Influence of growing systems and non-fertilizer ameliorants on microclimate and growth of Brazilian spinach

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Research Article

Influence of growing systems and non-fertilizer ameliorants on microclimate and growth of Brazilian spinach[1]

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ABSTRACT

Brazilian spinach is a leafy vegetable originating from the tropical region of South America. Despite the similarity in agroclimatic conditions, this nutrient-rich plant has not been widely cultivated in southeast Asia. Therefore, this research aimed to determine the most suitable growing technique (conventional, floating or bottom-wet culture systems) for Brazilian spinach grown in the wet tropical climate. Non-fertilizer ameliorants were applied to improve the physical characteristics of the growing substrate, making it more permeable. The addition of fine sand and/or rice husk biochar to the growing substrate did not increase the growth rate and the leaf yield of Brazilian spinach. The floating and bottom-wet culture systems performed better, when compared to the conventional system commonly practiced by farmers, particularly during the onset of the dry season. The harvest period lasted from 6 to 9 weeks after planting (WAP). The rate of leaf fresh weight to the total shoot weight gradually decreased during the harvest period, along with the percentage of marketable yield. Related to microclimate conditions, the floating and bottom-wet culture exhibited a higher substrate humidity, while the canopy temperature was not significantly affected by the air or substrate temperature. The substrate temperature measured at midday was significantly higher in the conventional system due to a decrease in the substrate water. In conclusion, Brazilian spinach is suitable for cultivation in wet tropical climate zones, with the application of floating or bottom-wet culture systems and harvest between 6 and 9 WAP emerging as the recommended approach.

KEYWORDS: *Alternanthera sissoo*, floating and bottom-wet culture systems, leafy green.

RESUMO

Influência de sistemas de cultivo e melhoradores não-fertilizantes no microclima e crescimento de espinafre-brasileiro

O espinafre-brasileiro é um vegetal folhoso originário da região tropical da América do Sul. Apesar da semelhança nas condições agroclimáticas, essa planta rica em nutrientes não tem sido amplamente cultivada no sudeste asiático. Objetivou-se determinar a técnica de cultivo mais adequada (convencional, flutuante ou por capilaridade) para o espinafre-brasileiro cultivado sob clima tropical úmido. Melhoradores não fertilizantes foram aplicados para melhorar as características físicas do substrato de cultivo, tornando-o mais permeável. A adição de areia fina e/ou biocarvão de casca de arroz ao substrato de cultivo não aumentou a taxa de crescimento e a produtividade foliar do espinafre-brasileiro. As técnicas flutuante e por capilaridade apresentaram melhor desempenho que o sistema de cultivo convencional comumente praticado pelos agricultores, especialmente no início da estação seca. O período de colheita durou de 6 a 9 semanas após o plantio (SAP). A taxa de massa fresca foliar, em relação à massa total da parte aérea, diminuiu gradativamente durante o período de colheita, assim como a porcentagem de produtividade comercial. Em relação às condições microclimáticas, as técnicas flutuante e por capilaridade mostraram maior umidade do substrato, enquanto a temperatura do dossel não foi significativamente afetada pela temperatura do ar ou do substrato. A temperatura do substrato medida ao meio-dia foi significativamente maior no sistema de cultivo convencional, devido a uma diminuição na umidade do substrato. Conclui-se que o espinafre-brasileiro é adequado para o cultivo em zonas de clima tropical úmido, bem como recomenda-se a utilização dos sistemas de cultivo flutuante ou por capilaridade e colheita entre 6 e 9 SAP como as abordagens mais recomendadas.

PALAVRA-CHAVES: *Alternanthera sissoo*, sistemas de cultivo flutuante e por capilaridade, vegetal folhoso.

INTRODUCTION

Brazilian spinach (*Alternanthera sissoo*) is a lesser-known plant, rarely cultivated by farmers in Indonesia. However, this plant has begun to be recognized as a leafy green in urban cuisine, with potential for cultivation, particularly considering the shared agroclimatic aspect between Indonesia and Brazil (Muda et al. 2022). Both countries are in a wet tropical climate zone, with high temperatures

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and heavy rainfall. Moreover, Brazilian spinach leaves contain several vitamins, minerals, dietary fiber, antioxidants and other natural compounds that are beneficial to human health (Ikram et al. 2022).

Brazilian spinach is easy to grow, does not require special treatment and exhibits a fast growth, allowing for frequent harvesting. Consequently, this plant is propagated using stem cutting and is suitable for cultivation in limited urban or peri-urban land area, for gardening hobbies or health-conscious consumption (Sani & Awang 2021). Muda et al. (2022) reported that stem cutting is the main planting material used in the Brazilian spinach cultivation due to its limited seed fertility. Furthermore, this plant can be cultivated using common soils or manufactured growing substrates, such as cocopeat, vermicompost and others (Sani & Awang 2021, Alam et al. 2022).

Several growing systems have been developed for limited land acreage in urban areas with minimum costs. Jaya et al. (2021) reported that floating growing systems promot plant growth by ensuring water availability through capillary forces. Some cultivars have also exhibited a robust performance under the floating growing system, such as water spinach, lettuce and rocket (Guo et al. 2019, Miceli et al. 2019). Meanwhile, other cultivars, including celery (Lakitan et al. 2021a) and chili pepper (Siaga et al. 2019a), have adapted well to the bottom-wet growing system.

This research aimed to determine the most promising cultivation system combined with non-fertilizer ameliorants for Brazilian spinach cultivation under urban tropical climate.

MATERIAL AND METHODS

The research was carried out at an outdoor agricultural facility in Jakabaring (104º46'44"E; 3º01'35'S), Palembang, South Sumatra, Indonesia, under urban tropical climate. Two-node stem cuttings were planted on March 12, 2022, at the near end of the rainy season in the country. The Brazilian spinach did not produce viable seeds due to infertility, and data were collected on May 21, 2022, or 10 weeks after planting (WAP). The number of rainy days per month during the transitional period from rainy to dry season was still relatively high, at around 15 days. The air humidity was consistently above 80 %, contributing to the warmer tropical climate and the large acreage of wetlands surrounding the research location (Figure 1).

The planting materials used were 5 cm long, with 2-node stem cuttings of *Alternanthera sissoo*. At the time of planting, the upper node had 2 adult leaves at opposite phyllotaxy and positioned above the surface of the growing substrate. Meanwhile, the lower node was buried at a depth of 1 cm below the surface of the growing substrate and there was a leaf bud in each axil. The cuttings were grown in 27.5 cm height and 27.5 cm upper diameter pots, filled with a mixture of 75 % of topsoil and 25 % of chicken manure as basic substrate (S0). The basic substrate was mixed with 25 % of fine sand (S1), 25 % of rice husk biochar (S2) and 12.5 % of fine sand plus 12.5 % of rice husk biochar (S3). The substrates were treated with a bio-sterilant at the concentration of 2 g L^{-1} and incubated for one week with sterilant containing the consortium of *Streptomyces* sp., *Geobacillus* sp. and *Trichoderma* sp.

The growing systems evaluated consisted of conventional, floating and bottom-wet culture. Water sources to meet the needs of plants originated from rainwater, and, when there was no rain for 3 consecutive days or more, the conventionally cultivated plants were watered manually to reach the level of water holding capacity. The source of water in the floating and bottom-wet culture was supplied through the continuously saturated lower layers of the growing substrate, maintaining direct contact with the surface of the water in the pond for the floating culture system or using stagnant water to wet the bottom of the substrate for the bottom-wet culture

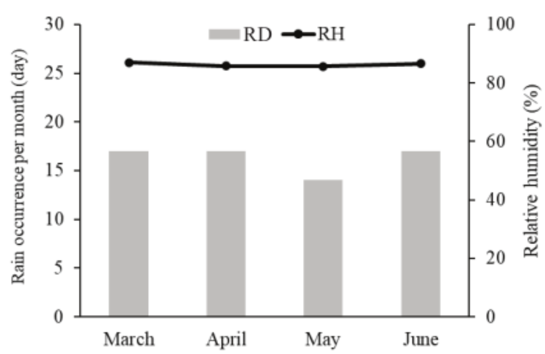


Figure 1. Rainy days per month (grey bar) and relative humidity (black solid line) from March to June 2022, at the research location. Source: Indonesian Agency of Meteorology, Climatology and Geophysics.

system. The last two systems were not watered manually. The movement of water to the upper part of the growing substrate was driven by capillary forces. Subsequently, the floating and bottom-wet culture systems were conducted in concrete ponds with inner dimensions of 4.0 m (length) x 2.0 m (width) x 0.45 m (depth).

The conventional cultivation system is a common practice by local farmers, in which water is given manually and with irregular frequency, based on rainfall and soil water conditions. In the floating culture system, 3 raft units were employed, each measuring 1.95 m (length) x 1.0 m (width) x 0.08 m (thickness). These rafts were constructed using 69 units of 1,500 mL of empty and water-sealed transparent plastic (polyethylene terephthalate, PET) bottles as the floater. The sturdy exoskeleton frame was designed using 1-inch diameter PVC pipe material with all the watertight joints. The depth of the water-substrate interface was adjusted to 1-2 cm.

In the bottom-wet culture system, the pots were placed directly in the experimental pond, but the depth of inundated water was continuously kept at 5 cm by opening the fixed 5 cm outlet valve. The water excess due to rain would flow directly out through the open valve and, at a higher evaporation rate, water was added into the concrete pond.

The experimental layout and data analysis adopted the split-plot design. The cultivation systems were assigned as the main plot, consisting of conventional, floating and bottom-wet culture. The subplots within the main plots consisted of varieties of the mixed substrates, including the control (S0), as well as the addition of 25 % of fine sand (S1), 25 % of rice husk biochar (S2), and 12.5 % of fine sand plus 12.5 % of rice husk biochar (S3).

The early Brazilian spinach growth was observed from 2 to 5 weeks after planting (WAP) and the data on canopy diameter, canopy area, canopy index and SPAD value (which served as an indicator of leaf nitrogen and chlorophyll concentrations) were collected. The midday canopy temperature, substrate water and substrate temperature were measured after the halting water supply for 4 days. Furthermore, data on the marketable leaf fresh weight, non-marketable leaf fresh weight and shoot dry weight were periodically collected from 6 to 10 WAP. The shoot dry weight of the bulky organ was thin-sliced before being oven-dried at 100 ºC, for 24 to 48 h, based on the size of the samples.

The SPAD value was measured using a chlorophyll meter (SPAD-502 Plus, Konica-Minolta Optics, Inc., Osaka, Japan) and the canopy area was determined with a digital image scanner for Android (Easy Leaf Area software, developed by Easlon & Bloom 2014). The canopy index was expressed as the ratio of canopy area measured using an image scanner to the calculated value with the widest diameter. Meanwhile, the canopy temperature was measured using a digital thermal camera (Teledyne FLIR C3-X Compact Thermal Camera, Mouser Electronics Inc., Mansfield, Texas, USA). The microenvironment measurements were also carried out using equipments for each measured parameter, namely substrate water using soil water meter (PMS-714, Lutron Electronics Canada, Inc., Pennsylvania, USA), substrate temperature with thermometer for semi-solid sample (Pen Thermometer KW0600308, Krisbow, Inc., Jakarta, Indonesia) and air temperature captured using digital thermometer (Thermometer KW0600278, Krisbow, Inc., Jakarta, Indonesia).

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

RESULTS AND DISCUSSION

The Brazilian spinach exhibited a positive response to the application of inorganic NPK, but the impact lasted only for 10-12 days. The floating culture system extended the positive impact of the NPK fertilizer application. However, the incorporation of the 25 % of rice husk biochar ameliorant into the growing substrate led to a fast decline in the NPK concentrations, as indicated by the accelerated reduction in the SPAD values (Figure 2).

The SPAD value has been used as an approach to determine the leaf nitrogen and chlorophyll status, in many cases exhibiting a positive correlation (Li et al. 2019). Furthermore, it was also used as a variable for plant response to environmental conditions in several leafy vegetables, such as lettuce (Mendoza-Tafolla et al. 2019) and rocket (Visconti et al. 2020). This value is affected by fertilization, which gradually increase after the application to *T. peniculatum* (Lakitan et al. 2021b). Therefore,

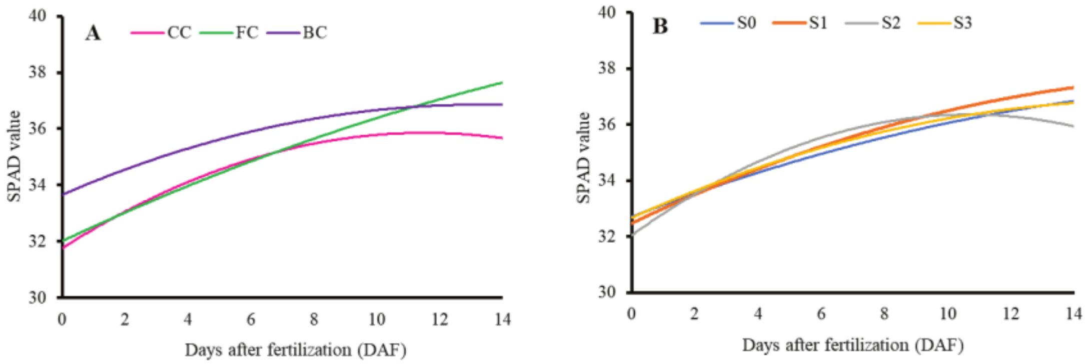


Figure 2. Response of Brazilian spinach grown under cultivation systems (A) and ameliorant mixtures (B) after being treated with NPK fertilization. CC: conventional cultivation; FC: floating culture; BC: bottom-wet culture. S0: control; S1: 25 % of fine sand; S2: 25 % of rice husk biochar; S3: 12.5 % of fine sand + 12.5 % of rice husk biochar.

the fertilizer should be reapplied after the SPAD value declined during the pre-fertilizer concentration period.

The adequate water availability in the substrate also played an important role in the SPAD value. This was because the drought stress lowers the SPAD value (Basal & Szabo 2020) and damages the leaf chlorophyll (Wang et al. 2018). Leafy vegetables were identified as the most sensitive plants to chlorophyll damage under drought stress. This phenomenon was reported in lettuce (Kiran 2019), as also observed in the conventionally cultivated Brazilian spinach in this research.

Excessive water conditions in the substrate also reduce the chlorophyll content (Zhang et al. 2019a), as confirmed by Brazel et al. (2021) in leafy vegetables such as kale (*Brassica oleracea*). However, the decrease in the SPAD value under excessive water conditions was caused by the lack of oxygen (anoxia) in the growing medium, because the air that the soil pores should contain was replaced by water excess.

For the treatments, the Brazilian spinach exhibited a consistent growth improvement along with the increasing plant age, without differing among them. The phenomenon was indicated by the canopy area in each treatment, and the growth mathematically followed an exponential curve (Figure 3).

The canopy area continued to increase at similar rates for the growing systems and substrate mixtures. The interactions of the cultivation system and substrate mixtures were only observed in the canopy area after the Brazilian spinach reached 5 WAP. A significant interaction effect at 5 WAP was observed between the bottom-wet culture system and substrate mixture with 25 % of fine sand (Figure 4).

The canopy densities were not affected by both treatments, namely cultivation systems and substrate compositions, but continuously increased as the plants grew. There was also an increase in the growth of individual leaf in each treatment, triggering an increase in canopy density at 5 WAP (Figure 5).

The specific shoot growth of the Brazilian spinach could be proxied by its dry weight and partitioned into leaves, branches and main stem (Figure 6). At the later growth stage (6 to 10 WAP), there was a change in the ratio between leaf weight and the weight of the other parts of the shoot, with the leaf being dominant during the early growth stage, specifically at 6-7 WAP.

The Brazilian spinach grown with the floating culture system exhibited a better leaf growth, when compared to the conventional and bottom-wet culture systems. The differences in the ameliorant composition of the growing substrates did not significantly affect the leaf growth rate. However, as the Brazilian spinach continued to grow, the old leaves began to experience senescence and eventually fell. The main branches and trunk also remained actively growing, leading to a gradual reduction in the proportion of leaf weight, while the weight of the main stem and branches continued to increase.

The harvested Brazilian spinach leaves were already at their maximum size, but remained young and healthy. Commercially, these leaves were

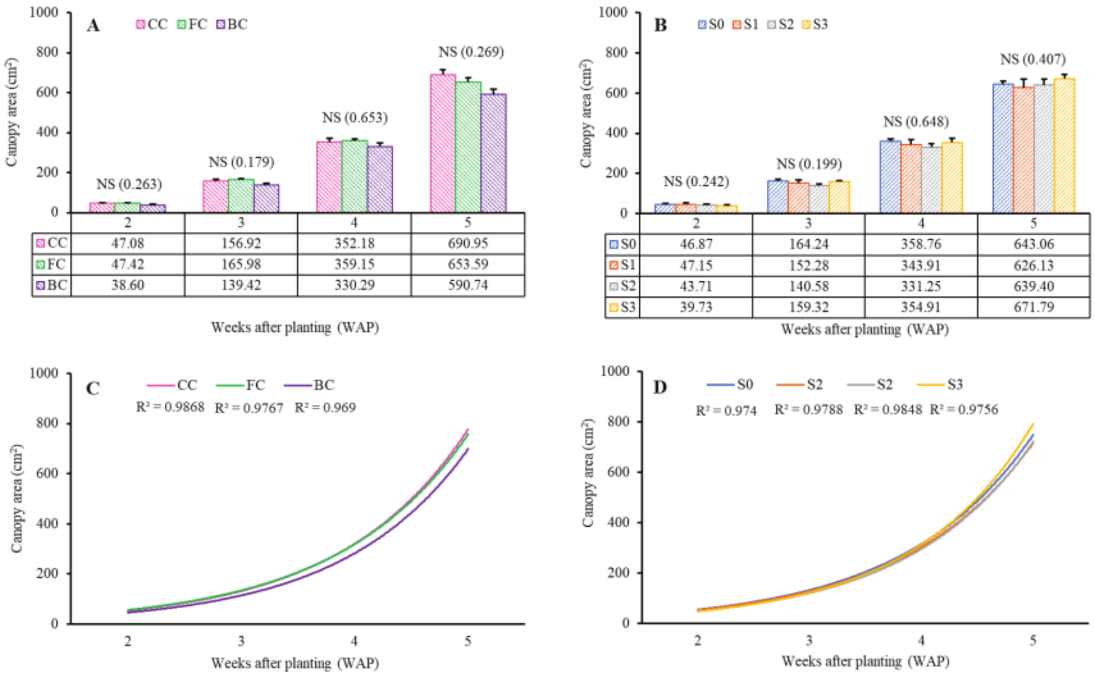


Figure 3. Growth of Brazilian spinach canopy area among cultivation systems (A) and ameliorant mixes (B), as well as increase in the weekly measured canopy areas during 2 to 5 weeks after planting (C and D). CC: conventional cultivation; FC: floating culture; BC: bottom-wet culture. S0: control; S1: 25 % of fine sand; S2: 25 % of rice husk biochar; S3: 12.5 % of fine sand + 12.5 % of rice husk biochar. NS: non-significant difference.

harvested periodically based on their readiness. As presented in Figure 7, the Brazilian spinach produced optimal and relatively stable results from 6 to 9 WAP. The Brazilian spinach cultivated under water-sufficiency conditions, namely floating and bottom-wet culture systems, provided better leaf yields, when compared to the conventional cultivation with water supplies that depended only on rain. Meanwhile, the application of rice husk biochar and fine sand as ameliorants or in combination had no significant impact on the leaf yields.

Based on the results, it was discovered that achieving the maximum harvest of Brazilian spinach leaves entailed ensuring sufficient water, without the need to add non-fertilizer ameliorants such as biochar or fine sand. Furthermore, replacing Brazilian spinach every 9 weeks was recommended. This was because when the plant kept growing older, the yield and quality of leaves decreased.

The growth of Brazilian spinach in each treatment showed a specific dynamics, as indicated by the growth parameters measured due to water availability in the substrate. Kartika et al. (2021) reported that the floating culture supplied and provided adequate water for tatsoy (*Brassica rapa* subsp. narinosa) growth in the early stages. Similarly, Siaga et al. (2019b) emphasized that the floating culture ensured adequate water availability through the capillary upward movement from the bottom layer of the substrate to root surfaces.

The substrate mixture created different pore sizes to provide adequate water. Liao & Thomas (2019) stated that larger pores increase the ability of the substrate to release excessive water within the wet substrate. Consequently, the differences in substrate mixtures were an alternative to improve the water-holding capacity to provide sufficient water for plant growth. Wang et al. (2019) also reported that biochar could enhance the water-holding capacity in the coarse substrate. This phenomenon was confirmed by Ndede et al. (2022), who stated that additional biochar to sandy substrate would increase the water-holding capacity. However, the substrate mixture with biochar and/or fine sand in this research showed no

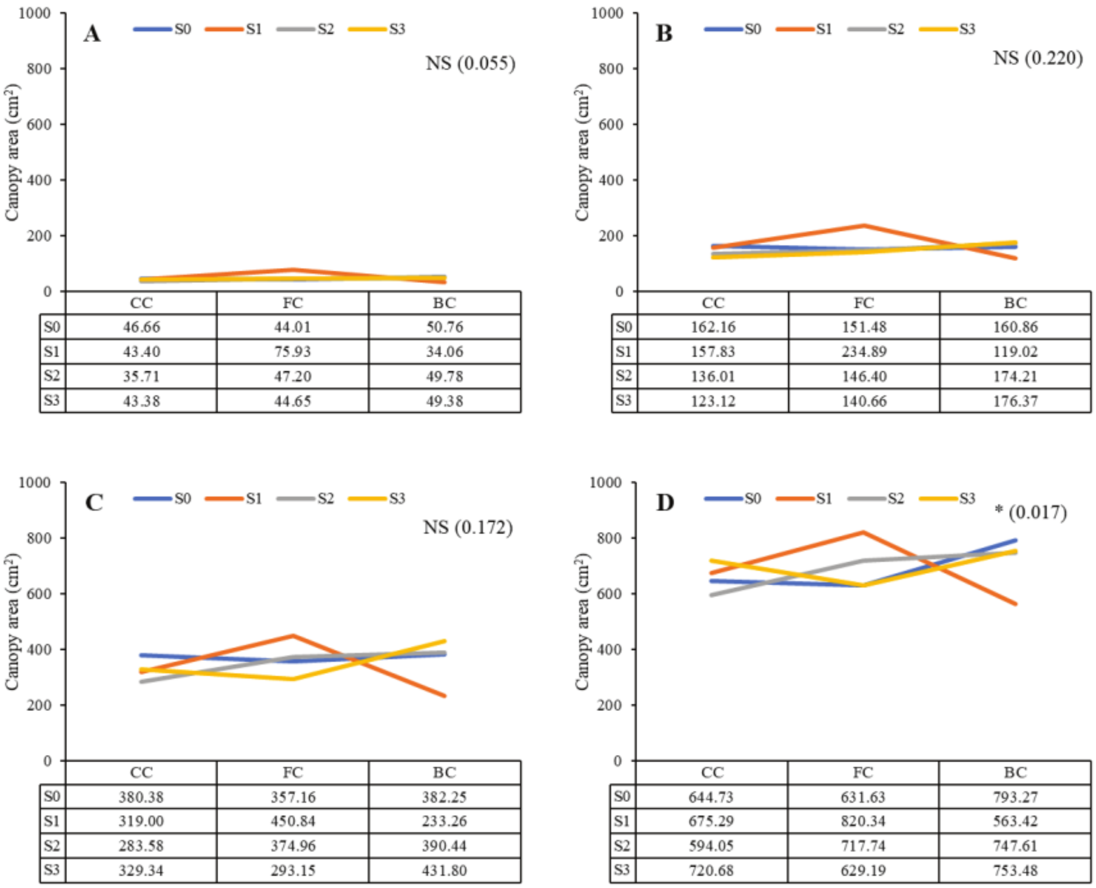


Figure 4. Interaction among growing systems and ameliorants mixtures for Brazilian spinach canopy area at 2 (A), 3 (B), 4 (C) and 5 (D) weeks after planting. CC: conventional cultivation; FC: floating culture; BC: bottom-wet culture. S0: control; S1: 25 % of fine sand; S2: 25 % of rice husk biochar; S3: 12.5 % of fine sand + 12.5 % of rice husk biochar. NS: non-significant difference; * significant difference at $p < 0.05$.

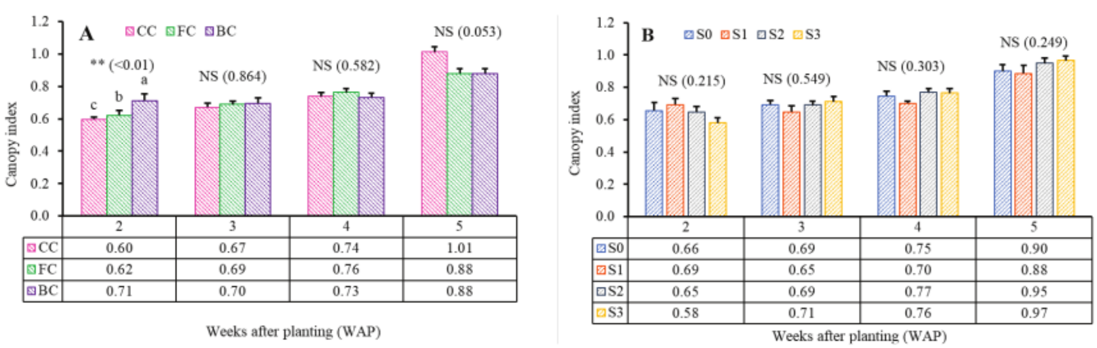


Figure 5. Comparison among cultivation systems (A) and ameliorant mixtures (B) for canopy density of Brazilian spinach, as indicated by the canopy index. CC: conventional cultivation; FC: floating culture; BC: bottom-wet culture. S0: control; S1: 25 % of fine sand; S2: 25 % of rice husk biochar; S3: 12.5 % of fine sand + 12.5 % of rice husk biochar. NS: non-significant difference; ** significant difference at $p < 0.01$.

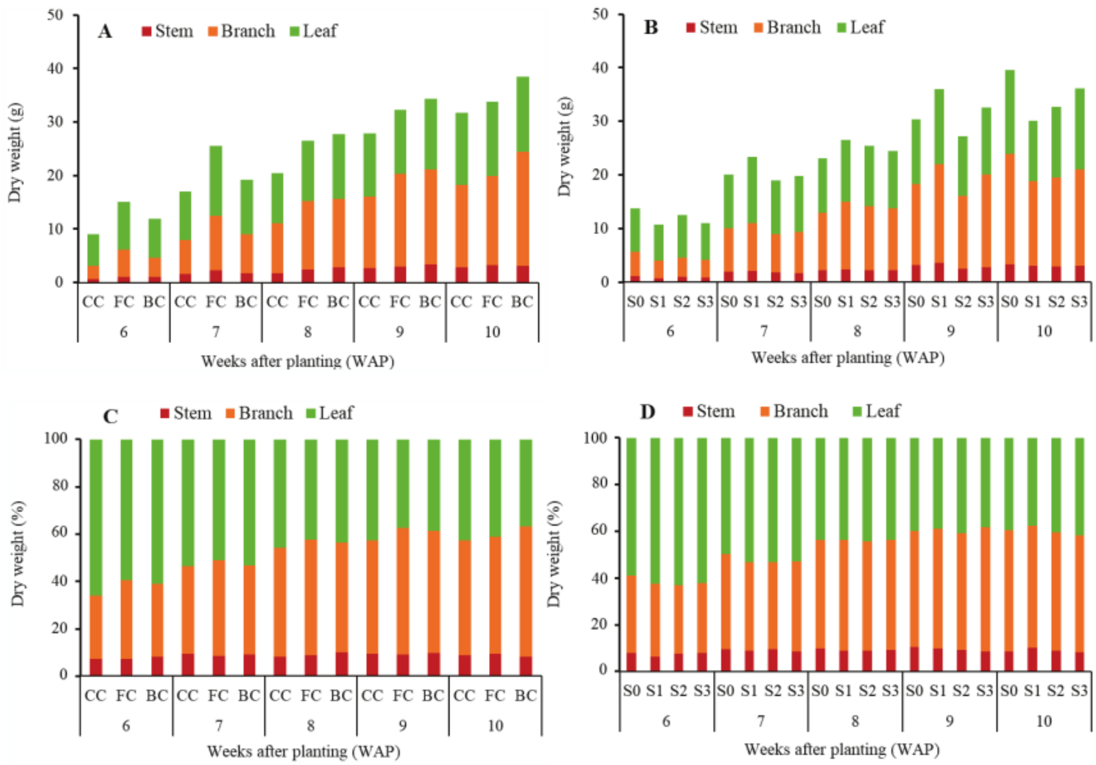


Figure 6. Comparison among cultivation systems (A-C) and ameliorant mixtures (B-D) for Brazilian spinach shoot organ, based on the absolute weight (A-B) and its percentage (C-D). CC: conventional cultivation; FC: floating culture; BC: bottom-wet culture. S0: control; S1: 25 % of fine sand; S2: 25 % of rice husk biochar; S3: 12.5 % of fine sand + 12.5 % of rice husk biochar.

significant difference among the treatments for the Brazilian spinach growth. The phenomenon of non-significant growth affected by ameliorant applications also occurred in other horticultural crops such as tomatoes and garlic (Gao et al. 2021).

Leafy vegetables are sensitive to the water stress conditions that can stem from water deficiency or excess (Parkash & Singh 2020). The plant would modify its metabolim under water deficiency conditions (Kapoor et al. 2020), inhibiting the nutrient solubility and hindering its availability for the plant (Soltanbeigi et al. 2021). Moreover, excessive water also has a negative impact on plant growth due to changes in metabolism (Hartman et al. 2021). This phenomenon was also experienced by the Brazilian spinach treated with the ameliorant compositions.

The substrate water availability was closely related to its water content. The floating and bottom-wet culture were more stable due to the consistent water supply driven by capillary force, vertically transporting water from the bottom to the upper surface of the growing substrate. The water supply in the conventional cultivation system depended only on rainfall. Meanwhile, in the substrate mixture treatment, there was no significant difference for water availability (Figure 8). The limited water source in the conventional cultivation system directly decreased the water availability in the substrate, needing watering on every non-rainfall day.

The physiological condition of the Brazilian spinach was associated with the canopy temperature, which was directly or indirectly affected by microenvironment conditions such as air temperature (direct) and substrate temperature (indirect). However, these effects were confounded with the water status of each organ (Figure 9).

The high water availability in the growing substrate, such as in the floating and bottom-wet culture, enhanced the water transport to all parts of the

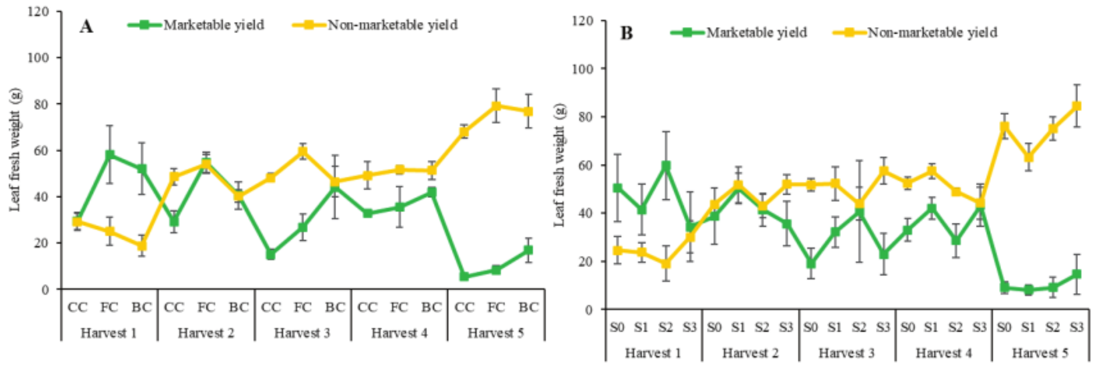
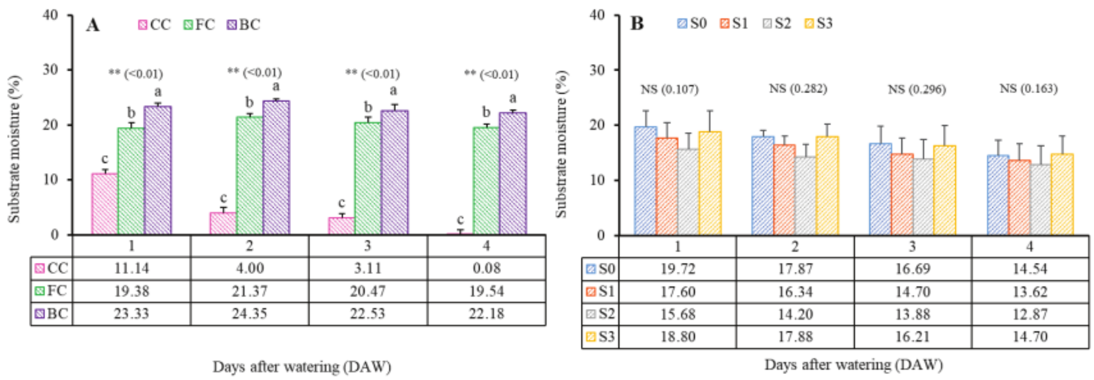


Figure 7. Weekly marketable and non-marketable yield for Brazilian spinach. The first harvest took place at 6 weeks after planting. CC: conventional cultivation; FC: floating culture; BC: bottom-wet culture. S0: control; S1: 25 % of fine sand; S2: 25 % of rice husk biochar; S3: 12.5 % of fine sand + 12.5 % of rice husk biochar.



	1	2	3	4
CC	11.14	4.00	3.11	0.08
FC	19.38	21.37	20.47	19.54
BC	23.33	24.35	22.53	22.18

	1	2	3	4
S0	19.72	17.87	16.69	14.54
S1	17.60	16.34	14.70	13.62
S2	15.68	14.20	13.88	12.87
S3	18.80	17.88	16.21	14.70

Figure 8. The water availability on the substrate was significantly lower in the conventional cultivation system (A), but there was no difference among the mixed ameliorants (B). CC: conventional cultivation; FC: floating culture; BC: bottom-wet culture. S0: control; S1: 25 % of fine sand; S2: 25 % of rice husk biochar; S3: 12.5 % of fine sand + 12.5 % of rice husk biochar. NS: non-significant difference; ** significant difference at $p < 0.01$.

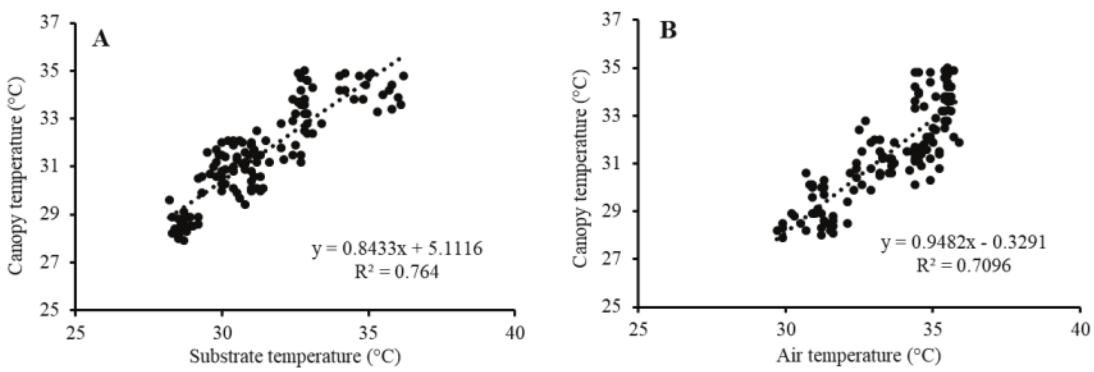


Figure 9. Substrate temperature (A) and air temperature (B) as related to the canopy temperature of Brazilian spinach. The temperatures were measured at the same time.

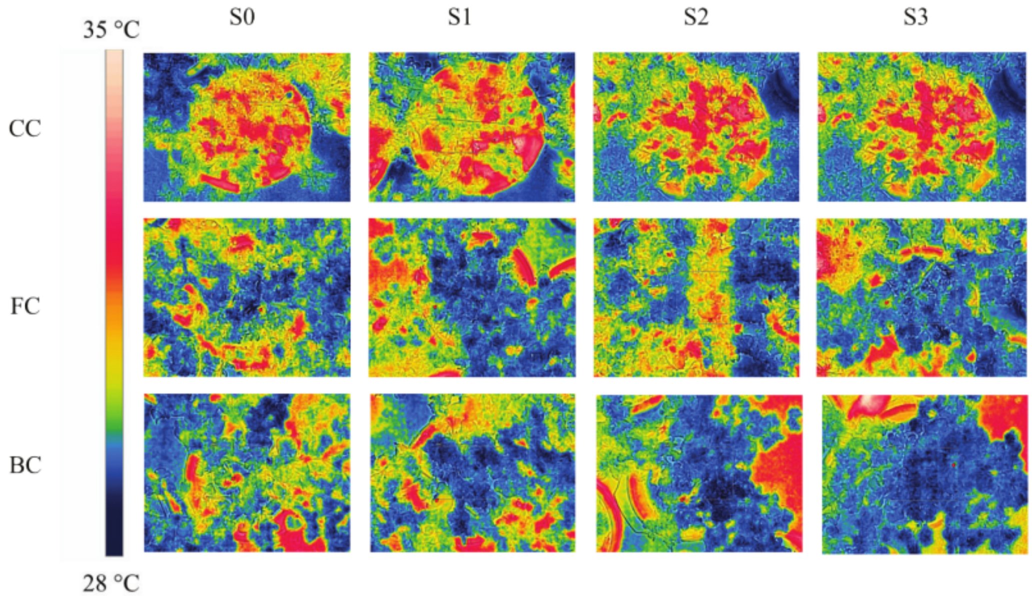


Figure 10. Brazilian spinach canopy temperature as revealed by the visual thermal capture. Bright red is for the highest temperature and dark blue is for the coldest temperature within the range of 28 to 35 ºC. CC: conventional cultivation; FC: floating culture; BC: bottom-wet culture. S0: control; S1: 25 % of fine sand; S2: 25 % of rice husk biochar; S3: 12.5 % of fine sand + 12.5 % of rice husk biochar.

canopy, particularly leaves. Furthermore, the higher leaf water content reduced the leaf temperature, as solar heat energy became predominantly used in the transpiration process. A significant amount of energy was expended in converting liquid water into vapor. However, the limited water in the growing substrate, as in the conventional cultivation, caused the leaf temperature to rise fast during the day, due to the lack of water availability to neutralize the incoming heat energy to leaves (Figure 10).

Water availability plays a significant role in the microenvironment. According to Tanure et al. (2019), the water availability in the substrate is positively related to soil water. Wang et al. (2022) argued that the decline in the substrate temperature is regulated by the ability of water to absorb the heat, while Li et al. (2020) reported a negative correlation.

Atmospheric and substrate microenvironments contributed to the Brazilian spinach growth and development. The canopy temperature was used in monitoring the plant physiological condition. Zhang et al. (2019b) reported that there was a strong correlation between canopy temperature and stomatal conductance under water stress. According to Uz-Zaman et al. (2022), heat stress decreases plant growth and changes the physiological role of spinach. This was also observed in water spinach, which changed its physiological activities under heat stress (Guo et al. 2020). Onwuka (2016) stated that substrate factors, including temperature and water, had an impact on plant conditions, while Sarker & Oba (2018) reported a significant impact on spinach physiology.

CONCLUSIONS

1. The Brazilian spinach growth and yield perform better under floating and bottom-wet culture, when compared to the conventional cultivation system. Meanwhile, the advantages associated with both methods are related to the continuity of water availability in the growing substrate;
2. The addition of fine sand and/or rice husk biochar as non-fertilizer ameliorants do not increase the growth rate and leaf yield of Brazilian spinach;
3. The Brazilian spinach harvest period lasts from 6 to 9 weeks after planting, but the percentage of marketable yield gradually decreases over time;
4. The substrate temperature measured at midday is significantly higher under conventional cultivation systems, due to a fast decrease in water content.

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