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Biochar, a potential hydroponic growth substrate, enhances the nutritional status and growth of leafy vegetables

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A B S T R A C T

A hydroponics system developed using a nutrient film technique was used to evaluate the effectiveness of rice husk biochar (RB) alone or in combination with perlite (PL) as substrates for increasing the growth of leafy vegetables compared with that of PL. Seedlings of cabbage, dill, mallow, red lettuce, and tatsoi were grown hydroponically in PL, RB, and PL + RB (1:1 ratio of PL to RB, v/v) substrates for a 30-d under optimal environmental conditions in a greenhouse. Shoot length and fresh/dry masses of cabbage, dill, and red lettuce plants grown in RB substrate were decreased by 49% on average compared to those plants grown in PL substrate. In contrast, PL + RB substrate led to approximately 2-fold increases in shoot length, number of leaves, and fresh/dry masses of leafy vegetable plants compared with those grown in PL substrate. Foliar nutritional composition (Ca, Mg, K, Na, Mn, Fe, and Zn) and nitrogen status (SPAD index) of plants grown in PL + RB and PL substrates suggested the presence of optimal growth conditions for ensuring optimum yield with high quality. In addition, RB substrate contributed to respective increases of 1.2–3.5-fold in leaf K, Mg, Mn, and Zn contents in most vegetable plants compared with those grown in PL substrate. The RB alone or in combination with PL substrates decreased algal growth in the nutrient solutions as confirmed by scanning electron micrographs of microalgae on the RB surface. The results also indicated that use of PL + RB hydroponic substrate could be an alternative and effective technology for the better management of unwanted algal growth in nutrient solutions and high production of leafy vegetables.

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1. Introduction

Water shortage is a great threat to crop production sustainability and food security (Power and Jones, 2016). To cope with this challenge, hydroponics is a rapidly developing eco-industrial technology for the production of commercial crops in nutrient-rich solutions, instead of soil (Jones Jr., 2016; Nhut et al., 2006; Resh, 2012). Hydroponics offers several benefits such as improved yield and good-quality products, precise nutrient and disease management, short cultivation times, and safe food and growth environments (Nhut et al., 2006; Resh, 2012). Inert substrates such as coconut coir, peat, perlite (PL), and vermiculite have commonly been used to support plant root systems and maintain an appropriate concentration of the nutrient solution around the roots (Ok et al., 2015b).

As associated problems with hydroponics substrates, recirculation of nutrient solution in hydroponics systems provides a favorable condition for algal growth (Coosemans, 1993; Schwarz and Gross, 2004). Microalgae were easily found in hydroponics containers and had an adverse effect on the water supply system and nutrient uptake by plants, leading to a remarkable reduction in crop yield (Schwarz and Gross, 2004). Further, the odor and appearance of crops grown in a hydroponics system containing algae might reduce their value of product, and such algae may also secrete toxins that are harmful to human health (Corbel et al., 2014; Magee et al., 2013; Sayre, 2010). In terms of food safety, some types of algae found in hydroponics containers secrete certain toxic compounds that inhibit the growth of organisms, including crops, by decreasing the light-dependent photosynthetic reactions (Corbel et al., 2014; Schwarz et al., 2005; Schwarz and Gross, 2004). Since algal toxins might be taken up by plants, they pose a health hazard to people who consume these plants (Burgoon and Bottino, 1976; Meriluoto and Spoof, 2008). Therefore, developing alternative industrial substrates for hydroponics that maintain the water quality by eliminating/reducing algal growth, thereby increasing crop production, is necessary for ensuring sustainable crop production in hydroponics (Jones Jr., 2016; Kaudal et al., 2016; Resh, 2012).

Biochar (BC) is a carbon-rich product of the pyrolysis of biomass such as wood, crop residues, and manure in a closed container with little or no oxygen (Lehmann and Joseph, 2015). Biochar increases plant growth by improving the physicochemical and biological properties of soil and has been known to retain soil fertility, and remediate organic/inorganic contaminants (Jeffery et al., 2015; Lehmann et al., 2015; Ok et al., 2015a; Van Zwieten et al., 2010). In particular, rice husk BC (RB) reduced the bioavailability of Pb and increased the germination rate and root elongation of lettuce in contaminated soil (Ahmad et al., 2012). From practical viewpoint, BC is stable and highly resistant to microbial degradation due to the recalcitrant nature of BC molecules (Kuzyakov et al., 2014; Singh et al., 2012); therefore, it can be efficiently used as a growth substrate in hydroponics systems. The physicochemical characteristics of BC are similar to those of the standard industrial substrate coir peat and can be used as an alternative growing medium (Kaudal et al., 2016). The agronomic applications of BC need to be better understood, as recommended in 2010 by the American Society of Agronomy–Soil Science Society of the American Environmental Quality Division (Ippolito et al., 2012). Notably, further research is needed to assess using BC as an inert substrate to produce vegetables in hydroponics systems.

We hypothesized that RB alone or in combination with PL as a commercial substrate might increase vegetable production because of its high capacity for binding nutrients while promoting plant growth in nutrient-rich solutions by enhancing root growth. Biochar may also optimize water quality and maintain nutrient solution around plant roots in hydroponics systems by reducing/eliminating algal growth in hydroponics containers. In this study, we investigated whether RB alone or in combination with PL as inert substrates could enhance the growth of leafy vegetables (cabbage, dill, mallow, red lettuce, and tatsoi) and eliminate the growth of algae in the nutrient solutions in a hydroponics system.

2. Materials and methods

2.1. Hydroponics substrates and vegetable cultivars

Perlite (PL), a hydroponics substrate (average particle size, 1.2 mm; GFC Co., Ltd., Korea) and RB produced at 500 °C (≤2 mm; DAEWON GSI Co., Korea) were purchased from commercial sources. The PL and RB substrates were characterized using scanning electron microscopy (SEM; Model S-4300, Hitachi, Tokyo, Japan) operated at 15 keV with energy dispersive X-ray spectroscopy (EDS). Scanning electron micrographs of PL and RB substrates showing their surface structures are shown in Fig. 1. The scanning electron microscopy (SEM) images revealed that the surface of PL is rugged, irregular, and porous, whereas that of RB is covered with well-aligned, small, irregularly shaped particles. Rice husk biochar was previously characterized by Kim et al. (2015) as follows: cation exchange capacity (CEC), total carbon (TC), and total nitrogen (TN) of 50.4 $cmol_c\ kg^{-1}$, 20.5%, and 0.26%, respectively. In addition, the pH and electrical conductivity (EC) of RB were 10.2 and 0.82 $dS\ m^{-1}$, respectively. Leafy vegetable cultivars of hybrid Chinese cabbage (Asia Alpine F1; *Brassica rapa* L. ssp. *pekinensis*), dill (*Anethum graveolens* L.), curled mallow (*Malva verticillata* L.), red lettuce (*Lactuca sativa* L.), and tatsoi (*Brassica rapa* var. *rosularis*) were obtained from commercial companies in Korea (Asia Seed Co., Ltd. and Jeil Seed & Agricultural Products Co., Ltd.).

2.2. Raising nursery seedlings

Cabbage, dill, mallow, lettuce, and tatsoi seeds were sown in 128-cell plug trays (Bumnong. Co., Ltd., Korea) filled with commercial growth substrate (BM2; Berger Group Ltd., Canada) on April 11, 2015. The BM2 growth substrate consisted of 70%–80% fine sphagnum peat moss, fine PL, and fine vermiculite. Seedling trays were fertilized by overhead irrigation twice a week with Wonder Grow fertilizer (Chobi Co., Ltd., Korea). Uniform and vigorous 30-day-old seedlings were transplanted to pots after the removal of the growth substrate by rinsing the roots with tap water.

2.3. Hydroponics experiment

Six nutrient film technique hydroponics systems were purchased from a commercial company (Easy-Farm, Korea). The hydroponics systems, with an area of 105 cm × 40 cm and a height of 167 cm, consisted of a nutrient solution container (30 L) for growing 48 plants and a 20 W Young IL water pump (model YI-20; Fig. S1). More details about the hydroponics systems are available from the Easy-Farm company website (http://www.easy-farm.com). Uniform seedlings of each vegetable crop were planted in plastic pots (4.5 cm × 3 cm × 4.5 cm), which were then filled with PL, RB, or a combination of both (PL: RB at a 1:1 ratio (v/v)). Pots with hydroponic growth systems were placed inside a greenhouse fitted with a roof that automatically opens and closes at the Agricultural Farm of the College of Agriculture and Life Sciences, Kangwon National University, Gangwon Province, Korea, for 30 days. Plants were grown under greenhouse conditions according to Siddiqui et al. (2001). Briefly, the mean temperature was maintained at 18 °C (night) and 25–28 °C (day), and humidity was maintained in the range of 70%–85%. The movable hydroponics units were arranged in a random fashion in the greenhouse, and the

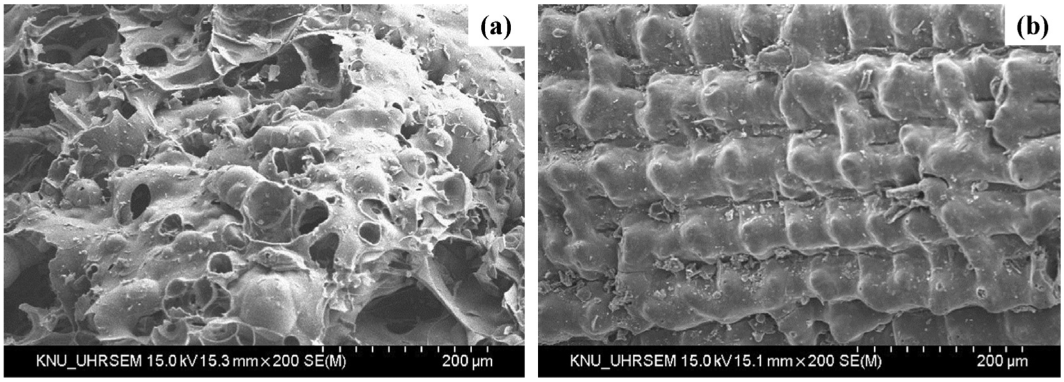


Fig. 1. Scanning electron micrographs of perlite (PL: a) and rice husk biochar (RB: b) substrates.

maximum temperature (approximately 32 °C at noon) was reduced by using fans and automatic shading with 50% white knitted shade cloth from 11:00 a.m. to 2:00 p.m. Two hydroponics systems were used as replicates for each growth substrate condition, and seedlings of the five vegetable cultivars were distributed randomly in each system. The hydroponics systems were operated continuously under a constant water flow rate of 2.5 L min^{-1}.

2.4. Nutrient solution

High EC-low pH nutrient solution was prepared from Wonder Grow fertilizer (Wonder Grow Fertilizers, Chobi Co., Ltd., Seoul, Korea) and used to grow the leafy vegetables in the hydroponics systems (Vu et al., 2014; Wortman, 2015). The pH of the nutrient solutions in RB and PL alone or in combination (PL + RB) were 5.8, 5.4, and 5.6, whereas the ECs were 1.6, 1.3, and 1.5 dS m^{-1}, respectively. The pH and EC of the nutrient solutions were maintained at 5.8 and 1.5 dS m^{-1}, respectively, by changing the solutions in the hydroponics containers every 10 d. The chemical characteristics of the nutrient solutions, used in the study, are given in Table 1.

2.5. Growth parameters

Leaf chlorophyll content was measured immediately before the final harvest following Richardson et al. (2002) by using a Minolta SPAD 502Plus chlorophyll meter (Konica Minolta. Inc., Co., Tokyo, Japan). All vegetable crops were carefully harvested 30 days after transplanting. Plants were washed with tap water, thoroughly rinsed with deionized water, and then divided into shoot and root. Selected growth parameters were measured in five plants (replicates; n = 5) per leafy vegetable/treatment. Shoot length was measured using a ruler. Fresh masses of shoots and roots were recorded. Leaves were separated from the shoots, and the number of leaves was counted. The total leaf area of each plant was measured using an area measurement system (ΔT area meter; Delta-T Devices Ltd. Co., Burwell, Cambridge, England). Shoots and roots were dried at 70 °C for 48 h in an electric oven (Yang et al., 2009), and the dry masses of shoots and roots were also recorded.

Table 1
Chemical characteristics of the nutrient solutions used in the study.

	Nutrient solution (mg·L^{-1})	Common range (mg·L^{-1}) (Ferguson et al., 1978; Jones Jr., 2016)
N	110	49 to 210
PO_4-P	48	15 to 192
K	150	–
Ca	110	80 to 200
Mg	30	24 to 60
Fe	0.35	–
Mn	0.35	–
Zn	0.06	0 to 0.146
B	0.1	–

2.6. Chemical analysis

The pH and EC values of the hydroponics nutrient solution were measured using a pH-EC meter (Orion 3 Star; Thermo, USA). Dried leaf samples with three replicates per vegetable/treatment were finely ground and digested using 10 mL 60% HNO_3 and 2 mL 30% H_2O_2 in a microwave oven (1600 W) at 175 ± 5 °C according to EPA Method 3052 (USEPA, 1995). The concentrations of calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), iron (Fe), manganese (Mn), and zinc (Zn) in leaf tissue were then analyzed using inductively coupled plasma/atomic emission spectroscopy (ICP-AES; Perkin Elmer Optima, USA).

2.7. Scanning electron microscopy

At harvest, samples of each hydroponics substrate were collected. Particles having a green color owing to algal growth were selected and frozen. The absorption of microalgae on each hydroponics substrate surface was confirmed by using ultra-high resolution scanning electron microscopy (HI-9116-0002; Hitachi, Tokyo, Japan); the microscope was operated at 15 keV with energy dispersive spectra (EDS) to quantify the elemental composition of the hydroponics substrates.

2.8. Statistical analysis

The mean growth parameters (n = 5) and foliar nutrient compositions (n = 3) of leafy vegetables were compared using one-way and two-way factorial analysis of variance and Tukey's honestly significant differences tests at a significance level of $P < 0.05$ (SAS, 2004). Data were analyzed using SAS/STAT 9.1. The standard error of the means was calculated from five and three replicates for each growth and foliar nutritional composition, respectively.

3. Results

3.1. Growth of leafy vegetables

Different substrates significantly affected the root and shoot fresh and dry mass and shoot length of all tested leafy vegetables; leaf area of dill and lettuce; and leaf chlorophyll content of dill, mallow, and lettuce (Table 2). However, the leaf number in all tested leafy vegetables; leaf area of cabbage, mallow, and tatsoi; and leaf chlorophyll content of cabbage and tatsoi were not significantly affected (Table 2).

The shoot length and the fresh and dry masses of shoots and roots of plants grown hydroponically in PL + RB substrate were remarkably increased; there was a 1.7-fold increase in these attributes compared to that in plants grown in PL substrate alone (Table 2). Mallow plants grown in RB substrate showed a significant increase in shoot length, fresh mass of shoots, and fresh/dry mass of roots by 1–1.4-fold more than those of plants grown in PL substrate. Furthermore, RB substrate increased the fresh and dry masses of tatsoi roots by 36.5% and 42.1%, respectively, compared with those of plants grown in PL substrate.

In contrast, cabbage, dill, and lettuce plants grown in RB substrate showed significant reduction in shoot length (0.1%–47.8%), fresh mass of shoots (16.3%–91.7%), dry mass of shoots (55.8%–87.1%), fresh mass of roots (22.7%–63.6%/), and dry mass of roots (19.4%–65.9%) compared to those of plants grown in PL substrate. Lettuce plants grown in hydroponics RB substrate showed the highest reduction in fresh/dry mass of shoots, which was 7.7-/12.1-fold less than that of plants grown in PL substrate.

Leaf area of mallow plants grown in PL + RB was increased by 1.6-fold compared to that in plants grown in PL substrate. The leaf number, leaf area (except for mallow), and leaf chlorophyll content of the investigated vegetables grown in PL + RB substrate were not significantly different from those of plants grown in PL substrate (Table 2). Further, the total leaf area of leafy vegetables grown in RB substrate was not different from that of plants grown in PL substrate, except 56% and 92.5% decrease in the leaf area of dill and lettuce plants, respectively.

The SPAD values showed that the chlorophyll contents of dill, mallow, and red lettuce leaves grown in RB substrate were significantly lower (by 17.4%–24.4%) than those of plants grown in PL substrate. The chlorophyll content of cabbage and tatsoi leaves grown in RB substrate was not significantly different from that of plants grown in PL substrate (Table 2). These results suggested that RB substrate decreased the growth and yield of cabbage, dill, and red lettuce plants, but not of mallow or tatsoi plants (Table 2).

Thus, the combination of perlite and rice husk biochar (PL + RB) substrate produced a remarkable increase in leafy vegetable yield compared to that of plants grown in PL substrate alone (Table 2).

3.2. Foliar nutrient composition

Changes in the contents of leaf nutrients (Ca, Mg, K, Na, Fe, Mn, and Zn) with the investigated plants and substrates of RB, PL, and their combination are shown in Table 3. Different substrates substantially affected the leaf Ca content in mallow and lettuce; leaf Mg content in cabbage, dill, and mallow; leaf K content in cabbage and mallow; leaf Fe content in dill, lettuce, and tatsoi; leaf Mn content in all tested leafy vegetables; leaf Zn content in cabbage, dill, mallow, and tatsoi; and leaf Na content in cabbage and dill (Table 3). However, the different substrates did not significantly affect leaf Ca content in cabbage and dill; leaf Mg content in lettuce and tatsoi; leaf K content in dill, lettuce, and tatsoi; leaf Fe content in cabbage and mallow; leaf Zn content in lettuce; and leaf Na content in mallow, lettuce, and tatsoi (Table 3).

The foliar content of Ca in plants grown in the investigated substrates showed similar trends, except mallow and lettuce grown in RB substrate. A respective 1.1-fold increase and 2.2-fold decrease in the Ca content of mallow and lettuce plants grown in RB substrate were observed compared to that of plants grown in PL substrate (Table 3). The levels of leaf Mg content in cabbage,

Table 2
Growth parameters of leafy vegetables grown in substrates of perlite, rice husk biochar, and a combination of the two in hydroponics systems (Mean ± standard error; n = 5).

Vegetable/substrate	Fresh mass (g)			Dry mass (g)			Shoot length (cm)	Number of leaves	Leaf area (cm^2)	Leaf chlorophyll content (SPAD)
	Shoot	Roots	Total	Shoot	Roots	Total				
Cabbage										
PL [a]	69.63 ± 8.62 b	2.28 ± 0.24 b	71.91 ± 8.77 b	4.41 ± 0.49 b	0.27 ± 0.03 b	4.68 ± 0.51 b	23.00 ± 0.95 b	19.80 ± 0.86 a	1035.80 ± 111.36 a	40.02 ± 1.27 a
RB	34.08 ± 2.61 c	1.76 ± 0.13 c	35.84 ± 2.67 c	1.95 ± 0.11 c	0.19 ± 0.02 c	2.14 ± 0.12 c	18.38 ± 0.63 c	15.25 ± 0.25 a	481.50 ± 39.63 a	35.15 ± 1.74 a
PL + RB	100.31 ± 7.45 a	4.04 ± 0.32 a	104.35 ± 7.70 a	5.23 ± 0.49 a	0.38 ± 0.04 a	5.61 ± 0.53 a	28.40 ± 1.29 a	18.80 ± 0.80 a	1401.80 ± 185.92 a	37.12 ± 1.04 a
Dill										
PL	46.18 ± 5.47 b	3.85 ± 0.61 c	50.03 ± 6.00 b	3.21 ± 0.37 b	0.43 ± 0.08 b	3.64 ± 0.45 b	44.30 ± 2.02 b	26.80 ± 1.07 a	374.80 ± 49.78 b	32.98 ± 2.17 a
RB	20.30 ± 2.92 c	3.93 ± 0.81 b	24.23 ± 3.23 c	1.38 ± 0.22 c	0.35 ± 0.07 c	1.73 ± 0.28 c	20.13 ± 1.91 c	26.75 ± 2.66 a	164.50 ± 30.18 c	24.93 ± 2.34 b
PL + RB	80.32 ± 5.97 a	13.41 ± 1.29 a	93.73 ± 7.04 a	4.76 ± 0.46 a	0.92 ± 0.10 a	5.68 ± 0.55 a	56.60 ± 3.64 a	31.20 ± 0.73 a	625.00 ± 38.92 a	32.62 ± 1.35 a
Mallow										
PL	18.34 ± 2.03 c	4.63 ± 0.64 a	22.97 ± 2.59 c	2.13 ± 0.24 b	0.39 ± 0.05 c	2.52 ± 0.29 b	13.40 ± 0.87 c	9.60 ± 0.40 a	474.80 ± 59.66 b	40.78 ± 0.89 a
RB	20.80 ± 1.34 b	5.13 ± 0.31 a	25.93 ± 1.62 b	1.97 ± 0.17 c	0.41 ± 0.03 b	2.38 ± 0.20 c	19.40 ± 1.34 b	12.60 ± 1.29 a	493.00 ± 24.31 b	33.70 ± 0.97 b
PL + RB	31.78 ± 1.81 a	7.64 ± 0.47 a	39.42 ± 2.19 a	2.95 ± 0.14 a	0.59 ± 0.03 a	3.55 ± 0.17 a	29.67 ± 1.94 a	9.83 ± 0.31 a	739.33 ± 41.37 a	35.75 ± 1.05 ab
Red Lettuce										
PL	55.30 ± 5.37 b	4.93 ± 0.34 b	60.23 ± 5.59 b	2.56 ± 0.27 b	0.36 ± 0.03 b	2.92 ± 0.30 b	30.00 ± 1.76 b	23.40 ± 1.91 a	1300.80 ± 116.23 a	33.96 ± 1.52 a
RB	4.58 ± 1.93 c	1.79 ± 0.53 c	6.37 ± 2.46 c	0.33 ± 0.13 c	0.12 ± 0.04 c	0.45 ± 0.17 c	15.67 ± 1.48 c	8.00 ± 1.15 b	98.00 ± 44.31 b	26.53 ± 1.89 b
PL + RB	90.34 ± 8.28 a	10.09 ± 0.64 a	100.43 ± 8.83 a	4.68 ± 0.28 a	0.64 ± 0.04 a	5.32 ± 0.31 a	31.40 ± 1.17 a	23.40 ± 0.68 a	2125.20 ± 159.82 a	36.32 ± 1.14 a
Tatsoi										
PL	56.17 ± 8.17 b	2.74 ± 0.40c	58.91 ± 8.55 b	2.94 ± 0.49 b	0.27 ± 0.04 c	3.21 ± 0.52 c	22.20 ± 1.93 b	32.00 ± 2.92 a	433.80 ± 70.42 a	51.30 ± 1.85 a
RB	47.02 ± 4.24 c	3.74 ± 0.49 b	50.76 ± 4.18 c	2.93 ± 0.20 c	0.38 ± 0.07 b	3.31 ± 0.23 b	17.70 ± 0.73 c	31.20 ± 2.13 a	422.20 ± 35.46 a	50.92 ± 2.40 a
PL + RB	84.25 ± 4.85 a	4.77 ± 0.42 a	89.02 ± 5.05 a	4.38 ± 0.24 a	0.39 ± 0.03 a	4.77 ± 0.26 a	23.80 ± 1.33 a	31.80 ± 1.07 a	754.80 ± 52.39 a	53.29 ± 2.48 a

Note: Different letters, for a vegetable, in each column indicate significant differences at $P \leq 0.05$.
[a] PL: perlite substrate, as a control; RB: rice husk biochar substrate; PL + RB: 1:1 ratio of PL to RB, v/v.

Table 3
Foliar nutrient composition (%) of leafy vegetables grown in substrates of perlite, rice husk biochar, and a combination of the two in hydroponics systems (Mean ± standard error; n = 3).

Substrate/vegetable	Ca	Mg	K	Fe	Mn	Zn	Na
Cabbage							
PL [a]	1.80 ± 0.090 a	0.45 ± 0.005 c	5.84 ± 0.149 b	0.008 ± 0.001 a	0.034 ± 0.003 b	0.011 ± 0.002 b	0.193 ± 0.019 a
RB	1.89 ± 0.123 a	0.54 ± 0.013 a	6.31 ± 0.114 b	0.008 ± 0.001 a	0.065 ± 0.004 a	0.034 ± 0.001 a	0.116 ± 0.014 b
PL + RB	2.08 ± 0.114a	0.49 ± 0.009 b	7.45 ± 0.063 a	0.008 ± 0.001a	0.058 ± 0.004 a	0.019 ± 0.002 b	0.106 ± 0.005 b
Dill							
PL	0.82 ± 0.043 a	0.29 ± 0.010 b	7.60 ± 0.210 a	0.008 ± 0.001 a	0.033 ± 0.001 c	0.009 ± 0.0002 c	0.231 ± 0.024 a
RB	0.93 ± 0.074 a	0.38 ± 0.023 a	7.47 ± 0.379 a	0.004 ± 0.0001 b	0.057 ± 0.002 a	0.030 ± 0.001 a	0.149 ± 0.008 b
PL + RB	0.93 ± 0.032 a	0.29 ± 0.010 b	8.48 ± 0.479 a	0.006 ± 0.0004 a	0.046 ± 0.002 b	0.013 ± 0.001 b	0.135 ± 0.010 b
Mallow							
PL	1.60 ± 0.050 b	0.34 ± 0.015 b	5.05 ± 0.248 b	0.008 ± 0.001 a	0.030 ± 0.001 c	0.007 ± 0.0003 b	0.067 ± 0.011 a
RB	2.15 ± 0.067 a	0.55 ± 0.016 a	6.42 ± 0.227 a	0.007 ± 0.0005 a	0.119 ± 0.010 a	0.029 ± 0.002 a	0.072 ± 0.006 a
PL + RB	1.58 ± 0.029 b	0.40 ± 0.020 b	6.58 ± 0.092 a	0.007 ± 0.001 a	0.061 ± 0.005 b	0.010 ± 0.001 b	0.057 ± 0.0003 a
Red Lettuce							
PL	0.87 ± 0.022 a	0.41 ± 0.009 a	6.62 ± 0.162 a	0.010 ± 0.001 a	0.038 ± 0.002 b	0.008 ± 0.001 a	0.087 ± 0.008 a
RB	0.62 ± 0.053 b	0.39 ± 0.029 a	5.98 ± 0.241 a	0.007 ± 0.0004 b	0.029 ± 0.004 b	0.010 ± 0.002 a	0.057 ± 0.004 a
PL + RB	0.86 ± 0.017 a	0.37 ± 0.023 a	6.60 ± 0.173 a	0.009 ± 0.0005 a	0.060 ± 0.002 a	0.010 ± 0.0004 a	0.081 ± 0.011 a
Tatsoi							
PL	1.57 ± 0.051 a	0.57 ± 0.040 a	7.16 ± 0.229 a	0.008 ± 0.0004 b	0.051 ± 0.002 b	0.021 ± 0.004 b	0.157 ± 0.007 a
RB	1.61 ± 0.104 a	0.57 ± 0.024 a	7.11 ± 0.172 a	0.006 ± 0.0001 c	0.076 ± 0.006 a	0.037 ± 0.006 a	0.102 ± 0.014 a
PL + RB	1.69 ± 0.161 a	0.55 ± 0.028 a	8.50 ± 0.620 a	0.010 ± 0.0002 a	0.067 ± 0.002 ab	0.027 ± 0.003 b	0.193 ± 0.081 a

Note: Different letters in each column, for a vegetable, indicate significant differences at $P \leq 0.05$.
[a] PL: perlite substrate, as a control; RB: rice husk biochar substrate; PL + RB: 1:1 ratio of PL to RB, v/v.

dill, and mallow plants grown in RB substrate were significantly increased by 1.2–1.6-fold compared to those of plants grown in PL or PL + RB. Cabbage and mallow plants grown in PL + RB substrate showed increased (by 1.3-fold) leaf K content compared to that of plants grown in PL substrate. Plants grown in RB and PL + RB substrates had more foliar content of Mn (a mean increase of 2- and 1.6-fold) compared with that in plants grown in PL substrate, respectively. The foliar content of Zn in cabbage, dill, and mallow plants grown in RB was significantly higher (by a mean of 3.5-fold) than that of plants grown in PL substrate, whereas the Na content was less in the leaves of cabbage and dill grown in RB and PL + RB substrates (Table 3).

3.3. Surface micromorphology of substrates

Surface micromorphology of PL, RB, and PL + RB substrates was investigated at the time of harvest by using SEM-EDS. Elliptical and spherical microalgae grew on the surface of PL substrate and in the nutrient solution (Fig. 2 and Table 4). Microalgal cells were clustered together and adsorbed on the surface of the PL substrate and in the micropores of RB particles (Fig. 2, black arrows). Salts were also precipitated as white clusters on the substrate surfaces. Freezing and drying of hydroponics substrates conserved algal cell shape and induced flattening into confluent homogeneous layers. Microalgal growth was greater on PL substrate than on RB substrate. Further, the PL + RB substrate induced the growth of some beneficial fungi/microorganisms (Table 4).

4. Discussion

The results of the present study indicated that we could obtain optimal yield and foliar nutritional composition of leafy vegetables grown in PL substrate (Tables 2 and 3). The PL is a well-drained and well-aerated growth substrate that supplies sufficient amount of O_2 for root respiration and helps improve root growth by preventing oxygen deficiency and/or CO_2 excess (Ok et al., 2015b; Schwarz, 2012). This allows the harvesting of high-quality vegetables with better yields (Tables 2 and 3). For instance, the hydroponic growth substrate and environmental conditions for hydroponically grown vegetables are the key factors that control the development of root systems (Schwarz, 2012). Root function in hydroponics systems depends on the growth substrate, which mechanically supports the plant root system and maintains the nutrient solution around the roots (Ok et al., 2015b). However, microalgae grew in hydroponics containers associated with PL substrate (Fig. S2). As reported by Schwarz and Gross (2004), microalgae such as *Chlamydomonas* spp. and *Scenedesmus* spp. were found in hydroponics containers. Algae are photosynthetically more efficient than crop plants, leading to the production of high yields of algal biomass at the expense of nutrients and oxygen in the hydroponics systems (Corbel et al., 2014; Magee et al., 2013; Sayre, 2010). Algal growth is an associated problem with PL substrate, which may reduce the economic value of vegetables due to the generation of toxins or odor.

Nonetheless, the combination of RB and PL as a hydroponics substrate was very effective in improving the yield of leafy vegetables, which was up to 2-fold higher than that of plants grown in PL substrate (Table 2). This might be attributed to the favorable buffering of the nutrient solution around plant roots in the PL + RB substrate similar to that in the commercial growing media (Kaudal et al., 2016). In addition, RB inhibited algal growth in hydroponics containers (Fig. S2). The adsorption capacity, porous structure, and surface area of RB likely increased nutrient availability to plants (Ahmad et al., 2014; Jeffery et al., 2015) and inhibited algal growth in the nutrient solution, thereby improving water quality and enhancing vegetable growth in the hydroponics systems. For instance, Beck et al. (2011) reported that BC improved the quality of runoff water by decreasing the discharge of total N and P and facilitating the retention of nutrients in the greenroof soil. For instance, water-holding capacity, cation exchange capacity, air void space, and stability are considered as important criteria for ideal hydroponics substrates (Ok et al., 2015b; Savidov et al., 2005; Van Os, 2000). It is noteworthy that the combination of RB and PL is an ideal growth substrate.

Foliar nutritional composition (Ca, Mg, K, Na, Fe, and Zn), chlorophyll content, and foliar nitrogen condition (represented by SPAD values) indicated optimal growth conditions and similar yield quality across PL + RB and PL substrates. The foliar nutrient compositions were in the range that allowed sufficient and adequate growth in accordance with the average concentration of mineral nutrients in plant dry matter, as reported by Epstein (1972) and

Jones Jr. (2016). When BC was used as a substrate for hydroponic growth, high-value tomato production was possible, suggesting a sustainable means of supporting the growth of food crops (Dunlop et al., 2015). The similar physicochemical properties of urban BC amended with coir peat substrate up to 60% (v/v) substrate and coir peat substrate alone (Kaudal et al., 2016) are consistent with the results for PL + RB substrate obtained in the present study. The release of nutrients from biochar may be one of the possible mechanisms for improving K, Mg, Mn, and Zn uptake by plant root systems in PL + RB substrate. Specifically, RB induced the growth of beneficial microorganisms on its surfaces, as verified by the SEM images (Fig. 2d), which may enhance the uptake of nutrients by plant. Similar mechanism for improving the uptake of macronutrients by maize plants in soil treated with biochar was described by Kim et al. (2017), Lee et al. (2015) and Rehman et al. (2016). Furthermore, RB in combination with PL substrate maintained favorable moisture and aeration around the plant root systems. Biochar increased the plant root biomass of maize under field conditions and micronutrients in bean plants, as reported by Abiven et al. (2015) and Puga et al. (2015).

SEM images indicated that RB inhibited the growth of algae by absorption in addition to reducing toxicity (Fig. 2). Microalgal cells formed clusters and were adsorbed on RB substrates, which restricts the nutrient uptake and subsequent algal growth. Inhibition of algal development can be a viable option for food safety, since it avoids the uptake of toxic compounds in hydroponically grown crops. The RB-induced decrease in the growth of microalgae in the nutrient solution in hydroponics containers might be attributed to their adsorption on the surface of RB. SEM images (Fig. 2) clearly indicate that RB absorbed algae on its surface and inhibited their growth in hydroponics containers, as has been reported by Magee et al. (2013). This might also contribute to the enhanced uptake of microelements such as K, Mg, Mn, and Zn by plant root systems of some leafy vegetables compared to that in PL substrate (Table 3).

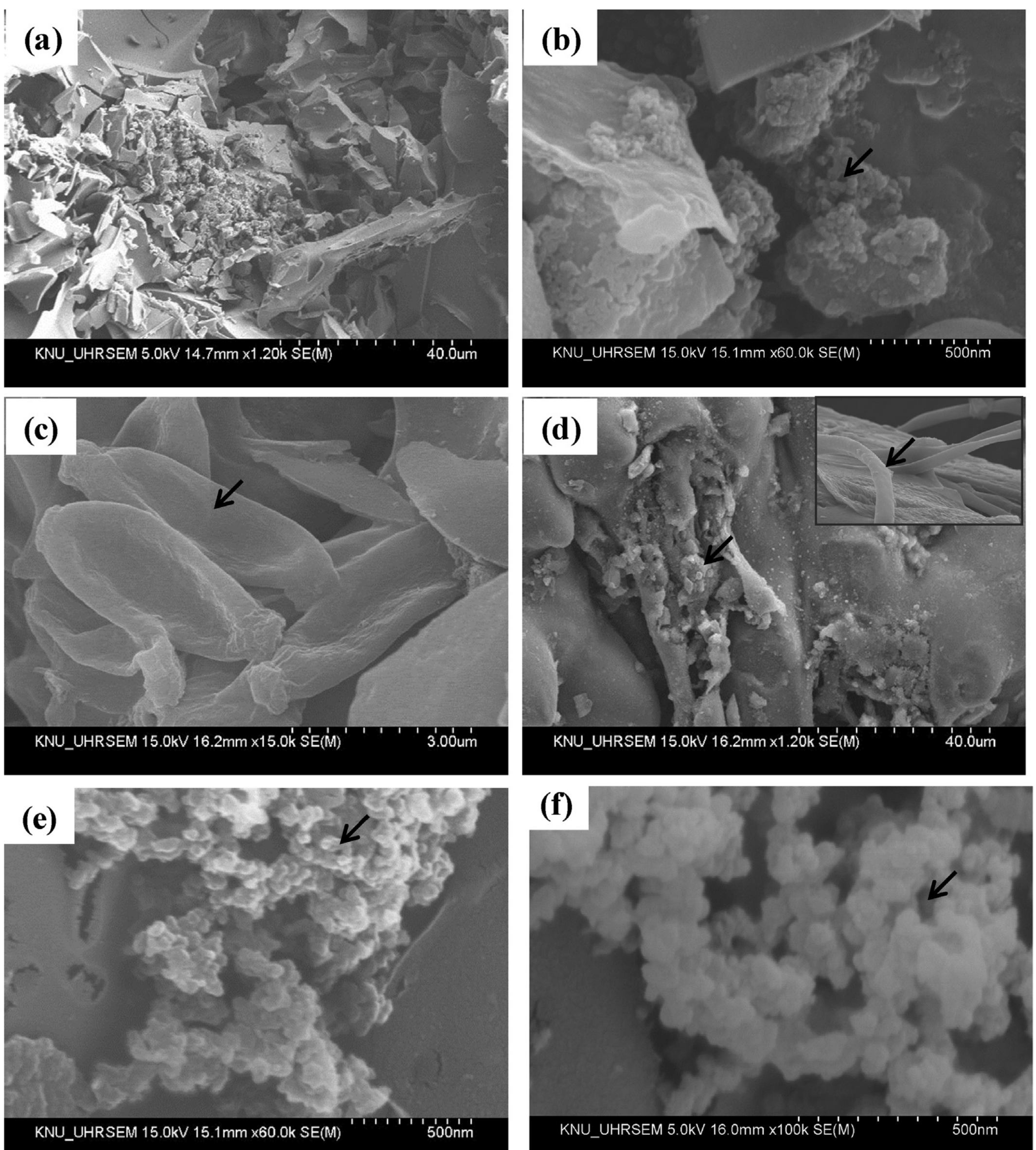
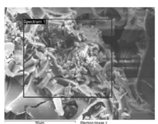
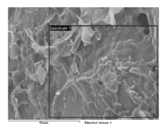
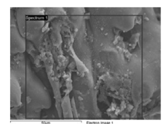
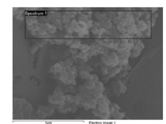
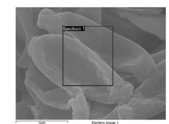


Fig. 2. Scanning electron micrographs of perlite (PL: a, b, c) and rice husk biochar (RB: d, e, f) substrates after a hydroponics system was run for 30 days to produce leafy vegetables. Various microalgal cells (as shown by black arrows) adsorbed on PL (b and c), and RB (e and f) substrates. Beneficial microorganisms on the surface of RB (d) are shown by black arrows.

Table 4
Elemental composition (%) determined using scanning electron microscopy-energy dispersive spectra analysis of perlite, rice husk biochar, and a combination of the two substrates after 30 days of hydroponics experiment.

Element	PL[a] alone	PL in PL + RB	RB alone	RB in PL + RB	microalgae
C	–	28.16	26.56	23.56	36.66
O	40.56	39.85	36.12	13.43	39.47
Na	1.56	1.26	–	–	1.11
Mg	0.26	–	–	0.13	0.26
Al	11.71	4.04	0.20	1.23	2.98
Si	35.89	23.23	35.77	56.12	16.45
S	1.87	–	–	–	0.68
K	5.04	2.39	0.37	1.98	1.87
Ca	2.15	0.55	0.41	0.64	0.52
Mn	–	–	–	1.29	–
Fe	0.96	0.52	0.57	1.62	–
SEM image					

[a] PL: perlite growth substrate, as a control; RB: rice husk biochar substrate; PL + RB: 1:1 ratio of PL to RB, v/v.

This better management of algal growth in the nutrient solutions in hydroponics system is recommended as a promising technology for ensuring food safety and high yield of healthy vegetables. For instance, Patterson and Harris (1983) reported a decrease of 1.2- to 6.2-fold in the dry mass of common bean plants grown in hydroponics culture with toxin concentration of 5 and 10 *Volvox* units per mL, respectively.

However, the yields of cabbage, dill, and red lettuce plants grown in RB substrate decreased because of the relatively high pH of the nutrient solution compared to those of plants grown in PL and PL + RB substrates, as mentioned above. This may be due to the low air void space available for root respiration compared to that in PL and PL + RB substrates. In addition, plant N availability might decrease in RB substrate because of the high adsorption of N on the RB surface (Rajkovich et al., 2012). Biochar application has been reported to hinder plant growth by reducing the amount of available inorganic N in soil owing to the high NO_3^- absorption efficiency of BC (Novak et al., 2010; Rajkovich et al., 2012). This mechanism was confirmed by the lower SPAD values (nitrogen status) of dill, mallow, and red lettuce leaves grown in RB substrate compared to those of plants grown in PL substrate. For instance, leaf N status (SPAD index) is an important indicator for optimal photosynthetic N-use efficiency (Moon et al., 2015). This might be the major reason for the significant reduction in the growth parameters and yield of dill, mallow, and red lettuce vegetables (Table 2).

Further, PL + RB substrate increased the contents of micronutrients in cabbage, dill, and mallow. Unlike for RB or PL substrate alone, Mn was one of the major elements on the RB surface when it was in combination with PL based on the quantitative elemental composition determined using SEM-EDS analysis (Table 3). Human consumption of vegetables containing high concentrations of these elements can enhance immune responses and contribute to oxidative stress resistance, because Mn and Zn act as coenzymes for antioxidant enzymes (MatÉs et al., 1999; Sen and Chakraborty, 2011). Antioxidant enzymes such as superoxide dismutases, catalases, glutathione peroxidases, reductases, and transferases ensure cell survival by protecting the mitochondria and DNA from oxidative stress (MatÉs et al., 1999; Sen and Chakraborty, 2011). Ensuring adequate amount of trace elements in vegetables grown hydroponically in the PL + RB substrate is essential for biofortification for ensuring sustainable global food supply (Dubey et al., 2016; Garnett, 2014).

5. Conclusions

The combination of PL and RB as a hydroponic growth substrate increased the yield of leafy vegetables by approximately 2-fold compared with that of plants grown in PL substrate alone. However, the use of RB substrate alone showed the lowest growth parameters of cabbage, dill, and red lettuce plants. The foliar nutritional composition of plants grown in PL + RB and PL substrates suggested the presence of optimal growth conditions for ensuring high yield. In addition, the RB substrate led to 1.2–3.5-fold higher leaf K, Mg, Mn, and Zn contents in most vegetable plants compared to those grown in PL substrate. Furthermore, RB decreased algal growth and thus ensured the safety of vegetables for human consumption. Hydroponic production of cabbage, mallow, and red lettuce vegetable in PL + RB substrate can be recommended using the current nutrient solution. The PL + RB substrate can be recommended as a promising hydroponics substrate that can facilitate the management of algal growth in nutrient solutions and ensure the high yield of safe and healthy vegetables. To our knowledge, this is the first study to examine the use of PL + RB as an alternative and effective hydroponics substrate for the better management of algal growth in nutrient solutions and high production of leafy vegetables. Further research on applications of different biochars in combination with industrial hydroponics substrates for the better management of algal growth and plant pathogens in the nutrient solutions and high production of leafy vegetables is needed.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jclepro.2017.04.070.

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