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Article

Effect of biochar as a hydroponic substrate on growth, colour and nutritional content of red lettuce (*Lactuca sativa* L.)

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ABSTRACT: Hydroponic is a technique of growing plants in a soilless medium by using a sponge, rock wool, hydroton, coconut coir, and perlite as an alternative for crop production systems. Recently, biochar has been reported to be a potential substrate for hydroponic cultivation techniques due to its physicochemical properties which can help increase vegetable production. A greenhouse study was conducted to investigate the effect of biochar as hydroponic substrate on the growth, colour and nutritional content of red lettuce (Lactuca sativa L.). This study utilized the nutrient film technique (NFT) hydroponic system and was conducted using a randomized complete block design with five treatments, including T1 (hydroton only), T2 (perlite only), T3 (palm kernel biochar), T4 (palm kernel biochar + hydroton), and T5 (palm kernel biochar + perlite). Data analysis revealed the treatment, which consists of both palm kernel biochar and hydroton (T4) resulted in a significant increase in plant growth and yielded the highest plant height (19.86 cm), leaf width (14.16 cm), plant fresh weight (68.19 g), and dry weight (8.29 g). The leaves nutrient content (N, P, K, Mg, Ca) of red lettuce grown in palm kernel biochar and hydroton (T4) substrates suggested the presence of optimal growth conditions for ensuring optimum yield with high quality. The application of palm kernel biochar and hydroton (T4) as substrate also showed a higher lightness value ($L^* = 66.67$). Besides, the methanolic leaf extracts from the red lettuce grown in palm kernel biochar and hydroton (T4) showed the highest bioactive metabolite content. In addition, the application of palm kernel biochar (T3) as a hydroponic substrate decreased the algal density in the nutrient solution. In conclusion, the combination of palm kernel biochar and hydroton as a substrate was observed to be the best in enhancing the growth performance, colour, and nutritional content of red lettuce (*Lactuca sativa* L.), and it is therefore recommended to be used as the growth substrate in the NFT system.

Key words: bioactive metabolites, biochar, growth, hydroponic, nutrient content, substrate.

INTRODUCTION

Soil-based agriculture is facing challenges that include urbanization, natural disasters, climate change, as well as the indiscriminate use of herbicides and pesticides, which degrade soil fertility (Sharma et al. 2018). As an alternative, hydroponic cultivation is gaining popularity all over the world due to its efficient natural resource management and good yield quality (Swain et al. 2021). With hydroponics, plants are grown in a soilless medium that serves as a source of nutrients and is made up of a solution of water and fertilizers. It has been shown that hydroponics is an excellent alternative crop production system and it is a highly exacting and demanding system that ensures better production of crops (Savvas 2003, Rahman et al. 2018). Growing plants without soil as a planting medium has been introduced to address these issues and sustain food production in order to feed the world's growing population (Gwynn-Jones et al. 2018). Hydroponic crops grow at a rate that is 30-50% faster than soil crops (Haddad and Mizyed 2011).

Several media have been identified to be suitable for hydroponic cultivation techniques such as a sponge, rock wool, hydroton, coconut coir, perlite, and vermiculite (Kumar and Saini 2020). The choice of hydroponic medium or substrate is

important because it has advantages and disadvantages. For example, according to previous research, materials such as coconut coir or peat moss should be avoided because they absorb too much nutrient solution and suffocate the plants (Kumar and Saini 2020), and it was discovered that replacing perlite substrate during each growing season of the crop was costly (Hanna 2009).

Recently, biochar has been reported to be a potential substrate for hydroponic cultivation techniques due to its physical and chemical characteristics that can help increase vegetable production (Huang and Gu 2019). Biochar is a product of pyrolysis, which is carbon-rich from agricultural wastes such as manure, oil palm waste and dried banana leaves in a closed container with little or no oxygen (Lehmann and Joseph 2015). Biochar as a hydroponic substrate is stable and highly resistant to microbial degradation because of the recalcitrant nature of biochar molecules (Singh et al. 2012, Kuzyakov et al. 2014). Therefore, it can be efficiently used as a substrate in hydroponic systems. Biochar can be utilized as an alternative growing media since its physicochemical properties are comparable to those of the common commercial substrate, i.e., coir peat (Kaudal et al. 2016).

Red lettuce (*Lactuca sativa* L.) belongs to the family Compositae (Asteraceae) and is a popular salad vegetable (Mampholo et al. 2016). Consuming red lettuce has numerous health advantages, such as its increased dietary fibre content, which aids in digestion, and its high levels of β-carotene, which are linked to a lower risk of cancer, cataracts, heart disease, and stroke (Mampholo et al. 2016). Many people consumed red lettuce (*Lactuca sativa* L.) as part of their daily diet. It is commonly planted using hydroponic systems. However, in Malaysia, leafy lettuce is commonly cultivated in open fields, as well as under greenhouse conditions. Hence, this study was conducted to determine the effect of biochar as a hydroponic substrate in improving the growth performance, colour, and nutritional content of red lettuce (*Lactuca sativa* L.).

MATERIALS AND METHODS

Experimental design and sample preparation

This study was arranged using randomized complete block design, which consisted of 20 sets of nutrient film technique (NFT) hydroponic systems that were purchased from a commercial source. Each channel had seven planting plastic pots. Red lettuce seedlings of similar size were planted in plastic pots, filled with hydroton (T1), perlite (T2), palm kernel biochar (T3), palm kernel biochar + hydroton at ratio 1:1 (v:v) (T4), and palm kernel biochar + perlite at ratio 1:1 (v:v) (T5). Pots with hydroponic systems were placed in the greenhouse at ZS Hidro Farm, Kampung Jalan Kebun, Seksyen 30, Shah Alam, Malaysia, for 45 days. The greenhouse was 24 feet × 10 feet in size and was built using the greenhouse ultraviolet (UV) transparent plastic. Plants were grown under greenhouse conditions.

The nutrient solution for the hydroponic system was prepared using AB fertilizer, which was comprised of chemical fertilizers that were divided into two sets, namely Set A and Set B. Set A contained 100-g·L^{-1} calcium nitrate and 7-g·L^{-1} EDTA Fe, while Set B contained 58-g·L^{-1} potassium nitrate, 26 monopotassium phosphate, 51-g·L^{-1} magnesium sulfide, 0.61-g·L^{-1} manganese sulfide, 0.33 boric acid, 0.05-g·L^{-1} zinc sulfide, 0.04-g·L^{-1} copper (II) sulfide, and 0.04-g·L^{-1} sodium molybdate. Hydroponic substrates used in this study were hydroton (average particle size range from 8-16 mm), perlite (average particle size range from 3-6 mm), and palm kernel biochar produced at 250 °C (≥ 10 mm). In addition, the pH of palm kernel biochar was 7.71, while the total carbon, total nitrogen, and total hydrogen were 75.43, 0.23 and 1.50%, respectively.

After harvesting, the samples were collected and washed with distilled water. For nutrient analysis, the samples were blot dried before being put into an oven (Memmert, Germany) at 105 °C until they were completely dried. The dried plant samples were finely ground and kept in air-tight containers before analysis. For bioactive metabolites analysis, the harvested samples were subjected to freeze drying process using Labconco freeze dryer (Labconco Corporation, MO 64132, United States of America) at -50 °C. About 1 g of freeze-dried samples were ground on a chilled mortar and pestle. Homogenized samples were soaked in 30 mL of absolute methanol and incubated in the dark for 48 hours at 4 °C. After 48 hours, the homogenates were filtered using filter paper before being centrifuged at 9,000 rpm for 5 minutes, at 4 °C (Universal 32 R centrifuge Hettich Zentrifugen, D-78532, Germany). The clear coloured supernatants were transferred into new tubes. A portion of these supernatants were used instantly for subsequent pigment analysis to measure the total chlorophyll, carotenoid, and anthocyanin contents. Meanwhile, the remaining portion of the supernatants were concentrated under

reduced pressure at 45 °C using rotary evaporator (Rotavapor R-3 BÜCHI Labortechnik, 9230, Switzerland) to remove excess solvent. The resultant dried extracts were weighed and redissolved in absolute methanol to a concentration of 20 mg/mL and used for total phenolics, total flavonoids and antioxidant analysis.

Plant growth performance

The growth performance of red lettuce was measured weekly and after harvest. The parameters of growth performance for weekly measurements include the plant height, leaf length, number of leaves, leaf width, and relative chlorophyll content for each plant. Plant height was measured using a meter ruler from the bottom of the shoot, which was at the surface of the hydroponic substrates to the tip of the tallest leaf in the foliage of the plant (Mensah and Frimpong 2018). The number of leaves was counted manually, while the leaf width and leaf length were measured using a meter ruler. The relative chlorophyll content of the leaves was determined using a chlorophyll meter (SPAD-502 Plus, Minolta, Japan). The parameters of growth performance for after harvest measurement include the fresh weight and dry weight for shoot and root, as well as the shoot and root length. The fresh weight of each plant was measured using a weighing balance after harvesting, meanwhile the dry weight was obtained by wrapping the plant in an aluminum foil and drying it in an oven at 105 °C for 24 hours. The root and shoot length were measured using a meter ruler.

Determination of algal density in nutrient solution

Determination of algal density in the nutrient solution was done in triplicates using a Multiskan™ Go plate reader (Thermo Scientific, Waltham, MA, United States of America) to measure the absorbance at 684 nm (Rodrigues et al. 2011). The nutrient solution from the hydroponic system was taken weekly, and the algae density in the nutrient solution was calculated based on Eq. 1:

$$\text{Cell Density} = e^{\{[\ln(absorbance_{684}) + 16.439] \div 1.0219\}} \quad (1)$$

Determination of colour by CIELAB analysis

The plant leaves (0.5 g) were subjected to solvent extraction using 40-mL methanol and filtered through 0.45-µm filters before the CIELAB analysis (Anuar et al. 2017). The variation of colours was determined using chromameter (Chromameter CR-410, Konica Minolta, Japan). The three readings for each treatment were averaged, and the colour for each sample was expressed in terms of CIE values for lightness (L*), red-green (a*), yellow-blue (b*), chroma (C), and hue (H°). a* takes a positive value depicts a shift towards red, whereas negative a-value depicts a shift towards green. b* takes a positive value depicts a shift towards yellow, whereas a negative b-value depicts a shift towards blue. The chroma and hue were calculated based on Eqs. 2 and 3:

$$\text{Chroma: } C = \sqrt{(a*)^2 + (b*)^2} \quad (2)$$

$$\text{Hue-Angle: } H° = \tan^{-1}(b* \div a*) \quad (3)$$

Determination of nutrient content: N, P, K, Mg, and Ca

The total nitrogen (N) was determined using the in-house elemental analyzer method (Jimenez and Ladha 1993). The sample was placed in the tin container, and the sample was weighed. Then, the sample was loaded in the autosampler. The analysis process was started on the instrument using software. Data was automatically calculated using the provided software from the elemental analyzer.

For the determination of the phosphorus, potassium, magnesium, and calcium (P, K, Mg, and Ca) content, the dry ashing method was used (Baker et al. 1964). A gram of sample was weighed in a crucible and placed in the furnace for 1 hour at

300 °C. Then, the temperature was increased to 500 °C for 5 hours until white or greyish ash was obtained in the crucible. The crucible was taken out from the furnace and left to cool. After that, the ash was moistened by adding a few drops of distilled water. The sample in the crucible was then poured with 2 mL of concentrated hydrochloric acid (HCl) before being heated on the hot plate. The sample was heated until it was dried. Next, the dried sample was added with 10 mL of 20% nitric acid and placed in the water bath for 1 hour at 100 °C. The sample in the crucible was transferred to the 100-m·L^{-1} volumetric flask by filtering the sample through filter paper. The crucible was rinsed several times, and the volume was made up of distilled water. Then, the sample was transferred into the plastic vials for the phosphorus, potassium, magnesium, and calcium (P, K, Mg, and Ca) content analysis by using inductive coupled plasma mass optical emission spectrometry (ICP-OES).

Determination of total chlorophyll and carotenoid content

The leaf extracts were analyzed in triplicate using a spechtrophotometer (Multiskan™ Go, Thermo Scientific, Waltham, MA, United States of America) at 652.4 and 665.2 nm for photometric determination of chlorophyll a and b, as well as carotenoid. The concentration of chlorophyll a, chlorophyll b and carotenoids content were calculated based on Eqs. 4, 5 and 6, described by Lichtenthaler and Buschmann (2001):

$$\text{Chlorophyll a: } C_a\,(\mu g/mL) = 16.72\,A_{665.2} - 9.16\,A_{652.4} \quad (4)$$

$$\text{Chlorophyll b: } C_b\,(\mu g/mL) = 34.09\,A_{652.4} - 15.28\,A_{665.2} \quad (5)$$

$$\text{Carotenoid: } C_{(x+c)}\,(\mu g/mL) = \frac{1000\,A_{470} - 1.63\,Ca - 104.96\,Cb}{221} \quad (6)$$

Determination of total anthocyanin content

The pH differential method was used to determine the amount of monomeric anthocyanin pigment (cyanidin-3-glucoside) of the samples (Giusti and Wrolstad 2001) with some modifications. The leaf extract was separately diluted with two types of buffer: potassium chloride (0.025 M at pH 1) and sodium acetate [pH 4.5 using the ratio 1:4 (one-part test portion and four parts of buffer)], and the absorbance values were taken in triplicate at 520 and 700 nm using a spectrophotometer (Multiskan™ Go, Thermo Scientific, Waltham, MA, United States of America). The Eq. 7 was used to measure the anthocyanin pigment concentration of samples:

$$\text{Anthocyanin pigment content (mg/L)} = \frac{(A \times MW \times DF \times 1000)}{(\varepsilon \times 1)} \quad (7)$$

in which: *A*: ($Abs_{520} - Abs_{700}$) pH 1.0 – ($Abs_{520} - Abs_{700}$) pH 4.5; *MW* (molecular weight of cyanidin - 3 - glucoside): 449.2 g/mol; *DF*: dilution factor; ε: 26,900.

Determination of total phenolic content

Determination of total phenolic content was conducted using the method described by Yusof et al. (2018), but with several changes. About 10 µL of 20 mg/mL leaf extracts were added with 75 µL of diluted Folin-Ciocalteu reagent (FCR) and incubated for 10 minutes at room temperature. Prior to the mixture incubation, FCR was diluted with deionized water. A 75 µL of 2% sodium carbonate (Na_2CO_3) were added to the mixture and incubated for another 45 minutes in the dark condition. The absorbance was taken at 765 nm in triplicate using a spectrophotometer (Multiskan™ Go, Thermo Scientific, Waltham, MA, United States of America). A standard curve was prepared using gallic acid with six different concentrations (ranging from 0.10 to 0.60 mg/mL, $r^2 = 0.99$). Total phenolic content was expressed as mg of gallic acid equivalents/g dry extract (mg GAE/g DE) of leaf extracts.

Determination of total flavonoid content

Total flavonoid content of leaf extracts was determined based on aluminum chloride colorimetric method (Yusof et al. 2018) with minor modifications. A total of 15 µL of 20 mg/mL leaf extracts were mixed with 45 µL of 70% methanol, 3 µL of 10% aluminum chloride hexahydrate ($AlCl_3.6H_2O$), 3 µL of 1 M sodium acetate ($NaC_2H_3O_2.3H_2O$), and 84 µL of distilled water. The mixture was incubated for 40 minutes, and the absorbance was taken at 415 nm in triplicate using a spectrophotometer (Multiskan™ Go, Thermo Scientific, Waltham, MA, United States of America). A quercetin standard curve with concentrations ranging from 0.10 to 0.60 $mg{\cdot}mL^{-1}$ was prepared to calculate the total flavonoid content of the leaf extracts, and the results were expressed as quercetin equivalents in mg per gram of dry extract (mg QE/g DE) of leaf extracts.

Antioxidant content by DPPH radical scavenging activity assay

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging activity assay of leaf extracts was analyzed using a microplate analytical assay following the standard procedure (Yusof et al. 2018). A 50 µL of leaf extracts at a series of concentrations (ranging 0.50–6 mg/mL) were added to 150 µL of DPPH solution (1 mM) in each well of a 96-well plate and incubated for 30 minutes at room temperature. A spectrophotometer (Multiskan™ GO, Thermo Scientific, Waltham, MA, United States of America) was used to measure the absorbance at 515 nm. All leaf extracts were assayed in triplicate, and the data were used to determine the sample concentration required to scavenge 50% of the DPPH free radicals (IC_{50}) (Eq. 8).

$$\text{DPPH radical scavenging activity (\%)} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100 \quad (8)$$

in which: A_0: absorbance of the control; A_1: absorbance of samples.

Antioxidant content by ABTS radical scavenging activity assay

Decolorization assay were used to determine the scavenging activity of 2,2-Azinobis (3-Ethylbenzothiazoline-6-Sulfonic Acid) (ABTS) free radical (Rajurkar and Hande 2011). Initially, the solution of ABTS was prepared by mixing 10 mL of 2.6 mM potassium persulfate and 10 mL of 7.4 mM ABTS solution, and the mixture was incubated for 12 hours in the dark condition at room temperature. After 12 hours, double distilled water (ddH_2O) was used to dilute the mixture until the absorbance produced was 0.70 ± 0.2 at 734 nm. A 200-mL diluted ABTS solution was mixed with 20 µL of leaf extract of six different concentrations ranging from 0.50 to 6 mg/mL and incubated for 30 minutes at room temperature. Absorbance was taken in triplicates at 734 nm using a spectrophotometer (Multiskan™ Go, Thermo Scientific, Waltham, MA, United States of America). The data were used to determine the sample concentration required to scavenge 50% of the ABTS free radicals (IC_{50}) (Eq. 9).

$$\text{ABTS scavenging activity (\%)} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100 \quad (9)$$

in which: A_0: absorbance of the control; A_1: absorbance of samples.

Antioxidant content by ferric reducing antioxidant power assay

Ferric reducing antioxidant power (FRAP) assay of leaf extract was conducted by following the standard protocol with slight changes (Benzie and Strain 1999). FRAP reagent was prepared fresh during the assay by mixing 300 mM of acetate buffer (C_2H_3NaOO and C_2H_4O), pH 3.6, 10 mM 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ) solution, and 20 mM iron (III) chloride hexahydrate ($FeCl_3.6H_2O$) in the ratio of 10:1:1, respectively. Then, FRAP reagent was incubated at 37 °C in the water bath for 30 minutes. Ten µL of each leaf extract were mixed with 300 µL of FRAP reagent, and the mixture was incubated for 30 minutes at room temperature. Absorbance was taken in triplicate at a 593 nm using a spectrophotometer

(Multiskan™ Go, Thermo Scientific, Waltham, MA, United States of America). A series of stock solution at 0.50, 1, 2, 3, 4, 5, and 6 mg/mL of ferrous sulphate ($FeSO_4$) were prepared to generate a standard curve ($r^2 = 0.99$). The FRAP value was expressed as ferrous equivalent Fe (II) in gram per gram of dry weight of samples.

Statistical analysis

The collected data were statistically analyzed using IBM Statistical Package for the Social Sciences (SPSS) software version 26 (IBM Corporation, Armonk, NY, United States of America). The differences in mean values were evaluated by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Correlations among data were analyzed using Pearson's correlation coefficient in bivariate correlations, in which it was necessary to measure the degree of relationship between parameters, and the principal component analysis was also performed. All diagrams were drawn using Microsoft Excel 365 (Microsoft Corporation, Redmond, Washington, D.C., United States of America) and XLSTAT (Addinsoft, New York, United States of America).

RESULTS AND DISCUSSION

Effect of biochar as a hydroponic substrate on plant growth performance

Figure 1a shows the application of different hydroponic substrates has positively affected the height of red lettuce. Data analysis revealed that red lettuce planted with palm kernel biochar and hydroton (T4) grew taller than the plants planted with the combination of palm kernel biochar and perlite (T5), palm kernel biochar (T3), hydroton (T1), and perlite (T2) with mean plant heights of 19.86, 19.49, 17.47, 17.08, and 15.17 cm, respectively, at Week 5. The height of red lettuce was also found to be not significantly different between T1, T3, T4 and T5, but it was significantly different with T2. The adsorption capacity, porous structure, and surface area of biochar likely increased the nutrient availability to the plants, whilst the hydroton, which is a well-aerated growth substrate, enhanced the plant's growth (Ahmad et al. 2014, Jeffery et al. 2015).

Figure 1b shows the application of different hydroponic substrates has positively affected the number of leaves of red lettuce for five-week observation. Data analysis revealed that red lettuce planted with hydroton (T1) provide more leaves than the plants planted with the palm kernel biochar (T3), the combination of palm kernel biochar and perlite (T5), the combination of palm kernel biochar and hydroton (T4), and perlite (T2), with mean number of leaves of 20, 18, 18, 17 and 15, respectively, on Week 5. The number of red lettuce leaves was found to be significantly different between T1 and T2, while no significant difference was observed between T3, T4, and T5. Hydroton has a neutral pH and possesses exceptional water retention capacities, which provide better drainage for plants (Hamid et al. 2022). The red lettuce leaves might receive more nutrient availability through a nutrient solution, besides the environmental condition such as light, temperature, humidity and oxygen in the greenhouse supporting the plant growth (Schwarz 2012).

Figures 1c and 1d show the application of different hydroponic substrates has positively affected the leaf length and leaf width of red lettuce for five-week observation. An increasing trend was observed though there were no significant differences in the leaf length and leaf width of red lettuce. The leaf length of red lettuce planted with the palm kernel biochar and hydroton (T4) achieved the highest increment (14.16 cm), whilst the red lettuce planted with perlite (T2) exhibited the least increase (12.69 cm) throughout the planting period. For the leaf width, the red lettuce planted with the palm kernel biochar and hydroton (T4) also achieved the highest increment (11.28 cm), whilst the red lettuce planted with perlite exhibited the lowest increment at 10.75 cm throughout the planting period.

Figure 1e shows that the relative chlorophyll content of red lettuce in all treatments decreased in general, though there were no significant differences between each treatment. The relative chlorophyll content of red lettuce, which was planted with perlite (T2), decreased the lowest, which was about 10.76 $\mu mol/m^2$, whilst the relative chlorophyll content of red lettuce planted with biochar (T3) achieved the highest increment, 11.29 $\mu mol/m^2$, on the Week 5. The amount of chlorophyll content in leaf tissue is influenced by nutrient availability and environmental stresses, such as drought, salinity, cold, and heat (Palta, 1990). Besides, the optimum temperature for growing lettuce ranged 20 °C/15 °C to 24 °C/20 °C (day/night). However, the temperature of

planting location was quite high, ranging from 27 °C/24 °C to 34 °C/25 °C (day/night). As surrounding temperature rise above optimal levels, the rates of photosynthesis fall due to a decline in the affinity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) for carbon dioxide (CO_2), reducing carboxylation and increasing oxygenation in plants (Haworth et al. 2018).

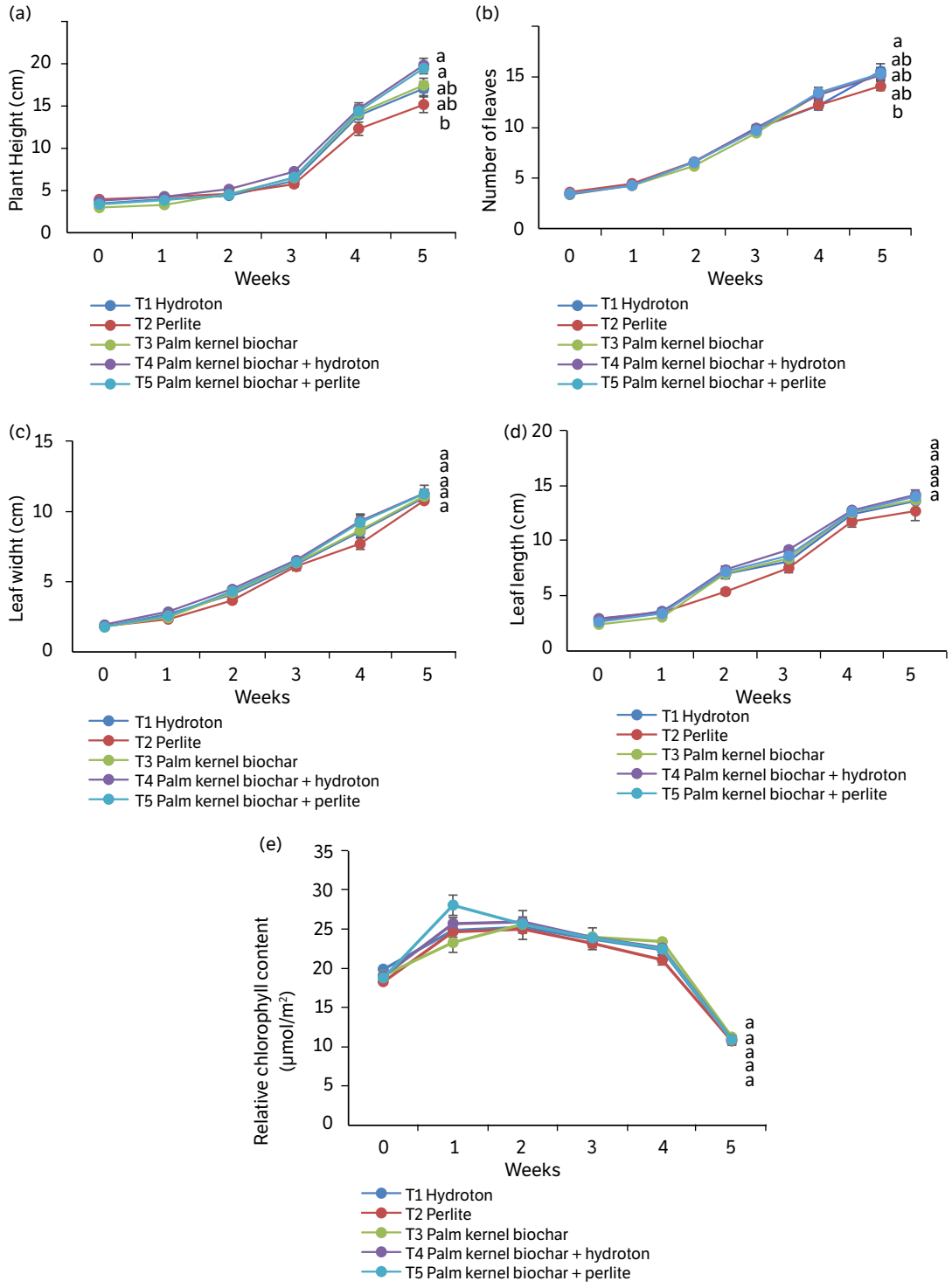


Figure 1. Effect of different hydroponic substrates on (a) plant height, (b) number of leaves, (c) leaf width, (d) leaf length, and (e) relative chlorophyll content for the five-week observation. Each data item represents the mean of replicates, and the vertical bar represents the standard error. Mean values followed by different letters were significantly different ($p < 0.05$).

The fresh weight and dry weight of the red lettuce after harvest were positively affected by the different treatments. Red lettuce planted with the combination of palm kernel biochar and hydroton (T4) showed the highest shoot fresh weight among other treatments (50.69 g), whilst the red lettuce planted with perlite (T2) had the lowest shoot fresh weight (43.87 g). The shoot fresh weight was significantly different between T4 and T2. For shoot dry weight, red lettuce planted with the combination of palm kernel biochar and hydroton (T4) had the highest dry weight (5.23 g), whilst the red lettuce with perlite (T2) had the lowest dry weight (3.32 g). The red lettuce planted with palm kernel biochar and hydroton (T4) also had the highest root fresh weight at 17.50 g, while the red lettuce planted with perlite (T2) had the lowest at 11.68 g. Similar trends were observed with plant biomass of shoot and root, whereby the highest fresh weight and dry weight, as well as the length of shoot and root, were observed in the red lettuce grown in the application of palm kernel biochar and hydroton (T4), which showed a significant difference with other treatments, particularly T2. This was likely caused by the combined application of biochar and hydroton (T4), which helped improve the root elongation since the roots are the main plant organs that are in direct contact with a nutrient solution in hydroponics, in which a nutrient solution has a direct effect on the growth of crops (Both et al. 1999). The particle size of biochar and hydroton is also bigger, thereby providing high pore space, which might encourage the elongation of the root of red lettuce.

Root is responsible for absorbing water and nutrients from nutrient solution which are then transported to the rest of the plant through xylem, which in turn would indirectly increase the shoot growth. Root function in hydroponic systems depends on the substrate, which mechanically support the plant root system and maintain the nutrient solution around the roots (Ok et al. 2015). Biochar increased the plant root biomass of maize under field condition, as reported by Abiven et al. (2015). As observed in Table 1, the shoot length and root length of the red lettuce after harvest were also shown to be positively affected by the different treatments.

Table 1. Effect of different hydroponic substrates on the red lettuce biomass of shoot and root, as well as the shoot and root length*.

TREATMENTS	Fresh weight of shoot (g)	Dry weight of shoot (g)	Fresh weight of root (g)	Dry weight of root (g)	Shoot length (cm)	Root length (cm)
T1 Hydroton	44.35 ± 2.49^{ab}	4.00 ± 0.27^{b}	12.48 ± 0.85^{c}	2.26 ± 0.02^{b}	22.03 ± 0.74^{bc}	22.40 ± 1.44^{b}
T2 Perlite	43.87 ± 1.40^{b}	3.32 ± 0.01^{c}	11.68 ± 0.28^{c}	1.49 ± 0.16^{d}	20.46 ± 0.84^{c}	21.81 ± 0.81^{b}
T3 Palm kernel biochar	45.97 ± 1.53^{ab}	4.06 ± 0.22^{b}	14.22 ± 0.75^{bc}	1.85 ± 0.09^{c}	22.70 ± 0.04^{bc}	23.16 ± 0.49^{b}
T4 Palm kernel biochar + hydroton	50.69 ± 2.31^{a}	5.23 ± 0.16^{a}	17.50 ± 0.56^{a}	3.06 ± 0.01^{a}	25.62 ± 1.27^{a}	28.43 ± 0.47^{a}
T5 Palm kernel biochar + perlite	49.27 ± 1.33^{ab}	4.30 ± 0.01^{b}	16.23 ± 0.84^{ab}	2.30 ± 0.01^{b}	23.23 ± 0.72^{ab}	24.17 ± 0.70^{b}

*Mean ± standard error. Mean values followed by different letters within a column were significantly different ($p < 0.05$).

Effect of biochar as a hydroponic substrate on algal density in nutrient solution

In general, the algal density in nutrient solution with the application of different hydroponic substrates showed an increasing trend in Fig. 2. Data analysis revealed that the algal density with the application of biochar (T3) was lower than the application of palm kernel biochar and hydroton (T4), hydroton (T2), palm kernel biochar and perlite (T5), and also perlite only (T1), with mean algal densities of 73.82×10^4 cells/mL, 74.04×10^4 cells/mL, 78.83×10^4 cells/mL, 85.84×10^4 cells/mL, and 94.99×10^4 cells/mL, respectively. The nutrient solution with the application of palm kernel biochar (T3), as well as the combination of palm kernel biochar and hydroton (T4) as the substrate, was observed to be cleaner and clearer compared to the other treatments in this study, which indicated that palm kernel biochar was able to inhibit the algal growth. Nevertheless, no significant difference was observed between T3 and T4.

These findings are in agreement with the results reported by other studies in which rice husk biochar has been found to inhibit the growth of algae by absorption in addition to reducing toxicity (Awad et al. 2017). Inhibition of algal growth can be an alternative option for food safety, since it avoids the uptake of toxic compounds in hydroponically grown crops

(Awad et al. 2017). The odour and appearance of plants grown in a hydroponic system containing algae might reduce the product quality, and the algae may also secrete toxins that are harmful to human health (Corbel et al. 2014). A biochar-induced decrease in the growth of algae in the nutrient solution in hydroponic containers might be attributed to the algae adsorption on the surface biochar, in which the algal cells formed clusters and were adsorbed on biochar substrates, thereby blocking nutrient uptake and algal growth (Awad et al. 2017).

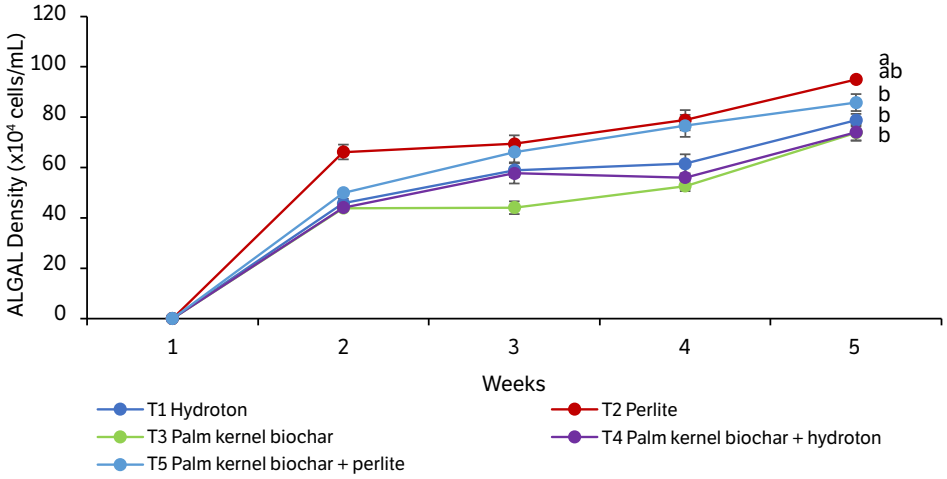


Figure 2. Effect of different hydroponic substrates on the algal density in the nutrient solution for the five-week observation. Each data item represents the mean of replicates, and the vertical bar represents the standard error. Mean values followed by different letters were significantly different ($p < 0.05$).

Effect of biochar as a hydroponic substrate on red lettuce colour

The CIELAB values for the red lettuce in Table 2 were expressed in terms of five parameters:

- $H°$: hue, which is the actual colour recognized by human eyes;
- L^*: lightness;
- C: chroma or saturation;
- a^*;
- b^*.

Both the hue and chroma were defined from two-colour coordinates, that were a^*, which took a positive value for red and a negative value for green, and b^*, which took a positive value for yellow and a negative value for blue. The red lettuce planted with the combination of palm kernel biochar and hydroton (T4) had a higher lightness value ($L^* = 66.67$) compared to the red lettuce planted with other treatments. The colour value a^* was negative whilst the colour value b^* was positive for the red lettuce planted with each treatment, indicating that there was a shift in the colour of the lettuce leaf towards green and yellow. The more intense colour (high chroma) was obtained in the red lettuce planted with palm kernel biochar and perlite (T5) ($C = 53.14$), less colour intensity was obtained in the red lettuce planted with the palm kernel biochar and hydroton (T4) ($C = 48.87$), and intermediate chroma values ($C = 52.78, 51.94, 49.74$) were observed in red lettuce planted with perlite only (T2), palm kernel biochar (T3) and hydroton (T1). The value of $H°$ for the red lettuce were close to 120°, and further confirmed that the lettuce leaves also contained pigments ranging from yellow to green colours.

For this study, all colour parameters were found to be correlated well with total carotenoid content and total flavonoid content, which served as the main contributors that affect the colour parameter. These results were in agreement with the observation reported by Alonso et al. (2003), who evaluated the colour of Spanish saffron that the lightness, a^* (red-green), and b^* (yellow-blue) correlated well with their colouring power.

Table 2. Effect of different hydroponic substrates on the colour of red lettuce#.

TREATMENT	*L**	*a**	*b**	*C*	*H*°
T1 Hydroton	65.40 ± 0.12^{b}	-24.07 ± 0.13^{a}	43.53 ± 0.16^{b}	49.74 ± 0.20^{c}	118.94 ± 0.06^{c}
T2 Perlite	64.03 ± 0.13^{c}	-26.15 ± 0.04^{c}	45.84 ± 0.08^{a}	52.78 ± 0.09^{ab}	119.70 ± 0.02^{a}
T3 Palm kernel biochar	63.86 ± 0.52^{c}	-25.38 ± 0.17^{b}	45.32 ± 0.35^{a}	51.94 ± 0.39^{b}	119.25 ± 0.04^{b}
T4 Palm kernel biochar + hydroton	66.67 ± 0.03^{a}	-23.71 ± 0.05^{a}	42.73 ± 0.26^{b}	48.87 ± 0.24^{c}	119.03 ± 0.14^{bc}
T5 Palm kernel biochar + perlite	64.41 ± 0.28^{c}	-26.29 ± 0.24^{c}	46.18 ± 0.49^{a}	53.14 ± 0.53^{a}	119.65 ± 0.13^{a}

#Mean ± standard error. Mean values followed by different letters within a column were significantly different ($p < 0.05$); *L**: lightness; *a**: red-green; *b**: yellow-blue; *C*: chroma; *H*°: hue.

Effect of biochar as a hydroponic substrate on nutrient content of red lettuce

Data on the red lettuce nutrient contents taken after five weeks of transplanting are presented in Table 3. Data analysis revealed that there was a significant difference among all nutrients content determined in the leaf of red lettuce after planted in the different treatments. The red lettuce planted with the combination of palm kernel biochar and hydroton (T4) showed higher nitrogen (N) (3.35%), phosphorus (P) (0.75%), potassium (K) (7.85%), magnesium (Mg) (0.29%), and calcium (Ca) (1.37%). Biochar has been reported to improve the quality of runoff water by decreasing the discharge of total N and facilitating the retention of nutrients in the greenhouse soil (Beck et al. 2011). Similar trends were found in the total phosphorus content (P), total potassium content (K), total magnesium content (Mg), and total calcium content (Ca), and the highest percentage of P, K, Mg and Ca were exhibited by the red lettuce leaves grown with palm kernel biochar and hydroton (T4).

A previous study has shown that biochar induced the growth of beneficial microorganisms on its surface, which may enhance the uptake of nutrients by plants, and it may be one of the possible mechanisms for improving P, K, Mg, and Ca uptake by plant root systems (Awad et al. 2017). This is in agreement with Karakaş et al. (2017), who reported a similar mechanism for improving the uptake of macronutrients by tomato seedlings in hydroponic system treated with biochar. Furthermore, biochar as organic material has further decomposition, which was caused by further basic cation release such as K^{+}, Mg^{2+}, and Ca^{2+} contents of organic materials (Ch'ng et al. 2019), which can dissolve in water (Smider and Singh 2014), thus might enhancing the nutrient uptake of plant.

Table 3. Effect of different hydroponic substrates on the total nutrient content of red lettuce*.

TREATMENTS	N (%)	P (%)	K (%)	Mg (%)	Ca (%)
T1 Hydroton	2.84 ± 0.13^{b}	0.67 ± 0.04^{ab}	7.62 ± 0.04^{ab}	0.27 ± 0.02^{b}	1.28 ± 0.07^{ab}
T2 Perlite	2.63 ± 0.05^{b}	0.65 ± 0.00^{b}	7.48 ± 0.05^{b}	0.27 ± 0.00^{b}	1.23 ± 0.00^{b}
T3 Palm kernel biochar	2.86 ± 0.01^{b}	0.71 ± 0.00^{ab}	7.77 ± 0.03^{ab}	0.28 ± 0.00^{ab}	1.35 ± 0.04^{ab}
T4 Palm kernel biochar + hydroton	3.35 ± 0.15^{a}	0.75 ± 0.01^{a}	7.85 ± 0.18^{a}	0.29 ± 0.00^{a}	1.37 ± 0.03^{a}
T5 Palm kernel biochar + perlite	3.00 ± 0.15^{ab}	0.74 ± 0.00^{a}	7.61 ± 0.04^{ab}	0.28 ± 0.01^{ab}	1.31 ± 0.02^{ab}

*Mean ± standard error. Mean values followed by different letters within a column were significantly different ($p < 0.05$); N: nitrogen; P: phosphorus; K: potassium; Mg: magnesium; Ca: calcium.

Effect of biochar as a hydroponic substrate on total chlorophyll and carotenoid content of red lettuce

The total chlorophyll and total carotenoid content in the red lettuce leaf extract with the application of different hydroponic substrates were measured in Table 4. The amounts of chlorophyll a (C*a*) and b (C*b*) showed similar trends. The extract of red leaf letttuce planted with palm kernel biochar and hydroton (T4) exhibited the highest value for C*a* (0.76 mg/g DW). Significantly higher values of C*b* content (0.60 mg/g DW) were also obtained in the red lettuce leaf extract with the application of palm kernel biochar and hydroton (T4). The lowest values were also observed in the leaf

extracts with the application of different treatment which is the extract of red lettuce leaf with the application of hydroton (T1) (0.36 mg/g DW) for C*a* and (0.26 mg/g DW) for C*b*. The chlorophyll a content was found to be higher than chlorophyll b, maybe due to salt stress in nutrient solution in which the electrical conductivity reading for nutrient solution in hydroponic system was observed to increase weekly until harvesting day.

The application of biochar increased the amount of chlorophyll a and b, mainly due to improving the uptake of Mg and N as essential macronutrients for chlorophyll biosynthesis (Chan et al. 2008, Zargar Shooshtari et al. 2020). For the total chlorophyll content of the red lettuce leaf extract, the highest mean value (1.36 mg/g DW) were recorded in the red lettuce planted with palm kernel biochar and hydroton (T4) compared from other treatments. The highest total carotenoid content value was obtained in the leaf extract planted with perlite only (T2) (0.20 mg/g DW), and the lowest was in the leaf extract planted with hydroton (T1) (0.13 mg/g DW), in which the values were significantly influenced by the different hydroponic substrates.

These results indicate that the addition of perlite and biochar did not significantly influence the carotenoid content of the red lettuce leaves. In previous study conducted on tomato plants, it was found that K fertilization can affect carotenoid biosynthesis, particularly lycopene (Serio et al. 2007, Almeselmani et al. 2009, Afzal et al. 2015). It has been reported that the relationship between chlorophyll and carotenoid contents in plants are highly influenced by K status on the plant (Trudel and Ozbun 1970). However, the ratio of chlorophyll and carotenoid content changes during ripening in which chlorophyll content will decrease as carotenoid increase as fruit ripens (Trudel and Ozbun 1971).

Table 4. Comparison of chlorophylls and carotenoid content from methanolic extract of red lettuce*.

TREATMENT	C*a* (mg/g DW)	C*b* (mg/g DW)	C*a*/C*b* ratio
T1 Hydroton	0.36 ± 0.02^{c}	0.26 ± 0.01^{d}	1.37 ± 0.05^{b}
T2 Perlite	0.65 ± 0.03^{b}	0.41 ± 0.01^{c}	1.63 ± 0.05^{a}
T3 Palm kernel biochar	0.75 ± 0.03^{a}	0.48 ± 0.03^{b}	1.57 ± 0.03^{a}
T4 Palm kernel biochar + hydroton	0.76 ± 0.02^{a}	0.60 ± 0.03^{a}	1.29 ± 0.05^{b}
T5 Palm kernel biochar + perlite	0.59 ± 0.03^{b}	0.36 ± 0.02^{c}	1.66 ± 0.02^{a}
TREATMENT	**C*a*+C*b* (mg/g DW)**	**C(*x*+*c*) (mg/g DW)**	**C*a*+C*b*/ C(*x*+*c*) ratio**
T1 Hydroton	0.63 ± 0.02^{e}	0.13 ± 0.01^{c}	4.83 ± 0.15^{d}
T2 Perlite	1.08 ± 0.03^{c}	0.20 ± 0.00^{a}	5.51 ± 0.07^{c}
T3 Palm kernel biochar	1.23 ± 0.02^{b}	0.19 ± 0.00^{a}	6.60 ± 0.23^{b}
T4 Palm kernel biochar + hydroton	1.36 ± 0.05^{a}	0.16 ± 0.01^{b}	9.40 ± 0.07^{a}
T5 Palm kernel biochar + perlite	0.99 ± 0.02^{d}	0.19 ± 0.01^{a}	5.17 ± 0.20^{d}

*Mean ± standard error. Mean values followed by different letters within a column were significantly different ($p < 0.05$); C*a*: chlorophyll a; C*b*: chlorophyll b; C*a*+C*b*: total chlorophyll content; C*a*/C*b* ratio: chlorophyll a and b ratio; C(*x*+*c*): total carotenoid content; C*a*+C*b*/ C(*x*+*c*) ratio: chlorophyll and carotenoid ratio; DW: dry weight.

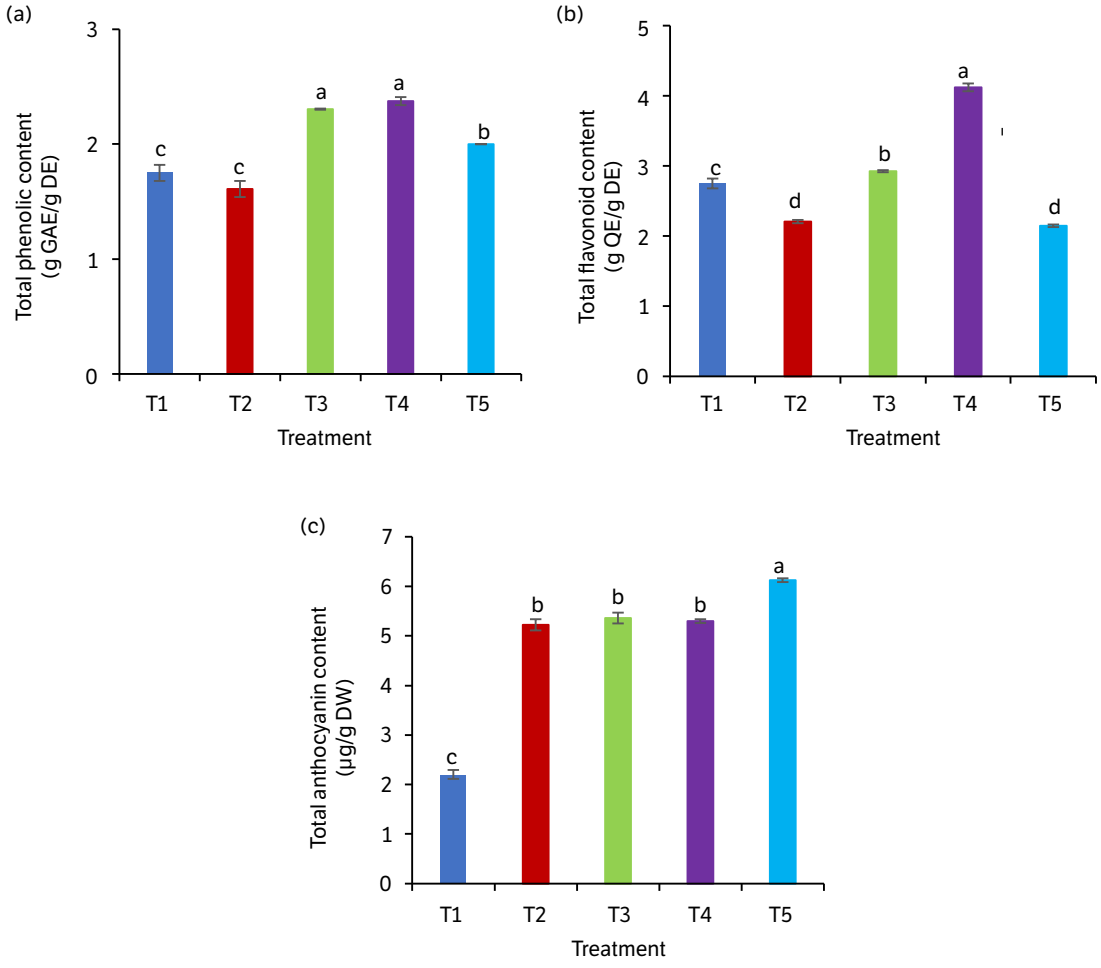
Effect of biochar as a hydroponic substrate on total phenolic, flavonoid and anthocyanin content of red lettuce

The total phenolic (TPC), total flavonoid (TFC) and total anthocyanin (TAC) contents in the red lettuce leaf extracts with the application of the different hydroponic substrates were measured. Based on Fig. 3a, the highest TPC was obtained in the red lettuce planted with palm kernel biochar (T3) (2.31 g GAE/g DE) and the combination of palm kernel biochar and hydroton (T4) (2.38 g GAE/g DE), whilst the lowest TPC was recorded in the red lettuce leaf planted with hydroton only (T1) (1.75 g GAE/g DE) and perlite only (T2) (1.61 g GAE/g DE). However, there were no significant differences observed between both treatments (for the highest and lowest TPC). Total phenolic content was generally higher in red lettuce varieties compared to green red lettuce (Mampholo et al. 2016).

This is also in agreement with a previous study reported by Petruccelli et al. (2015), in which the total phenolic content was higher in fruits grown in substrate amended with straw biochar and olive residues biochar. The TFC of red lettuce leaf

ranged from 2.16 to 4.23 g QE/g DE, in which the red lettuce leaf from the treatment of palm kernel biochar and hydroton (T4) (4.12 g QE/g DE) showed the highest TFC, compared to the other treatments. The particle size of perlite might influence the level of flavonoid compounds.

Based on a previous study by Ahmadi et al. (2021), it was shown that the increase in perlite particle size leads to a decrease of total flavonoid content. No significant differences in TFC were observed between the palm kernel biochar and perlite (T5) and perlite only (T2) treatments. For the TAC of the red lettuce leaf extract, the highest mean value (6.12 µg/g DW) were recorded in the red lettuce planted with palm kernel biochar and perlite (T5) compared from other treatments. Based on a previous study, the genetics, temperature, and light influence anthocyanin accumulation in lettuce (Gazula et al. 2005). It can be supported that the combination of palm kernel biochar and perlite might affect the level of anthocyanin because of both substrates were well-aerated (Ok et al. 2015, Obia et al. 2018), therefore supporting root respiration and, in turn, decreased the temperature of nutrient solution in hydroponic system.



*Mean values followed by different letters were significantly different ($p < 0.05$); T1: hydroton (control); T2: perlite; T3: palm kernel biochar; T4: palm kernel biochar + hydroton; T5: palm kernel biochar + perlite; GAE: gallic acid; QE: quercetin; DE: dried extract; DW: dry weight.

Figure 3. Effect of different hydroponic substrates on the (a) total phenolic content, (b) total flavonoid content, and (c) total anthocyanin content of red lettuce. Each data item represents the mean of replicate,s and the vertical bar represents the standard error*.

Effect of biochar as a hydroponic substrate on antioxidants potential of red lettuce

The intake of anthocyanin rich vegetables has shown many health benefits, such as visual improvement, anti-carcinogenic, and anti-mutagenic, especially due to the strong antioxidant property (Mampholo et al. 2016). There, in this study, the radical

scavenging activities and reducing power of the red lettuce leaf extracts were also evaluated to determine the effects of the different treatments on the plants' antioxidant properties. Table 5 shows the IC_{50} values for DPPH assay and ABTS assay, as well as FRAP values of the methanolic extract of red lettuce leaves. Three different antioxidant assays (DPPH, ABTS, FRAP) were carried out to determine the antioxidant activity of the red lettuce leaves planted with different hydroponic substrates due to the complexity of the phytochemicals (Mampholo et al., 2016).

For the DPPH assay, the leaf extract from the red lettuce planted with palm kernel biochar and hydroton (T4) showed the strongest scavenging activity (denoted by the lowest IC_{50} value) against DPPH radicals compared to the red lettuce planted with other treatments. Similar observation was recorded for ABTS assay, in which the red lettuce leaf extracts planted with palm kernel biochar and hydroton (T4) possessed the highest scavenging activity against ABTS radicals, with the lowest IC_{50} value of 0.94 mg/mL. In general, the scavenging activity of the leaf extracts against ABTS radicals in increasing order is T2 < T1< T3 < T5 <T4. This line is in agreement with a previous study reported by Kim et al. (2018), who reported that red lettuce cultivars had higher DPPH and ABTS free radical scavenging activities than green cultivars. In addition, a FRAP assay was also conducted to estimate the antioxidant power of the extracts, which is the reducing ability of the substances involved in the transfer of electron in the reaction. Perlite (T2) was observed to have the lowest FRAP reducing activity (56.71 g of $FeSO_4$ equivalent/g of dried extract), whilst the red lettuce leaf extract planted with palm kernel biochar (T3) exhibited significantly highest FRAP antioxidant capacity, with the mean value 73.70 g of $FESO_4$ equivalent/g of dried extract.

Table 5. Effect of different hydroponic substrates on the antioxidant activities from the extract of red lettuce leaves*.

TREATMENT	DPPH IC_{50} (mg/mL)	ABTS IC_{50} (mg/mL)	FRAP (g FE/g DE)
T1 Hydroton	31.67 ± 1.10^{b}	2.62 ± 0.05^{a}	62.65 ± 1.03^{c}
T2 Perlite	38.78 ± 1.54^{a}	2.66 ± 0.11^{a}	56.71 ± 0.18^{d}
T3 Palm kernel biochar	15.18 ± 0.61^{d}	2.16 ± 0.07^{b}	73.70 ± 1.00^{a}
T4 Palm kernel biochar + hydroton	9.03 ± 0.02^{d}	0.94 ± 0.09^{d}	69.31 ± 0.25^{b}
T5 Palm kernel biochar + perlite	25.89 ± 1.22^{c}	1.86 ± 0.10^{c}	61.86 ± 0.19^{c}

*Mean ± standard error. Mean values followed by different letters within a column were significantly different ($p < 0.05$); FE: ferric equivalent; DE: dried extract.

Correlation analysis between plant growth and bioactive metabolites of red lettuce

A Pearson's correlation analysis was conducted to determine the relationship between the plant growth parameters and content of bioactive metabolites of red lettuce (Table 6). Plant height demonstrated a significant negative correlation with the antioxidant activity of red lettuce extracts against DPPH and ABTS radical scavenging activities, with r-values of -0.610 and 0.702, respectively ($p \leq 0.01$). Root fresh weight also demonstrated a significant negative correlation with DPPH and ABTS radical scavenging activities, with r-values of -0.722 ($p \leq 0.01$) and -0.825 ($p \leq 0.05$). ABTS assay negatively correlated with shoot length ($r = -0.699$) and root length ($r = -0.917$) (significant at $p \leq 0.05$ level). These indicate that taller plants with higher shoot length, root length and root fresh weight had higher scavenging activities against DPPH and ABTS radicals. In addition, the shoot length showed a positive correlation with total phenolic and total flavonoid contents, indicating that red lettuce plants with longer shoot length contained higher amounts of TPC and TFC. Moreover, data analysis revealed a strong negative correlation between TPC and TFC of the extracts with DPPH antioxidant activity, with r-value of -0.976 ($p \leq 0.05$) and -0.784 ($p \leq 0.01$). These indicate that the antioxidant activity against DPPH radicals would significantly increase with increasing concentrations of total phenolic and total flavonoid.

Table 6. Correlations between plant growth parameters and bioactive metabolites content of red lettuce.

	Plant height	No. of leaves	Leaf length	Leaf width	FWR	DWR	FWS	DWS	Shoot length	Root length	Chl A	Chl B	Total Chl	TCC	TAC	TPC	TFC	DPPH	ABTS	FRAP
Plant height	1																			
No. of leaves	0.936**	1																		
Leaf length	0.925**	0.953**	1																	
Leaf width	0.946**	0.979**	0.983**	1																
FWR	0.645*	0.458	0.591	0.653	1															
DWR	0.564	0.465	0.408	0.589	0.772**	1														
FWS	0.371	0.322	0.554	0.782**	0.914**	0.817**	1													
DWS	0.555	0.453	0.508	0.670*	0.863**	0.954**	0.904**	1												
Shoot length	0.429	0.468	0.397	0.742**	0.901**	0.894**	0.900**	0.921**	1											
Root length	0.532	0.194	0.472	0.568	0.837**	0.844**	0.767**	0.885**	0.888**	1										
Chl A	0.065	-0.446	0.007	0.234	0.427	-0.154	0.214	0.008	0.300	0.474	1									
Chl B	0.258	-0.060	0.115	0.436	0.628	0.226	0.386	0.371	0.571	0.733*	0.902**	1								
Total Chl	0.156	-0.288	0.053	0.331	0.530	0.006	0.297	0.164	0.431	0.599	0.981**	0.968**	1							
TCC	-0.287	-0.800	-0.151	-0.215	0.056	-0.670	0.085	-0.474	-0.121	-0.124	0.720**	0.406	0.602*	1						
TAC	0.289	-0.639	0.197	0.077	0.461	-0.056	0.425	0.135	0.181	0.391	0.863**	0.667*	0.802**	0.956**	1					
TPC	0.600	0.349	-0.419	0.621	0.695	0.635	0.366	0.671	0.812*	0.489	0.616	0.726*	0.678*	0.327	0.453	1				
TFC	0.424	0.343	0.366	0.558	0.637	0.800*	0.416	0.835*	0.679*	0.838*	0.312	0.631*	0.457	-0.403	-0.200	0.655	1			
DPPH	-0.610*	-0.235	-0.468	-0.289	-0.722*	-0.445	-0.421	-0.643	-0.642*	-0.630	-0.551	-0.736*	-0.640	0.011	-0.367	-0.976**	-0.784*	1		
ABTS	-0.702*	-0.268	-0.392	-0.348	-0.825**	-0.679*	-0.599	-0.817**	-0.699**	-0.917**	-0.585	-0.839**	-0.712*	0.015	-0.524	-0.786	-0.768*	0.760**	1	
FRAP	0.427	0.419	0.387	0.217	0.406	0.322	0.160	0.424	0.478	0.358	0.409	0.565	0.490	-0.114	0.183	0.886*	0.709*	-0.912**	-0.490	1

*Correlation was significant at the 0.01 level (2-tailed); **correlation was significant at the 0.05 level (2-tailed); FWR: root fresh weight; DWR: root dry weight; FWS: shoot fresh weight; DWS: shoot dry weight; Chl a: chlorophyll a; Chl b: chlorophyll b; total Chl: total chlorophyll content; TCC: total carotenoid content; TAC: total anthocyanin content; TPC: total phenolic content; TFC: total flavonoid content.

Principal component analysis

Principal component analysis (PCA) was also used to determine the relationship between the growth performance, colour properties and nutritional value of red lettuce (Fig. 4). The red lettuce planted with palm kernel biochar (T3) and the combination with palm kernel biochar and perlite (T5) shared the same quadrant in the PCA biplot whilst the other treatments did not share any quadrant, which showed that the samples were different in some ways. The red lettuce planted with palm kernel biochar (T3) and the combination of palm kernel biochar and perlite (T5) were clustered on the right with high positive PC1 value and characterized by more bioactive metabolites factors. The red lettuce planted with palm kernel biochar and hydroton (T4) was clustered in the upper part of the PC2 axis with a positive PC2 value and characterized by high-growth performance and nutrient content, which could be explained by the higher levels of growth performance parameters and the total nutrient (N, P, K, Mg, Ca) content of the red lettuce. The four principal components have eigenvalues greater than 1: 19.24 for component 1, 7.13 for component 2, 9.70 for component 3, and 2.53 for component 4.

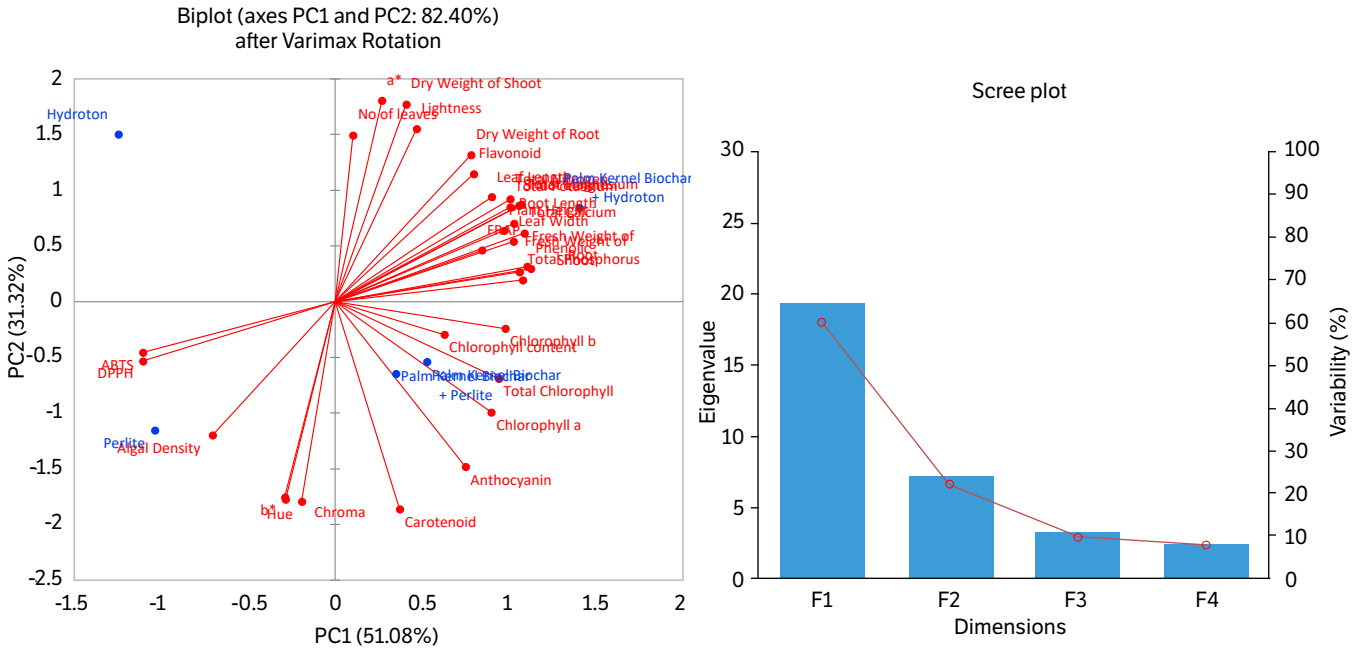


Figure 4. Principal component analysis, eigenvalue and variability (%) of growth, colour parameters, nutrient content, and bioactive metabolites in red lettuce.

CONCLUSION

In conclusion, the different types of hydroponic substrates used in the hydroponic system had positively affected the plant growth, colour, nutrient, as well as bioactive metabolites content. Furthermore, the combination of palm kernel biochar and hydroton (T4) increased the growth performance of red lettuce except for the number of red lettuce leaves, whereby the application of hydroton (T1) produced more leaves compared to other treatments. In terms of the colour of red lettuce, T4 showed the highest lightness, while T5 provided the highest chroma for the red lettuce leaves. The combination of palm kernel biochar and hydroton (T4) also improved the nutrient content (N, P, K, Mg, Ca) and bioactive metabolites content of red lettuce such as total chlorophyll content, total flavonoid content, DPPH and ABTS radical scavenging activities. Besides, the application of palm kernel biochar (T3), as well as the combination of palm kernel biochar and hydroton (T4) as a hydroponic substrate decreased the algal density in the nutrient solution and improved the total phenolic content compared to the other treatments.

This study also showed that palm kernel biochar (T3) enhanced the FRAP antioxidant capacity of red lettuce. Taken together, the use of palm kernel biochar, either alone or in combination with hydroton as the hydroponic substrates in the NFT hydroponic system, can be recommended for production of high-quality red lettuce.

AUTHORS' CONTRIBUTION

Conceptualization: Rosli, N. S. M., Abdullah, R. and Yaacob, J. S.; **Methodology:** Rosli, N. S. M., Abdullah, R. and Yaacob, J. S.; **Investigation:** Rosli, N. S. M., Abdullah, R., Yaacob, J. S. and Razali, R. B. R.; **Writing – original draft:** Rosli, N. S. M.; **Writing – review and editing:** Abdullah, R. and Yaacob, J. S.; **Funding acquisition:** Abdullah, R.; **Supervision:** Abdullah, R. and Yaacob, J. S..

DATA AVAILABILITY STATEMENT

All data analyzed during this study are included in this published article.

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