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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.

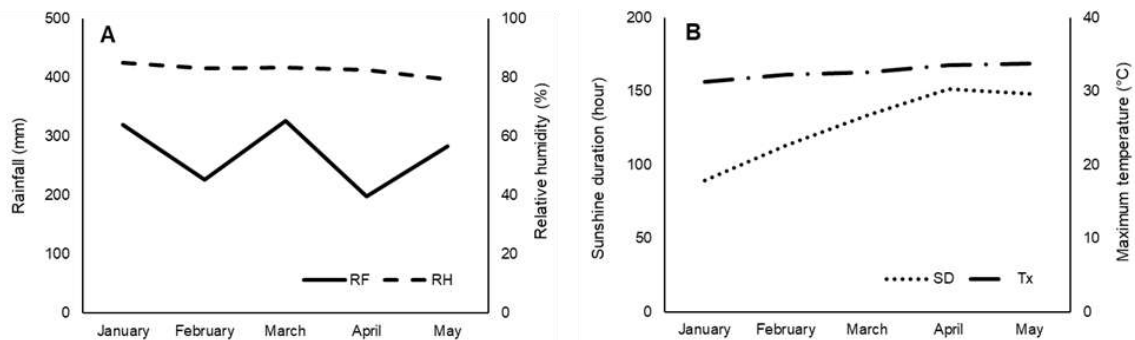


Figure 1. Agroclimatic characteristics in research location as indicated by total monthly rainfall (RF) and average humidity (RH) (A), and total monthly sunshine duration (SD) and average maximum temperature (Tx) (B). (Source: Indonesian Agency for Meteorology, Climatology and Geophysics).

### 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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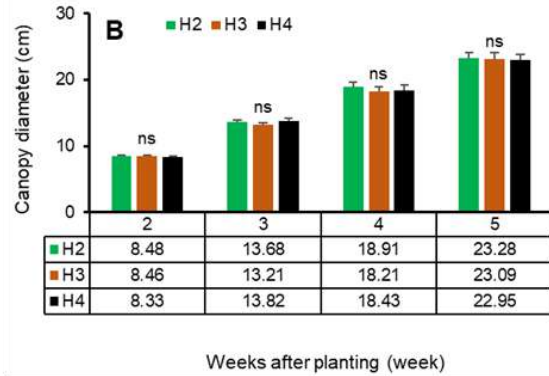
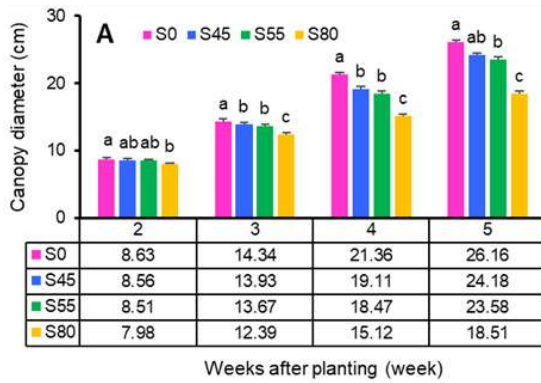
## 153 **RESULTS AND DISCUSSION**

### 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).

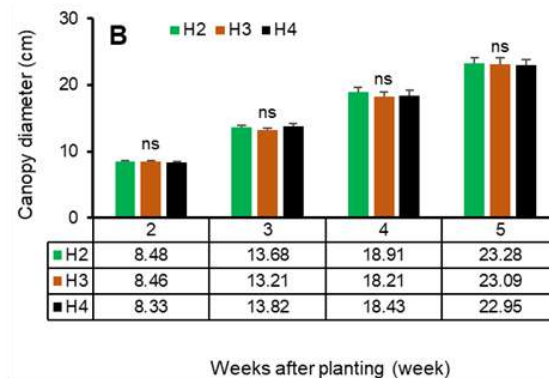
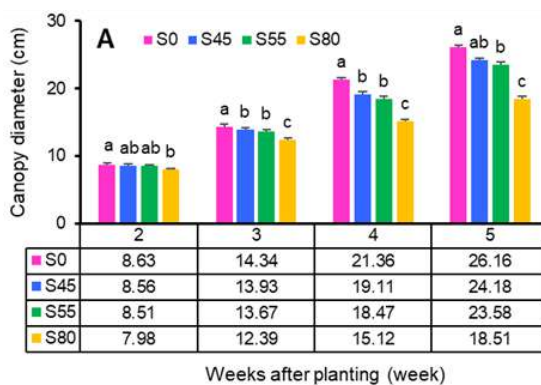
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168

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).



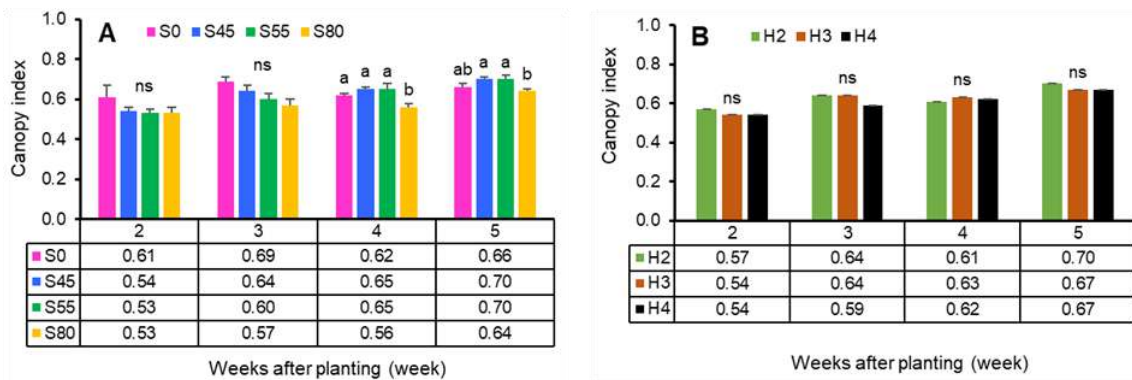
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179 Figure 3. Brazilian spinach canopy diameter on early vegetative growth on different  
 180 shading (A) and harvest period (B) treatment. The shading consists of no shading (S0),  
 181 45% shading (S45), 55% shading (S55), and 80% shading (S80). 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).

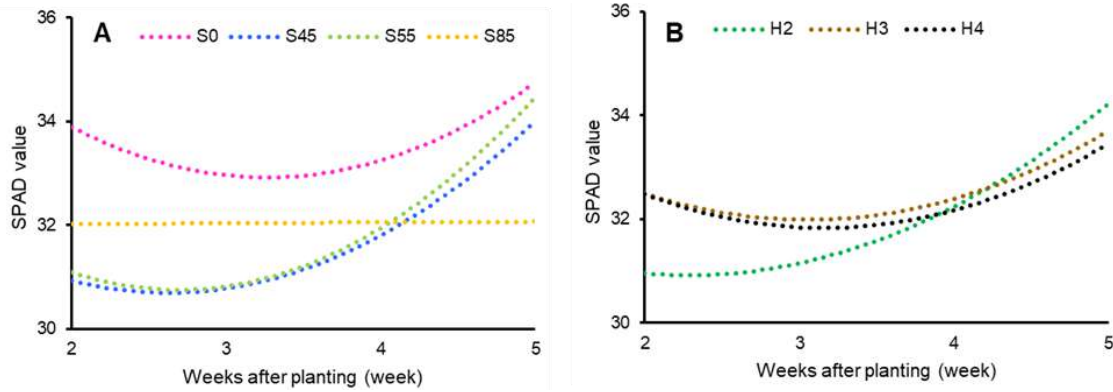


189  
 190 Figure 4. Canopy index on early vegetative growth on different shading and harvest  
 191 period as treatment. The shading consists of no shading (S0), 45% shading (S45), 55%  
 192 shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent harvest  
 193 period per 2 weeks, 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  
 219 Figure 5. The SPAD value on early vegetative growth on different shading (A) and  
 220 harvest period (A) treatment. The shading consists of no shading (S0), 45% shading  
 221 (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent  
 222 harvest period per 2 weeks, 3 weeks.

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 <sub>0.05</sub>             | 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 <sub>0.05</sub>             | 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 <sub>0.05</sub>             | 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 <sub>0.05</sub>             | 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 <sub>0.05</sub>             | 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 <sub>0.05</sub>             | 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<br>(g) | Branch dry weight<br>(g) | Leaf dry weight<br>(g) | Root dry weight<br>(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 <sub>0.05</sub>             | 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 <sub>0.05</sub>             | 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 <sub>0.05</sub>             | 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.

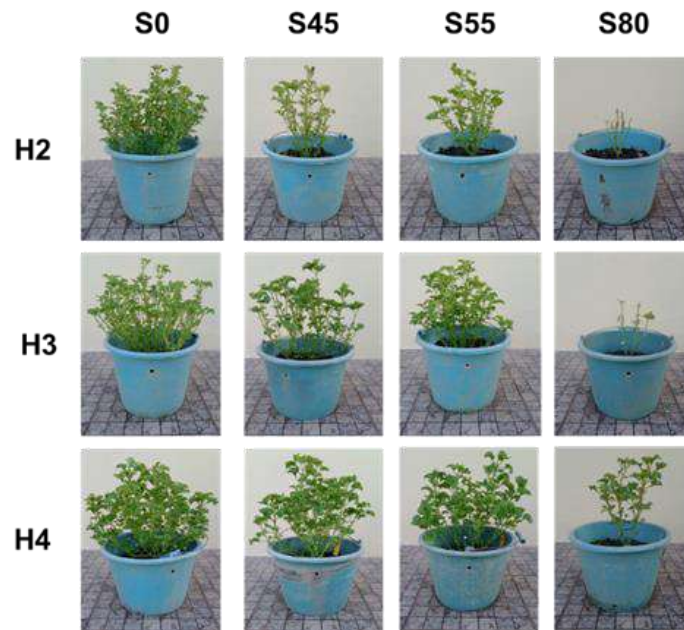


Figure 6. Visualization of Brazilian spinach shoot on different shading and harvest period at 13 weeks after planting (WAP). Shading treatment consist of S0: no-shading, S45: 45% shading, S55: 55% shading, and S80: 80% shading. Meanwhile, the harvest period consists of H2: per 2 weeks, H3: per 3 weeks,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

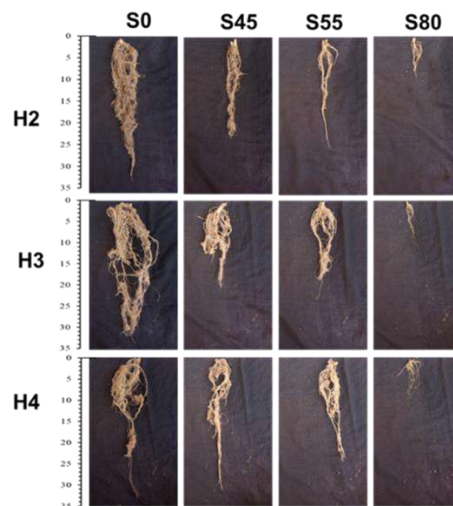


Figure 7. Visualization of Brazilian spinach root on different shading and harvest period at 13 weeks after planting (WAP). Shading treatment consist of S0: no-shading, S45: 45% shading, S55: 55% shading, and S80: 80% shading. Meanwhile, the harvest period consists of H2: per 2 weeks, H3: per 3 weeks,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

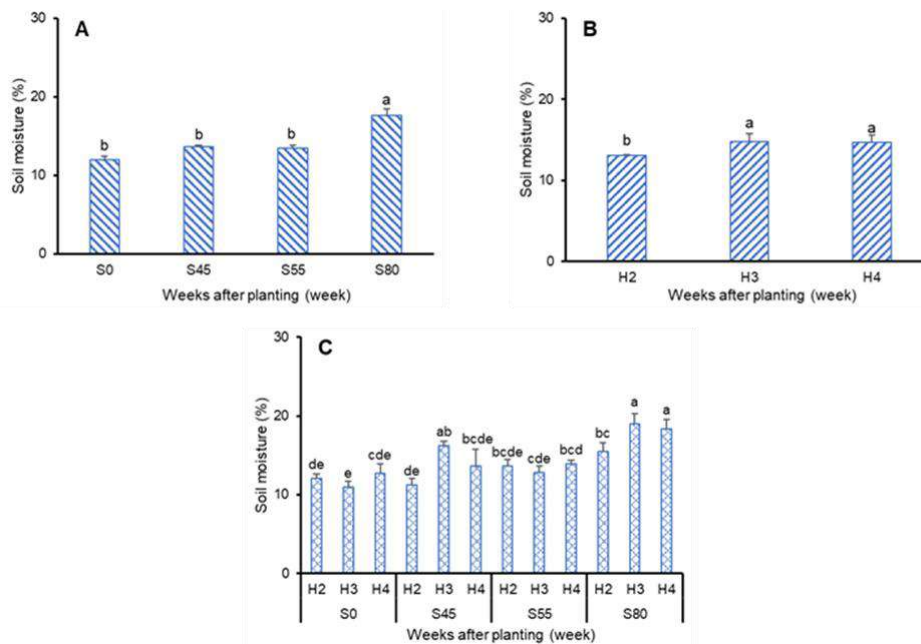


Figure 8. Substrate moisture on different shading (A), harvest period (B), and their interaction (C). Shading treatment consist of S0: no-shading, S45: shading 45%, S55: shading 55%, and S80: shading 80%. Meanwhile, the harvest period consists of H2: every 2 weeks, H3: every 3 weeks, and H4: every 4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

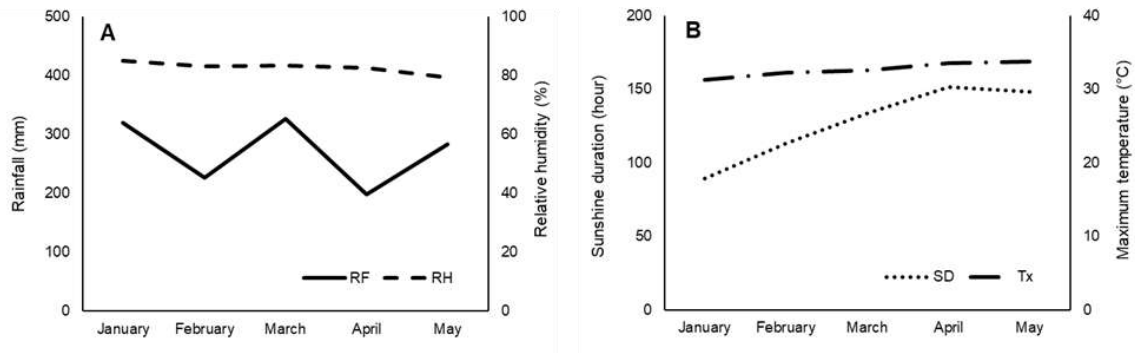


Figure 1. Agroclimatic characteristics in research location as indicated by total monthly rainfall (RF) and average humidity (RH) (A), and total monthly sunshine duration (SD) and average maximum temperature (Tx) (B). (Source: Indonesian Agency for Meteorology, Climatology and Geophysics).

#### *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).

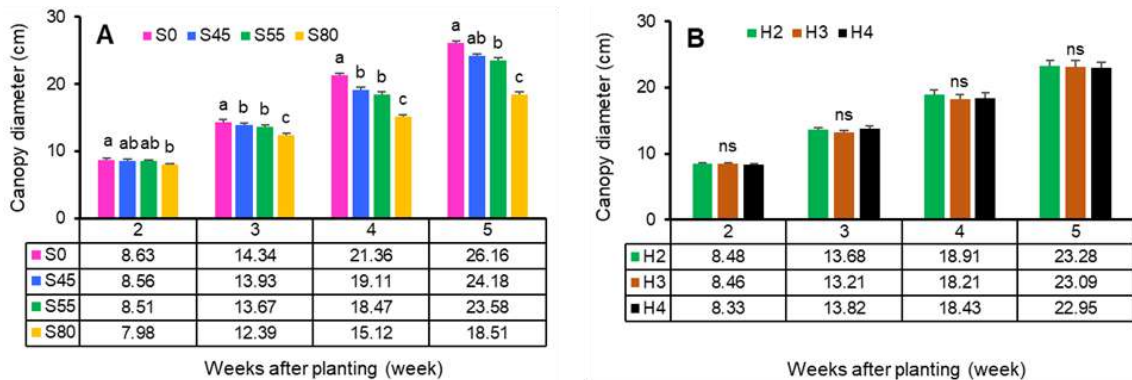


Figure 2. Brazilian spinach canopy area on early vegetative growth on different shading (A) and harvest period (B) treatment. The shading consists of no shading (S0), 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent harvest period per 2 weeks, 3 weeks,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

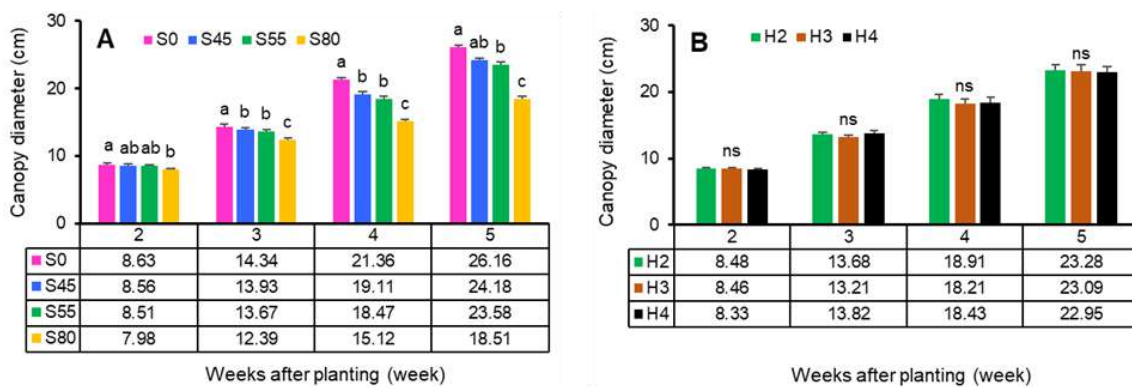


Figure 3. Brazilian spinach canopy diameter on early vegetative growth on different shading (A) and harvest period (B) treatment. The shading consists of no shading (S0), 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent harvest period per 2 weeks, 3 weeks,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



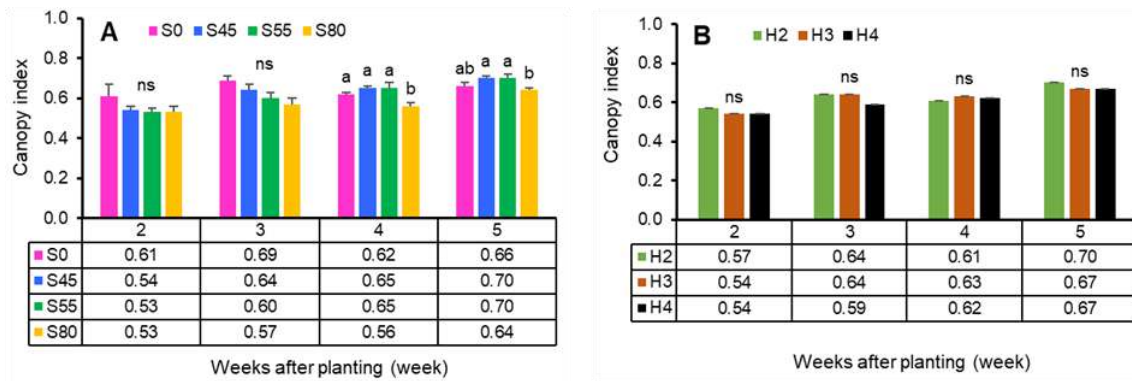


Figure 4. Canopy index on early vegetative growth on different shading and harvest period as treatment. The shading consists of no shading (S0), 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent harvest period per 2 weeks, 3 weeks, and 4 weeks, respectively.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

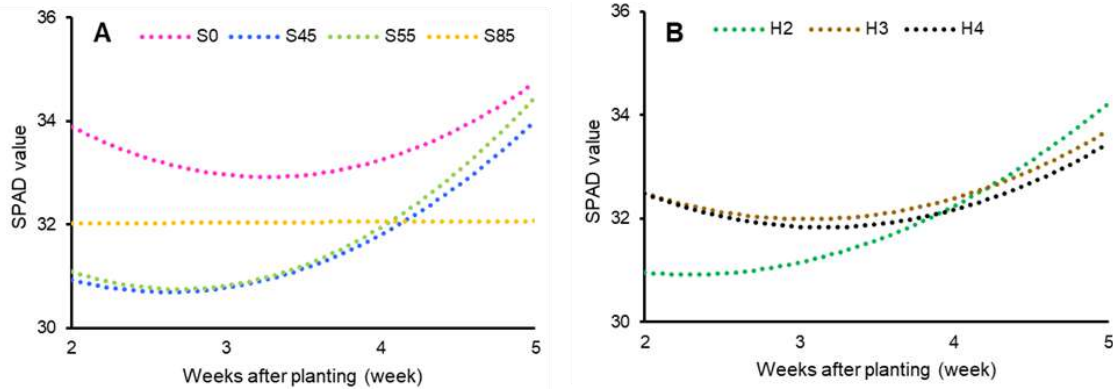


Figure 5. The SPAD value on early vegetative growth on different shading (A) and harvest period (A) treatment. The shading consists of no shading (S0), 45% shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent harvest period per 2 weeks, 3 weeks.

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 <sub>0.05</sub>             | 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 <sub>0.05</sub>             | 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 <sub>0.05</sub>             | 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 <sub>0.05</sub>             | 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 <sub>0.05</sub>             | 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 <sub>0.05</sub>             | 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 <sub>0.05</sub>             | 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 <sub>0.05</sub>             | 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 <sub>0.05</sub>             | 0.572               | 2.457                 | 2.234               | 3.744               |

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

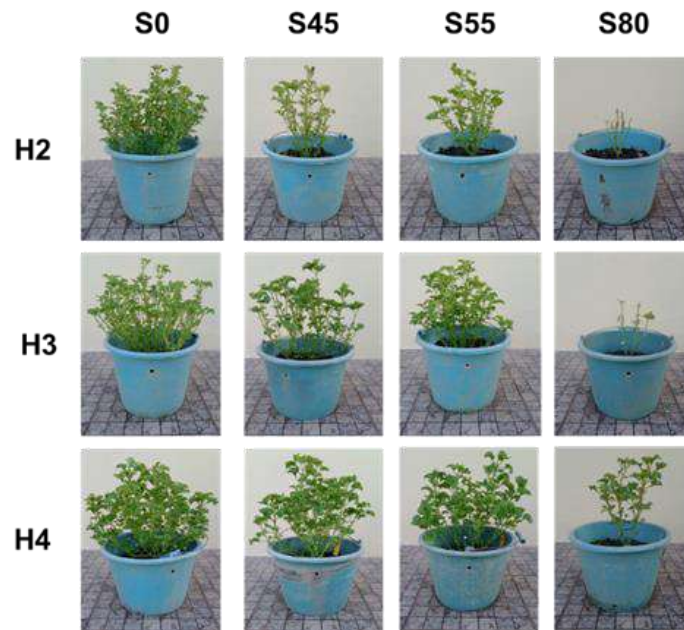
83

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).

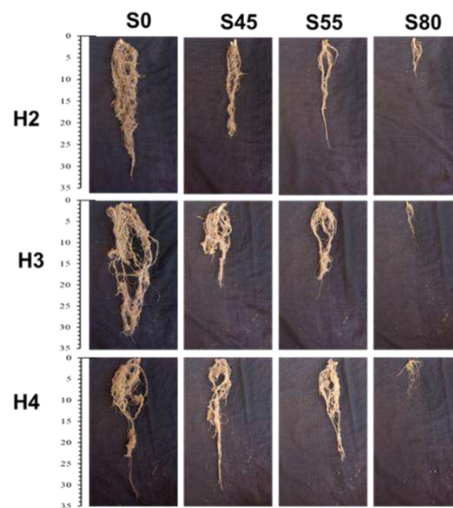
92         Brazilian spinach showed varying morphological traits under different treatments.  
93 Shading causes alterations in plant organs, as shown on soybean stems, which experience  
94 inhibited growth (Castronuovo et al., 2019). Cao et al. (2022) reported that *Cynodon*  
95 *dactylon* shoot organs 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

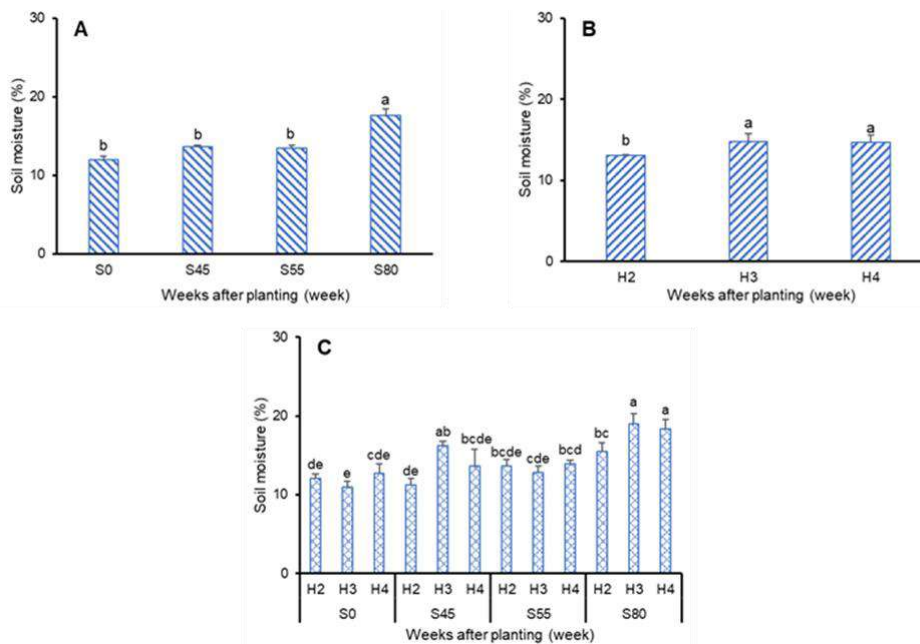
112 Figure 7. Visualization of Brazilian spinach root on different shading and harvest period  
 113 at 13 weeks after planting (WAP). Shading treatment consist of S0: no-shading, S45: 45%  
 114 shading, S55: 55% shading, and S80: 80% shading. Meanwhile, the harvest period  
 115 consists of H2: per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

## [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

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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.

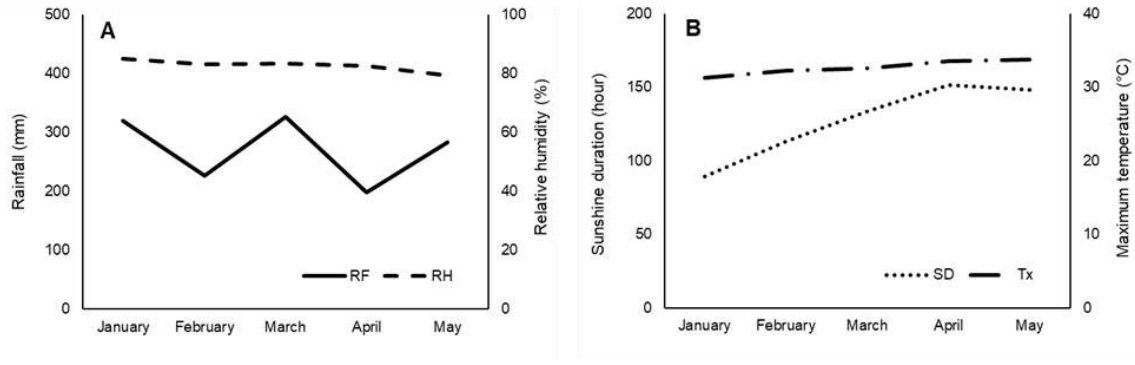
341 The cultivation of Brazilian spinach is characterized by its simplicity, since it may  
342 be easily grown. Muda et al. (2022) reported that the propagation of Brazilian spinach  
343 can be achieved via stem cuttings. There is an insufficient amount of research pertaining  
344 to the adaptability 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  $p < 0.05$ .

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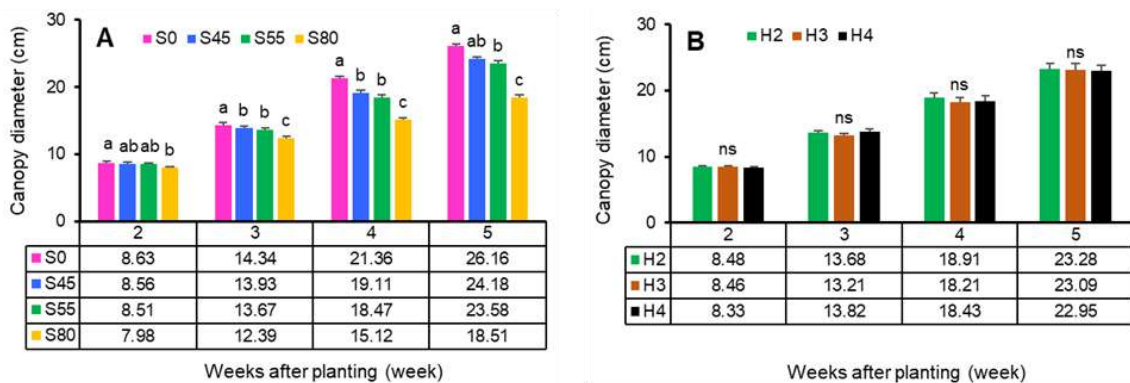
**RESULTS AND DISCUSSION**

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).

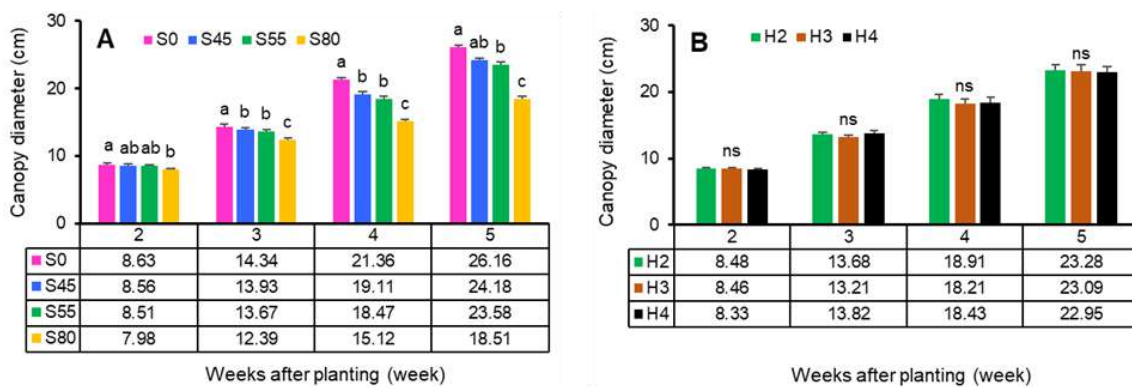
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436 Figure 2. Brazilian spinach canopy area on early vegetative growth on different shading  
 437 (A) and harvest period (B) treatment. The shading consists of no shading (S0), 45%  
 438 shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, 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  $p < 0.05$ .

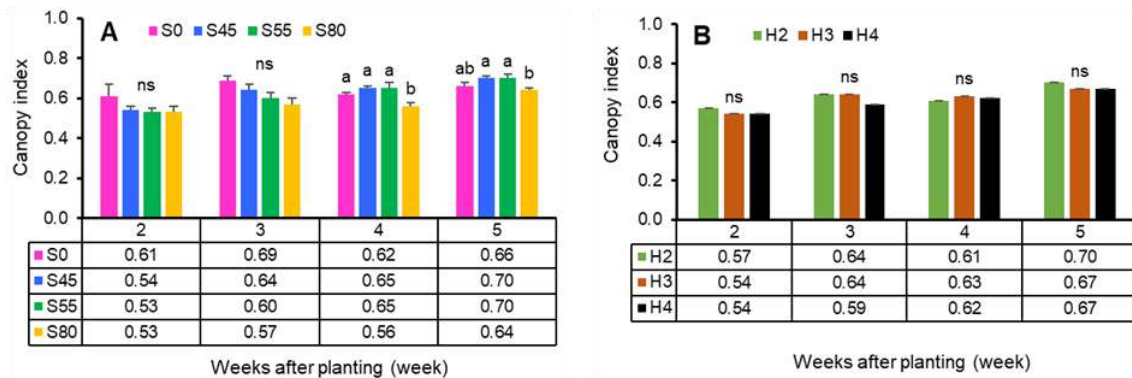
441 Brazilian spinach branches significantly influence canopy diameter, with  
 442 elongation affecting canopy expansion. Shading treatment inhibits branch growth. Full  
 443 sunlight cultivation leads to a wider canopy than canopies grown under different levels  
 444 of shading (S45, S55, and S80)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



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





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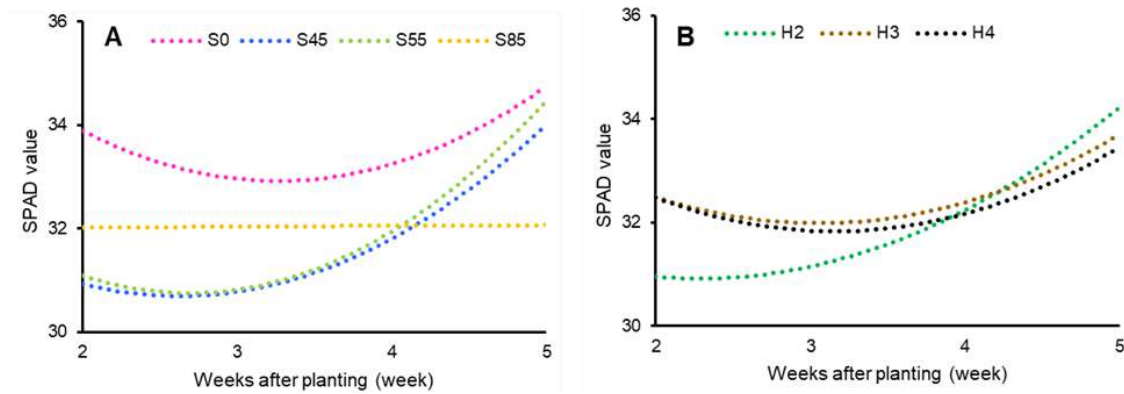
457 Figure 4. Canopy index on early vegetative growth on different shading and harvest  
 458 period as treatment. The shading consists of no shading (S0), 45% shading (S45), 55%  
 459 shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4 represent harvest  
 460 period per 2 weeks, 3 weeks, 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 <sub>0.05</sub>             | 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 <sub>0.05</sub>             | 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 <sub>0.05</sub>             | 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 <sub>0.05</sub>             | 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 <sub>0.05</sub>             | 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 <sub>0.05</sub>             | 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<br>(g) | Branch dry weight<br>(g) | Leaf dry weight<br>(g) | Root dry weight<br>(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 <sub>0.05</sub>             | 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 <sub>0.05</sub>             | 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 <sub>0.05</sub>             | 0.572                  | 2.457                    | 2.234                  | 3.744                  |

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

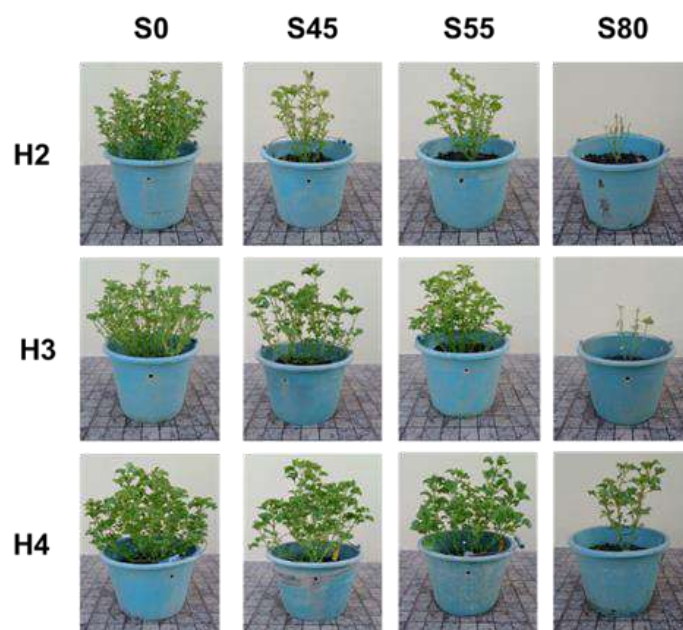
610

611 *Visual appearance of Brazilian spinach on different treatment*

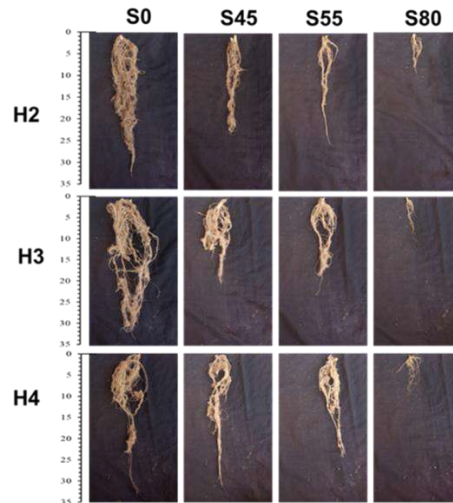
612 The study analysed the shoot appearance of Brazilian spinach under different  
613 shading conditions and harvesting periods. Unshaded areas had a denser appearance,  
614 while based on the harvesting period, treatments tend to show similarities with each other  
615 (Figure 6). Furthermore, Brazilian 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.

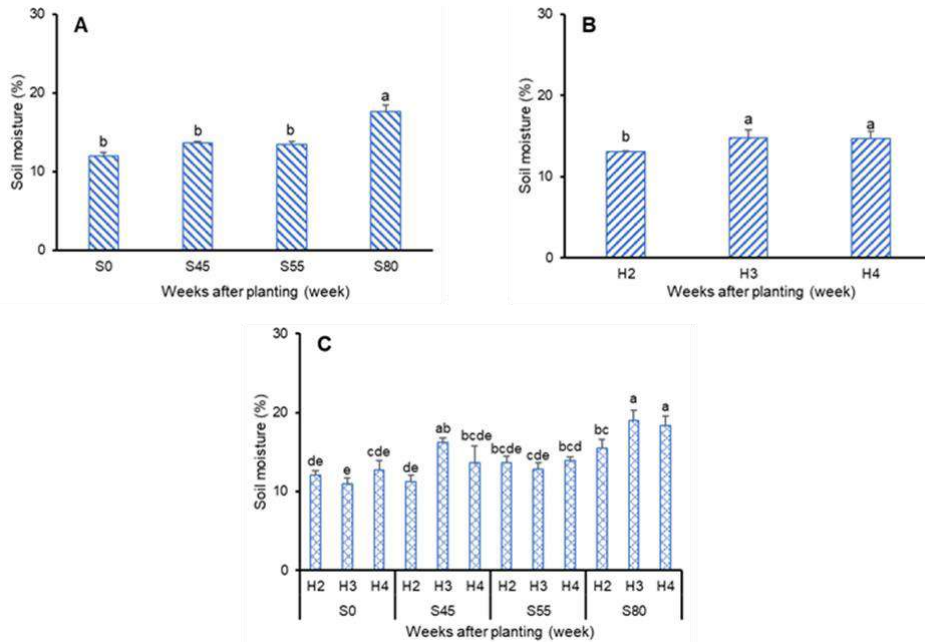
644

#### 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.

### 666 CONCLUSION

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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677

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**Reviewer 2**

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## **BRAZILIAN SPINACH GROWTH UNDER DIFFERENT SHADING**

815

## **INTENSITIES AND HARVESTING PERIODS IN LOWLAND TROPICAL**

816

## **URBAN ECOSYSTEM**

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818

### **ABSTRACT**

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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**

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

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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.

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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.

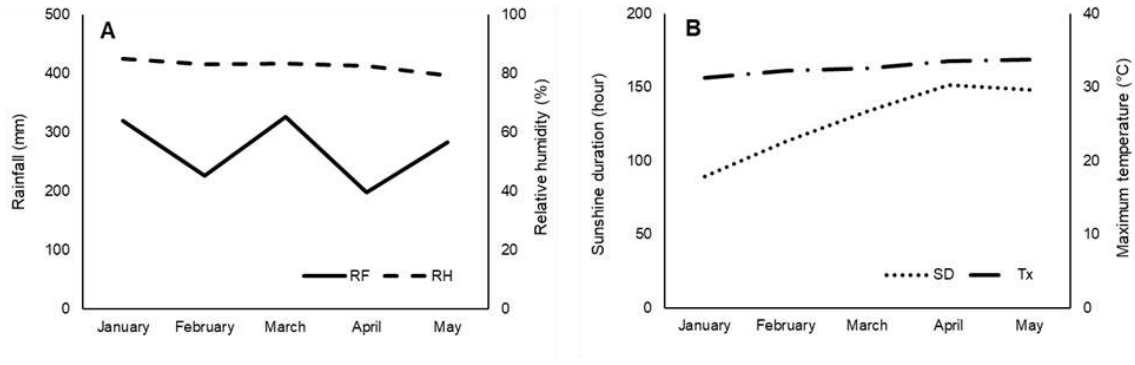
865 The cultivation of Brazilian spinach is characterized by its simplicity, since it may  
866 be easily grown. Muda et al. (2022) reported that the propagation of Brazilian spinach  
867 can be achieved via stem cuttings. There is an insufficient amount of research pertaining  
868 to the adaptability 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  $p < 0.05$ .

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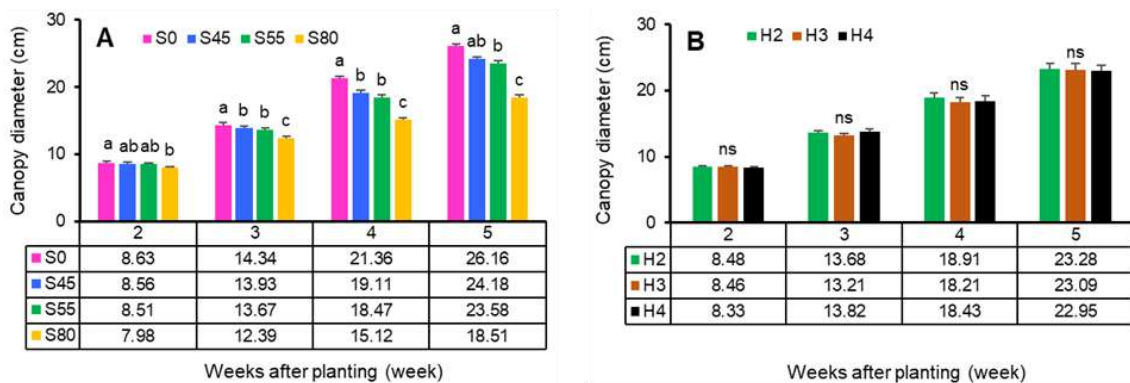
**RESULTS AND DISCUSSION**

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).

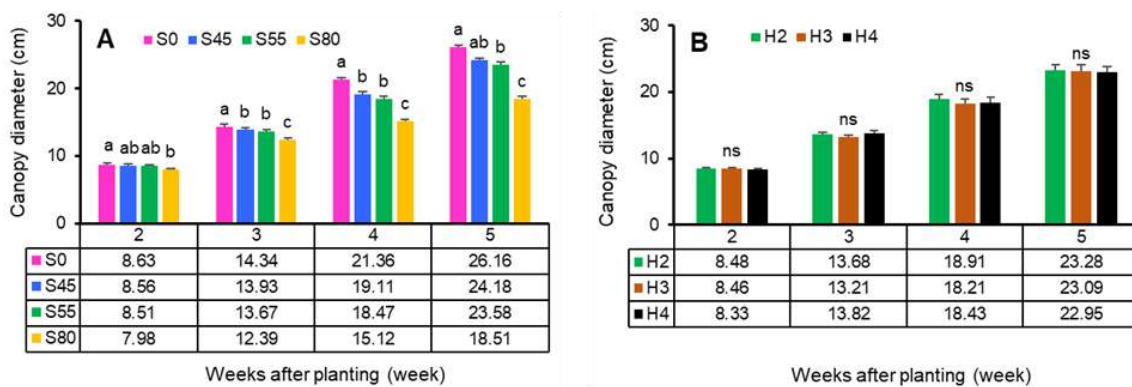
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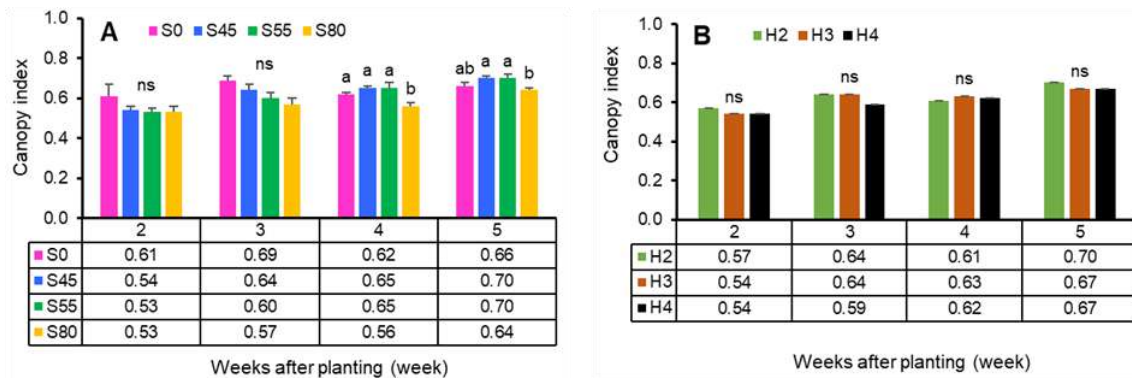
960 Figure 2. Brazilian spinach canopy area on early vegetative growth on different shading  
 961 (A) and harvest period (B) treatment. The shading consists of no shading (S0), 45%  
 962 shading (S45), 55% shading (S55), and 80% shading (S80). Meanwhile, H2, H3, and H4  
 963 represent harvest period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

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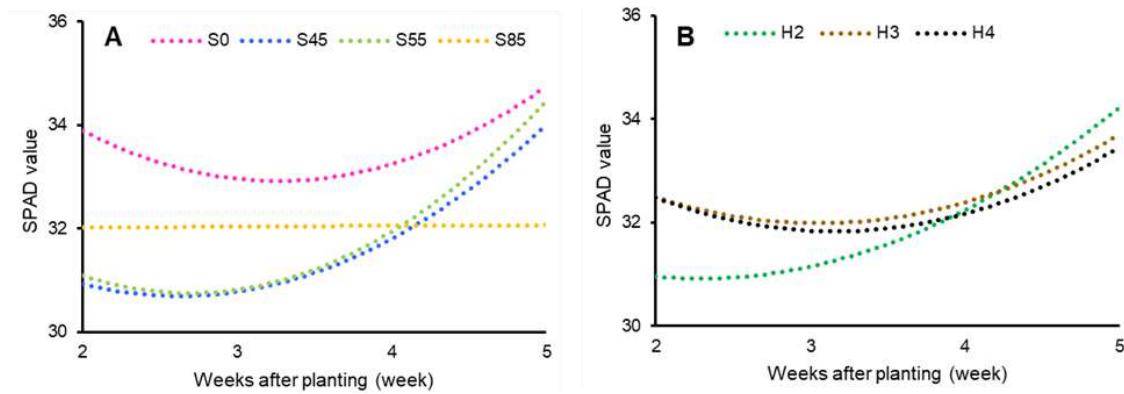
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The SPAD value is a method for evaluating leaf nitrogen and chlorophyll content, with a positive relationship between these factors. Brazilian spinach leaf's SPAD value was affected by shading treatments, with differences observed within each shading 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



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 <sub>0.05</sub>             | 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 <sub>0.05</sub>             | 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 <sub>0.05</sub>             | 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 <sub>0.05</sub>             | 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 <sub>0.05</sub>             | 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 <sub>0.05</sub>             | 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 <sub>0.05</sub>             | 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 <sub>0.05</sub>             | 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 <sub>0.05</sub>             | 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).

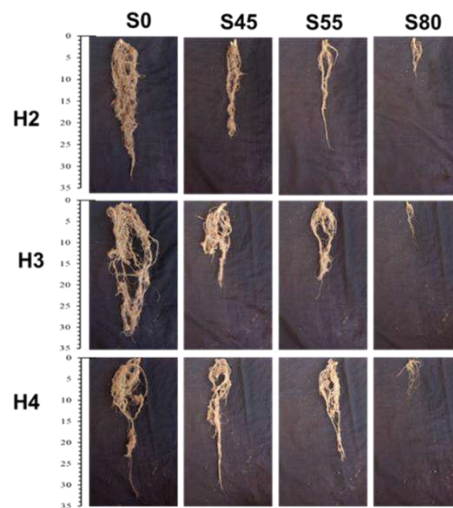
1142         Brazilian spinach showed varying morphological traits under different treatments.  
1143 Shading causes alterations in plant organs, as shown on soybean stems, which experience  
1144 inhibited growth (Castronuovo et al., 2019). Cao et al. (2022) reported that *Cynodon*  
1145 *dactylon* 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

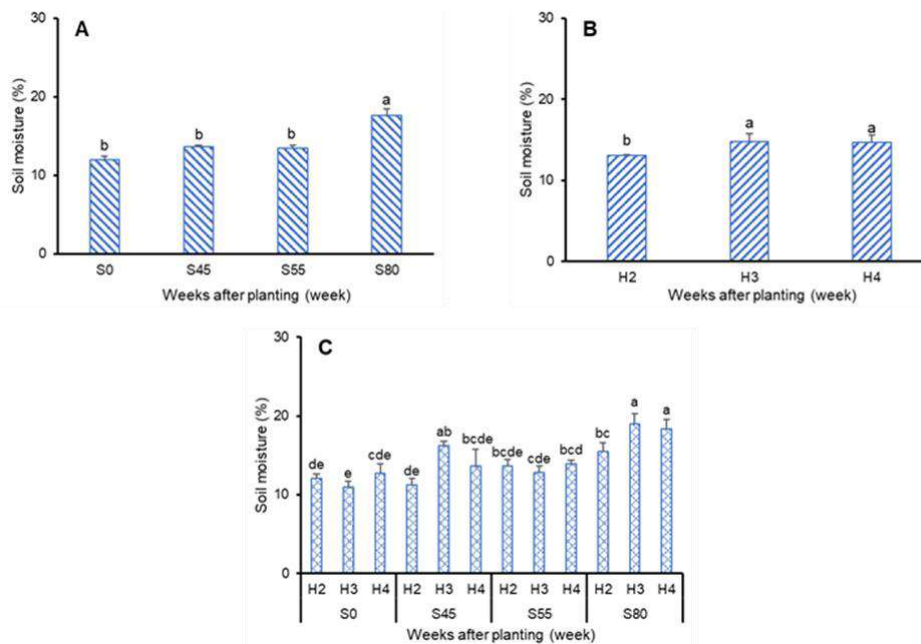
1162 Figure 7. Visualization of Brazilian spinach root on different shading and harvest period  
 1163 at 13 weeks after planting (WAP). Shading treatment consist of S0: no-shading, S45: 45%  
 1164 shading, S55: 55% shading, and S80: 80% shading. Meanwhile, the harvest period  
 1165 consists of H2: 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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**Revised version**

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## **BRAZILIAN SPINACH GROWTH UNDER DIFFERENT SHADING**

1338

## **INTENSITIES AND HARVESTING PERIODS IN LOWLAND TROPICAL**

1339

## **URBAN ECOSYSTEM**

1340

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### **ABSTRACT**

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

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**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

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

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

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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

**cm<sup>2</sup>), 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.

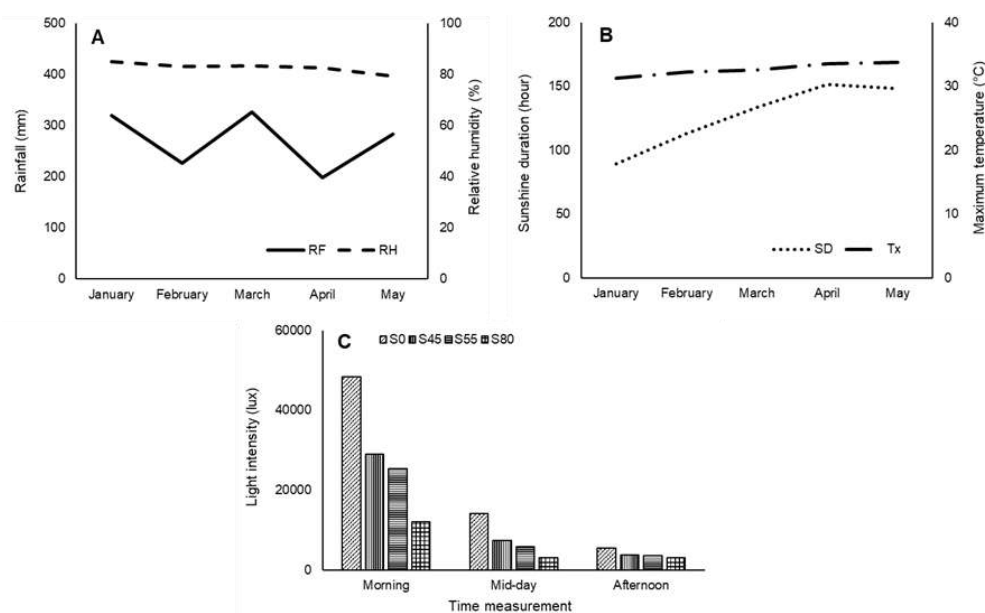
1402 The cultivation of Brazilian spinach is characterized by its simplicity, since it may  
1403 be easily grown. Muda et al. (2022) reported that the propagation of Brazilian spinach  
1404 can be achieved via stem cuttings. There is an insufficient amount of research pertaining  
1405 to the adaptability 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.

1410

## 1411 MATERIALS AND METHODS

### 1412 *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 I<sub>2</sub>, I<sub>3</sub>, and I<sub>4</sub>,  
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).

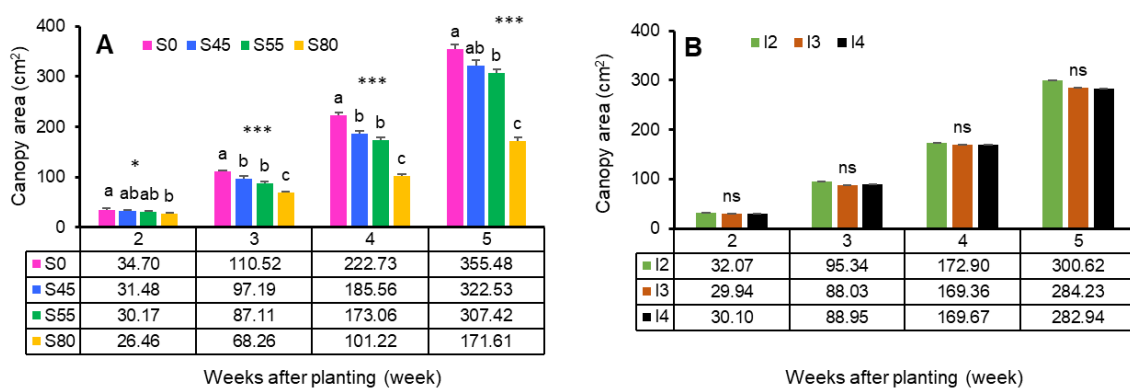
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## RESULTS AND DISCUSSION

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).

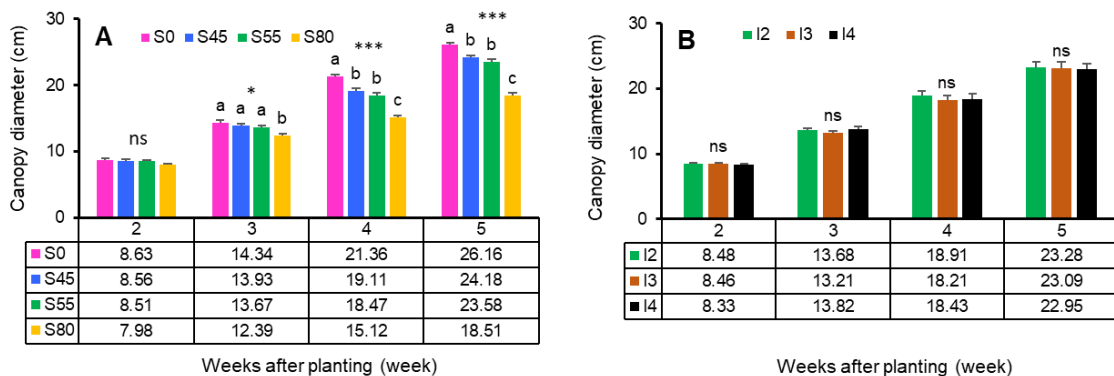


1507

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-

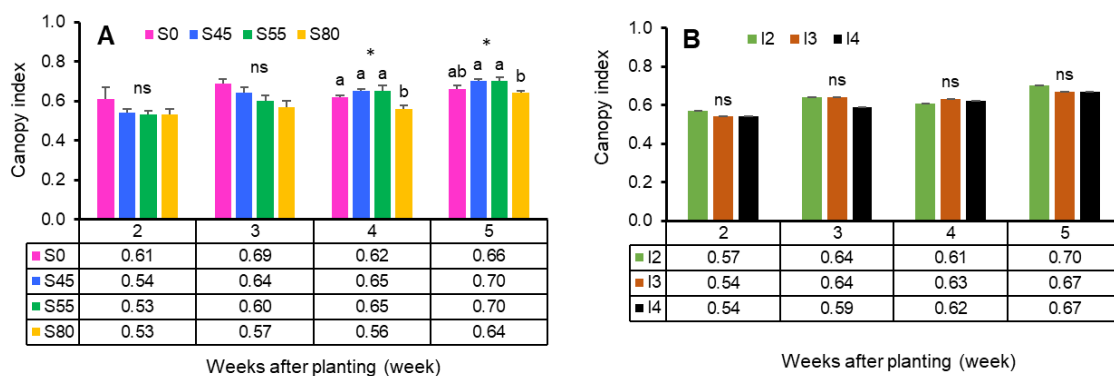
1512 significant difference at  $P < 0.05$ ; \*= significant difference at  $P < 0.05$ ; \*\*\*= significant  
 1513 difference at  $P < 0.001$ .

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).



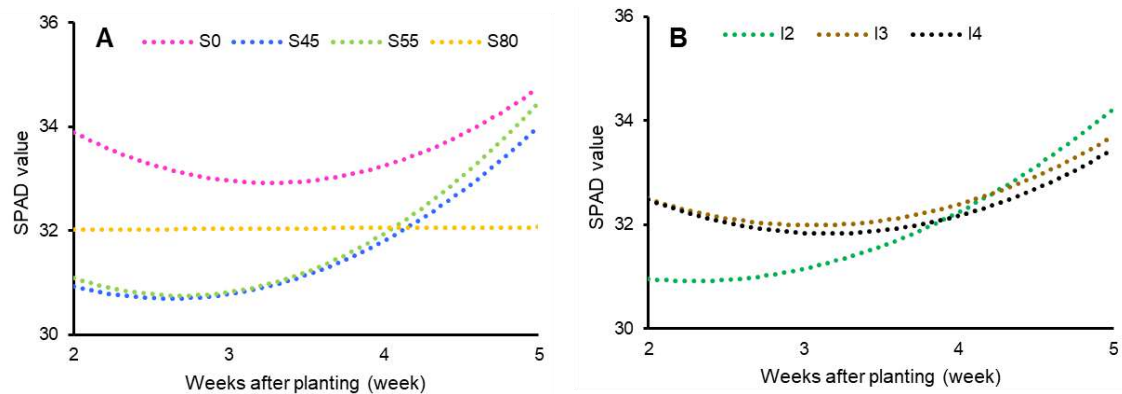
1530

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.

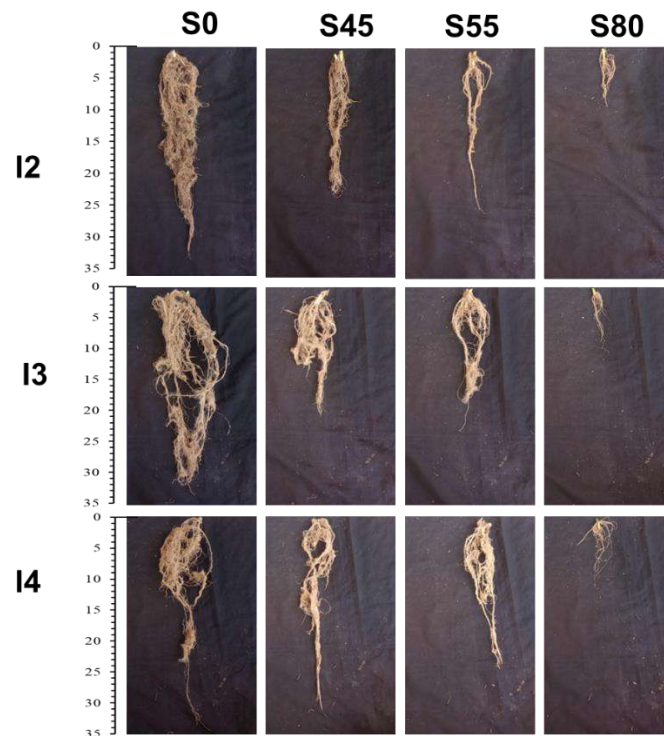


Figure 7. Visualization of Brazilian spinach root on different shading and harvest period at 13 weeks after planting (WAP). Shading treatment consist of S0: no-shading, S45: 45% shading, S55: 55% shading, and S80: 80% shading. Meanwhile, the harvest period consists of **I2**: per 2 weeks, **I3**: per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

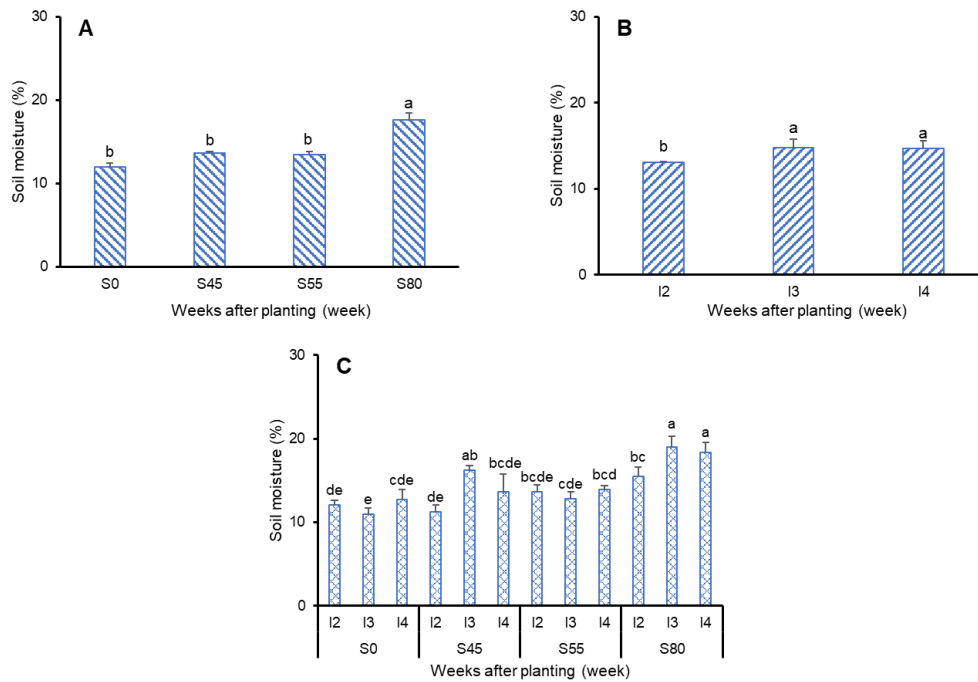


Figure 8. Substrate moisture on different shading (A), harvest period (B), and their interaction (C). Shading treatment consist of S0: no-shading, S45: shading 45%, S55: shading 55%, and S80: shading 80%. Meanwhile, the harvest period consists of I2: per 2 weeks, I3: per 3 weeks, and I4: per 4 weeks.

## 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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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".

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

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## **ABSTRACT**

Brazilian spinach (*Alternanthera sissoo*) is a less prominent perennial leafy vegetable growing in the tropical ecosystem. Therefore, this research aimed to investigate the effects of different shading intensities and harvesting periods on the growth and yield of Brazilian spinach in a tropical lowland urban ecosystem. The investigation used a split-plot design, with different shading intensities (no-shading, 45%, 55%, and 80% shading) as the main plot and harvesting periods (every 2, 3, and 4 weeks) as the subplot. The results showed that Brazilian spinach growth was significantly enhanced under no-shading conditions compared to shading treatments. Furthermore, 80% shading negatively impacted plant growth during early stages, as evidenced by alterations in parameters including canopy area (26.47 cm<sup>2</sup>), 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 cm<sup>2</sup>), 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.

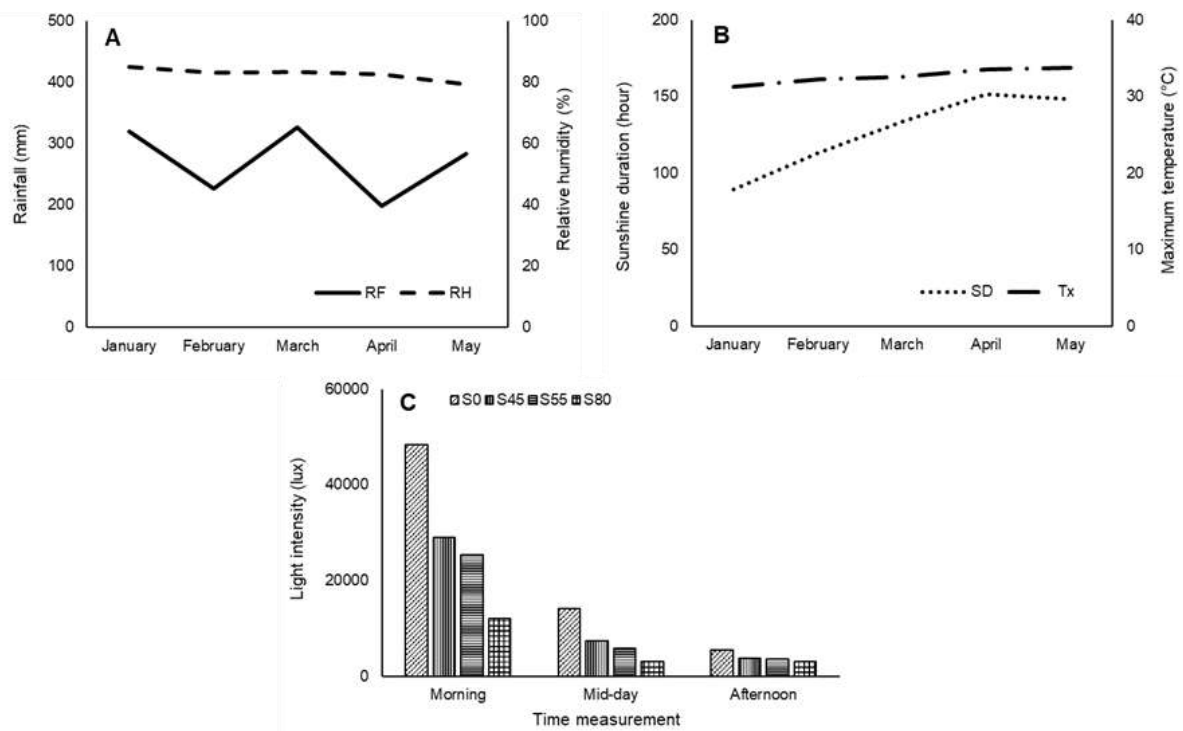


Figure 1. Agroclimatic characteristics of the research site include total monthly rainfall (RF) and average humidity (RH) (A), sunshine duration (SD) and average maximum temperature (Tx) (B), and average light intensity of each treatment (C). (Source: Indonesian Agency for Meteorology, Climatology, and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

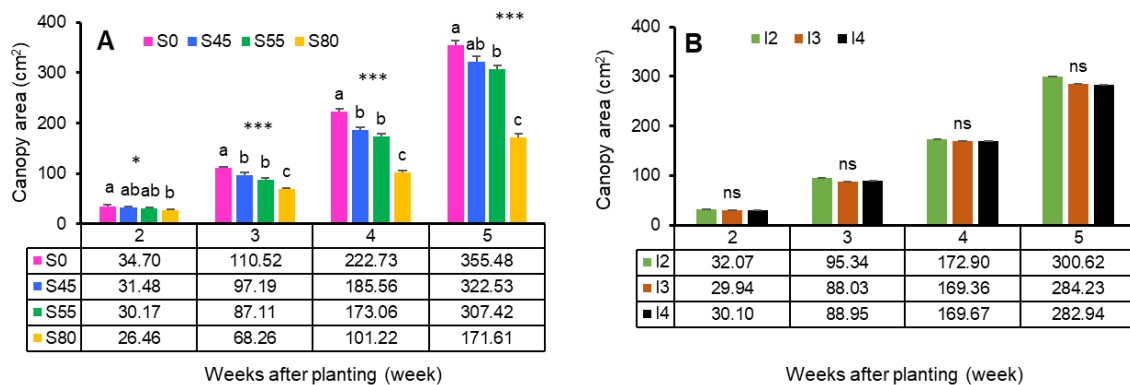


Figure 2. Brazilian spinach canopy area during early vegetative growth under different shading (A) and harvest period (B) treatments. The shading intensities consist of S0, S45, S55, and S80, while harvest periods include I2, I3, and I4. The ns= non-significant difference at  $P < 0.05$ ; \*= significant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

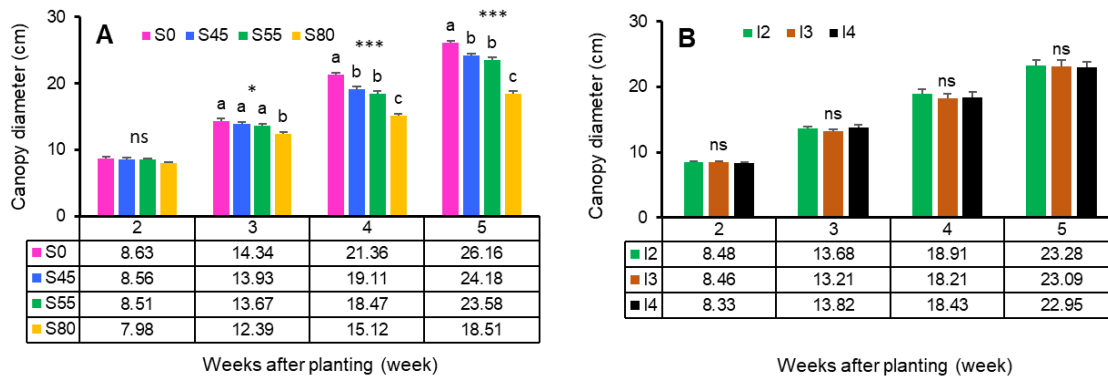


Figure 3. Brazilian spinach canopy diameter during early vegetative growth under different shading (A) and harvest period (B) treatments. Shading intensities consist of S0, S45, S55, and S80, while harvest periods include I2, I3, and I4. The ns= non-significant difference at  $P<0.05$ ; \*= significant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

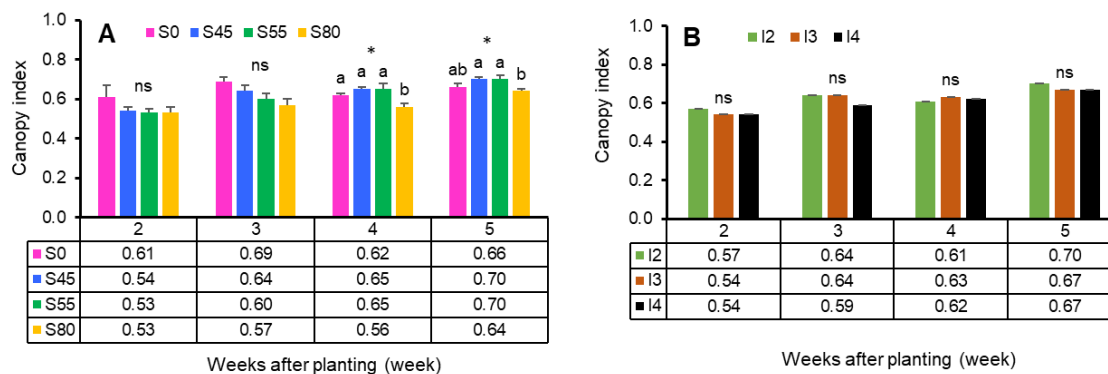


Figure 4. Canopy index for early vegetative growth at different shading and harvest period treatments. The shading intensities consist of S0, S45, S55, and S80, while harvest periods include I2, I3, and I4. The ns= non-significant difference at  $P<0.05$ ; \*= significant difference at  $P<0.05$ .

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).

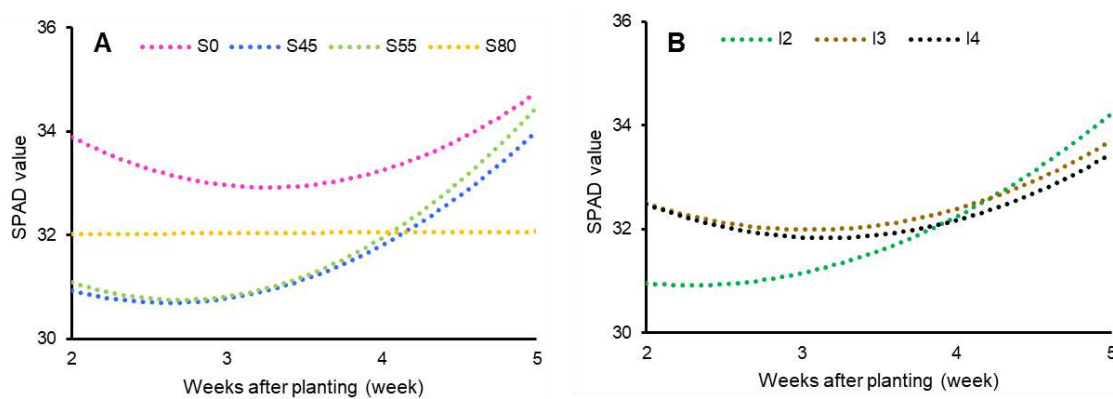


Figure 5. The SPAD value of Brazilian spinach leaves during early vegetative growth under different shading (A) and harvest period (A) treatments. The shading intensities consist of S0, S45, S55, and S80, while harvest periods include I2, I3, and I4.

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.

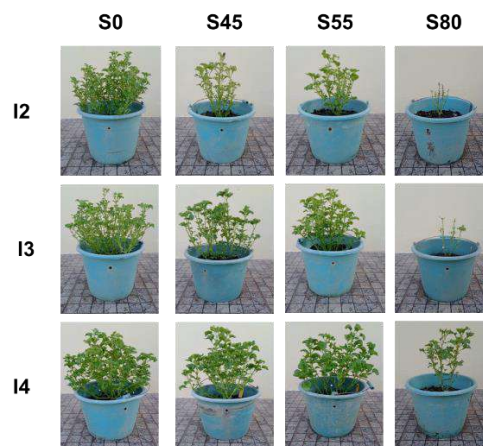


Figure 6. Visualization of Brazilian spinach shoots under different shading and harvest period treatments at 13 WAP. Shading intensities consist of S0, S45, S55, and S80, while the harvest periods include I2, I3, and I4. Photos: Strayker Ali Muda.

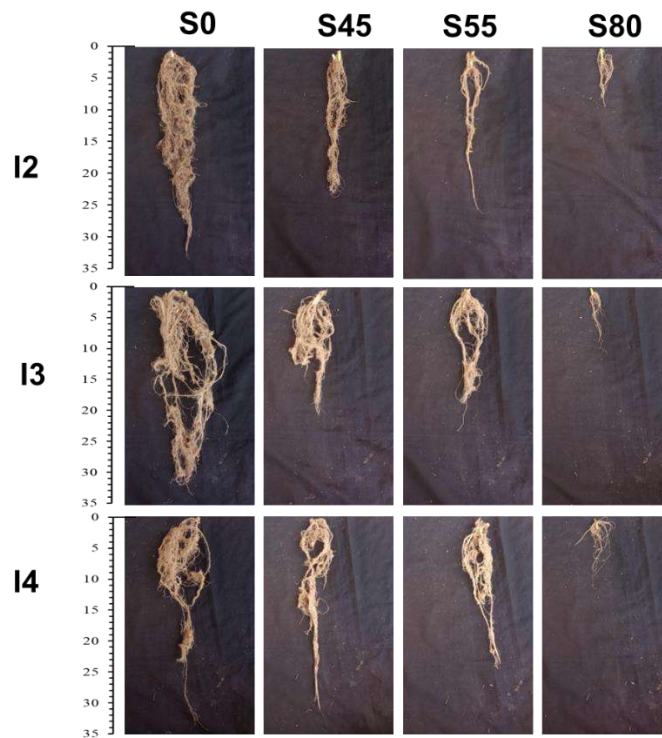


Figure 7. Visualization of Brazilian spinach roots under different shading and harvest period treatments at 13 WAP. Shading intensities consist of S0, S45, S55, and S80, while the harvest periods include I2, I3, and I4. Photos: Strayker Ali Muda.

#### *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.

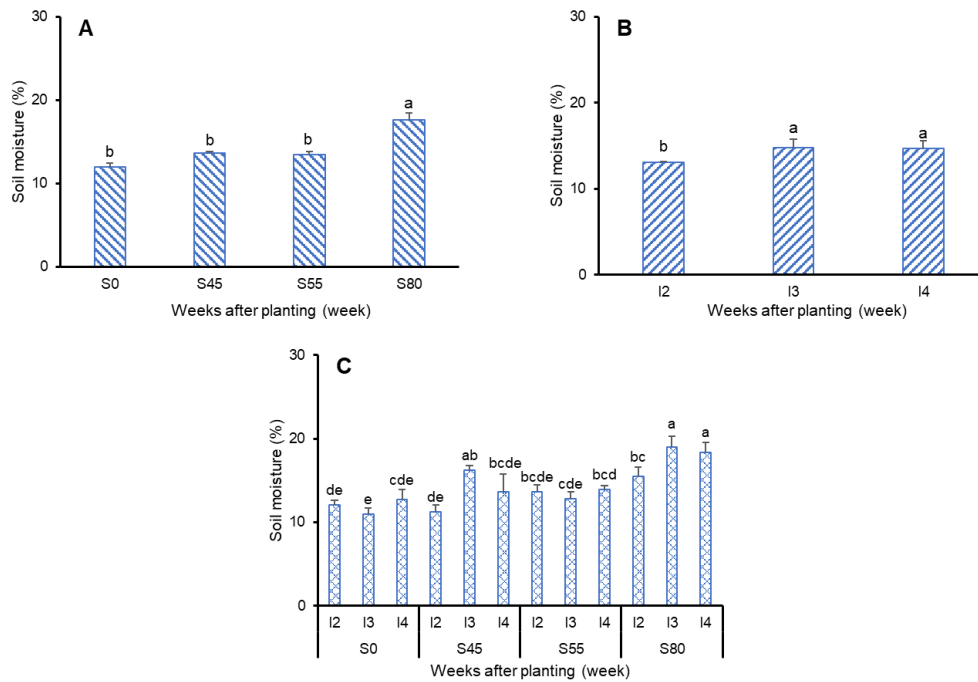


Figure 8. Substrate moisture at different shading intensities (A), harvest periods (B), and the interactions between both treatments (C). Shading intensities consist of S0, S45, S55, and S80, while the harvest periods include I2, I3, and I4.

## 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 cm<sup>2</sup>), 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 cm<sup>2</sup>), 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.

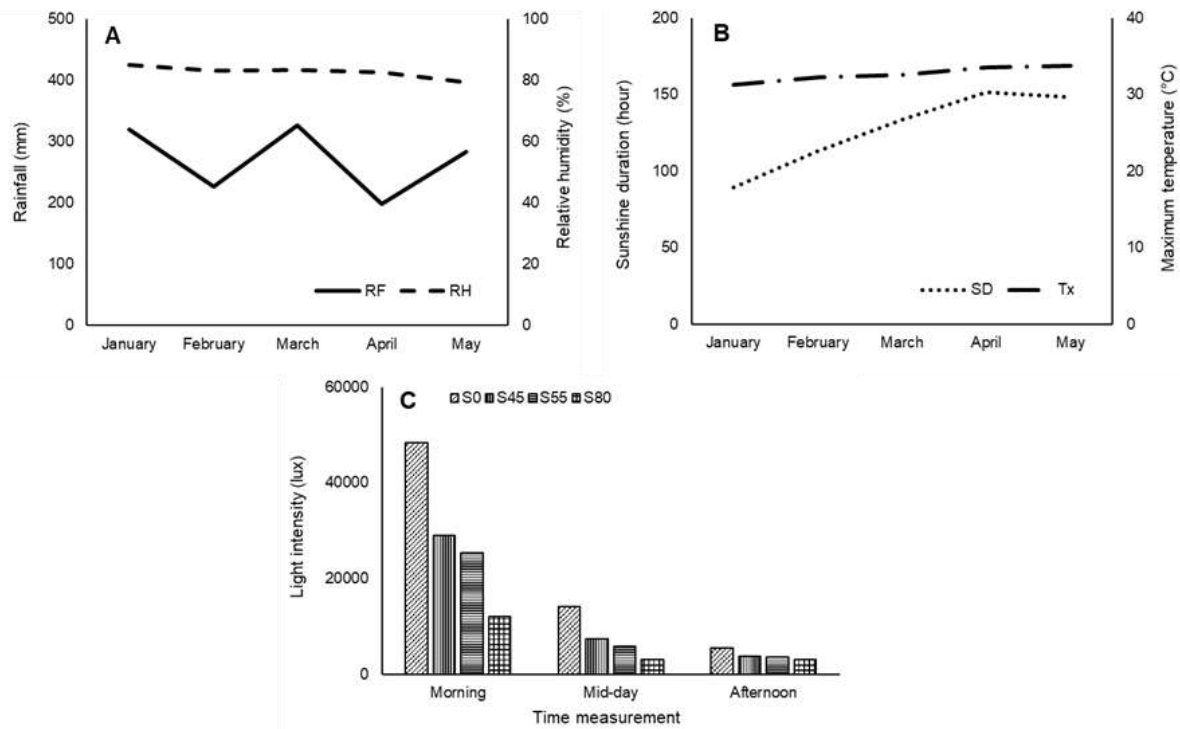


Figure 1. Agroclimatic characteristics of the research site include total monthly rainfall (RF) and average humidity (RH) (A), sunshine duration (SD) and average maximum temperature (Tx) (B), and average light intensity of each treatment (C). (Source: Indonesian Agency for Meteorology, Climatology, and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

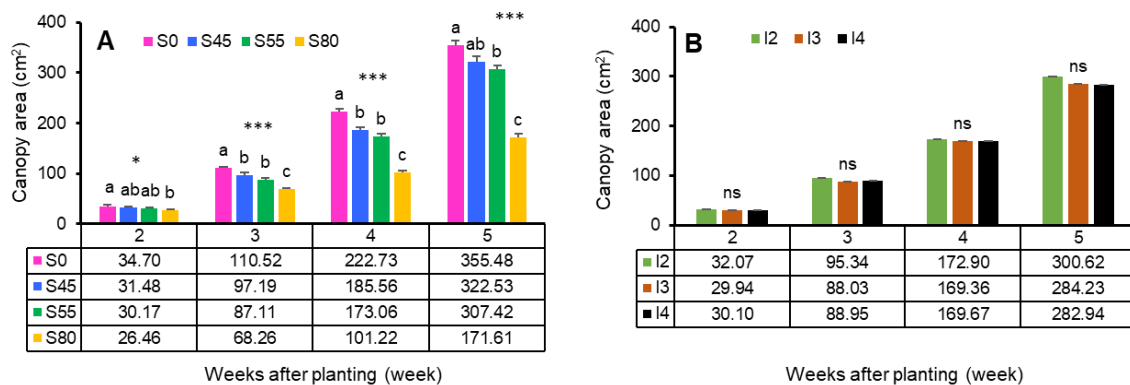


Figure 2. Brazilian spinach canopy area during early vegetative growth under different shading (A) and harvest period (B) treatments. The shading intensities consist of S0, S45, S55, and S80, while harvest periods include I2, I3, and I4. The ns= non-significant difference at  $P<0.05$ ; \*= significant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

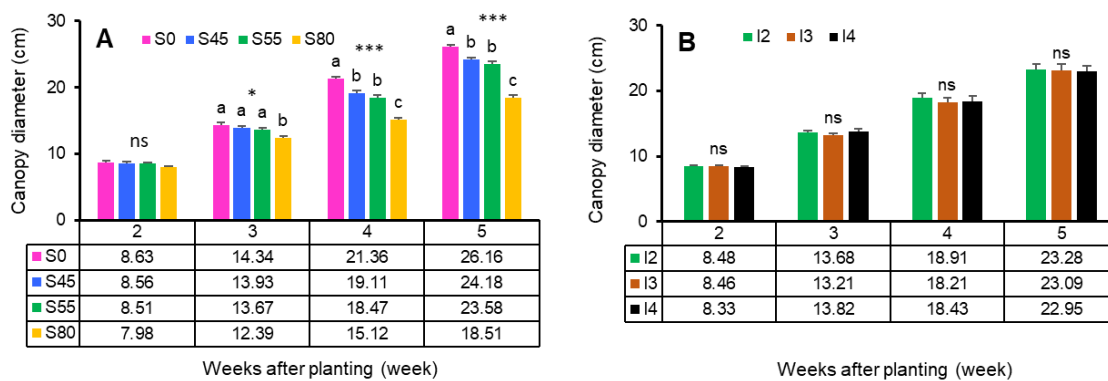


Figure 3. Brazilian spinach canopy diameter during early vegetative growth under different shading (A) and harvest period (B) treatments. Shading intensities consist of S0, S45, S55, and S80, while harvest periods include I2, I3, and I4. The ns= non-significant difference at  $P<0.05$ ; \*= significant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

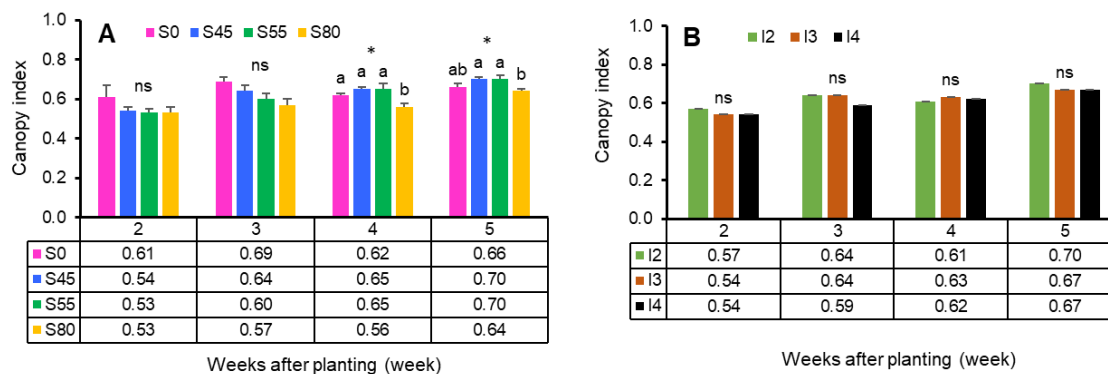


Figure 4. Canopy index for early vegetative growth at different shading and harvest period treatments. The shading intensities consist of S0, S45, S55, and S80, while harvest periods



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).

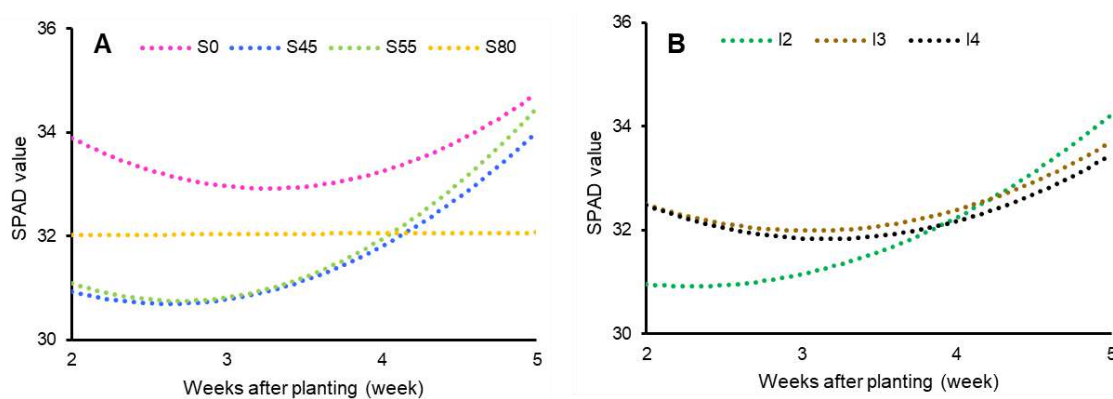


Figure 5. The SPAD value of Brazilian spinach leaves during early vegetative growth under different shading (A) and harvest period (A) treatments. The shading intensities consist of S0, S45, S55, and S80, while harvest periods include I2, I3, and I4.

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.

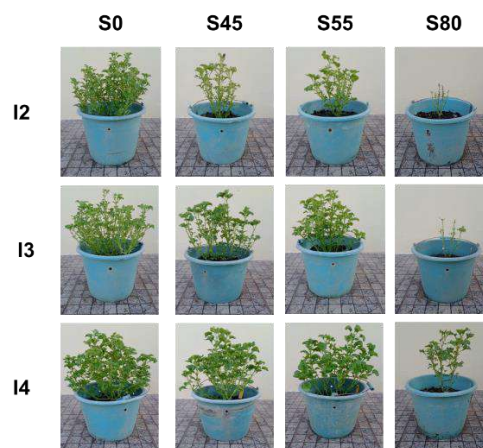


Figure 6. Visualization of Brazilian spinach shoots under different shading and harvest period treatments at 13 WAP. Shading intensities consist of S0, S45, S55, and S80, while the harvest periods include I2, I3, and I4. Photos: Strayker Ali Muda.

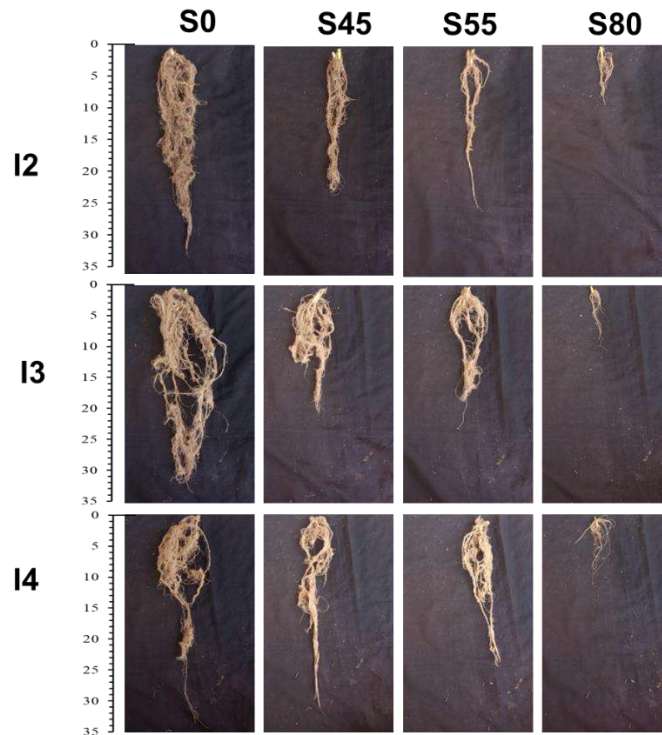


Figure 7. Visualization of Brazilian spinach roots under different shading and harvest period treatments at 13 WAP. Shading intensities consist of S0, S45, S55, and S80, while the harvest periods include I2, I3, and I4. Photos: Strayker Ali Muda.

#### *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.

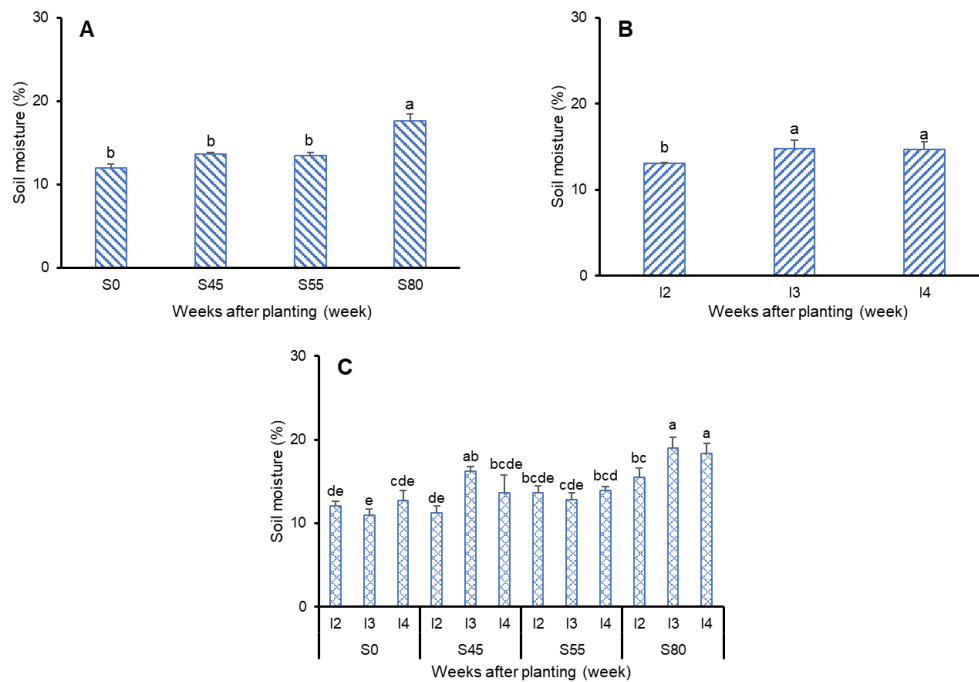


Figure 8. Substrate moisture at different shading intensities (A), harvest periods (B), and the interactions between both treatments (C). Shading intensities consist of S0, S45, S55, and S80, while the harvest periods include I2, I3, and I4.

## 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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