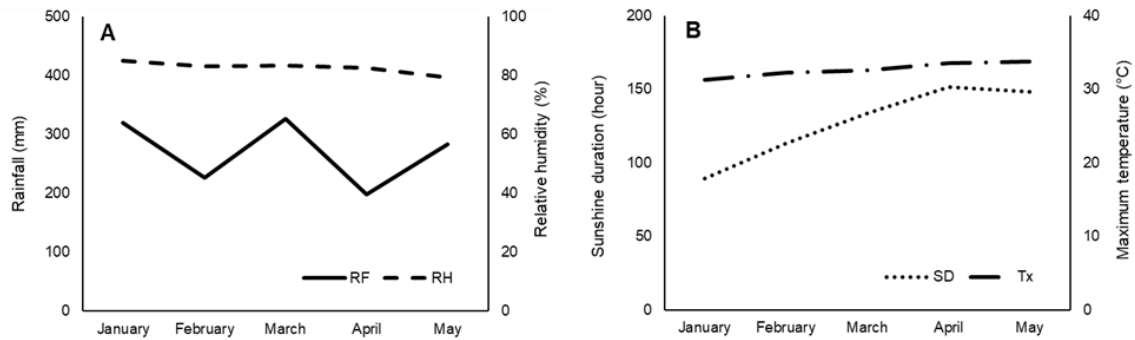
341 The cultivation of Brazilian spinach is characterized by its simplicity, since it may
342 be easily grown. Muda et al. (2022) reported that the propagation of Brazilian spinach
343 can be achieved via stem cuttings. There is an insufficient amount of research pertaining
344 to the adaptability of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

349 MATERIALS AND METHODS

350 *Research site and agroclimatic characteristic*

351 The research was carried out in the Jakabaring research facility located in
352 Palembang (104°46'44"E, 3°01'35"S), South Sumatra, Indonesia. This research began

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



357

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

362 *Cultivation and treatment procedures*

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

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

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

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

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

391 *Data collection*

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

404 The SPAD value was monitored using chlorophyll meters (SPAD-502 Plus,
405 Konica-Minolta Optics, Inc., Osaka, Japan). Canopy area was calculated using a digital
406 image scanner for Android (Easy Leaf Area software, developed by Easlou & Bloom
407 2014). Substrate moisture (SM) was measured using a soil moisture meter (PMS-714,
408 Lutron Electronics Canada, Inc., Pennsylvania, USA). The carbon, nitrogen, and C:N
409 ratios were examined using Kjeldahl-Titrimetry

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

414 *Data analysis*

415 All data collected was analysed using the RStudio software version 1.14.1717 for
 416 Windows (developed by RStudio team, PBC, Boston, MA). Significant differences
 417 among treatments were tested using the least significant difference (LSD) procedure at
 418 $p < 0.05$.

419

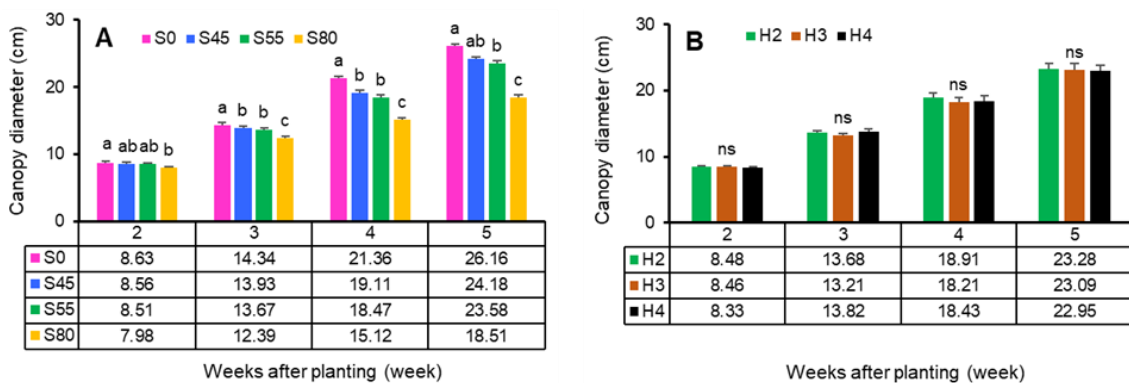
420 **RESULTS AND DISCUSSION**

421 *The Brazilian spinach growth during early vegetative growth before harvested*

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

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

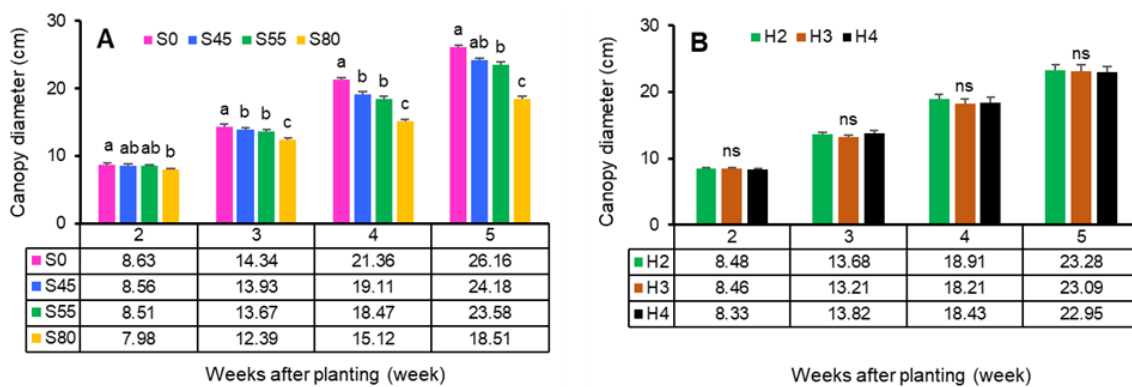
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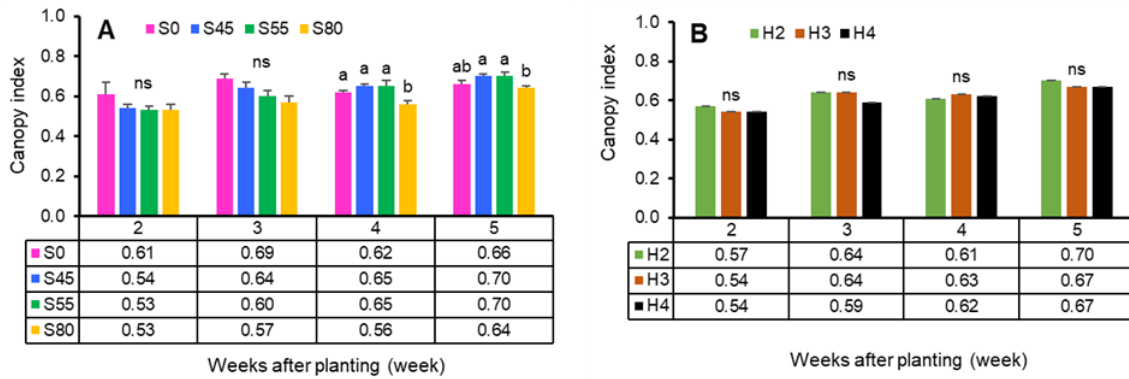
436 Figure 2. Brazilian spinach canopy area on early vegetative growth on different shading
 437 (A) and harvest period (B) treatment. The shading consists of no shading (S0), 45%
 438 shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4
 439 represent harvest period per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

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



445
 446 Figure 3. Brazilian spinach canopy diameter on early vegetative growth on different
 447 shading (A) and harvest period (B) treatment. The shading consists of no shading (S0),
 448 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3,
 449 and H4 represent harvest period per 2 weeks, 3 weeks, and 4 weeks, respectively. The ns
 450 mean non-significant difference at $p < 0.05$.

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



456

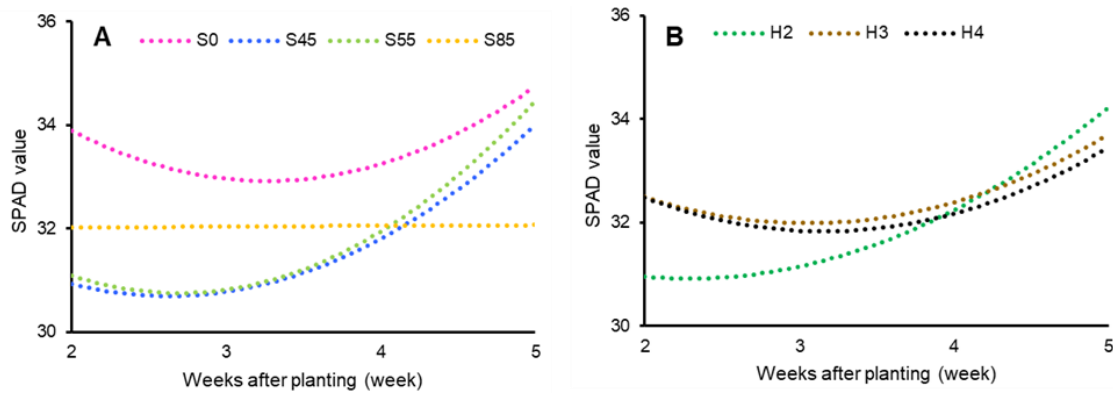
457 Figure 4. Canopy index on early vegetative growth on different shading and harvest
 458 period as treatment. The shading consists of no shading (S0), 45% shading (S45), 55%
 459 shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent harvest
 460 period per 2 weeks, 3 weeks, and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

462 According to this research's findings, Brazilian spinach's canopy growth was more
 463 hindered under greater shading (S80) than it was unshaded. The constituent organs of the
 464 canopy, such as the leaves and branches, endure stunted growth, which prevents the
 465 canopy from growing. According to Fadilah et al. (2022), denser shading intensity was
 466 shown to inhibit purple pakchoy leaf growth. According to the findings of Wan et al.
 467 (2020), plants planted in the shading area produce less photosynthetic performance than
 468 plants grown in full sunlight. Moreover, Liang et al. (2020) have underscored the
 469 significance of shading for plants, noting that it leads to a decrease in photosynthesis,
 470 resulting in a reduction in carbon flow. The inhibition of vegetative organ growth,
 471 particularly the canopy, in Brazilian spinach is attributed to the decreased carbon flow.
 472 This reduction in carbon flow occurs throughout the early growth cycle. The phenomenon
 473 of reduced vegetative organ development due to shading during the early growth phase
 474 has been documented in various vegetable crops, including chili (Kesumawati et al.,
 475 2020).

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

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

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



485
 486 Figure 5. The SPAD value on early vegetative growth on different shading (A) and
 487 harvest period (A) treatment. The shading consists of no shading (S0), 45% shading
 488 (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent
 489 harvest period per 2 weeks, 3 weeks.

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

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

503 *Brazilian spinach growth after harvested*

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

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

511 The elongation of the Brazilian spinach branch was influenced by the harvesting
512 period. Less frequent harvesting leads to the highest branch elongation, especially from
513 7 to 11 weeks after planting. An interaction between shading level and harvesting period
514 treatment was observed, starting within 9 weeks after planting. This highlights the
515 importance of harvesting frequency in influencing Brazilian spinach growth.

516 The study revealed a decrease in branch elongation in Brazilian spinach at S80,
517 indicating a decrease in the allocation of photosynthetic products. This is due to reduced
518 levels of non-structural carbohydrates, which are essential for growth (Yamashita et al.,
519 2020). However, in the S0 treatment, photosynthesis is optimised, leading to increased
520 branch growth.

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

527 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
<i>Shading</i>							
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
<i>Harvest period</i>							
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
<i>Shading x harvest period</i>							
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

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

529

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

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

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

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

562 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)
<i>Shading</i>				
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
<i>Harvest period</i>				
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
<i>Shading x harvest period</i>				
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 ± 2.70 cd	3.21 ± 0.36 ef
S55H3	58.36 ± 4.61 cd	5.88 ± 0.59 c	44.67 ± 2.41 a	4.69 ± 0.48 cde
S55H4	46.47 ± 4.24 d	4.78 ± 0.52 c	25.30 ± 1.38 d	2.69 ± 0.15 fg
S80H2	15.54 ± 1.30 e	1.17 ± 0.14 d	3.27 ± 0.19 e	0.37 ± 0.02 h
S80H3	17.09 ± 1.32 e	1.48 ± 0.13 d	2.37 ± 1.07 e	0.28 ± 0.18 h
S80H4	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
LSD _{0.05}	14.781	1.587	9.092	1.587

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

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

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

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

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

Treatment	Carbon (%)	Nitrogen (%)	C-N ratio
<i>Shading</i>			
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
<i>Harvest period</i>			
H2	35.85	4.38	8.74
H3	33.90	4.42	8.10
H4	32.20	4.07	8.30
<i>Shading x harvest periode</i>			
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

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

593 The presence of shading in Brazilian spinach is linked to biomass production.
 594 Under unshaded conditions, it enhances photosynthesis, leading to increased biomass
 595 production. However, under intense shading conditions, it reduces biomass in various
 596 parts of the plant. Harvesting over extended periods (H3 and H4) results in increased
 597 biomass accumulation, particularly in the stem and branch in the final observation.
 598 Brazilian spinach, when grown under shading conditions and extended harvesting,
 599 showed inhibited growth due to restricted photosynthetic activity. This caused the
 600 restricted allocation of photosynthetic products to individual plant organs. Studies have
 601 shown that shading reduces biomass accumulation and alterations in plant morphological
 602 traits (Xu et al., 2020b). Additionally, there is a distribution of photosynthetic activity that
 603 utilizes plant organs beyond just leaves. This aligns with Yu et al. (2019) finding that
 604 when plants age and their organs undergo senescence, photosynthetic flux redirects
 605 towards the stem. This highlights the importance of considering the allocation of
 606 photosynthetic products to plant growth through periodic harvesting.

607 Table 4. Dry weight of Brazilian spinach organs on different shading, harvest period, and
 608 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)
<i>Shading</i>				
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
<i>Harvest period</i>				
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
<i>Shading x harvest period</i>				
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

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

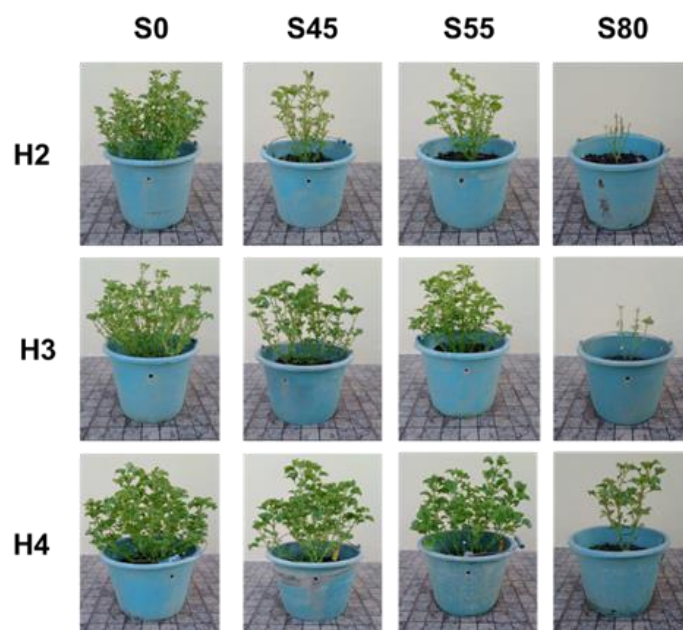
610

611 *Visual appearance of Brazilian spinach on different treatment*

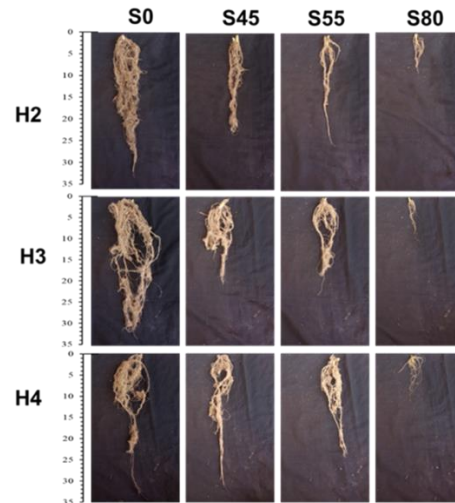
612 The study analysed the shoot appearance of Brazilian spinach under different
613 shading conditions and harvesting periods. Unshaded areas had a denser appearance,
614 while based on the harvesting period, treatments tend to show similarities with each other
615 (Figure 6). Furthermore, Brazilian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

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

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



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



638
639 Figure 7. Visualization of Brazilian spinach root on different shading and harvest period
640 at 13 weeks after planting (WAP). Shading treatment consist of S0: no-shading, S45: 45%
641 shading, S55: 55% shading, and S80: 80% shading. Meanwhile, the harvest period
642 consists of H2: per 2 weeks, H3: per 3 weeks, and H4: per 4 weeks. Photos: Strayker Ali
643 Muda.

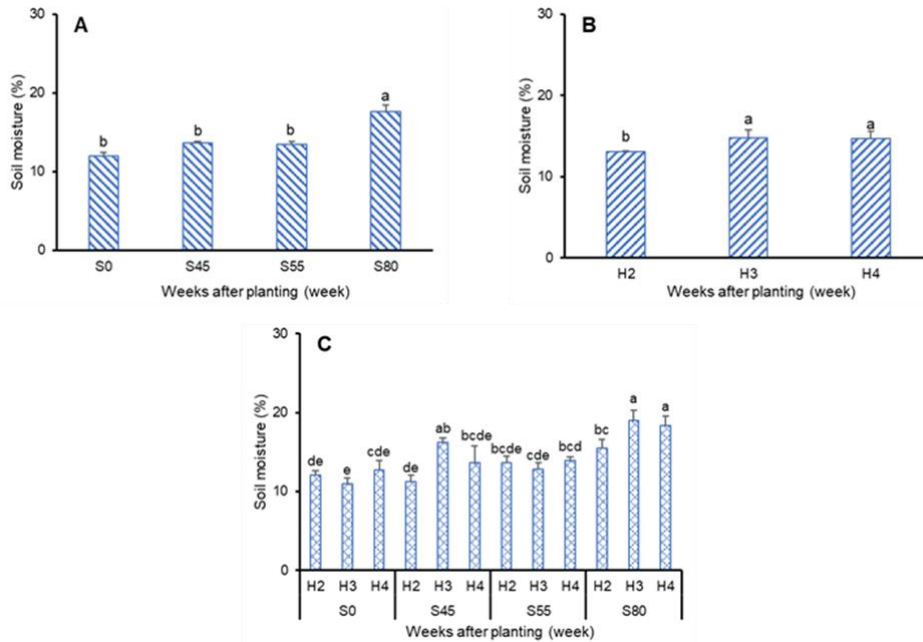
644

645 *Water status on different treatment*

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

655 The more frequently Brazil spinach is harvested, the wider the substrate surface is
656 not covered by the canopy, causing higher evaporation rates and reduced water

657 availability. This phenomenon aligns with the findings of Huang et al. (2020), who
 658 provided empirical evidence that plants with lower canopy density exhibit higher rates of
 659 water loss via evaporation.



660
 661 Figure 8. Substrate moisture on different shading (A), harvest period (B), and their
 662 interaction (C). Shading treatment consist of S0: no-shading, S45: shading 45%, S55:
 663 shading 55%, and S80: shading 80%. Meanwhile, the harvest period consists of H2: every
 664 2 weeks, H3: every 3 weeks, and H4: every 4 weeks.

665 CONCLUSION

666
 667 The adoption of shading led to a decrease in the growth and yield of Brazilian
 668 spinach through an alteration in the morphological traits of its root, stem, branch, and
 669 leaf. In addition, the implementation of a 2-week harvesting period led to increased
 670 Brazilian spinach growth and yield. Interactions between shading and harvest periods
 671 primarily pertain to the length of branches, yields (both marketable and non-marketable),
 672 dry weight of organs (namely branch and leaf), and substrate moisture.

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674
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677

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812

813

Reviewer 2

814

BRAZILIAN SPINACH GROWTH UNDER DIFFERENT SHADING

815

INTENSITIES AND HARVESTING PERIODS IN LOWLAND TROPICAL

816

URBAN ECOSYSTEM

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818

ABSTRACT

819

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.

833

KEYWORDS

834

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

836

837

INTRODUCTION

838

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.

844

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

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

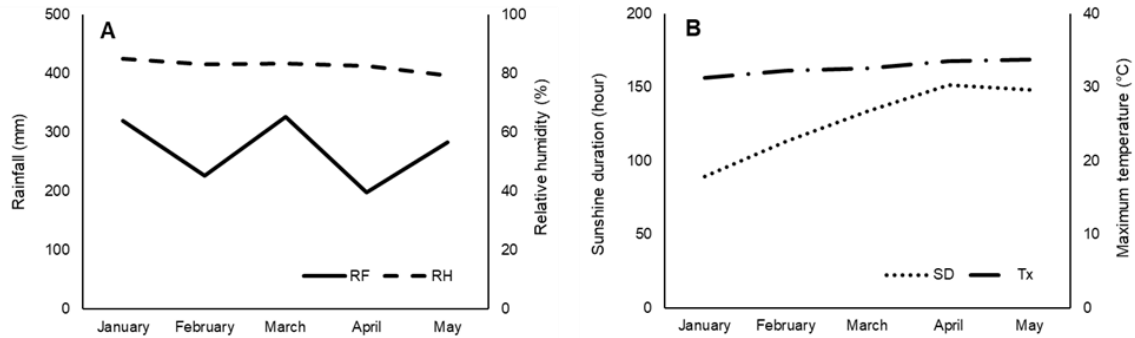
865 The cultivation of Brazilian spinach is characterized by its simplicity, since it may
866 be easily grown. Muda et al. (2022) reported that the propagation of Brazilian spinach
867 can be achieved via stem cuttings. There is an insufficient amount of research pertaining
868 to the adaptability of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

873 **MATERIALS AND METHODS**

874 *Research site and agroclimatic characteristic*

875 The research was carried out in the Jakabaring research facility located in
876 Palembang (104°46'44"E, 3°01'35"S), South Sumatra, Indonesia. This research began

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



881

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

886 *Cultivation and treatment procedures*

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

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

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

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

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

915 *Data collection*

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

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

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

938 *Data analysis*

939 All data collected was analysed using the RStudio software version 1.14.1717 for
 940 Windows (developed by RStudio team, PBC, Boston, MA). Significant differences
 941 among treatments were tested using the least significant difference (LSD) procedure at
 942 $p < 0.05$.

943

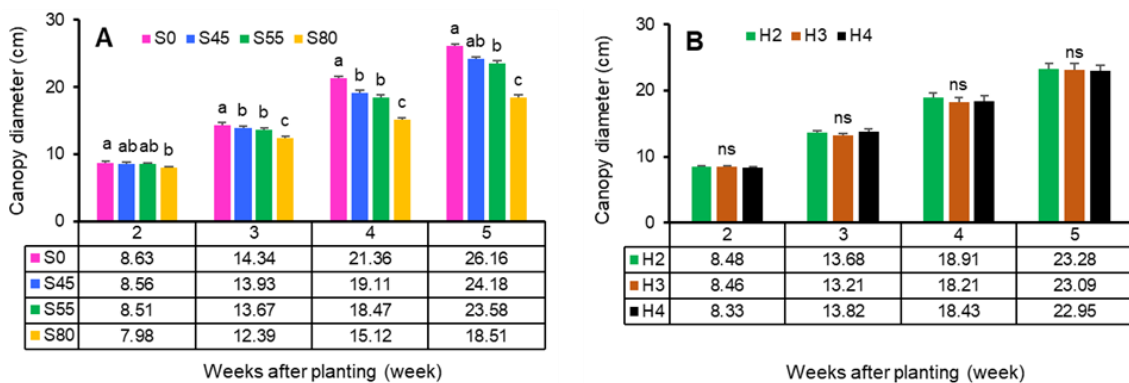
944 **RESULTS AND DISCUSSION**

945 *The Brazilian spinach growth during early vegetative growth before harvested*

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

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

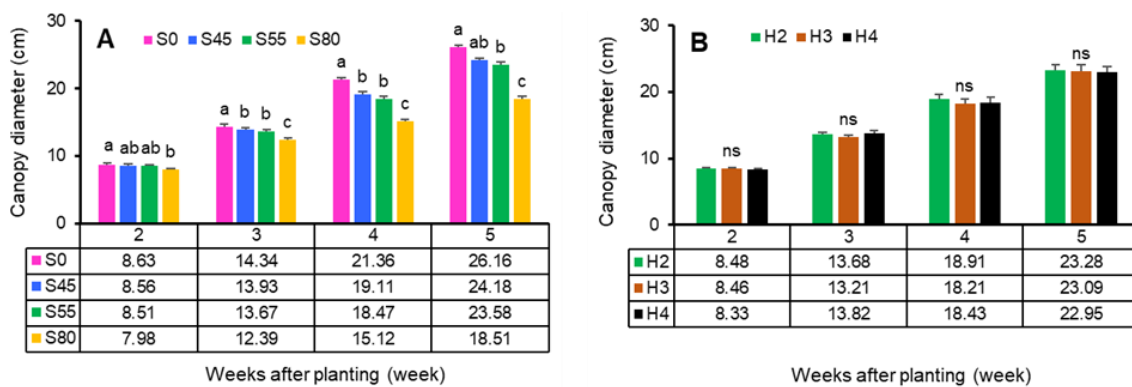
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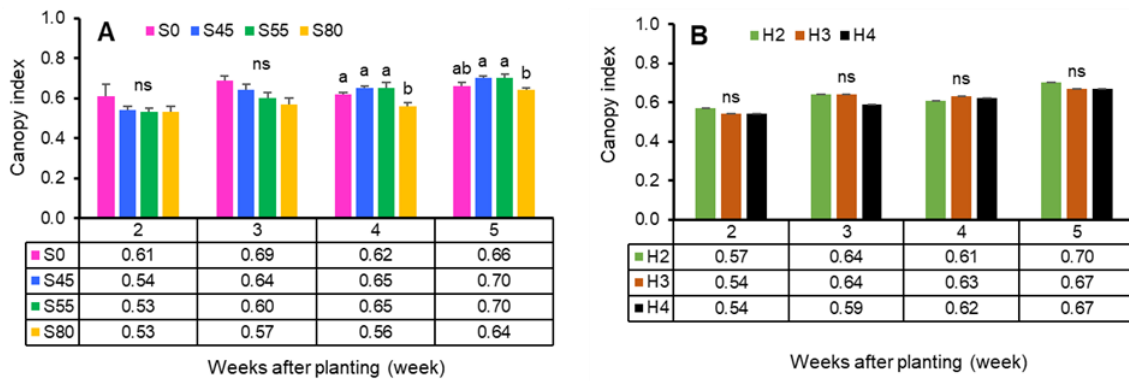
960 Figure 2. Brazilian spinach canopy area on early vegetative growth on different shading
 961 (A) and harvest period (B) treatment. The shading consists of no shading (S0), 45%
 962 shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4
 963 represent harvest period per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

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



969 Figure 3. Brazilian spinach canopy diameter on early vegetative growth on different
 970 shading (A) and harvest period (B) treatment. The shading consists of no shading (S0),
 971 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3,
 972 and H4 represent harvest period per 2 weeks, 3 weeks, and 4 weeks, respectively. The ns
 973 mean non-significant difference at $p < 0.05$.

974 The growth of leaf and branch significantly affects canopy density, with dominant
 975 growth resulting in a denser canopy as represented by canopy density. The effect is most
 976 noticeable between 4 and 5 weeks after planting. Brazilian spinach grown under shading
 977 conditions, especially S80, showed reduced leaf size and branch elongation, resulting in
 978 lower canopy density (Figure 4).
 979



980

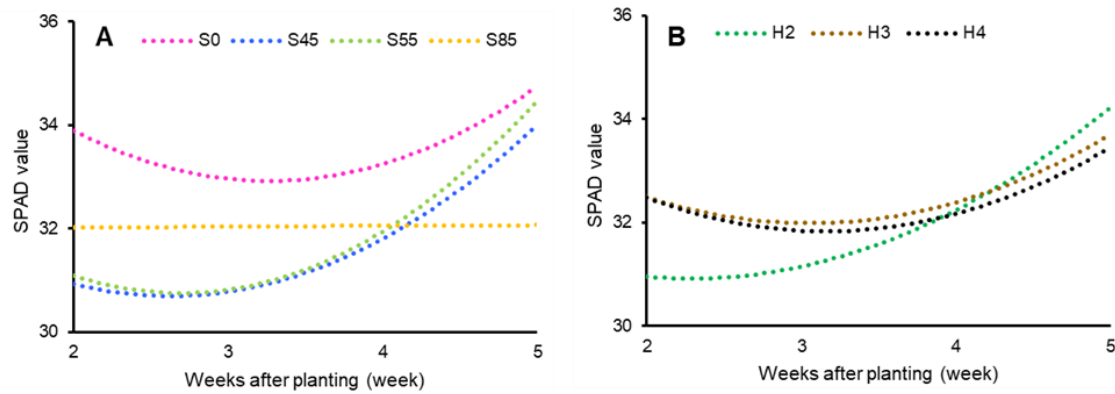
981 Figure 4. Canopy index on early vegetative growth on different shading and harvest
 982 period as treatment. The shading consists of no shading (S0), 45% shading (S45), 55%
 983 shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent harvest
 984 period per 2 weeks, 3 weeks, and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

986 According to this research's findings, Brazilian spinach's canopy growth was more
 987 hindered under greater shading (S80) than it was unshaded. The constituent organs of the
 988 canopy, such as the leaves and branches, endure stunted growth, which prevents the
 989 canopy from growing. According to Fadilah et al. (2022), denser shading intensity was
 990 shown to inhibit purple pakchoy leaf growth. According to the findings of Wan et al.
 991 (2020), plants planted in the shading area produce less photosynthetic performance than
 992 plants grown in full sunlight. Moreover, Liang et al. (2020) have underscored the
 993 significance of shading for plants, noting that it leads to a decrease in photosynthesis,
 994 resulting in a reduction in carbon flow. The inhibition of vegetative organ growth,
 995 particularly the canopy, in Brazilian spinach is attributed to the decreased carbon flow.
 996 This reduction in carbon flow occurs throughout the early growth cycle. The phenomenon
 997 of reduced vegetative organ development due to shading during the early growth phase
 998 has been documented in various vegetable crops, including chili (Kesumawati et al.,
 999 2020).

1000 The SPAD value is a method for evaluating leaf nitrogen and chlorophyll content,
 1001 with a positive relationship between these factors. Brazilian spinach leaf's SPAD value
 1002 was affected by shading treatments, with differences observed within each shading
 1003 treatment from the early growth 2 weeks after planting (WAP) (Figure 5).

1004 Brazilian spinach grown without shading (S0) showed a higher SPAD value
 1005 compared to under different shading levels, with a notable rise starting 4 weeks after

1006 planting. This trend was also observed in S45 and S55. On the other hand, Brazilian
 1007 spinach grown at S80 showed a stagnation trend, persisting until the end of the early
 1008 growth, specifically 2 to 5 weeks after planting.



1009
 1010 Figure 5. The SPAD value on early vegetative growth on different shading (A) and
 1011 harvest period (A) treatment. The shading consists of no shading (S0), 45% shading
 1012 (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent
 1013 harvest period per 2 weeks, 3 weeks.

1014 The SPAD value is a widely used method for assessing leaf chlorophyll and
 1015 nitrogen content, with its reliability well established. It has been found to have a positive
 1016 correlation with both chlorophyll content and leaf nitrogen content (Song et al., 2021;
 1017 Farnisa et al., 2023). Prior research had provided confirmation on the capacity of specific
 1018 leafy vegetables, namely *Talinum paniculatum* (Lakitan et al., 2021b) and spinach
 1019 (Mendoza-Tafolla et al., 2019), to proficiently evaluate and track the quantities of leaf
 1020 chlorophyll and nitrogen content.

1021 Brazilian spinach grown under full sun (S0) has a higher SPAD value than that
 1022 grown under shading, indicating that shading reduces the solubility of chlorophyll and
 1023 nitrogen. Wang et al. (2020a) found that shading affects the solubility of nitrogen, leading
 1024 to a decrease in leaf nitrogen content. Li et al. (2020) highlighted the biochemical
 1025 alterations caused by shading stress. This condition is due to Brazilian spinach,
 1026 particularly in plants subjected to the 80% shading treatment (S80).

1027 *Brazilian spinach growth after harvested*

1028 The vegetative growth of Brazilian spinach after harvest was conducted at 5 weeks
 1029 after planting. The growth of branch was compared under different shading conditions,
 1030 harvest periods, and their interaction effects. Brazil spinach grown under S80 exhibited
 1031 shorter branches as early as 11 weeks after planting. However, different shading

1032 treatments (S0, S45, and S55) showed comparable levels of branch elongation until 9
1033 weeks after planting. Brazilian spinach grown in S45 had an increased rate of branch
1034 elongation, particularly at 10 and 11 weeks after planting (Table 1).

1035 The elongation of the Brazilian spinach branch was influenced by the harvesting
1036 period. Less frequent harvesting leads to the highest branch elongation, especially from
1037 7 to 11 weeks after planting. An interaction between shading level and harvesting period
1038 treatment was observed, starting within 9 weeks after planting. This highlights the
1039 importance of harvesting frequency in influencing Brazilian spinach growth.

1040 The study revealed a decrease in branch elongation in Brazilian spinach at S80,
1041 indicating a decrease in the allocation of photosynthetic products. This is due to reduced
1042 levels of non-structural carbohydrates, which are essential for growth (Yamashita et al.,
1043 2020). However, in the S0 treatment, photosynthesis is optimised, leading to increased
1044 branch growth.

1045 The increased frequency of harvesting inhibits branch growth, and it is possible
1046 that the distribution of photosynthetic products changes, potentially causing a heightened
1047 initiation process of new leaves (Oliveira et al., 2021). Additionally, Raza et al. (2019)
1048 reported that maize plants with a higher number of eliminated leaves have an increased
1049 allocation of photosynthetic resources towards expanded leaves, as evidenced by an
1050 enhanced leaf area.

1051 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
<i>Shading</i>							
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
<i>Harvest period</i>							
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
<i>Shading x harvest period</i>							
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

1052 Remark: the ns mean non-significant difference at p<0.05.

1053 Brazilian spinach showed significant differences in leaf growth when treated with
1054 different shading and harvesting periods. Cultivated without shade (S0), it tends to
1055 dominate leaf growth compared to cultivated under different levels of shading (S45, S55,
1056 and S80) (Table 2). However, this method also demonstrated a significant proportion of
1057 non-marketable leaves. This indicates that early leaf growth is achieved without shading,
1058 but it also leads to accelerated leaf aging and increased pest susceptibility, resulting in a
1059 higher proportion of non-marketable leaves compared to those cultivated under shading.

1060 The frequent harvesting of Brazilian spinach leads to the initiation of young
1061 leaves, resulting in more marketable leaves. This is evident in the yield of commercially
1062 viable leaves throughout the H2 and H3 periods. However, during the H3 harvesting
1063 period, a significant proportion of non-marketable leaves are produced due to leaf aging.
1064 In contrast, extended harvesting periods (H4) often lack the capacity for leaf initiation,
1065 resulting in decreased yields of both marketable and non-marketable leaves. The
1066 interaction impact of shading and harvesting period significantly showed on leaf growth,
1067 with the most significant impact observed under 80% shade, especially during the longer
1068 harvesting period (H4).

1069 Brazilian spinach's leaf initiation is higher in conditions without shade compared
1070 to shading conditions, affecting both marketable and non-marketable leaves. This is due
1071 to reduced carbohydrate accumulation and allocation (Hussain et al., 2020). Shading
1072 conditions inhibited plant growth, while without shade, leaf senescence accelerated due
1073 to enhanced photosynthesis. Meanwhile, direct sunlight exposure accelerates ageing
1074 processes in plants, similar to sweet basil (Castronuovo et al., 2019). However, without
1075 shade, spinach is more susceptible to pest infestation, leading to an increased prevalence
1076 of non-marketable leaves. Implementing shading at a specific density is a viable pest
1077 control strategy.

1078 Brazilian spinach harvesting, similar to leaf and shoot pruning, has been found to
1079 increase yield. Dheeraj et al. (2022) found that pruning at the apical meristem increased
1080 growth-promoting hormones, specifically cytokinin. Xu et al. (2020a) reported that
1081 pruning tomato plants also increases cytokinin hormone levels. Cytokinin hormones
1082 influence cell division processes, including those during leaf cell development.
1083 Harvesting Brazilian spinach at H2 and H3 treatment results in elevated levels of
1084 cytokinin hormones, enhancing leaf initiation and resulting in a greater marketable yield.

1085 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)
<i>Shading</i>				
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
<i>Harvest period</i>				
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
<i>Shading x harvest period</i>				
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 ± 2.70 cd	3.21 ± 0.36 ef
S55H3	58.36 ± 4.61 cd	5.88 ± 0.59 c	44.67 ± 2.41 a	4.69 ± 0.48 cde
S55H4	46.47 ± 4.24 d	4.78 ± 0.52 c	25.30 ± 1.38 d	2.69 ± 0.15 fg
S80H2	15.54 ± 1.30 e	1.17 ± 0.14 d	3.27 ± 0.19 e	0.37 ± 0.02 h
S80H3	17.09 ± 1.32 e	1.48 ± 0.13 d	2.37 ± 1.07 e	0.28 ± 0.18 h
S80H4	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
LSD _{0.05}	14.781	1.587	9.092	1.587

1086 Remark: the ns mean non-significant difference at p<0.05.

1087 The metabolism of Brazilian spinach was influenced by shading and harvesting
 1088 periods. Brazilian spinach grown without shade (S0) increased metabolism activity
 1089 compared to the shading areas (S45, S55, and S80). This is represented by the carbon and
 1090 nitrogen levels (Table 3). Higher metabolism correlates with enhanced nitrogen usage,
 1091 which is crucial for plant metabolic processes. Therefore, increasing fertilization
 1092 frequency is necessary for plants exposed to sunlight. Leaf nitrogen concentration
 1093 remains consistent across harvesting periods, suggesting no significant differences in
 1094 nitrogen across different harvesting periods.

1095 The carbon-nitrogen ratio calculation can be used to determine leaf hardness in
 1096 Brazilian spinach leaves. The study showed that unshaded (S0) areas yield more tough

1097 leaves, decreasing with increased shading levels. Despite this, Brazilian spinach
1098 consistently showed comparable levels of leaf hardness across harvesting periods.

1099 Shading significantly impacts the carbon reduction and nitrogen enrichment of
1100 leaves, affecting the process of photosynthesis. Light intensity and photosynthesis are
1101 linked, with studies showing a reduction in carbon and nitrogen buildup in plants exposed
1102 to shading. Tang et al. (2022) found that plants exposed to modest levels of irradiation
1103 exhibit reduced concentrations of leaf non-structural carbohydrates, leading to a reduction
1104 in carbon content. Nitrogen accumulation in shaded Brazilian spinach leaves is due to
1105 limited light availability, hindering the conversion of nitrogen into organic nitrogen
1106 compounds essential for plant metabolic processes. Gao et al. (2020) found that prolonged
1107 shading reduces nitrogen utilization efficiency in plant. Wang et al. (2020b) demonstrated
1108 that the procedure of removing leaves of plants results in an increase in non-structural
1109 carbohydrates in leaf. On the other hand, an increased frequency of harvesting triggers
1110 the growth of new leaves, leading the movement of nitrogen toward younger leaves.
1111 Jasinski et al. (2021) reported that nitrogen mobilization occurs from older to younger
1112 leaves.

1113 Table 3. Carbon, Nitrogen and C-N ratio of Brazilian spinach leaf on different shading,
 1114 harvest period, and their interaction.

Treatment	Carbon (%)	Nitrogen (%)	C-N ratio
<i>Shading</i>			
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
<i>Harvest period</i>			
H2	35.85	4.38	8.74
H3	33.90	4.42	8.10
H4	32.20	4.07	8.30
<i>Shading x harvest periode</i>			
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

1115 Remark: the ns mean non-significant difference at $p < 0.05$.

1116 The presence of shading in Brazilian spinach is linked to biomass production.
 1117 Under unshaded conditions, it enhances photosynthesis, leading to increased biomass
 1118 production. However, under intense shading conditions, it reduces biomass in various
 1119 parts of the plant. Harvesting over extended periods (H3 and H4) results in increased
 1120 biomass accumulation, particularly in the stem and branch in the final observation.
 1121 Brazilian spinach, when grown under shading conditions and extended harvesting,
 1122 showed inhibited growth due to restricted photosynthetic activity. This caused the
 1123 restricted allocation of photosynthetic products to individual plant organs. Studies have
 1124 shown that shading reduces biomass accumulation and alterations in plant morphological
 1125 traits (Xu et al., 2020b). Additionally, there is a distribution of photosynthetic activity that
 1126 utilizes plant organs beyond just leaves. This aligns with Yu et al. (2019) finding that
 1127 when plants age and their organs undergo senescence, photosynthetic flux redirects

1128 towards the stem. This highlights the importance of considering the allocation of
 1129 photosynthetic products to plant growth through periodic harvesting.

1130 Table 4. Dry weight of Brazilian spinach organs on different shading, harvest period, and
 1131 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)
<i>Shading</i>				
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
<i>Harvest period</i>				
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
<i>Shading x harvest period</i>				
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

1132 Remark: the ns mean non-significant difference at p<0.05.

1133

1134 *Visual appearance of Brazilian spinach on different treatment*

1135 The study analysed the shoot appearance of Brazilian spinach under different
1136 shading conditions and harvesting periods. Unshaded areas had a denser appearance,
1137 while based on the harvesting period, treatments tend to show similarities with each other
1138 (Figure 6). Furthermore, Brazilian spinach grown unshaded (S0) showed greater root
1139 growth and a higher density of root hairs than other shading, while samples subjected to
1140 varying harvesting periods (H2, H3, and H4) showed similar root morphology without
1141 any significant differences (Figure 7).

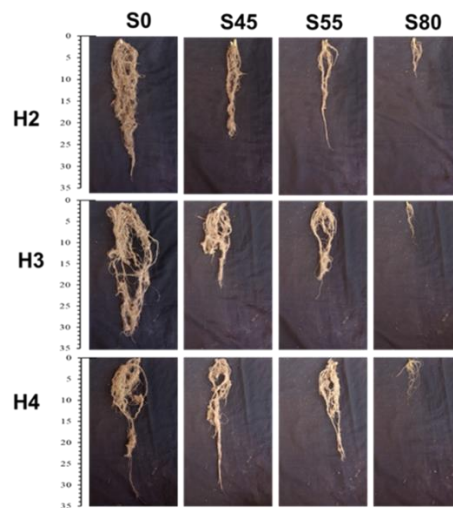
1142 Brazilian spinach showed varying morphological traits under different treatments.
1143 Shading causes alterations in plant organs, as shown on soybean stems, which experience
1144 inhibited growth (Castronuovo et al., 2019). Cao et al. (2022) reported that *Cynodon*
1145 *dactylon* shoot organs also experience alterations. Root development also shows a distinct
1146 reaction to shading, with a decline in root growth under shading stress. Fu et al. (2020)
1147 found reductions in root volume and length, indicating a decline in root growth under
1148 these conditions.

1149 Brazilian spinach with a longer harvesting period (H4) showed a rise in branches
1150 and stems, with a higher presence of mature leaves. Pruning at longer intervals increased
1151 plant height and branches in *Talinum Paniculatum*, while extending harvesting intervals
1152 hindered fresh leaf commencement (Purbajanti et al., 2019). Bessonova et al. (2023)
1153 found that removing leaves and branches from plants led to the development of shoot
1154 features with a greater number and area of leaves.



1155

1156 Figure 6. Visualization of Brazilian spinach shoot on different shading and harvest period
 1157 at 13 weeks after planting (WAP). Shading treatment consist of S0: no-shading, S45: 45%
 1158 shading, S55: 55% shading, and S80: 80% shading. Meanwhile, the harvest period
 1159 consists of H2: per 2 weeks, H3: per 3 weeks, and H4: per 4 weeks. Photos: Strayker Ali
 1160 Muda.



1161

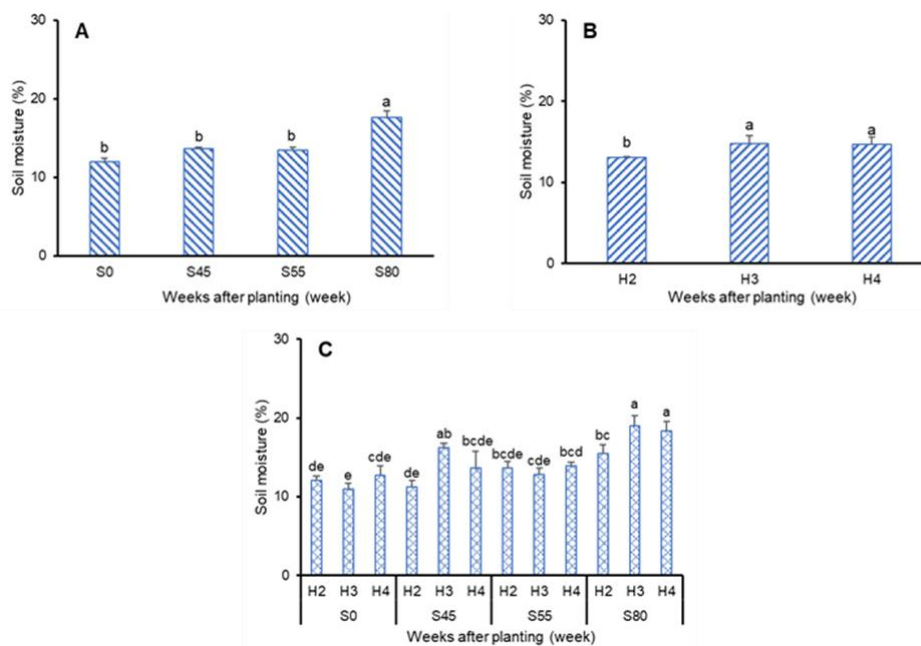
1162 Figure 7. Visualization of Brazilian spinach root on different shading and harvest period
 1163 at 13 weeks after planting (WAP). Shading treatment consist of S0: no-shading, S45: 45%
 1164 shading, S55: 55% shading, and S80: 80% shading. Meanwhile, the harvest period
 1165 consists of H2: per 2 weeks, H3: per 3 weeks, and H4: per 4 weeks. Photos: Strayker Ali
 1166 Muda.

1167

1168 *Water status on different treatment*

1169 The water availability for Brazilian spinach growth was represented by substrate
 1170 moisture. Increased shading intensity (S80) leads to higher moisture content, reducing
 1171 direct sunlight exposure and reducing evaporation, resulting in reduced water loss.
 1172 Conversely, Brazilian spinach grown in areas with lower shading or without shading
 1173 showed higher evaporation rates, indicating more water loss, as shown by substrate
 1174 moisture levels (Figure 8). The use of shading can effectively adjust microclimate
 1175 conditions, such as substrate moisture levels (Bollman et al., 2021). The study found that
 1176 shaded growing media had higher humidity levels than unshaded media, and the addition
 1177 of shading reduced evaporation rate, as confirmed by Khawam et al. (2019).

1178 The more frequently Brazil spinach is harvested, the wider the substrate surface is
 1179 not covered by the canopy, causing higher evaporation rates and reduced water
 1180 availability. This phenomenon aligns with the findings of Huang et al. (2020), who
 1181 provided empirical evidence that plants with lower canopy density exhibit higher rates of
 1182 water loss via evaporation.



1183 Figure 8. Substrate moisture on different shading (A), harvest period (B), and their
 1184 interaction (C). Shading treatment consist of S0: no-shading, S45: shading 45%, S55:
 1185 shading 55%, and S80: shading 80%. Meanwhile, the harvest period consists of H2:
 1186 every 2 weeks, H3: every 3 weeks, and H4: every 4 weeks.

1188

CONCLUSION

1189

1190 The adoption of shading led to a decrease in the growth and yield of Brazilian
1191 spinach through an alteration in the morphological traits of its root, stem, branch, and
1192 leaf. In addition, the implementation of a 2-week harvesting period led to increased
1193 Brazilian spinach growth and yield. Interactions between shading and harvest periods
1194 primarily pertain to the length of branches, yields (both marketable and non-marketable),
1195 dry weight of organs (namely branch and leaf), and substrate moisture.

1196

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1197

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1200

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1336

Revised version

1337

BRAZILIAN SPINACH GROWTH UNDER DIFFERENT SHADING

1338

INTENSITIES AND HARVESTING PERIODS IN LOWLAND TROPICAL

1339

URBAN ECOSYSTEM

1340

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ABSTRACT

1349

Brazilian spinach, a lesser-known **perennial** leafy vegetable, **growing in the tropical**

1350

ecosystem. **The** study was conducted to evaluate the growth of Brazilian spinach in

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tropical lowland urban ecosystem under different levels of shading intensities and

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harvesting periods. The research used a split-plot design, **with** different levels of shading

1353

intensities (no-shading, shading 45%, shading 55%, and shading 80%) as the main plot

1354

and harvesting periods **(per 2 weeks, per 3 weeks, and per 4 weeks)** as the sub-plot. The

1355

results showed that Brazilian spinach growth was more favourable when exposed to

1356

treatment without shading compared to shading conditions. Shading treatment, **especially**

1357

at shading 80%, had a negative impact on plant growth was observed during the early

1358

stages of growth, as indicated by alterations in canopy parameters **(canopy area (26,47**

1359

cm²), canopy diameter (7.98 cm), and canopy index (0.52)) and SPAD values trend.

1360

Shading 80% has reduced branch elongation, yield **(marketable (14.76 g) and non-**

1361

marketable (4.68)), stem dry weight (0.25 g), branch dry weight (0.40 g), leaf dry weight

1362

(0.85 g), and root dry weight (0.44 g). Conversely, Brazilian spinach grown on no-shading

1363

increased the carbon content **(34.64 %)** and reduced nitrogen content **(2.83 %)** of

1364

marketable leaves. More frequent harvesting **(per 2 weeks)** increased in marketable yield

1365

(67.22 g), but suppressed the growth of stem **(1.05 g)**, branches **(4.39 g)**, and root **(1.73)**.

1366

Therefore, it is recommended to cultivate Brazilian spinach in an unshaded area with a

1367

biweekly harvesting routine.

KEYWORDS

1368

1369 Harvest time, leafy green, lesser-known vegetable, plant acclimatization, solar irradiation
1370 intensity.

INTRODUCTION

1371

1372 Brazilian spinach (*Alternanthera sissoo*) is a leafy vegetable originating from
1373 Brazil. As reported by Ikram et al. (2022), Brazilian spinach is rich in flavonoids,
1374 vitamins, minerals, and other antioxidants, which have been found to have positive effects
1375 on human health. The cultivation and use of this particular plant by the Indonesian
1376 population are infrequent, leading to its classification as a rather rare plant species.
1377 Indonesia's ecosystem exhibits similarities to its indigenous location, hence indicating the
1378 potential for cultivating this plant within the country.

1379

1380 Urban cultivation faces several challenges, especially in regard to the availability
1381 of light for plants. Shaded areas in urban environments tend to prevail, impeding the
1382 penetration of light into plant development. Consequently, the amount of light received
1383 by plants decreases, leading to disruptions in certain aspects of plant metabolism. This
1384 phenomenon is particularly observed in horticultural crops characterized by compact
1385 growth, such as Brazilian spinach. Based on Shafiq et al. (2021), regulating alterations
1386 occur in plants as a means to enhance the efficiency of photosynthesis. The tolerance of
1387 plants to the intensity of light they receive varies depending on the specific plant species.
1388 Certain vegetable crops have been reported as being capable of growing under shaded
1389 conditions. In this regard, Sifuentes-Pallaoro et al. (2020) revealed that *Lactuca*
1390 *canadensis* exhibits favourable growth under shading, while Lakitan et al. (2021a) found
1391 that celery also demonstrates similar adaptability. Furthermore, Gomes et al. (2023)
1392 reported that in the Brazilian ecosystem cultivated plants will grow well at full or at less
1393 70% light intensity.

1393

1394 Brazilian spinach is a perennial leafy vegetable, enabling its continuous growth
1395 throughout the year. Additionally, this suggests that regular harvesting is required.
1396 Annual plants undergo periodic harvesting that involves a defoliation mechanism.
1397 According to the findings of Raza et al. (2019), the implementation of a defoliation
1398 treatment on plants has been observed to enhance overall plant growth, particularly in
1399 terms of leaf growth, especially during the vegetative phase. Further experimentation is
1400 required to enhance the output of Brazilian spinach, a plant species characterised by its

1400 commercially valuable leaf organs, which are described as young and acceptable damage
1401 by pests and diseases.

1402 The cultivation of Brazilian spinach is characterized by its simplicity, since it may
1403 be easily grown. Muda et al. (2022) reported that the propagation of Brazilian spinach
1404 can be achieved via stem cuttings. There is an insufficient amount of research pertaining
1405 to the adaptability of Brazilian spinach to shading environments. The capacity of
1406 Brazilian spinach to acclimatise to shading environments for a specific duration will
1407 ensure the availability of sustainable vegetable nutrition. The study was aimed to
1408 evaluating the adaptability of Brazilian spinach to shading conditions via various
1409 harvesting periods.

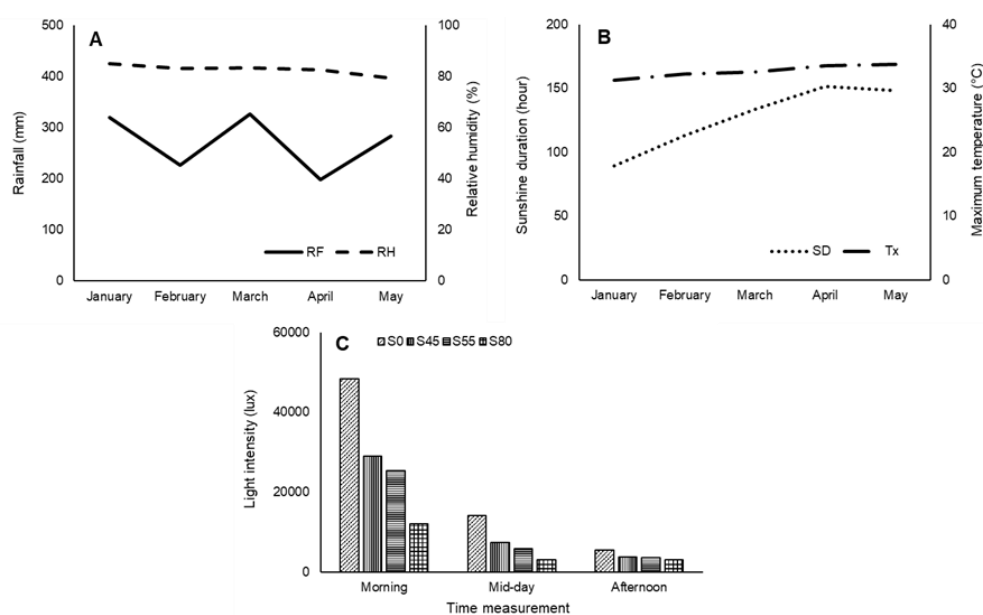
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MATERIALS AND METHODS

Research site and agroclimatic characteristic

1413 The research was carried out in the Jakabaring research facility located in
1414 Palembang (104°46'44"E, 3°01'35"S), South Sumatra, Indonesia. This research began
1415 with initiating the propagation of stem cuttings on 30 January 2023, and concluded the
1416 data collection on 02 May 2023. The research site is situated in a tropical urban lowland
1417 area with an elevation of 8 meters above sea level (masl). The agroclimatic characteristics
1418 of the area are shown in Figure 1.



1419

1420 Figure 1. Agroclimatic characteristics in research location as indicated by total monthly
1421 rainfall (RF) and average humidity (RH) (A), sunshine duration (SD) and average

1422 maximum temperature (Tx) (B), and average light intensity of each treatment (C).
1423 (Source: Indonesian Agency for Meteorology, Climatology and Geophysics). S0= no-
1424 shading; S45= shading 45%; S55= shading 55%; S80= shading 80%.

1425 *Cultivation and treatment procedures*

1426 The propagation material used was stem cuttings with two leaves that were taken
1427 from healthy mother plant. Mother plant used as a source for stem cuttings was a 3-month-
1428 old plant and in healthy condition. The planting materials were planted in pots with
1429 dimensions of 27.5 cm in diameter and 20 cm in height. The pots were filled with a
1430 growing medium that was a mixture of top soil and chicken manure (3:1 v/v). Prior to
1431 planting, the growing medium had a bio-sterilization treatment (2 g/l), with the addition
1432 of live microorganisms including *Streptomyces thermovulgaris*, *Thricoderma virens*, and
1433 *Geobacillus thermocatenuatus*. The growing medium was subsequently subjected to a
1434 one-week incubation period before planting. The bio-sterilization application aims to
1435 prevent the pathogen infestation from substrate to cultivated plants.

1436 The growing medium that had been incubated was used for the planting of
1437 Brazilian spinach cuttings, which were subsequently arranged in accordance with the
1438 principles of a split-plot design. The main plot of the study focused on the intensity of
1439 shading, whereas the subplot examined the harvest period. The treatment involved
1440 selecting shading intensity at three separate levels, namely no-shading (S0), 45% shading
1441 (S45), 55% shading (S55), and 85% shading (S80). Furthermore, treatment for the harvest
1442 period started after the simultaneous harvesting, which was carried out at 5 weeks after
1443 planting (WAP). The designated harvest period has three different intervals, namely
1444 appearing per 2 weeks, per 3 weeks, and per 4 weeks, symbolised as I₂, I₃, and I₄,
1445 respectively. The first harvest was carried out at five week after planting (WAP).

1446 The plants were systematically positioned within shadow houses measuring 4
1447 meters in length, 2 meters in width, and 2 meters in height. These shadow houses are
1448 constructed using knockdown frames made of 1.5-inch PVC pipes. The entire perimeter
1449 of the shadow house is enveloped with a shade material, specifically a black polyethylene
1450 net, which has been tested for its density to ensure optimal shading.

1451 The leaves of the Brazilian spinach cuttings get trimmed when it reaches one week
1452 after planting (WAP) in order to maintain uniformity in the planting material. Meanwhile,
1453 fertilization was applied using compound NPK fertilizers (16:16:16) at 1 and 5 WAP at a

1454 dose of 3 g/plant. The watering of each plant was normally carried out every day around
1455 08:00 a.m and 05:00 p.m.

1456 *Data collection*

1457 The data collection covered Brazilian spinach growth and yield data. The growth
1458 data that was obtained is categorised into two categories of measurements, such as non-
1459 destructive and destructive. The dataset for non-destructive growth measurement includes
1460 several variables, including SPAD values, canopy width, canopy diameter, canopy index,
1461 branch length, and stem diameter. In addition, the destructive measurements included the
1462 stem fresh weight, branch fresh weight, root fresh weight, stem dry weight, branch dry
1463 weight, and root dry weight. Destructive observation was carried out at 13 weeks after
1464 planting. The collected data concerning Brazilian spinach yield covers several
1465 parameters, including the fresh weight of marketable leaf, fresh weight of non-marketable
1466 leaf, dry weight of marketable leaf, dry weight of non-marketable leaf, the carbon content
1467 of marketable leaf, nitrogen content of marketable leaf, and the C:N ratio of marketable
1468 leaf. Marketable leaf is young, healthy, and easily-breakable. Meanwhile, non-marketable
1469 leaf is aged, damaged by pests and/or diseases, and high fiber content. The moisture
1470 content of the planting medium was also examined in order to determine the water content
1471 of the substrate.

1472 The SPAD value was monitored using chlorophyll meters (SPAD-502 Plus,
1473 Konica-Minolta Optics, Inc., Osaka, Japan). Canopy area was measured using a digital
1474 image scanner for Android (Easy Leaf Area software, developed by Easlon & Bloom
1475 2014). Canopy diameter was measured using a measuring tape on the widest side of the
1476 canopy. Canopy index was the ratio of the measured canopy area to circular area with
1477 widest diameter. Meanwhile, substrate moisture (SM) was measured using a soil moisture
1478 meter (PMS-714, Lutron Electronics Canada, Inc., Pennsylvania, USA). Light intensity
1479 was measured using a lux meter (GM1030, Benetech, Inc., Illinois, USA). Carbon content
1480 was analysed using the furnace method, while nitrogen content was analysed using the
1481 Kjeldahl-Titrimetry method.

1482 The dry weight of each plant organ was determined by treating it to a drying
1483 process in an oven set at a temperature of 100°C for a duration of 24 hours. Prior to being
1484 placed in the oven, the plant's organs are trimmed to a reduced thickness to accelerate the
1485 drying process.

1486 *Data analysis*

1487 The effect of shading intensities and harvest periods were revealed by analysis of
1488 variance (ANOVA). Significant differences among treatments were tested using the
1489 Tukey's honestly significant difference (HSD) procedure at $P < 0.05$. All data was
1490 analysed using the RStudio software version 1.14.1717 for Windows (developed by
1491 RStudio team, PBC, Boston, MA).

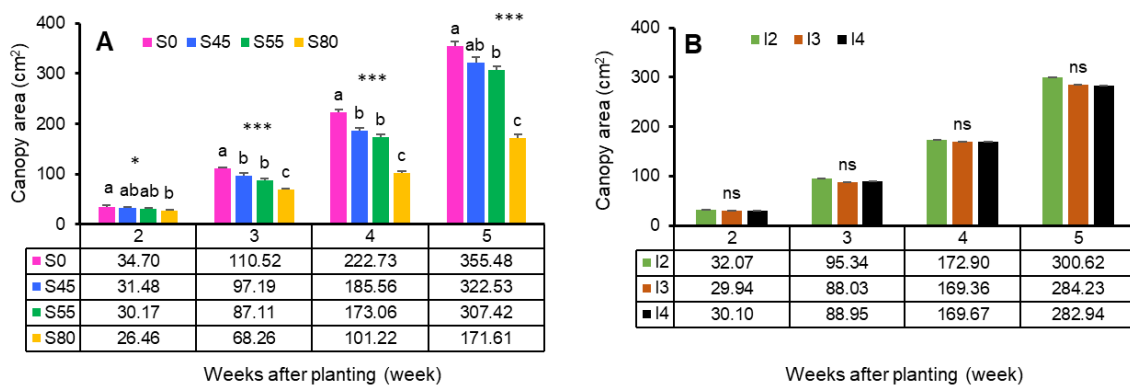
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RESULTS AND DISCUSSION

1494 *The Brazilian spinach growth during early vegetative growth before harvested*

1495 The early vegetative growth of Brazilian spinach is analysed by considering its
1496 unique characteristics, such as canopy growth and SPAD value. This approach involves
1497 non-destructive observation, allowing plants to grow naturally. The canopy
1498 characteristics selected were: canopy area, canopy diameter, and canopy index.

1499 Brazilian spinach grown in unshaded conditions (S0) had a higher leaf initiation
1500 and larger individual leaf area compared to in shading conditions. More and larger leaves
1501 contribute to the increase in canopy area. This leads to a broader canopy compared to
1502 those grown under shade. The Brazilian spinach canopy area growth increased
1503 significantly in the S0 condition, particularly 2 to 5 weeks after planting, compared to
1504 shade conditions (S45, S55, and S80). However, no significant leaf growth was observed
1505 in the S45 and S55 shades. The shading with the highest density (S80) showed suppressed
1506 growth, starting 2 weeks after planting (Figure 2).

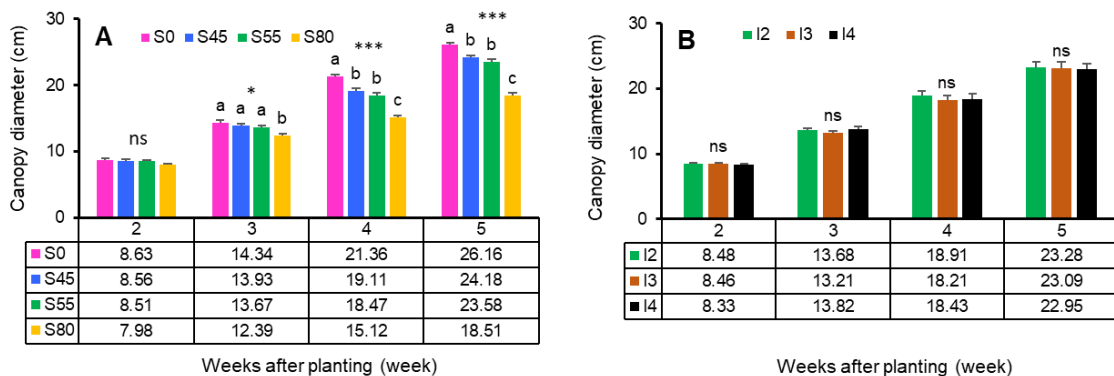


1507

1508 Figure 2. Brazilian spinach canopy area on early vegetative growth on different shading
1509 (A) and harvest period (B) treatment. The shading consists of no-shading (S0), 45%
1510 shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, I2, I3, and I4
1511 represent harvest period per 2 weeks, 3 weeks, and 4 weeks, respectively. The ns= non-

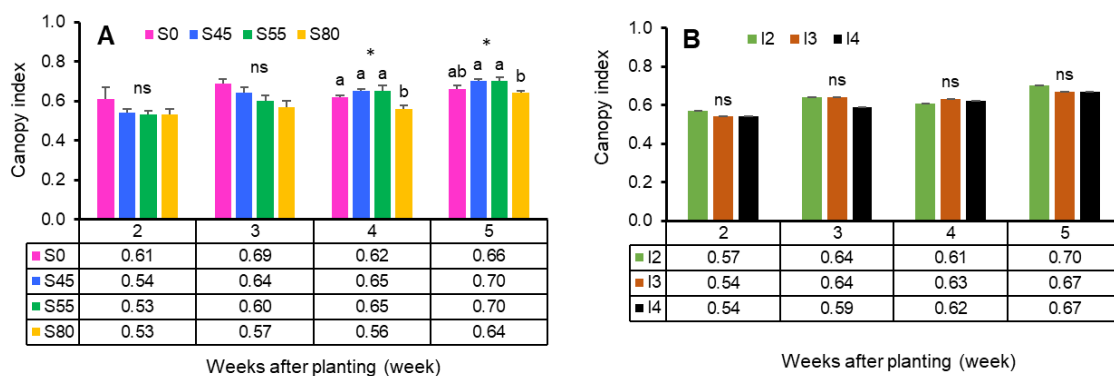
1512 significant difference at $P < 0.05$; *= significant difference at $P < 0.05$; ***= significant
 1513 difference at $P < 0.001$.

1514 Brazilian spinach branches significantly influence canopy diameter, with
 1515 elongation affecting canopy expansion. Shading treatment inhibits branch growth. Full
 1516 sunlight cultivation leads to a wider canopy than canopies grown under different levels
 1517 of shading (S45, S55, and S80) (Figure 3).



1518
 1519 Figure 3. Brazilian spinach canopy diameter on early vegetative growth on different
 1520 shading (A) and harvest period (B) treatment. The shading consists of no-shading (S0),
 1521 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, I2, I3, and
 1522 I4 represent harvest period per 2 weeks, 3 weeks, and 4 weeks, respectively. The ns= non-
 1523 significant difference at $P < 0.05$; *= significant difference at $P < 0.05$; ***= significant
 1524 difference at $P < 0.001$.

1525 The growth of leaf and branch significantly affects canopy density, with dominant
 1526 growth resulting in a denser canopy as represented by canopy density. The effect is most
 1527 noticeable between 4 and 5 weeks after planting. Brazilian spinach grown under shading
 1528 conditions, especially S80, showed reduced leaf size and branch elongation, resulting in
 1529 lower canopy density (Figure 4).

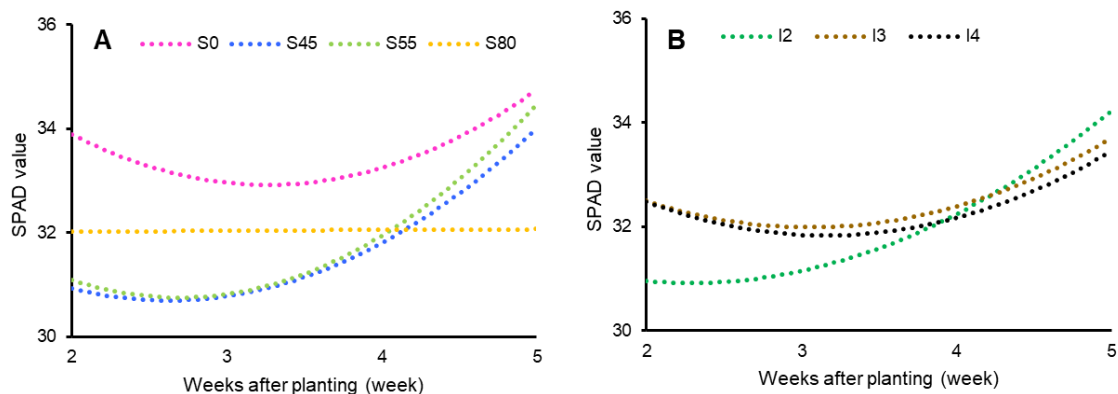


1530

1531 Figure 4. Canopy index on early vegetative growth on different shading and harvest
1532 period as treatment. The shading consists of no-shading (S0), 45% shading (S45), 55%
1533 shading (S55), and 80% shading (S80). Meanwhile, I2, I3, and I4 represent harvest period
1534 per 2 weeks, 3 weeks, and 4 weeks, respectively. The ns= non-significant difference at
1535 $P < 0.05$; *= significant difference at $P < 0.05$.

1536 According to this research's findings, Brazilian spinach's canopy growth was more
1537 hindered under greater shading (S80) than it was unshaded. The constituent organs of the
1538 canopy, such as the leaves and branches, endure stunted growth, which prevents the
1539 canopy from growing. According to Fadilah et al. (2022), denser shading intensity was
1540 shown to inhibit purple pakchoy leaf growth. According to the findings of Wan et al.
1541 (2020), plants planted in the shading area produce less photosynthetic performance than
1542 plants grown in full sunlight. Moreover, Liang et al. (2020) have underscored the
1543 significance of shading for plants, noting that it leads to a decrease in photosynthesis,
1544 resulting in a reduction in carbon flow. The inhibition of vegetative organ growth,
1545 particularly the canopy, in Brazilian spinach is attributed to the decreased carbon flow.
1546 This reduction in carbon flow occurs throughout the early growth cycle. The phenomenon
1547 of reduced vegetative organ development due to shading during the early growth phase
1548 has been documented in various vegetable crops, including chili (Kesumawati et al.,
1549 2020).

1550 The SPAD value is a method for evaluating leaf nitrogen and chlorophyll content,
1551 with a positive relationship between these factors. Brazilian spinach leaf's SPAD value
1552 was affected by shading treatments, with differences observed within each shading
1553 treatment from the early growth 2 weeks after planting (WAP). The Brazilian spinach's
1554 leaf grown no-shading (S0) showed a higher SPAD value compared to under different
1555 shading levels, with a notable rise starting 4 weeks after planting. This trend was also
1556 observed in S45 and S55. On the other hand, Brazilian spinach grown at S80 showed a
1557 stagnation trend, persisting until the end of the early growth, specifically 2 to 5 weeks
1558 after planting (Figure 5).



1559

1560 Figure 5. The Brazilian spinach's leaf SPAD value on early vegetative growth on different
 1561 shading (A) and harvest period (A) treatment. The shading consists of no-shading (S0),
 1562 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, I2, I3, and
 1563 I4 represent harvest period per 2 weeks, 3 weeks.

1564 The SPAD value is a widely used method for assessing leaf chlorophyll and
 1565 nitrogen content, with its reliability well established. It has been found to have a positive
 1566 correlation with both chlorophyll content and leaf nitrogen content (Song et al., 2021;
 1567 Farnisa et al., 2023). Prior research had provided confirmation on the capacity of specific
 1568 leafy vegetables, namely *Talinum paniculatum* (Lakitan et al., 2021b) and spinach
 1569 (Mendoza-Tafolla et al., 2019), to proficiently evaluate and track the quantities of leaf
 1570 chlorophyll and nitrogen content.

1571 Brazilian spinach grown under no-shading (S0) has a higher SPAD value than that
 1572 grown under shading, indicating that shading reduces the solubility of chlorophyll and
 1573 nitrogen. Wang et al. (2020a) found that shading affects the solubility of nitrogen, leading
 1574 to a decrease in leaf nitrogen content. Li et al. (2020) highlighted the biochemical
 1575 alterations caused by shading stress. This condition is due to Brazilian spinach,
 1576 particularly in plants subjected to the 80% shading treatment (S80).

1577 *Brazilian spinach growth after harvested*

1578 The vegetative growth of Brazilian spinach after harvest was conducted at 5 weeks
 1579 after planting. The growth of branch was compared under different shading conditions,
 1580 harvest periods, and their interaction effects. Brazil spinach grown under S80 exhibited
 1581 shorter branches as early as 11 weeks after planting. However, different shading
 1582 treatments (S0, S45, and S55) showed comparable levels of branch elongation until 9
 1583 weeks after planting. Brazilian spinach grown in S45 had an increased rate of branch
 1584 elongation, particularly at 10 and 11 weeks after planting (Table 1).

1585 The elongation of the Brazilian spinach branch was influenced by the harvesting
1586 period. Less frequent harvesting leads to the highest branch elongation, especially from
1587 7 to 11 weeks after planting. An interaction between shading level and harvesting period
1588 treatment was observed, starting within 9 weeks after planting. This highlights the
1589 importance of harvesting frequency in influencing Brazilian spinach growth.

1590 The study revealed a decrease in branch elongation in Brazilian spinach at S80,
1591 indicating a decrease in the allocation of photosynthetic products. This is due to reduced
1592 levels of non-structural carbohydrates, which are essential for growth (Yamashita et al.,
1593 2020). However, in the S0 treatment, photosynthesis is optimised, leading to increased
1594 branch growth.

1595 The increased frequency of harvesting inhibits branch growth, and it is possible
1596 that the distribution of photosynthetic products changes, potentially causing a heightened
1597 initiation process of new leaves (Oliveira et al., 2021). Additionally, Raza et al. (2019)
1598 reported that maize plants with a higher number of eliminated leaves have an increased
1599 allocation of photosynthetic resources towards expanded leaves, as evidenced by an
1600 enhanced leaf area.

1601 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
<i>Shading</i>							
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
<i>Harvest period</i>							
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
<i>Shading x harvest period</i>							
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

1602 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)
<i>Shading</i>				
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
<i>Harvest period</i>				
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
<i>Shading x harvest period</i>				
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 ± 4.61 cd	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 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
<i>Shading</i>			
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
<i>Harvest period</i>			
I2	35.85	4.38	8.74
I3	33.90	4.42	8.10
I4	32.20	4.07	8.30
<i>Shading x harvest period</i>			
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)
<i>Shading</i>					
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
<i>Harvest period</i>					
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
<i>Shading x harvest period</i>					
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.

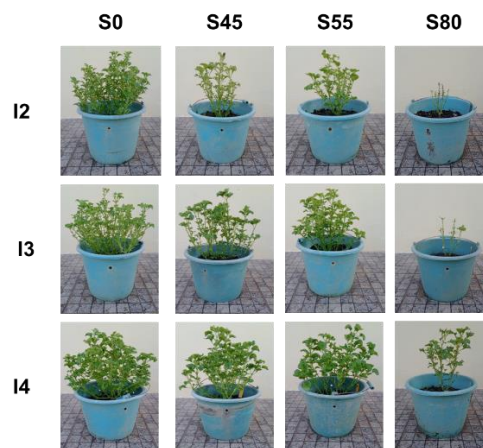


Figure 6. Visualization of Brazilian spinach shoot on different shading and harvest period at 13 weeks after planting (WAP). Shading treatment consist of S0: no-shading, S45: 45% shading, S55: 55% shading, and S80: 80% shading. Meanwhile, the harvest period consists of I2: per 2 weeks, I3: per 3 weeks,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

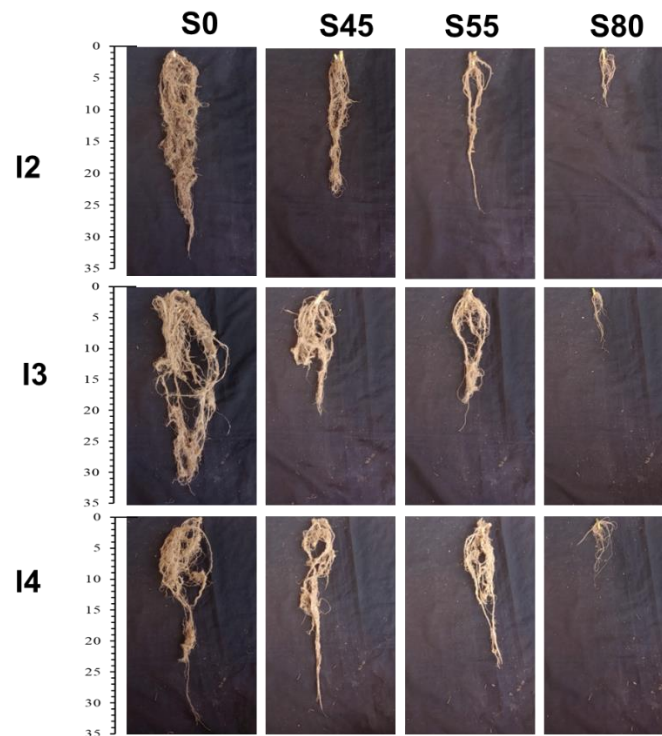


Figure 7. Visualization of Brazilian spinach root on different shading and harvest period at 13 weeks after planting (WAP). Shading treatment consist of S0: no-shading, S45: 45% shading, S55: 55% shading, and S80: 80% shading. Meanwhile, the harvest period consists of **I2**: per 2 weeks, **I3**: per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

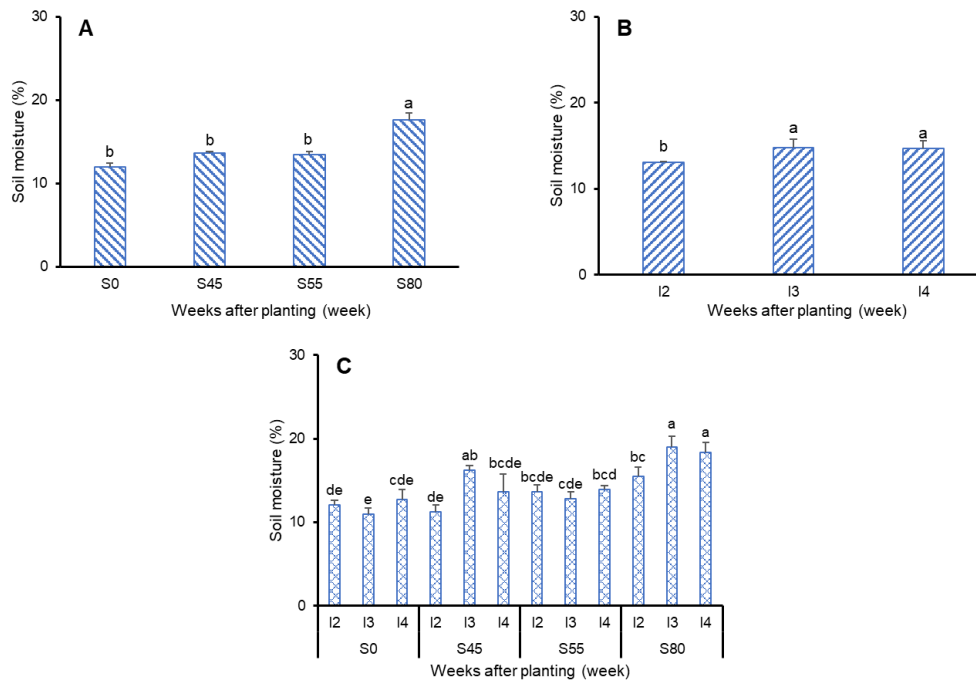


Figure 8. Substrate moisture on different shading (A), harvest period (B), and their interaction (C). Shading treatment consist of S0: no-shading, S45: shading 45%, S55: shading 55%, and S80: shading 80%. Meanwhile, the harvest period consists of I2: per 2 weeks, I3: per 3 weeks, and I4: per 4 weeks.

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.

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4. Editor Decision: Accepted (22 Mei 2024)

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[REAN] Editor Decision

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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

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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 cm²), 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, aclimação 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 (104°46'44"E, 3°01'35"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.

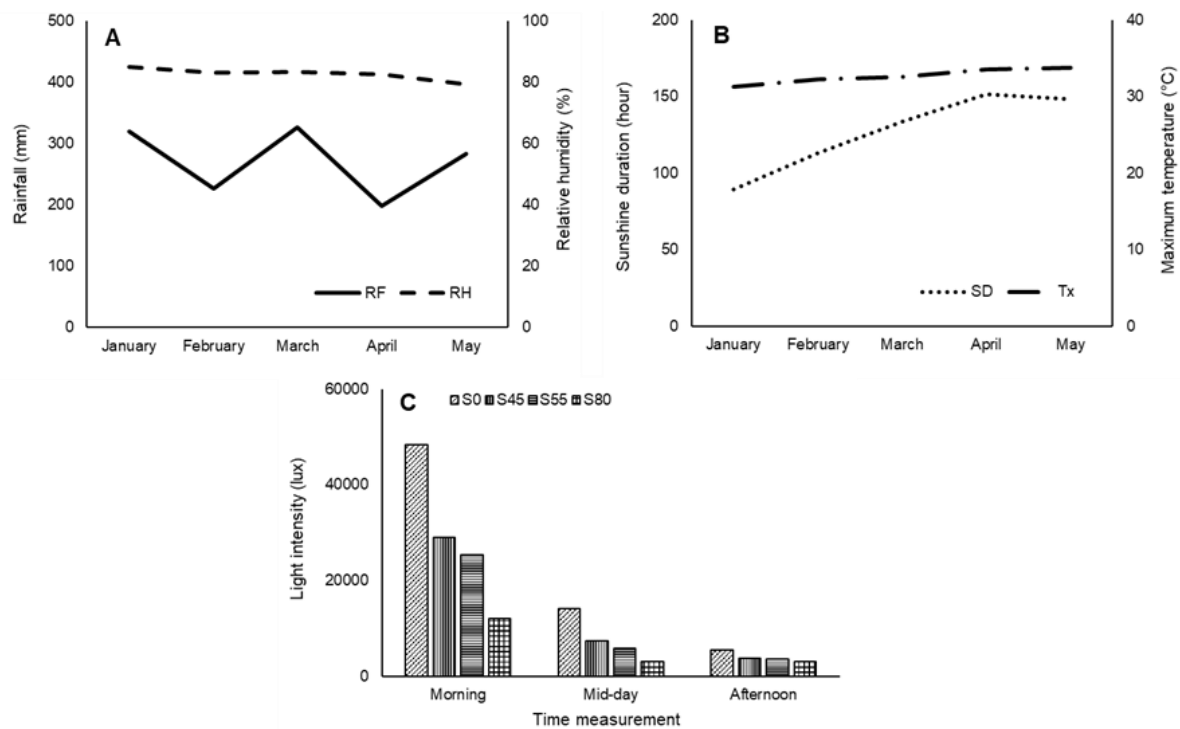


Figure 1. Agroclimatic characteristics of the research site include total monthly rainfall (RF) and average humidity (RH) (A), sunshine duration (SD) and average maximum temperature (Tx) (B), and average light intensity of each treatment (C). (Source: Indonesian Agency for Meteorology, Climatology, and 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 thermocatenuatus*. 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 Easlou & 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.

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).

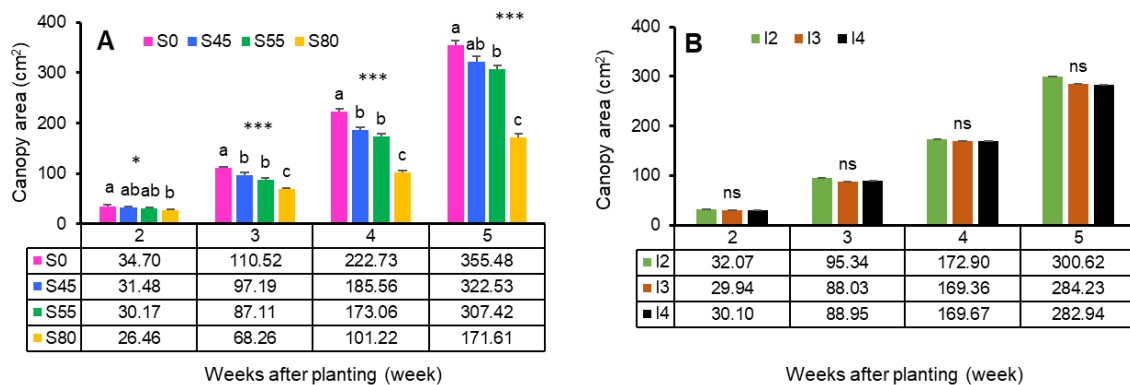


Figure 2. Brazilian spinach canopy area during early vegetative growth under different shading (A) and harvest period (B) treatments. The shading intensities consist of S0, S45, S55, and S80, while harvest periods include I2, I3, and I4. The ns= non-significant difference at $P < 0.05$; *= significant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

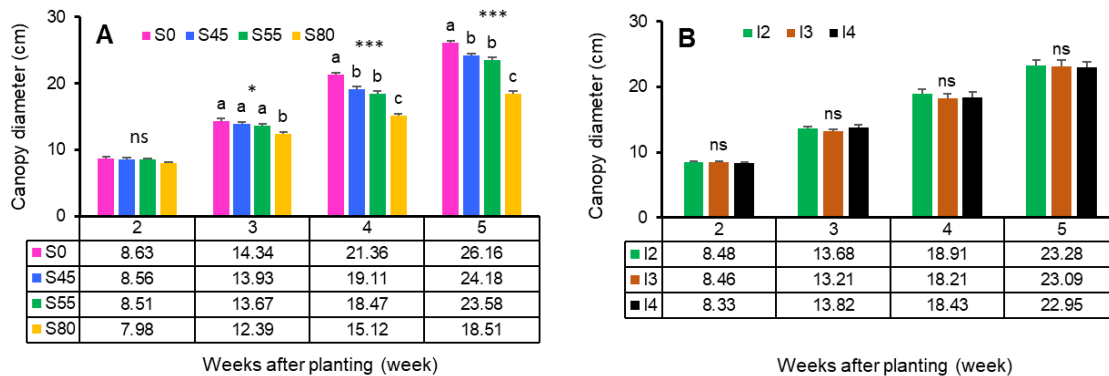


Figure 3. Brazilian spinach canopy diameter during early vegetative growth under different shading (A) and harvest period (B) treatments. Shading intensities consist of S0, S45, S55, and S80, while harvest periods include I2, I3, and I4. The ns= non-significant difference at $P<0.05$; *= significant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

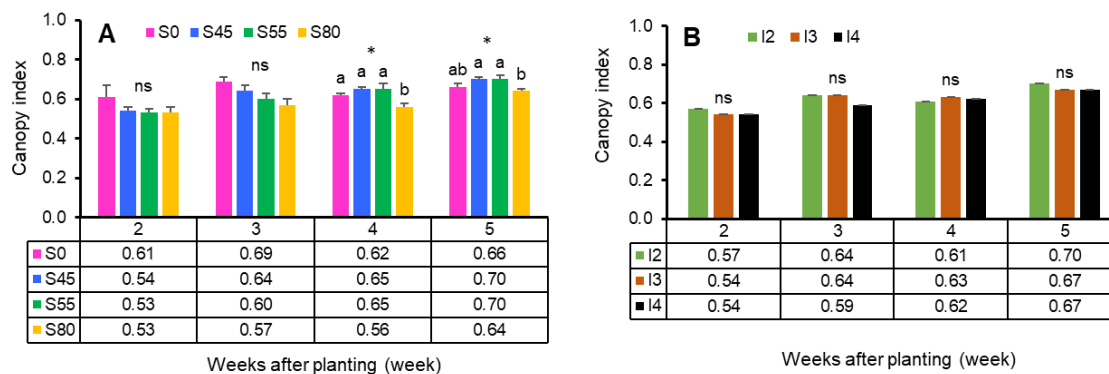


Figure 4. Canopy index for early vegetative growth at different shading and harvest period treatments. The shading intensities consist of S0, S45, S55, and S80, while harvest periods include I2, I3, and I4. The ns= non-significant difference at $P<0.05$; *= significant difference at $P<0.05$.

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).

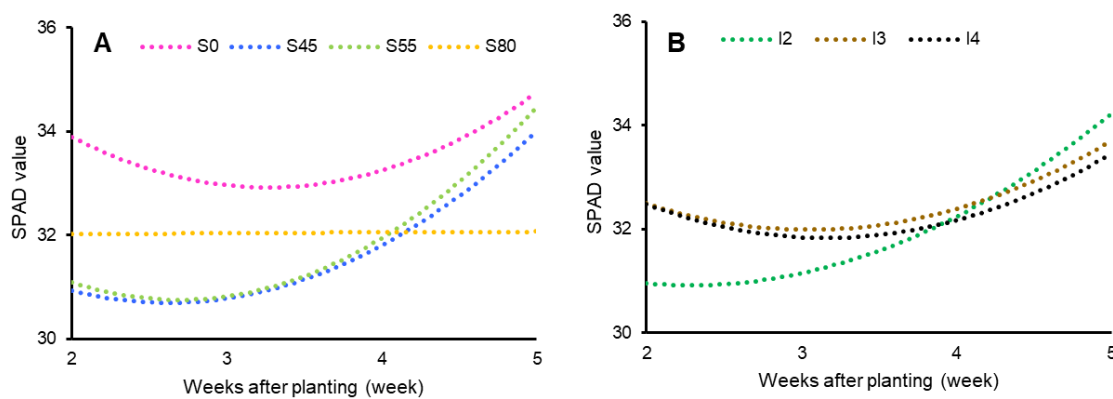


Figure 5. The SPAD value of Brazilian spinach leaves during early vegetative growth under different shading (A) and harvest period (A) treatments. The shading intensities consist of S0, S45, S55, and S80, while harvest periods include I2, I3, and I4.

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.

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

Treatment	Weeks after planting (week)						
	5	6	7	8	9	10	11
	<i>Shading</i>						
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
	<i>Harvest period</i>						
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
	<i>Shading x harvest period</i>						
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

- 3 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)
<i>Shading</i>				
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
<i>Harvest period</i>				
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
<i>Shading x harvest period</i>				
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 ± 4.61 cd	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. 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
<i>Shading</i>			
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
<i>Harvest period</i>			
I2	35.85	4.38	8.74
I3	33.90	4.42	8.10
I4	32.20	4.07	8.30
<i>Shading x harvest period</i>			
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)
<i>Shading</i>					
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
<i>Harvest period</i>					
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
<i>Shading x harvest period</i>					
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.

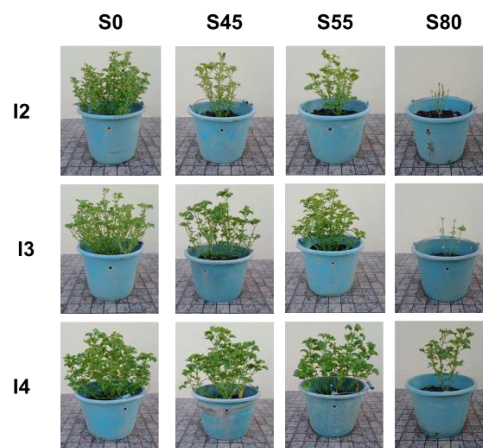


Figure 6. Visualization of Brazilian spinach shoots under different shading and harvest period treatments at 13 WAP. Shading intensities consist of S0, S45, S55, and S80, while the harvest periods include I2, I3, and I4. Photos: Strayker Ali Muda.

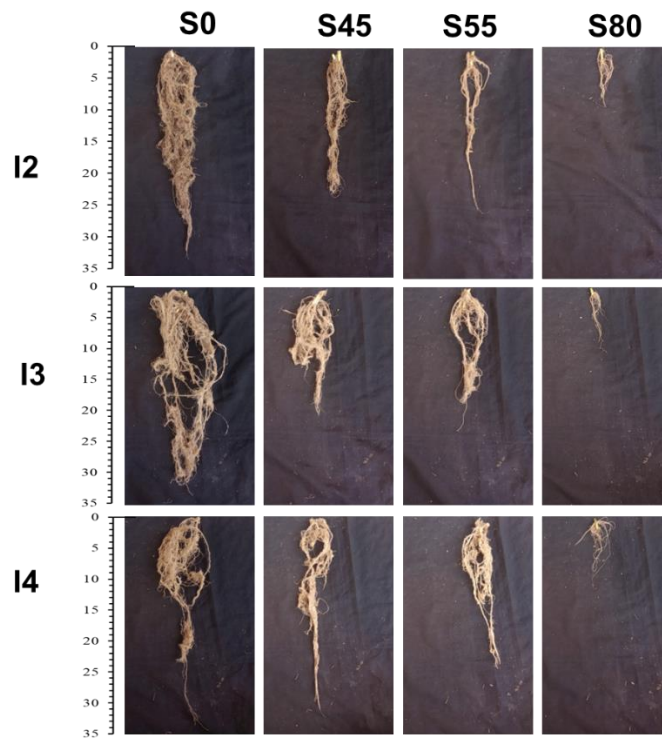


Figure 7. Visualization of Brazilian spinach roots under different shading and harvest period treatments at 13 WAP. Shading intensities consist of S0, S45, S55, and S80, while the harvest periods include I2, I3, and I4. Photos: Strayker Ali Muda.

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.

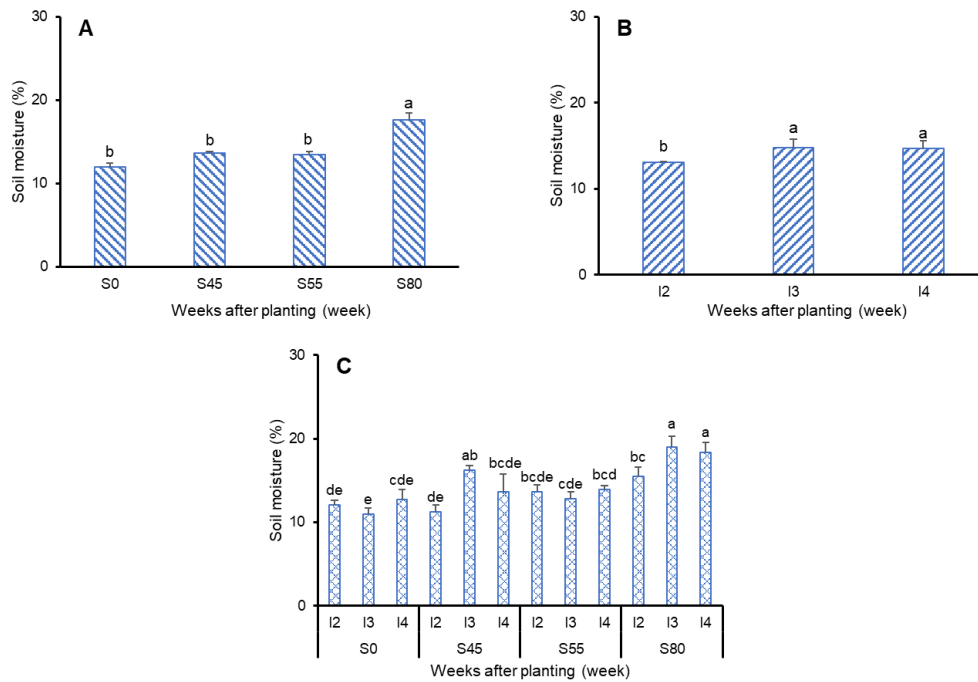


Figure 8. Substrate moisture at different shading intensities (A), harvest periods (B), and the interactions between both treatments (C). Shading intensities consist of S0, S45, S55, and S80, while the harvest periods include I2, I3, and I4.

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.

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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 cm²), 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, aclimação 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 (104°46'44"E, 3°01'35"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.

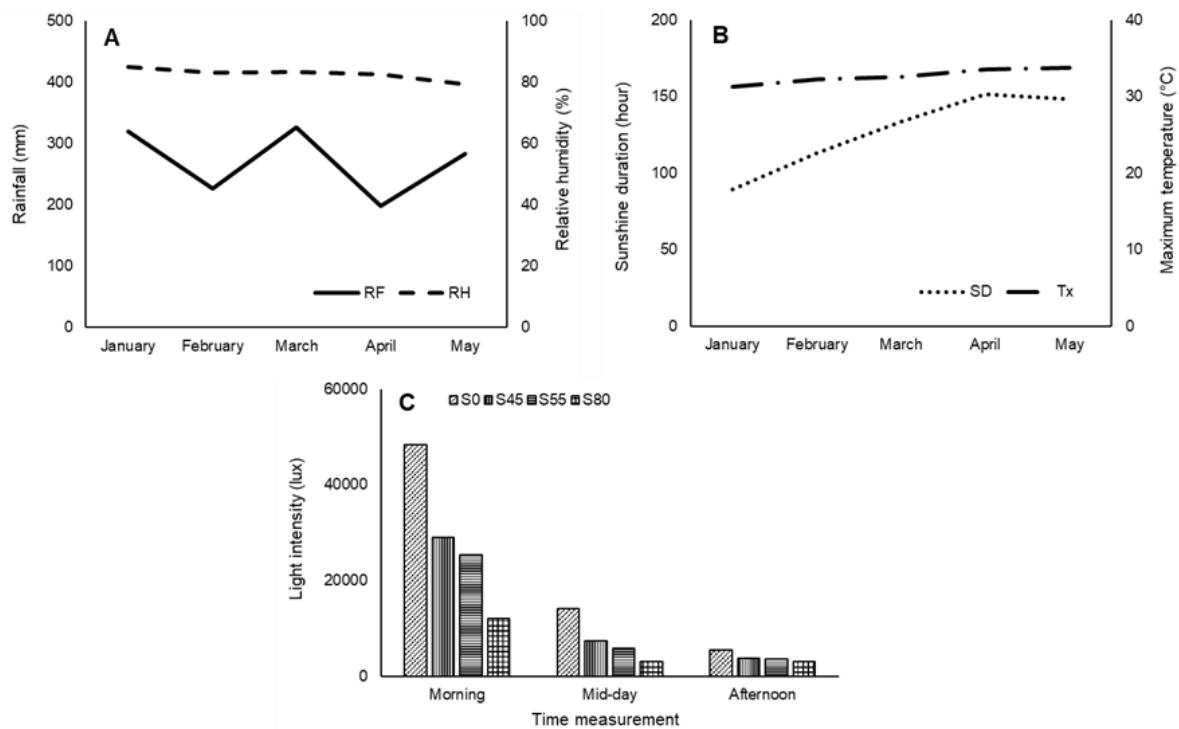


Figure 1. Agroclimatic characteristics of the research site include total monthly rainfall (RF) and average humidity (RH) (A), sunshine duration (SD) and average maximum temperature (Tx) (B), and average light intensity of each treatment (C). (Source: Indonesian Agency for Meteorology, Climatology, and 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 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.

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).

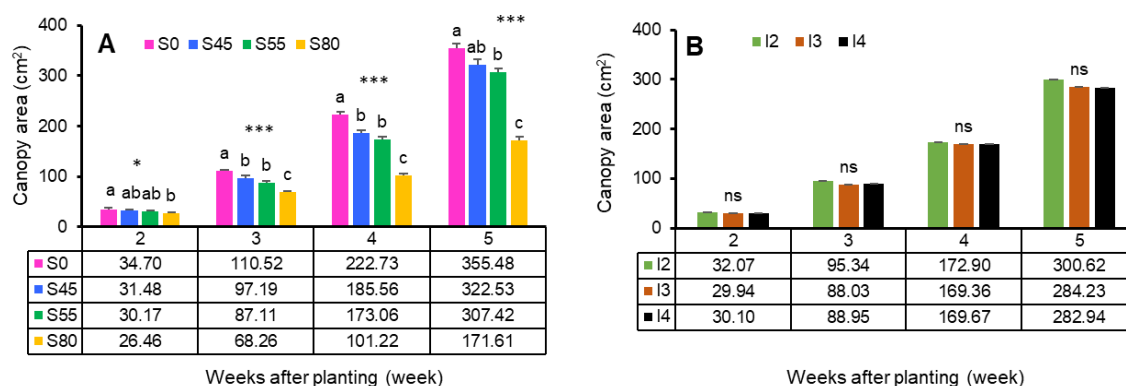


Figure 2. Brazilian spinach canopy area during early vegetative growth under different shading (A) and harvest period (B) treatments. The shading intensities consist of S0, S45, S55, and S80, while harvest periods include I2, I3, and I4. The ns= non-significant difference at $P<0.05$; *= significant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

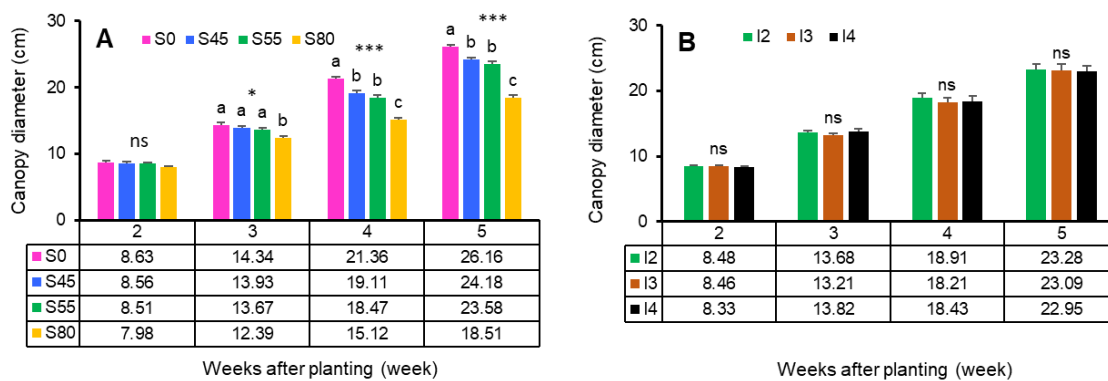


Figure 3. Brazilian spinach canopy diameter during early vegetative growth under different shading (A) and harvest period (B) treatments. Shading intensities consist of S0, S45, S55, and S80, while harvest periods include I2, I3, and I4. The ns= non-significant difference at $P<0.05$; *= significant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

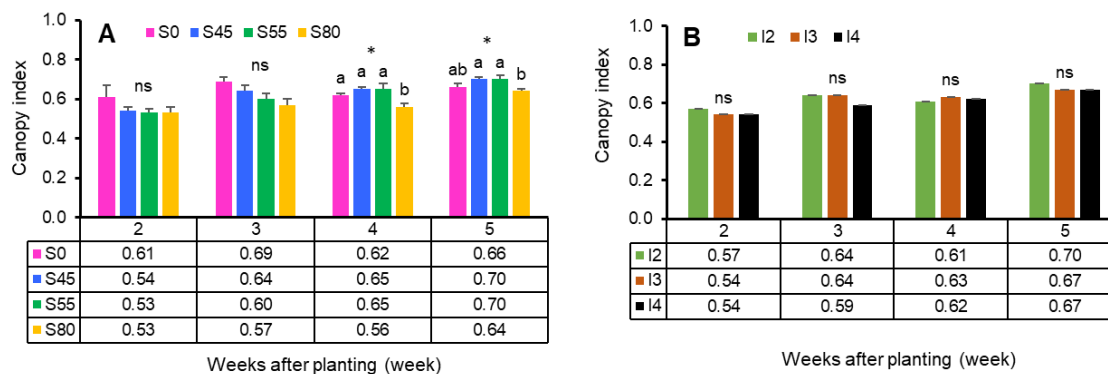


Figure 4. Canopy index for early vegetative growth at different shading and harvest period treatments. The shading intensities consist of S0, S45, S55, and S80, while harvest periods

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).

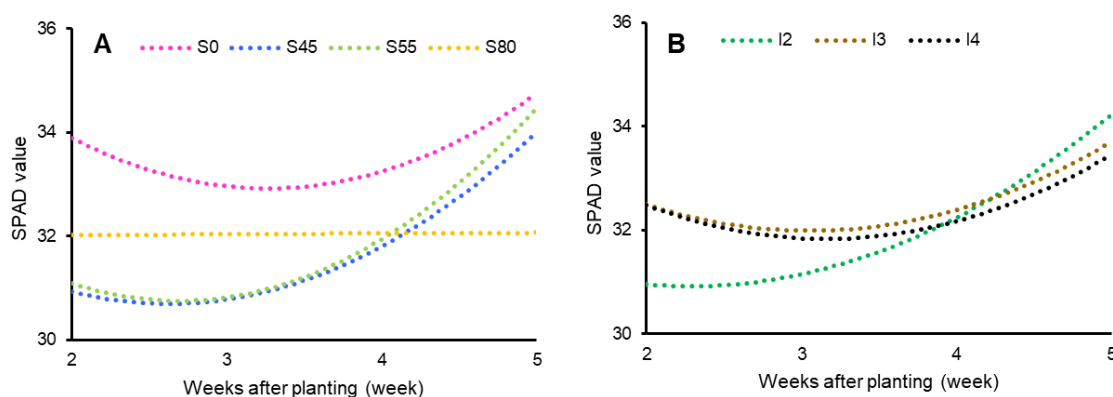


Figure 5. The SPAD value of Brazilian spinach leaves during early vegetative growth under different shading (A) and harvest period (A) treatments. The shading intensities consist of S0, S45, S55, and S80, while harvest periods include I2, I3, and I4.

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.

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

Treatment	Weeks after planting (week)						
	5	6	7	8	9	10	11
	<i>Shading</i>						
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
	<i>Harvest period</i>						
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
	<i>Shading x harvest period</i>						
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

- 3 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)
<i>Shading</i>				
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
<i>Harvest period</i>				
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
<i>Shading x harvest period</i>				
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 ± 4.61 cd	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. 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
<i>Shading</i>			
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
<i>Harvest period</i>			
I2	35.85	4.38	8.74
I3	33.90	4.42	8.10
I4	32.20	4.07	8.30
<i>Shading x harvest period</i>			
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)
<i>Shading</i>					
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
<i>Harvest period</i>					
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
<i>Shading x harvest period</i>					
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.

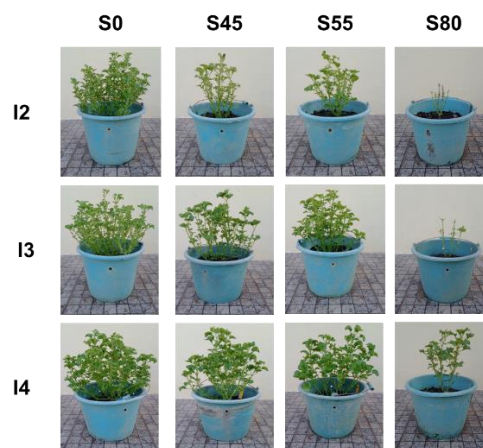


Figure 6. Visualization of Brazilian spinach shoots under different shading and harvest period treatments at 13 WAP. Shading intensities consist of S0, S45, S55, and S80, while the harvest periods include I2, I3, and I4. Photos: Strayker Ali Muda.



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.

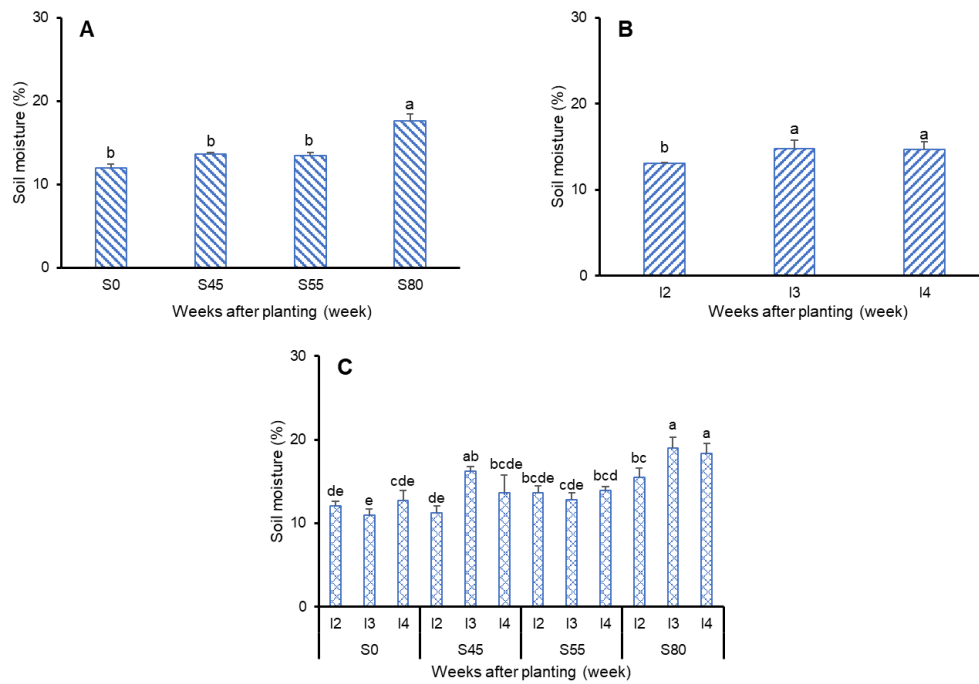


Figure 8. Substrate moisture at different shading intensities (A), harvest periods (B), and the interactions between both treatments (C). Shading intensities consist of S0, S45, S55, and S80, while the harvest periods include I2, I3, and I4.

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.

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